

USMLE Step 1

Microbiology and Immunology Notes

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MICROBIOLOGY

Author

Louise Hawley, Ph.D.
Assistant Professor
Department of Medical Microbiology and Immunology
University of Minnesota
Duluth School of Medicine
Duluth, MN

Executive Editor and Coauthor

Don Dunn, Ph.D.
Professor, Microbiology and Immunology
Oklahoma University Health Science Center
Oklahoma City, OK

Contributors

Richard Hyde, Ph.D.
Professor Emeritus
Department of Microbiology
University of Oklahoma
College of Medicine
Oklahoma City, OK

Mary Ruebush, Ph.D.
Microbiology/Immunology
Adjunct Professor of Microbiology
Montana State University
Bozeman, MT

Kenneth H. Ibsen, Ph.D.
Director of Academic Development
Kaplan Medical
Professor Emeritus Biochemistry
University of California-Irvine
Irvine, CA

IMMUNOLOGY

Author/Executive Editor

Don Dunn, Ph.D.
Microbiology/Immunology
Professor, Microbiology and Immunology
Oklahoma University Health Science Center
Oklahoma City, OK

Contributors

Richard Hyde, Ph.D.
Microbiology/Immunology
Professor Emeritus, Department of Microbiology
University of Oklahoma, College of Medicine
Oklahoma City, OK

Thomas Lint, Ph.D.
Immunology
Professor of Immunology/Microbiology
Rush Medical College
Chicago, IL

Mary Ruebush, Ph.D.
Microbiology/Immunology
Adjunct Professor of Microbiology
Montana State University
Bozeman, MT

Kenneth H. Ibsen, Ph.D.
Director of Academic Development
Kaplan Medical
Professor Emeritus Biochemistry
University of California-Irvine
Irvine, CA

Executive Director of Curriculum

Richard Friedland, M.D.

Director of Publishing and Media

Michelle Covello

Director of Medical Illustration

Christine Schaar

Medical Illustrators

Rich LaRocco and Christine Schaar

Managing Editor

Kathlyn McGreevy

Production Editor

Ruthie Nussbaum

Production Artist

Michael Wolff

Cover Design

Joanna Myllo

Cover Art

Christine Schaar

Table of Contents

Preface vii

Section I: Microbiology

Chapter 1: General Microbiology 1

Chapter 2: Medically Important Bacteria 9

Chapter 3: Medically Important Fungi 111

Chapter 4: Medical Parasitology 127

Chapter 5: Medically Important Viruses 145

Chapter 6: Microbial Genetics/Drug Resistance 205

Chapter 7: Clinical Infectious Disease 239

Chapter 8: Comparative Microbiology 263

Chapter 9: Flow Charts/Clue Sheets 277

Section II: Immunology

Introduction 293

Chapter 1: Innate Immunity 295

Chapter 2: Acquired (Adaptive) Immunity 305

Chapter 3: Immunoglobulins and T-Cell Receptors 311

Chapter 4: Immunoglobulin and T-Cell Receptor (TCR) Genes 329

Chapter 5: Antigens and Immunogens 337

Chapter 6: Major Histocompatibility Complex (MHC)	341
Chapter 7: The Lymphoid System	349
Chapter 8: The Immune Response	365
Chapter 9: T-Cell Subsets and Interleukins	375
Chapter 10: Cell-Mediated Immunity	385
Chapter 11: Complement	395
Chapter 12: Failures of the Immune System: Hypersensitivity	401
Chapter 13: Failures of the Immune System: Immunodeficiency	419
Chapter 14: Failures of the Immune System: Autoimmunity	429
Chapter 15: Regulation and Tolerance	435
Chapter 16: Transplantation Immunology	437
Chapter 17: Immunology Laboratory Procedures	441

Preface

These seven volumes of Lecture Notes represent a yearlong effort on the part of the Kaplan Medical faculty to update our curriculum to reflect the most-likely-to-be-tested material on the current USMLE Step 1 exam. Please note that these are Lecture Notes, not review books. The Notes were designed to be accompanied by faculty lectures—live, on video, or on the web. Reading these Notes without accessing the accompanying lectures is not an effective way to review for the USMLE.

To maximize the effectiveness of these Notes, annotate them as you listen to lectures. To facilitate this process, we've created wide, blank margins. While these margins are occasionally punctuated by faculty high-yield “margin notes,” they are, for the most part, left blank for your notations.

Many students find that previewing the Notes prior to the lecture is a very effective way to prepare for class. This allows you to anticipate the areas where you'll need to pay particular attention. It also affords you the opportunity to map out how the information is going to be presented and what sort of study aids (charts, diagrams, etc.) you might want to add. This strategy works regardless of whether you're attending a live lecture or watching one on video or the web.

Finally, we want to hear what you think. What do you like about the notes? What do you think could be improved? Please share your feedback by E-mailing us at medfeedback@kaplan.com.

Thank you for joining Kaplan Medical, and best of luck on your Step 1 exam!

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SECTION I

Microbiology

General Microbiology

1

What the USMLE Requires You to Know

- Differences among viruses, fungi, bacteria, and parasites
- Differences between eukaryotic and prokaryotic cells
- Important normal flora
- Major mechanisms of pathogenicity

MAJOR MICROBIAL GROUPS

Table I-1-I. Comparison of Medically Important Microbial Groups

Characteristic	Viruses*	Bacteria	Fungi	Parasites
Diameter**	Minute (0.02–0.3 μ)	Small (0.3–2 μ)	3–10 μ	15–25 μ (trophozoites)
Cell type	Acellular No nucleus (not cell)	Prokaryotic cells Nucleoid region: no nuclear membrane	Eukaryotic cells Nucleus with nuclear membrane	Eukaryotic cells
	DNA or RNA 1 nucleocapsid except in segmented or diploid viruses	DNA and RNA 1 chromosome No histones	DNA and RNA More than 1 chromosome	
	Replicates in host cells	DNA replicates continuously	G and S phases	
		Exons, no introns	Introns and exons	
	Some have poly- cistronic mRNA*** and post translational cleavage	Mono- and poly- cistronic mRNA	Monocistronic RNA	
	Uses host organelles; obligate intracellular parasites	No membrane bound organelles	Mitochondria and other membrane-bound organelles	
	No ribosomes	70s ribosomes (30s+50s)	80s ribosomes (40s+60s)	
Cellular membrane	Some are enveloped: but no membrane function	Membranes have no sterols except Mycoplasmas , which have cholesterol	Membrane ergosterol is major sterol.	Sterols such as cholesterol
Cell wall	No cell wall	Peptidoglycan	Complex carbo- hydrate cell wall : chitin, glucans, or mannans	No cell wall
Replication	Make and assemble viral components	Binary fission (asexual)	Cytokinesis with mitosis / meiosis	Cytokinesis with mitosis / meiosis

*Besides viruses, two other acellular forms exist:

- Viroids: obligate intracellular but acellular parasites of plants; naked RNA; no human diseases.
- Prions: acellular particles associated with Kuru, etc.; insensitive to nucleases.

Abnormal prion proteins (PrP) modify folding of normal prion-like proteins found in the body (coded for by human genes).

**If the diameter of a cell described in a clinical case is >2 μ, then it is probably a eukaryotic cell.

***Polycistronic mRNA carries the genetic code for several proteins. (It has multiple Shine-Dalgarno sites.)

Epidemiology

Normal Flora

- Is found on body surfaces contiguous with the outside environment
- Is semi-permanent, varying with major life changes
- Can cause infection
 - misplaced, e.g., fecal flora to urinary tract or abdominal cavity, or skin flora to catheter
 - or, if person becomes compromised, normal flora may overgrow (oral thrush)
- Contributes to health
 - protective host defense by maintaining conditions such as pH so other organisms may not grow
 - serve nutritional function by synthesizing: vitamin K and B vitamins

In A Nutshell

Definitions

Carrier: person colonized potential pathogen without overt disease.

Bacteremia: bacteria in bloodstream without overt clinical signs.

Septicemia: bacteria in bloodstream (multiplying) with clinical symptoms.

Table I-1-2. Important Normal Flora

Site	Common or Medically Important Organisms	Less Common but Notable Organisms
Blood, internal organs	None, generally sterile	
Cutaneous surfaces including urethra and outer ear	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus aureus</i> , <i>Corynebacteria</i> (diphtheroids), streptococci, anaerobes, e.g., peptostreptococci, yeasts (<i>Candida</i> spp.)
Nose	<i>Staphylococcus aureus</i>	<i>S. epidermidis</i> , diphtheroids, assorted streptococci
Oropharynx	Viridans streptococci including <i>Strep. mutans</i> ¹	Assorted streptococci, nonpathogenic <i>Neisseria</i> , nontypeable ² <i>Haemophilus influenzae</i>
Gingival crevices	Anaerobes: <i>Prevotella</i> , <i>Fusobacterium</i> , <i>Streptococcus</i> , <i>Actinomyces</i>	
Stomach	None	
Colon (microaerophilic/anaerobic)	Babies; breast-fed only: <i>Bifidobacterium</i> Adult: <i>Bacteroides</i> (Predominant organism) <i>Escherichia</i> <i>Bifidobacterium</i>	<i>Lactobacillus</i> , streptococci <i>Eubacterium</i> , <i>Fusobacterium</i> , <i>Lactobacillus</i> , assorted Gram-negative anaerobic rods, <i>Streptococcus faecalis</i> and other streptococci
Vagina	<i>Lactobacillus</i> ³	Assorted streptococci, Gram-negative rods, diphtheroids, yeasts

¹*S. mutans* secretes a biofilm that glues it and other oral flora to teeth, producing dental plaque.

²(Nontypeable for *Haemophilus* means no capsule.)

³Group B streptococci colonize vagina of 15–20% of women and may infect the infant during labor or delivery, causing septicemia and/or meningitis (as may *E. coli* from fecal flora).

Pathogenicity (Infectivity and Toxicity)

Major Mechanisms

Colonization

(Important unless organism is traumatically implanted.)

Adherence to cell surfaces involves

- **Pili/fimbriae:** primary mechanism in most gram-negative cells.
- **Teichoic acids:** primary mechanism of gram-positive cells.
- **Adhesins:** colonizing factor adhesins, pertussis toxin, and hemagglutinins.
- **IgA proteases:** cleaved Fc portion may coat bacteria and bind them to cellular Fc receptors.

Adherence to inert materials, biofilms: *Staph. epidermidis*, *Streptococcus mutans*

Avoiding immediate destruction by host defense system:

- **Anti-phagocytic surface components** (inhibit phagocytic uptake):
 - Capsules/slime layers:
 - Streptococcus pyogenes* M protein
 - Neisseria gonorrhoeae* pili
 - Staphylococcus aureus* A protein
- **IgA proteases**, destruction of mucosal IgA: *Neisseria*, *Haemophilus*, *S. pneumoniae*

“Hunting and gathering” needed nutrients:

- Siderophores steal (chelate) and import iron.

Ability to Survive Intracellularly

- **Evading intracellular killing by professional phagocytic cells** allows intracellular growth:
 - *M. tuberculosis* survives by inhibiting phagosome-lysosome fusion.
 - *Listeria* quickly escapes the phagosome into the cytoplasm **before** phagosome-lysosomal fusion.

Invasins: surface proteins that allow an organism to bind to and invade normally non-phagocytic human cells, escaping the immune system. Best studied invasin is on *Yersinia pseudotuberculosis* (an organism causing diarrhea).

Damage from viruses is largely from intracellular replication, which either kills cells, transforms them or, in the case of latent viruses, may do no noticeable damage.

Inflammation or Immune-Mediated Damage

Examples

- **Cross-reaction of bacterial induced antibodies with tissue antigens** causes disease. Rheumatic fever is one example.
- **Delayed hypersensitivity and the granulomatous response** stimulated by the presence of intracellular bacteria is responsible for neurological damage in leprosy, cavitation in tuberculosis, and fallopian tube blockage resulting in infertility from *Chlamydia* PID (pelvic inflammatory disease).
- **Immune complexes** damage the kidney post streptococcal acute glomerulonephritis.

Note

Mnemonic

Streptococcus pneumoniae
Klebsiella pneumoniae
Haemophilus influenzae
Pseudomonas aeruginosa
Neisseria meningitidis
Cryptococcus neoformans

(Some Killers Have Pretty Nice Capsules)

Note

Intracellular organisms

- Elicit different immune responses
- Different pathology
- Different antibiotics
- Different cultural techniques

- **Peptidoglycan-teichoic acid** (large fragments) of Gram-positive cells:
 Serves as a structural toxin released when cells die.
 Chemotactic for neutrophils.

Physical Damage

Swelling from infection in a fixed space damages tissues; examples: meningitis and cysticercosis.

Large physical size of organism may cause problems; example: *Ascaris lumbricoides* blocking bile duct.

Aggressive tissue invasion from *Entamoeba histolytica* causes intestinal ulceration and releases intestinal bacteria, compounding problems.

Toxins

Toxins may aid in invasiveness, damage cells, inhibit cellular processes, or trigger immune response and damage.

Endotoxin (Lipopolysaccharide = LPS)

- LPS is part of the **Gram-negative outer membrane**.
- **Toxic portion is lipid A**: generally not released (and toxic) until death of cell.
 Exception: *N. meningitidis*, which over-produces outer membrane fragments.
- LPS is **heat stable** and not strongly immunogenic so it **cannot be converted to a toxoid**.
- Mechanism
 - LPS **activates macrophages**, leading to release of TNF-alpha, IL-1, and IL-6.
 - IL-1 is a major mediator of fever.
 - Macrophage activation and products lead to tissue damage.
 - Damage to the endothelium from **bradykinin-induced vasodilation** leads to shock.
 - **Coagulation (DIC)** is mediated through the **activation of Hageman factor**.

Exotoxins

- are **protein toxins**, generally quite toxic and **secreted by bacterial cells** (some Gram +, some Gram -)
- can be **modified** by chemicals or heat to produce a **toxoid** that still is **immunogenic**, but **no longer toxic** so can be used as a vaccine
- A-B (or "two") **component** protein toxins
 - B component binds to **specific cell receptors** to facilitate the internalization of A.
 - A component is the **active (toxic) component** (often an enzyme such as an ADP ribosyl transferase).
 - Exotoxins may be subclassed as enterotoxins, neurotoxins, or cytotoxins.
- **Cytolysins**: lyse cells from outside by damaging membrane.
 - *C. perfringens* alpha toxin is a **lecithinase**. *makes gangrene*
 - *Staphylococcus aureus* alpha toxin inserts itself to form **pores** in the membrane.

Table I-1-3. Major Exotoxins

	Organism (Gram)	Toxin	Mode of Action	Role in Disease
Protein Inhibitors	<i>Corynebacterium diphtheriae</i> (+)	Diphtheria toxin	ADP ribosyl transferase; inactivates EF-2; 1' targets: heart/nerves/epithelium	Inhibits eukaryotic cell protein synthesis
	<i>Pseudomonas aeruginosa</i> (-)	Exotoxin A	ADP ribosyl transferase; inactivates EF-2; 1' target: liver.	Inhibits eukaryotic cell protein synthesis
	<i>Shigella dysenteriae</i> (-)	Shiga toxin	Interferes with 60s ribosomal subunit	Inhibits protein synthesis in eukaryotic cells. Enterotoxic, cytotoxic, and neurotoxic
	Enterohemorrhagic <i>E. coli</i> (EHEC)	Verotoxin (a shiga-like toxin)	Interferes with 60s ribosomal subunit	Inhibits protein synthesis in eukaryotic cells
Neurotoxins	<i>Clostridium tetani</i> (+)	Tetanus toxin	Blocks release of the inhibitory transmitters glycine and GABA	Inhibits neurotransmission in inhibitory synapses
	<i>Clostridium botulinum</i> (+)	Botulinum toxin	Blocks release of acetylcholine	Inhibits cholinergic synapses
Endotoxin Enhancers	<i>Staphylococcus aureus</i> (+)	TSST-1	Pyrogenic, decreases liver clearance of LPS, superantigen	Fever, increased susceptibility to LPS, rash, shock, capillary leakage
	<i>Streptococcus pyogenes</i> (+)	Exotoxin A, a.k.a.: erythrogenic or pyrogenic toxin	Similar to TSST-1	Fever, increased susceptibility to LPS, rash, shock, capillary leakage, cardiotoxicity
cAMP Inducers	Enterotoxigenic <i>Escherichia coli</i> (-)	Heat labile toxin (LT)	LT stimulates an adenylate cyclase by ADP ribosylation of GTP binding protein	Both LT and ST promote secretion of fluid and electrolytes from intestinal epithelium
	<i>Vibrio cholerae</i> (-)	Cholera toxin	Similar to <i>E. coli</i> LT	Profuse, watery diarrhea
	<i>Bacillus anthracis</i> (+)	Anthrax toxin (3 proteins make 2 toxins)	EF = edema factor = adenylate cyclase LF = lethal factor PA = protective antigen (B component for both)	Decreases phagocytosis; causes edema, kills cells
	<i>Bordetella pertussis</i> (-)	Pertussis toxin	ADP ribosylates G _i , the negative regulator of adenylate cyclase → increased cAMP	Histamine-sensitizing Lymphocytosis promotion Islet activation
Cytolysins	<i>Clostridium perfringens</i> (+)	Alpha toxin	Lecithinase	Damages cell membranes; myonecrosis
	<i>Staphylococcus aureus</i> (+)	Alpha toxin	Toxin intercalates forming pores	Cell membrane becomes leaky

Chapter Summary

The size, cell type, cellular membrane, cell walls, and modes of replication of viruses, bacteria, fungi, and protozoan parasites are compared, as are the differences between prokaryotes and eukaryotes. There is also a brief consideration of the properties and pathogenicity of prions and plant viroids.

The important normal microflora typically associated with various body sites are described.

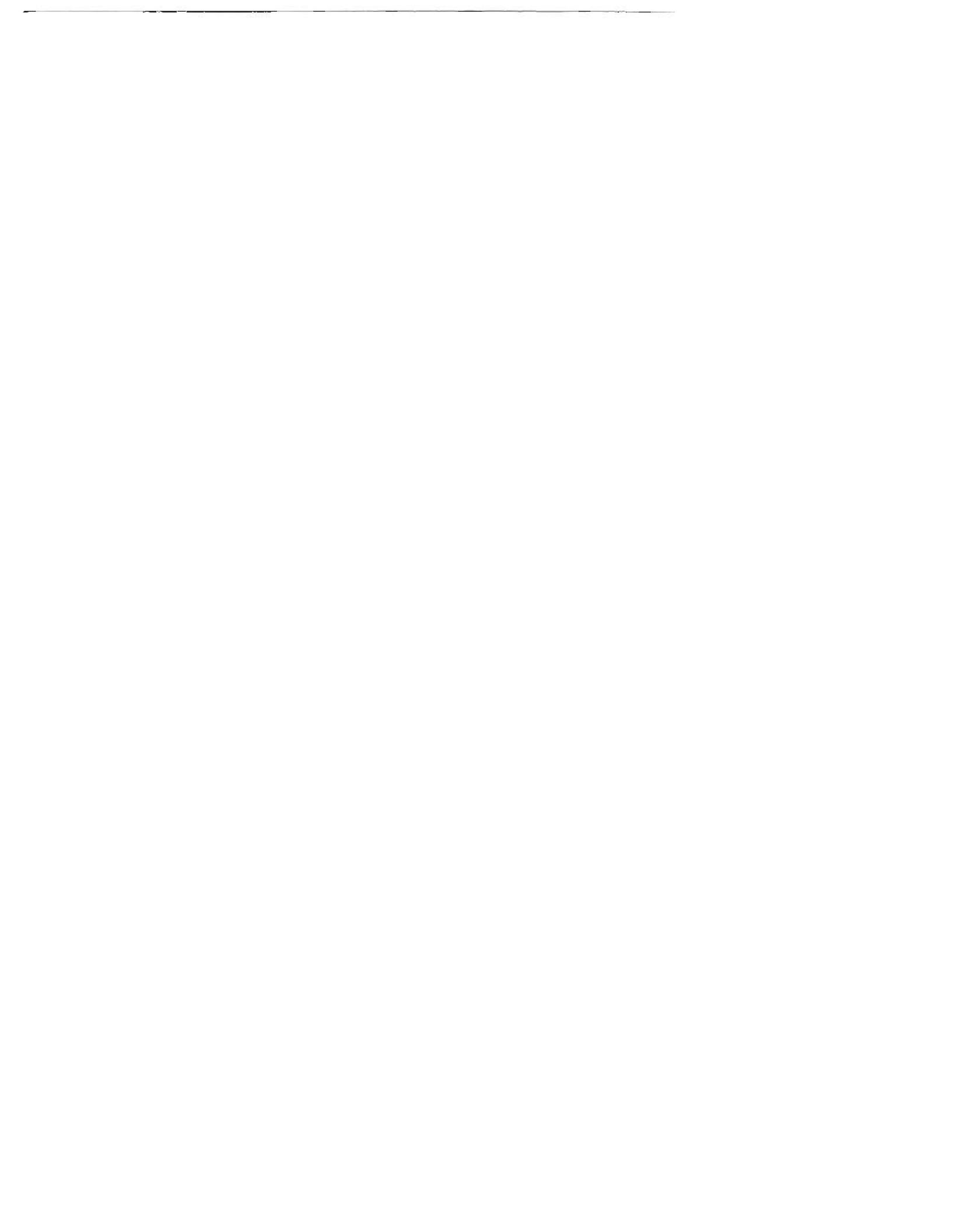
For the successful induction of pathogenicity, microorganisms must be infective and toxic. Infectivity requires colonization, which in turn requires adhesion, the avoidance of destruction by the host, and the invasion of cells. Modes of toxicity include damage caused by immune-mediated inflammation, physical-damage-associated tissue invasion, and/or production of endo- or exotoxins.

Review Questions

1. Your laboratory isolates an entirely new and unknown pathogen from one of your patients, which has all the characteristics of an aerobic filamentous fungus except that the ribosomes are prokaryotic. Unfortunately, your patient with this pathogen is very ill. Which agent would most likely be successful in treating your patient?
 - A. Third generation of cephalosporins
 - B. Isoniazid
 - C. Metronidazole
 - D. Careful limited usage of Shiga toxin
 - E. Tetracycline
2. Mitochondria are missing in
 - A. Filamentous fungi
 - B. Protozoan parasites
 - C. Viruses
 - D. Yeasts
 - E. Cestodes

Answers

1. **Answer: E.** The cephalosporin that inhibits prokaryotic cell peptidoglycan cross linkage will not likely be effective against the complex carbohydrate cell wall. Isoniazid, which appears to inhibit mycolic acid synthesis, also would not likely work. Metronidazole would not work on an aerobic organism. Shiga toxin is only effective against eukaryotic ribosomes. Tetracycline (the correct answer) would have the greatest chance of success. However, it may not be taken up by the cell, or the cell could have an effective pump mechanism to get rid of it quickly.
2. **Answer: C.** Mitochondria are found only in eukaryotic organisms so both viruses and bacteria lack them.



Medically Important Bacteria

2

What the USMLE Requires You to Know

The type of disease (major diseases) from presenting symptoms

- You must know the common etiologic agents of the disease and be able to determine the causative agent of the particular case from case clues.
- No distinguishing clues given? Know most common agent(s).
- Epidemiologic clues, symptomatic clues, or organism information given? Know the specific agent.
- Be able to answer basic science questions about disease or organism, predisposing conditions, epidemiology, mechanism of pathogenicity, and major tests used in identification.

The basic science used as clues or tested directly

Morphology

- Gram reaction, basic morphology, motility (*Listeria*), spore formation (*Bacillus* and *Clostridium*)

Physiology

- Obligate aerobes/anaerobes
- A few specific fermentations
- A few specific enzymes (oxidase, urease, catalase, coagulase, superoxide dismutase, hemolysins)
- How bacterial cells grow, divide, and die

Bacterial structures

- Their composition, function, and role in disease

Determinants of pathogenicity

- Toxins
- Factors aiding in invasiveness, pathogenicity, or immune system evasion
- Intracellular parasites (obligate and facultative)

Epidemiology/transmission

- Know how each major disease is acquired
- Particularly important for organisms with animal or arthropod vectors

Note

Nomenclature

Latin bacterial family names have "-aceae," e.g., Enterobacteriaceae.

Genus and species names are italicized and abbreviated, e.g., *Enterobacter aerogenes* = *E. aerogenes*.

(Continued)

What the USMLE Requires You to Know (continued)

Laboratory diagnosis

- Serologic/skin tests: specific serology for Syphilis
- Stains: acid fast and Gram
- Unusual growth requirements
- Specific media
- Steps in Gram stain and acid fast stain

Diseases

- Common presenting symptoms
- Stages of multistage diseases
- Common complications

Treatment (drug of choice – pharmacology)/prevention

(vaccination, public health, and prophylaxis, where regularly used)

(Basically the same for all other pathogens, too!)

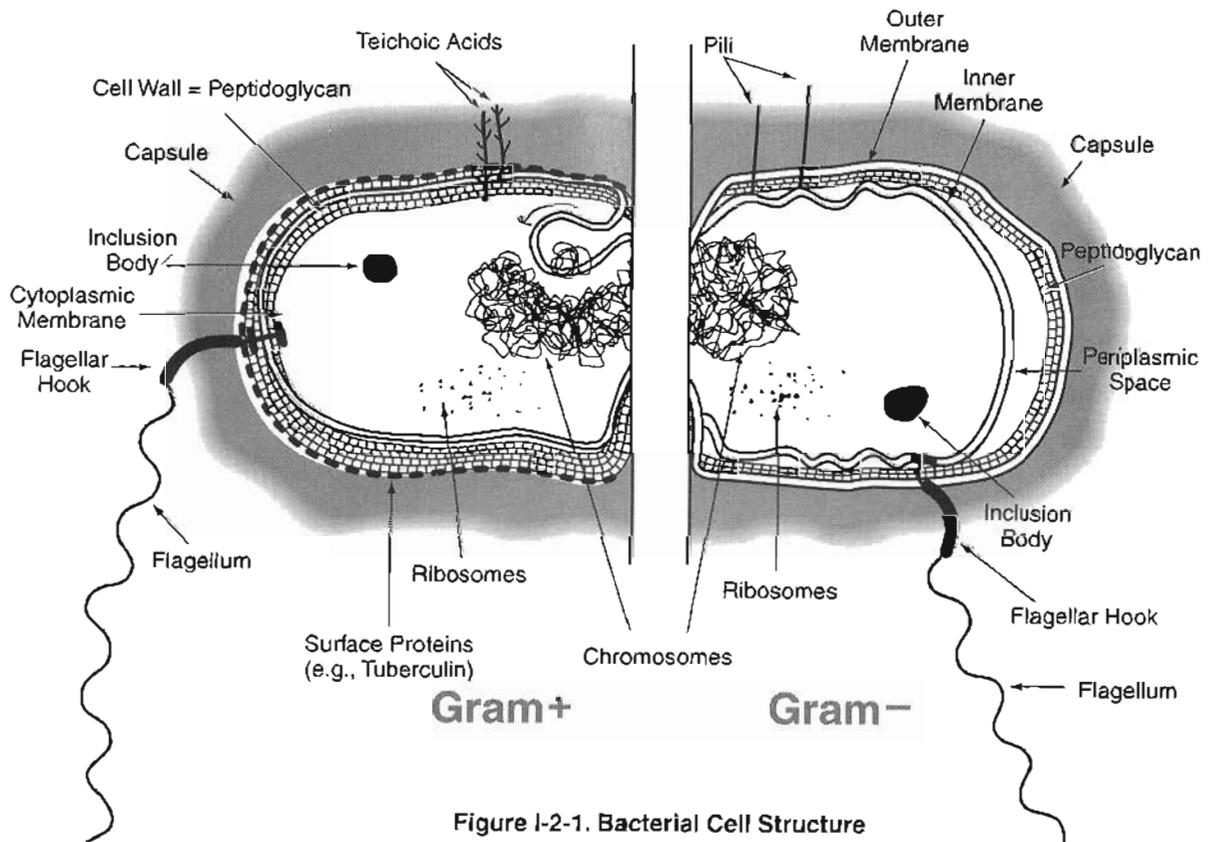


Figure I-2-1. Bacterial Cell Structure

Table I-2-1. Bacterial Envelope (All the Concentric Surface Layers of the Bacterial Cell)

Envelope Structure	Gram + or -	Chemical Composition	Function
Capsule (Non-essential) = Slime = Glycocalyx	Both Gram + Gram -	Polysaccharide gel*	Pathogenicity factor protecting against phagocytosis until opsonized; immunogenic**
Outer membrane	Gram-negative only	Phospholipid/proteins: Lipopolysaccharide Lipid A Polysaccharide	Hydrophobic membrane: LPS = endotoxin Lipid A = toxic moiety PS = immunogenic portion
		Outer membrane proteins	Attachment, virulence, etc.
		Protein porins	Passive transport
Cell wall = peptidoglycan	Gram + (thick) Gram - (thin)	Peptidoglycan—open 3-D net of: N-acetyl-glucosamine N-acetyl-muramic acid amino acids (DAP)	Rigid support, cell shape, and protection from osmotic damage Synthesis inhibited by penicillins and cephalosporins Confers Gram reaction
	Gram-positive only	Teichoic acids***	Immunogenic induces TNF-alpha, IL-1 Attachment
	Acid-fast only	Mycolic acids	Acid-fastness Resistance to drying and chemicals
Periplasmic space	Gram-negative only	“Storage space” between the inner and outer membranes	Enzymes to break down large molecules, (β-lactamases) Aids regulation of osmolarity
Cytoplasmic membrane = inner membrane = cell membrane = plasma membrane	Gram + Gram -	Phospholipid bilayer with many embedded proteins	Hydrophobic cell “sack” Selective permeability and active transport Carrier for enzymes for: Oxidative metabolism Phosphorylation Phospholipid synthesis DNA replication Peptidoglycan cross linkage Penicillin Binding Proteins (PBPs)

* Except *Bacillus anthracis*, which is a polypeptide of poly D-glutamate.

** Except *S. pyogenes* (hyaluronic acid) and type B *N. meningitidis* (sialic acid), which are nonimmunogenic.

*** Teichoic acid: polymers of ribitol or glycerol, bound to cell membrane or peptidoglycan.

Table I-2-2. Other Surface Structures of the Bacterial Cell

Pilus or fimbria 1. Common 2. Sex 3. Virulence	Primarily Gram - *	Glycoprotein (pilin)	Adherence to cell surfaces, including attachment to other bacteria during conjugation
Flagellum	+ and -	Protein (flagellin)	Motility
Axial filaments (internal flagellum)	Spirochetes Gram -	Protein	Motility

*M-protein of group A Strep described as diffuse fimbriate layer or fimbriae.

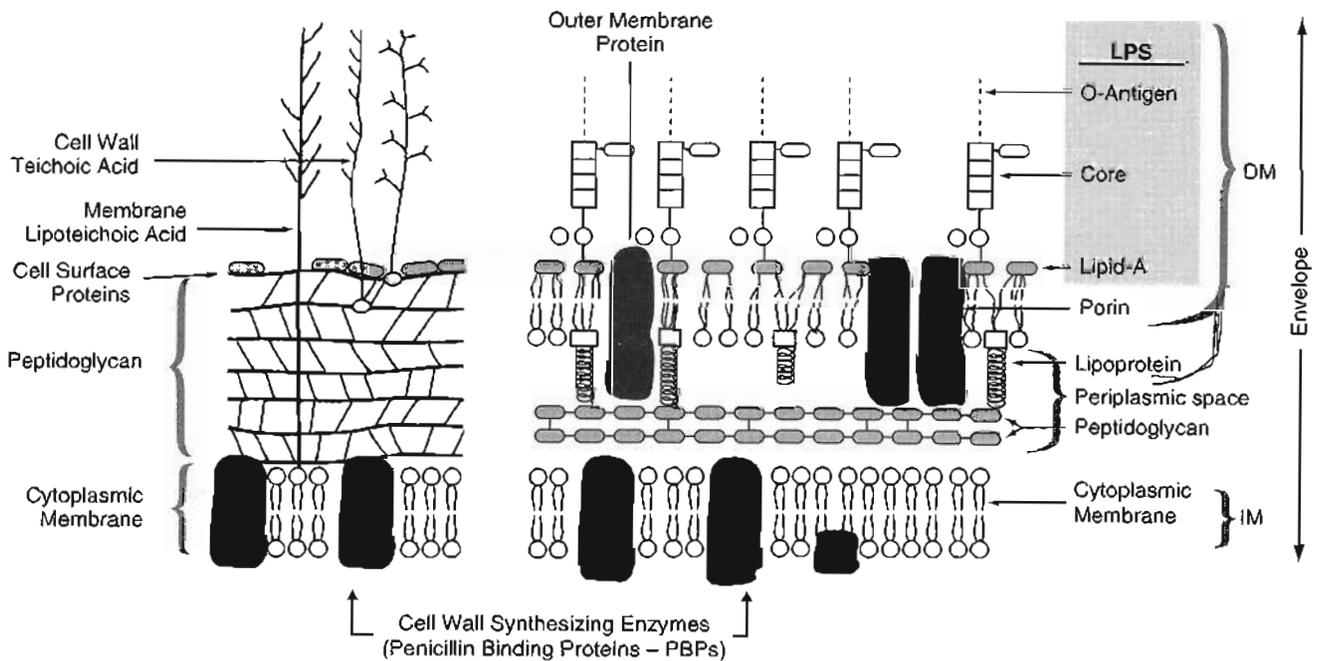


Figure I-2-2. Details of Cell Envelope and Peptidoglycan

STAINS

Table I-2-3. Gram Stain

Reagent	Gram-Positive	Gram-Negative
Crystal Violet (a very intense purple, small dye molecule)	Purple/Blue	Purple/Blue
Gram's Iodine	Purple/Blue (a large dye complex)	Purple/Blue (a large dye complex)
Acetone or Alcohol	Purple/Blue	Colorless
Safranin (a pale dye)	Purple/Blue	Red/Pink

All cocci are Gram-positive except *Neisseria* and *Moraxella*.

All spore formers are Gram-positive.

Background in stain modified for tissues will be pale red.

Table I-2-4. Ziehl-Neelsen Acid Fast Stain (or Kinyoun)

Reagent	Acid Fast	Non-Acid Fast*
Carbol Fuchsin with heat**	Red (Hot Pink)	Red (Hot Pink)
Acid Alcohol	Red	Colorless
Methylene Blue***	Red	Blue

* *Mycobacterium* is acid fast. *Nocardia* is partially acid fast. All other bacteria are non-acid fast except *Legionella micdadei*. Two protozoan parasites (*Cryptosporidium* and *Isospora*) have acid fast oocysts.

** Without the heat, the dye would not go in the mycobacterial cells.

*** Sputa and human cells will be blue.

Processing Sputa

(In labs with fluorescent microscopes)

Screen Sputum Using Auramine-Rhodamine Fluorescent Stain

- Mycobacteria, *Nocardia*, and some other bacteria fluoresce a bright apple green on a black background.
- No antibody is involved.
- Test is sensitive (picks up high percent of AFB) but not very specific (picks up others, too).
- Negatives can be screened in 5 minutes (as opposed to 20 minutes for negative acid fast stains).

All positives are confirmed by acid fast stain.

Table I-2-5. Internal Bacterial Structures*

Structure	Cell Type	Chemical Composition	Function
Nucleoid region No membrane No histones No introns	Gram + and Gram -	DNA RNA Proteins	Genetic material (all essential genes) Primers, mRNA Linker proteins, polymerases
Plasmids	Gram + and Gram -	DNA	Non-essential genetic material Roles in conjugation, drug resistance, toxin production
Ribosomes	Gram + and Gram -	70s (protein/RNA) 30s (16S RNA) 50s (23 and 5s)	Protein synthesis
Granules (various types)	Gram + and Gram -	Glycogen, lipids, polyphosphate, etc.	Storage: polymerization of molecules present in high numbers in cells reduces osmotic pressure. Volutin granules of <i>Corynebacterium diphtheriae</i> are used in clinical identification.
Endospores	Gram + <i>only</i>	Keratin coat, calcium dipicolinate	Resistance to heat, chemicals, and dehydration

*Note that there are no mitochondria or membrane-bound structures like chloroplasts.

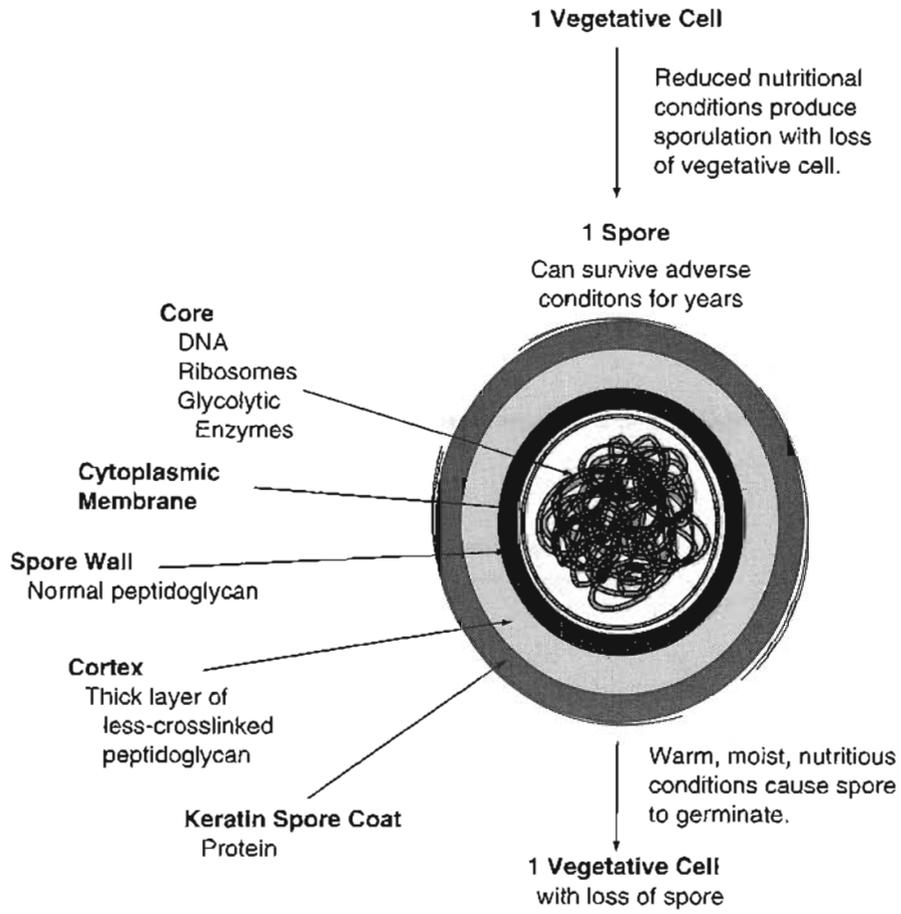


Figure I-2-3. Endospore

Note

Spores of fungi have a reproductive role.

ENDOSPORES

Organisms: *Bacillus* and *Clostridium*

Function

- Survival not reproductive (1 bacterium → 1 spore)
- Resistance to chemicals, dessiccation, radiation, freezing, and heat

Mechanism of Resistance

- New enzymes (i.e., dipicolinic acid synthetase, heat-resistant catalase)
- Increases or decreases in other enzymes
- Dehydration: calcium dipicolinate in core
- Keratin spore coat

BACTERIAL GROWTH AND DEATH

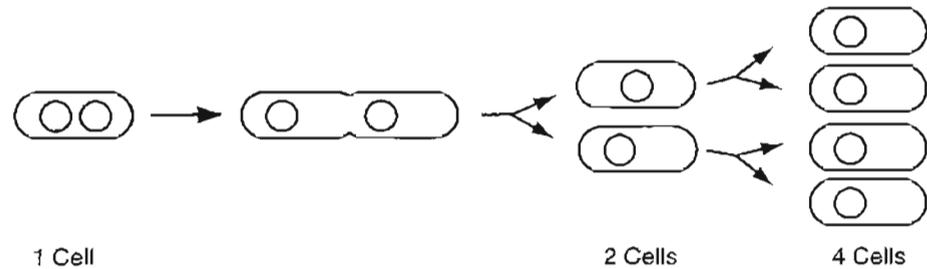


Figure I-2-4. Exponential Growth by Binary Fission

In A Nutshell

Lag Phase

- Initial Phase (only 1 lag phase)
- Detoxifying medium
- Turning on enzymes to utilize medium
- For exam, number of cells at beginning equals number of cells at end of lag phase.

Log Phase

- Rapid exponential growth
- Generation time = time it takes one cell to divide into two. This is determined during log phase.

Stationary Phase

- Nutrients used up
- Toxic products like acids and alkalis begin to accumulate.
- Number of new cells equals the number of dying cells.

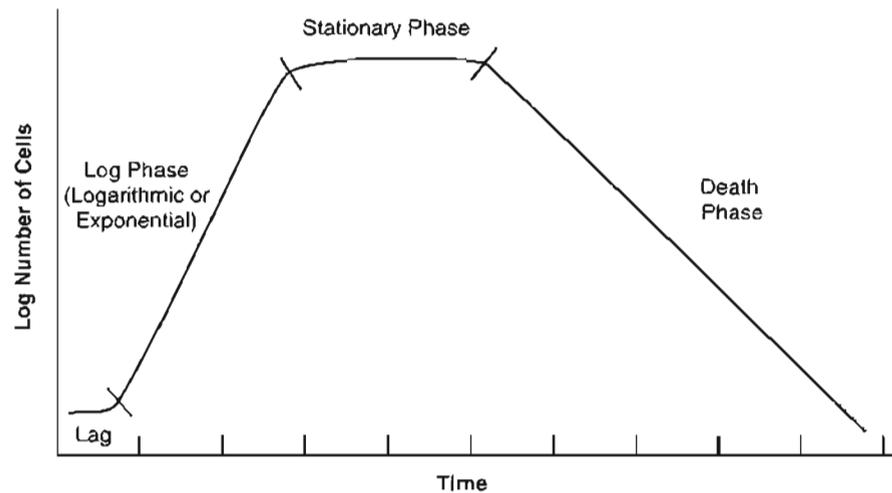


Figure I-2-5. Bacterial Growth Curve

Typical question:

A flask is inoculated to a density of 3×10^3 cells/ml. What is the density of cells in the culture after 50 minutes if the generation time is 20 minutes and the lag time is 10 minutes?

CULTURE OF MICROORGANISMS

- Obligate intracellular pathogens (viruses, rickettsias, chlamydias, etc.): Tissue cultures (cell cultures), eggs, animals, or not at all
- Facultative intracellular or extracellular organisms: Inert lab media (broths and agars)
 - Selective medium (S): A medium that selects for certain bacteria by inclusion of special nutrients and/or antibiotics.
 - Differential medium (D): A medium on which different bacteria can be distinguished by differences in colonial morphology or color.

Table I-2-6. Special Media for Selected Organisms

Organism	Medium
Anaerobes	Thioglycolate
<i>Corynebacterium</i>	Löffler's coagulated serum medium (S) Tellurite agar (D)
Enteric bacteria	Eosin methylene blue (D) MacConkeys (D)
Enteric pathogens	Hektoen enteric agar (D) Xylose-lysine-deoxycholate agar
<i>Vibrio cholerae</i> (likes alkaline growth medium)	TCBS (Thiosulfate Citrate Bile Salts Sucrose agar) (S)
<i>Legionella</i>	Charcoal-yeast extract agar (CYE agar) (S)
<i>Mycobacterium</i>	Löwenstein-Jensen medium (S)
<i>Neisseria</i> from normally sterile sites, <i>Haemophilus</i>	Chocolate agar
<i>Neisseria</i> from sites with normal flora	Thayer-Martin selective medium* (S)

*Thayer-Martin media is a chocolate agar supplemented with vancomycin, nystatin and colistin to inhibit the normal flora, including nonpathogenic *Neisseria*.

Table I-2-7. Miscellaneous Growth Requirements

Cholesterol and purines and pyrimidines	<i>Mycoplasma</i>
Cysteine*	<i>Francisella, Brucella, Legionella, Pasteurella</i>
X (protoporphyrin) and V (NAD)	<i>Haemophilus (influenzae and aegypticus</i> require both)

*The four Sisters Ella and the Cysteine Chapel.

ANAEROBIC AND AEROBIC

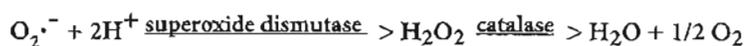


Table I-2-8. Oxygen Requirements and Toxicity

Classification	Characteristics	Important Genera
Obligate aerobes	Require oxygen Have no fermentative pathways Generally produce superoxide dismutase	<i>Mycobacterium</i> <i>Pseudomonas</i> (<i>Bacillus</i>)
Microaerophilic	Requires low but not full oxygen tension	<i>Campylobacter</i> <i>Helicobacter</i>
Facultative anaerobes	Will respire aerobically until oxygen is depleted and then ferment or respire anaerobically	Most bacteria, i.e., <i>Enterobacteriaceae</i>
Obligate anaerobes	1. Lack superoxide dismutase 2. Generally lack catalase 3. Are fermenters 4. Cannot use O ₂ as terminal electron acceptor	<i>Actinomyces</i> * <i>Bacteroides</i> <i>Clostridium</i>

*ABCs of anaerobiosis = *Actinomyces, Bacteroides, and Clostridium*.

BACTERIAL VACCINES

Childhood Vaccines

DTaP

- Totally acellular
- Components of *B. pertussis*:
 - Pertussis toxoid
 - ± Filamentous hemagglutinin
 - ± pertactin (adhesin)
- D-T portions are still toxoids.

DTP

- Diphtheria: diphtheria toxoid
(= inactivated toxin that no longer causes disease but produces immunity)
- Tetanus: tetanus toxoid
- Pertussis: killed *Bordetella pertussis* cells

HIB (*Haemophilus influenzae* type b)

H. influenzae capsular polysaccharide conjugated to protein (diphtheria toxoid or *Neisseria meningitidis* outer membrane proteins), making it a T-cell–dependent vaccine that infants respond to.

Senior Citizens or Asplenic

Streptococcus pneumoniae

Capsular polysaccharides of 23 different *Pneumococcus* strains

Limited Usage

Neisseria meningitidis

- Four capsular polysaccharides: Y, W-135, C, and A
- B serotype about 50% of cases in USA but capsule is sialic acid so not good immunogen.
- Used in outbreaks along with antibiotic prophylaxis
- Routine usage is in military recruits.

Salmonella typhi (ty21)

- Attenuated bacterium for travelers to endemic typhoid areas

In A Nutshell

Vaccine Efficacy

Neisseria vaccine

50% of cases are serotype B. It is not in the vaccine, so 50% are vaccine failures

Haemophilus HIB vaccine

95% of cases are invasive disease caused by type B. The vaccine is against type B and is 95% effective

Yersinia pestis

- Killed cellular vaccine (F-1 antigen)
- Military in endemic areas and *Y. pestis* laboratory workers

Bacillus anthracis

- Supernatant of partially purified proteins
- Military or occupational usage

BCG = Bacille Calmette Guerin (BCG)

- Attenuated (living) strain of *Mycobacterium bovis*
- Doesn't prevent pulmonary tuberculosis but reduces dissemination

GRAM-STAINING REACTIONS

(†Marked organisms have high numbers of questions in the pool.)

Table I-2-9. Gram-Positive Bacteria

Cocci	
	<i>Staphylococcus</i> † <i>Streptococcus</i> †
Rods	
Aerobic or facultative anaerobic	
	<i>Bacillus</i> <i>Listeria</i> <i>Corynebacterium</i> † <i>Nocardia</i> <i>Mycobacterium</i> †
Anaerobic	
	<i>Clostridium</i> † <i>Actinomyces</i> <i>Eubacterium</i> <i>Propionibacterium</i> <i>Lactobacillus</i>

*Spore formers are *Bacillus* and *Clostridium*.

Table I-2-10. Non-Gram-staining Bacteria*

Mycoplasmataceae	
	<i>Mycoplasma</i> † <i>Ureaplasma</i>

*Note also:

Poorly visible on traditional Gram stain: *Mycobacterium* does not stain well with the Gram stain due to its waxy cell wall. It is considered Gram-positive.

Most spirochetes, chlamydiae, and rickettsias are so thin that the color of the Gram stain cannot be seen. All have Gram-negative cell walls.

Legionella (Gram-negative) also does not stain well with the traditional Gram stain unless counterstain time is increased.

Table I-2-11. Gram-Negative Bacteria

Aerobic	
Cocci	<i>Neisseria</i> † <i>Moraxella</i>
Rods	<i>Pseudomonas</i> <i>Legionella</i> <i>Brucella</i> <i>Bordetella</i> † <i>Francisella</i>
Helical (and microaerophilic)	
	<i>Campylobacter</i> <i>Helicobacter</i>
Facultative anaerobic rods	
Enterobacteriaceae †	
	<i>Escherichia</i> † <i>Shigella</i> <i>Salmonella</i> † <i>Citrobacter</i> <i>Klebsiella</i> <i>Enterobacter</i> <i>Serratia</i> <i>Proteus</i> <i>Yersinia</i> †
Vibrionaceae	<i>Vibrio</i>
Pasteurellaceae	<i>Pasteurella</i> <i>Haemophilus</i> †
Anaerobic straight to helical rods	
	<i>Bacteroides/Prevotella</i> <i>Fusobacterium</i>
Spirochetes	
	<i>Treponema</i> † <i>Borrelia</i> <i>Leptospira</i>
Rickettsiaceae and relatives	
	<i>Rickettsia</i> † <i>Bartonella (Rochalimea)</i> <i>Coxiella</i> <i>Ehrlichia</i>
Chlamydiaceae	
	<i>Chlamydia</i> †

GENUS: STAPHYLOCOCCUS

- Gram-positive
- Cocci arranged in clusters
- Catalase positive

Table I-2-12. Medically Important *Staphylococci**

Species	Coagulase/ Hemolysis	Additional Virulence Factors and Lab ID	Common Diseases
<i>Staphylococcus aureus</i>	Coagulase positive/ β-hemolysis	Protein A TSST-1 Enterotoxins Exfoliatins Cytolysins Osteomyelitis	Infective endocarditis (dominant cause in IV drug abusers) Abscesses Toxic shock syndrome Gastroenteritis Suppurative lesions
<i>Staphylococcus epidermidis</i>	Coagulase negative/ no hemolysis	Susceptible to novobiocin Normal skin flora Adherence: biofilm	Catheter & prosthetic device and infections Endocarditis in IV drug abusers
<i>Staphylococcus saprophyticus</i>	Coagulase negative/ no hemolysis	Resistant to novobiocin	Urinary tract infections in newly active adolescent women

*All members of the genus *Staphylococcus* are catalase positive.

Staphylococcus aureus

Distinguishing Characteristics

- β-hemolytic, yellow colonies of Gram + cocci (clusters) on blood agar (BA)
- Catalase-positive, coagulase-positive, PYR test +
- Salt tolerant; ferments mannitol on mannitol salt agar

Reservoir

- Normal flora on nasal mucosa and skin.

Transmission

- Spread via the hands and sneezing
- Surgical wounds; lungs of cystic fibrosis patients
- Foods associated with food poisoning:
Ham or canned meats, custard pastries, and potato salad

Predisposing Factors for Infections

- Surgery or any break in skin, surgical packing or sutures or any foreign body (e.g., tampons); ventilators
- Severe neutropenia (<500/μL); cystic fibrosis
- IV drug abuse (IV drug abusers in general have more *S. aureus* on skin than *S. epidermidis*)
- Chronic granulomatous disease (CGD) Staph are catalase +

Pathogenesis

- Numerous enzymes and exotoxins
 - **Protein A inhibits phagocytosis**; binds Fc portion of antibody.
 - **Enterotoxins A-E** (heat stable 60°C, 10 min); fast 2–6 hours
 - **TSST-1** causes toxic shock syndrome. **Decreases normal liver clearance of endotoxin**; **superantigen** nonspecifically activates large numbers of T helper cells without processed antigen.
 - **Coagulases: convert fibrinogen to a fibrin clot**
 - **Cytolytic toxins, e.g., Staphylococcal alpha toxin**, a pore-forming toxin that damages human cell membranes
 - **Exfoliatins** involved in Scalded Skin Syndrome (SSS) and formation of the bullae seen in Staphylococcal impetigo

Table I-2-13. Staphylococcal Diseases

Diseases	Clinical Symptoms	Pathogenicity Factors
Gastroenteritis (food poisoning) - toxin ingested preformed in food	2–6 hours after ingesting toxin: nausea, abdominal pain, vomiting, followed by diarrhea	Enterotoxins A–E preformed in food
Infective endocarditis	Fever, malaise, leukocytosis, heart murmur (may be absent initially)	Fibrin-platelet mesh, cytolytic toxins
Abscesses/furuncles/carbuncles	Subcutaneous tenderness, redness and swelling; hot	Coagulase, probably the cytolytins
Toxic shock syndrome	Fever, hypotension, scarlatiniform rash which desquamates (particularly on palms and soles), multiorgan failure	TSST-1
Impetigo	Erythematous papules to bullae	Coagulase, exfoliatins
Pneumonia	Productive pneumonia with rapid onset, high rate of necrosis and high fatality; nosocomial, ventilator, post-influenza, IV drug abuse, CF, CGD*, etc.	all
Surgical infections	Fever with cellulitis and/or abscesses	Coagulase, exfoliatins, ± TSSTs

*CF = cystic fibrosis; CGD = chronic granulomatous disease.

Treatment

- Early on *S. aureus* (now known as methicillin-sensitive *S. aureus*, MSSA) acquired a multiple drug resistant plasmid with resistance to early beta-lactams (via a beta-lactamase) and most other antibiotics.
- Methicillin (and nafcillin) were developed.
- Methicillin-resistant *S. aureus* (MRSA) (due to changes in major penicillin-binding proteins) is **commonly resistant to all antibiotics except vancomycin and fusidic acid**.
- Topical mupirocin used to reduce nasal colonization

Prevention

- Basic hospital infection control

GENUS: STREPTOCOCCUS

- Gram-positive
- Chains or pairs of cocci
- Catalase negative

Hemolysis varies by species: Beta = clear; alpha = partial (green); gamma = non-hemolytic.

Streptococci are serogrouped using known antibodies to the cell wall carbohydrates

(Lancefield's Groups A-O)

Streptococci are serotyped using known antibodies to the

- Capsules for *Streptococcus pneumoniae*
- M-protein for *Streptococcus pyogenes*

Table I-2-14. Medically Important Streptococci¹

Species	Lancefield Group	Typical Hemolysis	Important Lab Characteristics
<i>S. pyogenes</i>	A	beta	Bacitracin-sensitive (A disk) PYR test ² positive
<i>S. agalactiae</i>	B	beta	Bacitracin-resistant (P disk) Hippurate utilized cAMP test positive
<i>Enterococcus faecalis</i> ³	D	alpha, beta or none	Growth in 6.5% NaCl PYR test positive
<i>S. bovis</i> ⁴	D	alpha or none	No growth in 6.5% NaCl
<i>S. pneumoniae</i>	Not groupable ⁵	alpha	Bile-soluble Inhibited by optochin
Viridans group	Not groupable	alpha	Not bile-soluble Not inhibited by optochin

¹All streptococci are catalase negative.

²PYR test demonstrates the presence of pyrrolidonyl arylamidase. *S. pyogenes* is the one beta hemolytic *Streptococcus* that is positive; enterococci are positive.

³*Enterococcus faecalis* = *Streptococcus faecalis*. The enterococci belong to the Streptococcaceae family.

⁴*S. bovis* is a non-enterococcal group D organism.

⁵Not serogrouped because they lack the carbohydrate cell wall antigens.

***Streptococcus pyogenes* (Group A Streptococcus)**

Distinguishing Characteristics

- Beta-hemolytic colonies inhibited by bacitracin on BA
- Gram positive cocci in chains, catalase-negative, PYR+

Reservoir

Human throat and skin

Transmission

Spread by respiratory droplets or direct contact

In a Nutshell

S. pyogenes

- group A
- beta hemolytic
- bacitracin sensitive

Pathogenesis

- **Hyaluronic acid capsule** (a polysaccharide) is **non-immunogenic**; inhibits phagocytic uptake.
- **M-protein**: virulence factor, **antiphagocytic**, used to **TYPE** group A Strep; **M12 strains**-associated with **acute glomerulonephritis**

Toxins

- **Streptolysin O**: **immunogenic**, hemolysin/cytolysin
- **Streptolysin S**: not immunogenic, hemolysin/cytolysin

Spreading factors:

- **Streptokinase**: breaks down fibrin clot
- **Streptococcal DNase**: liquefies pus, extension of lesion
- **Hyaluronidase**: hydrolyzes the ground substances of the connective tissues; important to spread in cellulitis

Exotoxins A–C

(Pyrogenic or erythrogenic exotoxins.)

- **Phage-coded** (e.g., the cells are lysogenized by a phage.)
- Cause **fever and the rash** of Scarlet fever
- **Inhibit liver clearance of endotoxin** (from normal flora), creating shock-like conditions
- **Superantigens**: activate many helper T cells by bridging T cell receptors and MHC class II markers without processed antigen

Diseases

- *Streptococcus pyogenes* causes a wide variety of acute infections; some have immunologic sequelae.

Table I-2-15. Acute (Suppurative) *Streptococcus Pyogenes* Infections

Disease	Clinical Symptoms (Sx)
Pharyngitis	Abrupt onset of sore throat, fever, malaise and headache; tonsillar abscesses and tender anterior cervical lymph nodes
Scarlet fever	Above followed by a blanching, “sandpaper” rash; circumoral pallor; palms and soles are generally spared; strawberry tongue; nausea/vomiting
Pyoderma/impetigo	Pus-producing skin infection (honey-crusted lesions)

Also, cellulitis/necrotizing fasciitis (flesh-eating bacteria!), puerperal fever, lymphangitis, pneumonia, a toxic shock-like syndrome, etc.

Table I-2-16. Non-suppurative Sequelae to Group A Streptococcal Infections

Disease	Sequelae to	Mechanism/Symptoms (Sx)
Rheumatic fever	Pharyngitis with Group A strep (not Group C)	Antibodies to heart tissue; mean = 19 d: fever, joint inflammation, carditis, erythema nodosum (chorea later) (Type II)
Acute glomerulonephritis (M12 serotype)	Pharyngitis or cutaneous	Immune complexes bound to glomeruli/pulmonary edema and hypertension, dark urine (Type III)

Lab Notes

- For Strep throat: **rapid antigen test** (misses about 25% of the Strep throats); culture all “negatives.”
- **ASO titer for rheumatic fever** (>200 is significant)

Treatment

- Beta-lactam drugs or erythromycin

Prevention

- Penicillin in rheumatic fever patients to prevent recurrent *S. pyogenes* pharyngitis

Streptococcus agalactiae = Group B Streptococci (GBS)

Distinguishing Characteristics

- **Beta-hemolytic**, bacitracin-resistant on BA
- **Gram-positive cocci in chains**
- **Group B** (determined by antiserum against cell wall carbohydrate in precipitin test)
- Catalase-negative, **hydrolyze hippurate**
- **cAMP test-positive**: **cAMP factor** is a **polypeptide** that “compliments” a *Staph aureus* sphingomyelinase to make an area of new complete beta-hemolysis.

Reservoir

- Colonizes human vagina (15–20% of women)

Transmission

- Newborn infected during birth
- Increased risk with **prolonged rupture of membranes**

Pathogenesis

- **Capsule**
- **Beta-hemolysin** and **cAMP factor** (an incomplete hemolysin)

Diseases

Neonatal septicemia and meningitis

- Group B Strep is the most common causative agent.

Treatment

- Ampicillin with cefotaxime or gentamicin

Prevention

- Treat mother prior to delivery if she has had a previous baby with GBS, has documented GBS colonization, or prolonged rupture of membranes.

In a Nutshell

S. agalactiae

- group B
- beta hemolytic
- bacitracin resistant
- hydrolyzes hippurate

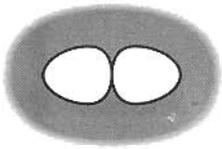


Figure I-2-6.
Streptococcus pneumoniae

In a Nutshell

S. pneumoniae

- alpha hemolytic
- bile soluble
- optochin sensitive

In A Nutshell

Typical Pneumonia

Bacterial pneumonia such as *Streptococcus pneumoniae* elicits neutrophils; arachidonic acid metabolites (acute inflammatory mediators) cause pain and fever.

Pneumococcus produces a lobar pneumonia with a productive cough, grows on blood agar, and usually responds well to penicillin treatment.

***Streptococcus pneumoniae* (Pneumococcus)**

Distinguishing Characteristics

- Alpha-hemolytic colonies **inhibited by optochin** on BA
- Gram-positive, lancet-shaped diplococci (or short chains)
- Lysed by bile

Reservoir

Human upper respiratory tract

Transmission

- **Respiratory droplets**; not considered highly communicable
- Often colonizes without causing disease.

Predisposing Conditions for Pneumonia

- Antecedent influenza or measles infection: damage to mucociliary elevator
- Chronic obstructive pulmonary disorders
- Congestive heart failure
- Alcoholism
- Asplenia predisposes to septicemia

Pathogenesis

- IgA protease: colonization
- Teichoic acids: attachment
- **Polysaccharide capsule: major virulence factor; retards phagocytosis** through inhibition of antibody-independent opsonization via the alternative complement pathway.
- **Quellung reaction** positive (swelling of capsule with type-specific antiserum)
- **Latex particle agglutination test** for capsular antigen in spinal fluid diagnostic for meningitis
- **Antibody to the capsule (>80 serotypes) provides type-specific immunity.**
- **Pneumolysin O: hemolysin/cytolysin**
 - **Damages respiratory epithelium** (hemolysin similar to streptolysin O, which damages eukaryotic cells).
 - (Inhibits leukocyte respiratory burst and inhibits classical complement fixation.)
- **Pneumococcus in alveoli** stimulate release of fluid and red and white cells producing “rusty sputum.”
- **Peptidoglycan/teichoic acids highly inflammatory** in CNS.

Diseases

Bacterial Pneumonia

- Most common bacterial cause, especially after 65 years but also in infants
- Sx: “big” shaking chill, high fever, lobar with productive blood-tinged sputum

Adult Meningitis

Most common cause. CSF generally has very high white cell count, low glucose.

Otitis Media and Sinusitis in Children

Most common cause

Treatment

- Penicillin G
- Resistance (both low level and high level) is chromosomal (altered penicillin-binding proteins); major concern in meningitis. (vancomycin ± rifampin used.)

Prevention

Vaccine 23 serotypes of capsule

Viridans Streptococci (*S. sanguis*, *S. mutans*, etc.)

Distinguishing Characteristics

- Alpha-hemolytic, resistant to optochin
- Gram-positive cocci in chains

Reservoir

Human oropharynx (normal flora)

Diseases

Dental Caries

S. mutans dextran-mediated adherence glues oral flora onto teeth, forming plaque and causing caries.

Infective Endocarditis

- Sx: malaise, fatigue, anorexia, night sweats, weight loss
- Predisposing conditions: damaged (or prosthetic) heart valve and dental work without prophylactic antibiotics or extremely poor oral hygiene

Pathogenesis

Dextran (biofilm)-mediated adherence onto tooth enamel or damaged heart valve and to each other (vegetation). Growth in vegetation protects organism from immune system.

Treatment

Penicillin G with aminoglycoside for endocarditis

Prevention

For individuals with damaged heart valve: prophylactic penicillin prior to dental work

In a Nutshell

Viridans streptococci

- alpha hemolytic
- bile resistant
- optochin resistant

In a Nutshell

S. faecalis

- group D
- bile esculin positive
- grows in 6.5% salt

GENUS: ENTEROCOCCUS

- Catalase negative
- PYR+

Enterococcus faecalis = *Streptococcus faecalis*

Distinguishing Characteristics

- Group D Gram-positive cocci in chains
- PYR test +
- Catalase-negative, varied hemolysis
- Hydrolyzes esculin in 40% bile and 6.5% NaCl (bile esculin agar turns black)

Reservoir

Human colon, urethra ± and female genital tract

Pathogenesis/ Predisposing Conditions

- Bile/salt tolerance allows survival in bowel and gall bladder.
- During medical procedures on GI or GU tract: *E. faecalis* → bloodstream → previously damaged heart valves → endocarditis

Diseases

Urinary, biliary tract infections

Infective (subacute) endocarditis in persons (often elderly) with damaged heart valves

Treatment

- All strains carry some drug resistance.
- Some vancomycin-resistant strains of *Enterococcus faecium* or *E. faecalis*: no reliably effective treatment.
- (VanA strains have UDP-N-acetylmuramyl pentapeptide with the terminal D-alanyl-D-alanine replaced with D-alanyl-D-lactate, which functions in cell wall synthesis but is not blocked by vancomycin.)

Prevention

Prophylactic use of penicillin and gentamicin in patients with damaged heart valves prior to intestinal or urinary tract manipulations

GENUS: BACILLUS

- Gram-positive rods
- Spore-forming
- Aerobic

Gram-Positive Rods

Table I-2-17. Medically Important Gram-positive Rods

	Spore Formation	Aerobic Growth	Motility	Exotoxin	Intracellular Growth	IC Host?***
<i>Bacillus</i>	+	+	+*	+	-	No
<i>Clostridium</i>	+	-	+**	+	-	No
<i>Corynebacterium</i>	-	+	-	+	-	No
<i>Listeria</i>	-	+	+ tumbling, actin "jetting"	-	+	Yes

* Most *Bacillus* spp. are motile (except *Bacillus anthracis*, which is nonmotile).

** Most clostridia are motile (except for *Clostridium perfringens*, which is nonmotile).

*** Is organism a significant problem in immunocompromised (IC) hosts?

Bacillus anthracis

Distinguishing Characteristics

- Large, boxcar-like, Gram-positive, spore-forming rods
- Capsule is polypeptide (poly-D-glutamate) and the only non-polysaccharide one. It is immunogenic.

Reservoir

Animals, skins, soil

Transmission

Contact with infected animals or inhalation of spores from animal hair and wool. Spores survive long after animal dies.

Pathogenesis

Capsule: Polypeptide, antiphagocytic

Anthrax toxin

Includes three protein components:

- Protective antigen (B component)—mediates entry of LF or EF into eukaryotic cells
- Lethal factor—kills cells
- Edema factor is an adenylate cyclase (calmodulin-activated like pertussis adenylate cyclase).

Disease

Anthrax (rare in humans)

Cutaneous anthrax: Papule → papule with vesicles (malignant pustules) → central necrosis (eschar) with erythematous border often with painful regional lymphadenopathy; fever in 50%

Pulmonary (Wool Sorter's Disease): Life-threatening pneumonia; cough, fever, malaise, and ultimately facial edema, dyspnea, diaphoresis, cyanosis, and shock with **mediastinal hemorrhagic lymphadenitis**

Treatment

Ciprofloxacin or doxycycline

Prevention

Vaccine: cell free vaccine for people in high-risk occupations

Bacillus cereus

- Spores found widely in nature, including food, and are not killed by boiling.
- **Food poisoning** associated with food held warm (not hot)
- Two possible toxins:
 - **Emetic toxin: fast (1–6 hours)**, similar to *S. aureus* with vomiting and diarrhea; associated with **fried rice**
 - **Diarrheal toxin (meats, sauces): 18 hours**, similar to *E. coli*; LT: increasing cAMP → watery diarrhea

GENUS: LISTERIA

- Gram-positive, nonspore-forming rods
- Facultative intracellular
- Tumbling motility

Listeria monocytogenes

Distinguishing Characteristics

- Small Gram-positive rods
- Beta hemolytic, nonspore-forming rod on BA
- Tumbling motility in broth; actin jet motility in cells
- Facultative intracellular parasite
- Cold growth

Reservoir

- Widespread: animals (gastrointestinal and genital tracts), unpasteurized milk products, plants, and soil
- Cold growth: soft cheeses, deli meats, cabbages (coleslaw)

Transmission

Foodborne, across the placenta, or by contact during delivery

Pathogenesis

- Listeriolysin O, a β -hemolysin: facilitates rapid egress from phagosome into cytoplasm, thus evading killing when lysosomal contents are dumped into phagosome; “jets” directly (by actin filament formation) from cytoplasm to another cell.
- Immunologic immaturity predisposes to serious infection.

Diseases

Listeriosis (human, peaks in summer)

- Healthy adults and children: generally asymptomatic or diarrhea with low % carriage
- Pregnant women: symptomatic carriage, septicemia characterized by fever and chills; can cross the placenta in septicemia.

Neonatal Disease

Early-onset: (granulomatosis infantiseptica) *in utero* transmission; sepsis with high mortality; disseminated granulomas with central necrosis.

Late-onset: 2–3 weeks after birth from fecal exposure; meningitis with septicemia.

Immunocompromised Patients (IC pts)

- Septicemia and meningitis (most common clinical presentation)
- *Listeria* meningitis is the most common cause of meningitis in renal transplant patients and adults with cancer.

Treatment

Ampicillin with gentamicin added for IC patients

Prevention

Precautions with food may reduce incidence.

GENUS: CORYNEBACTERIUM

- Gram-positive rods
- Nonspore-forming, nonmotile
- Aerobic

Corynebacterium diphtheriae

Distinguishing Characteristics

- Gray to black colonies of club-shaped Gram-positive rods arranged in V or L shapes on tellurite medium.
- Granules (volutin) produced on Loeffler's coagulated serum medium stain metachromatically
- Aerobic, nonspore-forming
- Toxin-producing strains have β -prophage carrying genes for the toxin (lysogeny, β -corynephage). The phage from one patient with diphtheria can infect the normal nontoxigenic diphtheroid of another person and cause diphtheria.

Transmission

Bacterium or phage via respiratory droplets from oropharynx of infected person

Pathogenesis

Organism **not invasive**; colonizes epithelium of oropharynx or skin in cutaneous diphtheria.

Diphtheria toxin (A-B component)—inhibits protein synthesis by adding ADP-ribose to EF-2.

- Effect on oropharynx:
 - Dirty gray pseudomembrane (made up of dead cells and fibrin exudate, bacterial pigment)
 - Extension into larynx/trachea → obstruction
- Effect of systemic circulation → heart & nerve damage

Disease

Diphtheria

Sore throat with pseudomembrane, bull neck, potential respiratory obstruction, myocarditis, cardiac dysfunction, recurrent laryngeal nerve palsy, and lower limb polyneuritis.

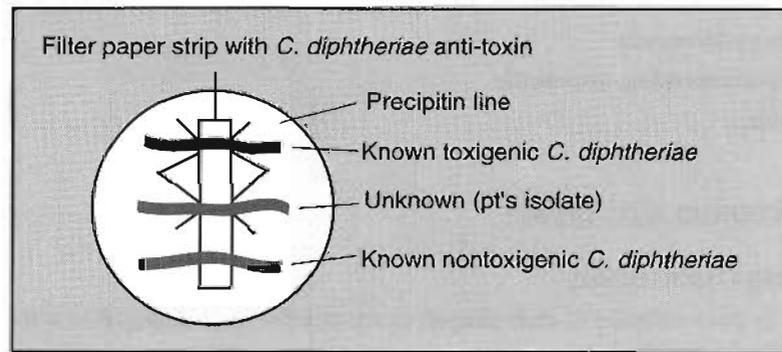


Figure I-2-7. Elek Test

Lab Notes

- Elek test to document toxin production.
- Toxin produced by Tox+ strains diffuses away from growth.
- Antitoxin diffuses away from the strip of filter paper.
- Precipitin lines form at zone of equivalence.

Treatment

Erythromycin and antitoxin

Prevention

Toxoid vaccine (formaldehyde-modified toxin is still immunogenic but with reduced toxicity), part of DTaP, DTP, or Td

GENUS: *ACTINOMYCES*

- Anaerobic BACTERIA
- Gram-positive rods to branching filaments
- Not acid fast

Actinomyces israelii

Distinguishing Characteristics

Anaerobic, Gram-positive branching rods

Reservoir

Human; normal flora of gingival crevices and female genital tract

Transmission

Endogenous

Pathogenesis

Invasive growth in tissues with compromised oxygen supply; anaerobic growth

Disease

Actinomycosis

- Generally not painful but **very invasive**, penetrating all tissues including bone
- Tissue swelling → draining abscesses (sinus tracts) with “sulfur” granules (hard yellow microcolonies) in exudate that can be used for microscopy or culture
- Only in tissues with low oxygenation (E_h)

Forms

- Cervicofacial (lumpy jaw): dental trauma or poor oral hygiene
- Pelvic: from thoracic or sometimes IUD's
- Thoracic: aspiration with contiguous spread
- Abdominal: surgery or bowel trauma
- CNS: solitary brain abscess most common

Treatment

Ampicillin or penicillin G and surgical drainage

In a Nutshell

Actinomyces

- Gram +
- Not acid fast
- Anaerobic
- Endogenous infection
- Penicillin

In A Nutshell

Nocardia

- Gram +
- Partially acid fast
- Aerobic
- Exogenous
- Sulfa drugs

GENUS: NOCARDIA

- BACTERIA
- Gram-positive filaments breaking up into rods
- Aerobic
- Partially acid-fast (some areas of smear will be blue and some red)

Nocardia asteroides

Distinguishing Characteristics

- Aerobic
- Gram-positive branching rods
- Partially acid-fast

Reservoir

Soil, dust

Transmission

Airborne or traumatic implantation

Pathogenesis

- No toxins or virulence factors known
- Immunosuppression and cancer predispose to pulmonary infection.

Diseases

Nocardiosis

- Cavitary bronchopulmonary nocardiosis
 - Sx: cough, fever, dyspnea
 - May spread hematogenously to brain (brain abscesses)
- Cutaneous/subcutaneous nocardiosis
 - Starts with traumatic implantation
 - Sx: cellulitis with swelling → draining subcutaneous abscesses with granules (mycetoma)

Treatment

Sulfonamides (high dose) or trimethoprim/sulfamethoxazole

GENUS: MYCOBACTERIUM

- Acid fast rods with waxy cell wall
- Obligate aerobe

Cell Wall

- Unique: high concentration of lipids containing long chain fatty acids called mycolic acids
- Structural organization shown in Figure II-8.

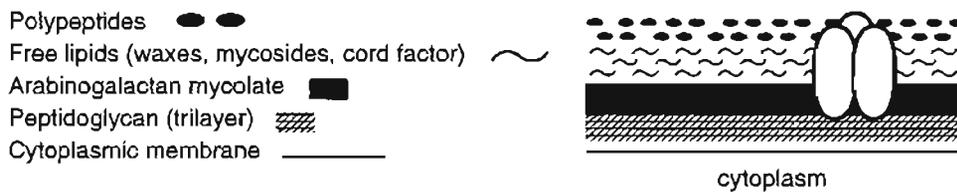


Figure I-2-8. Mycobacterial Cell Wall

- Wall makes mycobacteria highly resistant to
 - Desiccation
 - Many chemicals (including NaOH used to kill other bacteria in sputa before neutralizing and culturing)
- Mycobacteria are sensitive to UV

Table I-2-18. Mycobacteria and Close Relatives

Genus	Aerobic Growth?	Acid Fast?	Morphology (Don't memorize!)
<i>Corynebacterium</i>	Yes	Not AF	Rods
<i>Actinomyces</i>	No, anaerobe	Not AF	Rods, filaments
<i>Nocardia</i>	Yes	Partially AF	Rods, filaments
<i>Mycobacterium</i>	Yes, obligate aerobe	Yes, AF	Rods

Mycobacterium tuberculosis

Distinguishing Characteristics

- Auramine-rhodamine staining bacilli (fluorescent apple green); no antibody involved (sensitive but not specific)
- Acid fast
- Aerobic, slow growing on Lowenstein-Jensen medium; new culture systems (broths with palmitic acid) faster
- Produces niacin

- Produces a **heat-sensitive catalase**:
 - Catalase negative at 68°C (standard catalase test)—(other mycobacterial catalases are heat insensitive)
 - Catalase active at body temperature.

Reservoir

Human lungs

Transmission

Respiratory droplets and droplet nuclei

Predisposing Factor

For active disease is poverty, HIV infection, or any CMI system immunosuppression.

Pathogenesis

Facultative Intracellular Organism

Sulfatides (sulfolipids in cell envelope)

- Inhibit the phagosome-lysosomal fusion allowing intracellular survival. (If fusion occurs, waxy nature of cell envelope reduces killing effect.)

Cord factor (trehalose di-mycolate)

- Causes serpentine growth *in vitro*
- Inhibits leukocyte migration; disrupts mitochondrial respiration and oxidative phosphorylation

Tuberculin (surface protein) along with mycolic acid → delayed hypersensitivity and CMI

- Granulomas and caseation mediated by cell-mediated immunity (CMI)
- No exotoxins nor endotoxin; damage done by immune system

Disease

Tuberculosis

- Causative agents: *Mycobacterium tuberculosis* and *M. bovis*
- Complex disease: pulmonary, urinary tract, any organ or miliary (disseminated) (see pathology notes).
- Primary infection: organisms replicate in naïve macrophages, killing macrophages until CMI is set up.
- Most people heal without disease; some organisms walled off in the Ghon complex remain viable unless treated.
- Post primary (reactivational TB) erosion of granulomas into airways (high oxygen) later in life under conditions of reduced T-cell immunity leads to mycobacterial replication and disease symptoms.



Figure I-2-9.
Cord Factor

Diagnosis

- Microscopy on specimen: auramine-rhodamine stain (fluorescent apple green); no antibody involved; very sensitive; if positive followed by acid fast stain.
- **PPD skin test (Mantoux): measure zone of induration at 48–72 hours; positive if zone of induration at 48–72 hours is:**
 - ≥5 mm in HIV+ or anyone with recent TB exposure; AIDS patients have reduced ability to mount skin test.
 - ≥10 mm in high-risk population: IV drug abusers, people living in poverty, or immigrants from high TB area.
 - ≥15 mm in low-risk population.
- **Positive skin test indicates only exposure but not necessarily active disease.**
- Slow-growing (3–6 weeks) colony on Lowenstein-Jensen medium (faster new systems)
- Organisms produce **niacin** and are **catalase-negative** (68°C).
- **No serodiagnosis**

Treatment

- **Multiple drugs critical to treat infection**
- Standard observed short-term therapy for uncomplicated pulmonary TB (rate where acquired <4%):
 - First 2 months: isoniazid + rifampin + pyrazinamide
 - Next 4 months: isoniazid and rifampin
- Ethambutol or streptomycin added for possible drug-resistant cases until susceptibility tests are back (if area acquired has >4% DRM TB)

Prevention

- **Isoniazid** taken for 6–9 months can prevent TB in persons with infection but no clinical symptoms.
- Bacille-Calmette-Guérin (BCG) **vaccine** containing live, attenuated organisms may prevent disseminated disease. Not commonly used in the U.S.
- UV lights or HEPA filters used to treat potentially contaminated air.

Mycobacteria Other Than Tuberculosis (MOTTs)

- (MOTTs) = Non-tuberculous Mycobacteria = atypical **Mycobacteria**
- **Non-contagious!**
- Found in **surface waters, soil**, cigarettes; most common in southeastern U.S.
- Runyon terminology still used, particularly for pigmented ones. Dr. Runyon grew two tubes of each in the dark and then exposed one of the pair to bright light for 2 hours after the cultures were grown up.

Table I-2-19. Runyon Grouping of MOTTs

Species*	Runyon Grouping	Pigment in Dark**	Pigment in Light	Growth
<i>M. kansasii</i>	Photochromogen	-	+	slow
<i>M. scrofulaceum</i>	Scotochromogens	+	+	slow
<i>M. avium-intracellulare</i>	Non-chromogens	-	-	slow

* Not classified: *M. tuberculosis*, *M. bovis*, and *M. ulcerans*. None produce pigments. Also not included is *M. leprae*, which cannot be grown in the lab.

** Pigments are carotenoids.

Note: Speciation now uses biochemical methods; some strains of *M. avium-intracellulare* complex (MAI or MAC) may produce pale yellow pigments.

Diseases

Pulmonary/Gastrointestinal/Disseminated

- Patients: AIDS (prophylaxis < 75 CD4+ cells/mm³), cancer, chronic lung disease
- *M. avium-intracellulare*, *M. kansasii*.

Mycobacterial lymphadenitis

- Usually solitary cervical lymph nodes (surgically removed) in kids.
- *M. scrofulaceum*.

Soft-Tissue Infections

M. marinum: cutaneous granulomas in tropical fish enthusiasts (fish tank granuloma) or scuba divers from abrasions on coral

Mycobacterium leprae

Distinguishing Characteristics

- Acid fast rods (seen in punch biopsy)
- Obligate intracellular parasite (cannot be cultured *in vitro*)
- Optimal growth at less than body temperature

Reservoir

- Human mucosa, skin, and nerves are the only significant reservoir.
- Some infected armadillos in Texas and Louisiana

Transmission

Nasal discharge from untreated lepromatous leprosy patients

Pathogenesis

- Obligate intracellular parasite
- Cooler parts of body, e.g., skin, mucous membranes, and peripheral nerves

Note

M. tuberculosis and *M. avium-intracellulare* complex (MAI) are major problems in HIV-infected and AIDS patients.

M. tuberculosis with normal CD4 count or with low CD4 count (disseminated).

MAI only late with low CD4 count.

Disease

Leprosy: A continuum of disease, which usually starts out with an indeterminate stage called "borderline."

Table I-2-20. Two Extreme Forms of Leprosy

	Tuberculoid		Lepromatous
Cell-mediated immune system	Strong CMI	B	Weak CMI
Lepromin skin test	Lepromin test +	o	Lepromin test -
Number of organisms in tissue	Low	r	High (foam cells totally filled)
Damage from	Immune response (CMI killing infected cells) Granuloma formation → nerve enlargement/damage Loss of sensation → burns and trauma	d	Large number of intracellular organisms Nerve damage from overgrowth of bacteria in cells Loss of sensation → burns and trauma
Number of lesions and other symptoms	Fewer lesions: macular; nerve enlargement, paresthesia	e	Numerous lesions becoming nodular; loss of eyebrows; destruction of nasal septum Paresthesia Leonine facies

Laboratory Diagnosis

- Punch biopsy or nasal scrapings; acid fast stain
- Lepromin skin test is positive in the tuberculoid but not in the lepromatous form.
- (No cultures)

Treatment

Multiple-drug therapy with dapsone and rifampin, with clofazimine added for lepromatous

Prevention

Dapsone for close family contacts

GENUS: *CLOSTRIDIUM*

- Gram-positive rod
- Spore-forming
- Anaerobic

Clostridium tetani

Distinguishing Characteristics

- Large Gram-positive, spore-forming rods
- Anaerobes
- Produces tetanus toxin

Reservoir

Soil

Transmission

- Puncture wounds/trauma
- Requires low tissue oxygenation (E_h)

Disease

Tetanus; sx: risus sardonicus, opisthotonus

Pathogenesis

Spores germinate in the tissues producing tetanus toxin (an exotoxin also called tetanospasmin).

- Carried intra-axonally to CNS
- Binds to ganglioside receptors
- Blocks release of inhibitory mediators (glycine and GABA) at spinal synapses
- Excitatory neurons are unopposed → extreme muscle spasm
- One of the most toxic substances known

Diagnosis

Primarily a clinical diagnosis, organism is rarely isolated

Treatment

Of actual tetanus:

- Hyperimmune human globulin (TIG) to neutralize toxin, + metronidazole or penicillin
- Spasmolytic drugs (diazepam), debride, delay closure

Prevention

Toxoid Vaccines (DTP, DTaP, Td)

- Toxoid is formaldehyde-inactivated toxin.
- Important because disinfectants have poor sporucidal action
- Care of wounds: proper wound cleansing and care plus treatment

Table I-2-21. Wound Management

	Not Tetanus Prone linear, 1 cm deep cut, without devitalized tissue, without major contaminants, less than 6 hours old	Tetanus Prone blunt/missile, burn, frostbite, 1 cm deep; devitalized tissue present + contaminants (e.g., dirt, saliva), any wound 6 hours old
Not completed primary or vaccination history unknown	Vaccine	Vaccine and TIG*
Completed primary series	Vaccine if more than 10 years since last booster	Vaccine if more than 5 years since last booster

* TIG = tetanus immunoglobulin (human).

Clostridium botulinum

Distinguishing Characteristics

- Anaerobic, Gram-positive, spore-forming rods

Reservoir

- Soil/dust

Pathogenesis

Spores survive in soil and dust; germinate in moist, warm, nutritious but non-acidic and anaerobic conditions.

Botulinum toxin

- A-B polypeptide neurotoxin (actually a series of 7 antigenically different; Type A and B most common)
- Coded for by a prophage (lysogenized *Clostridium botulinum*).
- Highly toxic
- Heat labile (unlike staph), 10 minutes 60°C
- Mechanism of action:
 - Absorbed by gut and carried by blood to peripheral nerve synapses
 - Blocks release of acetylcholine at the myoneuronal junction resulting in a reversible flaccid paralysis

Table I-2-22. Forms of Botulism

Disease	Adult/food borne	Infant	Wound
Acquisition	Preformed toxin ingested (toxicosis) Poorly canned alkaline vegetables (green beans) Smoked fish	Spores ingested: household dust, HONEY Toxin produced in gut (toxi-infection)	Traumatic implantation of spores; <i>in vivo</i> production of toxin (toxi-infection) Debridement, no closure
Symptoms	1–2 day onset of Sx: weakness, dizziness, blurred vision, flaccid paralysis (reversible); ±diarrhea, nausea or vomiting	Constipation, limpness/flaccid paralysis (reversible): diplopia, dysphagia, weak feeding/crying; may lead to respiratory arrest	As for food without GI symptoms
Toxin demonstrated in	Suspected food or serum	Stool or serum	Serum
Treatment	Respiratory support Polyvalent antitoxin	Respiratory support in monitored intensive care; Hyperimmune human serum Antibiotics generally not used as may worsen or prolong	Rx: amoxicillin and antitoxin (respiratory support)
Prevention	Proper canning; Heat all canned foods	No honey first year	

Clostridium perfringens

Distinguishing Characteristics

- Large Gram-positive, spore-forming rods (spores rare in tissue), nonmotile
- Anaerobic: “stormy fermentation” in milk media
- Double zone of beta hemolysis

Reservoir

Soil and human colon

Pathogenesis

- Spores germinate under anaerobic conditions in tissue.
- Vegetative cells produce:
Alpha toxin (a.k.a., phospholipase C) is a lecithinase. It disrupts membranes, damaging RBC, platelets, WBC, endothelial cells → massive hemolysis, tissue destruction, hepatic toxicity.
- Identified by Naegler’s reaction: egg yolk agar plate—one side with anti-α-toxin. Lecithinase activity is detected on side with no antitoxin.
- 12 other toxins damage tissues.
- Enterotoxin produced in intestines in food poisoning: disrupts ion transport → watery diarrhea, cramps (similar to *E. coli*); resolution < 24 hours.

Diseases

Gas Gangrene (Myonecrosis)

- Contamination of wound with soil or feces
- Acute and increasing pain at wound site
- Tense tissue (edema) and exudate
- Systemic symptoms include fever and tachycardia (disproportionate to fever), diaphoresis, pallor, etc.
- Rapid, high mortality
- Prevention: extensive debridement of the wound plus administration of penicillin decreases probability
- Treatment: debridement, delayed closure, clindamycin and penicillin, hyperbaric chamber

Food Poisoning

- Reheated meat dishes, organism grows to high numbers; 8–24 hour incubation
- Enterotoxin production in gut; self-limiting non-inflammatory, watery diarrhea
- Treatment: supportive for food poisoning

Clostridium difficile

Antibiotic-associated (clindamycin, cephalosporins, amoxicillin, ampicillin) diarrhea, colitis, or pseudomembranous colitis (yellow plaques on colon)

Two Toxins

Toxin A: Enterotoxin damaging mucosa leading to fluid increase; granulocyte attractant

Toxin B: Cytotoxin: cytopathic

Treatment

Metronidazole (vancomycin): use only if no other drug available; to avoid selecting for vancomycin-resistant normal flora.

Note

Oxidase

Oxidase (cytochrome C oxidase) test: flood colony with phenylenediamine; in presence of oxidase, phenylenediamine turns black. Rapid test.

Major oxidase-negative Gram-negative group is Enterobacteriaceae.

GENUS: NEISSERIA

- Gram-negative
- Diplococci with flattened sides
- Oxidase positive

Table I-2-23. Medically Important *Neisseria*

Species	Capsule	Vaccine	Portal of Entry	Glucose Utilization	Maltose Fermentation	β-lactamase Production
<i>N. meningitidis</i>	+	Yes	Respiratory	+	+	Rare
<i>N. gonorrhoeae</i>	-	No	Genital	+	-	>16%

***Neisseria meningitidis* (Meningococcus)**

Distinguishing Characteristics

- Gram-negative kidney bean-shaped diplococci
- Large capsule; latex particle agglutination (or CIE; counter immunoelectrophoresis) to identify *N. meningitidis* capsular antigens in CSF
- Grows on chocolate (not blood) agar in 5% CO₂ atmosphere
- Ferments maltose in contrast to gonococci

Reservoir

Human nasopharyngeal area

Transmission

- Respiratory droplets; oropharyngeal colonization, spread to the meninges via the bloodstream
- Disease occurs in only small percent of colonized.

Pathogenesis

Important Virulence Factors

- Polysaccharide capsule: antiphagocytic, antigenic, 5 common serogroups: B is not strongly immunogenic (sialic acid), B strain is most common strain in USA. Used for: serogrouping, detection in CSF, and vaccine
- IgA protease allows oropharynx colonization.
- Endotoxin (LPS): fever, septic shock in meningococemia, overproduction of outer membrane
- Pili and outer membrane proteins important in ability to colonize and invade
- Deficiency in late complement components (C5-8) predisposes to bacteremia.

Disease

Meningitis and Meningococemia

- Abrupt onset with fever, chills, malaise, prostration, and a rash that is generally petechial; rapid decline
- Fulminant cases: ecchymoses, DIC, shock, coma, and death (Waterhouse-Freiderichsen syndrome)

Treatment

- Penicillin G or ceftriaxone
- Some β -lactamase production beginning to be seen

Prevention

- **Vaccine:** capsular polysaccharide of strains Y, W-135, C, and A
 - Type B (50% of the cases in USA) capsule not a good immunogen
- Prophylaxis of close contacts: rifampin (or ciprofloxacin)

***Neisseria gonorrhoeae* (Gonococcus)**

Distinguishing Characteristics

- Gram-negative kidney bean-shaped diplococci
- (Intracellular Gram (-) diplococci in PMNs from urethral smear from symptomatic male is suggestive of *N.g.*)
- Commonly: diagnosis by genetic probes with amplification
- Culture (when done) on Thayer-Martin medium
 - Oxidase-positive colonies
 - Maltose not fermented
 - No capsule

Reservoir

Human genital tract

Transmission

Sexual contact, birth; sensitive to drying and cold.

Pathogenesis

Pili

- Attachment to mucosal surfaces
- Inhibit phagocytic uptake
- Antigenic (immunogenic) variation: >1 million variants

Outer Membrane Proteins

- OMP I: structural, antigen used in serotyping
- Opa proteins (opacity): antigenic variation, adherence
- IgA protease: aids in colonization and cellular uptake

Organism invades mucosal surfaces and causes inflammation.

Newborn meningitis:

- ① group B Streptococ
- ② E. Coli
- ③ Listeria

Adult meningitis:

- ① *N. Meningitidis*
- ② *S. pneumonia*

most common bacterial

STD

- ① Chlamydia
- ② *N. gonorrhoeae*

Disease

Gonorrhea

- Male: urethritis, proctitis
- Female: endocervicitis, PID (contiguous spread), arthritis, proctitis
- Infants: ophthalmia (rapidly leads to blindness if untreated)

Treatment

- Ceftriaxone
- Test for *Chlamydia trachomatis* or treat with a tetracycline
- Penicillin-binding protein mutations led to gradual increases in penicillin resistance from the 50s to the 70s.
- Plasmid-mediated β -lactamase produces high-level penicillin resistance.

Prevention

- Adult forms: no vaccine; condoms
- Neonatal: silver nitrate or erythromycin ointment in eyes at birth

Moraxella catarrhalis

- Gram-negative diplococcus (close relative of neisseriae)
- Normal upper respiratory flora
- Otitis media
- Causes bronchitis and bronchopneumonia in elderly with COPD
- Drug resistance a problem; most strains produce a β -lactamase

Otitis media causes
① S. pneumoniae
② non-typable H. influenzae
③ Moraxella

GENUS: PSEUDOMONAS

- Gram-negative rod
- Oxidase-positive
- Aerobic

Pseudomonas aeruginosa

Distinguishing Characteristics

- Oxidase-positive, Gram-negative rods, non-fermenting
- Pigments: pyocyanin (blue-green) and fluorescein
- Grape-like odor
- Slime layer
- Non-lactose-fermenting colonies on EMB or MacConkey

Reservoir

Ubiquitous in water

Transmission

Water aerosols, raw vegetables, flowers

Pathogenesis

- Endotoxin causes inflammation in tissues and Gram-negative shock in septicemia.
- *Pseudomonas* exotoxin A ADP ribosylates EF-2, inhibiting protein synthesis (like diphtheria toxin)
- Liver is primary target.
- Capsule/slime layer: allows formation of pulmonary microcolonies; difficult to remove by phagocytosis

Compromising Condition/Opportunistic Infections

Normal People

- Transient GI tract colonization: loose stools (10% pop.)
- Hot tub folliculitis
- Eye ulcers: trauma, coma, or prolonged contact wear

Burn Patients: GI tract colonization → skin → colonization of eschar → cellulitis (blue-green pus) → septicemia

Neutropenic Patients: Pneumonia and septicemias—often superinfections (infections while on antibiotics)

Chronic Granulomatous Disease (CGD): Pneumonias, septicemias (*Pseudomonas* is catalase positive)

Note

Pseudomonas medical ecology

Pseudomonas aeruginosa is an ubiquitous water and soil organism that grows to very high numbers overnight in standing water (distilled or tap).

Sources for infections include:

- Raw vegetables, respirators, humidifiers, sink drains, faucet aerators, cut and potted flowers, and, if not properly maintained, whirlpools.
- Transient colonization of colons of about 10% of people. Bacteria get on skin from fecal organisms. Requires exquisitely careful housekeeping and restricted diets in burn units.

Note

Drug Resistance in
P. aeruginosa

Susceptibilities important.
Drug resistance very common:

Inherent resistance
(missing high affinity porin
some drugs enter through);
Plasmid-mediated β -
lactamases and acetylating
enzymes.

Septicemias: Fever, shock \pm skin lesions (black necrotic center, erythematous margin (**ecthyma gangrenosum**))

Catheterized Patients: Urinary tract infections (UTI)

Cystic Fibrosis: Early pulmonary colonization, recurrent pneumonias. Always high slime-producing strains

Treatment

Antipseudomonal penicillin, third-generation cephalosporins

Prevention

- Pasteurization or disinfection of water-related equipment, hand washing; prompt removal of catheters.
- No flowers or raw vegetables in burn units.

GENUS: *LEGIONELLA*

- Weakly Gram-negative
- Pleomorphic rods requiring cysteine and iron
- Water organisms

Legionella pneumophila
(and other legionellae)

Distinguishing Characteristics

- Stain poorly with standard Gram stain; **Gram-negative**
- **Fastidious** requiring increased iron and cysteine for laboratory culture (CYE, Charcoal Yeast Extract)
- **Facultative intracellular pathogens**
 - Diagnosis: DFA (Direct Fluorescent Antibody) on biopsy, (+) by Dieterle silver stain
 - Antigen urine test for serogroup 1 only
 - Fourfold increase in antibody

Reservoir

Rivers/streams/amoebae; air-conditioning water cooling tanks

Transmission

- Aerosols from contaminated **air-conditioning**
- **No human-to-human transmission**

Predisposing Factors

- **Smokers over 55 years with high alcohol intake**
- **Immunosuppressed patients**, e.g., renal transplant patients

Pathogenesis

- **Facultative intracellular pathogen**
- **Endotoxin**

Diseases

Seasonal: Associated with air-conditioning systems, now routinely decontaminated

Legionnaires' Disease ("Atypical Pneumonia")

- Pneumonia
- Mental confusion
- Diarrhea (no *Legionella* in gastrointestinal tract)

Pontiac Fever

- Pneumonitis
- No fatalities

Treatment

- Fluoroquinolone or azithromycin or erythromycin with rifampin for immunocompromised patients
- Drug must penetrate human cells.

Prevention

Routine decontamination of air-conditioner cooling tanks

GENUS: *BORDETELLA*

- Gram-negative small rods
- Strict aerobes

Bordetella pertussis

Distinguishing Characteristics

- Small Gram-negative, aerobic rods
- Fastidious/delicate: Regan-Lowe or Bordet-Gengou media; either direct cough plates or nasopharyngeal cultures.
- Difficult to culture from beginning of paroxysmal stage on
- Direct immunofluorescence (DFA) on nasopharyngeal smear
- PCR and serologic tests available

Reservoir/Transmission

Human (vaccinated); respiratory droplet

Pathogenesis

B. pertussis Is a Mucosal Surface Pathogen

Attachment to nasopharyngeal ciliated epithelial cells

- Filamentous hemagglutinin
- Pertussis toxin (on outer membrane) aids in attachment

Toxins damage respiratory epithelium.

- Adenylate cyclase toxin: impairs leukocyte chemotaxis → inhibits phagocytosis and causes local edema
- Tracheal cytotoxin: interferes with ciliary action; kills ciliated cells
- Endotoxin
- Pertussis toxin (A and B component, OM protein toxin):
ADP ribosylation of G_i (inhibiting negative regulator of adenyl cyclase) interferes with transfer of signals from cell surface to intracellular mediator system;
 - Lymphocytosis promotion
 - Islet-activation → hypoglycemia
 - Blocks immune effector cells
 - Increased histamine sensitivity

In A Nutshell

B. pertussis immunity

- Vaccine immunity lasts 5–10 years (and is primarily IgA)
- Babies born with little immunity.
- Vaccinated humans >10 yrs serve as reservoir.
- 12–20% of afebrile adults with cough >2 weeks have pertussis.
- Immunity to actual pertussis is life long.
- New vaccines (DTaP)
 - Acellular: components:
 - Immunogens vary by manufacturer
 - Pertussis toxoid
 - Filamentous hemagglutinin
 - pertactin (OMP)
 - 1 other

Disease

Whooping Cough (Pertussis)

Three stages after a 7–10 day incubation; contagious

- (1–2 weeks) **catarrhal**: rhinorrhea, malaise, fever, sneezing; contagious
- (2–4 weeks) **paroxysmal**: repetitive cough with whoops, vomiting; anoxia and severity of cough cause neurological damage and eye hemorrhages; organism present at beginning disappears
- (>3 weeks) **convalescence**: less cough, secondary complications manifest: pneumonia, seizures, encephalopathy

Treatment

Supportive care; hospitalization if <6 months old, erythromycin

Prevention

- Vaccine: DTaP; immunity wanes 5–7 years
- Babies born with little or no immunity (IgA) from mom

GENUS: *FRANCISELLA*

- Gram-negative small rods
- Facultative intracellular pathogen

Francisella tularensis

Distinguishing Characteristics

- Small Gram-negative rod
- Serodiagnosis; culture is hazardous

Reservoir

Many species of wild **animals**, especially rabbits, deer, and rodents

Transmission

- **Tick bite** (*Dermacentor*) → **ulceroglandular** disease, characterized by fever, ulcer at bite site, and regional lymph node enlargement and suppuration
- **Traumatic implantation** while skinning rabbits → ulceroglandular disease
- Aerosols (skinning rabbits) → pneumonia
- Ingestion (of undercooked, infected meat or contaminated water) produces typhoidal tularemia.

Pathogenesis

- **Facultative intracellular pathogen** (localizes in reticuloendothelial cells)
- Granulomatous response

Disease

Tularemia

- Endemic in every state of U.S.
- Arkansas and Missouri highest

Treatment

Streptomycin

Prevention

- Protect against tick bites, gloves while butchering rabbits
- Live, attenuated vaccine for persons in high-risk occupations

Note

Zoonotic organisms

- *Brucella*
- *Bacillus anthracis*
- *Listeria monocytogenes*
- *Salmonella enteritidis*
- *Campylobacter*
- Q fever (*Coxiella burnetii*)
- *Chlamydia psittaci*
- *Francisella tularensis*

GENUS: BRUCELLA

- Gram-negative rods
- Zoonotic
- Facultative intracellular pathogen

Brucella species

Distinguishing Characteristics

- Small Gram-negative rods, aerobic
- Facultative intracellular
- Serological confirmation of disease most common
- Culture is hazardous.

Reservoir

Domestic livestock

Transmission

- Unpasteurized dairy products
- Direct contact with the animal, work in slaughterhouse

Pathogenesis

- Endotoxin
- Facultative intracellular parasite (localizes in cells of reticuloendothelial system, RES) → septicemia
- Granulomatous response with central necrosis

Disease

Brucellosis (undulant fever)

- *B. abortus*: cattle
- *B. suis*: pigs
- *B. melitensis*: goats

Acute Septicemias

- Fever 100–104°F (often in evening)
- Influenza-like symptoms, including arthralgias, myalgia, back pain
- Sweating
- Hepatomegaly

Undulant Form: Milder, often a result of incomplete treatment

Chronic Form

(Disease for more than one year.)

- Usually in older people (veterinarians)
- Cyclic bouts of depression and sweating
- Fever rare
- Ocular complications (uveitis) in 5–10%

Prevention

- Vaccinate cattle; vaccinate high-risk humans.
- Pasteurize milk, especially goat milk.

GENUS: *CAMPYLOBACTER*

- Gram-negative curved rod with polar flagella
- Microaerophilic



Campylobacter jejuni

Distinguishing Characteristics

- Motile, curved Gram-negative rods (“gulls’ wings”)
- Microaerophilic, grows well at 42°C on selective media (Campy medium or Skirrow’s agar); oxidase positive.

Figure I-2-10. *Campylobacter*

Reservoir

Intestinal tracts of humans, cattle, sheep, dogs, cats, **poultry**

Transmission

Fecal-oral, primarily from **poultry**

Pathogenesis

- **Low infectious dose** (as few as 500)
- **Invades** mucosa of the colon, destroying mucosal surfaces; blood and **pus** in stools (inflammatory diarrhea)
- Rarely penetrates to cause septicemia

Disease

- Common cause of infectious diarrhea worldwide
- In U.S., *Campylobacter* enteritis > (*Salmonella* + *Shigella*)
- **Ten to more stools/day, may be frankly bloody**
- Abdominal pain, fever, malaise, nausea, and vomiting
- Generally **self-limiting in 3–5 days** but may last longer
- Complications:
 - **Guillain-Barré syndrome** (~30% of the GBS in the U.S.).
 - Reactive arthritis

Treatment

Erythromycin, fluoroquinolones, penicillin-resistant

Helicobacter pylori

Distinguishing Characteristics

- Gram-negative spiral gastric bacilli with flagella
- Microaerophilic, 37°C growth (Campy medium or Skirrow's agar); oxidase positive.

Reservoir

Humans

Transmission

Fecal-oral, oral-oral

Pathogenesis

- **Motile**
- **Urease-positive:** ammonium cloud neutralizes stomach acid, allowing survival in stomach acid during transit to border.
- **Mucinase** aids in penetration of mucous layer (rapid shift down to neutral as it penetrates).
- **Invasive** into stomach lining where pH is neutral.
- Inflammation is prominent.
- Two biotypes (I and II); type I produces vacuolating cytotoxin.

Disease

- Causes chronic **gastritis and duodenal ulcers**.
- Associated with several forms of **stomach cancer**. (Atrophic gastritis rather than duodenal ulcers correlates with risk.)
- Now classed by WHO as **Type I carcinogen**.

Lab Diagnosis

- Serologic test
- Biopsy with culture; histology with Giemsa or silver stain
- Breath test: ¹³C-urea swallowed; ammonia + ¹³C-CO₂ produced

Treatment

(Myriad of regimens.)

Omeprazole + amoxicillin + clarithromycin

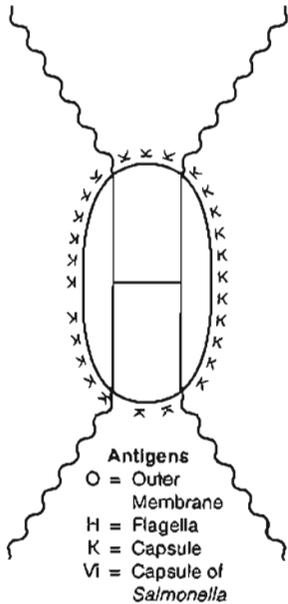


Figure I-2-11. Antigens of Enterobacteriaceae

FAMILY: ENTEROBACTERIACEAE

- Gram-negative rods
- Facultative anaerobes
- Ferment glucose
- Cytochrome C oxidase negative
- Reduce nitrates to nitrites
- Catalase positive

Pathogenesis (overall for family)

- Endotoxin
- Some also produce **exotoxins**.
- Antigens
 - O = cell envelope or O antigen
 - H = flagellar (motile cells only) antigen
 - K = capsular polysaccharide antigen
 - Vi = *Salmonella* capsular antigen (virulence)

Lab Diagnosis

Grow on two media:

- Blood
- Eosin-methylene blue or MacConkey agar (differentiate lactose fermentation)

Genera in Family

- Lactose fermenters: CEEK (colored colonies)
 - Citrobacter*
 - Escherichia*
 - Enterobacter*
 - Klebsiella*
- Nonlactose fermenters: ShYPS (colorless colonies)
 - Shigella* } nonmotile
 - Yersinia* } non H₂S producers
 - Protetis* } motile
 - Salmonella* } H₂S producers

Slow lactose fermenter: *Serratia*, which produces a salmon red pigment and is an opportunist. Each genus is characterized by a whole series of chemical tests to detect the presence of certain enzymes or pathways.

GENUS: *ESCHERICHIA*

- Gram-negative rod
- *Enterobacteriaceae*
- Ferments lactose

Escherichia coli

Distinguishing Characteristics

- Gram-negative rod
- Facultative anaerobic, oxidase negative
- *E. coli* is lactose-fermenter: colonies with iridescent green sheen on EMB

Reservoirs

- Human colon; may colonize vagina or urethra
- Contaminate crops where human fecal fertilizer is used
- Enterohemorrhagic strains: bovine feces

Diseases

Urinary Tract Infections (UTIs)

- *E. coli* is most common cause.
- Transmission: from own fecal flora → urethra
Predisposing factors: female anatomy; strictures, stones, or abnormality in urine flow; indwelling urinary catheters
- Pathogenesis
 - Motility aid
 - Adherence to uroepithelium important: P-pili: pyelonephritis-associated pili (PAP or P-pili); X-adhesins
 - β -hemolytic (many)
- Treatment: ampicillin or sulfonamides for UTI

Neonatal Septicemia and Meningitis

- Second most common cause after Group B streptococci
- Maternal fecal flora → vagina contaminates baby
 - **Capsule:** most strains have K1 serotype. Capsule allows the organism to evade phagocytic uptake in the blood and spleen so organism can get to blood/brain barrier.
 - **Endotoxin:** triggers shock; inflammation at blood/brain barrier facilitates invasion.

Septicemia

- Indwelling IV lines predispose to invasion from skin.
- Cytotoxic drugs damage intestinal mucosa, allowing escape.
- Endotoxin triggers Gram-negative shock.

Note

***E. coli* Identification from Stool**

- Isolation of *E. coli* from feces is not by itself significant.
- Sorbitol MacConkey screen.
- Most *E. coli* ferment sorbitol.
- Most EHEC do not (colorless).

Animal models and tissue culture assays may be used, but other methods of differentiating pathogenic *E. coli* from normal flora are more commonly:

- Immunoassay looking for specific protein antigens (on or excreted by the bacterium)
- Serotyping since certain serotypes are more often pathogenic
- DNA probes for specific genes in a culture
- PCR on clinical specimen

Note

Mnemonic:

Toxins ↑cAMP

- c = cholera
- A = anthrax
- Σ = *E. coli* LT
- P = pertussis

Gastroenteritides/Diarrheas

- **Most strains:** fecal-oral spread; poor sanitation/water; use of human feces on vegetables or fruits.
- **Enterohemorrhagic strains:** bovine fecal contamination: undercooked hamburger, raw milk, apple juice (from fallen apples), alfalfa sprouts.

ETEC = Enterotoxigenic *E. coli*

Major cause of “Traveler’s Diarrhea” and diarrhea in <3 year olds in developing countries.

- Two enterotoxins are produced:
 - LT: The **heat labile toxin** stimulates adenylate cyclase by **ADP ribosylation** of G_s resulting in increased cAMP causes outflow of chloride ions and water in small intestine → watery diarrhea.
 - ST: The **heat stable toxin** causes diarrhea by stimulating guanylate cyclase.
- Enterotoxin production identified by immunoassay, bioassay, or DNA probe assays.
- **Capsule** impedes phagocytosis
- **Colonizing factor adhesins (CFAs):** bind to small intestine.

EPEC = Enteropathogenic *E. coli*

- Second most common cause of infantile diarrhea after rotavirus
- **Prolonged, watery diarrhea** in babies <1 year in developing countries
- Plasmid containing virulence genes called the *E. coli* **adherence factor (EAF)**; EPEC adhere to M cells, causing rearrangement of actin and effacement of the brush border microvilli.

EIEC = Enteroinvasive *E. coli*

- **Invades large bowel**, similar to shigellosis, including the formation of actin “jet trails”
- Often manifests as **watery diarrhea** (with **excess of leukocytes**, however) with **fever and abdominal pain**. Only about 10% progress on to dysentery.

EHEC = Enterohemorrhagic *E. coli*

- Also known as **verotoxin producing *E. coli* (VTEC)**
- **O157:H7** is the most common serotype.
- EHEC disease ranges from **mild diarrhea** without blood to **hemorrhagic colitis ± hemolytic uremic syndrome (HUS)**. **Fever is generally absent**, distinguishing it from shigellosis. HUS most common in kids <5 years old.
- Plasmid-associated **verotoxins = Shiga-like toxin 1 and 2**. **Both toxins inhibit protein synthesis in the large intestine by nicking the 60S ribosome.**
- Not invasive, no inflammatory response; afebrile
- Focus of infection is the large intestine.
- **Excess fecal leukocytes are not seen**. Damage is due to toxin activity so does not promote inflammatory response.
 - Lab screen: most EHEC are sorbitol non-fermenters so have colorless colonies on sorbitol MacConkey; DNA probe for verotoxin genes.
- Antibiotics may increase risk of HUS and kidney damage, especially early and in children. Check before using.

EaggEC = Enteroaggregative *E. coli*

- Important cause of **persistent diarrhea** with vomiting, low-grade fever in the developing world.
- **Fimbriae** create a “stacked brick-like” thick biofilm by attaching to each other and enterocytes.
- Also produce enterotoxin (EAST1).

Treatment

- Depends on strain, severity of diarrhea, presence of fever, blood, and pus
- Rehydration for traveler’s diarrhea; trimethoprim-sulfamethoxazole may shorten duration of symptoms
- No antibiotic currently recommended for O157:H7 (generally bloody diarrhea without fever)
- Fluoroquinolones for bloody diarrhea with pus and fever >101°F and tenesmus
- Antibiotic resistance primarily mediated by plasmid-encoded enzymes, e.g., β-lactamase, and aminoglycoside modifying enzymes

GENUS: *SHIGELLA*

- Gram-negative rod
- *Enterobacteriaceae*
- Nonlactose fermenter
- Nonmotile

Shigella species

S. dysenteriae (most severe disease), *S. sonnei* (most common in U.S.), etc.

Distinguishing Characteristics

- Gram-negative rods, non-motile
- Facultative anaerobes, non-lactose fermenting (colorless colonies on EMB or MacConkey)
- Identified by biochemical reactions or by serology with anti-O antibody in agglutination test.

Reservoir

Human colon only (no animal carriers)

Transmission

- Fecal-oral spread, person to person
- As with all fecal-oral spread, the “Four Fs”: fingers, food, feces, flies

Pathogenesis

- Endotoxin triggers inflammation.
- No H antigens
- Shigellae invade M cells (membrane ruffling and macropinocytosis); get into the cytoplasm, replicate and then polymerize actin jet trails to go laterally without going back out into the extracellular milieu. This produces very shallow ulcers and rarely causes invasion of blood vessels.
- Shiga toxin:
 - Produced by *S. dysenteriae*, type 1
 - Three activities: neurotoxic, cytotoxic, enterotoxic
 - AB component toxin is internalized in human cells; inhibits protein synthesis by clipping 60 ribosomal subunit.

Disease

Enterocolitis/Shigellosis (most severe form is dysentery)

- Few organisms required to start infection (1–10) (extremely acid resistant)
- 1–4 day incubation
- Organisms invade producing bloody diarrhea.
- **Fever (generally >101°F), lower abdominal cramps, tenesmus; diarrhea first watery, then bloody, invasive but rarely septicemia; shallow ulcers.**
- Severity depends on the age of patient and the strain; *S. dysenteriae* Type 1 with toxin most severe.

Treatment

- Mild cases: fluid and electrolyte replacement only
- Severe cases: antibiotics
- Resistance is mediated by plasmid-encoded enzymes

Prevention

- Proper sanitation (sewage, clean drinking water, hand washing)

Note

Comparative Microbiology

- Invasive bacteria: PMN in stool: *Shigella*, *Salmonella*, *Campylobacter*, EIEC.
- Toxigenic bacteria: ETEC, *V. cholera*, *Cl. perfringens*, EHEC.

In A Nutshell

Comparative Microbiology:
Major encapsulated organisms

Some Killers Have Pretty Nice Capsules:

Strep pneumoniae

Klebsiella pneumoniae

Haemophilus influenzae Type b (a-d)

Pseudomonas aeruginosa

Neisseria meningitidis

Cryptococcus neoformans (the yeast)

(Not a complete list, just the big ones!)

GENUS: *KLEBSIELLA*

- Gram-negative rod
- *Enterobacteriaceae*
- Major capsule

Klebsiella pneumoniae

Distinguishing Characteristics

- Gram-negative rods with large polysaccharide capsule
- **Mucoid**, lactose-fermenting colonies on MacConkey agar
- Oxidase negative

Reservoir

Human colon and upper respiratory tract

Transmission

All commonly from own flora

Pathogenesis

- Capsule: impedes phagocytosis
- **Endotoxin**: causes fever, inflammation, and shock (septicemia)

Transmission/Disease

Pneumonia

- Community-acquired, most often in older males; most commonly in **patients with either chronic lung disease or alcoholism, or diabetes** (but not the most common cause of pneumonia in alcoholics! *S. pneumoniae* is.)
- Endogenous; assumed to reach lungs by inhalation of respiratory droplets from upper respiratory tract.
- Frequent **abscesses** make it hard to treat; fatality rate high
- **Sputum is generally thick and bloody (currant jelly)** but not foul smelling as in anaerobic aspiration pneumonia.

