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CHAPTER 4

Mechanisms of Microbial Infections[1]

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Key Readings Index

The goal of this chapter is to provide a mechanistic overview of the key steps involved in understanding the pathogeneses of infectious diseases caused by microbes (i.e., bacteria, viruses, fungi, protozoa, and prions). Coverage is not intended to be encyclopedic; specific diseases have been selected either because they illustrate a basic mechanism or because they are of primary importance to the practice of veterinary medicine. Because the knowledge base for some veterinary diseases is limited, certain sections of this chapter are conditional and are based on (1) extrapolations from known experimental systems, (2) established mechanisms of injury covered in the basic pathology chapters of this book, and (3) assumptions anchored in the characteristics of macroscopic and microscopic lesions that arise with each disease. This chapter will also discuss and illustrate selected "especially dangerous and contagious microbes" because diseases caused by these pathogens can have catastrophic impact on livestock health and production and on the economies of affected countries. The locations in this textbook of coverage of these diseases considered by the United States Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS) and the World Organisation for Animal Health (OIE) as "Foreign Animal Diseases" or "OIE Reportable Diseases," respectively, are listed in E-Table 4-1.

Chronologic Sequence of Steps in Microbial Diseases

The following is a list in chronological order of the "typical" sequence of steps[2] leading to disease caused by microbes (Fig. 4-1):

1. Acquire access to a portal of entry
2. Encounter "targets" in mucosae, mucocutaneous junctions, or skin such as epithelial cells, tissue-associated leukocytes, or tissue-associated substances like mucus
3. Colonize targets to sustain and/or amplify the encounter[3] or cross the barrier system formed by mucosae, mucocutaneous junctions, or skin to gain access to targets located locally in the lamina propria, submucosa, or dermis/subcutis

[1]For a glossary of abbreviations and terms used in this chapter see E-Glossary 4-1.

[2]Depending on the microbe, only the first two or three steps may be required to cause a specific disease.

[3]Some microbes do not spread beyond cells encountered at portals of entry because these cells are their "final" target cells within mucosae, mucocutaneous junctions, or skin.

E-Glossary 4-1 Glossary of Abbreviations and Terms

ATALT–Auditory tube–associated lymphatic tissue
BAD1–Blastomyces adhesion factor
BALT–Bronchial-associated lymphoid tissue
Bap–Biofilm-associated protein
BRDC–Bovine respiratory disease complex
BRSV–Bovine respiratory syncytial virus
BVD–Bovine viral diarrhea virus
C3b–Complement fragment 3b
CMG2–Capillary morphogenesis protein 2
CNS–Central nervous system
DIC–Disseminated intravascular coagulation
DNA–Deoxyribonucleic acid
DNT–Dermonecrotic toxin
ECM–Extracellular matrix
EF–Edema factor
EHEC–Enterohemorrhagic Escherichia coli
EIA–Equine infectious anemia
EPE–Equine proliferative enteropathy
EPEC–Enteropathogenic E. coli
ER–Endoplasmic reticulum
ETEC–Enterotoxigenic E. coli
FAE–Follicle-associated epithelium
FeLV–Feline leukemia virus
FIP–Feline infectious peritonitis
GALT–Gut-associated lymphoid tissue
HA–Hemagglutinin
IBR–Infectious bovine rhinotracheitis
IBRv–Infectious bovine rhinotracheitis virus
IFN-γ–Interferon-γ
IgG–Immunoglobulin G
IL-1–Interleukin 1
IL-6–Interleukin 6
IL-10–Interleukin 10
IL-12–Interleukin 12
LALT–Larynx-associated lymphoid tissue
LAM–Lipoarabinomannan
LDL–Low-density lipoprotein
LF–Lethal factor
LKT–Mannheimia leukotoxin
LOS–Lipooligosaccharide
LppQ–Bacterial membrane lipoprotein (Mycoplasma mycoides)
LPS–Lipopolysaccharide (endotoxin)
LsaA–Lawsonia surface antigen
LT–Heat labile enterotoxin
Mac-1–Macrophage-1 antigen
MALT–Mucosa-associated lymphoid tissue
M cell(s)–Microfold cell(s)
MHC–Major histocompatibility complex
MPS–Mononuclear phagocyte system
MRSA–Methicillin-resistant Staphylococcus aureus
NA–Neuraminidase
NADPH–Nicotinamide adenine dinucleotide phosphate
NO–Nitric oxide
NS3–Nonstructural protein 3
PA–Protective antigen
PABA–para-aminobenzoic acid
PAI–Pathogenicity islands
PAMP–Pathogen-associated molecular pattern
PBP–Penicillin-binding proteins
PCV2–Porcine circovirus type 2
PED–Porcine epidemic diarrhea
PEDV–Porcine epidemic diarrhea virus
PI–Parainfluenza
PI–Persistently infected
PI3–Parainfluenza virus
PMT–Pasteurella multocida toxin
PMWS–Postweaning multisystemic wasting syndrome
PNS–Peripheral nervous system
PRDC–Porcine respiratory disease complex
PRR–Pattern recognition receptor
PRRSV–Porcine reproductive and respiratory syndrome virus
PRSP–Penicillin-resistant Streptococcus pneumoniae
SAG–Glycosylphosphatidylinositol-linked surface proteins
SCV–Salmonella-containing vacuole
SIV–Swine influenza virus
spp.–Species
subsp.–Subspecies
ST–Heat stable enterotoxin
Stxs–Shiga toxins
SzP–Surface M-like protein (a virulence factor with anti-phagocytic characteristics)
TEM8–Tumor endothelial marker 8
TGF-α–Transforming growth factor-α
TGF-β–Transforming growth factor-β
TLR–Toll-like receptor
TLR3–Toll-like receptor 3
TLR4–Toll-like receptor 4
TNF-α–Tumor necrosis factor α
TRAP-C1–Thrombospondin-related adhesive protein Cryptosporidium 1
UPEC–Uropathogenic E. coli
VAP–Virulence-associated protein
VRE–Vancomycin-resistant enterococci

E-Table 4-1 Locations in This Textbook of the Coverage of Especially Dangerous and Contagious Microbes

Disorders of	Chapters
HORSES	
African horse sickness	4, 9, and 10
Anthrax	4, 7, 9, 10, and 13
Glanders	4, 9, and 10
RUMINANTS (CATTLE, SHEEP, AND GOATS)	
Anthrax	4, 7, 9, 10, and 13
Brucellosis	4, 18, and 19
Contagious bovine pleuropneumonia	4 and 9
Foot-and-mouth disease	4, 7, and 17
Rinderpest	4 and 7
Sheep and goat pox	4 and 17
PIGS	
African swine fever	4 and 10
Anthrax	4, 7, 9, 10, and 13
Brucellosis	4, 18, and 19
Classic swine fever	4 and 10
Foot-and-mouth disease	4, 7, and 17
Porcine epidemic diarrhea	4 and 7

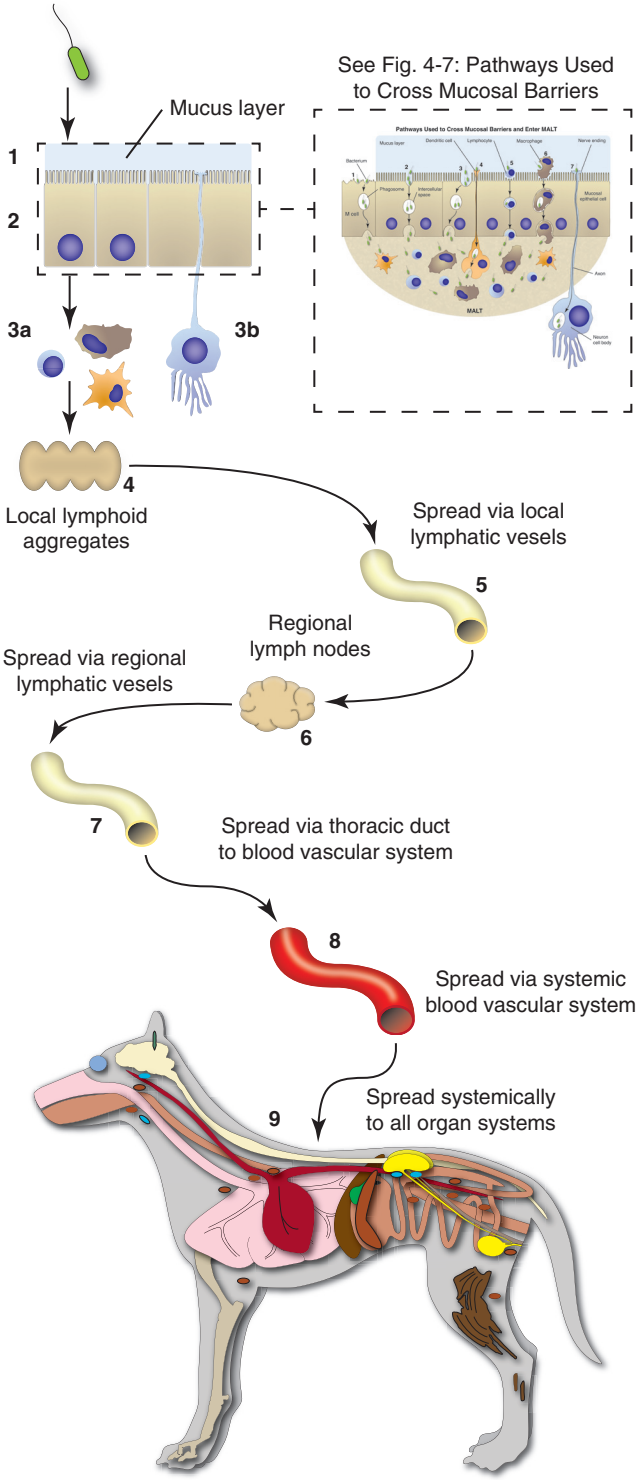


Figure 4-1 Spread of Microbes to Organ Systems. *1*, Microbes (bacteria used herein for illustration) must penetrate the mucus layer if present. *2*, Microbes cross mucosal, serosal, or integumentary barriers (see Fig. 4-7). *3a*, Microbes encounter mucosa-associated cells (e.g., lymphocytes, macrophages, and dendritic cells). *3b*, Microbes encounter receptors of the nervous system embedded in barrier systems. *4*, Microbes spread locally to lymphoid tissues (e.g., mucosa-associated lymphoid tissue [MALT] such as tonsils, Peyer's patches) in barrier system. *5*, Microbes spread regionally in afferent lymphatic vessels. *6*, Microbes encounter cells in regional lymph nodes. *7*, Microbes spread systemically in efferent lymphatic vessels to the thoracic duct and anterior vena cava. *8*, Microbes spread systemically in the blood vascular system. *9*, Microbes encounter target cells in systemic organ systems. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

4. Spread locally in the extracellular matrix (ECM) to encounter and colonize new populations of target cells, including lymphocytes, macrophages (monocytes), and dendritic cells, as well as blood and lymphatic vessels and their circulating cells
5. Enter blood and/or lymphatic vessels
   a. Travel inside lymphocytes, macrophages (monocytes), or dendritic cells within these vessels protected from the animal's defense mechanisms[4]
   b. Travel as "cell-free" microbes (i.e., not within or associated with a cell) within these vessels
6. Spread to regional lymph nodes and/or then systemically within the blood vascular system to encounter, colonize, and invade new populations of target cells that are unique to a specific organ system
7. Cause dysfunction and/or lysis of target cells and disease

These steps and thus the ability of microbes to cause disease (pathogenicity) are controlled by "virulence factors" expressed by their genes. The acquisition of new and/or more "virulent" genes through recombination and/or natural selection of mutated genes allows microbes to (1) complete one or more of the listed steps more rapidly and/or efficiently, (2) evade or reduce the effects of an animal's defense mechanisms, and/or (3) develop resistance to specific antibiotics. These outcomes may result in greater cell and tissue injury (and thus disease) within a targeted organ system of an individual animal or greater pathogenicity of a disease within a herd. Gene recombination also appears to account in part for "breakouts" of diseases thought to be contained by vaccination programs in farm and urban settings and, as an example, was also used as the scientific premise for the plot of the movie *Contagion*.

Portals of Entry

The portals of entry for microbes are the alimentary, respiratory, urogenital, and integumentary systems and the ear and eye (Fig. 4-2; Essential Concept 4-1). Microbes gain access to these portals via ingestion (alimentary system), inhalation (respiratory system), ascending entry (urogenital system), penetration (integumentary system, eye), and direct contact (integumentary system, ear, and eye). Following the initial entry, microbes may then gain access to (1) broader expanses of mucosa via normal physiologic processes such as ingestion (swallowing, peristalsis), inhalation (centrifugal forces, turbulence), ascension (reflux pressures, simple brownian movement [i.e., urogenital tract]), or direct contact (blink reflex, lacrimation) or to (2) deeper (and/or broader) expanses of skin and mucocutaneous junctions via traumatically induced abrasions or by penetration caused by insect bites or scratch and bite wound as examples.

A concept central to pathogeneses of infectious diseases is the ability of microbes to reach a site in the body that has "target cells or substances" suitable for their growth and replication. They will be discussed in a later section. Additionally, a second concept central to pathogeneses of infectious diseases is the phrase "virulence factor." Because this phrase will be used extensively in subsequent sections, its meaning needs to be summarized here but will be discussed in greater detail in sections that follow. Virulence factors are molecules (and thus genes) of microbes that enable them to replicate and cause disease. They include glycoprotein, glycolipid, or other types of molecules that are present in the structure of microbes, as well as molecules derived from transcription and/or translation of the microbial genome. Some of these virulence factors are integral to the biologic structure of microbes; other factors are synthesized by microbes using the metabolic processes of the target cell as needed to replicate. These factors serve to do the following:

- **Colonize** (e.g., adhesins) target substances, cells, and/or tissues at portals of entry
- **Invade** (e.g., invasins) target substances, cells, and/or tissues at portals of entry
- **Evade** (e.g., enzymes, toxic molecules) barrier systems and defense mechanisms
- **Suppress** (e.g., enzymes, toxic molecules) innate and adaptive immune responses
- **Acquire** (e.g., siderophores) nutrition from target substances, cells, and/or tissues.

The ability of microbes to replicate and cause disease is the result of their interactions with substances, cells, and/or tissues at portals

[4]Some microbes enter nerve endings at portals of entry to gain access to the peripheral nervous system (PNS) and central nervous system (CNS).

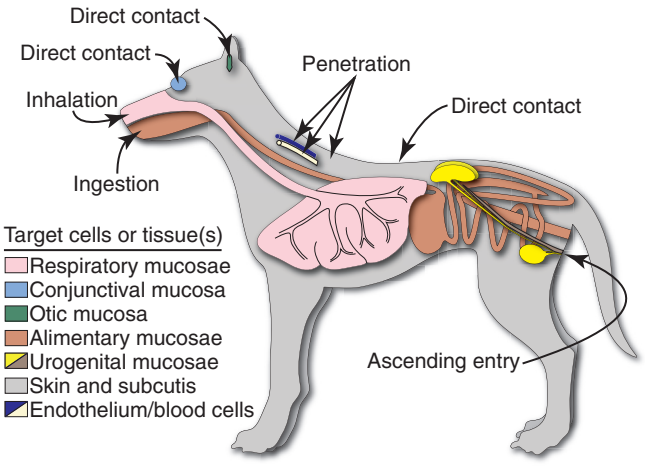


Figure 4-2 Portals of Entry. Microbes enter the body through ingestion, inhalation, direct contact, cutaneous penetration, and ascending infection and then encounter epithelial cells, macrophages, dendritic cells, and lymphocytes of barriers formed by mucosae, mucocutaneous junctions, or skin. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

ESSENTIAL CONCEPT 4-1 Portals of Entry

Portals of entry are the gateways used by microbes to gain access to and enter an animal's body. They include the alimentary, respiratory, and urogenital systems; the skin; and the ear and eye. The locations of initial encounters within these portals consist of the following:

1. Mucosae and mucocutaneous junctions of the oral cavity (alimentary system), nasal cavity (respiratory system), and urethral orifice (urogenital system)
2. Epidermis (also dermis and/or subcutis via penetration) of the skin (integumentary system)
3. Epidermis of the external acoustic meatus of the ear (auditory system)
4. Corneal and conjunctival epithelia of the eye (ocular system)

The cells and substances (i.e., epithelia, immune cells, nerve endings, lamina propria, extracellular matrix proteins, and mucus) at these sites of initial encounters form "barrier systems" that function as defense mechanisms to protect the animal's cells against colonization and infection by microbes.

of entry. These interactions are facilitated by virulence factors under the control of the microbial genome.

Depending on the biologic behavior of the microbe (virulence factors expressed by their genes), a portal of entry and its target cell(s) or substance(s) may be located in the following areas:

- Locally (i.e., cells and tissues initially encountered by microbes at the portal of entry)
- Regionally (i.e., neighboring cells and tissues encountered by microbes as they spread in regional lymphatic vessels and lymph nodes that drain the portal of entry)
- Systemically (i.e., cells and tissues encountered by microbes as they spread distally in the circulatory and/or lymphatic systems to other organ systems)

Management practices and physical contact often place susceptible animals in close proximity to "contagious (carrier)" animals, where microbes can be spread in water droplets, aerosols, and body fluids such as snot, sputum, urine, and feces through direct contact, grooming, licking, scratch and bite wounds, sneezing, and other physiologic body processes. Except for contact with carrier animals, the chronologic sequence of events that leads to disease caused by microbes is not a random event. These events are well designed (virulence factors) and serve to colonize cells and tissues by inhibiting, altering, or evading defense mechanisms and barrier systems (see later section) that protect animals against infectious diseases.

Within each portal of entry, numerous sites (along the length of the entire system) contain potential target cells for "initial encounters" with microbes. Which target cells and what location in the portal of entry are colonized by what microbe depends upon the genes (virulence factors—see later section) of the microbe and the availability of target cells with appropriate substrates and/or receptors for the microbe and its microbial products. As an example, the alimentary system has diseases that occur in the oral cavity, tonsils, esophagus, stomach, small intestine, cecum, and large intestine. Therefore some microbes must travel within the alimentary system, often for a great distance, to reach their "initial encounter" target cells. Swine dysentery (*Brachyspira hyodysenteriae*) is a bacterial disease that colonizes the mucus layer and goblet cells of the colon and cecum. The microbe's portal of entry is the alimentary system, but its primary target cells are in distal segments of the system and therefore the microbe must travel a great length to reach them.

When microbes initially encounter cells and tissues at portals of entry, colonization (infection) depends on creating an initial "beachhead" to establish, sustain, amplify, and if needed spread the microbe. In these beachheads, some microbes attach to, enter, and replicate on or within mucosal, mucocutaneous, and cutaneous epithelial cells, whereas others pass through via endocytosis (phagocytosis) or between cells within intercellular junctions. In other cases, they encounter mucosa-associated leukocytes and dendritic cells, and via phagocytosis (or endocytosis) they are carried across the mucosa or skin. Through one of these mechanisms, microbes reach the basal side of epithelial cells and then encounter other mucosal, mucocutaneous, and cutaneous cells and tissues, including different tissue macrophages, lymphocytes, and dendritic cells, ECM, and nerve endings where they may again replicate to sustain, amplify, and/or spread the microbe. It is from these beachheads that microbes then spread locally (submucosa and dermis and associated lymphoid tissues), regionally (lymph nodes), and/or systemically (organ systems) to other target cells and cause disease.

Most commonly, the initial beachhead is established in mucosae or skin:

Mucosae (also mucocutaneous junctions)

- Alimentary system (oral cavity, oral pharynx, esophagus, stomach, small and large intestines [see Chapter 7])
- Respiratory system (nasal cavity, nasal pharynx, conductive component [see Chapter 9])
- Lower urinary system (urethra, bladder, ureters [see Chapter 11])
- Reproductive systems (reproductive tracts [see Chapters 18 and 19])
- Ear (external acoustic meatus [see Chapter 20])
- Eye (cornea, conjunctiva [see Chapter 21])

Skin (also mucocutaneous junctions)

- Epidermis/dermis, endothelial cells, blood and lymphatic vessels (see Chapters 10, 13, and 17)
- Mucocutaneous junctions, endothelial cells, blood and lymphatic vessels (see Chapters 7, 9, 10, 13, and 17)
- Subcutaneous ECM and immune system cells such as macrophages, lymphocytes, and dendritic cells (see Chapters 3, 5, 13 and 17)
- Subcutaneous muscle cells, endothelial cells, blood and lymphatic vessels (see Chapters 10, 13, 15, and 17)

Mucosae of the alimentary and respiratory systems are covered by a protective mucus gel secreted by goblet cells that forms a barrier system against colonization by microbes. This important barrier is discussed in more detail in a later section and in the following sections covering the alimentary and respiratory systems.

Alimentary System (Ingestion)

Microbes enter the alimentary system (see Chapter 7) through ingestion and gain access to mucosae, most commonly tonsillar epithelium, villus epithelium, crypt epithelium, and epithelium containing microfold cells (M cells) overlying Peyer's patches by chewing, swallowing, and peristalsis. They are trapped in the mucus layer of mucosae of the oral pharynx and intestines and must penetrate this layer to reach targets such as epithelial cells, macrophages, and dendritic cells. M cells of the small intestinal mucosa lack a mucus covering and therefore offer a unique portal to enter the alimentary system (see later section on Target Cells). Mucus in the alimentary system is produced by goblet cells distributed among mucosal epithelial cells in the villi and crypts where it covers and protects microvilli. The mucus layer is a (1) physical and (2) biologic barrier protecting the intestine against microbes via (1) its thickness and viscosity, (2) binding to bacterial adhesins, (3) serving as a reservoir for immunoglobulin A (IgA) and lysozyme, and (4) acting as a free radical scavenger. Additionally, the mucus layer is a favorable habitat for beneficial and competitive enteric microflora.

Generally there are more goblet cells in the large intestine than in the small intestine, more in crypts than in villi, and more in the ileum than in the jejunum or duodenum. It appears that mucus covers all intestinal epithelial surfaces to varying degrees of thickness and viscosity and is composed of an inner gel layer and an outer soluble layer. The mucus layer is thickest in the colon (≈830 μm) and thinnest in the jejunum (≈123 μm). It is less prominent over absorptive enterocytes with microvilli when compared to crypt enterocytes. A mucus layer does not cover M cells; therefore microbes can readily interact with their cell membranes. Once entrapped in the mucus layer, microbes must then penetrate it to gain access to target cells for infection. Additionally, microbes also encounter mucosal fluids, such as gastric acid, mucins, secretions such as lysozyme, and humoral mediators such as immunoglobulins, and must also compete with normal microflora for resources and for receptors expressed on target cells.

Mucosa-associated lymphoid tissue (MALT) is a general term used to categorize lymphoid nodules located in mucosae and submucosae of many organ systems. MALTs are important defense mechanisms of mucosae and are discussed in greater detail later. In the

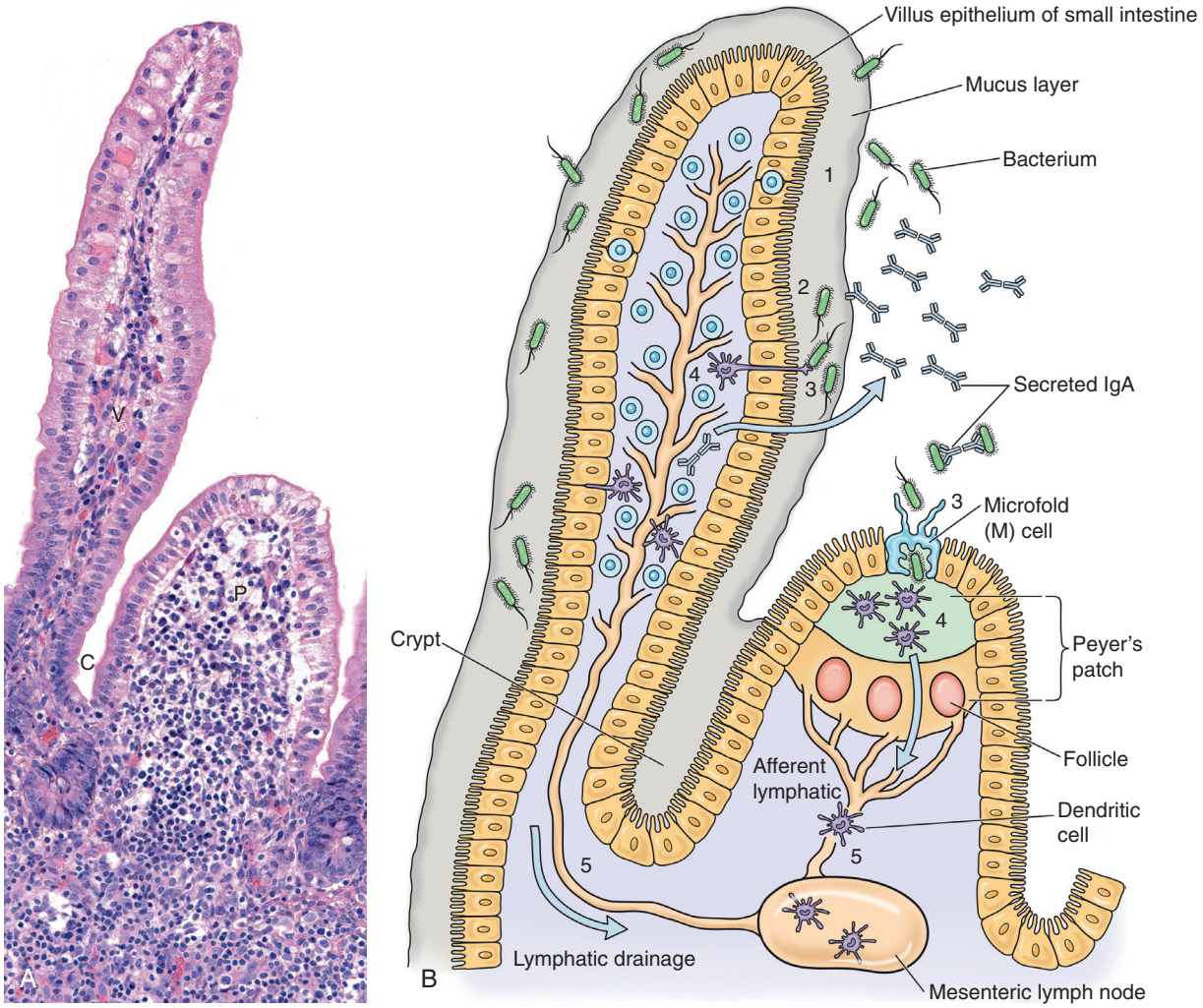


Figure 4-3 Microbial Interactions with a Barrier System: Intestinal Mucosa. A, Mucosa that cover intestinal villi *(V)* and Peyer's patches *(P)* and line crypts *(C)* form a barrier system that attempts to prevent the spread of microbes into the underlying lamina propria. H&E stain. **B,** Schematic diagram of the responses of bacteria (or viruses) trapped in the mucus layer *(1)*. Bacterial proteins (virulence factors) act to allow them to penetrate the mucus layer and come into contact with the mucosal epithelium *(2)*. Immunoglobulin (IgA) secreted by mature plasma cells in the lamina propria passes through mucosal epithelial cells into the lumen and can act as an "opsonizing" defense mechanism, thus preventing infection. Bacteria then interact with mucosal epithelial cells, dendritic cells, or microfold cells (M cells) *(3)*. They then encounter lymphoid cells in the lamina propria or Peyer's patches *(4)* and spread in lymphocytes or as free virus in lymph from this location via afferent lymphatic vessels to regional lymph nodes *(5)*. Note the absence of a mucus layer over M cells and follicle-associated epithelium. Also see Figure 4-7 for an example of barrier system: respiratory mucosae. (**A** courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

alimentary system as an example, submucosal lymphoid nodules located in the distal jejunum and ileum that surround groups of intestinal crypts are assigned a specific name, gut-associated lymphoid tissue (GALT), but are also commonly known as Peyer's patches (Fig. 4-3). These nodules are composed of lymphocytes, macrophages, and dendritic cells. In GALT (Peyer's patches), nodules are covered by modified epithelial cells of intestinal crypts called follicle-associated epithelium (FAE) and its microfold cells (M cells). M cells are the interface between materials in the lumen of intestinal crypts and the lymphoid nodules and function to transfer antigens in the lumen of the intestine across the mucosa to dendritic cells and immune cells like macrophages and lymphocytes in the nodule. Peyer's patches (GALT) have afferent lymphatic vessels that drain to regional mesenteric lymph nodes. Cells similar to M cells likely cover lymphoid nodules in most mucosae and perform a similar function at the luminal interface.

Respiratory System (Inhalation)

In the respiratory system (see Chapter 9), microbes are inhaled through the nostrils (see Fig. 4-2) and are deposited on mucosae of the nasal turbinates, nasal pharynx, and/or the conductive system (trachea, bronchi) based on physical properties of the agent such as size, shape, weight, and electrostatic charge (Fig. 4-4). Groups of microbes ranked from smallest to largest include viruses (≈5 to 300 nm [1×10^{-9} m] in diameter), prions (≈16 nm in diameter), bacteria (≈0.5 to 5 µm [1×10^{-6} m] in diameter), fungi (≈5 to 60 µm in diameter), and protozoa (≈1 to 300 µm in diameter). Although it is convenient to compare microbes based on size, rarely are they inhaled as free organisms. Most commonly they are enclosed in fomites (i.e., inanimate objects or substances capable of carrying microbes) such as dust particles, soil, septum, or body fluids. Thus the physical properties of infectious fomites (i.e., fomites containing microbes) determine where they are deposited on mucosal surfaces

of the respiratory system and cause disease. When inhaled, larger fomites, such as those containing bacteria and fungi, are deposited and trapped in the nasal turbinates, whereas in a gradient of descending size, smaller ones are able to reach the pharynx, larynx, trachea, and bronchi before they are deposited and trapped in mucosae. The nasal cavity and turbinates trap 70% to 80% of particulate matter approximately 3 to 5 μm or greater in diameter and 60% of particulate matter 2 μm or greater but cannot trap particles with sizes below 1 μm in diameter. In a normal functioning respiratory system, only fomites of approximately 1 μm or less in diameter, such as viruses and some bacteria, can be inhaled into bronchioles, alveolar ducts, and alveoli, which are the oxygen–carbon dioxide (O_2-CO_2) exchange portion of the respiratory system.

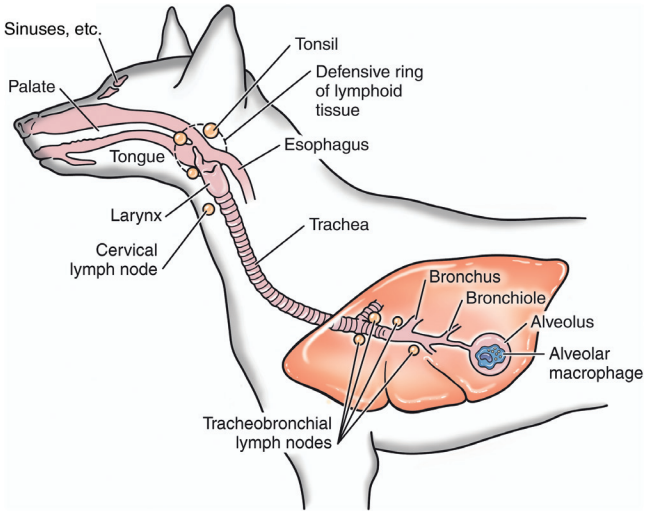


Figure 4-4 Deposition of Microbes. Microbes inhaled through the nostrils are deposited on mucosa of the nasal turbinates, nasal pharynx, and/or the conductive system of the respiratory tract. The site of deposition depends on the physical properties of the agent such as size, shape, weight, and electrostatic charge.

When infectious fomites are inhaled, they first encounter the nasal turbinates. The movement of air through the turbinates causes centrifugal turbulence that forces them against mucosae, where they are trapped in the overlying mucus layer for removal by the mucociliary apparatus. If the size of fomites allows them to pass through the turbinates and be carried to the pharynx, larynx, trachea, or bronchi, inertial turbulence forces fomites against airway mucosae, where they are trapped in the mucus layer for removal. Inertial turbulence occurs when a laminar stream of airflow is disrupted by a septum within the conductive portion of the respiratory system when airways branch. When the flow is split by a septum, the flow rotates centrifugally on either side of the septum and forces fomites against mucosae. Depending on the species, airways can branch 23 times en route from the trachea to the alveoli. The O_2-CO_2 exchange portion of the respiratory system (the bronchioles, alveolar ducts, and alveoli) is not ciliated and has no protective mucus layer because of its gas exchange function. The outcome of these turbulence mechanisms is to trap infectious fomites in the mucus layer overlying ciliated mucosal epithelial cells. When infectious fomites are trapped in the mucus layer, they are (1) acted on by other components of the innate immune system, such as phagocytes like alveolar macrophages and neutrophils, and microbicidal molecules, such as lysozyme and immunoglobulins, and (2) removed by the mucociliary apparatus (see Chapter 9).

The mucociliary apparatus is composed of the mucus layer and ciliated mucosal epithelial cells and is an important defensive mechanism in the respiratory system (Fig. 4-5). The mucus layer, produced by goblet cells and submucosal glands, is biphasic and consists of a luminal viscoelastic or gel layer used to trap infectious fomites and a serous inner layer in which the cilia of ciliated mucosal epithelial cells beat. The tips of the cilia just slightly enter the gel layer and their beating moves the gel and fomites. In the nasal cavity and sinuses, cilia move mucus and debris downward toward the pharynx for swallowing; in the conductive portion of the respiratory system, cilia move mucus and debris upward toward the pharynx for swallowing. The directionality of mucous flow is determined by the rhythmic unidirectional beating pattern of the cilia. If the mucus layer and/or the mucociliary apparatus are dysfunctional, gravity

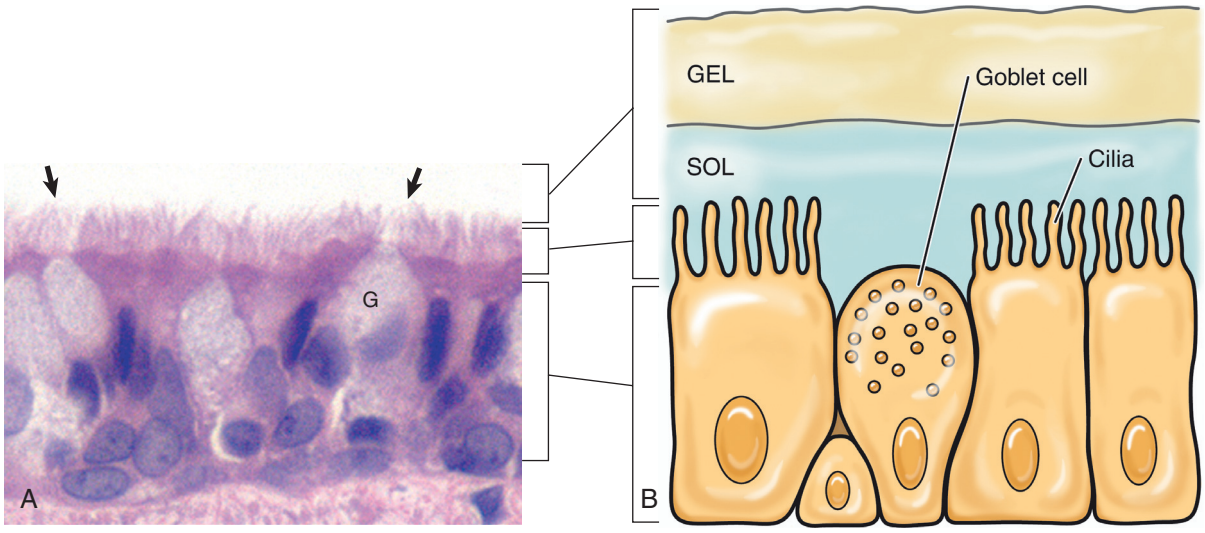


Figure 4-5 Mucociliary Apparatus. A, Cilia *(arrows)* of the bronchiole mucosal epithelial cells and the mucus layer (not visible) form the mucociliary apparatus of the conductive component of the respiratory system. The mucus layer is not visible because it has been removed during histologic processing of tissue. H&E stain. **B,** Diagram of the mucociliary apparatus. The mucus layer is biphasic and consists of a luminal viscoelastic or gel layer used to trap bacteria and a serous inner layer in which the cilia of ciliated mucosal epithelial cells beat unidirectionally to move bacteria upward in the airways to be swallowed or expectorated. G, Goblet cell; *SOL*, colloidal solution. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

influences the deposition of infectious fomites. The conductive portion of the respiratory system has an anterior and ventral flow pattern of distribution based on the effects of gravity. Therefore injury to mucosal epithelial cells by specific microbes, such as influenza and bovine rhinotracheitis viruses, can disrupt the function of the mucociliary apparatus, thereby exacerbating an existing disease or creating an opportunity for a secondary microbial infection of the dependent lung via settling attributable to gravity that is usually prevented by this clearance mechanism. The pathogenesis of many bronchopneumonias is based on this mechanism. Swallowing of infectious microbe-infected mucus may also be a mechanism to clear certain bacteria from the conductive portion of the respiratory system; however, it provides bacteria, such as *Rhodococcus equi*, the opportunity to gain access to the alimentary system and cause disease.

Mucosa of the conductive portion of the respiratory system contain dendritic cells and alveolar and tissue macrophages that commonly migrate through the mucosa and the mucus layer during their normal patterns of leukocyte trafficking (see Chapters 5, 9, and 13). Because these cells can phagocytose and kill microbes, they serve as a primary defense mechanism against infections. However, certain microbes have virulence factors that allow them to evade killing by phagocytes and use them like a "Trojan horse" to spread the agent and infect other cells and tissues. These cells are common targets for microbes and along with mucosal epithelia serve as the initial beachhead infection before microbes spread locally often to the tonsil, regionally to lymph nodes, and systemically to other organ systems. Bronchial-associated lymphoid tissues (BALTs) are submucosal lymphoid nodules located subjacent to ciliated mucosae, usually in areas in which inertial turbulence deposits foreign material on mucosae. Nodules are composed of lymphocytes, macrophages, and dendritic cells and function much like Peyer's patches. BALTs have afferent lymphatic vessels that drain to regional tracheobronchial lymph nodes.

Urogenital System (Ascending Infection)

Microbes can enter the lower urinary system and reproductive systems and encounter mucosae via ascending infection from coitus or the use of contaminated instruments, insemination straws, or semen. Traumatic injury of mucosae resulting in abrasions or penetrating wounds increases the likelihood of colonization by microbes. The mechanisms of encounter, colonization, infection, and spread are similar to those discussed earlier and in the later section on Pathways of Spread.

Skin (Direct Contact and Cutaneous Penetration)

For simplicity, this chapter uses the word "skin" in discussions. However, it should be remembered that the skin consists of epidermis, dermis, adnexa (hair follicles, sebaceous glands, sweat glands), and subcutis. Different microbes may use one or more of these components as portals of entry and will be discussed in sections covering specific diseases. The skin is a (1) thick and tenacious physical (especially the epidermis) and (2) biologic barrier protecting the body against microbes via (1) its dryness and acidity, (2) sebum (oils), and (3) normal bacterial flora, which compete against microbes for resources and for receptors expressed on target cells. Microbes encounter the skin (also mucocutaneous junctions) via direct contact and the dermis and subcutaneous tissues (see Chapter 17) via penetration through abrasions, scratches, and bite wounds or through bites (proboscis) of insect vectors like mosquitoes that spread the agent into subcutaneous tissues such as muscle, blood and lymphatic vessels, and ECM and connective tissue. In these tissues, microbes encounter a limited range of target cells such as epithelial cells in the skin, dendritic cells (Langerhans cells), tissue macrophages, nerve endings, endothelial cells of the vascular and lymphatic systems, and connective tissues and muscle of the dermis and subcutis. Microbes may also be deposited directly in the blood vascular system via penetration of a capillary, venule, or lymphatic vessel by an insect proboscis. Additionally in ECM of these tissues, microbes encounter body fluids, such as blood and plasma proteins, that serve as resources for survival, infection, and replication. The mechanisms of encounter, colonization, infection, and spread are similar to those discussed earlier and in the later section on Pathways of Spread.

Ear and Eye (Direct Contact and Cutaneous Penetration)

Microbes encounter the eye via direct contact with the cornea and conjunctiva (also lacrimal duct via its connection with the conjunctiva) and occasionally penetration, whereas they encounter the ear via direct contact with the external acoustic meatus. The mechanisms of encounter, colonization, infection, and spread are similar to those discussed earlier and in the later section on Pathways of Spread.

Target Cells and Substances

Microbes commonly colonize and injure specific populations of cells called "target cells" (and/or the biologic substances they synthesize and release into their surroundings) that are unique to a specific organ system (e.g., ciliated epithelial cells in the respiratory system) or to a cell lineage (e.g., M cells in the alimentary system) (Fig. 4-6; Essential Concept 4-2). The specific cells and substances used as targets by microbes are often based on ligand-receptor interactions in which proteins (ligands) expressed on the surface of microbes bind to receptors on membranes of target cells; mucus associated with these cells; vascularized ECM enclosing these cells; or macrophages, lymphocytes, and dendritic cells supporting these cells. Receptors expressed on target cells are usually those involved in normal function of cells and as examples may include receptors for complement, phospholipids, and carbohydrates. Once the proteins are bound to receptors, a sequence of steps facilitated by virulence factors is initiated that results in the colonization of the surface of these cells or invasion of the cells often through endocytosis/phagocytosis. The microbe then establishes control of the normal metabolic systems of these cells and uses them or their resources to replicate in and/or spread to other cells and/or organ systems. The outcome of this process is usually dysfunction and/or lysis of infected cells and thus clinical disease, and injury of specific targeted populations of cells in organ systems is often reflected in the results of serum biochemical analyses (e.g., elevated liver enzyme concentrations) and hematologic evaluations (e.g., leukopenia).

Target cells that are exploited by microbes can be placed into three functional groups:

- Target cells initially encountered at the portals of entry.
- Target cells used to spread microbes locally, regionally, or systemically.
- Target cells located systemically within other organ systems.

Microbes may use cells in one, some, or all of these groups to successfully complete their life cycles. As a general rule, target cells initially encountered at portals of entry (mucosae, mucocutaneous junctions, and skin) tend to be epithelial cells and mucosa-associated macrophages (monocytes), lymphocytes, dendritic cells, and nerve endings. Target cells used to spread microbes locally, regionally, or systemically tend to be macrophages (monocytes), lymphocytes, or dendritic cells that migrate through the body and encounter other

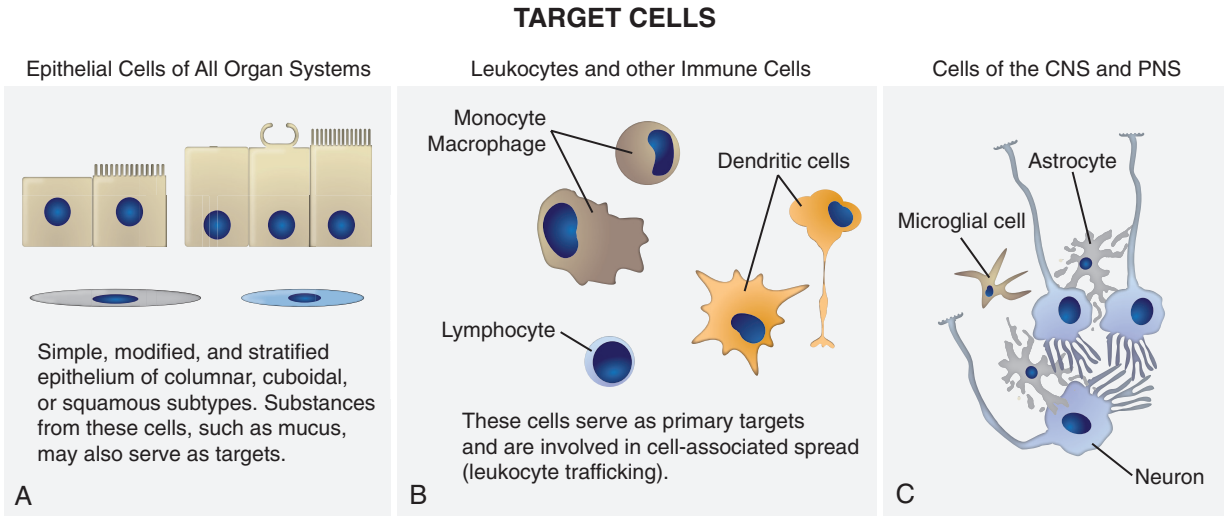


Figure 4-6 Target Cells. A, Target cells forming "barrier systems." Target cells encountered at portals of entry (i.e., barrier systems) and within organ systems of the body following systemic spread include simple or stratified epithelium (squamous, cuboidal, or columnar types). These cells cover and line surfaces (pleurae, pericardium, airways, ducts), form the parenchyma of organ systems such as hepatocytes in the liver, line organs (endothelium, synovium), and form glands (endocrine, exocrine [e.g., adrenal, pancreas]). Additionally, biologic substances, such as mucus, synthesized and released from these cells into their surroundings can be used by some microbes as target substances (not shown in figure). **B,** Target cells used to spread microbes. Target cells used by microbes to spread via leukocyte trafficking, regionally or systemically, to other organ systems include cells of the monocyte-macrophage system, lymphocytes, and dendritic cells. These cells may also be primary target cells for microbes in addition to being used as cells to travel in throughout the body. **C,** Target cells unique to the nervous system. Some microbes can enter nerve endings of the peripheral nervous system (PNS) (cranial and spinal nerves) in mucosa and dermis/subcutis at portals of entry and use retrograde and anterograde axonal transport to spread to and through neurons of the central nervous system (CNS). Other cells, including microglial cells (as part of the monocyte-macrophage system), and astrocytes can then become targets for infection. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

ESSENTIAL CONCEPT 4-2 Target Cells and Substances

Each kind of microbe requires a specific type of cell or substance in which to successfully replicate and complete its life cycle. Therefore the labels "target cells" and "target substances" are applied for the convenience of creating defined groups. Simple and stratified epithelial cells (squamous, cuboidal, or columnar types) are the cells most commonly used as targets by microbes at portals of entry. In organ systems these cells function as covering, lining, and glandular (including the liver) epithelia. Additionally, mucosa-associated leukocytes (lymphocytes, macrophages [monocytes], and dendritic cells) that form lymphoid aggregates and nodules (mucosa-associated lymphoid tissue [MALT]) in these organ systems can also serve as (1) "primary" target cells for diseases caused by some microbes or as (2) "secondary" targets used to spread microbes via leukocyte trafficking locally, regionally, or systemically to encounter epithelial cells (or substances) in other organ systems. Nerve endings embedded in epithelium may also be used as targets to enter the central nervous system (CNS) and peripheral nervous system (PNS). Substances, such as mucus produced by goblet cells, are often strong chemoattractants for specific types of microbes because they serve as physical matrices and chemical substrates for colonization. Colonization, invasion, and injury of target cells and/or substances are based on ligand-receptor interactions in which proteins (ligands) expressed on the surface of microbes bind to receptors on target cells or substances. Receptors on cells are usually transmembrane proteins involved in normal cell functions; receptors on substances are generally structural molecules. Once a microbe is bound, a sequence of steps is initiated that may result in colonization or invasion of cells (or substances). Thereafter the microbe may acquire control of some or all of the cell's metabolic systems, replicate, and complete its life cycle. The outcomes of successful microbial infections on target cells include cell death, directed cellular dysfunction, inflammation, structural injury, persistent infection, latent infection, cell proliferation, and malignant transformation.

organ systems. This mechanism of spread is called "leukocyte trafficking." From local sites, infected lymphocytes and macrophages spread via lymphatic vessels to regional lymph nodes, where additional cells are infected and then spread via lymphatic vessels to the thoracic duct and the circulatory system. From here, infected cells spread systemically to other organ systems in which specific target cells are infected, including lymphoid organs such as the spleen, lymph nodes, and bone marrow. Additionally, microbes can use nerve endings and fibers (that travel to and throughout all organ systems via the central nervous system [CNS] and peripheral nervous system [PNS]) to spread within the nervous system and/or systemically. Lastly, target cells located systemically within other organ systems tend to be epithelial cells, macrophages (monocytes), lymphocytes, dendritic cells, and/or neural cells. In some diseases, leukocyte trafficking ultimately returns the microbe back to mucosae, mucocutaneous junction, or skin initially encountered at the portal of entry. However, it is important to remember that the area of mucosa or skin involved in the initial beachhead encounter represents a very small percentage of the entire area of these organ systems; therefore there is always a large population of new target cells available for infection.