Urinary Tract Infections: Catheter-related (nosocomial) from fecal contamination of catheters

Septicemia: In immunocompromised patients may originate from bowel defects or invasion of IV lines

Treatment

Antibiotic sensitivity testing must be done

Prevention

Good catheter care; limit catheter use

GENUS: *SALMONELLA*

- Gram-negative rod (*Enterobacteriaceae*)
- Non-lactose fermenter
- Motile

2,000 serotypes of salmonellae; serotype names are still in use.

Diseases

Enteric or Typhoid Fever (*S. typhi*)

Gastroenteritis

Septicemia

Salmonella typhi

Distinguishing Characteristics

- Gram-negative rods, highly motile with the Vi capsule
- Facultative anaerobe, non-lactose fermenting
- Produces H₂S
- Species identification with biochemical reactions
- Sensitive to acid

Reservoir

- Humans only; NO ANIMAL RESERVOIRS
- Only the typhoid Mary's of the world!

Transmission

- Fecal-oral route from human carriers (gall bladder)
- Decreased stomach acid or impairment of mononuclear cells such as in sickle cell disease predisposes to *Salmonella* infections

Pathogenesis/Disease

Typhoid Fever (Enteric Fever), *S. typhi*

(milder form: paratyphoid fever; *S. paratyphi*)

- Organism ingested (large number if stomach acid is normal).
- Infection begins in ileocecal region; constipation common.
- Host cell membranes "ruffle" from *Salmonella* contact.
- *Salmonella* reach basolateral side of M cells then mesenteric lymph nodes and blood (transient 1st septicemia)
- At 1 week: patients have 80% positive blood cultures; 25% have rose spots (trunk/abdomen)
- Liver and spleen are infected with additional release of bacteria to bloodstream → signs of septicemia (mainly fever)

- *S. typhi* survives intracellularly and replication in macrophages; **resistant to macrophage killing due to:**
 - Decreased fusion of lysosomes with phagosomes
 - Defensins (proteins) allow it to withstand oxygen-dependent and -independent killing.
- Released from the macrophages. The Vi capsular antigen (*S. typhi* only) withstands complement mediated killing.
- **Biliary system** (liver, gall bladder) is infected, organisms enter intestinal tract in bile.
- **By week 3: 85% of stool cultures** are positive.
- Symptoms: **fever**, headache, abdominal pain, constipation more common than diarrhea
- Complications of untreated: **necrosis of Peyer's patches** with perforation (local endotoxin triggered damage), thrombophlebitis, cholecystitis, pneumonia, abscess formation, etc.

Treatment

Ciprofloxacin or ceftriaxone in seriously ill; some drug resistance

Prevention

Sanitation; 3 vaccines: attenuated oral vaccine of *S. typhi* strain 21 (Ty21a), parenteral heat killed *S. typhi*, and parenteral Vi polysaccharide capsular vaccine

Nontyphoidal Salmonellae: *S. enteritidis*, *S. typhimurium*, and Other Species

Distinguishing Characteristics

- Facultative Gram-negative rods, non-lactose-fermenting on EMB, MacConkey medium
- Produces H₂S, **motile** (unlike *Shigella*)
- Speciated with biochemical reactions and serotyped with O, H, and Vi antigens
- **Antibodies** to O, Vi, and H antigens **in patient's serum** can be detected by agglutination (**Widal test**).

Reservoir

Enteric tracts of human and domestic animals, e.g., chickens and turtles

Transmission

Largely through chicken products (raw chicken and eggs) in the kitchen

Pathogenesis

- Sensitive to stomach acid (infectious dose 10⁵ organisms)
- Lowered stomach acidity (antacids or gastrectomy) increases risk.
- Endotoxin in cell wall; no exotoxin
- **Invades** the mucosa in the ileocecal region, invasive to lamina propria → inflammation → increased PG → increased cAMP → loose diarrhea; shallow ulceration.
- Spread to septicemia not common with *S. enteritidis* (the most common) but may occur with others.

Diseases

Enterocolitis/Gastroenteritis

Second most common after *Campylobacter*. 6–48 hour incubation; nausea, vomiting, only occasionally bloody, loose stools, fever, abdominal pain, myalgia, headache

Septicemia

- *S. cholerae-suis*, *S. paratyphi*, and *S. dublin*
- When it occurs, it is usually in very young or elderly.
- Endocarditis or arthritis complicate about 10%.

Osteomyelitis: Sickle cell disease predisposes to osteomyelitis. *Salmonella* is the most common causative agent of osteomyelitis in sickle cell disease (not trait) patients (>80%).

Treatment

- Antibiotics generally not effective so not recommended for uncomplicated enterocolitis.
- Antibiotics for septicemia depend on sensitivity tests.

Prevention

Proper sanitation (sewage, clean drinking water, hand washing, particularly food handlers)

GENUS: *YERSINIA*

- Gram-negative rod
- *Enterobacteriaceae* (oxidase negative)

Yersinia pestis (*Enterobacteriaceae*)

Distinguishing Characteristics

- Small Gram-negative rods with bipolar staining
- Facultative intracellular parasite
- Coagulase +
- Clinical specimens and cultures are hazardous
- Serodiagnosis or direct immunofluorescence

Reservoir

U.S.: desert southwest, rodents, e.g., prairie dogs, chipmunks, squirrels, field mice, and voles

Transmission

- Wild rodents flea bite → sylvatic plague
- Human-to-human transmission by respiratory droplets

Pathogenesis

- Coagulase-contaminated mouth parts of flea
- Endotoxin, an exotoxin
- Two antigens (V and W)
- Envelope antigen (F-1) inhibits phagocytosis

Disease

Bubonic Plague

- Flea bites infected animal and then later uninfected human-coagulase role-contaminated mouth parts
- Symptoms:
 - Rapidly increasing fever
 - Regional buboes
 - Conjunctivitis
 - Leads to septicemia and death if untreated

Pneumonic Plague

- Arises from septic pulmonary emboli in bubonic plague or inhalation of organisms from infected individual.
- Highly contagious!

Treatment

- Streptomycin with tetracycline
- **Strict quarantine for 72 hours** after starting antibiotics

Prevention

- Animal control; avoid sick and dead animals.
- **Killed vaccine** is available for high-risk occupations.

Yersinia enterocolitica

- **Zoonotic, unpasteurized milk, pork**
- Enterotoxin
- **Multiplies in the cold**
- Enterocolitis in northern climates (Michigan, Scandinavia)
- Presentations may vary with age
 - Very young: febrile diarrhea (blood and pus)
 - Older kids/young adults: **pseudoappendicitis**
 - Adults: enterocolitis with post-infective sequelae like reactive arthritis
- **Blood transfusion-associated infections**

GENUS: *PROTEUS*

- Gram-negative rod
- *Enterobacteriaceae*
- Peritrichous flagella
- Non-lactose-fermenting
- Urease positive

Proteus mirabilis, *Proteus vulgaris*

Distinguishing Characteristics

- Gram-negative rods
- **Highly motile**; “swarming” motility on surface of blood agar
- Urease produced
- Facultative anaerobe (*Enterobacteriaceae*), oxidase negative

Reservoir

Human colon and environment (water and soil)

Disease

Urinary tract infection and septicemia

Pathogenesis

- Urease raises urine pH to cause kidney stones (staghorn renal calculi)
- **Motility** may aid entry into bladder.
- Endotoxin causes fever and shock when septicemia occurs.

Treatment

Do susceptibilities.

Prevention

Promptly remove urinary tract catheters.

Note

Weil-Felix test antigens of OX strains of *Proteus vulgaris* cross-react with rickettsial organisms.

GENUS: *VIBRIO*

- Gram-negative curved rod with polar flagella
- Oxidase positive (*Vibrionaceae*)

Vibrio cholerae

- *Vibrio cholerae* O1 divided into biotype El Tor (predominant now) and Cholerae (classical)
- *Vibrio cholerae* O139 also produces cholera toxin.

Distinguishing Characteristics

- “Shooting star” motility inactivated by specific serum.
- Oxidase-positive, which distinguishes them from *Enterobacteriaceae*
- Growth on alkaline but not acidic media (TCBS = Thiosulfate Citrate Bile salt Sucrose medium)

Reservoir

Human colon; no vertebrate animal carriers. (Copepod or shellfish may be contaminated by water contamination.) Human carriage may persist after untreated infection for months after infection; permanent carrier state rare.

Transmission

- Fecal-oral spread; sensitive to stomach acid
- Requires high dose ($>10^7$ organisms), if stomach acid is normal.

Pathogenesis

- Motility, mucinase, and toxin co-regulated pili (Tcp) aid in attachment to the intestinal mucosa.
- Cholera enterotoxin (cholera toxin) similar to *E. coli* LT. ADP ribosylates (G_s alpha) activating adenylate cyclase → increased cAMP → efflux of Cl^- & H_2O (persistent activation of adenylyl cyclase).

Disease

Cholera: Rice water stools, tremendous fluid loss; hypovolemic shock if not treated.

Treatment

- Fluid and electrolyte replacement
- Doxycycline or ciprofloxacin shortens disease and reduces carriage.

Prevention

Proper sanitation; new vaccine; tetracycline to reduce transmission

Vibrio parahaemolyticus

- Food poisoning associated with **undercooked or raw seafood**
- **Marine**
- 5–94 hours incubation (mean 24 hours)
- Self-limiting watery diarrhea with cramping, abdominal pain

Vibrio vulnificus

- Brackish water; oysters; warm months
- **Cellulitis** when it gets into cuts; hard to treat
- **Gastroenteritis** when ingested
- **Septicemia** in patients with preexisting liver disease (50% fatal)

GENUS: PASTEURELLA

- Small Gram-negative rods
- Facultative anaerobic rods

Pasteurella multocida

Distinguishing Characteristics

Small Gram-negative rods

Reservoir

Mouths of many animals, especially cats and dogs

Transmission

Animal bites; particularly from cat bites

Disease

Wound infection leading to **cellulitis with lymphadenitis**

Pathogenesis

Endotoxin, capsule; spreads rapidly within skin, no exotoxins known

Lab Diagnosis

Rarely cultured because routine prophylaxis is common.

Treatment/Prevention

Amoxicillin/clavulanate for cat bites. Amoxicillin/Clavulanate is standard prophylaxis and treatment for most bites (human included), along with thorough cleaning.

GENUS: HAEMOPHILUS

- Gram-negative, pleomorphic rod
- Requires growth factors

Haemophilus influenzae

Distinguishing Characteristics

- Encapsulated, Gram-negative rod
- Fastidious: requires factors X (hemin) and V (NAD) for growth on nutrient or blood agar
- Grows near *S. aureus* on BA = "Satellite" phenomenon
- Chocolate agar provides both x and y factor

Reservoir

Human nasopharynx

Transmission

Respiratory droplets, shared toys

Pathogenesis

- Polysaccharide capsule (type b capsule is polyribitol phosphate) most important virulence factor
- 95% of invasive disease is caused by capsular type b. Capsule important in diagnosis. Antigen screen on CSF (e.g., latex particle agglutination); serotype all isolates by Quellung.
- IgA protease is a mucosal colonizing factor.

Diseases

Meningitis: *H. influenzae*, type b encapsulated strains.

Epidemic in unvaccinated children ages 3 months to 2 years

- After maternal antibody has waned
- Before the immune response of the child is adequate
- *H. influenzae* was most common cause of meningitis in 1 to 5-year-old children (mainly younger than 2) up to 1990.
- Still a problem if child is <2 years and not vaccinated.

Vaccination effective to prevent type b disease.

- Polyribitol capsule conjugated to protein: (diphtheria toxoid or *N. meningitidis* outer membrane proteins) making it a T cell dependent vaccine.
- Vaccine: 2, 4, 6 months; booster 15 months; 95% effective

Otitis Media

Usually nontypeable strains

Bronchitis

Exacerbations of acute bronchitis in smokers with COPD

Pneumonia

1–24 months; smokers

Epiglottitis

Rare in vaccinated kids; seen in unvaccinated toddlers. *H. influenzae* was the major causative agent.

Treatment

Cefotaxime or ceftriaxone for empirical therapy of meningitis. Check nasal carriage before releasing; use rifampin if still colonized.

Prevention

- Conjugate capsular polysaccharide-protein vaccine
- Rifampin reduces oropharynx colonization and prevents meningitis in unvaccinated, close contacts <2 years.

Haemophilus ducreyi

- Sexually transmitted disease.
- Chancroid (genital ulcers): **soft, painful chancre** (“You do cry with ducreyi.”)
- **Slow to heal without treatment**
- **Open lesions increase transmission of HIV**
- Diagnosis: **DNA probe**

GENUS: *BACTEROIDES*

- Gram-negative rod
- Anaerobic
- Modified LPS with reduced activity

Bacteroides fragilis

Distinguishing Characteristics

- Anaerobic, Gram-negative rods
- Anaerobes are identified by biochemical tests and gas chromatography.

Reservoir

Human colon; the genus *Bacteroides* is the predominant anaerobe.

Transmission

Endogenous from bowel defects (e.g., from cytotoxic drug use, cancer), surgery, or trauma

Pathogenesis

- Modified LPS (missing heptose and 2-keto-3 deoxyoctonate) has reduced endotoxin activity.
- Capsule is antiphagocytic.

Disease

Septicemia, peritonitis (often mixed infections), and abdominal abscess

Treatment

- Metronidazole, clindamycin, or cefoxitin. Abscesses should be surgically drained.
- Antibiotic resistance is common (penicillin G, some cephalosporins, and aminoglycosides).

Prevention

Prophylactic antibiotics for gastrointestinal or biliary tract surgery

Bacteroides melaninogenicus = *Prevotella melaninogenica*

- Melanin-producing (black) *Bacteroides*
- Normal gingival flora
- Anaerobe
- Oral abscesses
- Heparinase leads to clotting in brain.

GENUS: *TREPONEMA*

- Spirochetes: spiral with axial filament (endoflagellum)
- Poorly visible on Gram stain but basically Gram-negative

Treponema pallidum

Distinguishing Characteristics

- Thin spirochete, not reliably seen on Gram stain
- Basically a Gram-negative cell envelope
- Outer membrane has endotoxin-like lipids.
- Axial filaments = endoflagella = periplasmic flagella
- Cannot culture in clinical lab; serodiagnosis
- Is an obligate pathogen (but not intracellular)

Reservoir/Transmission

Human genital tract; transmitted sexually or across the placenta.

Pathogenesis

Disease characterized by endarteritis resulting in lesions. Strong tendency to chronicity.

Disease

Syphilis: Progression in untreated syphilis: incubation: 10–90 days.

Primary

- Nontender chancre(s) at site of inoculation
- Margins generally “clean,” distinctly indurated edge
- Contagious (but you still cannot culture!)
- Chancre is good source of material for microscopy. (This is important as only 50% of those with chancres will be positive by nontreponemal serologic tests.)
- Heals spontaneously in 3–6 weeks

Secondary

- 1 to 3 months later (*T. pallidum* has spread early via bloodstream.)
- Maculopapular (often copper colored) rash on skin including palms and soles ± patchy alopecia
- Flat wart-like perianal condylomata lata and mucous membrane lesions, both highly infectious
- May “heal” spontaneously, regress (several times), and finally heal.
- Serology is almost always strongly reactive.

Latent syphilis

- Positive serology only

Tertiary

- May be years later in about one-third of the untreated patients
- Tertiary lesions consist of gummas, aortitis, or central nervous system inflammation.
- VDRL may be negative.

Congenital syphilis

- Commonly in babies of IV drug abusing women
- Sx: stillbirths, multiple fetal abnormalities (keratitis, 8th nerve deafness, notched teeth), or sometimes asymptomatic (or snuffles) at birth until two.

Laboratory Diagnosis

Visualize organisms by immunofluorescence or dark-field microscopy.

Serology important: Two types of antibody:

1. Nontreponemal antibody (= reagin)

- Binds to cardiolipin
 - An antigen found in mammalian mitochondrial membranes and in treponemes (but probably are from the host since treponemes don't make).
 - Cheap source of antigen is cow heart, which is used in screening tests (VDRL, RPR, ART).
- Screening tests: Nontreponemal antibody tests:
 - Venereal Disease Research Lab (VDRL)
 - Rapid plasma reagin (RPR)
 - Automated Reagin Test (ART)
 - Any one may be used for screening (inexpensive).
 - Very sensitive in primary (except early) and secondary; titer may decline in tertiary and with treatment.
 - But not specific; confirm with FTA-ABS

2. Treponemal antibody

Earliest antibody; binds to spirochetes

Specific tests for *Treponema* (more expensive)

- Fluorescent Treponemal Antibody-Absorption (FTA-ABS; most widely used test)
- *Treponema pallidum* Microhemagglutination
- These tests are more specific and positive earlier; usually remains positive for life. But positive in patients with other treponeme disease (bejel) and may be positive in Lyme disease.

TORCH screen for neonates no longer includes syphilis test; must order separately.

Treatment

Benzathine penicillin (long-acting form) for primary and secondary syphilis (no resistance to penicillin)

Prevention

Benzathine penicillin is given to contacts, no vaccine is available.

Jarisch-Herxheimer Reaction

- Starts generally during the first 24 hours of antibiotic treatment
- Increase in temperature, decrease in blood pressure; rigors, leukopenia
- May occur during treatment of any of the spirochete diseases

GENUS: BORRELIA

- Larger spirochetes
- Gram-negative
- Microaerophilic

Borrelia burgdorferi

Distinguishing Characteristics

Spirochete, not seen well on Gram-stained smear; can be cultured

Reservoir

Two reservoirs: white-footed mice (nymphs) and white-tailed deer (adult ticks)

Transmission

By *Ixodes* (deer) ticks and nymphs; worldwide but in three main areas in the U.S.:

- *Ixodes scapularis* (*I. dammini*) in Northeast (e.g., Connecticut), Midwest (e.g., Wisconsin)
- *Ixodes pacificus* on West Coast (e.g., California)

Pathogenesis

- *B. burgdorferi* invades skin and spreads via the bloodstream to involve primarily the heart, joints, and central nervous system.
- Arthritis is caused by immune complexes.

Disease

Lyme Disease

Initial symptoms:

- Erythema (chronicum) migrans: spreading annular skin lesion with an erythematous leading edge and central clearing, “bull’s eye” seen in 85% of cases.
- Malaise, headache, severe fatigue, fever, chills
- Musculoskeletal pain, lymphadenopathy

One to several weeks dissemination:

- Neurologic: severe headache, meningitis, cranial nerve palsies (Bell’s palsy)
- Cardiac: arrhythmias, myocarditis, pericarditis

Late and lasting for months or years:

- Arthralgias, arthritis (sx in 80% within few weeks to 2 years)

Laboratory Diagnosis

- Serodiagnosis by detecting IgM or IgG antibody (many false negatives/some false positives)
- Amplification/probes and cultures (Kelly medium) available

Treatment

- Doxycycline, amoxicillin, or azithromycin/clarithromycin (primary)
- Ceftriaxone for secondary
- Doxycycline or ceftriaxone for arthritis

Prevention

- DEET; avoid tick bite
- New vaccine: ospA flagellar antigen

GENUS: LEPTOSPIRA

- Spirochetes: thin, with hooks
- Too thin to visualize, but Gram-negative cell envelope

Leptospira interrogans

Distinguishing Characteristics

- Spirochetes with tight terminal hooks
 - seen on dark-field microscopy but not light microscopy
 - can be cultured *in vitro*; aerobic
- Generally diagnosed by serology

Reservoir

Wild and domestic animals

Transmission

- Via animal urine in water
- In U.S., via dog, livestock, and rat urine through contaminated recreational waters (jet skiers) or occupational exposure (sewer workers)

Pathogenesis

No toxins or virulence factors known.

Disease

Leptospirosis

- Influenza-like disease ± GI tract symptoms
- Progressing on to hepatitis and renal failure if not treated

Laboratory Diagnosis

Serodiagnosis and dark-field microscopy

Treatment

Penicillin G or doxycycline

Prevention

- Doxycycline effective for short-term exposure
- Vaccination of domestic livestock and pets; rat control

Table I-2-24. Comparison of the Genera *Rickettsia*, *Chlamydia*, and *Mycoplasma* with Typical Bacteria

	Typical Bacteria (<i>S. aureus</i>)	Chlamydia	Rickettsia	Mycoplasma
Obligate intracellular parasite?	Most no	Yes	Yes	No
Make ATP?	Normal ATP	No ATP	Limited ATP	Normal ATP
Peptidoglycan layer in cell envelope?	Normal peptidoglycan	Modified* peptidoglycan	Normal peptidoglycan	No peptidoglycan

*Chlamydial peptidoglycan lacks muramic acid and is considered by some as modified, by others as absent.

Table I-2-25. Infections Caused by Rickettsiae and Close Relatives

Group Disease	Bacterium	Arthropod Vector	Reservoir Host
<u>Spotted Fevers:</u>			
Rocky Mountain Spotted Fever	<i>Rickettsia rickettsii</i>	Ticks	Dogs, rodents, ticks
Rickettsial pox	<i>Rickettsia akari</i>	Mites	Mice
<u>Typhus Group:</u>			
Epidemic	<i>Rickettsia prowazekii</i>	Human louse	Humans
Endemic	<i>Rickettsia typhi</i>	Fleas	Rodents
Scrub	<i>Rickettsia tsutsugamushi</i>	Mites	Rodents
<u>Others:</u>			
Q fever	<i>Coxiella burnetii</i>	None	Cattle, sheep, goats
Bacillary angiomatosis in AIDS	<i>Bartonella quintana</i> (<i>Rochalimaea</i>)	Human louse	Humans
Cat Scratch Fever, Septicemia in homeless	<i>Bartonella henselae</i> (<i>Rochalimaea</i>)	None	Cats
Human granulocytic or monocytic Ehrlichiosis*	<i>Ehrlichia</i>	<i>Ixodes</i> ticks + ?	?

* New tick-borne rickettsial disease caused by *Ehrlichia*.

Note: Co-infections from a single tick infected with more than one agent may occur: N. East U.S.A.: *Babesia* and *B. burgdorferi*. N. Central U.S.A.: *B. burgdorferi* and *Ehrlichia*.

GENUS: *RICKETTSIA*

Obligate intracellular bacteria

Rickettsia rickettsii

Distinguishing Characteristics

- Obligate intracellular bacteria that divide by binary fission and cannot make sufficient ATP.
- Not seen well on Gram-stained smear (too small), but have Gram-negative cell envelope.
- Cross-reaction of *Rickettsia* antigens with OX strains of *P. vulgaris* (Weil-Felix reaction)

Reservoir

Small wild rodents and larger wild and domestic animals (dogs)

Transmission

Hard ticks: *Dermacentor* (also reservoir hosts because of transovarian transmission)

Pathogenesis

Rickettsia invades endothelial lining of capillaries, causing vasculitis.

Disease

Rocky Mountain Spotted Fever

- Prevalent on East Coast (2–12 day incubation)
- Headache, fever, malaise, myalgias, toxicity, vomiting, and confusion
- Rash (maculopapular → petechial) starts (by day 6 of illness) on ankles and wrists and then spreads to the trunk, palms, soles, and face (centripetal rash).
- Ankle and wrist swelling also occur.
- Dx may be confused by gastrointestinal symptoms, periorbital swelling, stiff neck, conjunctivitis and arthralgias.

Diagnosis

- Clinical symptoms (above) and tick bite
- Start treatment without laboratory confirmation.
- Serodiagnosis by complement fixation or Weil-Felix test

Treatment

Doxycycline

Prevention

Tick protection and prompt removal; doxycycline effective in exposed persons

GENUS: *COXIELLA*

- Obligate intracellular bacteria
- Two antigenic phases, one resistant to drying

Coxiella burnetii

Distinguishing Characteristics

- Obligate intracellular bacterium
- Not seen well on gram-stained smear

Reservoir

Domestic livestock: pregnant animals have high titers.

Transmission

- Inhalation of aerosols of urine, feces, amniotic fluid, or placental tissue
- Survives drying; can be infective miles away
- No significant arthropod vector in human infection

Disease

Q fever

- Febrile illness with NO RASH
- Pneumonia with hepatitis

Pathogenesis

Intracellular

Lab Diagnosis

Serodiagnosis; Weil-Felix test is negative.

Treatment

Doxycycline or erythromycin

Prevention

Vaccine for high-risk occupations

GENUS: EHRlichIA

- Obligate intracellular bacteria
- Rickettsial family

Ehrlichia chaffeensis

- Reservoir: ticks and deer
- Transmitted by the Lone Star Tick (*Amblyoma*)
- Infects monocytes and macrophages
- Human monocytic ehrlichiosis

Ehrlichia (near relative of *E. equi*)

- Reservoir
- Transmitted by the *Ixodes* tick
- Infects primarily neutrophils
- Human granulocytic ehrlichiosis

Disease

Ehrlichiosis

- Similar to Rocky Mountain spotted fever but generally without rash
- Leukopenia or low platelets
- Morulae (mulberry-like structures inside infected cells)

GENUS: *CHLAMYDIA*

- Obligate intracellular bacteria
- Elementary body/reticulate body
- Not seen on Gram stain
- Cannot make ATP
- Cell wall lacks muramic acid

Chlamydia trachomatis

Distinguishing Characteristics

- Obligate intracellular bacterium; cannot make ATP.
- Found in cells as metabolically active, replicating reticulate bodies.
- Infective form: inactive, extracellular elementary body
- Not seen on Gram stain; peptidoglycan layer lacks muramic acid.

Reservoir

Human genital tract and eyes

Transmission

Sexual contact and at birth. Trachoma is transmitted by hand-to-eye contact. Flies (trachoma).

Pathogenesis

Infection of nonciliated columnar or cuboidal epithelial cells of mucosal surfaces leads to granulomatous response and damage.

Diseases

STDs in U.S.

- Serotypes D-K (This is the most common bacterial STD in U.S. Herpes and HPV are more common.)
- Nongonococcal urethritis, cervicitis, PID, and major portion of infertility (no resistance to reinfection)
- Inclusion conjunctivitis
- Inclusion conjunctivitis and/or pneumonia in neonates/infants (staccato cough)

Lymphogranuloma venereum

- Serotypes L1, 2, 3
 - STD is prevalent in Africa, Asia, and South America
 - Swollen lymph nodes leading to genital elephantiasis in late stages
 - Tertiary: ulcers, fistulas, genital elephantiasis.

Trachoma

- Leading cause of preventable infectious blindness, Serotypes A, B, Ba, and C
- Follicular conjunctivitis leading to conjunctival scarring and inturned eyelashes leading to corneal scarring and blindness.

Lab Diagnosis

- DNA probes in U.S.
- **Cytoplasmic inclusions seen on Giemsa-, iodine-, or fluorescent-antibody-stained smear or scrapings**
- **Cannot be cultured on inert media**
- Is cultured in **tissue cultures or embryonated eggs**
- Serodiagnosis: complement fixation or microimmunofluorescence test

Treatment

Doxycycline or azithromycin

Prevention

- Erythromycin is effective in infected mothers to prevent neonatal disease.
- Treat neonatal conjunctivitis with systemic erythromycin to prevent pneumonia.

Chlamydia pneumoniae

- Respiratory infections. Atypical pneumonia (single lobe) is very common.
- Infect smooth muscle, endothelial cells of coronary artery and macrophages.
- Potential association with atherosclerosis.

Chlamydia psittaci

Distinguishing Characteristics

- Chlamydia is associated with birds.
- No glycogen in the inclusion bodies.

Reservoir

Birds: parrots (psittacine), turkeys, others

Transmission

- Dust of dried bird secretions and feces
- Turkeys are a major U.S. reservoir.

Pathogenesis

Intracellular growth

Disease

Psittacosis (Atypical Pneumonia)

- **Pneumonia often occurs with hepatitis.** Fever, chills, rash, myalgia but generally mild/moderate while X-ray may look severe.
- Cough may be absent; when present, non-productive initially; then scant mucopurulent.

Laboratory Diagnosis

- Cytoplasmic inclusions seen on Giemsa or fluorescent-antibody-stained sputum or biopsy
- Organism can be isolated from sputum in tissue culture, but rarely done.
- Serodiagnosis by complement fixation test

Treatment

Doxycycline

Prevention

No vaccine or drug is available.

GENUS: *MYCOPLASMA*

- Smallest free-living (extracellular) bacteria
- Missing peptidoglycan (no cell wall)
- Sterols in membrane

Mycoplasma pneumoniae (Eaton's agent)

Distinguishing Characteristics

- Extracellular, tiny, flexible
- No cell wall. Not seen on Gram-stained smear
- Membrane with cholesterol but **does not synthesize cholesterol**
- Requires cholesterol for *in vitro* culture

Reservoir

Human respiratory tract

Transmission

Respiratory droplets; close contact: families, military recruits, medical school classes, college dorms

Pathogenesis

- Surface parasite: not invasive
- **Attaches to respiratory epithelium via P1 protein**
- **Inhibits ciliary action**
- **Produces hydrogen peroxide, superoxide radicals, and cytolytic enzymes**, which damage the respiratory epithelium, leading to necrosis and a bad hacking cough (walking pneumonia).

Diseases

Pneumonia

- **Pharyngitis**
- May develop into an atypical pneumonia with persistent hack (little sputum produced)
- **Most common pneumonia (along with viruses) in young adults**

Lab Diagnosis

- Primarily clinical diagnosis; PCR/nucleic acid probes
- Microscopy not useful
- Fried **egg colonies** on Mycoplasma or Eaton's media (have sterols); 10 days
- **Positive cold agglutinins** (autoantibody to red blood cells) test is not very specific and positive in only 65%.
- Complement fixation test for antibodies to *Mycoplasma pneumoniae* is more specific.

Treatment

Erythromycin, azithromycin, clarithromycin. **No cephalosporins nor penicillins.**

Prevention

None

Ureaplasma urealyticum

Distinguishing Characteristics

- Belongs to the *Mycoplasma* family
- Produces a urease

Diseases

- Urethritis (half of the non-Neisserial, non-Chlamydial), prostatitis
- Can cause renal calculi

Chapter Summary

The morphology of Gram-positive and Gram-negative bacteria is compared. Only the Gram-negative cells have an outer membrane and as a consequence a periplasmic space. The cell wall of Gram-positive cells is relatively thick and has teichoic acid filaments bound to it. Acid-fast cells contain mycolic acid in their cell walls.

Gram's stain is a four-step procedure. Cells are treated with crystal violet and then with Gram's iodine. Cells are then washed with acetone or alcohol, which removes the purple/blue stain from Gram-negative cells. Safranin is used as a counter stain, leaving Gram-positive cells purple/blue but staining Gram-negative cells red/pink.

The Ziehl-Neelsen (Kinyoun) acid-fast stain is used to identify cells that retain a carbol-fuchsin red dye after washing with acetone or alcohol. *Mycobacterium* and *Legionella micdadei* are acid fast, *Nocardia* is partially acid fast, and all other bacteria are nonacid fast. Cysts of *Cryptosporidium* and *Isospora* are also acid fast.

In processing sputa for *Mycobacterium*, samples are first screened using an auramine-rhodamine fluorescent stain. Samples showing fluorescence are then stained with the Ziehl-Neelsen stain to confirm the presence of acid-fast bacteria.

The internal structure of bacterial cells includes the circular, intron- and histone-free, DNA-containing nucleoid region; 70S ribosomes, consisting of 50S and 30S subunits; various granules; and, in many cases, DNA plasmids. Bacterial cells have no mitochondria, chloroplasts, or any other membrane-bound organelle.

Two Gram-positive genera, *Bacillus* and *Clostridium*, are endospore producers. These spores have no reproductive function but permit survival under adverse conditions.

Bacterial cells reproduce by binary fission. When cultured, they initially enter a short lag phase with no cell division and then enter a logarithmic (exponential) growth phase, followed by a stationary and finally a death phase.

The types of culture media required for growth of the various microbes are summarized in a series of tables (Tables I-2-6, I-2-7, and I-2-8).

Children should be vaccinated against diphtheria, tetanus, and pertussis with either the DTP or the newer DTaP (acellular) vaccine and against *Haemophilus influenzae* type b with the Hib vaccine.

It is recommended that senior citizens get vaccinated against *Streptococcus pneumoniae*. Other vaccines with specialized uses include those against *Neisseria meningitidis*, *Salmonella typhi*, *Yersinia pestis*, *Bacillus anthracis*, and Bacille Calmette Guerin (BCG).

Table I-2-9 lists the Gram-positive pathogens, Table I-2-10 lists the non-Gram staining pathogens, and Table I-2-11 lists the Gram-negative pathogens.

The remainder of this chapter is an inventory of the various medically important pathogenic bacteria arranged by genus. The diseases associated with each genus and the important properties for the various species in each genus are succinctly described.

Review Questions

1. Staphylococci are routinely differentiated from streptococci by
 - A. Coagulase test
 - B. Test with hydrogen peroxide
 - C. Polymerase chain reaction
 - D. Protein A immune assay
 - E. Growth in 6.5% sodium chloride

2. An atherosclerotic 80-year-old man develops a pelvic abscess following a ruptured appendix. What is/are the most likely causative agent(s)?
 - A. *Bacteroides* species and microaerophilic streptococci
 - B. *Candida albicans*
 - C. *Enterobacter aerogenes*
 - D. *Haemophilus influenzae* Group B
 - E. *Streptococcus viridans*

3. A 21-year-old student was seen by his family physician with complaints of pharyngitis. Examination of the pharynx revealed patchy erythema and exudates on the tonsillar pillars. Throat smear showed Gram-positive cocci in chains and Gram-negative diplococci. He admitted having been sexually active. What is the significance of the Gram stain smear in this case?
 - A. It provides a rapid means of diagnosing the infection
 - B. It should have been examined by an experienced microbiologist
 - C. It is not useful as it is not possible to make a diagnosis this way
 - D. It strongly suggests gonococcal pharyngitis
 - E. It is evidence of infection with hemolytic streptococci and neisseriae

4. With an appropriately performed acid-fast staining procedure, *Staphylococcus epidermidis* will appear
 - A. Blue
 - B. Red
 - C. Purple
 - D. Colorless
 - E. Brown

5. *Treponema pallidum* can be identified from a syphilitic lesion (either primary or secondary stage) by
 - A. Culture on Fletcher's serum semi-solid medium
 - B. Immunofluorescent stain of smear made from the active lesion
 - C. Gram stain
 - D. Special culture using hemoglobin and yeast extract
 - E. Rapid plasma reagin (RPR) assay

6. Which of the following properties is shared by *Legionella pneumophila* and *Mycobacterium avium-intracellulare*, an atypical *Mycobacterium*?
 - A. Common cause of venereal disease
 - B. Acid fast
 - C. Requiring iron and cysteine for growth
 - D. Not readily transmitted from person to person
 - E. Inability to grow in laboratory culture media

7. What laboratory test is most useful for diagnosis of Lyme disease?
 - A. Blood culture on sheep blood agar plate
 - B. Spinal fluid culture on Thayer-Martin agar
 - C. Detection of IgM/IgG antibodies to the spirochete
 - D. Detection of specific antibody to *Ixodes* tick
 - E. Documentation of fever and arthritis

8. With which of the following diseases is strict isolation indicated for the hospitalized patient?
 - A. Botulism
 - B. *Y. pestis* pneumonia
 - C. Pneumococcal pneumonia
 - D. *Mycobacterium kansasii* pulmonary infection
 - E. Cervical-facial actinomycosis

9. A previously healthy 5-month-old infant now with apparent upper body weakness including droopy eyes, head lag, drooling, and inability to sit unassisted. The most likely infectious form is
 - A. Elementary body
 - B. Reticulate body
 - C. Endospore
 - D. Exotoxin
 - E. Vegetative cell

10. Sixteen residents in a retirement home have fever, malaise, and anorexia. These residents have taken their meals prepared by the same kitchen. Blood cultures from 11 of these residents grow *Salmonella typhi*. The primary reservoir of this organism is
 - A. Hen's egg
 - B. Dogs and cats
 - C. Turkeys
 - D. People
 - E. Water

11. If a culture is inoculated to a density of 5×10^2 cells/ml at time 0 and has both a generation time and lag time of 10 minutes, how many cells/ml will there be at 40 minutes?
- 1.5×10^3
 - 2×10^3
 - 4×10^3
 - 6×10^3
 - 4×10^6
12. A 6-year-old girl crashed on a toboggan ride and complained of pain in the perineal area. Exam showed only bruising of the area. Two days later, she developed fever, prostration, discoloration of the buttock and blebs of the skin in the area. After admission to the hospital, she developed progressive involvement of the leg, thigh, and buttock with extension to the lower abdomen. She went into shock and died before surgery could be performed. At autopsy, a 1-inch piece of wood was found in the perineum, which had perforated the anus. The most likely causative agent
- Requires an elevated oxidation reduction potential
 - Is a Gram-negative coccobacillus
 - Is a marked lecithinase producer
 - Is non-hemolytic on blood agar
 - Is non-fermentative
13. A 71-year-old male was admitted from his extended care facility (nursing home) because of recent aggravation of an exfoliative skin condition that has plagued him for several years. He had been receiving a variety of topical antibiotic regimens over the last year or two. He now has a temperature of 38.9°C (102°F). The skin of upper chest, extremities, and neck shows erythema with diffuse epidermal peeling and many pustular lesions. Cultures obtained from these lesions were reported back from the laboratory as yielding a Gram-positive organism that is highly salt (NaCl) tolerant. What lab result is used to confirm the species of the causative agent?
- Bacitracin sensitivity
 - Bile solubility
 - Catalase production
 - Coagulase production
 - Optochin sensitivity
14. Eight of 10 family practice residents who had a potluck 4 days ago now have diarrhea with abdominal cramps, general malaise, and fever ranging from 37.5° to 38.7°C . Stools from three are blood tinged. Laboratory studies revealed the causative agent was a microaerophilic Gram-negative, curved rod with polar flagella often in pairs to give a "seagull" appearance. It grew on special media at 42°C . The original contamination probably was found in
- Poultry
 - Improperly canned food
 - Fried rice
 - Fish
 - Vegetables

15. In the screen for bacterial meningitis (most commonly a latex particle agglutination test), what chemical component are we searching for in the cerebrospinal fluid?
 - A. Cellular proteins
 - B. Endotoxin
 - C. Immunoglobulins
 - D. Polysaccharide
 - E. Ribosomal RNA

16. What percentage of the time does tetracycline resistance accompany methicillin resistance in methicillin-resistant *Staphylococcus aureus*?
 - A. Less than 5% of the time
 - B. 5–24% of the time
 - C. 25–49% of the time
 - D. 50–74% of the time
 - E. Greater than 75% of the time

17. The structure that is found in Gram-negative but not in Gram-positive bacteria is
 - A. Capsule
 - B. Cell wall
 - C. Cytoplasmic membrane
 - D. Endospore
 - E. Outer membrane

18. Since procaryotes do not possess mitochondria, oxidative phosphorylation and electron transport in these cells take place in association with the
 - A. Polysaccharide gel layer
 - B. Lipopolysaccharide layer
 - C. Peptidoglycan layer
 - D. Periplasmic space
 - E. Lipoprotein bilayer

19. A 5-year-old child of an Eastern European immigrant family is brought to your pediatric clinic. The child is afebrile, but weak and exhausted from a week of paroxysmal coughing with inspiratory whoops, frequently associated with vomiting. The parents profess religious objections to childhood vaccinations, but permit withdrawal of a blood sample, which reveals a lymphocytosis of 44,000/mm³. Production of lymphocytosis, insulin secretion, and histamine sensitization are all results of which attribute of this organism?
 - A. Filamentous hemagglutinin
 - B. Adenylate cyclase toxin
 - C. Beta-hemolysin
 - D. Anaerobic growth
 - E. Pertussis toxin
 - F. Tracheal cytotoxin
 - G. Motility throughout the circulation

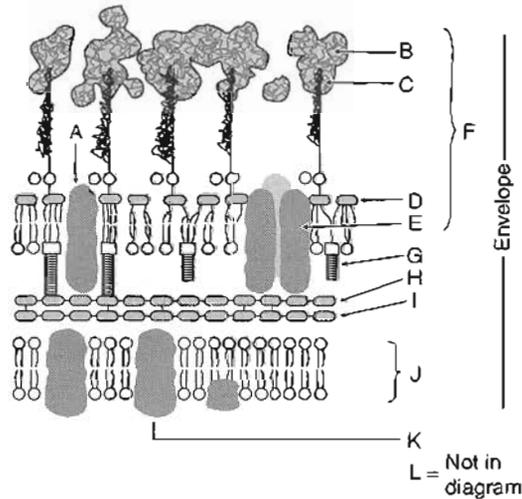
20. The earliest definitive diagnosis of shigellosis in the U.S. is routinely made by clinical findings and
- Culture of the stool
 - Identification of Shiga toxin in stools
 - Positive blood cultures
 - Isolation of a sorbitol fermenting Enterobacteriaceae either from stool or blood
 - Demonstration of fecal PMNs
 - PCR for H antigens
21. Which of the following bacterial structures or products is notoriously anti-phagocytic?
- Teichoic acid of *Streptococcus pyogenes*
 - Bound coagulase of *Staphylococcus aureus*
 - Lipopolysaccharide (LPS)
 - Pili of gonococci
 - Peptidoglycan of rough strains of pneumococci
22. What structure is most responsible for triggering Gram-negative shock?
- Capsule
 - Heat shock proteins
 - Outer membrane
 - Periplasmic space
 - Peptidoglycan-teichoic acid fragments
 - Sex pili
23. Pneumococcal pneumonia or meningitis rarely occurs in the absence of what virulence factor?
- Capsule
 - Heat shock proteins
 - Outer membrane
 - Periplasmic space
 - Peptidoglycan-teichoic acid fragments
 - Sex pili
24. What antigen is most useful in identifying nephritogenic strains of Group A streptococci which may induce glomerulonephritis?
- Capsular antigen
 - Cell wall carbohydrates
 - M proteins
 - Outer membrane proteins
 - P protein
 - Teichoic acids

25. What toxin stimulates adenylate cyclase by catalyzing the transfer of ADP-ribose to the inhibitory subunit of the G protein?
- A. Pertussis toxin
 - B. Cholera toxin
 - C. Diphtheria toxin
 - D. *Escherichia coli* labile toxin
 - E. *Escherichia coli* verotoxin
 - F. *Shigella dysenteriae* Shiga toxin
26. What toxin blocks the function of the inhibitory neurotransmitter at synapses in the spinal cord leading to spasm?
- A. *Bordetella pertussis* neurotoxin
 - B. Cholera toxin
 - C. *Clostridium botulinum* toxin
 - D. Diphtheria toxin
 - E. *Escherichia coli* verotoxin
 - F. Shiga toxin
 - G. Tetanus toxin
27. What toxin inhibits acetylcholine release at the neuromuscular junction?
- A. *Bordetella pertussis* neurotoxin
 - B. Cholera toxin
 - C. *Clostridium botulinum* toxin
 - D. Diphtheria toxin
 - E. *Escherichia coli* verotoxin
 - F. Shiga toxin
 - G. Tetanus toxin
28. What toxin inhibits protein synthesis in mammalian cells by catalyzing the ADP-ribosylation of the elongation factor 2 (EF2)?
- A. Pertussis toxin
 - B. Cholera toxin
 - C. Botulinum toxin
 - D. Diphtheria toxin
 - E. *Escherichia coli* verotoxin
 - F. Shiga toxin
 - G. Tetanus toxin

29. What toxin continually stimulates adenylate cyclase to overproduce cAMP by catalyzing the binding of ADP-ribose to the Gs protein leading to severe fluid loss?
- Pertussis toxin
 - Cholera toxin
 - Escherichia coli* stable toxin
 - Escherichia coli* verotoxin
 - Shiga toxin
 - Tetanus toxin
30. Which organism grows best in low O₂ concentrations but requires oxygen?
- Bacteroides melaninogenicus* (*Prevotella melaninogenica*)
 - Campylobacter jejuni*
 - Escherichia coli*
 - Mycobacterium leprae*
 - Mycobacterium tuberculosis*
31. What is the major chemical component giving bacterial cells protection from osmotic damage?
- Lipopolysaccharide-phospholipid
 - Peptidoglycan
 - Phospholipid
 - Polysaccharide
 - Protein
 - Teichoic acid
32. A 27-year-old female, returning home from her honeymoon, has developed urinary frequency, dysuria, and urgency. Her urine is grossly bloody. Which lab data are most likely to define the causative agent?
- A Gram-negative diplococcus, which is oxidase positive but does not ferment maltose
 - A Gram-positive coccus, which is catalase positive and coagulase negative
 - An optochin-resistant, catalase-negative, Gram-positive coccus
 - A Gram-positive bacillus grown on a low oxidation-reduction medium
 - A Gram-negative bacterium capable of reducing nitrates to nitrites
33. Two days after eating a meal that included home-canned green beans, three people developed various degrees of visual problems, including double vision and difficulties focusing. Describe the Gram reaction of the organism most likely to be isolated from the left-over beans and lab findings, which will be used in its identification.
- A Gram-positive coccus, which is catalase-positive and grows in a high salt environment
 - A Gram-positive aerobic bacillus, which sporulates
 - A Gram-positive coccus, which is catalase-negative and optochin-resistant
 - A Gram-positive bacillus grown on a low oxidation-reduction medium
 - A Gram-negative bacillus capable of reducing nitrates to nitrites

34. A 16-year-old has pneumonia with a dry, hacking cough. The X-ray pattern shows a light, diffuse infiltrative pattern. The most likely organism producing these symptoms is
- A non-Gram-staining bacterium requiring sterols
 - A bacillus showing granules when stained with methylene blue
 - A bacitracin-sensitive, catalase-negative Gram-positive coccus
 - A coagulase positive, Gram-positive coccus in clusters; catalase positive
 - A Gram-positive bacillus grown on a low oxidation-reduction medium
35. Because of cold growth of this fairly common animal fecal bacterium potentially contaminating some deli meats and soft cheeses, renal transplant patients should only have hot deli sandwiches. What is this causative agent of meningitis in transplant patients?
- Brucella* spp
 - Francisella tularensis*
 - Leptospira interrogans*
 - Listeria monocytogenes*
 - Streptococcus pneumoniae*
36. A 55-year-old woman had her rheumatic heart valve replaced with a prosthetic valve. Six blood cultures became positive after 3 days of incubation. An optochin-resistant, catalase-negative Gram-positive coccus that was alpha-hemolytic was isolated. What was the most likely causative agent?
- Candida albicans*
 - Pseudomonas aeruginosa*
 - Serratia marcescens*
 - Staphylococcus aureus*
 - Streptococcus pneumoniae*
 - Streptococcus pyogenes*
 - Streptococcus viridans*
37. Which of the following organisms is killed by oxygen and ferments in absence of oxygen?
- Bacteroides melaninogenicus* (*Prevotella melaninogenica*)
 - Campylobacter jejuni*
 - Escherichia coli*
 - Mycobacterium leprae*
 - Mycobacterium tuberculosis*
38. A virulence factor that causes *Mycobacterium tuberculosis* to clump together and grow in a "serpentine-like" fashion is
- Endotoxin
 - M protein
 - PPD (purified protein derivative)
 - Slimy capsule
 - Trehalose-6,6-dimycolate
 - Wax D

39. From the diagram below, pick the structure that is associated with a passive transport across the membrane.



40. Calcium dipicolinate is found in
- Aspergillus*
 - Bacillus*
 - Escherichia*
 - Mycobacterium*
 - Rickettsia*
 - Vibrio*
41. What are the trimeric structures involved in transport of materials across the outer membrane of the Gram-negative bacteria?
- GTP-binding proteins
 - Lipopolysaccharides
 - Outer membrane proteins
 - Periplasmic space
 - Porin proteins
 - Prion proteins
42. What is the function of penicillin-binding proteins when there is no penicillin present in the bacterium's environment and the cell is actively replicating?
- They are involved in microtubule formation and cell division
 - They have enzymatic activity: transpeptidases and carboxypeptidases
 - They are involved in protein elongation
 - They are involved in the supercoiling of DNA
 - They are transcriptional regulators

43. What is an organism called that respire in the presence of oxygen and ferments in the absence of oxygen?
- A. Aerobe
 - B. Anaerobe
 - C. Facultative aerobe
 - D. Facultative anaerobe
 - E. Microaerophile
 - F. Obligate aerobe
44. A 15-day-old male presents with purulent conjunctivitis. Iodine staining bodies are seen in conjunctival scrapings. The most likely infectious form is
- A. Elementary body
 - B. Reticulate body
 - C. Endospore
 - D. Exotoxin
 - E. Vegetative cell
 - F. Virus resistant to alcohol
 - G. Virus sensitive to alcohol
45. What organism is most likely responsible for bacterial pneumonia in persons with alcohol intoxication?
- A. *Haemophilus influenzae*
 - B. *Proteus vulgaris*
 - C. *Pseudomonas aeruginosa*
 - D. *Staphylococcus aureus*
 - E. *Streptococcus pneumoniae*
 - F. *Streptococcus viridans*
46. What organism is most likely responsible for bacterial meningitis in infants during the first month of life?
- A. *Enterococcus faecalis* (*Streptococcus faecalis*)
 - B. *Haemophilus influenzae*
 - C. *Staphylococcus aureus*
 - D. *Streptococcus agalactiae*
 - E. *Streptococcus pneumoniae*
 - F. *Streptococcus pyogenes*

47. What organism is most likely responsible for bacterial endocarditis in men following urological instrumentation?
- Enterococcus faecalis* (*Streptococcus faecalis*)
 - Pseudomonas aeruginosa*
 - Streptococcus pyogenes*
 - Streptococcus viridans*
 - Ureaplasma urealyticum*
48. An AIDS patient with septicemia and a target-shaped necrotic lesion on the buttock with a black center and an erythematous margin. Which causative agent is most likely?
- Bacillus anthracis*
 - Enterococcus faecalis* (*Streptococcus faecalis*)
 - Pseudomonas aeruginosa*
 - Staphylococcus aureus*
 - Streptococcus pyogenes*
49. What causative agent is most likely responsible for edema, hematuria, proteinuria in a patient who had impetigo 3 weeks ago?
- Clostridium perfringens*
 - Pseudomonas aeruginosa*
 - Staphylococcus aureus*
 - Staphylococcus epidermidis*
 - Streptococcus agalactiae*
 - Streptococcus pyogenes*
50. Patient was admitted to the hospital because of bleeding duodenal ulcer. Culture at 37°C grew urease-positive curved bacteria. The most likely causative agent is
- Campylobacter jejuni*
 - Entamoeba histolytica*
 - Enterococcus faecalis* (*Streptococcus faecalis*)
 - Helicobacter pylori*
 - Pseudomonas aeruginosa*

Answers

1. **Answer: B.** The catalase test is carried out with hydrogen peroxide. The other four tests do not differentiate.
2. **Answer: A.** Atherosclerosis leads to poor circulation to the lower extremities, which in turn lowers the oxidation-reduction potential of the tissues. All this predisposes to infections caused by anaerobic M-Os, in this case, *Bacteroides* and *Streptococci*. The patient is suffering from anaerobic cellulitis or possibly myonecrosis.
3. **Answer: C.** Gram-positive cocci (alpha hem. Strep) and Gram-negative cocci (neisseriae) are normally present in the throat. There is no way to differentiate pathogens from non-pathogens by the Gram stain.
4. **Answer: A.** Students need to remember that the primary stain in the acid-fast stain is carbolfuchsin, which stains acid-fast organisms red. The counterstain is methylene blue, which stains everything else blue. They also need to know the three important genera of acid-fast organisms: *Mycobacterium*, *Nocardia*, and *Cryptosporidium*.
5. **Answer: B.** Treponema cannot be cultured. Fletcher's medium is for *Leptospira*. The spirochete that will take Gram stain is *Borrelia*. RPR is for detection of antibody.
6. **Answer: D.** Neither one is common for venereal disease. *Legionella* is not acid fast. Iron and cysteine requirement refers to *Legionella*. Both can grow in artificial media.
7. **Answer: C.** *Borrelia burgdorferi*, the causative agent of Lyme disease, grows in a complex medium, not on sheep blood agar. Thayer-Martin is for pathogenic neisseriae. There is no purpose to detect antibody to the tick. ELISA or some rapid test for IgG/IgM is currently used for diagnosis of Lyme disease.
8. **Answer: B.** *Y. pestis* causes bubonic plague, which is not contagious through respiratory droplets; however, the bubonic form can progress via septic emboli to pneumonia, which is contagious. Adult botulism is a toxemia, thereby not contagious. Pneumococcal pneumonia is caused by *S. pneumoniae*, which is not considered highly infectious and colonizes many people without causing disease. Choice D (*Mycobacterium kansasii*) and choice E (*actinomyces*) are both environmental M-O: not contagious.

Remember that as a rule-of-thumb, M-O that have the environment (water or soil) as reservoirs, and those that are zoonotic or arthropod-borne, and those that are normal human flora are generally not contagious from person to person. *Y. pestis* is an exception.
9. **Answer: C.** Infant botulism is a toxicoinfection started by the ingestion of *Clostridium botulinum* endospores from the environment. The spores germinate in the immature flora of the GI tract and the toxin is produced *in vivo* in contrast to adult botulism where the preformed toxin is ingested.
10. **Answer: D.** The reservoir for *S. typhi* is people (humans). Other species of *Salmonella* have animals as their reservoirs.
11. **Answer: C.** Explanation: Remember that each cell divides into two at each generation following the single lag phase. So at the end of the first 10 minutes there is still 5×10^2 , and then at the end of the first 20 minutes (total) there are 10×10^2 . At the end of 30 minutes total time there will be 20×10^2 , and at the end of the total time, 40×10^2 , which is written 4×10^3 in proper scientific notation.

12. **Answer: C.** The description suggests strongly that she has myonecrosis. Therefore, the causative agent (at least one) is *C. perfringens*. *C. perfringens* is an anaerobe, therefore choice A is wrong. Clostridia are all Gram-positive, therefore choice B is wrong. *C. perfringens* have concentric areas of beta hemolysis, therefore choice D is wrong. *C. perfringens* is a marked lecithinase producer; therefore choice C is correct.
13. **Answer: D.** The patient has the “scalded skin” syndrome caused by *S. aureus*. The GENUS *Staphylococcus* would be distinguished from Strep by Staphylococcal production of catalase. But the SPECIE (*S. aureus*) would be distinguished from *S. epidermidis* on the basis of *S. aureus* production of coagulase. Bacitracin sensitivity and bile solubility are specie characteristics of Strep pneumoniae.
14. **Answer: A.** The clue is Gram-negative curved rods with polar flagella often in pairs to give a “seagull” appearance and the microaerophilic on special media and growing at 42°C. That description is most compatible with *Campylobacter jejuni*. Poultry are one of the most important reservoirs so choice A is the correct response.
15. **Answer: D.** The diagnostic test looks for capsular material, which is a polysaccharide (choice D). The only non-polysaccharide one is anthrax, which is a polypeptide. Immunoglobulins would not be found this early in the CSF and may never be formed in some severely immunocompromised patients.
16. **Answer: E.** Methicillin-resistant strains of *Staph aureus* are generally resistant to all available antibiotics except for vancomycin, teicoplanin, and fusidic acid. Therefore, the answer is generally 100%, making choice E the best answer.
17. **Answer: E.** Capsules, cell wall, and cytoplasmic membranes are found in both Gram-positive and Gram-negative bacteria. Endospore (choice D) occurs with certain Gram-positive bacteria, e.g., *Bacillus* and *Clostridium*. Only Gram-negatives have an outer membrane.
18. **Answer: E.** The site of most metabolic processes in the prokaryotic cell is the cytoplasmic membrane, which is best described as a lipoprotein bilayer (choice E). Of the distractors: Polysaccharide (choice A) describes the capsule layer, which is antigenic and antiphagocytic and outside the cell; lipoprotein (choice B) describes the outer membrane of Gram-negative bacteria, which also is basically outside of the cell; peptidoglycan (choice C) refers to the cell wall layer of all bacteria and periplasmic space; choice D refers to the space between the inner membrane and cell wall of Gram-negative bacteria. None of the distractors (A)–(D) are significant sites of action of any of the metabolic enzymes.
19. **Answer: E.** The disease here is whooping cough, caused by *Bordetella pertussis*. The pertussis toxin (also known as the lymphocytosis-promoting toxin) is not believed to be directly cytotoxic, but stimulates adenylate cyclase by ribosylating regulatory proteins. It causes a variety of effects depending on the cell type involved: insulin secretion, lymphocytosis, and alteration of immune effector cells. Of the distractors: filamentous hemagglutinin (choice A) mediates attachment; the adenylate cyclase toxin (choice B) stimulates local edema; the organism produces only a small zone of hemolysis around its colonies, so choice C is not true; it is an aerobe and does not grow anaerobically (choice D); and is non-motile (choice G). All systemic manifestations of the disease arise from the circulation of the toxins, not the organism itself.

20. **Answer: A.** Shigellosis is an invasive disease confined to the intestine. Definitive diagnosis is made by prompt stool culture (usually positive by week 2). The finding of PMNs is not definitive for *Shigella*. In the U.S., the most common organism is *Shigella sonnei* or *flexneri*, neither of which produce Shiga toxin, making choice B false. Although *Shigella* is invasive, it does not invade the vasculature so blood cultures would not be positive. Sorbitol fermentation is used in isolation of most strains of VTEC. Remember that *Shigellae* are nonmotile; thus, there are no H antigens.
21. **Answer: D.** The correct answer to this question is the pili of *Neisseria gonorrhoeae*. Another antiphagocytic component is any bacterial capsule (missing in rough strains of *Pneumococcus* and the peptidoglycan does not inhibit phagocytosis, making choice E not a proper choice). Other correct answers would have been M-protein of *Strep pyogenes*, and the A proteins of *Staph aureus*.
22. **Answer: C.** Lipid A is the actual component of LPS, which is responsible for triggering Gram-negative shock. Since it is not listed, then you need to think about where it is found as it is a structural toxin. It is located in the outer membrane, and thus this is the correct answer. Peptidoglycan-teichoic acid fragments are found in Gram-positive bacteria only and are responsible for triggering septic shock when a Gram-positive organism is in the bloodstream, or inflammatory response when it is in the CSF or in tissues.
23. **Answer: A.** The most important virulence factor for *S. pneumoniae* is the capsule. Without it the strains are avirulent, except in seriously immunocompromised patients.
24. **Answer: C.** Remember first that Group A Strep is also called *Streptococcus pyogenes* and that it has the nonimmunogenic capsule of hyaluronic acid. The important antigen here is a surface protein called the M protein; it is used in “typing” GAS. Certain M types are more commonly involved in glomerulonephritis with M12 the most common in AGN. The large number of M-protein types and nonimmunogenic capsules (hyaluronic acid) are why many people get repeated Strep infections.
25. **Answer: A.** Pertussis toxin activates adenylate cyclase by turning off G_i protein via ADP-ribosylation. Cholera toxin and the labile toxin of *E. coli* are the other two that ADP ribosylate G proteins, but their ADP ribosylation is of G_s . Both verotoxin and Shiga toxin clip the 60S ribosomal subunit.
26. **Answer: G.** Tetanus toxin blocks the release of inhibitory factor (glycine) at the spinal cord. If one forgets the mode of action, think of the clinical presentation of tetanus, which is spasm rather than paralysis, as in botulism.
27. **Answer: C.** This is the mode of action of botulism toxin, and the result is paralysis of muscles.
28. **Answer: D.** Diphtheria toxin inactivates EF2 and thus inhibits protein synthesis. Students need to remember *Pseudomonas* exotoxin will do the same—a question frequenting USMLE exams.
29. **Answer: B.** Cholera toxin locks G_s protein in the “on” position via ADP-ribosylation. Adenylate cyclase is thus continually being stimulated. Most severe fluid loss leading to hypovolemic shock occurs with cholera.
30. **Answer: B.** *Campylobacter* is the only microaerophile on the list.

31. **Answer: B.** Although the outer membrane (which is chemically a lipopolysaccharide-phospholipid) plays a minor role in protecting Gram-negative bacteria from osmotic damage, it is not as important as the cell wall. CW is the peptidoglycan net structure that offers major protection from osmotic damage and confers cell shape.
32. **Answer: E.** *E. coli*-induced cystitis is highest in sexually active females. It generally reduces nitrates and is also a lactose fermenter. Choice A = *Neisseria gonorrhoeae*; choice B = *Staphylococcus saprophyticus*; choice C = *Enterococcus faecalis* is one possibility; choice D = *Clostridium*.
33. **Answer: D.** This case history describes botulism (key words: home-canned green beans and visual problems). Foods classically associated are those with a neutral or alkaline pH. *C. botulinum*, the agent of botulism, is an anaerobe and thus has a low oxidation-reduction requirement. The other Gram-positive bacillus (aerobic) would be *Bacillus cereus*. Choice A = *Staph aureus*; choice B = *Bacillus cereus*; choice C = *S. pneumoniae*; choice D = *Clostridium botulinum*; choice E = *E. coli*.
34. **Answer: A.** The disease is most likely mycoplasma pneumonia caused by *Mycoplasma pneumoniae*, which is non-Gram staining and requires cholesterol for growth. Choice B = *C. diphtheriae*; choice C = *S. pneumoniae*; choice D = *Staph aureus*; choice E = *Clostridium*.
35. **Answer: D.** Meningitis in renal transplant patients and many cancer patients is frequently *Listeria* acquired from foods. This is also a problem if pregnant women are infected; it may cause meningitis in neonates.
36. **Answer: G.** A patient with a history of rheumatic fever and blood cultures growing an alpha hemolytic coccus, which is catalase-positive and optochin-resistant.
37. **Answer: A.** This is one of the dark-pigmented *Bacteroides*, now called *Prevotella*. They are obligate anaerobes.
38. **Answer: E.** Cord factor (trehalose dimycolate) causes serpentine-like clumps of TB organism.
39. **Answer: E.** This refers to the porins that passively (with the aid of electrostatic charges) allow entry of materials into the periplasmic space, from whence they are actively transported across the cytoplasmic membrane.
40. **Answer: B.** Calcium dipicolinate is found only in the core of bacterial spores. It plays a role in the dehydration and stabilization of the DNA in the spores. The two bacterial genera that form endospores are *Bacillus* and *Clostridium*.
41. **Answer: E.** The trimeric structures spanning the outer membrane are porins, a series of proteins forming a pore, permitting passive transfer of materials into the periplasmic space. They are outer membrane proteins. Please remember that this is a “best answer test,” and if an answer is more specific to the question, it would be better.
42. **Answer: B.** Penicillin-binding proteins (PBPs) are involved in the final cross linkage of new pieces of peptidoglycan. PBPs are cellular transpeptidases and carboxypeptidases. They are located in the cytoplasmic membrane.
43. **Answer: D.** This describes a facultative anaerobe. Facultative aerobe is a term that does not exist.

44. **Answer: A.** The patient has inclusion conjunctivitis caused by *Chlamydia trachomatis*. The only form of this bacterium that has the ability to bind to the membranes and infect is the elementary form.
45. **Answer: E.** *S. pneumoniae* is the most common cause of community-acquired pneumonia. One of the predisposing factors is alcohol intoxication; another one is influenza virus infection.
46. **Answer: D.** *Strep agalactiae* is one of the most prominent neonatal pathogens. The other common causative agent is *E. coli*, which is not on the list.
47. **Answer: A.** Several organisms on this list can cause endocarditis under various conditions. *E. faecalis* is the one associated with urological manipulation. *S. viridans* is associated with oral manipulation.
48. **Answer: C.** *Pseudomonas aeruginosa*. Target-shaped necrotic lesion with a black center and an erythematous margin depicts Ecthyma Gangrenosum caused by *Pseudomonas* associated with immunodeficiency.
49. **Answer: F.** Edema, hematuria, and proteinuria are pathognomonic for acute glomerulonephritis following *S. pyogenes* skin infections. Impetigo can be caused by *S. aureus* and *S. pyogenes* but only *S. pyogenes* leads to acute glomerulonephritis.
50. **Answer: D.** This patient had Helicobacter, which is urease positive. *Campylobacter jejuni* also grows at 42°C, but it is urease negative.

Medically Important Fungi

3

What the USMLE Requires You to Know

- Basic morphology of fungi (hyphae, yeast, dimorphic, and various types of conidia)
- Basic chemistry, particularly that involved in antifungals or that distinguishes fungi from other groups
- Scientific names of the fungal pathogens and opportunists found in the United States
 - What are the four dimorphic fungi?
Recognize their tissue and environmental forms.
 - What are the three dermatophytes and what tissues do they invade?
 - What is the most common cause of meningitis in AIDS patients?
 - What does it look like? How do you diagnose?
 - What is a common cause of interstitial pneumonitis in AIDS patients?
 - Why is it considered a fungus?
 - Which medically important fungus has a capsule?
 - Which is found most commonly inside cells of the reticuloendothelial system?
- The diseases they cause and:
 - How acquired (geography, route)
 - Common presenting symptoms, most common sites of dissemination (if they disseminate commonly)
 - What two fungi are a problem in IV lines?
 - Most common cause of fungal septicemia and the clues used in the cases (germ tube test positive, pseudohyphae and true hyphae as well as yeast forms)

MYCOLOGY

Mycology is the study of fungi (molds, yeasts, and mushrooms).

All fungi are

- Eukaryotic (e.g., true nucleus, 80S ribosomes, mitochondria, as are humans).
- Complex carbohydrate cell walls: chitin, glucan, and mannan.
- Ergosterol = Major membrane sterol
 - Imidazole antifungals inhibit synthesis of ergosterol.
 - Polyene antifungals bind more tightly to ergosterol than cholesterol.
- Heterotrophic (require organic carbon)
 - Saprophytic or saprobic (fungus living on dead organic material)
 - Parasitic (fungus living on another living organism)

FUNGAL MORPHOLOGY

Hyphae = filamentous cellular units of molds and mushrooms



Figure I-3-1.
Nonseptate Hyphae

Nonseptate Hyphae

- No cross walls
- Broad hyphae with irregular width
- Broad angle of branching

Septate Hyphae

- With cross walls
- Width is fairly regular (tube-like).

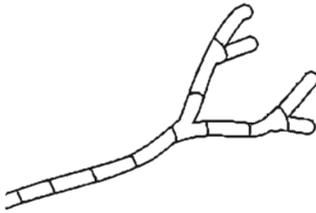


Figure I-3-2.
Septate Hyphae

Hyphal Coloration

- Dematiaceous: dark colored (gray, olive)
- Hyaline: clear

Mat of hyphae = mycelium

Yeasts = single celled (round to oval) fungi



Figure I-3-3. Yeasts

Dimorphic Fungi

- Fungi able to convert from hyphal to yeast or yeast-like forms.
- Thermally dimorphic: in the "cold" are the mold form.

Key Dimorphic Fungi

- Histoplasma*
- Blastomyces*
- Coccidioides*
- Sporothrix*

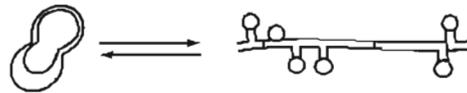


Figure I-3-4. Dimorphic Fungi

Pseudohyphae (Candida albicans)

Hyphae with constrictions at each septum

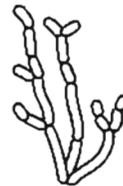


Figure I-3-5. *Candida*
Pseudohyphae

Spore Types

Conidia

- Asexual spores
- Formed off of hyphae
- Common
- Airborne

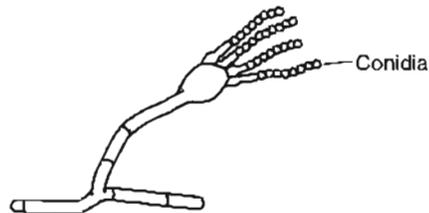


Figure I-3-6. Conidia

Blastoconidia: “Buds” on yeasts (asexual budding daughter yeast cells)



Figure I-3-7. Blastoconidia

Arthroconidia: Asexual spores formed by a “joint”

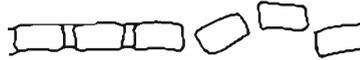


Figure I-3-8. Arthroconidia

Spherules and Endospores (Coccidioides): Spores inside the spherules in tissues

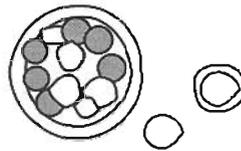
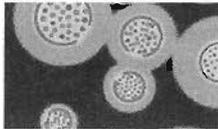


Figure I-3-9. Endospores and Spherules

Diagnosis

Table I-3-1. Microscopic Methods/Special Fungal Stains

Preparation	Fungal Color	Notes
KOH wet mount (KOH degrades human tissues releasing hyphae and yeasts)	Colorless (hyaline) refractive green or light olive to brown (dematiaceous) fungal elements	Heat gently; let set 10 minutes; dissolves human cells
PAS	Hot pink	
Silver stain	Old rose gray to black	
Calcofluor white (Can be done on wet mounts.)	Bright blue-white on black	Scrapings or sections, fluorescent microscope needed
India ink wet mount of CSF sediment	Colorless cells with halos (capsule) on a black particulate background (<i>Cryptococcus neoformans</i>)	Only “rules in.” Insensitive; misses 50%.  Figure I-3-10. <i>Cryptococcus neoformans</i>

Culture

(May take several weeks.) Special fungal media: inhibitory mold agar is modification of Sabouraud's with antibiotics.

- Sabouraud's agar
- Blood agar
- Both of the above with antibiotics

Identification from Cultures

- Fungal morphology
- PCR with nucleic acid probes

Serology

(E.g., antibody screen, complement fixation, etc.) Looking for patient antibody.

Fungal Antigen Detection: (CSF, serum)

Cryptococcal capsular polysaccharide detection by latex particle agglutination (LPA) or counter immunoelectrophoresis

Skin Tests

- Most useful for epidemiology or demonstration of anergy to an agent you know patient is infected with (grave prognosis)
- Otherwise, like tuberculosis, a skin test only indicates exposure to the agent.

NONSYSTEMIC FUNGAL INFECTIONS

Superficial Infections (Keratinized Tissues)

Malassezia furfur (Fungus Name)

Normal skin flora (lipophilic yeast)

Diseases

- Pityriasis or tinea versicolor
 - Superficial infection of keratinized cells
 - Hypopigmented spots on the chest/back (blotchy suntan)
 - KOH mount of skin scales: spaghetti and meatballs
Yeast clusters & short curved septate hyphae
 - Treatment is topical selenium sulfide; recurs.
- Fungemia in premature infants on intravenous lipid supplements

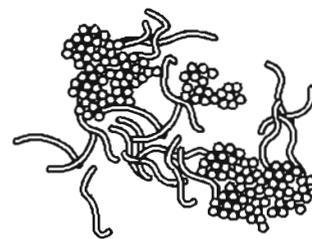


Figure I-3-11.
Malassezia furfur

Cutaneous Fungal Infections (without systemic disease)

Yeast or dermatophytic infections.

Yeast Skin Infections

- Commonly cutaneous or mucocutaneous candidiasis
- May disseminate in compromised patients
- Discussed with opportunistic fungi

Dermatophytes (Group of Fungi)

- Filamentous fungi (monomorphic)
- Infect only skin and hair and/or nails (do not disseminate)
- Three genera:
 - Trichophyton* - Infects skin, hair and nails
 - Microsporum* - Infects hair and skin
 - Epidermophyton* - Infects nail and skin



Figure I-3-12. Dermatophyte

Diseases

Dermatophytic Infections = Tineas (Ringworms)

- If highly inflammatory, generally from animals (zoophilic) (i.e., *Microsporum canis*: cats or dogs)
- If little inflammation, generally from humans (anthropophilic tinea capitis: *M. audouinii*)
- Tinea capitis = ringworm of the scalp
- The most serious of the tinea capitis is favus (tinea favosa), which causes permanent hair loss and is very contagious.
- Tinea barbae = ringworm of the bearded region
- Tinea corporis = dermatophytic infection of the glabrous skin
- Tinea cruris = jock itch
- Tinea pedis = athlete's foot

Diagnosis

- *Microsporum fluoresces* (Wood's lamp)
- KOH mount of nail or skin scrapings should show arthroconidia and hyphae.

Treatment

- Topical imidazoles or tolnaftate
- Oral imidazoles or griseofulvin where hairs are infected, or skin contact hurts
- Keep areas dry.
- ID reaction (Dermatophytid) = Allergic response to circulating fungal antigens

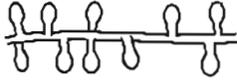


Figure I-3-13.
Sporothrix Hyphae

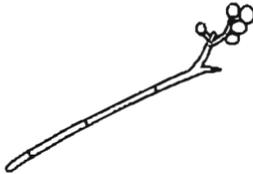


Figure I-3-14.
Sporothrix

Subcutaneous Mycoses

Sporothrix schenckii

Dimorphic Fungus

- Environmental form: on plant material, world wide as hyphae with rosettes and sleeves of conidia
- Traumatic implantation (rose or plum tree thorns, wire/sphagnum moss)
- Tissue form: cigar-shaped yeast in tissue

Diseases

- Sporotrichosis (rose gardener's disease): subcutaneous or lymphocutaneous lesions. Treatment: itraconazole or potassium iodide in milk
- Pulmonary (acute or chronic) sporotrichosis. Urban alcoholics, particularly homeless (alcoholic rose-garden-sleeper's disease)

DEEP FUNGAL INFECTIONS

Classical Pathogens

Three important classical pathogens in the U.S.A.:

Histoplasma

Coccidioides

Blastomyces

All three cause

- Acute pulmonary (asymptomatic or self-resolving in about 95% of the cases)
- Chronic pulmonary, or
- Disseminated infections

Diagnosis

(Most people never see a doctor.)

- Sputum cytology (calcofluor white helpful)
- Sputum cultures on blood agar and special fungal media (inhibitory mold agar, Sabouraud's)
- Peripheral blood cultures are useful for *Histoplasma* since it circulates in RES cells.

Histoplasma capsulatum

Dimorphic Fungus

- Environmental form: hyphae with microconidia and tuberculate macroconidia
- Endemic region: Eastern Great Lakes, Ohio, Mississippi, and Missouri River beds

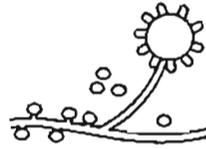


Figure I-3-15. *Histoplasma* Environmental Form

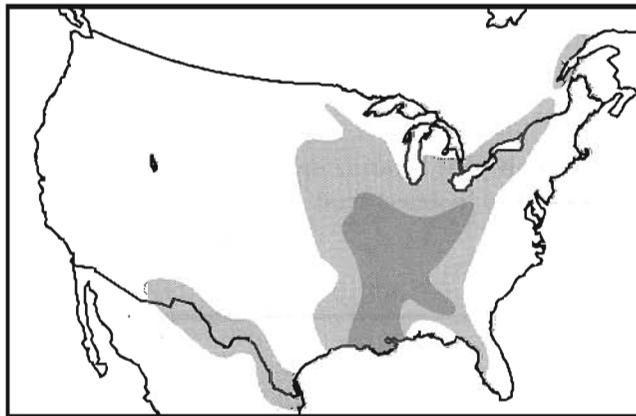


Figure I-3-16. *Histoplasma* Endemic Region

- Found in soil (dust) enriched with bird or bat feces
- Spelunking (cave exploring), cleaning chicken coops, or bulldozing starling roosts
- Tissue form: small intracellular yeasts with narrow neck on bud; no capsule
- Facultative intracellular parasite found in reticuloendothelial (RES) cells (tiny; can get 30 or so in a human cell)

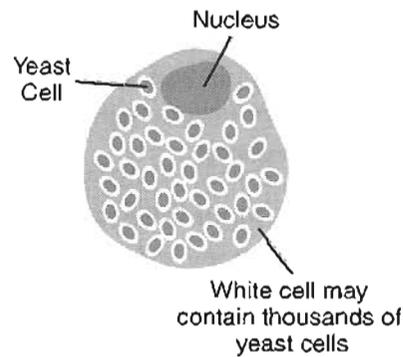


Figure I-3-17. Human RES Cell

Disease

Fungus flu (a pneumonia)

- Asymptomatic or acute (but self-resolving) pneumonia with flu-like symptomatology
- **Hepatosplenomegaly may be present even in acute pulmonary infections** (facultative intracellular RES)
- Very common in summer in endemic areas: kids or newcomers (80% of adults are skin-test positive in some areas)
- Lesions have a tendency to **calcify as they heal**.
- Relapse potential increases with T cell immunosuppression.
- **Disseminated infections:** Mucocutaneous lesions are common; also common in AIDS patients in endemic area.

Coccidioides immitis

Dimorphic Fungus

- **Environmental form:** hyphae breaking up into arthroconidia found in desert sand.

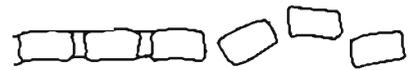


Figure I-3-18. *Coccidioides immitis*

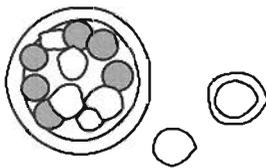


Figure I-3-20. *Coccidioides immitis* Spherules

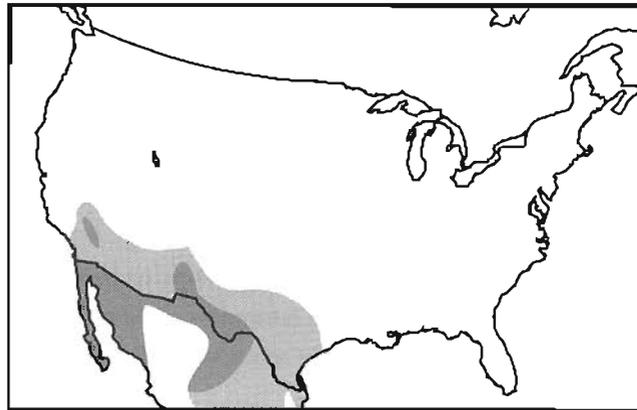


Figure I-3-19. *Coccidioides* Endemic Region

- Endemic region: **Southwestern United States–Southern California** (especially San Joaquin Valley), Arizona, New Mexico, Texas, Nevada
- Arthroconidia are inhaled, round up, and enlarged, becoming spherules inside which the cytoplasm wall off, forming endospores.
- Tissue form: **spherules with endospores**

Disease: Valley Fever (asymptomatic to self-resolving pneumonia)

- **Desert bumps** (erythema nodosum) and arthritis are generally good prognostic signs.
- Very common in endemic region
- **Pulmonary lesions have a tendency to calcify as they heal**.
- **Systemic infections are a problem in AIDS and immunocompromised patients in endemic region** (meningitis, mucocutaneous lesion).
 - Cocci has a tendency to **disseminate in third trimester of pregnancy**.

Blastomyces dermatitidis

Dimorphic Fungus

Environmental form: hyphae with nondescript conidia (i.e., no fancy arrangements)

- Association not definitively known, appears to be associated with rotting wood such as beaver dams
- Endemic region: Upper Great Lakes, Ohio, Mississippi River beds plus the southeastern seaboard of the U.S. and northern Minnesota into Canada

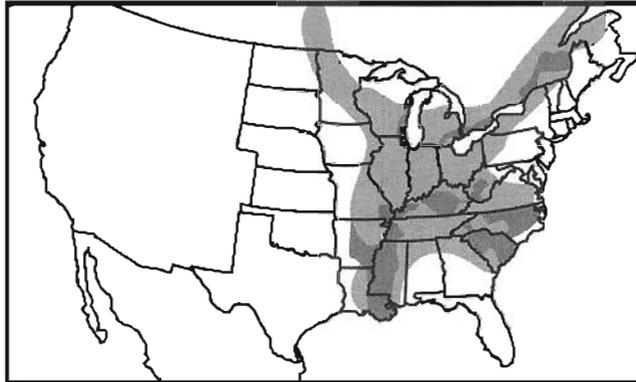


Figure I-3-22. Blastomycosis Endemic Region

Tissue form: broad-based budding yeasts and a double refractile cell wall (not capsule)

Disease: Blastomycosis

- Acute and chronic pulmonary disease
- Considered less likely to self-resolve than *Histoplasma* or *Coccidioides*. So many physicians will treat even acute infections with ketoconazole.
- Disseminated disease



Figure I-3-21. *Blastomyces dermatitidis* Hyphae with Conidia

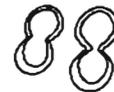


Figure I-3-23. *Blastomyces dermatitidis* Broad-Based Budding Yeasts

Opportunistic Fungi

Aspergillus fumigatus

Monomorphic filamentous fungus

- Dichotomously branching
- Generally acute angles
- Septate
- One of our major recyclers: compost pits, moldy

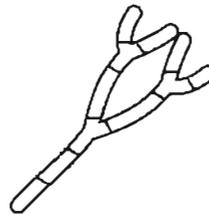


Figure I-3-24. *Aspergillus* Showing Monomorphic Filamentous Fungus

Diseases/Predisposing Conditions

- Allergic bronchopulmonary aspergillosis/asthma, allergies (growing in mucous plugs in the lung but not penetrating the lung tissue)
- Fungus ball: free in preformed lung cavities (surgical removal to reduce coughing, which may induce pulmonary hemorrhage)
- Invasive aspergillosis/severe neutropenia, CGD, CF, burns
Invades tissues causing infarcts and hemorrhage.
Nasal colonization → pneumonia or meningitis
Cellulitis/in burn patients; may also disseminate.

Treatment

- Depends on severity of disease and underlying conditions: Itraconazole or amphotericin B

***Candida albicans* (and other species of *Candida*)**

- Yeast endogenous to our mucous membrane normal flora
- *C. albicans* yeasts form germ tubes at 37°C in serum.
- Form pseudohyphae and true hyphae when it invades tissues (nonpathogenic *Candida* do not).

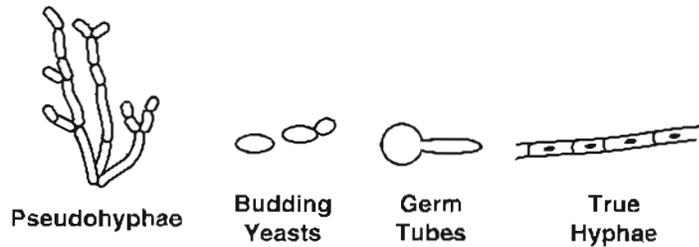


Figure I-3-25. *Candida albicans*

Diseases/Predisposing Conditions

- Perleche: crevices of mouth/malnutrition
- Oral thrush/prematurity, antibiotic use, immunocompromised (IC) host, AIDS
- Esophagitis/antibiotic use, IC host, AIDS
- Gastritis/antibiotic use, IC host, AIDS
- Septicemia (with endophthalmitis and macronodular skin lesions)/immunocompromised, cancer and intravenous (IV) patients
- Endocarditis (with transient septicemias)/IV drug abusers
- Cutaneous infections/obesity and infants; patients with rubber gloves
- Yeast vaginitis/particularly a problem in diabetic women
- Chronic mucocutaneous candidiasis/endocrine defects; anergy to *Candida*

Diagnosis

- KOH: pseudohyphae, true hyphae, budding yeasts
- Septicemia: culture lab identification: biochemical tests/formation of germ tubes

Treatment

- Topical imidazoles or oral imidazoles; nystatin
- Disseminated: Amphotericin B or fluconazole

Cryptococcus neoformans

Encapsulated Yeast (Monomorphic)

Environmental Source: Soil enriched with pigeon droppings

Diseases/Predisposing Conditions

- Meningitis/Hodgkin's, AIDS (the dominant meningitis)
- Acute pulmonary (usually asymptomatic)/pigeon breeders

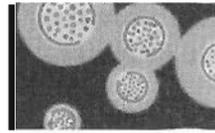


Figure I-3-26. *Cryptococcus neoformans*

Diagnosis of Meningitis: CSF

- Detect capsular antigen in CSF (by latex particle agglutination or counter immunoelectrophoresis)
- India ink mount (misses 50%) of CSF sediment to find budding yeasts with capsular "halos"
- Cultures (urease positive yeast)

Treatment: AMB+5FC until afebrile and culture negative, then fluconazole

Mucor, Rhizopus, Absidia (Zygomycophyta)

Nonseptate filamentous fungi

Environmental Source: Soil; sporangiospores are inhaled

Disease

- Rhinocerebral infection caused by *Mucor* (or other zygomycophyta)
- (Old names: Mucormycosis = Phycomycosis = Zygomycosis)
- Characterized by paranasal swelling, necrotic tissues, hemorrhagic exudates from nose and eyes, and mental lethargy
- Occurs in ketoacidotic diabetic patients and leukemic patients.
- These fungi penetrate without respect to anatomical barriers, progressing rapidly from sinuses into the brain tissue.

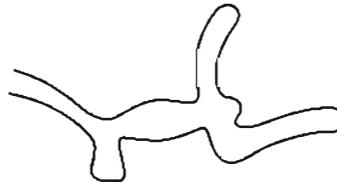


Figure I-3-27. Nonseptate Hyphae with Broad Angles

Diagnosis: KOH of tissue; broad ribbon-like nonseptate hyphae with about 90° angles on branches.

Treatment

- Debride necrotic tissue and start Amphotericin B fast
- High fatality rate because of rapid growth and invasion

Pneumocystis carinii

Fungus (based on molecular techniques like ribotyping)

- Obligate extracellular parasite
- Silver stained cysts in tissues



Figure I-3-28. *Pneumocystis*

Disease: Interstitial pneumonia

- Pneumonia in AIDS patients even with prophylaxis (mean CD4+/mm³ of 26), malnourished babies, premature neonates, and some other IC adults and kids
- Symptoms: fever, cough, shortness of breath; sputum nonproductive except in smokers
- *Pneumocystosis* attaches to and kills Type I pneumocytes, causing excess replication of Type II pneumocytes and damage to alveolar epithelium. Serum leaks into alveoli, producing an exudate with a foamy or honeycomb appearance on H & E stain. (Silver stain reveals the holes in the exudate are actually the cysts and trophozoites, which do not stain with H & E.)
- X-ray: patchy infiltrative (ground glass appearance), the lower lobe periphery may be spared.

Diagnosis: Silver-staining cysts in bronchial alveolar lavage fluids or biopsy

Treatment: Trimethoprim/sulfamethoxazole

Chapter Summary

Mycology is the study of fungi. The fungi include molds, yeasts, and mushrooms. All fungi are eukaryotic, have complex cell walls, have ergosterol in their cell walls, and are heterotrophic, either as free-living saprophytes or as parasites.

Yeasts are single-celled fungi. Some fungi grow as extended filamentous units called hyphae and can be classified according to the presence (septated-types) or absence (nonseptated-types) of cross walls and color. A mat of hyphae is called a mycelium.

Dimorphic fungi can convert between hyphal and yeast forms. Important examples of such fungi are *Histoplasma*, *Blastomyces*, *Coccidioides*, and *Sporothrix*.

Pseudohyphae forms present in *Candida albicans* have constrictions in the hyphae that resemble budding yeasts.

Most fungi reproduce by forming asexual spores called conidia. Variant forms of conidia include blastoconidia and arthroconidia. Some form sexual endospores inside of spherules in host tissues.

Cutaneous fungi are generally diagnosed by using KOH treatment of skin. Preparations also may be stained using periodic acid-Schiff (PAS) stain, silver stain, calcofluor, or India ink.

Fungi may also be cultured on Sabouraud's or blood agar with antibiotics and then identified by cell morphology or by polymerase chain reaction (PCR) using specific nucleic acid probes.

Previous or present fungal infections may also be diagnosed serologically or by skin tests.

Nonsystemic fungal infections include pityriasis (tinea) versicolor or fungemia in premature infants, caused by *Malassezia furfur*; cutaneous or mucocutaneous candidiasis; and nail and hair infections caused by species of *Trichophyton*, *Microsporum*, or *Epidermophyton*.

Sporotrichosis is a subcutaneous or lymphatic (rose gardener's disease) or pulmonary (alcoholic rose-garden-sleeper's disease) mycosis.

In the United States, deep fungal infections are caused by *Histoplasma capsulatum* (endemic eastern Great Lakes, Mississippi, and Missouri river beds); *Coccidioides immitis* (endemic southwestern United States, northern Mexico), or *Blastomyces dermatitidis* (endemic Great Lakes, Saint Lawrence, Ohio, and Mississippi river beds, southeastern United States). The disease states, modes of recognition, and other salient properties of each of these pathogens are described.

Opportunistic fungi include *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, *Mucor* species, *Rhizopus* species, *Absidia* species, and *Pneumocystis carinii*. The compromising condition leading to infection, the diseases caused, the modes of identification, and other significant features describing each of these organisms are described.

Review Questions

1. An obese 32-year-old diabetic woman presents with complaint of red and painful skin in her abdominal skin folds. Examination reveals a creamy white material at the base of the fold. It is erythematous underneath and extends beyond the creamy material. Microscopic examination of the exudate reveals oval budding structures ($3 \times 6 \mu\text{m}$) mixed with more budding elongated forms. The most likely causative agent is

 - A. *Aspergillus fumigatus*
 - B. *Candida albicans*
 - C. *Epidermophyton floccosum*
 - D. *Microsporum canis*
 - E. *Sporothrix schenckii*
2. What fungus causes tinea capitis or ringworm infection of the scalp?

 - A. *Aspergillus fumigatus*
 - B. *Microsporum canis*
 - C. *Epidermophyton floccosum*
 - D. *Candida albicans*
 - E. *Sporothrix schenckii*
3. An 18-year-old high school student in rural north Mississippi develops fever, cough, and chest pain. The cough, associated with weight loss, persisted. Because of poor performance at football practice he was advised to see a physician. Lymph node biopsies stained with H and E studies revealed granulomatous inflammation and macrophages engorged with oval structures measuring 2–4 μm . Cultures incubated at room temperature grew powdery white colonies, which on microscopic study had tuberculate spores. The high school student most likely acquired the infection from

 - A. Desert sand
 - B. Cat feces
 - C. Soil enriched with bird excrement
 - D. Another human via respiratory secretions
 - E. Contaminated drinking water
4. The most common portal of entry in *Blastomyces dermatitidis* infection is

 - A. Mouth
 - B. Circulatory system
 - C. Skin
 - D. Respiratory tract
 - E. Central nervous system

5. For which pathogen is infection triggered by traumatic contact with plants on which the organism is growing?
 - A. *Candida albicans*
 - B. *Coccidioides immitis*
 - C. *Cryptococcus neoformans*
 - D. *Histoplasma capsulatum*
 - E. *Sporothrix schenckii*

6. Hematoxylin and eosin stain of a biopsy specimen from an AIDS patient shows spherules with endospores. The most likely organism is
 - A. *Blastomyces dermatitidis*
 - B. *Candida albicans*
 - C. *Coccidioides immitis*
 - D. *Cryptococcus neoformans*
 - E. *Histoplasma capsulatum*
 - F. *Sporothrix schenckii*

Answers

1. **Answer: B.** Cutaneous candidiasis is a problem in skin folds of obese individuals. It is an even greater problem in diabetic patients because of the high sugar levels. Only the members of the genus *Candida* would produce a creamy surface growth. The erythematous base is due to the production of a cytotoxin. *Aspergillus*, *Epidermophyton*, and *Microsporum* are all monomorphic filamentous fungi and would not fit the description. *Sporothrix* is found as cigar-shaped budding yeasts but would not clinically present like this. It is traumatically implanted to start subcutaneous infections.

2. **Answer: B.** Tinea capitis or fungal infection of the scalp hair is caused by two genera of the dermatophytes: *Microsporum* and *Trichophyton*. *Epidermophyton* causes infections of the skin and nails but not the hair. *Microsporum* (the correct answer here) infects hair and nails. The other three fungi do not cause hair infection.

3. **Answer: C.** The clues here are the geography, weight loss, granulomatous inflammation, and macrophages engorged with oval structures (RES disease). The colonial appearance and tuberculate spores strongly suggests the causative agent to be *Histoplasma capsulatum*. *Histoplasma* is acquired from dusty environments containing bird (most often chicken or starling) or bat feces. The areas of highest endemicity are in the great central river beds with bat caves, chicken coops, and starling roosts having extremely high levels.

4. **Answer: D.** Like most of the systemic fungal agents, this fungus is transmitted by respiratory route. Direct inoculation via skin is possible but not the most common route, even though the species name of this fungus suggests skin.

5. **Answer: E.** Transmission of *Sporothrix* is by break or cut of the skin, whereby the fungus in the contaminated plants or wood is introduced.

6. **Answer: C.** *C. immitis* is the only fungus with endosporulating spherules, demonstrated by either KOH prep or histopathologic stain.

Medical Parasitology

4

What the USMLE Requires You to Know

The USMLE generally does not have many parasitology questions but you will be expected to know the following.

- Name of organism (scientific and common) and major parasite type (e.g., nematode or flagellate).
- Name of disease (common names are frequently used).
- Route of spread, including vector names and reservoir hosts.

For the following organisms you should also know symptoms and understand the pathogenicity:

Entamoeba

Giardia

Plasmodium

Toxoplasma

Cryptosporidium

Enterobius

Ascaris

Hookworms: *Necator* and *Ancylostoma*

Trichinella

Schistosoma

- Review additional bolded material in the following tables.

CLASSIFICATION OF PARASITES

Medical Parasitology is the study of the invertebrate animals and the diseases they cause. Parasites are classified as protozoans or metazoans. The most important organisms in the U.S.A. are identified in the following two tables in boldface type.

Table I-4-1. Protozoans

Common Name	Amebae	Flagellates	Ciliates	Apicomplexa
Important Genera	<i>Entamoeba</i> <i>Naegleria</i> <i>Acanthamoeba</i>	LUMINAL (GUT, UG) <i>Trichomonas</i> <i>Giardia</i> HEMOFLAGELLATES <i>Leishmania</i> <i>Trypanosoma</i>	<i>Balantidium</i>	BLOOD/TISSUE <i>Plasmodium</i> <i>Toxoplasma</i> <i>Babesia</i> INTESTINAL <i>Cryptosporidium</i> <i>Isospora</i>

Pneumocystis, which was formerly classified as a protozoan, has been determined to be a fungus through ribotyping and other molecular biologic techniques.

Table I-4-2. Metazoans: Worms*

Phylum	Flat worms (Platyhelminthes)		Roundworms
Classes: Common name:	Trematodes (flukes)	Cestodes (tapeworms)	Nematodes ** (roundworms)
Genera:	<i>Fasciola</i> <i>Fasciolopsis</i> <i>Paragonimus</i> <i>Opisthorchis</i> (<i>Clonorchis</i>) <i>Schistosoma</i>	<i>Diphyllobothrium</i> <i>Hymenolepis</i> <i>Taenia</i> <i>Echinococcus</i>	<i>Necator</i> <i>Enterobius</i> (<i>W</i>) <i>Uchereria/Brugia</i> <i>Ascaris</i> and <i>Ancylostoma</i> <i>Toxocara</i> , <i>Trichuris</i> & <i>Trichinella</i> <i>Onchocerca</i> <i>Dracunculus</i> Eye worm (<i>Loa loa</i>) <i>Strongyloides</i>

* Metazoans also include the Arthropoda, which serve mainly as intermediate hosts (the crustaceans) or as vectors of disease (the Arachnida and Insecta).

**Nematodes mnemonic.

HOSTS

The infected host is classified as

- Intermediate host in which larval stages develop.
- Definitive host in which the adult parasite reaches sexual maturity.

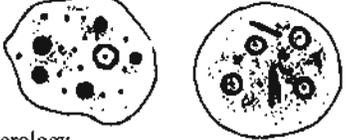
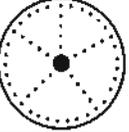
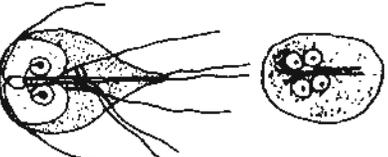
VECTORS

Vectors are living transmitters (e.g., a fly) of disease and may be

- Mechanical, which transport the parasite but there is no development of the parasite in the vector.
- Biologic, in which some stages of the life cycle occur.

IMPORTANT PROTOZOAN PARASITES

Table I-4-3. Protozoan Parasites

Species	Disease /Organs Most Affected	Form/Transmission	Diagnosis	Treatment
<i>Entamoeba histolytica</i>	Amebiasis: dysentery Inverted flask-shaped lesions in large intestine with extension to peritoneum and liver, lungs, brain, and heart. Blood and pus in stools. Liver abscesses.	Cysts Fecal-oral transmission—water, fresh fruits, and vegetables	Trophozoites:  or cysts in stool: Serology Nuclei have sharp central karyosome and fine chromatin “spokes”. 	Metronidazole followed by iodoquinol
<i>Giardia lamblia</i>	Giardiasis: Ventral sucking disk attaches to lining of duodenal wall, causing a fatty, foul-smelling diarrhea (diarrhea → <i>malabsorption</i> duodenum, jejunum)	Cysts Fecal (human, beaver, muskrat, etc.), oral transmission—water, food, day care, oral-anal sex	Trophozoites or cysts in stool or fecal antigen test (replaces “string” test)  When seen, “falling leaf” motility	Metronidazole
<i>Cryptosporidium</i> sp.	Cryptosporidiosis: transient diarrhea in healthy, severe in immunocompromised hosts	Cysts Undercooked meat, water; not killed by chlorination	Acid fast oocysts in stool: Biopsy shows dots (cysts) in intestinal glands 	
<i>Balantidium coli</i>	Dysentery: infection of colon with penetration	Cysts Contaminated food or water	Ciliated trophozoites, cysts in feces 	Tetracycline
<i>Trichomonas vaginalis</i> (urogenital)	Trichomoniasis: often asymptomatic or frothy vaginal discharge	Trophozoites Sexual	Motile trophozoites in methylene blue wet mount: May give positive “whiff” test in KOH 	Metronidazole

Free Living Amebae

- Occur in polluted water or soil (*Naegleria*, *Acanthamoeba*)
- Occur in contact lens saline solutions (*Acanthamoeba*): cysts from dust contaminate

Table I-4-4. Free Living Amebae That Occasionally Infect Humans

Species	Disease / Locale	Form / Transmission	Diagnosis	Treatment
<i>Naegleria</i>	Primary amebic meningoencephalitis (PAM): severe pre-frontal headache, nausea, high fever, often an altered sense of smell; often fatal.	Free-living amebae picked up while swimming or diving in very warm fresh water.	Motile trophozoites in CSF Culture on plates seeded with Gram-negative bacteria. Amebae will leave trails.	Amphotericin B (rarely successful)
<i>Acanthamoeba</i>	Keratitis; Granulomatous amebic encephalitis (GAE) in immunocompromised patients: insidious onset but progressive to death.	Free living amebae in contaminated contact lens solution (airborne cysts) Not certain for GAE: inhalation or contact with contaminated soil or water.	Star-shaped cysts on biopsy; rarely seen in CSF. Culture as above.	Keratitis: topical miconazole and propamidine isothionate GAE: sulfadiazine (rarely successful)

Plasmodium Species

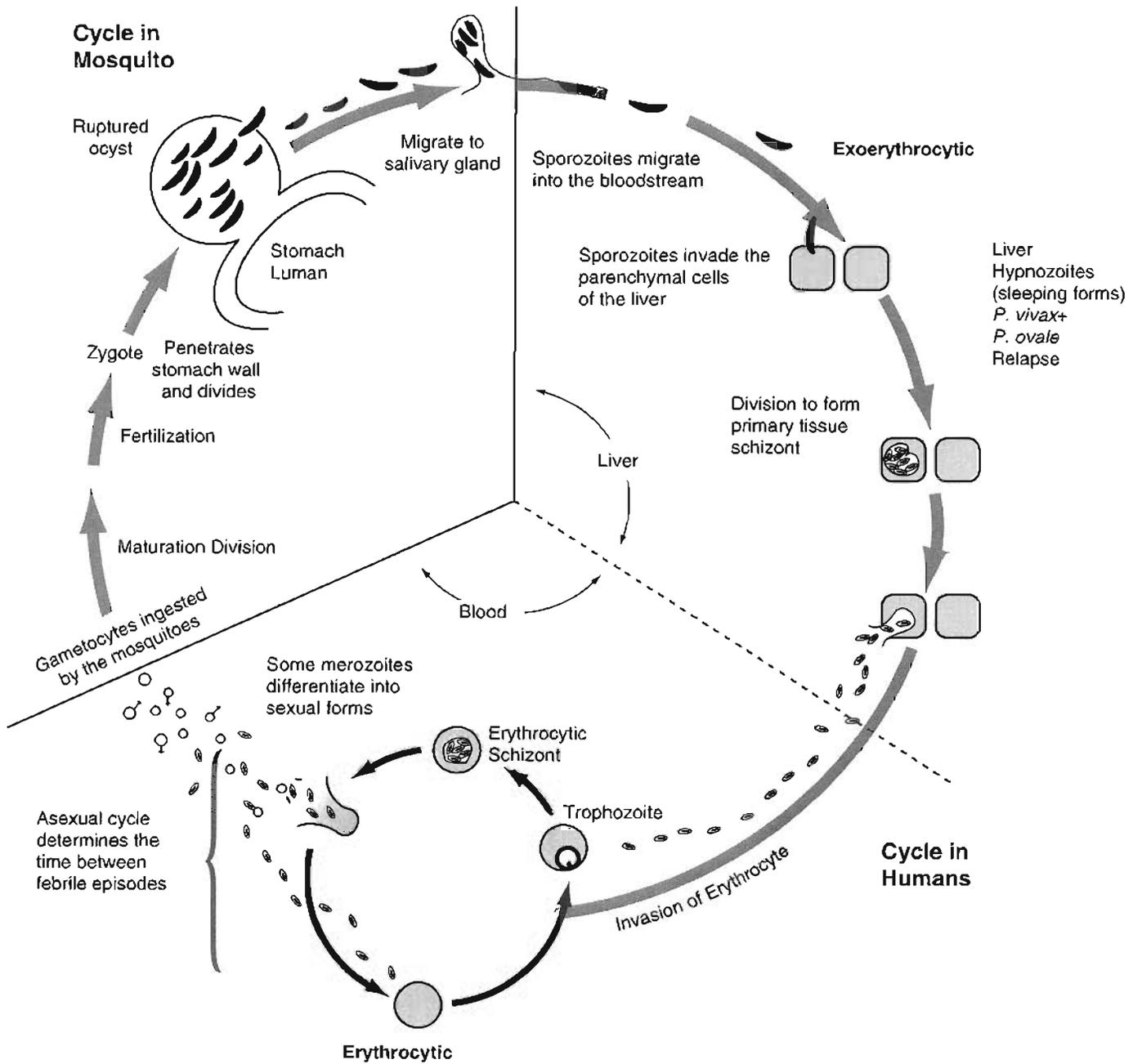


Figure I-4-1. *Plasmodium* Life Cycle

Each *Plasmodium* has two distinct hosts.

- A vertebrate such as the human where asexual phase (schizogony) takes place in the liver and red blood cells.
- An arthropod host (*Anopheles* mosquito) where gametogony (sexual phase) and sporogony take place.

Cause disease by a wide variety of mechanisms, including metabolism of hemoglobin and lysis of infected cells leading to anemia and to agglutination of infected RBC.

Cause paroxysms (chills, fever spike, and malarial rigors) when the infected RBC are lysed, liberating a new crop of merozoites.

Table I-4-5. *Plasmodium* Species

Species	Disease	Important Features	Blood Smears	Liver Stages	Treatment**
<i>Plasmodium vivax</i>	Benign tertian	48-hour fever spikes	Enlarged host cells; ameboid trophozoites	Persistent hypnozoites Relapse*	Chloroquine PO ₄ then primaquine
<i>Plasmodium ovale</i>	Benign tertian	48-hour fever spikes	Oval, jagged, infected RBCs	Persistent hypnozoites Relapse	Chloroquine PO ₄ then primaquine
<i>Plasmodium malariae</i>	Quartan or malarial	72-hour fever spikes; recrudescence*	Bar and band forms; rosette schizonts	No persistent stage*	Chloroquine PO ₄ (no radical cure necessary)
<i>Plasmodium falciparum</i>	Malignant tertian	Irregular fever spikes; causes cerebral malaria	Multiple ring forms crescent-shaped gametes	No persistent stage*	Chloroquine resistance a problem***

*Recrudescence is a reoccurrence of symptoms from low levels of organisms remaining in red cells (recrudescence).

Relapse is an exacerbation from liver stages (hypnozoites).

**Treatment:

1. Suppressive (to avoid infection)
2. Therapeutic (eliminate erythrocytic)
3. Radical cure (eliminate exoerythrocytic)
4. Gametocidal (destruction of gametocytes)

Successful treatment is accomplished with chloroquine followed by primaquine. Chloroquine therapy is suppressive, therapeutic, and gametocidal, whereas primaquine eliminates the exoerythrocytic form.

***Use quinine sulfate plus pyrimethamine-sulfadoxine.

Hemoflagellates (Trypanosomes and Leishmanias)

Hemoflagellates infect blood and tissues.

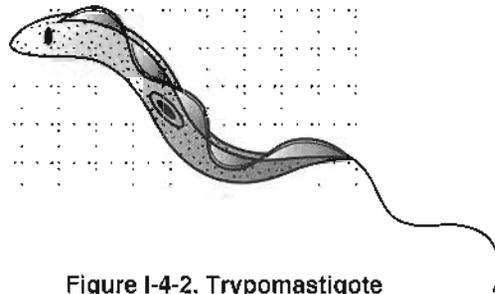


Figure I-4-2. Trypomastigote



Figure I-4-3. Amastigote

Trypanosomes are found

- In human blood as trypomastigotes with flagellum and undulating membrane
- In tissues as amastigotes (oval cells having neither the flagellum nor undulating membrane)

Leishmania found always as amastigotes in macrophages.

Table I-4-6. Hemoflagellates

Species	Disease	Vector/Form/Transmission	Reservoirs	Diagnosis	Treatment
<i>Trypanosoma cruzi</i> *	Chagas' disease (American trypanosomiasis) Latin America Swelling around eye: (Romaña's sign) common early sign Cardiac muscle, liver, brain often involved	Reduviid bug (kissing or cone bug; genus <i>Triatoma</i>) passes trypomastigote (flagellated form) in feces as it bites. Scratching implants in bite site.	Cats, dogs, armadillos, opossums Poverty housing	Blood films	Nifurtimox
<i>Trypanosoma brucei</i> <i>Trypanosoma b. gambiense</i> <i>Trypanosoma b. rhodesiense</i>	African sleeping sickness (African trypanosomiasis) Antigenic variation	Trypomastigote in saliva of tsetse fly contaminates bite	Humans, some wild animals	Blood films, CSF High immunoglobulin levels in CSF	Acute: suramin Chronic: melarsoprol
<i>Leishmania donovani</i> ** complex	Visceral Leishmaniasis Kalazar	Sandfly bite	Urban: humans Rural: rodents and wild animals	Amastigotes in macrophages in bone marrow, liver, spleen	Stibogluconate sodium (from CDC)
<i>Leishmania</i> (About 15 different species)	Cutaneous Leishmaniasis (Oriental sore, etc.)	Sandfly bite	Urban: humans Rural: rodents and wild animals	Amastigotes in macrophages in cutaneous lesions	Stibogluconate sodium
<i>Leishmania braziliensis</i> complex	Mucocutaneous Leishmaniasis	Sandfly bite	Urban: humans Rural: rodents and wild animals	Same	Stibogluconate sodium

**T. cruzi*: An estimated 1/2 million Americans are infected, creating some risk of transfusion transmission in U.S. In babies, acute infections often serious involving CNS. In older children and adults, mild acute infections but may become chronic with the risk of development of cardiomyopathy and heart failure.

***Leishmania* all: Intracellular, sandfly vector, stibogluconate.

Miscellaneous Apicomplexa Infecting Blood or Tissues

Table I-4-7. Miscellaneous Apicomplexa Infecting Blood or Tissues

Species	Disease/Locale of Origin	Transmission	Diagnosis	Treatment
<i>Babesia</i> (primarily a disease of cattle) Humans: <i>Babesia microti</i> , WA1, & MO1 strains	Babesiosis (hemolytic, malaria-like) Same range as Lyme NE, N Central, California and NW USA	<i>Ixodes</i> tick Co-infections with <i>Borrelia</i>	Giemsa stain of thin smear or hamster inoculation	Clindamycin + quinine
<i>Toxoplasma gondii</i>	See below	Cat is essential definitive host. Many other animals are intermediate host. Mode: 1) Raw meat in US #1 = pork 2) Contact with cat feces	Serology High IgM or rising IgM acute infection	Pyrimethamine + sulfadiazine

Toxoplasmosis

Most common parasitic disease.

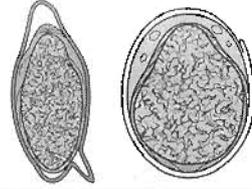
- *Toxoplasma* acquired **after** birth is most commonly asymptomatic or mild, nonspecific with lymphadenopathy and fever. May mimic infectious mononucleosis.
- Once infected, as immunity develops, bradyzoites encyst, but generally remain viable as evidenced by a positive serotiter.
- Unless prophylactic drugs are given, AIDS patients who are seropositive for *Toxoplasma* will have reactivational infections.
- Produces severe disease in AIDS or immunocompromised patients.
- Maternal antibodies protect the fetus, even if the mother is reinfected during pregnancy.
- If primary maternal infection occurs during pregnancy, the fetus may be infected.
- If *Toxoplasma* crosses placenta early, severe congenital infections: intracerebral calcifications, chorioretinitis, hydro- or microcephaly, convulsions.
- If later, may be inapparent; untreated inapparent congenital infections lead to progressive blindness.

IMPORTANT METAZOAN PARASITES

Trematodes

- Are commonly called flukes.
- Are leaf-shaped worms, which are generally flat and fleshy.
- Are hermaphroditic except for *Schistosoma*, which has separate male and female.
- Have complicated life cycles occurring in two or more hosts.
- Have operculated eggs (except for *Schistosoma*), which contaminate water, perpetuating the life cycle, and which are also used to diagnose infections.
- The first intermediate hosts are snails.

Table I-4-8. Trematode (Fluke) Diseases

Organism	Common Name	Reservoir Host	Acquisition	Progression in Humans	Important Ova	Treatment
<i>S. mansoni</i> <i>Schistosoma japonicum</i>	Intestinal schistosomiasis	Cats, dogs, cattle, etc.	Contact with water; skin penetration	Skin penetration (itching) → mature in veins of mesentery → eggs cause granulomas in liver (liver enlargement in chronic cases)		Praziquantel
<i>Schistosoma haematobium</i>	Vesicular schistosomiasis	Primates	Contact with water; skin penetration	Skin penetration (itching) → Mature in bladder veins; chronic infection has high association with bladder carcinoma in Egypt and Africa		Praziquantel
Non-human schistosomes	Swimmer's itch	Birds (Great Lakes U.S.)	Contact with water; skin penetration	Penetrate skin producing dermatitis without further development in humans; itching is most intense at 2 to 3 days		Trimeprazine Calamine Sedatives
<i>Clonorchis sinensis</i>	Chinese liver fluke	Dogs, cats, humans	Raw fish ingestion		Operculated eggs	Praziquantel
<i>Fasciola hepatica</i>	Sheep liver fluke	Sheep, cattle, humans	Ingestion of aquatic plants: water cress		Operculated eggs	Praziquantel
<i>Fasciolopsis buski</i>	Giant intestinal fluke	Pigs, dogs, rabbits, humans	Ingestion of aquatic plants: water chestnuts		Operculated eggs	Praziquantel
<i>Paragonimus westermani</i>	Lung fluke	Humans, cat family, canines, pigs	Raw crabs, crayfish		Operculated eggs	Praziquantel

Cestodes

- Are the tapeworms.
- Consist of three basic portions: the head or scolex; a “neck” section, which produces the proglottids; and the segments or proglottids, which mature as they move away from the scolex. (The combination of the neck and proglottids is called the strobila.)
- Are hermaphroditic with each proglottid developing both male and female reproductive organs, and mature eggs developing in the most distal proglottids.
- Adhere to the mucosa via the scolex, which is knobby looking and has either suckers or a sucking groove.
- Have no gastrointestinal (GI) tract, they absorb nutrients from the host's GI tract.
- Are diagnosed by finding eggs or proglottids in the feces.
- Have for the most part complex life cycles involving extraintestinal larval forms in intermediate hosts. When humans are the intermediate host, these infections are generally more serious than the intestinal infections with adult tapeworms.

Gastrointestinal Cestodes (Tapeworms)

Table I-4-9. Gastrointestinal Cestodes (Tapeworms)

Cestode (Common name) Intermediate Host (IH) Definitive Host (DH)*	Form/Transmission	Humans Are:	Disease/Organ Involvement/Symptoms (Sx)	Diagnosis	Treatment
<i>Taenia saginata</i> (beef tapeworm) IH: cattle DH: humans	Rare beef containing the cysticerci is ingested	DH	Intestinal tapeworm/Small intestine Sx: Asymptomatic or vague abdominal pains	Proglottids or eggs in feces	Praziquantel
<i>Taenia solium</i> (pork tapeworm) IH: swine; Rarely: humans DH: humans Developing and Slavic countries	Water, vegetation, food contaminated with eggs Auto infection	IH	Cysticercosis/eggs → larva develop in brain, eye, heart, lung, etc.	Biopsy	Praziquantel: surgery in some sites
	Rare/raw pork containing the cysticerci is ingested by humans	DH	Intestinal tapeworm Sx: as for <i>Taenia saginata</i>	Proglottids or eggs in feces	Praziquantel
<i>Diphyllobothrium latum</i> (fish tapeworm) IH (2): crustaceans → fish; Rare: humans DH: humans/mammals Cool lake regions	Drinking pond water w/ → copepods (crustaceans) carrying the larval forms or frog/snake poultices	IH	Sparganosis/larvae penetrate intestinal wall and encyst	Biopsy	Praziquantel
	Rare, raw, pickled fish → containing sparganum	DH	Intestinal tapeworm (up to 10 meters)/Small intestine megaloblastic anemia	Proglottids or eggs in feces	Praziquantel
<i>Echinococcus granulosus</i> IH: herbivores; Rare: humans DH: carnivores in sheep- raising areas	Ingestion of eggs	IH	Hydatid cyst disease liver & lung where cysts containing brood capsules develop.	Imaging; Serology	Surgery; albendazole
<i>Echinococcus multilocularis</i> IH: rodents DH: canines and cats Northern areas	Ingestion of eggs	IH	Alveolar hydatid cyst disease	Tough, as above but no protoscolices	Surgical resection

* Definitive host = adult tapeworm develops in; intermediate host = cysticerci or larvae develop in; cysticerci = encysted larvae found in intermediate host.