Epithelial Cells as Microbial Targets

Epithelial cells (and their ECM), the most common cell type used as targets by microbes, are categorized into two groups, simple epithelium (one layer of cells) or stratified epithelium (two or more

layers of cells) (E-Fig. 4-1). Each group can be further subdivided into squamous, cuboidal, or columnar types based on morphologic structure (E-Table 4-2). However, for a better understanding of the mechanisms of microbial infections, epithelial cell targets can also be classified as follows:

Covering epithelium

- Integumentary system (skin, mucocutaneous junctions)
- Respiratory system (serosa: mesothelium [pleurae])[5]
- Alimentary system (serosa: mesothelium [peritoneum])[5]
- Cardiovascular system (serosa: mesothelium [pericardium/epicardium])
- Nervous system (serosa: mesothelium [meninges])
- Muscle (synovium of tendon sheaths)
- Eye (cornea)

Lining epithelium

- Alimentary system (mucosae: oral cavity, pharynx, esophagus, stomach, intestines)
- Respiratory system (mucosae: nasal cavity, pharynx, larynx, trachea, bronchi, lungs)
- Urinary system (mucosae: urethra, bladder, ureter, pelvis, tubules)
- Cardiovascular system (endothelium [blood vessels, endocardium, lymphatic vessels])[5]
- Reproductive system (mucosae: tracts)
- Skeletal system (synovium of joint capsules)[5]
- Eye (mucosa: conjunctiva)
- Ear (mucosa: external acoustic meatus)

Glandular epithelium

- Endocrine system (endocrine glands)
- Hepatobiliary system (liver [hepatic plates forming lobules], gallbladder)
- Alimentary system (exocrine [salivary] glands)
- Reproductive system (gonads, exocrine glands)

Mucosa-Associated Lymphoid Tissues as Microbial Targets

MALT is a general term used to categorize lymphoid nodules composed of lymphocytes, macrophages, and dendritic cells that are located in mucosae and submucosae of many organ systems. Each, some, or all of these cells types can serve as target cells for specific diseases. In some cases, these cells are the primary targets in which the microbe successfully completes its life cycle, whereas in other cases they serve as target cells used to spread microbes (leukocyte trafficking [see later section]) locally, regionally, or systemically and encounter cells and tissues in other organ systems. More specifically, MALT includes BALT, conjunctiva-associated lymphoid tissue (CALT), nose-associated lymphoid tissue (NALT), larynx-associated lymphoid tissue (LALT), and auditory tube–associated lymphatic tissue (ATALT), as well as lymphoid nodules (unnamed) in the genital tract, mammary gland, and urinary bladder mucosa. In GALT, nodules are covered by modified epithelial cells of intestinal crypts called microfold cells (M cells). M cells are the interface between materials in the lumen of intestinal crypts and the lymphoid nodules and function to transfer antigens in the lumen of the intestine across the mucosa to dendritic cells and immune cells like macrophages and lymphocytes in the nodule. Cells similar to M cells likely cover lymphoid nodules in most mucosae and perform a similar function at the luminal interface. MALT-like arrangements with similar functions to those in mucosae also exist in the skin.

Biologic Substances as Microbial Targets

In a few diseases, targets for infection are substances produced by epithelial cells, such as mucus by goblet cells of the alimentary system. As an example, swine dysentery (*B. hyodysenteriae*) is a bacterial disease in which the mucus layer and thus goblet cells of the colon and cecum (portal of entry) are targets for the spirochetes. Mucus is a strong chemoattractant and is also important as a physical matrix and chemical substrate for colonization. Mucus and swine dysentery will be discussed in several of the following sections and in the section that covers specific bacterial diseases.

Pathways of Spread

Microbes exploit a limited number of pathways to: (1) colonize cells, tissues, and/or substances at the site of initial encounter at the portal of entry and cause disease or (2) cross a "barrier system" such as mucosae of the alimentary or respiratory systems to reach and colonize cells, tissues, and/or substances located locally in mucosae and cause disease or to spread regionally or systemically to cells, tissues, and/or substances located in other organ systems and cause disease (Essential Concept 4-3; E-Figs. 4-2 and 4-3). Crossing a mucosal (or cutaneous) barrier system is an important step in the process, and microbes may use one or more of seven distinct mechanisms shown in Fig. 4-7 to accomplish this task:

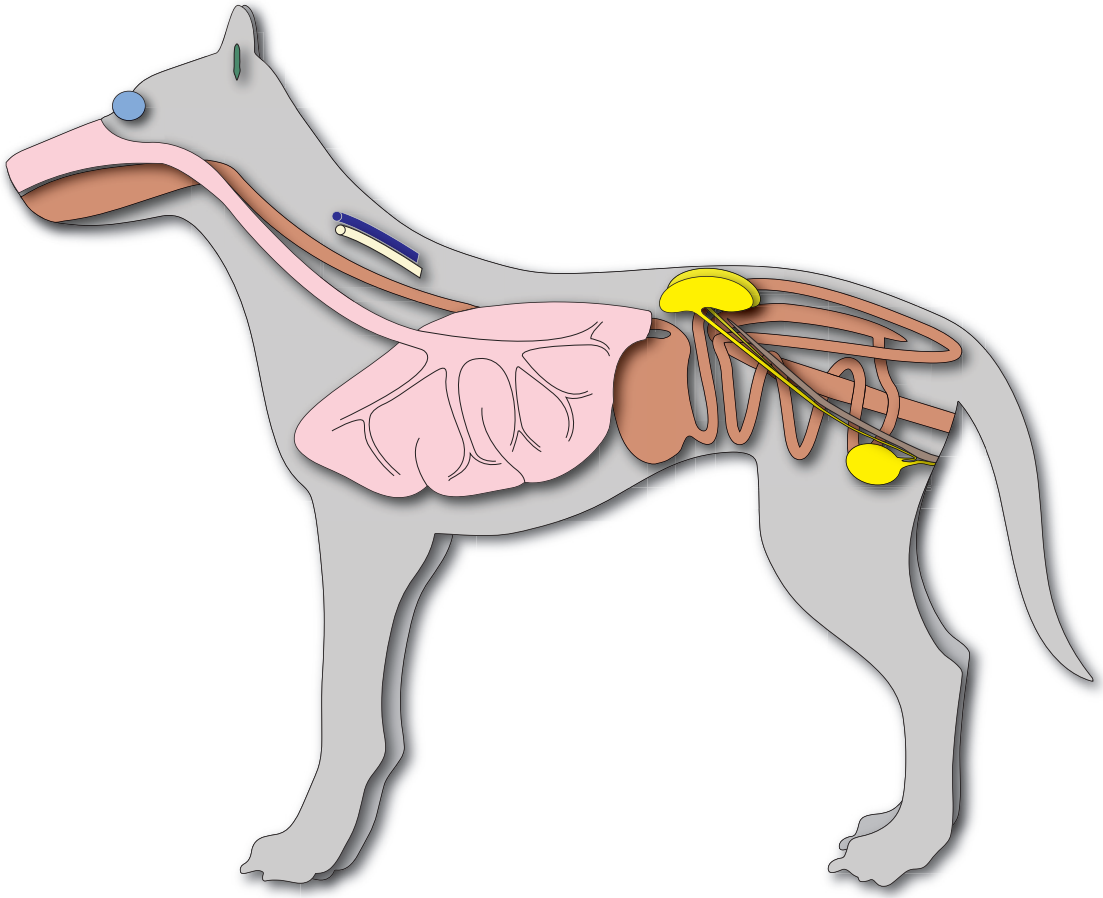
1. Endosomes and transcytosis via M cells
2. Intercellular junctions
3. Endosomes and transcytosis via other types of epithelial cells (ciliated, microvillus border)
4. Mucosa-associated dendritic cells
5. Migrating mucosa-associated lymphocytes

ESSENTIAL CONCEPT 4-3 Pathways of Spread

Pathways of spread are the routes used by microbes to reach target cells or substances they require to replicate and complete their life cycles. For some microbes these pathways may begin and end at the portal of entry, whereas for others they may end in a distant organ system. In general, most pathways of spread follow this pattern: portal of initial encounter → cross a barrier system → encounter mucosa-associated leukocytes → spread to local lymphoid aggregates → afferent lymphatic vessels → regional lymph nodes → efferent lymphatic vessels → thoracic duct and anterior vena cava → blood vascular system → target cell in a systemic organ system.[9] Along these pathways, microbes encounter a variety of barrier systems, defense mechanisms, and cells and/or substances. As a result, they have acquired virulence factors that allow them to cross barrier systems (see later); evade defense mechanisms such as those involved in phagocytosis and microbial killing; and colonize and invade cells and/or substances via ligand-receptor interactions at the site of the initial encounter, at sites distant from portals of entry along pathways of spread, and in a targeted organ system. Mechanisms used to cross barrier systems include passage in M cells, microbial motility through intercellular junctions, passage through cells via endosomes (transcytosis), leukocyte trafficking, passage in blood and lymphatic vascular systems as cell-free microbes, and passage in nerve endings and processes.[10]

[5]Endothelium and mesothelium (both derived from mesoderm) are considered epithelia by histologists, but in pathology such cell types are not considered true epithelium in the area of tumor diagnostics and thus are classified as sarcomas, not carcinomas.

[9]See text for variations and exceptions.
[10]See text and Fig. 4-7 for greater detail.



Target tissue(s) or cells	Types of cells targeted by infectious microorganisms
Respiratory mucosae	Ciliated and nonciliated cuboidal/columnar epithelial cells, goblet cells, and squamous epithelial cells (localized and not shown)*
Conjunctival mucosa	Squamous epithelial cells and goblet cells
Otic mucosa	Squamous epithelial cells, goblet cells, and cuboidal/columnar epithelial cells (localized and not shown)
Alimentary mucosae	Squamous epithelial cells (esophagus) and cuboidal/columnar epithelial cells with microvilli and goblet cells (stomach and lower intestine)*
Urogenital mucosae	Transitional epithelial cells (cuboidal/columnar shape), goblet cells, and squamous epithelial cells (localized and not shown)
Epidermal/dermal cells (mucocutaneous junction)	Squamous epithelial cells and dermal stromal cells**
Serosal coverings/linings (not shown in illustration)	Mesothelial cells of the meninges, pericardium and epicardium, pleura, peritoneum, synovia, and tendon sheaths
Capillary endothelium Lymphatic endothelium	Monocytes, endothelial cells, endothelium-associated (vascular) macrophages, tissue macrophages

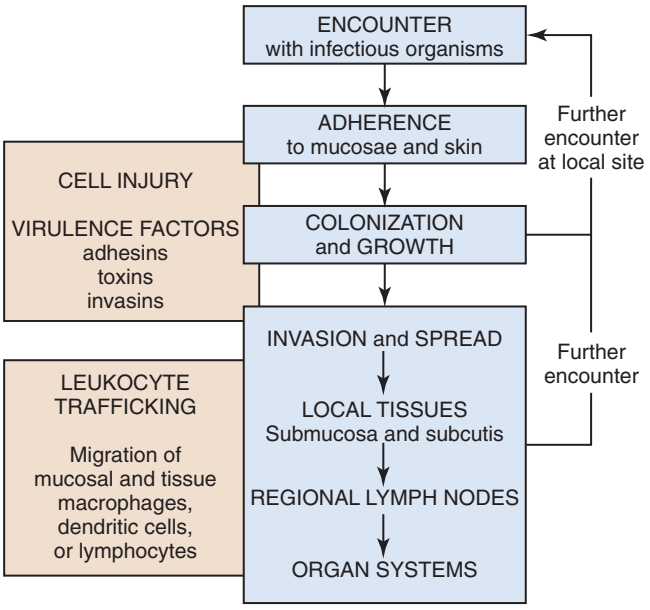
Where applicable:
* Mucosa-associated macrophages, lymphocytes, and dendritic cells are dispersed within the applicable tissues.
** Tissue macrophages, dendritic cells (Langerhans cells) are dispersed within the applicable tissues.

E-Figure 4-1 Types of Cells and Tissues Targeted by Microbes and Their Locations in the Body. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

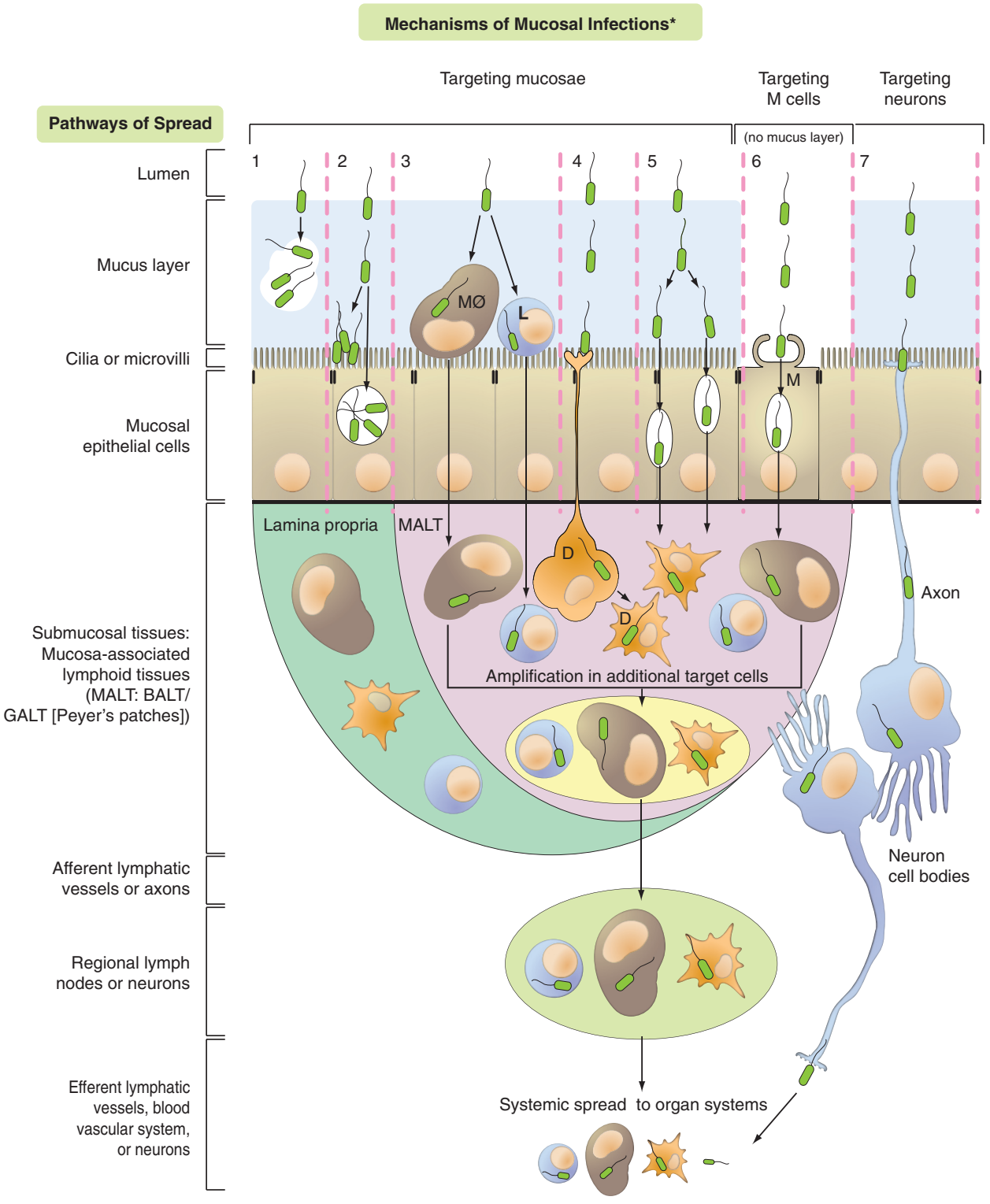
E-Table 4-2 Morphologic Types of Epithelium and Structural Examples

Type of Epithelium	Examples
SINGLE LAYER OF CELLS	
Simple squamous	Vascular and lymphatic endothelia
	Type I pneumocytes of alveoli
	Pleurae, pericardium, peritoneum, and meninges (serosae)
	Parietal layer of Bowman's layer of kidney
	Loop of Henle of kidney
Simple cuboidal	Renal convoluted tubules and collecting ducts
	Pigmented epithelium of the eye
	Chorioid plexuses of the CNS
	Ducts and secretory parts of glands such as the thyroid gland
Simple columnar (nonciliated)	Mucosae of alimentary system (stomach to anus)
	Gallbladder
	Oral cavity
	Auditory meatus
	Ducts and secretory parts of some glands
Simple columnar (ciliated)	Small bronchi
	Uterine tubes and uterus
Pseudostratified (nonciliated)	Epididymis
	Ductus deferens
	Ducts of some glands
Pseudostratified (ciliated)	Nasal cavity, trachea, and upper respiratory tract (larger bronchi)
MULTIPLE LAYERS OF CELLS	
Stratified squamous (noncornified)	Oral mucosa, esophagus
	Cornea and conjunctiva
Stratified squamous (cornified)	Skin
Stratified cuboidal	Salivary, mammary, and sweat glands
Stratified columnar	Portions of male urethra and of some glands (very rare type)
Transitional	Mucosa of urinary bladder (squamous when distended, cuboidal when empty)

CNS, Central nervous system.



E-Figure 4-2 Sequence of Events in Infection. The chronologic sequence of events used by infectious microorganisms to colonize and invade mucosae and skin, spread to local tissues and regional and systemic organ systems, and cause disease.



E-Figure 4-3 Mechanisms of Microbial Infections and Pathways of Spread. *Pathway 1*, Bacteria target the mucus layer. *Pathway 2*, Bacteria target cilia or microvilli and/or mucosal epithelial cells. *Pathway 3*, Bacteria target mucosa-associated lymphoid tissue (MALT) via mucosal macrophages (MØ) and/or lymphocytes (L). *Pathway 4*, Bacteria target MALT via dendritic cells (D). *Pathway 5*, Bacteria target MALT via transcytosis or intercellular (junctional complexes) spread. *Pathway 6*, Bacteria target MALT via microfold cells (M cells) and transcytosis. *Pathway 7*, Bacteria target nerve endings in mucosa and enter the brain via retrograde axonal transport. *BALT*, Bronchial-associated lymphoid tissue; *GALT*, gut-associated lymphoid tissue.

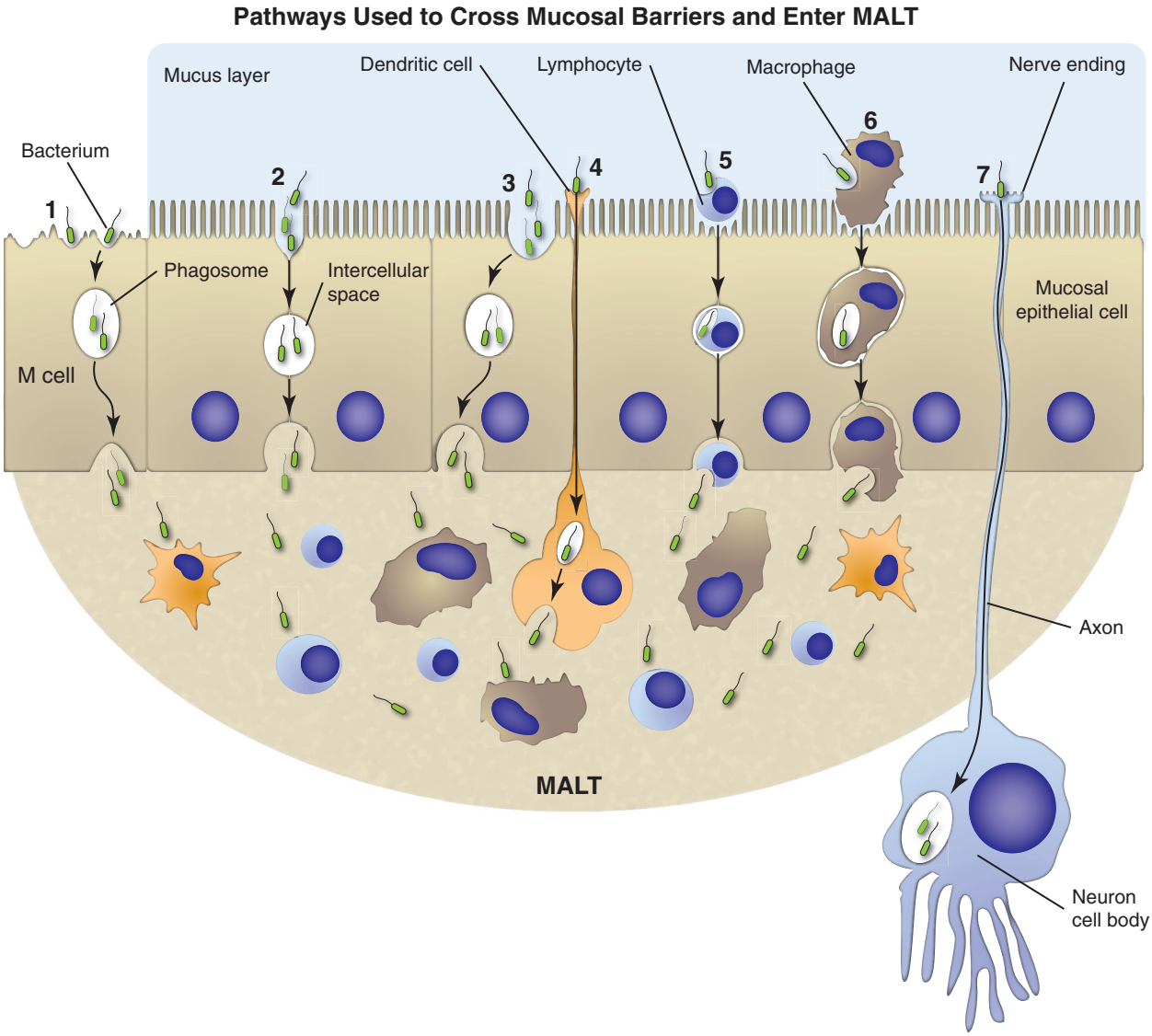


Figure 4-7 Mechanisms Used to Cross Mucosal Barrier Systems. *1*, Transcytosis (M cells, which lack a mucus layer). *2*, Microbial motility via intercellular junctions. *3*, Transcytosis. *4*, Processes of dendritic cells embedded in mucosae. *5*, Leukocyte trafficking (lymphocytes) via intercellular junctions. *6*, Leukocyte trafficking (monocytes or macrophages) via intercellular junctions. *7*, Nerve endings and nerve processes via axonal transport in cranial or spinal nerves. Once within MALT, microbes can interact with and/or be phagocytized by leukocytes to continue the processes of colonization and spread. Examples illustrated here are also, in general, applicable to skin and mucocutaneous junctions. Specific viruses, fungi, protozoa, or prions may use some of these mechanisms. *MALT*, Mucosa-associated lymphoid tissue; *M cell*, microfold cell. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

6. Migrating mucosa-associated macrophages
7. Mucosa-associated nerve endings

Transcytosis and endosomes (microvesicles) are covered in greater detail in the section on Transcytosis and Endocytosis/Exocytosis and in Chapter 1.

As examples, the pathways of spread for the alimentary and respiratory systems are shown in Fig. 4-8 and for the integumentary system (skin) in Fig. 4-9. For some microbes the mechanisms and pathways of colonization, replication, and spread occur at one location, usually restricted to mucosae or skin (also mucocutaneous junctions) at the site of the initial encounter; for others the processes involve multiple locations, including mucosae or skin at the site of the initial encounter, as well as tissues and cells located locally, regionally, and systemically. Although these latter processes occur at multiple locations, microbes have a limited number of "entry points" such as through M cells, within mucosa-associated leukocytes and dendritic cells (Trojan horse), via transcytosis (within endosomes [microvesicles]) within epithelial cells, or through nerve endings. Some motile microbes can also enter mucosae by moving directly through epithelial cells or between them through intracellular junctional complexes to spread to subjacent MALTs. Microbes use one or more pathways to reach their primary target cells or substances (i.e., cell or biologic substances in which they replicate) (see Figs. 4-1 and 4-7). These pathways and target cells and substances are discussed in greater detail later.

Mechanisms Used to Colonize Mucosae (or Biologic Substances) at Portals of Entry

Colonize Mucus (Goblet Cells)

Swine dysentery (*B. hyodysenteriae*) (see Figs. 7-171 and 7-172) is an example of a bacterial disease in which the mucus layer and thus goblet cells of the colon and cecum (portal of entry) are the primary targets for microbial colonization and replication. The encounter results in a mucohemorrhagic necrofibrinous colitis and typhlitis

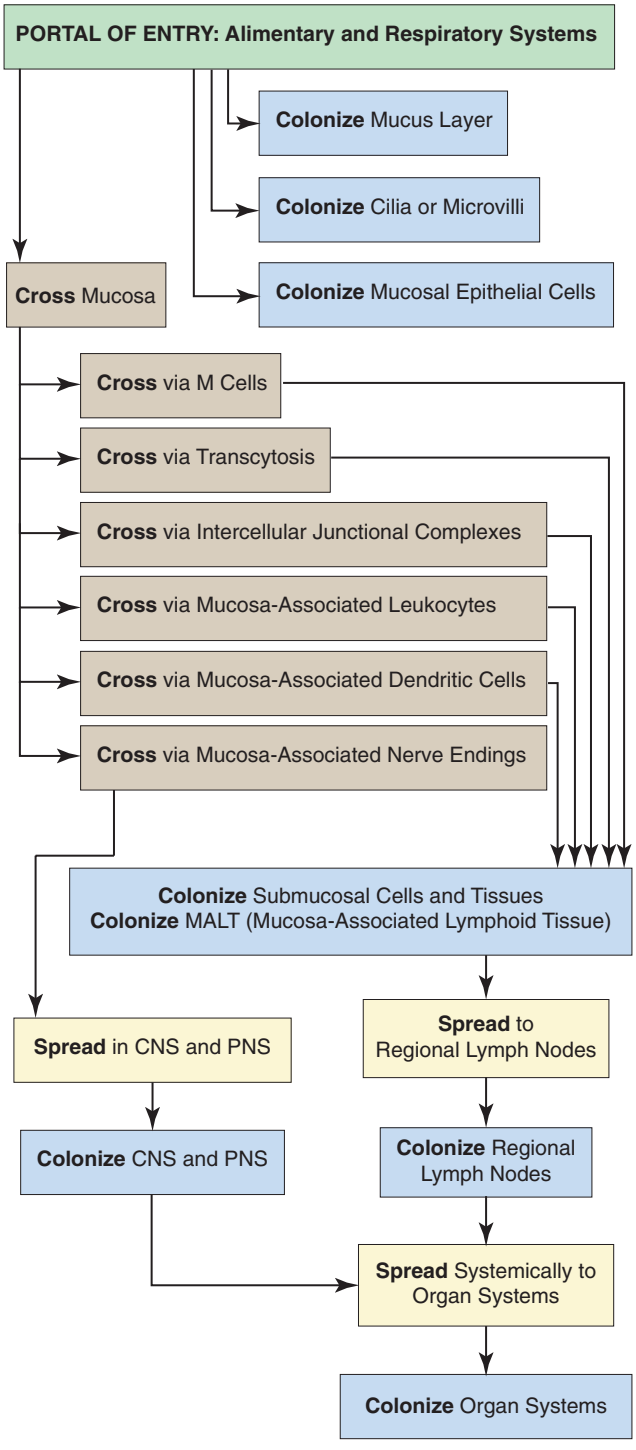


Figure 4-8 Pathways of Spread Used by Microbes in the Alimentary and Respiratory Systems. *CNS*, Central nervous system; *PNS*, peripheral nervous system. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

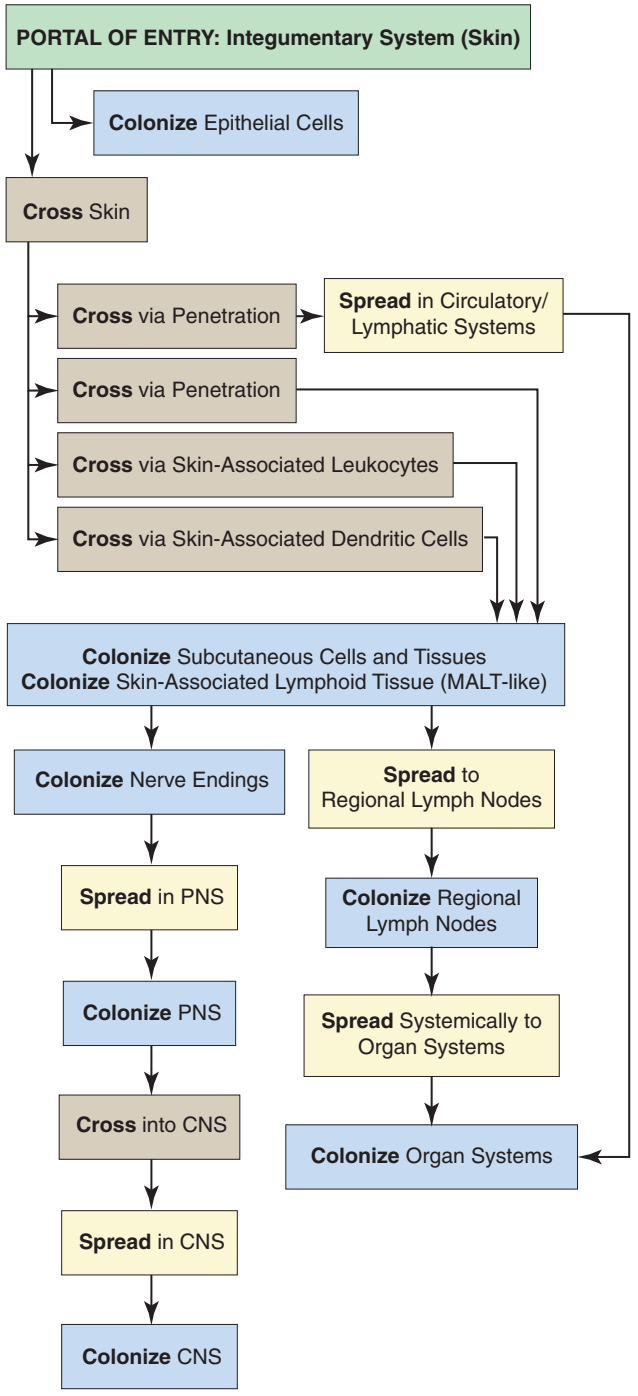


Figure 4-9 Pathways of Spread Used by Microbes in the Integumentary System. *CNS*, Central nervous system; *MALT*, mucosa-associated lymphoid tissue; *PNS*, peripheral nervous system. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

caused by bacterial hemolysins and proteases and from inflammation and its mediators and degradative enzymes. Mucus is a strong chemoattractant for spirochetes and is also important as a physical matrix and chemical substrate for colonization. This disease is discussed in greater detail in the section on Bacterial Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Pigs; Swine Dysentery.

Colonize Cilia (or Microvilli) of Mucosal Epithelial Cells

Porcine enzootic pneumonia (*Mycoplasma hyopneumoniae*) (see Fig. 9-96 and E-Fig. 9-23) is an example of a bacterial disease in which cilia of ciliated mucosal epithelial cells in the respiratory system (or with a different bacterium the microvilli of the alimentary system) are the primary targets for microbial colonization and replication. The bacterium attaches to cilia of epithelial cells of the bronchi and

bronchioles, and this interaction results in dysfunction of cilia (ciliostasis), lysis of the epithelial cells, reduced function of the mucociliary apparatus, and bronchopneumonia. Other bacteria such as *Mannheimia* (*Pasteurella*) *haemolytica* accomplish the same outcome after colonizing ciliated mucosal epithelial cells by producing toxins like neuraminidase (virulence factors) that injure and destroy the cilia and kill the ciliated epithelial cells. Porcine enzootic pneumonia is discussed in greater detail in the section on Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Pigs; Porcine Enzootic Pneumonia (*Mycoplasma hyopneumoniae*).

Colonize the Cell (Endocytosis)

Proliferative enteritis/hemorrhagic bowel syndrome of pigs (*Lawsonia intracellularis*) (see Fig. 7-173) is an example of a bacterial disease in which the bacterium enters via endocytosis (phagocytosis) and colonizes epithelial cells of intestinal crypts located in the proliferative zone of the ileum. It resides in a phagosome within the cytoplasm but escapes from the phagosome before phagosome-lysosome fusion occurs and resides free in cell cytoplasm to then initiate the mechanism that causes cell proliferation. Proliferative enteritis/hemorrhagic bowel syndrome is discussed in greater detail in the section on Bacterial Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Pigs; Porcine Proliferative Enteritis/Hemorrhagic Bowel Syndrome (*Lawsonia intracellularis*).

Mechanisms Used to Cross Mucosae at Portals of Entry

M Cell Entry

Postweaning multisystemic wasting syndrome (porcine circovirus type 2) (see Chapters 9 and 13) is an example of a viral disease in which the virus uses the alimentary system as its portal of entry to subsequently be physiologically transported through the system to reach the small intestine and encounter, enter, and cross the mucosa using M cells that overlie Peyer's patches (GALT). Via M cells the virus then gains access to and infects mucosa-associated lymphocytes and lymphocytes in GALT. Leukocyte trafficking spreads the virus systemically via lymphatic vessels to regional lymph nodes and then systemically through postcapillary venules or lymphatic vessels and the thoracic duct to the circulatory system to lymphocytes in the spleen, lymph nodes, and other lymphoid tissues. Postweaning multisystemic wasting syndrome (porcine circovirus type 2) is discussed in greater detail in the section on Viral Diseases of Organ Systems; Bone Marrow, Blood Cells, and Lymphatic System; Disorders of Pigs; Postweaning Multisystemic Wasting Syndrome (Porcine Circovirus Type 2, Nonenveloped DNA Virus).

Leukocyte Trojan Horse Entry

Rhodococcal pneumonia (*R. equi*) (see Fig. 9-82) is an example of a bacterial disease in which the bacterium crosses the mucosa and enters the respiratory system using leukocyte (Trojan horse) entry and subsequent trafficking. When inhaled, the bacterium is deposited in the mucus layer of mucosa of airways and then is phagocytosed by mucosa-associated macrophages (and likely mucosa-associated dendritic cells), carried via leukocyte trafficking to local lymphoid tissues, such as BALT (within lung tissue), and then to tracheobronchial lymph nodes (regional) via afferent lymphatic vessels. Rhodococcal pneumonia (*R. equi*) is discussed in greater detail in the section on Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Horses; Rhodococcal Pneumonia (*Rhodococcus equi*).

Dendritic Cell Entry

Sheeppox and goatpox (poxviruses) (see Fig. 17-64) are examples of viral diseases in which each virus uses dendritic cells within mucosae of the respiratory and alimentary systems as portals of entry. Virus encounters oronasal mucosae via inhalation or ingestion, infects mucosa-associated dendritic cells, and then uses these cells to transfer across the mucosa into MALT and its leukocytes, including macrophages. Via leukocyte trafficking, virus spreads to regional lymph nodes, systemically to other lymph nodes, spleen, and bone marrow. Sheeppox and goatpox are discussed in greater detail in the section on Viral Diseases of Organ Systems, Integumentary System, Disorders of Ruminants (Cattle, Sheep, and Goats), Pox (Cowpox [Orthopoxvirus], Sheeppox and Goatpox [Capripoxvirus], Swinepox [Suipoxvirus], Enveloped DNA Virus).

Transcytosis Entry

Diamond skin disease of pigs (*Erysipelothrix rhusiopathiae*) (see Fig. 10-80) is an example of a bacterial disease that likely, but not fully proven, uses transcytosis entry. The bacterium likely interacts with the cell membrane at the luminal surface of the cell, enters a vesicle formed by an invagination of the membrane, crosses the interior of the cell in the vesicle, fuses with the basolateral membranes of the cell, and is then ejected from the vesicle into the tissues that surround the basolateral areas. The bacterium, a commensal organism that resides in a biofilm of the pharyngeal mucosae, replicates in sufficient numbers to colonize mucosae when pigs experience farm stress. It is spread via inhalation or ingestion to epithelia of the pharyngeal mucosae and the tonsil and to cells of MALT in susceptible pigs. The bacterium then spreads locally, regionally, and systemically via leukocyte trafficking to infect blood vascular endothelial cells of the skin and cause diamond skin disease. Diamond skin disease (*E. rhusiopathiae*) is discussed in greater detail in the section on Bacterial Diseases of Organ Systems, Integumentary System, Disorders of Pigs, Diamond Skin Disease (*Erysipelothrix rhusiopathiae*).

Direct Entry (Motility)

Leptospirosis (*Leptospira* spp.) (see Fig. 11-66) is an example of a bacterial disease that uses direct entry (motility) to cross mucosae. The bacterium is a highly motile spirochete and is able to move directly through mucosae or skin epithelial cells or between the cells via intracellular junctional complexes and reach well-vascularized ECM tissues. In ECM these spirochetes use invasive motility (a virulence factor) to penetrate endothelial cells and vascular walls of capillaries and postcapillary venules and gain access to the circulatory system and cause disease. Leptospirosis (*Leptospira* spp.) is discussed in greater detail in the section on Bacterial Diseases of Organ Systems, Urinary System, Disorders of Domestic Animals, Renal Leptospirosis (*Leptospira* spp.).

Nerve Ending Entry

Bovine herpesvirus meningoencephalitis (bovine herpesvirus 5) is an example of a viral disease that uses nerve endings located in mucosae to cross mucosae. Initially the virus is inhaled or ingested and deposited on mucosae of the oral, nasal, and pharyngeal cavities and the conductive component of the respiratory system. The virus enters nerve endings that extend onto the luminal surface of mucosae between epithelial cells. Through these nerve endings, virus enters neurons, such as the trigeminal and olfactory nerves, and spreads via retrograde axonal transport to other neurons and glial cells within the nervous system. Bovine herpesvirus meningoencephalitis is discussed in greater detail in the section on Viral Diseases of Organ Systems, Nervous System, Disorders of Ruminants

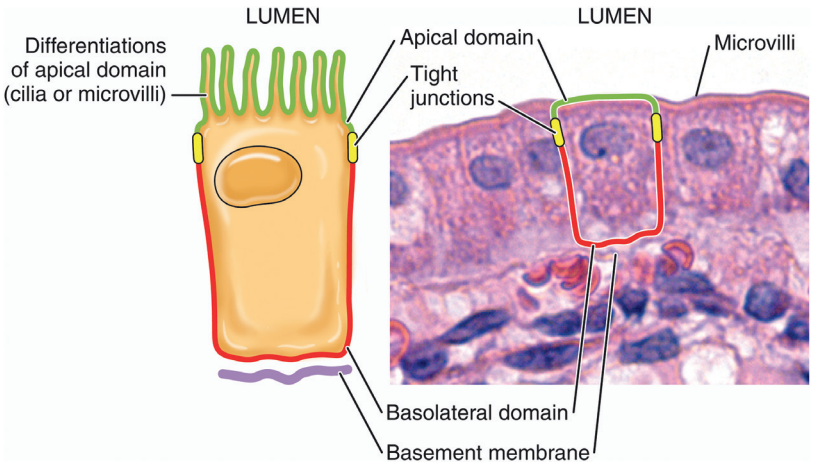


Figure 4-10 Domains of Polarized Epithelial Cells in Mucosal Barriers. Microbes use the apical or basolateral domains of mucosal epithelial cells to enter and exit from these cells. Apical or basolateral cell surface receptors may also facilitate entry into the cell. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

(Cattle, Sheep, and Goats), Bovine Herpesvirus Meningoencephalitis (Bovine Herpesvirus 5: Alphaherpesvirus, Enveloped DNA Virus).

Mechanisms of Adhesion, Colonization, Invasion, and Replication

Later in this chapter, diseases are grouped in sections under the headings Bacterial Diseases, Viral Diseases, Fungal Diseases (Mycoses), Protozoan Diseases, and Prion Diseases. At the beginning of each of these sections, the mechanisms used by microbes within each group to colonize cells and complete their life cycles are discussed and illustrated in greater detail.

Cell Polarity

In the alimentary and respiratory systems (and likely in other mucosae), the surface of an epithelial cell located above its junctional complexes and exposed to the lumen is called the *apical domain*, whereas the surface below junctional complexes on the sides and base make up the *basolateral domain* (Fig. 4-10). This relationship establishes a polarity to the cell, and it has been shown experimentally that such polarity is often reflected in the expression of different sets of cell membrane receptors that can potentially be used by microbes to attach to and enter the cells and also leave cells. As examples, parvoviruses use receptors expressed only on the basolateral surfaces to infect small intestinal crypt cells by spreading from cells located in Peyer's patches, whereas influenza viruses use receptors expressed only on apical surfaces to infect respiratory epithelial cells via the airway.

Transcytosis and Endocytosis/Exocytosis

Transcytosis is a normal function of epithelial cells, endothelial cells, and many other cell types of the body and is used to move macromolecules across cells in microvesicles (also known as endosomes) (see Chapter 1). By using specific virulence factors, microbes enter cells through a process called *receptor-mediated endocytosis*, most commonly at the apical surface, and exit the cell from the basolateral surface via a mechanism called *exocytosis* into the lamina propria and/or dermis and encounter mucosa- or dermis-associated lymphoid tissue (MALT) or other ECM tissues (see Fig. 4-7).

Systemic Spread

Systemic spread may occur (1) in a passive manner by dispersal of cell-free microbes in lymph via the lymphatic system or in plasma via the circulatory system to randomly encounter appropriate target cell(s) or (2) in an active manner through infection of mucosal or submucosal (also dermal/subcutaneous) macrophages, lymphocytes, and/or dendritic cells with dispersal of cell-associated microbes in the lymphatic or circulatory systems to randomly encounter appropriate target cell(s). This latter means of spread is called *leukocyte trafficking* (Fig. 4-11) and occurs as these cells migrate through all organ systems during their normal surveillance activities for the lymphoid (immune) system. When, during their migration, trafficking leukocytes encounter the appropriate target cell(s), a series of steps occur that allow cell-associated microbes to escape (often by lysing the trafficking cell) and then infect appropriate target cell(s) via ligand-receptor interactions (discussed later). For microbes that use leukocyte trafficking as a means of spread, the initial encounter with cells in mucosae, mucocutaneous junctions, or skin may be an initial and very limited beachhead step, where the sole purpose is to replicate microbes in sufficient numbers to infect appropriate leukocyte targets through phagocytosis or endocytosis for subsequent trafficking. An additional means of systemic spread for specific microbes is within the nervous system. Microbes can interact with nerve endings within mucosae, mucocutaneous junctions, or skin during the initial beachhead encounter, enter these nerve endings likely via endocytosis, acquire protection from the animal's defense mechanisms within the neuron, and then use retrograde and anterograde axonal transport mechanisms to be spread throughout the nervous system protected within neurons to reach their destination target cell (usually but not always located within the CNS or PNS).

Defense Mechanisms

At portals of entry, microbes run into a variety of structural, functional, physiologic, and innate defense mechanisms (Essential Concept 4-4).

Barrier Systems

Structural (Physical) Barriers

Structural barriers prevent microbes from gaining access to target cells and tissues. Although there are many important macroscopic structural barriers in the body, such as those formed by bone (calvarium, vertebral column) and meninges (dura mater) as examples, this section will focus on the microscopic structural barriers formed by mucosa, mucocutaneous junctions, and skin. Mechanisms such as trauma and penetrating wounds that allow microbes to cross

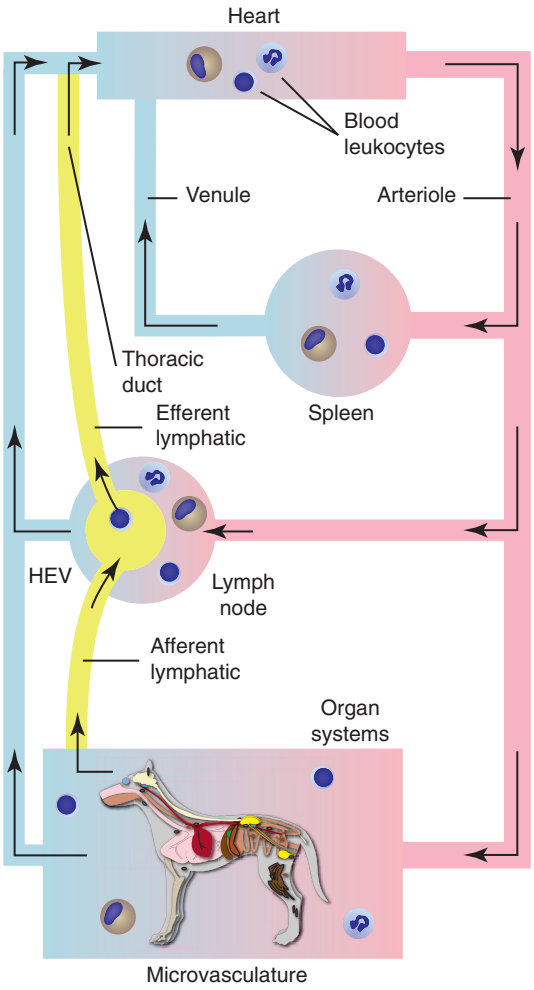


Figure 4-11 Leukocyte Trafficking. Microbes often use macrophages, lymphocytes, neutrophils, and/or dendritic cells to spread to other organ systems as these cells migrate through these systems as part of their normal immunologic surveillance activities. High endothelial venules (HEVs) are lined by specialized endothelial cells that allow lymphocytes circulating in the blood to enter the lymph node by crossing through the HEVs. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

structural barriers are relatively straightforward; other mechanisms used to cross mucosa, mucocutaneous junctions, and skin are much more stealthlike and are discussed herein.