Nematodes

- Are the roundworms
- Cause a wide variety of diseases (pinworms, whipworms, hookworms, trichinosis, threadworms, filariasis, etc.)
- Have round unsegmented bodies
- Are transmitted by:
 - ingestion of eggs (*Enterobius*, *Ascaris*, or *Trichuris*);
 - direct invasion of skin by larval forms (*Necator*, *Ancylostoma*, or *Strongyloides*);
 - ingestion of meat containing larvae (*Trichinella*); or
 - infection involving insects transmitting the larvae with bites (*Wuchereria*, *Loa loa*, *Mansonella*, *Onchocerca*, and *Dracunculus*).

Table I-4-10. Round Worms (Nematodes) Transmitted by Eggs

Species	Disease/Organs Most Affected	Form/Transmission	Diagnosis	Treatment
<i>Enterobius vermicularis</i> Most frequent helminth parasite in U.S.	Pinworms, large intestine, perianal itching	Eggs/person to person autoinfection	Sticky swab of perianal area Ova have flattened side with larvae inside 	Albendazole Treat entire family
<i>Trichuris trichiura</i>	Whipworm cecum, appendicitis, and rectal prolapse	Eggs ingested	Barrel-shaped eggs with bipolar plugs in stools 	Albendazole
<i>Ascaris lumbricoides</i> Most common helminth worldwide Largest roundworm	Ascariasis Ingest egg → larva migrate thru lungs (cough) and mature in small intestine; may obstruct intestine or bile duct	Eggs ingested	Bile stained, knobby eggs Adults 6–12" roundworms 	Supportive therapy during pneumonitis; surgery for ectopic migrations; albendazole
<i>Toxocara canis</i> or <i>cati</i> (dog/cat Ascarids)	Visceral Larva Migrans Larvae wander aimlessly until they die, cause inflammation	Eggs ingested/from handling puppies or from eating dirt in yard (pica)	Clinical findings and serology	Albendazole; self-limiting in most cases

Table I-4-11. Roundworms (Nematodes) Transmitted By Larvae

Species	Disease/Organs	Form/Transmission	Diagnosis	Treatment
<i>Necator americanus</i> New World hookworm	Hookworm infection Lung migration → pneumonitis bloodsucking → anemia	Filariform larva penetrates intact skin of bare feet	Fecal larvae (up to 13 mm) and ova: oval, transparent with 2–8 cell-stage visible inside Occult blood fecal may be present	Mebendazole and iron therapy
<i>Ancylostoma braziliense</i> <i>Ancylostoma caninum</i> (dog and cat hookworms)	Cutaneous Larva Migrans/intense skin itching	Filariform larva penetrates intact skin but cannot mature in humans	Usually a presumptive diagnosis; exposure	Thiabendazole
<i>Strongyloides stercoralis</i>	Threadworm strongyloidiasis: Early: pneumonitis, abdominal pain, diarrhea Later: malabsorption, ulcers, bloody stools	Filariform larva penetrates intact skin; Autoinfection leads to indefinite infections unless treated.	Larvae in stool, serology	Thiabendazole
<i>Trichinella spiralis</i>	Trichinosis: larvae encyst in muscle → pain	Viable encysted larvae in meat are consumed: wild game meat.	Muscle biopsy; clinical findings: fever, myalgia, splinter hemorrhages, eosinophilia	Steroids for severe symptoms + mebendazole

Wuchereria bancrofti and *Brugia* are filarial worms causing elephantiasis. Both are transmitted by mosquitoes.

Loa loa is the eye worm transmitted by biting flies.

Onchocera volvulus causes river blindness characterized by itchy “leopard” rash and worms in eye. Black fly transmits.

Dracunculus medinensis is the guinea worm. It is transmitted by drinking infected copepods (cyclops) in water.

Matching

- (A) *Ancylostoma braziliensis*
- (B) *Ascaris lumbricoides*
- (C) *Balantidium coli*
- (D) *Diphyllobothrium latum*
- (E) *Clonorchis sinensis*
- (F) *Cryptosporidium parvum*
- (G) *Dermacentor andersoni*
- (H) *Echinococcus multilocularis*
- (I) *Entamoeba coli*
- (J) *Entamoeba histolytica*
- (K) *Enterobius vermicularis*
- (L) *Giardia lamblia*
- (M) *Ixodes scapularis* (*I. dammini*)
- (N) *Leishmania braziliensis*
- (O) *Plasmodium vivax*
- (P) *Pneumocystis carinii*
- (Q) *Pediculus humanus*
- (R) *Sarcoptes scabiei*
- (S) *Schistosoma haematobium*
- (T) *Strongyloides stercoralis*
- (U) *Toxoplasma gondii*
- (V) *Trichinella spiralis*
- (W) *Trichuris trichiura*
- (X) *Trichomonas vaginalis*
- (Y) *Trypanosoma cruzi*
- (Z) *Wuchereria bancrofti*

- _____ 1. Invasive amebae causing dysentery, which is noted for causing extraintestinal abscesses
- _____ 2. Chronic infections associated with bladder carcinoma
- _____ 3. Vector for *Borrelia burgdorferi*
- _____ 4. Untreatable or at least poorly treatable causative agent of chronic diarrhea in AIDS patients, which is diagnosed by finding acid fast cysts in the stool
- _____ 5. By ribotyping, now considered to be a fungus
- _____ 6. Critically careful surgery is major component of therapy
- _____ 7. Ciliate causative agent of diarrhea
- _____ 8. Filarial worm maturing in the lymphatics and causing elephantiasis
- _____ 9. Adult females live inside adult male groove
- _____ 10. Carrier of epidemic typhus and Trench Fever
- _____ 11. Infection results in enteritis and eosinophilia with flu-like symptoms, periorbital swelling, petechial hemorrhages, and ultimately muscle pain; later in life X-ray may show fine calcifications in the muscle
- _____ 12. Fatty diarrhea associated with malabsorption syndrome

Answers

1. J The organism causing diarrhea but most noted for its ability to leave the gastrointestinal tract is *Entamoeba histolytica*. Remember that *Entamoeba coli* is not a pathogen but rather a commensal organism.
2. S Chronic infections with *Schistosoma haematobium* are notably associated with bladder carcinoma.
3. M The vector for Lyme disease is the *Ixodes* tick, *Ixodes scapularis* (*I. dammini*) in the midwest and east, with *Ixodes pacificus* in the western United States.
4. F *Cryptosporidium* treatment is still experimental and not highly effective.
5. P *Pneumocystis carinii* has always stained like a fungus but until ribotyping was considered a protozoan parasite.
6. H Hydatid cyst disease requires delicate surgery to remove them without breaking and releasing the larvae.
7. C *Balantidium* is the only ciliate on the list (and the only one you need to know).
8. Z *Wuchereria* is one causative agent of elephantiasis. *Brugia* is another.
9. S Schistosomes are not hermaphroditic like other flukes (trematodes). They instead have separate sexes, but they live permanently together with the female in a groove of the male.
10. Q The human body louse is a *Pediculus* and carries both epidemic typhus and Trench Fever.
11. V Classical description of trichinosis, causative agent *Trichinella spiralis*.
12. L *Giardia* is probably most noted among the parasites for causing diarrhea with fat malabsorption.

Chapter Summary

Table I-4-1 identifies the important genera in each of the four groups of protozoa, and Table I-4-2 identifies the important genera in each of the three phyla of metazoa.

Table I-4-3 describes the diseases, affected organs, cellular forms, methods of transmission, modes of diagnosis, and treatments for the important pathogenic protozoan species. Table I-4-4 does the same for free-living species that occasionally infect humans.

The life cycle of malaria-causing species is illustrated in Figure I-4-1, and the properties of individual *Plasmodium* species are described in Table I-4-5.

The morphology and properties, as well as the diseases caused by, the vectors responsible for transmission of, the modes of transmission of, the reservoirs for, the diagnoses of, and the treatments for infection by the various species of the *Trypanosoma* and *Leishmania* hemoflagellates are described in Table I-4-6 and Figures I-4-2 and I-4-3.

The most common parasitic disease, toxoplasmosis, is caused by *Toxoplasma gondii* and is transmitted by raw meat or contact with cat feces. It and *Babesia* are described in Table I-4-7.

The trematode organisms, the common names of the diseases they cause, their reservoir hosts, their modes of acquisition, the progression of the diseases they cause in humans, their ova, and treatments for their infections are described in Table I-4-8.

The tapeworms (Cestodes) are described in Table I-4-9; the roundworms (Nematodes) transmitted by eggs are described in Table I-4-10, and the roundworms transmitted by larvae are described in Table I-4-11.

Review Questions

1. B.F., a 44-year-old, returns home to New York following a 2-week camera safari to East Africa. She started chloroquine anti-malarial prophylaxis 2 weeks prior to her departure for Kenya and continued throughout her foreign travel. She stopped taking the pills on her arrival home because they made her nauseated. Two weeks after her return she develops paroxysmal fever and diaphoresis and is quickly hospitalized with febrile convulsions, jaundice, and anemia. Blood smears reveal red blood cells multiply infected with delicate ring-like trophozoites and rare sausage-shaped gametocytes. The stage of the parasite life cycle that is responsible for the appearance of the parasites 2 weeks after departure from the malarious area is the
 - A. Hypnozoite
 - B. Sporozoite
 - C. Exoerythrocytic schizont
 - D. Erythrocytic schizont
 - E. Merozoite

2. At a school nurse's request, a clinic in rural South Carolina sees a 9-year-old girl who appears listless and inattentive, although hearing and visual testing has been within normal limits. The physician finds the child thin, with the "potbelly" of malnutrition, and orders a fecal exam and CBC. The CBC reveals a microcytic, hypochromic anemia, and the fecal exam detects brown, oval nematode eggs approximately 65 microns in size, too numerous to count. What was the most likely means by which this child was infected?
 - A. Ingestion of eggs
 - B. Ingestion of larvae
 - C. Ingestion of cysts in muscle
 - D. Skin penetration by larvae
 - E. Mosquito transmission of sporozoites

3. An HIV-positive patient with a CD4+ count of 47 presents with diarrhea. Acid-fast oocysts are found in the stool. From this finding, what is the proper care and prognosis with that care?
 - A. Infection is short lasting and self-resolving and requires no treatment
 - B. If treated with antibiotics, the infection should resolve in 3–6 days
 - C. Infection will resolve only with a combination of anti-tuberculous drugs and then it may take weeks
 - D. Infection could have been prevented by avoiding cat feces and undercooked or raw meat
 - E. Even with the best treatment, the infection may be unrelenting

4. A 24-year-old primiparous woman in her eighth month of gestation develops a positive IgM titer to *Toxoplasma gondii* for the first time. She should be advised by her physician
 - A. That this child and all future fetuses are likely to be infected
 - B. That a newborn with a positive anti-*Toxoplasma* IgG response should be treated with anti-parasitics
 - C. That future infections can be avoided by proper vaccination and worming of cats
 - D. That retinochoroiditis can be prevented by drug treatment of an infant with a positive IgM response
 - E. That major organ damage can be reversed by prompt treatment of the newborn

5. A 35-year-old Captain in the Army Reserves has been plagued by a painful, erosive lesion near his ear lobe since his return from Operation Desert Storm several years ago. He denies exposure to the toxic by-products of burning oil fields. Punch biopsy of the leading edge of the erosion reveals macrophages distended with oval amastigotes. How was this infection acquired?
 - A. Contact with contaminated drinking water
 - B. Bite of infected *Anopheles* mosquito
 - C. Bite of infected reduviid bug
 - D. Fecal contamination of food
 - E. Direct human contact in barracks
 - F. Bite of sandfly
 - G. Bite of tsetse fly

6. A group of six college students undertake to climb Mt. Rainier outside Seattle on their spring break. They pack food and camping provisions except for water, which they obtain from the many fresh water mountain streams that arise at the summit. The adventure takes a little over a week to accomplish, and all return safely and in good spirits to their classes the following week. Within the first week after their return, 5 of the 6 students report to the infirmary with profuse diarrhea and tenesmus. Each affected student experiences weakness and weight loss and stool samples submitted to the lab are yellow, greasy, and foul smelling. What attribute of this parasite imparts its pathogenicity?
- Lytic enzymes
 - Flagella
 - Ventral sucking disc
 - Encystment
 - Toxic metabolites

Answers

- Answer: C.** B.F. is suffering from *Plasmodium falciparum* malaria acquired shortly before her departure from Kenya. Liver stages of *plasmodium* are not susceptible to chloroquine killing. Because she did not continue the prophylaxis after her return to the States, this allowed those parasites to initiate all of the erythrocytic stages of the life cycle. Any erythrocytic stages generated out of the liver phase of the life cycle while she remained on prophylaxis would have been killed. Thus, the late onset of her symptoms was due to survival of exoerythrocytic stages that had not yet left the liver at the time she ceased prophylaxis. Hypnozoites are responsible for relapse of symptoms in *P. vivax* and *P. ovale* malaras, but do not exist in *P. falciparum*, and it is clear that she has *falciparum* malaria due to the delicate ring forms multiply infecting erythrocytes and the sausage-shaped gametocytes. Sporozoites are the infectious forms injected by mosquitoes and would not have been available in this country to initiate the symptoms on the time course described. Erythrocytic schizonts and merozoites would have been killed by prophylaxis before she left Africa and could not be responsible for the late onset of symptoms.
- Answer: D.** This child has the typical symptoms of hookworm disease, caused in this country usually by *Necator americanus*. The infection is acquired by penetration of the filariform larvae through the skin of the feet or buttocks, after contamination of soil with the eggs of the agent deposited in human feces. Of the other distractors, choice A would be most likely if the infection were due to ascarids, pinworms, or whipworms. Choice C would describe infection with either *Taenia* or *Trichinella*, and choice E would be the means of infection with *Plasmodium*.
- Answer: E.** The described infection is most likely to be *Cryptosporidium*, which is a very difficult infection in AIDS patients even though it is self-resolving in normal noncompromised individuals. In AIDS patients it is most commonly unrelenting, even with treatment. *Cryptosporidium* is usually acquired from water; it is *Toxoplasma* that's from cats.
- Answer: D.** The positive IgM titer arising in the eighth month means that this woman has become acutely infected with *Toxoplasma*. Infections acquired at this time have a high likelihood of infecting the fetus and are most likely to be manifested by the development of retinochoroiditis. A mother can transmit this parasite to her fetus only during an acute infection; therefore, all future fetuses will be protected from the disease. Since IgG antibodies cross the placenta, presence of the anti-*Toxoplasma* antibodies of this class in the neonate may simply reflect the infection of the mother—only a positive IgM response in

the neonate is proof of the child's infection, which should therefore be treated. There is no way to reverse major organ damage when it occurs in utero, but it would not be expected to occur with an acute infection beginning in the third trimester.

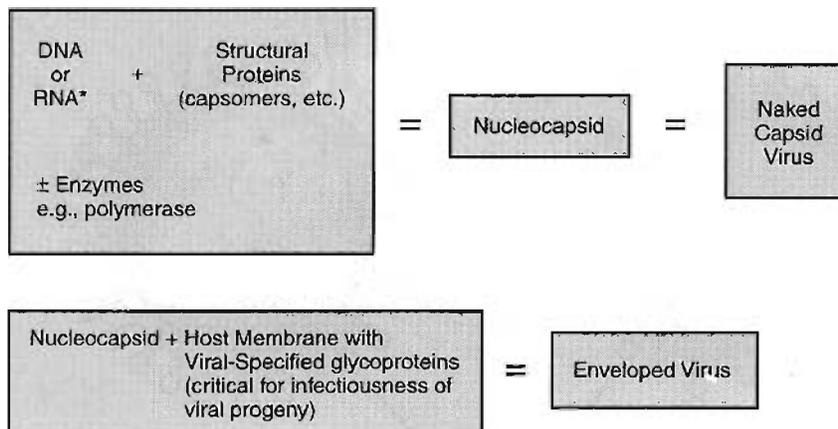
5. **Answer: F.** *Leishmania* spp. are transmitted by the bite of sandflies. They cannot be transmitted from person to person by trivial means, so unless organ transplantation is occurring in the barracks, direct human contact (choice E) is not a possibility. To survive outside the human host, they must be in the vector (sandfly), so transmission by food or water (choices A or D) are not possible. Of the distractors that involve true vectors: *Anopheles* mosquitoes (choice B) transfer malaria; reduviid bugs (choice C) transfer American trypanosomiasis (Chagas' disease); and tsetse flies (choice G) transmit African trypanosomiasis (sleeping sickness).
6. **Answer: C.** *Giardia* is common in mountain streams throughout the U.S., and the presentation of prolonged fatty diarrhea and weight loss is pathognomonic. It causes its pathology by its adherence to the mucosa of the upper small intestine with its ventral sucking disc. No toxic metabolites or lytic enzymes are involved in the pathology, which apparently results from blockage of normal digestive absorption. The organism is a flagellate, and thus has flagella, but migration into extraintestinal sites is not a well known problem associated with pathology. And although the organism does encyst as it passes along the intestine, this is not known to produce symptoms.

Medically Important Viruses

What the USMLE Requires You to Know

- Major concepts of host and tissue specificity
- Major concepts of viral replication
- How viruses cause disease
- Basics of viral diseases (as for bacterial diseases)
- Plus for each virus
 - Nucleic acid (and generalities about how it replicates)
 - Nucleocapsid shape
 - Whether or not it is enveloped

STRUCTURE AND MORPHOLOGY



*Positive sense RNA = (+) RNA
(can be used itself as mRNA)

ss = Single stranded
ds = Double stranded

*Negative sense RNA = (-) RNA

- Complementary to mRNA
- Cannot be used as mRNA
- Requires virion-associated RNA-dependent RNA polymerase (as part of the mature virus)

Figure I-5-1. The Basic Virion

Note

SITV*1 and 2
(Simplified imaginary teaching virus):

- The codon for phenylalanine is UUU.
- Phenylalanine is represented in Figure V-2 by the Greek letter Φ (phi).
- The SIT viruses (SITV + RNA and SITV - RNA) both have a single gene that codes for their capsids, which is made up entirely of phenylalanine.
- Look at the genome in Figure V-2. Which is the positive RNA virus?

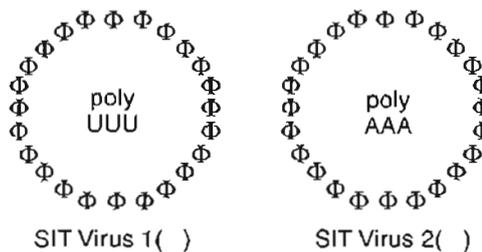


Figure I-5-2. Simplified Imaginary Teaching Viruses

VIRAL STRUCTURE

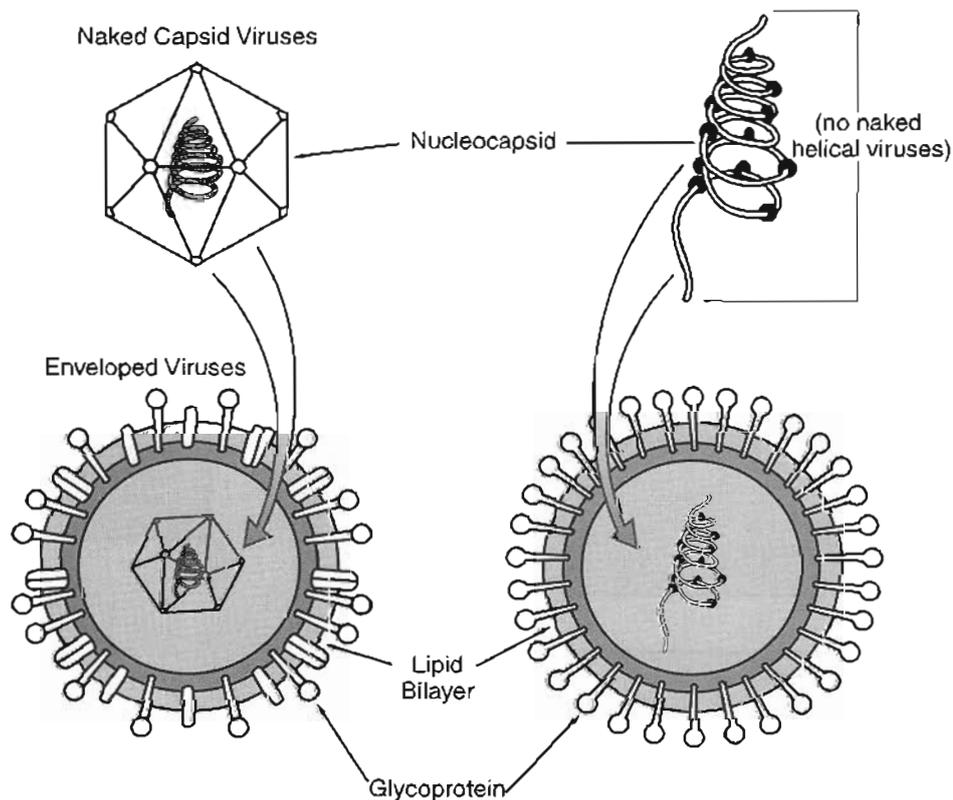


Figure I-5-3. Morphology of Viruses

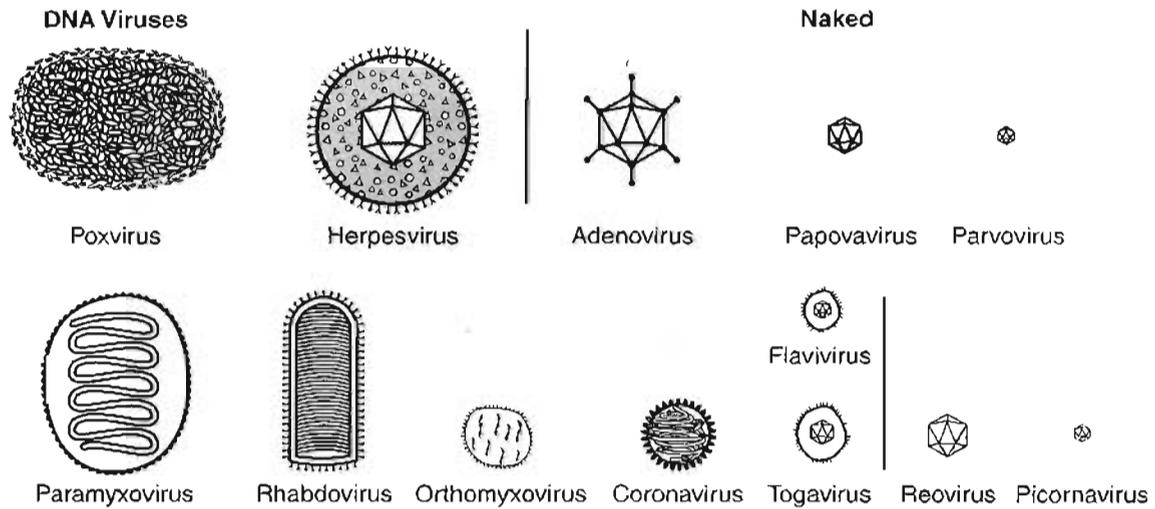


Figure I-5-4. Relative Sizes and Shapes of Different Viruses

VIRAL REPLICATION

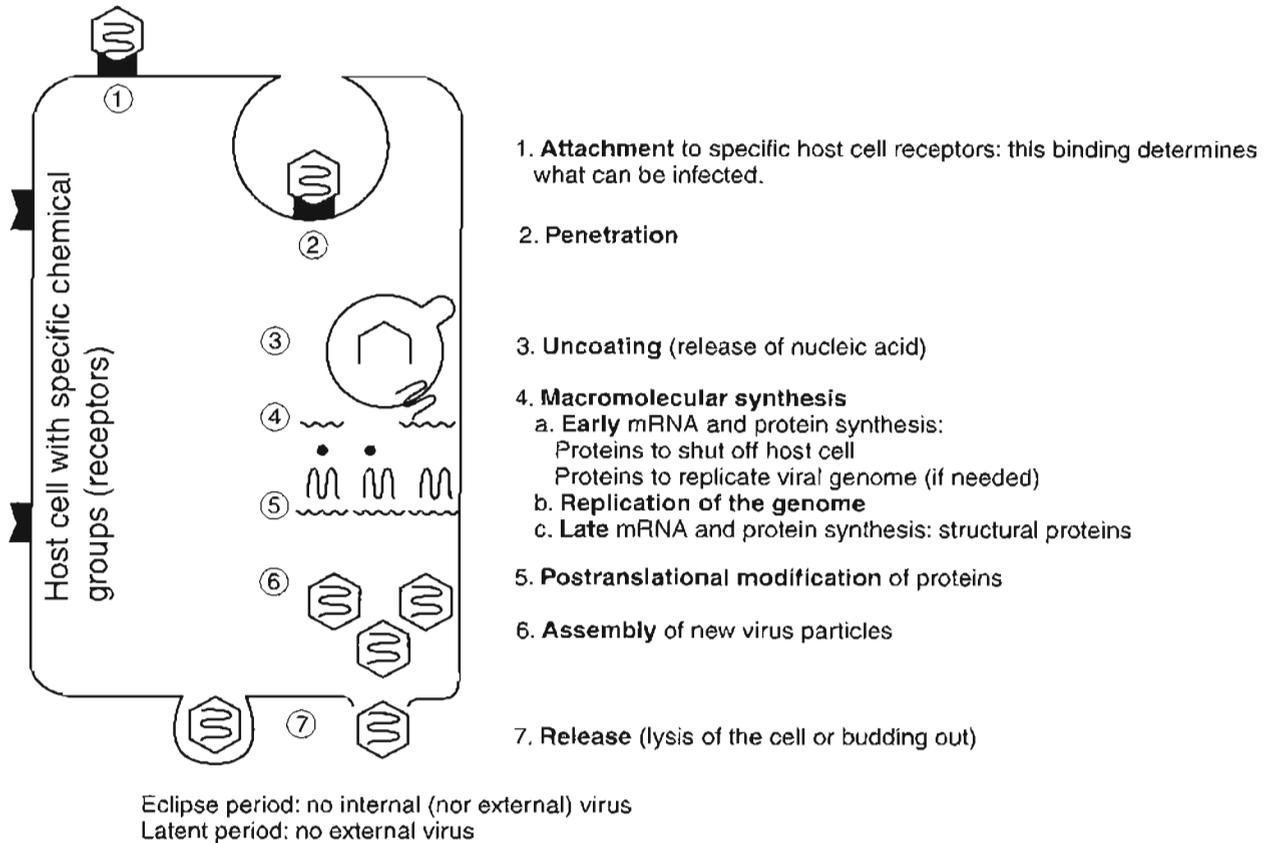
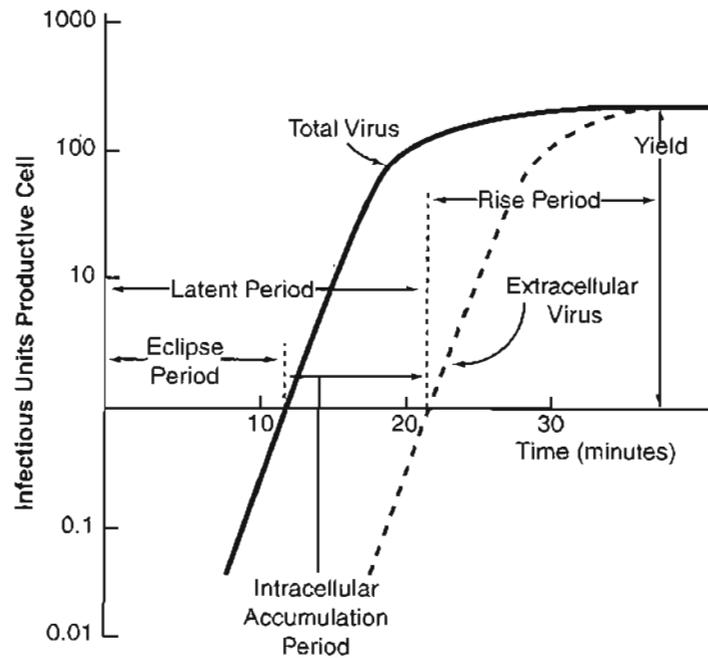


Figure I-5-5. Generalized Viral Replication Scheme



Internal virus is present after the end of the eclipse period.
 External virus is present after the end of the latent period.

Figure I-5-6. One Step Viral Growth Curve

IMPORTANT STEPS IN VIRAL REPLICATION

Spread

Viruses are spread basically by the same mechanisms (e.g., respiratory droplets or sexually) as other pathogens.

Arthropod-borne viruses are referred to as **arboviruses**.

Most belong to three formal taxonomic groups

- Togavirus encephalitis viruses (a.k.a. alphaviruses)
- Flavivirus
- Bunyavirus

Vectors

- Mosquitoes are most common vectors.
- Ticks, biting midges, and sandflies are less common.

Attachment

Viruses bind through specific interaction with the host cell surface components and

- Specific viral surface glycoproteins of enveloped viruses, or
- Specific viral surface proteins of naked viruses.

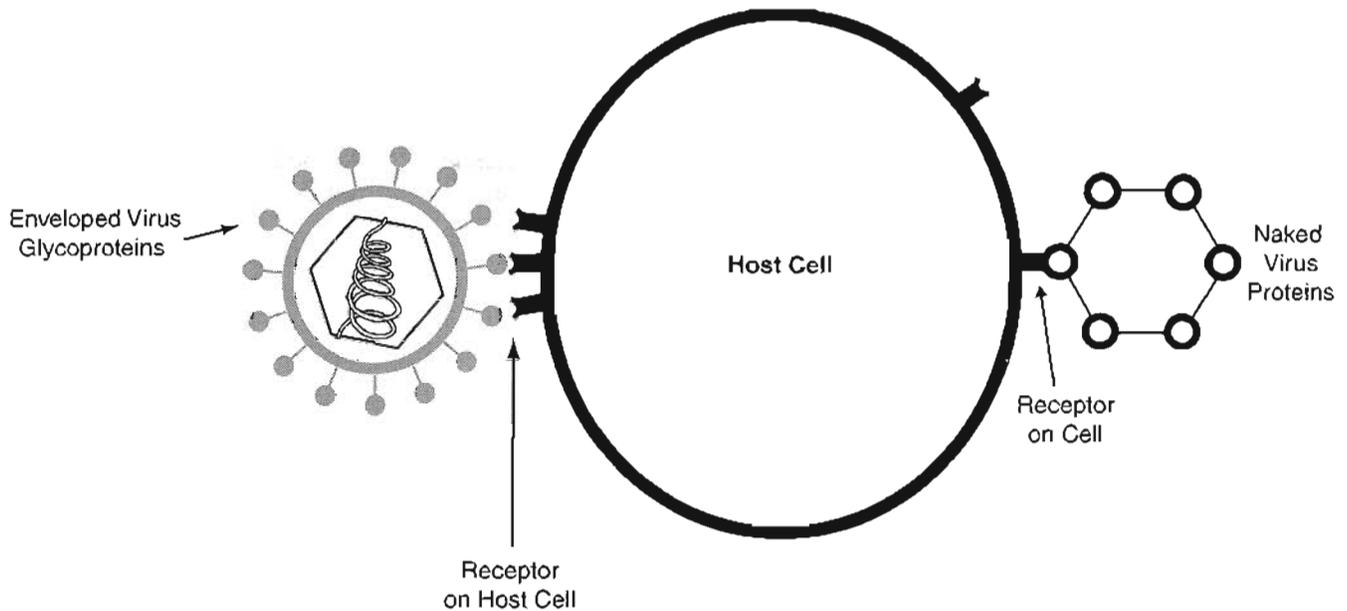


Figure I-5-7. Attachment

These interactions (and the distribution of the receptors) determine viral

1. Host range (e.g., horses or humans)
2. Tissue specificity (e.g., liver versus heart)

Table I-5-1. Specific Viral Receptors to Know

Virus	Target Cell	Receptor on Host Cell
HIV	Th cells, macrophages, microglia	CD4
EBV	B lymphocytes	CD21= CR2
Reovirus	Neurons	β -adrenergic receptor
Rabies	Neurons	Acetylcholine receptor

Table I-5-2. Difference Between Naked and Enveloped Viruses

	Naked	Enveloped
Inactivated by heat, detergents, and organic solvents like ether and alcohols?	No	Yes, since the lipid envelope holds the glycoproteins essential for attachment. Dissolving the envelope inhibits attachment and therefore uptake.
Immune response	Prominently antibody	Antibody and prominent cell-mediated immunity

Viral Entry Into Host Cell

Viral entry is by

- Receptor-mediated endocytosis
- Uptake via coated pits
- Or for those enveloped viruses with fusion proteins via fusion of the cell membrane with the viral envelope

Macromolecular Synthesis

How do the various viruses make their mRNA? mRNA production is diagrammed below.

- The major types of viral genomes are shown on the right.
- The replication intermediates necessary to make mRNA are shown in the gray area.

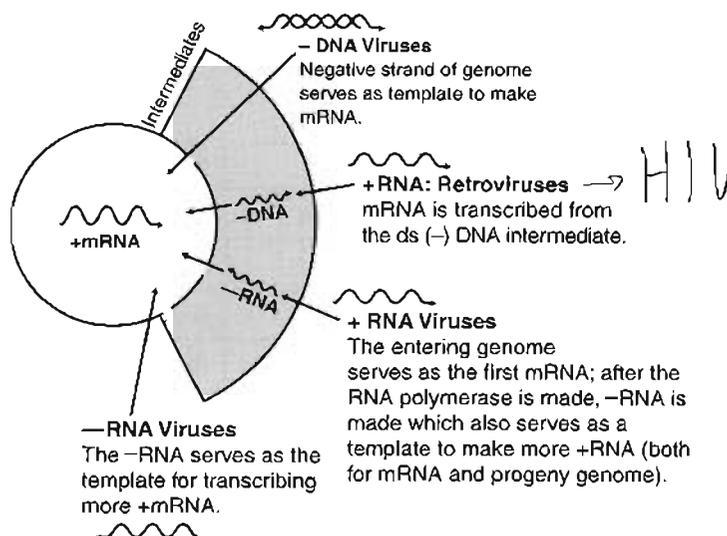


Figure I-5-8. Viral Messenger RNA

Replication of the Genomic Nucleic Acid (NA)

Progeny viruses have a nucleic acid sequence identical to the parent virus.

All single-stranded RNA viruses replicate through a replicative intermediate.

Going back to the Simplified Imaginary Teaching Viruses:

- If the parental genomic sequence is UUUUUUUUU, then the progeny must have the same sequence.
- (Poly AAA would make a polylysine capsid instead.)
- To make more poly UUU, a replicative intermediate of AAAAAAAAAA would be required.
- The replicative intermediate is used to make new poly UUU.

Table I-5-3. Strategy for Viral Genome Replication

Virus Type	Parental Genome	Intermediate Replicative Form	Progeny Genome
Most +ssRNA viruses	+ssRNA	-ssRNA	+ssRNA
Retroviruses	+ssRNA →	dsDNA	+ssRNA
-ssRNA viruses	-ssRNA	+ssRNA	-ssRNA
Most dsDNA viruses	dsDNA		dsDNA
Hepatitis B	dsDNA	ssRNA →	dsDNA

+ means an RNA which can serve as mRNA (or for the retroviruses has the same sequence.)

→ = RNA-dependent DNA polymerase

- Called reverse transcriptase for the retroviruses.
- Called the DNA polymerase for hepatitis B.
- Both actually make the first strand of the DNA using the RNA template and then breakdown the RNA and use the single strand of DNA as template to make the second strand.

Release of Viruses

Naked viruses lyse the host cells. Thus, there are no persistent productive infections with naked viruses (only cytolytic productive or latent infections).

Release of enveloped viruses: Budding leads to cell senescence (aging), but cells may produce a low level of virus for years as occurs in chronic hepatitis B.

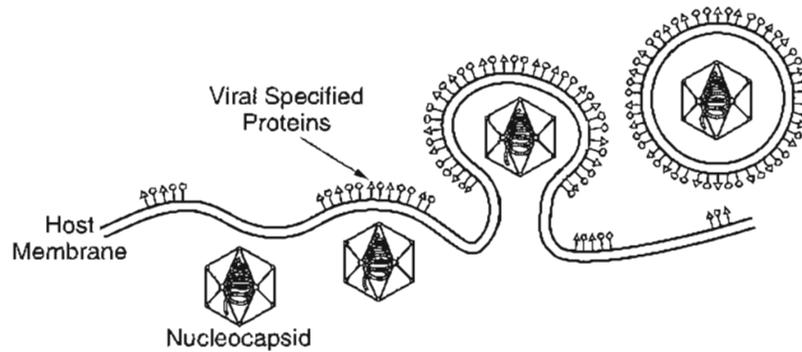


Figure I-5-9. Release of Enveloped Virus

The glycoproteins on the enveloped viral surface are essential for viral infectivity.

PATTERNS OF VIRAL INFECTIONS

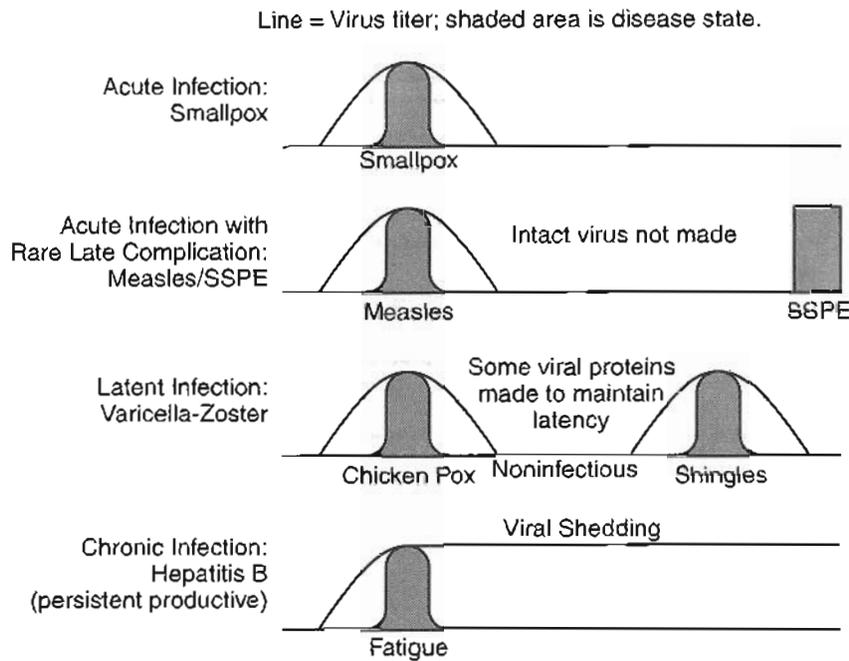


Figure I-5-10. Time Courses: Acute and Persistent Viral Infections

Table I-5-4. Cellular Effects

Infection Type	Virus Production	Fate of Cell
Abortive	-	No effect: No virus is made nor is latency established; Virus is terminated
Cytolytic Naked viruses lyse host cells. Some enveloped viruses also are cytolytic, killing the cell in the process of replication.	+	Lysis of the host cell (death)
Persistent Productive (enveloped viruses)	+	Senescence (premature aging)
Latent	-	No overt damage to host; no production of virus, but viral production may be turned on later.
Transforming	±	Immortalization

In Utero	At Birth	Infants	Children	Adolescents and Young Adults	Adults	Senior Citizens
Cytomegalovirus	→				→	
Rubella	→		Rubella	→		
HSV 2	→			HSV 2	→	
HIV	→			HIV	→	
	Hepatitis B			Hepatitis B	→	
	HSV 1					
		Respiratory Syncytial (bronchiolitis)				
		Parainfluenza (croup)				(colds)
		Rotavirus (infant diarrhea)				
		Influenza				
			Measles			
			Mumps			
			Hepatitis A			
			Epidemic Gastroenteritis (Norwalk virus)			
			Varicella (chicken pox)			Zoster
						St. Louis Encephalitis

Figure I-5-11.

HOST RESISTANCE TO VIRAL INFECTION

Primary Defenses

- Skin barrier (dead keratinized cells impervious to viruses)
- Skin has acids and other inhibitors produced by normal bacterial flora
- Mucociliary elevator

Immune Defense

Innate immune response

- Interferon
- Complement
- Natural killer cells

Adaptive immune response

- Antibody
- Lymphocyte-mediated response

Interferon Production

Interferons (IFNs) are a family of eukaryotic cell proteins classified according to the cell of origin. IFN-alpha and IFN-beta are produced by a variety of virally infected cells. They:

- Act on target cells to **inhibit viral replication**.
- Do not act directly on the virus.
- Are **not virus-specific**.
- Are **species-specific** (e.g., mouse IFN versus human IFN).

Interferon inhibits viral protein synthesis

- Through activation of an RNA endonuclease, which digests viral RNA.
- By activation (by phosphorylation) of protein kinase that inactivates eIF2 inhibiting viral protein synthesis.

Exogenous human IFN (produced by recombinant DNA technology) may be used in antiviral therapy for chronic, active HBV and HCV infections.

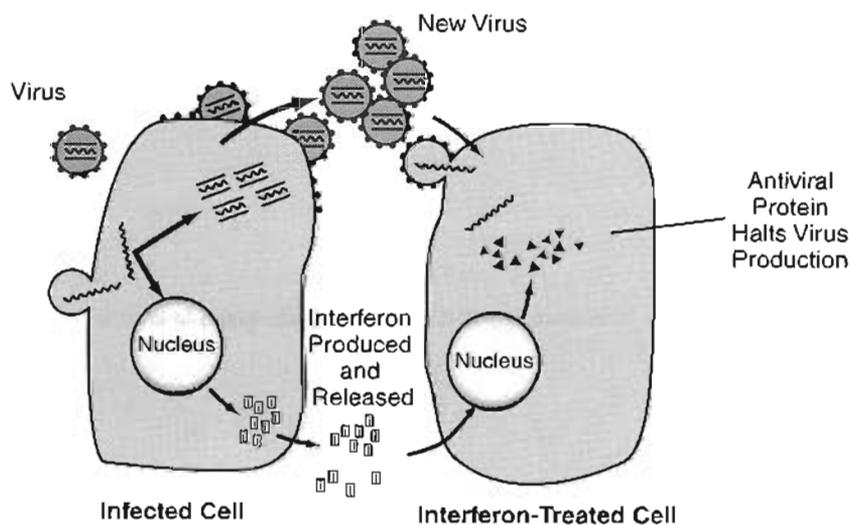


Figure I-5-12. Interferon Production

IMMUNOPROPHYLAXIS

Table I-5-5. Vaccines

	Attenuated (Live)	Killed	Component
Can it revert to a pathogenic form?	Possibly	No	No
Can it cause infections in immunocompromised hosts?	Sometimes	No	No
Immunogenicity?	High	Lower	Middle
Special storage?	Yes; viable organisms	No	No
Potential for contamination with other viruses?	Yes; high	Reduced	No

Active Immunization—Killed Vaccines

RIP-A (Rest In Peace Always—the killed viral vaccines):

- Rabies (killed human diploid cell vaccine) and immunoglobulins
- Influenza
- Polio (Salk)
- A Hepatitis

Active Immunization—Live Viral Vaccines

All but adenovirus are attenuated

(mnemonic: Mr. V.Z. Mapsy)

- Mumps
- Rubella
- Varicella - Zoster
- Measles
- Adenovirus (pathogenic [not attenuated] respiratory strains given in enteric coated capsules)
- Polio (Sabin)
- Small Pox
- Yellow Fever

Active Immunization—Component Vaccines

Hepatitis B

Passive Immunotherapy: Transfer of Immunoglobulins

- Hepatitis A
- Hepatitis B
- Measles
- Rabies
- Varicella-Zoster

VIRAL HEPATITIS

Symptoms of Hepatitis

Fever, malaise, headache, anorexia, vomiting, dark urine, jaundice.

Table I-5-6. Hepatitis Viruses (Hepatotropic)

Virus	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E
	“Infectious” (HAV)	“Serum” (HBV)	“Post transfusion Non A Non B” (HCV)	“Delta” (HDV)	“Enteric” (HEV)
Family Features	Picornavirus RNA Naked Capsid	Hepadnavirus DNA Enveloped	Flavivirus RNA Enveloped	Defective Circular RNA Enveloped	Calicivirus RNA Naked capsid
Transmission	Fecal-oral	Parenteral, sexual	Parenteral, sexual	Parenteral, sexual	Fecal-oral
Disease No chronic	Mild acute No chronic No sequelae	Acute is occasionally severe Chronic: 5–10% adults 90% infants 1' hepatocellular carcinoma, cirrhosis	Acute is usually subclinical 80% become chronic Primary hepatocellular carcinoma, cirrhosis	Co-infection with HBV: occasionally severe; Superinfection with HBV: often severe Cirrhosis, fulminant hepatitis	Normal patients mild Pregnant patients: severe No chronic
Mortality	<0.5%	1–2%	0.5–1%	High to very high	Normal patients 1–2%; pregnant patients: 20%
Diagnosis acute hepatitis-symptoms &:	IgM to HAV	HBsAg, IgM to HBcAg	Antibody to HCV ELISA	Hepatitis D Ab, HBsAg	Antibody to HEV ELISA

Remember that hepatitis also may occur in other viral diseases (e.g., CMV and EBV infections, congenital rubella, and yellow fever).

Hepatitis B (HBV) Terminology and Markers

Dane particle = infectious HBV

HBsAg

- Surface antigen
- Found during acute disease and persistent infections.
- Presence of HBsAg past 6 months indicates chronic infection.

HBsAb = Antibody to the surface antigen; provides immunity to HBV.

HBs window is the period between:

1. **the end of detection of surface antigen and**
2. **the beginning of the detection of surface antibody.**

The absence of these two markers is why the diagnostic test for core antibody is so important.

HBcAg = HBV core antigen

HBcAb = antibody to HBcAg:

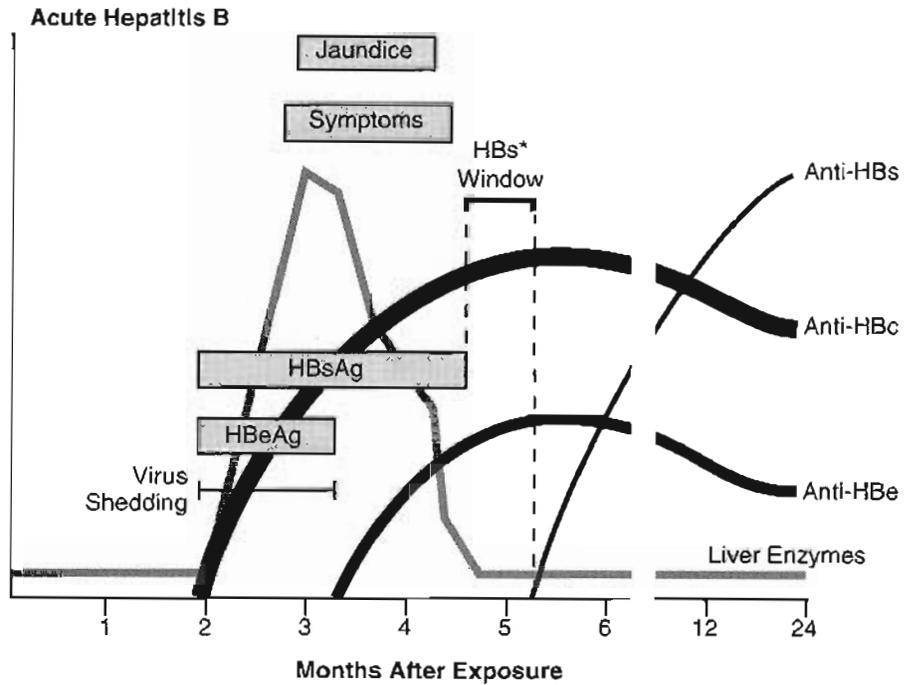
- First antibody to appear
- IgM antibody to core is important test in screening for recent infection.

HBeAg = a second antigenic determinant found in the core of the virus. Its presence correlates with:

- Active viral production
- Infectivity

HBeAb = antibody to HBeAg:

- Generally is detectable after virus is no longer detectable.
- Used to suggest lower risk of transmission.



*The window is the time between the disappearance of the HB surface antigen and before antibody to the surface antigen is detected.

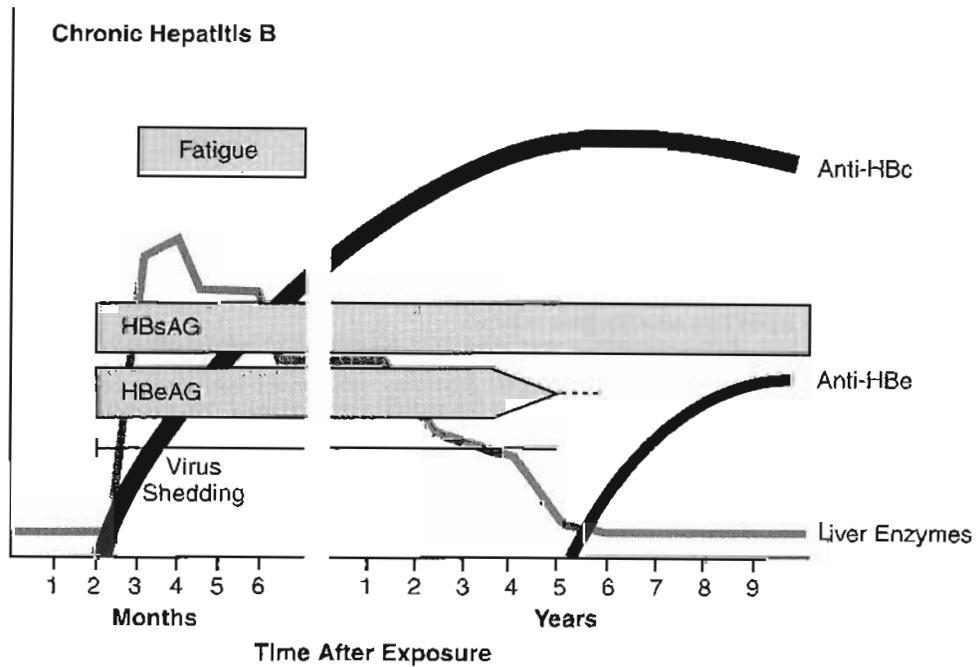


Figure I-5-13. Hepatitis B

DNA VIRUSES: CHARACTERISTICS

All DNA viruses:

- Are double-stranded, except parvovirus
- Are icosahedral, except poxviruses, which are brick-shaped “complex”
- Replicate their DNA in the nucleus, except poxvirus

Table I-5-7. DNA Viruses*

Virus Family	DNA type	Virion-Associated Polymerase	Envelope	DNA Replicates in:	Major Viruses
Parvovirus	ssDNA	No	Naked	Nucleus	B-19
Papovavirus	dsDNA circular	No	Naked	Nucleus	Papilloma Polyoma
Adenovirus	dsDNA linear	No	Naked	Nucleus	Adenoviruses
Herpes virus	dsDNA linear	No	Enveloped (nuclear)	Nucleus; virus assembled in nucleus	HSV Varicella-Zoster Epstein-Barr Cytomegalovirus
Poxvirus	dsDNA linear	Yes**	Enveloped	Cytoplasm	Variola Vaccinia Molluscum contagiosum
Hepadnavirus	partially dsDNA circular	Yes***	Enveloped	Nucleus, RNA intermediate	Hepatitis B

*Mnemonic: **Parva's Papa Adds Her Poxes to Hepa's**

** Poxviruses have a virion-associated transcriptase (DNA dependent RNA polymerase) so it can transcribe its own DNA in the cytoplasm and make all of the enzymes and factors necessary for replication of the poxvirus DNA in the cytoplasm.

*** Hepadnaviruses: DNA viruses that carry a DNA polymerase with reverse transcriptase activity to synthesize an RNA intermediate that is then used to make the genomic DNA. Hepatitis B is partially double-stranded with one complete strand.

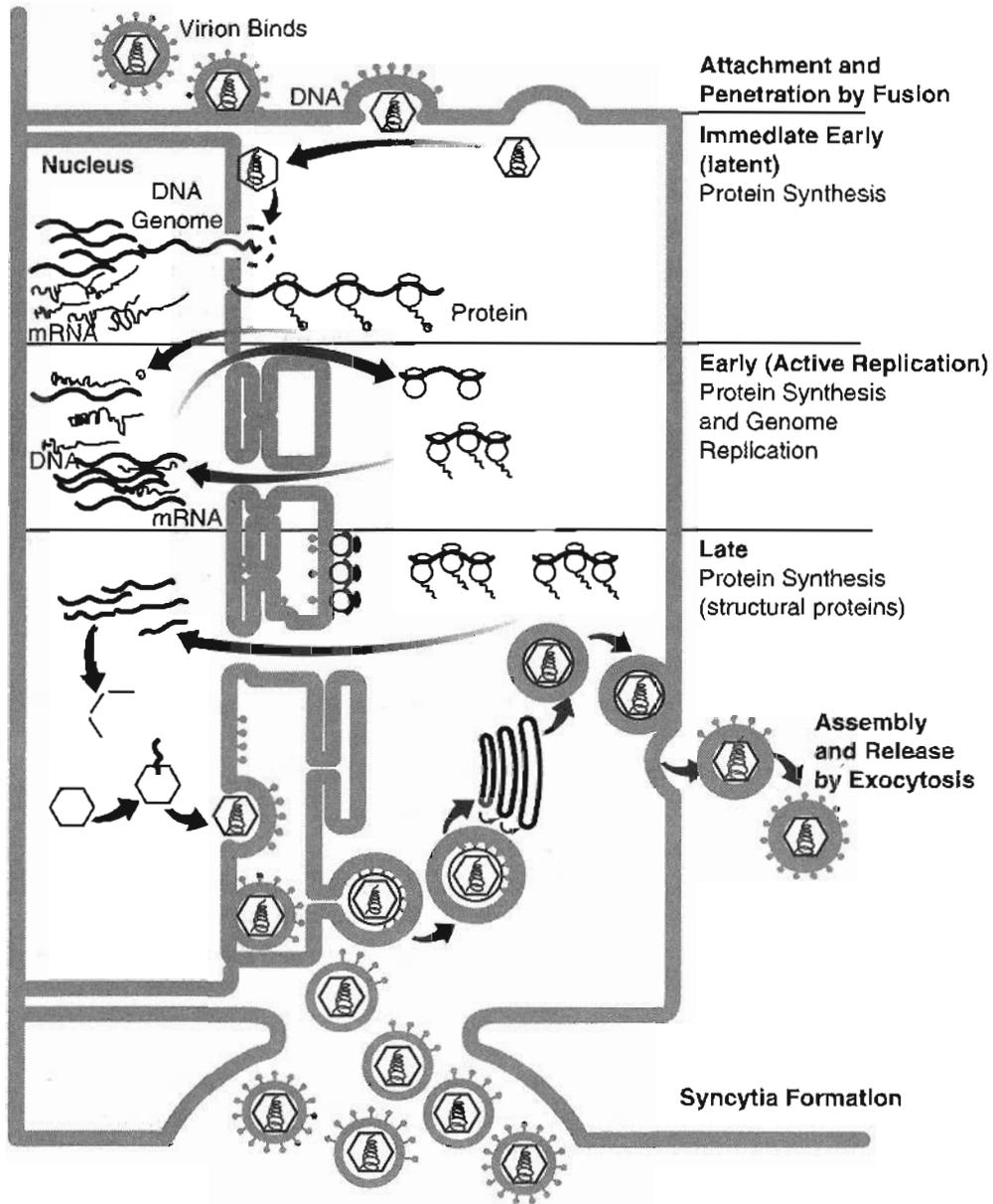


Figure I-5-14. DNA Virus: Life Cycle of Herpes

DNA VIRAL DISEASES

Parvoviridae

- ssDNA (linear)
- Naked icosahedral



Figure I-5-15. Parvovirus

B-19 Virus

- Fifth disease = erythema infectiosum = slapped cheek fever:
A mild, febrile disease with facial rash followed by lacy body rash
- Linked to aplastic crises in Sickle Cell Anemia patients.
B-19 infects only immature red cells causing lysis, hence the anemia, which is only clinically significant in Sickle Cell Disease.
- Potential cause of hydrops fetalis

Papovaviridae

- Circular dsDNA
- Naked icosahedral



Figure I-5-16. Papovavirus

Human Papilloma Virus

- Contact
- Plantar warts: HPV 1 & 4
- Anogenital (condyloma acuminatum) and laryngeal papillomas; HPV types 6 and 11 are most common (considered benign).
- Cervical intraepithelial neoplasia (HPV 98%)
HPV 16 and 18 are most common.
U.S.A. deaths 4,000–5,000/year
- Pathological findings: koilocytic cells on Pap smear (perinuclear cytoplasmic vacuolization and nuclear enlargement)
- Some strains of HPV associated with cancer; these have two genes which inactivate tumor suppressor genes.

Polyoma Viruses

- BK virus: in immunocompromised hosts, causes renal disease
- JC virus: causes progressive multifocal leukoencephalopathy (slow conventional virus).

Adenoviruses

- dsDNA, naked
- Hexons, penton bases, and fibers
- Fibers bind host cell receptors (will bind to most human cells and many animal cells) and also act as hemagglutinins. Fibers, when purified, are toxic to human cells.

Adenovirus Diseases Include:

- Upper respiratory disease in kids.
- Pharyngoconjunctivitis (“pink eye” or “swimming pool” conjunctivitis), non-purulent (unlike *Haemophilus aegyptius*).

- Epidemic keratoconjunctivitis (“shipyard” conjunctivitis: patients having foreign particles removed acquired these viruses from equipment used on the eyes).
- Acute respiratory disease (ARD) and pneumonia:
 - Major problem in young military recruits
 - Serotypes 4, 7, and 21.
 - Three separate vaccines are used by the U.S. military
 - live non-attenuated
 - serotypes 4, 7, and 21
 - administered separately by enteric-coated capsules
- Adenovirus 40 and 41 cause infantile diarrhea.
- Adenovirus serotypes 12, 18, and 31 are common in *normal* human feces and are not known to cause human disease. These same strains cause malignant transformation of hamster cells. The adenoviruses are the standard examples of a **permissive host** (where virus is produced) and **nonpermissive host** (where the virus is not produced but transforms the cells).

Herpesviridae

Enveloped icosahedral nucleocapsids with dsDNA

DNA is synthesized in the nucleus.

Can enter the **latent state** in the host:

- HSV in neurons
- EBV in B lymphocytes

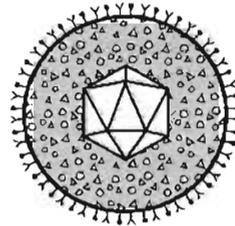


Figure I-5-17. Herpesvirus

Herpes viruses synthesize their own enzymes to synthesize DNA.

Acyclovir (the prodrug form of a nucleoside analog) is effective only in Herpes virus–infected cells because the herpes thymidine kinase is required to activate it, then it inhibits only the herpes polymerase, leaving the host polymerase functioning. No current drug can remove the latent DNA.

Herpes **envelope is from the host nuclear membrane** (virus is assembled in the nucleus).

Herpes form **intranuclear inclusion bodies**.

Herpes produce **distinctive cytopathology**.

Herpes Simplex Type 1 (HSV-1)

- Gingivostomatitis and recurrent cold sores (skin or lip);
latent in trigeminal root ganglion
- Keratoconjunctivitis generally with lid swelling and vesicles; dendritic ulcers may be seen; untreated repeated attacks may result in visual impairment.
- Meningoencephalitis
 - Characterized by: fever, headache, and confusion (focal temporal lesions; perivascular cuffing)
 - Diagnosis/treatment STAT: PCR on CSF; RBCs in CSF; acyclovir: early diagnosis reduces mortality

Herpes Simplex Type II (HSV-2)

- Meningitis: mild self-limiting disease
- Herpes genital infections: painful vesicular lesions of genitals and anal area; **latent in sacral nerve ganglia**
- Neonatal herpes may be one of three presentations:
 1. Disseminated with liver involvement; high mortality;
 2. Encephalitis; high mortality;
 3. Skin, eyes, or mouth.
- Diagnosis:
 - PCR (CSF for encephalitis)
 - Viral culture with fluorescent antibody stain to identify virus
- The Tzanck smear (Giemsa stain to show multinucleated giant cells) has been largely replaced by immunofluorescent staining, which can distinguish HSV-1 from HSV-2.

Varicella-Zoster

- Chickenpox
 - Asynchronous rash
 - **Latent in dorsal root ganglia** → shingles in adults (severe nerve pain)
- Associated with Reye's syndrome
- Disseminated infections in immunocompromised hosts
- Attenuated vaccine
- Passive transfer of immunity with Varicella-Zoster immunoglobulin

Epstein-Barr Virus (EBV)

- Selectively **infects B cells binding to CD21 = CR2**
- Many inapparent infections; common, worldwide 90% of the population seropositive
- Infectious mononucleosis, "Kissing disease"
 - **Heterophile positive mononucleosis**, fatigue, fever, sore throat, lymphadenopathy, and splenomegaly
 - Atypical reactive T lymphocytes (Downey Type II cells) may become as high as 70% WBC.
 - Positive for heterophile antibodies that cross-react with Paul-Bunnell antigen on sheep and bovine RBC (only mono that is heterophile antibody positive).
 - Antigens produced by productive cells:
 - EA = early antigen
 - VCA = viral capsid antigen used in diagnostic tests
 - EBNA = Epstein-Barr nuclear antigen
 - MA = membrane antigen
- Burkitt lymphoma, nasopharyngeal cancer, thymus carcinoma: EBNA found in all transformed B lymphoid cells.

Cytomegalovirus (CMV)

- Herpesviridae infecting fibroblasts; common 80% worldwide.
- Owl's eyes = CMV "Sightomegalovirus" basophilic intranuclear inclusion bodies with smaller eosinophilic cytoplasmic inclusion bodies.

Table I-5-8. Acquisition Routes for CMV

Transmission	Patient	Resulting Disease
In utero (most common in utero infection)	Fetuses	Ranges from infected but no obvious defects to severe cytomegalic inclusion disease (jaundice, hepatosplenomegaly, thrombocytic purpura, pneumonitis, and CNS damage [periventricular calcifications] to death)
Birth process, milk	Babies	Serious disease uncommon, heterophile negative mononucleosis
Sex, transfusions	Healthy adults	Serious disease uncommon, heterophile negative mononucleosis
Reactivation in transplanted organ	Transplant patients	Interstitial pneumonitis and systemic disease
Reactivation or new acquisition	<u>AIDS patients</u>	CMV retinitis, pneumonitis, and systemic disease

Human Herpesvirus 6

Exanthem subitum (Roseola): common infant disease; fever followed by rash.

Human Herpesvirus 8

Probable cofactor in Kaposi sarcoma

Poxviridae

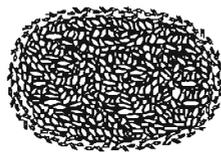


Figure I-5-18. Poxvirus

Complex morphology: Brick-shaped large viruses with ds linear DNA

Virus-coded lipid on surface

Virion-associated DNA-dependent RNA polymerase allows replication in cytoplasm.

Variola

- Small pox
- Extinct in 1977
- Live attenuated Vaccinia vaccine
- Guarnieri bodies found in infected cells (intracytoplasmic)

Vaccinia

Immunogen in smallpox vaccine

Molluscum contagiosum

- Small pink benign wart-like tumors
- Molluscum bodies in central caseous material: oval, eosinophilic cytoplasmic inclusion bodies
- Problem in some immunocompromised hosts

Hepadnaviridae

Hepatitis B virus: see earlier notes.

RNA VIRUSES

Generalizations

All RNA viruses are single stranded (ss) except reovirus.

ss (-) RNA viruses carry an RNA-dependent RNA polymerase.

A virion-associated polymerase is also carried by

- Reovirus
- Arenavirus
- Retrovirus (reverse transcriptase)

Most are enveloped; only **naked** ones are

- Picornavirus
- Calicivirus
- Reovirus

Some are **segmented** (different genes on different pieces of RNA)

- Reovirus
- Orthomyxovirus
- Bunyavirus
- Arenavirus
(ROBA sounds like robot pieces)

Table I-5-9. Positive-Sense RNA Viruses*

Virus Family	RNA Structure	Virion-Associated Polymerase	Envelope	Shape	Multiplies in	Major Viruses
Calicivirus	ss(+)RNA Linear Non-segmented	No polymerase	Naked	Icosahedral	Cytoplasm	Norwalk agent Hepatitis E
Picornavirus	ss(+)RNA Linear Non-segmented	No polymerase	Naked	Icosahedral	Cytoplasm	Polio** ECHO Enteroviruses Rhino Coxsackie Hepatitis A
Flavivirus	ss(+)RNA Linear Non-segmented	No polymerase	Enveloped	Icosahedral	Cytoplasm	Yellow fever Dengue St. Louis encephalitis Hepatitis C
Togavirus	ss(+)RNA Linear Non-segmented	No polymerase	Enveloped	Icosahedral	Cytoplasm	Rubella WEE, EEE Venezuelan encephalitis
Coronavirus	ss(+)RNA Linear Non-segmented	No polymerase	Enveloped	Helical	Cytoplasm	Corona-viruses
Retrovirus-	Diploid ss(+)RNA Linear Non-segmented	RNA dep. DNA polymerase	Enveloped	Icosahedral or truncated conical	Nucleus	HTV HTLV Sarcoma

*Mnemonic: (+) RNA Viruses: Call Pico and Flo To Come Rightaway

**Mnemonic: Picornaviruses PEE Co Rn A Viruses

Polio, Entero, Echo, Coxsackie, Rhino, Hep A

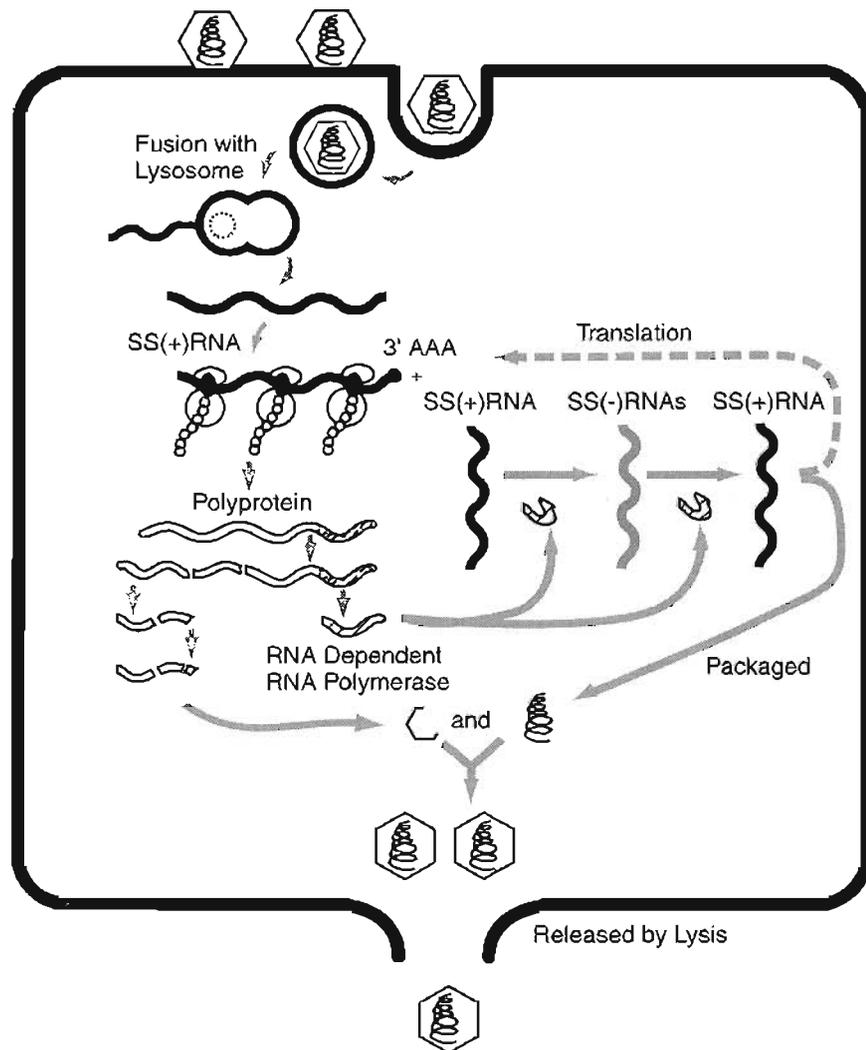


Figure I-5-19. Positive Sense RNA Virus Life Cycle

Abbreviations:

- H = Hemagglutinin—surface glycoproteins that bind to sialic acid (N-acetylneuraminic acid) receptors
- N = Neuraminidase—clips off sialic acids, thus aiding in release of virus
- M = Matrix protein—membrane stabilizing protein underlying the viral envelope
- F = Fusion protein—destabilizes host membrane
- P = Polymerase associated with virion

Caliciviridae

Small (slightly larger than picornaviridae) but similar to picornaviridae

Norwalk Agent

- Described as calicivirus-like.
- Naked icosahedral
- Epidemic viral gastroenteritis in school-age kids and adults

Hepatitis E

See hepatitis notes.

Picornaviridae

- Small ss (+) RNA viruses
- Naked



Figure I-5-20. Picornavirus

Enteroviruses (group)

- Summer-fall peak incidence
- Fecal-oral transmission but do not cause diarrhea
- Peak age group <9 years for most
- Stable at pH3
- Resistant to alcohol, detergents because there is no envelope

Polio Virus

- Most infections are asymptomatic; small % cause fever (viremia).
- Smaller % cause aseptic meningitis.
- Poliomyelitis (paralysis) (even smaller %) results from viral damage to **anterior horn motor neurons**.
- **Vaccines:** both are trivalent
 - Sabin (live/oral/best gut immunity)
 - Salk (killed/injectable)

Coxsackie A

- Herpangina (vesicles on soft palate and fauces)
- Hand-foot-and-mouth disease (oral lesions primarily in the anterior buccal mucosa)
- Aseptic meningitis
- Acute lymphoglandular pharyngitis
- Common cold (aachoo's)

Coxsackie B

- Bornholm's disease (a.k.a., pleurodynia or Devil's grip; severe intercostal pain, fever)
- Aseptic meningitis
- Severe systemic illness of newborns
- Possible link to acute-onset, insulin-dependent diabetes in young children
- Myocarditis

Hepatitis A Virus

- ss (+) RNA
- Infectious hepatitis
- Inactivated vaccine
- Hyperimmune serum for post-exposure prophylaxis

Echoviruses and Most Enteroviruses: Aseptic meningitis

Rhinoviruses

- The common cold
- Not stable under acidic conditions
- Peaks summer and fall

Flaviviridae

ss (+) RNA icosahedral capsid with envelope

Yellow Fever Virus

- Mosquito-borne (Aedes)
- Liver, kidney, heart, and gastrointestinal mucosa damage
- Attenuated vaccine

St. Louis Encephalitis Virus

- Mosquito-borne (summer)
- Elderly (especially blacks or individuals with hypertension), most likely to have severe disease

Dengue Virus

- Dengue hemorrhagic shock syndrome in previously infected children who are reinfected. Immune enhancement of entry into macrophages.
- Dengue (bonebreak disease): mild disease with rash and joint or muscle pain
- Mosquito-borne (Aedes)

Hepatitis C

Discussed with hepatitis viruses.

Togaviridae

- ss (+) RNA viruses
- H, no P



Figure I-5-21. Togavirus

Alpha Viruses (group)

Equine Encephalitis Viruses: Western, Eastern, and Venezuelan

- All mosquito-borne
- Wild birds are reservoirs.
- Horses are also hosts.

Rubella

- Crosses placenta and is teratogenic.
- Most serious during first 16 weeks gestation
- Congenital rubella = patent ductus arteriosus, pulmonary stenosis, cataracts, microcephaly, deafness
- Attenuated vaccine; single strain—part of MMR

Coronaviridae

- ss (+) RNA, enveloped helical virus
- The large surface glycoprotein spikes give a crown appearance.
- Second most common cause of common cold (peak winter and early spring)

Retroviridae

Diploid ss (+) RNA viruses

Virion-associated reverse transcriptase

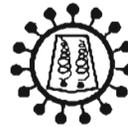


Figure I-5-22. Retrovirus

Oncovirus Group

Human T-cell Leukemia/Lymphotropic (HTLV)

- Adult T-cell leukemia
- C-type particle (most oncoviruses, centrally located electron dense nucleocapsid)
- Japan, Caribbean, southern U.S.

Lentivirus Group

Human Immunodeficiency Virus (HIV); acquired immunodeficiency syndrome (see next section)

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Structure and Genes of HIV

Positive sense (ss) RNA virus, diploid, non-segmented

Lentivirus in the retrovirus family (not oncogenic)

The HIV virion contains

- Enveloped truncated conical capsid (type D retrovirus)
- Two copies of the ss (+) RNA
- RNA-dependent DNA polymerase (reverse transcriptase)
- Integrase
- Protease

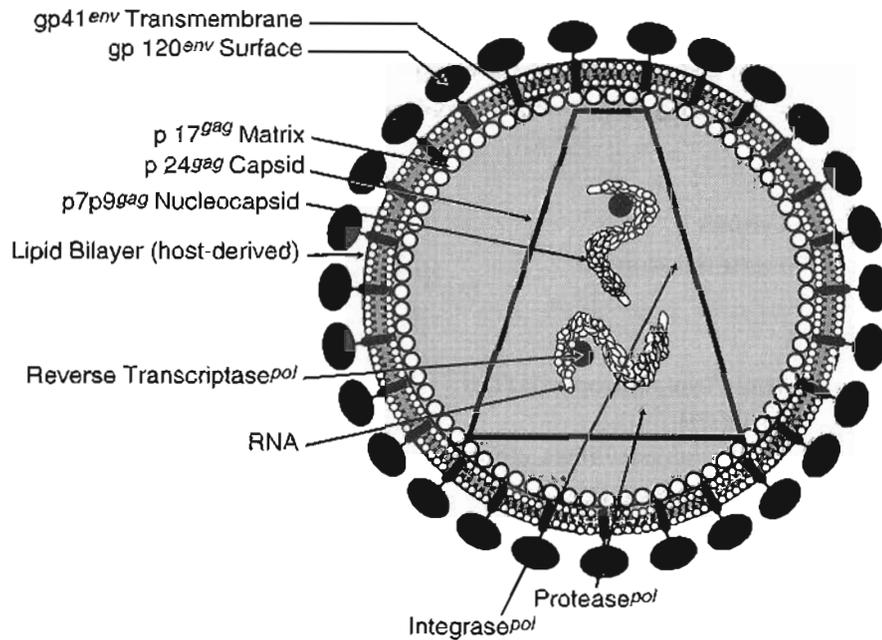


Figure I-5-23. Structure and Genes of HIV

Table I-5-10. Important HIV Genes and Their Functions

Gene	Product(s)	Function
Structural Genes		
Gag	Group specific antigens p24 p7p9 p17	Structural proteins capsid protein core nucleocapsid proteins matrix proteins (stabilizes envelope)
Pol	Reverse transcriptase Integrase Protease	Produces dsDNA provirus Proviral dsDNA integration into host DNA Cleaves polyprotein
Env	gp120 gp41	Surface protein that binds to CD4 on host cell responsible for tropism; genetic drift transmembrane protein for cell fusion
Regulatory Genes		
LTR (U3 U5)	DNA long terminal repeats	Integration and virus gene expression
Tat	Transactivator proteins	Transactivator of transcription; (upregulation); spliced gene
Rev	Regulator virion proteins	Upregulates transport of unspliced and spliced transcripts to the cell cytoplasm; a spliced gene
Nef	Negative factor*	Multiple functions, one of which is to decrease MHCI on infected T cells

*Initially thought to downregulate HIV production; now thought to enhance the pathogenicity of HIV.

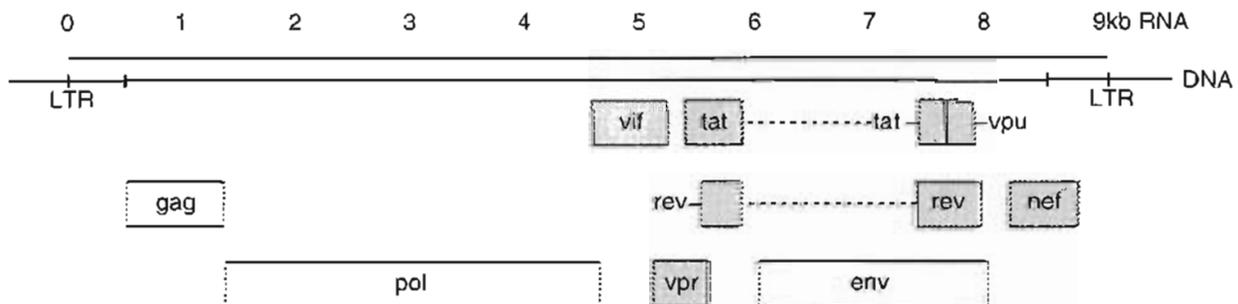
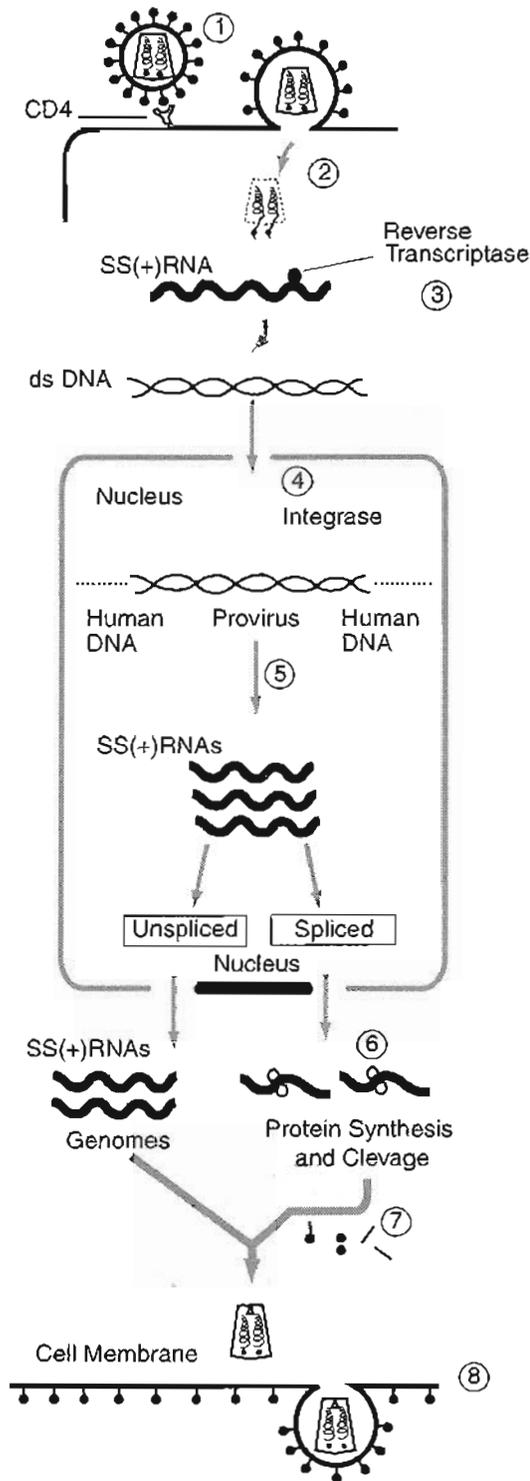


Figure I-5-24. Genetic Map of HIV



1. Surface gp120 of HIV binds to CD4 of T helper cells, macrophages, and microglia.
2. HIV is taken into the cell, losing the envelope; the RNA is uncoated.
3. The RNA is copied using the virion-associated reverse transcriptase; ultimately dsDNA with long terminal repeats is made.

4. The DNA and integrase migrate to nucleus and the DNA is integrated into host DNA forming the provirus.

The provirus remains in the host DNA.

The rate of viral replication is regulated by the activity of the regulatory proteins (tat/rev, nef, etc).

Tat upregulates transcription.

Rev regulates transport of RNAs to cytoplasm.

Co-infections (e.g., mycobacterial) stimulate the HIV-infected cells to produce more virus.

5. Transcription produces ss (+) RNAs, some spliced and some remain intact.
 - Spliced RNAs will be used as mRNA.
 - Whole RNA is used as genomic RNA.
6. Translation produces the proteins some of which are polyproteins that are cleaved by the HIV protease.
7. Assembly
8. Maturation/release of virus

Figure I-5-25. Retrovirus Life Cycle: HIV

HIV INFECTION

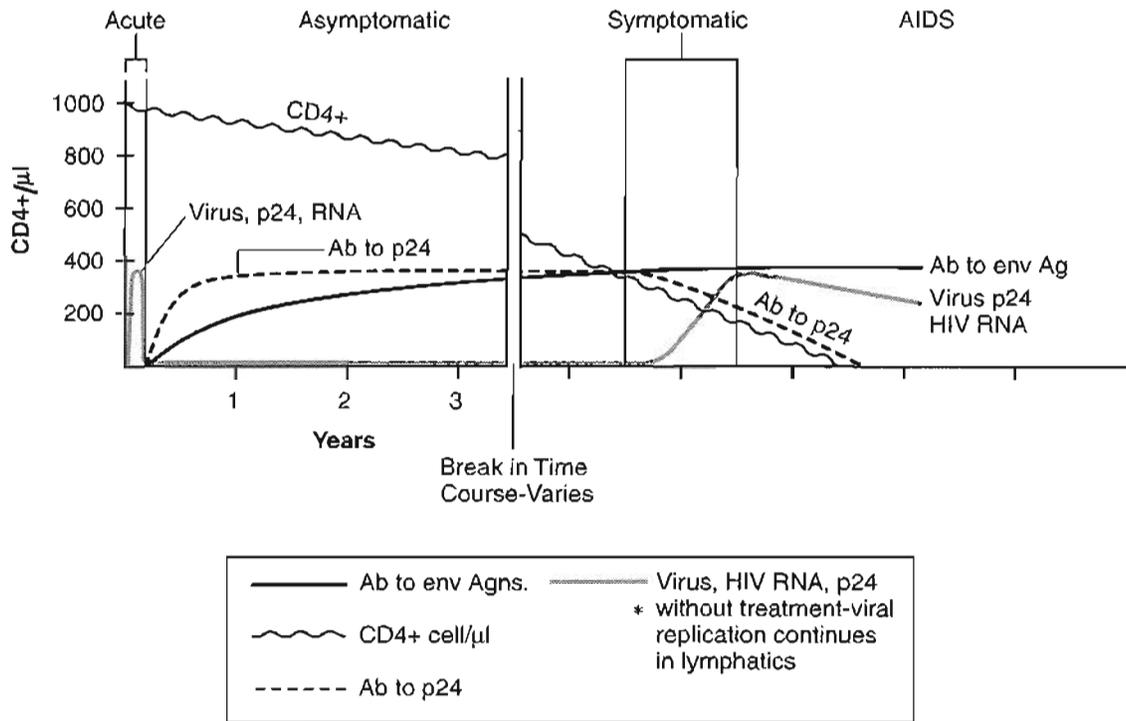


Figure I-5-26. Clinical Stages of HIV Infection

Table I-5-11. CDC Categories*

	Category A	Category B	Category C
CD4+ T cells/μL	(excludes conditions in B & C) Acute (primary) or asymptomatic HIV infection Persistent generalized lymphadenopathy	Symptomatic but not conditions in C Condition attributed to HIV infection (list below) or are indicative of a defect in cell-mediated immunity	AIDS defining conditions (See following list)
>500	A1	B1	C1
200-499	A2	B2	C2
<200	A3	B3	C3

AIDS = A3, B3, or C1-3

Acute phase has high level of viral production and mononucleosis-like symptoms: fever, sore throat, rash, malaise, lymphadenopathy, diarrhea, etc.

*1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS among Adolescents and Adults. MMWR December 18, 1992/41(RR-17)

CDC Category B (Symptomatic)

- Bacillary angiomatosis (disseminated Bartonellosis)
- Candidiasis (oral or persistent vulvovaginal)
- Cervical dysplasia or carcinoma *in situ*
- Constitutional sx (fever 38.5°C or diarrhea lasting >1 month)
- Hairy leukoplakia
- Idiopathic thrombocytopenic purpura
- Listeriosis
- Pelvic inflammatory disease (especially with abscess)
- Peripheral neuropathy

AIDS Defining Conditions (C)

- Encephalopathy, HIV-related
- Pneumonia, recurrent (leading cause of death)

Fungal Infections

- Candidiasis of esophagus, bronchi, trachea, or lungs
- Coccidioidomycosis, disseminated, or extrapulmonary
- Cryptococcosis, extrapulmonary
- Histoplasmosis, disseminated, or extrapulmonary
- *Pneumocystis carinii* pneumonia

Carcinomas

- Invasive cervical
- Kaposi's sarcoma; Burkitt's, immunoblastic, or primary CNS lymphoma

Viral Infections

- Cytomegalovirus retinitis (with loss of vision) or disease (other than liver, spleen, or nodes)
- Herpes simplex: chronic ulcer(s) (>1 month); or bronchitis, pneumonitis, or esophagitis
- Progressive multifocal leukoencephalopathy
- Wasting syndrome due to HIV

Parasitic Infections

- Cryptosporidiosis, chronic intestinal (>1 month)
- Isosporiasis, chronic intestinal (>1 month)
- Toxoplasmosis of brain

Bacterial Infections

- *Mycobacterium tuberculosis*, any site (pulmonary or extrapulmonary)
- *Mycobacterium avium* complex or *M. kansasii* or other species or unidentified species, disseminated or extrapulmonary
- *Salmonella* septicemia, recurrent

Diagnosis of HIV Infection

Screening

ELISA (most commonly done) to detect HIV antibodies in patient's serum. (Most tests include the antigens p24, p17, gp160, gp120, and gp41.)

- Anti-p24 is the first reliably detected antibody but declines as viral titers rise in late infection.
- Envelope antibodies rise more slowly but stay high at end. (Env antigens show major antigenic variation.)
- ELISA for p24 antigen useful early.

Confirmation (Using a Second Blood Sample)

- Western blot for antibodies specific for HIV (electrophoretically separated HIV antigens react with the patient's antibody; detection by enzyme-labeled anti-human IgG) or
- Immunofluorescence

HIV DNA PCR

- Qualitative to detect HIV infection in newborns of mothers are HIV+
- Quantitative HIV DNA PCR to determine viral load to assess treatment

Culture for HIV (with Antigen Detection in Culture)

- HIV infection in newborns whose mothers are HIV positive
- To assess drug resistance

Table I-5-12. HIV's Mechanisms of Immunologic Evasion

Characteristic	Function
Multiplication in lymphocytes and macrophages	Eliminates cell-mediated and antibody-mediated immunity
Nef and tat gene products down-regulate class I MHC expression.	Makes infected cells less susceptible to cytotoxic T-cell killing
Destruction of CD4 T cells	Elimination of immune enhancement response
Antigenic drift of the gp120 Heavy glycosylation of gp120	Evade antibody-mediated effector mechanisms

Table I-5-13. Negative Sense RNA Virus

Virus	RNA Structure	Virion-Associated Polymerase	Envelope	Shape	Multiplies in	Major Viruses
Paramyxovirus	ss(-) RNA Linear Non-segmented	Yes	Yes	Helical	Cytoplasm	Mumps Measles Respiratory syncytial Parainfluenza
Rhabdovirus	ss(-) RNA Linear Non-segmented	Yes	Yes	Bullet-shaped helical	Cytoplasm	Rabies Vesicular stomatitis
Filovirus	ss(-) RNA Linear Non-segmented	Yes	Yes	Helical	Cytoplasm	Marburg Ebola
Orthomyxovirus	ss(-) RNA Linear 8 segmented	Yes	Yes	Helical	Cytoplasm & nucleus	Influenza
Bunyavirus	ss(-) RNA Linear → Circular 3 segments Ambisense	Yes	Yes	Helical	Cytoplasm	California encephalitis La Crosse encephalitis Hantavirus
Arenavirus	ss(-) RNA Circular 2 segments 1 (-) sense 1 ambisense	Yes	Yes	Helical	Cytoplasm	Lymphocytic chorio-meningitis Lassa fever

Mnemonic for the ss(-) RNA viruses: Pairing Rats Fight Over Bunny's Area or Pain Results From Qur Bunions Always.

You can remember these are the negative ones since fighting is a negative thing to do.

Note that all are enveloped, all have virion-associated polymerase, and all have helical nucleocapsids.

The oddballs are the last three:

The orthomyxoviruses are linear (ortho) but with eight (ortho/octo) segments, which is one of the reasons they can genetically "mix" it up. The orthomyxoviruses are also odd in that they replicate in both the nucleus and cytoplasm. The bunyaviruses are somewhat contortionists (circular): California playboy bunnies in a ménage à trois?

The arenaviruses have one negative sense and one ambisense strand of RNA.

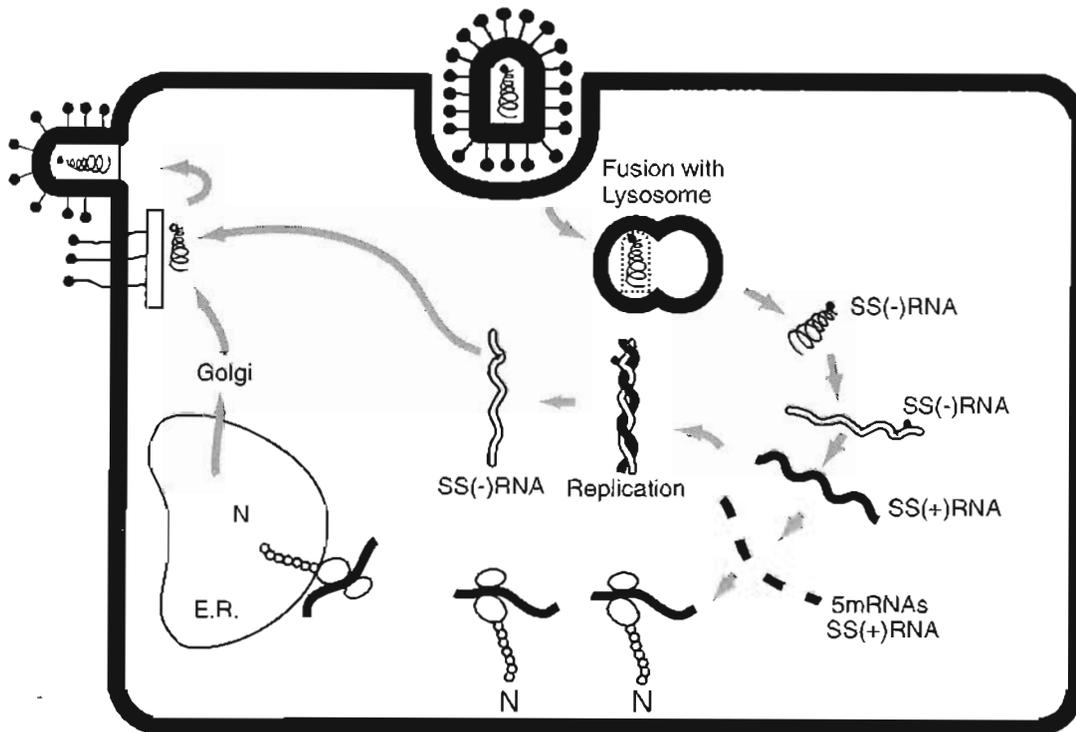


Figure I-5-27. Negative Sense RNA Virus Life Cycle

NEGATIVE SENSE RNA VIRUSES – DISEASES

Paramyxoviridae

- ss(-) RNA strand
- Enveloped helical nucleocapsids

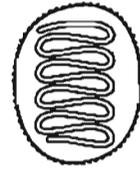


Figure I-5-28. Paramyxovirus

Parainfluenza Virus

- Single HN glycoprotein, also fusion protein (F)
- Croup (laryngotracheobronchitis)
- Common cold, bronchitis

Mumps

- ss(-) RNA
- Single HN glycoprotein, also F protein
- Live vaccine
- Parotitis
- Pancreatitis
- Orchitis in adult males
- Meningoencephalitis

Measles (Rubeola)

- ss(-) RNA
- H glycoprotein and fusion protein, no neuraminidase
- Measles:
 - Presentation generally the three C's with photophobia: Cough, coryza, and conjunctivitis
 - Koplik spots → maculopapular rash from the ears down → Giant cell
 - Pneumonia (Warthin-Finkeldy cells)
 - Rare complication: subacute sclerosing panencephalitis (chronic CNS degeneration)
- Live vaccine (single strain)

Respiratory Syncytial Virus

- ss(-) RNA
- No H nor N glycoproteins; only F protein
- Major cause of bronchiolitis and pneumonia in infants
- Common cold

Rhabdoviridae

- ss(-) RNA
- Bullet shaped
- Enveloped

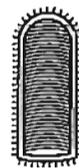


Figure I-5-29. Rhabdovirus

Rabies Virus

- Negri bodies—intracytoplasmic inclusion bodies
- Inactivated vaccines; passive immunization
- Spread to humans by bites of rabid dogs; contact with bats
- Eastern U.S. reservoirs: foxes & raccoons; western U.S.: skunks

Vesicular Stomatitis Virus

Foot and mouth disease

Filoviridae

- ss(-) RNA
- Helical capsid with envelope
- Virion-associated RNA-dependent RNA polymerase

Marburg Virus

Acute hemorrhagic fever, frequently fatal

Ebola Virus

Acute hemorrhagic fever, frequently fatal

Orthomyxoviridae

Influenza



Figure I-5-30. Orthomyxovirus

- ss(-) RNA
- Segmented (8)
- Enveloped nucleocapsids
- Separate H and N glycoproteins
- Can lead to Guillain-Barré (A or B) or Reye's syndrome (primarily B)
- Inactivated vaccine, H1N1 and H3N2
- Influenza A & B (and many other viruses, most notably HIV) undergo **genetic drift** = slight changes in antigenicity due to mutations (in influenza responsible for epidemics)
- Influenza A has rare **genetic shift** (genetic reassortment) - major changes from new combinations of RNA segments or recombination between the segments in co-infections causing new pandemics

Bunyaviridae

- Segmented single (-) RNA
- Enveloped helical capsids (circularize) ambisense
- California group

California Encephalitis and LaCrosse Encephalitis Viruses

- Mosquito-borne
- Young (<15 years) have more severe cases

Hanta Virus (Sin Nombre)

- Hanta virus pulmonary syndrome (cough, myalgia, dyspnea, tachycardia, pulmonary edema and effusion, and hypotension [mortality 50%])
- High endemic area: Four Corners region (UT, AZ, NM, CO), but all of North America
- Associated with rodent feces

Arenaviridae

- Segmented RNA (2 pieces)
- ss(-) RNA, ss (ambisense) RNA
- Enveloped
- Helical

Lymphocytic Choriomeningitis Virus

- Influenza-like with meningeal signs
- Imported from South America

Lassa Fever Virus

- West Africa
- Hemorrhagic fever
- 50% fatal

DOUBLE-STRANDED RNA VIRUS–DISEASE

Table I-5-14. Double-Stranded RNA Viruses

	RNA Structure	Virion-Associated Polymerase	Envelope	Shape	Major Viruses
Reovirus	Linear, dsRNA 10–11 segments	Yes	Naked	Icosahedral double shelled	Reovirus Rotavirus

Reoviridae

Reoviruses

Upper respiratory tract infections

Rotaviruses

- GI tract infection especially <2 years old, a prolonged diarrhea
- Major cause of infant mortality worldwide



Figure I-5-31. Reovirus

ONCOGENIC VIRUSES

Definitions

Malignant Transformation of Cells

- Dedifferentiation
- Loss of growth control
- Immortalization
- Appearance of new surface antigens (“T” antigens)

Provirus

Viral DNA inserted into host DNA

Oncogenes

Genes with the potential to cause malignant transformation

Cellular Oncogenes (abbreviated c-onc)

These are normal cellular genes whose products control regulation of cell growth and division (e.g., kinases, growth factors and their receptors, G proteins and nuclear regulatory proteins).

Viral Oncogenes (abbreviated v-onc)

Genes carried by certain viruses causing cancer. Viral oncogenes are homologs of cellular oncogenes.

Tumor Suppressor Genes

These genes suppress, or constrain, cell growth and replication.

Major Concepts of Tumorigenesis

Mutation of a c-Oncogene or Tumor Suppressor Gene

- Mutation in one of these control genes may result in unregulated growth of cells.
- Example of mutated oncogene—ras
- Retinoblastoma (Rb) is an example of mutation in tumor suppressor gene.

Dosage Effects

- Oncogenes in amplified DNA—increased number of copies results in overexpression of gene.
- Translocation, which links an oncogene with a more active enhancer and/or promoter resulting in overexpression (Burkitt’s lymphoma).
- Provirus insertional mutagenesis—for example, a retrovirus with its very active transcriptional promoter/enhancer region, the LTR (long terminal repeat) may integrate (insert) near a cellular oncogene. This is one of the mechanisms by which retroviruses that do not have v-onc cause carcinoma.

- Infection with a virus carrying a v-onc: e.g., infection with a retrovirus carrying viral oncogenes such as src. The gene was probably picked up by a provirus inserted near a cellular oncogene picking up copies of c-onc. Viral progeny then contain the new oncogene now called v-onc. When a new cell is infected with the recombinant virus, the oncogene is now under the transcriptional control of the viral enhancer/promoter.
- Interaction between the products of oncogenes and tumor suppressor genes. Proteins E6 and E7 of the human papilloma virus combine with and inactivate the p53 and p110 (Rb), respectively.

Specific Viruses Associated with Human Cancers

EBV

- Burkitt's lymphoma, nasopharyngeal, and thymic carcinoma
- BL occurs only in malarial regions; the plasmodia are thought to produce a slight immunosuppression.
- EBV stimulates B-cell replication and eventually, if a translocation of *c-myc* to the DNA region where genetic rearrangements involved in antibody synthesis occurs, BL develops.

Chronic HBV

Primary hepatocellular carcinoma

Chronic HCV

Primary hepatocellular carcinoma

HPV

- Cervical carcinoma
- Mechanism: inactivation of tumor suppressor gene

HTLV-1

- CD4+ T-cell leukemia/lymphomas
- Provirus insertion or capture

PRION DISEASES

Table I-5-15. Prion Diseases

Disease	Infectious agent	Host	Comments
Kuru	Prion	Human	Subacute Spongiform Encephalopathy (SSE); Fore Tribe - New Guinea; cannibalism
Creutzfeldt-Jakob Disease (and variant)	Prion	Human	SSE Genetic predisposition. Ingestion of infected cow brains
Gerstmann-Straussler	Prion	Human	SSE
Fatal Familial Insomnia	Prion	Human	SSE
Scrapie	Prion	Sheep	SSE—scraping their wool off on fences

Table I-5-16. Slow Conventional Viruses (Viruses)

Disease	Infectious agent	Host	Comments
Measles SSPE	Virus	Human having had measles	Subacute sclerosing panencephalitis
AIDS dementia	HIV	Human	Dementia
PML	JC Virus	Human	Progressive multifocal leukoencephalopathy

VIRAL GENETICS

Phenotypic Mixing

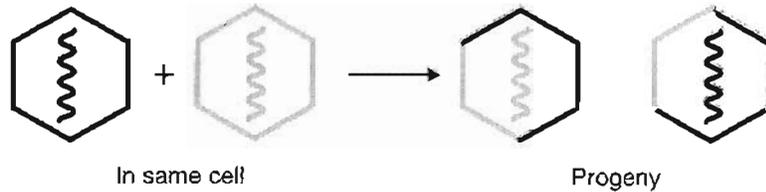


Figure I-5-32. Phenotypic Mixing

- Related viruses coinfect cell (virus A and virus B).
- Resulting proteins on the surface are a mixture capsid of AB around nucleic acid of either A or B.

Phenotypic Masking

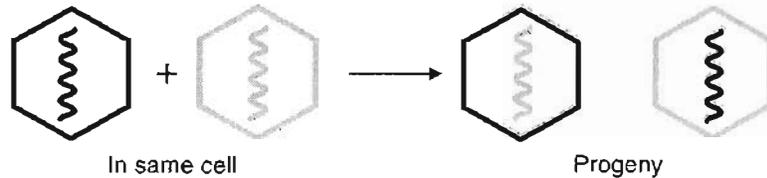


Figure I-5-33. Phenotypic Masking

- Related viruses coinfect cell (virus A and virus B).
- Capsid of proteins of virus A form around nucleic acid of B.

Complementation

- Two related defective viruses infect the same cell. If they are defective in different genes, viral progeny (still with mutated DNA) will be formed.

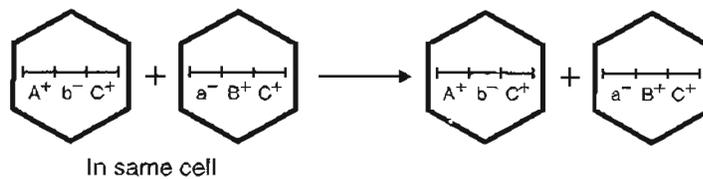


Figure I-5-34. Complementation: Mutations in Different Genes

If they are defective in the same gene, no progeny will be formed.

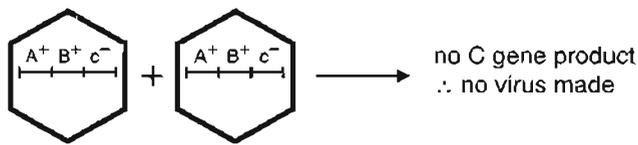


Figure I-5-35. Complementation: Mutations In Same Genes

- Coinfection of hepatitis B and D is a clinical example of complementation where HBV supplies the needed surface antigen for hepatitis D.

Genetic Reassortment = Genetic Shift

- Two different strains of a segmented RNA virus infect the same cell.
- Major new genetic combinations are produced through “shuffling,” resulting in stable and dramatic changes.

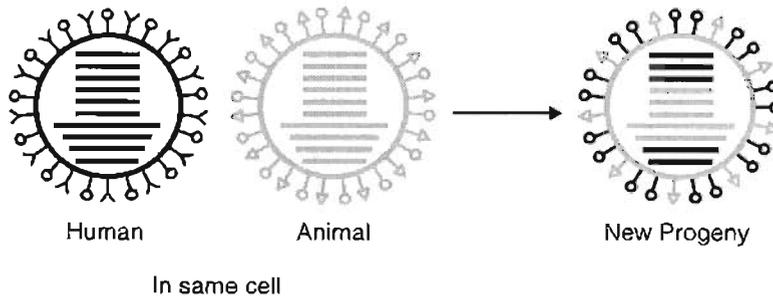


Figure I-5-36. Genetic Reassortment

Genetic Drift

- Minor antigenic changes from mutation
- Occurs in many viruses, particularly RNA ones
- Most noted in HIV and influenza

Viral Vectors

- Recombinant viruses are produced that have combinations of human replacement genes with the defective viral nucleic acid.

Chapter Summary

The virus structure consists of a core of RNA or DNA associated with structural proteins and perhaps key enzymes. This is called the nucleocapsid and constitutes a naked capsid virus. The nucleocapsid plus host membranes, including viral-specified glycoproteins, make up an enveloped virus.

The various viruses have characteristic sizes and shapes, as illustrated in Figure I-5-4.

The first step in viral replication is attachment. This is accomplished by the recognition of specific receptor sites on the host cell. This is followed by replication, release of the nucleic acid core (uncoating), and then macromolecular synthesis. Finally, new virus particles are assembled and released.

The eclipse period is the time it takes to replicate the first viral particles intracellularly. The latent period is the time between infection and the first release of virus from the infected cell.

Viruses are spread like any other pathogen. Arboviruses are those borne by arthropods, most commonly mosquitoes but also ticks, biting midges, and sandflies. Most arboviruses belong to the Togavirus, Flavivirus, or Bunyavirus families.

Virus attachment is mediated by an interaction between specific receptor sites on the host cell and either a specific viral surface glycoprotein on enveloped viruses or a specific surface protein on naked viruses.

Heat, detergents, or organic solvents inactivate enveloped (but not naked) viruses by destroying the lipid envelope.

The distribution of the required host receptors determines the species and tissue specificity for infection by a specific virus. Table I-5-1 summarizes the relationship among four important viruses, their targeted cells, and the cell receptors used for attachment by each of these viruses.

Once attached, the virus may invade the host cell by receptor-mediated endocytosis, by uptake via coated pits, or by the fusion of a viral envelope with the cell membrane.

The initial chore facing the invading virus is to synthesize protein that will replace normal host-cell function with those essential for viral survival and replication. To do so, the virus must use mRNA that will be recognized and used by the host cell's ribosomes.

Double-stranded DNA viruses directly transcribe mRNA using the negative strand as a template.

Retroviruses use their positively stranded RNA genome as templates to reverse transcribe double-stranded DNA using an RNA-dependent DNA polymerase (reverse transcriptase). The negative DNA strand is then used to transcribe mRNA.

Positively stranded RNA viruses can use their genome directly as mRNA.

Negatively stranded RNA viruses make the complementary positive strand for use as mRNA.

Once reproduced, the naked viruses lyse the host cell. Enveloped viruses acquire host-cell membrane as an outer surface as they leave the cell.

The various patterns of viral infection are shown in Figure I-5-10 and Table I-5-5. They may be abortive, resulting in no infection; cytolytic, generally resulting in an acute infection; or persistent, resulting in a chronic infection, latent with possible later emergence of disease, or transforming resulting in cancer.

(Continued)

Chapter Summary (continued)

Figure I-5-11 summarizes the ages at which various viral diseases may be expected to occur.

Immune defense against viruses utilizes the innate and adaptive pathways in a fashion similar to that used for protection from bacterial infection. However, a component of the innate system, interferon, is of unique importance as a protective mechanism against viral infection. Interferon is produced and released by virally infected cells and is transmitted to noninfected cells where it inhibits nascent viral protein synthesis (Figure I-5-12).

Viral vaccines are developed using killed viruses, attenuated viruses (treated to lose virulence), and viral components. The properties and uses of each type of vaccine are summarized.

The responsible viral family, molecular features, transmission mode, disease characteristics, mortality rates, and diagnostic techniques associated with the hepatitis A, B, C, D, and E viruses are summarized in Table I-5-6. The characteristics of infection by the hepatitis B virus are discussed in greater detail on the following page and illustrated in Figure I-5-13.

The medically important DNA viruses include the Parvoviruses, Papovaviruses, Adenoviruses, Herpes viruses, Poxviruses, and the Hepadnaviruses. The properties and associated disease states of important members of each of these families are summarized in Tables I-5-7 and -8, Figures I-5-14, -15, -16, -17, and -18, and the accompanying text.

The medically important RNA viruses include the Calciviruses, Picornaviruses, Flaviviruses, Togaviruses, Coronaviruses, and Retroviruses. The properties and associated disease states of important members of each of these families are summarized in Tables I-5-9, -10, -11, -12, -13, and -14, Figures I-5-19, -20, -21, -22, -23, -24, -25, -26, -27, -28, -29, -30, and -31, and the accompanying text.

Table I-5-15 summarizes the characteristics of Kuru, Creutzfeldt-Jacob disease, Gerstmann-Strausler disease, fatal familial insomnia, and scrapie—all prion-associated diseases.

Table I-5-16 summarizes the slow effects of three conventional virus diseases (not prion). These are subacute sclerosing panencephalitis (SSPE) caused by latent measles virus, HIV-induced dementia, and progressive multifocal leukoencephalopathy (PML) provoked by the JC virus.

Natural genetic modification of viruses can occur by genetic mixing, phenotypic masking, complementation, genetic re-assortment (genetic shift), and genetic drift. In addition, the genetic characteristics of viruses can be altered by recombinant technology.