Lining and covering epithelia (see earlier sections) of the alimentary, respiratory, integumentary, and urogenital systems, as well as the eye and ear, are the interface ("structural barriers") between the outside[6] and inside of the body and are held together (to each other) by occluding junctions such as tight junctions, desmosomes, and adherens junctions and to the basement membrane and ECM by anchoring junctions.

Skin is protected from microbes by (1) its physical thickness (five strata of stratified and pseudostratified epithelium) anchored by junctional complexes; (2) the keratinization, acidity, and oiliness (sebum) of the outer stratum corneum and stratum lucidum (antibacterial and antifungal properties); and (3) sloughing of keratinized

[6]The alimentary, respiratory, and urogenital systems (also ear and eye) are functionally considered "outside" of the body because they have orifices that connect them with the outside.

ESSENTIAL CONCEPT 4-4 Defense Mechanisms and Barrier Systems

At portals of entry, along pathways of spread, and within organ systems, microbes encounter a variety of defense mechanisms and barrier systems. They are designed to prevent, limit, and/or delay microbial attachment to and/or colonization of cells and substances and then to isolate and contain their spread to allow acute inflammation and adaptive immunologic responses to control and eliminate them.

These defenses include the following:

1. Structural defenses such as those formed by the calvarium and vertebral column and the dura mater of the meninges
2. Structural and functional defenses such as those formed by mucosae, mucocutaneous junctions, and skin (epithelia) of barrier systems
3. Functional defenses such as mucus, bile, bacteriostatic/bactericidal molecules, lysozyme, defensins, surfactant, gastric acid, bile acids, and digestive enzymes
4. Physiologic defenses such as vomiting, exaggerated peristalsis (diarrhea), mucociliary clearance, lacrimation, and desquamation
5. Innate defenses such as those occurring in acute inflammation (e.g., phagocytosis, respiratory burst, antimicrobial granules, phagosome-lysosome fusion)

Acute inflammation dilutes, wall offs (isolates), and kills microbes via phagocytosis by neutrophils and macrophages (monocytes) recruited to the portals of entry or to sites along the pathways of spread. Additionally, these phagocytes present microbial antigens to lymphocytes, dendritic cells, and/or other cells involved in adaptive immune responses.

cells into the environment from the stratum corneum. In contrast, the physical (thickness) defensive attributes of mucosae are not as substantial as those of the skin. Skin (in a dog as an example) ranges from 0.5 to 5.0 mm in thickness, whereas mucosae range from 10 to 100 μm in thickness. Thus skin is a substantial physical barrier to microbes when compared to mucosae.

Functional (Biologic) Barriers

The functional (biologic) defense mechanisms of mucosae (alimentary, respiratory, and urogenital systems and the ear and eye) and of the skin are extensive. They include the physiologic functions of peristalsis (alimentary system, urinary system) and mucociliary clearance (respiratory system) and the biologic functions of substances such as mucus (alimentary, respiratory, and urogenital systems [discussed later]), bacteriostatic and bactericidal substances such as lysozyme, defensins, surfactant, gastric acid, bile acids, and digestive enzymes (alimentary and respiratory systems). Substances and processes such as tears (lacrimation [eye]), cerumen (ear), and desquamation of skin cells function to "flush" microbes off mucosae and skin and out of the organ system. Lastly, mucus provides nutrients for resident microflora that compete for resources needed by microbes and provides a suitable environment for mucosa-associated leukocytes that phagocytize and kill microbes.

Mucus Layer. Mucosae of the alimentary and respiratory systems are covered by a protective mucous gel composed predominantly of mucin glycoproteins synthesized and secreted by goblet cells (Fig. 4-12). The mucus layer forms a barrier system that attempts to do the following:

- Block microbes from reaching target cells
- Trap microbes so they can be phagocytosed by mucosal macrophages and neutrophils

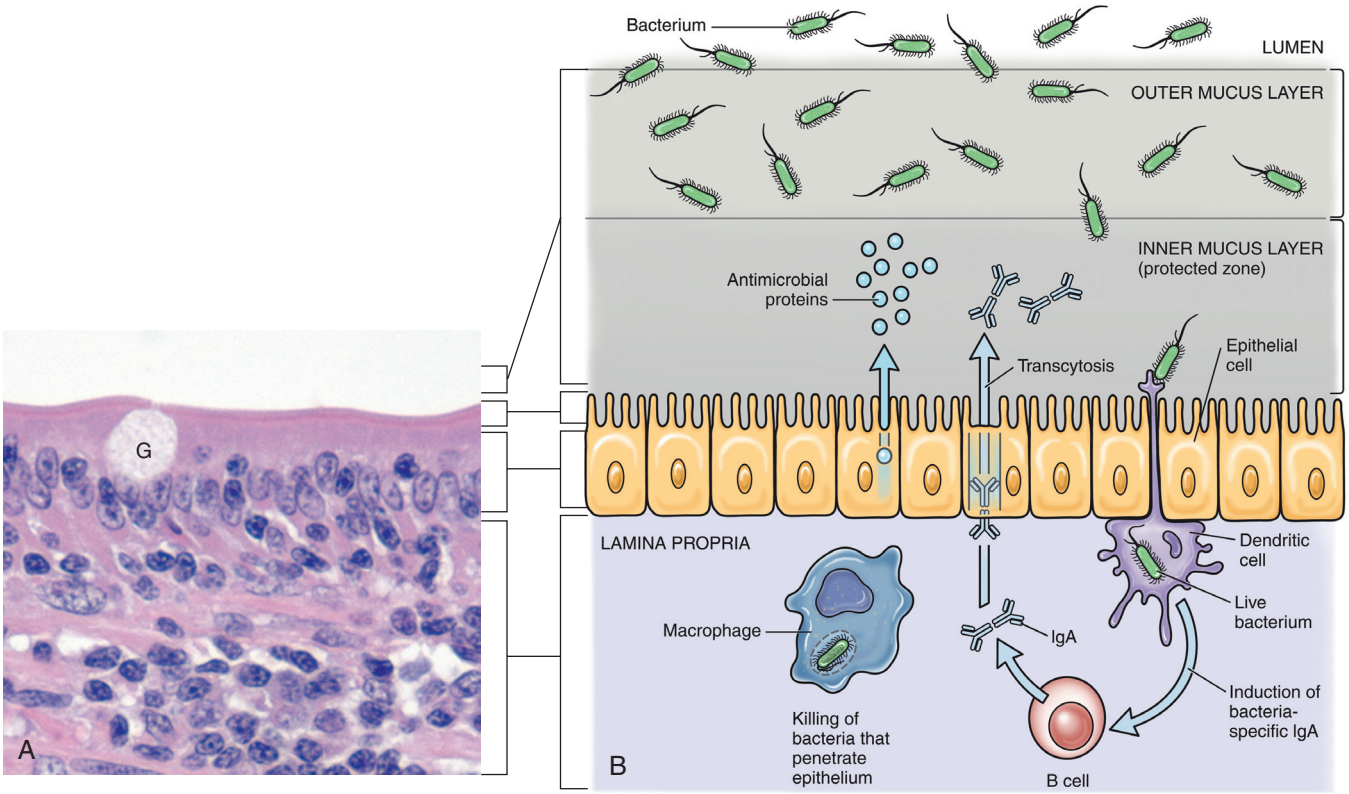


Figure 4-12 Mucus Layer of Alimentary and Respiratory Mucosae. A, Mucosae of the intestine (shown here) and of the conductive respiratory airways are covered by a mucus layer (not visible in H&E sections) secreted by goblet cells (*G*). The mucus covers the microvilli or cilia of these systems. H&E stain. **B,** The mucus layer has an outer layer that traps microbes (infectious and noninfectious) and other particles and an inner layer in which the cilia beat and which contains antimicrobial substances that diffuse into the outer layer. Dendritic cells and mucosa-associated macrophages and lymphocytes play central roles in preventing infection of mucosa. *IgA*, Immunoglobulin A. (**A** courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

- Trap microbes so they can be exposed to bacteriostatic and bactericidal molecules sequestered in the mucin matrix
- Facilitate phagocytosis of microbes via mucosa-associated macrophages, mucosal dendritic cells, and microfold (M) cells
- Deliver microbial antigens to local lymphoid tissues like Peyer's patches or BALT and then to regional lymph nodes via afferent lymphatic drainage

Normal microflora such as bacteria are observed within the outer luminal zone of the mucus layer, indicating the importance of mucous gel in preventing direct adherence of bacteria to epithelial cells. Changes in the function of goblet cells and the chemical composition of mucus can occur through the release of bioactive factors from microbes or by activation of immune cells. Additionally, predisposing management stressors, such as dehydration, shipping, humidity, and ventilation, combined with weather changes can also change the function of goblet cells and the chemical composition of mucus, making mucosae more susceptible to infection.

Microbes use three mechanisms to penetrate the mucus layer and gain access to target cells. More information is known about the interactions of bacteria with the mucus layer, when compared to other microbes, especially viruses. These mechanisms include penetrating motility, digestion of mucus via enzymes and the consumption of mucus as an energy source, and evasion of the mucus layer in areas around Peyer's patches and M cells, areas devoid of mucus. Mucus also provides pathogenic advantages to bacteria as follows: (1) mucin oligosaccharides represent a direct source of carbohydrates, peptides, and exogenous nutrients, including vitamins and minerals; (2) bacteria that colonize mucus avoid rapid expulsion out of the alimentary system by peristalsis; and (3) adhesion to specific molecules within the mucin facilitates colonization of the mucus layer by microbes. Microbial mucolysis, the ability to enzymatically degrade mucus, appears to be a common trait among bacteria (virulence factor) and provides access to readily available sources of carbon and energy and enables bacteria to reach the surface of epithelial cells. Mucins are classified as neutral and acidic types, with the latter being further categorized as sulfated (sulfomucins) or nonsulfated (sialomucins). These biochemical differences likely explain some of the segmental target cell specificity (i.e., localization in one area of the organ over another) of some diseases of the alimentary and respiratory systems. Localization and colonization of specific zones of mucus by certain microbes likely occurs through the expression of adherence molecules unique to specific types of mucins.

As an example in cattle, a disease of the respiratory system, mannheimiosis, is caused by the bacterium *Mannheimia haemolytica*. One of its virulence factors is neuraminidase (a glycoside hydrolase enzyme), which reduces the viscosity of mucus, making it a less dense and more fluidic layer. This change allows the bacterium better access to cell membranes via gravity and random brownian movement. Concurrently, neuraminidase also cleaves sialic acid from the surface of cell membranes, thus decreasing the net negative surface charge and allowing closer contact of the bacterium with membranes in a functionally degraded mucus layer.

Innate and Adaptive Immune Responses

The innate (acute inflammation) and adaptive immune responses are covered in detail in Chapters 3 and 5. In summary, acute

inflammation (see Chapter 3) is the first line of defense against the efforts of microbes to colonize and replicate in mucosa, mucocutaneous junctions, and skin (structural barriers). Acute inflammation is a response of vascularized tissue in these three structural barriers to cell injury and/or lysis and occurs when microbes attempt to colonize target cells. Molecules released from injured or dead cells (or cells in the area such as natural killer [NK] cells, basophils, mast cells, eosinophils, and platelets) initiate a cascade of humoral and cellular events designed to prevent or limit colonization by the microbe until phagocytes are recruited to the site and more effective defensive mechanisms are employed. Additionally, inflammasomes (see Chapters 3 and 5) are unique components of the innate immune system that detect microbes and are involved in the activation of inflammatory responses via pattern recognition receptors (PRRs), such as inflammasome sensor molecules. They activate a cascade of proinflammatory cytokines such as interleukin-1β (IL-1β) and IL-18.

With cell and tissue injury the fluidic phase (vascular phase) of acute inflammation dilutes, wall offs, and kills microbes via edema fluid, fibrin, and humoral factors of the complement and coagulation systems and other factors such as lactoferrin and transferrin, interferons, lysozyme, and IL-1. Some of these factors and microbial debris are chemotactic and recruit neutrophils and macrophages (monocytes) to the site. The fluidic phase hinders colonization and spread of microbes from the portal of entry and isolates them so they subsequently can be phagocytized and killed by neutrophils and macrophages (monocytes) during the cellular phase of the acute inflammatory response. Additionally, these phagocytes present microbial antigens to cells of the adaptive immune system such as macrophages (monocytes), lymphocytes, and dendritic cells.

Phagocytes interact with microbes using ligand-receptor interactions that are discussed in greater detail in sections covering specific classes of microbes such as bacteria, viruses, fungi, protozoa, and prions. Briefly, phagocytes bind (1) directly with microbes via ligands expressed specifically by the microbe or (2) indirectly with microbes by binding to biologic substances that coat the microbe during the fluidic phase of acute inflammation. In the first mechanism listed, microbial proteins called pathogen-associated molecular patterns (PAMPs) bind to PRRs (also known as Toll-like receptors [TLRs]) that are expressed on cell membranes of mucosa-associated phagocytes. PAMPs and PRRs play important roles in phagocytosis and killing ("activation") by phagocytes of specific types and classes of microbes. Additionally, these "activated" phagocytes release molecules such as inflammatory cytokines IL-1, IL-6, and tumor necrosis factor (TNF)-α that recruit additional phagocytic cells to the site. In the second mechanism listed , microbes become coated with a variety of biologic substances such as immunoglobulin G (IgG) antibody, C3b of complement, polyanions, and other molecules during the fluidic phase of acute inflammation. Phagocytes have cell membrane receptors for these ligands and attempt to phagocytose and kill these coated microbes.

The intended outcome of acute inflammation is to kill microbes and enable broad activation of an effective adaptive immune response. However, much of this chapter will discuss the processes used by microbes to block and evade innate and adaptive immune responses and to successfully complete their life cycles and cause disease in animals (Box 4-1).

Box 4-1 Mechanisms Used by Microbes to Block Defensive Activities of Phagocytes

Mechanism used by microbes	Effect on phagocytes
Avoidance	Gain access to tissues inaccessible to phagocytes
Stealth behavior	Avoid provoking an acute inflammatory (innate) response
Toxin	Inhibit chemotaxis by phagocytes
Polysaccharide capsule	Block encounter with phagocyte (prevents phagocytosis)
Opsonin	Block encounter with phagocyte (prevents phagocytosis)
Membrane trafficking molecules	Block fusion of phagosome with lysosome in phagocytic cell
Surface components or extracellular molecules	Block killing within the phagolysosome
Enzyme/toxin	Escape from phagosome or phagolysosome followed by replication in the cytoplasm
Toxin	Kill phagocyte before or after phagocytosis
Antioxidant	Resistance to killing by phagocytes

Monocyte-Macrophage System

The monocyte-macrophage system (also known as mononuclear phagocyte system [MPS]) is covered in detail in Chapter 5. Under normal conditions, tissue macrophages are derived from two sources: blood monocytes and tissue macrophage progenitor cells that are distributed throughout body tissues during organogenesis of the embryo. Precursor monocytes in bone marrow are capable of providing monocytes that migrate to and differentiate into macrophages in tissues. Tissue macrophages are also replenished locally and in large numbers by proliferation of tissue macrophage progenitor cells. These two populations of cells give rise to tissue macrophages that form the functional basis for innate and adaptive responses to microbes in tissues and organs (see Chapters 5 and 13).

In summary, cells of the monocyte-macrophage system are important in phagocytizing and killing microbes (Fig. 4-13) and then "presenting" microbial antigens to lymphocytes, dendritic cells, and/or other cells involved in the adaptive immune response. Cells of the monocyte-macrophage system originate in bone marrow and enter the circulatory system as blood monocytes. Monocytes (and macrophages) then (1) may be recruited into tissues along a chemotactic gradient during acute inflammation and differentiate into macrophages (see previous section) or multinucleated giant cells (fused macrophages) or (2) may migrate into the ECM and other types of supportive tissues in a wide variety of organ systems to establish and maintain a resident population of phagocytic cells. These latter cells include (1) lung (alveolar macrophages), (2) liver sinusoids (Kupffer cells), (3) lymph nodes (free and fixed macrophages), (4) spleen (free and fixed macrophages), (5) bone marrow (fixed macrophages), (6) connective tissue (histiocytes), (7) serous fluids (pleural and peritoneal macrophages), (8) skin (histiocytes), (9) mucosae (mucosa-associated macrophages), (10) brain (microglia cells), and (11) bone (osteoclasts) (Box 4-2). Monocytes and macrophages are also part of a systemic network of phagocytic and immune cells (lymphocytes, dendritic cells) that migrate through organs via the circulatory and lymphatic systems. This migratory process is called *leukocyte trafficking* (see Fig. 4-11), and these cells behave as "lookouts" for microbes and other matter such as cell debris (cells injured by microbes). When they encounter microbes, they facilitate responses like acute inflammation and "present" antigens for adaptive immunity to protect the animal against microbes. However, some microbes have acquired virulence factors that allow them to enter trafficking leukocytes and spread protected within them to target cells in other organ systems. This mechanism will be discussed in greater detail in later sections and in material covering individual diseases.

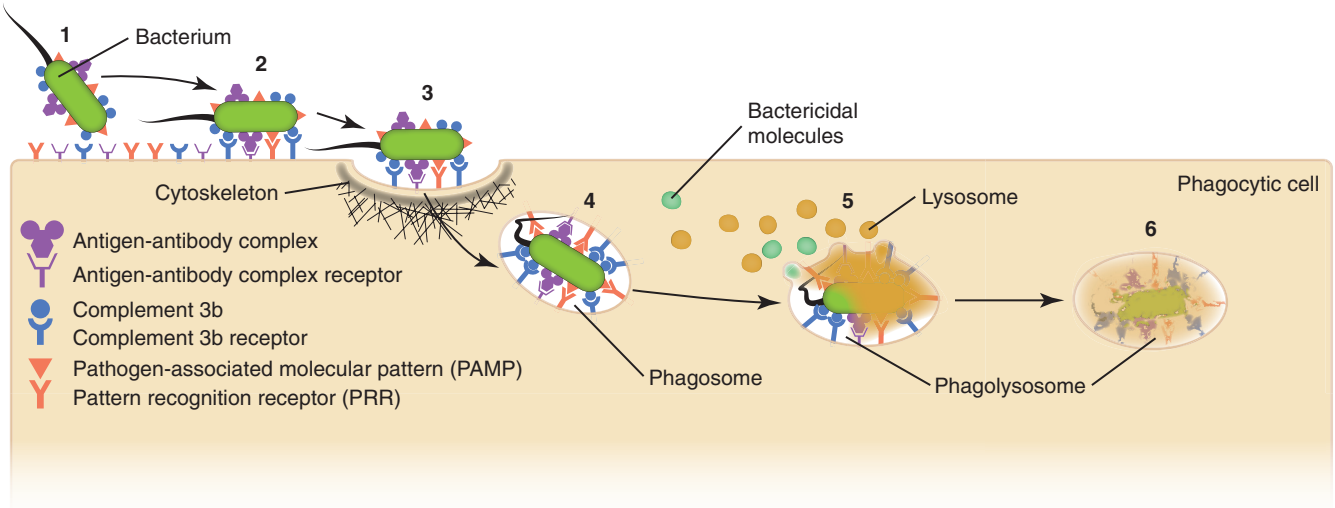


Figure 4-13 Phagocytosis. *1,* Microbes encounter phagocytic cells, such as macrophages and neutrophils, at portals of entry and extracellular matrix tissues. *2,* The surfaces of microbes contain a variety of ligands, such as opsonins, C3b, and pathogen-associated molecular patterns (PAMPs), that allow them to recognize and attach to phagocytes. Phagocytes have complementary receptors for these ligands. When ligand and receptors interact successfully, the microbe is firmly attached to the surface of the phagocyte. *3,* Ligand-receptor binding initiates a cascade of membrane second messengers and cytoskeletal elements (see Chapter 1) that begin the process of internalizing the microbe. *4,* The microbe is confined in the cytoplasm within a phagosome (phagocytic vacuole) and moved into the cell. *5,* The phagosome fuses with lysosomes and becomes a phagolysosome. Lysosomes contain a variety of enzymes and other microbiostatic and microbicidal molecules such as reactive oxygen species and nitric oxide. *6,* These molecules are released into the phagosome and act to kill the microbe. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

Box 4-2 Cells of the Monocyte-Macrophage System

Cell type	Location
Promonocyte	Bone marrow
Blood monocyte	Circulatory and lymphatic systems
Kupffer cell	Hepatic sinusoids
Mesangial cell	Renal glomerulus
Alveolar macrophage	Air-blood barrier of pulmonary alveolus
Tissue histiocyte	Extracellular matrix, surfaces covered by mesothelium
Microglial cell	CNS
Splenic macrophage	Red and white pulp of spleen
Tissue macrophage (histiocyte)	Medullary cords of lymph nodes
Mucosa-associated macrophages	Mucosae

CNS, Central nervous system.

Dendritic Cells

Dendritic cells are covered in detail in Chapters 3 and 5. In summary, dendritic cells are phagocytic antigen-processing and antigen-presenting cells that are commonly found intermixed with epithelial cells of mucosae and skin (e.g., Langerhans cells) as well as other tissues and organ systems. They play a central role in developing an adaptive immune response to microbes. However, because dendritic cells are phagocytic and migratory, microbes can also use them to complete their life cycles. Microbes use mechanisms similar to those used to infect macrophages to infect dendritic cells. Once infected, dendritic cells can migrate from mucosae and skin to local and regional lymphoid tissues via lymphatic vessels. Microbes through their surface proteins are able to bind to receptors expressed on the apical domains of these cells and infect them, then exit via the basolateral domain via exocytosis, gain access to lymphoid nodules (tissues commonly associated with dendritic cells), and establish a local infection in lymphocytes and macrophages. Infected lymphocytes and macrophages spread the agent via leukocyte trafficking from local sites to regional lymph nodes and then systemically to other organ systems.

Phagosome-Lysosome Fusion

Phagosome-lysosome fusion is an intracellular process used by phagocytes to kill microbes. Lysosomes are cellular organelles (see Chapter 1, Fig. 1-1, and Fig. 4-13) that contain an array of enzymes and toxic molecules. Microbes enter phagocytic cells via phagocytosis or endocytosis and are found in intracellular vesicles (phagosomes or endosomes). These vesicles fuse with lysosomes and these lysosomes release an array of degradative enzymes and toxic molecules into the fused vesicle, now called a phagolysosome that are designed to kill the microbe. Microbes have virulence factors that act to block phagosome-lysosome fusion or, if fusion occurs, to neutralize the effects of toxic molecules released from lysosomes and allow them to colonize cells and tissues, replicate, and complete their life cycles (Fig. 4-14). Examples of these mechanisms are discussed in the section covering Johne's disease (*Mycobacterium avium* subsp. *paratuberculosis*) and in other diseases in this chapter.

Genetic Resistance of Animals to Infectious Diseases

The resistance of animals to disease depends on the effective interplay of many structural and functional (physiologic) components of the body, including cutaneous and mucosal barrier systems and the immune system, respectively. Distinct networks of genes play central roles in structural and functional activities of the body. They control the development, maturation, and maintenance of epithelial cells, mucus, and supporting ECM tissues, such as collagen, that form the barrier systems. Additionally, they control similar structural activities for a variety of cell lineages of the innate and adaptive immune systems like T lymphocytes, macrophages, neutrophils, and dendritic cells and the expression of proteins that form PRRs in the membranes of these cells (see Chapter 5). These receptors recognize PAMPs expressed by microbes and are discussed

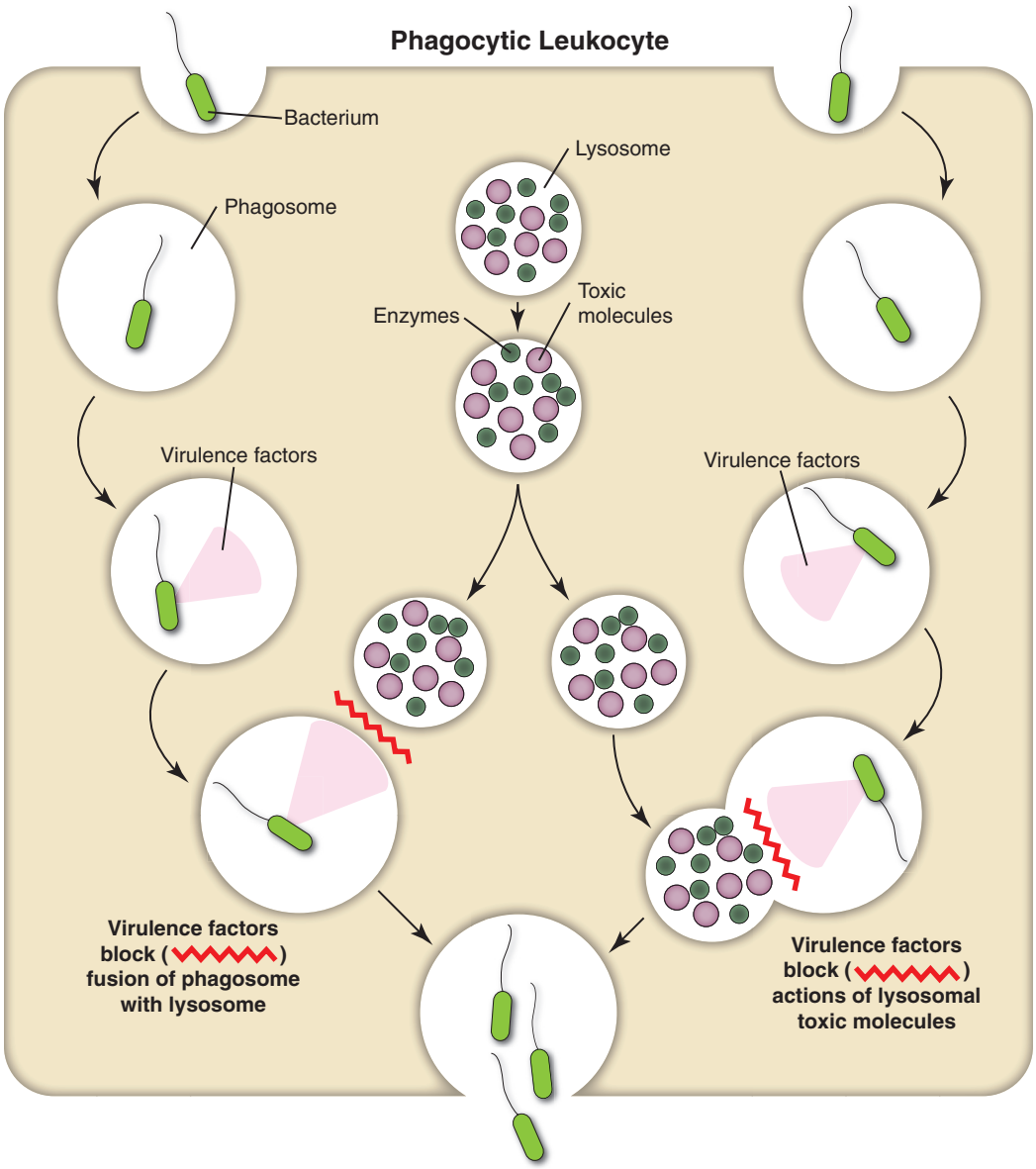


Figure 4-14 Virulence Factors Block Actions of Lysosomes in Phagocytes. Microbes enter leukocytes such as macrophages via phagocytosis and are protected within intracellular vesicles (phagosomes). As a defense mechanism, leukocytes have lysosomes that fuse with phagosomes and release an array of degradative enzymes and toxic molecules into the fused vesicle, now called a phagolysosome that are designed to kill the microbe. Microbes have virulence factors that act to block phagosome-lysosome fusion or if fusion occurs, to neutralize the effects of toxic molecules released from lysosomes and allow them to colonize cells and tissues, replicate, and complete their life cycles. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

in greater detail in Chapters 3 and 5. Genes also play a central role in functional processes of cells, including adhesion, chemotaxis, phagocytosis, phagosome-lysosome fusion, intracellular killing of microbes, and antigen processing (see Chapters 3 and 5) involved in innate and adaptive responses of the immune system. Thus genetic resistance to infectious diseases is a polygenic trait regulated mainly by the immune system and its interactions with barrier systems and environmental factors such as weather conditions and nutritional status.

In animals the genetics of disease resistance is most closely associated with the major histocompatibility complex (MHC), a tightly linked group of genes that encode proteins involved in immune responses. This genetic region in cattle has been given the abbreviated name *BoLA*, and similar regions have been identified in other animal species. Few specific genes or genetic markers related to disease resistance have been identified in domestic animals; however, genes involved in antigen processing appear to be important in resistance to infectious diseases.

Disorders of Barrier Systems

Barrier systems most commonly involved in infectious diseases of animals include the alimentary and respiratory mucosae and the skin and were discussed previously. Additionally, mucosae of the conjunctiva and urinary systems and skin of the ear also serve as barriers to microbes. These systems and their physical barriers develop embryologically under strict genetic control and when mature are functionally maintained, regulated, and repaired through processes dependent on transcription and translation of genes. Structural and/or functional alterations in these barriers can make animals more susceptible to microbes.

An example of a genetic disorder that predisposes animals to infectious disease that occurs because of an alteration in the development of the basic structure of a barrier is epitheliogenesis imperfecta. It is an autosomal recessive hereditary disease of young horses, cattle, and pigs characterized by loss of epithelium affecting the skin and mucosae of the oral cavity and tongue likely caused by alterations in the subbasal plate and its hemidesmosomes and laminin-5 (see Chapter 17). Loss of the skin or mucosae exposes underlying vascularized ECM tissues to environmental contamination with feces and other matter, allowing bacterial pathogens access to ECM and capillary beds.

An example of a genetic disorder that predisposes animals to infectious disease that occurs because of an alteration in the function of a barrier is primary ciliary dyskinesia of the dog. It appears to be an autosomal recessive hereditary disease of young dogs, but an autosomal dominant mutation has not been excluded. This disorder is caused by ciliary dysfunction attributable to immotile or dyskinetic cilia caused by defects of proteins in the outer and/or inner dynein arms of cilia, which give them their motility. This outcome leads to dysfunction of the mucociliary apparatus and the retention of microbes in the respiratory system leading to bacterial bronchitis and pneumonia. Other examples of genetic alterations of barrier systems that predispose animals to increased susceptibility to infectious disease are discussed in the organ system chapters of this book.

Disorders of the Innate Immune Response

The innate immune system (i.e., acute inflammation) provides animals with an immediate defense against microbes and is discussed in detail in Chapters 3 and 5 and in earlier sections of this chapter. In summary, this system involves the initial encounter of mucosae, mucocutaneous junction, and/or skin with microbes at a portal of entry. It is essentially acute inflammation and (1) the cellular and chemical mediators associated with the process, such as phagocytic cells like macrophages, neutrophils, and dendritic cells; (2) effector cells, such as T lymphocytes and mast cells; (3) chemical mediators of the complement system; and (4) the vascular system. The purpose of acute inflammation is to dilute and isolate microbes in edema fluid and fibrin, phagocytose and kill them, and process and present their antigens to effector cells of the adaptive immune response. When epithelial cells, endothelial cells, or mucosal or cutaneous macrophages of barrier systems are injured by or infected with microbes, they release large quantities of cytokines into the surrounding tissues. These cytokines recruit, via chemotaxis, inflammatory cells from capillaries in vascularized ECM tissues and cause vasodilation and increased permeability of these blood vessels (i.e., edema fluid and fibrin). Inflammatory cells also release cytokines and other chemical mediators that act to recruit additional inflammatory cells, activate the complement cascade to identify bacteria and kill microbes, promote phagocytosis of dead cells and microbes by phagocytic cells, and activate the adaptive immune system through antigen processing and presentation to immune cells such as T and B lymphocytes. Phagocytic and effector cells of the acute inflammatory response are recruited from capillaries and migrate along a chemoattractant gradient formed by chemical mediators and molecules released from microbes to the inflammatory focus. In inflammatory foci, these cells express TLRs, also known as *pattern recognition receptors* (PRRs), that recognize molecules on infectious agents called PAMPs (see Chapters 3 and 5). These cells also express IL-1 receptors that act in concert with PRRs to initiate and sustain the innate immune response through phagocytosis.

Genetic disorders can affect all of the steps involved in the innate immune response as summarized earlier from initial recognition of microbes to their phagocytosis and killing and are discussed in many chapters of this book. Examples of genetic disorders of the innate immune system that predispose animals to infectious disease, most commonly bacterial diseases, include leukocyte adhesion deficiencies and granulocytopathy syndromes. Leukocyte adhesion deficiency occurs in dogs and cattle and has an autosomal recessive mode of inheritance. It is characterized by alterations in the leukocyte adhesion cascade (see Chapter 3) involving deficiencies or dysfunction of integrins and selectins resulting in the inability of neutrophils to adhere to endothelial cells in the wall of blood vessels and migrate into sites of bacterial infection. Granulocytopathy syndrome occurs in dogs and cattle and has an autosomal recessive mode of inheritance. It is characterized by alterations in the ability of neutrophils to kill bacteria in phagosomes and is linked to reduced nicotinamide adenine dinucleotide phosphate (NADPH) concentrations that may arise from a metabolic anomaly in the hexose monophosphate shunt. This deficiency may lead to reduced concentrations of hydrogen peroxide in phagosome-lysosome fusion and the bactericidal capability of the neutrophil. The process of phagocytosis is usually normal. Affected animals have shortened life span, long-term febrile disease, dermatitis, oral ulcers, lymphadenitis, and poor healing, attributable to irresolvable and repeated bacterial infections.

Genetic disorders of the innate immune system can also be caused by failure of leukocytes to correctly develop and mature in the bone marrow. Cyclic hematopoiesis occurs in dogs and has an autosomal recessive mode of inheritance. It is caused by an abnormality of stem cells in the bone marrow resulting in periodic declines, every 10 to 12 days, in neutrophil concentrations followed by hyperplasia and a return to normal. Abnormal concentrations of purine and pyrimidine metabolites in affected stems cells suggest that a metabolic derangement in purine or pyrimidine metabolism may be the cause of this genetic disorder. This outcome increases susceptibility to bacterial infection, often leading to periodic fever, joint pain, or other signs of ocular, respiratory, or skin infections. Other examples of genetic alterations of the innate immune response that predisposes animals to increased susceptibility to infectious disease are discussed in the organ system chapters of this book.

Disorders of the Adaptive Immune Response

Genetic disorders of the adaptive immune response are disorders in which affected animals are incapable of generating antigen-specific immune responses (see Chapter 5). Such genetic diseases are closely associated with genes that regulate the expression of the MHC, especially those genes involved in antigen processing and presentation. Examples of this type of disorder include agammaglobulinemia (a B lymphocyte immunodeficiency) and severe combined immunodeficiency (a B and T lymphocyte immunodeficiency). T lymphocyte, macrophage, and complement immunodeficiencies also occur but are not discussed here. Agammaglobulinemia has an X-linked recessive mode of inheritance and thus is a disorder of young colts. It is likely caused by dysfunction of cytoplasmic tyrosine kinase resulting in blockage in the differentiation of B lymphocyte lineages and a nearly complete absence of B lymphocytes and plasma cells. Clinically, this type of immunodeficiency results in colts with chronic bacterial diseases leading to pneumonia, enteritis, dermatitis, arthritis, and laminitis. Severe combined immunodeficiency occurs in dogs and Arabian horses and has an autosomal recessive mode of inheritance. In dogs, an X chromosome–linked mode of inheritance has also been identified. Affected animals produce no antibodies after infection or immunization because of an absence of B lymphocytes and have no or nonfunctional T lymphocytes when present. This genetic disorder occurs when lymphocyte precursors

fail to differentiate into mature T or B lymphocytes, which is likely because of the result of mutations within recombinase-activating genes or within genes encoding DNA-dependent protein kinase or when differentiated lymphocytes are incapable of completing signal transduction pathways because of defects in cell surface receptors for interleukins. Other examples of genetic alterations of the adaptive immune response that predisposes animals to increased susceptibility to infectious disease are discussed in the organ system chapters of this book.

Bacterial Diseases

Pathogenicity

The pathogenicity (i.e., ability to cause disease) of a bacterium is regulated by its virulence factors. In summary, virulence factors are used by microbes to kill phagocytic cells, block phagocytosis, evade fusion with lysosomes, block killing within phagocytes, and enhance replication within phagocytes. Virulence factors are molecules, often glycoproteins or glycolipids, derived from bacterial genes. Their expression establishes the processes used by bacteria to successfully colonize mucosae, infect cells, grow and replicate, and cause cell lysis.

Important actions of virulence factors and the biologic substances with which they interact include the following:

- Production of bacterial toxins that kill phagocytes
- Synthesis of bacterial proteins that prevent phagocytosis by blocking the interaction of opsonins with phagosomes
- Synthesis of a bacterial capsule that can block contact with the microbe and prevent phagocytosis
- Inhibition of fusion of the phagosome containing microbes with lysosomes
- Facilitate escape of the microbe into the cytoplasm before the microbe is killed in the phagolysosome
- Production of bacterial antioxidants (i.e., catalase) that block killing in phagolysosomes

The interaction between an animal and a bacterial pathogen is back and forth, each acting to influence the activities and functions of the other. The outcome depends on the virulence (i.e., virulence factors) of the pathogen and the resistance or susceptibility (i.e., genes) of the animal. Resistance or susceptibility to disease in healthy animals is derived from (1) innate defenses such as cellular and mucous barrier systems, acute inflammation (including neutrophil phagocytosis), the monocyte-macrophage system (phagocytosis), and normal bacterial flora and (2) adaptive defenses provided by the immune system such as passive immunity via colostrum and active immunity via T and B lymphocytes. In general, bacterial pathogenicity is determined by two characteristics: (1) the bacterium's ability to colonize cells and (2) its ability to produce toxins and damage cells and their ECM tissues such as collagen. To colonize cells, bacteria use mechanisms such as adhesion, multiplication, colonization, tissue invasion, and circumvention of animal defense mechanisms. To damage cells via toxins, microbes use mechanisms such as cytolysis and invasion of vascularized ECM tissues (locally or systemically) incited by bacteria-derived exotoxins (Gram-positive bacteria) and endotoxins (Gram-negative bacteria). Virulence factors determine the sum of the characteristics that allow bacteria to cause disease and thus provide a pathogenicity profile or fingerprint for each bacterium.

Virulence Factors

Bacterial virulence factors are molecules that influence interactions between bacteria and target cells and/or substances, including processes such as adherence to cell membranes; colonization and invasion of skin or mucosae; endocytosis and/or phagocytosis; growth, replication, and other metabolic processes; local, regional, and systemic spread; and cell injury and/or lysis (Fig. 4-15; Table 4-1). Furthermore, they inhibit innate and adaptive immune responses, allowing the bacterium to evade defense mechanisms and also to proliferate in harsh environments. Bacterial virulence factors act as proteases, lipases, deoxyribonucleases (DNases), toxins, physiologic mediators (inhibitors or enhancers), lytic agents, adhesion factors, biofilms, bacterial capsules made of carbohydrates, and antiphagocytic factors (see Table 4-1). Bacterial virulence is determined in part by the type and number of factors the bacterium expresses to successfully complete its life cycle in an animal. In general, virulence factors are coded for by more than one bacterial gene. Other things that can indirectly influence the success of these virulence factors include physical and environmental stressors, such as weather, access to food and water, and management (shipping) or housing conditions (ventilation, humidity, or overcrowding).

Initial Encounters at Portals of Entry

Before infecting epithelial cells in mucosae (except areas with M cells), bacteria must penetrate the mucus layer to gain access to these cells. Once in the mucus layer, bacteria may be phagocytized by macrophages, lymphocytes, and/or dendritic cells as they migrate in, on, and through the mucus. As these phagocytes containing bacteria migrate, they interact with epithelial cells, phagocytotic and immune cells in the lamina propria and submucosa, ECM, and endothelial cells. These interactions allow bacteria, if they "escape" from phagocytes, to interact with all of these cell types and infect those required for them to complete their life cycles. However, in many diseases, it is unclear how bacteria ultimately invade or penetrate the mucus layer to gain access to mucosal epithelial cells (target cells). Several mechanisms, some regulated by virulence factors, are likely used to reach mucosal epithelial cells, including (1) motility, (2) digestion and consumption of the mucus layer, and (3) random discovery of mucosae lacking a mucus layer. As examples in the alimentary system, some bacteria, such as the spirochetes, are motile and can penetrate the mucus layer and reach target cells. Other bacteria, such as *Clostridium septicum*, digest the mucus layer with bacterial enzymes and then consume oligosaccharides such as *N*-acetylglucosamine, galactose, and *N*-acetylgalactosamine in the mucus layer as a carbon source during intense periods of replication. Finally, some bacteria use M cells to gain access to target cells; these cells are not covered by mucus, and their surface membranes and receptors are available to passing microbes.

Adhesion, Colonization, Toxigenesis, and Invasiveness

Adhesion, colonization, toxigenesis, and invasiveness are processes that occur during initial encounters between bacteria and cells of mucosa/skin at portals of entry.

- **Bacterial adhesion**—the process of bacteria attaching to cells, tissue, and biologic substances.
- **Bacterial colonization**—the adherence, multiplication, and establishment of bacteria at a portal of entry.
- **Bacterial toxigenesis**—the ability of bacteria to produce toxins.
- **Bacterial invasiveness**—the ability of bacteria to invade tissues.

These processes, facilitated by bacterial virulence factors, are also affected by other factors that act indirectly to minimize the actions of the animal's defense mechanisms by enabling resistance to antibiotics, enhancing antiphagocytic properties, and weakening or inhibiting immune responses.

Virulence factors, derived from membrane proteins, polysaccharide capsules, secretory proteins, cell wall and outer membrane

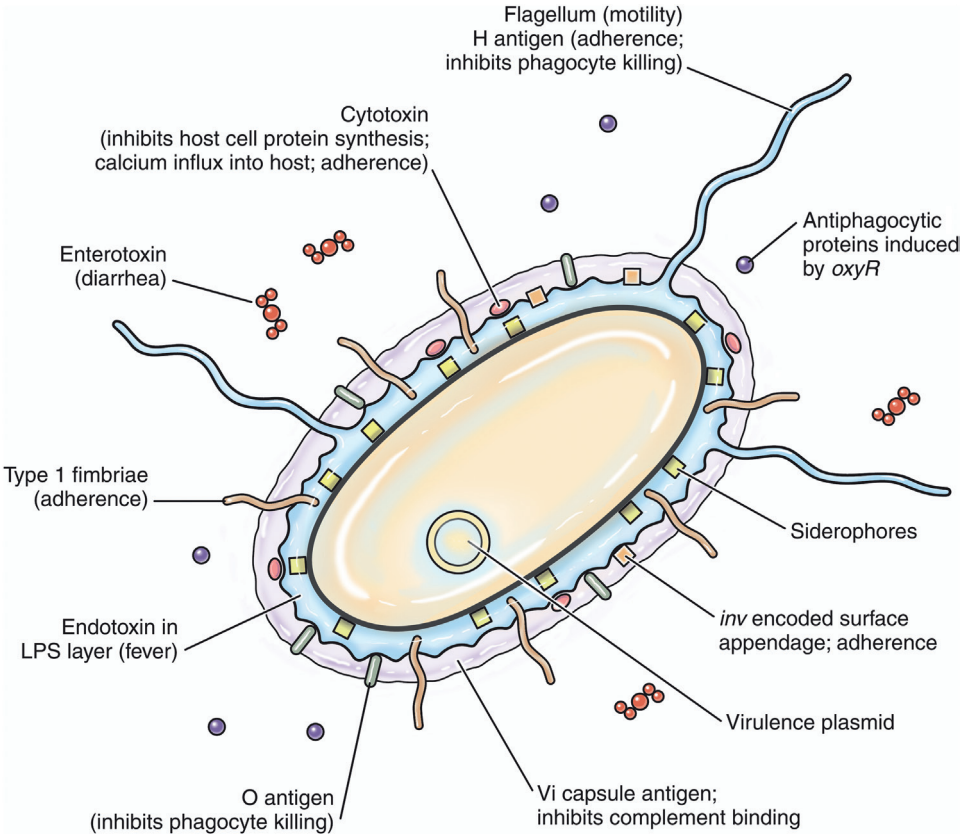


Figure 4-15 Virulence Factors Used by Bacteria to Cause Disease. *LPS,* Lipopolysaccharide.

Table 4-1 Examples of Virulence Factors and Their Biologic Actions

Virulence Factor(s)	Action
Adhesins	Attach to receptor(s) on cell membranes or on substances such as mucus, mucins, or ECM proteins; also facilitate entry into cell by endocytosis/phagocytosis
Invasins	Spread into and through cell membranes, cells, or tissue via ligand-receptor interactions, cell dysfunction and lysis, or breakdown of ECM
Endotoxins (lipopolysaccharides)	Stimulate macrophages and endothelial cells to secrete proinflammatory cytokines and nitric oxide; cause cell dysfunction and lysis
Exotoxins	Inhibit biochemical pathways within a cell
Excitotoxins	Dysfunction and lysis of neurons and other cell types
Mycotoxins	Dysfunction and lysis of cells
Immunoglobulin (Ig) proteases	Break down immunoglobins used in adaptive immune defense mechanisms
Hemolysins	Cell lysis
Lipases	Degrade cell lipids (cell membranes) and disrupt lipid metabolism
Hyaluronidases	Break down hyaluronan (hyaluronic acid) in ECM of mucosae, skin, connective tissue, and nervous tissue; some bacteria use hyaluronan as a carbon source for growth and replication; other bacteria may use hyaluronidase to spread though barrier systems and ECM
Collagenases	Break down collagen fibers of ECM, especially in muscle tissue
Neuraminidases	Degrade neuraminic acid (sialic acid) in cells and cell membranes; viral neuraminidase is used by influenza viruses to escape from target cells by budding from the cell membrane
Hemagglutinins	Attachment proteins located on the surface of influenza viruses that facilitate binding to cell membrane and entry into target cells
Kinases	Digest fibrin and prevent clotting of the blood needed to wall off bacteria
Lecithinases	Punch holes through or break down cell membranes
Phospholipases	Punch holes through or break down cell membranes

ECM, Extracellular matrix.