Review Questions

- Three genetically distinct and fairly stable serotypes have made this virus's vaccines so successful that worldwide eradication of this virus is anticipated in the next 5 to 10 years.
 - Adenovirus
 - Herpes virus
 - Influenza virus
 - Measles
 - Poliovirus
- Roommates of a 19-year-old college student become alarmed when he does not get up to go to swim practice in the morning and they are unable to wake him for his 11 AM class (he had complained of a headache and not feeling well the night before). The rescue squad finds a febrile, comatose young man with a petechial rash. In the emergency room, Kernig and Brudzinski's signs are present. No papilledema is seen so a spinal tap is done. Protein is high, glucose low. CSF WBC count is 9,000 (mainly PMNs) with few RBCs. The characteristics of the most likely causative agent are
 - An enveloped dsDNA virus
 - A naked (+) ssRNA virus
 - A Gram-negative bacillus with a polyribitol capsule
 - A Gram-negative, oxidase-positive diplococcus
 - A Gram-positive, lancet-shaped alpha-hemolytic diplococcus
- Serologic test results from a hepatitis patient reveal: anti-HBc positive, HBsAg positive, and anti-HBs negative. The correct interpretation of the patient's status is
 - No longer contagious
 - Immune to hepatitis B virus
 - Evidence of receiving hepatitis B vaccination
 - Hepatitis B virus chronic carrier state
 - Impossible to have both surface antigen and core antibody positive
- Two individuals become infected with HIV at approximately the same time. JT progresses rapidly to full-blown AIDS and dies within 3 years. AY is still asymptomatic after 10 years. One possible explanation is that the virus in JT is probably expressing a high amount of activity of which regulatory gene product?
 - vif*
 - vpr*
 - tat*
 - env*
 - pol*

5. The best prospects for treatment and cure of microbial diseases are always those unique factors of a pathogen's life cycle that can be altered without affecting the survival of the host's own cells. In HIV, one such therapeutic target would be the products of the *pol* gene, which codes for the reverse transcriptase unique to the retroviral life cycle. If it were possible to ablate expression of the HIV *pol* gene, what other aspect of the virus's life cycle would be directly altered?
 - A. Transcription of proviral DNA
 - B. Production of viral mRNA
 - C. Integration of proviral DNA
 - D. Nucleocapsid
 - E. Viral maturation

6. Interferons inhibit viral growth primarily by affecting
 - A. Host cytokine production
 - B. Host protein synthesis
 - C. Viral protein synthesis
 - D. Viral transcription process
 - E. Viral assembly and release

7. Transfection with the naked nucleic acid of which virus would result in active viral replication?
 - A. Bunyavirus
 - B. Coxsackie
 - C. Poxvirus
 - D. Retrovirus
 - E. Rhabdovirus

8. A chronic infection of hepatitis B is defined as having demonstrated the presence of
 - A. HBsAg for more than 6 months
 - B. HBsAg for more than 3 months
 - C. Antibody against HBsAg for more than 6 months
 - D. Antibody against HBcAg for less than 3 months
 - E. HBcAg for 3 months

9. Live, attenuated vaccines exist for human diseases caused by
 - A. Hepatitis A and B viruses
 - B. Hepatitis A and polioviruses
 - C. Rubella and rubeola viruses
 - D. Influenza viruses
 - E. Rabies virus and rotavirus

10. A boy with bilateral swelling of his salivary glands is found to have an elevated serum amylase. The most likely viral causative agent
 - A. Has equal amounts of adenine and thymine in its genome
 - B. May cause complications at the time of acute disease or during convalescence
 - C. Has multiple serotypes
 - D. Is characterized by being helical, circular, single-stranded RNA and naked

11. A 5-year-old female presents with a fever and a generalized macular rash that is most dense on the scalp and trunk of the body. Several waves of lesions appear, one after another, and evolve rapidly into vesicles and then pustules over several days. The most likely disease and causative agent is
 - A. Exanthem subitum due to cytomegalovirus
 - B. Chicken Pox due to the varicella-zoster virus
 - C. Whitlow's infection due to herpes simplex virus type 1
 - D. Herpetic gingivostomatitis due to the varicella-zoster virus
 - E. Infectious mononucleosis due to the Epstein-Barr virus

12. Infection of appropriate cells with a composite virus made up of Coxsackie virus capsid components and poliovirus RNA would yield progeny which would
 - A. Have the host cell range of Coxsackie virus
 - B. Also be composite viruses
 - C. Show phenotypic mixing
 - D. Have a recombinant genome consisting of both Coxsackie and poliovirus
 - E. Cross-react with Sabin-vaccine-induced antibodies

13. Positive-stranded viral RNA
 - A. Replicates most commonly through a double-stranded DNA intermediate
 - B. Binds to ribosomes and can be translated
 - C. Requires a special polymerase in the virion
 - D. Is not infectious
 - E. Replicates without an intermediate

14. To design a vaccine against HIV infection, a logical goal would be to alter some native molecule or product of the virion in order to make it highly immunogenic. If you wished to prevent the attachment of the virus to helper T lymphocytes, which molecule or family of molecules might best be targeted?
 - A. gp41
 - B. gp120
 - C. nucleocapsid protein
 - D. p17
 - E. p24

15. How do the oncogenes of the human papilloma viruses act?
- They act as tumor suppressor genes
 - They function identically to cellular oncogenes
 - They kill the infected cell when upregulated
 - We don't know how they act because they are present in every cell of every individual and yet only some become cancerous
 - They code for early viral proteins, leading to malignant transformation
16. To which of the following viruses is hepatitis A most closely related genetically?
- Hepatitis B
 - Poliovirus
 - Measles
 - Rubella virus
 - Influenza
17. A naked protein similar in amino acid sequence to a normal human protein, but which has no nucleic acid associated with it, can cause
- AIDS dementia
 - Mumps meningitis
 - Progressive multifocal leukoencephalopathy
 - Subacute sclerosing panencephalitis
 - Subacute spongiform encephalopathy
18. A 37-year-old ambitious executive for a local Health Maintenance Organization comes to your office because he has developed multiple blister-like lesions on his penis over the last 1–2 days. They are somewhat painful, and he is worried that he has AIDS. He denies homosexuality and intravenous drug abuse and had an HIV test prior to his marriage 3 years ago. He reports several similar episodes several years ago when he worked as a photographer in Nepal. He was never told what they were, and they resolved over several days without any treatment. His physical examination is remarkable only for the presence of 6–8 vesicular lesions 3–4 mm in diameter on the glans of the penis. There is no crusting, drainage or bleeding. The lesions are moderately tender and there is mild inguinal adenopathy bilaterally. How does the causative agent produce its messenger RNA?
- By producing a positive sense intermediate
 - By direct translation from the genome
 - By transcription from proviral DNA
 - By producing a negative sense intermediate
 - By transcribing the genomic DNA
 - By producing a double-stranded DNA intermediate
 - The genomic RNA is used directly on the ribosomes

19. The Tzanck test, which aids in the diagnosis of herpes simplex infection, is a search for
 - A. Antibodies to herpes simplex 1 or 2
 - B. Intracytoplasmic inclusion bodies
 - C. Virus shedding from pustular lesions
 - D. Multinucleated giant cells
 - E. Immunofluorescence of infected cells
 - F. Detection of viral RNA
 - G. Koilocytic cells

20. In the U.S., a baby has the greatest chance of acquiring which virus *in utero*?
 - A. Cytomegalovirus
 - B. Hepatitis B virus
 - C. Herpes simplex virus
 - D. Respiratory syncytial virus
 - E. Rubella virus

21. Which of these viruses has RNA for both its genome and replicative intermediate?
 - A. Cytomegalovirus
 - B. Hepadnavirus
 - C. Retroviruses
 - D. Togaviruses
 - E. Poxvirus

22. What is the most common lab testing method for diagnosing infectious mononucleosis?
 - A. The Monospot test to detect EBV-specific antibody
 - B. An assay for Epstein-Barr nuclear antigen
 - C. The presence of atypical lymphocytes in the blood establishes the etiology
 - D. A test for heterophile antibody, which cross-reacts with antigens found on a variety of animal red blood cells
 - E. A simple procedure is done to isolate EBV from saliva, blood, or lymphoid tissue

23. What virus is noted for genetic reassortment, which leads to major pandemics about once every 10 to 11 years?
 - A. Adenovirus
 - B. Herpes virus
 - C. Human immunodeficiency virus (HIV)
 - D. Influenza virus
 - E. Poliovirus

24. What virus is noted for such a high incidence of genetic drift that more than one antigenic variant can be isolated from most infected individuals who have high viral titers?
- Adenovirus
 - Herpes virus
 - Human immunodeficiency virus (HIV)
 - Influenza virus
 - Poliovirus
25. A 19-year-old male college student reports sore throat and extreme fatigue following even normal non-taxing tasks like getting dressed and going down to breakfast. He tells you that he has been sick for several weeks, that he has been feverish and that his girlfriend now appears to be getting the same thing. His tonsils are inflamed with a white exudate adhering; cervical lymphadenopathy is prominent, as is splenomegaly. The most likely causative agent is
- ss DNA, naked icosahedral virus
 - ds DNA, naked icosahedral virus
 - ds DNA, enveloped complex virus
 - ds DNA, enveloped icosahedral virus
 - ds RNA, naked segmented virus
 - ss RNA, segmented enveloped and helical virus
 - (-) ss RNA, bullet-shaped helical virus
 - (-) ss RNA, naked helical virus
 - (+) ss RNA, naked icosahedral virus
 - (+) ss RNA, enveloped icosahedral virus
 - (+) ss RNA, enveloped diploid virus
26. Cataracts and patent ductus arteriosus in a newborn suggest *in utero* infection with what viral family?
- Adenovirus
 - Paramyxovirus
 - Parvovirus
 - Picornavirus
 - Reovirus
 - Togavirus
27. What is the most dominant method of spread for measles?
- Animal bite
 - Fecal-oral
 - Fomite spread
 - Respiratory droplet spread
 - Sexual contact
 - Transfusion or intravenous drug abuse
 - Tick bite

28. How are human papilloma virus type 4 warts spread?
- A. Animal bite
 - B. Fecal-oral
 - C. Fomite spread
 - D. Respiratory droplet spread
 - E. Sexual contact
29. A 15-year-old member of the high school swim team notices painless, umbilicated cutaneous lesions on the toes. Large eosinophilic cytoplasmic inclusions are present in the affected epithelia. What is the most likely causative agent?
- A. Adenovirus
 - B. B-19 virus
 - C. Cytomegalovirus
 - D. Herpes simplex virus
 - E. Human papilloma virus
 - F. Molluscum contagiosum virus
 - G. Varicella-Zoster virus
30. A bone marrow transplant recipient becomes febrile and hypoxic and chest films demonstrate diffuse interstitial pneumonia. What is the most likely causative agent?
- A. BK virus
 - B. Cytomegalovirus
 - C. Herpes simplex virus
 - D. Molluscum contagiosum virus
 - E. Paramyxovirus
 - F. Varicella-Zoster virus
31. A 6-month-old infant presents with painless verrucous growths on the laryngeal folds. What is the most likely causative agent?
- A. B-19 virus
 - B. Cytomegalovirus
 - C. Herpes simplex virus
 - D. Human papilloma virus
 - E. Molluscum contagiosum virus

Answers

1. **Answer: E.** Poliovirus has three serotypes, which do not change significantly from year to year or within a person. The World Health Organization (WHO) hopes to have it eradicated within the next five years. (Note the clue of multiple vaccines.)
2. **Answer: D.** The most likely causative agent here is a bacterium. Viral meningitis is usually mild and would not fit the CSF values. Both the age of the patient and the petechial rash suggest it is most likely to be *Neisseria meningitidis*, which is a Gram-negative diplococcus that is oxidase-positive. The overproduction of outer-membrane fragments is what leads to the petechial rash, even prior to antibiotic treatment.
3. **Answer: D.** The positive test for hepatitis B surface antigen indicates that the patient still has the hepatitis B virus and hence is still contagious. The presence of hepatitis B surface antigen and the absence of the surface antibody (anti-HBs) indicate either an acute HBV infection (if patient has had the disease for only a short time) or a chronic carrier state (if the hepatitis has been going on for many months). Because acute HBV is not a choice, choice D then becomes the correct answer. Choice B would be a right answer if HBsAg is negative. Core antibodies would not be present if the person is only vaccinated. Also, HBs antigen should not be present in a detectable amount from vaccination.
4. **Answer: C.** The *nef* gene product suppresses transcription of viral DNA and tends to keep the infection in a latent state. Products of *tat* and *rev* genes increase viral maturation and act to decrease the latent period (J.T. probably had a high expression of those genes). The *vif* gene product affects viral infectivity, and *vpr* gene products increase the efficiency of budding. Another possible difference is that A.Y. may lack the invasion gene, which codes a cellular co-receptor for HIV. *Env* codes for envelope antigen and *pol* codes for reverse transcriptase.
5. **Answer: C.** The *pol* gene codes both for reverse transcriptase and the integrase, which allow the linear proviral DNA to be integrated, apparently at a random site, into a chromosome in the host cell. Of the distractors, both choices A and B are accomplished using the host cell's RNA polymerase. Choice D is a function of the *gag* gene, and choice E is controlled by *tat* and *rev* genes.
6. **Answer: C.** Interferons interfere with virus multiplication by blocking translation of viral protein (by inhibiting viral mRNA and hence inhibiting viral protein synthesis). They do not inhibit transcription, assembly, or release. They also do not inhibit host-protein synthesis.
7. **Answer: B.** Two of the viruses can be eliminated because they are negative RNA viruses and require polymerase accompanying the RNA to replicate. These two are the *Bunyavirus* and the rhabdoviruses. Since the retrovirus RNA requires the reverse transcriptase to replicate, it also cannot start an infection with just the RNA. Because of the locale of replication of the one DNA virus in the list (Poxvirus replicates in the cytoplasm), it must also bring in an enzyme to replicate: a transcribing enzyme.
8. **Answer: A.** Presence of HBsAg (surface antigen to hepatitis B virus) for 6 months (not 3 months) is considered chronic. Students need to know the abbreviations used for hepatitis serology.

9. **Answer: C.** Hepatitis A vaccine is a killed vaccine, and B is a component vaccine. The three important, live viral vaccines are MMR (mumps, measles, or rubeola, and rubella or German measles). The adenovirus vaccine (not in the question) is a live vaccine that is NOT attenuated but is enteric-coated and given orally to reduce respiratory infections in military personnel. Other live viral vaccines are attenuated and include Sabin's polio, varicella, and yellow fever.
10. **Answer: B.** Complications do occur. Choice A is wrong because the agent is the mumps virus, which is RNA (not DNA), and thereby should have uracil instead of thymine. Only one serotype exists, explaining why one gets mumps only once. Choice D is wrong because the genome of the mumps virus is linear and not circular.
11. **Answer: B.** The clinical presentation is consistent with chickenpox caused by VZV. Exanthem subitum is caused by human herpes virus 6, not by CMV. Herpetic gingivostomatitis refers to herpes simplex type 1, not VZV. Students need to know the typical clinical presentations of Whitlow's and infectious mononucleosis.
12. **Answer: E.** This question requires you to understand viral replication and viral genetics quite well. The only nucleic acid in the composite parental virus is the RNA belonging to poliovirus. Thus, only poliovirus is made. The only role the Coxsackie virus would play in the infection is to bind to the host cell and stimulate the uptake of the composite virus. Once uncoating takes place, the Coxsackie components play no further role. A perfect poliovirus will have been made.
- So choice A is incorrect—the progeny will have the host-cell range of polio because that is what they'll be.
- Choice B is incorrect because there is no genetic material coding for the Coxsackie components, so you cannot get a composite.
- Choice C is incorrect because no capsid components of Coxsackie will be made; there can be no mixing.
- Choice D is incorrect because there was only one type of RNA; there can never be recombination.
- Choice E is correct because Sabin is a polio-specific vaccine, and poliovirus will be produced.
13. **Answer: B.** Positive-stranded viral RNA is infectious because it can bind to ribosomes and be translated and therefore can make its polymerase (a RNA-dependent RNA polymerase). It does not need to carry it in its virion. It replicates by making a negative template of RNA, and only HIV replicates through a DNA intermediate. All single-stranded RNA viruses require a replicative intermediate, making choice E false.
14. **Answer: B.** Gp120 is the surface antigen of HIV that mediates its attachment to CD4 lymphocytes. Gp41 is a transmembrane glycoprotein, and p24, p17, and nucleocapsid protein are all internal molecules, which would rarely be accessible to the immune response.
15. **Answer: E.** Our normal tumor-suppressor genes are “anti-oncogenes,” which negatively regulate cell growth. The oncogenes of human papilloma viruses code for two early proteins: E6 and E7, which inactivate the tumor-suppressor genes of the infected cells, resulting in malignant transformation. Viral oncogenes are similar but *not identical* to cellular oncogenes. Expression of viral oncogenes may lead to malignant transformation but will not kill the infected cells. HPV oncogenes are not present in every cell and certainly not in every individual.

16. **Answer: B.** Hepatitis A is a picornavirus so that is the most likely choice. If you do not know the family but know that it is naked and positive RNA, you can eliminate hepatitis B because it is DNA. Measles, rubella, and influenza are all enveloped.
17. **Answer: E.** The definition fits a prion. Prions cause subacute spongiform encephalitis (e.g., Kuru, Creutzfeldt-Jakob disease, Gerstmann-Straussler disease, etc.). PML is caused by the JC virus. AIDS dementia is HIV. SSPE follows measles and perhaps is caused by hypermutated, defective forms of the virus.
18. **Answer: E.** The virus is HSV II, a herpesvirus, which are dsDNA viruses that use the mechanisms of our own cells to transcribe an RNA strand from their genomic DNA and use the transcribed RNA as a messenger RNA. Of the distractors: choice A is the technique used by the negative sense RNA viruses; choices B and G are used by the positive sense RNA viruses; choice C is used by the retroviruses; choice D is used during the genomic duplication of negative sense RNA viruses; and choice E is used in genomic replication by the retroviruses. Choice F would not produce RNA.
19. **Answer: D.** In the Tzanck test, a swab is taken from the exposed base of a lesion and observed microscopically by Giemsa staining for the presence of characteristic multinucleated giant cells. The Tzanck test is not an immunofluorescence test (choice E) or a test for patient antibodies (choice A) or a viral culture. (Both direct immunofluorescent staining of cells from lesions and viral culture with DFA confirmation are commonly used for diagnosis in large hospitals because the Tzanck test is not specific.) Herpes simplex virus produces intranuclear infection (thus choice B is not true), and the lesions are generally not pustular (choice C). Choice F is not true because herpesviruses are DNA viruses, and koilocytic cells (choice G) are hallmarks of human papilloma virus infection in PAP smears.
20. **Answer: A.** CMV is an extremely common virus and crosses the placenta oftentimes without causing obvious symptomology. Fortunately, Rubella, which is highly teratogenic particularly in early pregnancy, is generally prevented by routine vaccination in childhood or at least 16 weeks prior to pregnancy. Less than 5% of hepatitis B could possibly be *in utero*. HSV 2 will only cross the placenta if mom acquires Herpes for the first time during her pregnancy. RSV and other respiratory viruses will not. Other viruses that can cross the placenta include Coxsackie B and HIV.
21. **Answer: D.** Cytomegalovirus, Hepadna, and Poxviruses are all dsDNA viruses and not RNA. Retrovirus is an RNA virus but replicates through a dsDNA, so it also is not the correct answer. Toga is a positive RNA virus, which replicates through a negative RNA intermediate and has no DNA; therefore, it's the correct answer.
22. **Answer: D.** Monospot is the most commonly used test for the diagnosis of infectious mononucleosis caused by EBV. However, it does not detect EBV-specific antibody. It instead detects heterophile antibody, which is nonspecific in that it may be present in different organisms and individuals and it cross-reacts with many animal RBCs. Epstein-Barr nuclear antigen test is routinely run in the diagnosis of mononucleosis. Atypical lymphocytes are found in mononucleosis caused by EBV and CMV, but CMV is heterophile antibody-negative. Isolation of EBV, of course, can establish the diagnosis. However, the procedure is cumbersome and laborious, and would identify asymptomatic infected cases as well.
23. **Answer: D.** The segmented influenza viruses may undergo recombination with a similar animal virus. This leads to genetic change that negates everyone's prior immunity, leading to severe pandemics.

24. **Answer: C.** HIV. It is this genetic drift that makes it difficult for the body to fight off HIV and has complicated the development of an effective vaccine.
25. **Answer: D.** Both the symptomology, length of infection, and the epidemiological clues (college student, age 19, has given it to his girlfriend) strongly suggest that this is EBV, which is a herpes virus.
- Choice A = parvo; choice B = adeno; choice C = pox; choice D = papova/hepadna because there's no distinction as to circular or partial ds DNA; choice E = reovirus; choice F = arena virus, bunya, and orthomyxo; choice G = rabies; choice H = none; choice I = calci or picorna; choice J = flavi and toga; choice K = retro. *Note:* no envelope (-) ss RNA helical [= corona] choice.
26. **Answer: F.** The description fits congenital rubella, a togavirus, which is an enveloped positive RNA virus that is not segmented.
27. **Answer: D.** If you have any trouble, think about which of these viruses has respiratory symptoms (in this case, pneumonia).
28. **Answer: C.** Remember that type 4 strains cause plantars warts, and these are largely transmitted by shower room floors, towels, etc.
29. **Answer: F.** This describes the typical presentation of molluscum contagiosum, which is commonly acquired through small breaks in the skin in environments where moisture keeps the virus viable (swimming pools, showers).
30. **Answer: B.** CMV is the most common viral cause of death in bone-marrow transplant patients, causing an interstitial pneumonia.
31. **Answer: D.** Perinatal infection with human papilloma virus can cause infantile laryngeal warts.

Matching

A. Match disease with causative agent. Remember that choices may be used once, more than once, or not at all.

- A. Adenovirus
- B. Coronavirus
- C. Coxsackie virus
- D. Echovirus
- E. Gerstmann-Straussler
- F. Herpes 1
- G. Marburg agent
- H. Measles
- I. Norwalk agent
- J. Parvovirus
- K. Rabies
- L. Rotavirus
- M. Rubella
- N. Vaccinia
- O. Varicella-Zoster

- ___ 1. A common cause of acute coryza, a usually afebrile upper respiratory infection causing inflammation of the nose, paranasal sinuses, throat, larynx, and trachea.
- ___ 2. The patient has experienced a low-grade fever and stiff neck for the past day. This morning he awakened with difficulty and his wife noted distinct personality changes. He experienced several seizures and was brought into the emergency department. A non-traumatic spinal tap yielded CSF fluid with lymphocytes and RBCs. A diagnosis of encephalitis was made.
- ___ 3. An epidemic of diarrheal disease has occurred at Lincoln Grade School. Twenty percent of the children have been ill enough to stay at home and three teachers and a custodian have also called in sick.
- ___ 4. Several children in a daycare center have developed papulovesicular erythematous lesions over the buccal mucosa and palate. The children had experienced fever, headache, and sore throat a few days preceding the appearance of the vesicular lesions. Similar lesions are noted on the hands and feet and in the diaper area in the very young.
- ___ 5. Over fifty percent of the boys in Scout Troop #27 have developed a febrile disease during the first week after their camp out on the lake. They all complain of sore throat and itchy eyes; their conjunctiva are inflamed. A few other members of the troop have conjunctivitis but no signs of pharyngitis.
- ___ 6. This 17-year-old child has been bothered with insomnia for the past several weeks. Her schoolwork has suffered and she has been having hallucinations. The onset of seizures has prompted a neurologic examination. The presence of elevated levels of gamma globulin in the spinal fluid prompts the neurologist to order an EEG. A diagnosis of subacute sclerosing panencephalitis is made. What earlier viral infection has she had?

- ___7. The patient complains of easy fatigability and insomnia. He is somewhat apathetic and disoriented. Aphasia and other signs of abnormal higher cortical functions are noted. A neurological consult examines the patient and suggests that he might have a subacute spongiform encephalopathy.
- ___8. The patient is a well-developed young man of 16 years of age who has been working on a combine crew this summer. He has a sudden onset of malaise and fever and complains of a global headache and a stiff neck and back. CSF exam reveals an elevated opening pressure, normal glucose, but elevated proteins. The predominating cell in the spinal fluid is a lymphocyte.

Answers to A

1. B Because the rhinoviruses are not here, you have to choose the coronaviruses.
2. F Herpes 1 is a major causative agent of encephalitis.
3. I Communal eating may lead to food contaminated by a food preparer with Norwalk virus. Characteristically, infants are less likely to be involved not having eaten the food, even though they may have attended the event.
4. C This is hand, foot, and mouth disease caused by Coxsackie A virus.
5. A Adenovirus
6. H Late sequela to measles (rubeola)
7. E This is prion disease. The only prion listed is Gerstmann-Straussler.
8. D The most common causative agent of aseptic meningitis is echovirus.

B. Match viral feature with agent.

- A. Adenovirus
- B. Rubella
- C. Influenza A
- D. Herpes 1
- E. Human immunodeficiency virus
- F. Human papilloma virus
- G. Hepatitis B
- H. Variola

- ___1. Genetic shift important to epidemiology
- ___2. Highest transmissibility rate by needle stick
- ___3. Oncogenic virus
- ___4. Self-coded envelope
- ___5. Teratogenic

Answers to B

- 1. C Shift is in influenza A, while drift also is notable in HIV.
- 2. G Hepatitis B is acknowledged to be more infectious than HIV.
- 3. F HPV has the strongest evidence for being carcinogenic. HIV is not considered directly oncogenic.
- 4. H Small pox (variola) is the only one that makes its own envelope.
- 5. B Rubella is most noted for teratogenicity.

C. These are killers. If you can do these, you are in excellent shape! Write what you know about the virus, and then look on the list, rather than trying to do it in whatever order they have given you the characteristics.

Match features with viral types.

- A. Icosahedral, enveloped, double-stranded DNA
- B. Icosahedral, naked, double-stranded DNA
- C. Icosahedral, naked, single-stranded DNA
- D. Icosahedral, naked, positive single-stranded RNA
- E. Icosahedral, enveloped, positive single-stranded RNA
- F. Icosahedral, double-stranded, segmented DNA
- G. Helical, naked, negative single-stranded RNA
- H. Helical, enveloped, negative single-stranded RNA
- I. Helical, enveloped, negative, single-stranded, segmented RNA

- ___1. The baby was born at the 31st week of gestation. It was small for gestational age and showed jaundice. Petechiae were noted and the spleen and liver were enlarged. The pathology department reported that placental examination revealed chorioamnionitis, villitis, and owl's eye intranuclear inclusion bodies.
- ___2. The Peace Corp worker was bitten by a camp dog while helping in the construction of a dam near the village of Urundan in Central Africa. The animal was caged and 4 days after the attack, died. The head was sent to a reference laboratory and Negri bodies were found in Ammon's horn.
- ___3. A 4-year-old child of Mexican immigrant parents was brought to the hospital with a temperature of 101°F, a cough coryza, conjunctivitis, and a characteristic exanthem on the buccal mucosa opposite the first and second upper molars. The spots resemble tiny grains of white sand surrounded by inflammatory areolae.
- ___4. A 16-year-old high school dropout develops urticaria and arthralgia. He reports that his urine is dark in color and his sclera are yellow. He has not felt well for the past week or so and has quit smoking because the cigarettes started to taste funny.

Answers to C

- 1. A Owl's eye's inclusion bodies are found in cytomegalovirus, which is a Herpes virus. The viruses are DNA (double-stranded), enveloped, and icosahedral.
- 2. H Negri bodies are found in rabies, which is a negative single-stranded RNA virus, both helical and enveloped.
- 3. H Koplik spots are associated with measles, which is an enveloped, helical, negative, single-stranded RNA virus.
- 4. A Dane particles are the infectious particle of hepatitis B that are icosahedral, enveloped, double-stranded DNA viruses.

Microbial Genetics/ Drug Resistance

6

TERMINOLOGY

Polymerases

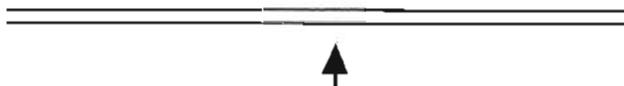
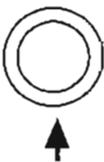
(major nucleic acid synthesizing polymerases)

_____ -dependent _____ polymerase
(template) (product)

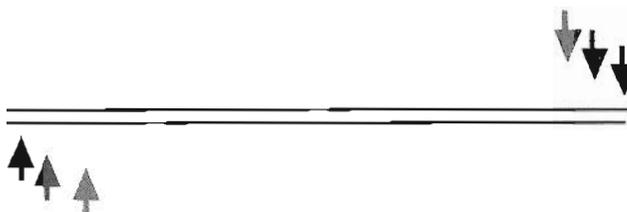
_____ -dependent _____ polymerase
(template) (product)

Nucleases

Endonucleases—cleave the nucleic acid backbone in the middle of the nucleic acid strand. Restriction endonucleases (a.k.a., site-specific endonucleases) recognize specific base sequences and make specific breaks. Bacterial cells methylate their own restriction nuclease sites so that their enzymes do not break down their own DNA. Endonucleases are not present in high concentration in the cells.



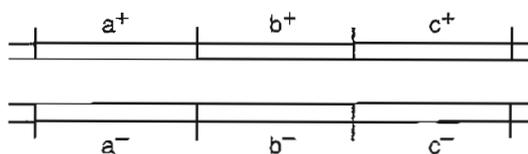
Exonucleases—remove end nucleotides sequentially from linear pieces of nucleic acid, ultimately totally breaking the piece of DNA down. Numerous exonucleases per cell.



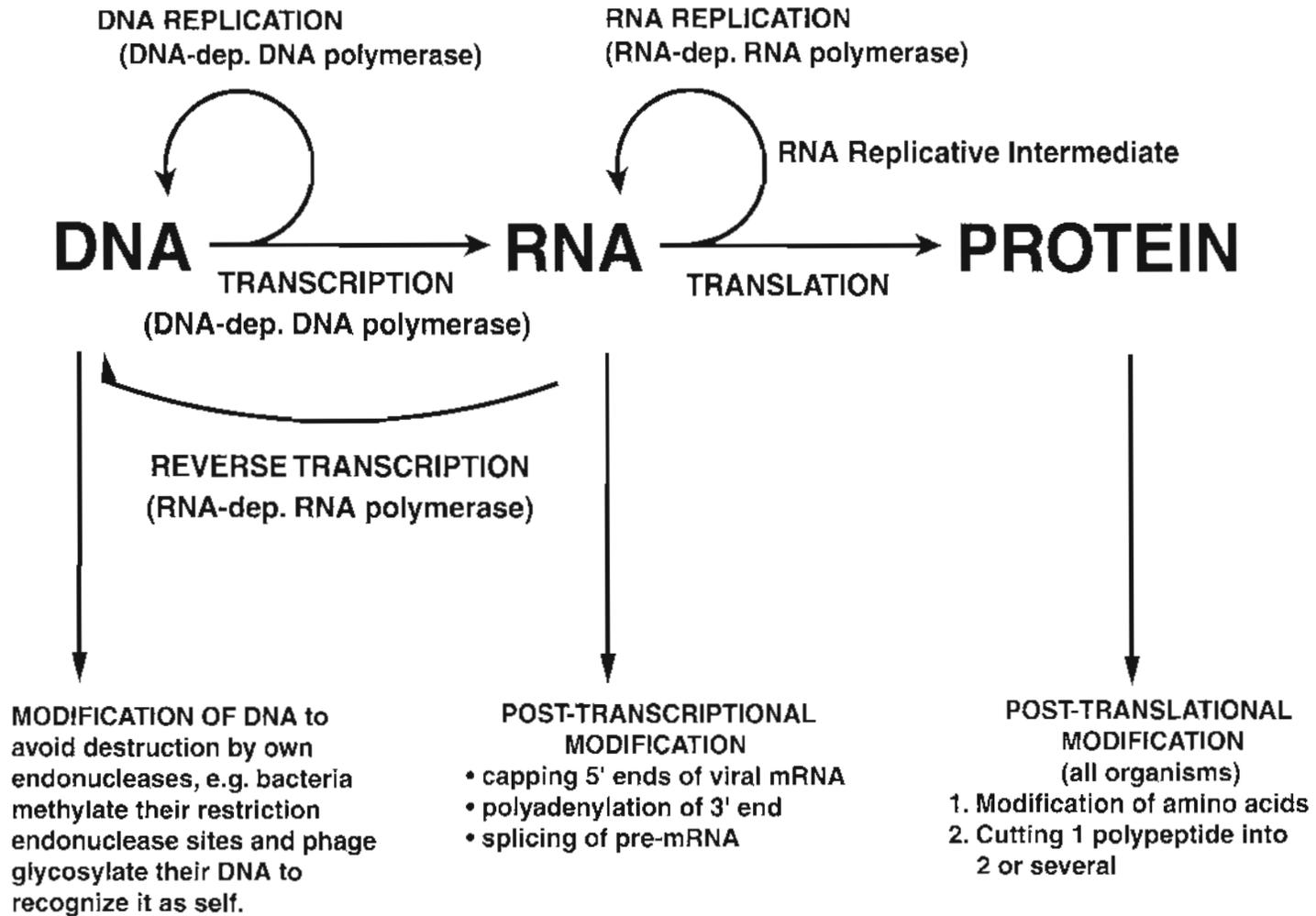
Alleles

Alternative forms of the same gene.

In the following two strands of DNA, *a*, *b*, and *c* are different genes but *a*⁺ and *a*⁻ are different alleles.



THE FLOW OF GENETIC INFORMATION

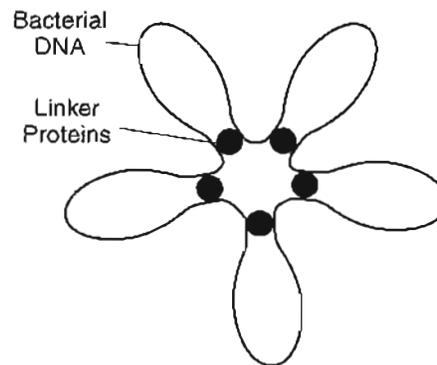


THE BACTERIAL GENETIC MATERIAL

Three different types of DNA may be found in a bacterial cell: bacterial DNA, plasmid DNA, or bacteriophage DNA.

Bacterial Chromosome (Genome)

- Most bacteria have only **one chromosome** but often **multiple copies** of it in the cell.
- Most bacterial chromosomes are a **large covalently closed, circular DNA molecule** (about 1,000 times the diameter of the cell).
- The chromosome is **organized into loops** around a proteinaceous center. A single-stranded topoisomerase (1 nick) will relax only the nicked loop, allowing DNA synthesis or transcription.
- Most have **around 2,000 genes**. (*E. coli* has about 4,500 kbases.)
- All **essential genes** are on the bacterial chromosome.



Plasmids

- Are **extrachromosomal genetic elements** found in bacteria (and eukaryotes)
- Are generally covalently closed, **circular DNA**
- Are **small** (1.5–400 kB)
- Can replicate autonomously in bacterial cells
- One subclass of plasmids, called **episomes**, may be integrated into the bacterial DNA. Episomes have insertion sequences matching those on the bacterial chromosome.
- Plasmids carry the genetic material for a variety of genes, e.g., the fertility genes directing conjugation (*tra* operon), many of the genes for antibiotic resistance, and most bacterial exotoxins.

Bacteriophage (= phage = bacterial virus) Genome

- Stable pieces of bacteriophage DNA may be present in the bacterial cell.
- These are generally repressed temperate phage DNA inserted into the bacterial chromosome (called a prophage).
- Besides the repressor protein, this prophage DNA may also direct synthesis of another protein. Most notable are gene products that make bacteria more pathogenic. This enhanced virulence is called lysogenic conversion.

INSERTION SEQUENCES/TRANSPOSONS

Transposons (Tn) and Insertion Sequences (IS)

- Are mobile genetic elements (DNA) that can move themselves or a copy from one molecule of DNA to another (“jumping genes”)
- Are found in eukaryotic and bacterial cells and viruses
- Have sequences of indirect repeats of bases on each end
- Have at least one gene for a transposase (enzyme[s] involved in the “movement”)
- Create additional mutations with their insertion in another totally unrelated gene
- May insert anywhere the transposase recognizes the specific sequence of nucleotides.
- Insertion creates direct repeats on each side of the transposon/IS.

Phage-Coded Pathogenic Factors or Lysogenic Conversion

O = O antigen of *Salmonella*

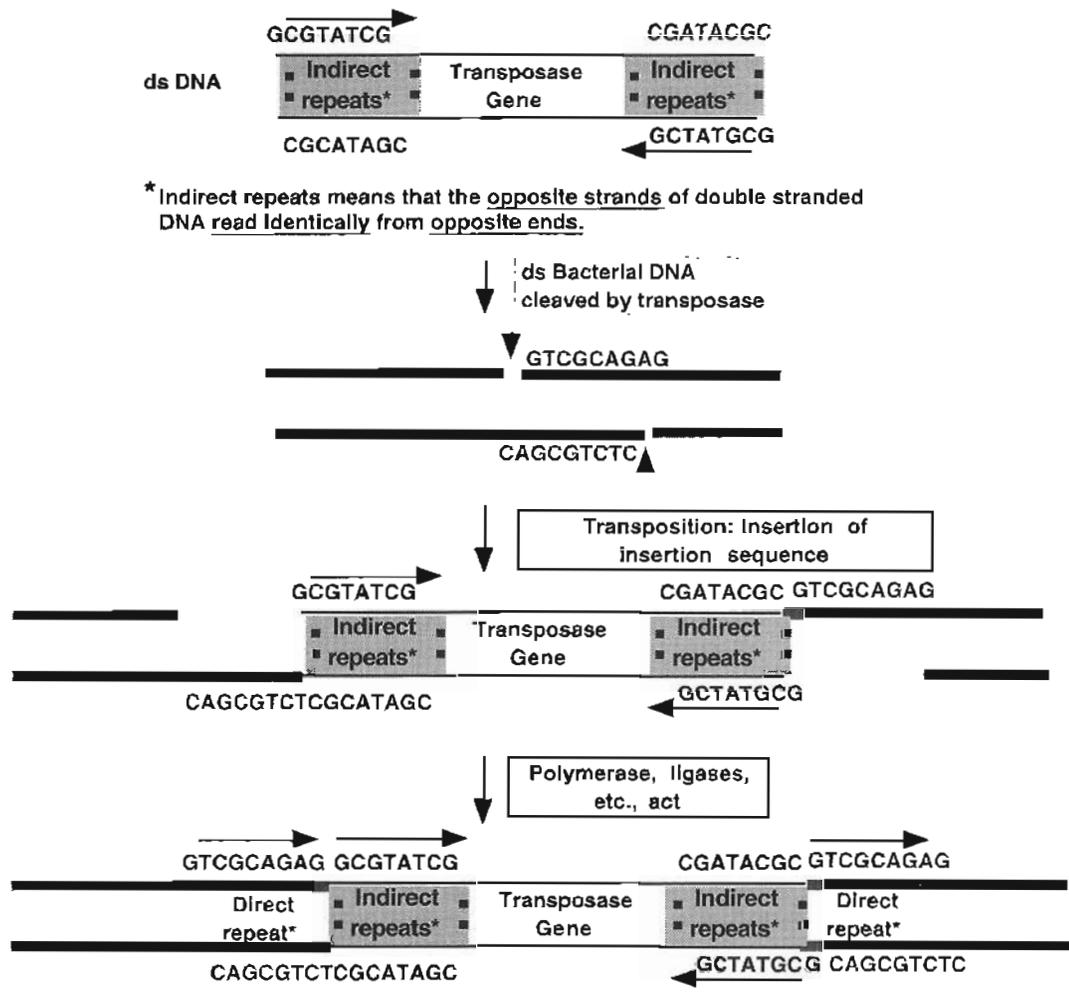
B = Botulinum toxin

E = Erythrogenic exotoxins of *S. pyogenes* (SPE-A, -B, and -C)

D = Diphtheria toxin
(OBED: a little bit pregnant with phage!)

- Insertion Sequences (IS)
- Minimal transposable elements (A, below)
 - Have just the one gene for transposase.
 - Have the terminal indirect repeats.
 - Have promoters and transcription and translation-termination signals.

Insertion Sequence Showing Terminal Indirect Repeats* + Genes for Transposition



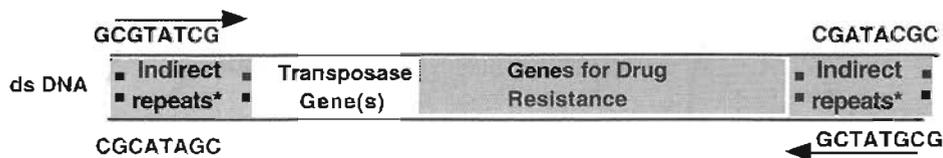
*Direct repeat means that the same strand read from the same direction is identical. It is created because a homologous strand was separated over a short space and then each strand used as a template.

Transposons (Tn)

Insertion sequences plus at least one other gene (B, below)

- Include some genes for drug resistance
- Transposons play an important role in building multiple drug resistance plasmids. They also (but not as frequently) may move to the chromosome.

Transposons have additional genes, e.g., drug resistance genes



REARRANGEMENT OF DNA WITHIN A BACTERIUM

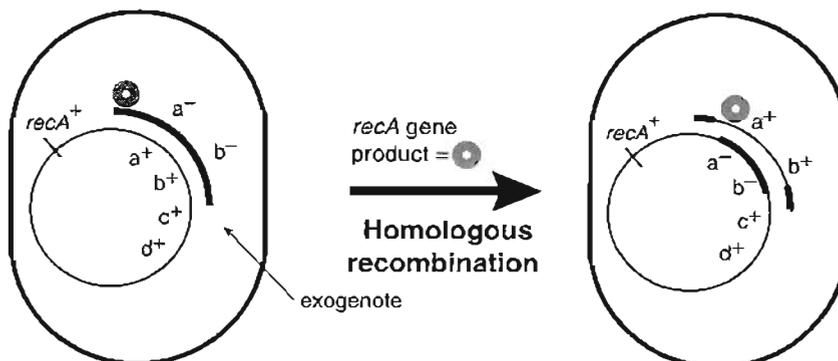
Homologous Recombination

Homologous recombination is a gene exchange process that may stabilize some genes introduced by transformation, conjugation, or transduction.

Imported bacterial DNA (transferred into a cell by transformation, conjugation or transduction) is on short linear pieces of DNA called exogenotes. Most linear DNA is not stable in cells because it is broken down by exonucleases.

Homologous recombination produces an “exchange” of pieces of DNA between the linear piece of DNA and it is near a homologous region on the stable (circular) bacterial chromosome. Homologous recombination requires:

- Several genes worth of homology or near homology between the DNA strands.
- A series of recombination enzymes/factors coded for by the recombination genes *recA*, *recB*, *recC*, and *recD* (with *recA* generally an absolute requirement).



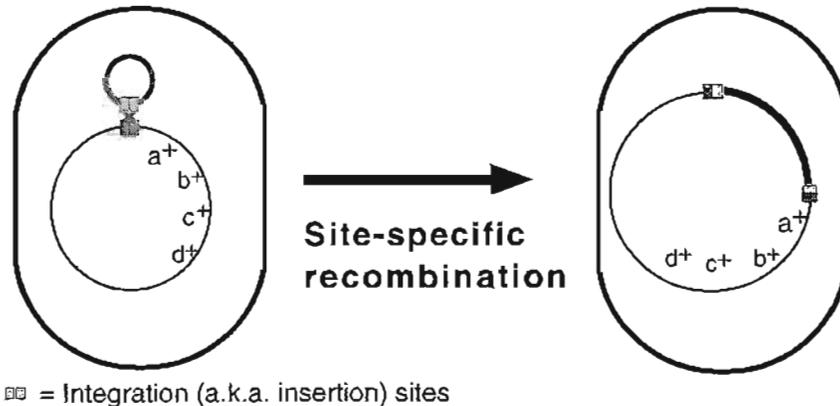
Genes ending up on the linear piece of DNA are lost.

Those on the circular molecule become part of the cell's permanent genetic make up.

Site-Specific Recombination

Site-specific recombination is the integration of a DNA molecule into a DNA with which it has no homology except for a small site on each DNA (called an attachment, integration or insertion site).

- Requires restriction endonucleases and restriction endonuclease sites on each DNA (often named an integration site or attachment site but basically insertion sequences) but DNA synthesis is not required.
- Since this process integrates rather than exchanging pieces of DNA, the end result is a molecule the sum of the two original molecules.



Three major roles of site-specific integration

- Integration of a fertility factor to make an Hfr cell
- Integration of temperate phage DNA into a bacterial chromosome to create a prophage
- Movement and insertion of transposons (transposition is the name of site-specific integration of transposons)

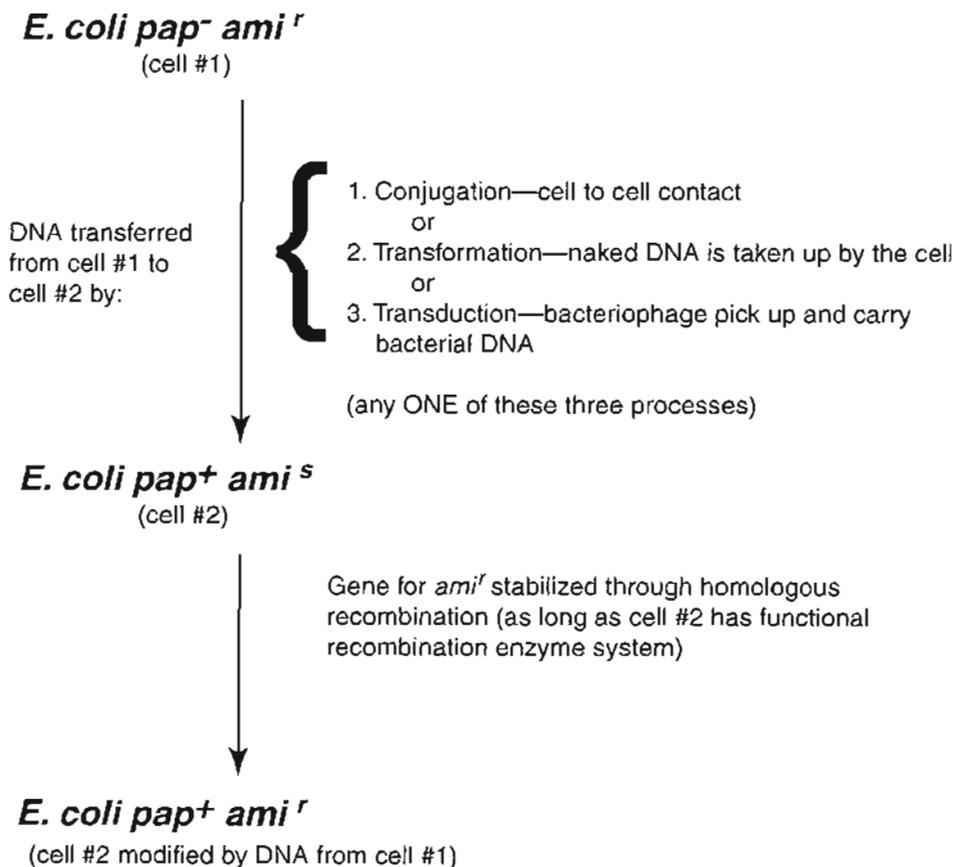
GENE TRANSFER

Overview

Bacterial reproduction is asexual so progeny are identical to parent cell with only rare mutations.

How do you get new genetic combinations in bacteria?

Answer: Gene transfer followed by stabilization of genes (recombination).

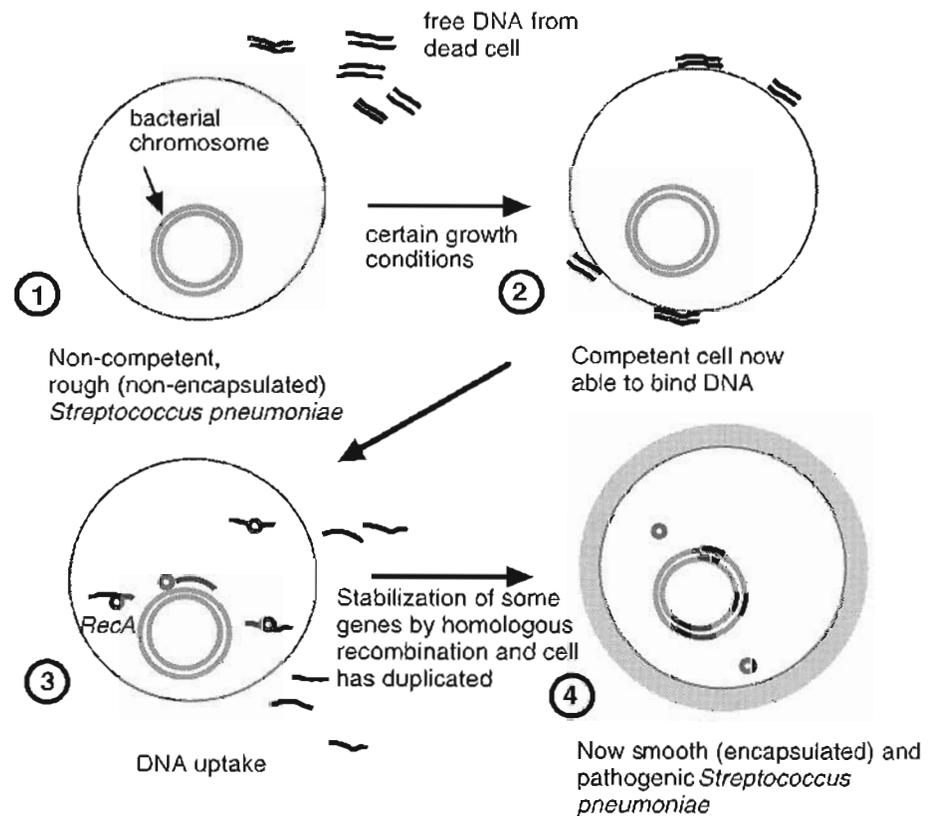


- Now *pap⁺* (initially linked to *ami^s*) is linked to *ami^r* instead, producing a new combination of genes and more significantly, a cell that can cause pyelonephritis and is amikacin resistant.
- (Could also have yielded *E. coli* that was *pap⁻ ami^s* or *pap⁻ ami^r* or the cell could have stayed *pap⁺ ami^s*.)

Transformation

Transformation is the uptake of naked DNA from the environment by competent cells.

- Cells become competent (able to bind short pieces of DNA to the envelope and import them into the cell) under certain environmental conditions (which you do not need to know).
- DNA (released from dead cells) is taken up.
- Newly introduced DNA is generally linear, homologous DNA from same type of cell but perhaps one that is genetically diverse.
- The steps of transformation of a non-encapsulated *Streptococcus pneumoniae* are shown below.



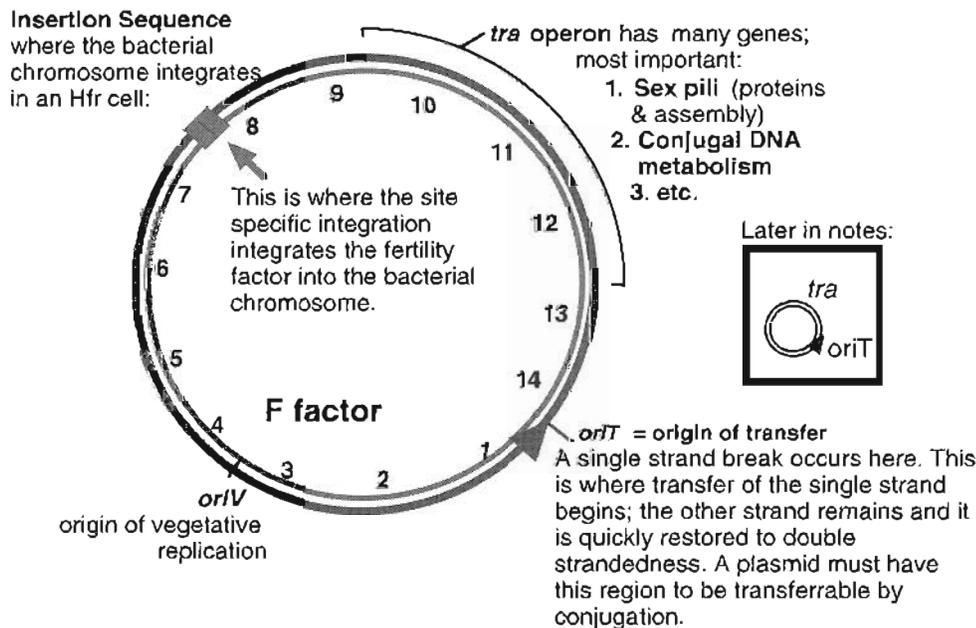
Conjugation

Conjugation is gene transfer from one bacterial cell to another involving direct cell-to-cell contact.

- **Fertility factors control conjugation**
- Sex pili (genes on F factor) play a role in establishing cell-to-cell contact.
- A single strand (or a portion thereof) of the double helix of DNA is transferred from the donor (or male) cell to the recipient or female cell.
- Bacterial genes transferred in by conjugation have to be stabilized by homologous recombination (i.e., in an Hfr × F⁻ cross). Plasmid genes transferred by conjugation circularize and are stable without recombination.
- Conjugation with recombination may produce new genetic combinations.

Donor (Male) Cells

- ALL have fertility plasmids known as F factors. F factors have a series of important plasmid "fertility" genes called the transfer or *tra* region which code for:
 - sex pili
 - genes whose products stabilize mating pairs
 - genes which direct conjugal DNA transfer, and other genes.
- Have a region called *oriT* (origin of transfer) where a single strand break in the DNA will be made and then *oriT* begins the transfer of one strand of the double helix.
- Many have insertion sequences where the plasmid can be inserted into the bacterial chromosome combining to make one larger molecule of DNA.
- A genetic map of an F factor is shown below.

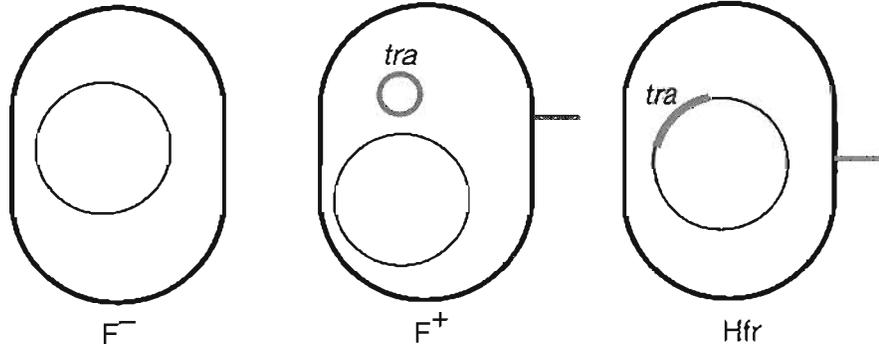


- Donor cells in which the fertility plasmid is in its free state are called F⁺ cells.
- Donor cells in which the fertility factor has inserted itself into the bacterial chromosome are called Hfr cells.

Recipient (Female) Cells: F⁻ Cells

- Recipient cells lack fertility factors and genes.
- In every cross, one cell must be an F⁻ cell.

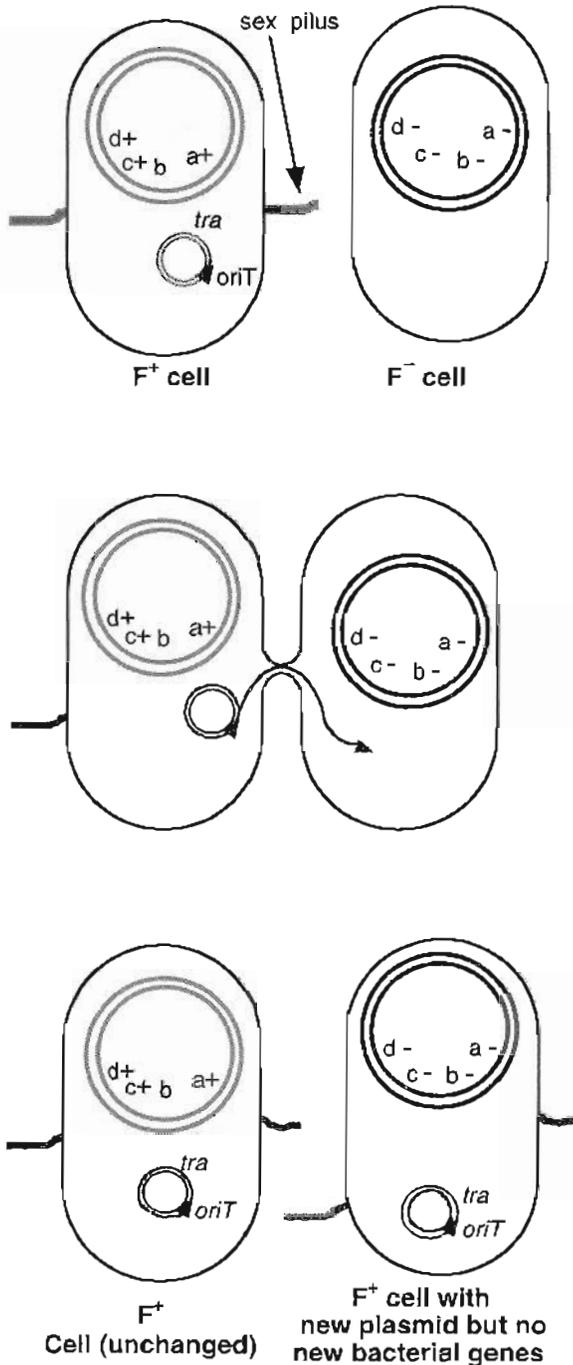
Mating Types



CONJUGAL CROSSES

There are two major types of crosses:

Conjugation: $F^+ \times F^-$ Mating



- 1 Important points: In the male or F^+ parent, the fertility factor is present but free from the bacterial chromosome. Transfer is uni-directional from male to female. *oriT*, as in every cross, will be transferred first and then the rest of the plasmid genes.
- 2 Note only a single strand of the plasmid DNA duplex is transferred. The area that is lost is reduplicated (shown as dotted lines) so that the donor always stays the same genotype. The last genes to be transferred are the *tra* region.
- 3 The transfer of the plasmid is fairly quick so assume that it is transferred in its entirety 100% of the time unless otherwise told. Note that the F^- cell undergoes a sex change becoming F^+ (male). These two F^+ cells can no longer mate. But no BACTERIAL genes are transferred.

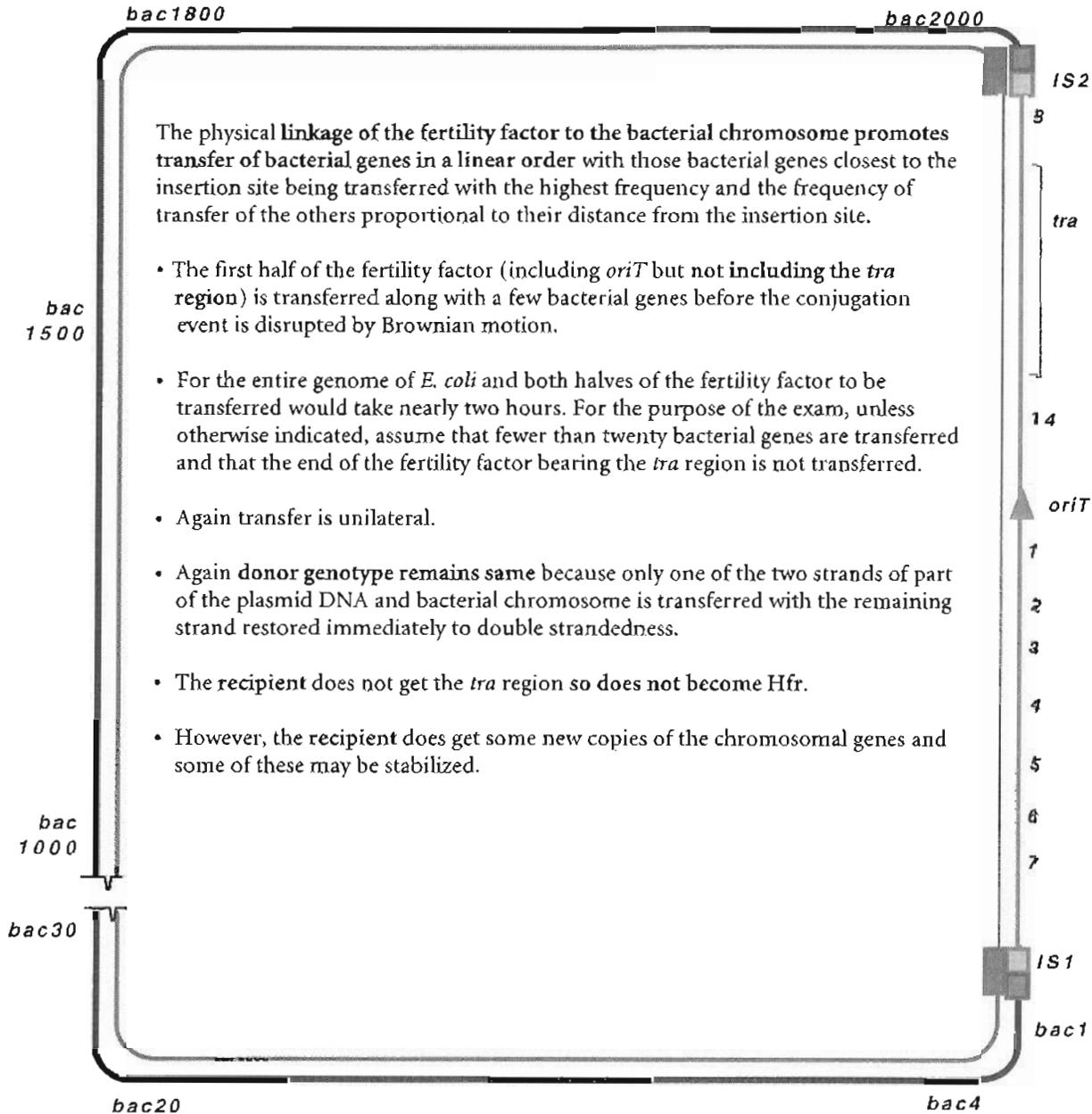
Integrated Fertility Factor

Hfr Chromosome

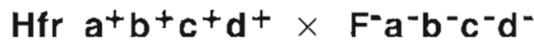
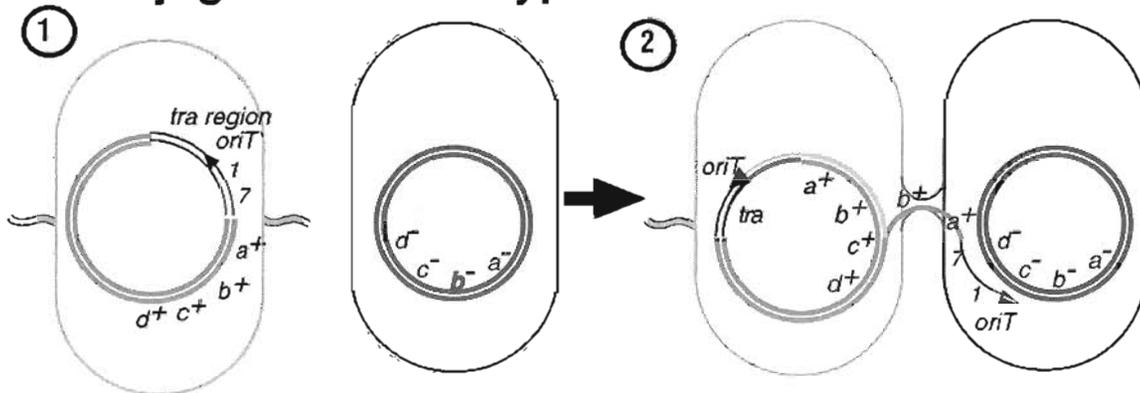
(Bacterial chromosome with integrated F factor)



Bacterial genes are represented as *bac##* to remind you that there are generally several thousand bacterial genes and that this molecule of DNA is very large.

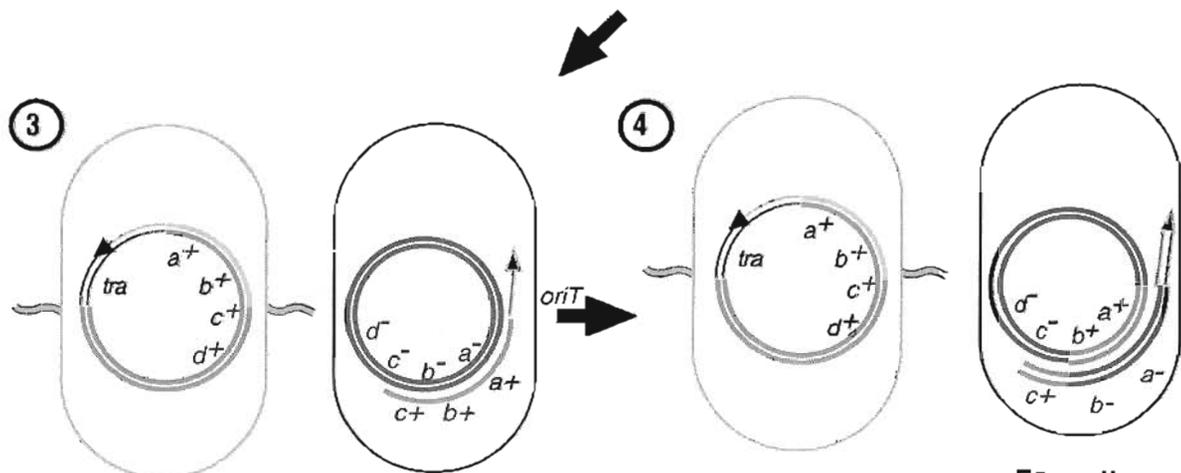


Conjugation: 2nd type of cross Hfr × F⁻



Important points: Fertility factor is integrated into the bacterial chromosome. In this cross *oriT* and the first half of the fertility factor (regions 1-7 on the F factor) will be transferred first (and in that order) and then the bacterial genes in the linear order away from the plasmid.

Note, that as with the F⁺ × F⁻ cross, only a single strand of the DNA duplex is transferred. The area that is lost is reduplicated so that the donor always stays the same genotype. The last genes to be transferred would be the *tra* region.



It takes approximately two hours for a complete transfer to occur. Because the cytoplasmic bridge and DNA strand is so fine, mating is normally interrupted before the transfer is complete. For the purpose of exam, assume that mating is interrupted and the recipient gets some new genes but does not become Hfr.

**Hfr cell
(unchanged)**

**F⁻ cell
with new
bacterial genes:
a⁺ and b⁺
(No sex change)**

Transduction

Transduction is the transfer of bacterial DNA by a phage vector.

The phage picks up the bacterial DNA through an error in phage production.

There are two types of transduction: generalized and specialized.

A generalized transducing phage is produced by the phage putting a piece of bacterial DNA into its head. All genes have an equal chance of being transduced.

- Specialized transduction is dependent on integration of phage DNA into the bacterial chromosome at a specific site and then an error being made in its excision as the phage begins to replicate lytically.

To understand transduction, you need first to understand how a phage replicates normally so that you can understand how the errors are made.

Phage

= bacteriophage = bacterial virus

Come in two major types:

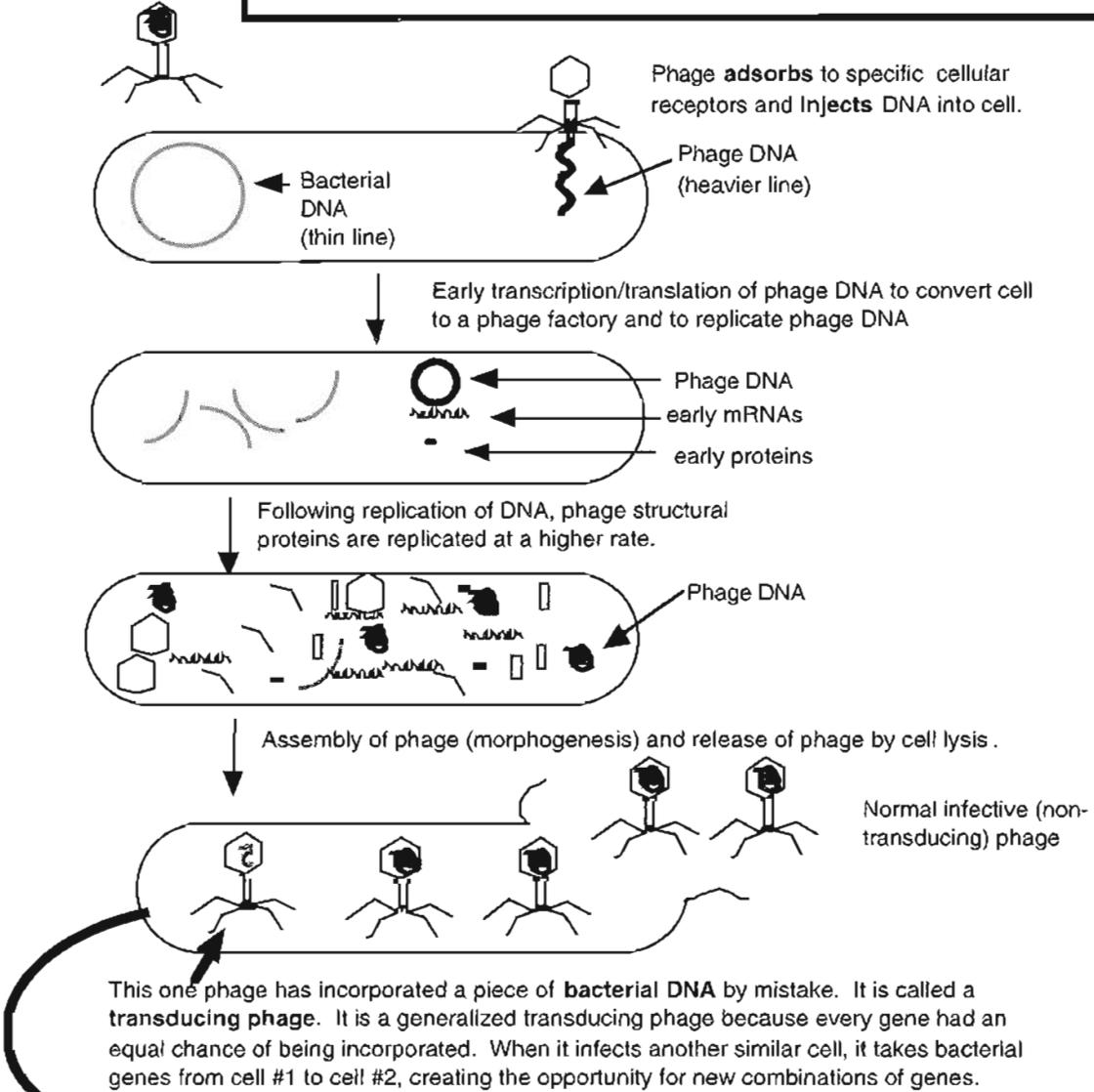
- **Virulent phage** infect bacterial cells, always making more virus and lysing the cells (lytic replication).
- **Temperate phage** often infect without lysing the cells because they have the ability to repress active phage replication and to stably integrate their DNA into the bacterial chromosome. In the absence of functional repressor protein, they also may replicate lytically.

Lytic Infection

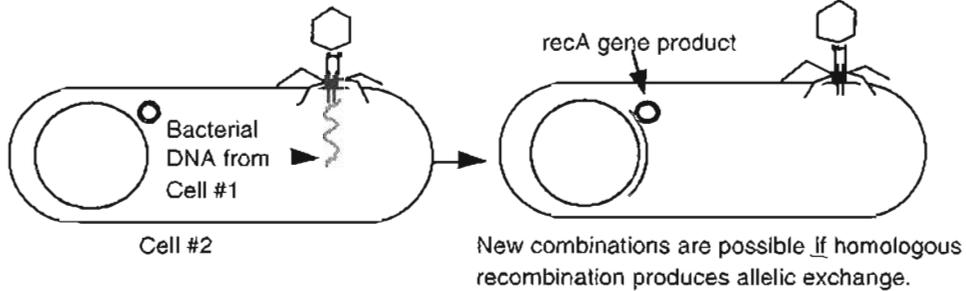
Lytic infection, by phage or viruses, leads to production of viruses and their release by cell lysis.

- **Virulent viruses can only go into lytic life and can only carry out generalized transduction.**
- The lytic (or productive) life cycle of virulent phage is shown below. It is entirely normal except for a mistaken incorporation of bacterial DNA into one phage head, creating a transducing virus, shown at the bottom of the next page. Transduction of another bacterial cell is shown following that.

Lytic cycle
Shown with the rare production of a generalized transducing phage.



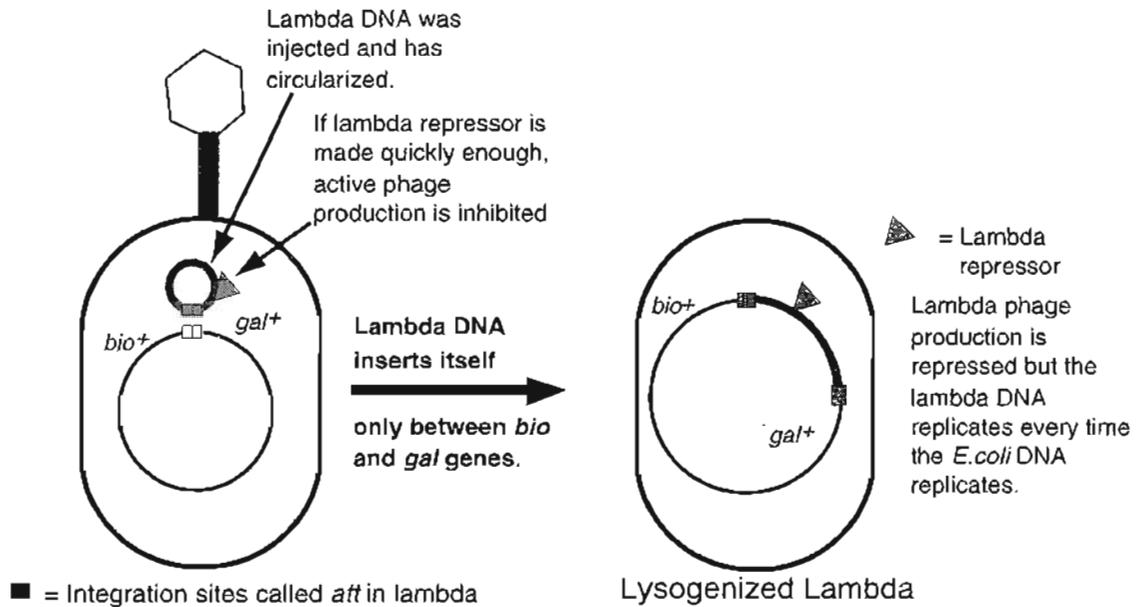
Generalized Transduction



Lysogeny and Specialized Transduction

Temperate phage may become prophage (DNA stably integrated) or replicate lytically.

- When repressor is made, temperate phage insert their DNA into the bacterial chromosome where it stably stays as a prophage.
- If the repressor gene gets mutated or the repressor protein gets damaged then the prophage gets excised from the bacterial DNA and is induced into the lytic production of virus. On rare occasions these temperate phage can produce either specialized or generalized transducing viruses. Lambda phage of *E. coli* is the best studied. Most temperate phages have only a single insertion site.
- Lambda inserts ONLY between *E. coli* genes *gal* and *bio* as shown below.



Lysogeny

Lysogeny is the state of a bacterial cell with a stable phage DNA (generally integrated into the bacterial DNA), **not undergoing lytic replication** either because it is repressed or defective. When the cell DNA replicates, the phage DNA also replicates and, as long as the repressor protein is not damaged, the lysogenic state continues ad infinitum. Defective phage (or defective viruses in the human equivalent) cannot go into an active replication unless a helper virus is present.

Phages that have both options (lytic replication or lysogeny) are called temperate phages. When a temperate phage first infects a cell there is a regulatory race that determines whether the repressor is made fast enough to prevent synthesis of phage components.

The lysogenized cell will replicate to produce two identical cells each with a prophage as long as the repressor gene product is present.

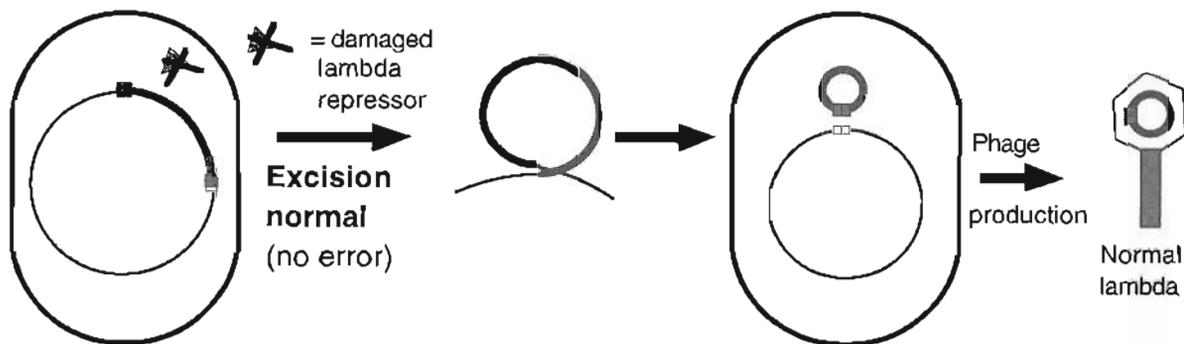
Significance of lysogeny:

- Can confer new properties to a genus such as toxin production or antigens:
 - O: Presence of specific prophage in *Salmonella* can affect O antigens.
 - B: Phage CE β or DE β cause *Clostridium botulinum* to produce Botulinum toxin.
 - E: Exotoxins A–C (erythrogenic or pyogenic) of *Streptococcus pyogenes*
 - D: Prophage beta causes *Corynebacterium diphtheriae* to make Diphtheria toxin.
- (Mnemonic for phage-mediated pathogenic factors = OBED)
- Model for retrovirus provirus
- Allows specialized transduction

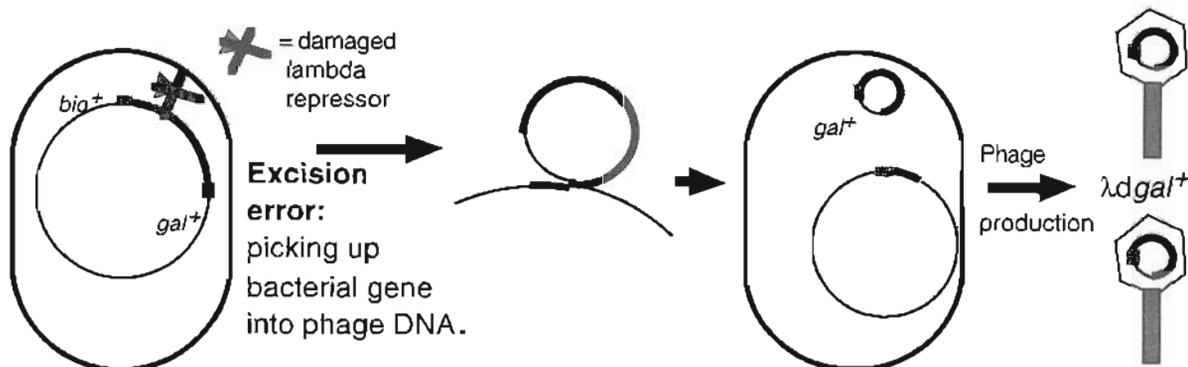
Induction

If the repressor is damaged (by UV, cold, or alkylating agents), then the prophage is excised and the cell goes into lytic replication of the phage. This process is called induction.

Most of the time this process is carried out perfectly as below and the cell produces perfect (non-transducing) normal phage.



Rarely, in the excision process, an excisional error is made and one of the bacterial genes next to the insertion site is removed attached to the lambda DNA and a little bit of lambda DNA is left behind.



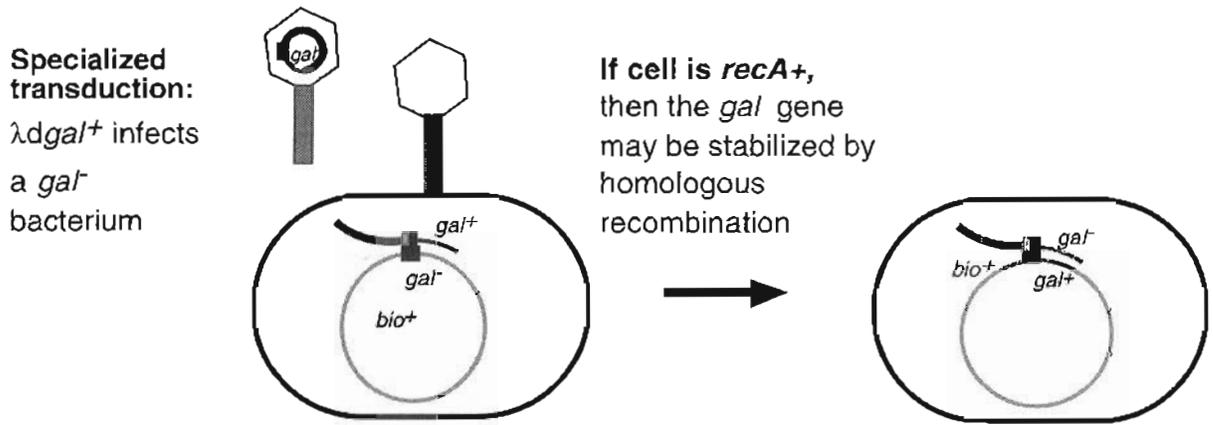
Because lambda has only one insertion site (between *gal* or *bio*), only *gal* or *bio* can be incorporated by excisional error.

Because all of the phage genes are still in the cell, phage are still made with the circular defective phage genome copied and put in each phage head. These are **specialized transducing phage** (only able to transduce *bio* or *gal*).

Induction with an excisional error is shown above.

Specialized Transduction

Bacterial genes picked up by error in the excision process are transferred to another generally closely related but often genetically distinct cell. If any genes on the exogenote are stabilized by recombinational exchange, then new genetic combinations occur.



Only those genes next to the phage insertion site can be transduced by specialized transduction.

Table I-6-1. Comparison of Transformation, Conjugation, and Transduction

Requirement	Transformation	Conjugation	Transduction
Is cell-to-cell contact required?	No	Yes	No
Does it require an antecedent phage infection?	No	No	Yes
Is competency required?	Yes	No	No
Is naked (free) DNA involved?	Yes	No	No
Is recombination required to stabilize new genes?	Yes	No for F ⁺ × F ⁻ Yes for Hfr × F ⁻	Yes

Table I-6-2. Comparison of Generalized and Specialized Transduction

	Generalized	Specialized
Mechanism	Error in assembly	<u>Error of excision</u> Requires stable insertion of prophage DNA (lysogeny)
What genes may be transferred?	Any	Only genes next to the insertional site

DRUG RESISTANCE

Overall problem

- Drug resistance is becoming such a significant problem that there are bacteria for which most antibiotics no longer work. Experts have begun to discuss the “post-antibiotic era.”
- Drug resistance can be transferred from one genus of bacteria to another, e.g., from your normal flora to a pathogen.
- Three general types of antibiotic resistance: intrinsic, chromosomal-mediated, and plasmid-mediated.

Intrinsic Drug Resistance

For example, a cell which has no mycolic acid will not be inhibited by isoniazid.

Chromosomal-Mediated Antibiotic Resistance

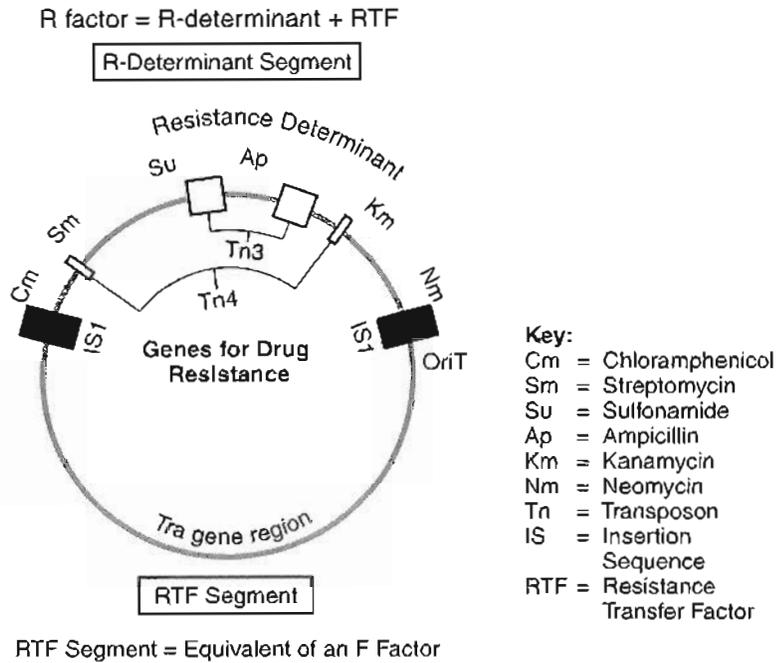
The genes that determine this resistance are located on the bacterial chromosome.

- Most commonly these genes modify the receptor for a drug so that the drug can no longer bind (e.g., a mutation in a gene for a penicillin binding protein [normal cell wall synthetic enzyme]).
- In general, causes low level drug resistance rather than high (exception: methicillin resistance in *Staphylococcus aureus* where a major PBP was mutated).
- But low level resistance may be clinically significant, e.g., in *Streptococcus pneumoniae* meningitis.

Plasmid-Mediated Drug Resistance

The genes that determine this resistance are located on plasmid.

- R factors are conjugative plasmids carrying genes for drug resistance.
 - One section of the DNA (containing *oriT* and the *tra* gene region) mediates conjugation.
 - The other section (R determinant) carries genes for drug resistance. Multiple genes seem to have been inserted through transpositional insertion into a “hot spot.”
 - A typical genetic map of an R factor (a conjugative drug-resistant plasmid) is shown on the next page:
- Plasmid-mediated resistance is created by a variety of mechanisms but often genes code for enzymes that modify the drug.



- Nonconjugative plasmids
 - Have lost their *tra* operon (genes) so have lost the ability to DIRECT conjugation.
 - But as long as they retain their *oriT*, nonconjugative plasmids may actually be transferred by conjugation as long as there is another fertility factor in the same cell with a functional *transfer* region.
 - The process may be referred to as **mobilization** and is able to occur because the genes in the *tra* region are soluble gene products that are **trans acting**. The region *oriT*, by contrast, is **cis acting**.

Table I-6-3. Plasmid-Mediated Mechanisms

Antimicrobial Agent	Mechanism
Penicillins and cephalosporins	Production of β -lactamase; cleavage of β -lactam rings
Aminoglycosides	Production of acetyltransferase, adenosyltransferase, or phosphotransferase; inactivation of drug by acetylation, adenosylation, or phosphorylation
Chloramphenicol	Production of acetyltransferase; inactivation of drug by acetylation
Tetracyclines	Increased efflux out of cell
Sulfonamides	Active export out of cell and lowered affinity of enzyme

Transfer of Drug Resistance

Neisseria gonorrhoeae

Nonconjugative plasmids: The segment with the *tra* operon has separated leaving a plasmid with the genes for drug resistance still linked to *oriT*.

Transferred by conjugation (mobilization): As long as the cell still has the *tra* region in the cell, it can direct conjugation, make the nick at *oriT* and mobilize the transfer of the nonconjugative plasmid.

Staphylococcus aureus (Methicillin Resistant = MRSA)

This is chromosomal (methicillin) transferred by transduction. Most of the antibiotic resistance is transferred by plasmids.

Gram-Negative Bacilli

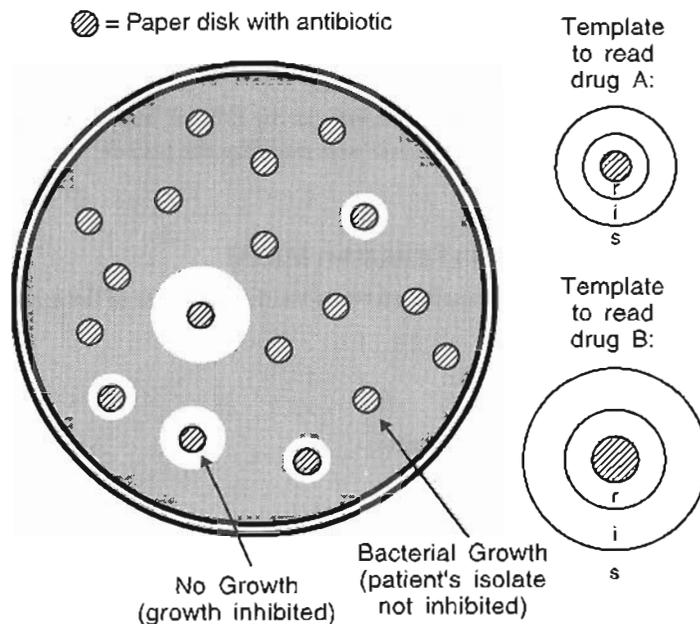
Plasmid mediated, transferred by conjugation.

ANTIBIOTIC SUSCEPTIBILITY TESTING

Kirby Bauer Agar Disk Diffusion Test

- Solid medium with patient's isolate swabbed on the entire plate surface
- Multiple paper disks, each with a single dried drug placed on plate
- Hydration and diffusion of drug set up a concentration gradient during incubation and growth of the bacteria.
- The diameter of the zones of inhibition must be measured to determine significance.
- Only qualitative (reported back as susceptible, intermediate, or resistant)
- Advantages: relatively cheap, easy, can test numerous antibiotics on one plate, wealth of information based on clinical correlation
- Disadvantage: qualitative

Antibiotic Susceptibility Testing Kirby Bauer agar diffusion plate



“Rapid” Methods

Testing for specific enzymes and a very few probes for genes determining drug resistance are currently available but still require a culture of the patient’s pathogen. One current example is β -lactamase testing, shown below.



Minimal Inhibitory Concentration (MIC)

MIC measures antibiotic inhibition.

- This is a dilution technique where each container (well of microtiter plate, test tube, or automated system bottle) has one concentration of an antibiotic with the patient’s isolate. Always one control container has just the patient’s isolate and growth medium with no antibiotic to make sure the inoculum is viable.
- Lowest concentration showing no visible growth is the MIC.
- In the example below, MIC = 2 $\mu\text{g/ml}$.
- This indicates levels needed to inhibit; it does not necessarily indicate killing levels, which is done with the MBC (see below).

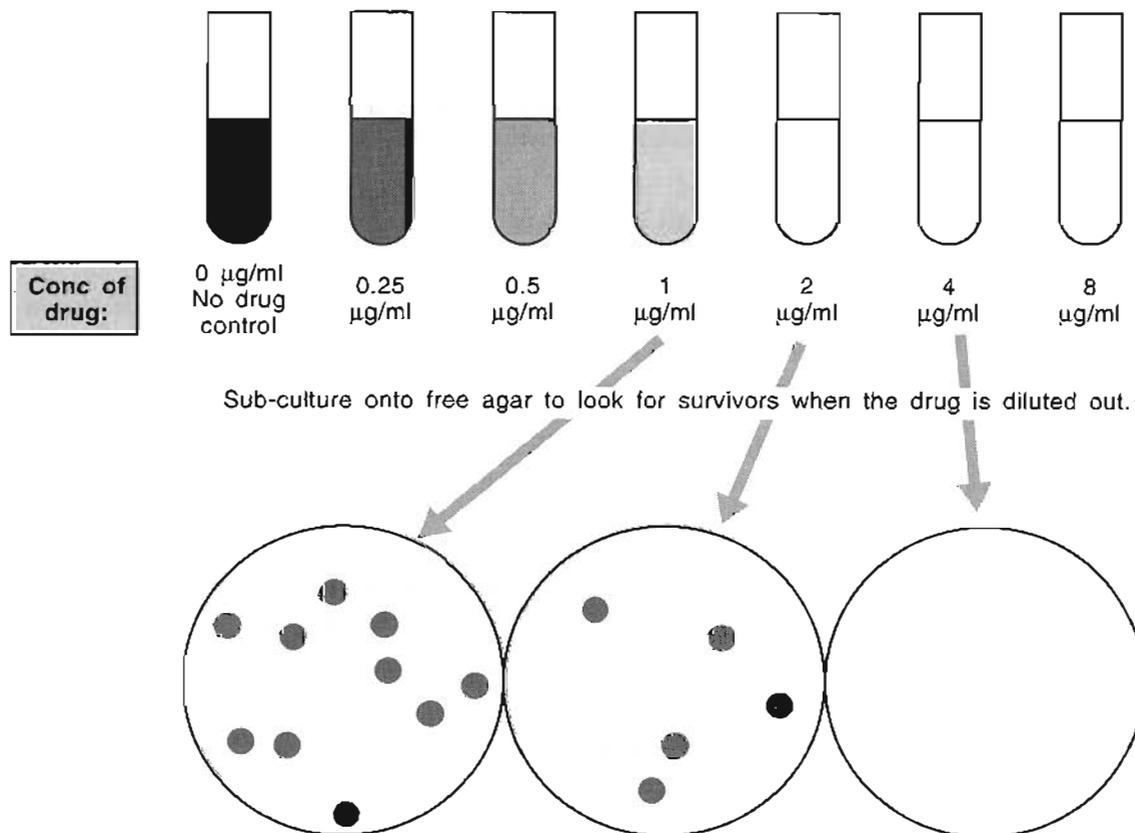
Minimal Bacteriocidal Concentration (MBC)

This measures the antibiotic killing (bacteriocidal activity).

- A dilution technique starting with the MIC containers and sub-plating onto solid medium. Because a small inoculum is used on the plate with a large volume of medium, this dilutes the drug way below the MIC and allows determination of viability of cells.
- Important to determine for treating immunocompromised patients whose immune system cannot kill the bacteria while they are inhibited.
- The **MBC is the lowest antibiotic concentration showing no growth on subculture to media without the antibiotic.** In the example below, the MBC would be 4 $\mu\text{g/ml}$.
- Formulas are increasingly replacing this test.

Minimal Inhibitory Concentration = MICs

1. Each container has one concentration of a drug.
2. Each container has identical inoculum of the patient's bacterial isolate
3. Must run a no drug control.
4. Lowest concentration showing no visible growth = MIC
(in example, MIC = 2 $\mu\text{g/ml}$)



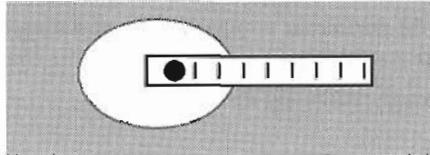
Minimal Bacteriocidal Concentration (MBC)

(Not routinely done in many hospitals but ordered when necessary)

The lowest drug concentration showing no growth on sub-culture to media without drugs = MBC
MBC in example would be 4 $\mu\text{g/ml}$.

E-Test (Agar Diffusion)

E-test uses a strip of plastic marked with a gradient unique to each antibiotic that has the dot of dried antibiotic on the underside. This is placed on an agar plate already swabbed with the patient's isolate and read after incubation. It produces a $\mu\text{g/ml}$ value that correlates fairly well with the Minimal Inhibitory Concentration.



Sterilization, Disinfection, Pasteurization

Sterilization: complete removal or killing of all viable organisms.

Disinfection: the removal or killing of disease-causing organisms. Compounds for use on skin: antiseptics.

Pasteurization: the rapid heating and cooling of milk designed to kill milk-borne pathogens such as *Mycobacterium bovis*, *Brucella*, and *Listeria*.

Physical Methods of Control

Heat = saturated steam

- Autoclaving (steam under pressure): 15 lbs pressure \rightarrow 121°C 15–20 min (sterilizing)
- Dry heat—2 hr 180°C

Radiation

- UV: formation of thymine–thymine pairs on adjacent DNA strands

Filtration

- HEPA (High Efficiency Particulate Air) filters for air
- Nitrocellulose or other known pore-size filters
 - 0.45 μm filters out most bacteria except Mycoplasmas and other cell wall-less forms.
 - 0.22 μm will filter out all bacteria and spores.

Chemical Methods of Control

Agents damaging membrane

- **Detergents:** (surface active compounds) most notable the quaternary ammonium compounds like benzalkonium chloride—interact with membrane through hydrophobic end disrupting membrane.
- **Alcohols:** disrupt membrane and denature protein.
- **Phenols and derivatives:** damage membrane and denature proteins.

Agents modifying proteins

- **Chlorine:** oxidizing agent inactivating sulfhydryl-containing enzymes
- **Iodine and iodophors** (which have reduced toxicity): also oxidation of sulfhydryl-containing enzymes
- **Hydrogen peroxide:** oxidizing agent (sulfhydryl groups); catalase inactivates
- **Heavy metals:** (silver and mercury) bind to sulfhydryl groups inhibiting enzyme activity
- **Ethylene oxide:** alkylating agent (sterilizing agent)
- **Formaldehyde and glutaraldehyde:** denatures protein and nucleic acids and alkylates amino and hydroxyl groups on both

Modification of nucleic acids

- **Dyes:** like crystal violet and malachite green whose positively charged molecule binds to the negatively charged phosphate groups on the nucleic acids

Chapter Summary

The definitions of polymerases, nucleases, and alleles and the flow of genetic information are briefly reviewed.

Three types of DNA may be found in a bacterial cell: chromosomal, plasmid, and bacteriophage DNA.

The chromosomal DNA contains all the essential bacterial genes. Most bacteria have one chromosome but may have multiple copies of it. The chromosomal DNA exists as a large covalently closed circular strand, looped around a proteinaceous center, and contains about 2,000 genes. Each loop can be transcribed independently.

Plasmids are small, covalently closed circular DNAs that replicate autonomously and may be transferred from one bacterium to another. Episomes are a type of plasmid integrated into the bacterial chromosome. Plasmids may code for fertility factors, antibiotic resistance, and exotoxins.

Bacteriophage (viral) DNA may be inserted into the bacterial chromosome as a prophage by a temperate virus. Such an inserted viral gene sometimes directs the synthesis of a virulence factor, making the bacterium more pathogenic.

Transposons are elements of DNA that independently move from one site to another on a plasmid or chromosome. Insertion may cause mutations in unrelated genes and may cause repeats on both sides of the transposon. Transposons may carry genes for drug resistance and play an important role in the development of multiple drug resistance.

Homologous recombination is a process in which linear DNA introduced by transformation, conjugation, or transduction is exchanged for near-homologous DNA on the chromosome. This stabilizes the newly introduced DNA, which as a consequence has now become an integrated part of the bacterial genome.

Site-specific recombination is the integration of a nonhomologous circular DNA molecule into the bacterial genetic material.

Excision is the reversal of site-specific recombination in which an integrated prophage or plasmid may be removed from the bacterial genome.

(Continued)

Chapter Summary (continued)

New genetic information is introduced into bacterial cells by gene transfer followed by recombination. The modes of gene transfer are transformation, conjugation, and transduction.

Transformation is the incorporation of naked DNA from the environment. This process is probably of minor biologic import but was used to transform rough Streptococcal cells into smooth ones in the now classic experiments that confirmed the genetic role of DNA.

Conjugation is the direct transfer of DNA from one bacterial cell to another.

Conjugation occurs by direct contact mediated by a fertility (F) plasmid. Donor (male) cells are F⁺, and receptor (female) cells are F⁻. A strand of the plasmid from the F⁺ cell is transferred across the conjugal bridge formed by a sex pilus. The net result is that both cells end up with a complete copy of the plasmid.

A second type of conjugation occurs when a high-frequency recombination (Hfr) donor cell, which has its F factor incorporated into its chromosome, contacts an F⁻ recipient cell. In this case, the attempt to transfer the whole genome is aborted before completion, and only a portion of the genetic material is transferred and incorporated into the recipient's chromosome.

Transduction is the transfer of bacterial DNA by a bacterial virus (phage). Phages are either virulent (lytic) or temperate (lysogenic). Virulent phages always replicate and lyse their hosts. Temperate phages can repress their lytic behavior and stably integrate their DNA into the bacterial chromosome. If anything interferes with the repressive mechanisms, they revert to a lytic form. Generalized transduction occurs when a lytic phage lyses a bacterium and some bacterial DNA accidentally recombines with phage DNA and is subsequently transferred into another bacterial cell. Specialized transduction occurs when the excision of integrated phage DNA (a prophage) includes some chromosomal DNA. This sometimes occurs when a temperate virus is induced to become lytic by damage to the repressor system or infection with a "helper" virus.

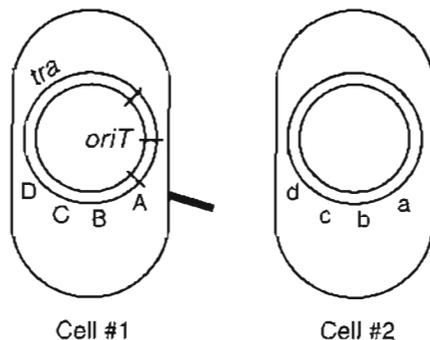
Drug-resistant bacteria have become a major medical problem. Drug resistance arises by three different mechanisms: intrinsic, chromosome-mediated, or plasmid-mediated. Intrinsic resistance is that which is normal for a species. Chromosome-mediated resistance is brought about by mutation, and plasmid-mediated resistance is transferred to a bacterium by the transduction or conjunction of plasmid DNA associated with an R-factor. Transfer of plasmid DNA is the most important way of dispersing drug resistance among bacteria.

Antibiotic susceptibility may be tested by the Kirby-Bauer agar diffusion test, by testing for specific resistance causing enzymes, by the minimal inhibitory concentration test, by the minimal bacteriocidal test, or by the E (agar diffusion) test.

Pathogenic contamination may be controlled by sterilization or disinfection. Methods of control include heating (e.g., autoclaving, dry heat, or pasteurization); radiation, filtration, or chemicals that destroy the membrane (e.g., detergents, alcohols, or phenols); denaturing proteins (e.g., chlorine, iodine and iodophors, H₂O₂, heavy metals, ethylene oxide, or formaldehyde); or modifying nucleic acids (dyes).

Review Questions

- What type of genetic material is created by repeated transpositional recombination events?
 - Chromosomal drug resistance genes
 - Genetic operon
 - Hfr chromosome
 - Insertion sequences
 - Multiple drug resistance plasmids
- Which genetic material is found in pathogenic *Corynebacterium diphtheriae* but not in nonpathogenic *C. diphtheriae*?
 - A diphthamide on EF-2
 - An episome
 - An F factor
 - An integrated temperate phage
 - Highly repetitive bacterial DNA
- How is a prophage created?
 - Through activation of the *recA* gene product of an exogenote
 - Through infection of a bacterial cell with a virulent bacteriophage
 - Through site-specific recombination of a temperate phage and bacterial DNA
 - Through infection of a bacterial cell with lambda phage, lacking the lambda repressor
 - Through excision of bacterial DNA and active lytic replication of a bacteriophage
- If one cell of type one (figure below) is mixed into a culture of 100 cells of type two (below), and culture conditions are optimized for conjugation BUT NOT for cell division, the cellular genotype that would predominate after overnight incubation would be that of
 - Cell #1
 - Cell #1 with new a, b, c, and d alleles
 - Cell #2 with new A, B, C, and D alleles
 - Cell #1 with a new a allele
 - Cell #2 with a new A allele
 - Cell #1 with new a and b alleles
 - Cell #1 with new A and B alleles



5. Assume the following cells have no other plasmids other than those mentioned. Which cell type would contain two molecules of DNA?
 - A. F⁺
 - B. F⁻
 - C. Hfr

6. Assume the cells whose genotype is listed below have no other plasmids than those indicated by the indicated genotype. Which bacterial cell is most likely to transfer chromosomal genes in linear order?
 - A. F⁺
 - B. F⁻
 - C. Hfr

7. What bacterial gene transfer process is most sensitive to extracellular nucleases?
 - A. Conjugation
 - B. Generalized transduction
 - C. Homologous recombination
 - D. Site-specific recombination
 - E. Specialized transduction
 - F. Transformation

8. Following specialized transduction, if any of the bacterial genes transferred in are to be stabilized, what process must occur?
 - A. Conjugation
 - B. Generalized transduction
 - C. Homologous recombination
 - D. Site-specific recombination
 - E. Specialized transduction
 - F. Transformation

9. The ability of a cell to bind DNA to its surface and import it is required for which genetic process?
 - A. Conjugation
 - B. Generalized transduction
 - C. Homologous recombination
 - D. Site-specific recombination
 - E. Specialized transduction
 - F. Transformation

10. Process by which bacterial or plasmid DNA may be mistakenly incorporated (during assembly) into one phage being produced by the lytic life cycle and then that DNA-transferred to another bacterial cell which may acquire some new genetic traits is called
- Conjugation
 - Generalized transduction
 - Homologous recombination
 - Site-specific recombination
 - Specialized transduction
 - Transformation
11. Recombination is required for stabilization of genetic material newly transferred by all of the following processes EXCEPT
- Movement of a transposon
 - Integration of a temperate bacteriophage
 - Transduction of a chromosomal gene
 - Conjugal transfer of an R factor
 - Transformation of a chromosomal gene
12. Lysogenic conversion
- is a change in pathogenicity due to the presence of a prophage.
 - is the induction of a prophage to its virulent state.
 - is the conversion of a virulent phage into a temperate phage.
 - refers to the incorporation of a prophage into the chromosome.
 - is the immunity that a prophage confers on a bacterium.
13. Which of the following events is most likely due to bacterial transformation?
- A formerly non-toxicogenic strain of *C. diphtheriae* becomes toxigenic.
 - A non-encapsulated strain of *Streptococcus pneumoniae* acquires a gene for capsule formation from an extract of an encapsulated strain.
 - A strain of *Neisseria gonorrhoeae* starts producing a β -lactamase encoded by a plasmid similar to a plasmid of another Gram-negative strain.
 - A gene for gentamicin resistance from an *E. coli* chromosome appears in the genome of a virulent bacteriophage that has infected it.
14. Which of the following mechanisms is most likely to be involved in multiple drug resistance transfer from one cell to another?
- Specialized transduction of a chromosomal gene for drug resistance
 - Transformation of chromosomal genes
 - Transposition
 - Conjugation with one parent with a free plasmid carrying drug resistance
 - Conjugation with one parent with chromosomal drug resistance

15. Which of the following agents, if introduced into a growing culture of bacteria, would halt growth but, if then removed, would allow growth to resume?
- A. Antiseptic
 - B. Bacteriocide
 - C. Bacteriostat
 - D. Disinfectant
 - E. Sterilizing Agent

Answers

1. **Answer: E.** Transposition or transpositional recombination is a form of site-specific recombination and is largely responsible for the creation of multiple drug resistant plasmids. Chromosomal drug resistance may arise by movement of a plasmid gene to the chromosome, but it is usually just a solitary gene and not a repetitive event. The Hfr chromosome arises through a single site-specific integration of a fertility factor with the bacterial chromosome.
2. **Answer: D.** This question is asking what carries the genetic code (or, more simply, codes) for diphtheria toxin, which must be some kind of DNA, which in turn means that the protein EF-2 can be immediately eliminated. The diphthamide on EF-2 is actually the substrate for the ADP-ribosylation done by the diphtheria toxin. Genes expressing the diphtheria toxin originally enter *C. diphtheriae* as part of the DNA of the temperate corynebophage. Integration of this temperate phage results in a stable prophage, which directs the production of the diphtheria toxin.
3. **Answer: C.** Site-specific recombination of phage DNA into bacterial cell DNA by the process of lysogeny creates a prophage. The RecA gene product is necessary for homologous recombination with an exogenote but does not create a prophage. A virulent bacteriophage causes lysis of the host cell and not the production of prophage. The lambda phage is a temperate phage, which can cause lysogeny of infected cells, but the lambda repressor is necessary in such cases to prevent the lytic life cycle. Choice E might be the pathway a prophage may choose to reinitiate its bacteriophage lytic lifestyle, but it would not be a means to create a prophage.
4. **Answer: E.** This hypothetical condition describes the mixing of one Hfr cell with 100 F⁻ recipients. Over time, with no cell division occurring, the one Hfr cell would repeatedly conjugate with the F⁻ cells and transfer one strand of its chromosomal DNA in sequence, beginning with oriT and theoretically ending with the tra genes. The most frequently transferred bacterial genes also have the greatest likelihood of successful recombination; they are those closest to oriT; in this example, the A allele. The entire chromosome is so large that it is virtually never transferred in its entirety and thus, the tra genes would not be transferred. (Even if tra genes were transferred, oriT and tra genes have no homologous regions in the recipient cell chromosome and so would not successfully recombine within.) Thus, the recipient cell acquires only new chromosomal alleles and NOT the whole fertility factor and never changes phenotype to become an Hfr cell. Therefore, any of the answers with cell one (the Hfr parent) as the dominant type would be wrong.

The genes are transferred in linear order, so choice A will always be transferred more frequently than any of the later genes.

Therefore, given sufficient time for conjugation, the cell type that would be most numerous is that of the recipient genotype with a newly acquired allele close to oriT. This means

that the best answer is choice F: cell two with a new A gene. The farther from oriT that the allele is, the less likely that it will be successfully transferred. The distractor, choice C, with all four alleles transferred in, is less likely.

5. **Answer: A.** The F^+ cell would contain both the bacterial chromosome and the fertility factor. The other two would just each have the bacterial chromosome (F^-) or the single DNA molecule of the chromosome with the integrated fertility factor.
6. **Answer: C.** Only F^+ and Hfr can donate genes to a recipient or F^- cell. The F^+ cell would transfer only plasmid genes. The Hfr would be the only one likely to transfer chromosomal genes.
7. **Answer: E.** The nucleic acid from the donor cell is not protected from the environment either by a cell or by a phage coat, but is instead naked and therefore subject to nucleases.
8. **Answer: C.** The DNA is transferred in as a linear piece and must be stabilized by homologous recombination.
9. **Answer: E.** The statement fits the definition of competency required for transformation.
10. **Answer: B.** This obviously is transduction, but what are your clues? First, it says "one phage" rather than all the phage in the cell (as for specialized). Then it also said plasmid DNA could be picked up. For specialized transduction, only episomal plasmid DNA (incorporated into the bacterial chromosome near an attachment site) could be picked up.
11. **Answer: D.** Transpositional movement actually involves a type of recombination called transposition that is a form of site-specific recombination. Site-specific recombination is also involved in integration of a temperate bacteriophage. Both transformation and transduction require homologous recombination as would transfer of Hfr DNA by conjugation. But either F factor or R factor DNA circularizes when it enters a new cell and thus is stable without recombination even as circular DNA is not subject to the cellular exonucleases.
12. **Answer: A.** D is a definition of lysogeny but lysogenic conversion is when lysogeny changes the characteristic of the lysogenized organism. In medicine this usually means an increased pathogenicity from the lysogeny.
13. **Answer: B.** A would require phage infection with a temperate coryneophage. B (the answer) is most likely to occur through transformation. C is most likely to take place through a conjugal transfer. D might occur by generalized transduction.
14. **Answer: D.** Multiple drug resistance is almost always plasmid-mediated, which rules out A, B, and E. Transposition is just generally within a cell moving a copy of the DNA to another molecule of DNA within the cell.
15. **Answer: C.** This is the classic description of a bacteriostatic agent.

Clinical Infectious Disease

7

These charts are designed for self-study after the organisms have all been reviewed in class. They represent the basics used in clinical scenarios on the USMLE.

Cover the last column on each chart and write the causative agent(s) on paper. Then think about how the organism causes disease. Is there a major virulence factor?

Abbreviations Used

→ means progressing on to

~ means about or approximately

HIV+ = patient with known human immunodeficiency virus infection

Can be used for anyone who is infected but often used for those who are HIV+ but do not have frank AIDS (in other words, CD4+ count >200)

AIDS = acquired immunodeficiency syndrome (CD4+ count <200)

abd = abdominal

CF = cystic fibrosis

CMI = cell-mediated immunity

CGD = chronic granulomatous disease

IC = immunocompromised

Infl'd = inflamed

Infl'n = inflammation

i.v. = intravenous

mo = month(s)

NF = normal flora

occ = occasional

PMNs = polymorphonuclear cells

pt = patient

RBCs = red blood cells

subQ = subcutaneous

If there are multiple causative agents, at the end of the description there may be a # with the abbreviation "CA." This means you should be able to list that number. If it specifically says "species," you should give species.

Table I-7-1. Diseases of Skin, Mucous Membranes, and Underlying Tissues

Type Infection	Case Vignette/Key Clues	Common Causative Agents
Furuncles, carbuncles	Neck, face, axillae, buttocks	<i>Staphylococcus aureus</i>
	Inflamed follicles from neck down	<i>Pseudomonas aeruginosa</i> (hot tub folliculitis)
Acne vulgaris	Inflammation of follicles and sebaceous glands	<i>Propionibacterium acnes</i>
Cutaneous lesions (may be from scratching mosquito bites, cats)	Initially vesicular; skin erosion; honey crusted lesions; catalase negative organism	<i>Streptococcus pyogenes</i>
	Initially vesicular but with longer lasting bullae; catalase positive organism	<i>Staphylococcus aureus</i>
Red raised butterfly facial rash	Dermal pain, edema, and rapid spread	<i>Streptococcus pyogenes</i> (Erysipelas)
Jaw area swelling with pain, sinus tract formation, yellow granules in exudate	Associated with carious teeth, dental extraction or trauma	<i>Actinomyces israelii</i> "lumpy jaw" (Actinomycosis)
Hot inflamed tissues	Deeper tissues from extension of skin lesions or wounds including surgical	Variety of bacteria: <i>S. aureus</i> , <i>S. pyogenes</i> , Gram - rods, <i>Clostridia</i> and anaerobes (cellulitis)
Vesicular lesions	Sometimes preceded by neurologic pain	Herpes
	Sometimes large	<i>Staphylococcus aureus</i>
SubQ granulomas/ulcers/cellulitis	Tropical fish enthusiasts; granulomatous lesion	<i>Mycobacterium marinum</i>
	Cellulitis following contact with saltwater or oysters	<i>Vibrio vulnificus</i>
	Solitary or lymphocutaneous lesions, rose gardeners or florists, sphagnum moss	<i>Sporothrix schenckii</i> (Rose Gardener's disease)
	Subcutaneous swelling (extremities, shoulders), sinus tract formation, granules; multiple CA	Bacteria: <i>Actinomyces</i> , <i>Nocardia</i> , Fungi: <i>Madurella</i> , <i>Pseudallescheria</i> (Mycetoma)
Malignant pustule	Pustule → dark red fluid-filled, tumor-like lesion → necrosis → black eschar surrounded by red margin	<i>Bacillus anthracis</i>
Enlargement from lymphatic blockage	Legs or genitalia with previous painless genital chancre	<i>Chlamydia trachomatis</i> L1-3
	Fever, headache, myalgia, inflammation and then lymphadenopathy and elephantiasis of limbs or genitalia	<i>Wuchereria</i> and <i>Brugia</i> ; mosquito spread
Burns, cellulitis	Blue-green pus, grape-like odor	<i>Pseudomonas aeruginosa</i>
Wounds	Surgical wounds (clean)	<i>Staphylococcus aureus</i>
	Surgical wounds (dirty)—list groups	<i>S. aureus</i> , <i>Enterobacteriaceae</i> , anaerobes
	Trauma - list groups	<i>Clostridium</i> , <i>Enterobacteriaceae</i> , <i>Pseudomonas</i>
	Animal bites	<i>Pasteurella multocida</i>
	Cat scratches resulting in lymphadenopathy with stellate granulomas	<i>Bartonella henselae</i>
	Shallow puncture wound through tennis shoe sole	<i>Pseudomonas aeruginosa</i>
Target lesion, generally with fever, headache	(Not necrotic) rashy border; bite site	<i>Borrelia burgdorferi</i>

Table I-7-2. Ear, Nose, Throat, Upper Respiratory System Infections

Type Infection	Case Vignette/Key Clues	Common Causative Agents
Acute otitis media	Red, bulging tympanic membrane, fever 102–103°; pain goes away if drum ruptures or if ear tubes are patent. -3CA	<i>Streptococcus pneumoniae</i> <i>H. influenzae</i> (often nontypeable, recurs) <i>Moraxella catarrhalis</i>
Otitis externa	Ear pain—list of organisms	Normal flora often involved Often mixed infections: <i>Staph aureus</i> (from NF)* <i>Candida albicans</i> (from NF) <i>Proteus</i> (water organism) <i>Pseudomonas</i> (water)
Malignant otitis externa	Severe ear pain in diabetic; life threatening	<i>Pseudomonas aeruginosa</i>
Sinusitis	Sinus pain; low-grade fever	As for acute otitis media
Oral cavity disease	Painful mouth—overgrowth of spirochetes and fusiform bacteria	<i>Fusobacterium</i> and treponemes (normal oral spirochetes)
	Sore mouth with thick white coating (painful red base under); increased risk: premature infants, AIDS, IC pts, pts on antibiotics, vitamin C deficiency	<i>Candida</i>
Sore throat	Inflamed tonsils/pharynx, which may be purulent and may develop abscesses; cervical lymphadenopathy, fever, ± stomach upset; ± sandpaper rash	<i>Streptococcus pyogenes</i> (Group A Strep) Rash indicates presence of erythrogenic exotoxin A
	White papules with red base on posterior palate and pharynx, fever	Coxsackie A
	Throat looking like Strep with severe fatigue, lymphadenopathy, fever ± rash	Epstein-Barr virus (Downey type II cells)
	Low-grade fever with a 1–2 day gradual onset of membranous nasopharyngitis and/or obstructive laryngotracheitis; bull neck from lymphadenopathy; elevated BUN; abnormal ECG; little change in WBC (toxin)	<i>Corynebacterium diphtheriae</i> (diphtheria)
Common cold	Rhinitis, sneezing, coughing; list CA with seasonal peaks	Rhinoviruses (Summer–Fall) Coronaviruses (Winter–Spring)

* NF = normal flora.