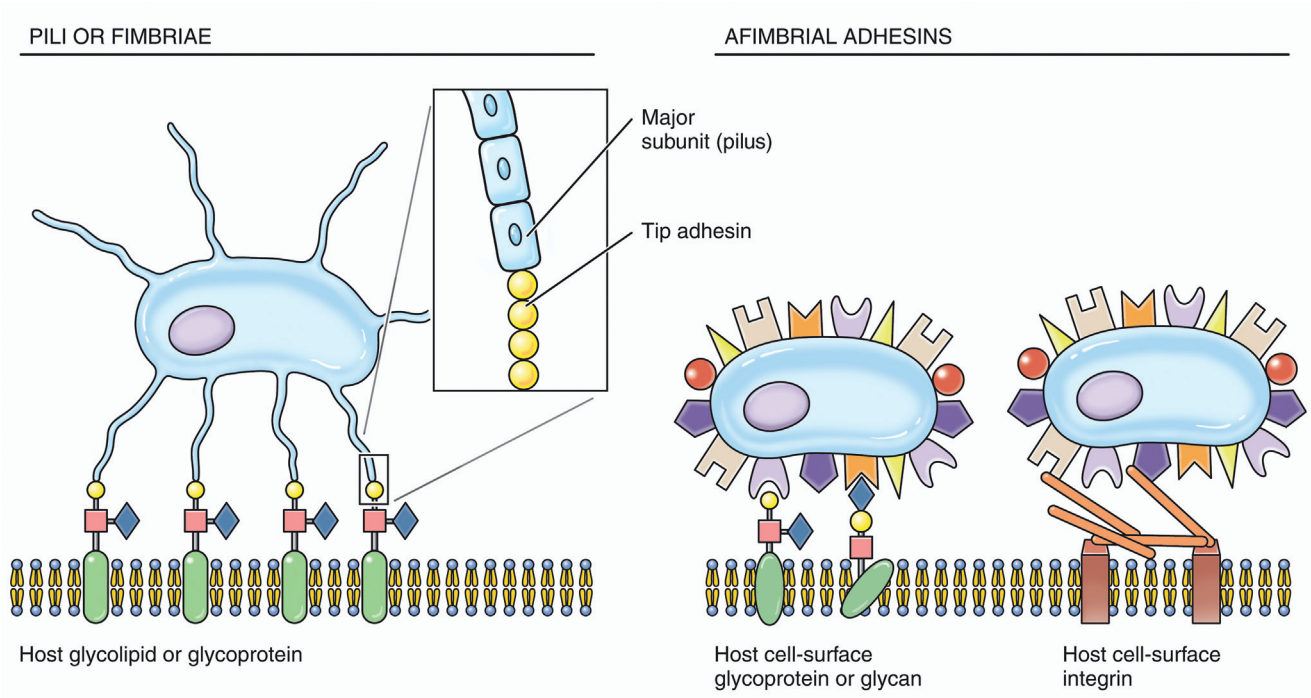


Figure 4-16 Fimbrial (Pilus) and Afimbrial Adhesins. These structures are used by microbes to attach and bind to protein receptors on membranes of target cells (especially mucosal epithelial cells) or to molecules of the mucus layer or vascularized extracellular matrix (connective) tissues.

components, and other miscellaneous proteins of bacteria, assist them in adhering to, colonizing, and invading epithelia at portals of entry (toxigenesis will be discussed in the next section). However, because epithelial cells of skin and mucosae are replaced continually (≈48-hour life span) and these systems have defensive mechanisms such as peristalsis, unidirectional mucociliary undulations, and micturition, bacteria must be able to adhere to, colonize (replicate), and/or invade into or between these epithelial cells in order to avoid being swept away. Attachment occurs when membrane proteins of bacteria called *adhesins* (a broad term) bind to receptors on cell membranes of mucosae and skin. Attachment is a typical ligand-receptor interaction; a protein on the bacterium binds to a receptor on a target cell. Some bacteria express adhesins, such as microbial surface cell recognition adhesion matrix molecules, that bind the bacterium to the surface of the cell. Other bacteria use extensions of their cell membranes called *fimbriae* or *pili* to bind to animal cells. Fimbriae and pili have adhesins, such as pilus-associated proteins, fimbrial antigens, or fimbrial adhesins that bind to receptors on microvilli of the glycocalyx or in the mucus layer (Fig. 4-16). Fimbriae and pili may also act to inhibit phagocytosis. For example, uropathogenic and enterotoxic *Escherichia coli*, causes of urinary tract infections and diarrhea in animals, express fimbrial (type 1, P, and S/F1C) and pilus (K99) adhesins, respectively. In the urinary tract, P fimbria is an important attachment adhesin and allows the bacterium to attach to the transitional epithelium (mucosa) of the bladder and cause the disease known as acute necrohemorrhagic urocystitis. Other virulence factors, such as α-hemolysin and cytotoxic necrotizing factor type 1, cause necrosis and hemorrhage later in the disease process. In the small intestine, K99 pilus adhesin allows *E. coli* to adhere to enterocytes and reduces their loss in number via intestinal peristalsis. When large numbers of *E. coli* are attached to the small intestine, they produce other virulence factors, such as enterotoxin, that act directly on enterocytes, leading to diarrhea.

Ligand-receptor interactions are likely common to most bacterial diseases; however, in many veterinary diseases, specific bacterial ligands and their target cell receptors have not been identified. The attachment of sufficient numbers of bacteria at the appropriate portal of entry initiates an early stage of bacterial infection termed *colonization*. After colonization, bacteria produce another group of virulence factors called invasins, or spreading factors. These factors include hyaluronidase, collagenase, kinases, lecithinase, and phospholipase and act to break down barrier systems formed by mucosae (mucus layer) and skin, cell junctional complexes, and ECM molecules like collagen. Additionally, other virulence factors that injure and/or kill cells include proteases and lipases; DNases, which break down DNA; and hemolysins that destroy cells, such as red blood cells. Invasiveness and invasins allow bacteria to spread rapidly into and through intercellular spaces and protect themselves in safe areas such lamina propria isolated from unfavorable host environments or host-derived defensive molecules. As examples, *Clostridium chauvoei*, the bacterium that causes blackleg in cattle, produces sufficient lecithinases and phospholipases to punch holes in cell membranes of skeletal muscle and cause lysis of myocytes and endothelial cells. *Listeria monocytogenes*, the cause of listeriosis in the nervous system of cattle, produces invasins that induce endocytosis of the bacterium for colonization by acting on target cell actin filaments. Other proteins of bacteria, such as surface components and polysaccharide capsules, are virulence factors that allow bacteria to avoid phagocytosis and evade recognition by cells of the innate and/or adaptive immune systems. They disrupt or block one or more steps used by neutrophils, monocytes, or macrophages in the phagocytic process such as initial contact, engulfment, phagosome formation, phagosome-lysosome fusion, and killing and digestion. As examples, *Streptococcus pyogenes*, a cause of bovine mastitis, uses M protein and a hyaluronic acid capsule to inhibit phagocytosis and the same hyaluronic acid capsule to evade recognition of the immune system. Finally, some bacteria

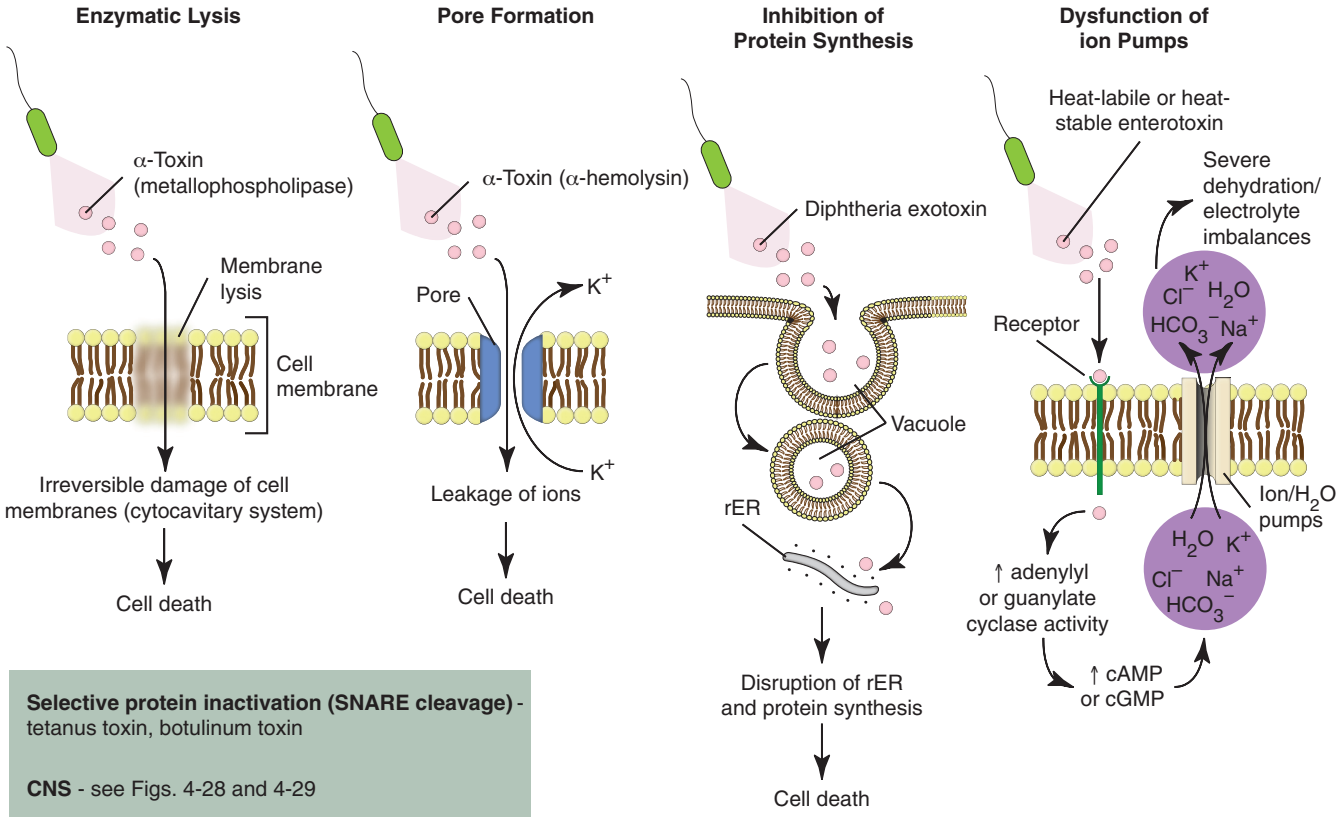


Figure 4-17 Actions of Bacterial Toxins (Virulence Factors) on the Structure and Function of Target Cells (see Fig. 4-6). Examples of such toxins in animals include the following (see text for greater detail): **enzymatic lysis:** clostridial myositis in horses (malignant edema; gas gangrene)—*Clostridium perfringens* α-toxin; **pore formation:** cutaneous superficial pyoderma in dogs—*Staphylococcus aureus* α-toxin; **inhibition of protein synthesis:** caseous lymphadenitis in ruminants—*Corynebacterium pseudotuberculosis* diphtheria-like toxin; **dysfunction of ion pumps:** enterotoxigenic enteritis in all domestic animal species—*Escherichia coli* heat-labile and heat-stable enterotoxins; **selective protein inactivation (SNARE cleavage):** tetanus in horses—*Clostridium tetani* tetanospasmin (neurotoxin); botulism in horses—*Clostridium botulinum* neurotoxin. *cAMP*, Cyclic adenosine monophosphate; *cGMP*, cyclic guanosine monophosphate; *rER*, rough endoplasmic reticulum; *SNARE*, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

have virulence factors that are immunoglobulin proteases. They break down immunoglobins involved in adaptive immune responses and thus depress defensive mechanisms.

Toxigenesis (Toxins)

Certain virulence factors are toxins, including exotoxins, lipoteichoic acid, and endotoxins (lipopolysaccharide [LPS]). Exotoxins are secreted by living Gram-positive bacteria; lipoteichoic acid is released from dead Gram-positive bacteria (i.e., bacteriolysis from bactericidal molecules and antibiotics); and endotoxins are released from dead Gram-negative bacteria (i.e., normal bacterial turnover, bacteriolysis from bactericidal molecules and antibiotics). These toxins activate a large variety of biochemical cascades involving cell membrane systems and organelles that result in cell dysfunction and/or death (Fig. 4-17; Box 4-3). They are expressed by Gram-negative and Gram-positive bacteria (Fig. 4-18) and injure and/or kill cells and damage their ECM proteins, such as collagen. Structurally, these actions enable colonization and invasiveness. Functionally, these molecules also act to kill cells directly via cytolysis (e.g., pore formation) or apoptosis or indirectly through activation of acute inflammation, often initiated via the complement pathway. In certain diseases these toxins are named (and grouped) according to their biologic activity such as leukotoxins (bovine pneumonic pasteurellosis/mannheimiosis) and neurotoxins (botulism and botulinum toxin [*Clostridium botulinum*], tetanus and tetanospasmin [*Clostridium tetani*]) (see sections covering specific bacterial and viral diseases). Fungi also have virulence factors that produce toxins that cause tissue injury and lysis. Examples include mycotoxins/aflatoxins.

Exotoxins and Lipoteichoic Acid. Exotoxins (usually from Gram-positive bacteria) are secreted from viable bacteria and are potent toxins. Some act directly on cells to cause cytolysis; others act via the A-B toxin system and bind to cell membranes with a receptor (B subunit) and deliver a second toxic molecule (A subunit) into the cytoplasm. As examples, A-B toxin systems are used in botulism (*C. botulinum*), tetanus (*C. tetani*), and diseases caused by *Corynebacterium* spp. Vacuolating toxin of *Helicobacter pylori*, *E. coli* hemolysin, and superantigens belonging to *S. pyogenes* and *Staphylococcus aureus* are surface-acting exotoxins. Surface-acting exotoxins bind to cell membranes and form pores through which cell lysis occurs. *S. aureus* also has pore-forming cytotoxins called α-toxin. Another virulence factor, lipoteichoic acid, binds to endothelial cells, interacts with circulating antibodies, activates the complement cascade, and triggers the release of reactive oxygen and nitrogen species, acid hydrolases, highly cationic proteinases, bactericidal cationic peptides, growth factors, and cytotoxic cytokines from neutrophils and macrophages. Lipoteichoic acid is also located in the cell wall of Gram-positive bacteria like *S. aureus*. It behaves as a Gram-positive endotoxin because its actions mimic LPS.

Endotoxins. Gram-negative bacteria, such as *E. coli*, *Salmonella* spp., *Pseudomonas* spp., *Haemophilus* spp., and *Bordetella* spp. can

Box 4-3 Examples of the Effects of Toxins (Virulence Factors) Causing Diseases in Animals (see Fig. 4-17)

Biologic outcome	Disease	Pathogenesis
Enzymatic lysis	Clostridial myositis in horses (malignant edema; gas gangrene)	*Clostridium perfringens* α-toxin has phospholipase C activity and causes lysis of cell membranes (cell death).
Pore formation	Cutaneous superficial pyoderma in dogs	*Staphylococcus aureus* α-toxin has membrane-disrupting activities via a hemolysin that creates membrane pores and causes cell lysis (cell death).
Inhibition of protein synthesis	Caseous lymphadenitis in ruminants	*Corynebacterium pseudotuberculosis* diphtheria-like toxin inhibits protein synthesis by functioning as an RNA translational inhibitor and causes cell death.
Dysfunction of ion pumps	Enterotoxigenic enteritis in all domestic animal species	*Escherichia coli* heat-labile and heat-stable enterotoxins cause increased activity of membrane adenylyl and guanylate cyclase, resulting in increased intracellular cAMP and cGMP concentrations, respectively, leading to activation of ion and water pumps and loss of electrolytes and water from affected cells. These enterotoxins behave in a manner similar to cholera and pertussis toxins.
Selective protein inactivation (SNARE cleavage)	Tetanus and botulism in horses	*Clostridium tetani* tetanospasmin (neurotoxin) cleaves a SNARE protein, components of the synaptic fusion complex, preventing the release of inhibitory neurotransmitters glycine and γ-aminobutyric acid (GABA) into the synaptic cleft. This outcome results in exaggerated and frequent muscle twitches and "tetanic" contractions because the effects of the excitatory neurotransmitter (acetylcholine) are not effectively counterbalanced by those of inhibitory neurotransmitters. *Clostridium botulinum* neurotoxin cleaves a SNARE protein, components of the synaptic fusion complex, preventing the fusion of neurotransmitter vesicles (acetylcholine) with terminal membranes of the neuron and myoneural junctions. This outcome leads to muscle weakness, flaccid paralysis, and death attributable to respiratory failure.

cAMP, Cyclic adenosine monophosphate; *cGMP*, cyclic guanosine monophosphate; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor

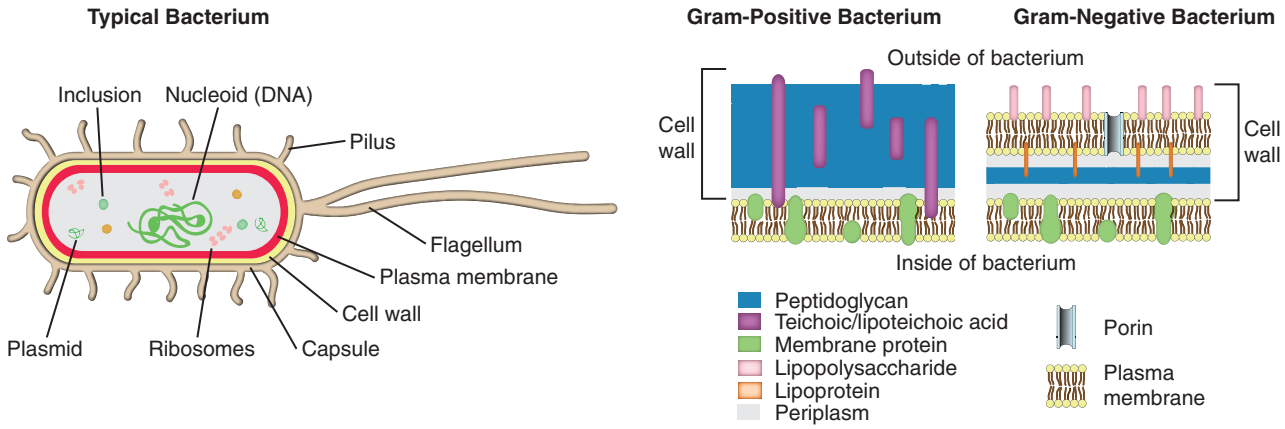


Figure 4-18 Morphologic Characteristics and Molecules of Gram-Positive and Gram-Negative Bacteria. The structure of a typical bacterium is shown on the left. The plasma membrane and cell wall of Gram-negative and Gram-positive bacteria contain molecules such as endotoxins (lipopolysaccharide [LPS]), exotoxins, and teichoic/lipoteichoic acid. They act as virulence factors (see Fig. 4-17) that damage target cells and their extracellular matrices such as collagen and thus are important in the pathogeneses of a wide variety of diseases. Gram-positive bacteria have a thick meshlike outer layer of peptidoglycan (also known as murein) that consists of sugars and amino acids. It provides structural strength in the formation of the cell wall. Gram-negative bacteria have a thin peptidoglycan layer and an outer membrane of LPS. Porins are cell membrane proteins that act as pores through which molecules can diffuse. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

release endotoxins into vascularized tissues when they die. Endotoxins is a general term used to characterize any outer membrane–associated toxin of the cell wall (see Figs. 4-15, 4-17, and 4-18). However, the term most commonly refers to LPS complex. Toxicity of LPS is attributable to the lipid A component of LPS, whereas immunogenicity (bacterin production [immunization]) is attributable to polysaccharide components of LPS. The outer membrane of the bacteria functions as a protective barrier against harmful large molecules and hydrophobic compounds in the environment such as bile salts, toxic molecules, lysozyme, and antimicrobial drugs. The membrane also functions to (1) impede phagocytosis by macrophages, (2) facilitate colonization of target cells, and (3) participate in the process of genomic variation (see section of viral diseases) in which the outer membrane acquires naïve polysaccharide components and evades host innate and acquired immune responses.

Endotoxins are released following destruction of the bacterial cell wall and are toxic to most animal cells (especially endothelial cells, platelets, and macrophages), tissues, and organs and can be lethal if large quantities are absorbed by or released into the circulatory system, causing the activation of proinflammatory cytokines and nitric oxide (NO) from macrophages and endothelial cells. This outcome leads to the activation of the complement and coagulation cascades and endotoxic shock (see Chapter 3) characterized by fever, hypoglycemia, thrombosis, (disseminated intravascular coagulation [DIC]), hypotensive shock, and death.

A-B Toxin. Some bacteria, such as *Bacillus anthracis* (anthrax) and *C. botulinum* (botulism), produce an exotoxin (virulence factor) called A-B toxin. A-B toxins are composed of two parts. Chronologically, the B part acts as a ligand and facilitates cell-surface recognition of target cells and entry of the A part into the cell via endocytosis. In the cell the A part carries out a toxic enzymatic reaction that interferes with one or more metabolic functions within the cell and allows the bacterium to colonize and replicate. For more detail see section on Bacterial Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Domestic Animals; Alimentary Anthrax (*Bacillus anthracis*).

Other Virulence Factors

Secretion Systems. Secretion systems, of which six types (type I to VI) have been described, are bacterial organelles that secrete or inject bacterial-derived toxins into the cytoplasm of target cells. The type III secretion system is best known and occurs in some Gram-negative bacteria such as *Salmonella* spp. and *E. coli*. It injects, like a needle, specialized bacterial protein toxins like exotoxins into the cytoplasm of cells. These protein toxins often disrupt cell signal transduction and other cellular processes, leading to cell lysis.

Siderophores. Some bacteria require iron for colonization of mucosae. Iron is plentiful in cells but unavailable to bacteria because it is tightly bound in heme, ferritin, transferrin, or lactoferrin molecules. Siderophores are virulence factors that mediate the release of iron from intracellular iron stores (see Fig. 4-15). One example is enterobactin from *E. coli* and *Salmonella* spp.; this molecule scavenges bound iron from animal cells and makes it available for the bacteria. In another example, siderophores also play a role in the pathogenesis of the disease anthrax (*B. anthracis*). The bacterium releases two siderophores, bacillibactin and petrobactin, into the ECM, where they acquire iron for use by the bacterium.

Biofilms/Intracellular Bacterial Communities. Bacterial colonization can occur through virulence factors that form an exopolysaccharide matrix called a *biofilm* on mucosal surfaces lining the oral and nasal cavities and the mammary duct system as examples. Bacteria embedded in biofilms are not susceptible to phagocytosis by macrophages, and they can become resistant to antibiotics. A surface protein, biofilm-associated protein (Bap), has been implicated in the formation of a *S. aureus* biofilm in chronic bovine mastitis. Similarly, infections caused by certain strains of uropathogenic *E. coli* can result in the formation of intracellular bacterial communities affecting mucosal epithelial cells of the urinary bladder, which behave functionally much like a biofilm.

Capsules. Bacterial capsules are virulence factors that protect bacteria from phagocytosis by cells such as neutrophils and macrophages during acute inflammatory and adaptive immune responses. Capsules are secreted by the bacterium and are tightly adhered to the bacterial cell wall. They also aid with adhesion to mucosae and skin and are a reserve of nutrients, including water. Capsules are common in Gram-negative bacteria like *E. coli* and *Salmonella* spp., but also occur on fungi like *Cryptococcus neoformans*.

Role of Bacterial Genes in Susceptibility and/or Resistance to Disease

Microbes acquire through gene recombination (see later section) virulence genotypes and gene products (virulence factors) that allow them to escape defense mechanisms and spread locally, regionally, and/or systemically to encounter new target cell(s) and cause disease.

Virulence Factors

Virulence factors are encoded in and translated from genes in chromosomal DNA, bacteriophage DNA, or plasmids of bacteria. They can be readily transferred horizontally between bacteria (e.g., virulence factors for antibiotic resistance) via pathogenicity islands (PAIs) and/or virulence plasmids. PAIs are clusters of genes that code for virulence factors found in bacterial chromosomes. Virulence plasmids are clusters of self-replicating extrachromosomal genes for virulence factors located in plasmids within the cytoplasm of the bacteria. Most bacteria have only one chromosome but may contain hundreds of copies of a specific virulence plasmid. Plasmids replicate independently of cell division, and when a bacterium containing plasmids divides, the plasmids distribute randomly between the two resulting bacteria. Chromosomal or plasmid genes express virulence factors such as bacterial adhesins, colonization factors, protein toxins like hemolysins, other types of toxins, and molecules that affect the innate and adaptive immune responses. Strains of bacteria lacking PAIs and/or virulence plasmids usually do not cause disease. The number and type of virulence factors in a given bacterial strain are constantly changing, usually through genomic selection of those factors that favor the survival of the bacterium in the animal host. Each bacterial genera and strains within genera have their own unique virulence factor profile; therefore the total number of determinates that have been identified in all bacteria genera combined are in the hundreds. As an example, strains of *R. equi* that cause disease have chromosomal virulence factors for capsular polysaccharide, cholesterol oxidase, phospholipase C, lecithinase, and cell wall mycolic acids and plasmid virulence factors for virulence-associated protein (VAP).

Antibiotic Resistance

Antibiotic resistance, the ability of bacteria to withstand the static or lytic effects of antibiotics, evolves via natural selection of randomly mutated bacterial genes. These genes code for bacterial molecules (i.e., virulence factors) that cause resistance through the following four key mechanisms (Fig. 4-19):

1. Enzymatic deactivation (antibiotic inactivation or modification) as occurs with β-lactamases and extended-spectrum β-lactamases (resistant to cephalosporins and monobactams) produced by bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E. coli*, and *Salmonella typhimurium*
2. Alteration of antibiotic binding sites (penicillin-binding proteins [PBPs]) such as occurs in infections with methicillin-resistant *S. aureus* (MRSA) and other penicillin/methicillin/oxacillin-resistant bacteria such as *Streptococcus pneumoniae*, vancomycin-resistant enterococci (VRE), and penicillin-resistant *S. pneumoniae* (PRSP)
3. Alteration of a metabolic pathway, such as occurs with some sulfonamide-resistant bacteria that use preformed folic acid in place of *para*-aminobenzoic acid (PABA), a precursor for the synthesis of folic acid in bacteria inhibited by sulfonamides

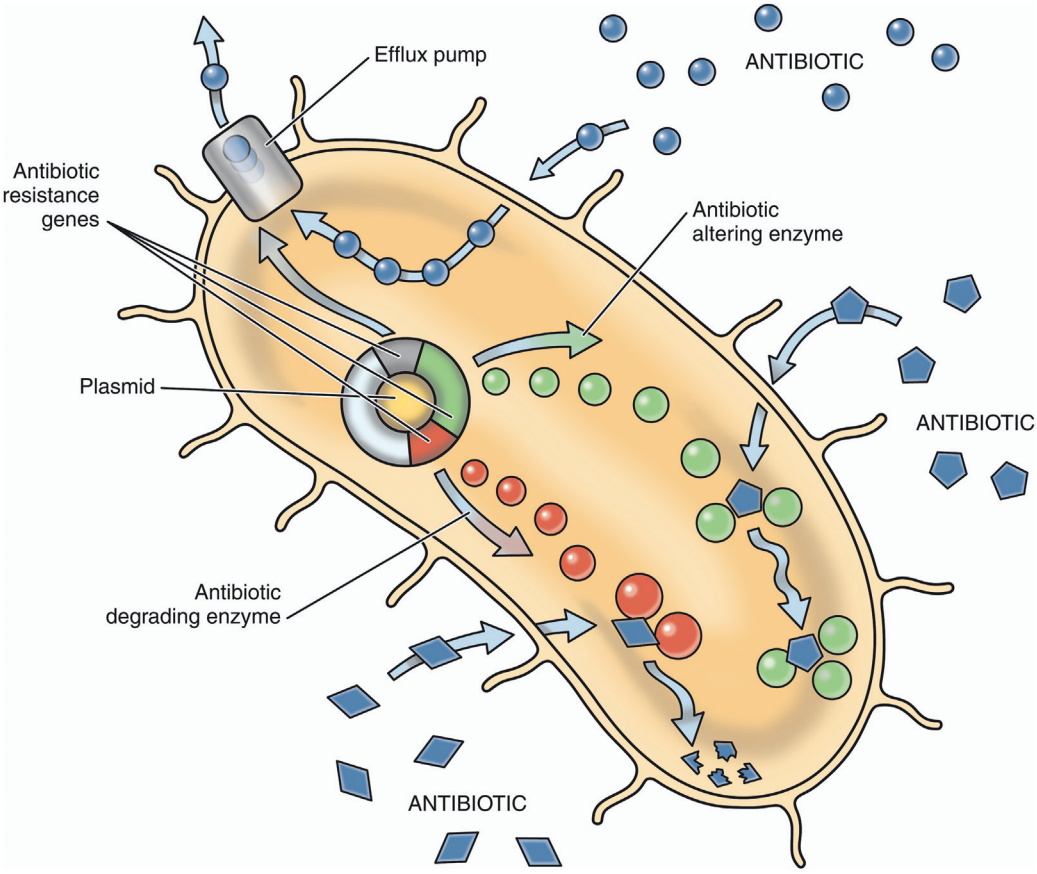


Figure 4-19 **Mechanisms Used by Bacteria to Establish Resistance to Antibiotics.**

4. Reduced antibiotic accumulation in bacteria through decreased membrane permeability to the antibiotic and/or enhanced efflux via membrane pumps

Bacterial Transfer of Antibiotic Resistance. The time required for bacteria to divide or a colony of bacteria to double in number is called the *generation time* and can be as short as 15 minutes. Although gene mutations for antibiotic resistance in bacteria are very rare steps, because of fast generation times and the ability to reach extremely high absolute numbers of bacteria via binary fission in short periods of time if unchecked, it does not take long before antibiotic resistance develops. The spontaneous mutation rate for antibiotic resistance is approximately 1×10^8 to 1×10^9. This means that one out of every 100 million to 1 trillion bacteria in an infection develops resistance through a mutation. The use of antibiotics is a form of environmental pressure on bacteria; those having a favorable genetic mutation (i.e., virulence factor for antibiotic resistance) survive therapy and continue to reproduce.

If a bacterium contains several antibiotic resistance genes, it is called a multiresistant microbe. Although a human disease, MRSA infections are beginning to appear in animals. Such pathogens have multiple resistance genes that protect them from most if not all broad-spectrum antibiotics commonly used to treat the disease. These resistance genes are transferred between and among bacteria of related and different genera by vertical and horizontal gene transfer.

Bacterial Gene Transfer

Vertical Gene Transfer. Vertical gene transfer is the process through which bacteria pass virulence factors such as antibiotic resistance to their offspring (asexual reproduction) during DNA replication. This transfer results in offspring fully resistant to an antibiotic. Because of this process, the overuse of broad-spectrum antibiotics in human beings and animals is a serious concern.

Horizontal Gene Transfer. Bacteria can also transfer antibiotic resistance genes between bacteria via horizontal gene transfer (Fig. 4-20) as follows:

1. Direct bacteria-bacteria contact (conjugation) via plasmids (the most common form)
2. Chromosomal DNA (transformation) in which pieces of DNA coded for antibiotic resistance and free in extracellular fluid as a result of lysis of its host bacterium are taken up by viable bacteria
3. Bacteria-specific viruses (bacteriophages) that transfer DNA (transduction) between two closely related bacteria

Mechanisms of Genomic Change

Mechanisms of genomic variation, antigenic drift (genetic drift), and antigenic shift are discussed in a later section on Viral Diseases, Mechanisms of Genomic Change. The concepts discussed are interchangeable with those that occur in bacterial diseases.

Bacterial Diseases of Organ Systems

Although the same bacterial disease often affects several different organ systems, diseases in this section are placed into a specific organ system based on which organ system demonstrates the primary gross lesion or lesions that are most commonly used to initially recognize and identify the disease. Bacterial diseases are identified by a primary mechanism of injury in E-Table 4-3.

E-Table 4-3 Mechanisms of Injury in Diseases Caused by Bacteria

Inflammation	Toxins	Structural Injury	Disruption of Neurotransmitter Exocytosis	Alterations of the Cell Cycle	Dysfunction of Electrolyte/ Fluid Pumps
ALIMENTARY SYSTEM AND THE PERITONEUM, OMENTUM, MESENTERY, AND PERITONEAL CAVITY					
Disorders of Domestic Animals					
• Enteric colibacillosis (enterohemorrhagic *Escherichia coli* [EHEC]) • Salmonellosis	• Enteric colibacillosis (EHEC) • Alimentary anthrax • Salmonellosis • Enterotoxemia	• Enteric colibacillosis (enteropathogenic *E. coli* [EPEC])			• Enteric colibacillosis (enterotoxigenic *E. coli* [ETEC])
Disorders of Horses					
• Rhodococcal enteritis					
Disorders of Ruminants (Cattle, Sheep, and Goats)					
• Johne's disease • Bovine intestinal tuberculosis • Wooden tongue	Alimentary anthrax				
Disorders of Pigs					
• Swine dysentery • Porcine polyserositis	• Swine dysentery • Hemorrhagic bowel syndrome			• Porcine proliferative enteritis	
HEPATOBILIARY SYSTEM AND EXOCRINE PANCREAS					
Disorders of Domestic Animals					
• Hepatic leptospirosis	• Hepatic leptospirosis	• Hepatic leptospirosis			
Disorders of Horses					
• Tyzzer's disease	• Tyzzer's disease				
Disorders of Ruminants (Cattle, Sheep, and Goats)					
	• Bacillary hemoglobinuria • Infectious necrotic hepatitis				
RESPIRATORY SYSTEM, MEDIASTINUM, AND PLEURAE					
Disorders of Domestic Animals					
• *Streptococcus zooepidemicus*	• *Streptococcus zooepidemicus* • Respiratory anthrax				
Disorders of Horses					
• Rhodococcal pneumonia • *Streptococcus zooepidemicus* • Strangles	• *Streptococcus zooepidemicus*				
Disorders of Ruminants (Cattle, Sheep, and Goats)					
• Bovine pneumonic pasteurellosis/ mannheimiosis • Pulmonary histophilosis • Bovine enzootic pneumonia • Contagious bovine pleuropneumonia • Bovine tuberculosis	• Bovine pneumonic pasteurellosis/ mannheimiosis • Pulmonary histophilosis • Bovine enzootic pneumonia				
Disorders of Pigs					
• Porcine pleuropneumonia • Porcine polyserositis	• Porcine pleuropneumonia • Atrophic rhinitis • Porcine polyserositis	• Porcine enzootic pneumonia			

Continued

E-Table 4-3 Mechanisms of Injury in Diseases Caused by Bacteria–cont'd

Inflammation	Toxins	Structural Injury	Disruption of Neurotransmitter Exocytosis	Alterations of the Cell Cycle	Dysfunction of Electrolyte/ Fluid Pumps
Disorders of Dogs					
	• Acute tracheobronchitis				
CARDIOVASCULAR SYSTEM AND LYMPHATIC VESSELS					
Disorders of Domestic Animals					
• Embolic vasculopathy/ vasculitis • Vascular leptospirosis	• Embolic vasculopathy/ vasculitis • Septicemic anthrax • Vascular leptospirosis	• Vascular leptospirosis			
Disorders of Horses					
• Glanders (Farcy, Malleus, Droes)					
Disorders of Pigs					
	• Edema disease				
URINARY SYSTEM					
Disorders of Domestic Animals					
• Necrohemorrhagic urocystitis • Renal leptospirosis	• Necrohemorrhagic urocystitis • Renal leptospirosis	• Renal leptospirosis			
Disorders of Ruminants (Cattle, Sheep, and Goats)					
• Contagious bovine pyelonephritis	• Contagious bovine pyelonephritis • Pulpy kidney (overeating) disease				
BONE MARROW, BLOOD CELLS, AND LYMPHATIC SYSTEM					
Disorders of Domestic Animals					
• Brucellosis					
Disorders of Horses					
• Strangles • Rhodococcal mesenteric lymphadenitis • Caseous lymphadenitis					
Disorders of Ruminants (Cattle, Sheep, and Goats)					
• Caseous lymphadenitis • Brucellosis					
Disorders of Pigs					
• Rhodococcal mesenteric lymphadenitis • Brucellosis					
Disorders of Dogs					
• Brucellosis					
NERVOUS SYSTEM					
Disorders of Domestic Animals					
• Meningitis			• Botulism • Tetanus		

E-Table 4-3 Mechanisms of Injury in Diseases Caused by Bacteria–cont'd

Inflammation	Toxins	Structural Injury	Disruption of Neurotransmitter Exocytosis	Alterations of the Cell Cycle	Dysfunction of Electrolyte/ Fluid Pumps
Disorders of Ruminants (Cattle, Sheep, and Goats)					
• Listeriosis	• Listeriosis				
• Thrombotic meningoencephalitis	• Focal symmetric encephalomalacia				
• *Mannheimia* meningoencephalitis	• *Mannheimia* meningoencephalitis				
Disorders of Pigs					
	• Edema disease				
SKELETAL MUSCLE					
Disorders of Ruminants (Cattle, Sheep, and Goats)					
	• Blackleg				
	• Malignant edema				
	• Big head, black disease				
BONE, JOINTS, TENDONS, AND LIGAMENTS					
Disorders of Ruminants (Cattle, Sheep, and Goats)					
• Lumpy jaw					
INTEGUMENTARY SYSTEM					
Disorders of Pigs					
• Greasy pig disease	• Greasy pig disease				
• Diamond skin disease	• Diamond skin disease				
Disorders of Dogs					
• Canine pyoderma	• Canine pyoderma				
FEMALE REPRODUCTIVE SYSTEM AND MAMMARY GLAND					
Disorders of Domestic Animals					
• Brucellosis					
Disorders of Ruminants (Cattle, Sheep, and Goats)					
• Bovine mastitis	• Bovine mastitis				
MALE REPRODUCTIVE SYSTEM					
Disorders of Domestic Animals					
• Brucellosis					

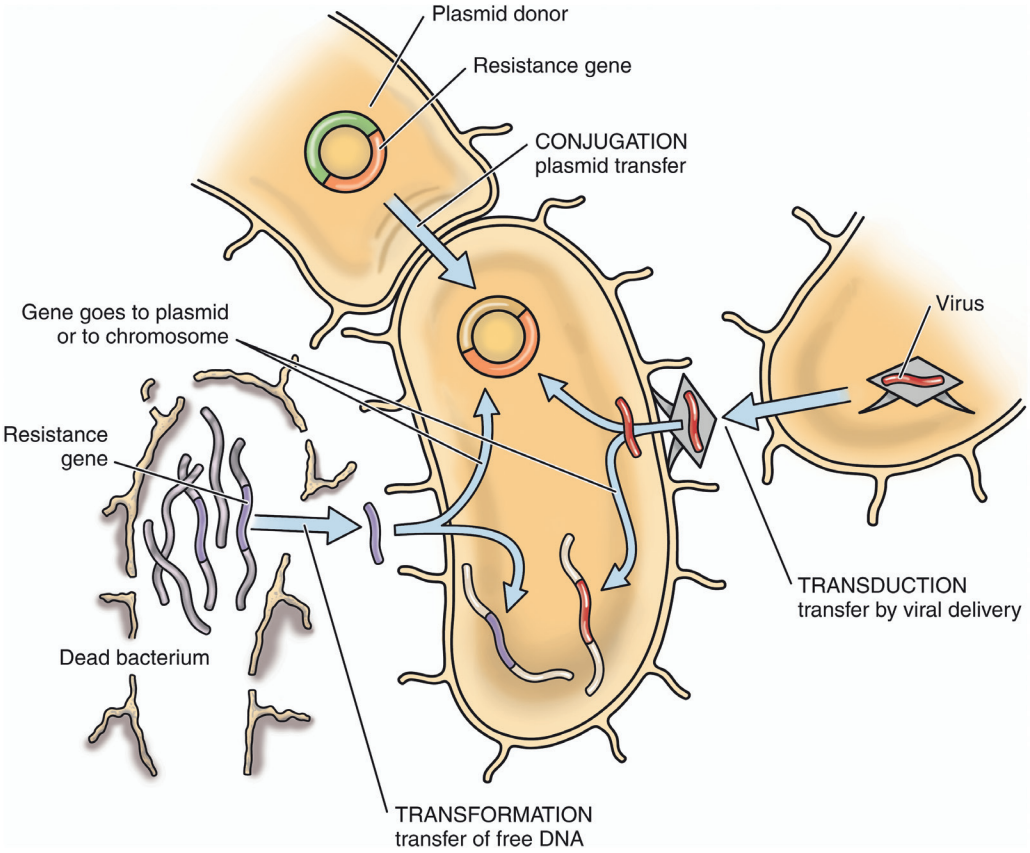


Figure 4-20 Horizontal Gene Transfer. Mechanisms used by bacteria to transfer resistance to an antibiotic to other bacteria.

Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity

Disorders of Domestic Animals

***Enteric Colibacillosis* (Escherichia coli).** *E. coli* strains that cause disease in animals have been named enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), and enterohemorrhagic *E. coli* (EHEC) based on the mechanisms and virulence factors that they use to cause diarrhea. In summary, the mechanisms of injury in enteric colibacillosis are (1) nonstructural alterations in the function of cell membrane ion and fluid transport systems (ETEC) and (2) structural alterations of cell membranes characterized by acute coagulative necrosis caused by bacterial toxins and by acute inflammation and its mediators and degradative enzymes (EPEC and EHEC). Enterotoxigenic and enteropathogenic strains do not invade mucosal enterocytes, whereas enterohemorrhagic strains do invade mucosal enterocytes. Enterotoxigenic strains secrete toxins that functionally, but not structurally, affect enterocytes, causing alterations in electrolyte and fluid secretion that result in a secretory diarrhea. Enteropathogenic strains structurally affect the microvillus border of enterocytes, causing alterations in electrolyte and fluid secretion that result in an osmotic diarrhea (malabsorption) and a less significant secretory diarrhea. Enterohemorrhagic strains structurally affect the enterocytes of the colon, causing cell lysis (necrosis), inflammation, and hemorrhage that lead to reduced absorption of colonic fluids and a malabsorption diarrhea. Endotoxins (LPS) likely directly or indirectly play a role in diseases caused by these three strains. There are no gross lesions in enterotoxigenic colibacillosis, whereas in enteropathogenic and enterohemorrhagic colibacillosis, mucosae are rough and granular (enterocyte necrosis, villus atrophy) with areas of hemorrhage, acute inflammation, and fibrin exudation.

Animals encounter *E. coli* through ingestion of bacteria in fomites contaminated with fecal material. The bacterium is swallowed and gains access via peristalsis to the mucus layer and mucosae of the intestines. It is likely that flagella expressed by some strains of *E. coli* facilitate their penetration of the mucus layer to gain access to microvilli of enterocytes.

Enterotoxigenic *Escherichia coli.* ETEC expresses K99 (F5) or F41 fimbrial adhesins that allow it to bind to receptor molecules in the mucus layer and to ganglioside and glycoprotein receptors on cell membranes of microvilli of enterocytes. When the mucosa is colonized, large numbers of bacteria are produced (Fig. 4-21), and they secrete heat labile (LT) and heat stable (ST) enterotoxins that diffuse in the mucus layer and microvilli, bind to specific receptors on the microvillus border of enterocytes, disrupt the function of cell membrane electrolyte and fluid transport systems, and cause secretory diarrhea. This process results in a functional lesion; no structural changes are observed grossly. LT and ST enterotoxins bind to glycolipid receptors on apical surfaces of enterocytes. After binding, these complexes are endocytosed and interact with a series of second messenger systems (epithelial cell signal transduction systems), which ultimately results in increased concentrations of intracellular cyclic adenosine monophosphate (cAMP, LT enterotoxin) and cyclic guanosine monophosphate (cGMP, ST enterotoxin). These molecules operate to open chloride channels (cystic fibrosis transmembrane regulator) in enterocyte cell membranes, thus acting irreversibly to move intracellular chloride ions extracellularly into the lumen of the intestine. Excessive chloride ion secretion also pulls water with it into the lumen of the intestine, thereby increasing the volume of fluid in the intestine. This volume ultimately exceeds the ability of the intestine to absorb the excessive fluid.

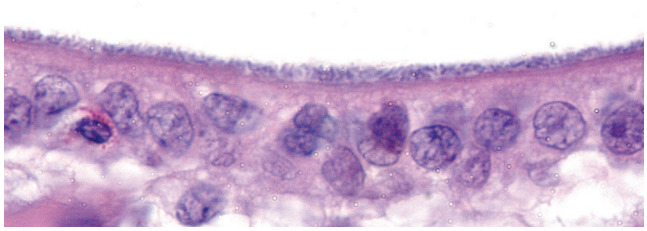


Figure 4-21 Colonization of Mucosa in Enteric Colibacillosis. *Escherichia coli* attach to microvilli, thus forming a uniform layer of blue-staining (hematoxylin) coccobacilli. Note the lack of epithelial cell injury. H&E stain. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

Enteropathogenic *Escherichia coli.* EPEC colonizes mucosae in a manner similar to that used by ETEC. EPEC does not produce LT or ST enterotoxins but does express adhesins such as P and S fimbriae, EPEC adherence factor, and intimin (nonfimbrial outer membrane protein). Integrins may serve as target cell membrane receptors for intimin, and this interaction appears to produce a tight bond between the bacterium and the enterocyte. After colonization and growth, bacterial virulence factors injure the brush border, leading to the loss of microvilli at the site of colonization. Such virulence factors appear to involve processes that disrupt cytoskeletal functions of the microvilli through interference with actin filaments, actin polymerization, and other cytoskeletal components and by causing alterations in intracellular calcium concentrations. This type of change has been called *attaching and effacing injury* and has resulted in naming the bacterium attaching and effacing *E. coli*. Injury to and loss of microvilli leads to decreased digestive enzyme activity in the glycocalyx (osmotic diarrhea) and disruption of ion transport systems (secretory diarrhea). EPEC also secretes bacterial proteins and likely injects them into the cytoplasm of enterocytes through a type III secretion system. These proteins, EspA, EspB, and EspD, activate a number of signal transduction pathways in the target cell, which appear to be involved in the pathogenesis of microvillus disruption. Additionally, acute inflammation occurs at the site of binding between the bacterium and the microvillus, likely contributing to the attaching and effacing lesion. Some attaching and effacing strains also secrete a virulence factor called *verotoxin*, which kills enterocytes and cells of the lamina propria (vascularized ECM tissues), leading to mucosal erosions and ulcers, intestinal edema and hemorrhage, and increased denuded mucosal surfaces for the absorption of endotoxins (LPS).