Table I-7-3. Eye Infections

Type Infection	Case Vignette/Key Clues	Common Causative Agents
Eyelid	Bilateral eye lid swelling, >10% eosinophilia, muscle pain; earlier GI Sx	<i>Trichinella</i>
	Stye; 2 CA	<i>Staphylococcus aureus</i> <i>Propionibacterium acnes</i>
	Unilateral inflammation at bite site often around eye or mouth; travel to Mexico, travel to Central or South America	<i>Trypanosoma cruzi</i>
Conjunctivitis neonate	Red itchy eye(s)/pus; onset 2–5 days	Bacterial pink eye
	Red itchy eye(s)/pus; onset 5–10 days Neonate with “sticky eye”	<i>Neisseria gonorrhoeae</i> <i>Chlamydia trachomatis</i> (serotype D–K U.S.) <i>Staphylococcus aureus</i>
Conjunctivitis	Red itchy eye(s), thin exudate; pain, photophobia	Viral pink eye: adenovirus (more common than bacterial pink eye)
	Red eye, pus 3 CA	<i>S. aureus</i> Group A Strep, <i>Strep pneumoniae</i> (all Gram +) <i>Haemophilus influenzae</i> (<i>H. aegyptius</i>)
	Red eye, pus, presence of inclusion bodies in scrapings; CA with serotypes in U.S.	<i>Chlamydia trachomatis</i> serotypes D–K (inclusion conjunctivitis)
	Granulomas and inturned eye lashes, corneal scarring, blindness; CA with serotypes	<i>Chlamydia trachomatis</i> serotypes A, B, Ba, C (trachoma)
Chorioretinitis	Neonate or AIDS; 2 CA	<i>Toxoplasma</i> , CMV
Retinopathy with keratitis in baby	Mom i.v. drug abuser	<i>Treponema pallidum</i> (congenital syphilis)

Table I-7-4. Cardiac Symptoms

Chills, fever, arthralgia, myalgia, back pain, acutely ill, Janeway lesions; emboli	Developing a heart murmur; i.v. drug user	<i>Staphylococcus aureus</i>
	Not i.v. drug user	<i>Staphylococcus aureus</i>
Fever with vague symptoms with insidious onset, fatigue, weakness, weight loss, night sweats, anorexia, myalgias; murmur may have been long present; emboli	Poor oral hygiene or dental work	Viridians streptococci (55% of cases in native hearts)
	Biliary or urinary tract infection gu manipulation in elderly men	<i>Enterococcus faecalis</i>
Endocarditis in i.v. drug user		<i>Staphylococcus aureus</i> Viridians streptococci <i>Staph. epidermidis</i> <i>Aspergillus</i> (branching <45°) <i>Candida</i> (pseudohyphae) <i>Pseudomonas</i>

Table I-7-5. Middle and Lower Respiratory System Infections

Type Infection	Case Vignette/Key Clues	Most Common Causative Agents
Respiratory difficulty or obstruction	Inflamed epiglottitis; patient often 2–3 and unvaccinated	<i>Haemophilus influenzae</i> (epiglottitis)
	Infant with fever, sharp barking cough, inspiratory stridor, hoarse phonation	Parainfluenza virus (Croup)
Laryngotracheitis laryngotracheobronchitis		Viral etiology
Bronchitis	Wheezy; infant or child ≤5 years	RSV
	>5 years	<i>Haemophilus influenzae</i> , <i>Mycoplasma pneumoniae</i> , <i>Chlamydia pneumoniae</i>
	With cough >2 weeks, afebrile; >9	<i>Bordetella pertussis</i>
Pneumonia	Poorly nourished, unvaccinated baby/child; giant cell pneumonia with hemorrhagic rash	Measles: malnourishment ↑ risk of pneumonia and blindness
	Adults (including alcoholics) Lobar pneumonia or less commonly, bronchopneumonia	<i>Streptococcus pneumoniae</i> (rusty sputum)
	Neutropenic pts, burn patients, CGD, CF	<i>Pseudomonas</i>
	Pneumonia teens/young adults; bad hacking cough; initially non-productive cough	<i>Mycoplasma pneumoniae</i> (most common cause of pneumonia in school age children)
	Atypical with air conditioning exposure especially >50 yr, heavy smoker, drinker	<i>Legionella</i> spp.
	Atypical with bird exposure ± hepatitis	<i>Chlamydia psittaci</i>
	Foul smelling sputum, aspiration possible	anaerobes, mixed infection (<i>Bacteroides Fusobacterium</i> , <i>Peptococcus</i>)
	Alcoholic, abscess formation, aspiration, facultative anaerobic, Gram-negative bacterium with huge capsule	<i>Klebsiella pneumoniae</i> (currant jelly sputum)
AIDS patients with staccato cough; “ground glass” x-ray; biopsy: honeycomb exudate with silver staining cysts	<i>Pneumocystis carinii</i>	
Pneumonia with influenza	Primary infection	Influenza virus pneumonia
	Secondary	<i>Streptococcus pneumoniae</i>
Acute pneumonia or chronic cough with weight loss, night sweats	Over 55, HIV+, or immigrant from developing country	<i>Mycobacterium tuberculosis</i>
	Dusty environment with bird or bat fecal contamination (Missouri chicken farmers)	<i>Histoplasma capsulatum</i>
	Desert sand SW U.S.A.	<i>Coccidioides immitis</i>
	Rotting contaminated wood	<i>Blastomyces dermatitidis</i>

Table I-7-6. Genitourinary Tract Infections

Type Infection	Case Vignette/Key Clues	Most Common Causative Agents
Urethritis	Gram-negative diplococci in PMNs in urethral exudate	<i>Neisseria gonorrhoeae</i>
	Culture negative, inclusion bodies	<i>Chlamydia trachomatis</i>
	Urease positive, no cell wall	<i>Ureaplasma urealyticum</i>
	Flagellated protozoan with corkscrew motility	<i>Trichomonas vaginalis</i>
	Frequent and painful urination, hematuria, and fever	(Cystitis) #1 <i>E. coli</i> , other Gram-negative enterics, <i>Pseudomonas</i> , <i>Proteus</i>
	Young, newly sexually active individual; Gram-positive cocci	<i>Staphylococcus saprophyticus</i>
	As above with flank pain and prominent fever	(pyelonephritis) <i>E. coli</i> , <i>Staphylococcus</i>
Cervicitis	Friable, inflamed cervix with mucopurulent discharge; probes or culture to distinguish	<i>Neisseria Gonorrhoeae</i> (Gram-negative diplococci) <i>Chlamydia trachomatis</i> (non-staining obligate intracellular parasite) Herpes simplex (virus)
Vaginal itching, pain, discharge odor	Adherent yellowish discharge, pH >5, fishy amine odor in KOH, clue cells; Gram-negative cells dominate	(Bacterial vaginosis) overgrowth of <i>Gardnerella vaginalis</i> and anaerobes
	Vulvovaginitis, pruritis, erythema discharge: consistency of cottage cheese	<i>Candida</i> spp.
	Foamy, purulent discharge, many PMNs and motile trophozoites microscopically (corkscrew motility)	<i>Trichomonas vaginalis</i>
Genital lesions	Genital warts	Human papilloma virus (most common U.S. STD), <i>Treponema pallidum</i>
	Multiple painful vesicular, coalescing, recurring	Herpes
	Nontender ulcer healing spontaneously 2–10 weeks	<i>Treponema pallidum</i>
	Non-indurated, painful ulcer, suppurative with adenopathy, slow to heal	<i>Haemophilus ducreyi</i>
	Initial papule heals; lymph nodes enlarge and develop fistulas; genital elephantiasis may develop	<i>Chlamydia trachomatis</i> L1-3

Diarrhea

Dysentery

- Abdominal cramps, tenesmus, and pus and blood in the stool
- Usually associated with invasive bacterial disease in the colon

Diarrhea

- Refers to profuse watery feces
- Most commonly associated with increased secretion of fluid across the mucosal surfaces of the small intestine in response to a toxin or a viral infection
- No inflammatory cells

Table I-7-7. Diarrhea by Intoxication

Most Common Sources	Common Age Group Infected	Incubation Period	Pathogenesis	Symptoms	Duration of Symptoms	Organism
Ham, potato salad, cream pastries	All	1–6 hours	Heat stable enterotoxin is produced in food contaminated by food handler with skin lesion; food sits at room temperature	abd. cramps, vomiting, diarrhea; sweating and headache may occur; no fever	<24 hours	<i>Staphylococcus aureus</i>
Rice	All	<6 hours	Heat stable toxin causes vomiting		8–10 hours	<i>Bacillus cereus</i> : emetic form
Meat, vegetables	All	>6 hours	Heat labile toxin causes diarrhea (similar to <i>E. coli</i> LT)	Nausea, abd cramps, diarrhea	20–36 hrs	<i>Bacillus cereus</i> : diarrheal form

Table I-7-8. Microbial Diarrhea: Organisms Causing Noninflammatory Diarrhea

Most Common Sources	Common Age Group Infected	Incubation Period	Pathogenesis	Symptoms	Duration of Symptoms	Organism
Day care, water, nosocomial, fecal-oral	Infants and toddlers, some older	1-3 days (fall, winter, spring)	Microvilli of small intestine blunted; mononuclear infiltrate in lamina propria; disaccharidase activity down; glucose coupled transport normal; lactose intolerance may cause build up and osmotic influx creating watery diarrhea	Noninflammatory watery diarrhea, vomiting, fever, and dehydration	5-7 days	Rotaviruses
Water, food, fecal-oral	Older kids and adults	18-48 hours	Jejunal biopsy shows blunting of microvilli; cytoplasmic vacuolization is seen along with mononuclear infiltrates of tissue; virus appears to decrease brush border enzymes causing malabsorption.	Diarrhea, nausea, and vomiting; fever in some	12-48 hours	Norwalk virus
Nosocomial	Young kids, IC	7-8 days	?	Diarrhea, fever, and vomiting	8-12 days	Adenovirus 40/41
Beef, poultry, gravies Mexican food	All	8-24 hours	Enterotoxin	abd cramps and watery diarrhea, rarely fever or vomiting	<24 hours	<i>Clostridium perfringens</i>
Water, food, fecal-oral	All ages	9-72 hours	Toxin stimulates adenylate cyclase and causes increase in cAMP in the small intestine without inflammation or invasion.	Profuse watery diarrhea with vomiting; fever may be present (rice water stools)	3-4 days	<i>Vibrio cholera</i>
Raw or undercooked shellfish prominent)	Anyone eating raw shellfish	5-92 hours	Self-limited gastroenteritis mimicking cholera; there is a severe, rarer dysentery form, no clear enterotoxin; hemolysins, phospholipase and lysophospholipase; tests for invasiveness are negative.	Explosive watery diarrhea along with headache, abdominal cramps, nausea, vomiting, and fever.	Up to 10 days	<i>Vibrio parahaemolyticus</i>
Water, uncooked fruits and vegetables	All ages	12-72 hours	Heat labile toxin (LT) stimulates adenylate cyclase resulting in efflux of water and ions into the small intestine stable toxin stimulates guanylate cyclase	Watery diarrhea with some vomiting and sometimes fever	3-5 days	Enterotoxigenic <i>E. coli</i>
Food, water, fecal-oral	Infants in developing countries	2-6 days	Adherence to enterocytes through pili causes damage to adjoining microvilli.	Watery to profusely watery diarrhea	1-3 weeks	Enteropathogenic <i>E. coli</i>
Food, fecal-oral (hamburger)	50% <10 yrs., all	3-5 days	Verotoxin, which is a cytotoxin, causes bloody diarrhea with no invasion of the organism.	Abdominal cramps, watery diarrhea with blood	7-10 days	Enterohemorrhagic <i>E. coli</i> .
Water, day care, camping, beavers, dogs, etc.	All, children	5-25 days	Cysts ingested; excyst in the duodenum and jejunum; multiply and attach to intestinal villi by sucking disk.	Loose, pale, greasy diarrhea; mild to severe malabsorption syndrome	1-2 weeks to years	<i>Giardia lamblia</i>
Day care, fecal-oral, animals, homosexuals	Children, AIDS patients	2-4 weeks	Sporozoites attach to the epithelial surface of the intestine and replicate.	Mild diarrhea in immunocompetent; severe chronic diarrhea in AIDS	4 days to 3 weeks in AIDS; indefinite	<i>Cryptosporidium parvum</i>

Table I-7-9. Microbial Diarrhea: Organisms Causing Inflammatory Diarrhea/Dysentery

Most Common Sources*	Common Age Group Infected	Incubation Period	Pathogenesis	Symptoms	Duration of Symptoms	Organism
Poultry, domestic animals, water, unpasteurized milk, day care, fecal-oral	All, especially <1 year and young adults	3–5 days	Multiply in the small intestine; invades epithelium resulting in inflammation and RBC and WBC in stools.	Diarrhea, abd pain, malaise enteritis with diarrhea, malaise, fever	1–2 days mild; <1 week normal self-limiting	<i>Campylobacter jejuni</i>
Poultry, domestic animals, water, day care, fecal-oral	All, especially infants and kids	8–48 hours	Adsorb to epithelial cells in terminal small intestine; penetrate to lamina propria of ileocecal region causing PMN response and PG response, which stimulates cAMP and watery diarrhea.	Diarrhea (occ bloody), abdominal cramps, abd tenderness, fever, and nausea w/occ vomiting osteomyelitis in sickle cell anemia	3–5 days; spontaneous resolution	<i>Salmonella</i> gastroenteritis
Water, day care, no animal reservoirs, fecal-oral	All, esp 6 mo to 10 yr.	1–7 days	<i>Shigella</i> colonize the small intestine producing at first an enterotoxin-induced watery diarrhea; ultimately the <i>shigellae</i> penetrate the colon mucosa producing shallow mucosal ulcerations and dysentery; septicemia rare.	Watery diarrhea at first → lower abdominal cramps, tenesmus and abundant pus and blood in the stools (dysentery)	4–7 days; antibiotics can reduce spread	<i>Shigella</i>
Milk, wild domestic animals water, fecal-oral	All, esp older kids and young adults	2–7 days	The terminal ileum is infected with enlargement of the mesenteric lymph nodes; produces focal necrosis difficult to distinguish from appendicitis; organism is able to grow in cold; produces heat insensitive enterotoxin. Arthritis may occur.	Fever, diarrhea (frequently with leukocytes & blood in stools), abdominal pain; also a noninflamm gastroenteritis	1 day – 3 weeks (avg. 9 days)	<i>Yersinia enterocolitica</i>
Associated with antibiotic use	Pt on antibiotics	NA	Intense inflammatory response creates the friable yellow plaque-like colonic lesions (pseudomembrane) associated with this disease	Mild diarrhea to severe colitis; abdominal cramps; spiking fever, systemic toxicity; blood, mucous, and pus in stools	Until antibiotic stopped; treat with metronidazole	<i>Clostridium difficile</i>
Food, water, fecal-oral	Adults	2–3 days	Similar to <i>Shigella</i> dysentery	Fever and cramps with blood and pus in the stools	1–2 weeks; fluid and electrolyte replacement	Enteroinvasive <i>E. coli</i>
Food, water, fecal-oral, tropical generally	All	2–4 weeks	Ingested cysts survive (trophozoites die) and multiply in the colon with invasion of the colon wall producing the characteristic flask-like lesions and extra-intestinal abscesses.	Gen. acute diarrhea with cramping; sometimes dysentery; ulceration of colon may produce peritonitis	Weeks to months Rx with metronidazole followed by iodoquinol	<i>Entamoeba histolytica</i>

Abbreviations: abd = abdominal; esp = especially; occ = occasional.

*Sources: water = those listed are the most common diarrhea diseases spread through water.
 Day care = organisms listed are ones which have caused outbreaks in day care facilities, but note that any organism spread by the oral-fecal route may be a problem in this setting.
 Milk = unpasteurized milk or dairy products.

Table I-7-10. Other Gastrointestinal or Liver Infections

Signs and Symptoms	Case Vignette/Key Clues	Most Common Causative Agents
Jaundice, anorexia, nausea, right upper quadrant pain on palpation, cigarettes taste foul, elevated liver enzymes*	Food-borne (possibly contaminated raw oysters or clams); 14–45 days; without chronicity; sturdy naked RNA virus	Hepatitis A (“infectious” hepatitis) (picornavirus)
	i.v. drug abuse, needle stick; chronic carrier state, cirrhosis, primary hepatocellular carcinoma; DNA virus easily inactivated by alcohol	Hepatitis B (“serum” hepatitis) neonatal transmission (Hepadnavirus)
	Transfusion or i.v. drug abuse; acute illness is less severe than hepatitis B but chronicity is higher, with 60% of those infected having chronic active hepatitis; RNA, enveloped virus	Hepatitis C (Flavivirus)
	Enterically transmitted with high fatality in pregnant women, no chronic form	Hepatitis E (Calicivirus)
Female with lower abdominal pain; onset often following menses	Adnexal tenderness, bleeding, deep dyspareunia, vaginal discharge, ± fever. Tenderness from cervical movement, possibly palpable inflammatory mass on bimanual exam	<i>Neisseria gonorrhoeae</i> or <i>Chlamydia trachomatis</i> or both or a variety of other organisms
Acute abdominal pain	Intestinal blockage	<i>Ascaris lumbricoides</i> or potentially <i>Diphyllobothrium latum</i>
Bile duct blockage following surgery (anesthesia)		<i>Ascaris lumbricoides</i>
Peritonitis		Mixed flora often involving anaerobic normal flora: <i>Bacteroides fragilis</i> and facultative anaerobes such as <i>E. coli</i>
Cirrhosis	Travel history: Puerto Rico, Peace Corps, etc.; egg granulomas block triads → fibrosis	<i>Schistosoma mansoni</i>
	i.v. drug use	Hepatitis viruses
Pancreatitis	Generally with swelling of salivary glands	Mumps virus

*Hepatitis may also occur with two other viruses: CMV and yellow fever virus or with toxoplasmosis or leptospirosis.

Table I-7-11. Changes in Blood Cells

Symptoms and Signs	Case Vignette/Key Clues	Most Common Causative Agents
Anemia	Megaloblastic	<i>Diphyllobothrium latum</i>
	Normocytic	Chronic infections
	Microcytic and hypochromic (iron deficiency anemia)	<i>Ancylostoma, Necator, Trichuris</i>
Patient with cyclic or irregular fever, decreased hemoglobin and hematocrit	Often foreign travel to tropics, schizonts in RBCs	<i>Plasmodium</i>
Reduced CD4 cell count		HIV
Increases in PMNs		Generally found in many extracellular bacterial infections
Increases in eosinophils		Allergy
		Helminths during migrations
Increases in mononuclear leukocytes (monocytes or lymphocytes)		Viral intracellular organisms <i>Listeria, Legionella, Leishmania, Toxoplasma, Pneumocystis</i>
Increases in lymphocytes (mononucleosis) Fever, fatigue, lymphadenopathy, myalgia, headache	Infectious mononucleosis Heterophile + Downey type II cells (reactive T cells) sore throat, lymphadenopathy, young adult	Epstein-Barr virus (EBV)
	Heterophile negative	CMV <i>Toxoplasma</i> <i>Listeria</i> (Listeriosis)
Lymphocytosis with hacking cough	Unvaccinated child hypoglycemic	<i>Bordetella pertussis</i>

Table 1-7-12. Central Nervous System Infections

Signs and Symptoms	Case Vignette/Key Clues	Most Common Causative Agents
Sepsis ± seizures, irritability, or lethargy; rarely bulging fontanelles or nuchal rigidity	Neonate to 2 months	<i>Streptococcus agalactiae</i> #1 (Gram-positive coccus) <i>E. coli</i> (Gram-negative rod) More rarely: <i>Listeria monocytogenes</i> (motile Gram-positive rod)
Headache, fever, confusion, lethargy, nuchal rigidity, vomiting	6 months to 2 years; no mention of <i>Haemophilus</i> vaccine; no indication that child is properly vaccinated	<i>Haemophilus influenzae</i> type B* (Gram-negative pleomorphic rod with polyribitol capsule)
	3 mo to young adult prodrome may be very rapid; child may be properly vaccinated; rash	<i>Neisseria meningitidis</i> (Gram-negative diplococcus with capsule; ferments maltose)
	<2 yrs Young adults to elderly	<i>Streptococcus pneumoniae</i> (Gram-positive coccus, catalase negative, alpha hemolytic, inhibited by optochin, lysed by bile)
	Renal transplant patient	<i>Listeria monocytogenes</i> (motile Gram-positive rod)
As above but less toxic and a more gradual onset (several days)		Viral: Enteroviruses (~70%): Coxsackie B, echovirus; poliovirus, Coxsackie A. Summer and fall but sporadically all year Mumps virus (now rare with vaccine) winter and spring most cases imported from Lymphocytic choriomeningitis (exposure to rodents, e.g., hamsters); most cases imported from S. America Herpes simplex type 2 or Varicella-Zoster
Several month prodrome (except in severely compromised) with signs of meningitis	Usually some underlying condition	Fungal, e.g., Cryptococcal, or if in Southwestern U.S.: <i>Coccidioides</i> If near U.S. great river beds with exposure to bird, bat feces: <i>Histoplasma capsulatum</i>
Prefrontal headache, high fever, disturbance of smell	Swimming and often diving in very warm polluted waters	<i>Naegleria</i>
Bell's palsy	Systemic disease	<i>Borrelia burgdorferi</i>
Guillaine-Barré	With GI tract problems	<i>Campylobacter jejuni</i>
	With respiratory problems	Influenza

*By 1990, with day care centers and the dramatic increase in *Haemophilus meningitis*, *Haemophilus meningitis* became overall the most common. Since late 1990, when the conjugated vaccine went into use, there has been a dramatic decrease in *Haemophilus meningitis* in vaccinated kids.

(Continued)

Table I-7-12. Central Nervous System Infections (*continued*)

Signs and Symptoms	Case Vignette/Key Clues	Most Common Causative Agents
Headache, and fever ± drowsiness, coma, hemiplegia, cranial nerve palsy, hallucinations, behavioral disturbances, and other focal neurological findings	Summer-fall, mosquito-borne from bird reservoirs (except for California encephalitis, which is a rodent reservoir)	Encephalitis with arboviruses: Western equine encephalitis (midwest and west U.S.) St. Louis encephalitis elderly most severe infections California encephalitis entire U.S. Eastern equine encephalitis all age groups but most common in young and old, highest morbidity of viral CNS infections; with mental retardation, seizures, personality changes in survivors
Headache, behavioral changes, lethargy, somnolence → focal deficit (especially frontal temporal lobe involvement)	Focal uptake of radionuclide, RBCs in CSF	Herpes simplex encephalitis (treatable)
Nerve palsies in a patient with tuberculosis and a low CSF glucose	Patient with low CMI	Tuberculosis meningitis
Meningoencephalitis in immunocompromised patients		<i>Acanthamoeba</i> or <i>Toxoplasma</i>
Mass lesion (symptoms dependent on location of mass) and elevated intracranial pressure along with headache, mental changes, nausea, vomiting, fever with chills, and seizure	Generally following: sinus, ear, or dental infection, infection at distant site, head trauma, etc.	Don't do lumbar puncture; CT generally shows ring enhancing lesion; 45% mixed infections; Streptococci and <i>Bacteroides</i> are the two most commonly identified groups of bacteria
Child following a viral illness with pernicious vomiting, lethargy and irritability, which may lead to brain swelling	Perhaps indication of aspirin usage, though the linkage is not definitive	Influenza or varicella infection (Reye's syndrome)

Table I-7-13. Cerebrospinal Fluid Finding in Meningitis

Pressure	CSF Appearance	Cell Count (cells/mm ³)	Dominant Cell Type	Glucose mg/dL	Protein mg/dL	Condition
<100 mm H ₂ O	Clear	0-5	Lymphocytes	40-70	<40	Normal
Normal or +	Clear	0-500	Early: PMNs Late: lymphocytes	Normal or -	Normal or +	Viral infection
++	Opaque	1-60,000	PMNs	-	++	Bacterial infection
+	Clear	10-500	Early: PMNs Late: lymphocytes	-	+ to ++	Fungal infection

- Below normal range, + above normal range

Table I-7-14. Selected Rashes (cover the last two columns)

Type Rash	Progression	Other Symptoms	Disease	Causative Agent/Toxin
Erythematous maculopapular rash (sandpaper-like rash)	Trunk and neck → extremities	Sore throat, fever, nausea	Scarlet fever	<i>Strep. pyogenes</i> Exotoxin A-C
Diffuse erythematous, macular sunburn-like rash	Trunk and neck → extremities with desquamation on palms and soles	Acute onset, fever >102°F, myalgia, pharyngitis, vomiting, diarrhea; hypotension leading to multi-organ failure	Toxic shock syndrome	<i>Staph. aureus</i> TSST-1
Perioral erythema, bullae, vesicles, desquamation	Trunk and neck → extremities, except tongue and palate; large bullae and vesicles precede defoliation	Abscess or some site of infection	Staphylococcal skin disease: scalded skin disease & scarletina	<i>Staph. aureus</i> Exfoliatin
Petechiae → purpura	Trunk → extremities; spares palms, soles, and face	Fever, rash, headache, myalgias, and respiratory symptoms	Epidemic typhus	<i>Rickettsia prowazekii</i> ? endotoxin
Petechiae → purpura	Ankles and wrists → generalized with palms and soles	Fever, rash, headache, myalgias, and respiratory symptoms	Rocky Mountain spotted fever (most common on East Coast)	<i>Rickettsia rickettsii</i> ? endotoxin
Petechiae → purpura	Generalized (all over)	Abrupt onset, fever, chills, malaise, prostration, exanthem → shock	Early meningococcemia	<i>N. meningitidis</i> endotoxin
Skin: maculopapular; mucous membrane: condyloma	Generalized involving the palms and soles	Fever, lymphadenopathy, malaise, sore throat, splenomegaly, headache, arthralgias	Secondary syphilis	<i>Trep. pallidum</i> endotoxin
Confluent erythematous maculopapular rash	Head → entire body	Cough coryza, conjunctivitis, and fever (prodrome), oral lesions, exanthem, bronchopneumonia and ear infections	Measles	Rubeola virus Rash from T cell destruction of virus-infected cells in capillaries

Table I-7-15. Osteomyelitis

Type Infection	Case Vignette/Key Clues	Most Common Causative Agents
Fever, bone pain with erythema and swelling, some patients (diabetic particularly, may have associated cellulitis)	Adults, children, and infants without major trauma or special conditions	<i>Staphylococcus aureus</i>
	Neonates (<1 mo)	<i>Staphylococcus aureus</i> Group B <i>Streptococcus</i> Gram-negative rods (<i>E. coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Pseudomonas</i>)
	Sickle Cell Anemia*	<i>Salmonella</i>
	Trauma	<i>Pseudomonas</i>

* Sickle Cell Anemia patients are functionally asplenic and may have defective opsonic and alternate complement pathway activities. The most common bacterial infections include

- Encapsulated organisms
 - Streptococcus pneumoniae*
 - Haemophilus influenzae* type b
 - Neisseria meningitidis*
 - Salmonella enteritidis*
- Osteomyelitis due to *Salmonella* sp
- Pneumonia, bacteremia, and meningitis are all a problem.

Table I-7-16. Arthritis Related to Infections

Type Infection	Case Vignette/Key Clues	Most Common Causative Agents
Pain, redness, low-grade fever, tenderness, swelling, reduced joint mobility	#1 overall except in the 15–40 age group where gonococcal is more prevalent	<i>Staphylococcus aureus</i>
	Multiple joints	From septicemia, e.g., staphylococci, gonococcal
	15–40 years; mono- or polyarticular	Gonococcal arthritis
	Prosthetic joint	Coagulase negative staphylococci
	Viral	Rubella and hepatitis B parvovirus
	Chronic onset, monoarticular	<i>M. tuberculosis</i> or fungal
	Large joint resembling Reiter's following tick bite or erythema migrans	<i>Borrelia burgdorferi</i>
Postinfectious (Reiter's)	Following gastrointestinal infection	<i>Salmonella</i> , <i>Shigella</i> , <i>Campylobacter</i> , or <i>Yersinia enterocolitica</i>

Chapter Summary

This is a self-study chapter consisting of 16 tables organized by organ system. Each table describes the major infectious disease associated with that organ. The information presented has particular relevance to the USMLE. Following these tables are 23 (A–Z) USMLE-style clinical vignettes with open-ended questions and answers. (Please note: This section is not reviewed on video.)

CASE HISTORIES

Case A: A 28-year-old known alcoholic man presents with fever and productive cough. He was basically well until 3 days ago when he noticed perspiration, cough, shaking, chills, and headache. His cough has been associated with the production of a yellowish-green sputum, which occasionally was tinged with brownish streaks, but was not foul smelling. A Gram stain shows Gram-positive cocci in pairs and short chains.

A. What laboratory tests could you use to identify the genus?

Answer: Catalase test (negative) to genus.

B. When plated on blood agar, what other bacterium might you isolate and confuse the causative agent with, and why? What test(s) could distinguish the two?

Answer: Viridans Strep; optochin and bile.

C. What procedure would you perform to type the isolate?

Answer: Quellung reaction with known antibodies to capsule (not antibodies to cell-wall antigens).

Case B: The patient in Case A developed meningitis and died.

A. What would be the expected CSF cell count?

Answer: High.

B. What would be the expected CSF protein and sugar values?

Answer: Protein high, sugar low.

Case C: A 60-year-old male presents with ulceration of the fingers and sensory loss.

A. What is the status of his CMI?

Answer: Leonine facial appearance and the multitude of lesions is strongly suggestive of lepromatous leprosy, so CMI is depressed and lepromin test is negative.

B. Does the organism grow intracellularly or extracellularly?

Answer: Intracellularly-obligate pathogen.

Case D: A patient presents with multiple, crusted and oozing, honey-colored lesions.

A. What is the skin infection?

Answer: Impetigo.

B. What two bacteria would you expect to isolate on culture?

*Answer: Strep pyogenes (often honey-colored crusted)
and/or Staph aureus (often longer-lasting vesicular or with bullae).*

C. How would you separate the two? Which child did this test come from?

Answer: Test is from child on the right with the bullous impetigo (Staphylococcus).

Case E: A patient had intermittent bouts of general malaise, fever with weight loss, and progressive anemia. She presents also with a heart murmur.

A. What is an additional physical sign and what caused it?

Answer: Splinter hemorrhages as seen in subacute bacterial endocarditis (also seen in trichinosis and sometimes without either of these diseases).

B. What is her underlying condition and the most commonly involved bacteria?

*Answer: Damaged heart valve;
Viridans streptococci (associated with bad oral hygiene or dental work) or
Enterococcus faecalis or E. faecium if she has had bowel surgery.*

C. How would you describe the colony on blood agar? The disk is a P disk.

Answer: Alpha hemolytic not inhibited by optochin; thus the agent is a viridans streptococcus.

Case F: A mother brings her 2-year-old son to the emergency room because of fever and a stiff neck. Examination reveals an acutely ill child with a temperature of 104°F. CSF is Gram stained, examined in a rapid test, and also cultured. A Gram stain of the isolated organism shows *Haemophilus influenzae*.

A. What laboratory test could confirm the identity of the isolate?

Answer: Meningitis screen, a series of immunologic rapid identification tests (usually EIAs using known antibodies), followed by growth of CSF sediment or filtrate on special media and drug susceptibilities.

B. What growth factors are required to grow the isolate on blood agar?

Answer: X = hemin and V = NAD.

Chocolate agar provides both X and V.

C. What is the drug of choice?

Answer: Cefotaxime or Ceftriaxone.

D. What part of his routine health care is he likely missing?

Answer: His Haemophilus vaccination and perhaps others.

Case G: A 23-year-old woman presents with lower back pain, fever, and dysuria of 3 days' duration. Urinalysis reveals many white blood cells (WBC) and WBC casts. Gram stain of the uncentrifuged urine reveals numerous Gram-negative bacilli per oil immersion field. *Proteus* was isolated on culture.

A. What is its most important biochemical characteristic? Why?

Answer: Urease producing Enterobacteriaceae; kidney stones induced.

Case H: A child developed a unilateral mucopurulent conjunctivitis 10 days after birth. A conjunctival specimen was sent to the laboratory and inoculated into tissue culture cells. The diagnosis is chlamydial conjunctivitis.

A. What would a microscope test show?

Answer: Inclusion bodies stained either with iodine or fluorescent antibody.

B. What is unusual about the chemical makeup of the organism?

Answer: ATP defective mutant, also muramic acid missing from peptidoglycan.

C. What are the two forms of the organism?

Answer: Elementary bodies (extracellular) and reticulate bodies = replicating forms.

D. What would you see on Gram stain?

Answer: Nothing in the cells—poorly Gram staining.

E. What serologic type caused the child's problem?

Answer: If U.S. kid, serotype D-K.

Case I: A 27-year-old physician is hospitalized. He was in excellent health until two days earlier when he noted malaise, fatigability, and profound anorexia. He remembers approximately 6–8 weeks ago of an accidental needle "stick". He is diagnosed with hepatitis B because of the strong acute response.

A. How would you confirm your clinical diagnosis?

Answer: HBsAg and IgM to HbcAg.

B. What is meant by the "window"?

Answer: A time period between the end of the detectable presence of HBsAg and the beginning of the production of antibody to HbsAg.

HBc antibody and HBeAg are present.

C. What antigen's presence in past 6 months is indicative that the patient is entering a carrier state?

Answer: HBsAg past 6 months.

D. What antigen correlates with viral production?

Answer: HbeAg.

E. Does the virus carry a virion associated polymerase? If so, what kind?

Answer: Yes, RNA-dependent DNA polymerase (Hepatitis B replicates through an RNA intermediate.)

Case J: A young man became ill with a sore throat and swollen tonsils, marked fatigue, cervical adenopathy, a palpable spleen, and a pruritic erythematous rash that started after self-administration of ampicillin.

- A. What is the most likely disease? What are the most common laboratory diagnostic tests? What does the antibody test measure?

Answer: Infectious mononucleosis; monospot test (measures heterophile antibody which is not specific to EBV antigen) plus CBC.

- B. What type of cells are the Downey type II cells?

Answer: T lymphocytes. (Reactive cells not infected.)

- C. What cells does the virus infect? Through what receptor does the lymphocytic infection begin?

Answer: EBV infects epithelia cells and B lymphocytes, whose receptor is CD21 = CR2.

Case K: A 35-year-old woman presents with a unilateral vesicular rash.

- A. The most likely diagnosis is

Answer: Shingles.

- B. Describe the virion's nucleic acid.

Answer: Linear dsDNA.

- C. Patient had a previous history of what other disease?

Answer: Chickenpox.

Case L: A 27-year-old man presents to the hospital emergency room with a cough, chest pain, and fever. Two days before admission he developed a nonproductive cough. Rales are heard. Gram stain of sputum was negative. Sputa cultures on blood agar were also negative. Culture on a special medium containing cholesterol, purines, and pyrimidines produced colonies in 10 days. Serology 3 weeks later (when he returned because of persistent cough but feeling better) showed cold agglutinins.

- A. What is the probable causative agent?

Answer: Mycoplasma pneumoniae.

- B. Why did the organism not show up on the Gram stain?

Answer: Organism does not have a cell wall and does not stain with either the primary or counterstain in the Gram stain.

- C. What antibiotics do you NOT use?

Answer: Penicillin/cephalosporin.

Case M: A 27-year-old woman presents to the hospital with a fever of 104°F, dyspnea, and cough. Approximately three days ago she noted a papular rash on the face and trunk. At the time of admission there were many skin lesions: some papular, some vesicular, and some umbilicate. She also has similar lesions in her mouth, and her exanthem is shown.

A. The most likely agent causing this condition is:

Answer: Varicella-Zoster.

B. What two viral coded enzymes made both by VZV and HSV make acyclovir a useful drug in treating severe active VZV infections?

Answer: Thymidine kinase and herpes coded DNA polymerase.

Case N: A markedly sick 3-year-old boy presents to the family physician because of fever, dyspnea, cough, and photophobia. In addition, he has a maculopapular eruption on the face and trunk. On examination, it is observed that he has pinpoint gray-white areas with a red base on his buccal mucosae.

A. What is the most probable disease and causative agent?

Answer: Measles; rubeola.

B. What type of vaccine is available and could have prevented these symptoms?

Answer: Attenuated, single serotype of rubeola.

C. To what viral family does the agent belong?

Answer: Paramyxoviruses.

D. Does the virion carry a polymerase? Why?

Answer: Yes, because it is a negative RNA virus.

Case O: A 35-year-old worker at a plant nursery seeks his physician for a suppurative lesion on one of his fingers. A smear is taken of the drainage and stained. He is diagnosed with *Sporotrichosis*.

A. What is the causative agent?

Answer: Sporothrix schenckii.

B. Is the fungus dimorphic or monomorphic?

Answer: This is DIMORPHIC FUNGUS consistent with Sporothrix. You can tell from cigar-shaped yeast (in tissues generally tough to visualize) and hyphae with sleeve and rosettes arrangement of conidia in culture.

C. Treatment

Answer: Itraconazole but oral KI in milk given will also clear up.

Case P: A 24-year-old female returned to the United States after spending six months in Mexico. Ten days ago she started to have attacks of diarrhea and developed abdominal distention. After lunch on the 10th day she noticed marked abdominal discomfort. While the pain was initially mid-abdominal, by 10:00 p.m. it became located predominantly in the right-lower quadrant. She went to the local emergency room where on physical examination it was noted that she had rebound tenderness and a fever of 37.9°C. She became nauseous and vomited.

Laboratory studies revealed a white blood cell count of 20,000/mm³ with a pronounced eosinophilia. The emergency room physician diagnosed the problem as acute appendicitis. An appendectomy was performed. A smear was made of the appendiceal exudate and knobby oval to barrel-shaped structures measuring 35 × 55 μm were observed.

A. What is the most likely cause of her appendicitis?

Answer: Ascaris lumbricoides migrating into the appendiceal orifice.

B. What major group does the organism belong to?

Answer: Nematodes

Case Q: A 50-year-old Missouri farmer was referred to the hospital because of malaise, weakness, weight loss, fever, and a palpable spleen. Examination of the mouth reveals a painless ulcerated lesion. A punch biopsy of the lesion is obtained and submitted for laboratory study. Histologic study revealed oval structures measuring 2–5 μm.

A. What is the most likely causative agent, and what are the distinctive forms?

Answer: Histoplasma capsulatum with the intracellular oval yeasts and the tuberculate macroconidia (and microconidia) in the hyphal state.

B. Where in nature will you find the fungus in large numbers?

Answer: Histoplasma capsulatum: Great central riverbed plains. Chicken coops in Missouri 100% infected. Indianapolis has had an ongoing outbreak and has major problems with it disseminating in their AIDS patients; NY City also high.

Case R: A 64-year-old male is hospitalized because of dementia. One month prior to admission he complained of headaches. On physical examination it is noted that he has nuchal rigidity, disorientation to time and place, and marked confusion. Lumbar puncture reveals 100 WBCs, which are predominantly lymphocytes, protein 85 mg/dl and sugar 45 mg/dl (concomitant blood sugar is 90 mg/dL).

Despite attempts to treat, three weeks after admission he died. An autopsy was performed. Brain sections were stained with H&E. He is diagnosed with cryptococcal meningitis.

A. How do you know?

Answer: Capsule present on yeast in tissues.

B. Who usually acquires the infection?

Answer: Immunosuppressed for meningitis; pulmonary generally only in pigeon breeders or people exposed to extremely high doses.

- C. If the India ink had been negative, what test should have been run?

Answer: The India ink test is very insensitive and cannot rule out Cryptococcal meningitis. Latex particle agglutination is much more sensitive. India ink is still frequently on the exam.

Case S: A 34-year-old accountant presents to the emergency room because of headache and fever of 3 days' duration. The day before admission his wife noted mild confusion and irritability. Lumbar puncture revealed an opening pressure of 300 mm, 200 cells, 90% of which are lymphocytes, sugar of 85 mg/dL (concomitant blood sugar of 110 mg/dL), and protein of 65 mg/dL. Bacteriologic smears (and ultimately also the bacterial cultures) were negative, as were India ink preparations. All latex particle agglutination tests for fungal and bacterial capsules done on the patient's CSF were also negative. The patient's condition did not improve despite appropriate therapy, and he died 10 days after hospitalization. An autopsy was performed. The most likely diagnosis is herpes simplex encephalitis.

- A. Does the virus have an envelope?

Answers: Icosahedral with envelope.

- B. Where within the cell does the virus replicate?

Answer: Nucleus for both DNA synthesis and assembly.

- C. What other members belong to the same family?

Answers: EBV, Varicella-Zoster, Cytomegalovirus.

Case T: A markedly dehydrated patient presents with diarrhea. His stool culture grew organisms only when grown on an alkaline medium. The isolate was oxidase-positive. He was diagnosed with *Vibrio cholerae*.

- A. What is the mechanism of the produced enterotoxin?

Answer: ADP ribosylation → activating adenylate cyclase → increased cAMP (leaves Gs locked in the active state).

- B. How would you describe his stool specimen?

Answer: Rice water.

Case U: A patient presents with warts.

- A. What is the virus that probably caused the tumors?

Answer: Human papilloma virus.

- B. What serotypes are most commonly associated with this clinical presentation?

Answer: 6 and 11

- C. Are they premalignant?

Answer: Rarely.

- D. What serotypes are most commonly associated with cervical intraepithelial neoplasia?

Answer: 16, 18, and 31. These are sexually transmitted.

Case V: A girl received a bone marrow transplant for the treatment of leukemia. Nine weeks after the transplant her temperature rose, she became dyspneic, and died. Impression smears were taken from the cut surface of the lower lobe of the left lung. The smears were stained with H&E. She was diagnosed with cytomegalovirus.

A. Why did the patient develop the pneumonia?

Answer: Immunocompromised—No T cells.

B. How would you describe what you would see (using only two words)?

Answer: Owl's eyes: cells with prominent basophilic intranuclear inclusion bodies.

C. What is the virion's nucleic acid type? To what viral family does it belong?

Answer: dsDNA; Herpes viruses.

Case W: A young woman developed a feverish illness with painful swelling of her knee, elbow, and wrist joints. She has a sparse rash on the distal parts of her limbs, consisting of small hemorrhagic pustules with an erythematous base. A smear was obtained from the exudate of the exanthem and Gram stained. The stain showed intracellular gram-negative diplococci.

A. What disease does she have?

Answer: Disseminated gonococcal infection.

B. Do pili play a role in the pathogenesis?

Answer: Yes, for attachment to epithelial surfaces—colonizing factor along with outer membrane proteins and antigenic variation.

Comparative Microbiology

8

MORPHOLOGY/TAXONOMY

Spore-Forming Bacteria (Have Calcium Dipicolinate)

Bacillus

Clostridium

Non-motile Gram-Positive Rods

Corynebacterium diphtheriae

Nocardia

Clostridium perfringens (rest of the pathogenic *Clostridia* are motile)

Bacillus anthracis (most other *Bacillus* species are motile)

Acid Fast Organisms

Mycobacterium

Nocardia (partially acid fast)

Cryptosporidium oocysts

Legionella micdadei

Isospora oocysts

Bacteria and Fungi That Characteristically Have Capsules

The “biggies” can be remembered by the mnemonic: Some Killers Have Pretty Nice Capsules!

Streptococcus pneumoniae

Klebsiella pneumoniae

Haemophilus influenzae

Neisseria meningitidis

Cryptococcus neoformans (only encapsulated fungal pathogen)

Pseudomonas aeruginosa—slime producer especially in cystic fibrotic patients' lungs

Other Important Capsule Producers

E. coli meningeal strains have capsule, mostly K₁

Bacillus anthracis—poly D-glutamate capsule

Salmonella typhi—(virulence) capsular antigen

Streptococcus pyogenes when first isolated; non-immunogenic (but anti-phagocytic) hyaluronic acid capsule

Biofilm Producers

Staphylococcus epidermidis (catheter-related infections)

Streptococcus mutans (dental plaque)

Pigment Production

Pseudomonas aeruginosa—pyocyanin (blue-green, fluorescein)

Serratia—red pigment

Staphylococcus aureus—yellow pigment

Photochromagenic and scotochromagenic *Mycobacteria*—Carotenoid pigments (yellow and orange)

Corynebacterium diphtheriae—black to gray

Unique Morphology/Staining

Metachromatic staining—*Corynebacterium*

Lancet-shaped diplococci—*Pneumococci*

Kidney bean-shaped diplococci—*Neisseriae*

Bipolar staining—*Yersinia pestis*

Gulls wings—*Campylobacter*

Table I-8-1. Viral Cytopathogenesis

Inclusion Bodies	Virus
Intracytoplasmic (Negri bodies)	Rabies
Intracytoplasmic acidophilic (Guarnieri)	Poxviruses
Intracytoplasmic and intranuclear (Owl's eye)	Cytomegalovirus
Intranuclear (Cowdry's)	Herpes simplex virus Subacute sclerosing panencephalitis (measles) virus
Syncytia formation	Virus
Present	Herpes simplex virus Varicella-zoster Paramyxovirus Respiratory syncytial virus HIV

PHYSIOLOGY

Table I-8-2. Metabolism*

Aerobes	Anaerobes	Microaerophilic
<i>Mycobacterium</i>	<i>Actinomyces</i>	<i>Campylobacter</i>
<i>Pseudomonas</i>	<i>Bacteroides</i>	<i>Helicobacter</i>
<i>Bacillus</i>	<i>Clostridium</i>	
<i>Nocardia</i>	<i>Fusobacterium</i>	
<i>Corynebacterium diphtheria</i>	<i>Prevotella</i>	
	<i>Propionibacterium</i> (aerotolerant)	
	<i>Eubacterium</i>	
	<i>Lactobacillus</i> (aerotolerant)	

*Most others are considered facultative anaerobes.

Enzymes

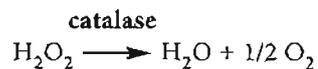
Oxidase

- All Enterobacteria are oxidase negative.
- All *Neisseria* are oxidase positive (as are most other Gram-negative bacteria).

Urease Positive

- *Helicobacter*
- All *Proteus* species produce urease; this leads to alkaline urine and may be associated with renal calculi.
- *Ureaplasma* (renal calculi)
- *Cryptococcus* (the fungus)
- *Nocardia*

Catalase



Staphylococci have catalase, *Streptococci* do not.

Most anaerobes lack catalase.

Catalase positive organisms are major problems in Chronic Granulomatous Disease (CGD):

- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Candida*
- *Aspergillus*
- Enterobacteriaceae, especially *Klebsiella*

Coagulase Positive

- *Staph aureus*
- *Yersinia pestis*

DETERMINANTS OF PATHOGENICITY

Genetics

Genes Encoding Pathogenic Factors Reside on:

- The bacterial chromosome
 - Cholera^gen
 - Endotoxin
 - Shiga toxin
 - Mnemonic (Chromosomal Encoded Somethings)
- A plasmid
 - Most toxins and multiple drug resistance
- A bacteriophage chromosome stably integrated into the host DNA as a prophage.
 - Virulence modified by the stable presence of phage DNA in bacterial cell = lysogenic conversion.

Examples:

- O = *Salmonella* O antigen
- B = Botulinum toxin (phage CE β and DE β)
- E = Erythrogenic toxin of *Streptococcus pyogenes*
- D = Diphtheria toxin (Corynephage β)
- Mnemonic: OBED (or a little pregnant with phage).

Antigenic Variation

- Neisseria gonorrhoeae* (pili)
- Borrelia recurrentis*
- Trypanosoma brucei*

Table I-8-3. Disease Due to Toxin Production

Bacterium	Disease	Activity of Toxin
<i>Corynebacterium diphtheriae</i>	Diphtheria	ADP ribosylation of EF-2 results in inhibition of protein synthesis
<i>Clostridium tetani</i>	Tetanus	Binds to ganglioside in synaptic membrane, blocks release of glycine
<i>Clostridium botulinum</i>	Botulism	Prevents release of acetylcholine
<i>Vibrio cholerae</i>	Cholera	Cholera ^g en stimulates adenylate cyclase
<i>E. coli</i> (ETEC)	Travelers' diarrhea	LT stimulates adenylate cyclase
<i>Clostridium difficile</i>	Diarrhea	Toxin A and B inhibit protein synthesis and cause loss of intracellular K ⁺

EF-2 = eukaryotic elongation factor-2.

Heat Stable Toxins

60°C

- *Staphylococcus aureus* enterotoxin
- ST toxin of *E. coli*
- *Yersinia enterocolitica* toxin

100°C

- Endotoxin

Toxins with ADP-Ribosylating Activity

Table I-8-4. Toxins with A-B ADP-Ribosylating Transferase Activity

Toxin	ADP-Ribosylated Host Protein	Effect on Host Cell
<i>Pseudomonas</i> Exotoxin A Exotoxin S	eEF-2 unknown	Inhibits translocation during protein synthesis
Diphtheria toxin	eEF-2	Inhibits translocation during protein synthesis
<i>E. coli</i> heat-labile toxin (LT)	G-protein (G _s)	Increases cAMP in intestinal epithelium causing diarrhea
Cholera toxin	G-protein (G _s)	Increases cAMP in intestinal epithelium causing diarrhea
Pertussis toxin	G-protein (G _i)	Increases cAMP causing lymphocytosis and increased insulin secretion

A is the ADP-ribosyl transferase.

B binds to cell receptor and translocates the A subunit into the cell.

Table I-8-5. Invasive Factors

Invasive Factor	Function	Bacteria
All capsules	Antiphagocytic	See earlier list with morphology
Slime layer (capsule or glycocalyx)	Antiphagocytic	<i>Pseudomonas</i>
M protein	Antiphagocytic	Group A Streptococci
A protein	Antiphagocytic	<i>Staph. aureus</i>
Lipoteichoic acid	Attachment to host cells	All Gram-positive bacteria
<i>N. gonorrhoeae</i> pili	Antiphagocytic	<i>N. gonorrhoeae</i>

Table I-8-6. Extracellular Enzymes

Enzyme	Function	Bacteria
Hyaluronidase	Hydrolysis of ground substance	Group A Streptococci
Collagenase	Hydrolysis of collagen	<i>Clostridium perfringens</i> <i>Bacteroides melaninogenicus</i>
Kinases	Hydrolysis of fibrin	<i>Streptococcus</i> <i>Staphylococcus</i>
Lecithinase (alpha toxin)	Damage to membrane	<i>Clostridium perfringens</i>
Heparinase	May contribute to thrombophlebitis	<i>Bacteroides fragilis</i>
IgA Proteases	Colonizing factor	<i>Neisseria</i> <i>Haemophilus</i> <i>Strep. pneumoniae</i>

Ability to Survive and Grow in Host Cell

Obligate Intracellular Parasites

Cannot be cultured on inert media. Virulence is due to the ability to survive and grow intracellularly where the organism is protected from many B-cell host defenses.

- Bacteria
 - All Rickettsiae
 - Chlamydia trachomatis*
 - Chlamydia psittaci*
 - Mycobacterium leprae*
- Viruses
 - All are obligate intracellular parasites.
- Protozoa
 - Plasmodium*
 - Toxoplasma gondii*
 - Babesia*
 - Leishmania*
 - Trypanosoma cruzi* (amastigotes in cardiac muscle)
- Fungi
 - None

Obligate Parasites That Are Not Intracellular

(e.g., cannot be cultured on inert media but are found extracellularly in the body)

- *Treponema pallidum*
- *Pneumocystis carinii*

Facultative Intracellular Parasites of Humans

- Bacteria
 - Francisella tularensis*
 - Listeria monocytogenes*
 - Mycobacterium tuberculosis*
 - Brucella* species
 - Non-tuberculous *mycobacteria*
 - Salmonella typhi*
 - Legionella pneumophila*
 - Yersinia pestis*
 - Nocardia*
 - Borrelia burgdorferi*
- Fungi
 - Histoplasma capsulatum*
- Protozoa
 - Trypanosoma*

EPIDEMIOLOGY/TRANSMISSION

Bacteria That Have Humans as the Only Known Reservoir

Mycobacterium tuberculosis
M. leprae (armadillos in Texas)
Shigella species
Salmonella typhi
Rickettsia prowazekii (epidemic typhus)
 Group A β -hemolytic streptococcus
Neisseria meningitidis and *N. gonorrhoeae*
Corynebacterium diphtheriae
Streptococcus pneumoniae
Treponema pallidum
Chlamydia trachomatis

Zoonotic Organisms

(Diseases of animals transmissible to humans)
Bacillus anthracis
Salmonella species except *S. typhi*
Leptospira
Borrelia
Listeria monocytogenes
Brucella species
Francisella tularensis

Pasteurella multocida (cat bites)
Vibrio parahaemolyticus (from fish)
Vibrio vulnificus (oysters)
Yersinia pestis, *Y. enterocolitidis*, *Y. pseudotuberculosis*
Campylobacter fetus, *C. jejuni*
Most Rickettsia
Chlamydia psittaci (birds)
Coxiella

Arthropod Vectors in Human Disease: Insects

- Lice
 - Epidemic or louse-borne typhus (*Pediculus h. humanus*)
 - Epidemic relapsing fever
 - Trench fever
- True bugs
 - Chagas' disease (American trypanosomiasis)—kissing bugs (*Reduviidae*)
- Mosquitoes
 - Malaria (*Anopheles* mosquito)
 - Dengue (*Aedes*)
 - Mosquito-borne encephalitides: WEE, EEE, VEE, SL
 - Yellow Fever (*Aedes*)
 - Filariasis
- Sandflies
 - Leishmanias
 - Sandfly fever (viral)
 - Bartonellosis
- Midges
 - Filariasis
- Blackflies
 - Onchocerciasis
- Deerflies and horse flies
 - Loaloasis
 - Tularemia
- Tsetse flies
 - African trypanosomiasis
- Fleas
 - Plague
 - Endemic typhus

Arthropod Vectors That Are Not Insects

- Ticks
 - Rocky Mountain spotted fever (*Dermacentor, Amblyomma*)
 - Colorado tick fever (*Dermacentor*)
 - Lyme disease (*Ixodes*)
 - Ehrlichia*
 - Babesiosis (*Ixodes*)
 - Tularemia
 - Recurrent fever or tick-borne relapsing fever (*Ornithodoros*, a soft tick)
- Mites
 - Scrub typhus (*Leptotrombium*) (transovarial transmission in vector)
 - Rickettsialpox

Parasitic Infections Transmitted by Eggs

Enterobius vermicularis (pin worms)
Ascaris lumbricoides (round worms)
Toxocara canis
Echinococcus granulosus
Taenia solium

Bacterial and Fungal Infections That Are Not Considered Contagious

(i.e., no human-to-human transmission)
 Nontuberculous mycobacterial infections, e.g., *Mycobacterium avium-intracellulare*
 Non-spore forming anaerobes
Legionella pneumophila
 All fungal infections except the dermatophytes

Infections That Cross the Placenta

Toxo
 Other (Syphilis)
 Rubella
 CMV
 Herpes and HIV
 <5% perinatal hepatitis B could possibly have been acquired by crossing placenta.

- Viruses
 - Cytomegalovirus*
 - Rubella*
 - Herpes II (in primary infection)
 - Coxsackie B
 - Polio
 - HIV

- Parasites
Toxoplasma gondii
- Bacteria
Treponema pallidum
Listeria monocytogenes

Spread by Respiratory Droplet

- | | |
|---|-----------------------------|
| <i>Streptococcus pyogenes</i> (Group A) | Influenza |
| <i>Streptococcus pneumoniae</i> | Rubella measles |
| <i>Neisseria meningitidis</i> | Chicken pox |
| <i>Mycobacterium tuberculosis</i> | <i>Pneumocystis carinii</i> |
| <i>Bordetella pertussis</i> | |
| <i>Haemophilus influenzae</i> | |
| <i>Corynebacterium diphtheriae</i> | |
| <i>Mycoplasma pneumoniae</i> | |

Spread by Inhalation of Organisms from the Environment

- Histoplasma*
- Coccidioides*
- Blastomyces*
- Nontuberculous mycobacteria, e.g., *M. avium-intracellulare* (MAC)
- Legionella*
- Chlamydia psittaci*
- Pseudomonas* (also spread by ingestion and contact)
- Coxiella burnetti* (the only Rickettsia that is stable in the environment)

Spread by Oral/Fecal Route

(Infections may be spread by oral sex.)

- Salmonella*
- Shigella*
- Campylobacter*
- Vibrio*
- Yersinia enterocolitica*
- Yersinia pseudotuberculosis*
- Bacillus cereus*
- Clostridium*
- Staphylococcus* (also other routes commonly)
- Enteroviruses
- Rotavirus
- Norwalk agent
- Hepatitis A
- Polio virus

Toxoplasma—cat feces

Entamoeba

Giardia

Balantidium

All nematodes of interest

Echinococcus—dog feces

Contact: (Person-to-Person) Nonsexual

Impetigo (*Strep* and *Staph*)

Staphylococcus

Herpes II

Epstein-Barr (kissing)

Hepatitis B (all body fluids)

Contact: Sexual

Chlamydia HPV

Neisseria HIV

Treponema Herpes II

Trichomonas CMV

PATHOLOGY

Organisms That Produce Granulomas (Persistent Antigen)

Fran Likes My Pal Bruce And His Blasted Cockerspaniel (in) Blessed Salt Lake City.
(Mnemonic by M. Free.)

(ic) = intracellular organism

Francisella (ic)

Listeria (ic)

Mycobacterium (ic)

Treponema pallidum

Brucella (ic)

Actinomyces

Histoplasma (ic)

Blastomyces

Coccidioides

Berylliosis

Schistosoma species, sarcoid

Lymphogranuloma venereum (ic)

Cat scratch fever

Infections Causing Intracerebral Calcifications

Toxoplasma

CMV

LABORATORY DIAGNOSIS

Special Stains

- Silver stains
 - Dieterle—*Legionella*
 - Gomori methenamine—*Pneumocystis*, fungi
- Acid fast (Ziehl-Neelson or Kinyoun)
 - Mycobacterium*, *Nocardia* (partially AF), *Legionella micdadei*, *Cryptosporidium*, and *Isospora*
- India ink—*Cryptococcus* (if negative not a reliable diagnostic method)
- Calcofluor white—fungi
- Giemsa
 - Blood protozoa (*Plasmodium*, *Babesia*, *Trypanosoma*, *Leishmania*)
 - Histoplasma capsulatum* in RES cells

Name Tests

<u>Tests</u>	<u>Disease</u>
PPD or Tuberculin (Mantoux)	TB
Lepromin	Leprosy
Fungal skin tests	Clinically valuable only to demonstrate exposure or anergy
cAMP test	<i>Streptagalactiae</i> carriers
Elek test	Toxin producing <i>C. diphtheriae</i> strains
Weil-Felix	Rickettsia (with <i>Proteus</i> strain OX antigens)

Unusual Growth Requirements

Haemophilus (most species require one or both)

- X factor = protoporphyrin IX, the precursor of hemin
- V factor = NAD (nicotinamide dinucleotide) or NADP

Mycoplasma

- Cholesterol

Salt

- *Staph aureus* will grow on high salt media.
- Group D enterococci will grow on 6.5% NaCl.
- *Vibrio parahaemolyticus* requires NaCl to grow and grows at 6.5%.

Cysteine requirement for growth

- Four Sisters Ella of the Cysteine Chapel (wonderful mnemonic by M. Free)
Francisella, *Legionella*, *Brucella*, and *Pasteurella*

Cultures that must be observed for a long time

- *Mycobacterium tuberculosis* and all non-tuberculosis mycobacteria except rapid growers
- *Mycoplasma pneumoniae*
- *Brucella* sp
- Systemic fungal pathogens (*Blastomyces*, *Histoplasma*, and *Coccidioides* in U.S.)

TREATMENT/PREVENTION

Treat Prophylactically

- *Neisseria meningitidis* (household and day care contacts—vaccination also used in outbreaks)
- *Mycobacterium tuberculosis* with a recent skin test conversion or known household (i.e., significant) exposure; or persons under 35 with a positive skin test who have never been treated
- *Haemophilus influenzae* B (unvaccinated household contacts <6 years old)—also vaccinate
- *Neisseria gonorrhoeae* (sexual contacts)
- *Treponema pallidum* (sexual contacts)
- *Yersinia pestis*

Vaccines Available in the U.S.

Inactivated Vaccines

- Pertussis (killed whole cell in DTP)
- *Vibrio cholera*
- Influenza virus
- Salk polio (killed)—for IC patient
- Rabies (HDC or RVA)
- Japanese encephalitis and several other encephalitis vaccines
- Hepatitis A

Live, Attenuated Vaccines

- *Francisella tularensis*
- Measles
- Rubella
- Mumps (killed vaccine available for IC patients)
- Sabin polio (oral)
- Smallpox
- Yellow fever
- Varicella-Zoster

Live, Pathogenic Virus (in enteric-coated capsules)

- Adenovirus

Toxoid: Chemically Modified Toxin—Vaccines

- Tetanus
- Diphtheria
- Pertussis toxoid (in DTaP)

Recombinant Vaccines or Subunit Vaccine

- Hepatitis B—HBsAg (produced in yeast)
- *Haemophilus*—purified capsular polysaccharide conjugated to protein
- *Neisseria meningitidis*—capsular polysaccharides
- Pneumococcal—capsular polysaccharide (23 serotypes)

Chapter Summary

The microorganisms, listed by their taxonomic classification, are divided into the following morphologic groups: spore-forming bacteria, nonmotile Gram-positive rods, acid-fast organisms, capsulated bacteria and fungi, biofilm producers, pigment producers, and bacteria with unique morphology or staining properties. Table I-8-1 lists viruses with inclusion bodies and those that form syncytia.

The micro-organisms are listed according to the following properties related to their physiology: aerobes, anaerobes, and microaerophiles and oxidase, urease, catalase, and coagulase activity.

The microorganisms are listed according to the following properties related to virulence: genetic coding of pathogenic factors, ability to undergo antigenic variation, ability to cause toxin-induced diseases, production of heat-stable toxins, production of toxins that have ADP-ribosylation activity, production of invasive factors, production of extracellular enzymes, and ability to survive and grow in host cells.

The micro-organisms are listed according to the following properties related to epidemiology: bacteria having only human reservoirs; microorganisms that normally cause disease in animals but also can cause human disease; organisms transmitted to humans by arthropod vectors; parasites transmitted to humans as eggs; parasites and bacteria that cause nontransmissible diseases; organisms that cross the placenta; and organisms spread by respiratory droplets, by inhalation, by the fecal/oral route, and by personal nonsexual or sexual contact.

The microorganisms also are listed according to the pathologic production of granulomas and intracerebral calcifications.

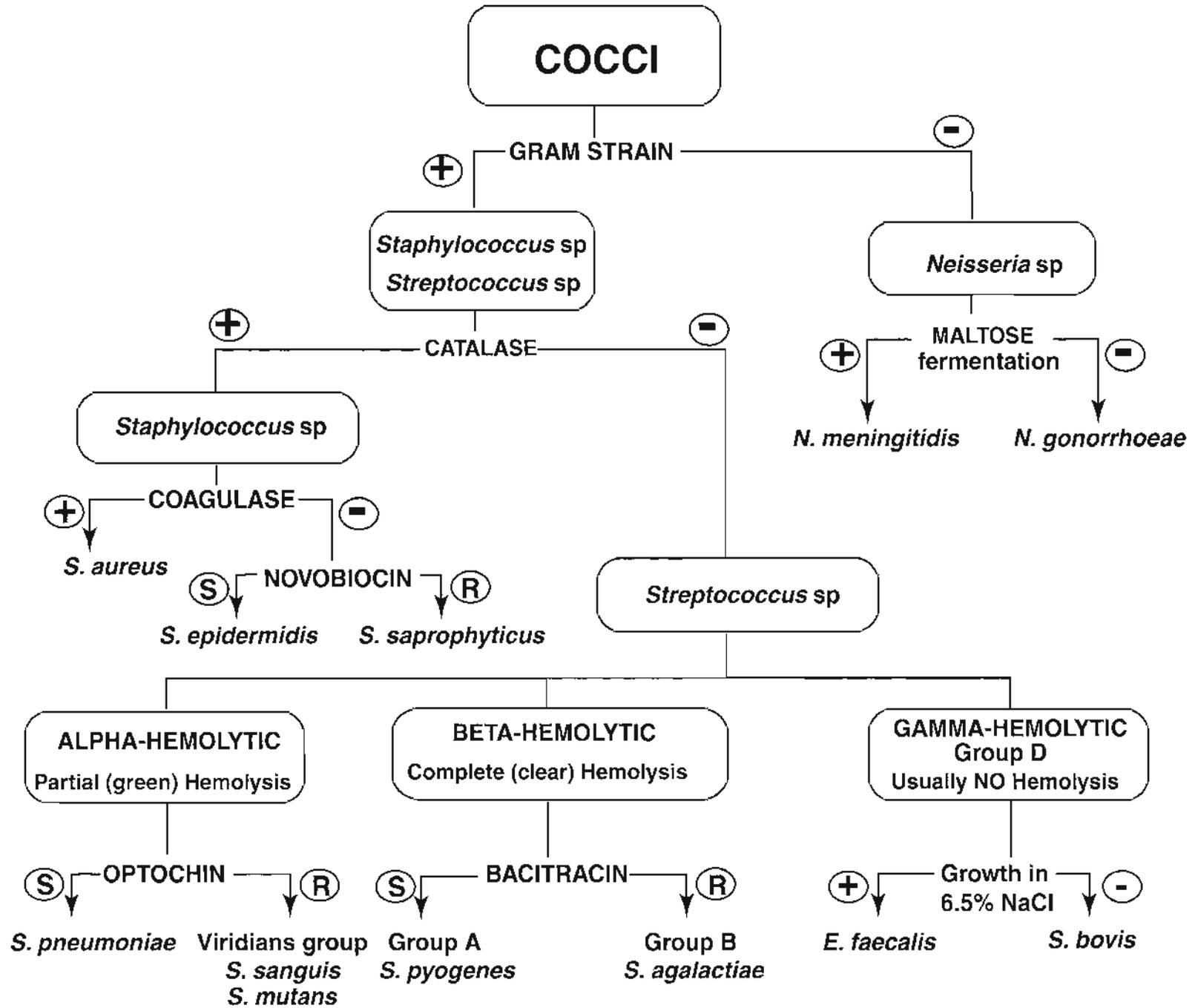
The organisms that can be identified by special stains or by named tests are cataloged.

Fastidious organisms and their unusual growth requirements are listed.

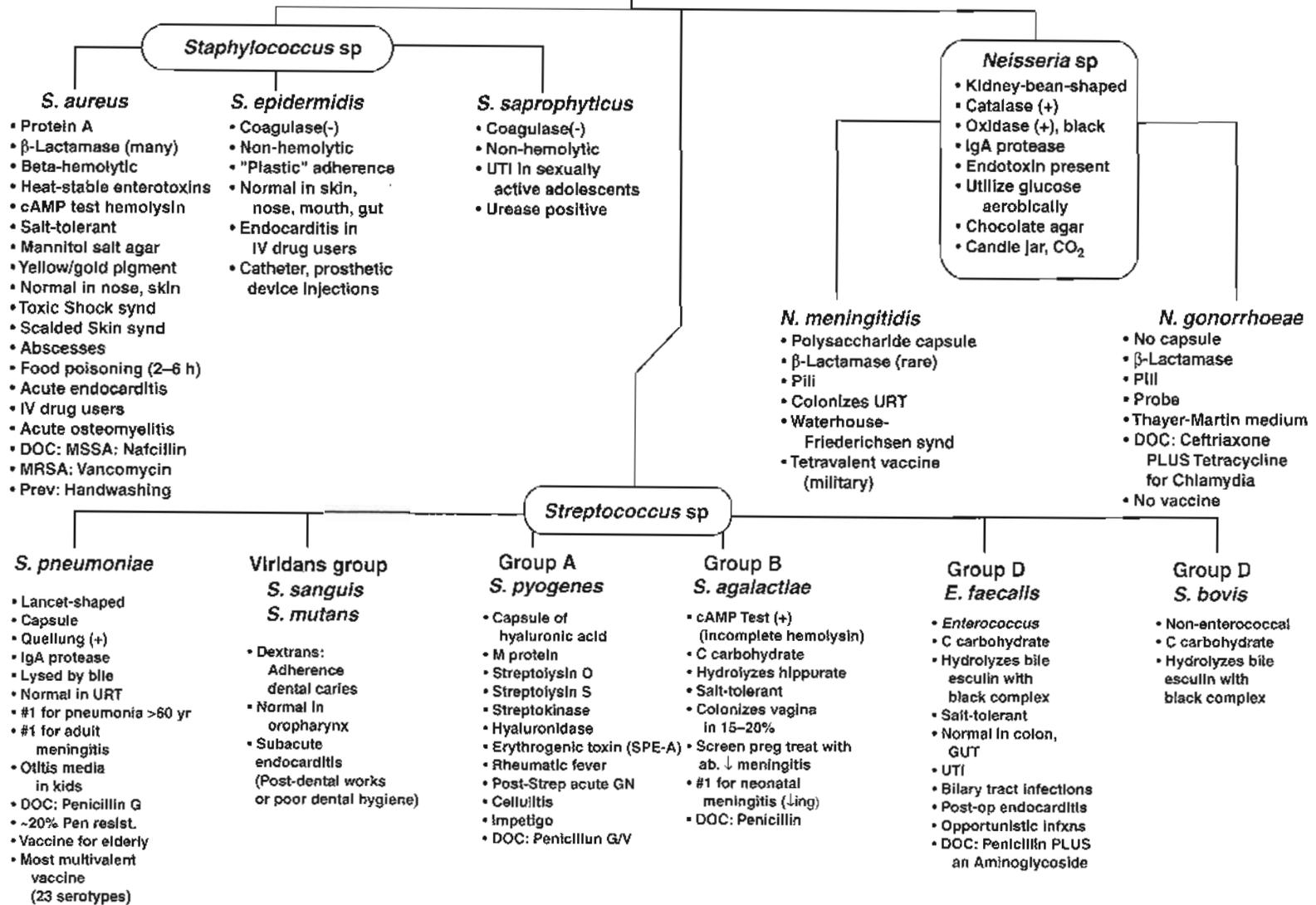
Organisms responding to prophylactic treatments (avoidance, etc.) and for which vaccines are available in the United States are listed. The list includes inactive, live attenuated, live pathogenic, toxoid, and recombinant or component (subunit) vaccines.

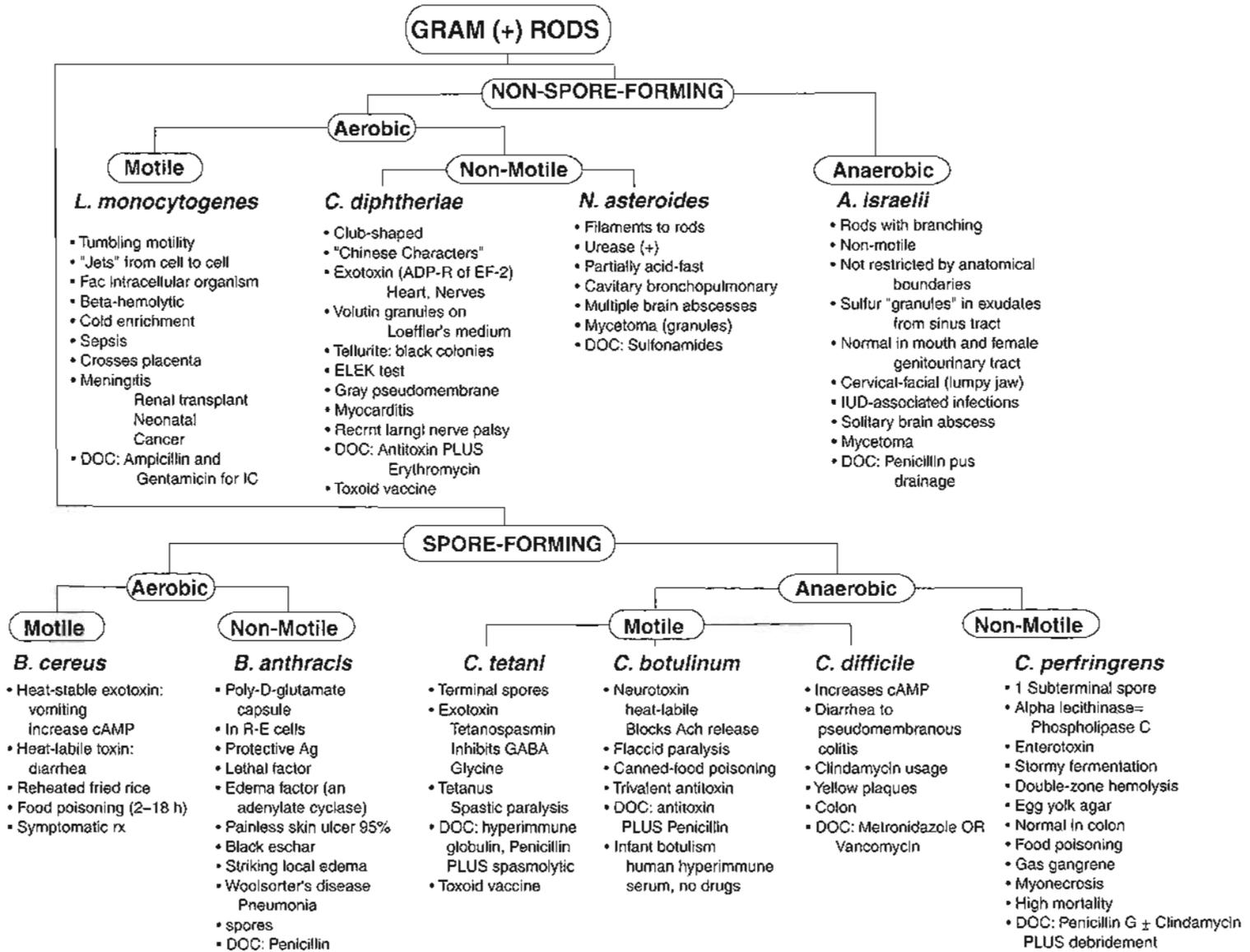
Flow Charts/Clue Sheets

9



COCCI





GRAM (-) RODS & SPIROCHETES

Facultative Anaerobes →

AEROBES

B. pertussis

- Adhesion to cell via hemagglutinin and pertussis toxin
- Adenylate cyclase txn (local edema)
- Tracheal toxin
- Dermanecrosis toxin
- Endotoxin - Lipid X, A ADP-R of GNB
- Bordet-Gengou agar
- Regan-Lowe agar
- Whooping cough
- DOC: Erythromycin
- Killed vaccine

Brucella sp

- In R-E cells
- Endotoxin
- Requires CYS, CO₂
- Unpasteurized milk
- Undulant Fever
Bang's disease
Malta fever
- *B. abortus*
cattle, mild
- *B. suis*
pigs
suppurative, chronic
- *B. melitensis*
goats
severe, acute

F. tularensis

- In R-E cells
- Requires CYS
- *Dermacentor* tick bite
Transovarian trans.
- Aerosol
- Rabbits, rodents
- Granulomatous rxn
- Tularemia - AK, MO, TX
- Live, attnd vaccine

L. pneumophila

- Water-loving
air conditioning
- Requires CYS & Fe
- Buffered Charcoal
Yeast agar
- Dieterle silver stain
- Stains poorly Gram (-)
- Atypical pneumonia
- Mental confusion
- Diarrhea
- DOC: Erythromycin
- Not contagious

P. aeruginosa

- Slime-layer
- Grape-like odor
- Exotoxin A:
ADP-R of EF-2
Liver
- Oxidase (+)
- Pigments
pyocyanin, pyoverdin
- Transient colonization
In 10% of normal pop
- Osteomyelitis in drug abusers
- Pneumonia in
cystic fibrosis
- Nosocomial infections
Burn patients
Neutropenic patients
- Ecthyma gangrenosum
- DOC: Carbencillin
PLUS Aminoglycoside

ANAEROBES

Bacteroides sp

- *B. fragilis* - obligate
- Modified LPS, capsules
- Predominant colonic flora
- Normal in oropharynx, vagina
- Predisposing factors:
surgery, trauma
chronic disease (cancer)
- Septicemia, peritonitis
aspiration pneumonia
- *B. melaninogenicus*
Human bite
- Fusobacterium (combined w/
Treponema microdentium)
Vincent's angina
Trench mouth
- DOC: Metronidazole OR
Clindamycin OR Cefoxitin

Treponema sp

- *T. pallidum* - Syphilis
Obligate parasite
- 1° - PAINLESS chancre, infectious
- 2° - Rash infectious
- 3° - Gummas, CVS, CNS
- Congenital: stillbirths, malformed
- VDRL & RPR - Screening tests
- Reagin ab - rxn with Cardiollpin
- FTA-ABS (immunofluorescence)
specific test
- Dark-field microscopy
- DOC: Benzathine Penicillin

SPIROCHETES

- Thin-Walled
- Spiral-Shaped
- Axial Filaments
- Jarisch-Herxheimer Rxn

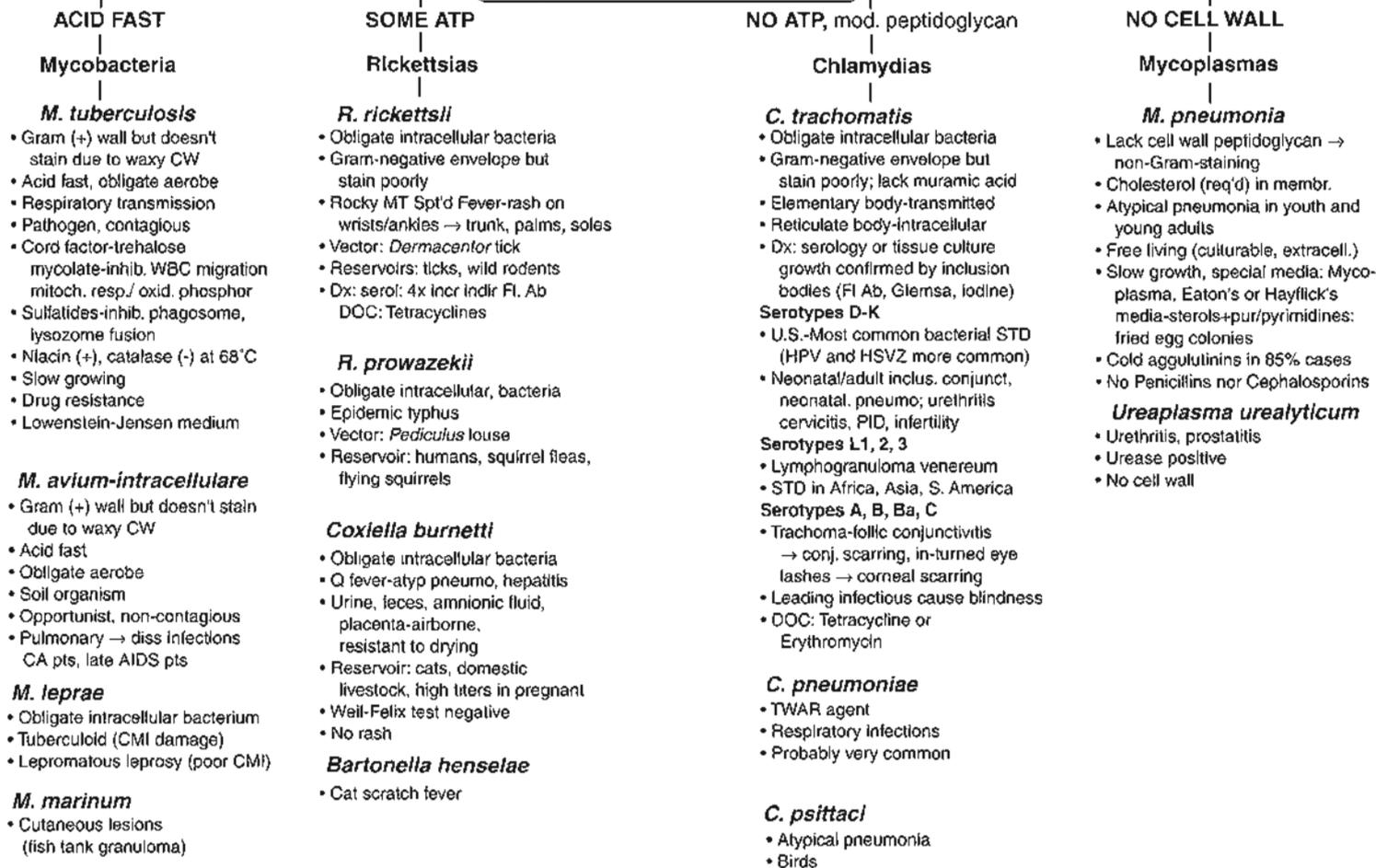
Borrelia sp

- Microaerophilic
- Giemsa stain
- *B. burgdorferi*
Lyme disease
(*I. scapularis*), *I. pacificus*
Reservoirs: mice, deer
CT, WI, CA
Erythema Migrans
Target lesions
- *B. recurrentis*
Relapsing fever
Vector: body louse
Antigenic variation
- DOC: Penicillin or Tetracycline

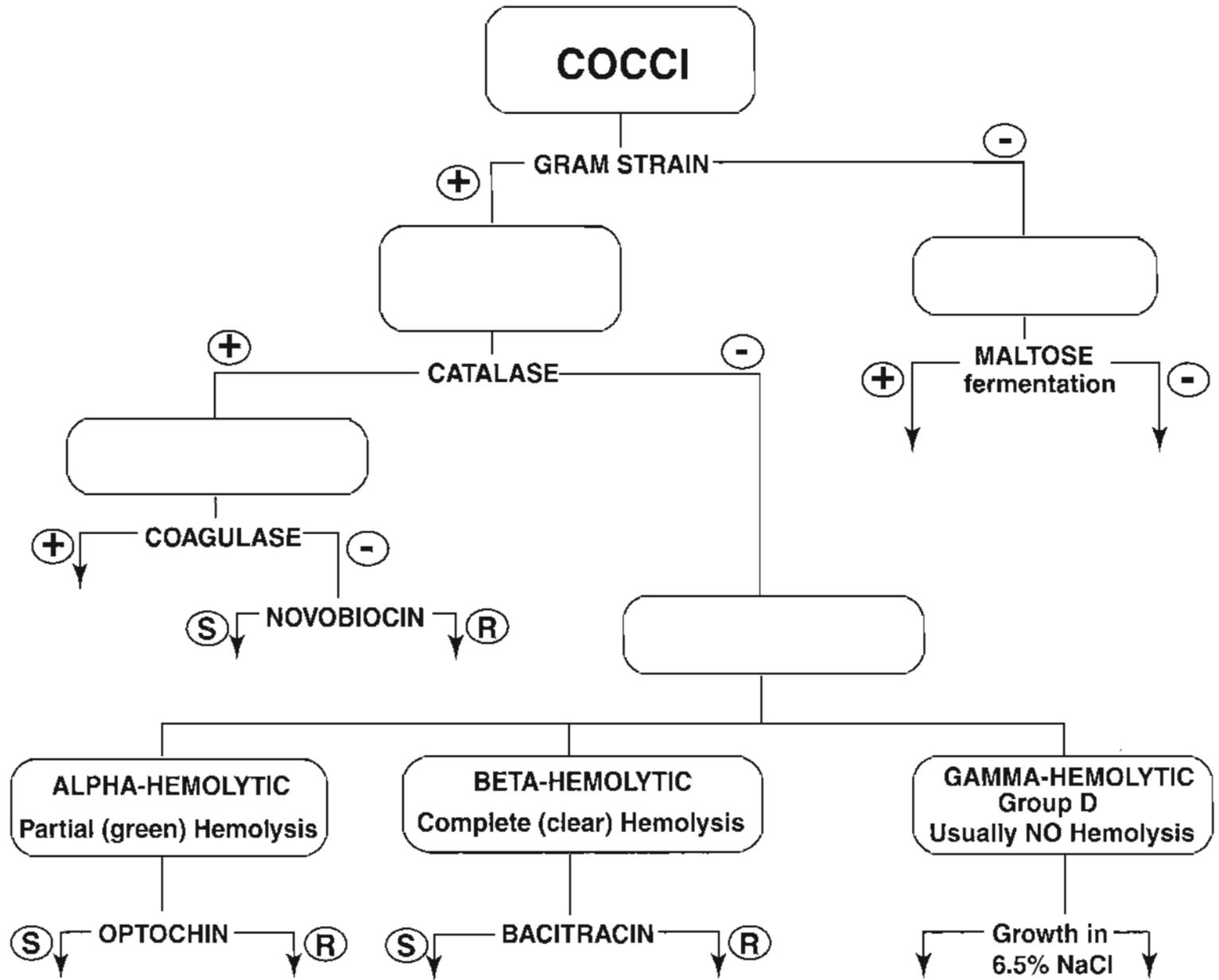
Leptospira sp

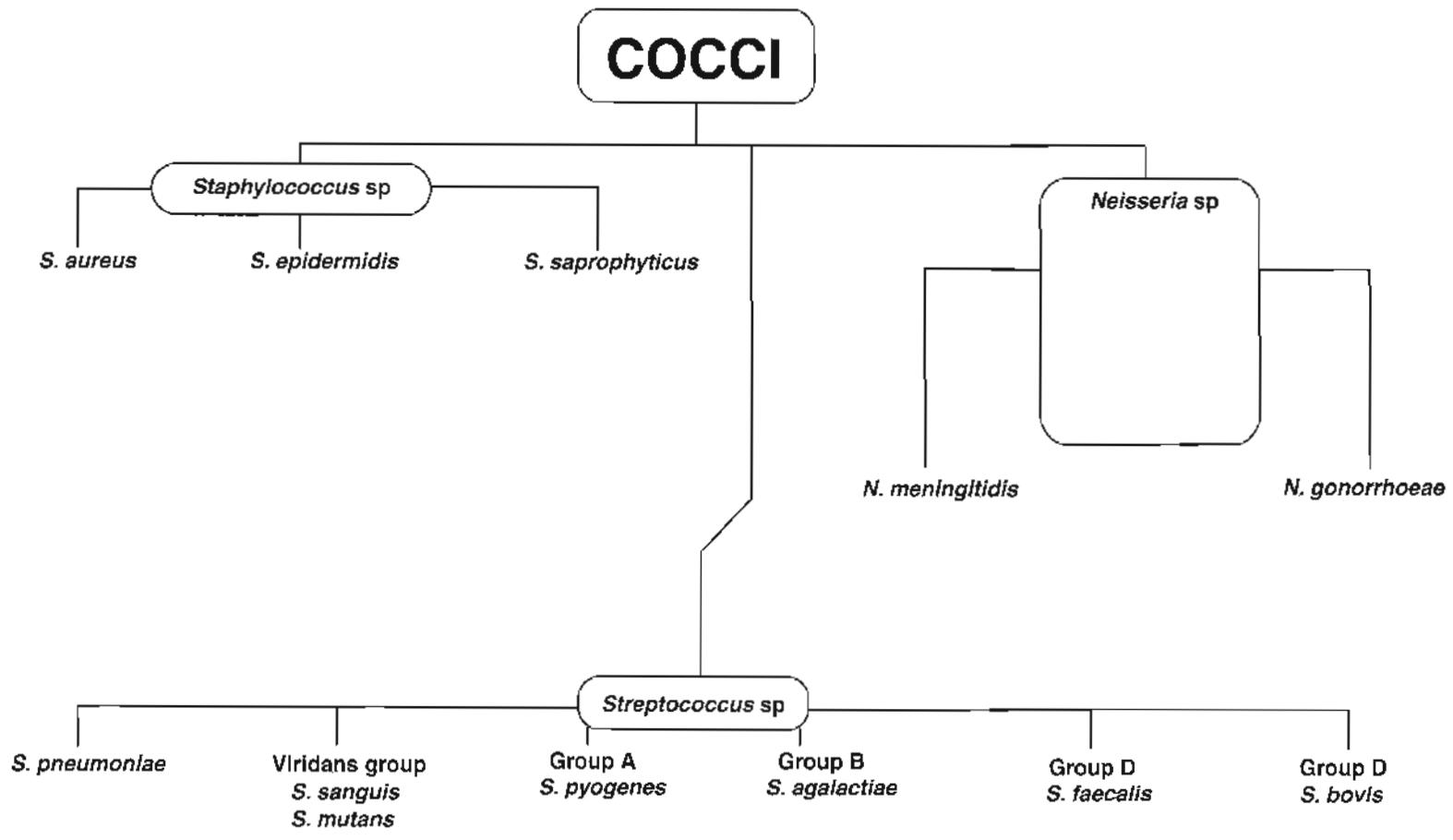
- Dark-field microscopy
- Contaminated water
Animal urine
- Fever, jaundice, uremia
- Non-icteric Leptospirosis
Meningitis - No PMN in CSF
uveitis, rash
- Icteric Leptospirosis
Weil's disease
Renal failure, myocarditis
- DOC: Penicillin G or Tetracycline

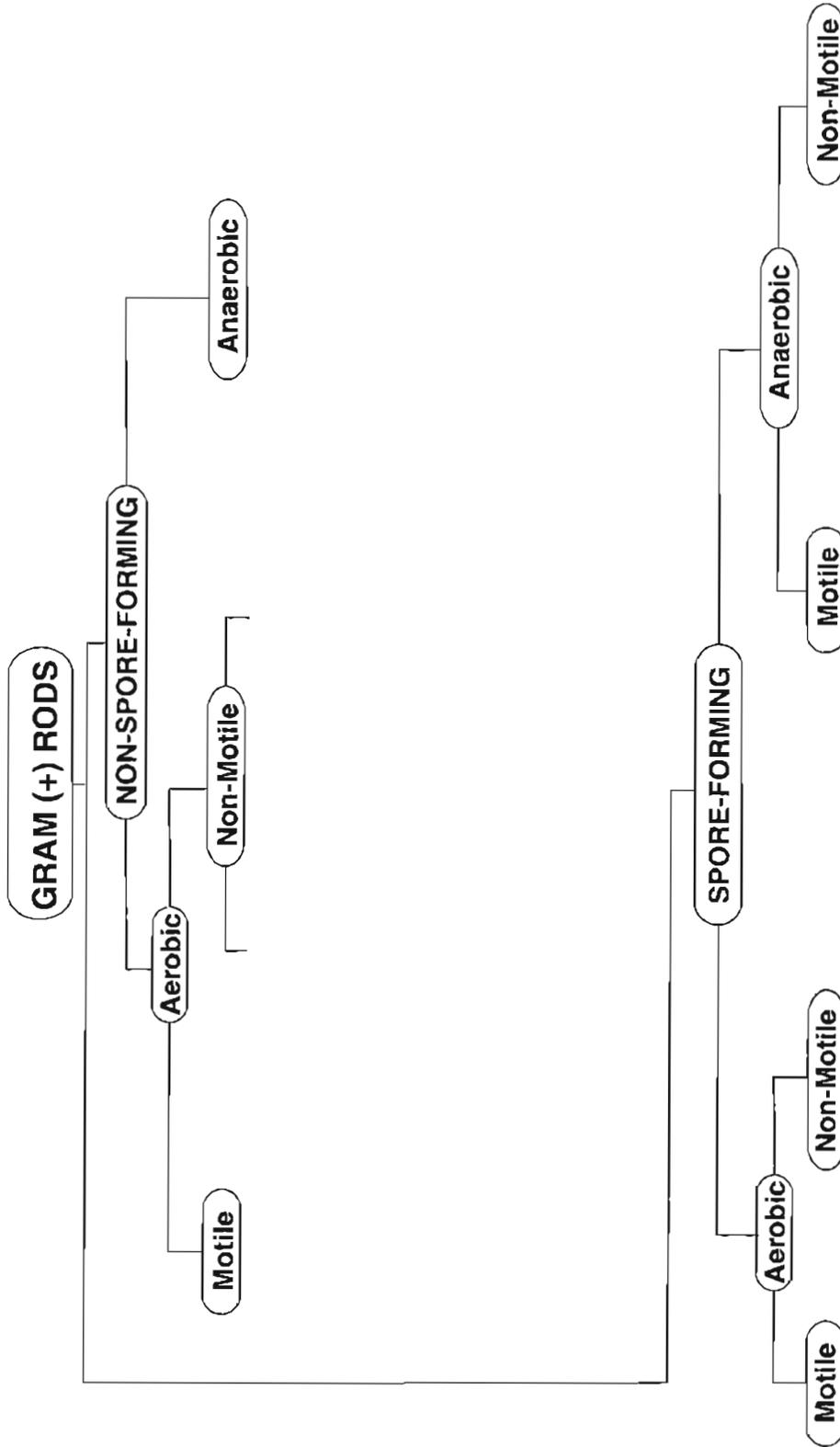
Poorly Gram-Staining Organisms*

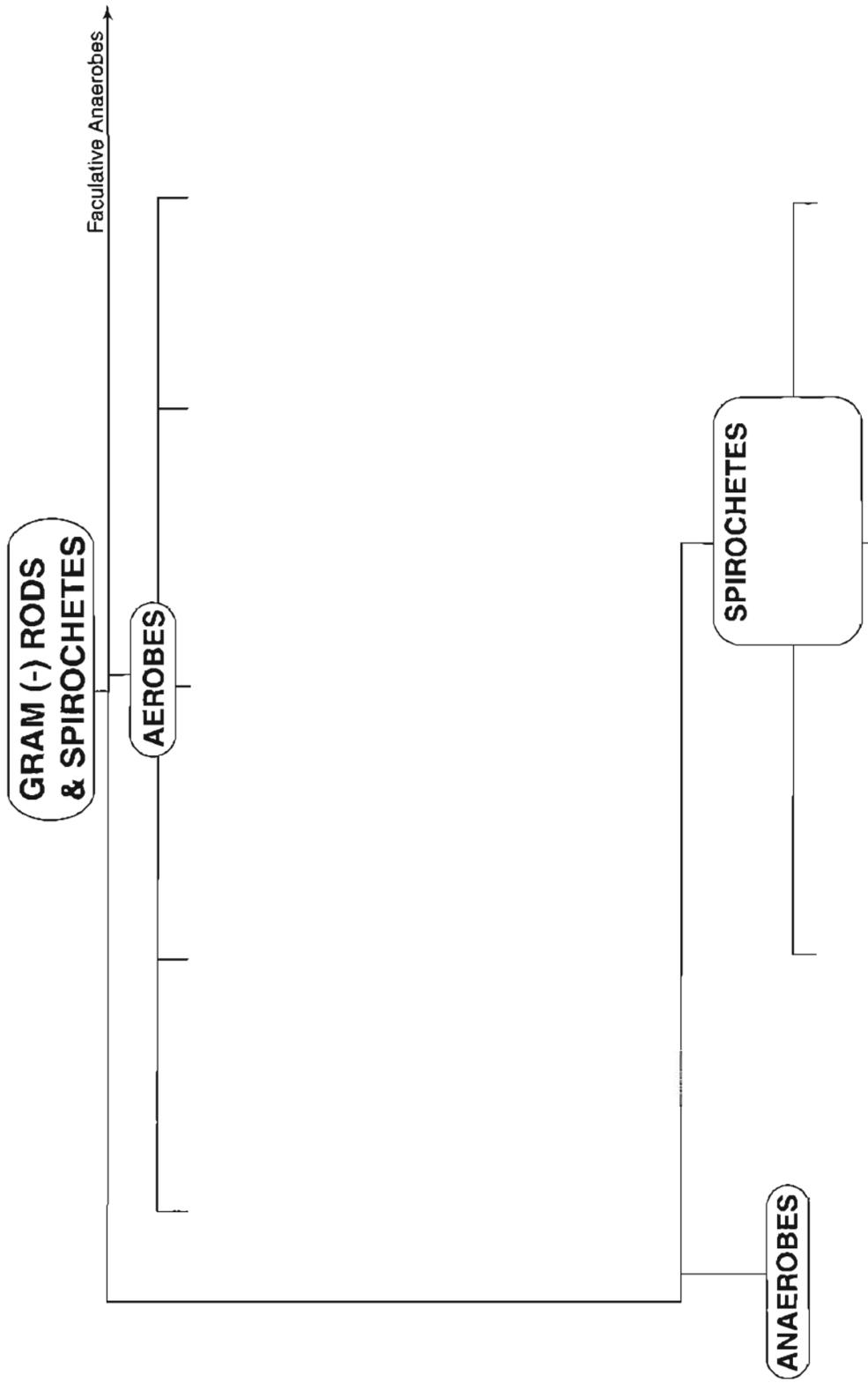


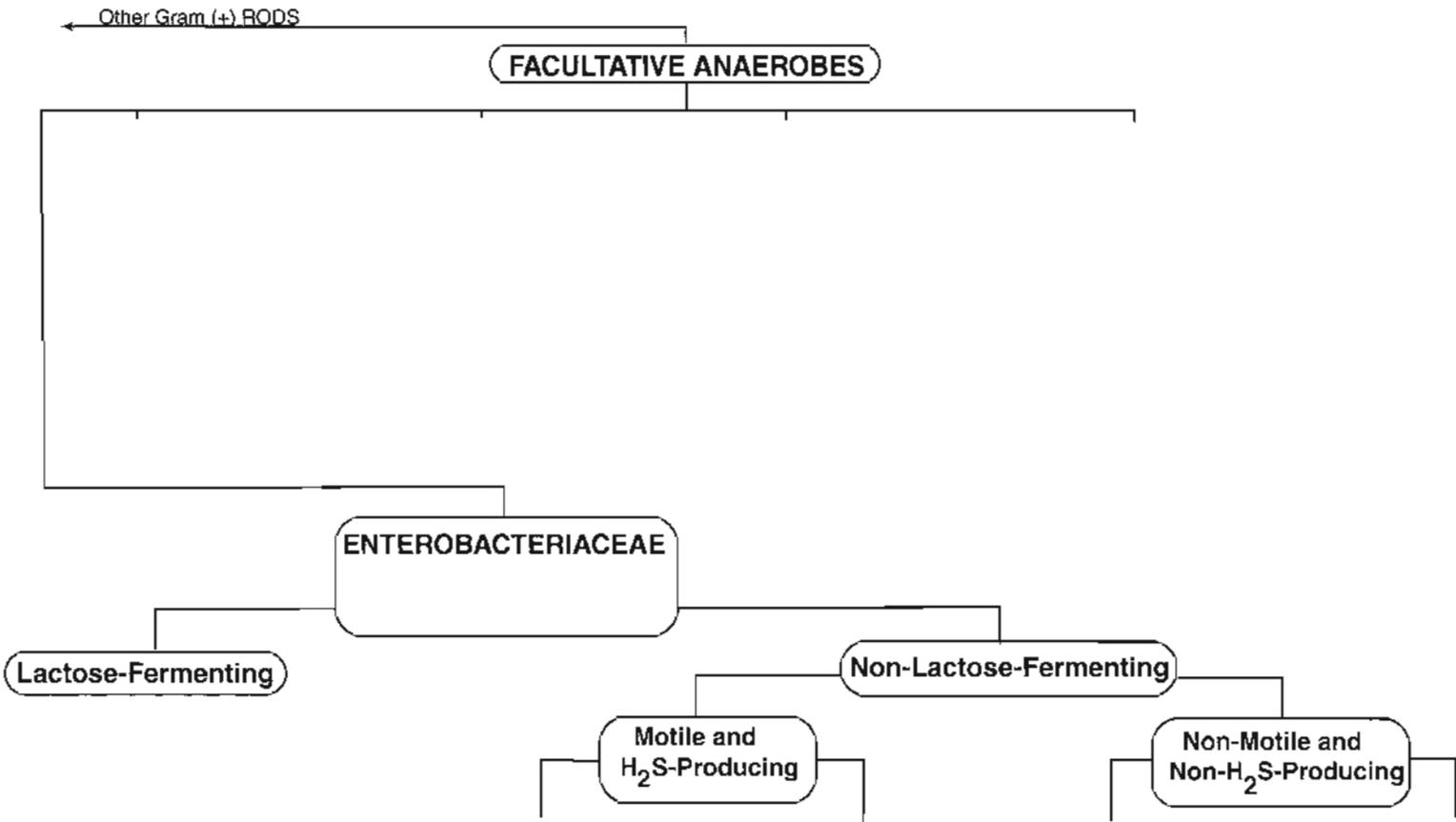
*Also note that *Legionella* and the spirochetes (*Treponema*, *Leptospira*, and *Borrelia*)—all Gram-negative—do not show up reliably with Gram stain.



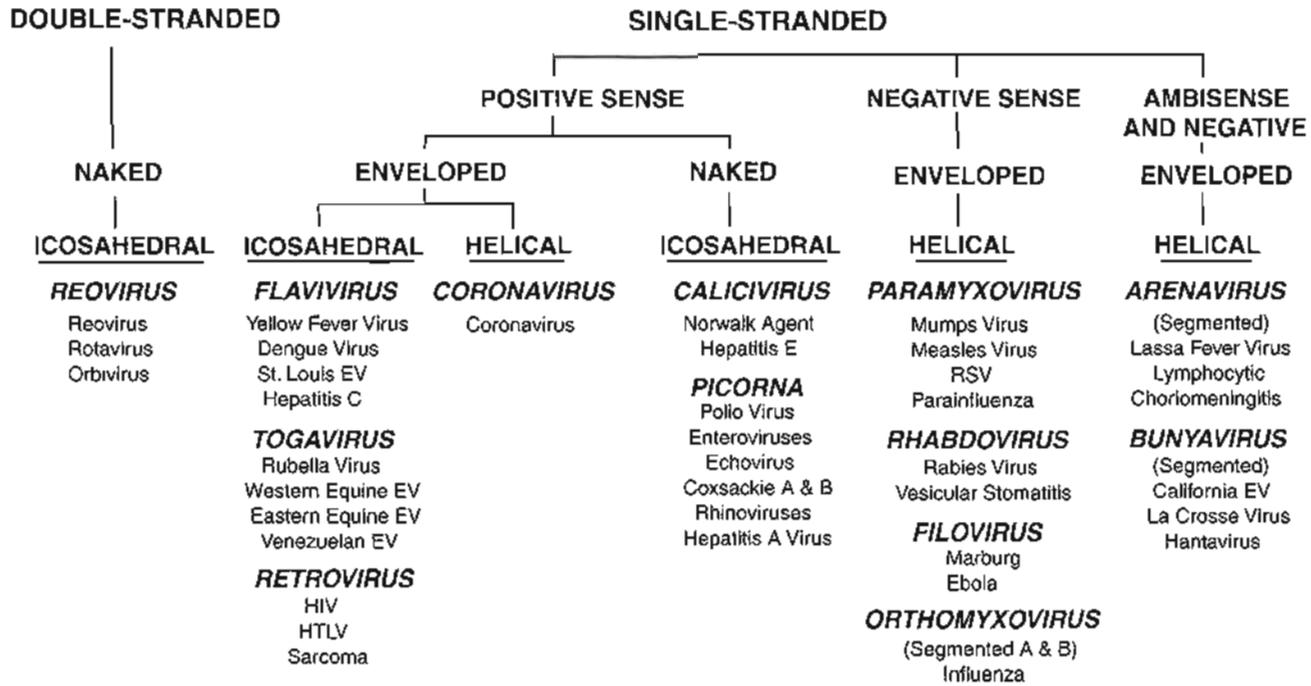




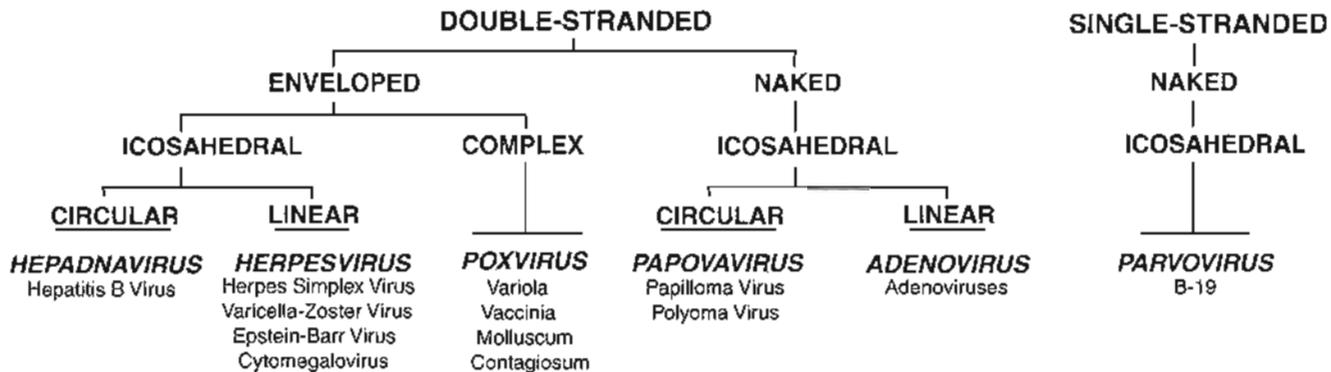


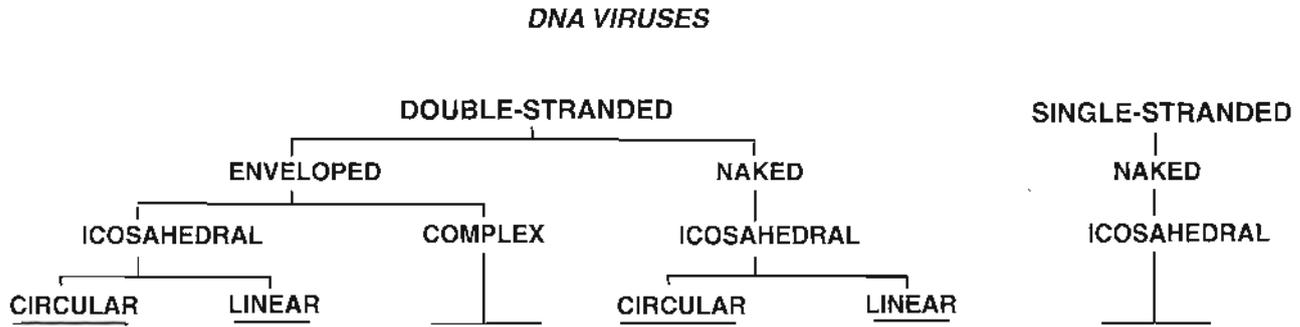
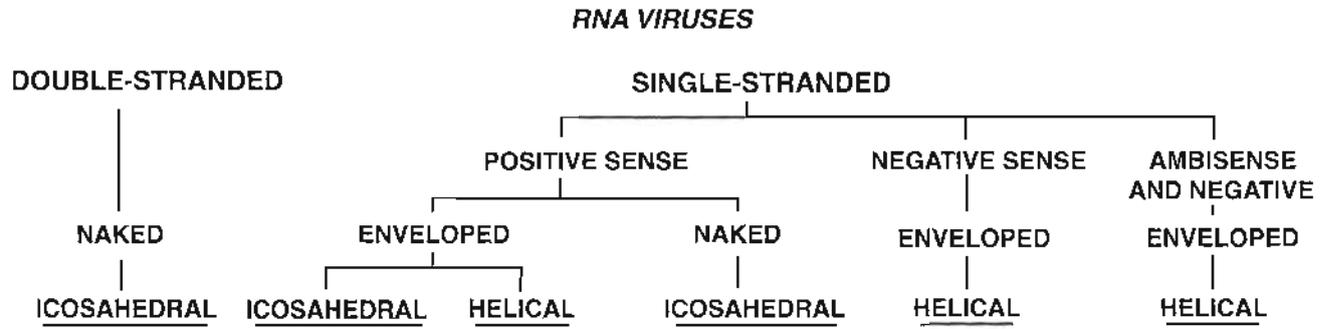


RNA VIRUSES



DNA VIRUSES





SECTION II

Immunology

Introduction

A. The Two Basic Branches of the Immune System

The immune system is an intricate collection of organs, tissues, cells, and soluble factors that allow individuals to defend against harmful agents such as viruses, bacteria, fungi, parasitic organisms, and tumor cells. The ultimate goal of this system is to prevent or limit infections or damage by these agents. The immune response involves recognizing any foreign material and mounting a reaction to eliminate it. The immune response is divided into two categories: the innate and the adaptive branches. These are compared in the table below.

In a Nutshell

- Innate immune system
- Adaptive immune system

Table 1. Innate Versus Adaptive Immunity

Characteristic	Innate Branch	Adaptive Branch
Synonyms	Nonspecific, natural	Specific, acquired
Mode of activation	Not applicable, present at birth	Acquired response to antigens
Inducible	No	Yes
Memory	No	Yes
Reaction time	Rapid 0-6 hours	Slow initiation, rapid thereafter
Phagocytosis	Yes	No
Includes various leukocytes	PMNs, monocytes, macrophages, eosinophils, NK cells	Specific B cells, specific T cells
Specific antibodies	No	Yes
Includes the complement system	Yes	No
Includes physical barriers, e.g., skin and mucous membranes	Yes	No
Produces interferons	Yes	No
Lysozyme in secretions	Yes	No
Induces fever	Yes	No
Causes inflammation	Yes	No

Note

A cytokine is a substance secreted by any type of leukocyte in response to a stimulus. Those cytokines that are produced by one class of leukocyte and affect the activity of a different class are called interleukins or lymphokines.

B. Interaction Between the Innate and Adaptive (Acquired) Branches

The interrelationship between the various parts of the innate and adaptive branches is summarized in the figure below. Communication between the two branches of the immune system is conducted via molecular messengers called cytokines.

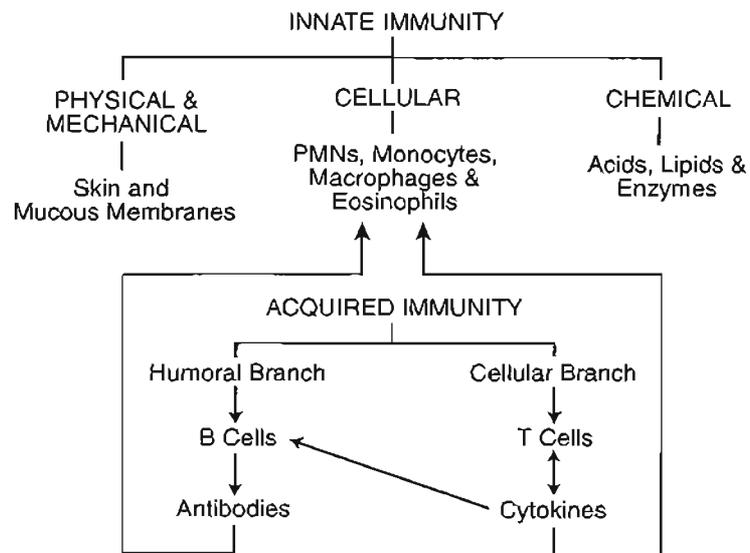


Figure 1. Innate and Acquired Immunity

Innate Immunity

1

A. Major Barriers for Entry of Pathogenic Organisms

These are illustrated in the figure below (note lysozymes are also found in other secretions such as tears and saliva).

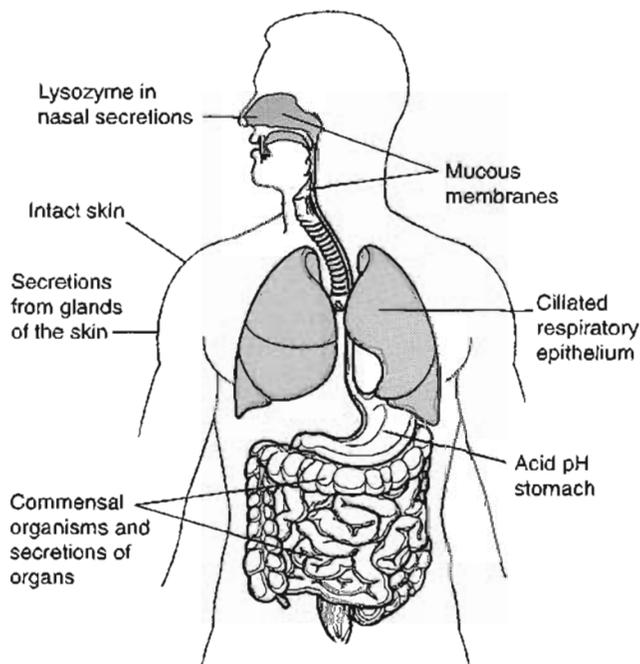


Figure II-1-1

B. Professional Phagocytic Cells

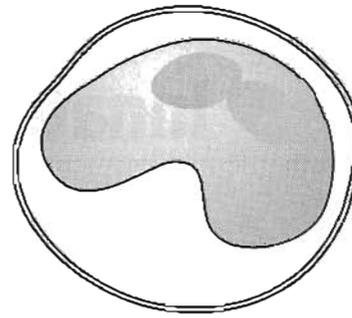
These cells have enzymatic constituents in their granules to oxidize, kill, digest, and destroy particulate material that they ingest. These include:

1. Mononuclear phagocytes

- a. Monocytes (found in the blood)

In a Nutshell

- Physical and mechanical barriers
- Chemical barriers

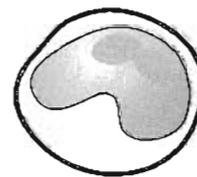


Horseshoe-shaped Nucleus

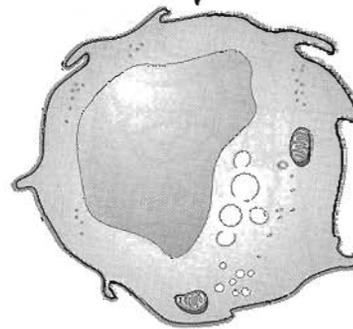
Figure II-1-2. Monocyte

In a Nutshell

- Phagocytic monocytes
- Phagocytic macrophages
- Phagocytic neutrophils



Blood Monocyte

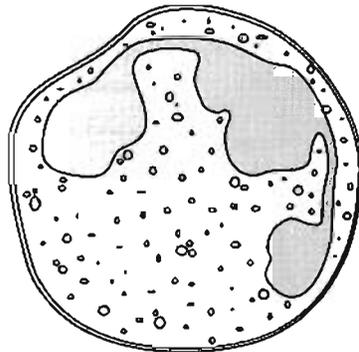


Tissue Macrophage

Figure II-1-3. Differentiation of a Blood Monocyte Into a Tissue Macrophage

- b. Tissue macrophages
 - i. Liver Kupffer cells
 - ii. Lung alveolar macrophages
 - iii. Kidney mesangial macrophages
 - iv. CNS microglial cells
 - v. Spleen and lymph nodes
 - vi. Peyer's patches and tonsils
 - vii. Peritoneal macrophages

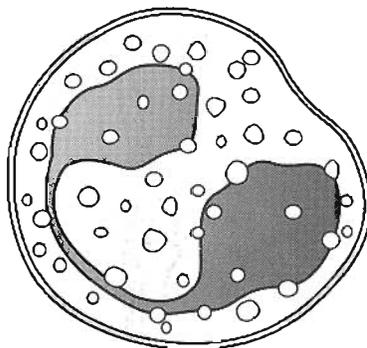
2. **Polymorphonuclear leukocytes (PMNs)**
 a. **Neutrophils** (most aggressive phagocyte)



Lobed Nucleus,
Small Granules

Figure II-1-4. Neutrophil

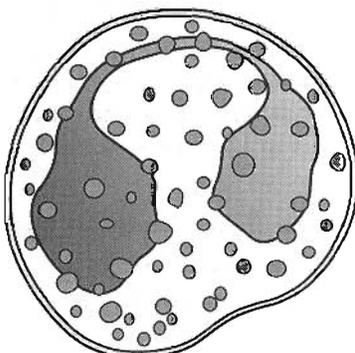
- b. **Eosinophils** (antiparasitic phagocyte)



Bilobed Nucleus,
Large Granules (Pink)

Figure II-1-5. Eosinophil

- c. **Basophils** (secretory cells)



Bilobed Nucleus,
Large Granules (Blue)

Figure II-1-6. Basophil

Clinical Correlate

Eosinophilia is a hallmark of:

- Atopic allergies
- Worm infections

It can also be seen in collagen vascular diseases, neoplastic disorders, and any skin rash.

In a Nutshell

Phagocytic RES cells

- Lung
- Spleen
- Lymph nodes
- Liver

In a Nutshell

- **Chemotaxis:** Cell migration toward an attractant (a chemotactic factor) along a concentration gradient
- **Selectins and integrins:** Molecules that enhance binding of leukocytes to specific endothelial sites and thereby control leukocyte "homing"
- **Diapedesis:** Migration of leukocytes through capillary walls
- **Leukotrienes:** Molecules formed from arachidonic acid via the lipoyxygenase pathway. Leukotrienes cause prolonged constriction of smooth muscle; these are sometimes still called by their older name, the slow-reacting substances of anaphylaxis (SRS-A)

3. Locations of macrophages

Collectively this system is called the reticuloendothelial system (RES).