Enterohemorrhagic *Escherichia coli.* EHEC appears to colonize the mucus layer and mucosae in a manner similar to that used by the other two strains of the bacterium; however, enterocytes of the colon are the primary target cells. This specificity could be mediated through ligand-receptor interactions, and bacterial fimbriae have been shown to act as adhesins and attach to enterocyte cell membranes. Chemical gradients, such as the concentration of iron in target cells, could also provide the basis for colonic specificity. Once attached to colonic enterocytes, the bacterium replicates in large numbers and secretes a verotoxin that elicits an intense acute inflammatory response. It is also able to invade enterocytes, and verotoxin kills the cells. EHEC does not produce LT or ST enterotoxins, but Shiga toxins (Stxs) or Shiga-like toxins are virulence factors in some bacterial strains. Thus mucosal lesions (hemorrhagic colitis) characteristic of this strain of *E. coli* appear to result from a combination of inflammatory enzymes and mediators and toxins, all of which cause cell lysis and expose the underlying denuded lamina propria to a variety of harmful luminal molecules such as LPS that are readily available for absorption. Endotoxin, especially when in the blood, can lead to inflammation, capillary damage, vasculitis, thrombosis, intravascular coagulation, tissue degradation and infarction, endotoxic shock, and lysis. These mechanisms likely underlie the occurrence of acute adrenal cortical hemorrhage and necrosis (see E-Fig. 12-21) observed in endotoxemia with *E. coli* and other coliform infections.

Enterotoxemic and Septicemic Colibacillosis. Septicemic colibacillosis, likely resulting from an enteropathogenic strain of *E. coli*, may begin as an alimentary manifestation and then progress to enterotoxemic colibacillosis or septicemic colibacillosis. In these forms the enterotoxins (and the bacterium) most likely gain access to the circulatory system via invasion into and absorption through capillary beds in the lamina propria of injured intestinal mucosae. Enterotoxemic colibacillosis and its toxins cause edema disease of the nervous system (see section on Nervous System; also see Chapter 14) leading to fibrinoid arteriopathy/arteriolopathy of the brain and ischemia and malacia, whereas septicemic colibacillosis and its bacterium and toxins cause disease of the cardiovascular system (see Chapter 10) leading to lysis via toxic and endotoxic shock and cardiovascular collapse.

Salmonellosis (*Salmonella* spp.). The mechanism of injury in salmonellosis is acute coagulative necrosis of cells caused by bacterial toxins and by acute inflammation and its mediators and degradative enzymes. Three forms of salmonellosis occur: peracute, acute, and chronic. Gross lesions in the peracute form include petechiation and a blue discoloration (cyanosis) of the skin, fibrinous polyserositis, and disseminated intravascular coagulopathy (see Fig. 7-118). These lesions are rooted in injury of the vascular system with vasculitis and thrombosis caused by bacterial toxins. In the acute form, lesions affect mucosae of the small intestine, large intestine, and cecum (fibrinonecrotic ileotyphlocolitis) and are characterized by a rough and granular mucosal surface (necrosis) mixed with mucus, fibrin, and occasionally blood (see Fig. 7-118). The content is malodorous (septic tank odor). This pattern of injury is caused by bacterial toxins, acute inflammation, and their effects on enterocytes and blood vessels within the lamina propria. Many of these processes are enabled by virulence factors encoded on "Salmonella pathogenicity islands" on its chromosome. Bacteria can spread via the portal vein to the liver, leading to the formation of foci of bacteria- and toxin-induced necrosis and inflammation (paratyphoid nodules) (see Fig. 8-54). This spread likely occurs via leukocyte trafficking and infection of Kupffer cells, but bacteremia may also occur. Leukocyte trafficking also spreads the bacteria to mesenteric and systemic lymph nodes and the gallbladder (fibrinous cholecystitis). In the chronic form, injury is associated with discrete foci of necrosis and ulceration of mucosae (button ulcers). These lesions are rooted in injury of the vascular system with vasculitis and thrombosis caused by bacterial toxins diffusing within the submucosa of the intestine.

Animals encounter *Salmonella* spp. through ingestion of bacteria in fomites contaminated with fecal material. The bacterium is swallowed and gains access via peristalsis to the mucus layer and mucosae of the intestines. *Salmonella* spp. appear to use two mechanisms to colonize mucosae and gain access to the lamina propria and its capillary beds. The first mechanism uses M cells in intestinal crypts. Because a mucus layer is absent over M cells, the bacteria have direct contact with apically positioned cell membranes of M cells. The second mechanism uses apically positioned cell membranes of enterocytes to gain access to these cells; however, these cells are covered with a mucus layer. Because *Salmonella* spp. are motile bacteria (flagella—virulence factor), they likely can penetrate through the mucus layer and gain access to these membranes. The

bacterium must adhere to the apical surfaces of M cells and enterocytes to begin the process of colonizing the intestine, which is a process mediated through ligand-receptor interactions. Although unproved, it is probable that one or some of the fimbriae possessed by *Salmonella* spp. are involved in the initial adherence between the bacterium and these cells. It is thought, though unconfirmed, that fimbriae may determine susceptibility of the various strains of *Salmonella* spp. for animal species and for specific target cells within each species. Which fimbriae (virulence factor) are used to bind to target cells may vary depending on whether the target cell is an M cell or an enterocyte. Additionally, PAMP and PRRs (also known as TLRs [Toll-like receptors]) are probably involved. Once bound to target cell membrane, a type III secretion system is used to inject bacterial proteins into the target cell that stimulate phagocytosis through the mobilization of actin filaments in the cytoplasm. Colonization of mucosae only occurs if LPS is present in the bacterial cell wall, and it likely plays a role in adherence to target cells through its participation in cell wall stability and resistance to bile salts, cell surface hydrophobicity, and the correct insertion and folding of membrane proteins such as those that occur in fimbriae.

Once the bacterium has reached the luminal (apical) surfaces of mucosal epithelial cells (villus epithelium or M cells), *Salmonella* spp. have several options in interacting with these cells. They can (1) colonize and replicate on apical surfaces, (2) enter via endocytosis and colonize and replicate within epithelial cells, or (3) migrate through cells via an endocytotic-exocytotic pathway to exit the basal side of the cell, enter the lamina propria, and colonize and replicate within cells such as those in GALT. Additionally, mucosa-associated macrophages and dendritic cells may interact with epithelial cells and bacteria and can phagocytize and carry bacteria via leukocyte trafficking to mucosa-associated lymphoid cells and in afferent lymphatic vessels to Peyer's patches.

Once the microbe is internalized via phagocytosis or endocytosis, *Salmonella* spp. survive and replicate within a "*Salmonella*-containing vacuole (SCV)". *Salmonella* spp. are able to inhibit phagosome-lysosome fusion, thereby blocking their killing within macrophages (see Fig. 4-14). If phagosome-lysosome fusion occurs, bacteria are able to block the effects of lysosomal enzymes, acidity, and free radicals. In fact, the bacterium resides and replicates in a phagosome and/or phagolysosome (SCV) until released from the macrophage after the lysis of the macrophage caused by toxins produced by the bacterium. Once in Peyer's patches, macrophages infected with bacteria die and release bacteria that will infect additional macrophages through ligand-receptor interactions. *Salmonella* spp. can kill macrophages via apoptosis using a type I secretory system that leads to activation of caspase-1 in macrophages. These macrophages are often recruited as monocytes from the systemic circulation as a component of the acute inflammatory response. Once infected with the bacterium, macrophages migrate in efferent lymphatic vessels via leukocyte trafficking to regional mesenteric lymph nodes using mechanisms similar to those described in Peyer's patches and then systemically via the thoracic duct and circulatory system. Macrophages also likely gain access to the systemic circulatory system through capillaries and postcapillary venules in lymph nodes. It also appears that dendritic cells in the intestinal mucosa are infected by the bacterium, and these cells likely spread the bacterium to Peyer's patches.

In all of these situations, acute inflammation occurs concurrently in response to bacterial toxins and antigens, resulting in vascular permeability changes and injury, and in the recruitment of neutrophils and their degradative enzymes, which can cause additional tissue injury. As part of this response, the interaction of M cells and enterocytes with bacteria appears to cause the release of chemokines and other chemoattractants for neutrophils into surrounding vascularized ECM. It appears that the form of disease (peracute, acute, or chronic) that occurs depends on which steps used in the chronology (as described previously) are emphasized through the expression of virulence factors by different strains of *Salmonella* spp. The peracute form likely favors spread to regional lymph nodes and then systemically with the release of toxins leading to vascular injury, failure of the circulatory system, and lysis. The acute form likely favors mucosal adherence and colonization leading to mucosal necrosis mediated by bacterial toxins. Through this process and the acute inflammation that ensues, capillary beds in the lamina propria are permeable and likely bacteria, bacterial toxins, and bacteria-infected macrophages enter the venous circulatory system and spread via the portal vein to the liver. The chronic form likely favors invasion of the lamina propria and submucosa (motile bacteria) with direct effects on the vasculature that supplies the intestine with blood. However, it is possible that button ulcers observed in this form are a manifestation of septicemia with attachment of bacteria to vascular endothelium resulting in vasculitis, thrombosis, ischemia, and infarction. The lesions and clinical signs that occur in diseases caused by *Salmonella* spp. are in part attributable to (1) an enterotoxin (exotoxin) that produces a secretory diarrhea, (2) a cytotoxin that inhibits protein synthesis, and (3) endotoxins and LPSs that cause membrane injury and cell lysis. Acute inflammation and cell and tissue injury that ensue are also important causes of the lesions.

Enterotoxemia (*Clostridium perfringens*). The mechanism of injury in enterotoxemia is acute coagulative necrosis of cells and tissues caused by bacterial toxins. Gross lesions include segments of the small intestine or the entire small intestine that are dark red to purple-black (hemorrhagic enteritis) and accompanied by mucosal, submucosal, and serosal edema and hemorrhage (see Figs. 7-121, 7-122, and 7-163). *Clostridium perfringens* attaches in a layered fashion to mucosal surfaces, and as toxins are released, it diffuses into the mucosa and lamina propria, causing in addition to necrosis, thrombosis of mucosal and submucosal vessels. Inflammation usually does not occur. C. *perfringens* causes disease syndromes that are categorized based on bacterial type (type A to E), toxin type (α-, β-, ε-, and ι-toxins), species affected, and/or age of the animal affected. These classification systems are always in a state of change as new toxins are identified in strains of the bacterium. At least 16 different toxins and enzymes (virulence factors) have been identified on chromosomes and plasmids of different clostridial strains; however, no single strain of the bacterium produces all of these factors. Further discussion of these classification systems is beyond the scope of this chapter.

Cattle, sheep, goats, pigs, and horses encounter C. *perfringens* through ingestion of bacterial spores in the soil or from contact with fomites contaminated with the vegetative form of the bacterium from carrier animals. The vegetative form of C. *perfringens* can be a normal inhabitant of the alimentary system of domestic animals. It appears that under the proper conditions in the intestine that are usually linked to changes in the diet or the ingestion of an energy source rich in carbohydrates, spores germinate into vegetative forms and proliferate or the ingested vegetative form proliferates. It has been shown experimentally that dietary trypsin can inactivate β-toxin, thus it is thought that diets deficient in trypsin may increase the likelihood of disease. Additionally, sudden dietary change can also alter the composition of normal intestinal microflora, providing opportunities for the vegetative form of the bacterium to proliferate and produce toxins. Vegetative forms are nonmotile and gain access to mucosae via random motion from peristalsis. They colonize the mucus layer by using bacterial proteases to expose receptors in mucus and then use bacterial adhesins to bind to these receptors. Within

the mucus layer, bacteria are protected from acids and enzymes in intestinal content. The bacterium also consumes mucus as an energy source for bacterial growth and replication, and this process is thought to activate bacterial genes that regulate the production of toxins. Once the mucus layer is colonized, bacteria then interact with microvilli of enterocytes by attachment and retraction of type IV pili (gliding motility) and eventually attach to the apical surfaces of enterocytes. Attachment is likely mediated by ligand-receptor interactions.

Experimental studies suggest that bacterial toxins that diffuse across mucosae may first injure endothelial cells in capillaries of the lamina propria before the attachment of bacteria to the apical surfaces of enterocytes and that attachment may require changes in the membranes of apical enterocytes that are induced directly by the effect of toxins at the apical surfaces of enterocytes and indirectly by ischemia. At this phase of the disease, injury is primarily limited to enterocytes. Once mucosae are colonized, bacteria replicate in immense numbers, and the disease enters a second phase characterized by the production of abundant potent cytotoxins, which spread via diffusion as a wave into the mucosa, lamina propria, submucosa, and muscle layers. Some clinical forms of enterotoxemia remain in the first phase of the disease; others progress into the second phase. Discussion of these various clinical forms is outside the scope of this chapter. Potent cytotoxins produced by the bacteria include α-, β-, ε-, and ι-toxins (alpha [CPA], beta [CPB], epsilon [ETX], and iota [ITX]), which behave as enterocyte membrane toxins (α, β), such as phospholipases, lecithinases, and pore-forming toxins, as well as ECM toxins, such as collagenase, hyaluronidase, and sialidase. ε-Toxin has the unique ability to increase enterocyte and endothelial cell permeability by acting on the cytoskeleton and probably altering the function of junctional complexes, thus affecting the absorption of toxins by the vascular system, resulting in systemic effects. ι-Toxin disrupts the cytoskeleton, which leads to cell lysis. Because of the abundance of toxins produced in the second phase of enterotoxemia, toxins freely move in the intestinal lumen via peristalsis to interact with uncolonized normal enterocytes; thus the lesions are quickly spread to other areas within the intestine. Toxins cause lysis and sloughing of enterocytes of villi and crypts followed by further colonization by bacteria, additional proliferation and toxin production, and toxin-induced massive necrosis resulting in structural breakdown and hemorrhage of the entire intestinal wall. Because ε-toxin is a permease that alters cell permeability, the vascular beds in affected intestinal tissues readily absorb toxins from the lumen of the intestine into the circulatory system. Toxins are then carried to the brain, kidney, and other tissues in which the increase in vascular permeability leads to the release of blood plasma containing toxins into the interstitium and body cavities, resulting in edema and effusions. In the brain and kidney these toxins cause focal symmetric encephalomalacia (see Fig. 14-96) and pulpy kidney disease (see Fig. 11-49), respectively. However, it appears that the mechanisms leading to these two diseases occur in the first phase or early in the second phase of enterotoxemia before toxin-induced massive necrosis of the intestine occurs.

Alimentary Anthrax (*Bacillus anthracis*). The mechanism of injury in alimentary anthrax is acute coagulative necrosis of cells caused by bacterial toxins. Gross lesions include segments of the small intestine or the entire small intestine that are dark red to purple-black (hemorrhagic enteritis) and accompanied by mucosal, submucosal, and serosal edema and hemorrhage (see Fig. 7-124). Additionally, mesenteric lymph nodes can be enlarged, edematous, and hemorrhagic. The bacterium replicates in large numbers and is closely linked with mucosae of the small intestine; however, the mechanisms of adherence and colonization are uncertain. It produces an A-B toxin that diffuses into the mucosa and lamina propria, causing in addition to necrosis, thrombosis of mucosal and submucosal vessels. This latter lesion leads to ischemic necrosis (acute coagulative necrosis) of tissues supplied by these blood vessels. Inflammation usually does not occur.

Animals, most commonly cattle, encounter *B. anthracis* through the ingestion of fomites contaminated with endospores and/or vegetative forms of the bacterium. The bacterium exists most commonly in soil and water as an endospore, a dormant, nonreproductive form that is resistant to ultraviolet radiation, dehydration, extremely cold and hot temperatures, and chemical disinfectants. These conditions are harmful to the vegetative form, the form of the bacterium that produces toxin and causes disease. Animals can ingest endospores that subsequently germinate into vegetative forms in the alimentary tract. However, animals can also ingest vegetative forms as the result of environmental conditions that allow the vegetative form to persist for a limited period of time. Heavy rain after a drought can cause the germination of endospores in areas contaminated with endospores and the multiplication of vegetative forms. Endospores present in undercooked or poorly processed meat wastes and by-products can germinate into vegetative forms, persist for a limited period of time, and then be ingested when fed to animals.

Endospores and/or vegetative forms are swallowed, evade destruction by gastric acidity, and gain access to mucosae of the small intestine via peristalsis. The sequence of steps from ingestion of endospores or vegetative forms to the occurrence of lesions is largely unknown. It has been suggested that endospores can gain access to mucosae and lamina propria via ulcers, cuts, and puncture wounds of the alimentary system, geminate into vegetative forms, colonize the tissue, and secrete toxins causing disease. It is also likely that vegetative forms gain access to regional mesenteric lymph nodes via afferent lymphatic vessels as cell-free bacteria or within macrophages via phagocytosis that migrate via leukocyte trafficking in lymphatic vessels to these nodes. Vegetative forms probably use mechanisms discussed later to cause disease in the injured mucosae. Alternatively, the need for mucosal injury as an initiating step in the disease is conceivable. Hypothetically, three possible mechanisms could result in the production of vegetative forms, toxins, and lesions:

1. Endospores could be trapped in the mucus layer of the mucosa, endospores germinate to vegetative forms, vegetative forms use mucus to grow and replicate, and vegetative forms produce toxins that diffuse into the mucosa and submucosa, resulting in lesions.
2. Endospores could be trapped in the mucus layer of the mucosa, phagocytosed by macrophages or dendritic cells, and carried to Peyer's patches, where they germinate into vegetative forms, and vegetative forms produce toxins that diffuse into the mucosa and submucosa, resulting in lesions.
3. Endospores are phagocytosed by M cells and carried to Peyer's patches, where they germinate into vegetative forms, and vegetative forms produce toxins that diffuse into the mucosa and submucosa, resulting in lesions.[7]

Primary virulence factors produced by *B. anthracis* are in plasmid genes and include those that form the capsule and anthrax toxins. The capsule is important in establishing the infection, whereas anthrax toxins cause the lesions, disease, and lysis. The capsule consists of poly-D-glutamic acid that is nontoxic, protects the bacterium from destructive antibodies and bactericidal components of

[7]If vegetative forms are ingested, three similar mechanisms are proposed to occur in animals, but the time course of disease would likely be shortened.

plasma, and inhibits phagocytosis, killing, and digestion of vegetative forms of the bacterium by macrophages and neutrophils. Anthrax toxins behave as an A-B toxin system and consist of three exotoxins that act together to cause cell lysis. One exotoxin, called *protective antigen* (PA), the B part of the A-B toxin, facilitates the entry of itself into cells via endocytosis and then creates a pore in the cell membrane through which the remaining two toxins, *edema factor* (EF) and *lethal factor* (LF), the A component of the A-B toxin, can enter the cell. Exotoxins must first bind to receptors on target cells. PA binds to two different cell surface receptors, tumor endothelial marker 8 (TEM8) and capillary morphogenesis protein 2 (CMG2). These receptors appear to explain the vascular orientation of the disease and the circulatory system collapse that results. In addition to vascular tissues, these receptors are also commonly expressed on cells in many other organ systems, likely accounting for the various forms (inhalation, cutaneous, and gastrointestinal) of anthrax. Once inside cells, PA combines with edema factor to form edema toxin, which disrupts cell membrane water and electrolyte transport systems, resulting in edema, and also blocks phagocytosis of vegetative forms by neutrophils and macrophages. Additionally, PA combines with lethal factor to form lethal toxin. This toxin stimulates the production of a variety of cytokines that act to cause cell lysis, especially affecting phagocytic cells such as macrophages and endothelial cells of capillaries. Because of injury to mucosae and lamina propria, capillary beds in the ECM can absorb edema and lethal toxins, as well as a variety of intestinal endotoxins. Cytokines, anthrax toxins, and endotoxins have profound systemic effects on the cardiovascular system, all of which contribute to cardiogenic and circulatory shock and lysis. Chromosomal genes of the vegetative form of the bacterium also express a capsule virulence factor, which makes it resistant to phagocytosis by mucosa-associated tissue macrophages. Additionally, the bacterium has several chromosomal virulence factors for hemolysins, phospholipases, and iron acquisition proteins that can contribute to or cause cell lysis.

Disorders of Horses

Rhodococcal Enteritis (*Rhodococcus equi*). The pathogenesis of *R. equi* infection is also discussed in the section on Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Horses. An overview is provided herein. Gross lesions include (1) ulcerative enteritis (see Fig. 7-138) characterized by discrete foci of ulceration and hemorrhage centered over Peyer's patches and (2) chronic active pyogranulomatous lymphadenitis (see Fig. 7-139) characterized by enlarged firm lymph nodes that on a cut surface have discrete and coalescing areas of yellow-white exudate infiltrating and compressing contiguous parenchyma.

In the alimentary system, foals encounter *R. equi* by swallowing mucus (sputum), exudate, and cellular debris contaminated with bacteria that move into the oral pharynx via the positive pressure of coughing and the upward rhythmic movement of cilia in the mucociliary apparatus. The bacterium then gains access to the alimentary system via intestinal peristalsis. Bacteria probably bind to receptors on luminal surfaces of M cells and are then transported in endocytotic vesicles to the basal membranes of the cell and released into the Peyer's patches, where they can be phagocytosed by tissue macrophages or dendritic cells. Unlike most other regions of the intestine, the luminal surface of M cells lacks a covering of mucus. Therefore bacteria have direct access to M cells. It is likely that ligand-receptor mechanisms (as discussed in the section on the Respiratory System, Mediastinum, and Pleurae) are applicable to M cells and tissue macrophages in Peyer's patches. Once tissue macrophages are infected, the pathogenesis of the disease appears to progress much like that which occurs in the lung resulting in pyogranulomatous enteritis and lymphadenitis. Mechanistically, *R. equi* has virulence factors in a PAI and in a plasmid that block (1) the fusion of phagosomes with lysosomes (virulence-associated proteins [Vaps]), (2) the actions of lysosomal enzymes and toxins, and (3) the respiratory burst used by macrophages to kill the bacterium. The bacterium can then replicate within the phagosome of macrophages. Pyogranulomatous enteritis is attributable to repeated cycles of phagocytosis, dysfunction of phagosomes, bacterial growth and replication, lysis of macrophages, release of large numbers of new bacteria, recruitment of additional naïve inflammatory cells, and reparative responses like fibrosis that likely perpetuate and expand the scope of the disease process. Ulcerative enteritis, characteristic of the alimentary form of the disease, occurs over affected Peyer's patches. Although unknown, it is probable that mediators and degradative enzymes from inflammation diffuse into contiguous tissues, causing direct injury to the mucosa or indirectly via vascular injury and occlusion leading to infarction and ulceration. Bacteria-infected tissue macrophages can also spread via leukocyte trafficking in afferent lymphatic vessels within the intestinal mesentery to mesenteric lymph nodes leading to a pyogranulomatous lymphadenitis and then systemically to lymph nodes and lymphoid tissues such as the spleen.

Tyzzer's Disease (*Clostridium piliforme* [*Bacillus piliformis*]). See Bacterial Diseases of Organ Systems, Hepatobiliary System and Exocrine Pancreas, Disorders of Horses, Tyzzer's Disease (*Clostridium piliforme* [*Bacillus piliformis*]).

Disorders of Ruminants (Cattle, Sheep, and Goats)

Johne's Disease (*Mycobacterium avium* ssp. *paratuberculosis* [MAP]). The mechanisms of injury in Johne's disease are (1) dysfunction and lysis of the epithelial cells and ECM proteins forming the junctional barrier systems of mucosae of the small intestine, (2) dysfunction of the drainage of afferent lymphatic vessels in the lamina propria of villi of the small intestine, and (3) lysis of cells of the monocyte-macrophage system and of all cell populations in the lamina propria of intestinal villi from chronic inflammation and its mediators and degradative enzymes. Gross lesions include granulomatous enteritis and mesenteric granulomatous lymphadenitis, lymphangitis, and lymphangiectasia (see Fig. 7-162). Granulomatous enteritis is characterized by a thickened intestinal wall, most commonly affecting the ileum and ileal-cecal junction with a yellow-white exudate exemplified by infiltrating granulomatous inflammatory cells. Mesenteric granulomatous lymphadenitis is characterized by enlarged mesenteric lymph nodes that on cut surfaces have discrete and coalescing areas of yellow-white caseous exudate, occasionally mineralized, infiltrating, and compressing contiguous parenchyma.

The young of cattle, sheep, and goats encounter *M. avium* ssp. *paratuberculosis* through ingestion of the bacterium in manure-contaminated fomites in the environment. It is unknown why young animals are more susceptible to this microbe. It has been suggested that the "open gut" that occurs during the first 24 hours after birth, where immunoglobulins in colostrum are absorbed by pinocytosis, is a mechanism that may also be used by the bacterium to cross the mucosal barrier and enter the submucosa. Young animals may also be more susceptible to infection because of immature innate and/or adaptive immune responses. Susceptibility is also probably strongly influenced by management and environmental factors and to a lesser extent by the genes of the animal.

The bacterium is swallowed and gains access via peristalsis to the alimentary system. Bacteria appear to bind to receptors on luminal (apical) surfaces of M cells and are likely translocated in endocytotic vesicles or phagosomes to the basal membranes of the cell and are released into Peyer's patches, where they can be phagocytosed by

tissue macrophages. Unlike most other regions of the intestine, the luminal surface of M cells lacks a covering of mucus. Therefore bacteria have direct access to M cells. Lesions appear to have a segmental pattern of occurrence most commonly affecting the ileocecal intestine. Although attachment and phagocytosis of the bacterium by M cells likely involves ligand-receptor interactions, this mechanism does not explain why the lesions are most severe in ileocecal intestine. A second, but less likely, pathway of spread can also result from ingestion. Chewing of foodstuffs places *Mycobacterium*-infected fomites in contact with the palatine tonsils. Experimentally it has been shown that the bacterium can infect mucosal and submucosal cells of the tonsil, likely spread via leukocyte trafficking to regional lymph nodes, and then spread via leukocyte trafficking in efferent lymphatic vessels to mesenteric lymph nodes (ileocecal lymph nodes) and the mucosa and submucosa of the ileum and ileal-cecal junction areas.

M. avium ssp. *paratuberculosis* requires iron for growth inside phagosomes of tissue macrophages. For an unknown reason the concentration and availability of iron is greatest in tissue macrophages of the ileocecal intestine when compared to the concentration in other types of tissue macrophages. Therefore this gradient of iron appears to establish tissue specificity for lesions in Johne's disease. In macrophages, iron is stored as ferritin, but it is not accessible by the bacterium. Mycobacteria secrete iron-chelating proteins called exochelins, iron-reductases, and potentially siderophores (i.e., virulence factors) and use these enzymes to acquire iron from ferritin stored in macrophages. Additionally, as the severity of inflammation increases, there is a concurrent increase in the concentration of ferritin available for use by the bacterium in cells and tissues in the areas of inflammation. Mycobacterial siderophores or reductases may also serve to block iron-dependent bactericidal reactions of tissue macrophages such as Fe^{3+}-dependent conversion of H_2O_2 into highly toxic hydroxyl radicals.

In ileocecal tissues, phagocytosis of the bacterium by tissue macrophages likely involves ligand-receptor interactions. TLRs may also be involved in attachment and phagocytosis. The cell walls of mycobacteria contain a variety of complex lipoglycans, glycoproteins, and lipoproteins such as lipoarabinomannan (LAM), 19-kDa lipoprotein, and the mycolyl-arabinogalactan-peptidoglycan complex that can serve as ligands. The cell membranes of tissue macrophages express receptors for these specific molecules, and they are probably involved in the recognition, attachment, and adherence of the bacterium to the macrophage cell membrane. Additionally, complement receptors and other receptors, including mannose and CD14 receptors, expressed on tissue macrophages are the major receptors involved in phagocytosis of the bacterium, whereas integrin receptors, TLRs, mannose receptors, CD14 receptors, scavenger receptors, and immunoglobulin Fc receptors are involved in early recognition and cell signaling in response to interaction with the bacterium. Generally, these signaling pathways initiate production of a variety of cytokines, chemokines, and antimicrobial metabolites that control mycobacterial infections; however, the bacterium through these signaling pathways is able to attenuate macrophage activation responses induced by interferon-γ (IFN-γ) and the secretion of IFN-γ. Type 1 T helper lymphocytes, attempt to enhance the killing of intracellular mycobacterial organisms by releasing cytokines such as IFN-γ that activate macrophages to kill the bacterium.

These interactions do not involve opsonin-mediated phagocytosis; thus the induction of a respiratory burst to kill the internalized bacteria does not occur, and the bacterium persists in the phagosome. Receptors for opsonins expressed by tissue macrophages might also play a role in phagocytosis of the bacterium. Fibronectin may bind to the surface of macrophages and serve as a ligand to facilitate phagocytosis by macrophages. However, when employing opsonization as a means of entry into a phagosome, the bacterium must also use a mechanism to inhibit the respiratory burst to prevent its lysis.

The time from initial encounter with the bacterium to the expression of clinical disease is usually 12 months or longer. An explanation for this extended delay is unknown; it may simply be a slow-growing bacterium. However, bacterial growth likely involves the (1) interplay of bacterium-infected tissue macrophages and cells of the immune system mediated by proinflammatory and antiinflammatory cytokines, (2) migration of tissue macrophages locally from Peyer's patches into the lamina propria and submucosal tissues, (3) time it takes the bacterium to replicate in sufficient quantities to activate the adaptive immune response, and (4) progression of severity resulting from the lysis of bacterium-infected macrophages leading to the recruitment of additional macrophages.

After phagocytosis by tissue macrophages in Peyer's patches, the bacterium is confined within phagosomes and phagolysosomes (see Fig. 4-13). It appears to be able to disrupt phagosome-lysosome fusion and if fusion occurs, block the degradative actions of lysosomal enzymes and molecules via the structure and composition of its cell envelope and through the production of peroxidases. When a phagolysosome forms, the fused lysosome releases an acidic cytosol, proteases, and antibacterial substances, such as defensins and toxic oxygen and nitrogen intermediates, into the phagosome, all of which can injure and kill the bacterium. In general, mycobacterial species can (1) inhibit acidification of the phagosome, phagosome-lysosome fusion, and lysosomal enzyme activities; (2) block injury from toxic oxygen and nitrogen intermediates; and (3) suppress the ability of macrophages to be activated by cytokines such as INF-γ. Although highly probable, it is not known which or if any of these mechanisms are used by *M. avium* ssp. *paratuberculosis*. Tissue macrophages, once infected with the bacterium, are activated and begin to secrete proinflammatory cytokines that act to recruit and activate additional macrophages. Additionally, because the life span of fully differentiated tissue macrophages is approximately 10 to 30 days, lysis of these cells related to aging and bacterial-induced injury releases bacterium into adjacent tissue, where they are phagocytosed by newly recruited macrophages only to endlessly repeat this process. Granulomatous inflammation ensues, and multinucleated giant cells are noted histologically in the exudate (see Chapters 3 and 5).

The severity and extent of the inflammatory response, concurrently with tissue injury, grows through the recruitment of additional monocytes and tissue macrophages from the circulatory system and regional lymph nodes. This repetitive process accounts for the marked thickening of the mucosa and submucosa of the ileum and ileal-cecal junction characteristic of the gross lesion of Johne's disease. It also destroys the integrity of the mucosal barrier system, as well as lymphatic drainage, often resulting in a protein-losing enteropathy. This process is also probably an important factor contributing to severe malabsorption, diarrhea, weight loss, and emaciation that ensue. Bacteria-infected tissue macrophages can also spread via leukocyte trafficking in afferent lymphatic vessels within the intestinal mesentery to mesenteric lymph nodes (ileocecal lymph nodes), leading to a pyogranulomatous lymphadenitis and lymphangiectasia through the same progressive mechanism of inflammation.

Bovine Intestinal Tuberculosis (*Mycobacterium bovis*). The pathogenesis of *Mycobacterium bovis* infection is also discussed in the section on Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants (Cattle, Sheep, and Goats). An overview is provided herein. The pathogenesis and lesions of bovine intestinal tuberculosis are identical to those

observed in the pneumonic form. Also, see Johne's disease in the previous section because its pathogenesis is similar to that of intestinal tuberculosis. It appears that intestinal tuberculosis commonly begins as the pneumonic form and is spread to the intestine by (1) coughing up and swallowing sputum containing macrophages infected with bacteria and/or "free" bacteria and (2) hematogenous or lymphatic spread of infected macrophages via leukocyte trafficking to intestinal lymph nodes and Peyer's patches. Via the alimentary route, intestinal M cells and possibly dendritic cells are used to phagocytose bacteria and then release them via exocytosis from basolateral surfaces into Peyer's patches, where they are phagocytosed by macrophages and granulomatous inflammation and granuloma formation follow. Intestinal tuberculosis is associated with mucosal ulceration overlying Peyer's patches. Ulceration appears to result from vasculitis, thrombosis, ischemia, and infarction secondary to inflammation in Peyer's patches but could also be caused directly by inflammatory mediators released from granulomas diffusing to and acting on blood vessels or mucosae.

Wooden Tongue (*Actinobacillus lignieresii*). The mechanisms of injury in wooden tongue are persistent pyogranulomatous inflammation and fibrotic reparative responses. Gross lesions include a firm enlarged tongue that protrudes from the oral cavity. Cut surfaces have numerous randomly distributed yellow-white granulomas intermixed with broad bands of fibrous connective tissue (see Figs. 7-51 and 52). *Actinobacillus lignieresii* is a normal commensal bacterium of mucosae of the oral cavity of cattle and sheep, the species in which this disorder occurs most commonly. However, it has, although very rarely, been reported to occur in horses, pigs, and dogs. During chewing the bacterium is carried through the mucosa into submucosal connective tissues via penetrating wounds such as those caused by sharp foreign bodies like sticks or wires. The bacterium colonizes submucosal connective tissue, and LPS of the bacterial cell wall, in part, likely plays a role in the pyogranulomatous inflammatory and concurrent fibrotic responses that occur. Little is known about how virulence factors, ligand-receptor interactions, target cells, toxins, capsule antiphagocytic molecules, or other factors contribute to the pathogenicity of this bacterium. However, it appears likely that the bacterium is able to evade killing by neutrophils and macrophages, thus colonizing itself in abscesses in tissues of the tongue and oral cavity. Repeated cycles of phagocytosis, bacterial growth and replication, lysis of macrophages, release of large numbers of new bacteria, recruitment of additional naïve inflammatory cells, and reparative responses like fibrosis likely perpetuate and expand the scope of the disease process. Fibrosis and encapsulation occur concurrently and appear to represent a last-ditch attempt to isolate and wall off the bacterium from the vascularized tissue in the tongue and oral cavity. *A. lignieresii* can spread via lymphatic vessels to regional lymph nodes and cause a similar inflammatory response and lesion in these nodes.

Alimentary Anthrax (*Bacillus anthracis*). See Bacterial Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Domestic Animals; Alimentary Anthrax (*Bacillus anthracis*).

Disorders of Pigs

Porcine Proliferative Enteritis/Hemorrhagic Bowel Syndrome (*Lawsonia intracellularis*). Although proliferative enteritis/hemorrhagic bowel syndrome is most commonly recognized as a disease of pigs, a syndrome resembling the proliferative form of this disease in pigs also occurs in horses (equine proliferative enteropathy [EPE]), and the pathogenesis is likely similar to that described in pigs. *L. intracellularis* appears to cause two distinct disease syndromes in a single disease continuum. The mechanism of injury of the first syndrome is characterized by pathologic processes that result in cell proliferation (i.e., proliferative enteritis), whereas the mechanism in the second syndrome is characterized by processes that result in cell lysis and hemorrhage (i.e., hemorrhagic bowel syndrome). It seems that the first syndrome can occur and resolve without transitioning into the second syndrome. The reverse scenario does not appear to occur. The proliferative form of this disease is also known as *porcine intestinal adenomatosis*, *proliferative ileitis*, *regional ileitis*, *garden hose disease*, and other similar names. The hemorrhagic form of this disease is also known as *necrotic enteritis* or *acute proliferative hemorrhagic enteropathy*. The mechanism of injury is initially cellular hypertrophy and hyperplasia (proliferation) that can be followed by cell lysis resulting from ischemia and necrosis. Gross lesions in proliferative enteritis include a circumferentially firm and thickened ileum with a mucosa that fills the lumen and bulges into the lumen on cut surface (see Fig. 7-173). Gross lesions in hemorrhagic bowel syndrome include segmental necrosis and hemorrhage with fibrinous diphtheritic membranes attached to the mucosa, luminal blood clots, and thinned intestinal walls (see Fig. 7-175).

Pigs encounter *L. intracellularis* through ingestion of the bacterium in manure-contaminated fomites from the environment. It is swallowed and gains access via peristalsis to mucosae of the small intestine. It is unclear how the bacterium initially colonizes the mucus layer and mucosae and why it targets epithelial cells in the ileum. Ligand-receptor interactions are likely involved in this ileal specificity and *Lawsonia* surface antigen (LsaA) may act as an adhesin or invasin early in colonization. Other specific bacterial adhesins or cell membrane receptors have not been identified. Alternatively, possibly in conjunction with ligand-receptor interactions, ileal specificity may be attributed to unidentified metabolic or growth factors required by the bacterium that are provided only by intestinal crypt cells (see later section for more detail). In addition, it is unclear how the bacterium is able to penetrate the mucus layer and gain direct access to the luminal membrane of epithelial cells. *L. intracellularis* is regarded as a nonmotile bacterium; however, there is scant experimental evidence suggesting that the bacterium develops a transient bacterial appendage that behaves as a flagellum and could provide motility into and through the mucus layer. Colonization also appears to be enhanced by the presence of other anaerobic bacterial species in the mucus layer. The significance of this finding is unclear but could be related to these species providing molecules required by *L. intracellularis* for colonization and replication.

L. intracellularis infects cells of the crypts located in the proliferative zone (Fig. 4-22). Following colonization, the bacterium interacts with the brush border and then is taken into the cell via endocytosis and resides in a phagosome within the cytoplasm. The bacterium rapidly escapes from the phagosome before phagosome-lysosome fusion occurs and resides free in cell cytoplasm. A phospholipase bacterial virulence factor, mediated through a type III secretion system, may be involved in the escape mechanism. Once free in the cytoplasm, bacteria remain in close proximity to the apical (luminal) cell membrane, where they grow and replicate within the cytoplasm (see Fig. 7-176). In this location, bacteria appear to aggregate near mitochondria; thus it has been suggested that they may need preformed triphosphates for growth.

A unique feature of the pathogenesis of proliferative enteritis is the finding that proliferation of bacteria intracellularly (growth and replication) occurs concurrently with proliferation of crypt enterocytes (hypertrophy and hyperplasia) (see Fig. 7-174). One process does not occur without the other. Under normal conditions, crypt cells within the proliferative zone are dividing cells that differentiate into nondividing cells of the differentiative zone as these cells migrate along basement membrane up the villus to its apex (see Fig.

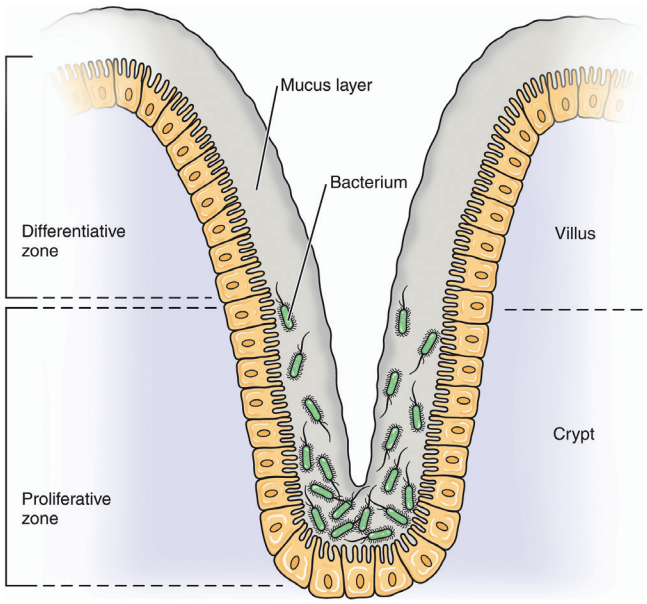


Figure 4-22 Pathogenesis of Proliferative Enteritis in Pigs. *Lawsonia intracellularis* infects cells of the crypts located in the proliferative zone.

4-22). It appears that once crypt cells of the proliferative zone are infected with bacteria, the bacterium is able to inhibit the normal maturation of crypt cells, probably through disruption of the cell cycle. Additionally, infection of crypt cells by bacteria dramatically increases the rate of crypt cell division.

Therefore, when crypt cells are infected, they do not mature but remain in an undifferentiated proliferative state and divide continuously, thus resulting in massive thickening of the mucosal surface by proliferating crypt cells. Proliferating cells continue to migrate to the apices of villi, where they die and their contents, including large numbers of bacteria, are extruded into the lumen of the intestine. This outcome provides a source of bacteria to infect additional uninfected crypt enterocytes and spread through feces into the environment. Normal villus structure is lost and replaced by a branching glandular pattern, where cells lining the hyperplastic glands are crowded into a mucosal layer of up to 10 to 15 epithelial cells in thickness. Mitotic activity is prominent. Inflammation does not occur.

The veterinary literature does not provide any discussion of the relationship between proliferative enteritis and hemorrhagic bowel syndrome and how the former transitions into the latter or if it does. Histologically, it has been shown that lesions occurring in necrotic enteritis (hemorrhagic bowel syndrome) include acute coagulative necrosis of the proliferating crypt epithelium. This lesion is consistent with ischemia or the direct effects of toxins (burnlike injury). Necrotic enteritis may simply be a manifestation of proliferating cells outgrowing an available blood supply, becoming ischemic, and then dying via acute coagulative necrosis. No cell in the body can survive if it is farther than 100 µm away from a source of oxygen, either from a capillary or highly oxygenated body fluid. Additionally, *L. intracellularis* is a Gram-negative bacterium, and endotoxin or other toxic molecules may directly cause injury and cell lysis consistent with acute coagulative necrosis. Acute inflammation with hemorrhage and fibrinogenesis commonly occur concurrently with acute coagulative necrosis.

Swine Dysentery *(Brachyspira hyodysenteriae)*. The mechanism of injury in swine dysentery is lysis of mucosal epithelial cells of the colon and cecum caused by bacterial hemolysins and proteases and from inflammation and its mediators and degradative enzymes. Gross lesions include a mucohemorrhagic necrofibrinous colitis and typhlitis with diphtheritic membranes covering intestinal mucosae formed by abundant mucus, hemorrhage, fibrin, plasma proteins, and cellular debris arising from necrotic mucosal epithelial cells and inflammatory cells (see Fig. 7-171).

Pigs encounter *B. hyodysenteriae* (previously named *Serpulina hyodysenteriae* and *Treponema hyodysenteriae*) through ingestion of the bacterium in manure-contaminated fomites in the environment. The bacterium is swallowed and gains access via peristalsis to the intestine, especially the cecum and colon. Virulence factors that block its destruction, by bile and digestive enzymes as examples, during transit in the small intestine to the cecum and colon are unknown. Goblet cell mucus is important as a physical matrix and as a chemical substrate for colonization by the bacterium; thus goblet cells play a central role in the pathogenesis of lesions affecting mucosal epithelial cells and their junctional complexes. *B. hyodysenteriae*, an anaerobic motile spirochete, is able to actively move through the mucus layer to gain access to mucosal epithelial and goblet cells. It is unclear why the bacterium infects the cecum and colon, but it appears that it prefers to initially replicate in mucigen droplets within goblet cells. Mucigen droplets fill the apical cytoplasm of goblet cells, and the nucleus is displaced to the basal region of the cell. Because the relative number of such cells is much greater in the cecum and colon when compared to other segments of the alimentary system, this quantitative difference may account for the location of the lesions. Additionally, mucins (e.g., the fucose and L-serine components) are strong chemoattractants for spirochetes, and because there are significant biochemical and pH differences in mucins, such as those that are synthesized and released from goblet cells, it is plausible that the chemical composition of the mucins may account for the locations of the lesions. Once the bacterium infects mucigen droplets within goblet cells, it appears to be able to activate the goblet cell to increase the production of mucus. Thus there is a large increase in the volume of mucus secreted by these cells so that mucosal surfaces are covered with a thick grayish gelatinous layer. It is unclear how the bacterium activates the goblet cell to produce and release large quantities of mucus. It is plausible that one or more bacterial virulence factors influence the cellular processes of transcription, translation, assembly, and packaging of mucigen droplets, thereby producing abundant quantities of mucus to enhance its opportunity to colonize the mucosa. Concurrently with infecting goblet cells, the bacterium begins the process of colonizing the thickened mucus layer covering the mucosal epithelium. It appears that mucus and its mucins are central to both the colonization and replication processes and result in the accumulation of large numbers of bacteria in close proximity to cell membranes and junctional complexes of mucosal epithelial cells.

Recently it has been demonstrated experimentally that more virulent strains of the bacterium express increased numbers of genes for carbohydrate and amino acid metabolism and transport that potentially could be linked to energy and carbon sources that are available in the mucus layer. Additionally, highly fermentable feeds favor colonization of the mucus layer by bacteria. Fermentation may provide the bacterium with an energy source or other molecules required for colonization and replication. Colonization is also enhanced by the presence of other anaerobic bacterial species in the mucus layer. The significance of this finding is unclear, but again could be related to these anaerobic species providing molecules required by *B. hyodysenteriae* for colonization and replication. Finally, because the bacterium is an anaerobe, it synthesizes high concentrations of nicotinamide adenine dinucleotide hydrogen (NADH) oxidase (a virulence factor) that is used to protect itself

from oxidative stress and toxic oxygen molecules in the oxygen-rich environment of the mucus layer.

The bacterium does not attach to luminal (apical) membranes of colonic and cecal epithelial cells; however, experimental studies have reported that the bacterium invades the epithelium and the lamina propria because it has been identified in these areas. The largest quantity of bacteria appears to exist in the mucus layer just overlying the epithelium. Therefore it is unclear as to whether this invasion is a direct and targeted process or merely an innocent bystander phenomenon, in which the motility of the bacterium carries it into these locations to cause injury. Injury and lysis of colonic and cecal epithelial cells (enterocytes), as well as penetration through junctional complexes into the superficial lamina propria, are likely caused by one or more proteases and hemolysins and endotoxic effects of lipooligosaccharide (LOS) from the cell wall of the bacterium. Lysis and loss of the mucosal epithelium results in hemorrhage and the opportunity for other microbes, such as other anaerobic bacteria and the protozoan *Balantidium coli*, to invade the lamina propria. Denuded mucosa also provides a mechanism for the absorption of endotoxins, cytotoxins from inflammatory cells, and other toxic molecules that could cause endotoxic shock locally and systemically via the blood vascular system.

Porcine Polyserositis (*Haemophilus suis/parasuis, Actinobacillus suis, Streptococcus suis*, or *Escherichia coli*). See Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Pigs; Porcine Polyserositis (*Haemophilus suis/parasuis*, *Actinobacillus suis*, *Streptococcus suis*, or *Escherichia coli*).