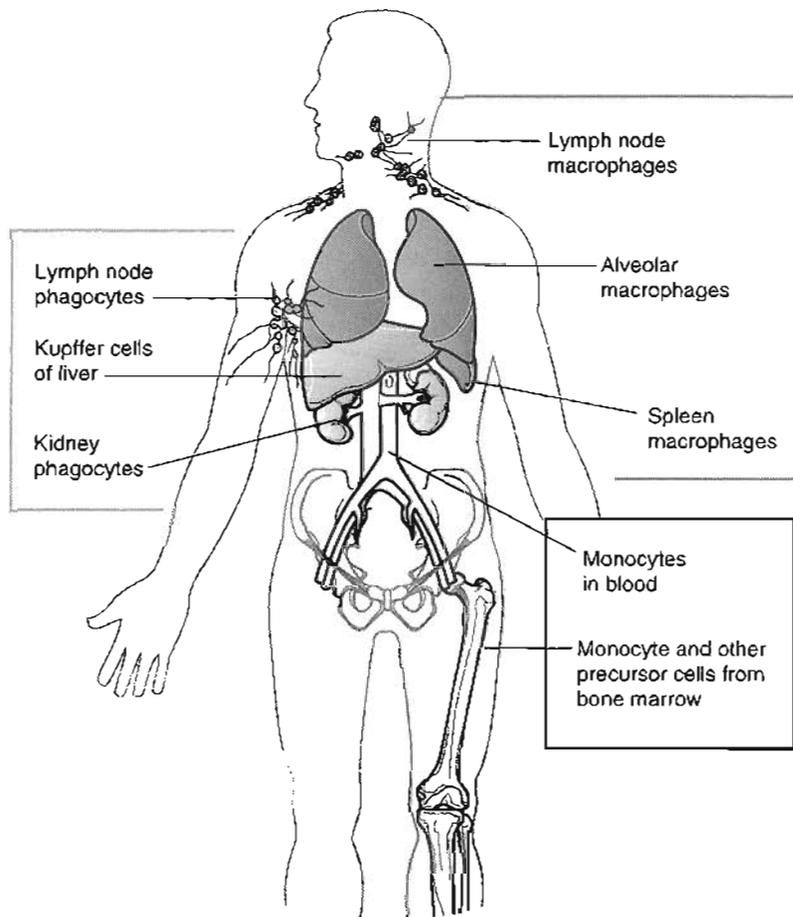


Figure II-1-7. Distribution of Macrophages Within the Body

4. Stages of phagocytic cell function

- a. **Production:** Bone marrow (14-day maturation time)
- b. **Mobilization:** Release from bone marrow reserve. Most phagocytes in the body are found as fully mature cells held in reserve in the bone marrow. On infection, these cells can be rapidly mobilized to increase phagocyte concentrations in the blood and provide defense more quickly (4 to 12 hours, instead of 14 days).
- c. **Chemotaxis**
 - i. Endothelial cell adherence (E- and L-selectins, integrin-ICAM interactions)
 - ii. Diapedesis—crossing the endothelial barrier
 - iii. Directed migration toward the highest chemotactic factor concentration
 - iv. Activation of phagocytes
 - v. Chemotactic factors

Table II-1-1. Source of Chemotactic Factors

Source	Chemotaxin
Bacteria	f-Met-Peptides
Serum	Complement 5a (C5a)
Macrophages	Interleukin 8 (IL-8)
Neutrophils	Leukotriene B ₄

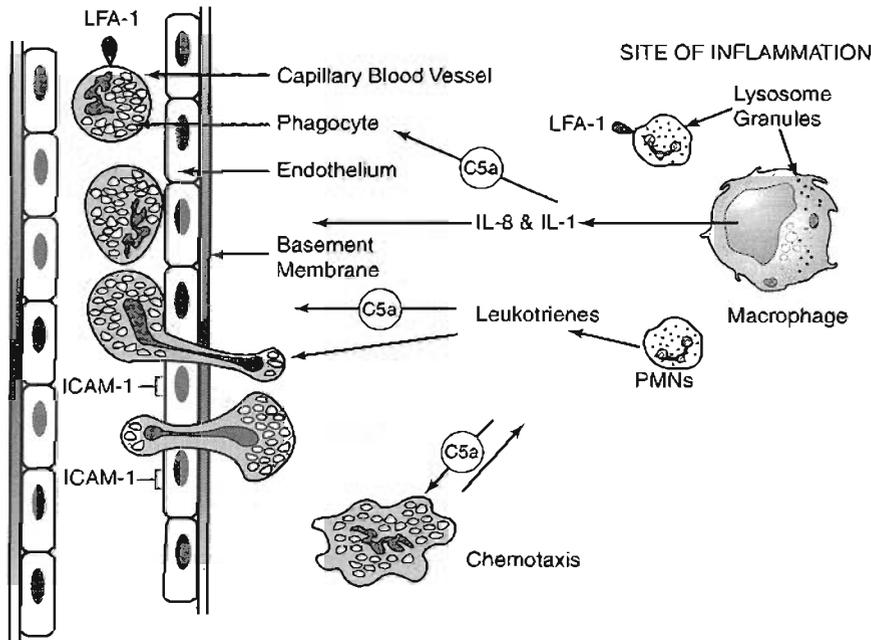


Figure II-1-8. Chemotaxis

ICAM-1 = intercellular adhesion molecule-1; LFA-1= lymphocyte function-associated antigen.

d. Opsonization: Coating of particles to improve digestion.

- i. IgG (via Fc receptors)
- ii. C3b (via CR3 complement receptors)

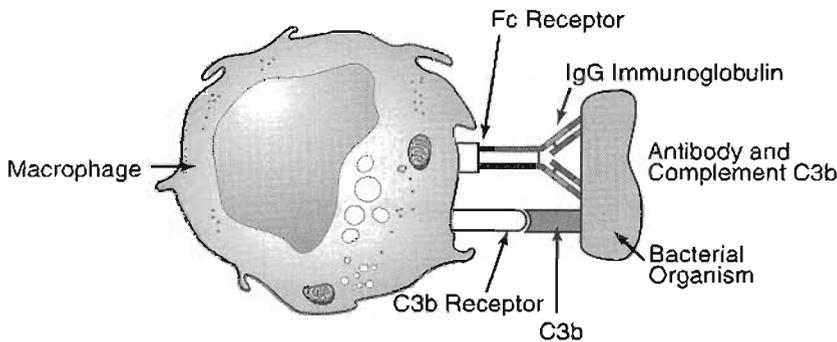
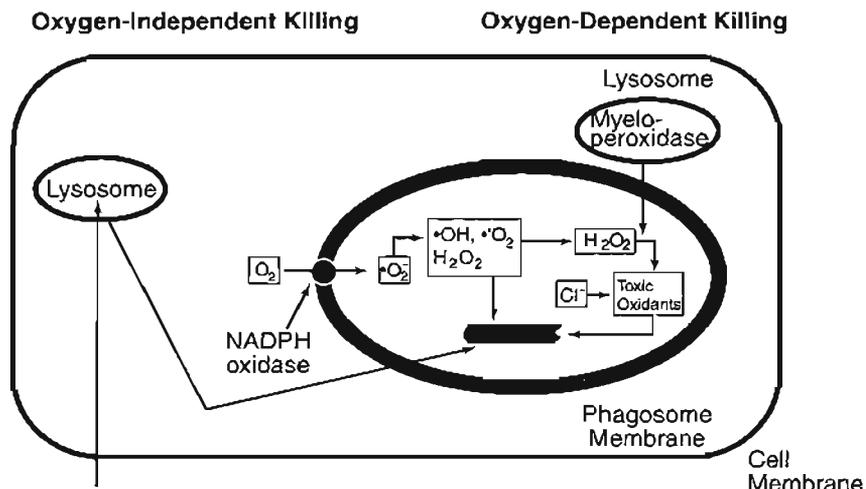


Figure II-1-9. Antibody and Complement C3b

Note

- Macrophages and neutrophils both phagocytose bacteria that are coated with antibody and complement.
- The C3b fragment of complement binds to bacteria opsonized by antibody, then binds to receptors on phagocytic cells and signals them to phagocytose the organisms.
- The Fc receptors on macrophages are also useful for opsonization of bacteria by antibody. They react with the Fc region of IgG antibody molecules and hold the microbe close to the phagocytic cell membrane, thus facilitating the engulfment process.

- e. **Engulfment:** Formation of phagocytic vacuole (**phagosome**).
 - i. Energy required to generate ATP is essential.
 - ii. Intracellular actin-myosin polymerization causes invagination of the phagocyte membrane at the site of attachment of the opsonized particle and eventual entrapment in a vacuole called a phagosome.
- f. **Metabolic stimulation and killing:** Within minutes of ingestion of an opsonized particle, there are massive alterations in the metabolic pathways used by phagocytes. There is oxygen-dependent killing and oxygen-independent killing occurring within the phagocyte.



OXYGEN-INDEPENDENT KILLING OCCURS INSIDE LYSSOSOME

1. Hydrolytic Enzymes
2. Cationic Proteins
3. Lysozyme
4. Lactoferrin

Figure II-1-10. Metabolic Stimulation and Killing Within the Phagocyte

- g. **Metabolic events associated with phagocytosis**
 - i. Increased glycolysis via hexose monophosphate shunt pathway
 - ii. Increased lactic acid: decreased pH in phagosome
 - iii. Increased oxygen consumption with production of toxic metabolites
 - H_2O_2
 - Superoxide anion (O_2^-)
 - Singlet oxygen ($^1\text{O}_2$)
 - Hydroxyl radical ($\cdot\text{OH}$)
 - Hypochlorite (via myeloperoxidase) (HOCl)

C. Lytic Cells: Natural Killer Cells

In addition to the phagocytic cells of the innate immune system, there is another very important cell type: the **natural killer (NK) cell**. These **lymphoid cells** are classified as **large granular lymphocytes (LGL)**. The NK cells are found in the spleen, lymph nodes, bone marrow, and peripheral blood. These cells can lyse a variety of target cells, including **virus-infected cells**, **antibody-coated cells**, and **different tumor cells**.

Clinical Correlate

Chronic granulomatous disease (CGD) is an inherited deficiency in one subunit of NADPH oxidase. This results in the inability to generate the toxic oxygen metabolites. Patients get severe infections with catalase-positive organisms, such as *Staphylococcus aureus*, because the catalase they produce breaks down H_2O_2 and removes the use of the second intracellular killing technique using MPO to generate toxic halide radicals. Catalase-negative organisms, and multiple other intracellular processes, produce H_2O_2 as a waste product, and phagocytes can use it to produce all of the toxic halide metabolites for killing.

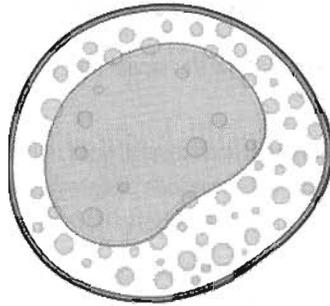


Figure II-1-11. Natural Killer Cell With Granules

1. **Mechanism of killer cell lysis of targets**
 - a. Both cytotoxic T cells and NK cells have granules
 - b. Steps include:
 - Cell-cell binding
 - Granule mobilization (release of perforins)
 - Exocytosis of granules
2. **Mechanism of killer cell-independent lysis:** pores are formed in target cell membrane

In a Nutshell

Natural killer cells

- Kill virus-infected cells
- Antibody-coated cells
- Tumor cells

Chapter Summary

There are two branches to the immune system: the innate (aka nonspecific or natural) and the adaptive (aka specific or acquired).

The innate system is functional at birth, responds rapidly, does not use antibodies, and has no memory. Modalities used by the innate system include phagocytosis and lysis by leukocytes, the complement system, physical barriers, interferons, lysozyme, fever, and inflammation.

The adaptive system is specifically induced by response to antigens. It is initially slow to develop, but, because it has memory, it is rapid thereafter. This system includes antibodies and specific B and specific T lymphocytes but does not include the other classes of leukocytes. However, lymphocytes of the adaptive branch use cytokines and antibodies to communicate with and further activate the leukocytes of the innate branch.

The major barriers preventing the entry of pathogens into the body include intact skin, mucous membranes, respiratory epithelial cilia, gastric acidity, commensal flora in the intestine, secretions of the skin and digestive tract, and lysozyme, which is an enzyme capable of breaking peptidoglycan bonds in bacterial cell walls. Lysozyme is found in tears, mucus, and saliva.

The cell types functioning as part of the innate system include phagocytic and lytic cells. These cells are found in the blood, spleen, lymph nodes, lung, kidney, liver, and bone marrow.

The phagocytes include monocytes, tissue macrophages, polymorphonuclear leukocytes (neutrophils), eosinophils, and basophils.

The phagocytes develop in the bone marrow over a period of approximately 14 days. However, reserve cells are also maintained in the bone marrow and are rapidly released (within 4 to 12 hours) in response to chemotactic stimuli generated at the site of infection. These chemotactic factors include f-met-peptides released by bacterial lysis, serum complement component 5a, macrophage interleukin 8, and leukotriene B₄.

Phagocytosis by macrophages and neutrophils is facilitated by opsonization, a process in which the foreign body is coated with antibody and complement. The particle is then engulfed into a phagosome generated by invagination of the phagocyte's membrane. Oxygen-dependent and oxygen-independent processes of killing start within minutes of ingestion. This process is ATP-dependent.

Large granular lymphocytes are not phagocytic but act by lysing cells. These natural killer (NK) cells are found in the spleen, lymph nodes, bone marrow, and peripheral blood. They can lyse virus-infected cells, tumor cells, and antibody-coated cells.

Review Questions

1. The cell that is considered to be one of the basic cells of the innate immune response is which one of the following?
 - A. B lymphocyte
 - B. CD4⁺ T-helper 1 lymphocyte
 - C. CD4⁺ T-helper 2 lymphocyte
 - D. CD8⁺ T-cytotoxic lymphocyte
 - E. Macrophage
2. Pathogenic bacteria can be killed intracellularly in phagocytic cells by different mechanisms. Which one of the following is part of the non-oxidative killing pathway?
 - A. Lysozyme
 - B. Superoxide ions
 - C. Hydrogen peroxide
 - D. Hypochlorous acid
 - E. Myeloperoxidase
3. An active inflammatory reaction is occurring, and large numbers of neutrophils are being attracted to the inflammation site. Which one of the following is a major chemotactic factor involved in this reaction?
 - A. IL-2
 - B. IgM
 - C. C5a
 - D. C3b
 - E. Lysozyme
4. Which pair of molecules are both opsonins?
 - A. IgG and C5a
 - B. IgA and IgG
 - C. IgE and IgG
 - D. IgG and IgM
 - E. IgG and C3b
5. All of the following describe the process of neutrophil chemotaxis except:
 - A. It can be mediated by either C5a or leukotriene B₄.
 - B. It requires a chemotactic factor gradient.
 - C. It begins with adherence of cells to endothelium.
 - D. It is also called random migration or mobilization.
 - E. It can also include activation of the neutrophil.

Answers

1. **Answer: E.** The macrophage is a phagocytic cell that engulfs foreign material. It does not have to be induced, it is not specific, and it has no memory. This cell is one of our most important phagocytic and antigen-presenting cells.
2. **Answer: A.** Lysozyme is present in tears, saliva, mucus, vaginal secretions, and several other body fluids. This material lyses the peptidoglycan layer of the cell wall of bacteria, and it does not require the oxidative killing pathway.
3. **Answer: C.** In this active inflammatory site, complement system has been activated and the production of C5a is a strong chemoattractant to neutrophils and other phagocytic cells.
4. **Answer: E.** Opsonins are molecules that bind to particles and interact with receptors on phagocytic cell surfaces, leading to increased particle adherence and eventually particle ingestion. The Fc receptors on PMN and macrophage surfaces bind **all** subclasses of IgG, but not other antibody classes. Phagocytes also use C3b receptors for binding complement-coated particles. IgM, IgA, and IgE are not opsonins.
5. **Answer: D.** Factors that induce random migration stimulate a process called **chemokinesis**. **Chemotaxis** refers to directed (**not** random) migration.

Acquired (Adaptive) Immunity

2

A. Functional Units

The acquired immune system can be conveniently divided into four functional units:

1. **Recognition**, which
 - Is specific
 - Has memory
2. **Amplification**, which is due to
 - Cell division
 - Activation of enzyme cascades
3. **Regulation**, or control
4. **Effector mechanisms**, by which foreign structures are actually eliminated

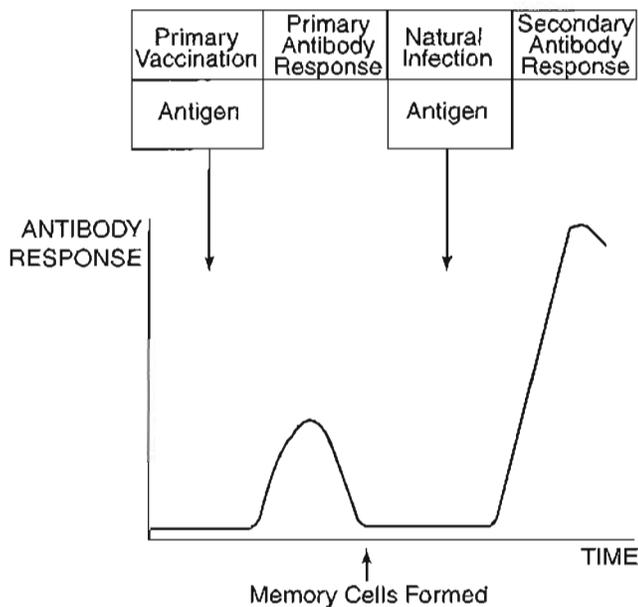


Figure II-2-1. Antibody Production Response to Introduction of Antigen as in Vaccination

In a Nutshell

- Active immunity: produce our own Ab
- Passive immunity: Ab acquired from another source

B. Modes of Acquisition of Specific Immunity

1. **Naturally acquired**
 - a. **Passive:** Placental Ab transfer
 - b. **Active:** Recovery from disease, e.g., ***Bordetella pertussis*** or influenza virus
2. **Artificially acquired**
 - a. **Passive:** Antitoxin administration, e.g., rabies or tetanus
 - b. **Active:** Vaccination, e.g., MMR, DPT, or DaPT

C. Induction of the Acquired (or Adaptive) Immune Response

1. The specific immunologic response that follows first exposure to a foreign substance (antigen) triggers a chain of events that induces a response. This foreign substance induces lymphocytes to:
 - a. **Proliferate**
 - b. **Differentiate**
 - c. **Produce soluble substances**
 - Antibodies
 - Cytokines
 - d. **Establish memory**
2. **Three major types of cells participate in this adaptive system: macrophages, and the two types of lymphocytes, T cells and B cells.**
 - a. Macrophages are nonspecific and are involved in **processing and presentation of peptides** to T cells.
 - b. B cells **synthesize and secrete antibodies** with the ability to react with specific antigen.
 - c. T cells **collaborate** with B cells to stimulate the **production of antibody**. They are also involved in many other immune mechanisms including **regulation of immune responses**.
3. **Differentiation of B lymphocytes:** B lymphocytes differentiate into **plasma cells** that secrete large amounts of antibody (immunoglobulin). As illustrated below, B cells use surface antibody to bind to antigen. This causes them to become activated and secrete more immunoglobulin.

In a Nutshell

Adaptive immune system cells

- Macrophages
- T cells
- B cells

Clinical Correlate

Absence of circulating B cells diagnostic of Bruton X-linked hypogammaglobulinemia

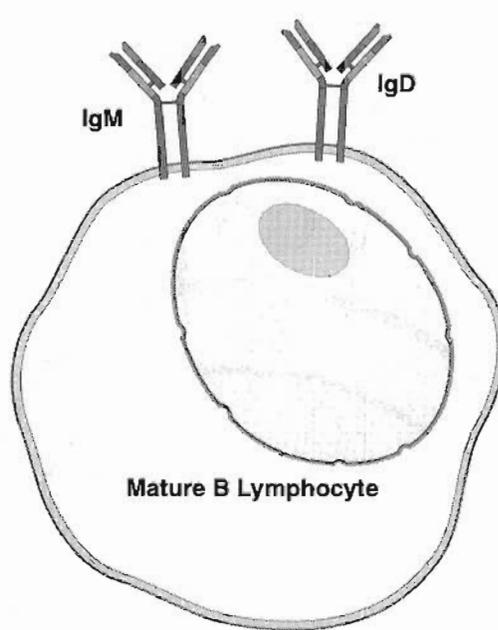


Figure II-2-2. Antibody Binding to a Mature B Lymphocyte (Plasma Cell)

In a Nutshell

- Th cells are CD4⁺
- Tc cells are CD8⁺

4. **Differentiation of T lymphocytes:** Cellular differentiation (CD) proteins are present on many cells of the body. They reflect the function of the cell and are specific markers for particular cells. Two major types of T cells exist: CD4 (helper) and CD8 (cytotoxic) T lymphocytes. These are classified on the basis of expression of cell-surface CD proteins.
 - a. CD4⁺ T lymphocytes: **Helper T cells are CD4 positive.** They enhance both humoral and cell-mediated immunity.
 - b. CD8⁺ T lymphocytes: **Cytotoxic T cells are CD8 positive.** They are a part of the cell-mediated immune response against virus-infected cells or tumor cells.
5. **The T-cell receptor (TCR):** A specific surface molecule of the T cell that recognizes and reacts with foreign antigen presented to the T cells by a variety of antigen-presenting cells, including:
 - a. Macrophages
 - b. Dendritic cells
 - c. B cells
 - d. Langerhans cells

The following figure below illustrates a T lymphocyte with a TCR, CD4, and CD8 molecule. Remember: Mature T cells do not have BOTH CD4 and CD8 molecules on the same cell.

In a Nutshell

APC (antigen-presenting cells)

- Macrophages
- Dendritic cells
- B cells
- Langerhans cells

In a Nutshell

Humoral immunity = B cells = antibodies

Cellular immunity = CD4⁺ & CD8⁺ lymphocytes = cytokines

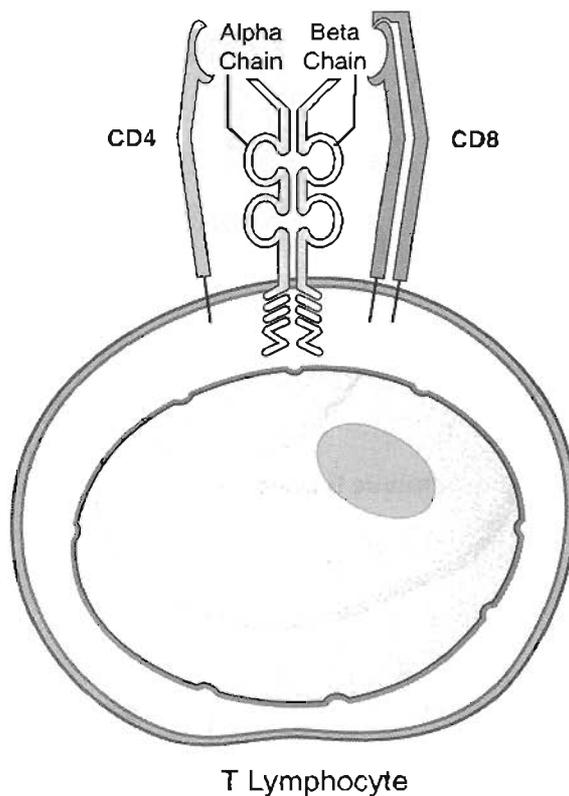


Figure II-2-3. T-Cell Receptor (TCR)

Chapter Summary

Acquired immunity requires the recognition, amplification, regulation, and elimination of the foreign substance. It may be acquired passively or actively. Passive acquisition occurs by the transfer of preformed products manufactured by another host. Active acquisition occurs in response to exposure to an antigen, either artificially by administration of a vaccine or naturally via recovery from an infection. In naturally acquired immunity, the first exposure results in the primary response, which is relatively weak. However, this exposure produces memory cells, which promote a rapid, more profound response on subsequent exposure to the same antigen, the secondary response.

B cells bind antigen to the immunoglobulin molecules on their surfaces and differentiate into plasma cells. T cells bind antigen presented to them and differentiate into CD4⁺ helper cells or CD8⁺ cytotoxic cells.

The three T-cell surface molecules that are essential for this process are the T-cell receptor, which reacts with foreign antigens presented to it, and either—but never both—the CD4 or the CD8 surface glycoprotein.

Review Questions

1. Which one of the following cells is found in the adaptive (acquired) immune system?
 - A. Macrophage
 - B. Neutrophil
 - C. B cell
 - D. Langerhans cell in the skin
 - E. Kupffer cell in the liver
2. The administration of the DPT shot would stimulate which one of the following types of immunity?
 - A. Adaptive immunity
 - B. Natural active immunity
 - C. Natural passive immunity
 - D. Artificial active immunity
 - E. Artificial passive immunity
3. Immunological memory is a characteristic of both
 - A. T cells and NK cells
 - B. B cells and dendritic cells
 - C. T cells and B cells
 - D. Macrophages and B cells
 - E. B cells and LAK cells
4. Which one of the following pairs of immunoglobulins is found on the surface of the mature B cells?
 - A. IgG and IgD
 - B. IgG and IgA
 - C. IgM and IgE
 - D. IgM and IgD
 - E. IgE and IgA

Answers

1. **Answer: C.** The B cell is part of the adaptive immune system. This cell has in its membrane IgM and IgD immunoglobulins that react specifically with only one epitope. This cell had to be induced to be formed. It has specificity and memory against this one epitope. This cell can be stimulated to divide rapidly by interleukins, and it divides and forms a larger clone than before, following its stimulation.
2. **Answer: D.** The administration of the diphtheria toxoid-pertussis products and tetanus toxoid to an individual stimulates our own immune system to produce antibody and memory cells against this mixture. Active immunity is when we produce our own antibody. Artificial refers to the fact that the stimulus was a vaccination with the antigen.
3. **Answer: C.** Only cells that specifically recognize antigen, through antigen receptors, can exhibit memory, and only B and T cells are antigen-specific. NK cells, lymphokine-activated NK (LAK) cells, macrophages, and dendritic cells are nonspecific and therefore are not increased in number or function upon antigen challenge.
4. **Answer: D.** IgM and IgD are both found on mature B cells.

Immunoglobulins and T-Cell Receptors

3

A. Structure of an Immunoglobulin

1. **Basic structure:** Immunoglobulins act as antigen-specific receptors on B cells and, when secreted by plasma cells, mediate humoral responses. The basic structural unit of an immunoglobulin molecule is a glycoprotein **monomer**, consisting of four polypeptide chains linked covalently by disulfide bonds. The molecule contains **two identical light chains** and **two identical heavy chains**. The structure has two identical antigen binding areas consisting of both light and heavy chains. Heavy and light chains have **variable regions** on their most N terminal ends.

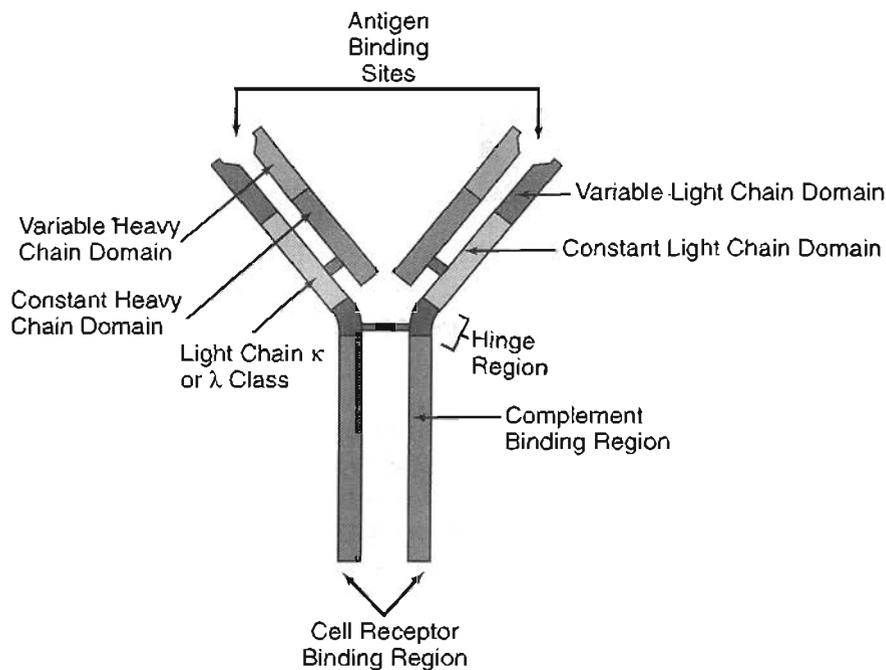


Figure II-3-1. The Basic Structure of IgG

2. **Heavy and light chains:** These chains are subdivided into **variable** and **constant regions**. In addition to the disulfide bonds linking the chains together, there are intra-chain disulfide links that divide each chain into areas called **domains**. The **light chains** have two domains, one **variable** and one **constant**. The **heavy chains** have **four to five domains**, one **variable** and **three or four constant** depending on the type of immunoglobulin. The light chains may be of two different classes, **kappa (κ)** or **lambda (λ)**, on the basis of structural differences in the constant domain.

In a Nutshell

Immunoglobulin monomer

- 2 light chains
- 2 heavy chains

Note

The immunologic specificity of antibody molecules is specified by the variable regions of both light and heavy chains.

The biologic effector function of antibody molecules is controlled by the heavy chain constant regions.

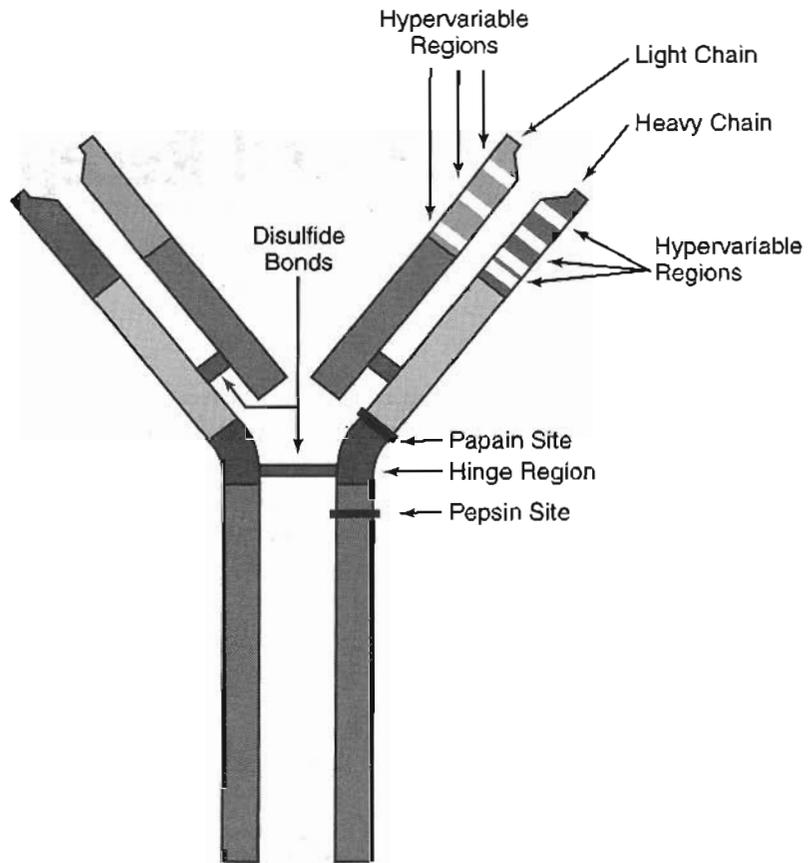


Figure II-3-2. Schematic Structure of Immunoglobulin Molecule

Note

1. There are two parts to the variable region
 - The framework
 - The hypervariable or complementarity determining regions (CDRs)
2. Antigenic specificity is dictated by the amino acids in the CDRs

3. **Proteolytically derived fragments:** The functions of antibody molecules were defined using two different enzymatic digestions. The action of **papain** resulted in the identification of two Fab fragments and one Fc fragment. The action of **pepsin** results in the identification of one large F(ab')₂ fragment and many different low molecular weight peptides coming from digestion of the heavy chains. Enzymatic cleavage of the IgG molecule is illustrated in Figure III-3.

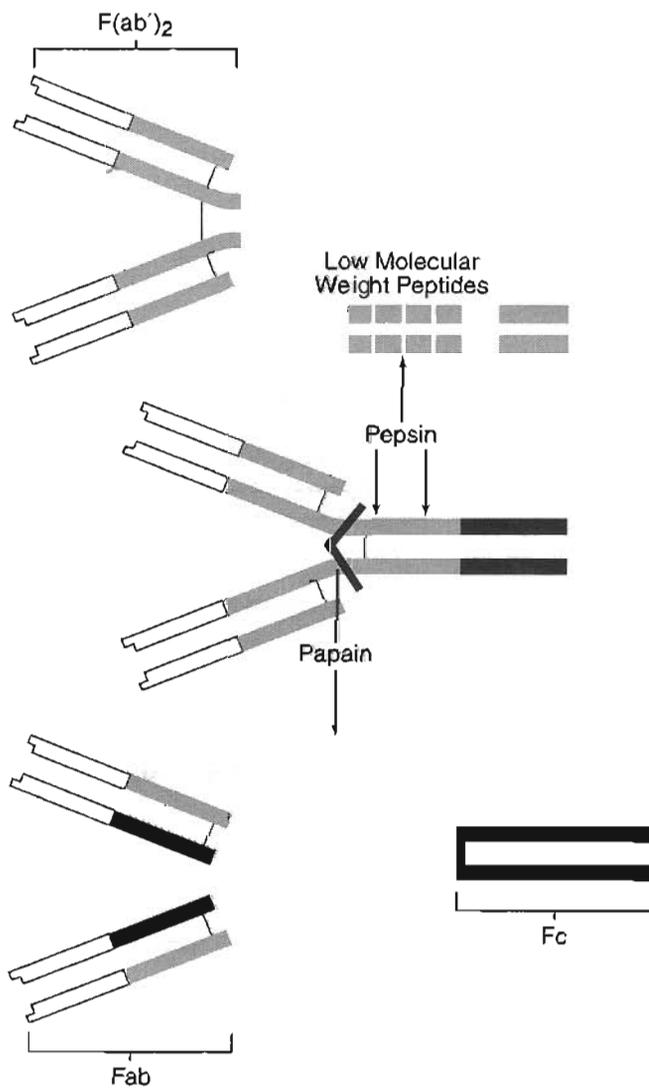


Figure II-3-3. Proteolytically Derived Fragments of IgG

B. Major Immunoglobulin Classes

There are **five classes** or isotypes of human immunoglobulins. These are designated by the type of heavy chain they have.

1. **IgG**: gamma (γ) H (heavy) chain
2. **IgA**: alpha (α) H chain
3. **IgM**: mu (μ) H chain
4. **IgE**: epsilon (ϵ) H chain
5. **IgD**: delta (δ) H chain

C. Subclasses

1. **IgG** is further divided into four subclasses: IgG1, IgG2, IgG3, and IgG4.

Clinical Correlate

$F(ab')_2$ is still bivalent and can bind Ag and precipitate it or agglutinate it. Fab fragments are monovalent and can bind but cannot agglutinate or precipitate Ag.

In a Nutshell

Immunoglobulin classes and subclasses are named on the basis of chemistry of H (heavy) chains.

2. **IgA:** There are also two subclasses of IgA, called IgA1 and IgA2.

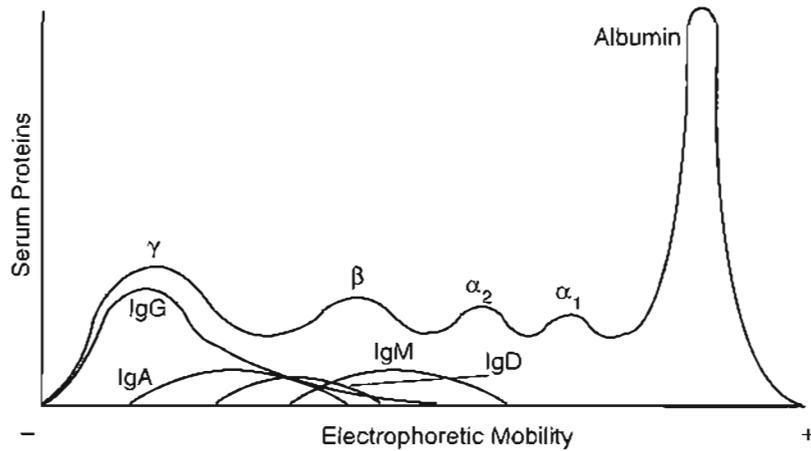
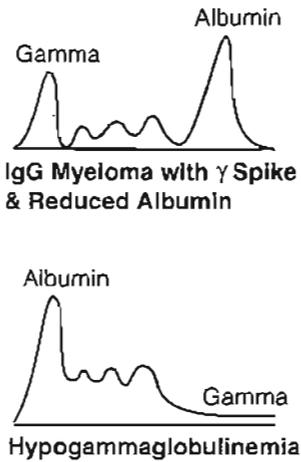


Figure II-3-4. Serum Proteins and Immunoglobulins

D. Characteristics of the Five Classes

1. **IgG**
 - a. It is the Ig present at the highest concentration in plasma
 - b. It is actively transported across the placenta
 - c. It activates complement
 - d. It opsonizes
 - e. It is the Ig characteristically produced during the secondary immune response
 - f. It is bound by *Staphylococcus* protein A
 - g. It mediates antibody-dependent cellular cytotoxicity (ADCC)
2. **IgM** (Figure III-5)
 - a. In serum it is a pentameric molecule (five sets of four-chain IgG-like units) held together with a J-chain as well as disulfide bonds
 - b. It is the Ig characteristically produced during the primary immune response
 - c. It is the first antibody that an infant makes; an increase signals an in utero infection
 - d. It is the most efficient Ig at activating complement
 - e. The monomeric form (one four-chain unit) serves as the antigen receptor on the B-cell surface

In a Nutshell

- IgG 650–1,500 mg/dL
- IgM 40–345 mg/dL
- IgA 76–390 mg/dL

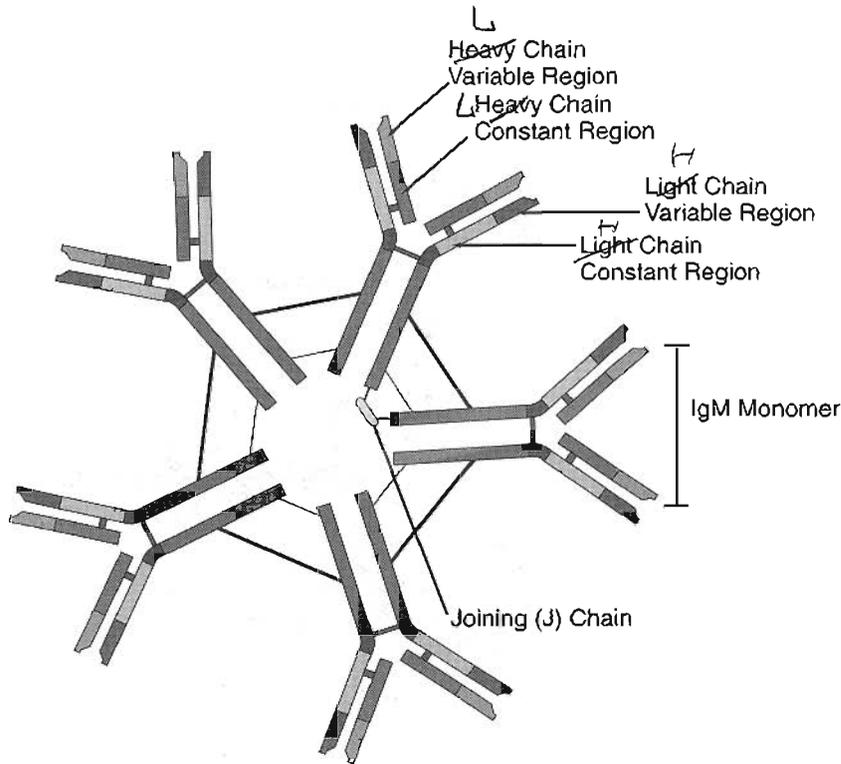


Figure II-3-5. IgM Pentamer

3. IgA (Figure III-6)

- a. It is the main Ig in external secretions such as milk, saliva, tears, and respiratory and intestinal mucus
- b. It protects mucosal surfaces
- c. It is the major protective factor in colostrum
- d. It is present in secretions as a dimer with a J (joining) chain and secretory piece (which it picks up during passage through epithelial cells; called sIgA; see Figure III-7). J chain is made by B or plasma cell; secretory piece made by epithelial cell.
- e. There are two subclasses: IgA1 and IgA2

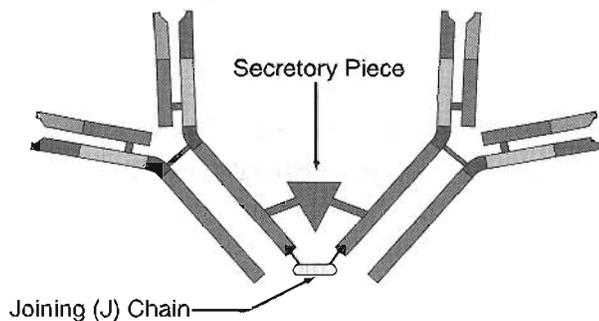


Figure II-3-6. The IgA Dimer

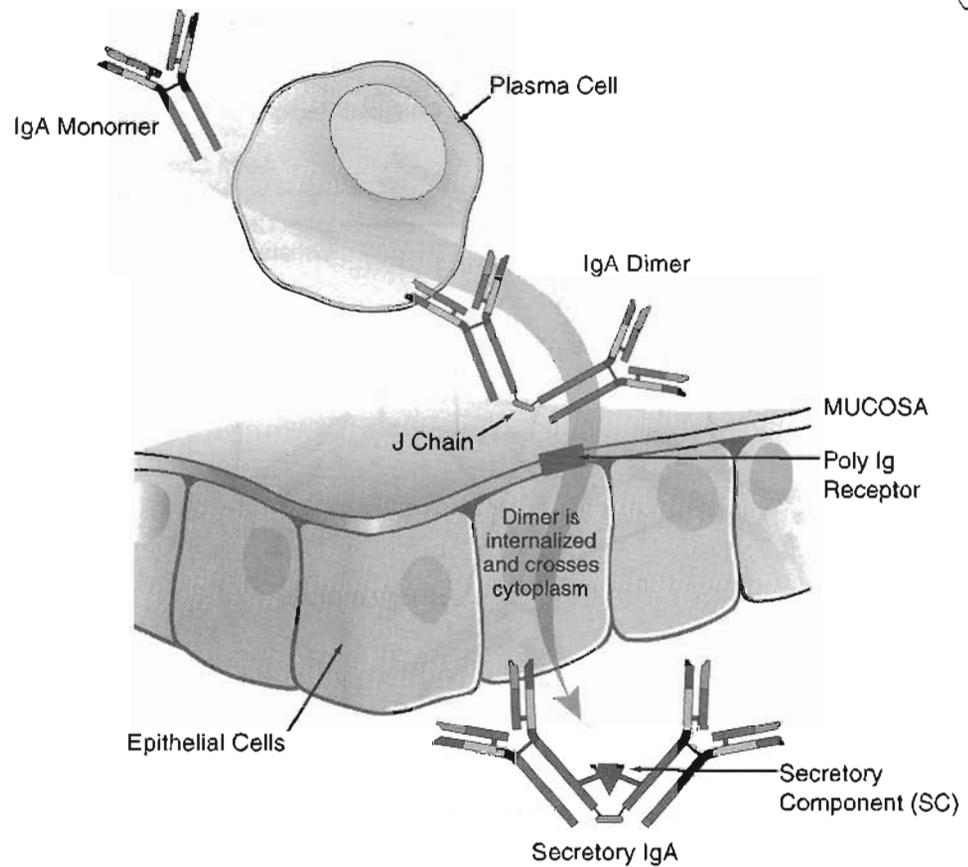


Figure II-3-7. Secretory IgA

4. IgE
 - a. It is present in plasma as a monomer in very low levels
 - b. It is homocytotropic to (binds to) mast cells and basophils that have Fc receptors for epsilon heavy chains.
 - c. The bridging of two IgE molecules on cell membranes causes release of granule contents
 - d. It is important in providing immunity against metazoan parasites
 - e. It mediates Type I hypersensitivity (allergic) reactions
5. IgD
 - a. It is present in plasma at very low concentrations
 - b. It is present in the membrane of mature B cells at very high levels
 - c. It functions as an antigen receptor (with IgM monomers) on B cells

Table II-3-1. Summary of the Biological Functions of the Antibody Classes

Function		IgG	IgM	IgA	IgD	IgE
Complement activation, classic pathway		+	+	-	-	-
Opsonization		+	-	-	-	-
Antibody-dependent cell-mediated cytotoxicity (ADCC)		+	-	-	-	-
Placental transport		+	-	-	-	-
B-cell antigen receptor	Mature (naive)	-	+	-	+	-
	Memory (one only)	+	-	+	-	+
Trigger mast cell granule release		-	-	-	-	+
Antigen binding		+	+	+	+	+

E. Antibody Variants

Three terms are used to describe variants among antibody molecules:

1. **Isotype:** The class of an antibody heavy chain or light chain. IgM, IgE, and IgA are all examples of different isotypes. Light chains also have isotypes; these are called **kappa** (κ) and **lambda** (λ). Each isotype is the product of a **different** gene, and the isotype is determined only by the **structure of the constant domains** of the immunoglobulin molecule. Immunoglobulin subclasses are also examples of different isotypes (e.g., IgG1 versus IgG4; IgA1 versus IgA2). Thus, there are **nine** human immunoglobulin heavy chain isotypes: IgG1, IgG2, IgG3, and IgG4; IgA1 and IgA2; IgM; IgD; and IgE.

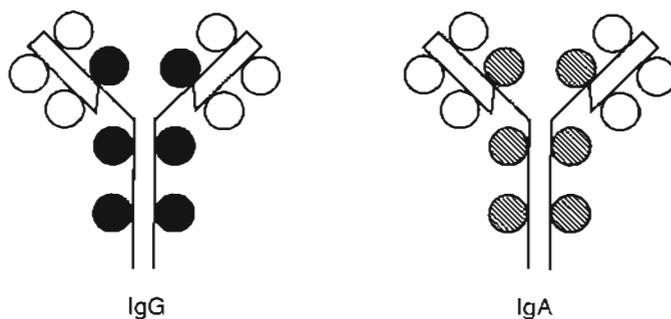


Figure II-3-8. Isotypes of IgG and IgA

In a Nutshell

- Light chain isotypes 2, κ and λ .
- Heavy chain isotypes: 9
- Total isotypes: 18

2. **Allotype:** A genetically determined difference in a molecule between two members of the **same species**; also called genetic **polymorphisms**. Allotypes result from the substitution of only one or two amino acids in the constant regions (usually) of heavy or light chains and have **no biological significance**.

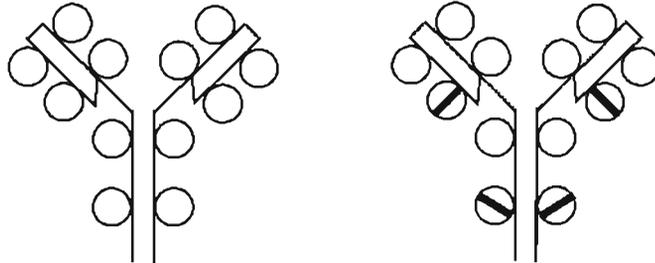


Figure II-3-9. IgG Allotypes

3. **Idiotypic:** The **individual, unique** differences between antibodies of different **antigen-binding specificities**. Idiotypes are determined by the structure of the **variable regions** of antibodies. Each person's serum will contain millions of different idiotypes because our antibodies have millions of different antigen-binding specificities. Both **heavy and light chains contribute to the antibody idiotypic**, because both are required to make an antigen binding site. T-cell **antigen receptors** also have idiotypes.

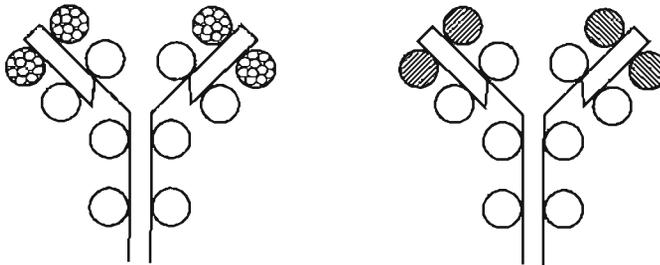


Figure II-3-10. IgG Idiotypes

In a Nutshell

TCR isotypes

- Mostly α and β
- Some δ and γ

P. The T-Lymphocyte Antigen Receptor

T lymphocytes also specifically recognize antigen, but they do not synthesize immunoglobulin molecules. Instead, they express a surface-bound T-cell antigen receptor (TCR). TCRs share several structural and functional characteristics with immunoglobulins. They have two chains, but they are not called heavy and light chains because they are about the same molecular weight. Instead the two chains are called **alpha (α)** and **beta (β)** chains on most T cells. TCRs also have **isotypes**. Besides the most common isotype, the $\alpha\beta$ TCR, there is a second isotype composed of **gamma (γ)** and **delta (δ)** chains. The $\gamma\delta$ T cells are found mainly in the skin and at mucosal surfaces. All TCRs have a domain structure similar to antibody light chains (i.e., both chains have a **variable domain** with **hypervariable loops** and one **constant domain**). The TCR is always cell associated; it is not designed to be secreted.

The TCR is noncovalently linked to the CD3 molecule, and engagement of the TCR by antigen stimulates CD3 to transmit biochemical signals into the interior of the cell. These signals are required to trigger the T cell. CD3 is composed of four to five different polypeptide chains: γ , δ , ϵ , ξ , and η . Intracellular signals are transmitted through two ζ chains or a ζ and η chain.

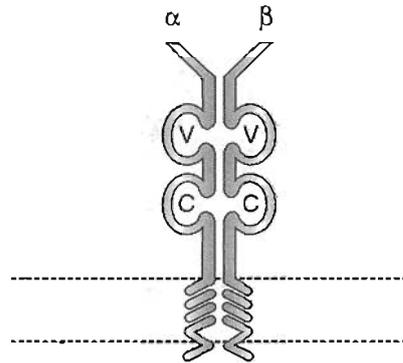


Figure II-3-11. T-Cell Receptor

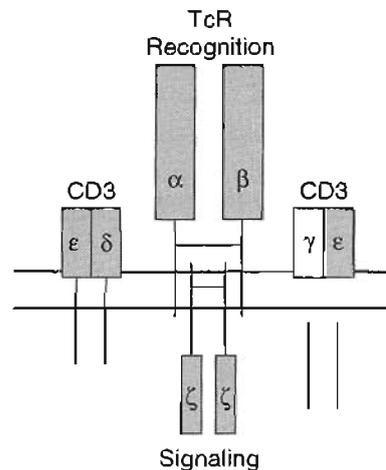


Figure II-3-12. T-Cell Receptor – CD3 Complex

In a Nutshell

The TCR

- Molecule of two chains ($\alpha\beta$ or $\gamma\delta$)
- Variable and constant domains
- Always cell associated
- Never secreted
- Associated with signal transduction complex (CD3)

G. Signal-Transduction Molecules Associated With Immunoglobulin Signaling

Immunoglobulin is noncovalently associated in B-cell membranes with Ig- α (CD79a) and Ig- β (CD79b). The cytoplasmic portions of these are in turn coupled to kinases within the B cell. These kinases phosphorylate specific proteins leading to transcription of several genes and thereby actualizing B-cell activation. This process, by which a signal induced by antigen binding to external membrane-bound immunoglobulin is passed into the interior of the cell, is called **signal transduction**.

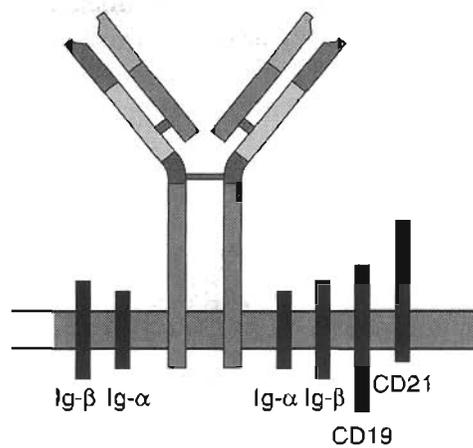


Figure II-3-13. B Cell-Signal-Transduction Complex

Table II-3-2. Comparison of B-Cell Antigen Receptor and T-Cell Antigen Receptor

	B-Cell Antigen Receptor (Antibody)	T-Cell Receptor (TCR)
Molecules/lymphocyte	100,000	100,000
Idiotypes/lymphocyte	1	1
Isotypes/lymphocyte	2 (IgM & IgD)	1 ($\alpha + \beta$ or $\gamma + \delta$)
Is secretion possible?	Yes	No
Valence	2	1
Mobility	Flexible (hinge region)	Rigid
Type of antigen recognized	Soluble unprocessed any chemical composition	ONLY processed peptides presented on a major histocompatibility (MHC) molecule
Associated signal-transduction molecules	Ig- α , Ig- β , CD19, CD20, CD21	CD3 complex (γ , δ , ϵ , and ζ chain dimers)

Chapter Summary

Immunoglobulins are Y-shaped proteins having two identical light (L) and two identical heavy (H) chains, which are held together by disulfide bridges. Both the low-molecular-weight L and the high-molecular-weight H chains have constant and variable regions. These regions are subdivided into segments called domains. L chains have one variable and one constant domain. H chains have one variable and three or four constant domains. The variable domains are responsible for antigen binding, and the constant chains are responsible for various other functions. Amino-acid differences in the constant region divide the L chains into either a κ or a λ type.

Early studies on immunoglobulin structure used cleavage into smaller fragments by papain and pepsin. The peptide fragments obtained were isolated, named, and assigned functions. Two important peptides identified in this way are the Fab fragment, which contains the antigen binding sites, and the Fc fragment, which is involved in placental transfer, complement fixation, and attachment to cells.

There are five immunoglobulin classes (IgG, IgM, IgA, IgE, and IgD), which are identified by differences in their heavy chains. The γ H chain is expressed on IgG, the α on IgA, the μ on IgM, the ϵ on IgE, and the δ on IgD.

IgG is normally present in serum at higher levels than any of the other four classes of immunoglobulin. It is the only maternal immunoglobulin that crosses the placental barrier. It activates complement, opsonizes, is bound by *Staphylococcus* protein A, and mediates antibody-dependent cellular cytotoxicity. It characteristically is produced during the secondary immune response.

IgM is a pentameric molecule: It has five Y-shaped molecules joined by a J chain. It is the major immunoglobulin produced during the primary antibody response, it is the first to be produced in infancy, and it can be produced by the fetus in utero as a defense against infection. As a pentamer it has 10 antigen binding sites, giving it the highest avidity for antigen. Its five heavy chains with Fc complement binding sites also make it the most efficient complement-activating immunoglobulin. The monomeric form (one four-chain Y structure) serves as an antigen receptor on B-cell surfaces.

IgA is the primary immunoglobulin found in secretions such as milk colostrum, saliva, tears, and respiratory and intestinal mucus. It prevents the attachment of microorganisms to mucous membranes. It is secreted as a dimer with the monomeric forms joined by a J chain and a secretory piece called sIgA, which is added during passage through the mucosa. Some IgA is found in serum in a monomeric form.

Normally, IgE monomer is present at very low concentrations in serum. It binds to mast cells and basophils, triggering the release of granules. It provides protection against metazoan parasites but is also responsible for immune hypersensitivity (allergic) reactions in sensitive individuals.

IgD is primarily found bound to the membrane of mature B cells where it functions in antigen recognition.

The immunoglobulins can be further subdivided on the basis of antigenic differences. Three different classes of antigenic variations are recognized: isotypes, allotypes, and idiotypes.

The isotype of an antibody is determined by the structure of its constant region. Thus, each class of antigen is of a different isotype (e.g., IgM has a m heavy chain and IgG has a g heavy chain). The immunoglobulins can also be further subdivided into isotypic subclasses on the basis of antigenic determinants on the H chain. There are four IgG subclasses (IgG 1–4) and two IgA subclasses (IgA 1 and 2).

Allotypes are minor differences in the amino-acid composition of the constant regions of the molecule. They have no biological relevance to function but are used in population genetic analysis.

(Continued)

Chapter Summary (continued)

Idiotypes are the individual, antigen-specific binding areas of immunoglobulins formed by the variable regions of both the L and H chains. Each person has millions of idiotypes.

The T-lymphocyte receptor also binds antigen and has millions of idiotypes. Although they have structural and functional properties analogous to the immunoglobulins, there are important differences. Structurally they have two chains of similar molecular weight. The most common isotype has chains called α and β ; a less common isotype primarily expressed in skin and at mucosal surfaces has γ and δ chains. Functionally, the T-cell antigen receptor differs from the immunoglobulins in that it recognizes antigen only in conjunction with specific MHC proteins and that it is noncovalently associated with a CD3 molecule. Engagement of the antigen-activated receptor with the CD3 molecule transmits signals (signal transduction) to the T cell's interior and starts the reactions leading to T-cell activation.

A similar series of events occurs on B cells. Here, however, the antibody binds directly to the cell's membrane. This, in conjunction with associated molecules Ig- α , Ig- β , CD19, CD20, and CD21, transmits signals to the B cell's interior.

Review Questions

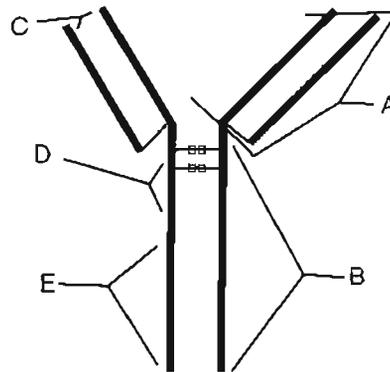


Figure II-3-14

For Questions 1–4, identify the labeled segments in Figure III-14 (above).

1. Fc portion of IgG
2. Idiotype is found here
3. Complement (C1q) binding site
4. Fab fragment
5. IgG2 and IgG4 are examples of different
 - A. Idiotypes
 - B. Allotypes
 - C. Genotypes
 - D. Isotypes

6. Which of the following is not characteristic of secretory component?
- It is synthesized by plasma cells.
 - It is an epithelial transport protein.
 - It is the poly-Ig receptor on epithelial cells.
 - It protects IgA from degradation.
 - It binds dimeric IgA.
7. All of the following statements about $\alpha\beta$ T-cell antigen receptors are true **except**:
- They recognize soluble foreign antigen.
 - They consist of two different (heterologous) chains.
 - They have variable and constant domains.
 - They have idiotypes.
 - They have different isotypes (classes).
8. IgG is the only antibody isotype that can cross the placenta. The basis for this is that
- It is smaller than plasma IgM, so it diffuses more easily.
 - The poly-Ig receptor on the placental epithelial cells recognizes it and transports it.
 - Fc receptors on macrophages bind the IgG and carry it across the placental barrier.
 - Placental Fc receptors recognize and bind the constant (carboxyterminal) end of the light chains.
 - Placental Fc receptors bind to the CH3 domain.
9. Idiotypic determinants are present on all of the following **except**:
- Fab fragments of IgA
 - T-cell antigen receptor beta chains
 - CH2 domains of IgM
 - Kappa light chains
 - F(ab)₂' fragments of IgG4
10. An IgG2 molecule is composed of:
- One gamma 1 chain and two kappa chains
 - Two gamma 2 chains and two kappa chains
 - Two gamma 1 chains and two kappa chains
 - Two alpha, one gamma 2, and two kappa chains
 - Two gamma 1 chains, one kappa and one lambda chain
11. The idiotype of an immunoglobulin molecule is defined by
- Kappa chain
 - Lambda chain
 - Heavy chain
 - Fc fragment
 - Complimentary determining regions (antigen-binding region)

12. The B cell can be identified from other cells in a flow cytometric analyzer. Which one of the following markers is present on B cells for its specific identification?
 - A. CD16 and CD56
 - B. CD14
 - C. CD3
 - D. CD19 and CD20
 - E. CD8

13. Which one of the following is correct about an IgG immunoglobulin molecule?
 - A. The constant heavy 2 domain binds to C1 complement molecules.
 - B. The constant heavy 1 domain binds to antigen epitopes.
 - C. The variable light domain binds to cell receptors on cell membranes.
 - D. The constant heavy 3 domain binds to antigen epitopes.
 - E. The variable light domain binds to J chains.

14. Which one of the components of the TCR (T-cell receptor) is responsible for transmitting signals into the interior of the cell?
 - A. Zeta chain
 - B. Alpha chain
 - C. Beta chain
 - D. Gamma chain
 - E. Variable region

15. Which one of the following immunoglobulin designations would be found in only certain humans and not in all human species?
 - A. Kappa types
 - B. Lambda types
 - C. Isotypes
 - D. Idiotypes
 - E. Allotypes

16. A 28-year-old man was brought into court for nonpayment of child support. A 20-year-old woman insists that he is the father of her child. The court suggests before hearing the paternity case that various genetic tests be performed on the man, woman, and child. One of the sets of tests was for genetic immunoglobulin identification. Which immunoglobulin would be helpful in this paternity case?
 - A. IgA2
 - B. Isotypes
 - C. Idiotypes
 - D. Allotypes
 - E. IgM

17. A 26-year-old obstetric patient becomes acutely ill during the first trimester of pregnancy with infectious mononucleosis-like symptoms, except her heterophil antibody test was negative. From the family history the OB determined that the family had two cats that were house animals. The OB also determined that the family routinely eats meat that is not thoroughly cooked. The appropriate laboratory tests determined the expectant mother has *Toxoplasma gondii* during her first trimester of pregnancy. The woman delivers a full-term baby with no apparent signs of *in utero* infection with the protozoan parasite. The best test to diagnose acute infection in the neonate would be a parasite specific for ELISA for which isotype of immunoglobulin?
- IgM
 - IgE
 - IgA
 - IgG1
 - IgG4
18. If a patient were genetically unable to make J chains, which immunoglobulins would be affected?
- IgG and IgA
 - IgM and IgE
 - IgA and IgE
 - IgG and IgM
 - IgM and IgA
19. IgA dimers are protected from catabolism directed toward polysaccharide-rich portions of the molecule by secretory component. Secretory component is produced by
- B cells
 - T cells
 - Plasma cells
 - Epithelial cells
 - Dendritic cells
20. The antigen-recognition molecules of T and B lymphocytes are similar in that they are both
- Monovalent
 - Rigid
 - Secreted
 - Created by somatic hypermutation
 - Have three complementarity-determining (hypervariable) regions per chain
21. In all subpopulations of T lymphocytes, the T-cell antigen receptor is found closely associated with
- CD2
 - CD3
 - CD4
 - CD8
 - CD19

Answers

1. Answer: B.
2. Answer: C.
3. Answer: D.
4. Answer: A.

Answer: A. Choice B shows the Fc region. Choices D and E are only parts of the Fc region. Choice C is the best answer for Question #2. Although choice A includes the antigen-binding site (idiotype), it also includes a good deal of the constant regions of the heavy and light chains, which have nothing to do with the idiotype. Similarly, choice D is the best answer for Question #3. Even though choice B includes the complement-activating C₂ domain, it also encompasses a lot more of the molecule.

5. Answer: D. Idiotype refers to antibody specificity, determined by the variable domains. Allotype refers to different genetic variants (polymorphisms) in a molecule encoded by the same allele in two different individuals of the same species. Isotypes are different antibody classes/products of different genes, which is the case for IgG2 and IgG4.
6. Answer: A. All are true statements about secretory component, except choice A. It is not synthesized by plasma cell or B cells, but by epithelial cells. The three functions of secretory component are that 1) it is the epithelial cell poly Ig receptor (receptor for dimeric IgA); 2) it transports IgA across the epithelial barrier (choice C); and 3) it protects IgA from degradation.
7. Answer: A. T-cell antigen receptors **cannot** recognize free (soluble) foreign antigen. They only recognize antigen that has been processed by a host cell and presented on its surface in association with a self MHC class I or class II molecule. Structurally, they are similar to antibody in that they have V and C domains, antigen specificity (idiotypes), heavy and light chains, and isotypes ($\alpha\beta$ and $\gamma\delta$).
8. Answer: E. This is an active transport process, so choice A is wrong. Choice B describes secretory IgA transport at mucosal surfaces. Macrophages (choice C) are not antibody transport cells, even though they do possess Fc receptors. The placental Fc receptor binds the constant region of the heavy (choice E) chain, not the light chain (choice D).
9. Answer: C. The determinants that make up the idiotype of an antigen-recognition molecule (either an antibody or a T-cell antigen receptor) are found on the variable domains. Of the choices listed, only choice C does not include a variable domain.
10. Answer: B. The 2 in IgG2 indicates in which subclass the molecule belongs. It must have two gamma 2 chains, as this cell can only form this one type of heavy chain, and it can form either two kappa or two lambda chains, but never one of each.
11. Answer: E. The idiotype of an immunoglobulin has all the specificity of the antibody against the antigen. This is the variable region of the light and heavy chain. Within the variable region, there are even more specific areas that bind the antigen, and they are called CDRs. The light and heavy chain variable regions each have three CDRs.

12. **Answer: D.** The B cells have IgM and IgD on their surfaces, and they are excellent markers for B cells. They also have CD19, 20, and 21 markers that help in their identification.
13. **Answer: A.** The constant heavy domain #2 binds to the C1 complement component to activate the classic complement pathway.
14. **Answer: A.** The CD3 complex associated with the TCR receptor contains two zeta chains that function for transmitting intracellular signaling.
15. **Answer: E.** Certain humans have allotypic differences in immunoglobulin heavy or light chains. These differences are minor genetic changes that do not interfere with the functioning of the molecules. These minor genetic differences can be used to establish paternity cases.
16. **Answer: D.** There are genetic allotypic markers found on different immunoglobulin molecules. The best examples are kappa light chains and IgG1, IgG2, and IgG3 heavy chains. These are distinctive markers that, when present, can be helpful in these cases. If this man had these markers and the child had the same markers, then this would be possible evidence of the correct father. There would be other genetic tests performed in this case because if mother, father, and child had the same markers, then the markers could have come from the mother.
17. **Answer: A.** The IgM immunoglobulin isotype is the only one of these answers that would prove infection in the newborn baby. This is the only antibody that a baby can form with an acute infection. This is the first antibody that is formed in an infection, and it is also the antibody that is present on the surface of immature and mature B cells.
18. **Answer: E.** IgM and IgA contain J chains (joining chains).
19. **Answer: D.** The secretory component is part of the immunoglobulin receptor of epithelial cells, which is internalized with the Ig on the serosal surface and passed through the cell to the mucosal surface. Plasma cells and B cells produce the J chain that holds IgA dimers together, but these cells do not produce secretory component.
20. **Answer: E.** Both immunoglobulin and the TCR possess three CDRs per chain. Only the TCR is monovalent and rigid, while only immunoglobulin is secreted and enhanced by somatic hypermutation.
21. **Answer: B.** CD3 and zeta chain are found on the surface of all T cells and form part of the signal transduction mechanism following antigen binding to the TCR. CD2 is an adhesion molecule on thymocytes and mature T cells, but is not physically associated with the TCR. CD4 and CD8 are the molecules that act as coreceptors for MHC class II and class I antigens, respectively, and CD19 is a marker for B lymphocytes.

Immunoglobulin and T-Cell Receptor (TCR) Genes

4

A. Immunoglobulin and T-Cell Receptor (TCR) Genes

The human immune system is capable of producing a vast number of different **antibody molecules** and **TCRs**, each with its own **antigenic specificity**. To produce such diversity without requiring excessive numbers of genes, it uses special **somatic recombination (DNA rearrangement and deletion)** followed by **RNA splicing**. This results in a large variety of antigen-recognizing molecules.

1. The genes for the light chain of immunoglobulin molecules are composed of several different **V (variable) segments** and **J (joining) segments**. There is a single κ **constant region gene** and a single λ **constant region gene**.
2. The genetic region that contains the heavy chain genes has different **V genes** and **J genes**, along with an additional set of **D (diversity) genes**. Following these exons are several **C (constant) genes**, which encode the heavy chain constant regions.

In a Nutshell

- There are many variable region genes
- There are a few D region genes
- There are a few J region genes
- There are nine constant region genes (μ , δ , γ , etc.)
- Through DNA rearrangements and recombinations, a complete heavy chain is produced
- The enzyme terminal deoxynucleotidyl transferase (Tdt) inserts bases randomly at the junctions of J with D and D with V; this creates additional diversity

B. Gene rearrangement for heavy chain production (1st DNA recombination event)

The gene for the heavy chain variable domain contains three gene segments that provide the DNA that encodes for the variable domain amino acids. The important characteristics of the heavy chain genome are:

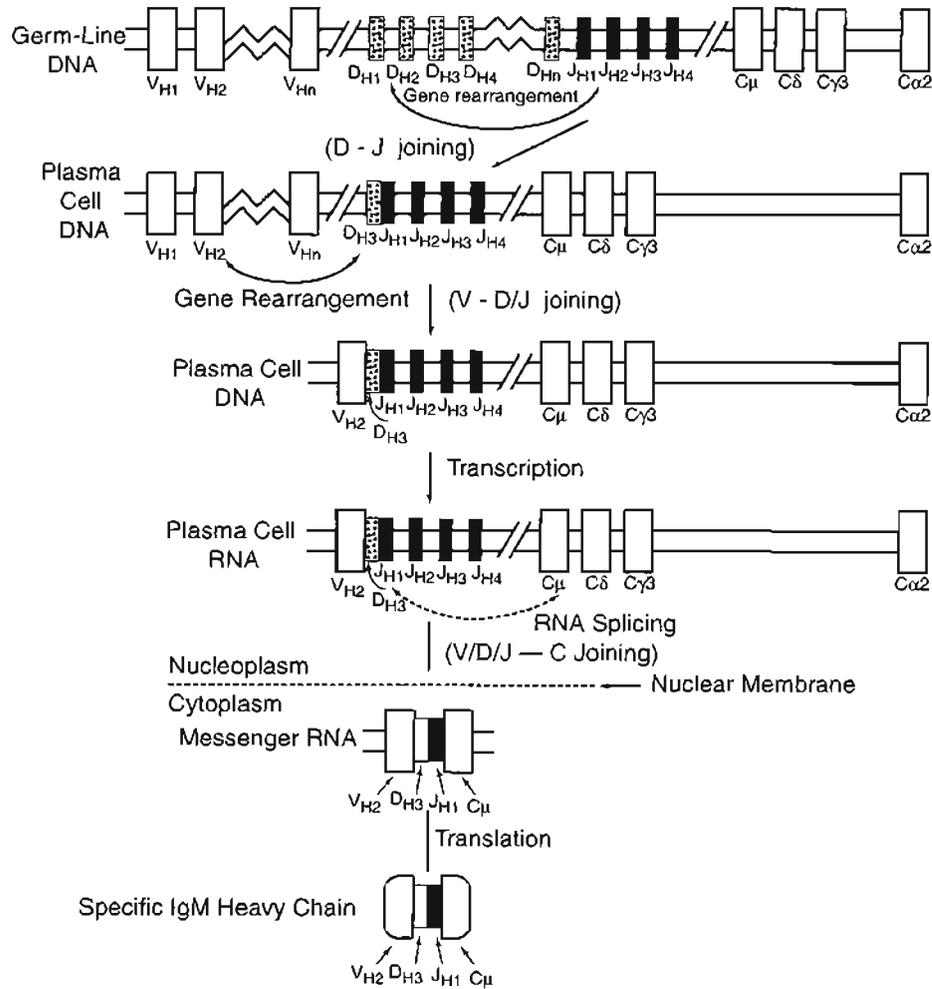


Figure II-4-1. Production of a Heavy Chain

1. Gene rearrangement for light chain production (2nd DNA recombination event)

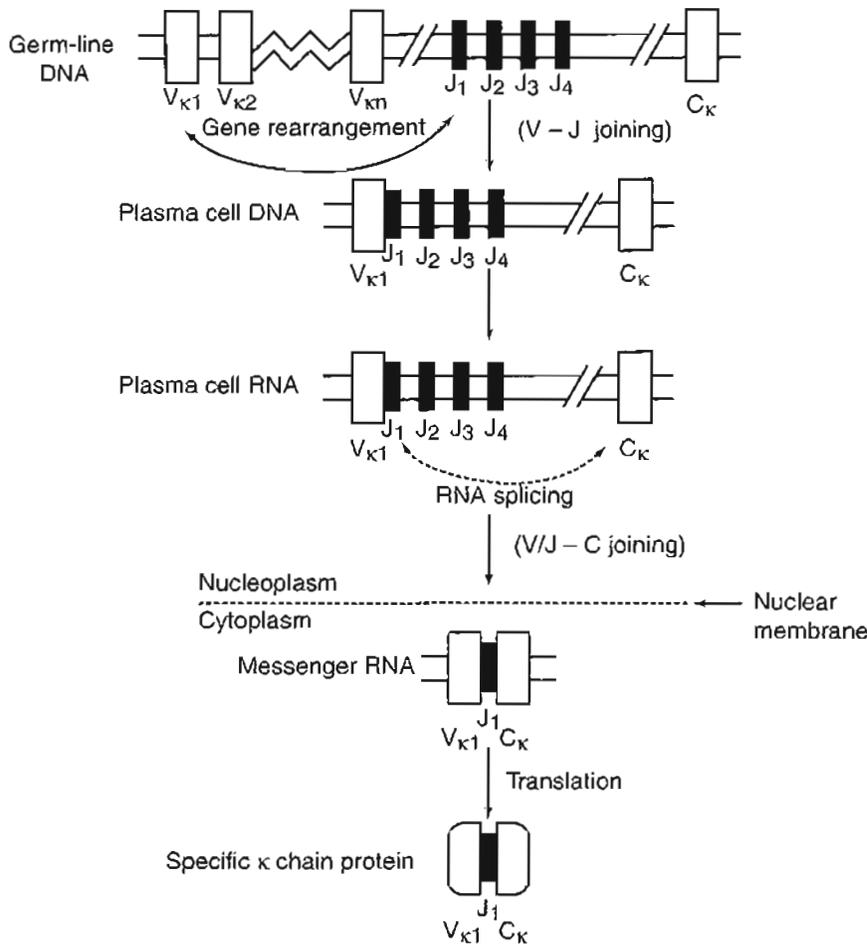


Figure II-4-2. Production of a κ Light Chain

In a Nutshell

- There is inheritance of many germ-line V regions
- There are few J region genes
- There is one κ constant region gene
- Through DNA rearrangements and recombinations, a complete κ light chain is produced

2. Recombinational inaccuracies

While heavy chain variable regions are undergoing recombination (D with J first, followed by V with DJ), the enzyme tdt is active. This enzyme inserts bases randomly (without a template on the complementary strand) at the junctions of J with D and D with V. This creates additional diversity in the messenger RNAs transcribed from this DNA. When the light chains rearrange later, tdt is not active so this mechanism generates variability only in heavy chains. For TCRs, tdt is active during all V gene rearrangements.

3. Random assortment of heavy and light chains

Any heavy chain variable region can be used with virtually any possible light chain V domain, so one can multiply the number of heavy chains times the number of light chains to give the total number of possible combining sites that can be generated. In T cells, any α chain can be paired with any β chain, or any δ chain can be used with any γ chain.

When one heavy chain gene rearranges to produce a functional gene product, it **shuts off** the rearrangement and expression of the other allele (on the homologous chromosome). This same phenomenon also occurs with the light chain genes. This process,

In a Nutshell

Antibody Diversity Is Due To:

- Inheritance of multiple germ-line V regions
- Recombination of immunoglobulin genes for:
 - Variable region
 - Joining region
 - Diversity region (H chains only)
- Random assortment of heavy chains with light chains
- Addition of nucleotides to DNA during genetic recombination by the enzyme tdt
- Somatic mutations
- Somatic mutations only occur after exposure to antigen

In a Nutshell

- First membrane immunoglobulin on B cell is mIgM
- Second membrane immunoglobulin on B cell is mIgD
- Class switching produces new class of immunoglobulin
- There are switch regions that permit formation of loops
- The information in the loop is excised and degraded
- Class switching is one-way
- Class switching does not affect the variable domain

called allelic exclusion, is essential to ensure that each B cell synthesizes only one heavy chain variable domain and only one light chain, and therefore makes only one specific antibody. T cells also exhibit allelic exclusion in the expression of TCR genes.

4. Somatic mutation of V region genes

Because the B cells divide rapidly in germinal centers of the lymph nodes, there are many opportunities for mutations. Although these mutations can be detrimental to individual B cells (these cells are then eliminated), mutations that increase antibody affinity are preferentially selected, and mutations thus play a role in affinity maturation of B-cell clones. Somatic mutation, however, plays essentially no role in T-cell receptor diversity.

The affinity of an antibody refers to the strength of association between one antigenic determinant and one antibody binding site.

C. Mature B-cell Antigen Receptor Expression

The first membrane immunoglobulin found on the B cell is membrane IgM (mIgM). Very soon thereafter, mIgD is also detected. For the switch from IgM to IgD, the rearranged DNA for the heavy chain contains the VDJ region followed by the constant regions for μ and δ . The primary RNA transcript can terminate at two different sites. The first termination site produces mRNA for IgM synthesis. The second site produces the transcript for IgD that must be processed to remove the μ constant region exon (alternative RNA splicing). As the immune response proceeds, the cell will switch from making IgM to a different class or subclass of immunoglobulin.

The ability of a cell to express a new class of immunoglobulin is termed isotype or class switching. During the antibody response, signals from helper T cells instruct the B cell to switch class. It does this by rearranging the DNA that encodes for the constant region of the heavy chain. The information in the loop is excised and degraded, so this information is lost. That is why class switching is a one-way street. Once a switch is made, the cell can never go back in the 5' direction because the genes are gone. Note that class switching does not affect the variable domain of the heavy or light chains. Thus the specificity of the antibody stays the same even as the cell switches which isotype is expressed.

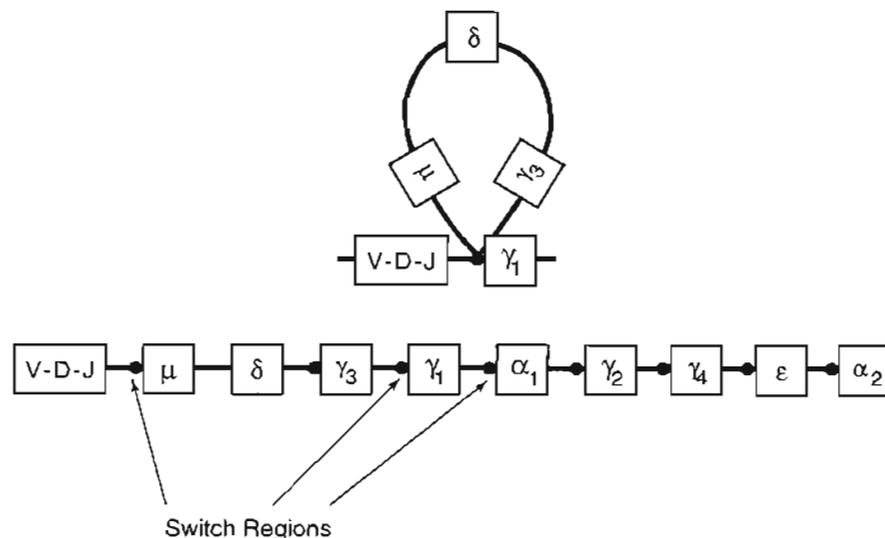


Figure II-4-3. Immunoglobulin Heavy Chain Switching

Chapter Summary

The human immune system produces millions of different immunoglobulins and T-cell receptors. It can do this because of the ability to recombine segments of germ-line DNA of both chains of the antibody and T-cell receptor. This random assortment of segments creates the three-dimensional structure of the hypervariable region (idiotype) of the antibody or T-cell receptor. In addition, terminal deoxynucleotide transferase (tdt) randomly inserts nucleotides at the junction between each of these segments.

Further diversity can be obtained by mutations. B cells are subject to mutation because they divide rapidly. These mutation-induced changes generally increase the affinity of the antibody for antigen because cells expressing more effective antibodies are preferentially selected. T-cell receptors are not subjected to mutation-induced diversity.

The first antibody expressed on B cells is IgM. IgD is expressed concomitantly due to alternative RNA splicing. Signals from helper T cells instruct B cells to make class switches to other isotypes. To accomplish class switching, a loop is formed on the DNA coding for the constant region of the heavy chain. This loop is then excised. This is an irreversible phenomenon because the excised segment is discarded.

Review Questions

- Which of the following is **not** a mechanism involved in generating antibody diversity?
 - Heavy chain class switching
 - Random assortment of heavy and light chains
 - Heavy chain VDJ gene rearrangement
 - Somatic mutation of V region genes
 - Usage of multiple inherited germline V genes
- Which of the following DNA sequences would be most likely to occur in a pre-B cell?
 - V1, V2...Vn; D1, D2...Dn; J1, J2...J6; mu, delta, gamma3
 - V2, D2, J5, mu, delta, gamma3
 - V1, D4, J2, gamma3, gamma1
 - V1, V2...Vn; D1-Dn; J1, J2...J6, kappa1, kappa2, lambda1, lambda2
 - V1, J3, kappa1, kappa2, lambda1, lambda2
- When stem cells start to rearrange gene segments to become B cells, what happens first?
 - D gene rearranges with a V gene.
 - V gene rearranges with the C gene.
 - D gene rearranges with a J gene.
 - V gene rearranges with a J gene.
 - D gene rearranges with the C gene.

4. Which one of the following pairs of genes in the synthesis of immunoglobulin is linked on a single chromosome?
 - A. C gene for gamma chain, and C gene for alpha chain
 - B. V gene for lambda chain, and C gene for kappa chain
 - C. V gene for kappa chain, and C gene for the epsilon chain
 - D. C gene for gamma chain, and C gene for kappa chain
 - E. V gene for lambda chain, and V gene for heavy chain

5. A germline B lymphocyte that possesses 200 distinct V region genes, 5 J region genes, and 7 isotopic possibilities to rearrange for its selection of light-chain synthesis. Assuming no recombinational inaccuracies, how many distinct idiotypes could be produced by combining this coding sequence with one heavy chain?
 - A. 205
 - B. 1,000
 - C. 212
 - D. 7,000
 - E. 1,400

6. Isotype switching during B-cell ontogeny dedicates mature B cells to production of a single, heavy-chain isotope, except in the case of IgM and IgD, which can be expressed concomitantly. This expression of both isotopes simultaneously is possible due to
 - A. Allelic exclusion
 - B. Allelic codominance
 - C. Affinity maturation
 - D. Alternative RNA splicing
 - E. Somatic hypermutation

7. The genetic diversity of T-cell antigen receptors is very similar to that of the B-cell antigen receptor. Which of the following does **not** contribute to the development of antigenic repertoire in T cells?
 - A. 50–100 choices of variable alpha and beta genes
 - B. Multiple J gene segment codons
 - C. Junctional variability in alpha and beta molecules
 - D. Somatic hypermutation
 - E. Random combination of alpha and beta chains

Answers

1. **Answer: A.** Heavy chain class switching involves rearrangement and usage of different constant regions of the antibody molecule, but does not affect the light chain or the variable region of the heavy chain, so it has no effect on antibody specificity.
2. **Answer: B.** Pre-B cells are defined as cells in which the mu heavy chain has been successfully rearranged, but the light chain genes have not yet been rearranged. Of the choices, choice B reflects a successful rearrangement of the heavy chain to express the mu isotope. Choice A is the germ-line configuration in earlier B cells that have not yet begun to rearrange their immunoglobulin genes. Choice C reflects a B cell that has not only undergone successful VDJ rearrangement, but has switched isotype to production of IgG3. Choice D is not a meaningful distractor because D regions do not occur in light chain genes, and choice E shows a cell that has undergone VJ rearrangement of light chain genes and should express kappa1 light chains, as well as heavy chains.
3. **Answer: C.** The first rearrangement in immunoglobulin genetics is the D and J rearrangement in the early pro-B cell.
4. **Answer: A.** The genes for the synthesis of the entire heavy chain are present on human chromosome 14. From the above choices, the only genes that are present on one chromosome are for the C (constant regions) of the heavy chains. Remember the heavy chains have the determining traits for the isotypes IgG, IgM, IgA, IgD, and IgE.
5. **Answer: B.** The idiotype of an antibody is composed of V region and J region gene products randomly combined. Thus, 5×200 different specificities in the light chain could be produced. The seven isotypic possibilities (which encode for constant regions) will not affect the idiotype of the antibody produced.
6. **Answer: D.** Alternative RNA splicing allows a mature B cell to attach either delta or mu chains on a single idiotype that has been generated by germ-line DNA rearrangements. Allelic exclusion refers to the expression of either parental chromosome type but not both, and codominance does not occur in the alleles for Ig synthesis. Affinity maturation refers to the increase of affinity of a population of antibodies over time of immunization, and somatic hypermutation is the phenomenon that allows affinity maturation to occur.
7. **Answer: D.** Somatic hypermutation, which is important in the development of affinity maturation in B-cell populations, is not present in T cells undergoing selection of their antigenic repertoire. This is very critical for the immune response because T cells are subjected to rigorous positive and negative selection in the thymus for the ability to correctly recognize self and non-self, and hypermutation after encountering antigen (such as occurs in B cells) would be a potential mechanism for the development of autoreactivity. All of the other mechanisms to increase antigen-recognizing diversity are used by both T and B cells.

Antigens and Immunogens

5

A. Immunogenicity and Antigenicity

The immune response is characterized by the production of both antibodies and specific reactive T lymphocytes. **Immunogenicity** is the inherent ability of a substance (immunogen) to induce a specific immune response. **Antigenicity** or **specific reactivity** is the property of a substance (antigen) that causes it to react specifically with the antibody or lymphocyte. A single isolated immunogenic determinant is called a **hapten**. Haptens are not **immunogenic** because they are too small to cause elicitation of an immune response. When they are coupled with a **body protein (carrier)** they become **immunogenic**.

The three characteristics required for immunogenicity are:

- **Size:** Minimal molecular weight
- **Foreignness:** Phylogenetic distance; normally will not recognize "self"
- **Chemistry:** Certain degree of complexity required

A structural definition of an antigen can be achieved by considering the portion of the antibody that actually contacts the antigen. Because this is limited in size to the hypervariable loops of the heavy and light chains, it follows that only a limited portion of a large antigen will actually be recognized by the antibody. This is called the **antigenic determinant** or **epitope**. The larger the antigen, the more determinants it will have. The maximal size for an antigenic determinant or a protein antigen is about five to six amino acids; for a carbohydrate antigen, the maximal size is about four to five hexose units. TCRs recognize a somewhat larger structure of 10 to 20 amino acids.

Note

Because in most cases the immunogen that induces a specific immune response is identical to the antigen that will react with the antibody, the word antigen is often loosely used in place of immunogen.

Clinical Correlate

Penicillin may act as a hapten.

Poison ivy, penicillin, and many other drugs may act as haptens.

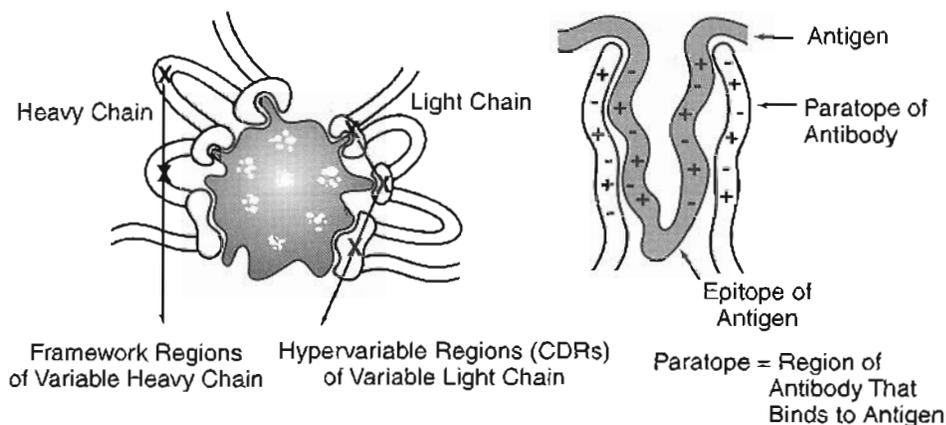


Figure II-5-1. Interaction of the Antigenic Determinant With Antibody

B. Affinity and Avidity

How good is the fit between the antibody combining site and the antigenic determinant? It is important to remember that the binding is **reversible**, so that the antibody can **dissociate** from the antigen. Two terms are used to describe the strength of association between antibody and antigen: **affinity** and **avidity**.

1. Affinity

Affinity refers to the strength of association between **one antigenic determinant** and **one antibody binding site**. In considering this one-to-one interaction, the only important factor is how good the complementarity (fit) is between the antigenic determinant (epitope) and the binding site (paratope).

2. Avidity

Avidity refers to the strength of association between **multiple antibody binding sites** and **multiple antigenic determinants**. Not only is the goodness of the fit important, but the **valence** also becomes a crucial determining factor. For antigen and antibody to dissociate, all the binding sites must dissociate at the same instant. Thus, a relatively low-affinity antibody (poor fit between the binding site and the determinant) can still bind tightly to an antigen if multiple sites are engaged simultaneously.

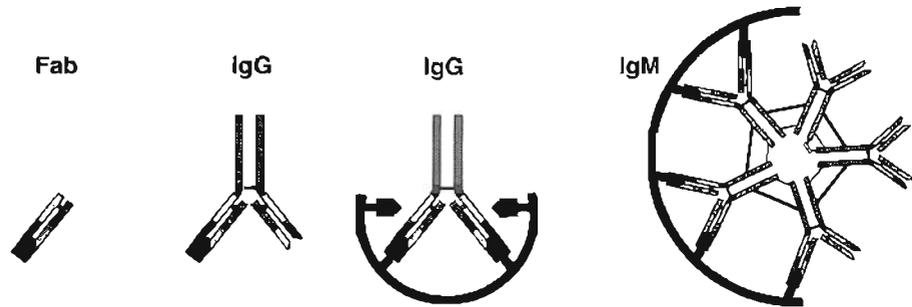


Figure II-5-2. Affinity and Avidity

Clinical Correlate

In most immune responses, avidity decreases as affinity increases.

Table II-5-1. Valence as a Determinant of Avidity

Antibody	Fab	IgG	IgG	IgM
Effective antibody valence	1	1	2	Up to 10
Antigen valence	1	1	n	n
Definition of binding	Affinity	Affinity	Avidity	Avidity

C. Adjuvants

Adjuvants are nonspecific **immunostimulants** that increase the immune response by their inflammatory action. They also prolong contact with the immunogen by acting as a depot of antigen deposition. This increases the strength of the immune response but cannot make a nonimmunogenic molecule into an immunogen.

Chapter Summary

Immunogenicity is the ability of a substance to induce an immune reaction. In contrast, antigenicity is the ability of a substance to react with a preformed antibody or receptor.

Immunogens must be somewhat complex, with an appreciable molecular weight, and must be considered foreign by the immune system.

The relatively small area of antigen that reacts with an antibody or receptor is called the antigenic determinant, or epitope. A single epitope is called a hapten. Haptens are too small to be immunogens in their own right but may become immunogenic when associated with a larger molecule. The penicillins may act as haptens.

Two terms, affinity and avidity, are used to describe the strength of the noncovalent, reversible interactions between antigen and antibody. Affinity describes the strength of association between one epitope and one idiotype. Avidity describes the strength of association between multiple sites, such as those formed by the polyvalent IgM and its antigens. A low-affinity antibody can thus have a high avidity if multiple binding sites are engaged simultaneously.

Adjuvants are compounds that nonspecifically increase the immune response to immunogens.

Review Questions

For questions 1–3, match the following terms with the description that best fits them.

- A. Epitope
 - B. Isotope
 - C. Hapten
 - D. Immunogen
 - E. Antigen
1. Molecule that possesses only one antigenic determinant
 2. The portion of an antigen to which antibody binds
 3. Molecule that is foreign to the host in which it is injected; has at least two antigenic determinants
 4. Immunogens are
 - A. Always proteins
 - B. Always peptides
 - C. Self molecules that react with class I MHC molecules
 - D. Same as haptens
 - E. Always composed of at least two determinants

5. During WWII, when quinine was used as a prophylactic against malaria infections in U.S. personnel on long-term assignment to the South Pacific, a small proportion of soldiers developed blackwater fever: chronic kidney damage from the autoimmune effects of complement-mediated hemolysis of quinized RBCs. In this case, the quinine played the role of
- A. An autoantigen
 - B. A hapten
 - C. An immunogen
 - D. A carrier
 - E. A reagin
6. The antibiotic gentamicin is a hapten that can be covalently bound to a carrier protein, e.g., bovine serum albumin (BSA). When the hapten/carrier complex is injected into a rabbit, the antibodies produced will
- A. Be specific only for gentamicin
 - B. Be only of the IgG isotype
 - C. Bind to both BSA epitopes and to gentamicin
 - D. Consist only of pentameric IgM
 - E. Not fix complement

Answers

1. Answer: C.

2. Answer: A.

3. Answer: D.

A hapten is an isolated (free) antigenic determinant, too small (by definition) to trigger an immune response on its own. *Epitope* is a synonym for antigenic determinant, the part of an antigen to which antibody binds. An immunogen is a molecule that actually does produce an immune response upon injection; it must be both foreign to the host, present at the proper concentration, and large enough to have at least two antigenic determinants.

4. Answer: E. Carbohydrates, lipids, and even nucleic acids or organic molecules can induce an immune response and are therefore *immunogens*. Thus, choices A and B are incorrect. Immunogens are *foreign*, not self, so choice C is wrong. A hapten is a univalent, single antigenic determinant and is never an immunogen unless coupled to a carrier. Choice E is part of the definition of an immunogen, along with "foreign."

5. Answer: B. Quinine binds to the surface of the infected and uninfected RBCs and is recognized in association with RBC surface antigens. Therefore, it is serving as a hapten, the RBCs are the carrier, and together they are an immunogen against which the immune response occurs. Because IgE antibodies are not mentioned in this pathology, reagins are not involved.

6. Answer: C.

Major Histocompatibility Complex (MHC)

6

A. The MHC

The MHC is a collection of highly **polymorphic** genes encoding the proteins that regulate immune responses. These genes include, most notably, the **class I** and **class II** cell surface proteins and the **class III** genes that encode some soluble **complement molecules** and **cytokines**.

In humans, the MHC gene products are termed **human leukocyte antigens (HLA)**, and their corresponding genes are found on the short arm of **chromosome 6**. The HLA are glycoproteins present on cell surfaces that enable T cells to **recognize** and **bind antigenic peptides**. They function in **immune recognition**.

In a Nutshell

- MHCs are highly polymorphic
- MHCs are codominant
- MHCs function in immune recognition

Table II-6-1. Molecular Products of the Human MHC on Chromosome 6

Class I Gene Products			Class II Gene Products			Class III Gene Products	
HLA-A	HLA-B	HLA-C	HLA-DP	HLA-DQ	HLA-DR	Complement C2, C4, B	TNF- α & β *

* TNF= tumor necrosis factor. Note: TNF- α is sometimes called TNFA, and TNF- β is sometimes called TNFB.

B. HLA Gene Class I Antigens

Class I proteins are membrane glycoproteins on the surface of all **nucleated cells** and **platelets**. They bind peptides processed from protein synthesized in the cell's cytosol and are necessary for antigen recognition by **CD8⁺ cytotoxic T lymphocytes**. In humans, the three types of MHC class I genes are referred to as **HLA-A**, **HLA-B**, and **HLA-C** genes, which code for their respective antigenic molecular products, namely **HLA-A**, **HLA-B**, and **HLA-C**. The expression of MHC genes is **codominant**. That is, each cell that expresses MHC class I molecules expresses six different gene products: **HLA-A**, **HLA-B**, and **HLA-C** from the **maternal chromosome 6** and **HLA-A**, **HLA-B**, and **HLA-C** from the **paternal chromosome 6**.

Table II-6-2. Tissue Distribution of Human MHC Gene Products

MHC Region	Gene Products Expressed	Tissue Distribution
Class I	HLA-A, HLA-B, HLA-C	All nucleated cells; platelets

Currently, 147 HLA-A, 200 HLA-B, and 90 HLA-C genes have been recognized. This leads to the extensive **polymorphism** that exists for their molecular products.

All class I molecules share common structures. These include:

1. An **α heavy chain**
 - a. It has three extracellular domains, two of which form the peptide-binding groove.
 - b. The carboxy terminus lies within the cytoplasm.
2. A **single transmembrane domain**
3. A **light β chain, called β 2-microglobin**
 - a. It is not encoded by a gene in the MHC region.
 - b. It functions to transport MHC class I molecules to the cell surface.

In a Nutshell

Class I Molecules

- An α chain with three extracellular domains
- Single transmembrane domain
- Separately encoded β chain (β_2 microglobulin)

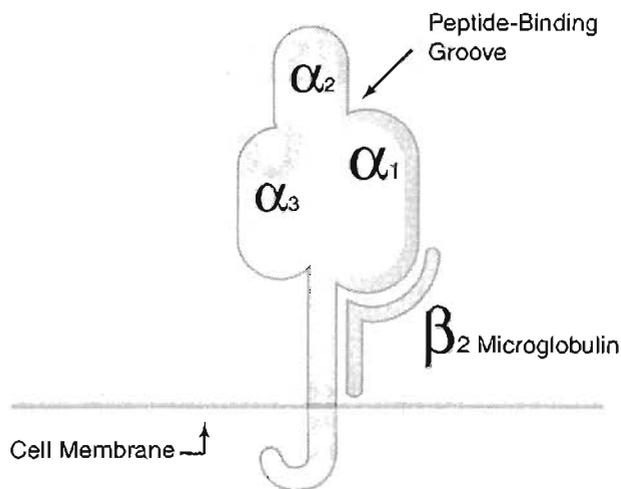


Figure II-6-1. MHC Class I Molecule

Proteins synthesized in the cell cytosol are routinely degraded by proteases, and the peptides from these proteins are transported through a peptide transporter, known as the TAP complex, into the endoplasmic reticulum, where they have an opportunity to bind to freshly synthesized HLA class I proteins (see Figure VI-2). These are **endogenously** produced peptides from **viruses, intracellular bacteria**, intracellular parasites, or neoantigens made by transformed tumor cells.

Cytotoxic CD8⁺ T lymphocytes recognize the peptides that are associated with the class I molecules. Thus the ability of a CD8⁺ cytotoxic T cell to recognize antigen depends on association of the antigen with a class I protein. This is termed **MHC restriction**. A cytotoxic T cell that kills a virus-infected cell will not kill a cell infected with the same virus if the cell does not also express the appropriate class I protein.

In a Nutshell

Cytotoxic T Cells

- CD8⁺, MHC class I restricted
- Lyse target cells that are seen as foreign
 - tumor cells
 - virus-infected cells
 - grafts
- Lysis occurring via release of perforins and nucleases, for example, from granules

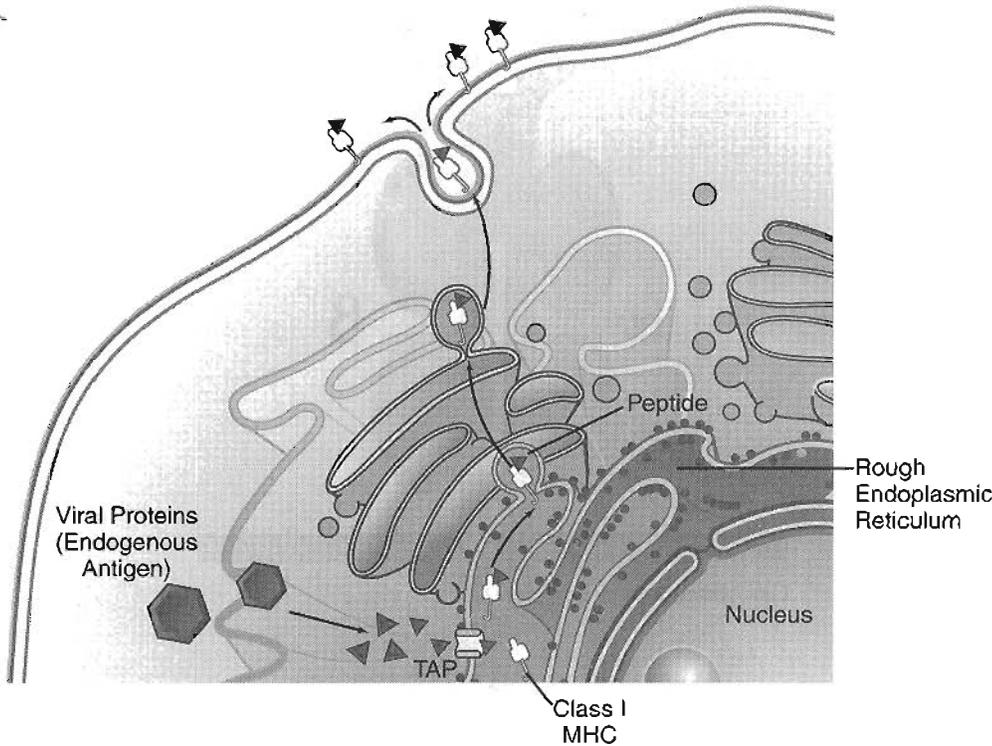


Figure II-6-2. The Endogenous Pathway (Class I MHC)

In a Nutshell

TAP: Transporters associated with antigen processing. They are proteins involved in transporting short peptides from cytosol into lumen of the endoplasmic reticulum.

C. HLA Gene Class II Antigens

HLA class II proteins are expressed on a more restricted set of cells, including antigen-presenting cells (dendritic cells, Langerhans cells, activated macrophages, B cells, activated T cells, and activated endothelial cells).

These proteins bind exogenous peptide epitopes that have been endocytosed and processed and are necessary for antigen recognition by CD4⁺ helper T lymphocytes. Class II genes code for cell-surface glycoproteins with two polypeptide components called α and β . In humans, the class II genes include HLA-DR, HLA-DQ, and HLA-DP. As with the class I system, the class II genes are highly polymorphic; there are approximately 500 total class II gene products. They are also codominantly expressed.

Table II-6-3. Tissue Distribution of Human MHC Class II Gene Expression

MHC Region	Gene Products	Tissue Distribution
Class II	HLA-DP, HLA-DQ, HLA-DR	Monocytes, macrophages, B lymphocytes, dendritic cells, Langerhans cells, activated T lymphocytes, activated endothelial cells

In a Nutshell

Class II molecules

- Internalized and digested exogenous antigens; the resultant peptide fragments complex with class II molecules to be presented on the cell surface.
- Both α and β chains with two extracellular domains.
- Two transmembrane domains.
- Recognized by CD4⁺ helper T cells.

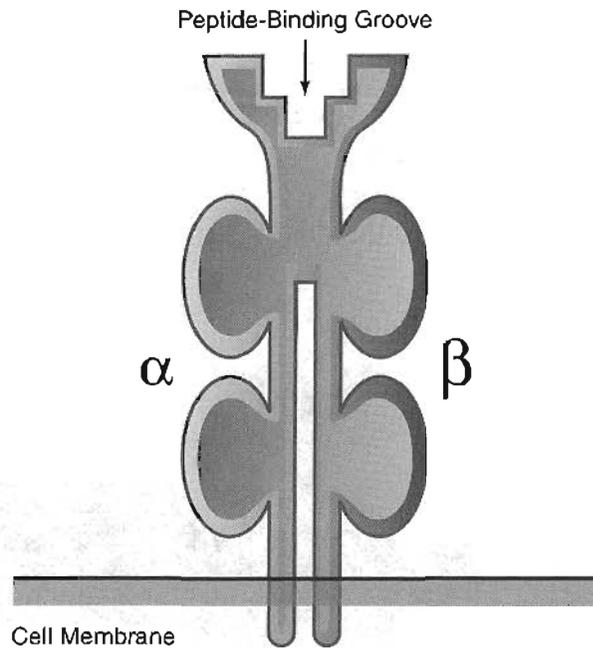


Figure II-6-3. Class II MHC Molecule

Proteins from outside these **antigen-presenting cells** are nonspecifically internalized by **endocytosis**. In the endocytic vacuoles in these cells, enzymes degrade (**process**) the proteins into small (about 10–20 amino acid) peptides. The MHC class II α and β chains are synthesized by these cells, along with a third chain, called the **invariant chain**. The invariant chain blocks access to the peptide-binding groove of the MHC class II molecule as it is transported through the cell. Once the vesicle containing the MHC class II molecule fuses with the endocytic vacuole containing the digested, internalized peptides, the invariant chain is released and degraded, leaving the MHC class II molecule accessible to the peptides in the vacuole. The **MHC class II–peptide complex** is then transported to the cell surface, where it can be recognized by helper T cells, which have antigen receptors that recognize only peptides bound to MHC class II molecules. **This is termed MHC restriction.**

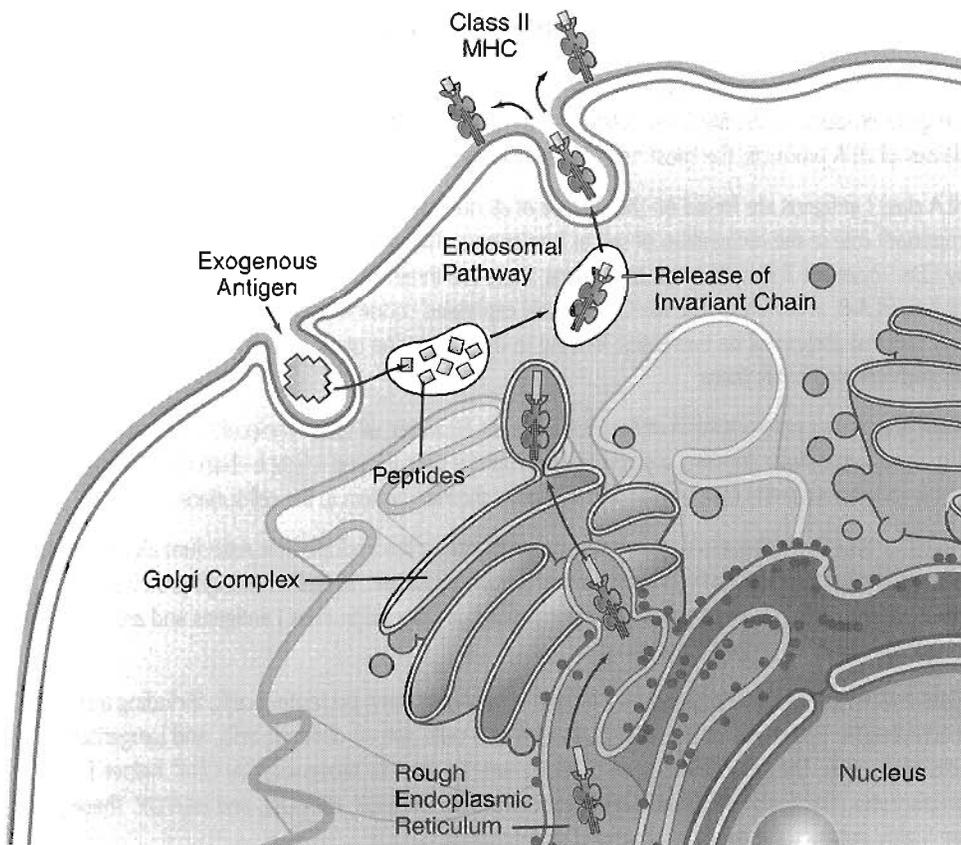


Figure II-6-4. Binding of Peptides to Class II MHC Molecules

Table II-6-4. Human MHC Summary

	MHC Class I	MHC Class II
Names	HLA-A, HLA-B, HLA-C	HLA-DP, HLA-DQ, HLA-DR
Tissue distribution	All nucleated cells; platelets	B lymphocytes, monocytes, macrophages, dendritic cells, Langerhans cells, activated T cells, activated endothelial cells
Recognized by	Cytotoxic T cells (CD8)	Helper T cells (CD4)
Peptides bound	Endogenously synthesized	Exogenously processed
Function	Elimination of abnormal (infected) host cells by cytotoxic T cells	Presentation of foreign antigen to helper T cells
Invariant chain	No	Yes
β 2-microglobulin	Yes	No

Chapter Summary

The major histocompatibility complex (MHC) is a collection of highly polymorphic genes. In humans, the gene products of the MHC are called the human leukocyte antigens (HLAs). There are several classes of HLA products, the most notable being classes I, II, and III.

HLA class I antigens are found on the surface of all nucleated cells and platelets, where they play an important role in the recognition of self or foreignness. They are also essential for antigen recognition by CD8⁺ cytotoxic T lymphocytes. In humans, there are three MHC class I genes, which code for HLA-A, HLA-B, and HLA-C. The MHC genes are expressed codominantly. Therefore, both maternal and paternal alleles will be expressed, leading to the expression of six different HLA class I products on each cell that bears them.

There is extensive polymorphism of the HLA products. However, all class I molecules share an α chain with three extracellular domains, a single transmembrane domain, and a light β chain (β -2 microglobulin) that is coded outside the MHC region and transports the HLA protein to the cell surface.

Protein products of intracellular processes are produced in the endoplasmic reticulum along with HLA class I proteins. Peptides derived from normal cellular processes, as well as foreign proteins, intracellular organisms, or neoplastic transformations, are bound to class I antigens and are presented to cytotoxic CD8⁺ T lymphocytes.

Class II MHC genes are expressed by a limited variety of antigen-presenting cells, including activated macrophages, T cells and epithelial cells, as well as B cells, spleen dendritic cells, and Langerhans cells of the skin. The HLA class II gene products are required for recognition by CD4⁺ helper T lymphocytes. There also are three class II gene products: HLA-DR, HLA-DQ, and HLA-DP. These again are highly polymorphic and are expressed codominantly.

These antigen-presenting cells endocytize proteins, proteolytically degrade them into small peptides, and then transport them to the cell surface in association with a class II HLA molecule. Once on the surface the complex is recognized by helper T cells, which recognize only antigens bound to a class II HLA molecule, another example of MHC restriction.

The class III MHC genes code for some soluble complement and cytokine molecules that are not cell surface bound.

Review Questions

- All are features of cell-surface HLA/B molecules, except:
 - They are associated with $\beta 2$ microglobulin.
 - They bind exogenous peptides.
 - They are polymorphic.
 - They are expressed on B lymphocytes.
 - They can be bound by CD8 molecules.
- Toxoplasma gondii* is an intracellular parasite that lives inside phagocytic and nonphagocytic cells by generating its own intracellular vesicle. This may allow it to avoid recognition and killing by CD8⁺ lymphocytes, which require the presentation of foreign peptides transported into the endoplasmic reticulum and loading them onto MHC molecules that have
 - A peptide-binding groove
 - A single transmembrane domain
 - Two similar chains
 - Invariant chains
 - A $\beta 2$ domain instead of a $\beta 2$ microglobulin
- There are several ways that tumors can escape immune surveillance. Loss of which class of molecules on the surface of the tumor cell target would result in loss of susceptibility for killing?
 - MHC class II
 - MHC class I
 - CD4
 - CD8
 - CD3
- Which one of the following is true of class II MHC molecules?
 - They consist of an alpha chain of three domains and a $\beta 2$ microglobulin.
 - They are found in all nucleated cells of our body.
 - They are involved in antigen presentation to CD8⁺ cytotoxic lymphocytes.
 - They consist of DR, DQ, and DP molecules.
 - They are located on the X chromosomes.
- Which one of the following is true of class I MHC molecules?
 - They are found only on immunocompetent cells of our body.
 - They are involved in antigen (epitope) presentation to CD4⁺ T-helper lymphocytes.
 - They consist of an alpha chain with two domains and a beta chain with two domains.
 - Their formation is regulated by DR, DP, and DQ genes on chromosome 6.
 - Their formation is regulated by A, B, and C genes on chromosome 6.

6. When antigens are processed by the exogenous route of antigen presentation, they are associated with which one of the following for presentation?
- A. Fc receptors
 - B. IgG heavy chains
 - C. MHC class I molecules
 - D. MHC class II molecules
 - E. TCR (T-cell receptors)

Answers

1. **Answer: B.** HLA-B are one of the MHC class I molecules that are expressed on all nucleated cells, are always associated with β 2-microglobulin, and act as a target for elimination of infected or tumor-transformed host cells by CD8-bearing cytotoxic T cells. Both class I and class II MHC molecules are polymorphic, which is the basis for graft rejection of tissue transplanted to a non-MHC identical recipient. The MHC class I molecules bind endogenous peptides during their synthesis by host cells. The MHC class II molecules bind exogenous peptides produced during lysosomal enzyme degradation of ingested proteins internalized by antigen-presenting cells.
2. **Answer: B.** This pathway of antigen presentation requires the MHC class I antigen that is described in choice B. All the other choices refer either to both MHC class I and II (**choice A**) or only to MHC class II (**choices C, D, and E**).
3. **Answer: B.** The MHC class I molecules present the peptide fragments from the tumor in a groove of the MHC class I molecule after it has moved to the surface of the tumor cell. They present this peptide fragment to cytotoxic CD8 T cells. The cytotoxic CD8 T cells are able to recognize the MHC class I molecules on the cell surface and kill the tumor cell.
4. **Answer: D.** The class II molecules DR, DQ, and DP are located on immune cells that interact with T helper CD4⁺ lymphocytes. The genes for these molecules are located on chromosome 6. They are highly polymorphic and codominant so there are many different haplotypes.
5. **Answer: E.** The class I MHC genes are located on chromosome 6 and they consist of A, B, and C genes involved in producing the class I products. There is one A, one B, and one C gene on each chromosome 6 from each parent. These genes and products are polymorphic and codominant.
6. **Answer: D.** When pathogenic organisms are phagocytosed and degraded from an exogenous route they are presented on the surface of the antigen-presenting cell by MHC class II molecules to a CD4⁺ T lymphocyte with a specific TCR for this antigen epitope.

The Lymphoid System

7

A. Structure and Function

The lymphoid system is composed of the primary lymphoid organs, in which **hematopoiesis**, **lymphopoiesis**, and **education** occur, and the secondary lymphoid organs, in which **immune responses** occur.

1. Primary lymphoid organs

The **primary**, or **central**, **lymphoid organs** are the anatomical locations in which lymphocytes develop the ability to specifically recognize foreign antigen and to distinguish self from nonself. These lymphoid organs include the **bone marrow** and **thymus**. **Hematopoiesis** occurs in the bone marrow, producing **erythrocytes**, **platelets**, **monocytes**, **granulocytes**, and **B cells**, as well as precursors for **T cells**, **natural killer (NK) cells**, **dendritic cells**, and **mast cells**. The **T cells** finish maturation in the **thymus**, whereas the rest finish differentiation in the periphery. Two cytokines play a central role in these early developmental stages. **Interleukin (IL)-7** is produced by **stromal cells** in the **bone marrow** and is important in **lymphoid cell development**. **IL-3** and various other **colony-stimulating factors** are essential for **myeloid cell development** (granulocytes and monocytes).

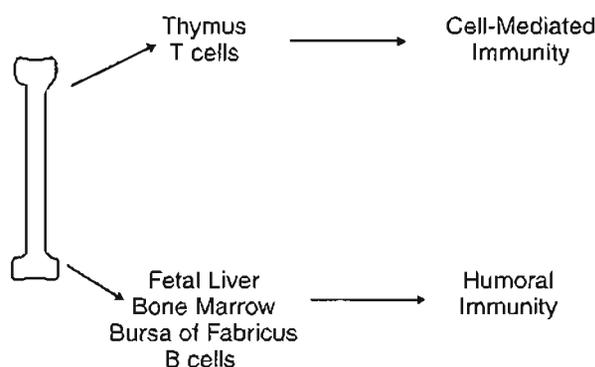


Figure II-7-1. Ontogeny of the Immune Response

2. Secondary lymphoid organs

The major **secondary**, or **peripheral**, **lymphoid organs** and tissues include the **lymph nodes**, **spleen**, **tonsils**, and the **mucosa-associated lymphoid tissue (MALT)**. This system includes the **gut-associated lymphoid tissue (GALT)** and **bronchus-associated lymphoid tissue (BALT)**, as well as **submucosal lymphoid tissues** of **genitourinary tract**. The **secondary lymphoid organs** are the sites for **presentation of foreign antigens to cells of the immune system**.

In a Nutshell

- IL-7 = B and T cell development in bone marrow
- IL-3 = myeloid-cell development in bone marrow

In a Nutshell

Primary Lymphoid Organs

Bone marrow

Thymus

Secondary Lymphoid Organs

Lymph nodes

Spleen

Tonsils

Mucosa-associated lymphoid tissue (MALT)

- Gut-associated lymphoid tissue (GALT)
- Bronchus-associated lymphoid tissue (BALT)

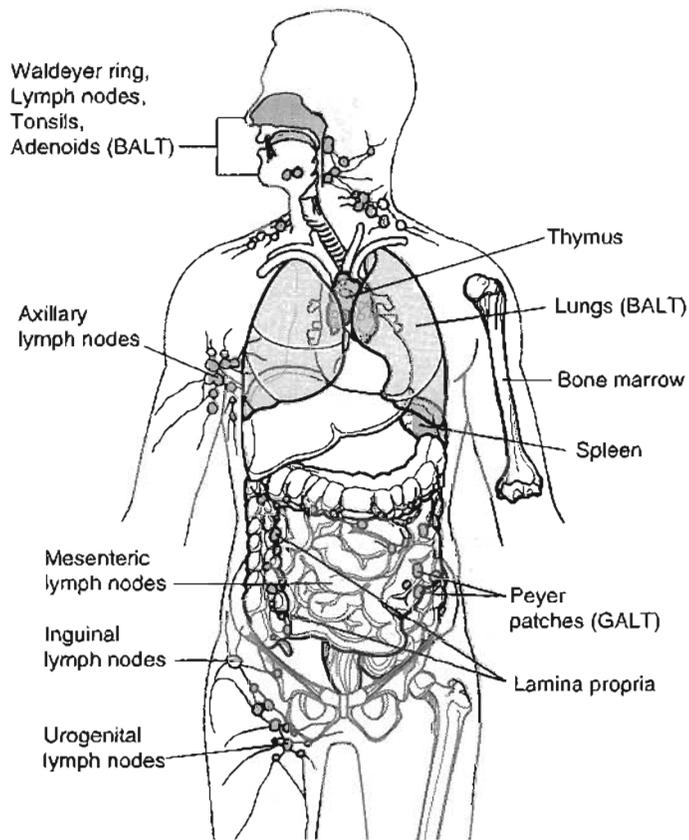


Figure II-7-2. Locations of the Lymphoid Organs

In a Nutshell

Cellular differentiation (CD): Cluster of antigens with which antibodies react that characterizes cell-surface molecules

- CD19 = B cell
- CD21 = B cell

B. B-Lymphocyte Differentiation

B lymphocytes differentiate from lymphoid precursors in the bone marrow. Unlike T cells, however, which must migrate from the bone marrow to the thymus to attain functional maturity, the B cells leave the bone marrow with the full ability to recognize and respond to antigen. They then migrate to the secondary lymphoid organs to await the arrival of antigen. Like T cells, B cells also express cluster determinant (CD) markers, which are useful in their identification. However, the best markers of B-cell development are the immunoglobulin chains that they make.

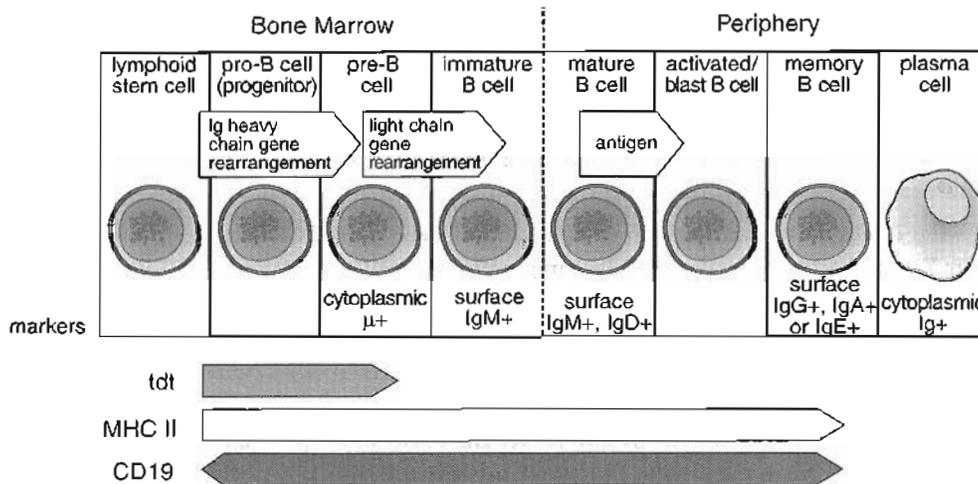


Figure II-7-3. B-Cell Differentiation

In a Nutshell

- Pre B cell = cytoplasmic μ chain
- Immature B cell = surface IgM
- Mature B cell = IgM and IgD on surface of cell

1. The important B-cell CD markers include:
 - a. CD19: used clinically to enumerate B cells in blood.
 - b. CD21: complement receptor type 2 (CR2) that binds cleaved C3 (iC3b and C3dg) fragment; also the receptor for the Epstein-Barr virus (EBV).
 - c. CD40: required for class switch signals from T cells.
2. All B cells also express MHC class II molecules, which are essential in their interaction with helper T cells.

Table II-7-1. The Stages of B-Cell Development

Stage	Ig Genes	Ig Synthesis	Comment
Precursor B cell	Germ line (not rearranged)	None	Found in bone marrow
Pre-B cell	Only heavy chain V region rearranged	IgM heavy chain (μ) only in cytoplasm	Not able to recognize antigen
Immature B cell	Heavy and light chain V regions rearranged	IgM expressed on surface membrane	Antigen responsive, easily tolerated
Mature B cell	Heavy and light chain V regions rearranged	IgM and IgD expressed on surface membrane	Short-lived, difficult to tolerate
Memory B cell	Constant region: heavy chain gene rearrangement, class switch	IgG (subclass 1, 2, 3, or 4) or IgA or IgE on surface	Long-lived
Plasma cell		Rapid synthesis and secretion of one Ig class	Lives 1–2 weeks

In a Nutshell

T-cell development

- Development occurs in the thymus
- Gene rearrangements occur during development of the T cells that generate diversity in the T-cell populations
- Cells are selected to be nonreactive to self
- Thymic cortical epithelial cells help select T cells that recognize antigen in association with MHC class I or class II antigens
- Surface molecules (antigenic markers) change on T-cell subsets, and these confer different activities to the cell

In a Nutshell

- Positive selection of a CD4⁺ or CD8⁺ cell
- Negative selection results in apoptosis and elimination of T-cells

C. T-Cell Differentiation

T lymphocytes must undergo differentiation in the thymus to function. When the pre-T cells leave the bone marrow they cannot recognize antigen. These cells are transported to the thymus, migrate to the cortex, and, as they mature, move deeper into the medulla. **Intimate contact with thymic epithelial cells, macrophages, and dendritic cells is essential in T-cell maturation.**

As the T cells develop, they begin to express T-cell receptors (TCRs) but only one specificity per T cell, chosen by random VDJ recombinational events. The thymic epithelial cells, dendritic cells, and macrophages present self-antigens (but not foreign antigen) to the developing T cells in the cortex. Those that recognize self-MHC molecules, but not strongly, will get a **positive selection** signal to continue dividing and will establish a clone that will eventually mature in the medulla, followed by exit into the periphery to the **secondary lymphoid organs**. The T cells that fail to effectively recognize self-MHC molecules will not receive this signal and will die in the thymus (**failure of positive selection**).

T cells expressing antigen receptors with too high an affinity to self-MHC molecules have the potential to cause **autoimmune disease**. The thymus induces these autoreactive T cells to undergo **apoptosis** (programmed cell death). This process is called **negative selection**.

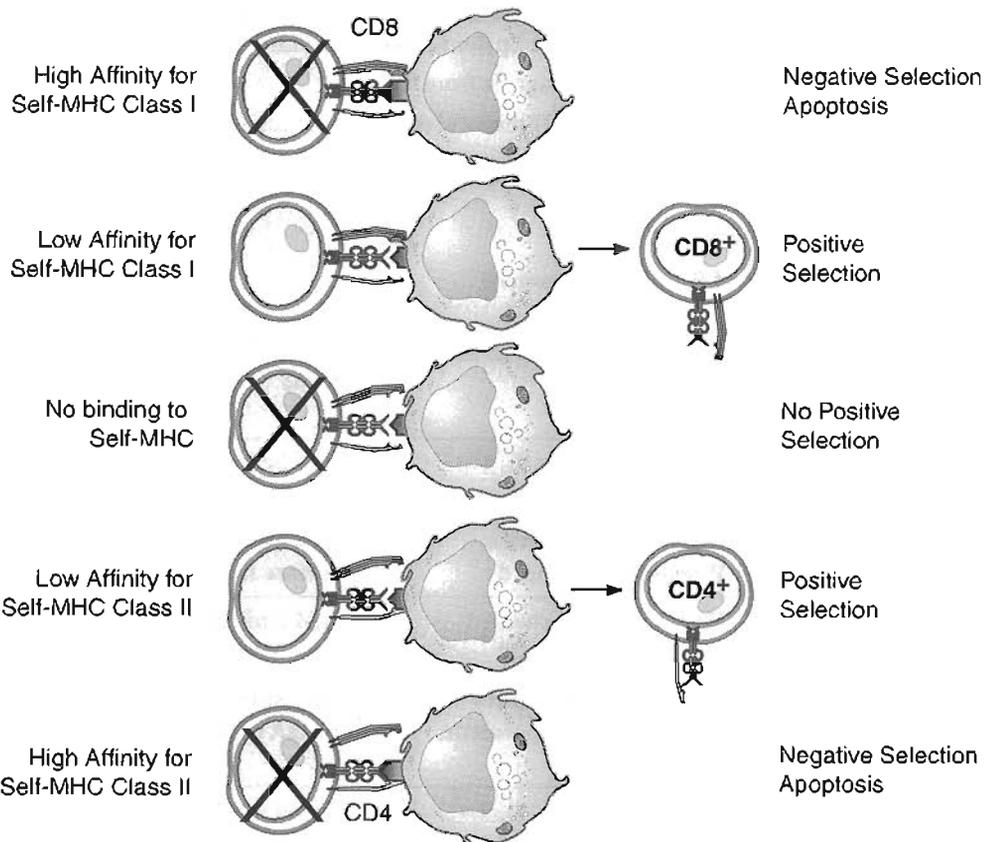


Figure II-7-4. T-Cell Selection in the Thymus

D. T-Lymphocyte Maturation Markers

During the development of T cells in the thymus, several important surface molecules (or surface markers) are expressed. These molecules were often first identified by monoclonal antibodies, and their functions were unknown. As was the case for the B-cell markers, the surface molecules on the cells were called CD (for **cluster of differentiation, cellular differentiation**, or cluster determinant) markers.

Tolerance is the absence of specific immune responses in an otherwise fully immunocompetent person. This unresponsiveness can be either autotolerance (naturally acquired) or specifically induced acquired tolerance.

1. Autotolerance

Autotolerance is to one's own antigens. This tolerance is naturally acquired during fetal life in the central lymphoid organs and involves deletion of autoreactive clones.

Immature B cells are also inactivated by contact with antigen. Depending on the nature of the antigen, the inactivated B cells either die immediately (**clonal deletion**) or they persist in a nonfunctional state (**clonal anergy**). For this reason, tolerance in B cells is not as complete as it is in T cells.

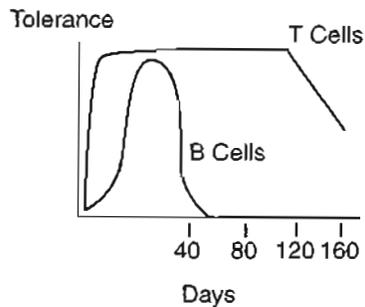


Figure II-7-5. T Cells Are More Sensitive to Tolerance Induction

Markers	Prethymic	Thymic Cortex	Thymic Medulla	Circulating T Cells
tdt	High	High	High	High
CD2	High	High	High	High
CD3	High	low	high	high
TCR	High	low	high	high
CD4	High	High	High	High
+	High	High	High	High
CD8	High	High	High	High

Figure II-7-6. Human T-Cell Differentiation

In a Nutshell

T-cell markers

- CD2
- CD3
- CD4 or CD8
- CD40
- CD28

In early thymocyte development, the CD4 and CD8 molecules are coexpressed. As the cell matures and expresses an antigen receptor, only one of these molecules is retained.

If the T cell's antigen has an affinity for MHC class I molecules, the CD8 marker will be retained, the CD4 will stop being produced, and the cell will become a mature cytotoxic T cell.

Conversely, if the antigen receptor has an affinity for the MHC class II molecule, the CD4 marker will be retained, the CD8 will be lost, and the cell will mature into a helper T cell. The essential CD markers known on T cells are:

Table II-7-2. CD Markers on T Cells

Marker	Expressed by	Functions
CD3	All T cells	TCR-associated signal-transduction molecule
CD4	Helper T cells	Interaction with MHC class II molecules
CD8	Cytotoxic T cells	Interaction with MHC class I molecules
CD2	All T cells	Adherence to other cells, binds to LFA-3
CD40 ligand	Activated helper T cells	Binds to CD40 on B cells; essential for Ab isotype switching
CD28	Helper T cells & most CD8 ⁺ T cells	Costimulatory molecule needed for T-cell activation; binds B7 on B cell, macrophage, or dendritic cell

LFA, leukocyte (or lymphocyte) function-associated antigen; TCR, T-cell receptor.

In a Nutshell

Events of T-cell and B-cell maturation

- Mature B cells migrate from bone marrow to cortex of lymph nodes
- Mature T cells migrate from thymus to paracortex of lymph nodes
- Antigen-trapping macrophages and dendritic cells found throughout the lymph node; antigen epitopes are presented to T cells by antigen-presenting cells
- T cells cooperate with B cells
- B cells produce antibody
- B cells differentiate into plasma cells

E. Secondary or Peripheral Lymphoid Organs

1. Overview

The T lymphocytes and B lymphocytes migrate from the central lymphoid tissues to the peripheral lymphoid tissues, where they can respond to foreign antigens. The peripheral lymphoid tissues are compartmentalized into areas rich in T or B cells.

The lymph fluid that bathes the cells of the body is collected into afferent lymphatic vessels that bring antigen into the lymph nodes. Immunocompetent B cells move from the bone marrow after maturing to the follicles in the cortex of the lymph nodes. Immunocompetent T cells move from the thymus to the paracortex of the lymph nodes. Macrophages and dendritic cells are found throughout the node to trap foreign antigen that enters via afferent lymphatic vessels.

2. The lymph nodes

Lymph nodes are the major antigen-trapping sites of the body designed to filter foreign substances from the tissue fluids and lymph. Soluble antigens that are carried to the lymph node are picked up nonspecifically by macrophages and dendritic cells that process them and present them to T lymphocytes. The activated T lymphocytes can now cooperate with B cells to stimulate an antibody response. Specific B cells in the follicles are stimulated to divide, producing clones of cells that make antibody specific for the unprocessed, native antigen. The cell division that takes place in the follicles produces a germinal center. Stimulation of B cells by antigen induces their terminal differentiation into plasma cells that are the main antibody-secreting cells of the body.

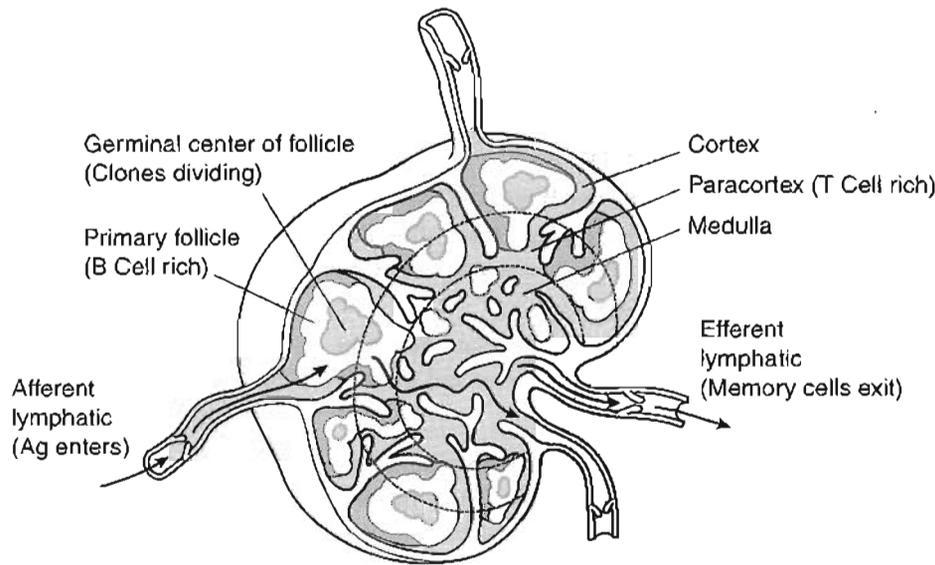


Figure II-7-7. Compartmentalization of a Lymph Node

Some of the antigen-stimulated B cells and T cells differentiate into **memory cells**, which provide the host long-lived memory for the antigen. Memory cells may leave the lymph node via efferent lymphatic vessels that deliver them to the bloodstream, permitting the cells to move to various other secondary lymphoid locations in the body.

3. The spleen

The spleen consists of red and white pulp. **Immune cells** are located in the **white pulp**, which consists of lymphatic tissue arranged in sheaths around arterioles. These **T-cell-rich areas** are called **periarteriolar sheaths (PALS)**. The **B cells cluster** peripherally to form **primary follicles**. After antigenic stimulation, these follicles develop germinal centers of rapidly dividing B cells.

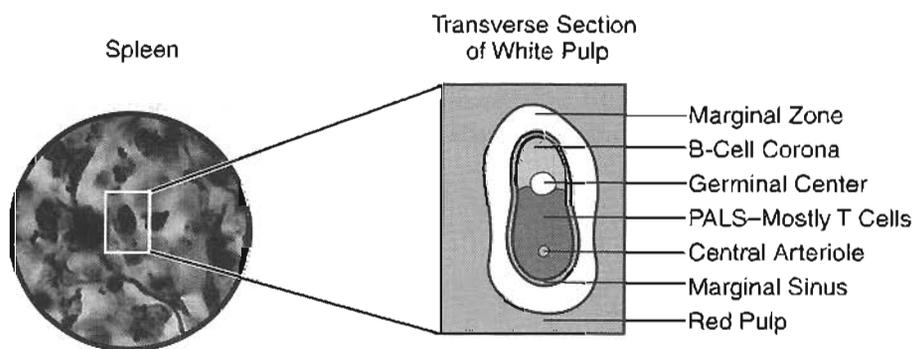


Figure II-7-8. T- and B-Cell Dependent Areas of the Spleen

Note

Immunologic memory

- B cells will differentiate into memory cells
- T cells will differentiate into memory cells

In a Nutshell

Immune cells of the spleen

- Immunocompetent cells are located in white pulp of spleen
- T cells are located in PALS region of spleen
- B cells are located within primary follicles

In a Nutshell

Antigen clearing

- Lymph nodes sample antigen that arrives parenterally
- The spleen samples antigen that is intravenous
- MALT samples antigen that enters via mucosa

Note

Lymphocyte homing is controlled by selectins in the lymphocyte membrane that react specifically with addressins found in the lymphatic vasculature.

4. **Mucosa-associated lymphoid tissue (MALT)**

These are organized collections of lymphoid tissue scattered throughout the submucosa and lamina propria. The GALT (gut-associated lymphoid tissue) is not encapsulated and is present in the gastrointestinal tract. It includes large follicular aggregates in the small intestine called Peyer patches.

The BALT (bronchus-associated lymphoid tissue) includes the lymphoid tissue beneath the respiratory mucosa and the aggregates of nodular lymphatic tissues called tonsils. The tonsils are composed of nodular aggregates of B lymphocytes and diffuse areas that contain mostly T lymphocytes.

Lymphocyte recirculation is an important function, as the lymphocytes continually move from the blood out into the tissues and then return via lymphatics.

5. **Cellular attraction and adhesion**

Several important groups of molecules are involved in the adhesion of cells to each other or with the extracellular environment. Three major “families” of proteins are involved: (1) cell adhesion molecules (CAMs) of the immunoglobulin superfamily (IgCAMs); (2) integrins; and (3) selectins.

Definitions:

CAMs—Cell adhesion molecules that have a domain structure similar to immunoglobulins (~110-amino acid-long globular units with one intrachain disulfide bond) are widely distributed in tissues. They include the CD2 and LFA-3 molecules that aid in helper T cell interactions with antigen-presenting cells and B cells, as well as intercellular adhesion molecule-1 (ICAM-1) and ICAM-2.

Integrins—These are two-chain (α and β) molecules that are expressed as two major families that share a common β chain. The β_1 integrins are called VLA (very late antigen) molecules because of the length of time required for their expression. They mediate leukocyte interaction with the extracellular matrix. The β_2 integrins mediate tight leukocyte adhesion to endothelial cells and play a very important role in migration of cells out of the blood and into tissues during an immune response. They also help mediate the passage of lymphocytes into secondary lymphoid tissues at the high endothelial venules. Two of the β_2 integrins are also receptors for activated complement fragments (C3b fragments) that mediate phagocytosis.

Selectins—As their name implies, these molecules are lectins (carbohydrate-binding proteins) that allow cells to bind to the carbohydrate regions of glycoproteins or oligosaccharides on cells. They mediate the initial (low-affinity) binding between lymphocytes and high endothelial venules, between neutrophils and inflammatory cytokine-activated endothelial cells, or between activated platelets and endothelial cells.

Table II-7-3. Summary of Adhesion Molecules

Adhesion Molecule Type	Name (Synonyms)	Ligand(s)	Function(s)
Selectin	E-selectin	Carbohydrates on PMNs, monocytes, lymphocytes	Leukocyte migration and homing
	L-selectin	Vascular carbohydrates	Initial binding to endothelium
	P-selectin	Carbohydrates on PMNs, monocytes, lymphocytes	Leukocyte migration to inflammatory site
Integrin	VLA family (β_1)	Fibronectin, laminin, collagen	Migration through extracellular matrix
	<u>LFA-1</u>	<u>ICAM-1, -2, -3</u>	Tight binding to endothelium
	<u>CR3 (Mac-1)</u>	<u>ICAM-1, C3b</u>	Tight binding to endothelium Phagocytosis
	<u>CR4</u>	<u>C3b</u>	Phagocytosis (opsonin receptor)
Ig CAM	<u>ICAM-1, -2, and -3</u>	<u>LFA-1</u>	Homing to lymph node, migration to inflammatory sites
	<u>LFA-3</u>	<u>CD2</u>	T-cell interactions
	<u>CD2</u>	<u>LFA-3</u>	Lymphocyte interactions

Chapter Summary

Erythrocytes, platelets, monocytes, granulocytes, and B cells, as well as immature forms of T cells, natural killer (NK) cells, dendritic cells, and mast cells, develop in the bone marrow. T lymphocytes move to the thymus where they complete the training and selection required to recognize self from nonself. The primary lymphoid organs (bone marrow and thymus) are free of foreign antigen challenge. IL-7 is required for lymphoid cell development and IL-3 for myeloid (granulocytes and monocytes) development.

The secondary lymphoid system organs are sites for the presentation of foreign antigens to immune system cells. These organs include the spleen, lymph nodes, tonsils, the gut-associated lymphoid tissue (GALT), the bronchus-associated lymphoid tissue (BALT), and the mucosal lymphoid cells of the genitourinary tract.

Mature B cells leave the bone marrow and are transported to the secondary lymphoid organs to await the arrival of antigen. They may be recognized by the antibody produced or by cluster determinant (CD) markers. CD19 is used to count B cells in the blood; CD21, also known as complement receptor type 2, binds cleaved C3 fragment (C3d) and is the receptor for the Epstein-Barr virus; CD40 is required for class switch signals. B cells also express HLA class II molecules.

T-cell maturation starts in the thymic cortex and finishes in the medulla. Thymic epithelial and dendritic cells present self-antigens to the T cells in the cortex. T cells that weakly recognize self-antigens in conjunction with HLA class I or II molecules are selected to mature further in the medulla. Those cells that do not recognize self-antigens or do so too strongly, having a potential to cause autoimmune disease, are destroyed.

Both CD4 and CD8 markers are expressed during early T-cell development. Cells that develop an affinity for HLA class I markers retain the CD8 marker; those with an affinity for HLA class II markers retain the CD4 molecule.

The secondary lymphoid organs are the major antigen-trapping sites of the body. The lymph nodes filter lymph, and the spleen filters the blood. Immunocompetent B and T cells move from the bone marrow or thymus, respectively, to these organs. B cells in lymph nodes are found in germinal centers, and T cells are found in the paracortical areas. The medullary cords are rich in antigen-presenting cells.

In the white pulp of the spleen, T cells are found in the periarteriolar lymphoid sheaths (PALS). B cells cluster peripherally, forming primary follicles that develop centers of rapidly dividing B cells when stimulated.

The GALT, mucosal-associated lymphoid tissue (MALT), and BALT are loosely organized aggregates of T cells as well as IgA-secreting B lymphocytes and plasma cells.

Three families of proteins are involved in the adhesion of cells to each other or to the extracellular environment. These are cell adhesion molecules (CAMs), integrins, and selectins.

CAMs have domain structures similar to immunoglobulins, are widely distributed, and aid in helper T-cell interaction with antigen-presenting cells and B cells.

Integrins mediate tight leukocyte binding to endothelial cells and play a role in the migration of cells from the blood into tissues during an immune response.

Selectins are carbohydrate-binding proteins (lectins) that permit cells to bind to carbohydrates of other cell membranes.

Review Questions

1. The best marker for enumerating total peripheral blood T cells is
 - A. CD4
 - B. CD8
 - C. CD11b
 - D. CD14
 - E. CD3

2. The high endothelial venules (HEV) are the sites at which
 - A. Memory T cells enter the lymph node.
 - B. Plasma cells leave the lymph node.
 - C. Foreign antigen enters the lymph node.
 - D. Mature B cells leave the lymph node.
 - E. Secreted antibody leaves the lymph node.

3. Which of the following correctly describes a role of the thymus in lymphocyte development?
 - A. T cells are differentiated from B cells in this organ.
 - B. Foreign antigen presentation leads to the development of antigen-specific T cell clones.
 - C. The CD4 and CD8 markers that distinguish helper from cytotoxic T cells are found primarily on cells in the thymic cortex.
 - D. The CD3 marker appears on only a subset of the T cells that eventually leave the thymus.
 - E. T cell antigen receptors that are expressed during thymic development must recognize self MHC class I or class II molecules.

4. Secondary lymphoid organs are sites in which
 - A. CD4 and CD8 are coexpressed on most T cells.
 - B. Antigen receptor gene rearrangement occurs.
 - C. Foreign antigen processing occurs.
 - D. IL-7 mediates stem cell development.
 - E. Terminal deoxyribonucleotidyl transferase (TdT) in lymphocytes is active.

5. Which of these is the earliest marker to appear during T-cell development?
 - A. CD3
 - B. CD4
 - C. TdT
 - D. T-cell antigen receptor alpha chain
 - E. HLA-DR

6. Functions of the thymus include all the following **except**
 - A. Elimination of T cells that have low affinity for MHC class I molecules.
 - B. Elimination of T cells that have high affinity for MHC class II molecules.
 - C. Stimulation of T cells that have low affinity for MHC class I molecules.
 - D. Stimulation of T cells that have low affinity for MHC class II molecules.

7. A lymph node biopsy of a 6-year-old boy shows markedly decreased numbers of lymphocytes in the paracortical areas. Analysis of his peripheral blood leukocytes is likely to show normal to elevated numbers of cells expressing surface
 - A. CD4
 - B. CD8
 - C. CD3
 - D. CD2
 - E. CD19

8. Epstein Barr Virus (EBV) is associated with malignant transformation of mature B cells (Burkitt lymphoma) and dendritic cells (Hodgkin disease). In the lymph node, dendritic cells would be found in the
 - A. Paracortical area
 - B. Primary follicle
 - C. Germinal center
 - D. Outer cortex
 - E. Medulla

9. The homing of leukocytes into mucosal lymphoid tissue is initiated by which molecules present on the surface of the leukocyte:
 - A. L-selectins
 - B. E-selectins
 - C. Vascular addressin
 - D. Integrins
 - E. Interleukins

10. T cells that have a low affinity for MHC class I molecules differentiate in the thymus to become which type of cell?
 - A. T-helper 1 cell
 - B. CD8⁺ cytotoxic lymphocyte
 - C. T-helper 2 cell
 - D. Gamma-delta T cell
 - E. Natural killer cell

11. The blood from an 8-year-old boy was analyzed by flow cytometric measurements. The exact number of B cells was counted. Which one of the following cell surface markers was used to identify the B cells in this blood sample?
- A. CD4
 - B. CD8
 - C. CD56
 - D. CD3
 - E. CD19
12. The differentiation of human B cells occurs in the bone marrow. Which one of the following events would occur first in this differentiation sequence?
- A. Immunoglobulin heavy chain rearrangement
 - B. Surface IgM present on the B cell
 - C. Surface IgD and IgM on the B cell
 - D. Cytoplasmic μ chain present in the B cell
 - E. Immunoglobulin light chain rearrangement
13. T-cell maturation occurs in:
- A. The lymph nodes
 - B. The spleen
 - C. A central lymphoidal organ
 - D. A peripheral lymphoidal organ
 - E. Bone marrow
14. Before 1960, children with enlarged thymus glands were frequently irradiated to functionally ablate this organ, whose role was not yet known. Over the lifetime of such individuals, which of the following conditions was likely to develop?
- A. Depressed immune surveillance of tumors
 - B. Depressed primary response to soluble antigens
 - C. Depressed oxygen-dependent killing by neutrophils
 - D. Increased tendency toward atopy
 - E. Increased cellularity of lymph node paracortical areas
15. During the same period of time, tonsillectomies were routinely performed on children with swollen tonsils. This procedure has lost its widespread appeal as we have learned the important role of mucosal-associated lymphoid tissue (MALT) in the protective immune response. The major immunoglobulin produced by MALT is
- A. IgA
 - B. IgD
 - C. IgE
 - D. IgG
 - E. IgM

Answers

1. **Answer: E.** CD3 is known as the “pan-T cell marker.” It is found on all T cells, in association with the T-cell antigen receptor. The CD3 molecular complex is responsible in part for the signal-transducing activity following antigen recognition. CD4 and CD8 mark only subsets of T cells (helper and cytotoxic/suppressor, respectively). CD11b is a C3 receptor that is widely distributed on leukocytes, and CD14 is a marker for monocytes. CD2 would also be a correct answer to this question.
2. **Answer: A.** The HEV are specialized endothelial cells that have homing receptors for lymphocytes only (both T and B cells). Thus, it is via the HEV that T and B cells *enter* the lymph node. Lymphocytes *leave* the lymph node via the efferent lymphatic ducts. Antigen enters via the afferent lymphatics, while antibody exits via the capillary beds. Plasma cells do not normally leave the lymph node.
3. **Answer: E.** Self MHC recognition by T-cell antigen receptor is an essential feature of the T cell recognition process. There are no (or very few) B cells in the thymus. The split between B and T cells occurs in the bone marrow. Foreign antigen is not present in the thymus, and is not needed for the generation of antigen specificity. CD4 and CD8 as distinguishing markers are found mainly on T cells in the medulla, and in the periphery. CD3 is on *all* T cells.
4. **Answer: C.** CD4 and CD8 are coexpressed on T cells that have not yet expressed an antigen receptor, which is a description of early thymocytes in the thymic cortex, a primary lymphoid tissue. Similarly, TdT is only active when a lymphocyte is rearranging its antigen receptor genes, processes that also occur in the thymus (for T cells) or in the bone marrow (for B cells). IL-7 is produced by stromal cells in bone marrow, and plays a role in the initial development of T and B cells. Foreign antigen is *excluded* from primary lymphoid organs, and is only processed and presented to T cells in secondary lymphoid tissue.
5. **Answer: C.** Terminal deoxyribonucleotidyl transferase (TdT) is active before the T or B cell expresses its antigen receptor, and is one of the earliest lymphocyte markers (choice C). Choices A, B, and D appear later, as the antigen receptor is being expressed. MHC class II molecules, such as HLA-DR, are markers of *antigen-activated* T cells, and appear very late in T cell differentiation.
6. **Answer: A.** The thymus *eliminates* (negative selection) T cells that express antigen receptors with too high an affinity for MHC class I or class II molecules (choice B), since such T cells could produce autoimmunity. The thymus stimulates division of T cells with low affinity for MHC molecules (choices C and D), as such recognition is necessary for T cell function.
7. **Answer: E.** The paracortex of the lymph node is the T cell-dependent area. Thus this patient is likely to lack circulating T cells. The T-cell markers will be low, but if any markers are elevated, it will be B-cell markers. CD19 (choice E) is the only B cell-specific marker in the list. All the others are T cell markers. CD4 (choice A) is found on helper T cells, CD8 (choice B) is on cytotoxic T cells, while CD3 (choice D) is found on all T cells.
8. **Answer: A.** Dendritic cells will be found in association with T cells in the paracortical area of a lymph node. Choices B–D describe B cell areas that would not typically have dendritic cells present (except for specialized follicular dendritic cells, which do not express MHC class II and are not able to present antigen to helper T cells). Choice E is the area of the lymph node that will have macrophages and plasma cells.

9. **Answer: A.** L-selectins on leukocytes initiate the interaction with endothelium. E-selectins are complementary molecules present on endothelial cells. Vascular addressins are mucin-like molecules on endothelium that are the ligands for L-selectins. Integrins bind to CAMs of the immunoglobulin superfamily and the extracellular matrix. Interleukins are intracellular communication molecules, and are not involved in adhesion or homing.
10. **Answer: B.** CD8⁺ cytotoxic T lymphocytes are positive-selected from the thymus cells because they have low affinity for MHC class I molecules. If they had possessed high affinity for the MHC class I molecules they would have been eliminated (negative selection) because of the danger of autoimmune disease. Also, cells that had no affinity for MHC class I molecules were negative-selected (eliminated).
11. **Answer: E.** The best markers for identification of B cells are CD19, CD20, and CD21. The CD21 marker is a receptor for EBV (Epstein Barr Virus).
12. **Answer: A.** The first event that occurs in the pro-B cell (progenitor) is the gene rearrangement of the heavy chain. The D gene and J gene recombination event occurs, followed by the V recombination with the D-J region.
13. **Answer: C.** The central lymphoid organs in man are the bone marrow and thymus. The T cell maturation occurs in the thymus after the precursor cells have moved from bone marrow to thymus.
14. **Answer: A.** The only choice on this list that is exclusively a function of T cells is immune surveillance of tumors. Many soluble antigens can elicit an IgM response without T cell help, and intracellular killing by phagocytes is innate, and does not require T cell help absolutely. Atopy (immunologic unresponsiveness) can be expressed in both T and B cell populations, and since paracortical areas are T-cell areas, they would have decreased cellularity in a thymectomized individual.
15. **Answer: A.** Secretory IgA is the major immunoglobulin of the mucosal surfaces. Choices B–E are found predominantly either in the serum or tissue spaces (IgM and IgG). The role of secreted IgD (if any) is not known.

The Immune Response

8

A. Antigen Processing

The first cell involved in the immune response is the **antigen-presenting cell (APC)**. The usual APCs are **macrophages**, **dendritic cells**, **Langerhans cells**, and **B cells**. Except for the B cell, APCs are **nonspecific**. Macrophages can ingest, process, and present the antigen, but the other APCs pinocytose materials. Once the antigen is in the endocytic compartment, the vacuoles fuse with lysosomal granules that contain proteases that cleave the protein antigens into peptide fragments. This is referred to as **antigen processing**. Class II MHC molecules are produced in the endoplasmic reticulum along with class I MHC molecules. Class II molecules should not combine with endogenous peptides, and they are prevented from doing so by an invariant chain. To keep them from picking up the endogenous peptides, they are blocked from this action by an **invariant chain**.

Class II MHC molecules are transported into the same endocytic compartment, where they encounter the processed exogenous peptides. As they move from endoplasmic reticulum through the Golgi complex and into the endocytic compartment, they lose the invariant chain. The peptides then bind into the groove on the MHC class II molecules, and the resultant **peptide–class II complex** is transported to the cell surface. Here this peptide or epitope will be recognized by a **specific T-cell receptor (TCR)** on a specific helper T lymphocyte. It is recognized as **nonself** and will elicit an immune response.

B. Activation

The second cell involved in the immune response is the **CD4⁺ helper T lymphocyte**. When a helper T lymphocyte with an antigen receptor that is specific for the peptide in the groove of the MHC class II molecule comes in contact with the APC, the cells undergo a mutual exchange of **activating signals**. These include secreted proteins (**cytokines**), new cell surface proteins, and DNA-binding molecules that regulate cell division. The cytokines include **interleukin (IL)-4**, which activates B cells, and **interferon- γ** , which activates macrophages. Macrophages in turn secrete **IL-1**, **IL-6**, and **tumor necrosis factor- α** , which activate T cells. The activated T cells express **IL-2 receptors** and secrete **IL-2**, which acts as an autoactivator and stimulates B cells. **Costimulatory molecules** are also important in the process. The T-cell surface molecule **CD28** interacts with a molecule on the APC surface called **B7**. **Adhesion molecules**, such as leukocyte-function-associated antigen (LFA)-1, intercellular adhesion molecule (ICAM)-1, LFA-3, and CD2, play an accessory role in the interaction.

In a Nutshell

APCs

- Macrophages
- Dendritic cells
- Langerhans cells
- B cells

In a Nutshell

Activation

- IL-4 activates B cells
- Gamma interferon activates macrophages
- IL-1 and IL-6 activate T cells

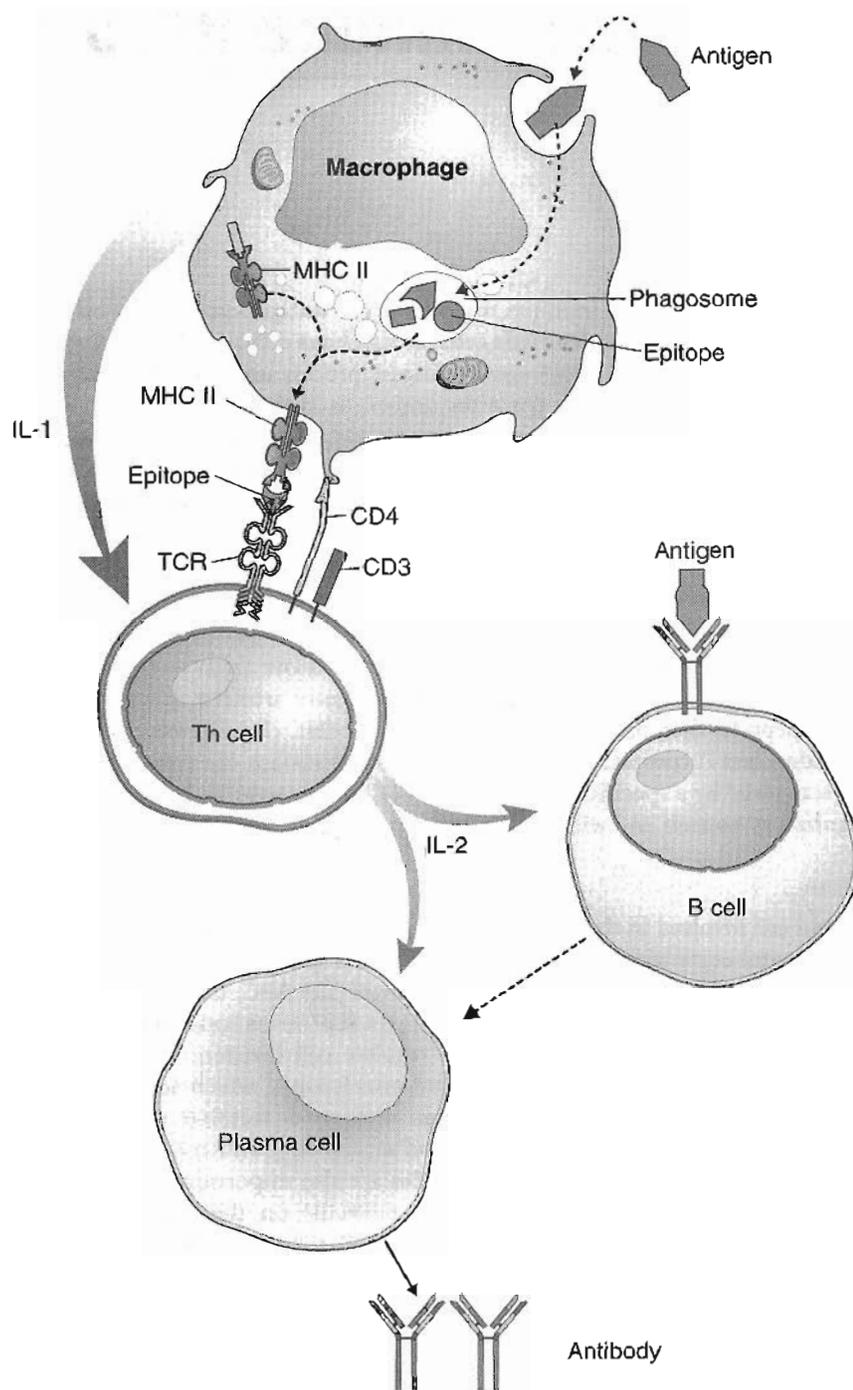


Figure II-8-1. Overview of Activation

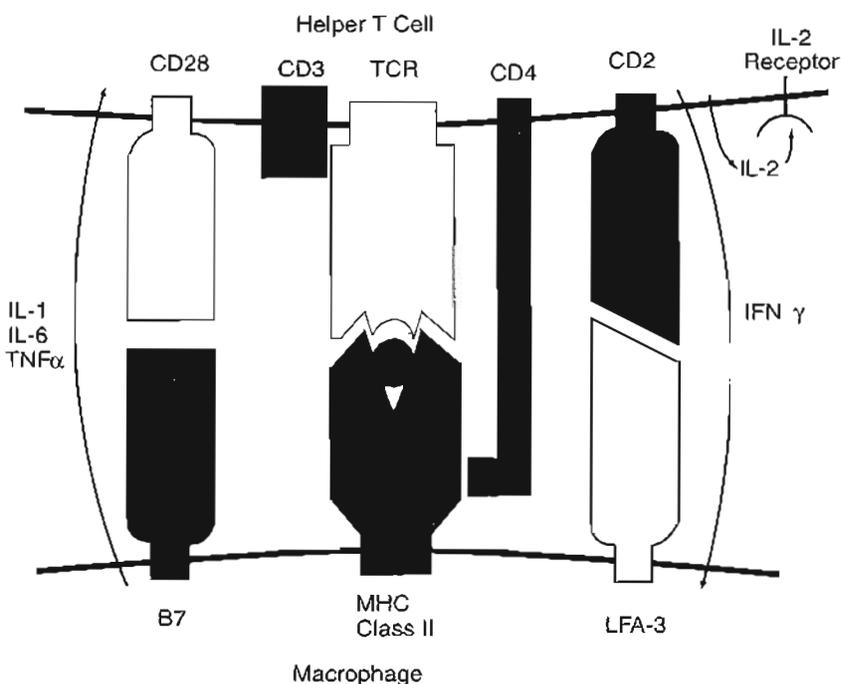


Figure II-8-2. Helper T Cells and Macrophage Adhesion

Note

Legend to figure (left)

- Interaction of antigen with the T-cell receptor (TCR)
- Interaction of CD4 with class II molecule
- Secretion of IL-1 by the APC
- Interaction of IL-2 with the IL-2 receptor
- Interaction of adhesion molecules on APC with the T cell

In a Nutshell

Interaction of antigen with TCR

- CD3 signal transduction
- TCR-CD4 cell reactivating epitope MHC class II molecules
- CD40 ligand: CD40 molecule for class switching
- CD2 with LFA-3
- Interaction of costimulatory CD28 on T cell with B-7 on B cell

C. T Cell–B Cell Cooperation

The third type of cell involved in the immune response is the B cell. Mature B cells bind and internalize antigen by using their antigen receptors, the surface immunoglobulins (IgM and IgD). This binding is antigen specific, and B cells can recognize, bind, and internalize unprocessed antigen.

T cells are quite different from B cells, as they recognize only processed antigenic peptides bound in the groove of an MHC molecule. Like the APCs previously described, the B cells internalize the antigen, process the antigenic peptides, and present them on the B-cell surface bound in the groove of their own MHC class II molecules.

When an activated helper T cell that is specific for the peptide MHC class II complex on the B-cell surface comes along, it can bind to the B cell. This interaction signals the helper T cell to release a wide variety of cytokines that are essential for B-cell division and differentiation to antibody-producing plasma cells. The interleukins from the helper T cell include IL-2, IL-4, and IL-5. CD28 and B7 are important for costimulation. CD40 and the CD40 ligand are required for differentiation and class switching.

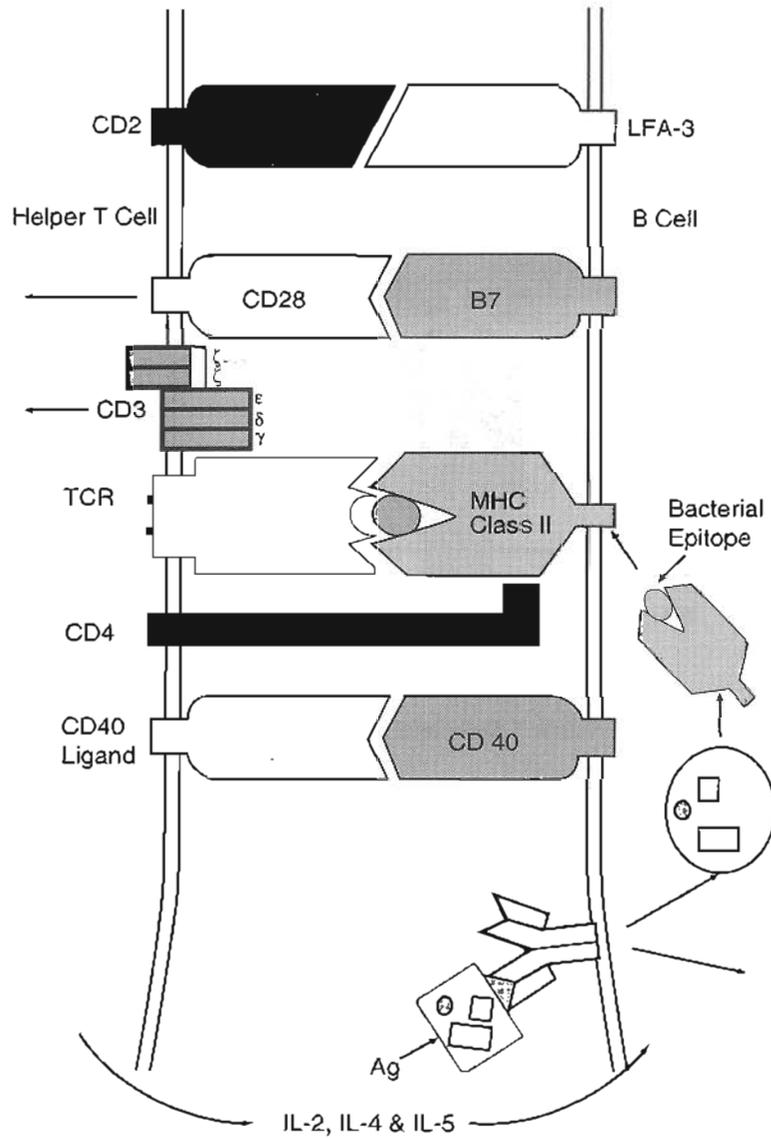


Figure II-8-3. T Cell–B Cell Cooperation

In a Nutshell

Antigen concentration
 As antigen levels decrease during an immune response that successfully eliminates them, there is less of a stimulus for continued proliferation and differentiation of lymphocytes, so immune responses decline. However, small amounts of antigen may persist in the lymph node follicles, which are attached to dendritic cell surfaces, for prolonged periods of time. Other mechanisms are therefore also needed to turn off the response.

After the three cell types involved in the immune response are stimulated, antibody is produced in response. The first exposure to the antigen results in the primary immune response.

D. Clonal Selection

The immune system has no prior knowledge of which antigens it will be challenged with in the future. Yet the B and T cells that are produced generate specific responses to an almost infinite number of antigenic structures. How could this happen? The problem is solved by clonal selection. First, a few rules:

1. **One B cell, one antibody specificity; one T cell, one T-cell antigen-receptor specificity.** This is an essential feature of an immune response. If the B or T cell made more than one antigen-receptor specificity, there would be no way to regulate the immune response.

2. All specificities that will ever be needed are made all the time by **random selection** of hypervariable region structures. Thus, although each B or T cell expresses only one antigen-receptor specificity on its surface, there are enough B and T cells available to represent all the necessary specificities.
3. Antigen selects the cells that have complementary receptors by binding to them, and only those cells undergo clonal expansion (cell division) and differentiation.

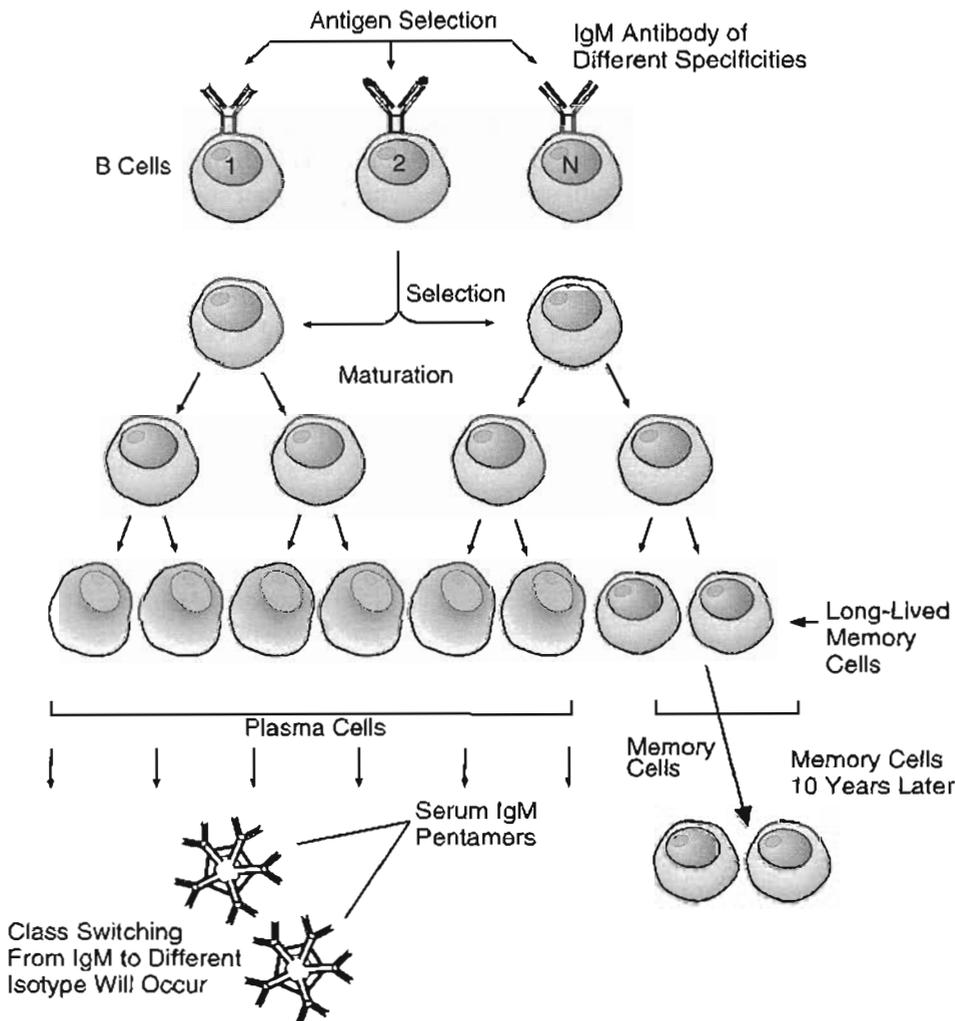


Figure II-8-4. Clonal Selection

E. Secondary Immune Response

A secondary immune response occurs on subsequent exposure to an antigen that has previously been encountered. The secondary response may also be called a memory response or an **anamnestic** response.

There are fundamental differences between primary and secondary immune responses. In a **secondary** response:

1. More antibody is produced.
2. The lag phase is always shorter.
3. Less antigen is required to trigger a response.
4. The antibody isotype (class) differs as follows: In the primary response, IgM always appears first. In the secondary response to an injected (parenteral) antigen, IgG is the predominant antibody; for an antigen administered via a mucosal route (oral, inhaled), IgA is usually the predominant antibody isotype.
5. Antibody affinity changes in the immune response (affinity maturation), as described below.

In an immune response, many B lymphocytes may respond to an antigen. Whereas IgM affinity for some might be high, others will be lower. Nevertheless, all will be stimulated to produce antibody because antigen concentration is relatively high. The average antibody affinity will be low. To differentiate, B cells need constant stimulation by antigen. Later in the immune response, as antigen levels decrease, competition for the diminishing antigen ensures that only the B cells with the highest affinity for antigen will continue to be stimulated; thus, only high-affinity antibody will be produced. This increase in antibody affinity with time occurs for the IgG antibody and is called **affinity maturation**. This process is accelerated by somatic mutation.

In a Nutshell

Primary response

- After first exposure to antigen
- Small amounts of antibody
- Usually IgM antibody
- Memory cells generated

Secondary response

- Subsequent exposure to antigen
- Larger amounts of antibody
- Class switching IgM to IgG, IgA, for example
- Affinity maturation

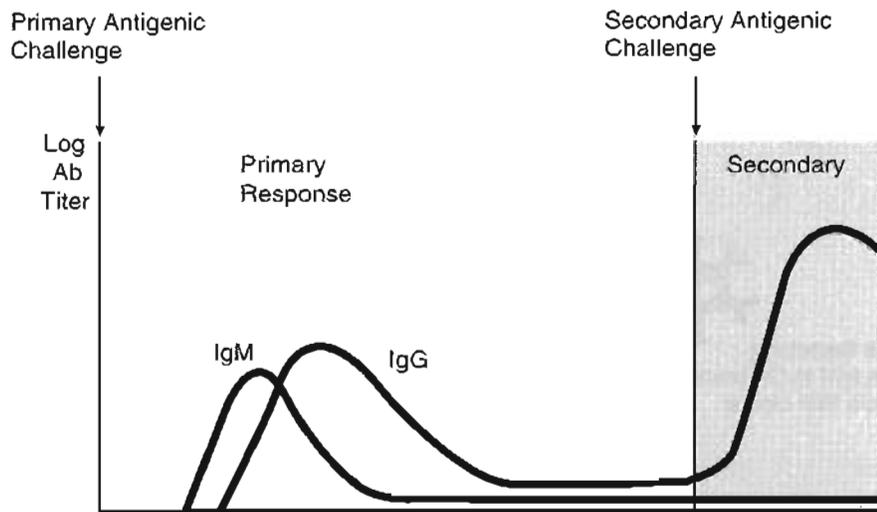


Figure II-8-5. Primary and Secondary Antibody Responses

Table II-8-1. Summary of Humoral and Cellular Events in the Primary Immune Response

	Phase	Antibody Response	Cellular Response
Primary immune response	Lag	None detected in serum	Antigen trapping by macrophage (Mφ) Antigen processing by Mφ Antigen presentation by Mφ Helper T-cell activation T-B cell interaction B-cell activation B-cell clonal expansion B-cell differentiation to plasma cells
	Log	Rapid increase in serum antibody levels	Antigen-stimulated increase in B-cell number, isotype switching, and differentiation into plasma cells
	Plateau	Near constant antibody levels in serum	Depletion of antigen, no further clonal expansion or differentiation of B cells to plasma cells; continued antibody secretion by plasma cells
	Decline	Antibody levels drop because of catabolism and lack of further synthesis	As plasma cells reach end of life span, they die but are not replaced because of absence of antigen stimulation; B and T cells that have begun to differentiate become memory cells

Table II-8-2. Summary of Humoral and Cellular Events in the Secondary Immune Response

	Phase	Antibody Response	Cellular Response
Secondary immune response	Lag (short!)	Little detected in serum	Antigen trapping by macrophage (Mφ) or dendritic cell Antigen processing by Mφ Antigen presentation by Mφ Memory helper T-cell activation T-B cell interaction Memory B-cell activation Memory B-cell clonal expansion Rapid memory B-cell differentiation to plasma cells
	Log	Very rapid increase in serum antibody levels	Antigen-stimulated increase in memory B-cell number; differentiation into plasma cells
	Plateau	Near constant (higher) antibody levels in serum	Depletion of antigen; no further clonal expansion or differentiation into plasma cells; continued antibody secretion by plasma cells
	Decline	Antibody levels drop because of catabolism and lack of further synthesis	As plasma cells reach end of life span, they die but are not replaced because of absence of antigen stimulation; memory B and T cells renewed

Chapter Summary

Antigen is taken up and processed by antigen-presenting cells (APCs)—macrophages, dendritic cells, Langerhans cells, or B cells. B cells recognize and internalize unprocessed antigen via their surface IgM and IgD immunoglobulins. In either case, the processed peptide is complexed to a class II MHC gene product. There it is presented to a specific CD4⁺ T-helper cell. This activates T cells, causing them to express IL-2 receptors and to secrete IL-2. The IL-2 acts in an autocrine fashion to stimulate T-cell proliferation. Interferon- γ is also produced and activates macrophages. The activated macrophages secrete IL-1, IL-6, and tumor necrosis factor.

CD28 and B7 act as costimulatory molecules, and LFA-1, ICAM-1, LFA-3, and CD2 act as adhesins.

T cells further produce IL-4, -5, -6, and -10, which stimulate B cells to proliferate and differentiate.

The primary immune response results from exposure to antigen after the three cell types involved (APCs, helper T cells, and B cells) have successfully worked together to produce antibody. Proof of such cell cooperation may be obtained by the use of haptens. Haptens are too small to be immunogenic. However, when covalently bound to a carrier protein, helper T cells can activate B cells to produce antibody against the hapten.

To produce a secondary response to a hapten alone (e.g., against penicillin), the B cells must have been exposed to the hapten twice, T cells must have been exposed to the carrier twice, and the hapten and carrier must have been covalently coupled.

A specific B or T cell responds to only one specific antigen, yet responses can be made against an almost infinite number of antigenic substances. These are then selected and amplified by clonal selection.

The secondary response usually results in the production of more antibody, using less antigen, and is characterized by having a shorter lag phase and primary production of IgG rather than IgM (unless antigen is administered by a mucosal route, in which case IgA is the predominant product).

Affinity maturation occurs with time as the most effective idiotypes are selected.

Review Questions

- The macrophages are involved in many different immune activities. What is the role of the macrophage during the formation of antibody?
 - Making antibody
 - Delayed hypersensitivity reactions
 - Lyse virus-infected cells
 - Process antigen and present it to T-helper CD4 cells
 - Activate cytotoxic CD8 T cells
- Which one of the following is the most important costimulatory signal provided to a T cell from an antigen-presenting cell?
 - ICAM-1 interacting with LFA-1
 - B7 molecules interacting with CD28
 - B7 molecules interacting with LFA-1
 - LFA-3 interacting with CD28
 - MHC class II interacting with T cell receptor

3. Which one of the following is correct about the secondary immune response?
 - A. Class switching from IgM to IgG will occur and the IgG antibody titer will rise.
 - B. The IgM titer will rise above the IgG level for a period of 6 months.
 - C. The IgG titer will continue to fall and IgA titer will increase due to class switching from IgG to IgA.
 - D. The IgM and IgG titers will continue to both rise during the secondary immune response.
 - E. The IgM titer will continue to increase above its level of the primary immune response.
4. In the process of B cell differentiation in the bone marrow and movement of the B cells to the lymph node, which event would be the last (latest) in the process?
 - A. Class switching
 - B. Presence of surface IgM
 - C. Presence of surface IgM and IgD
 - D. Light chain gene rearrangement
 - E. Heavy chain gene rearrangement
5. Antigen recognition by the helper T-cell antigen receptor occurs after
 - A. Interaction with the CD8 molecule on the T cell
 - B. Peptide binding to the HLA-DQ molecule
 - C. Carbohydrate binding to the HLA-B27 molecule
 - D. Carbohydrate binding to the HLA-DP molecule
 - E. Peptide binding to the HLA-B27 molecule

Answers

1. **Answer: D.** The macrophage has the role of phagocytosis of exogenous antigen (i.e., a bacteria) and degrading this antigen into small epitopes and presenting them on MHC class II molecules on its surface to CD4 T-helper cells.
2. **Answer: B.** The B7 molecule on the cell surface of the antigen-presenting cell reacts with the CD28 molecule on the T-cell surface for maximum costimulatory signals.
3. **Answer: A.** In the primary immune response the first immunoglobulin formed is IgM and for a short period of time its level will be high, but the B cell class switches to IgG and this titer rises. On secondary exposure to the antigen, the memory cells start dividing and produce large volumes of IgG immunoglobulin and the clone size continues to increase.
4. **Answer: A.** Choices B, C, D, and E occur while the B cell is still developing in the bone marrow before it ever reaches the lymph node. The class switching occurs after the B cell IgM has reacted with its specific epitope in the lymph node. Thus the antigen that it is specific for is present and it reacts with this antigen.
5. **Answer: B.** Helper T cells are CD4 positive (making choice A wrong) and recognize peptides (not carbohydrate determinants, as in choices C and D) bound to MHC class II molecules (choice B). HLA-B27 (choices C and E) is an MHC class I molecule.

T-Cell Subsets and Interleukins

A. Helper T-Cell Subsets

In recent years helper T (Th) cells have been divided into three functional subsets, recognized by the cytokines they secrete.

Th0 cells differentiate into one of two different classes of Th cells. Th1 subset cells are responsible for initiating **delayed-type hypersensitivity** reactions and helping the development of CD8⁺ **cytotoxic lymphocytes**. Th2 subset cells help produce antibody by stimulating B-cell division and differentiation.

The Th1 and Th2 subsets have cross-regulatory activities. Interleukin (IL)-4 and IL-10 secreted by Th2 inhibit the Th1 response, and interferon- γ from Th1 inhibits Th2 activity.

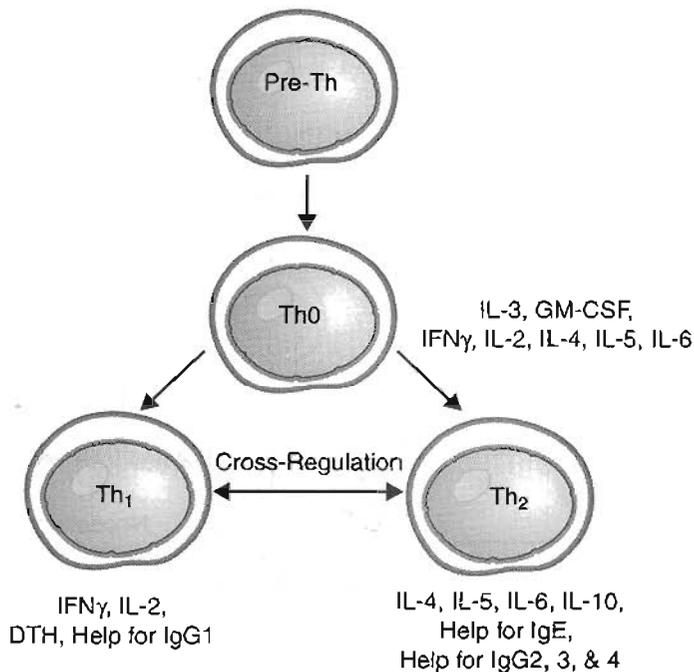


Figure II-9-1. Subsets of Th Cells

In a Nutshell

- Th1 subset regulates
 - DTH cells
 - CH8⁺ cells
- Th2 subset regulates
 - B cells

1. Th0

- a. Secretes IL-3 and GM-CSF (granulocyte macrophage–colony stimulating factor)
- b. Secretes interferon- γ and IL-2
- c. Secretes IL-4, IL-5, and IL-6

2. Th1
 - a. Proliferates Th1 and Tc (cytotoxic T)
 - b. Secretes IL-2 and interferon- γ
 - c. Secretes tumor necrosis factor (TNF)- β , IL-3, and GM-CSF
 - d. Downregulates Th2 responses via interferon- γ
3. Th2
 - a. Develops antibody secretion
 - b. Class switching (i.e., IgE)
 - c. Proliferates eosinophil precursors
 - d. Proliferates mast cell precursors
 - e. Secretes IL-4, IL-5, IL-6, and IL-10
 - f. Secretes IL-3 and GM-CSF
 - g. Downregulates Th1 responses via IL-10

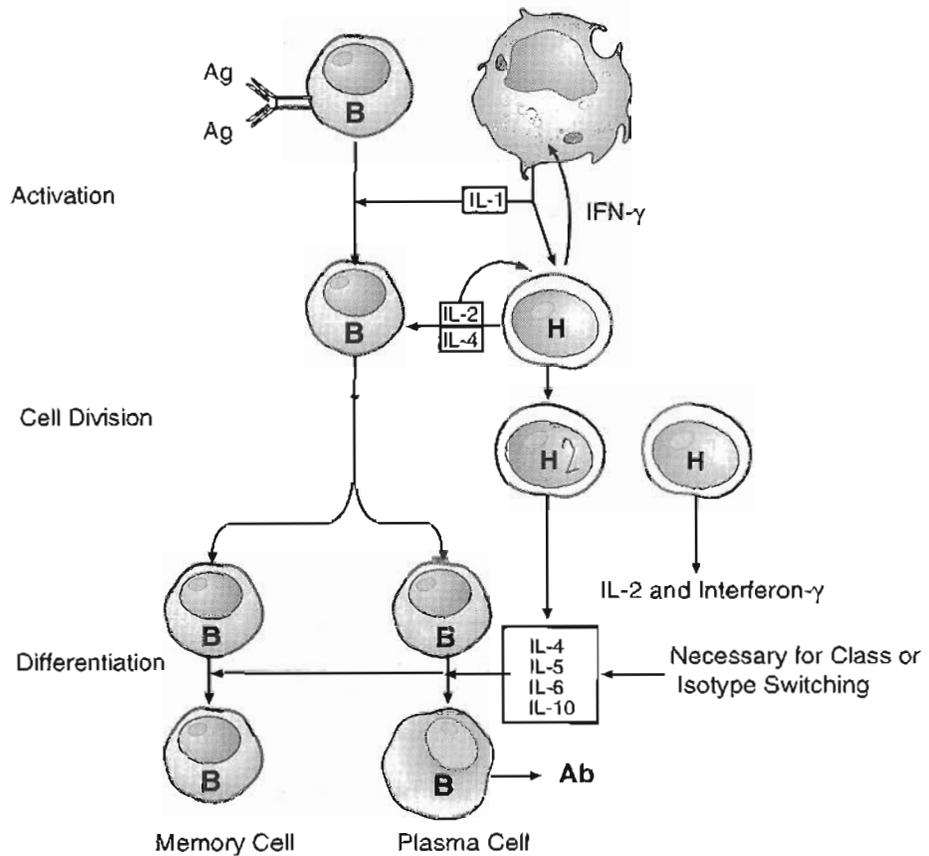


Figure II-9-2. Cytokine-Driven B-Cell Differentiation

B. Interleukins (Cytokines)

1. IL-1
 - a. Produced by macrophages
 - b. Stimulates IL-2 secretion
 - c. Pyrogenic (fever-inducing)
2. IL-2
 - a. Produced by activated Th1, natural killer (NK) cells, and Tc
 - b. Stimulates B cells
 - c. A T-cell growth factor
3. IL-3
 - a. Secreted by activated T cells
 - b. Stimulates bone marrow stem cells
4. IL-4
 - a. Secreted by Th2 cells and mast cells
 - b. Stimulates B cells
 - c. Increases IgG and IgE
5. IL-5
 - a. Secreted by Th2 cells
 - b. Promotes B-cell proliferation
 - c. Increases IgA synthesis
 - d. Increases eosinophils
6. IL-6
 - a. Stimulates production of acute-phase proteins
 - b. Stimulates B cells
7. IL-7
 - a. Stimulates pre-B and pre-T cells
8. IL-8
 - a. Produced by macrophages
 - b. Stimulates chemotaxis of neutrophils
 - c. Stimulates adhesion of neutrophils
9. IL-10
 - a. Downregulates cell-mediated immunity (CMI)
 - b. Inhibits cytokine release from macrophages
10. IL-12
 - a. Activates NK cells
 - b. Induces Th0 to Th1
 - c. Increases numbers of cytotoxic T lymphocyte and delayed-type hypersensitivity cells
11. TNF- α
 - a. Produced by cytotoxic T cells
 - b. Produced by macrophages
 - c. Increases MHC class I expression
 - d. Increases inflammatory processes

12. TNF- β
 - a. Produced by cytotoxic T cells
 - b. Increases MHC class I expression
 - c. Increases inflammatory processes
13. Interferon- γ
 - a. Stimulates macrophages
 - b. Stimulates NK cells
 - c. Inhibits Th2 cells
 - d. Produced by Th1 cells

Clinical Correlate

- Polyclonal mitogens are used to assess lymphocyte function in patients by stimulation of lymphocyte subsets.
- Heterophile antibodies in infectious mononucleosis arise as a result of B-cell polyclonal activation (mono-spot test).

C. Polyclonal Lymphocyte Activation

Specific antigen stimulates only a small portion of B and T cells to divide and differentiate. In the study of lymphocyte function, however, some molecules have been discovered that stimulate a large proportion of cells without antigen specificity. These have proven to be very useful in the understanding of immune function.

1. Polyclonal activators

- a. **Lectin mitogens:** Some plant lectins stimulate T-cell division. The best known of these are **phytohemagglutinin (PHA)** and **concanavalin A (CON A)**. **Pokeweed mitogen (PWM)** is also a polyclonal B-cell stimulator.
- b. **Epstein-Barr virus (EBV)** is a polyclonal B-cell activator.
- c. **Endotoxin (LPS)** is a polyclonal B-cell and T-cell stimulator.
- d. **Superantigens** are a class of molecules that are polyclonal activators of Th cells. They function by cross-linking the **variable domain** of the TCR β chain to the **MHC class II molecule** on an antigen-presenting cell (APC). Even though no specific peptide antigen is recognized by the TCR in this encounter, the nonspecific, tight binding induced by the superantigen will trigger T-cell activation. Two well-characterized superantigens are **toxic shock syndrome toxin (TSST-1)** and the **streptococcal exotoxins**.

In a Nutshell

- Superantigens are mitogenic for CD4⁺ cells bearing particular V-region β chain TCRs.
- Each superantigen stimulates a particular T-cell subset.
- Activation causes release of large amounts of cytokines.
- Examples include TSST-1, staphylococcal enterotoxins, and streptococcal pyrogenic exotoxins A-C.

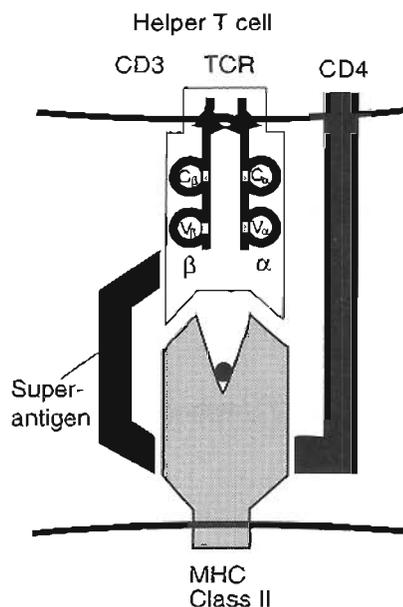


Figure II-9-3. Superantigen Activation of a Macrophage

D. Thymus-Dependent versus Thymus-Independent Antibody Response

The thymus-dependent response to complex antigens described previously produces the memory response characterized by high levels of high-affinity IgG antibodies. Antigens that activate B cells in the absence of significant T-cell help are called **T-independent antigens**. These antigens primarily induce IgM, and the titer of the IgM does not increase on repeated exposure. T-independent antigens are generally large polymers with repeating antigenic epitopes, such as **polysaccharides**. The multiple, identical epitopes can crosslink surface immunoglobulin on B cells and stimulate proliferation and IgM production.

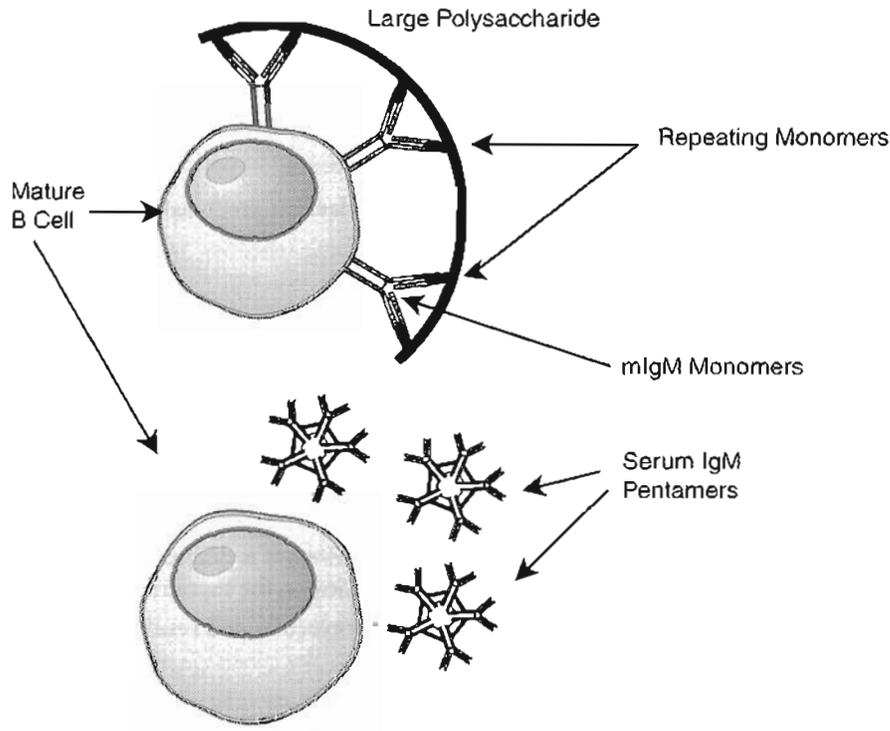


Figure II-9-4. B-Cell Activation by Thymus-Independent Antigens

In a Nutshell

Thymus-independent antigens

- IgM is the only immunoglobulin
- They have no memory
- There is no CD40-CD40 ligand interaction
- Antigens are usually polysaccharide

Note

T-cell help can be delivered only if the antigen has a determinant that can be recognized by the T cell. Because T-cell receptors recognize peptide epitopes in the groove of an MHC molecule, antigens that have no peptides cannot elicit T-cell help.

Chapter Summary

The naive helper T cell, Th0, is capable of producing all T-cell cytokines. It may differentiate into either a Th1 or a Th2 cell. The Th1 cell assists cell-mediated immunity by producing interferon- γ and by activating macrophages and cytotoxic CD8 cells. The Th2 cell produces IL-4, IL-5, and IL-6, provoking plasma cell differentiation from B cells. There is also cross-regulation and mutual inhibition between Th1 and Th2 cells mediated by IL-10 and IFN- γ .

Specific antigens stimulate a small specific portion of the B and T cells to divide and differentiate. However, there are some compounds that act as polyclonal activators, stimulating a large proportion of cells without having antigenic specificity. These include some plant lectins, the Epstein-Barr virus, endotoxin (LPS), and the superantigens. Particularly notable among the latter are the streptococcal enterotoxins and toxic shock syndrome toxin.

Some antigens activate B cells in the absence of significant T-cell help. These are usually polysaccharides with repeating antigenic epitopes that cannot elicit T-cell help because T-cell receptors recognize only peptides associated with an MHC II molecule. These antigens induce primarily IgM with a titer that does not increase with repeated exposure.

Review Questions

1. Which one of the following is one of the major interleukins produced by CD4⁺ T-helper 1 lymphocytes?
 - A. IL-1
 - B. IL-2
 - C. IL-4
 - D. IL-8
 - E. IL-6
2. Which one of the following is true of thymus-independent antibody response?
 - A. It is like the thymus-dependent antibody response but is different because it did not have to go through positive and negative selection in the thymus.
 - B. It is a response that is made to large protein molecules and results in only IgG production.
 - C. It results in a greater than normal number of memory cells produced.
 - D. It results in only the production of IgM molecules with no memory cells.
 - E. It is an example of where T-suppressor cells are presented antigen from the macrophages instead of T-helper cells being presented the antigen.
3. Which one of the following best describes a superantigen?
 - A. Bacterial product that binds to beta chain of TCR and MHC class II molecules of APC stimulating T-cell activation.
 - B. Bacterial product that binds to CD4⁺ molecule causing T-cell activation
 - C. Bacterial product that binds to B7 and CD28 costimulatory molecules
 - D. Bacterial product that stimulates massive amounts of IgG antibody due to its large size
 - E. Bacterial product that is presented by macrophages to larger than normal number of T helper CD4⁺ lymphocytes.

4. A 36-year-old farmer has been exposed to poison ivy on several different occasions and he usually gets very severe skin lesions. Since this is a delayed hypersensitivity type reaction, a flow cytometric measurement was made for T cells. The values for these cells were within normal range. The cytokines were then analyzed in a serum sample by specific ELISA assays. Which one of the following would inhibit T helper 1 cells and thus increase his inability to perform this delayed hypersensitivity reaction in a normal fashion?
- A. IL-10
 - B. IL-8
 - C. IL-4
 - D. Gamma interferon
 - E. IL-2
5. When a Th0 (T helper 0) cell matures in the presence of IL-4 and IL-5 they become
- A. Cytotoxic T cell
 - B. Suppressor T cell
 - C. T-helper 1 cell
 - D. T-helper 2 cell
 - E. Thymic T cell
6. Which of the following cytokines are associated with the development of cell-mediated immunity?
- A. IL-4
 - B. IL-5 and IL-10
 - C. Gamma interferon and IL-12
 - D. IL-4 and IL-3
 - E. IL-10 and IL-3

For Questions 7–12, match the description with the cytokine that best matches it from the list below.

Interferon γ
Interleukin 2
Interleukin 1
Interferon β
Interleukin 5
Interleukin 8

- 7. Produced by a variety of cells; main function is to inhibit viral replication.
- 8. Produced by helper T cells; main role is in antibody class switching and B cell differentiation.
- 9. Produced mainly by macrophages; activates helper T cells.
- 10. Produced by helper T cells; activates macrophages (and B cells).
- 11. Growth factor triggering T and B cell division.
- 12. Chemotactic for neutrophils.

Match the type of antigen with its mechanism of cell activation.

- A. T-dependent antigen
 - B. T-independent antigen
 - C. Mitogen
 - D. Immunogen
 - E. Superantigen
13. binds variable-beta region of TCR to MHC class II.
 14. plant lectin crosslinks sugars on cell surface.
 15. crosslinks immunoglobulin receptors causing capping.

Answers

1. Answer: B. This IL-2 stimulates NK cells, and CD8⁺ T cytotoxic lymphocytes. It also combines with its own IL-2 receptors and stimulates itself. It is involved in down regulating CD4⁺ T-helper 2 lymphocytes.
2. Answer: D. A thymus-independent antibody response is one where the B cells response to large molecular weight carbohydrate molecules has a large number of repeating monomers. The antibody is only IgM and there is no memory cells produced in this response.
3. Answer: A. The superantigen such as TSST-1 or Staphylococcal enterotoxin crosslinks the variable domain of the TCR beta chain to the MHC class II molecule and specifically induces massive T cell activation.
4. Answer: A. The IL-10 cytokine is produced by T-helper 2 cells and inhibits T-helper 1 cells. The cytokines from T-helper 2 cells stimulate B cells to produce antibody. The cytokines from T-helper 1 cells stimulate cell-mediated immunity and delayed hypersensitivity.
5. Answer: D. The IL-4 and IL-5 interleukins stimulate the T cells that are more involved in stimulating future antibody production. These T cells that are involved in stimulating antibody production are T-helper 2 cells.
6. Answer: C. Gamma interferon from T-helper 1 CD4⁺ lymphocytes and IL-12 from macrophages are major stimulators of the development of a cell-mediated immune response to intracellular organisms such as tuberculosis, leishmaniasis, histoplasmosis and many other organisms.
7. Answer: D.
8. Answer: E.
9. Answer: C.
10. Answer: A.
11. Answer: B.

12. Answer: E.

T-helper cells are the major source for the synthesis of IL-2, IFN- γ , and IL-5. IL-2 is mainly involved in cell division; it was originally called "T cell growth factor". Interferon- γ has only weak anti-viral properties; its main function is in immune regulation, particularly as a macrophage-activating factor. In contrast, interferon- β is produced by a wide variety of cell types (i.e., fibroblasts) and is a potent inhibitor of viral replication. IL-5 is mainly involved in the differentiation of IgA-secreting B cells. IL-1 is a major product of macrophages (a monokine) and has a long list of biological activities, including the activation of T and B cells. Several of these are chemotactic, but the best answer is IL-8. Its main action is as a chemotactic factor. IL-1 and IFN- γ have many other roles.

13. Answer: E. Superantigen

14. Answer: C. Mitogen

15. Answer: B. T-independent antigen

Cell-Mediated Immunity

10

A. Role of Cell-Mediated Immunity in Host Defense

Host defenses against **extracellular** infectious agents, such as extracellular bacteria, protozoa, worms, fungi, and viruses (before they infect a cell), are **mediated by antibody, complement, and phagocytes**. However, once an infectious agent invades a host cell, these defenses are virtually useless. Recovery from **intracellular infection** requires an entirely different defense system: **cell-mediated immunity (CMI)**.

In CMI, the first goal of the immune system is to destroy the intracellular infectious agent by killing the host cell that harbors it. In many cases, this will also kill the pathogen, which may require the host cell for its own reproduction. Virus infection virtually **always** requires the induction of cell-mediated cytotoxicity, mediated by **cytotoxic T cells, natural killer (NK) cells, or activated macrophages**, for recovery. The same cytotoxic mechanisms may be effective against (most) tumor cells.

In the special case where intracellular bacteria, parasites, or fungi infect a macrophage, **activation** of macrophage cellular metabolism by **helper T cell (Th1)-derived cytokines** may be sufficient for destruction of the pathogen. This results in the process called **delayed-type hypersensitivity (DTH)**, which is a very important protective mechanism when directed against an intracellular pathogen.

B. Delayed-Type Hypersensitivity (DTH)

Macrophages are often **targets** for infection with intracellular pathogens. Examples are infections with *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Toxoplasma gondii*, and *Leishmania* species. The release of cytokines, particularly **interferon (IFN)- γ** , from fully differentiated Th1 cells (**T_{DTH}** cells) activates these macrophages, allowing them to kill any ingested organisms. **Activated macrophages** can also kill some **abnormal (infected or tumor-transformed) host cells**. They are nonspecific and, unlike the lymphocytes, do not kill target cells by releasing pore-forming molecules (perforin). Instead, they use **oxygen metabolites (H₂O₂, O₂⁻)**, **nitric oxide (NO)**, **tumor necrosis factor (TNF)- α** , and enzymes to produce cytotoxicity. **CD14**, the endotoxin receptor, is the best marker for macrophages.

C. Induction of Cell-Mediated Immunity (CMI)

Like antibody production in humoral immunity, CMI requires T-cell help. Th1 cells must first **recognize antigen** presented by an **MHC class II molecule** on an antigen-presenting cell (**dendritic cell or macrophage**). The activated Th1 cell secretes **IL-2** and **IFN- γ** , which activate the cytotoxic effector cells (see list below). Even when the effector cell is not antigen-specific (macrophage or NK cell), the essential trigger for the response (activation of the Th1 cell) is antigen-specific.

D. Effector Cells in CMI

1. **Cytotoxic T-lymphocyte (CTL)** recognition requires recognition of both the antigenic peptide being synthesized by the infected cell and the **MHC class I molecule** to which it is bound. Thus, killing by CTLs is said to be both **antigen-specific** and **MHC-restricted**.

In a Nutshell

Defense against extracellular organisms

- Antibody
- Complement
- Phagocytosis

Defense against intracellular organisms

- DTH
- CD8⁺ cytotoxic cells
- NK cells
- ADCC cells
- Activated macrophages

Cytotoxic T cells express the CD8 molecule, which helps them recognize the MHC class I molecule on the infected target cell. Clonal expansion and activation of the antigen-specific CTL precursor cells by IL-2 derived from antigen-stimulated Th1 cells is essential for the generation of the cytotoxic effector CTL, which actually does the killing. How do cytotoxic T cells kill their targets? They use:

- a. **Perforin:** Fully activated (effector) CTLs contain granules that are released (exocytosed) when the CTL contacts a target cell. The granules contain perforin, a membrane-puncturing, pore-forming molecule that polymerizes in the membrane of the target cell. These pores damage the cell membrane, causing lysis.
 - b. **Granzymes:** The granules also contain enzymes that damage the target cell, possibly by passing through the perforin pores into the target cell.
 - c. **Cytokines:** IFN- γ and TNF- β are secreted by CTLs and act together on target cells that express receptors for them. These cytokines induce metabolic changes in the target cell, inducing it to produce enzymes that lead to its own death. This process is called apoptosis and often ends with the target cell digesting its own nucleus.
 - d. **Fas and Fas ligand:** Activated, effector CTLs express a surface molecule called Fas ligand. When the CTL specifically binds antigen presented by the MHC class I molecule on the target cell, Fas ligand interacts with the Fas molecule on the target cell surface, inducing apoptosis.
2. **NK cells.** Morphologically, NK cells are **large granular lymphocytes (LGLs)**. They are **non-T, non-B lymphocytes** that lack the marker molecules found on other lymphocytes. They lack CD3, CD4, CD8, and CD19 molecules, and do not make immunoglobulin or T-cell antigen receptors. Thus they are not antigen-specific, and MHC class I actually inhibits their killing function. The best markers for NK cells are CD16 and CD56. They kill by releasing **perforin, granzymes, and cytokines (IFN- γ and TNF- α)**, in a manner similar to T cells.
 3. **Lymphokine-activated killer (LAK) cells.** These are also LGLs that are **non-T, non-B lymphocytes**. They are simply an activated form of NK cells that have been stimulated in vitro by IL-2 and IFN- γ . They have been used in treatment of some tumors, and kill some tumor cells more effectively than NK cells. Just like NK cells, they are nonspecific.
 4. **NK-ADCC.** NK cells are also the major cell type that carries out **antibody-dependent cellular cytotoxicity (ADCC)**. NK cells have **Fc receptors** that recognize the **Fc domains of IgG**. When IgG is bound to foreign antigen on the surface of an infected cell, NK cells can recognize it, bind, and deliver a lethal hit. NK cells that mediate ADCC use their **Fc receptors (the CD16 molecules)** to **identify target cells**. ADCC can also be carried out by other cells that express Fc receptors and have the machinery to kill targets. Thus neutrophils, eosinophils, monocytes, and macrophages also carry out ADCC, although they use different effector molecules than NK cells.

In a Nutshell

CMI effector cells

- CD8 cytotoxic T cells
- NK cells
- LAK cells
- NK-ADCC cells

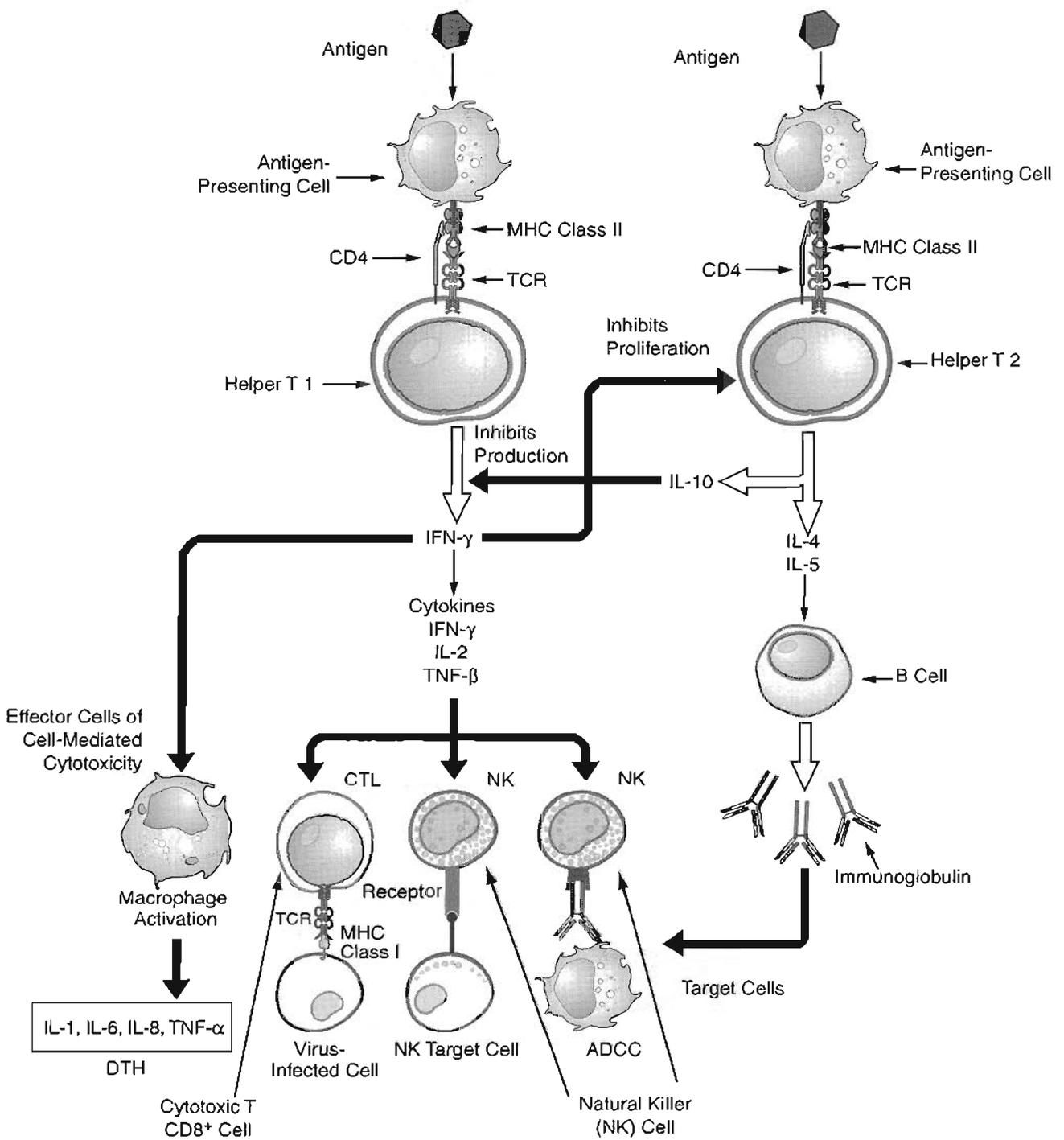


Figure II-10-1. Overview of Cell-Mediated Immunity

Table II-10-1. Effector Cells in Cell-Mediated Immunity

Effector Cell	CD Markers	Antigen Recognition	MHC Recognition Required for Killing	Effector Molecules
CTL	TCR, CD3, CD8, CD2	Specific TCR	Yes, class I	Perforin, cytokines (TNF- β , IFN- γ)
NK cell ADCC	CD16, CD56, CD2	Specific IgG	No	Perforin, cytokines (TNF- α , IFN- γ)
NK cell	CD16, CD56, CD2	Nonspecific	No	Perforin, cytokines (TNF- α , IFN- γ)
LAK cell	CD16, CD56, CD2	Nonspecific	No	Perforin, cytokines (TNF- α , IFN- γ)
Macrophage	CD14	Nonspecific	No	TNF- α , enzymes, NO, oxygen radicals

In a Nutshell

Cytotoxic T Cells

- CD8⁺, MHC class I restricted
- Lyse target cells that are seen as foreign, such as:
 - Tumor cells
 - Virus-infected cells
 - Grafts
- Lysis occurs via release of perforins and enzymes from granules

Table II-10-2. Major Cytotoxic Products of Activated Cytotoxic Cells

Product	Effect on Target Cell
Perforins	Polymerize in the cell membrane to form polyperforin channels that allow cytosol out of and toxic molecules into the cell
Serine proteases	Degrade proteins in cell membrane
Nucleases	Degrade DNA and RNA in the cell
TNF- β	Depresses protein synthesis; causes production of toxic free radicals
Fas ligand	Initiates apoptosis

Chapter Summary

Defense against intracellular pathogens is provided by cell-mediated immunity (CMI). The goal of CMI is to kill the host cell and hopefully the foreign agent it hosts.

Delayed-type hypersensitivity is a manifestation of one type of CMI. Release of cytokines, particularly interferon- γ , from Th1 cells activates macrophages, permitting them to kill any cell internalized. The active macrophages generate H₂O₂, \cdot O₂⁻, nitric oxide, and tumor necrosis factor (TNF)- α to accomplish this killing.

Cytotoxic T lymphocytes and natural killer (NK) cells also carry out CMI when activated by Th2-cell-generated cytokines. Cytotoxic T lymphocytes are both antigen specific and MHC restricted, and they express the CD8 marker. They use perforin and granzymes to directly kill, and they secrete interferon- γ , TNF- β , and Fas ligand to induce apoptosis in the target cell.

NK cells are large granular, non-T, non-B lymphocytes. NK cells are not antigen specific, but they also kill by using perforin, granzymes, interferon- γ , and TNF- β .

Lymphokine-activated killer cells are NK cells activated in vitro with IL-2 or interferon- γ .

Antibody-dependent cellular cytotoxicity (ADCC) is carried out by NK-ADCC cells and other cell types that use their Fc receptors (CD16) to recognize foreign antigens via IgG bound to their surfaces.

Review Questions

1. Which immune effector mechanism does not utilize pore-forming molecules to damage target cell membranes?
 - A. NK cells
 - B. Cytotoxic T cells
 - C. Activated macrophages
 - D. K cells (mediating ADCC)
 - E. Complement
2. The Th1 subset of helper T cells
 - A. Mediates cellular, as opposed to humoral, immunity
 - B. Expresses CD3, CD2, and CD8
 - C. Secretes IL-5, IL-6, and IL-10
 - D. Stimulates Th2 T cells
 - E. Can be identified by the expression of CD14
3. The T lymphocytes from a patient with a deficiency of b2-microglobulin in bone marrow stem cells would be unable to
 - A. Synthesize IL-2 or interferon gamma
 - B. Express CD3
 - C. Express the T-cell antigen receptor
 - D. Recognize antigenic peptides presented by a B cell
 - E. Specifically kill virus-infected macrophages
4. All of the following are true of antibody-dependent cellular cytotoxicity (ADCC) except
 - A. It can be mediated by macrophages.
 - B. It requires effector cell recognition of specific antigen on MHC class I.
 - C. It can involve pore formation perforin.
 - D. It can be mediated by large granular lymphocytes.
 - E. It requires binding of antibody to Fc receptors on the effector cell.
5. In antibody-dependent cell-mediated cytotoxicity (ADCC) the specificity of killing of target cells by the effectors is a function of
 - A. Large granular lymphocytes
 - B. Natural killer cells
 - C. Macrophages
 - D. Cytotoxic T lymphocytes
 - E. IgG

6. There is evidence that the immunological pathway that distinguishes the selection between the two polar forms of leprosy depends on the initial means of antigen presentation as well as individual differences in response. If early events of antigen recognition elicit production of IL-4, IL-5, and IL-10, lepromatous leprosy is more likely to result, with the outcome of failure to mount a protective delayed-type hypersensitivity response. What differential characteristic of the lepromatous form is predicted based on the fact of overproduction of IL-4, IL-5, and IL-10 in lepromatous lesions?
 - A. Hypergammaglobulinemia
 - B. Autoimmunity
 - C. Inflammation
 - D. Granuloma formation

7. Which one of the following would be considered cellular immunity, but antibody molecules would still be involved?
 - A. ADCC killing of a viral-infected cell.
 - B. T cytotoxic CD8⁺ killing of a viral-infected cell.
 - C. NK killing of a viral-infected cell.
 - D. NK killing of a tumor cell.
 - E. Macrophage activation and killing of intracellular TB

8. A 42-year-old Nigerian man who is in the United States visiting with his brother comes into the hospital clinic. He complains of several months of weight loss, night sweats, mild sputum production and sometimes he spits up blood. You run a PPD skin test and the results were positive. This positive tuberculin test indicates which one of the following?
 - A. A humoral immune response to TB is positive.
 - B. A cell-mediated immune response has occurred.
 - C. The B cell system is functional.
 - D. The phagocytic neutrophilic system is functional
 - E. The B and T cell systems are functional.

9. A 56-year-old homeless alcoholic man is brought to the ER febrile after a difficult night during which his coughing kept everyone at the shelter awake. On arrival his pulse is rapid and his breathing labored with diffuse rales. Endotracheal aspirates produce a mucopurulent discharge containing numerous gram-positive cocci in chains. His serum contains high titers of IgM antibodies specific for the polysaccharide capsule of *Streptococcus pneumoniae*. The effector mechanism most likely to act in concert with this early IgM production to clear infection is
 - A. Natural killer cells
 - B. ADCC (antibody-dependent cell-mediated cytotoxicity)
 - C. Cytotoxic T lymphocytes
 - D. Complement-mediated opsonization
 - E. LAK cells

10. There are several ways that tumors can escape immune surveillance. Loss of which class of molecules on the surface of a tumor cell target would result in loss of susceptibility to killing by CD8⁺ cells?
- A. β 2 microglobulin
 - B. CD8
 - C. MHC class I
 - D. MHC class II
 - E. CD3
11. Human infections with *Mycobacterium leprae* express a spectrum of clinical presentations depending on the extent and expression of their immune response to the intracellular organism. On one end of the spectrum, patients with tuberculoid leprosy produce an effective DTH response, which is successful at killing the intracellular organisms and, unfortunately, produces tissue damage due to his immune response. Patients with tuberculoid leprosy have granulomas that have elevated amounts of IL-2, IFN- γ , and TNF- β . The immune cell responsible for this latter pattern is the
- A. Epithelioid cell
 - B. Macrophage
 - C. Th1 cell
 - D. Th2 cell
 - E. CTL
12. Your patient has a severe case of poison oak contracted while hiking this past weekend. She asks what causes it. Which one of the following is most appropriate to say?
- A. Plant oils activate the alternate pathways of complement that causes inflammation in the skin.
 - B. Plant oils combine with skin proteins and stimulate helper T cells to react against the oil-protein complex.
 - C. You form antibodies against plant oils that crossreact with your skin cells.
 - D. Plant oils cause the release of enzymes, e.g., hyaluronidase, from phagocytes, which degrade subcutaneous tissue.
13. Increased susceptibility to viral and fungal infections would be expected with a deficiency of which one of the following cells?
- A. B cells
 - B. T cells
 - C. Neutrophils
 - D. Killer cells
 - E. Macrophages
14. All of the following characterize T lymphocytes **except**
- A. They are found in the paracortical areas of lymph nodes.
 - B. They have CD3 proteins on their surface.
 - C. They constitute 65 to 85% of the peripheral blood lymphocytes.
 - D. They are involved in Type II hypersensitivity reactions.
 - E. They are formed in the thymus.

Answers

- Answer: C.** Natural Killer (NK) cells, and Killer (K) cells are both large granular lymphocytes that kill targets by releasing perforin, a pore-forming molecule that polymerizes in the target cell membrane. Cytotoxic T cells also utilize perforin to kill. Complement also kills targets by producing holes (pores) in the membrane, using the C5b-9 complex. The macrophage, however, has no pore-forming effector molecule. It kills by releasing granule enzymes, oxygen radicals, or cytokines (TNF- α).
- Answer: A.** Choice A is true, and the Th2 subset of helper T cells mediates humoral immunity. Choice B is wrong because all helper T cells express CD4, not CD8. Choice C lists cytokines typically secreted by Th2 cells, not Th1 cells. Choice D is wrong because Th1 cells inhibit the function of Th2 cells (and Th2 cells inhibit the function of Th1 cells).
- Answer: E.** β 2 microglobulin is a part of the MHC class I molecule, and is required for MHC class I expression. Since MHC class I molecules provide the peptide-binding site for cytotoxic T-cell killing of a virus-infected cell, a virus-infected macrophage (bone marrow derived) could not be recognized by a cytotoxic T cell (choice E). Choices B and C are other molecules expressed by T cells, and would not be affected by absence of MHC class I. Choices A and D are functions of helper T cells, which recognize antigenic peptides bound to MHC class II, and would thus be unaffected by absence of MHC class I.
- Answer: B.** ADCC can be mediated by any effector cell that expresses IgG (or, for eosinophils, IgE) Fc receptors (choices A, D, or E). NK cells carry out ADCC by releasing perforin (choice C). ADCC does not involve MHC recognition (choice B).
- Answer: E.** The effectors of ADCC are probably NK cells that have receptors for the Fc portion of IgG. Therefore, the specificity of interaction with the pathogen is a function of the antibody molecule and not the innate cytotoxicity of the effector cell. Cytotoxic T lymphocytes are not involved in ADCC and mediate the specificity of their killing through the TCR.
- Answer: A.** IL-4 and IL-5 activate B cells and encourage class switching. IL-4 and IL-10 are suppressors of macrophage activity and cell-mediated immunity. Therefore, elaboration of these cytokines should enhance the antibody response to the infectious agent, which, unfortunately, is not the most protective response.
- Answer: A.** The ADCC is referred to as antibody-dependent cellular cytotoxicity. This type of killing involves a viral-infected cell and the viral epitopes are presented on the cell surface. This virus has been seen before and there are antibody molecules reacting with the cell surface. The Fc portion of the antibody reacts with a NK cell and the NK cell releases its granules into the inside of the viral infected cell, killing it.
- Answer: B.** The tuberculin skin test positive is a delayed type hypersensitivity test. This Type IV hypersensitivity is a cell-mediated immune response involving CD4 T-helper 1 lymphocytes. This individual is TB positive and should now have cultures and x-rays to confirm an active case of tuberculosis.
- Answer: D.** IgM is a very strong activator of complement. The remaining choices on the list are not involved with antibody at all (choices A, C, and E) or involve IgG (choice B).

10. **Answer: C.** Cytotoxic T cells ($CD8^+$ cells) recognize their targets by virtue of peptide antigen association with a groove in the class I MHC molecule. $\beta 2$ microglobulin is a component of the MHC class I molecule, but is not involved directly in antigen presentation. CD8 stabilizes the interaction between the TCR and the MHC molecule, MHC class II antigens are involved in antigen recognition for helper cells, and CD3 is part of the signal transduction complex, not directly involved in antigen recognition.
11. **Answer: C.** IL-2 is elaborated by Th1 cells, IFN- γ by Th1 cells, and NK cells and TNF- β by Th1 cells and CTLs. The only cell in the list that has all three capacities is the Th1 cell.
12. **Answer: B.**
13. **Answer: B.**
14. **Answer: D.**

Complement

11

A. Overview

Inflammation is an integral part of all immune responses. One of the major mechanisms for initiating inflammation is activation of the complement cascade, which results in the production of powerful **opsonins**, **chemoattractants**, and **anaphylatoxins** and can directly mediate cell killing through lysis.

The complement system is a cascade of interacting proteins found in the blood. Many of the complement proteins are made by the liver. The complement system can be divided into the classic pathway, which is activated by **antigen-antibody complexes**, and the **alternative pathway**, which is activated by components of various **pathogen surfaces**.

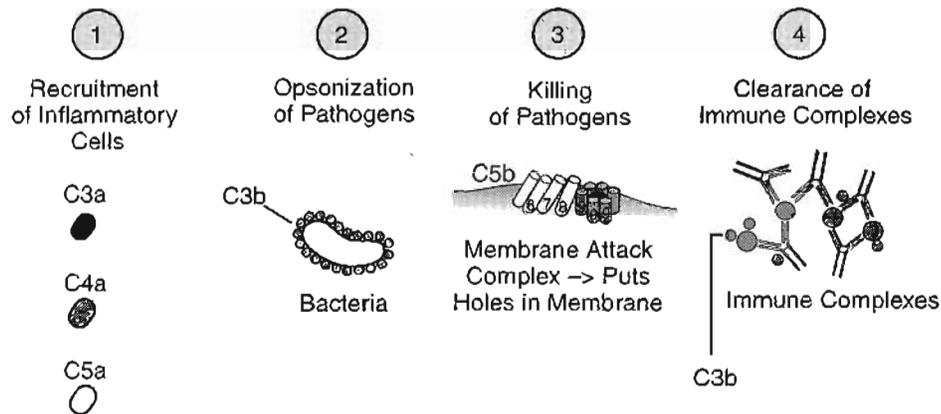


Figure II-11-1. Four Functions of the Complement System

In a Nutshell

Complement system

- It consists of at least 20 serum proteins and glycoproteins
- It generates products that facilitate antigen clearance and inflammation
- It has two pathways of activation
- After activation, it is a highly regulated enzymatic cascade
- Both pathways share a terminal reaction called the membrane-attack complex
- The alternative pathway protects body from pathogens in absence of antibody
- Bacterial surfaces can activate the alternative pathway
- Red blood cells, phagocytes, mast cells, and basophils have receptors for various complement fragments

In a Nutshell

- IgM or IgG Ab
- C1
- C4
- C3
- C5, C6, C7, C8, C9

B. The Classic Pathway

The classic pathway is the **most rapid and efficient pathway**. The immunoglobulins that participate are **IgG and IgM**. IgM is the most efficient.

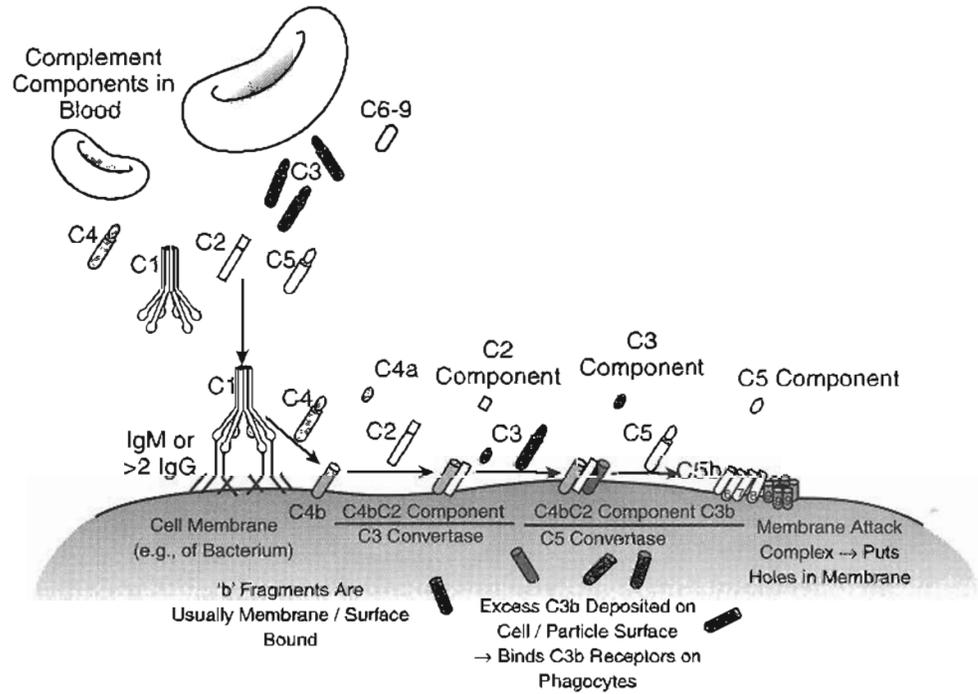


Figure II-11-2. The Classic Complement Pathway

C. The Alternative Pathway

The alternative pathway is activated as a result of:

1. Complement binding directly on surface of infectious agents
 - a. Bacterial polysaccharides
 - b. Lipopolysaccharide of cell wall of gram-negative bacteria
2. Aggregated immunoglobulins
3. Spontaneous degradation of C3 at a low rate, producing C3b

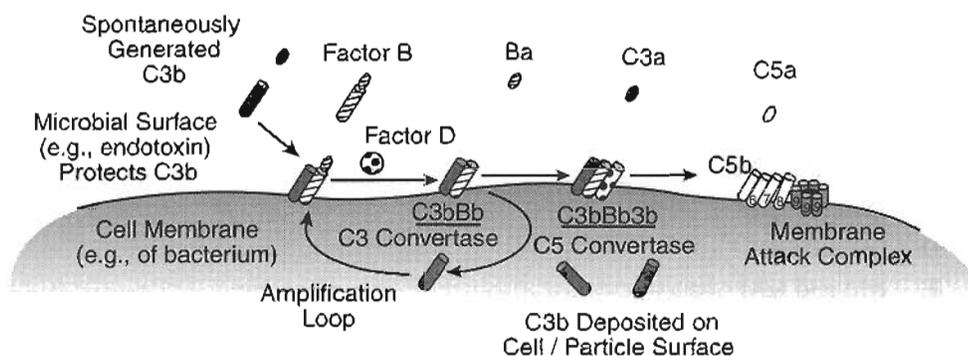


Figure II-11-3. The Alternative Complement Pathway

Table II-11-1. Activities of Activated Complement Components

Component	Activity
C3a, C5a, & C4a	Anaphylatoxins
C3b (iC3b, C4b)	Immune clearance and opsonization
C5a	Chemotaxis
C5-C9	Membrane damage

Chapter Summary

The complement system is a cascade of approximately 20 serum proteins that recruit inflammatory cells, opsonize and kill pathogens, and clear immune complexes. There are two pathways: the classic one (activated by antigen-antibody complexes) and the alternative one (activated by pathogen surfaces).

The classic pathway is the more rapid and efficient one and is activated by IgG or IgM, with IgM being more efficient.

The alternative pathway may be activated by complement binding to bacterial polysaccharides, aggregated immunoglobulins, or the spontaneous degradation of C3 to produce C3b.

Some components (and their biologic activities) are C3a, C5a, and C4a (anaphylatoxins); C3b, iC3b, and C4b (immune clearance and opsonization); C5a, (chemotaxis); and, C5 through C9 (membrane damage resulting in cell death).

Clinical Correlate

CD21 on B lymphocytes serves as receptor for Epstein-Barr virus. Biologically it is a complement receptor.

Review Questions

1. The classic complement pathway is activated by antibody-antigen complexes. The antibody isotypes that activate include both
 - A. IgG1 and IgA2
 - B. IgG4 and IgG3
 - C. IgM and IgG3
 - D. IgE and IgG2
 - E. IgM and IgA1
2. A 60-year-old alcoholic male who has smoked 2 to 3 packs of cigarettes a day since he was young was brought to the ER after he was found at 4 AM out behind the neighborhood bar. The outside temperature was freezing. On arrival at the ER his pulse was rapid, his breathing labored with diffuse rales. He was coughing and had an elevated temperature. He had periodic chills and was generally very lethargic. Endotracheal aspirates produced a blood-tinged mucopurulent discharge containing numerous gram-positive cocci. From the gram stain results and laboratory data it was determined he had *Streptococcus pneumoniae*. The laboratory data was alpha hemolytic colonies on blood agar, optochin sensitivity, and bile solubility. His serum also contained IgG and IgM antibodies to the polysaccharide capsule of *Streptococcus pneumoniae*. The man was treated with penicillin and his condition improved over the next few days. Which immune effector mechanism would be involved in completely clearing this infection?
 - A. Natural Killer cells
 - B. ADCC (Antibody Dependent Cell Cytotoxicity)
 - C. Cytotoxic T cell lymphocytes
 - D. Complement-mediated opsonization
 - E. Can be identified by the expression of CD14
3. A three-year-old boy has had several bouts with pneumonia. The *Streptococcus pneumoniae* organism was isolated and identified. The child was treated with penicillin and his condition resolved. It was felt that he perhaps had some sort of immune deficiency. It was determined his phagocytic mechanisms were intact. He had normal levels of immunoglobulins but it appeared his complement system was not working correctly. The complement system is a series of several different serum proteins that function to increase inflammation, lyse bacteria, and opsonize (coat bacteria) for the purpose of phagocytosis by macrophages and other phagocytic cells. Which one of the following is the primary opsonization fragment in the complement system?
 - A. C5a
 - B. C1q
 - C. C3b
 - D. Factor B
 - E. C5

4. A deficiency of the complement protein C4 would inhibit which one of the following complement activities?
 - A. Formation of C3b for opsonization
 - B. Formation of C5a for chemotactic attractant for neutrophils
 - C. Formation of the membrane attack complex
 - D. Completion of the classical pathway to the splitting of C3
 - E. Formation of C5 convertase via the alternative pathway

5. Complement components are involved in which types of hypersensitivities?
 - A. Type II and Type IV
 - B. Type I and Type II
 - C. Type III and Type IV
 - D. Type I and Type III
 - E. Type II and Type III

6. Which one of the following does not occur when the alternate complement pathway is activated?
 - A. Breakdown of C5 into C5a and C5b
 - B. Breakdown of C4 into C4a and C4b
 - C. Breakdown of C3 into C3a and C3b
 - D. Activation of the membrane attack complex
 - E. Generation of anaphylatoxins

Answers

1. **Answer: C.** The classic pathway of complement is activated by immune complexes containing IgM, IgG1, IgG2, or IgG3. IgG4, IgA (either subclass) IgE and IgD do not activate this pathway. If the question had specified the alternative complement pathway, choice A or B would be correct, since all subclasses of IgG and IgA activate the alternative pathway (but not IgM or IgD. IgE is controversial.).