Hepatobiliary System and Exocrine Pancreas

Disorders of Domestic Animals

***Hepatic Leptospirosis* (*Leptospira* spp.).** The pathogenesis of hepatic leptospirosis begins as vascular leptospirosis caused by *Leptospira* spp. The pathogenesis is discussed in the section on Bacterial Diseases of Organ Systems, Cardiovascular System and Lymphatic Vessels, Disorders of Domestic Animals. The mechanisms used by *Leptospira* spp. to infect the liver are likely similar to those used in the kidney and are covered in the Urinary System section of this chapter. Gross lesions include discrete and coalescing white-to-gray foci of hepatic necrosis scattered at random throughout hepatic parenchyma that are intermixed with hemorrhage.

Disorders of Horses

Tyzzer's Disease (*Clostridium piliforme* [*Bacillus piliformis*]). The mechanism of injury in Tyzzer's disease is acute coagulative necrosis of hepatocytes, intestinal mucosal epithelial cells, and adjacent vascular and stromal tissues and from inflammation and its mediators and degradative enzymes. Gross lesions include hepatomegaly and numerous white-gray-yellow foci (<2-mm diameter) of hepatocyte necrosis distributed at random, usually throughout all lobes of the liver (see Fig. 8-53). In severe cases the center of these foci may be depressed and red (hemorrhage).

The young of all animal species can contract Tyzzer's disease; however, foals appear to be the most susceptible and encounter *Clostridium piliforme*, an obligate intracellular bacterium, through ingestion of spores present in soil or vegetative forms in fecal fomites from infected animals. The disease is less common in dogs, cats, and calves. Although the bacterium uses the intestinal mucosa as an initial beachhead, it ultimately infects, replicates in, and injures the liver. The mechanism of spread from ingestion to the liver is unclear. In other diseases caused by *Clostridium* spp., such as blackleg, cells of the monocyte-macrophage system and M cells are probably used to spread spores and/or vegetative forms and hide (sequester) from innate and adaptive immune responses. After ingestion, it is likely that spores or vegetative forms are carried by normal peristaltic activities through the oral pharynx, esophagus, and stomach to their final destination, the small intestine (ileum). It is unknown if and how the bacterium interacts with and gains access to intestinal mucosal epithelial cells and/or mucosal macrophages. Vegetative forms of the bacterium are motile and may be able to penetrate the mucus layer and encounter mucosal epithelial cells of the small intestine. How they enter these cells is unknown, although direct penetration or receptor-mediated endocytosis through ligand-receptor interactions could be involved. Spores could be taken up through endostosis by intestinal mucosal epithelial cells, but how the spores penetrate the mucus layer and gain access to epithelial cells is unknown. They could be phagocytosed by mucosal macrophages in the mucus layer and carried to or through the mucosa by leukocyte trafficking. Additionally, spores could bind with receptors on the surface of M cells, which lack a mucus layer, enter though endocytosis, germinate into vegetative forms, infect and replicate in these cells, and then spread to adjacent mucosal epithelial cells.

In either case, spores or vegetative form are able to infect mucosal epithelial cells of the intestine through their apical surfaces and then replicate in the cells. It is not known what type of ligand-receptor interactions are involved in this process of entry into the cell. The bacterium appears to adhere to the apical cell membrane, be phagocytosed, and then escape the phagosome to reside and replicate in the cytoplasm of the cell.

It has not been shown how the bacterium spreads from mucosal epithelial cells or from M cells systemically to the liver. Because C. *piliforme* is a motile bacterium, it has been suggested that it leaves epithelial cells of the intestine (possibly from their basal surfaces), enters subjacent lamina propria, encounters and penetrates capillaries, enters the circulatory system, and is carried in blood plasma via the portal vein to the liver. The bacterium could also potentially be transported to the liver within phagosomes in macrophages (leukocytic trafficking). If the bacterium is able to infect and replicate in M cells, the integration of M cells with Peyer's patches provides the opportunity for the bacterium to interact with macrophages or gain access to capillaries within submucosal ECM tissues. Macrophages could phagocytose the bacterium using ligand-receptor interactions and carry it via leukocyte trafficking in afferent lymphatic vessels to mesenteric lymph nodes and then systemically via the thoracic duct and venous system into the circulatory system and ultimately the liver via the hepatic artery. However, in a mouse model of Tyzzer's disease, depletion of macrophages did not change the course of infection. This outcome suggests that leukocyte trafficking may not be involved in the spread of the bacterium from the intestine to the liver.

Once in the liver, the bacterium encounters endothelial cells lining hepatic sinusoids. As a motile bacterium, it is free in the circulatory system and could (1) directly penetrate the endothelium and enter and infect hepatocytes, (2) infect and replicate in endothelial cells and then spread to adjacent hepatocytes, or (3) infect and replicate in Kupffer cells and then spread to adjacent hepatocytes. Hemorrhage occurs in Tyzzer's disease, and this lesion suggests that vascular injury occurs either from direct penetration of the blood vessels or by lysis of the endothelial cells after replication of the bacterium. Although direct penetration of endothelial cells and hepatocytes is a possible mechanism of entry into these cells, typical ligand-receptor interactions may also be involved. The bacterium enters hepatocytes probably via receptor-mediated endocytosis and then escapes the phagosome to reside and replicate in the cytoplasm. The replication of C. *piliforme* in hepatocytes eventually results in hepatocellular necrosis. The mechanisms causing necrosis are

unknown. Bacterial cytotoxic proteins and several cellular cytokines like interleukin and TNF have been implicated as the cause of hepatocellular necrosis, but experimental results are inconclusive. It appears that the bacterium first causes acute hepatocyte necrosis, which then incites an acute inflammatory response with abundant neutrophils and occasional macrophages in affected tissues. In the same mouse model of Tyzzer's disease, the number of bacteria in hepatocytes and the severity of lesions were much worse in mice depleted of neutrophils and NK cells. This outcome suggests that the acute inflammation plays an important role as an innate defense mechanism in the disease.

Disorders of Ruminants (Cattle, Sheep, and Goats)

Bacillary Hemoglobinuria (*Clostridium haemolyticum*). The mechanism of injury in bacillary hemoglobinuria has a local component and a systemic component. Local injury is cell lysis (acute coagulative necrosis) of hepatocytes (necrotizing hepatitis), whereas systemic injury is lysis of erythrocytes in the blood vascular system. Injury in both components is caused by phospholipase C and other toxins released from *Clostridium haemolyticum*. Gross lesions include vasculitis, infarction, coagulative necrosis, and hemorrhage in the liver (see Fig. 8-73) and hemoglobinuria in the urinary system.

Cattle and sheep probably encounter *C. haemolyticum* through ingestion of spores present in the soil. Although the bacterium ultimately resides in and injures the liver, the mechanism of spread from ingestion to the liver is unknown. In other diseases caused by *Clostridium* spp., such as blackleg, cells of the monocyte-macrophage system, M cells, and dendritic systems are likely used to spread and to hide spores from immune responses and other defense mechanisms. It is plausible that after ingestion, spores are carried by normal peristaltic activities through the oral pharynx, esophagus, abomasum, and rumen to their final destination, the small intestine. It is unknown how spores interact with and gain access to epithelial cells and mucosal macrophages. The mucus layer of the small intestine probably presents a significant barrier to spores; therefore spores could bind with receptors on the surface of M cells or dendritic cells and through transcytosis gain access to macrophages and lymphocytes located in Peyer's patches contiguous with these cells. Mucosal-associated macrophages could also phagocytose spores using ligand-receptor interactions and carry the spores via leukocyte trafficking in afferent lymphatic vessels to mesenteric lymph nodes and then systemically via the thoracic duct into the circulatory system. Although unproved, spores are likely the form of the bacterium that spreads systemically to liver. Trafficking macrophages having spores in phagosomes could enter the sinusoids of the liver and transfer spores to Kupffer cells embedded in the endothelium. Spores then hide in Kupffer cells until they are activated to germinate and produce vegetative bacteria. Tropism for Kupffer cells is probably mediated by ligand-receptor interactions.

The occurrence of bacillary hemoglobinuria follows injury to the liver caused by migration of liver flukes (*Fasciola hepatica*, *Fascioloides magna*). Thus bacillary hemoglobinuria occurs only in geographic locations that have these flukes. The flukes migrate through the liver and injure intrahepatic veins, causing thrombosis, ischemia, and infarction of associated hepatocytes. Infarcted areas of liver are anaerobic and have a lowered oxidation-reduction (redox) potential required for germination of spores released from dead Kupffer cells. Spores germinate into vegetative bacteria, and they produce large quantities of phospholipase C (also known as *lecithinase C*, an α-toxin) and hemolysins that destroy cell membranes and cause hepatocyte lysis. These toxins are also absorbed into the venous system within viable liver, resulting in entry into the systemic circulation, leading to erythrocyte membrane injury, lysis of erythrocytes, release of hemoglobin, and hemoglobinuria.

Infectious Necrotic Hepatitis (*Clostridium novyi*). The pathogenesis and lesions of infectious necrotic hepatitis are similar to those of bacillary hemoglobinuria discussed in the previous section; however, the disease lacks hemoglobinuria, which is likely attributable to the absence of toxins that injure and lyse erythrocyte membranes.

Disorders of Pigs

Porcine Polyserositis (*Haemophilus suis/parasuis, Actinobacillus suis, Streptococcus suis*, or *Escherichia coli*). See Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Pigs; Porcine Polyserositis (*Haemophilus suis/parasuis*, *Actinobacillus suis*, *Streptococcus suis*, or *Escherichia coli*).

Respiratory System, Mediastinum, and Pleurae

Disorders of Domestic Animals

Strep Zoo (*Streptococcus equi* subsp. *zooepidemicus*). The mechanism of injury in "strep zoo" is injury and lysis of mucosal and serosal epithelial cells and vascular endothelial cells from bacterial toxins and from inflammation and its mediators and degradative enzymes. Gross lesions include vasculitis leading to (1) a lung with a firm texture attributable to the leakage from injured blood vessels of variable quantities of fibrin into alveoli and alveolar septa (fibrinous pneumonia) and (2) the appearance of variable quantities of a gray-white friable material (fibrin) often mixed with hemorrhage on serosal surfaces (fibrinous polyserositis) of the lungs (fibrinous pleuritis), heart (fibrinous pericarditis), and abdominal cavity (fibrinous peritonitis) (see Fig. 10-60). The body cavities formed by these anatomic structures may also contain a fibrinous exudate and edema fluid mixed with hemorrhage. Facing serosal surfaces are often loosely attached to each other by fibrinous exudate, making normal physiologic processes such as respiration more difficult. The serosa and cavities of meninges, joints, and testis can also be affected. Information about the mechanisms used by this bacterium is limited. Some of this section is conditional and based on (1) what is known about virulence factors used by other members of the Streptococcaceae family, especially *Streptococcus equi* subsp. *equi*, to cause disease (see the section in this chapter on Bone Marrow, Blood Cells, and Lymphatic System, Disorders of Horses, Strangles [*Streptococcus equi* subsp. *equi*]) and (2) a reasonable probability that inflammation, responses to injury, and lesions that have been described in strep zoo are the result of underlying and known pathobiologic mechanisms.

S. equi subsp. *zooepidemicus* is a zoonosis. Horses and dogs likely encounter *S. equi* subsp. *zooepidemicus* through inhalation of the bacterium in fomites or fluid droplets from "carrier" or infected animals. The bacterium appears to be a commensal organism of mucous membranes of the nasal and oral pharynxes, probably existing in biofilms of healthy animals. Environmental stressors, such as overcrowding, poor ventilation and humidity, or abrupt changes in ambient air temperature, alter the mucus layer and the commensal relationship, allowing the bacteria to replicate in sufficient numbers to colonize the respiratory mucosae and spread the bacterium to other animals. Preceding or concurrent viral infections could also damage the mucociliary apparatus, allowing these bacteria to colonize the mucus layer or mucosae. In the respiratory system the bacteria are deposited on mucosae of the conductive component by centrifugal and inertial turbulence and trapped in the mucus layer. Although *S. equi* subsp. *zooepidemicus* can express many of the virulence factors expressed by *S. equi* subsp. *equi*, a causal link of these factors to disease is unclear. The bacterium is nonmotile, and it has

not been clearly shown how it penetrates the mucus layer; gains access to mucosal epithelial cells or cilia; expresses virulence factors such as adhesins, capsular molecules, fimbriae, and outer membrane proteins (e.g., fibronectin-binding protein) required for ligand-receptor interactions; and colonizes the mucosa. Some strains of the bacterium have SzP, a surface M-like protein, which may through receptor-ligand phenomena determine which organ system is colonized and what types of cells in that organ system are colonized. Other strains of the bacterium may have putative virulence factors such as C5a peptidase, invasins, and fibronectin-binding protein that are thought to contribute to biofilm formation and cell adhesion. It has been shown that some of these virulence factors can be transferred between strains of the bacterium via PAIs through horizontal gene transfer. Once mucosae are colonized and epithelial cells are injured, acute inflammation ensues, leading to lysis of these cells and loss of the ciliated mucosal barrier. Injury to ciliated epithelial cells alters the function of the mucociliary apparatus, allowing the bacterium to gain access to terminal bronchioles and alveoli by dependent settling due to gravity. Herein, bacteria colonize mucosae of the terminal bronchioles and alveoli, spread into vascularized ECM tissues, interact with and injure blood vessels of the air-blood barrier, and leak fibrinogen into the alveoli (fibrinous pneumonia). This process accounts for a fibrinous pneumonia but may not satisfactorily account for the fibrinous polyserositis so characteristic of this disease.

By an undetermined mechanism, bacteria likely gain access to the lamina propria of the respiratory system and have direct access to the vascularized ECM. It has not been determined how bacteria actually cross this altered barrier, reach the capillary beds, penetrate the endothelium, and spread into the blood vascular system. Mechanisms, such as a cell-free bacteremia or leukocyte trafficking in alveolar or intravascular macrophages, lymphocytes, or dendritic cells, are hypothetical possibilities. The characteristic gross lesions of fibrinohemorrhagic polyserositis suggest these bacteria may have a tropism for vascular endothelial cells of serosae. It is unclear why this occurs, but it is probably linked to the expression of bacterial virulence factors and ligand-receptor interactions with host endothelial cells in specific locations of body systems. Additionally, it is possible that bacterial toxins may contribute to vascular injury and permeability changes leading to the leakage of fibrinogen and its polymerization to fibrin on serosal surfaces and in some cases to microthrombus formation and disseminated intravascular coagulation in other organ systems.

Disease caused by *S. equi* subsp. *zooepidemicus* in dogs appears to follow the same chronologic sequence of steps as in horses, but virulence factors involved in the disease appear to cause more severe lesions to the vascular system and a greater degree of hemorrhage.

Respiratory Anthrax (*Bacillus anthracis*). See Bacterial Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Domestic Animals; Alimentary Anthrax (*Bacillus anthracis*) for detailed information on the pathogenesis and virulence factors.

The mechanism of injury in respiratory anthrax is cell lysis caused by bacterial toxins that act directly on cell membranes leading to acute coagulative necrosis. Gross lesions include pulmonary and lymph node edema, hemorrhage, and necrosis.

Animals encounter *B. anthracis* through inhalation of fomites contaminated with endospores from soil. Fomites containing endospores must be less than 5 µm in diameter to reach the O_2-CO_2 exchange portion of the respiratory system. Infected fomites are deposited on mucosae, where they are then phagocytosed either by alveolar macrophages migrating through and on the surface of mucosae or by dendritic cells. Infected macrophages and dendritic cells spread the bacterium to regional lymph nodes (bronchiolar and mediastinal) via afferent lymphatic vessels through leukocyte trafficking. During the migration process, endospores germinate into vegetative bacteria, so on arrival at the lymph nodes, bacteria are already producing anthrax toxins, which kill infected cells and release the bacteria into the ECM of the lymph nodes. In lymph nodes, bacteria continue to replicate and produce anthrax toxins killing additional lymphoid and endothelial cells, leading to edema and hemorrhage. The bacterium and its toxins enter lymphatic vessels and spread via the thoracic duct into the circulatory system as a septicemia, where endothelial cells and cells of other organ systems are injured, leading to edema, hemorrhage, and cell necrosis.

Disorders of Horses

Rhodococcal Pneumonia (*Rhodococcus equi*). See Bacterial Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Horses; Rhodococcus Enteritis (*Rhodococcus equi*) for additional information on the pathogenesis and virulence factors.

The mechanism of injury in rhodococcal pneumonia is lysis of cells of the monocyte-macrophage system and of all cell populations in the respiratory system secondary to inflammation and its mediators and degradative enzymes. Gross lesions include (1) cranioventral chronic active pyogranulomatous pneumonia characterized by consolidated firm yellow-white lung parenchyma attributable to infiltrating inflammatory cells, abscesses, and granulomas in the affected lung tissue (see Fig. 9-82) and (2) necrotizing pyogranulomatous lymphadenitis of tracheobronchial lymph nodes of the lung typified by enlarged firm lymph nodes that on cut surface have discrete and coalescing areas of yellow-white exudate infiltrating and compressing contiguous parenchyma (see Fig. 7-139). The latter lesion occurs through leukocyte trafficking of bacteria-infected alveolar macrophages as described later.

Foals encounter *R. equi* through inhalation of the bacterium in manure-contaminated fomites or water droplets from the environment. It is a common bacterium of the soil, grows optimally at 30° C in the manure of most animal species, and has a very rapid generation time. When inhaled, the bacterium is deposited on mucosae of the conductive and exchange systems by centrifugal and inertial turbulence. Here, bacteria encounter cells of the monocyte-macrophage system, including alveolar macrophages and dendritic cells, which phagocytose bacteria in the mucus layer of the mucociliary apparatus. These cells carry bacteria via leukocyte trafficking to local lymphoid tissues, such as BALT, through peribronchiolar and alveolar septal connective tissue, and to regional lymph nodes via afferent lymphatic vessels. *R. equi* replicates intracellularly in alveolar and other tissue macrophages. Alveolar macrophages phagocytize *R. equi* through ligand-receptor interactions. The bacterium must initially adhere to macrophages and the bacterium must be opsonized with either antibody or complement fragments that occur through complement fixation and the activation of the alternative complement pathway. In nonimmune foals, complement is the primary opsonin. The bacterium also expresses uncharacterized surface molecules that bind to membrane receptors expressed on alveolar macrophages such as leukocyte complement receptor, Mac-1, other complement receptors, mannose receptors, and potentially TLRs before phagocytosis can occur.

Opsonized bacteria and the products of complement fixation facilitate the process of adhesion and invasion through phagocytosis into alveolar macrophages. Phagocytosis is mediated by bacterial

virulence factors that appear to restrict tropism to specific types of phagocytic cells. After phagocytosis by alveolar macrophages, the bacterium is confined within phagosomes. Experimental results from studies on fusion of phagosomes with lysosomes to form phagolysosomes are contradictory. Some studies suggest that *R. equi* can block the fusion of lysosomes with phagosomes, which allows for the survival, persistence, and replication of bacteria intracellularly. Other studies suggest that *R. equi* is not able to block phagosome-lysosome fusion; however, the bacterium is able to produce molecules that suppress acidification of phagolysosomes, resulting in their survival and replication in alveolar macrophages. The mechanism used to block fusion is unknown but appears to involve bacterium-directed compartmentalization of the fusion process so that they are selectively isolated from lysosomal effector molecules, such as acid, reactive oxygen, NO compounds, and lysosomal hydrolases within phagosomes. Other proteins and molecules appear to contribute to persistence and replication in alveolar macrophages. For example, strains of *R. equi* that cause disease have chromosomal virulence factors for capsular polysaccharide, cholesterol oxidase, phospholipase C, lecithinase, and cell-wall mycolic acids and plasmid virulence factors (pathogenicity island) for VAP. It is also likely that mycolic acids from the bacterial cell wall are involved in the pathogenesis of the pyogranulomatous pneumonia characteristic of the disease.

Because *R. equi* is able to disrupt normal phagosome-lysosome killing (lysis) in alveolar macrophages and the generation of an oxidative burst that could kill the bacterium, it is able to persist and replicate. Studies suggest that rapid replication of bacterium within phagosomes and molecules such as cholesterol oxidase produced by the bacterium contribute to premature lysis of alveolar macrophages, leading to the release of large numbers of microbes into adjacent tissue. Additionally, because the life span of fully differentiated alveolar macrophages is approximately 10 to 30 days, lysis of these cells related to aging and bacteria-induced injury releases large numbers of bacteria into adjacent tissue, where they are phagocytosed by macrophages only to endlessly repeat the process. The severity and extent of the inflammatory response, concurrently with tissue injury, grows through the recruitment of additional monocytes and tissue macrophages from the circulatory system and regional lymph nodes.

Neutrophils are active in the acute inflammatory response against *R. equi*. They are able to phagocytose the bacterium, fuse the phagosome with the lysosome to form a phagolysosome, initiate an oxidative burst, and kill the bacterium. However, this process is an ineffective mechanism in controlling the disease and results in extensive tissue destruction through the release of lysosomal enzymes and reactive oxygen species, thus contributing to the cyclic and progressive destruction of pulmonary parenchyma. This damage allows large numbers of bacteria to gain access to the alveoli and bronchioles and encounter mucus of the mucous membranes and the mucociliary apparatus. In general, the mucociliary apparatus is not directly affected by *R. equi*; thus the bacterium is moved up the conductive system to the nasopharynx, where it is swallowed and gains access via peristalsis to the alimentary system (i.e., *Rhodococcus* enteritis).

Strep Zoo (*Streptococcus equi* subsp. *zooepidemicus*). See Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Domestic Animals; Strep Zoo (*Streptococcus equi* subsp. *zooepidemicus*).

Strangles (*Streptococcus equi* subsp. *equi*). See Bacterial Diseases of Organ Systems; Bone Marrow, Blood Cells, and the Lymphatic System; Disorders of Horses; Strangles (*Streptococcus equi* subsp. *equi*).

Disorders of Ruminants (Cattle, Sheep, and Goats)

Bovine Respiratory Disease Complex. Bovine respiratory disease complex (BRDC) is the term applied to a group of respiratory diseases caused by four viruses and three bacterial strains acting jointly and concurrently in various combinations (i.e., polymicrobial respiratory diseases) to cause a variety of respiratory diseases in cattle (ruminants). It occurs in cattle of varying ages and management practices; the microbes involved also vary based on age, management practices, and geographic locations. It is typified by the interactions of a primary viral pathogen and a secondary bacterial pathogen. A primary viral pathogen may disrupt the function of the mucociliary apparatus and/or disrupt phagocytosis and killing by alveolar and intravascular macrophages. These outcomes allow a secondary bacterial pathogen to colonize and replicate within the respiratory system and disrupt mucociliary and macrophage function, resulting in disease. Acute inflammation may also contribute to injury of cells and tissues within the respiratory system. Viruses involved include infectious bovine rhinotracheitis (IBR) virus, bovine viral diarrhea (BVD) virus, parainfluenza virus (PI3), and bovine respiratory syncytial virus (BRSV). Bacteria involved include *Pasteurella multocida*, *M. haemolytica*, and *Histophilus somni*. The pathogeneses and mechanisms of injury for each of these microbes are discussed individually in other sections of this chapter.

Bovine Pneumonic Pasteurellosis/Mannheimiosis (*Mannheimia* [*Pasteurella*] *haemolytica*). Also see bovine respiratory disease complex discussed earlier. The mechanism of injury in bovine pneumonic pasteurellosis/mannheimiosis is injury and lysis (coagulative necrosis) of all cell populations in the respiratory system. In addition to injury caused by bacterial toxins (leukotoxin), acute inflammation and its mediators and degradative enzymes significantly contribute to the pathogenesis of the disease. *M. haemolytica* can cause severe pneumonic disease independent of other contributory factors; however, the susceptibility to and severity of disease can be enhanced by environmental stressors and earlier or concurrent viral infection. Gross lesions include severe fibrinonecrotic (often hemorrhagic) pneumonia and vasculitis attributable to necrosis and apoptosis, especially affecting type I pneumocytes and capillary endothelial cells forming the air-blood barrier of alveolar septa and the vascular system (severe necrotizing vasculitis) (see Figs. 9-72, 9-85, and 9-86).

Cattle (and probably sheep and goats) encounter *M. haemolytica* through inhalation of the bacterium in fomites or fluid droplets. The bacterium is a commensal organism that resides in the nasopharynx and tonsils of healthy animals, but environmental stressors, such as weaning, adverse weather conditions, changes in diet, and shipping, can alter the commensal relationship, allowing the bacteria to replicate in sufficient numbers to colonize the respiratory mucosae and spread the bacterium to other animals. Colonization appears to be a two-stage process, first affecting the conductive component (terminal bronchioles and alveoli), then affecting the O_2-CO_2 (terminal bronchioles and alveoli) exchange component. When inhaled, bacteria are deposited on and trapped in the mucus layer of mucosae of the conductive component by centrifugal and inertial turbulence. *M. haemolytica* is a nonmotile bacterium, and it has not been clearly shown how the bacterium penetrates the mucus layer to gain access to cilia of mucosal epithelial cells.

Several virulence factors have been identified in the pathogenesis of mannheimiosis including leukotoxin (LKT), LPS, adhesins, capsular polysaccharides, outer membrane proteins, and various proteases such as neuraminidase. Neuraminidase reduces the viscosity of the mucus, making it less dense and a more fluidic layer, thus allowing the bacterium better access to cell membranes via gravity and random brownian movement. Additionally, neuraminidase

cleaves sialic acid from the surface of cell membranes, thus decreasing the net negative surface charge and allowing closer contact of the bacterium with membranes. Once in contact with cell membranes, bacteria adhere and bind to receptors using fimbria and pili adhesins via ligand-receptor interactions. This process results in bacterial colonization of mucosae. The types of adhesins (ligands) and receptors used in colonization have not been determined. Once colonization occurs, the bacteria replicate in large numbers in the conductive component of the respiratory system and produce enzymes (virulence factors), such as neuraminidase, and toxins (virulence factors), such as leukotoxin and LPS, that injure and disrupt the function of the mucociliary apparatus.

Additionally, polysaccharides of the bacterial capsule (virulence factor) inhibit phagocytosis of the bacterium by neutrophils and mucosal macrophages. Because of mucociliary dysfunction, bacteria spread via gravity to dependent portions of the lung, including terminal bronchioles and alveoli of the O_2-CO_2 exchange component. Once bacteria arrive in this location, the second stage of the process, which is more severe than the first stage, begins. The difference in severity is, in part, based on three factors: (1) extensive replication of bacteria in stage one that settle into the O_2-CO_2 exchange component and their subsequent amplification through replication, (2) the large surface area of lung tissue affected, and (3) the greater vulnerability of the air-blood barrier and septa in the O_2-CO_2 exchange component to injury. All of these factors contribute to the severity of the acute inflammatory responses and tissue injury.

The single most important virulence factor in bovine pneumonic mannheimiosis is leukotoxin. *Mannheimia* leukotoxin (LKT), a member of the RTX group of toxins, is a cytotoxin that causes lysis and apoptosis of alveolar macrophages and neutrophils. RTX toxins attach to cells through passive adsorption and cell surface β_2 integrin receptors, the latter being the transmembrane receptor CD18. At high concentrations, it causes necrosis by creating pores in cell membranes, leading to cell swelling and lysis (oncotic necrosis), whereas at lower concentrations, it causes apoptosis. Additionally, at lower concentrations, it activates neutrophils and induces the production of proinflammatory cytokines. When bacteria are phagocytosed by alveolar macrophages, leukotoxin is used to kill the macrophages and release the bacteria back into vascularized ECM tissue and alveolar spaces. Iron is also required for optimal growth of the bacterium and production of leukotoxin. LPS and leukotoxin also activate the complement system and the release of proinflammatory cytokines, resulting in vascular injury and severe acute inflammation. Vascular injury leads to permeability changes with edema and the release of fibrinogen that polymerizes to fibrin in alveolar spaces, interalveolar septa, interlobular and interlobar septa, and on pulmonary serosal surfaces (fibrinonecrotic pneumonia). Vascular injury can also lead to pulmonary hemorrhage.

Acute inflammation is characterized by the recruitment of large numbers of neutrophils from the circulation into affected lung tissue followed by activation of these cells via a respiratory burst and the release of degradative enzymes. The bacterium has several mechanisms (see later) to minimize the effects of neutrophils, but innocent bystander lung tissue, such as terminal bronchioles and cells that form the air-blood barrier, are severely injured by the molecules and enzymes released from activated neutrophils. Capsular polysaccharides, outer membrane proteins, and LPS of the bacterium are also important in the pathogenesis of the disease, especially as related to acute inflammation and vascular injury. Polysaccharides are virulence factors that facilitate adherence, colonization, and likely invasion of respiratory mucosae; inhibit phagocytosis by neutrophils; and disrupt complement-mediated lysis of bacteria. Outer membrane proteins are chemotactic for neutrophils, but when in contact with neutrophils, they disrupt phagocytosis and intracellular killing of bacteria. LPS binds with cell membrane CD14, β_2 integrins, and TLRs on alveolar macrophages, inducing the synthesis of proinflammatory cytokines, arachidonic acid metabolites, and NO that injure cells in inflammation. LPS may also injure endothelial cells directly and through molecules released from macrophages such as those listed in the previous sentence.

Pulmonary Histophilosis (*Histophilus somni*). Also see bovine respiratory disease complex discussed earlier.

The pathogenesis of pulmonary histophilosis is likely very similar to the mechanisms described earlier for bovine pneumonic pasteurellosis/mannheimiosis. Also see Bacterial Diseases of Organ Systems, Nervous System, Disorders of Ruminants (Cattle, Sheep, and Goats), Thrombotic Meningoencephalitis (*Histophilus somni*).

Bovine Enzootic Pneumonia (*Pasteurella multocida* subsp. *multocida* serogroup A). Also see bovine respiratory disease complex discussed earlier.

Mechanistically, the virulence factors and mechanisms used by *Pasteurella multocida* subsp. *multocida* to cause bovine enzootic pneumonia are very similar functionally to those used by M. *haemolytica* to cause bovine pneumonic mannheimiosis. However, in bovine enzootic pneumonia, bacterial pathogenicity is noticeably reduced and reflected by a slow onset and insidious inflammatory response and a nearly complete absence of cell necrosis, vasculitis, permeability changes, and fibrinogenesis. The mechanism of injury in bovine enzootic pneumonia is injury of all cell populations in the respiratory system attributable to inflammation and its mediators and degradative enzymes.

The susceptibility to and severity of bovine enzootic pneumonia caused by *P. multocida* subsp. *multocida* serogroup A can be enhanced by environmental stressors and a preceding or concurrent infection with a primary viral pathogen such as bovine respiratory syncytial virus, bovine viral diarrhea virus, infectious bovine rhinotracheitis virus, or parainfluenza III virus. Gross lesions include firm (consolidation) yellow-gray anterior-ventral lung lobes (see Fig. 9-69). Pleural surfaces are usually not involved, indicating that vascular injury and permeability changes and their association with the expression of bacterial virulence factors are not significant in the pathogenesis of the disease. In some cases, *Mycoplasma bovis* and *Mannheimia varigena* have been reported as secondary bacterial pathogens. The pathogenicity of the bacterial pathogen in bovine enzootic pneumonia/bovine respiratory disease complex is determined by its virulence factors, which may include protein adhesins, capsular polysaccharides, outer membrane proteins, iron-binding proteins, LPSs, LOSs, enzymes, and toxins.

Contagious Bovine Pleuropneumonia (*Mycoplasma mycoides* var. *mycoides* Small Colony). Little is known about the mechanisms used by *Mycoplasma mycoides* var. *mycoides* small colony (SC) to cause disease in the respiratory system of cattle; thus much of this section is conditional and based on a reasonable probability that lesions that occur are the result of underlying and known pathobiologic mechanisms. The mechanism of injury in contagious bovine pleuropneumonia is cell lysis likely caused by acute inflammation and its mediators and degradative enzymes and by vasculitis leading to thrombosis, ischemia, and infarction of lung tissue. Gross lesions include (1) fibrinous pleural effusion and fibrinous pleuritis with hemorrhage and (2) fibrinous pleuropneumonia with prominent interlobular septa filled with fibrinous effusion and fibrin thrombi (Fig. 4-23). Infarcts occur in affected lung tissues probably arising from vascular injury leading to permeability changes, vasculitis with activation of clotting cascades, thrombosis, and infarction. Infarcted lung often appears as sequestra likely arising from

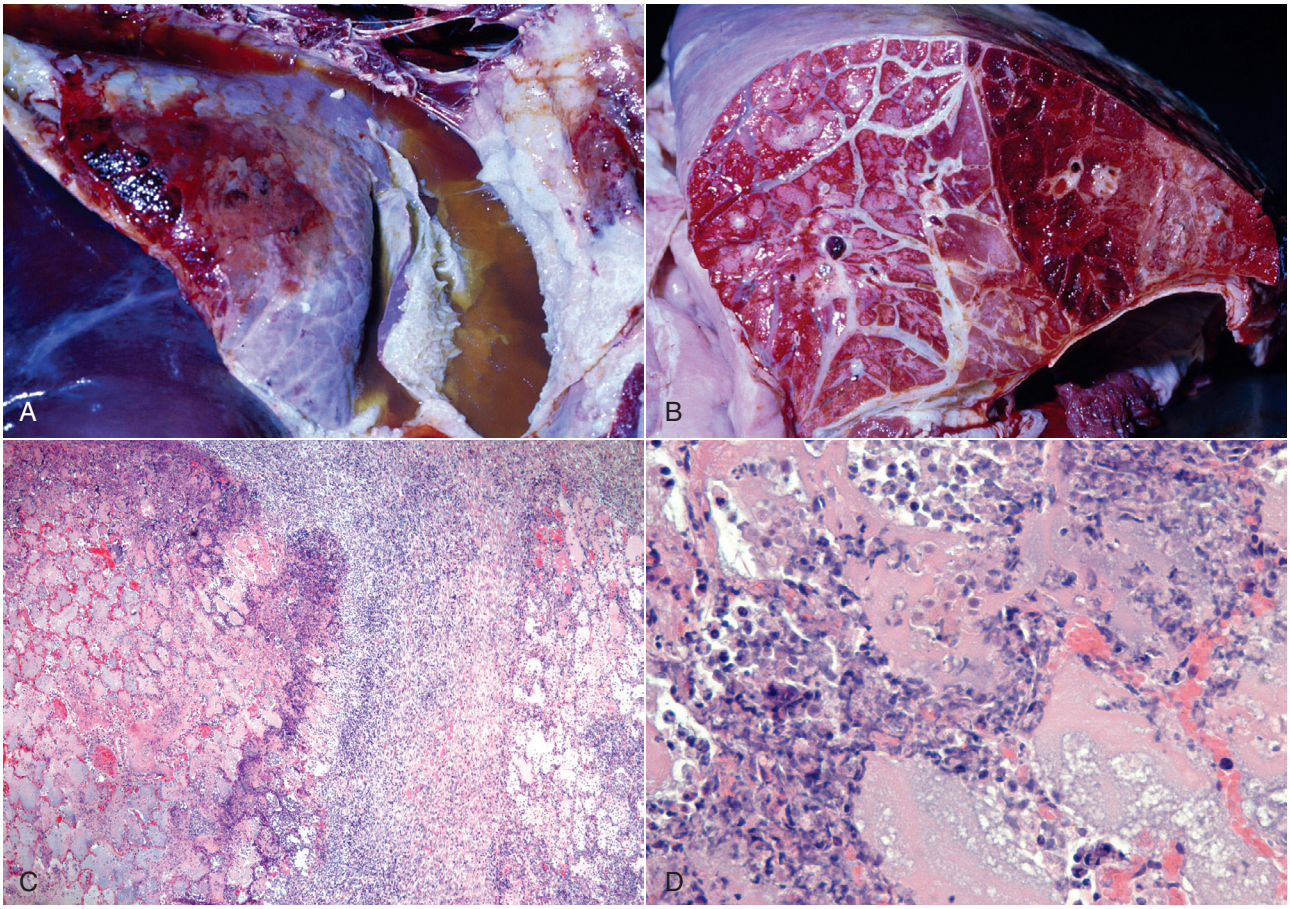


Figure 4-23 Contagious Bovine Pleuropneumonia. A, Thoracic cavity. The thoracic cavity is filled with a fibrinous pleural effusion, and the visceral and parietal pleurae are covered by fibrin (fibrinous pleuritis). Also note the areas of hemorrhage affecting the pleurae and the subjacent lung. **B,** Transverse section of lung. Note the prominent interlobular septa filled with fibrinous effusion and fibrin thrombi and additionally the area of hemorrhage *(right half of the section)*. Infarcts with pulmonary sequestra (not shown here) can occur in affected lung tissues, likely arising from vascular injury leading to infarction. **C,** The interlobular septum *(center)* is filled with acute inflammatory cells mixed with a fibrinous effusion. Alveoli are filled with highly proteinaceous edema fluid, fibrinous effusion, and acute inflammatory cells. There is extensive necrosis of all tissues at the interface between alveoli and the interlobar septum *(dark blue–staining band)*. H&E stain. **D,** Higher magnification of **C.** The dark blue color is attributable to necrosis of cells, including neutrophils, leading to escape and coagulation of nucleic acids from degenerate nuclei in the inflammatory exudate. Alveoli are filled with edema fluid and acute inflammatory cells. H&E stain. (**A** and **B** courtesy Dr. D. Gregg, Plum Island Animal Disease Center and Noah's Arkive, College of Veterinary Medicine, The University of Georgia. **C** and **D** courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

reparative mechanisms that isolate the dead infarcted tissue via fibrosis. It is unclear how infarcts occur in lung tissue when it has a dual blood supply, unless infarcts occur in areas that lack a dual supply or vasculitis and thrombosis concurrently affect vessels of each arterial source.

Cattle (and probably sheep and goats) encounter *M. mycoides* var. *mycoides* SC through inhalation of infected fomites and fluid droplets. These droplets are deposited on mucosae of the conductive component of the respiratory system by centrifugal and inertial turbulence where they are trapped in the mucus layer and are subsequently phagocytosed by alveolar macrophages. Ligand-receptor interactions are probably involved in target cell specificity via adhesins. Alveolar macrophages in all probability spread the bacterium to local lymphoid tissue such as BALT, in which the bacterium replicates in large numbers and kills infected macrophages, releasing bacteria into bronchiolar and alveolar interstitium, causing severe acute inflammation and the characteristic fibrinous lesions and vasculitis. How the bacterium evades killing by phagosome-lysosome fusion, produces toxic molecules that injure and kill cells, spreads to blood vessels, and causes vasculitis and thrombosis are unknown. Bacterial membrane lipoprotein LppQ, a common antigen of *Mycoplasma mycoides* var. *mycoides* SC, could be involved in some of these processes. Highly virulent strains of the bacterium are known to produce and release large quantities of H_2O_2 that are cytotoxic for all cells. H_2O_2 release appears to be correlated with adhesion of the bacterium to target cell membranes. It also appears that macrophages may spread the bacterium via leukocyte trafficking systemically to lymph nodes and synovium and joint spaces such as the carpus where inflammation characteristic of the disease also occurs. As presumed virulence factors, outer surface proteins and/or plasma membrane proteins of *Mycoplasma* spp. contain lipoproteins, capsular polysaccharides (e.g., galactan), and carbohydrate biofilms that likely function to protect the microbe from defense mechanisms and to cause the acute fibrinoid inflammatory response so characteristic of the disease. Unique to *Mycoplasma mycoides* var. *mycoides* SC is the apparent lack of virulence factors that can be characterized as toxins or invasins. In fact, virulence factors appear to arise from metabolic or catabolic pathways within the bacterium or from intrinsic components of the bacterial outer surface and/or cell membrane such as capsular polysaccharides.

Bovine Tuberculosis (*Mycobacterium bovis*). The mechanism of injury in bovine tuberculosis is lysis of cells of the

monocyte-macrophage system and of all cell populations in the lung and associated regional lymph nodes secondary to granulomatous inflammation and its mediators and degradative enzymes. Gross lesions include (1) enlarged lymph nodes that contain discrete and coalescing granulomas (tubercles) formed by dry and gritty (mineralized) yellow-white to green-white caseous exudate often encapsulated by fibrous connective tissue (see Fig. 1-18) and (2) lung parenchyma that contains similar granulomas distributed at random in some or all lung lobes (see Fig. 9-80 and E-Fig. 9-14).

Cattle (and probably sheep and goats) encounter *M. bovis* through inhalation of fomites and fluid droplets contaminated with the bacterium. These droplets are deposited on mucosae of the conductive component of the respiratory system by centrifugal and inertial turbulence where they are trapped in the mucus layer and are subsequently phagocytosed by alveolar and tissue macrophages. Ligand-receptor interactions are probably involved in target cell specificity via adhesins. Macrophages appear to use several routes to spread the bacterium across mucosal barriers and then to regional lymph nodes and the lung. In tonsillar mucosae, macrophages cross the mucosal barrier, migrate to the tonsil, and spread the bacterium to infect naïve macrophages in tonsillar tissues. In other mucosae of the pharynx, macrophages encounter and phagocytose bacteria and spread them to local lymphoid tissues and then via afferent lymphatic vessels to regional lymph nodes such as the retropharyngeal and parotid nodes to infect naïve macrophages in these nodes. Finally, in bronchi and bronchioles, bacteria that are deposited in the mucus layer of mucosae are phagocytosed by alveolar macrophages and spread to local lymphoid tissues (BALT) and then via afferent lymphatic vessels to regional lymph nodes, such as the tracheobronchial and mediastinal nodes, to infect naïve macrophages in these nodes.

The primary goal of *M. bovis* is to be phagocytized by macrophages. In the mucus layer, macrophages encounter trapped bacteria via random movement. Once bacteria are in contact with macrophages, bacteria adhere and bind to pathogen PRRs on macrophage cell membranes. The process used by macrophages to phagocytize *M. bovis* involves ligand-receptor interactions. In fact, the bacterium appears to use multiple membrane pathogen PRRs such as those for complement (CR1, 3, and 4), mannose, surfactant protein, and CD14 protein to enter macrophages. Some receptors are probably used in the early mucosal phases of infection when inflammation is minimal, whereas other receptors, such as those for complement, are used when vascular changes in lymph nodes and lung induced by acute inflammation result in permeability changes and the release of plasma proteins and complement into inflamed tissues.

M. bovis is able to activate the alternative pathway of complement and use C3b and C3bi fragments to opsonize its surface and then bind to complement receptors CR1, 3, and/or 4 on cell membranes of macrophages. This binding results in phagocytosis of the bacterium in phagosomes. Mannose receptors, surfactant proteins and receptors, and LAM and CD14 receptors are also involved in phagocytosis. It appears that the use of multiple ligands and pathogen PRRs ensures that the bacterium once inhaled or ingested can be phagocytosed by monocytes, macrophages, and/or even neutrophils that migrate to the site of local infection in response to chemokines secreted by infected macrophages. Subsequently, these cells can then be used to spread the infection to other regional or systemic sites, such as the liver, spleen, lymph nodes, and intestines, via leukocyte trafficking in the blood or lymphatic vascular systems.

Once present in a phagosome, *M. bovis* is able to disrupt phagosome-lysosome fusion and prevent activation of macrophage antimicrobial mechanisms such as production of reactive oxygen or nitrogen intermediates and phagosome acidification (see Figs. 4-13 and 4-14). The bacterium grows and replicates in phagosomes, but with cellular aging, infected macrophages die and release bacteria into the vascularized ECM tissues. This outcome results in repetitive cycles of inflammation and the recruitment of additional monocytes, macrophages, and neutrophils into the granuloma (tubercle). The formation of granulomas (tubercles) is covered in detail in Chapter 3, but components of the poorly digestible, waxy cell wall of the bacterium, such as sulpholipids and LAM, appear to contribute to the type of inflammatory response, chronic, that ensues and the formation of granulomas.

Ligand-receptor interactions are also important in initiating and prolonging the inflammatory response. PRRs on macrophages are activated by PAMPs on the bacterium. As a result, macrophages produce and release abundant proinflammatory cytokines and chemokines that act to recruit via chemotactic gradients additional macrophages/monocytes, neutrophils, and dendritic cells to the site. Because the life span of fully differentiated tissue macrophages is approximately 10 to 30 days, lysis of these cells related to aging and bacterial-induced injury releases bacterium into adjacent tissue, where they are phagocytosed by newly recruited macrophages only to endlessly repeat this process. Granulomatous inflammation ensues, and multinucleated giant cells are noted histologically in the exudate (see Chapters 3 and 5) as an attempt to degrade and eliminate the poorly digestible, waxy cell wall of the bacterium. Dendritic cells also phagocytose the bacterium, migrate to regional lymph nodes, and present mycobacterial antigens to lymphocytes for an adaptive immune response that ultimately is ineffective.

Disorders of Pigs

Porcine Respiratory Disease Complex. Porcine respiratory disease complex (PRDC) is the term applied to a group of respiratory diseases caused by three viruses and two bacterial strains acting jointly and concurrently in various combinations (i.e., polymicrobial respiratory diseases) to cause a variety of respiratory diseases in pigs. It occurs in pigs of varying ages and management practices; the microbes involved also vary based on age, management practices, and geographic locations. It is typified by the interactions of a primary viral pathogen and a secondary bacterial pathogen. A primary viral pathogen may disrupt the function of the mucociliary apparatus and/or disrupt phagocytosis and killing by alveolar and intravascular macrophages. These outcomes allow a secondary bacterial pathogen to colonize and replicate within the respiratory system and disrupt mucociliary and macrophage function, resulting in disease. Acute inflammation may also contribute to injury of cells and tissues within the respiratory system. Viruses involved include porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), and porcine circovirus type 2 (PCV2). Bacteria involved include *P. multocida* and *M. hyopneumoniae*. The pathogeneses and mechanisms of injury for each of these microbes are discussed individually in other sections of this chapter.

Porcine Pleuropneumonia (*Actinobacillus pleuropneumoniae*). The mechanism of injury in porcine pleuropneumonia is injury and lysis (coagulative necrosis) of all cell populations in the respiratory system, especially those of the vascular system (severe necrotizing vasculitis), secondary to the effects of bacterial toxins and to acute inflammation and its mediators and degradative enzymes. Gross lesions, attributable to vascular injury affecting lung and regional lymph nodes, include (1) edema and alterations in vascular permeability; (2) hemorrhage; and (3) fibrinous and hemorrhagic pneumonic, pleural, and pericardial effusions; and acute necrotizing inflammation and pneumonia (see Fig. 9-97). Although all lobes of the lung can be affected, a common site for lesions is the

dorsal area of the caudal lung lobes. In fact, a large area of fibrinohemorrhagic pleuropneumonia involving the caudal lobe of a pig's lung is considered almost diagnostic for this disease.