2. **Answer: D.** One of the most efficient mechanisms for eliminating extracellular pathogenic bacteria is by opsonization by phagocytic cells (macrophages). The IgG and IgM antibody that is produced in response to the organism reacts with the capsular structure and this stimulates the activation of the classic pathway of the complement system. This pathway produces large amounts of C3b and it coats the organism, allowing for maximum phagocytosis. This antibody, especially the IgG in the patient, was from a previous infection with the same organism and this is the reason the antibody was already present. However, in the few days he was in the hospital he started producing his own antibody.

3. **Answer: C.** This fragment is the most critical molecule in both the classical and alternative complement pathways. The C3 is cleaved by C4b2b (C3 convertase) and C3bBb (C3 convertase) of the 2 pathways. Since C3 is the most abundant protein of all the complement proteins it is broken up in large amounts into C3a and C3b. All of the C3b attaches to bacterial surfaces for opsonization by phagocytes.

4. **Answer: D.** The classic pathway to the point of splitting C3 would consist of V1, C4, C2, and then splitting C3 to C3b and C3a. A deficiency of C4 would have no effect on choices A, B, C, or E.
5. **Answer: E.** The Type II hypersensitivity is usually an antibody produced against a membrane, cell, or receptor. Upon the Ag-Ab reaction, complement system is activated and a lot of the damage is due to complement reactants. The Type III hypersensitivity is circulating Ag-Ab complexes. These are deposited in the kidney, joints, or blood vessels and with the activation of complement there is massive tissue damage.
6. **Answer: B.**

Failures of the Immune System: Hypersensitivity

12

As illustrated in the figure below, there are three basic ways in which the immune system may fail: **hypersensitivity**, **immunodeficiency**, and **autoimmunity**. As these types of failure cause clinical problems that you may encounter both on the USMLE and as future physicians, a whole chapter is devoted to each type.

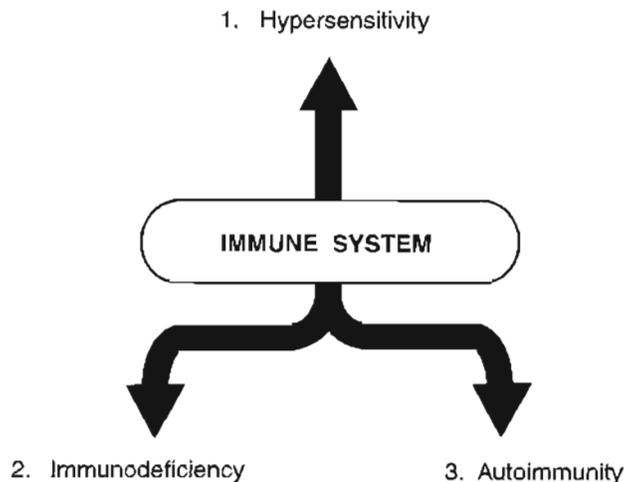


Figure II-12-1. Failures or Problems With the Immune System

A. Definition of Hypersensitivity

Hypersensitivity is a process of reactions of antigen with antibodies or sensitized lymphocytes that are harmful to the host. Hypersensitivity refers to processes in which the immune response itself is primarily responsible for the induction and/or exacerbation of disease. As in all immune responses, hypersensitivity requires **prior sensitization**, is **antigen-specific**, and depends on the participation of antibodies or lymphocytes.

B. Basic Characteristics of Hypersensitivity Reactions

- First contact with potential antigen produces no detectable reaction but it may sensitize
- If sensitized, reexposure to same antigen elicits a reaction
- The reaction is highly specific, elicited only by sensitizing antigen or a structurally related substance (**crossreacting**)
- Additional exposures to the same antigen may increase or sometimes decrease the severity of the reaction

C. Types of Hypersensitivity

There are four major types of hypersensitivity. Three are mediated by antibody, and the fourth is mediated by cellular mechanisms.

Table II-12-1. Gell and Coombs Classification

Type	Description	Effectors
I	Immediate reaction	IgE antibodies
II	Cytotoxic reaction	IgG & IgM antibodies
III	Immune complexes	IgG & IgM antibodies
IV	Delayed reaction	(48 hr) T cells

1. Type I: Immediate hypersensitivity

All individuals make an IgE response against parasitic infections. About 20% of the population, however, are also genetically predisposed to make an IgE response against relatively harmless environmental antigens, such as trees, grasses, weeds, and cat or dog proteins. These individuals are called **atopic**.

a. **Common allergens** in immediate-hypersensitivity reactions causing respiratory symptoms include:

- Tree, grass, and weed pollens
- Cat antigen and other animal dander antigens
- Dust mite fecal pellet antigens
- Mold spores

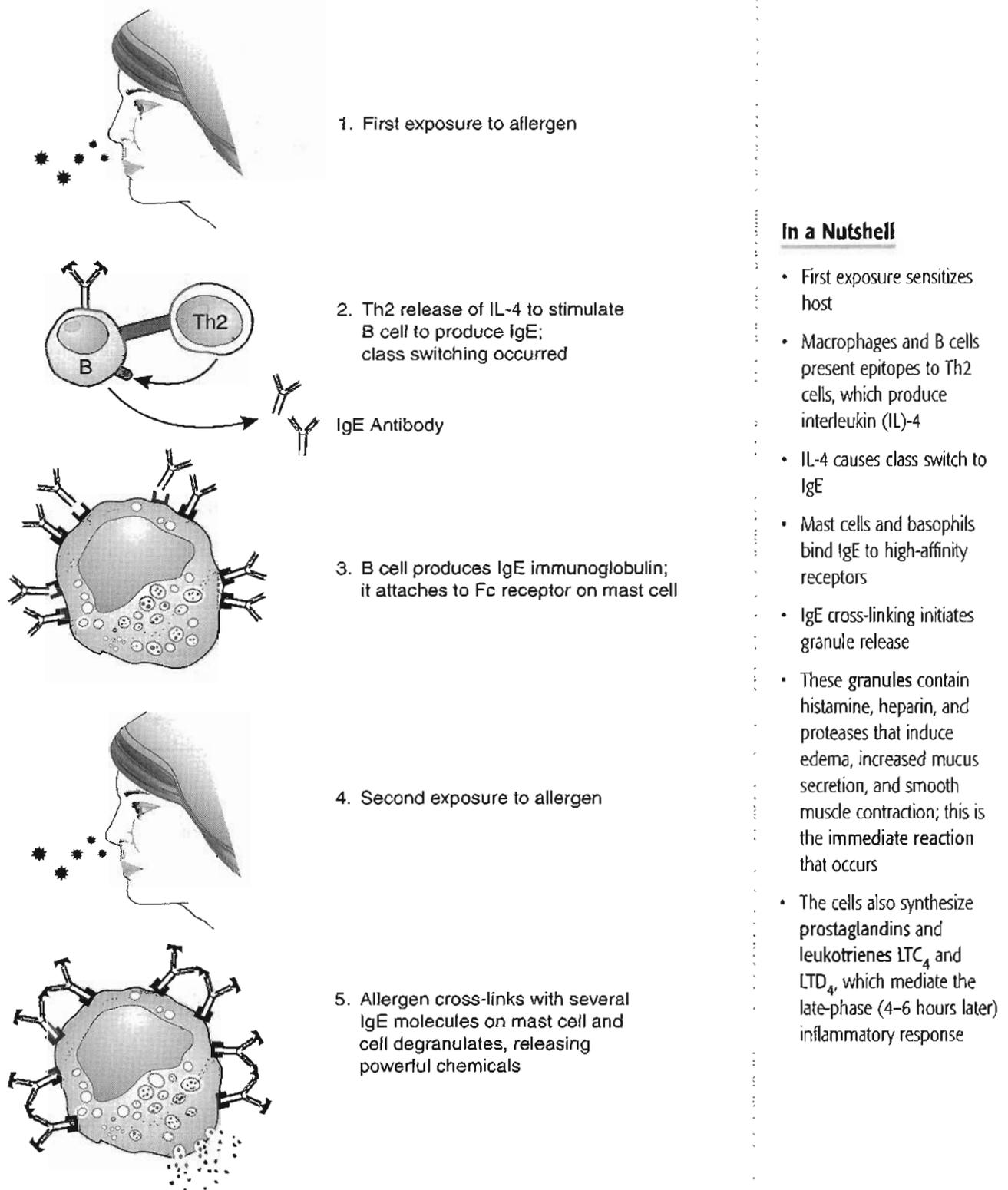
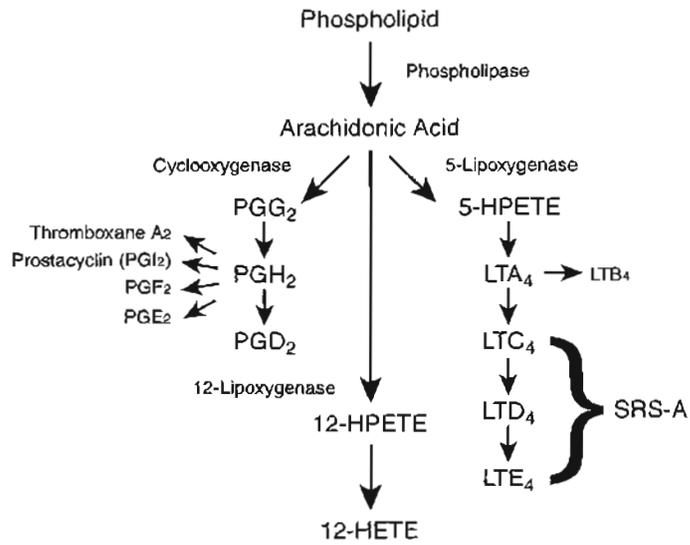


Figure II-12-2. Development of Immediate-Hypersensitivity Reaction



Eicosanoids are derived from arachidonic acid and mediate inflammation.

Figure II-12-3. Late Stage Mediation

Table II-12-2. Mast Cell Mediators

Mediators Stored and Released	Effect
Histamine	Smooth muscle contraction; increased vascular permeability
Heparin	Anticoagulant
ECF-A	Chemotactic
Mediators Newly Synthesized From Arachidonic Acid	Effect
Prostaglandin E ₂	Increased pain response and vascular permeability
Prostaglandin D ₂	Increased smooth muscle contraction and vascular permeability
Leukotrienes C ₄ , D ₄ , E ₄	Increased smooth muscle contraction and vascular permeability
Leukotriene B ₄	Chemotactic for neutrophils

ECF-A, Eosinophil chemotactic factor-A.

b. Eosinophils

Eosinophils represent about 1 to 3% of the circulating leukocytes. They are important in late inflammatory reactions, particularly in parasitic infections and allergies. They are attracted to the site by histamine, C5a, ECF-A, leukotriene B₄ (LTB₄), and platelet-activating-factor (PAF). Eosinophils contain such products as histaminase for degrading histamine, aryl sulfatase for degrading leukotrienes, and major basic protein, which is toxic for worms.

c. Basophils

Basophils represent about 1% of all leukocytes and have receptors for IgE. When basophils move from the blood stream into tissue they are termed mast cells (see Mast Cell Mediators table, above).

d. Physical and clinical findings

Depending on the allergen, there are a variety of physical or clinical findings. These are summarized in the table below.

Table II-12-3. Allergic Diseases Due to Specific Allergens and the Physical or Clinical Manifestations

Allergic Disease	Allergens	Clinical Findings
Allergic rhinitis (hay fever)	Trees, grasses, dust, cats, dogs, and mites	Edema, irritation, mucus in nasal mucosa
Food allergies	Milk, eggs, fish, cereals, & grains	Hives and GI problems
Wheal and flare	Insect bites, in vivo allergy, skin testing	Local skin edema, reddening, & vasodilation of vessels
Asthma	Inhaled materials	Bronchial and tracheal constriction, edema, mucus production, & massive inflammation
Systemic anaphylaxis	Insect stings, snake venoms, drug reactions	Bronchial and tracheal constriction and complete vasodilation and death

e. Therapies for immediate hypersensitivities

- Avoidance of the allergen
- Antihistamine (Benadryl) to block histamine receptors
- Cromolyn sodium to stabilize mast cell membranes
- Epinephrine to increase intracellular cyclic AMP
- Epinephrine to increase bronchial dilation and increase heart rate to raise blood pressure
- Theophylline (xanthines) to block phosphodiesterase enzyme that breaks down cyclic AMP
- Immunotherapy by injecting small amounts of allergen to switch the response from Th2 to Th1 lymphocyte type, resulting in decreased IgE production and increased IgG type
- Corticosteroids to reduce both inflammation and production of antibody
- β -Adrenergic agonists—bronchial dilators that relax smooth muscle in airways, e.g., albuterol or terbutaline

Clinical Correlate

Diagnosis of atopic disease

- In vivo skin testing with batteries of allergens
- In vitro RAST test (quantitate specific IgE levels)
- RAST = radioallergosorbent test

In a Nutshell

Prevention of type I reactions

- Stabilize mast cell membrane
- Keep cyclic AMP elevated
- Produce IgG to block (react with) allergen first
- Block more inflammation
- Prevent Ca²⁺ influx

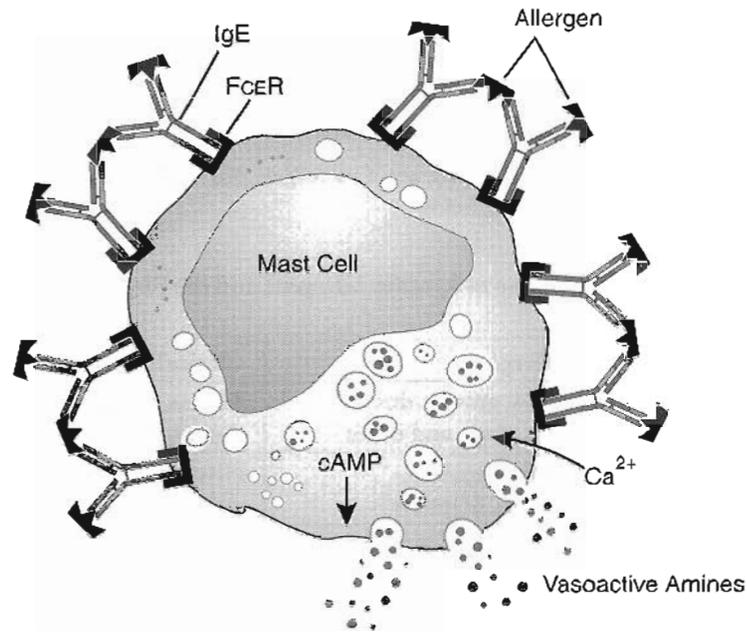


Figure II-12-4. Release of Vasoactive Amines From Mast Cells

2. Type II: Antibody-mediated hypersensitivity against our own cells or receptors or membranes

Type II or cytotoxic hypersensitivity is mediated by IgG or IgM antibodies against tissue antigens. The result is organ-specific antibody production. Antibody binds to cells or tissues and causes local complement activation, influx of leukocytes, and tissue destruction because of ADCC, degranulation by phagocytes, and production of oxygen radicals.

a. Hemolytic disease of the newborn

An important clinical example of type II cytotoxic hypersensitivity is hemolytic disease of the newborn, also known (in its severest form) as erythroblastosis fetalis. In the fetus this disease is due to the transport across the placenta of IgG specific for one of the Rhesus (Rh) protein antigens (RhD). About 85% of people are Rh⁺. If a pregnant woman is Rh⁻ and the father is Rh⁺, there is a chance that the fetus will also be Rh⁺. This situation will pose no problem in the first pregnancy, as the mother's immune system will not usually encounter fetal red blood cell antigens until placental separation at the time of birth. At that time, however, Rh⁺ fetal red blood cells will enter the maternal circulation and stimulate a T-dependent immune response, eventually resulting in the generation of memory B cells capable of producing IgG antibody against RhD. In a subsequent pregnancy with another Rh⁺ fetus, this maternal IgG can be transported across the placenta, react with fetal Rh⁺ red cells, and activate complement producing hemolytic disease.

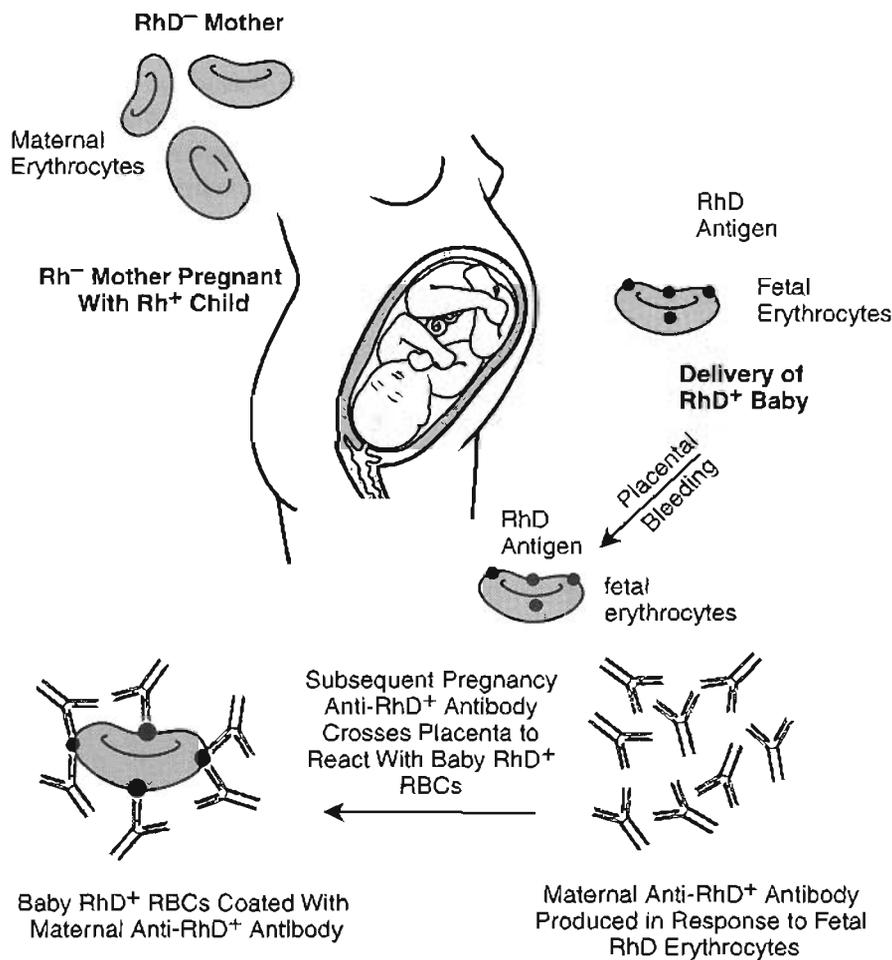


Figure II-12-5. Hemolytic Disease of the Newborn

Hemolytic disease of the newborn can be prevented by treating the Rh⁻ mother with RhoGAM™, a preparation of human anti-RhD IgG antibody, at 28 weeks of gestation and again within 24 hours after birth. This antibody effectively eliminates the fetal Rh⁺ red cells before they can generate RhD-specific memory B cells in the mother. Anti-RhD antibody should always be given to any Rh⁻ individual following termination of any pregnancy.

In a Nutshell

Antigen-antibody complexes
When antigen is free, it can bind to B cells and act as a stimulatory signal for antibody production; however, when most of the antigen is bound in complexes with IgG, these complexes deliver an inhibitory signal to B cells, preventing further differentiation or proliferation.

b. Noncytotoxic type II hypersensitivity

Noncytotoxic type II hypersensitivity is also due to the production of tissue-specific IgG autoantibody. Instead of causing cytotoxic tissue destruction, however, the antibody in these cases alters cellular structure or function. In Graves disease, an IgG autoantibody against the thyroid-stimulating hormone receptor mimics the hormone but stays bound for an excessive length of time, resulting in hyperthyroidism.

Clinical Correlate

Cytotoxic antibody disease

- Erythroblastosis fetalis
- Transfusion reactions
- Hyperacute graft rejection
- Acquired hemolytic anemia:
 - autoimmune
 - drug induced

Diagnosis of cytotoxic antibody diseases

- Detect immunoglobulins on affected cells or tissues:
 - Coombs serum
 - Immunofluorescence
- Detect complement in affected tissue
- Detect autoantibody or autoreactive T cells

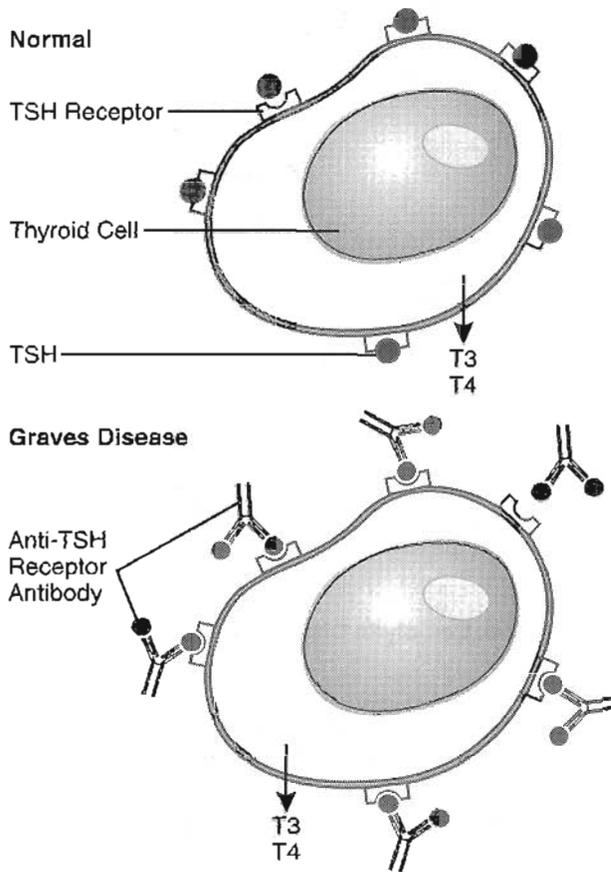


Figure II-12-6. Antibodies to the TSH Receptor In Hyperthyroidism

c. Other examples of type II hypersensitivity

- i. **Myasthenia gravis:** An autoantibody against the patient's own acetylcholine receptors in which the antibody removes and internalizes the receptors.

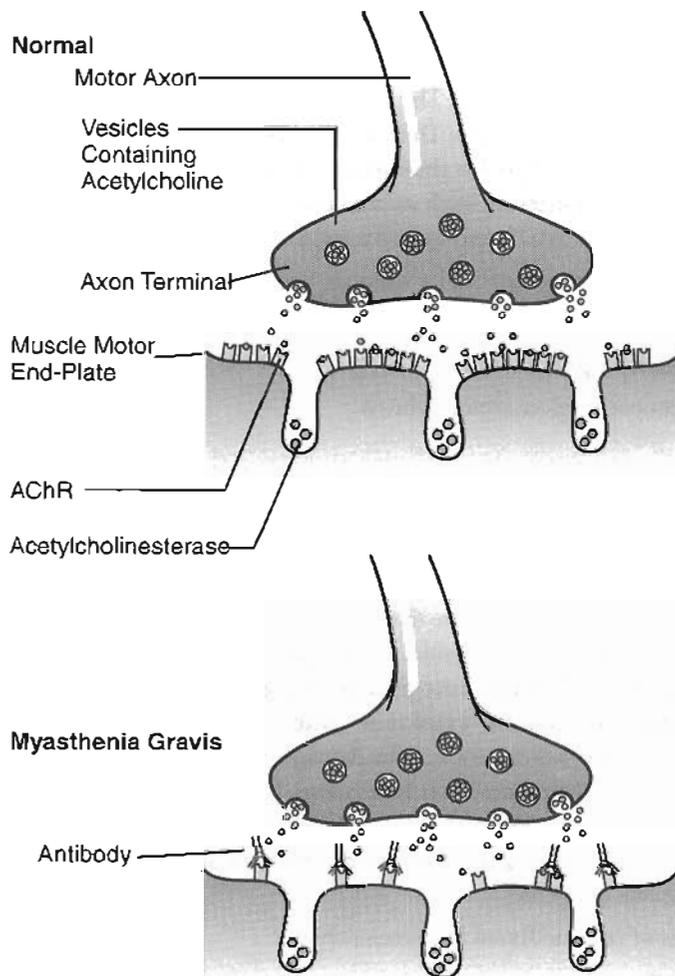


Figure II-12-7. Antibodies Against Acetylcholine Receptors in Myasthenia Gravis

- ii. **Goodpasture syndrome:** An autoantibody produced against the patient's own type IV collagen present in basement membranes of kidney and lung.
- iii. **Autoimmune hemolytic anemia:** An autoantibody produced against the patient's own red blood cell antigens (e.g., I antigen).
- iv. **Autoimmune thrombocytopenic purpura:** An autoantibody produced against the patient's own platelet integrin.
- v. **Hyperacute graft rejection:** The recipient of a graft already has pre-formed antibody against the graft; after receiving the graft it is rejected within hours.

Clinical Correlate

Therapy for type II and III hypersensitivities

- Withhold allergenic drug
- Remove antibodies:
 - Exchange transfusion
 - Plasmapheresis
- Use immunosuppressive agents:
 - Corticosteroids
 - Cyclosporin A

3. **Type III: Immune complex hypersensitivity**

Type III (immune complex) hypersensitivity is caused by high levels of circulating, soluble immune complexes containing IgG or IgM antibody. This results in systemic, rather than organ-specific, damage, as the circulating immune complexes overwhelm the ability of the mononuclear phagocyte system to remove them. The excess complexes then deposit in various tissues (e.g., skin, glomeruli, blood vessels, synovium, lungs) and activate complement. The subsequent attempt by neutrophils to remove them results in degranulation and tissue damage. The antigens can be host molecules that have triggered an autoantibody response, such as DNA and RNA–protein complexes (in systemic lupus erythematosus) or even IgG (in rheumatoid arthritis), or they can be foreign antigens that are produced persistently, such as hepatitis B surface antigen in chronic active hepatitis, HIV particles in AIDS, and mycobacterial antigens in leprosy.

Example of type III hypersensitivity immune complex diseases are:

- a. Serum sickness
- b. Hypersensitivity pneumonitis
- c. Post-streptococcal glomerulonephritis
- d. Lupus
- e. Rheumatoid arthritis
- f. Certain infectious diseases, e.g., hepatitis B, HIV, and mycobacterium

4. **Type IV: Delayed-type hypersensitivity (DTH)**

DTH is a T-cell-mediated response that gets its name from the long time (48 to 96 hours) that it takes for a skin reaction against the antigen to develop. Antibody and complement play no role in DTH. Th1 cells recognize the antigenic peptide presented by an antigen-presenting cell and secrete cytokines (interferon- γ) that activate macrophages. Macrophage mediators then produce the damage in tissues. Antigens may be intracellular bacterial pathogens (*Mycobacterium tuberculosis*, *Mycobacterium leprae*), viruses, fungi, or intracellular parasites. Contact dermatitis is caused by environmental substances (e.g., poison ivy, nickel), which, acting as haptens, enter the skin, attach to body proteins, and become complete antigens.

a. **Diagnosis of cell-mediated hypersensitivity**

- i. Skin test
- ii. Demonstrate T-cell mitogenicity by the antigen

b. **Characteristics of type IV hypersensitivity**

- i. Antigen-sensitized Th1 release cytokines after a secondary contact with the same antigen
- ii. Cytokines induce inflammatory reactions and attract and activate macrophages that release other mediators

c. **Common contact allergens**

- i. Poison ivy (catechols)
- ii. Nickel
- iii. Formaldehyde
- iv. Latex
- v. Chromium
- vi. Dyes in clothing and cosmetics

Table II-12-4. Hypersensitivity Summary

Type	Antibody	Complement	Effector Cells	Examples
I (immediate)	IgE	No	Basophil, mast cell	Hay fever, atopic dermatitis, insect venom sensitivity, anaphylaxis to drugs, some food allergies, allergy to animals and animal products
II (cytotoxic)	IgG, IgM	Yes	PMN, M ϕ , NK cell	Autoimmune or drug-induced hemolytic anemia, transfusion reactions, hemolytic disease of newborn, hyperacute graft rejection, Goodpasture disease, rheumatic fever
II (noncytotoxic)	IgG	No	None	Myasthenia gravis, Graves disease, type 2 diabetes mellitus
III (immune complex)	IgG, IgM	Yes	PMN, M ϕ	SLE, RA, polyarteritis nodosa, post-streptococcal glomerulonephritis, Arthus reaction, serum sickness
IV (delayed, DTH)	None	No	CTL, Th1, M ϕ	Tuberculin test, tuberculosis, leprosy, Hashimoto thyroiditis, poison ivy (contact dermatitis), acute and chronic graft rejection, graft-versus-host disease, type 1 diabetes mellitus

CTL, cytotoxic T lymphocyte; DTH, delayed-type hypersensitivity; M ϕ , macrophage; PMN, polymorphonuclear neutrophil; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

Chapter Summary

The immune system may fail in three basic ways: by hypersensitivity, immunodeficiency, or autoimmunity.

Hypersensitivity is an antigen-specific process that requires sensitization by contact with an antigen and the participation of antibodies or lymphocytes recruited by reexposure to the same or similar (cross-reacting) antigen, inducing a pathological response.

The Gell and Coombs classification distinguishes four major types of hypersensitivity.

Type I (immediate) sensitivity utilizes IgE. Normally, IgE provides protection against parasites, but about 20% of the population is genetically predisposed to mount an IgE response against harmless environmental antigens such as pollens, animal dander, dust-mite feces, or mold spores. The first exposure to an allergen activates Th2 cells to secrete IL-4. This induces class switching and the production of IgE by B cells. The IgE binds to the Fc receptor of mast cells, where it can be cross-linked by allergen on subsequent exposures. This causes the mast cell to release granules containing histamine, heparin ECF-A, and proteases. These induce the immediate reaction characterized by edema, mucus secretion, and smooth muscle contraction. An inflammatory late-phase reaction is induced by the release of prostaglandins and leukotrienes.

The type I allergic diseases include allergic rhinitis (hay fever), food allergies, wheal and flare (local skin reactions), asthma, and systemic anaphylaxis.

Therapies for type I reaction include avoidance of the allergen, antihistamines, mast-cell membrane stabilizers such as Cromolyn, raising cAMP levels with epinephrine or theophylline, switching from Th2 to Th1 response by injecting small amounts of allergen, antiinflammatory agents such as cortisone, and β -adrenergic agonists to relax smooth muscles.

Type II cytotoxic hypersensitivity is mediated by IgG or IgM immunoglobulins that cause local tissue destruction. A prime example is hemolytic disease of the newborn, known in its most severe form as erythroblastosis fetalis. In this disease, an Rh⁻ mother produces antibody in response to her Rh⁺ fetus. The first pregnancy sensitizes the mother to produce IgG antibodies that can cross the placenta to cause a hemolytic disease in subsequent pregnancies with an Rh⁺ fetus.

Type II noncytotoxic hypersensitivity is similar, but rather than causing tissue destruction using complement, an autoantibody that causes an alteration of tissue structure and/or function is formed. A prime example is Graves disease in which antibody bound to thyroid tissue simulates the TSH receptor and causes hyperthyroidism.

Other examples of type II hypersensitivity are myasthenia gravis, Goodpasture syndrome, autoimmune hemolytic anemia, autoimmune thrombocytic purpura, and hyperacute graft rejection.

Type III immune complex hypersensitivity is caused by circulating soluble immune complexes of IgG or IgM. The immune complexes are filtered out of the circulation in the small vasculature, and complement is activated, destroying the tissues. The inducing antigen is one that is produced constantly, such as a persistent foreign antigen or an autoantigen. Examples are the Arthus reaction, serum sickness, hypersensitivity pneumonia, post-streptococcal glomerulonephritis, lupus erythematosus, and rheumatoid arthritis. It is a common sequela to certain chronic infectious diseases such as hepatitis B, HIV, and leprosy.

(Continued)

Chapter Summary (continued)

Type IV (delayed-type) hypersensitivity is a T-cell-mediated response causing a reaction approximately 2 to 4 days after exposure. Th1 cells recognize a presented antigen and in response secrete interferon- γ , which activates macrophages. The inducing antigens may be intracellular pathogens (e.g., *Mycobacterium tuberculosis*) or molecules such as those causing contact dermatitis, which act as haptens by binding to skin cells. The Mantoux test (tuberculin skin test) is an example of a type IV hypersensitivity. Type IV hypersensitivity also causes Hashimoto thyroiditis, the contact allergies, acute and chronic graft rejection, graft-versus-host disease, and type 1 diabetes.

Review Questions

- Neutrophils are important mediators of tissue damage in which of the following hypersensitivity types?
 - Types I and IV
 - Types II and IV
 - Types II and III
 - Types III and IV
 - Types I and III
- Histamine release from basophil or mast cell granules
 - Requires that these cells have IgE of only one antigenic specificity per cell
 - Is Ca^{2+} independent
 - Requires IgG antibody crosslinking at the cell surface
 - Cannot occur in the absence of antigen-specific IgE
 - Follows IgE Fc receptor crosslinking
- Which are both examples of Type IV hypersensitivity?
 - Atopic eczema and contact dermatitis
 - Tuberculosis and graft-versus-host disease
 - Leprosy and myasthenia gravis
 - Poison ivy and the Arthus reaction
 - Chronic granulomatous disease and chronic allograft rejection
- Which of the following is not true of graft-versus-host disease?
 - It is an example of Type IV hypersensitivity.
 - It can occur when MHC mismatched macrophages are transfused into an immunocompromised host.
 - It can occur when bone marrow is transplanted between MHC non-identical twins as a treatment for leukemia.
 - It can occur following thymus transplantation between MHC non-identical siblings.
 - It can occur when a child with severe combined immunodeficiency receives a blood transfusion from an ABO compatible, unrelated donor.

5. All of the following are examples of hypersensitivity diseases **except**
 - A. Hereditary angioedema
 - B. Goodpasture's syndrome
 - C. Hay fever
 - D. Contact dermatitis
 - E. Rheumatoid arthritis

6. Your patient in the emergency room was stung by a bee about 15 minutes ago. She says that a few minutes after being stung she became very short of breath and saw hives (urticaria) appear in several places on her skin. Which one of the following is the **most** likely immunological mechanism for these clinical findings?
 - A. Sensitized helper T cells release large amounts of interleukin 2.
 - B. Immune complexes consisting of IgG and hapten precipitate in the lung and skin.
 - C. Activated macrophages produce large amounts of tumor necrosis factor.
 - D. Membrane attack complexes of complement damage cells in the lung and skin.
 - E. IgE-coated mast cells release histamine and leukotrienes.

7. Your patient has several attacks of sneezing, runny nose, and itchy eyes every spring, which you suspect is due to an allergy to some plant pollen. You refer the patient to an allergist who does skin tests with various allergens and gets a wheal-and-flare reaction with several pollens. What is the **most** likely sequence of events that produced the wheal-and-flare reactions?
 - A. Allergen binds to IgM in the plasma, which activates complement to produce C3b.
 - B. Allergen binds to IgM on the surface of B cells and histamine is released.
 - C. Allergen binds to IgE on the surface of mast cells, and histamine is released.
 - D. Allergen binds to IgE in the plasma and the allergen-IgE complex binds to the surface of macrophages and histamine is released.

8. Which of the following characterizes IgE?
 - A. It activates the classical complement pathway.
 - B. It crosses the placental membrane.
 - C. It contains a J chain in its structure.
 - D. It must be crosslinked in order to degranulate a mast cell.
 - E. It is involved in Type IV hypersensitivity reactions.

9. Erythroblastosis fetalis in future pregnancies can be prevented if the mother is injected, at parturition, with an antibody called
 - A. Anti-isotype antibody
 - B. Coombs antibody
 - C. Rho (D) antibody, RhoGAM
 - D. Antilymphocyte antibody
 - E. Antithymocyte antibody

10. In the 1960s it was quickly ascertained that Peace Corps workers sent to schistosome-endemic areas were exposed to massive initial doses of cercariae before this protective cutaneous response had any opportunity to develop. In these patients, IgG antibodies developed in response to the developing worms, and when the adults began their prodigious release of eggs into the circulation, the patients suffered acute and potentially life-threatening symptoms of fever, edema, arthralgia (joint pain), and rash. Another immunologically mediated condition that arises by this mechanism is
- Goodpasture syndrome
 - The tuberculin reaction
 - The Arthus reaction
 - The transfusion reaction
 - Atopic allergy
11. In native Egyptian populations, children are exposed to the cercariae (infectious forms) of the fluke *Schistosoma mansoni* in early childhood when they wade in irrigation ditches throughout the Nile Delta. On first exposure, the cercariae penetrate the skin and become schistosomules, which enter the circulation and eventually mature in the mesenteric veins. On subsequent exposures, schistosomules are frequently killed within minutes by an immune response in the skin manifested by intense itching, stinging, and urticaria. This protective immune response is a manifestation of
- Type I hypersensitivity
 - The Arthus reaction
 - Serum sickness
 - Contact dermatitis
 - Passive cutaneous anaphylaxis
12. A young newly-married woman, JT, goes to her physician with concerns about a “hereditary problem” described to her by her mother that was associated with her own birth in 1968. Her family was poor and her mother received no medical prenatal care before JT was born “blue” and covered with “splotches” and “bruises.” Although an earlier sibling had been born apparently normal, JT required multiple transfusions before her condition stabilized, and two further pregnancies of her mother ended in stillbirths. JT is concerned about the potential for development of similar problems in her own pregnancies. Blood tests ordered by the physician confirm his suspicions. He advises his patient:
- She is RhD⁺; there is no risk to a fetus.
 - She is RhD⁻ and should be treated postpartum with RhoGAM.
 - Her husband should be tested for Rh incompatibility.
 - She is RhD⁺ and should be treated postpartum with RhoGAM.
 - She is RhD⁻; there is no risk to a fetus.
13. Which of the following immunoglobulin classes is responsible for eliciting allergic reactions (Type I hypersensitivities)?
- IgG1
 - IgM
 - IgA
 - IgG
 - IgE

14. A group of college students were sitting in the stands at a football game and one of the students immediately started breathing hard and complaining of tightness in his chest. He started sweating and fainted. It was discovered a bee had just stung him. The medical personnel came and attended to the man and in a few minutes he recovered. Which one of the following drugs was administered in this case?
- A. Antihistamine (Benadryl)
 - B. Cromolyn sodium
 - C. Epinephrine
 - D. Theophylline (methylxanthines)
 - E. Blocking antibody
15. Type III hypersensitivities consist of antigen-antibody complexes being formed and deposited in joints, blood vessels, or kidney. Which one of the following diseases is considered to be a Type III reaction?
- A. Autoimmune hemolytic anemia
 - B. Graves disease
 - C. Goodpasture syndrome
 - D. Myasthenia gravis
 - E. Systemic lupus erythematosus
16. Which one of the following would be classified as a Type I hypersensitivity reaction?
- A. Allergic rhinitis
 - B. Contact dermatitis
 - C. Hemolytic disease of newborn
 - D. Hyperacute graft rejection
 - E. Post streptococcal glomerulonephritis
17. Immediate hypersensitivities (Type I) often require quick treatment to ensure stopping the reaction. Which one of the following would not allow the allergen to react with the IgE immunoglobulin present on the mast cell membrane?
- A. Antihistamine
 - B. Blocking antibody
 - C. Adrenalin
 - D. Cromolyn sodium
 - E. Theophylline (xanthines)

18. A 28-year-old mother delivered her first child. The genetics of the father was homozygous RhD positive. The mother was homozygous RhD negative. The baby was born without any complications but the mother was not administered any RhoGAM following the birth of the child. At the time of the delivery, large amounts of cord blood entered the mother's circulation and she received some of the infant's blood cells. One year later she delivered another child and immediately following birth the child had to receive a blood transfusion. The condition is termed Hemolytic Disease of Newborn (erythroblastosis fetalis). Which type of hypersensitivity best describes this condition?
- A. Delayed hypersensitivity
 - B. Immune complex disease
 - C. Cytotoxic disease
 - D. Atopic disease
 - E. Immediate hypersensitivity

Answers

1. **Answer: C.** Neutrophils are triggered to release their granule contents by IgG complexes and activated complement fragments (C3b, iC3b, C5a). These are characteristics of Types II and III hypersensitivity. In Type I, neutrophils play no role in the immediate (anaphylactic) response though they may be involved in the "late phase" response to allergens. They play no role at all in Type IV reactions.
2. **Answer: E.** Basophils and mast cells bind IgE via high affinity Fc receptors, so each cell has IgE of many different specificities on its surface. Ca^{2+} is required. **Choice C** would be correct if it said "IgE" instead of "IgG." **Choice D** is incorrect, since many non-IgE triggers can produce granule release, C3a and C5a, for example. **Choice E** describes the correct mechanism by which IgE binding to antigen triggers release.
3. **Answer: B.** Atopic eczema, as the name implies, is a Type I hypersensitivity disease. Myasthenia gravis is a Type II disease, and the Arthus Reaction is an experimental model of Type III. Chronic granulomatous disease is an enzyme deficiency, not a hypersensitivity disease. All others are Type IV.
4. **Answer: B.** Graft-versus-host (GVH) disease occurs when MHC non-identical lymphocytes are transplanted into an immunoincompetent host. The foreign lymphocytes recognize the new host as non-self and attack. This cannot happen when macrophages, or any non-lymphoid cell is transferred. Since this is purely a cell-mediated reaction, it is a Type IV hypersensitivity disease. Thymus, bone marrow, and even peripheral blood are all sources of immunocompetent lymphocytes.
5. **Answer: A.** **Choice B** is a Type II; **choice C** is a Type I; **choice D** is a Type IV; and **choice E** is a Type III hypersensitivity disease. **Choice A** is an inherited deficiency of C1-inhibitor, and thus is an immune deficiency disease, *not* a hypersensitivity disease.
6. **Answer: E.**
7. **Answer: C.**
8. **Answer: D.**
9. **Answer: C.**

10. **Answer: C.** The syndrome described is a classical example of Type III, immune complex-mediated hypersensitivity where an individual who has developed appreciable levels of IgG precipitins is subsequently challenged with large amounts of homologous antigen. The only choice in the list that is a Type III hypersensitivity is the Arthus reaction. Goodpasture syndrome and transfusion reactions are Type II (cytotoxic); the tuberculin reaction is Type IV (delayed-type hypersensitivity) and atopic allergy is Type I (anaphylactic).
11. **Answer: A.** Symptoms of itching, stinging, urticaria, and rapid onset on second exposure suggest anaphylactic (Type I) hypersensitivity mediated by IgE antibody bound to mast cells and basophils in the skin. Choices B and C are both Type III hypersensitivities that involve immune complex mechanisms. Choice D is a Type IV delayed-type hypersensitivity that is T cell-mediated, and choice E is simply a test to diagnose Type I hypersensitivity by transfer of serum; it is not a mechanism of protection.
12. **Answer: A.** The syndrome described is consistent with hemolytic disease of the newborn, caused when an RhD⁻ mother is sensitized to the RBC of her RhD⁺ fetus. Since JT was the child born with HDNB, she must be RhD⁺, so she cannot cause this problem for her own children.
13. **Answer: E.** This immunoglobulin is produced by class switching under the influence of IL-4 after the B cell has been stimulated by an allergen epitope. The IgE then attaches to a mast cell and on reexposure to the allergen the contents of the mast cell are released, causing the hypersensitivity reaction.
14. **Answer: C.** Epinephrine is the correct answer. This person already is having a major anaphylactic reaction and his tracheal-bronchial smooth muscle has contracted. Epinephrine is the only treatment in this group to reverse this reaction. It will relax the smooth muscle of the respiratory tree and stimulate the heart to beat stronger to raise the blood pressure.
15. **Answer: E.** In SLE we make antibody to our own double-stranded DNA and complement is activated via the classical pathway. These complexes are deposited in the above regions of our body.
16. **Answer: A.** The allergic rhinitis is a response to ragweed or some other type of allergen. This involves the production of IgE antibody against the allergen. The IgE antibody combines with mast cells in the nasopharyngeal area and on reexposure to the allergen the mast cells release products that cause an increase in fluid and mucus production.
17. **Answer: B.** Blocking antibody is an IgG antibody against the allergen that you have caused the allergic patient to make by administering small amounts of allergen over a period of time. This time when they are exposed to the allergen the IgG reacts with the allergen before it gets to the IgE coated mast cell.
18. **Answer: C.** This is Type II hypersensitivity where there is antibody produced against cells or receptors in the body. In this case of cytotoxic disease the mother produced antibody against the RhD positive cells she received upon delivery of her first child. This child must be RhD positive because the father is homozygous. The RhD cells are foreign to the mother since she is RhD negative and she mounted an antibody response against the antigen. The second child also had to be RhD positive and the mother's antibody crossed the placenta resulting in this disease.

Failures of the Immune System: Immunodeficiency

13

A. Primary Immunodeficiencies

1. Variants
 - a. Deficiencies of **phagocyte** cell function
 - b. Deficiency of a **complement** protein
 - c. Deficiencies of **B-cell** development or function
 - d. Deficiencies of **T-cell** development or function
 - e. Combined B- and T-cell deficiencies
2. Deficiencies of **phagocyte** cell function

Patients with enzyme deficiencies that affect **phagocytic** cell function get severe infections with **extracellular bacteria and fungi**, summarized in the table below. An example is **chronic granulomatous disease (CGD)**, which is an inherited deficiency in one subunit of **NADPH oxidase**. Patients get severe infections with catalase-positive bacteria and fungi, such as *Staphylococcus*, *Klebsiella*, *Serratia*, and *Aspergillus*. Diagnosis of CGD is done by the **nitroblue tetrazolium reduction test (NBT)**; in CGD, it is negative because of no generation of oxygen radicals.

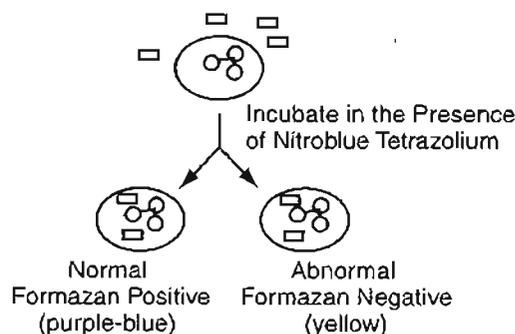


Figure II-13-1. Nitroblue Tetrazolium Reduction

Positive = Yellow, nitroblue tetrazolium is converted to purple-blue formazan if reactive oxygen intermediates are present.

Clinical Correlate

Associated characteristics of CGD

- Recurrent acute and chronic infections
- Pediatric age group
- Opportunistic pathogens
- Poor response to therapy
- Failure to thrive
- Hepatosplenomegaly

Treatment of CGD

- Antibiotics
- Neutrophil infusions
- Interferon (IFN)- γ to activate phagocytes

Table II-13-1. Phagocyte-Deficiency Diseases

Disease	Molecular Defect(s)	Symptoms
Chronic granulomatous disease (CGD)	Deficiency of NADPH oxidase (any one of four component proteins); failure to generate superoxide anion, other O ₂ radicals	Recurrent infections with catalase-positive bacteria & fungi
Chediak-Higashi syndrome	Granule structural defect	Recurrent infection with bacteria; chemotactic and degranulation defects; absent NK activity; partial albinism
Leukocyte adhesion deficiency (LAD)	Deficiency of CD18 = β chain of β 2 integrins: LFA-1, complement receptor (CR3), CR4	Recurrent infection with extracellular bacterial pathogens because of defective opsonization, adhesion, mobilization, and chemotaxis
Glucose-6-phosphate dehydrogenase (G6PD) deficiency	Deficiency of essential enzyme in hexose monophosphate shunt	Same as CGD
Myeloperoxidase deficiency	Granule enzyme deficiency	Mild or none

Clinical Correlate

Deficiencies in complement components predispose patients to certain diseases:

- C3 deficiency: Increased susceptibility to pyogenic infections
- C5–C8 deficiency: Recurrent *Neisseria* infections
- C1 inhibitor: Hereditary angioedema
- C2 deficiency: Increased incidence of connective tissue disorders (e.g., systemic lupus erythematosus)
- C1, C4, or C2 deficiency: Opsonization not efficient (leukocyte adhesion deficiency)

Clinical Correlate

B-cell deficiencies

- Increased susceptibility to bacterial infections
- X-linked hypogammaglobulinemia (Bruton)
- Transient hypogammaglobulinemia of infancy
- Common variable hypogammaglobulinemia
- Selective immunoglobulin deficiency

3. Complement deficiencies

Patients with complement deficiencies are susceptible to different diseases depending on which component is missing, as different components have different biological functions.

4. B-cell deficiencies

Patients with B-cell deficiencies usually present with recurrent pyogenic infections with extracellular pathogens. The absence of immunoglobulins for opsonization and complement activation is a major problem. The T-cell immune system is intact, and T-cell activities against intracellular pathogens, delayed hypersensitivity, and tumor rejection are intact.

a. Bruton X-linked hypogammaglobulinemia

- i. First appears in childhood (>6 months)
- ii. Immunologic findings
 - Low immunoglobulin—all classes
 - No circulating B cells (i.e., surface immunoglobulin [sIg] positive)
 - Pre-B cells in the bone marrow
 - Normal cell-mediated immunity
- iii. Primary defect: a block in maturation of the B cell due to a deficiency of a tyrosine kinase
- iv. Treatment: monthly γ -globulin replacement, antibiotics for infection

- b. Transient hypogammaglobulinemia of infancy
 - i. Delayed onset of normal IgG synthesis
 - ii. Seen in the fifth to sixth month of life
 - iii. Usually resolves by 16-30 months of age
 - iv. Treatment: antibiotics and sometimes γ -globulin replacement
- c. Treatment for the B-cell deficiencies is usually monthly infusions of immunoglobulin (exception for IgA deficiency)

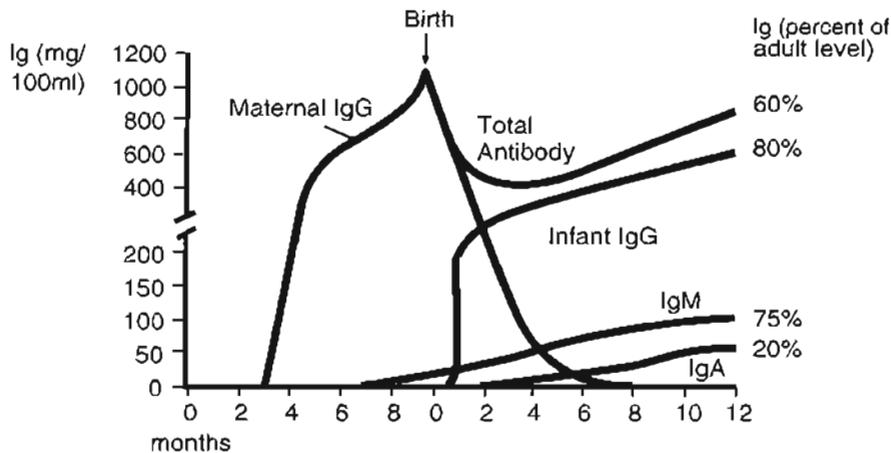


Figure II-13-2. Immunoglobulins in the Serum of the Fetus and Newborn Child

- d. Common variable (acquired) hypogammaglobulinemia
 - i. Immunoglobulin levels decrease with time
 - ii. First appears in late teens to early 20s
 - iii. Associated with autoimmunity in the patient or in the family
 - iv. A syndrome that is probably several different diseases
 - v. Low immunoglobulins—any class
 - vi. B cells present in the peripheral blood
 - e. Selective Ig deficiency (dysgammaglobulinemia)
 - i. Several different diseases described
 - ii. Selective IgA deficiency is the most common
 - Repeated sinopulmonary infection, gastrointestinal disease
 - Many with IgA deficiency have no symptoms
 - If both IgA and IgG2 subclass deficiencies, more likely to have infections
 - iii. Treatment: antibiotics, **not** Igs
5. T-cell deficiencies
- Patients with T-cell defects usually present with viral or fungal infections. Although B-cell function is compromised by lack of T-cell help, the major defect is handling intracellular pathogens. Patients receiving immunosuppressive drugs for treatment of allograft transplants may have similar problems with these organisms.

- a. DiGeorge syndrome
 - i. Failure of development of third and fourth pharyngeal pouches
 - Hypoplastic thymus
 - Hypoplastic parathyroid glands
 - ii. Clinical features
 - Thymic aplasia
 - Recurrent viral and fungal infections
 - Hypoparathyroidism—hypocalcemic tetany
 - Cardiac anomalies
 - Facial anomalies (fish mouth and flat face)

b. Chronic mucocutaneous candidiasis

This group of patients has severe chronic skin and mucous membrane infections with the fungal pathogen *Candida albicans*. Specific treatment in these cases is antifungal drugs.

6. Combined B-cell and T-cell deficiencies (SCID)

Patients with combined B-cell and T-cell deficiencies are susceptible to bacterial, viral, and fungal infections, but the T-cell deficiencies usually predominate.

a. Adenosine deaminase (ADA) deficiency

- i. This was the first human disease successfully treated with gene therapy.

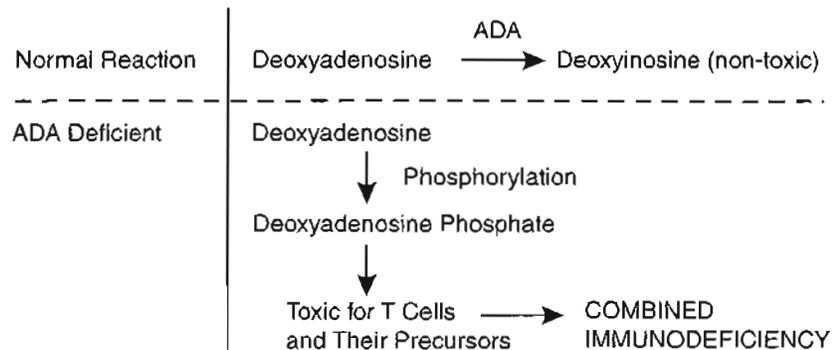


Figure II-13-3. Adenosine Deaminase Deficiency

- b. Severe combined immunodeficiency disease (SCID). SCID has many other X-linked and autosomal inheritance pattern problems. Some of the causes of SCID are deficiencies in class I molecules, class II molecules, T-cell receptors, cytokine receptors, and signal-transduction molecules.
- c. Wiskott-Aldrich syndrome is a complex immune deficiency in which there is a triad of symptoms. These include thrombocytopenia from birth, eczema, and immunodeficiency. It is an X-linked recessive disease. Patients are prone to the development of malignant lymphomas. Patients are also unable to mount a sufficient response to polysaccharide antigen.



Figure II-13-4. The Wiskott-Aldrich Triad

- d. Ataxia-telangiectasia (develops 1–2 years of age)
- i. Sinopulmonary infections
 - ii. Autosomal recessive
 - iii. Ataxia: uncoordinated muscle movements
 - iv. Telangiectasia: dilation of small vessels; seen in sclera of eye
 - v. Immunodeficiency
 - Selective IgA deficiency
 - Cell-mediated defects – variable
 - Other immunoglobulin – variable
 - vi. Treatment: antibiotics

In a Nutshell

Immune Deficiency	Predominating Opportunist
B cell	Pyogenic bacteria
T cell	Viruses and fungi; also mycobacteria
C3	Pyogenic bacteria
C5–8	<i>Neisseria</i>
Phagocytes	Pyogenic bacteria

Chapter Summary

The immunodeficiencies include aberrant phagocytic cell function, complement deficiencies, abnormal B-cell development or function, abnormal T-cell development or function, and combined B- and T-cell problems.

Phagocyte-deficiency diseases generally cause an inability to deal with pyogenic bacteria. They include chronic granulomatous disease (CGD), Chediak-Higashi syndrome, leukocyte adhesion deficiency (LAD), glucose 6-phosphate dehydrogenase (G6PD) deficiency, and myeloperoxidase deficiency.

The diseases associated with complement deficiency vary depending on which of the complement proteins are affected. C3 deficiency results in an inability to deal with pyogenic bacteria; C5 through C8 deficiencies result in an inability to fight *Neisseria* infections.

The B-cell deficiencies result in increased susceptibility to bacterial infection. B-cell deficiency may result in Burton X-linked hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, common variable hypogammaglobulinemias, and selective immunoglobulin deficiencies. The classic treatment has been monthly immunoglobulin infusions.

The major problem associated with T-cell deficiency is an inability to handle intracellular infections, such as those caused by viruses and fungi and the mycobacteria. Diseases associated with T-cell deficiency include DiGeorge syndrome (aplasia of the thymus and parathyroid glands) and chronic mucocutaneous.

Combined B- and T-cell deficient patients are unable to deal effectively with bacterial, viral, or fungal infections. Diseases associated with combined B- and T-cell deficiency include adenosine deaminase (ADA) deficiency, severe combined immunodeficiency disease (SCID), Wiskott-Aldrich syndrome, and ataxia-telangiectasia.

Review Questions

1. A 31-year-old man is treated for a fourth episode of disseminated *Neisseria gonorrhoeae* infection in the last 5 years. He had no previous history of unusual or recurrent infections. If he has an immunological defect, which of those below is most likely
 - A. Selective IgA deficiency
 - B. Severe combined immunodeficiency (SCID)
 - C. Common variable immunodeficiency
 - D. DiGeorge's syndrome
 - E. C8 deficiency
2. A patient with an inherited, homozygous C7 deficiency is most likely to present with
 - A. Staphylococcal abscesses
 - B. Recurrent *Strep. pneumoniae* infection
 - C. Disseminated *Neisseria gonorrhoeae* infection
 - D. Mucosal infection with *C. albicans*
 - E. Pneumocystis pneumonia
3. Patient JGR has been hospitalized 3 times for painful abdominal edema, and is complaining now of swollen lips. Laboratory findings in this patient would most likely include
 - A. Abnormal T cell function
 - B. Abnormal T cell numbers
 - C. Defective neutrophils chemotaxis
 - D. Reduced C4 levels
 - E. Abnormal superoxide anion production by neutrophils
4. A four-year-old girl presents with a severe *Staphylococcus aureus* abscess. Her history is significant for a previous infection with *Serratia marcescens*. If she has an enzyme deficiency, which of the following is most likely
 - A. C1-inhibitor
 - B. Adenosine deaminase (ADA)
 - C. Factor VIII
 - D. Superoxide dismutase (SOD)
 - E. NADPH oxidase
5. A patient with a 3-day history of a Staphylococcal skin abscess arrives in your office. On examination you find the abscess to be cold, and histology shows no leukocyte infiltrate. You would most likely suspect
 - A. A C5 deficiency
 - B. Defective synthesis of leukotriene B4
 - C. Reduced responsiveness of leukocytes to IL-8
 - D. An opsonic defect
 - E. Reduced leukocyte oxidative metabolism

6. There are many different types of immune deficiencies. Patients who have phagocytic defects are unable to generate strong oxidative killing. Which of the following would be considered to be a phagocytic defect?
- A. Hereditary angioedema
 - B. Bruton disease
 - C. DiGeorge syndrome
 - D. Chronic granulomatous disease
 - E. ADA (adenosine deaminase deficiency)
7. A new pediatrician has just opened his office next to the hospital and one of his first patients was a small four-year-old boy. His mother brought him into the office because he had several boil-like pyogenic lesions on his arm. His mother told the physician that the boy had these lesions on several different occasions. His other physician had prescribed antibiotics and the lesions resolved. His records indicate he has had all his immunizations. The pediatrician ordered several different laboratory tests and the following results were reported. Immunoglobulin levels were normal. B cell and T cell counts were normal. Complement levels were normal; calcium and parathyroid hormone (PTH) levels were normal. The NBT (Nitroblue Tetrazolium Test) for reactive oxygen intermediates in phagocytic cells was negative. The mother told the physician that she was not aware of any eczema or bleeding problems. Which disease is indicated by the above data?
- A. DiGeorge syndrome
 - B. Chronic granulomatous disease
 - C. Bruton sex-linked agammaglobulinemia
 - D. Wiskott-Aldrich syndrome (WAS)
 - E. Severe Combined Immunodeficiency Disease (SCID)
8. Which one of the following immune deficiency diseases has a triad of thrombocytopenia, eczema, and immunodeficiency?
- A. Ataxia-telangiectasia
 - B. Wiskott-Aldrich syndrome (WAS)
 - C. Severe Combined Immunodeficiency Disease (SCID)
 - D. Thymic hypoplasia
 - E. Multiple myeloma
9. Selective IgA deficiency is most commonly associated with which one of the following?
- A. Asthma
 - B. Recurrent sinopulmonary infections
 - C. Systemic lupus erythematosus
 - D. Myasthenia gravis
 - E. Ulcerative colitis

10. An acutely ill two-year-old boy was hospitalized with *Staphylococcus aureus pneumonia*, which was treated appropriately. The history indicated similar bouts of bacterial infections in the past. He recovered uneventfully from measles six months ago. Physical exam disclosed scant tonsillar tissue and no palpable lymphadenopathy. Quantitative immunoglobulins revealed absence of IgM and IgA, but a small amount of IgG. The NBT and chemiluminescence assays indicated normal phagocytic killing. Which of the following disorders is most likely responsible for this child's infections?
 - A. Common variable hypogammaglobulinemia
 - B. Wiskott-Aldrich syndrome
 - C. Bruton agammaglobulinemia
 - D. DiGeorge syndrome
 - E. Selective immunoglobulin deficiency

11. A two-year-old boy is brought to his family pediatrician suffering from repeated painful bouts of inflammation of mucosal surfaces, especially the lips. The mother remembers similar symptoms in previous generations of her family, and fears a heritable tendency toward food allergy. The laboratory finding that would best support the physician's suspicion of hereditary angioedema would be
 - A. Elevated C1
 - B. Elevated C1, C4, C2
 - C. Depressed C3
 - D. Depressed C4
 - E. Depressed C5

Answers

1. **Answer: E.** Unusual frequency or severity of *Neisseria* infections should always lead to a suspicion of a terminal complement component deficiency (C5, C6, C7, or C8). This patient would not have SCID because he would have had a long childhood history of severe viral, fungal, and bacterial infection. Similarly, DiGeorge syndrome (congenital thymic aplasia) would predispose to early viral and fungal infections, since it produces a profound T-cell deficiency. IgA deficiency, if symptomatic at all, would be expected to result in respiratory tract infections, GI tract disease, autoimmune disease or allergies. Common variable immunodeficiency certainly may be acquired in adulthood, but usually presents as pneumococcal, or other pyogenic bacterial infection, certainly not selective to *Neisseria*.

2. **Answer: C.** Deficiency of terminal complement components (C5, C6, C7, or C8) prevents complement-mediated lysis, which is crucial for defense against *Neisseria*. Thus, *N. meningitidis* would also be a correct answer for this question. The Gram-positive bacteria are all resistant to complement lysis, as are fungi and most parasites.

3. **Answer: D.** The description of painful abdominal edema and edema in the oral mucosa are absolutely typical of hereditary angioedema, a deficiency of C1-inhibitor. When this control protein is decreased, there is excessive utilization (consumption) of the classical complement pathway components, especially C4 (choice D). T cell abnormalities (choices A and B) would result in increased risk for viral or fungal infection. Defects in neutrophil movement or oxygen radical production (choices C and E) would predispose the patient to bacterial infection.

4. **Answer: E.** The infections are most characteristic of chronic granulomatous disease (CGD). While two-thirds of CGD patients are male, one-third have the autosomal recessive form of NADPH oxidase deficiency and can be female. ADA deficiency produces severe combined immune deficiency (SCID), and the infections are more likely to be due to the T-cell deficiency. For example, viral or fungal infections would be most likely, and of the bacteria, Streptococcal infections would be the biggest problem, and they do not pose difficulties for CGD patients. C1-inhibitor is not an enzyme, and its absence does not predispose to infection. SOD deficiency in leukocytes has not been reported, but would not likely lead to infection. Factor VIII deficiency causes hemophilia, but not infections.
5. **Answer: C.** The lack of an inflammatory response suggests a defect in leukocyte movement into the site of infection. Choices **D** and **E** would not result in such a finding, since they would affect leukocyte function after leukocyte accumulation at the infection site. A defect in an individual chemotactic factor (choices **A** and **B**) would be unlikely, as there are so many of these, and their functions are redundant. However, a defect in leukocyte responsiveness to chemotactic stimuli could be a general movement problem that would affect leukocyte movement in response to any chemotactic factor.
6. **Answer: D.** This disease is a deficiency in NADPH oxidase enzyme and the superoxide ions, hydrogen peroxide, and hypohalides cannot be formed. The NBT reduction assay is the test for the identification of this disease.
7. **Answer: B.** The fact that the boy had several different episodes with different pyogenic bacteria and they were eliminated only with antibiotics suggests a possible inability to kill the bacteria with normal intracellular phagocytic killing. The NBT test for reactive oxygen intermediates was negative. They were not being formed due to deficiency of NADPH oxidase enzyme.
8. **Answer: B.** This is the classic triad of this disease. The patient will have bleeding problems early in life. Later on the patient will develop a skin eczema condition, and finally they will develop immunodeficiency and neoplasia.
9. **Answer: B.**
10. **Answer: C.**
11. **Answer: D.** Hereditary angioedema is caused by a deficiency in C1 inhibitor, so early components in the classical pathway are used up more rapidly than normal, especially C4 and its levels would be depressed in the blood. Depressed C3 is not a normal sequela of this problem because there are multiple independent controls on its consumption, and this is also true of C5.

Failures of the Immune System: Autoimmunity

14

We usually exhibit tolerance to antigens that are recognized as **self**. A defect in this mechanism results in **autoimmune disease**. The most important step in the production of autoimmune disease is the **activation of self-reactive helper T CD4⁺ lymphocytes**. These Th1 or Th2 cells can induce either **cell-mediated (CMI)** or **antibody-mediated autoimmune reactions**.

A. Systemic Autoimmune Diseases (Collagen or Vascular)

1. Rheumatoid arthritis (RA)

- a. Inflammation of the joint
 - i. In synovial membrane
 - ii. Becomes proliferative
 - iii. Destroys cartilage and bone
- b. Immunologic features
 - i. Rheumatoid factor (IgM against own IgG)
 - ii. Immune complexes in joint fluid
 - iii. Antinuclear antibody (ANA): positive in some patients

2. Systemic lupus erythematosus (SLE)

- a. Multiorgan (skin, mucosa, kidney, brain, and CV system)
- b. Primarily young women
- c. Malaise, fever, lethargy, and weight loss
- d. Butterfly rash
- e. Renal involvement (lumpy-bumpy pattern of immune complex deposits)
- f. CNS manifestations
- g. Immunologic features
 - i. ANA
 - ii. Anti-dsDNA
 - iii. Many other autoantibodies
 - iv. Hypergammaglobulinemia
 - v. Decreased levels of complement

3. Goodpasture syndrome

- a. Young adult men
- b. Affects lungs and kidneys
- c. Linear deposits of IgG and complement in alveolar and glomerular basement membranes
- d. Antibody directed at type IV collagen

Clinical Correlate

Genetic predisposition of autoimmune diseases

Many autoimmune diseases exhibit a marked familial incidence. There is strong association of some diseases with certain HLA types:

- DR3/DR4 for type 1 diabetes
- B27 for ankylosing spondylitis
- DR4 for rheumatoid arthritis

Diagnostic facts important in autoimmune diseases

- Elevated serum levels of γ -globulin
- Autoantibodies
- Decreased levels of complement
- Immune complexes
- Lesions in tissues

In a Nutshell

Tolerance is antigen-specific and it can be broken with a crossreacting antigen. Remember that autoreactive (generally low-affinity) B cells are present but do not have either helper T cells or other signals required for immune response. The crossreacting antigen can provide these signals. Acquired tolerance can be induced by using large doses of protein antigens and maintenance of the antigen level in the host. This procedure is being used experimentally for MS (multiple sclerosis) patients by feeding large doses of myelin basic protein to make the body tolerant to the antigen.

B. Organ-Specific Autoimmune Diseases

1. Multiple sclerosis
 - a. Mononuclear infiltrates with demyelination of CNS
 - b. Immune response to myelin basic protein
 - c. Increased IgG in spinal fluid (oligoclonal)
 - d. Clustering of cases suggestive of responsible infection
 - e. Antibodies to measles virus
 - f. Motor weakness, ataxia, impaired vision, bladder dysfunction, paresthesias, and mental aberrations
2. Myasthenia gravis
 - a. Muscle weakness and fatigue
 - b. Faulty neuromuscular signal transmission
 - c. Interaction with acetylcholine blocked by antibody to ACh receptors
 - d. Loss of receptors due to endocytosis
3. Chronic thyroiditis (Hashimoto)
 - a. Enlarged thyroid gland
 - b. Lymphocyte and plasma cell infiltrate with varying amounts of fibrosis
 - c. Hypothyroidism: low circulating thyroid hormones
 - d. Antibodies versus
 - i. Thyroglobulin
 - ii. Microsomal antigen
4. Graves disease
 - a. Antibodies to thyrotropin receptors
 - i. Block binding of thyroid-stimulating hormone
 - ii. Cause proliferation of thyroid cells
 - iii. Thyroid-stimulating antibody—mimics thyrotropin
 - b. Hyperthyroidism, thyrotoxicosis
 - c. Overproduction of thyroid hormone
 - d. Fatigue, nervousness, sweating, palpitations, weight loss, heat intolerance
 - e. Increased B cells: correlates with disease severity
5. Type 1 diabetes: insulin-dependent diabetes mellitus (IDDM)
 - a. Inability to synthesize insulin
 - b. Destruction of β cells of the islets of Langerhans by Tc (cytotoxic T; CD8)
 - c. Fluctuations of blood glucose, ketoacidosis, polyuria, polydipsia, and polyphagia
 - d. Hyperglycemia: leads to cardiovascular disease, neuropathies, kidney disease, retinopathy
 - e. Treatment: dietary and insulin
 - f. Immunologic features: antibodies and T-cell responses
 - i. Anti-islet cell antibodies
 - ii. Anti-insulin antibodies
 - iii. Cytotoxic T cells—versus the islet cell
 - iv. Islets infiltrated with T and B cells and eventually destroyed

6. Pernicious anemia
 - a. Defective red blood cell maturation due to malabsorption of vitamin B₁₂
 - b. Block of B₁₂ transport across the small intestine by intrinsic factor
 - c. Parietal cells that make intrinsic factor are lost because gastric glands are destroyed
 - d. Autoantibodies
 - i. Intrinsic factor: may block attachment of B₁₂
 - ii. Parietal cell antigens
7. Idiopathic thrombocytopenic purpura (ITP)
 - a. Antibody-mediated platelet destruction
 - b. Petechiae and bleeding problems
 - c. Platelet counts suppressed
 - d. IgG antibodies to platelets: removed by the spleen
 - e. Can be drug induced: drug-antibody complexes adsorbed to a platelet with resulting complement activation—damage to platelet
8. Guillain-Barré syndrome
 - a. Immune reaction against myelin
 - b. T-cell mediated

C. Treatment of Autoimmune Disorders Depends on the Disease

- Anti-inflammatory drugs: SLE, RA
- Plasmapheresis: SLE, Guillain-Barré
- Splenectomy: ITP
- Anticholinesterase and thymectomy: Myasthenia gravis
- Metabolic control: Graves disease and pernicious anemia
- Hormone replacement: Hashimoto thyroiditis, type I diabetes

D. Immunosuppressive Agents: Two Categories

1. Lympholytic: Blocks initiation and/or expression of immunity.
 - Ionizing radiation
 - Antiserums
 - i. Antilymphocyte serum
 - ii. Antithymocyte serum (Ab versus CD3, CD4, etc.)
 - iii. Anti-RhD (RhoGAM)
2. Lymphocytotoxic: Blocks induction of immunity by interfering with cell DNA synthesis, for example.
 - Antimetabolites, e.g., base analogues and folic acid antagonists
 - Alkylating agents, e.g., cyclophosphamide, that cause base pair errors

Clinical Correlate

Several microbial diseases are associated with development of autoimmune sequelae:

- Measles: Subacute sclerosing panencephalitis (SSPE)
- Rubella: Type 1 diabetes
- Influenza and *Campylobacter*: Guillain-Barré syndrome

Chapter Summary

The autoimmune diseases result from a failure to recognize a cellular or tissue component as self and are generally associated with the activation of self-reactive CD4⁺ helper T cells. These cells can induce either cell-mediated or antibody-mediated autoimmune reactions. The unremitting production of antibody associated with some autoimmune diseases may also cause hypersensitivity reactions.

The systematic autoimmune diseases include rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Goodpasture syndrome.

Organ-specific autoimmune diseases include multiple sclerosis, myasthenia gravis, chronic (Hashimoto) thyroiditis, Graves disease, type 1 diabetes, pernicious anemia, idiopathic thrombocytopenic purpura (ITP), and Guillain-Barré syndrome.

Treatments for these diseases include antiinflammatory drugs for SLE and RA, plasmapheresis for SLE and Guillain-Barré syndrome, splenectomy for ITP, cholinesterase inhibitors and thymectomy for myasthenia gravis, metabolic control for Graves disease and pernicious anemia, and hormone replacement for Hashimoto thyroiditis and type 1 diabetes.

Immunosuppressive agents are either lympholytic or lymphocytotoxic.

Review Questions

1. Which one of the following diseases is characterized by decreased thyroid function, antithyroglobulin antibodies, antimicrosomal antibodies, and low thyroid hormone levels?
 - A. Graves disease
 - B. Goodpasture's syndrome
 - C. Hashimoto disease
 - D. Myasthenia gravis
 - E. Multiple sclerosis
2. The department of pathology at the university hospital received a call in the rheumatology section from a family practice physician. He had a 34-year-old male patient with hemoptysis (blood-stained sputum), uremia (blood-stained urine), radiographic evidence of focal pulmonary consolidations, and manifestations of glomerulonephritis. The patient was referred to the rheumatology section for further evaluation. It was determined that the patient had deposits of IgG and complement in alveolar and glomerular basement membranes. This was a major pathological disease with poor prognosis in this patient. Which one of the following diseases is characterized by these deposits in these two organs?
 - A. Rheumatoid arthritis
 - B. Systemic lupus erythematosus
 - C. Myasthenia gravis
 - D. Graves disease
 - E. Goodpasture's syndrome

3. Which of the following characterizes the classic rheumatoid factor?
 - A. IgM anti-IgG
 - B. IgG anti-IgG
 - C. IgA anti-IgM
 - D. IgG anti-IgM
 - E. IgM anti-IgA

4. All of the following suggest the autoimmune nature of insulin-dependent (Type I) diabetes mellitus, except
 - A. The association with DR3 and DR4 HLA antigens.
 - B. The presence of insulin autoantibodies in newly diagnosed patients.
 - C. The frequent finding of anti-islet cell antibodies early in the disease.
 - D. Lymphocyte infiltration of the pancreatic islets of Langerhans.
 - E. The finding of insulin receptor deficiency in the majority of patients.

5. Patients with systemic lupus erythematosus form autoantibodies most commonly against which of the following substances?
 - A. M protein of *Streptococcus pyogenes*
 - B. 60S ribosomal subunit
 - C. Double-stranded DNA
 - D. Myeloperoxidase
 - E. Muramic acid

Answers

1. **Answer: C.** This disease is characterized by an enlarged thyroid gland. Lymphocyte and plasma cell infiltrate with hypothyroidism. There are antibodies detected against thyroglobulin and microsomal antigens.

2. **Answer: E.** Goodpasture's syndrome is an autoimmune disease in which antibody is produced against our own Type IV collagen in the membranes of lungs and kidney. The antibody plus complement forms immune complexes in these organs. The immune complexes that are seen in fluorescent antibody tests produce a very characteristic linear design. In this design, the antibody and complement complexes are a constant staining pattern (linear). The antibody/complement complexes react with all of the Type IV collagen in the whole membrane.

3. **Answer: A.**

4. **Answer: E.**

5. **Answer: C.**

Regulation and Tolerance

15

A. Immune Regulation

Regulation of immune responses, which prevents overproduction of antibody or excessive proliferation of T cells and/or B cells, occurs at several levels.

1. **Antigen concentration:** As antigen levels decrease during an immune response that successfully eliminates them, there is less of a stimulus for continued proliferation and differentiation of lymphocytes, so immune responses decline. However, small amounts of antigen may persist in the lymph node follicles, which are attached to dendritic cell surfaces, for prolonged periods of time. Other mechanisms are therefore also needed to turn off the response.
2. **Antibody levels:** Free IgG antibody at high concentrations **can suppress immune responses**, but this is a less important mechanism than the others listed here.
3. **Antigen-antibody complexes:** When antigen is free, it can bind to B cells and act as a **stimulatory** signal for antibody production; however, when most of the antigen is bound in **complexes** with IgG, these complexes deliver an inhibitory signal to B cells, preventing further differentiation or proliferation.
4. **Anti-idiotypic antibodies:** As a particular clone of B cells expands in number, and the cells differentiate to plasma cells, the concentration of the particular idiotype of antibody produced by that clone will increase enormously. The **idiotype** itself will reach concentrations high enough that it can be recognized as an antigen, and an antibody response against it will be triggered. This anti-idiotypic will usually turn off the immune response of the initial B (or T) cell.
5. **Helper T-cell subset cytokine regulation:** This is one of the most important regulatory mechanisms. Antigen-stimulated helper T cells exist in two distinct subsets, **Th1** and **Th2**. Each helper subset **stimulates** one arm of the immune system, and **inhibits** the other, mainly through secretion of different cytokines.
 - a. The **interferon- γ** secreted by the Th1 cell **inhibits** Th2 cytokine production.
 - b. **Th2** cells, on the other hand, stimulate antibody production from **B cells (humoral immunity)** and direct it against extracellular pathogens (bacteria, extracellular parasites) or soluble foreign antigens (e.g., exotoxins) through the production of **interleukin (IL)-4, IL-5, IL-6, and IL-10**. **IL-4** is a potent **inhibitor** of macrophage function, whereas **IL-10** inhibits Th1-cell function.

B. Immune Tolerance

Tolerance is the absence of **specific immune responses** in an otherwise fully immunocompetent person. This unresponsiveness can be either autotolerance (naturally acquired) or specifically induced acquired tolerance.

1. Autotolerance

Autotolerance is to one's own antigens. This tolerance is naturally acquired during fetal life in the central lymphoid organs and involves deletion of autoreactive clones.

Immature B cells are also inactivated by contact with antigen. Depending on the nature of the antigen, the inactivated B cells either die immediately (**clonal deletion**) or they persist in a nonfunctional state (**clonal anergy**). For this reason, tolerance in B cells is not as complete as it is in T cells.

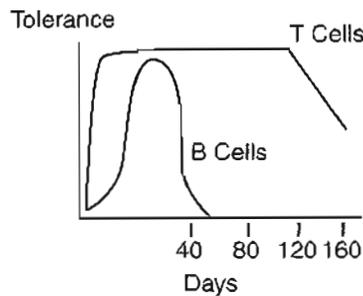


Figure II-15-1. T Cells Are More Sensitive to Tolerance Induction

Tolerance is **antigen-specific** and it can be **broken** with a cross-reacting antigen. Remember that autoreactive (generally low-affinity) B cells are present but do not have either helper T cells or other signals required for immune response. The crossreacting antigen can provide these signals. Acquired tolerance can be induced by using large doses of protein antigens and maintenance of the antigen level in the host. This procedure is being used experimentally for MS (multiple sclerosis) patients by feeding large doses of myelin basic protein to make the body tolerant to the antigen.

Chapter Summary

Immunoregulation prevents the overproduction of antibody or the excess proliferation of B or T cells. Five different mechanisms are succinctly described.

Immune tolerance is the absence of an immune response in a fully immunocompetent individual. Autotolerance is the normal tolerance to self. Failure of autotolerance mechanisms results in an autoimmune disease. Induced tolerance can be acquired using large doses of antigen to maintain a high level. This process is under trial as a potential treatment for multiple sclerosis.

Transplantation Immunology

16

The HLA is one of the most polymorphic gene systems known. Because the gene products are expressed codominantly from each parent, it is highly unlikely to find haplotypes from unrelated individuals.

The problem in transplantation is that if donor and recipient major histocompatibility (MHC) molecules are not identical, the recipient T cells will recognize the donor MHC molecules as self, but with some small differences in the peptide-binding groove. This difference will be sufficient enough to trigger both helper and cytotoxic T-cell responses against the transplanted tissue. Thus, lifelong general immunosuppression by drugs has been the only way to prevent rejection in all but identical twin transplants. Even in such twins, somatic mutations require some degree of suppression because total identity is lost over time. Cyclosporin A is an excellent immunosuppressive drug that acts in a limited way (inhibiting interleukin [IL]-2 production and IL-2 receptor expression) but is still a nonspecific immune suppressor.

A. Types of Grafts

1. **Allograft:** Transplant from one individual to another with a different genetic make-up, within the same species, e.g., kidney transplant from one person to any other (except an identical twin).
2. **Isograft or syngeneic graft:** Transplant between genetically identical, monozygotic twins, or between members of an inbred strain of animals.
3. **Autograft:** Transplant from one site to another on the same individual, e.g., transplanting a blood vessel from the leg to the heart during cardiac bypass surgery. This type of transplant does not require immunosuppressive therapy.
4. **Xenograft:** Transplant across species barriers, e.g., transplanting a heart from a baboon to a human. Xenografts have a very poor prognosis because of the presence of crossspecies reactive antibodies that will induce hyperacute rejection.

B. Graft-versus-Host Disease

Graft-versus-host disease can occur in the special case in which immunocompetent tissue (fresh whole blood, thymus, or bone marrow) is transplanted into an immunocompromised host. T cells from the transplant recognize the host MHC molecules as nonself and attack the host. This is a type IV hypersensitivity reaction, in which antibody plays no role at all.

Table II-16-1. Type and Tempo of Rejection Reactions

Type of Rejection	Time Taken	Cause
Hyperacute	Minutes to hours	Preformed antidonor antibodies and complement
Accelerated	Days	Reactivation of sensitized T cells
Acute	Days to weeks	Primary activation of T cells
Chronic	Months to years	Causes are unclear: antibodies, immune complexes, slow cellular reaction, recurrence of disease

Clinical Correlate

In kidney transplants, it is more important to match MHC class II than class I.

Clinical Correlate

Drugs used for postoperative immunosuppression

- Corticosteroids
- Antimetabolites
- Alkylating agents
- Cyclosporin A
- Antiserums

Chapter Summary

An immune response occurs if the HLA gene products of a transplant donor differ from those of a recipient. However, the HLA gene system is so highly polymorphic that it is essentially impossible to find full identity. Somatic mutation is likely even to produce a difference between identical twins. Therefore, immunosuppressive drugs, such as cyclosporin A, must be used to prevent rejection.

Grafts are classified according to the relationship between host and donor: an allograft is from one member of a species to another of the same species, an isograft or syngeneic graft is between genetically identical organisms, an autograft is from one site to another on the same organism, and a xenograft is between species.

Graft-versus-host disease occurs when T cells from the graft recognize the host as foreign and mount an attack against it. This occurs when immunocompetent cells, such as bone marrow cells, are transferred.

Hyperacute rejection reactions occur within minutes, accelerated rejection reactions within days, acute rejection reactions from days to weeks, and chronic rejection reactions from months to years after the transplant is done.

Review Questions

1. A 42-year-old auto mechanic has been diagnosed with end-stage renal disease. His twin brother is HLA identical at all MHC loci, and volunteers to donate a kidney to the brother. What type of graft transplant terminology is correct in this situation?
 - A. Allograft
 - B. Syngeneic graft
 - C. Xenograft
 - D. Autograft
 - E. Heterograft
2. The tempo of rejection of a graft depends upon many different factors. Which type of graft rejection depends on preformed antibodies present in the recipient of the graft?
 - A. Chronic
 - B. Acute
 - C. Accelerated
 - D. Hyperacute
 - E. Graft-versus-host
3. Which one of the following grafts would be successful and would not require any immunosuppressive drugs?
 - A. Graft with isoantigens
 - B. Graft with heterophile antigens
 - C. Allogeneic graft
 - D. Xenogeneic graft
 - E. Syngeneic graft

4. A patient with acute myelogenous leukemia (AML) undergoes irradiation and chemotherapy for his malignancy while awaiting bone marrow transplantation from a closely matched sibling. Six months after the transplant the immune response appears to be reconstituting itself well until at 9 months post infusion, symptoms of generalized rash with desquamation, jaundice, and bloody diarrhea begin to appear. A second more closely matched bone marrow donor is sought unsuccessfully and 10 months after the transfer, the patient dies. The immunological effector mechanism most closely associated with graft-versus-host rejection is
 - A. Activated macrophages
 - B. CD8⁺ lymphocytes
 - C. Antibodies and complement
 - D. NK cells
 - E. LAK cells

5. Which one of the following is the most important set of antigens to evaluate prior to organ allotransplantation in humans?
 - A. ABO and HLA
 - B. ABO and Gm
 - C. ABO and Rh
 - D. Rh and IL-2
 - E. Rh and gamma interferon

Answers

1. **Answer: B.** A syngeneic graft is the transfer of tissue between genetically identical individuals (identical twins). This type of graft is usually successful.

2. **Answer: D.** This type of reaction occurs within minutes following the transfer of the graft into the recipient. The preformed antibodies in the recipient will activate complement systems, coagulation systems, and neutrophils adherence, and degranulation occurs within minutes. This is a very fast form of Type II hypersensitivity reaction.

3. **Answer: E.** A syngeneic graft is a graft between genetically identical individuals such as identical twins. These grafts are successful and do not require any drug regime.

4. **Answer: B.** GVH is primarily a manifestation of sensitization of transplanted T cells against recipient tissues. The killing of mucosal and other epithelial cells is largely mediated through recognition of MHC class I incompatibility by transferred cytotoxic cells or their precursors, although, eventually, continuous priming by the host's own tissues will elicit immune responses at the level of all the cells of the immune system. NK and LAK cells are believed to be involved in rejection of bone marrow transplants by the recipient (host versus graft) but not in GVH disease.

5. **Answer: A.**



Immunology Laboratory Procedures

17

There are hundreds of different laboratory techniques used in immunology and disease diagnosis. The following discussions will simply illustrate some of the types of reactions with examples.

A. Agglutination Test

This is a test of the reaction of antibody with particulate antigen. The antigen could be red blood cells (RBCs), bacteria, or latex beads. Either the antibody or the antigen could be from the patient. An example of this type of test would be the standard ABO and Rh blood typing.

Blood Types

					
Type A Antigen B Antibody	 A Anti-B Antibodies	N	A	N	A
Type B Antigen A Antibody	 B Anti-A Antibodies	A	N	N	A
Type O No A or B Ag A & B Ab	 O Anti-A and Anti-B Antibodies	A	A	N	A
A & B Ag No A or B Ab	AB No Antibodies to A or B	N	N	N	N

A= Agglutination
N= No Agglutination

Figure II-17-1. Agglutination Test

1. The Coombs test for hemolytic anemia

The **Coombs test** is commonly used to detect **anti-RBC** antibodies. This can occur in **hemolytic anemia** and **Rh incompatibility**. Patients with hemolytic anemia due to the presence of autoantibodies on their surface directed against RBC antigens can be identified by using **anti-human γ -globulin**.

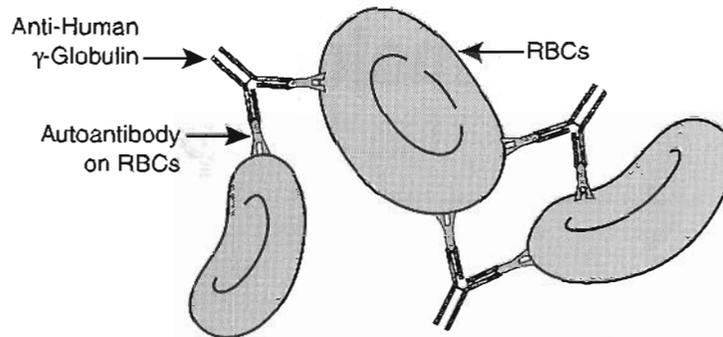


Figure II-17-2. Hemolytic Anemia

2. The Coombs test for Rh incompatibility

The Coombs test can be used to detect antibody on the surface of an infant's Rh⁺ RBC or to detect Rh antibody in the mother's serum against the infant's Rh⁺ cells. The **direct Coombs test** is used to detect the antibody already on the infant's RBCs. The anti-human γ -globulin would be added directly to the cells and they would agglutinate. The **indirect Coombs test** would be using mother's serum to detect antibody against the RhD⁺ cells. The reaction would involve known RhD⁺ cells + mother's serum + anti-human γ -globulin.

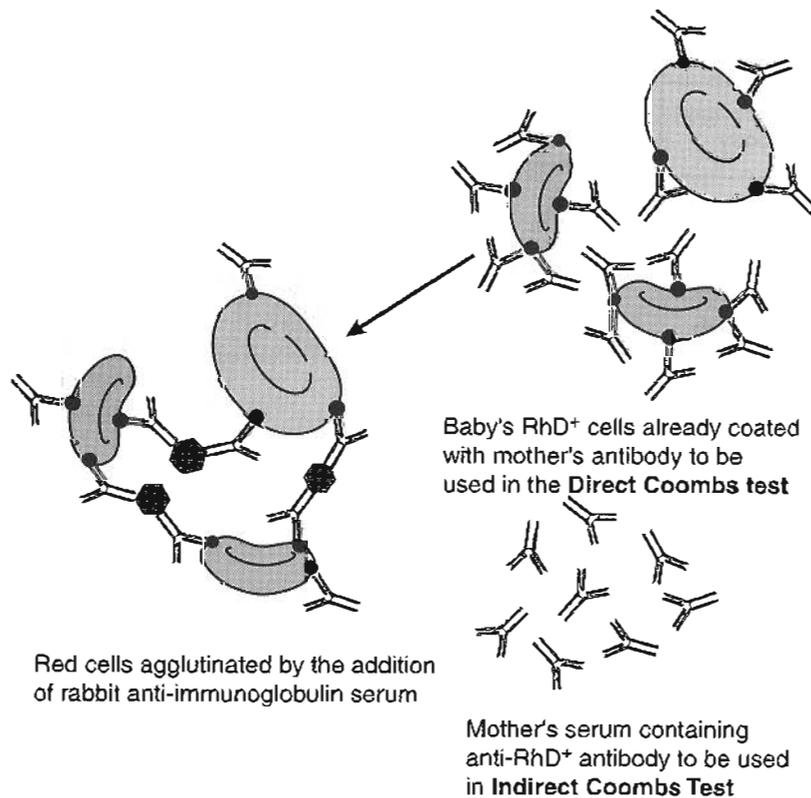


Figure II-17-3. Coombs Test

B. Precipitation Reactions

These are immunologic tests using a soluble **antigen** and soluble **antibody**. The reaction involves a lattice structure being formed and precipitation of Ag-Ab complexes occurring at an optimal concentration.

This type of reaction could be performed to detect **toxins, capsules, immunoglobulin (Ig) levels, or any soluble product from a pathogenic organism.**

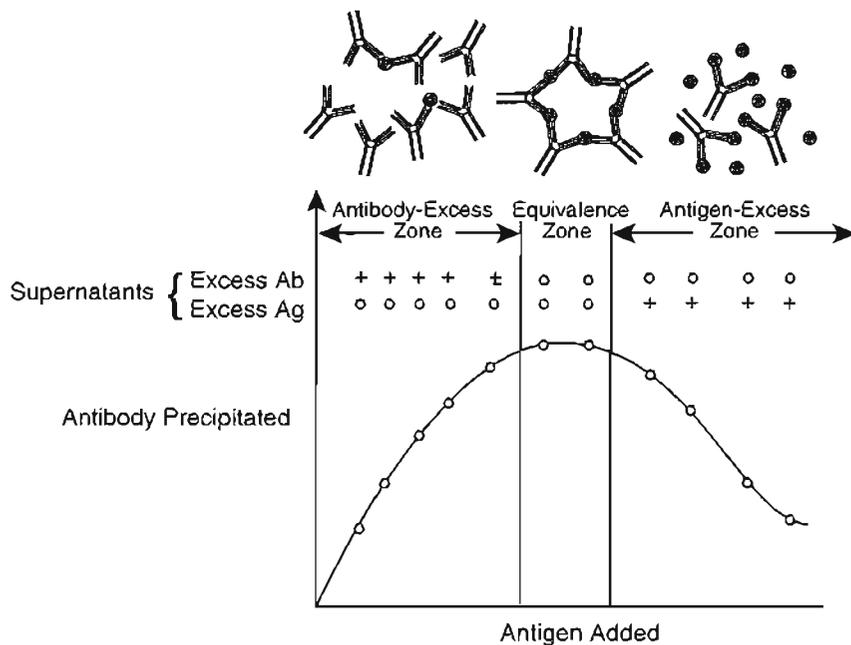


Figure II-17-4. Precipitation of Ag-Ab Complexes Caused by Titration of Ag With Ab

One of the most common precipitin tests used in clinical medicine is the **radial immunodiffusion (RID)** for **Ig** levels. The test is used to detect a **patient's serum level of IgG, IgM, and IgA**. The test involves putting anti-human Ig for the type you are testing into an agar medium, cutting wells in the medium, putting the patient's serum into the well, and allowing precipitation to occur between the reagents. A standard curve measuring the diameters of the zones of precipitation is used to determine the patient's Ig level.

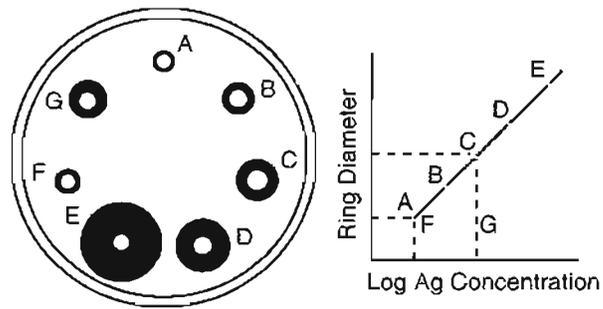


Figure II-17-5. RID Test

Clinical Correlate

Common uses of the DFA test in medicine

- RSV (respiratory syncytial virus)
- Primary syphilis chancre
- Pneumocystis

C. Fluorescent Antibody Tests

1. The **direct fluorescent antibody (DFA)** test

The DFA test is used to detect **antigen** in the patient. The sample could be any tissue suspected of infection with a specific organism.

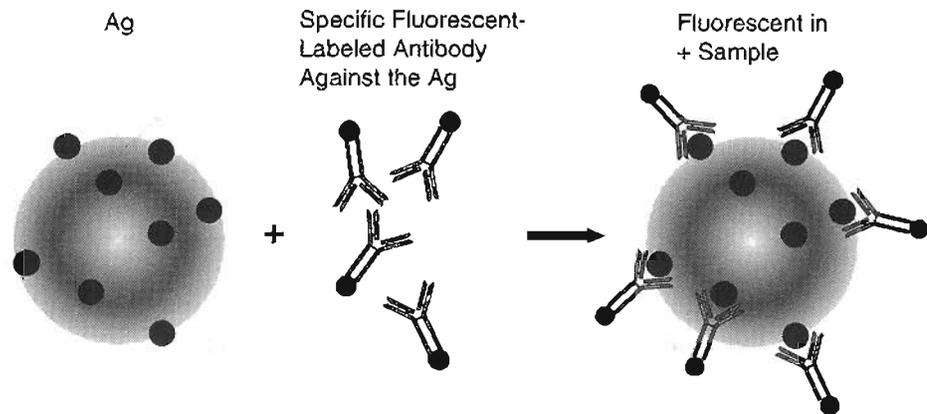


Figure II-17-6. DFA Test

Clinical Correlate

Common uses of the IFA test in medicine

- Systemic lupus erythematosus
- Secondary syphilis

2. The **indirect fluorescent antibody (IFA)** test

The IFA test is used to detect **antibody** in the patient.

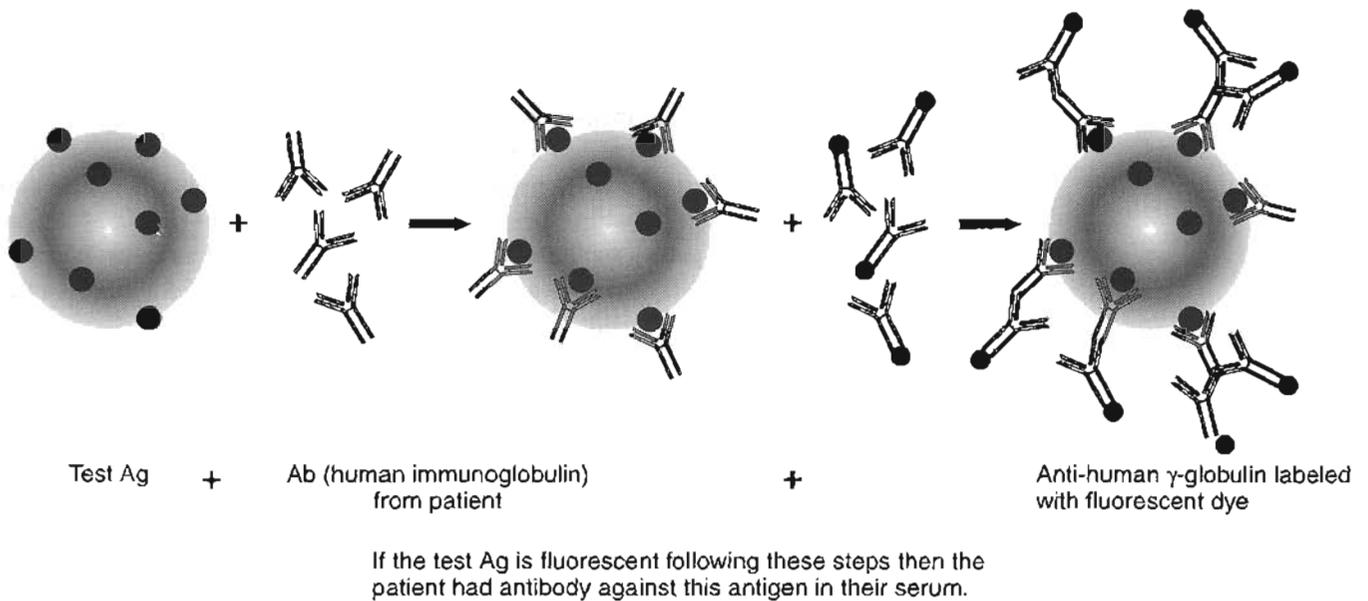


Figure II-17-7. Indirect FA Test

D. Radioimmunoassay (RIA) and Enzyme-Linked Immunosorbent Assay (ELISA, ELA)

RIA and ELISA are the typical assays used when **more sensitivity** is needed for **detection of antigen or antibody**. These assays can be used for hormones, drugs, antibiotics, serum proteins, organisms or organism products, and tumor markers.

ELISA is used as a **screening test for HIV**. The antigen is p24, which is purified and coated on microtiter plates. This test detects the anti-p24 IgG in the patient's serum. Some patients become positive (**seroconvert**) 6 weeks after initial infection. Many require months to seroconvert, with most becoming seropositive within 6 months of infection.

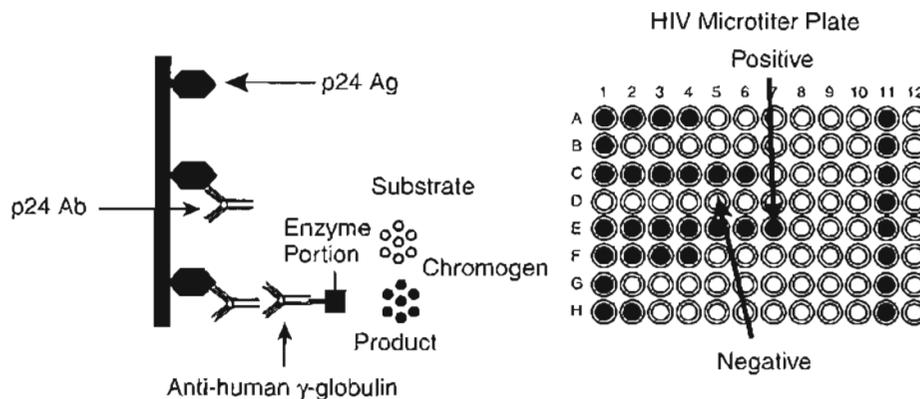
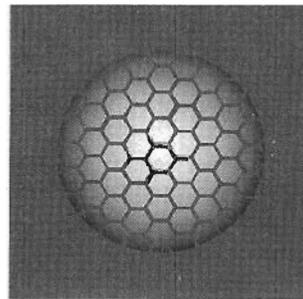


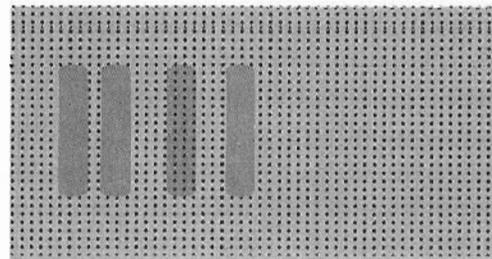
Figure II-17-8. ELISA Test

E. Western Blot or Immunoblot

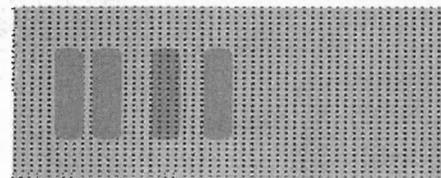
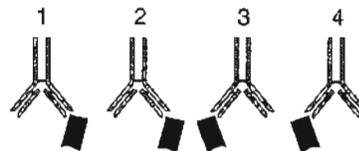
Western blot is used for **confirmation of HIV infection**. This procedure consists of separating viral antigens by SDS polyacrylamide gel electrophoresis and blotting them onto nitrocellulose paper. Patient's serum with immunoglobulins is then added, and, if specific HIV antibodies are present, they will bind. The bound IgG antibodies are detected by adding enzyme-coupled anti-human Ig. The substrate stains the paper when converted to a product. Antibodies are detected to numerous antigens, but the significant antigens are gp120, gp41, and p24. A positive test results when the patient has antibodies to 2 of the above listed antigens. When no response is observed, then the result is negative. If any activity to any antigen is observed that is not consistent with a positive result, the result is reported to be indeterminate.



Viral organism separated into constituent proteins



Proteins separated in SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis)



Separated proteins are transferred to nitrocellulose sheet and reacted with patient's serum. Next, anti-human γ -globulin labeled with an enzyme is reacted for color development and identification. Four different antisera were identified from this patient.

Figure II-17-9. Western Blot Test

F. Flow Cytometric Analysis

This procedure allows for rapid analysis of cell types present in a blood sample. By using specific, fluorescent-labeled antibody to different receptors on cells, it is possible to rapidly identify exact cell contents and types.

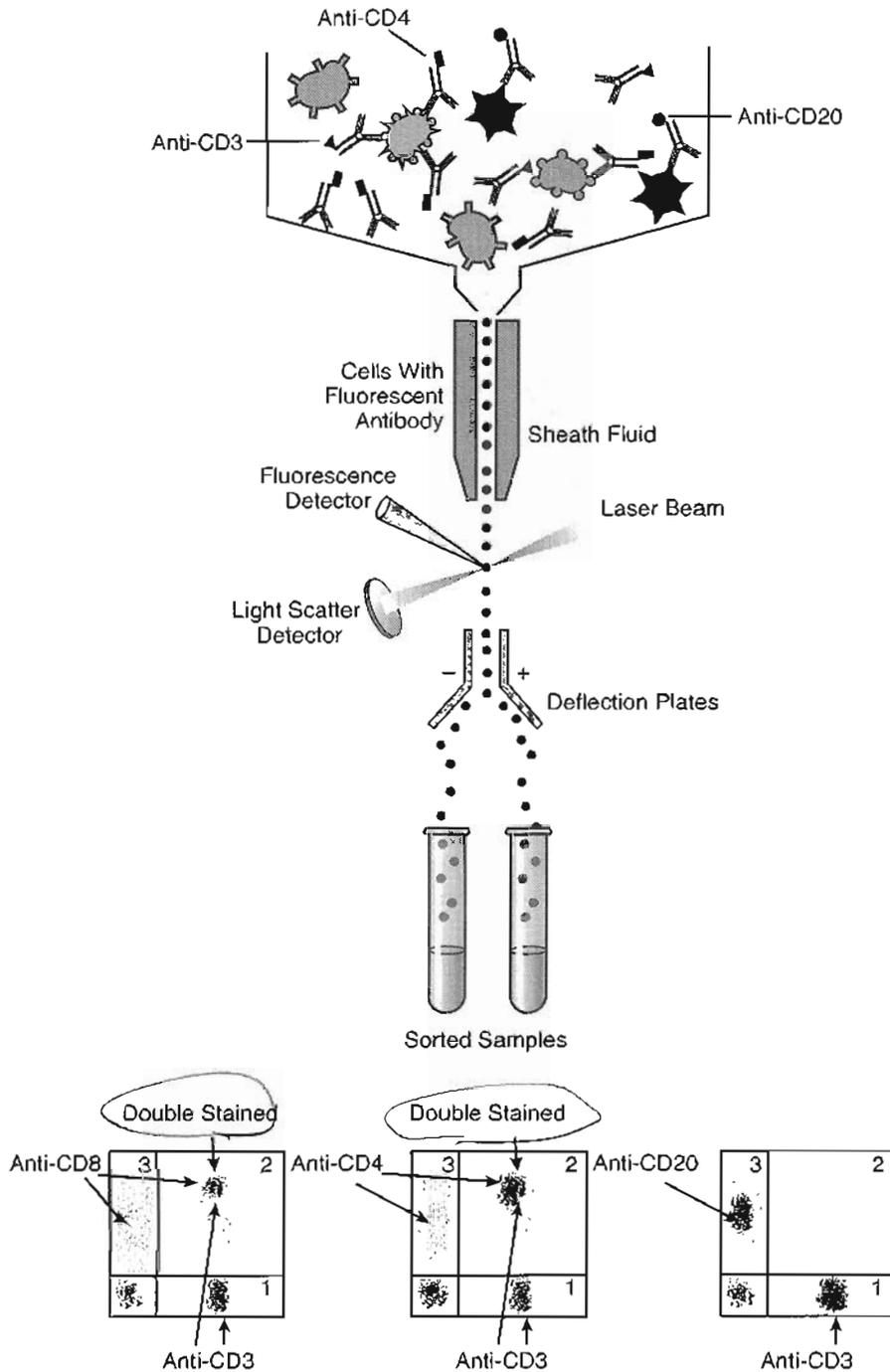


Figure II-17-10. Flow Cytometric Analysis

CD3, CD4 on helper T lymphocytes; CD3, CD8 on cytotoxic T lymphocytes; CD19, CD20, CD21 on B lymphocytes; CD16, CD56 on natural killer (NK) cells.

G. Histocompatibility Testing

The mixed lymphocyte assay is used to test reactions between donor and recipient cells. T cells are incubated with a B cell to check the response to class II (DR) antigens. The B cells act as the stimulator cell, and they are irradiated to prevent proliferation. The T cells respond because they recognize MHC molecules on the irradiated (stimulator) cell that are different from their own MHC class II molecules. Proliferation is measured by uptake of titrated thymidine in cellular DNA.

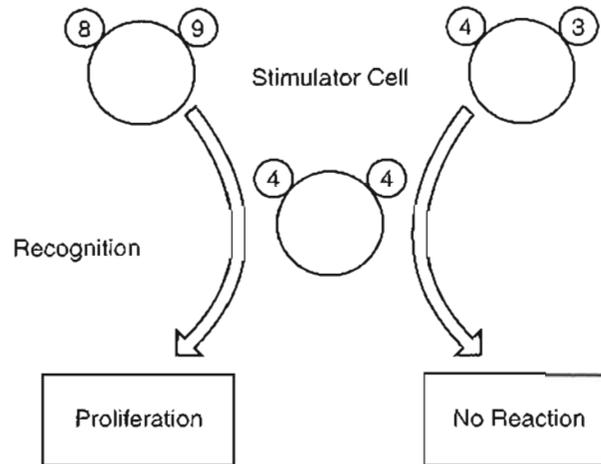


Figure II-17-11. Mixed Lymphocyte Assay

H. Microcytotoxicity Assay

The microcytotoxicity assay is used to identify class I MHC molecules. This test is performed using antisera against specific class I antigens, plus complement. If the test cell has class I antigen to match the antibody, then complement will be bound and the cell will be killed. The entry of the dye into the interior of the cell illustrates cell death.

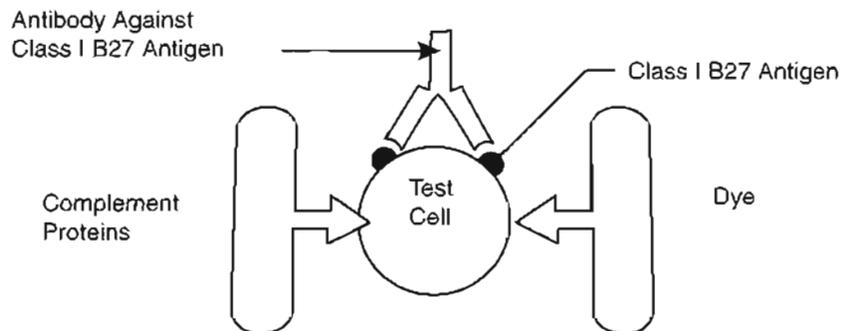


Figure II-17-12. Entry of Dye Into the Test Cell

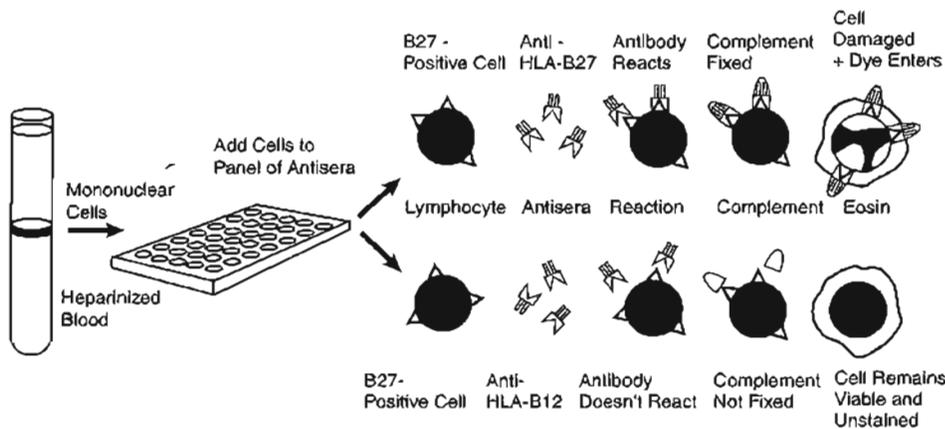


Figure II-17-13. Microcytotoxicity Assay

Chapter Summary

Some common laboratory procedures for determining immunologic responses are briefly described. The agglutination tests (including the direct and indirect Coombs' tests for hemolytic anemias and Rh compatibility), various precipitation reactions, fluorescent antibody, radioimmune assays, enzyme-linked immunosorbent assay (ELISA), the immunoblot (e.g., Western blot), flow cytometric analysis, histocompatibility testing, and the microcytotoxicity assay for identifying class I MHC gene products are described.

Review Questions

- AMG, a 16-year-old runaway heroin user visits a family planning/STD clinic irregularly to receive birth control pills. In April 1996, the standard HIV screen performed by this clinic reports back that her test was positive. The primary test for HIV infection uses
 - HIV antigen, patient serum, anti-immunoglobulin serum and enzyme substrate ligand
 - HIV antigen, patient serum, anti-immunoglobulin serum and radioactive ligand
 - HIV antigen covalently coupled to RBC and patient antiserum plus complement
 - HIV antigen covalently couple to RBC, patient serums, and anti-immunoglobulin
 - Electrophoresis of HIV antigens in polyacrylamide gel, transfer to nitrocellulose, followed by treatment with patient serum and then enzyme-labeled anti-Ig, followed by substrate.
- What is the significance of finding antibodies to p24, p31, and gp160 in the serum of a patient who has a positive ELISA screening assay for HIV?
 - It is inconclusive and requires a second confirmatory test.
 - It indicates a false positive ELISA screening test.
 - It indicates that the patient is infected with the HIV-1 virus.
 - It indicates that the patient has the acquired immunodeficiency syndrome (AIDS).
 - It indicates that the patient's immune system is intact.

3. The clinical immunology laboratory can easily measure levels of the most abundant immunoglobulins present in serum to detect a myeloma (increase) or immune deficiency. Which one of the following clinical immunology laboratory procedures would be used to routinely measure IgG, IgM, and IgA levels in patient's serum?
 - A. Radial immunodiffusion (RID)
 - B. Radioimmunoassay (RIA)
 - C. Microcytotoxicity assay
 - D. Complement fixation test
 - E. Serum protein electrophoresis

4. The screen test for HIV infection consists of which one of the following immunological laboratory procedures?
 - A. Western Blot for patient's p24, gp41, and gp120 antigen level
 - B. Western Blot for patient's antibody level to p24, gp41, and gp120
 - C. PCR test for p24 antigen
 - D. ELISA test for p24 antigen
 - E. ELISA test for p24 antibody

5. Direct Coomb's test was performed on a baby in its 7th month (30 weeks). The mother has had trouble with two early pregnancies and she has had no RhoGAM. The physician is concerned about a possible erythroblastosis case. What ingredients would be involved in a procedure to prove a positive Direct Coomb's test?
 - A. Rh⁺ RBCs + mother's serum + Coombs' reagent
 - B. Rh⁺ RBCs from the baby + Coombs' reagent
 - C. Mother's serum + Rh⁻ RBCs + Coombs' reagent
 - D. Mother's serum + RhoGAM + Coombs' reagent
 - E. RhoGAM + Rh⁺ RBCs from the baby

Answers

1. **Answer: A.** The screening test for HIV infection is the ELISA test, which is described in choice A. Choice B describes a radioimmunoassay; choice C describes a complement fixation test; and choice E describes the Western Blot, which is the confirmatory test for infection.

2. **Answer: C.**

3. **Answer: A.** The RID assay is an immunodiffusion test for doing quantitative immunoglobulin levels. This procedure is not as sensitive as RIA but the three immunoglobulins you measure in this procedure are the three most abundant serum immunoglobulins (mg levels). You cannot use this procedure for IgE or IgD because of the low levels of these materials.

4. **Answer: E.** The ELISA screen for HIV consists of analysis of the patient's serum for presence of antibody to p24 antigen.

5. **Answer: B.** The Rh⁺ RBCs from the baby would already be coated with anti-RhD antibody produced in the mother and transported across the placenta into the baby. If these cells are placed on a slide with Coombs' serum, which is antihuman gamma globulin, the cells will be agglutinated and lysed. This is a positive direct Coombs' test.