Pigs encounter *A. pleuropneumoniae* through inhalation of the bacterium in contaminated fomites or fluid droplets. In the respiratory system the bacterium appears to initially colonize mucosal epithelial cells of the tonsils (likely in a biofilm) and then via inhalation is deposited on mucosae of the conductive system and probably of the exchange system by centrifugal and inertial turbulence. The characteristic distribution pattern (dorsal-diaphragmatic) of gross lesions may reflect the fact that droplet size and inertial turbulence results in the initial deposit being near branch points of conductive airways. The bacterium must first colonize mucosae by adhering and binding to the membranes of epithelial cells using ligand-receptor interactions mediated through type 4 fimbriae and likely adhesins (multiple-step binding process). The bacterium also binds to mucus, but the purpose of this act is unclear. Following colonization, *A. pleuropneumoniae* requires iron to grow and replicate and is capable of using porcine transferrin as a source of iron. However, it also appears that iron is obtained from lysis of red blood cells caused by bacterial hemolysins and proteases. After the lysis of red blood cells, LPS and outer membrane proteins of the bacterial cell wall can bind hemoglobin and assist in the transfer of the iron molecule required for growth and replication into the bacterium. In part, this requirement may explain the severe hemorrhage that occurs in this disease. Other virulence factors (LPSs, exotoxins) appear to be involved in the acquisition of essential nutrients required for colonization and replication.

It has been shown that the bacterium binds poorly to cilia and the epithelium of the trachea and bronchi, whereas it binds strongly to cilia and membranes of terminal bronchioles and membranes of alveolar epithelial cells (type I pneumocytes). This selective pattern of binding and inertial turbulence (see previous discussion) may account for the unique distribution of gross lesions involving the caudal lung lobes observed in porcine pleuropneumonia.

LPS and LOS are known to play important roles in the pathogenesis of Gram-negative infections; however, their role in porcine pleuropneumonia is unclear and may involve acting as ligands for adherence to target cell surfaces. Glycosphingolipids present in membranes of epithelial cells may serve as receptors for these ligands. It has not been determined if and how *A. pleuropneumoniae* penetrates the mucus layer to gain access to cilia and cell membranes of epithelial cells. The suppression of mucus production and ciliary activity increase the severity of porcine pleuropneumonia by decreasing clearance of the bacteria through the mucociliary apparatus mechanism. Phagocytosis and immunoglobulins appear to be important defense mechanisms in response to this bacterium in pigs. The bacterium is able to produce proteases that degrade IgA and IgG; however, the significance of this defense mechanism in the pathogenesis of the disease is uncertain. Therefore it appears that neutrophils, cells of the monocyte-macrophage system, phagocytosis, and phagosome-lysosome fusion are very important defense mechanisms against this bacterium.

Once *A. pleuropneumoniae* is bound to cilia and cell membranes of terminal bronchioles and alveolar epithelial cells, the bacterium is positioned to be phagocytized by alveolar, interstitial, and intravascular macrophages. Although all of these types of macrophages are phagocytic, intravascular macrophages also have strong cytolytic activities that may also account, in part, for the hemorrhage characteristic of pulmonary vascular lesions.

Neutrophils are not involved in the initial phagocytic response to the bacterium, but once the process has been started by macrophages and cytokines and chemokines (interleukins and TNF) are released from activated macrophages, neutrophils are recruited from the vasculature into the acute inflammatory response to phagocytize the bacteria. After phagocytosis, it has been shown that neutrophils can immediately kill *A. pleuropneumoniae*, whereas macrophages cannot. In fact, the bacterium can survive for more than 90 minutes in a phagosome of a macrophage, during which it grows, replicates, and synthesizes and releases Apx toxins, leading to the lysis of these macrophages and release of the bacterium. Additionally, during this time, infected macrophages can move into alveolar and lobular septa, alveolar lumina, and perivascular and peribronchiolar tissues. Thus, when an infected macrophage is killed, large numbers of bacteria are released into vascularized ECM, leading to more acute inflammation, recruitment of additional neutrophils and macrophages, and exacerbation of injury to surrounding tissues.

A. pleuropneumoniae has several virulence factors that allow it to survive in phagosomes and resist the effects of phagosome-lysosome fusion (see Fig. 4-14), including a polysaccharide capsule, cell-wall LPS, copper-zinc superoxide dismutase, stress proteins, and ammonia. The capsule, cell-wall molecules, and superoxide dismutase participate in removing oxygen free radicals. The bacterium produces ammonia within phagosomes through the release of a potent urease, which inhibits phagosome-lysosome fusion and disrupts acid hydrolase activity in lysosomes. Finally, Apx toxins, pore-forming exotoxins that lyse cells, are important virulence factors in the pathogenesis of the disease. At low concentrations and probably early in the disease process, Apx toxins (ApxI to ApxIII) produced by the bacterium also impair chemotaxis and phagocytosis by macrophages and neutrophils by likely acting to disrupt actin-myosin–directed movements of the cell or its organelles. At higher concentrations, such as those that occur after the bacteria has gone through several replication and kill cycles in macrophages, ApxI and ApxIII are highly toxic and ApxII is moderately toxic for macrophages and neutrophils. Additionally, ApxI to ApxIII are highly toxic to surrounding tissues, such as blood and lymphatic vessels, and ECM tissues, resulting in loss of barrier systems, increased vascular permeability with fibrin leakage and polymerization, hemorrhage, and vasculitis. In fact, it should be remembered that all of these infected cells and innocent bystander cells and tissues are within several hundred micrometers of one another and the vascular system. In particular, vascular injury appears to result from the activation and killing of intravascular macrophages and endothelial cells by Apx toxins and LPS. Activation results in the release of oxygen free radicals (superoxide anion, hydrogen peroxide, and hydroxyl radical) as well as proteolytic enzymes and various cytokines, all of which can injure endothelial cells of capillaries and postcapillary venules. Injury leads to activation of the coagulation, fibrinolysis, and kinin systems (see Chapters 2, 3, and 5) with concurrent hemorrhage, edema, effusions, platelet activation, and the formation of thrombi, ischemia, and subsequent coagulative necrosis of lung.

Atrophic Rhinitis (*Bordetella bronchiseptica* and *Pasteurella multocida*). Although *Bordetella bronchiseptica* and *P. multocida* can each separately cause clinical forms of atrophic rhinitis in pigs, the classic form of the disease from a pathologist's perspective appears to be caused by these two bacteria interacting synergistically. Information about the mechanisms used by *B. bronchiseptica* and *P. multocida* to cause atrophic rhinitis in pigs is limited. Thus portions of this section are conditional and based on (1) what is known mechanistically about other diseases of the respiratory system caused by *Pasteurella* spp. and (2) a reasonable probability that inflammation, responses to injury, and lesions that have been described in atrophic rhinitis are the result of underlying and known pathobiologic mechanisms. The mechanism of injury is (1) lysis of ciliated epithelial and stromal cells of turbinate mucosae and (2) concurrent

activation and suppression of osteoclasts and osteoblasts, respectively, resulting in osteolysis of bone of the turbinates leading to turbinate atrophy. Gross lesions include varying degrees of loss (atrophy) and remodeling of turbinate scrolls and the nasal septum, with ventral scrolls usually being most severely affected (see Fig. 9-33).

Pigs encounter *B. bronchiseptica* and *P. multocida* through inhalation of the bacteria in fomites or fluid droplets. These bacteria are likely commensal microbes that reside in the nasopharynx of healthy pigs, but environmental stressors, such as overcrowding, poor ventilation and humidity, or abrupt changes in ambient air temperature, alter the commensal relationship, allowing the bacteria to replicate in sufficient numbers to colonize mucosae and spread the bacterium to other animals. Colonization appears to be a two-stage process, a *B. bronchiseptica* phase followed by a *P. multocida* phase. When inhaled, *B. bronchiseptica* is deposited on and trapped in the mucus layer of mucosae. The bacterium is nonmotile, and it has not been clearly shown how the bacterium penetrates the mucus layer, gains access to cilia of mucosal epithelial cells, and colonizes the nasal mucosae. *B. bronchiseptica* does produce a dermonecrotic toxin (DNT) (virulence factor) and possibly adenylate cyclase-hemolysin toxin that likely affect the mucus layer and ciliated epithelial cells, making them more susceptible to colonization. Eventually, the ciliated columnar epithelium of the nasal mucosa is replaced by stratified squamous epithelium. The types of adhesins (ligands) and receptors used in colonization have not been determined but may include adhesins such as filamentous hemagglutinin, pertactin, and fimbriae proteins. The bacterium has an outer membrane protein called pertactin that may act as an adhesin, allowing the bacterium to colonize turbinate mucosae that have been injured by DNT. Receptors for pertactin on mucosal epithelial cells have not been identified, but once in contact with cell membranes, bacteria likely adhere and bind to cell membrane receptors using fimbriae and pili. This process results in colonization of mucosae by *B. bronchiseptica*.

Under normal conditions, *P. multocida* has limited and weak virulence factors for attaching to and colonizing turbinate mucosae. Initial colonization by *B. bronchiseptica* leads to disruption of the mucus layer and the mucosal barrier system and results in mucosae that are more suitable for colonization by *P. multocida* in the second phase of infection. Mucosal lesions begin as focal erosions and ulcerations accompanied by acute inflammation (neutrophils), which subsequently spread into underlying lamina propria, ECM tissues, and bone of the turbinates. These lesions result in altered clearance mechanisms of ciliated epithelial cells and exposure of its lamina propria, where *P. multocida* can adhere to and colonize vascularized ECM tissues. Once mucosae and lamina propria are colonized, the primary virulence factor expressed by the bacterium is *P. multocida* toxin (PMT), a DNT. The toxin, a typical A-B toxin, causes turbinate atrophy and snout deformation through chronic inflammation leading to bone remodeling and fibrous osteodystrophy of periosteal fibroblast origin. Mechanistically, the toxin initially acts to stimulate osteoblasts, which in turn act to increase the numbers (hyperplasia) and activity of osteoclasts. As toxin concentrations increase, it acts by blocking the function of osteoblasts, and cell degeneration and lysis may ensue. Overall, PMT causes turbinate atrophy by increasing osteoclast numbers and activities through osteolysis of existing turbinate bone, as well as by inhibiting osteoblastic activities and the formation of new bone, both resulting in turbinate atrophy.

Porcine Enzootic Pneumonia (*Mycoplasma hyopneumoniae*). Also see porcine respiratory disease complex discussed earlier.

The mechanism of injury in porcine enzootic pneumonia is injury of ciliated epithelial cells of the bronchi and bronchioles, causing dysfunction and lysis of these cells. This outcome results initially in dysfunction of cilia, loss of cilia, and then cell lysis followed by a secondary bacterial infection leading to additional cell lysis of all cell types in the lung from chronic inflammation and its mediators and degradative enzymes. The susceptibility to and severity of porcine enzootic pneumonia can be enhanced by environmental stressors (abrupt or long-term changes in ventilation, temperature, and/or humidity) and earlier or concurrent viral (porcine reproductive and respiratory virus, swine influenza virus) or bacterial (*P. multocida*) infections. Gross lesions characteristic of this disease are firm (consolidation) yellow-tan-gray anterior-ventral lung lobes (see Fig. 9-96). Pleural surfaces are usually not involved, indicating that vascular injury and permeability changes and their association with the expression of bacterial virulence factors are not significant in the pathogenesis of the disease.

Pigs encounter *M. hyopneumoniae* through inhalation of the bacterium in fomites or fluid droplets. The bacterium is deposited on mucosae of the conductive component by centrifugal and inertial turbulence and trapped in the mucus layer. The bacterium is nonmotile, and it has not been clearly shown how it penetrates the mucus layer, gains access to cilia of mucosal epithelial cells, and colonizes mucosae. Colonization of the respiratory system appears to probably involve adherence and binding of the bacterium to cell membrane receptors, using fimbriae and pili by ligand-receptor interactions, because it has been shown that the bacteria attach to cilia and line up in parallel rows along the surface of the cells. A molecule called *cilium adhesin* expressed on the surface of the bacterium appears to be involved in the attachment and binding process, where it is thought to interact with glycosaminoglycan and heparin on cell membranes. Additionally, the bacterium probably expresses glycosaminoglycans, such as heparin, heparin sulfate, and chondroitin sulfate B, or coats itself with such molecules that bind to ECM molecules, such as fibronectin, vitronectin, laminin, and collagen, but little is actually known about this process. This interaction ultimately results in dysfunction of cilia (ciliostasis) and lysis of the epithelial cells and reduced function of the mucociliary apparatus. Secondly, there is an increase in mucus (from goblet cells) covering these ciliated epithelial cells. This outcome suggests the bacterium may use mucus in some as yet unknown manner to facilitate colonization of cilia, provide a source of nutrition, or protect itself against immune responses.

Virulence factors used by the bacterium to cause dysfunction and lysis of these cells have not been determined. *M. hyopneumoniae* does not produce toxins, but some mildly toxic molecules do occur. Because of dysfunction of the mucociliary apparatus, *M. hyopneumoniae* and other bacteria are able to reach distal aspects of terminal bronchioles and alveoli by dependent settling caused by forces of gravity. Bacteria replicate in these sites and cause chronic (active) anterior-ventral bronchopneumonia resulting from a continuum of concurrently occurring acute and chronic inflammation and their mediators and degradative enzymes.

Porcine Polyserositis (*Haemophilus suis/parasuis, Actinobacillus suis, Streptococcus suis*, or *Escherichia coli*). Several species of bacteria can cause porcine polyserositis, but it is most commonly associated with *Haemophilus suis/parasuis*, the bacterium that causes Glasser's disease. The mechanism of injury is vasculitis affecting serosal membranes and acute inflammation and its mediators and degradative enzymes. Gross lesions are characterized by variable quantities of a gray-white friable material (fibrin) on serosal surfaces (fibrinous polyserositis) of the lungs (fibrinous pleuritis), heart (fibrinous pericarditis), and abdominal cavity

(fibrinous peritonitis) (see Fig. 7-17). Body cavities may also contain a fibrinous exudate and edema fluid. Facing serosal surfaces are often loosely attached to each other by the fibrinous exudate, making normal physiologic processes like respiration and cardiac contraction more difficult. With chronicity and healing, these opposing surfaces may adhere to each by fibrosis and consequently restrict normal "gliding" movements of serosal surfaces of the thoracic and pericardial cavities, thus impeding normal respiratory or cardiac function. The serosa and cavities of meninges, joints, and testis can also be affected. Information about the mechanisms used by these bacteria to cause porcine polyserositis is limited. Thus portions of this section are conditional and based on (1) what is known mechanistically about other diseases of the respiratory system caused by the Pasteurellaceae family of bacteria and (2) a reasonable probability that inflammation, responses to injury, and lesions that have been described in porcine polyserositis are the result of underlying and known pathobiologic mechanisms.

Pigs likely encounter these species of bacteria through inhalation in contaminated fomites or fluid droplets. They appear to behave as commensal microbes of respiratory mucosae existing in biofilms in the nasopharynx and tonsil of healthy pigs. Environmental stressors, such as overcrowding, poor ventilation and humidity, or abrupt changes in ambient air temperature, alter the mucus layer and the commensal relationship, allowing bacteria to replicate in sufficient numbers to begin colonization of respiratory mucosae and to spread to other animals. Preceding or concurrent viral infections (i.e., PRRSV or SIV) could also damage the mucociliary apparatus, allowing bacteria to more extensively colonize the mucus layer or mucosae. In the respiratory system the bacteria are deposited on mucosae of the conductive component by centrifugal and inertial turbulence and are trapped in the mucus layer. These bacteria are nonmotile, and it has not been clearly shown how they penetrate the mucus layer; gain access to cilia of mucosal epithelial cells; express virulence factors such as adhesins, capsular molecules, fimbriae, and outer membrane proteins required for ligand-receptor interactions; and colonize mucosae. It has been suggested that receptors necessary for colonization may be exposed by a molecule that behaves like neuraminidase. Once the mucus layer and/or mucosae are colonized, nonciliated and ciliated epithelial cells are injured by LPS and possibly by a purported neuraminidase and bacterial toxin. Acute inflammation quickly ensues and is followed to a limited extent by lysis of these cells.

It has also been suggested that bacteria gain access to the lamina propria by altering the function of junctional complexes, allowing them to move between adjacent mucosal epithelial cells. The outcome of these processes is the loss of normal mucosal barriers, which provide the bacteria with direct access to the vascularized ECM of the lamina propria. It has not been determined how the bacteria actually cross these altered mucosal barriers, reach capillary beds in ECM, encounter and penetrate the endothelium, and spread within the blood vascular system. Mechanisms, such as a cell-free bacteremia or leukocyte trafficking via alveolar or intravascular macrophages, lymphocytes, or dendritic cells, are hypothetical mechanisms of spread. A study of *Haemophilus suis* has shown that mucosal macrophages contain structures resembling phagolysosomes that are indicative of phagocytic activity. Lesions suggest these bacteria may have a tropism for vascular endothelial cells of serosae. It is unclear why this occurs, but it is probably linked to the expression of bacterial virulence factors and ligand-receptor interactions with host endothelial cells. Additionally, it is thought that bacterial endotoxins (LPS) may contribute to vascular injury and permeability changes leading to the leakage of fibrinogen and its polymerization to fibrin on serosal surfaces and in some cases to microthrombus formation and disseminated intravascular coagulation in other organ systems.

Disorders of Dogs

Acute Tracheobronchitis (*Bordetella bronchiseptica*). The mechanism of injury in acute tracheobronchitis is lysis of ciliated epithelial cells of mucosae of the trachea, bronchi, and bronchioles and acute inflammation and its mediators and degradative enzymes. Gross lesions are characterized by reddened, rough, and granular mucosae (necrosis) that may, depending on severity of injury, be covered with mucus, fibrin, and occasionally blood.

B. bronchiseptica is inhaled, deposited on, and trapped in the mucus layer of mucosae of the conductive component of the respiratory system through centrifugal and inertial turbulence. The bacterium colonizes ciliated epithelium via fimbrial and nonfimbrial adhesins such as filamentous hemagglutinin and pertactin. It has not been determined if and how it penetrates mucus layers to gain access to epithelial cells or if mucosal macrophages and/or dendritic cells are involved. Once ciliated cells are colonized, *B. bronchiseptica* releases exotoxins, such as adenylate cyclase-hemolysin and DNT and endotoxins, that further impair function of the mucociliary apparatus, allowing for additional colonization of mucosae by the bacterium at new sites. These outcomes, especially dysfunction of the mucociliary apparatus, contribute to "dependent settling" via gravity of bacteria into bronchi of dependent lung lobes, resulting in secondary bronchopneumonia. This damage results in an acute inflammatory response that further injures mucosae throughout the lung. *B. bronchiseptica* toxins may also disrupt phagocytosis and/or killing of bacteria by alveolar macrophages and neutrophils and suppress cellular and humoral immune responses. The bacterium can also invade epithelial cells, evade immunologic defense mechanisms, and establish a persistent infection.

Canine infectious tracheobronchitis is a disease in which there is primary injury caused by canine parainfluenza virus leading to increased susceptibility secondarily to infection with *B. bronchiseptica* (or other bacteria). The pathogeneses and mechanisms of injury in this and other respiratory diseases caused by *B. bronchiseptica* are also discussed in other sections of this chapter; see Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Dogs; Canine Infectious Tracheobronchitis (Canine Cough, Kennel Cough; Canine Parainfluenza Virus, Enveloped RNA Virus); and also see Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Pigs; Atrophic Rhinitis (*Bordetella bronchiseptica* and *Pasteurella multocida*).

Cardiovascular System and Lymphatic Vessels

Disorders of Domestic Animals

Embolic Vasculopathy/Vasculitis (*Actinobacillus equuli, Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Fusobacterium necrophorum*). This section covers a variety of diseases in which the key component of the underlying pathogenesis is embolization through the blood vascular system, leading to vasculitis and potentially thrombosis and ischemia. Such embolic diseases most commonly begin in the skin/subcutis or mucosae but end in a wide variety of highly vascularized organ systems. Examples of embolic diseases include white spotted kidney disease (*E. coli*), embolic nephritis (foal shigellosis [*Actinobacillus equuli*]), milk spots in the liver (*E. coli*), bacterial endocarditis (*E. coli*), and bacterial hepatitis (*Fusobacterium necrophorum*). Embolization also occurs in diseases caused by angioinvasive fungi, and they are covered in the section on fungal diseases. The mechanism of injury in embolic vasculopathy/vasculitis is cell lysis, probably acute coagulative

necrosis, caused by bacterial toxins and inflammation and its mediators and degradative enzymes. Gross lesions include gray-white foci of necrosis and inflammation (aggregates of acute inflammatory cells) distributed at random (vascular embolization pattern) in tissue such as those, which occur in renal actinobacillosis of foals (see Fig. 11-35).

Bacteria are able to enter and spread in the vascular system by three mechanisms: (1) direct entry into a blood vessel; (2) establishment of a local infection followed by invasion of the vascular system; and (3) leukocyte trafficking in macrophages, lymphocytes, and/or dendritic cells. The first category usually results from penetration of blood or lymphatic vessels from trauma, bite wounds, or lacerations; the second category from penetrating traumatic injury leading to local inflammation and abscess formation followed by vascular entry; whereas the last category results from endocytosis or phagocytosis of microbes by leukocytes. In the direct entry mechanism, access to the vascular system, embolization, and entrapment in capillary beds are likely physical interactions based on the anatomy of vascular distribution patterns (i.e., sharp angles [90-degree] of curvature), physiology of vascular flow and pressures, and probably the distribution and number of appropriate endothelial cell surface receptor molecules at final destinations. As an example in the cerebral cortices, lesions caused by bacterial emboli tend to be observed at the interface between gray and white matter. Anatomically, at this location, capillaries penetrate through the gray matter from the overlying meninges and as they run into the white matter, they make abrupt turns (90-degree), so capillaries can run parallel to fiber tracts in the white matter. This flow change causes vascular turbulence and endothelial cell surface perturbations, and under the proper conditions, activation of Virchow's triad can result in the formation of vascular endothelial surfaces that may be sticky or have receptors or attached fibrin that can bind or entrap bacteria, respectively. Many of the bacterial virulence factors discussed throughout this chapter, as well as ligand-receptor interactions, probably are involved to some extent in the origination and entrapment and growth of bacterial emboli in the direct entry mechanism.

In the establishment of a local infection mechanism, contamination of the umbilicus at birth and the skin/subcutis through management practices, such as tail docking, castration, and ear notching, are common means of establishing local infections. Injury of mucosae, such as occurs in the abomasum from lactic acidosis in grain overload, also provides opportunities for bacteria (and fungi) to enter the portal blood vascular system and then embolize to and colonize the liver. Finally, bacteria that induce biofilms or cause irresolvable inflammatory processes, such as in dermatitis, otitis, cellulitis, periodontal disease, arthritis, or abscesses, can serve as a site for intermittent bacteremia and embolization. Many of the bacterial virulence factors discussed throughout this chapter, as well as ligand-receptor interactions, probably are involved to some extent in the origination and entrapment and growth of bacterial emboli in the local infection mechanism. The third mechanism of entry and spread in the vascular system is leukocyte trafficking and has been discussed in earlier sections of this chapter.

Vascular Leptospirosis (*Leptospira* spp.). The mechanism of injury in vascular leptospirosis is cell lysis caused by (1) physical properties (penetrating movements from motility) of bacteria that disrupt functions of endothelial cells and (2) bacterial toxins that act directly on membranes of endothelial cells of small blood vessels, including capillaries of the systemic vasculature in all organ systems, leading to coagulative necrosis of affected cells. Gross lesions include acute vasculitis (endothelial cell necrosis) with systemic petechial and ecchymotic hemorrhages, edema, and disseminated intravascular coagulation affecting all organ systems and serosal surfaces (see Fig. 2-18).

Animals encounter *Leptospira* spp. through direct contact of oral or conjunctival mucosae or skin with leptospira-infected urine or with water from reservoirs or ponds into which infected urine drains. Ingestion may also serve as a portal of entry if water contaminated with leptospira is consumed and the bacteria encounter mucosae of the intestine. During chewing and swallowing, it is likely that mucosae of the oral pharynx trap the bacteria in its mucus layer. After swallowing and through intestinal peristalsis, the bacteria are moved into contact with villi and crypts, where they are probably trapped in the mucus layer and encounter enterocytes. In the conjunctiva, it is also likely that mucosae trap the bacteria in a mucus layer. For infection to occur, it has been suggested that the skin and mucosae must have small cuts or abrasions that allow the bacteria to penetrate into the vascularized lamina propria, dermis, submucosal, or subcutaneous connective tissues and gain access to capillaries and/or postcapillary venules. However, *Leptospira* spp. are motile bacteria and are probably able to penetrate mucus layers and move across mucosae by going directly through mucosal epithelial cells or between the cells through intracellular junctional complexes. In all of these portals of entry, the goal is for the bacteria to reach well-vascularized ECM tissues. As a group, these spirochetes are highly motile and invasive, and using their invasive motility (virulence factor), they are able to penetrate the vascular wall and endothelial cells of capillaries and postcapillary venules to gain access to the circulatory system. *Leptospira* spp. may also invade lymphatic vessels and through afferent and efferent branches and the thoracic duct eventually gain access to the circulatory system. *Leptospira* spp. are able to grow and replicate in the circulatory system, spread systemically, and attach to endothelial cell membranes in other organ systems via adhesins before invading these cells and underlying ECM. These encounters and the penetration of blood vessels result in systemic petechial and ecchymotic hemorrhages characteristic of vascular leptospirosis.

Surface-associated proteins (outer membrane leptospiral protein) appear to be involved in ligand-receptor interactions that facilitate adhesion to receptors like cell-adhesion molecules and ECM proteins (Len protein family) on endothelial cells. Adhesion also appears to cause an increased expression of adhesion receptors such as E-selectin on endothelial cells, resulting in additional adhesion of bacteria, platelets, and neutrophils (acute inflammatory response). This response may be attributable to bacteria wall LPS, peptidoglycans, and outer membrane proteins, thus promoting inflammation in capillaries leading to vasculitis and hemorrhage. Bacterial LPS likely activates cells by binding to TLRs on target cell membranes. *Leptospira* spp. also produce pore-forming hemolysins, proteases, sphingomyelinases, and collagenases that may assist in this process, but their role in causing endothelial cell injury remains to be determined.

Septicemic Anthrax (*Bacillus anthracis*). The sections in this chapter on alimentary and inhalation anthrax should be reviewed for background information pertinent to understanding septicemic anthrax (see Fig. 7-124). Once vegetative forms of the bacteria enter the circulatory system from the respiratory or alimentary systems, septicemia ensues and vascular collapse occurs, resulting from massive release of toxins into the blood plasma. Septicemic anthrax is characterized by animals found dead unexpectedly, often in a classic sawhorse stance and with hemorrhage (unclotted) from body orifices (Fig. 4-24). If inadvertently opened, the spleen will be enlarged and unclotted blood will exit from cut surfaces; lymph nodes will be enlarged, edematous, and hemorrhagic; and body tissues and serosal surfaces will be edematous and hemorrhagic (see

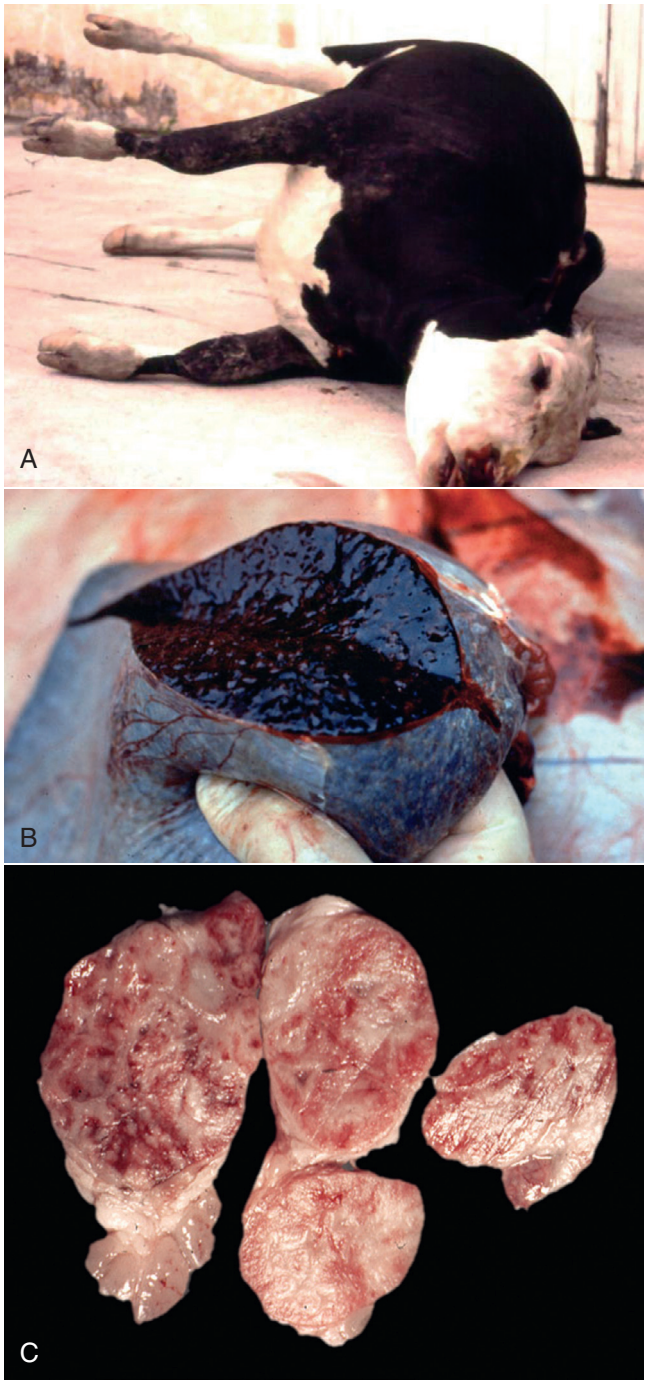


Figure 4-24 Anthrax, ox. A, Because of the high fever, cadavers of cattle dying of anthrax decompose rapidly with the usual result of excessive gas formation in the gastrointestinal tract, abdominal distention, and resultant "sawhorse" position of the legs. **B,** The spleen is enlarged and bloody (splenomegaly, bloody spleen). A postmortem examination should not be performed on an animal suspected of dying from anthrax. Air-dried impression smears of blood from external orifices or from an ear vein can be stained and the bacterium identified (see Fig. 7-124). **C,** Lymph nodes are also enlarged and bloody as a result of anthrax toxins that destroy vascular endothelial cells (see Fig. 13-57). Anthrax toxin can also cause severe injury to the intestines (see Fig. 7-124) and lungs. (**A** courtesy Dr. D. Driemeier, Federal University of Rio Grande do Sul, Brazil. **B** and **C** courtesy Dr. J. King, College of Veterinary Medicine, Cornell University.)

Fig. 4-24). Necropsies should not be performed on animals that are suspected of dying from anthrax because the vegetative form proliferates in large numbers in blood. When blood vessels are cut and blood drains onto the carcass or ground, the vegetative form quickly transforms into endospores, which contaminate the area long-term.

In the circulatory system, vegetative forms proliferate in large numbers and are arranged in long chains in the capillary beds of many organ systems, including the spleen (see Fig. 13-57). Large quantities of EF and LF toxins are released into the blood, causing dysfunction and lysis of endothelial cells and their barrier systems; thus the toxins increase the permeability of the capillary wall, leading to edema, vasodilation, and hemorrhage in affected organ systems. Anthrax toxins also disrupt the clotting cascade, likely through massive activation of disseminated intravascular coagulation and consumption of clotting factors. This outcome results in unclotted blood at body orifices (and within tissues and organs), a clinical (and gross) observation also characteristic of anthrax.

Disorders of Horses

Glanders (*Burkholderia mallei*; Farcy, Malleus, Droes). The mechanism of injury in glanders is cell lysis caused by pyogranulomatous inflammation and its mediators and degradative enzymes. It is a disease of lymphatic vessels (and local lymphoid tissues and skin) and of the respiratory system. Gross lesions include ulcers, pustules, and nodules that can affect skin of any part of the body but most frequently involves lymphatic vessels of the legs and flanks (cutaneous glanders), resulting in pyogranulomatous lymphangitis and lymphadenitis (Fig. 4-25). Nodules typically occur along the course of lymphatic vessels, resulting in a raised beaded appearance of the skin. They often rupture because of trauma to the skin or from pressure necrosis caused by an expanding volume of exudate within the nodules. This process results in craterlike ulcers of the skin that discharge a thick yellowish-white viscid and sticky purulent material containing abundant bacteria (see Fig. 4-25). In the respiratory system, pyogranulomas and ulcers occur in mucosae of the nasal cavity and in all lobes of the lungs (random pattern) (respiratory glanders).

Glanders is a zoonotic disease. Horses, mules, and donkeys probably encounter *Burkholderia mallei* most commonly via fomites arising from purulent exudate discharged from ulcerated lymphatic vessels of the skin. The skin and hair around draining ulcers becomes covered with exudate, which can be transferred to the skin of other animals via direct contact. Additionally, grooming behaviors may result in the bacterium being inhaled or ingested. Therefore the integumentary (skin), respiratory, and alimentary systems are portals of entry for the bacterium, whereas the integumentary and respiratory systems are final destinations for the bacterium. It is the intervening mechanisms involved in the potential pathways of spread that are unresolved.

At the skin, the epidermis and dermis impose structural and functional barriers blocking access to lymphatic vessels in the dermis and subcutis. It appears that the skin must be penetrated and the bacterium carried by direct extension into the dermis and subcutis for pyogranulomatous lymphangitis to develop. Thus the bacterium enters and acts locally. In the respiratory system, the potential pathway(s) of spread are more complicated. Following inhalation, the bacterium encounters mucus and mucosae of the nasal cavity and of the conductive component of the lung. The outcome of these interactions is likely controlled by virulence factors, which determine whether the nasal mucosa and lungs, lymphatic vessels of the skin, of both types of tissues are final targets for infection. In the nasal mucosa and lungs, the bacterium appears to enter and act

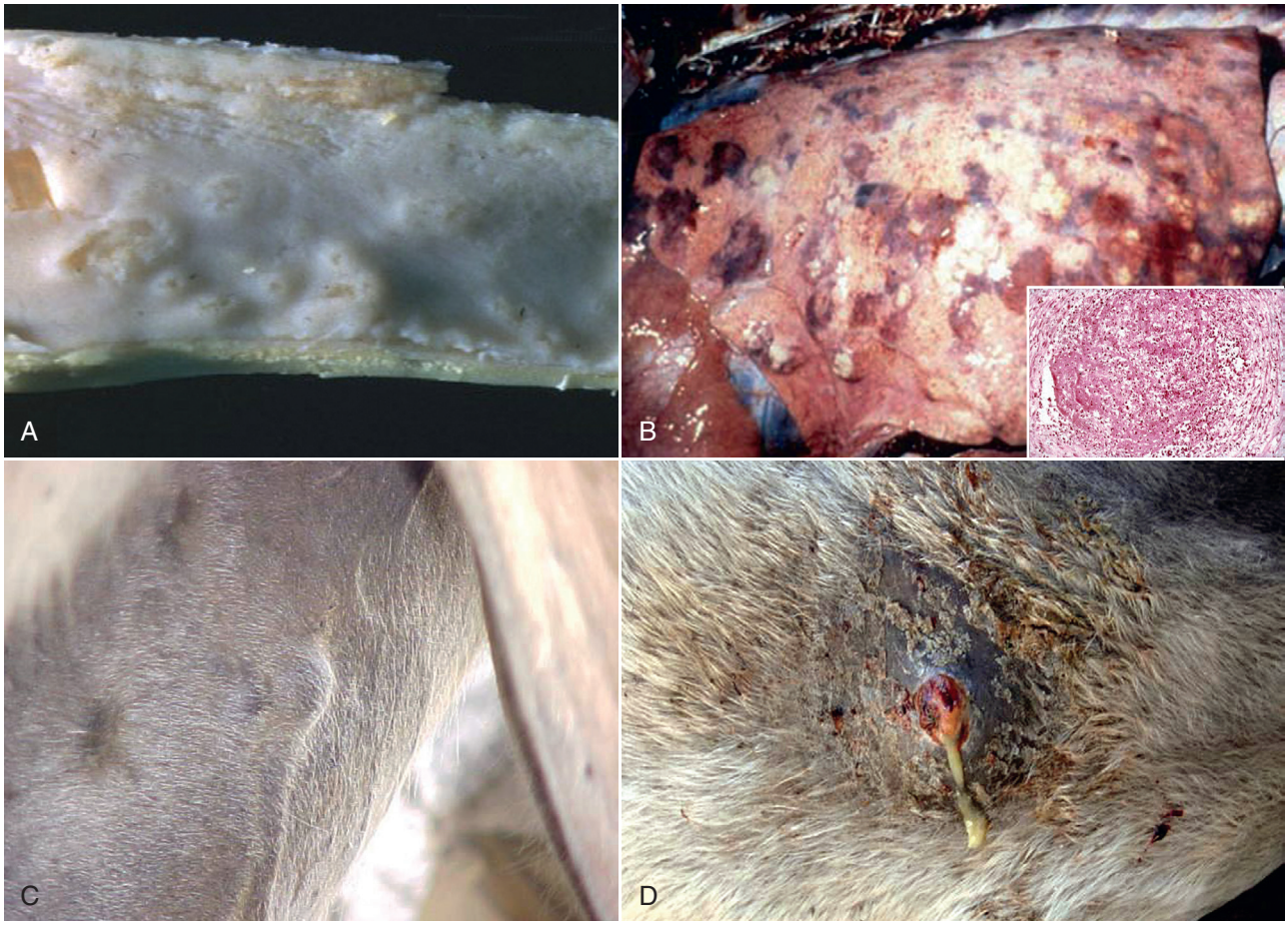


Figure 4-25 Glanders Disease. A, Mucosa, nasal turbinates, multiple nasal ulcers, and granulomas. *Burkholderia mallei* colonizes the nasal turbinates, resulting in pyogranulomatous inflammation, necrosis, and ulceration of the mucosa. **B,** When the bacterium colonizes the mucosa of the conductive system of the lung, it spreads into pulmonary parenchyma, resulting in the formation of pyogranulomas *(inset)* throughout the lung. *Inset,* H&E stain. **C,** When the bacterium spreads to the skin, it colonizes subcutaneous lymphatic vessels, resulting in the formation of pyogranulomatous nodules that typically occur along the course of lymphatic vessels (pyogranulomatous lymphangitis), resulting in a raised beaded appearance of the skin. **D,** These nodules frequently rupture because of trauma to the skin or from pressure necrosis due to the expanding volume of exudate within the nodules. This process results in crater-like ulcers of the skin that discharge a thick yellowish-white viscid and sticky purulent material containing abundant bacteria. (**A** courtesy Dr. D.D. Harrington, School of Veterinary Medicine, Purdue University; and Noah's Arkive, College of Veterinary Medicine, The University of Georgia. **B** courtesy United States Animal Health Association, St. Joseph, MO. Inset courtesy Dr. J. Tyler, College of Veterinary Medicine, University of Georgia and Noah's Arkive, College of Veterinary Medicine, University of Georgia. **C** courtesy Dr. D. Driemeier, Federal University of Rio Grande do Sul, Brazil. **D** courtesy Dr. R. Mota, Universidad Federal Rural de Pernambuco, Recife, Brazil, and Dr. M. Brito, Universidad Federal Rural do Rio de Janeiro, Brazil.)

locally. Whereas spread to the skin from the nasal mucosa requires a complicated series of steps, such as endocytosis, phagocytosis, and leukocyte trafficking (or cell-free bacteriemia) as examples, to cross the mucosa, spread, and reach and colonize target cells in cutaneous lymphatic vessels. Lastly, in the alimentary system, following ingestion and passage to the small intestine via swallowing and peristalsis, the potential pathway(s) of spread back to cutaneous lymphatic vessels (if they occur) are more complicated and largely unknown. Mechanisms potentially include virulence factors that facilitate crossing the mucus layer, crossing mucosal barriers at mucosal epithelial cells or M cells (endocytosis, phagocytosis, mucosa-associated macrophages), and leukocyte trafficking (or cell-free bacteriemia), as examples, to spread, reach, and colonize target cells in cutaneous lymphatic vessels.

Most of the mechanisms listed have been discussed and illustrated in earlier sections of this chapter and will not be detailed herein. However, a few important points will be discussed. It appears that the colonization process at mucosae may involve the development of a biofilm; adhesins, such as pili, have also been identified (virulence factors). An interaction of the bacterium with cell membranes is a prerequisite for infection to occur. A type IV pilin-like protein may be involved in the adherence of the bacterium to target cells. Secondly, the bacterium may have different sets of virulence factors that allow it to enter phagocytic cells (macrophages, dendritic cells) or nonphagocytic cells (mucosal and skin epithelial cells) via phagocytosis and endocytosis, respectively, and cross these barrier systems to gain access to BALT/GALT (MALT), and then spread systemically to the skin (lymphatic vessels) via leukocyte trafficking. In both macrophages and mucosal epithelial cells, type III and IV secretion systems (virulence factors) appear to be involved in cell invasion, actin-based motility, and transfer across cell membranes from cell to cell. Unique to nonphagocytic cells (mucosal and skin epithelial cells) are virulence factors that allow the bacterium to enter target cells through endocytosis, escape from

endocytotic vesicles and replicate in target cell cytoplasm, move in cytoplasm to the cell membrane (actin-based motility), and enter new target cells via membrane protrusions (actin-based motility) that move bacteria from one cell to another. This later mechanism may be important when the bacterium interacts with endothelial cells of cutaneous lymphatic vessels in the skin, resulting in ulcerative pyogranulomatous lymphangitis.

Cells of the monocyte-macrophage system and possibly mucosal dendritic cells probably play a role in colonization, replication, and spread of the bacterium. Once in MALTs the bacterium could spread to regional lymph nodes in lymphatic vessels as (1) a cell-free bacteremia or (2) via leukocytic trafficking in macrophages, and after colonization of and replication in lymphoid tissues, the bacterium could then spread systemically via lymphatic vessels and the thoracic duct to the circulatory system. Via the circulatory system the bacterium ultimately arrives (cell-free or in macrophages) at capillary beds of the skin, passes through the endothelial cells (leukocytic trafficking, endocytosis, or transcytosis), enters the subcutaneous tissues, encounters endothelial cells of cutaneous lymphatic vessels, and elicits a pyogranulomatous inflammatory response in these tissues. Additionally, it is possible that pyogranulomas in the lung could arise from spread of the bacterium through the circulatory system as a cell-free bacteremia or in macrophages as described earlier.

In macrophages and multinucleated giant cells, how and if the bacterium evades killing by phagosome-lysosome fusion (see Figs. 4-13 and 4-14), replicates, and spreads to cutaneous lymphatic vessels via the circulatory system are unknown. Type III and IV secretion systems (virulence factors) may be possible mechanisms for the invasion, escape from lysosomes or phagolysosomes, and survival in target cells. The bacterium is surrounded by a type I O-antigenic polysaccharide (capsular) antigen, a virulence factor, that may also block phagocytosis or phagosome-lysosome fusion (see Figs. 4-13 and 4-14). LPS, which likely contains a lipid A component, may also play a role in tissue injury. Multinucleated giant cells occur in the inflammatory exudate, and it is thought that the bacteria has virulence factors that causes fusion of macrophages, allowing the bacterium to evade adaptive immune responses and replicate within these fused cells.

Disorders of Pigs

Edema Disease (*Escherichia coli*). The pathogenesis of edema disease begins as an alimentary enterotoxemia and ends as a fibrinoid arteriopathy/arteriolopathy of the vascular system, especially of the brain, leading to ischemia and malacia. The enterotoxemia phase is discussed in the section on bacterial diseases of the alimentary system; the nervous system phase is discussed in the section on bacterial diseases of the nervous system. The mechanism of injury is lysis (coagulative necrosis) of endothelial and smooth muscle cells of arteries and arterioles caused by Shiga toxin 2e (also known as *verotoxin 2e*) produced by hemolytic strains of *E. coli*. After colonization of the intestinal mucosae, the toxin is absorbed from the alimentary system and circulates in the blood vascular system. Cells susceptible to the effects of this toxin include endothelial and smooth muscle cells of arteries and arterioles that express receptors for the toxin such as globotetraosylceramide, galactosylgloboside, and globotriaosylceramide. Toxin acts to disrupt protein synthesis leading to vascular permeability changes and cell lysis and thus edema of affected organs, most notably the eyelids, ventral neck (jowls), the gastric and colonic mesenteries, and the nervous system (see Figs. 7-169 and 7-170). Additionally, endothelial injury caused by this toxin may lead to hemorrhage, intravascular coagulation, microthrombosis, and infarction.

Urinary System

Disorders of Domestic Animals

Necrohemorrhagic Urocystitis (*Escherichia coli, Corynebacterium renale, Pseudomonas* spp., *Proteus vulgaris*, or *Klebsiella pneumoniae*). Necrohemorrhagic urocystitis is a term used herein to group bacteria whose virulence factors can cause acute inflammation and hemorrhage of the mucosa of the urinary bladder, especially affecting transitional epithelial cells and the lamina propria and its capillary beds. Because of the complicated nature of structure and function involved in this disease, a brief overview is provided (also see Chapters 1 and 11). The uroepithelium (urothelium), a mucosa formed by transitional epithelium, is a unique barrier system between urine and its components in the urinary space and the underlying well-vascularized lamina propria. The mucosal epithelium forms a barrier to ions, solutes, and water flux, as well as microbes. Transitional epithelium is composed of three layers, umbrella, intermediate, and basal cell layers. The outermost umbrella layer is a single layer of highly differentiated and polarized cells with distinct apical and basolateral domains demarcated by tight junctions. The intermediate and basal cell layers are connected to each other and the overlying umbrella cell layer by desmosomes and, likely, by gap junctions. The basal layer is connected to a basement membrane and its underlying lamina propria via substrate adhesion molecules.

Because more is known about virulence factors for uropathogenic *E. coli* (UPEC), it is discussed in greater detail; however, the other bacteria listed in this group likely use similar or related mechanisms to cause disease in the urinary bladder. The mechanism of injury is probably cell lysis (coagulative necrosis) caused by bacterial toxins that act directly on mucosal epithelial cells and capillaries in the lamina propria of the bladder and acute and chronic inflammation and their effector molecules and degradative enzymes. Gross lesions of the bladder include mucosal edema and mucosae that are rough and granular, red to dark red, and covered with white-gray flecks of fibrin mixed with cellular debris from inflammation (see Fig. 11-57). Blood vessels in the wall and serosa of the bladder are prominent; this change is due to active hyperemia of the fluidic vascular phase of acute inflammation.

Animals encounter these bacteria through contact with them in fomites or fluid droplets of urinary or fecal origin. They commonly become commensal microbes that reside in the mucous membranes of the vagina and prepuce, likely in biofilms. Physical changes in pressure across the tubular components of the urinary and reproductive systems caused by parturition and breeding appear to force these commensal bacteria via reflux mechanisms into the urethra and urinary bladder. The length of the urethra, in part, appears to determine why females have cystitis more commonly than males. Environmental stressors, such as peak lactation, traumatic mucosal injury, and a high-protein diet that increases the pH of the urine, make mucosae more susceptible to colonization and alter the commensal relationship, allowing the bacteria to replicate in sufficient numbers to colonize mucosae of the urinary and reproductive systems and spread bacteria to other animals.

Once in the lumen of the urinary bladder, bacteria gain access to mucosal surfaces via random movement of the urine. The urinary mucosa lacks goblet cells; thus there is no mucus layer to penetrate. These bacteria encounter the apical surface of transitional epithelial cells, and using ligand-receptor interactions characteristic of other bacterial diseases, begin the process of adherence, binding, and colonization of the mucosa. UPEC expresses adhesins, such as type 1 fimbriae, P fimbriae, and S fimbriae that are involved in this process. These fimbriae (also known as *pili*) bind to hexagonal arrays of mannosyl-glycoprotein receptors called uroplakins. They are

expressed on the apical (and luminal) surfaces of specialized transitional epithelial cells called *umbrella cells*. The tips of the type 1 fimbriae express a ligand called *FimH adhesin* that binds to uroplakin receptors. Uroplakins are integral membrane proteins of the umbrella layer, which can be used by microbes as adhesins (FimH adhesin protein) to colonize and invade the uroepithelium. Cells of the umbrella layer have one known receptor (UPIa) and other less well characterized receptors that are mannosylated proteins (integrins) for this adhesin (FimH). Similar processes of bacterial-induced internalization likely occur in the underlying intermediate and basal cell layers as a mechanism to cross the mucosal barrier. Once internalized, the bacterium apparently is able to replicate in endocytotic vesicles and can form intracellular bacterial communities where the bacteria are able to evade innate and adaptive immune defense mechanisms.

When uropathogenic *E. coli* adheres to and colonizes the apical surfaces of the umbrella cell layer, epithelial cells flatten and the bacterium is internalized via endocytosis. Endocytosis is a complicated process of entering and colonizing cells and mucosa, which occurs through a series of conformational changes in the apical surface of the umbrella cells and leads to cytoskeletal rearrangements and cell entry by a zipper mechanism. Bacterial flagella may also have a role in this mechanism. This process results in colonization of the mucosa and the development of a biofilm-like (or intracellular bacterial communities) arrangement affecting the mucosa. After formation of a biofilm, bacteria can kill infected umbrella cells via hemolysins that produce pores in membranes, thus releasing bacteria into the lumen of the bladder, where they colonize new umbrella cells and repeat the infective process or are released into the environment during urination.

The edema, hemorrhage, and necrosis characteristic of necrohemorrhagic urocystitis appear to be caused by acute inflammation and a variety of virulence factors in highly pathogenic strains of UPEC and likely the other bacteria listed earlier. Acute inflammation is probably induced via TLRs that recruit neutrophils from the vascular system into the lamina propria and mucosa and in response to cell necrosis, loss of the mucosal barrier, and interaction of the vascularized lamina propria with bacterial toxins. In umbrella cells infected by bacteria, bacterial toxins, such as LT and ST toxins, Shiga-like toxin, cytotoxins, and endotoxin, likely diffuse through the mucosa and cause membrane injury, leading to cell lysis (necrosis) and loss of the mucosal barrier system. These toxins may also stimulate apoptotic cell lysis, leading to release of bacteria into the urine. Once dead, these cells slough into the urine, and endotoxins and other toxic molecules can readily be absorbed into the highly vascularized lamina propria, resulting in injury to the capillaries and acute vasculitis with active hyperemia.

Other virulence factors that contribute to the pathogenesis of necrohemorrhagic urocystitis include bacterial surface molecules such as capsular K antigens and LPS that block phagocytosis and killing of the bacteria by neutrophils and macrophages. UPEC usually produces siderophores that play a role in iron acquisition for the bacteria during and after colonization. The lytic actions of hemolysins also increase the availability of iron and other nutrients for bacterial growth in colonized mucosa. Hemolysins also can kill lymphocytes and block phagocytosis and chemotaxis by phagocytic cells. Some strains of UPEC have a virulence factor for the production of urease, which hydrolyzes ammonia in urine into urea, resulting in alkaline urine that causes additional injury to mucosa. Finally, these bacteria can readily exchange genetic information with less virulent bacterial strains by transduction and conjugation using drug resistance, toxin, and other virulence plasmids. These factors are a few of the reasons why it is often difficult to treat and resolve certain types of acute and chronic bladder infections.

As a potential defensive mechanism, it has been shown that umbrella cells infected with bacteria undergo apoptosis, likely induced by LPS and TLRs, and affected cells are shed into the urine. This process may be a protective mechanism to remove infected cells; however, loss of apoptotic cells in the urine releases bacteria into the urine to encounter additional umbrella cells.

Renal Leptospirosis *(Leptospira* spp.*).* The pathogenesis of renal leptospirosis begins as vascular leptospirosis (see the section on Bacterial Diseases of Organ Systems, Cardiovascular System and Lymphatic Vessels, Vascular Leptospirosis [*Leptospira* spp.] for portals of entry) caused by *Leptospira* spp. The mechanism of injury in renal leptospirosis is cell lysis caused by (1) physical properties (penetrating movements) of bacteria that disrupt functions of endothelial cells, (2) bacterial toxins that act directly on membranes of renal tubular epithelial cells, and (3) acute and chronic inflammation and their effector molecules and degradative enzymes. Gross lesions include discrete and coalescing, often linear to radiating white to gray foci of acute tubular cortical necrosis and inflammation intermixed with hemorrhage (see Fig. 11-66). In chronic renal leptospirosis, lesions include discrete and coalescing, often linear to radiating white to gray foci of chronic inflammation and fibrosis (see Fig. 11-14).

In the kidney, primary target cells for infection appear to be epithelial cells of the proximal convoluted tubules (cortex) (Fig. 4-26, *A*) and then later, epithelial cells of the loops of Henle (medulla) (Fig. 4-26, *B*). Once *Leptospira* spp. gain access to the circulatory system, they disseminate in glomerular capillaries and then intertubular capillaries of proximal convoluted tubules. Bacteria could access proximal tubular cells via their apical or basolateral surfaces by two routes: (1) vascular by glomerular capillaries and migration into the lumen of the urinary space (apical) or (2) vascular via intertubular capillaries and migration into the interstitium (basolateral). Because glomerular changes are usually unremarkable and bacteria and inflammation are observed in the interstitium, it appears that epithelial cells of the proximal convoluted tubules are infected via the basolateral surfaces of the cells via migration through intertubular capillaries.

To infect tubular epithelial cells of the kidney, it appears that the bacterium must first attach to luminal (apical) surfaces of endothelial cell membranes, enter (endocytosis) and cross the cytoplasm (transcytosis), exit from basal surfaces (exocytosis), and gain access to underlying vascularized ECM adjacent to tubular epithelial cells. Bacteria likely attach to endothelial cell membranes of intertubular capillaries via adhesins then penetrate the vessel wall by moving directly through the cells or through their junctional complexes to gain access to the interstitium. In reality, the distance in the interstitium between capillaries and proximal tubular epithelial cells is probably no more than 100 μm, and it is likely that the flagella of these motile bacteria propel them to the tubular epithelial cells. It is unclear why the bacteria target proximal tubular epithelial cells for infection. *Leptospira* spp. initially spread through the vascular system to all tissues of the body and do not appear to specifically target the kidney via a tropism (attraction to a specific cell type or tissue) mechanism.

In the context of interacting with capillary endothelial cells and probably renal epithelium, surface-associated proteins (outer membrane leptospiral proteins) appear to be involved in ligand-receptor interactions that facilitate adhesion to target cell receptors like cell-adhesion molecules and ECM proteins (Len protein family). Adhesion to cells also appears to cause an increased expression of adhesion receptors such as E-selectin on endothelial cells, resulting in

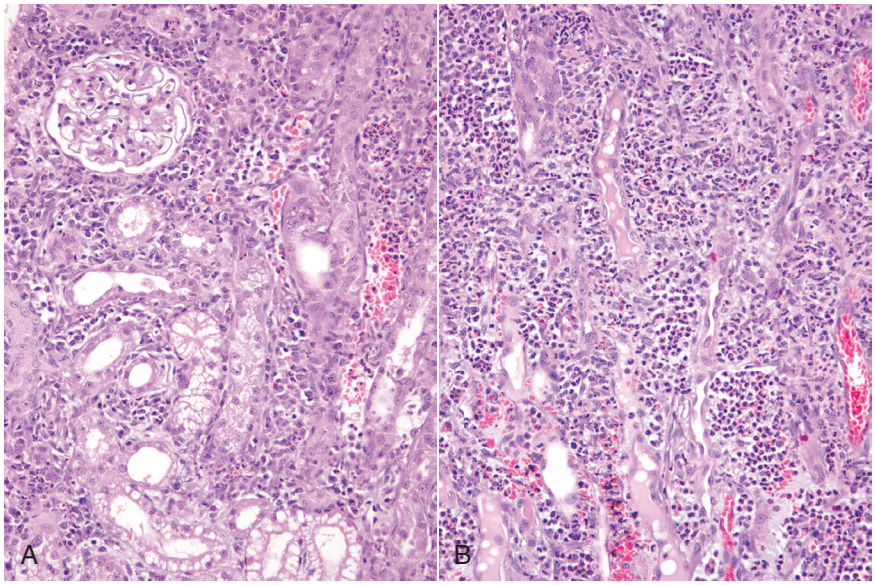


Figure 4-26 Renal Leptospirosis. A, Kidney, outer cortex. Note the infiltration of mononuclear cells, chiefly macrophages, lymphocytes, and plasma cells in the interstitium between the proximal convoluted tubules, the result of the leptospires infecting the proximal tubule cells after exiting the intertubular capillaries. H&E stain. **B,** Kidney (same as **A**), inner cortex. Numerous neutrophils distend the interstitium between the loops of Henle. This acute inflammatory response further down the nephron from the area in **A**, supports the concept that the cells of the loop of Henle are infected later than those in the proximal convoluted tubule. H&E stain. (**A** and **B** courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

additional adhesion of bacteria as well as platelets and neutrophils (acute inflammatory response). This response may be attributable to bacteria wall LPS, peptidoglycans, and outer membrane proteins, thus promoting inflammation in capillaries leading to vasculitis and hemorrhage. Bacterial LPS likely activates cells by binding to TLRs on target cell membranes. *Leptospira* spp. also produce pore-forming hemolysins, proteases, sphingomyelinases, and collagenases that may assist in this process, but their role in causing endothelial cell injury remains to be determined. However, once epithelial cells are infected, the reason for dominance of lesions in these organs is unclear and may be related to some essential trophism (nourishment of tissues) provided by these cells to the bacteria for colonization and proliferation. Additionally, although undetermined, such specificity could be attributed to ligand-receptor interactions or to a chemical gradient, such as an iron concentration, that could be required for bacterial growth and replication.

The cause of lysis of proximal tubular cells is probably multifactorial, involving vasculitis and ischemia, trauma from physical injury caused by bacterial motility, inflammatory mediators and degradative enzymes, and bacterial toxins (LPS). The bacteria are present in the cytoplasm of these cells; endocytosis and phagosome-lysosome fusion are not involved in cell entry. It appears that the bacteria are able to directly enter these cells via their motility. Inflammatory cells in the lesion progress from neutrophils (suppurative) to lymphocytes, macrophages, and plasma cells (chronic) and provide an array of molecules that could injure ands lyse tubular epithelial cells. Biofilms (virulence factor) formed by *Leptospira* spp. may also play a role in tubular injury. Epithelial cells lining the loop of Henle could also be infected by an intertubular capillary–interstitial route. This mechanism has not been confirmed. Additionally and based on inflammatory cell responses, it is unclear why cells of the proximal tubules appear to be infected at an earlier point in the disease than those of the loop of Henle. However, when proximal tubular cells die, they may release bacteria into the urinary space where they are carried in urine and spread into the environment via urination. During this luminal transit, the bacteria also encounter the apical surfaces of epithelial cells lining the loop of Henle. It is plausible that *Leptospira* spp. infect epithelial cells of the loop of Henle via their apical surfaces projecting into the urinary lumen, using mechanisms similar to those previously described. Infection appears to result in the same cascade of cell alterations and inflammatory responses as those described for proximal tubular cells. These outcomes in both proximal tubular and loop of Henle cells serve as the basis for characterizing this disease as tubulointerstitial nephritis.

Disorders of Ruminants (Cattle, Sheep, and Goats)

Contagious Bovine Pyelonephritis (*Corynebacterium renale, Trueperella pyogenes* [formerly *Arcanobacterium pyogenes*], or *Escherichia coli*). Contagious bovine pyelonephritis is caused by the *Corynebacterium renale* group (*C. renale*, *Corynebacterium cystitidis*, and *Corynebacterium pilosum*) of bacteria, but *Trueperella pyogenes* (formerly called *Arcanobacterium pyogenes*) and *E. coli* may also cause this disease. Depending on the region, *T. pyogenes* may be most common. These bacteria are likely commensal microbes that reside, probably in a biofilm, in the mucous membranes of the vagina and prepuce. Mechanisms that contribute to the occurrence of cystitis that precede pyelonephritis are discussed in the section on necrohemorrhagic urocystitis and UPEC. Information about the mechanisms used by *C. renale* or *E. coli* to cause contagious bovine pyelonephritis in cattle is limited. Thus portions of this section are conditional and based on (1) what is known mechanistically about other diseases caused by *Corynebacterium* spp. or *E. coli* and (2) a reasonable probability that inflammation, responses to injury, and lesions that have been described in contagious bovine pyelonephritis are the result of underlying and known pathobiologic mechanisms.

The mechanism of injury in contagious bovine pyelonephritis is probably cell lysis (acute coagulative necrosis) caused by (1) bacterial toxins that act directly on transitional epithelium of bladder mucosa and on renal pelvic and tubular epithelium and by (2) acute and chronic inflammation and their effector molecules and degradative enzymes. Gross lesions include white-tan streaks

often mixed with narrow red streaks (hemorrhage) that radiated from the pelvis, through the medulla, often extending to the cortical medullary junction or deeper into the cortex (see Fig. 11-46). In many ways, these lesions resemble inverted renal cortical infarcts with their bases against the pelvis and their apices extending into the medulla.

Cattle (and probably sheep and goats) encounter these bacteria through contact with contaminated fomites or fluid droplets of urinary or fecal origin. Environmental stressors such as parturition, peak lactation, traumatic mucosal injury, and a high-protein diet that increases the pH of the urine appear to alter the commensal (biofilm) relationship of bacteria in mucous membranes of the vagina and prepuce making them more suitable for colonization. It has been proposed that contagious bovine pyelonephritis occurs secondary to a chronic and often insidious cystitis (see section on Necrohemorrhagic Urocystitis [*Escherichia coli*, *Corynebacterium renale*, *Pseudomonas* spp., *Proteus vulgaris*, or *Klebsiella pneumoniae*]) likely resulting from reflux of bacteria into and up the urethra and then into the bladder via changes in urethral luminal pressures caused by parturition, breeding, or straining to defecate. Subsequently the bacterium must reach the renal pelvis via the ureters and then spread though the mucosal barrier formed by transitional epithelium of the renal pelvis to encounter the interstitium (vascularized ECM) of the renal medulla and renal tubules. How each of the steps occurs has not been specifically determined; however, ligand-receptor interactions characteristic of other bacterial diseases and their interaction with mucosae likely occur in contagious bovine pyelonephritis (see section on Necrohemorrhagic Urocystitis [*Escherichia coli*, *Corynebacterium renale*, *Pseudomonas* spp., *Proteus vulgaris*, or *Klebsiella pneumoniae*]).

Although C. *renale* is a nonmotile bacterium, some strains of *E. coli* are motile, and this virulence factor may help in ascension of the bacterium up the ureter to the kidney. It has been shown that pili are required for C. *renale* to adhere to transitional epithelium of the urinary system and to attach to and colonize mucosae of the reproductive system. Additionally, pili may serve to disrupt phagocytosis of the bacteria by neutrophils and macrophages. Putative adhesins such as FimH adhesin protein and other invasins are likely virulence factors (see section on Necrohemorrhagic Urocystitis [*Escherichia coli*, *Corynebacterium renale*, *Pseudomonas* spp., *Proteus vulgaris*, or *Klebsiella pneumoniae*]). Binding is strongest to mucosal epithelial cells of the vulva and vagina. This outcome allows for spread of the bacterium to other susceptible animals during breeding season or when other invasive management practices or examinations are performed. The receptors used by the bacterium to bind to mucosae have not been determined; however, once a bacterium is bound, colonization of mucosae begins. Once bacteria replicate in sufficient numbers, they spread by ascension to encounter and colonize mucosae of the urethra and bladder and then by vesicoureteral reflux to ascend to the ureters and renal pelvis. The development of a chronic insidious urocystitis is often an intervening stage in the disease that serves to produce large numbers of bacteria. After colonization of the renal pelvis, it is not known how bacteria cross mucosae to gain access to the medullary interstitium. Degradative enzymes and inflammatory mediators combined with bacterial virulence factors, such as Renalin, which is an extracellular cytolytic protein produced by C. *renale*, may facilitate spread across mucosal barriers and inflammation and cell lysis within the medulla. It has been suggested that lesions in the medulla (resembling inverted renal cortical infarcts) may actually begin as a vasculitis from inflammation resulting in thrombosis, ischemia, and necrosis. It has been shown that toxins (LPS) from *E. coli* may stimulate apoptotic cell lysis in renal tubular cells in pyelonephritis.

Pulpy Kidney (Overeating) Disease (*Clostridium perfringens*). The pathogenesis of pulpy kidney disease begins as an enterotoxemia of the alimentary system caused by C. *perfringens* and should be reviewed in the section on the alimentary system before reading this section. ε-Toxin appears to be an important virulence factor in the pathogenesis of pulpy kidney disease. Because ε-toxin is a permease that alters cell permeability (a pore-forming toxin), the vascular beds in affected intestinal tissues readily absorb toxins into the circulatory system. It appears that the sequence of steps leading to pulpy kidney disease occurs in the first phase or early in the second phase of alimentary enterotoxemia before toxin-induced massive necrosis of the intestine occurs. The mechanism of injury in pulpy kidney disease is cell lysis caused by ε-toxin that acts directly on renal endothelial cell membranes and tubular epithelial cell membranes, leading to vascular permeability changes and acute coagulative necrosis of tubular epithelial cells and likely endothelial cells. Microthrombosis and ischemia resulting from capillary endothelial injury are plausible but unproved mechanisms of tubular cell lysis. Gross lesions include soft pliable kidneys with hemorrhages; however, the lesions are often attributed to postmortem change. Experimental data suggest that vascular endothelial cells, such as those in the renal cortex supplying epithelial cells of renal tubules, express receptors (ligand-receptor interactions) for ε-toxin. Because ε-toxin is an angiotoxic permease, it increases the permeability of targeted endothelial cells, allowing plasma containing ε-toxin to leak into the ECM surrounding renal tubules. Renal tubular epithelial cells also express receptors for ε-toxin, and toxin binding may lead to membrane-mediated cytotoxicity and cell lysis.

Bone Marrow, Blood Cells, and Lymphatic System

Disorders of Domestic Animals

Brucellosis (*Brucella* spp.). The mechanism of injury in brucellosis is cell lysis caused by inflammation and its mediators and degradative enzymes. *Brucella* spp. do not have virulence factors for exotoxins or endotoxins that cause direct injury to cells. Gross lesions include chronic active pyogranulomatous lymphadenitis with enlarged firm lymph nodes that on a cut surface have discrete and coalescing areas of yellow-white exudate infiltrating and compressing contiguous parenchyma.

Animals (ruminants [cattle, sheep, and goats], pigs, and dogs) encounter *Brucella* spp. through inhalation or ingestion of bacteria in fomites contaminated with infected exudates from other organ systems such as the female reproductive tract. The bacterium encounters mucosae and their mucus layers through centrifugal turbulence and entrapment in the mucus layer of the nasal pharynx and through chewing, gravity, and entrapment in the mucus layer of the oral pharynx. Bacteria are phagocytosed by mucosa-associated macrophages or dendritic cells migrating through, on, or in mucosae and spread to local lymphoid tissues via leukocyte trafficking by lymphatic vessels, to regional lymph nodes via afferent lymphatic vessels, and then systemically to superficial and visceral lymph nodes and other organs such as spleen, liver, bone marrow, mammary glands, and reproductive organs. *Brucella* spp. can also enter and cross the mucosal barrier via endocytosis/transcytosis, exit the basal surface of mucosal epithelial cells through exocytosis, and spread cell-free in lymphatic vessels to local and regional lymph nodules and nodes, where they are phagocytosed by macrophages and then spread systemically as discussed earlier. Through ingestion, swallowing, and peristalsis, *Brucella* spp. can also reach the alimentary system where they encounter M cells. Bacteria infect M cells via endocytosis, undergo transcytosis, and exit the basal surfaces via exocytosis to gain access to macrophages within Peyer's patches. Macrophages within Peyer's patches are then infected with the

bacteria and used to spread bacteria systemically. The goals of these three types of mucosal encounters are to provide *Brucella* spp. with ample opportunity to infect macrophages and subsequently gain access to local, regional, and systemic lymph nodules and nodes.

In lymph nodes, bacteria-infected macrophages are killed by the bacterium or die through aging, and the bacteria are released into vascularized ECM. These bacteria elicit an acute inflammatory response that is quickly replaced by a pyogranulomatous inflammation because of LPS in the bacterial cell wall. LPS is not readily degradable, and macrophages (as monocytes) are recruited from the systemic circulation to phagocytose and degrade such material and kill the bacteria. *Brucella* spp. are able to evade the killing mechanisms when phagocytosed by neutrophils and macrophages. Additionally, they are able to grow and replicate in macrophages and dendritic cells. Once *Brucella* spp. encounter cell membranes of macrophages, they use ligand-receptor interactions to attach to and enter cells; however, the details remain unclear. Outer membrane protein of the bacterial cell wall and class A scavenger receptors on target cells are likely involved but not necessarily with each other. TLRs are also likely involved in attachment and entry into macrophages. Entry occurs via endocytosis through a phagosome, but phagosome-lysosome fusion does not occur because bacteria are able to block fusion through rapid acidification of the phagosome (see Figs. 4-13 and 4-14). LPS (a PAMP), a type IV secretion system, and a long list of other putative virulence factors such as cyclic β-1,2-glucan and heat shock proteins may also be involved in blocking phagosome-lysosome fusion and promoting bacterial growth and replication. The virulence of Brucella spp. strains appears related to the LPS composition of its capsule, with the encapsulated smooth phenotypes generally being more virulent. Additionally, the presence of a smooth capsule enhances bacterial growth and replication in phagosomes.

When *Brucella* spp. spread via leukocyte trafficking systemically in macrophages, they are able to gain access to tissues in the male and female reproductive systems and the mammary gland (Fig. 4-27; also see Fig. 19-18). In summary, infected macrophages interact with and infect placental trophoblasts in placentomas and epithelial cells of other reproductive tissues. Once such cells are infected, the bacteria likely infect fetal macrophage-like cells that serve to spread the bacteria through the fetus and to other lymphoid tissues in reproductive organs (see Fig. 4-27, *C* and *D*). *Brucella* spp. also survive in macrophages of these tissues by inhibiting the phagosome-lysosome fusion. Bacterial growth and replication with concurrent lysis of bacteria-infected macrophages results in pyogranulomatous inflammation of these tissues and organ systems.

Disorders of Horses

Strangles (*Streptococcus equi* subsp. *equi*). The mechanism of injury in strangles is lysis (coagulative necrosis) of cells of lymphatic vessels, lymph nodes, and the monocyte-macrophage system attributable to acute suppurative inflammation and its mediators and degradative enzymes. Gross lesions include the formation of abscesses within regional lymph nodes resulting in enlarged firm lymph nodes that on cut surface have discrete and coalescing areas of yellow-white suppurative exudate infiltrating and compressing contiguous parenchyma (see Fig. 13-77). Affected retropharyngeal and mandibular lymph nodes may also have draining fistulous tracts between affected nodes and the surface of the skin, the guttural pouches, and the nasal cavities and sinuses, resulting in release of bacteria into the environment. This outcome occurs because degradative enzymes released from dead neutrophils in abscesses digest the capsule of the lymph node and the structures of all contiguous tissues until a fistulous tract is formed.

Foals encounter *S. equi* subsp. *equi* through inhalation or ingestion of fomites or body fluids contaminated with the bacterium. It is deposited on mucosae of the nasal (centrifugal turbulence) and oral pharynx and trapped in the mucus layer. The bacterium is nonmotile, and it has not been clearly shown how it penetrates the mucus layer and gains access to mucosal epithelial cells. Mucosal epithelial cells of the tonsils and tonsillar crypts appear to be important target cells for adherence and binding, and this specificity may be determined by unique ligand-receptor interactions. Once in contact with cell membranes, several bacterial cell wall surface proteins, such as M-like proteins (SeM, SzPSe), may act as adhesins and attach to receptors expressed on the membranes of these cells. The characteristics of these receptors are unknown. It has not been determined if the bacterium needs to first colonize the mucosal surface before spreading into subjacent local lymphoid tissues. Additionally, it has not been determined how the bacterium crosses the mucosal barrier of the tonsil. Mucosal macrophages could phagocytose the bacteria in the mucus layer and carry it via leukocyte trafficking through the mucosal barrier into the local lymphoid tissues, or once bound to target cell receptors, it could be transported through the cell via transcytosis and released via exocytosis from the basal membranes of the cells into local lymphoid tissues of the tonsil. Dendritic cells could also be involved in the spread of bacteria across the mucosal barrier and into local and regional lymphoid tissues and lymph nodes. In local lymphoid tissues of the tonsil, the bacterium is able to evade destruction by the innate immune system, replicate extracellularly, and then spread to regional lymph nodes such as the mandibular or retropharyngeal. Although unknown, the bacterium could spread by means of lymphatic vessels to these regional nodes in macrophages via leukocyte trafficking or through a bacteremia (cell-free migration). The bacterium multiplies extracellularly in the lymph tissues and nodes, and the exudate contains large numbers of viable bacteria.

Mechanistically, several bacterial virulence factors contribute to the character of this exudate (suppurative) and the large number of viable bacteria. Bacterial virulence factors that act as chemoattractants for neutrophils and that disrupt phagocytosis and killing by neutrophils appear to explain the abundance of exudate and viable bacteria, respectively. Early in the sequence of steps when bacteria encounter mucosal and tissue macrophages in local and regional lymphoid tissues and nodes, a bacterial cell wall protein, SeeH, interacts with these cells, resulting in the release of proinflammatory cytokines, increased vascular permeability, and edema. The occurrence of acute suppurative inflammation is also facilitated by several virulence factors. When peptidoglycan of the bacterial cell wall interacts with C3 of complement in edema fluid via the alternative complement pathway, it produces complement-derived chemotactic factors that attract large numbers of neutrophils from capillary beds into local vascularized connective tissues (ECM). Furthermore, bacterial streptokinase interacts with plasminogen in edema fluid to form active plasmin, which hydrolyzes fibrin. This process appears to increase the spread and dispersion of bacteria in tissue. Normally fibrin confines bacteria by isolating them within its polymerized fibrillar meshwork so they can be phagocytosed and killed by neutrophils and macrophages, but when fibrin is hydrolyzed, large numbers of bacteria can accumulate in the exudate of an abscess and be readily available for release into the environment (see later). The outcomes of these processes also contribute to the initiation of the cellular (leukocytic) phase of acute inflammation and the accumulation of suppurative exudate characteristic clinically of strangles.

The surface of *S. equi* subsp. *equi* is coated with numerous protein virulence factors, such as hyaluronic acid, SeM, and Se18.9 proteins

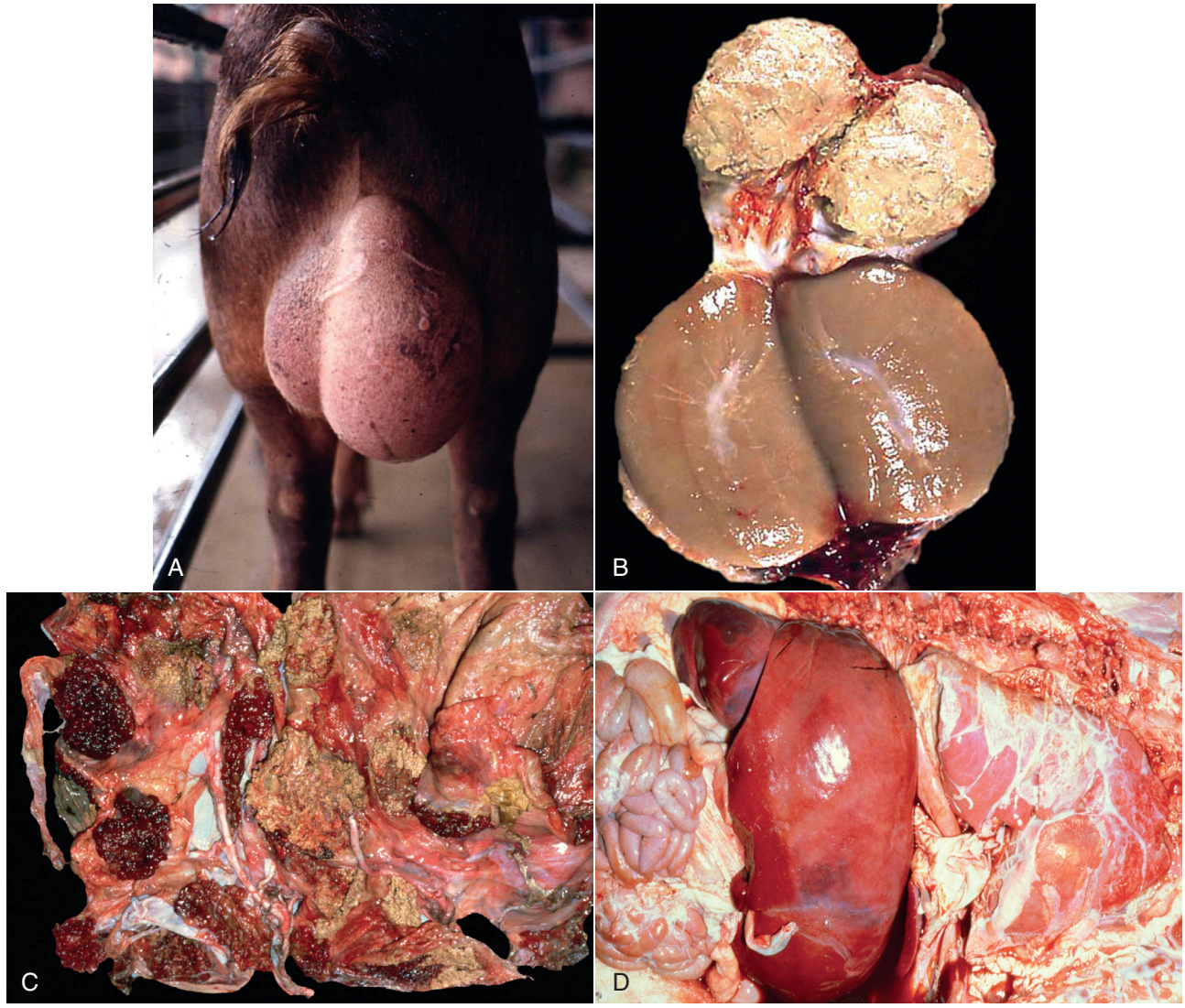


Figure 4-27 Brucellosis. Brucellosis is a disease in which the bacterium initially targets lymphocytes and macrophages in mucosa-associated lymphoid tissues, regional lymph nodes, systemic lymph nodes, and the spleen to replicate and grow in number. It uses macrophages to spread to and through these tissues and then systemically to infect cells and tissues in the placenta, sex organs of males and females, and fetuses. Therefore brucellosis is initially and long term characterized by a chronic active pyogranulomatous lymphadenitis with sequelae that affect the reproductive systems. **A,** Boar, swollen testis. The testis in enlarged due to chronic active pyogranulomatous inflammation. *Brucella* spp. spread in macrophages via leukocyte trafficking from lymphoid tissues systemically to the testis. **B,** The epididymis can be filled with pyogranulomatous exudate, which obstructs the flow of spermatozoa and causes infertility. Infected animals can also serve as carriers and spread the bacterium via sexual contact (also see Fig. 19-18). **C,** Fetal cotyledons. Note the roughened granular yellow-brown surface of cotyledons *(example: center of field)* infected with the bacterium. This lesion is caused by pyogranulomatous inflammation leading to severe necrosis of the affected cotyledons. Normal cotyledons are dark red and have a smooth shiny surface. **D,** Fetal brucellosis, hepatomegaly, and fibrinous polyserositis. The bacterium is thought to be spread from infected cotyledons to fetal organs via leukocytic trafficking in fetal macrophage-like cells. The bacterium causes extensive injury of the vascular system and organs through inflammatory responses induced in the fetus. (**A** courtesy Dr. C. Wallace, College of Veterinary Medicine, The University of Georgia; and Noah's Arkive, College of Veterinary Medicine, The University of Georgia. **B** courtesy Dr. K. McEntee, Reproductive Pathology Collection, University of Illinois; and Dr. J. King, College of Veterinary Medicine, Cornell University. **C** and **D** courtesy Dr. K. McEntee, Reproductive Pathology Collection, University of Illinois.)

that disrupt phagocytosis and killing. The bacterium also secretes leukocidal toxin and streptolysin S, a cell membrane pore-forming toxin, which kills leukocytes and disrupts phagocytosis. These processes lead to the accumulation of large numbers of viable bacteria in the exudate of lymphoid tissues and lymph nodes. Specific bacterial proteins are also chemoattractants and result in the recruitment of large numbers of neutrophils into the tissue and abscess formation. Additionally, hyaluronic acid in the capsule appears to block interactions between bacteria and neutrophils by increasing the negative charge and hydrophobicity of the bacterial surface and by producing a localized oxygen-reduced environment that protects the activity of oxygen-labile proteases and toxins such as streptolysin S. It is likely but undetermined that bastard strangles occurs because of spread of the bacterium to systemic lymph nodes and organ systems by leukocyte trafficking or bacteremia via efferent lymphatic vessels and/or capillaries or postcapillary venules in lymph nodes to gain access to the systemic circulatory system.

Rhodococcal Mesenteric Lymphadenitis (*Rhodococcus equi*). The pathogenesis of rhodococcal mesenteric lymphadenitis begins as an infection of the respiratory system (see section on

Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Horses), followed by infection of the alimentary system (see section on Bacterial Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Horses). The mechanism of injury in rhodococcal mesenteric lymphadenitis is lysis of cells of the monocyte-macrophage system and of all cell populations in the lymph node secondary to inflammation and its mediators and degradative enzymes. Gross lesions include chronic active pyogranulomatous lymphadenitis (see Fig. 7-139) with enlarged firm lymph nodes that on a cut surface have discrete and coalescing areas of yellow-white exudate infiltrating and compressing contiguous parenchyma. *R. equi* enters the alimentary system through M cells and is released into Peyer's patches, where it is phagocytosed by tissue macrophages. Bacteria-infected tissue macrophages spread via leukocyte trafficking in lymphatic vessels within the intestinal mesentery to mesenteric lymph nodes, leading to pyogranulomatous lymphadenitis, and then systemically via the thoracic duct and the blood vascular system to additional lymph nodes and lymphoid tissues such as the spleen. The pathogenesis of pyogranulomatous lymphadenitis appears to progress much like that which occurs in the lung (see section on the Respiratory System, Mediastinum, and Pleurae).

Caseous Lymphadenitis (*Corynebacterium pseudotuberculosis*). A disease similar to that which occurs in cattle, sheep, and goats also occurs in horses (see section on Bacterial Diseases of Organ Systems; Bone Marrow, Blood Cells, and Lymphatic System; Disorders of Ruminants [Cattle, Sheep, and Goats]; Caseous Lymphadenitis [*Corynebacterium pseudotuberculosis*]).

Disorders of Ruminants (Cattle, Sheep, and Goats)

Caseous Lymphadenitis (*Corynebacterium pseudotuberculosis*). The mechanism of injury in caseous lymphadenitis is cell lysis attributable to inflammation and its mediators and degradative enzymes affecting cells of the monocyte-macrophage system and cell populations in lymph nodes and other organ systems. Gross lesions include chronic active pyogranulomatous lymphadenitis (see Figs. 13-79 and 13-80) with enlarged firm lymph nodes that on a cut surface have discrete and coalescing areas of yellow-white caseous exudate infiltrating and compressing contiguous parenchyma and abundant connective tissue. In other organs, such as the lung, abscesses encapsulated by dense bands of fibrous connective tissue and containing yellow-white caseous exudate are common findings.

Sheep and goats encounter *Corynebacterium pseudotuberculosis* through penetrating wounds and potentially ingestion. The bacterium is a common contaminate of the environment usually from animals that have the cutaneous form of caseous lymphadenitis leading to fistulous tracts from draining cutaneous lymph nodes. Penetrating wounds most commonly involve the skin and mucous membranes of the oral cavity. Management practices, such as shearing, may cause skin abrasions, whereas objects like wire, sticks, and protruding barn or fence nails may puncture the skin. Similar types of injury may occur in the oral cavity by similar objects and mechanisms. Once the bacterium reaches the submucosa or dermis, it is phagocytosed by neutrophils and macrophages and spread via leukocyte trafficking to regional lymph nodes via afferent lymphatic vessels. The bacterium replicates in lymph nodes, and the inflammatory response results in multiple pyogranulomas (abscesses) that grow in size and with time, notably enlarge, and affect the entire lymph node. Macrophages infected with bacteria leave the lymph node via leukocyte trafficking and spread via efferent lymphatic vessels and probably the thoracic duct to the systemic circulation system or spread by entering the capillary or venous circulation within the node to gain access to the systemic circulatory system. Macrophages spread the bacterium to other visceral lymph nodes, especially the mediastinal and bronchial, and to tissues in a wide variety of organ systems, especially the lung. Because the bacterium is able to replicate in large numbers in macrophages and neutrophils, the lysis of these cells attributable to mycolic acid or cell aging results in the release of bacteria into vascularized ECM tissues. This process activates integrins and adhesins in vascular endothelium and causes massive recruitment of additional neutrophils and macrophages into the tissues as part of a chronic active inflammatory response, thus repeating the inflammatory process of forming pyogranulomas (abscesses).

The mechanisms used by *C. pseudotuberculosis* to gain access to lymph nodes via the alimentary system and ingestion (if it occurs) are unknown. Two potential pathways could be used, and both focus on macrophages and leukocyte trafficking. First, bacteria could interact with the mucus layer and mucosae of the oral pharynx, be phagocytized by mucosa-associated macrophages and carried to the tonsils, then to regional lymph nodes, and then systemically. Second, bacteria could be swallowed and via alimentary peristalsis encounter M cells of small intestinal crypts, spread via M cells to macrophages in contiguous Peyer's patches, then to regional lymph nodes, and then systemically. It is likely that the mechanisms and responses to injury described for the penetrating wounds portal of entry would also apply to these two scenarios.

C. pseudotuberculosis has two known bacterial virulence factors, phospholipase D and mycolic acid, that allow it to colonize tissues and produce pyogranulomas. Phospholipase D increases vascular permeability, which is thought to assist macrophages in migrating in and out of tissues infected with the bacterium, thus favoring the systemic spread of the bacterium. As a potent exotoxin, it also injures cell membranes, leading to macrophage and neutrophil dysfunction, disruption, and lysis, and interferes with neutrophil chemotaxis. *C. pseudotuberculosis* does not have a protective capsule but has a waxy mycolic acid coat on the cell wall surface. Mycolic acid induces acute inflammation, has a role in the formation of granulomas, is toxic for macrophages, and prevents killing of the bacterium with phagosome-lysosome fusion (see Fig. 4-14) likely by protecting against hydrolytic enzymes present within lysosomes.

Brucellosis (*Brucella* spp.). See the section on Bacterial Diseases of Organ Systems; Bone Marrow, Blood Cells, and Lymphatic System; Disorders of Domestic Animals; Brucellosis (*Brucella* spp.).

Disorders of Pigs

Rhodococcal Mesenteric Lymphadenitis (*Rhodococcus equi*). A disease similar to that which occurs in horses also occurs in pigs. See section on Bacterial Diseases of Organ Systems; Bone Marrow, Blood Cells, and Lymphatic System; Disorders of Horses; Rhodococcal Mesenteric Lymphadenitis (*Rhodococcus equi*).

Brucellosis (*Brucella* spp.). See section on Bacterial Diseases of Organ Systems; Bone Marrow, Blood Cells, and Lymphatic System; Disorders of Domestic Animals; Brucellosis (*Brucella* spp.).

Disorders of Dogs

Brucellosis (*Brucella* spp.). See section on Bacterial Diseases of Organ Systems; Bone Marrow, Blood Cells, and Lymphatic System; Disorders of Domestic Animals; Brucellosis (*Brucella* spp.).

Nervous System

Disorders of Domestic Animals

Botulism and Tetanus (*Clostridium botulinum, Clostridium tetani*). The mechanism of injury in botulism is disruption of

neurotransmitter vesicle exocytosis at *myoneural junctions* by botulinum toxin resulting in flaccid paralysis. The mechanism of injury in tetanus is disruption of neurotransmitter vesicle exocytosis at *neural-neural junctions* by tetanospasmin toxin resulting in spastic paralysis. These toxins, categorized as neurotoxins, are produced in anaerobic microenvironments (a lowered oxidation-reduction [redox] potential) such as occur in necrotic tissue resulting from traumatic wounds (e.g., nail penetrating sole of the hoof, gastric ulcers, necrotic muscle). Gross or microscopic lesions are not observed in the nervous systems of animals with these diseases.

Animals encounter these bacteria through contact with bacterial endospores present in soil and resting on environmental objects. Spores are carried into wounds and with the appropriate anaerobic conditions they germinate to vegetative forms. Neurotoxins are produced by the vegetative form and are released into surrounding tissues. Examples of such wounds include penetration of the skin or sole of the hoof or gastric ulcers. In addition, botulinum neurotoxin can also be released from lysed vegetative forms in the anaerobic environment of decaying vegetable matter (e.g., spoiled silage, hay [equine grass sickness], grain) and decomposing carcasses and absorbed into the circulatory system from the alimentary system with their ingestion. From a wound (or the alimentary system), neurotoxins access myoneural (botulinum neurotoxin) and neural-neural (tetanus neurotoxin) junctions by two routes, either hematogenously (botulinum neurotoxin) or via retrograde axonal transport (tetanus neurotoxin).

Botulinum neurotoxin enters the blood from (1) wounds as it diffuses via a concentration gradient to the periphery of the wound to areas with adequate circulation in which it is absorbed into the blood via capillaries and (2) absorption through intestinal villi and transfer to capillary beds within the lamina propria of the villi. Botulinum neurotoxin gains access to myoneural junctions via capillary beds that supply muscular tissues. On its release from capillaries, the neurotoxin diffuses in interstitial fluids until contacting the cell membrane of peripheral nerves (e.g., lower motor neuron), where it enters the cytoplasm of the neuron through the formation of endocytotic vesicles.

In contrast, tetanus neurotoxin (tetanospasmin) enters the nervous system and gains access to neural-neural junctions by initially entering the cytoplasm of distal processes of neurons through the formation of endocytotic vesicles in viable nerve endings located in tissue surrounding the site of the wound. The endocytotic vesicles are transported into the CNS by retrograde axonal transport, and tetanus neurotoxin is released into the interstitial fluid of the neural-neural junctions by exocytosis. Free tetanus neurotoxin then binds to the cell membrane of inhibitory interneurons of the spinal cord, is internalized via endocytosis, and acts to disrupt the release of inhibitory neurotransmitters via the same mechanism used by botulinum toxin: disruption of the synaptic fusion complex. Presynaptic neurons (upper motor neurons) excite postsynaptic neurons (lower motor neurons) on a nearly continuous basis. Inhibitory interneurons acting on lower motor neurons serve to counterbalance and smooth the excitatory effects of acetylcholine released from presynaptic neurons (upper motor neurons) to excite the same lower motor neurons. Thus skeletal muscle groups (opposing flexor and extensor muscles) are given time to relax; as a result, skeletal muscle contractions initiated by lower motor neurons are well regulated and coordinated. Failure to have adequate inhibitory interneuron regulation of lower motor neurons leads to the spastic paralysis observed in tetanus.

Although botulinum and tetanus toxins gain access to targets in the nervous system by different mechanisms, from this point forward in the pathogenesis, both botulinum and tetanus neurotoxins share a common mechanism of injury, the disruption of neurotransmitter vesicle exocytosis by disrupting the synaptic fusion complex. The mechanisms are demonstrated in Figures 4-28 and 4-29 for botulinum toxin and tetanus toxin, respectively. Thus diseases (clinical signs) that occur are the direct result of disruption of the function of myoneural (flaccid paralysis) and neural-neural (spastic paralysis) junctions. Botulinum and tetanus toxins have heavy and light chains and behave as typical A-B toxins composed of two units: a binding B-domain (heavy chain) that mediates transport via endocytosis and exocytosis and an enzymatically active A-domain (light chain) that serves to cleave proteins within the target cell. The heavy chain binds to the neuronal membrane of myoneural junctions (botulinum toxin) and nerve endings (tetanus toxin), and the entire toxin molecule enters the neuron via receptor-mediated endocytosis. The A-domain is cleaved from the B-domain within the target cell endocytotic vesicle and then released into the cytoplasm where it is active. The A-domain (light chain), a zinc-containing endopeptidase, leaves the endocytotic vesicle and enters the cytoplasm of the neuron and acts to cleave proteins that form the synaptic fusion complex. This complex, formed by fusion of synaptic vesicle proteins with presynaptic plasma membrane proteins, serves to bring neurotransmitter vesicles into contact with the neuronal cell membrane at the myoneural (botulinum toxin) and neural-neural (tetanus toxin) junctions and facilitates membrane fusion and release of excitatory (acetylcholine) and inhibitory neurotransmitters (glycine and γ-aminobutyric acid [GABA]), respectively.

Different types of glycosylphosphatidylinositol-anchored protein(s) may be expressed on different types of neurons, which may explain why B-domain of tetanus toxin binds only to inhibitory interneurons and not other types of neurons. Disruption of the synaptic fusion complex prevents neurotransmitter vesicles from fusing with the membrane, which in turn prevents release of neurotransmitters into the synaptic cleft. Proteins that form the synaptic fusion complex (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor [SNARE] proteins) include neurotransmitter vesicle proteins (such as vesicle-associated membrane proteins [VAMPs]/synaptobrevin) and presynaptic plasma membrane proteins (syntaxin, synaptosomal-associated protein [SNAP-25]). Different types of C. *botulinum* produce different types of toxins (toxin type A to G), and these types target and cleave specific types of SNARE proteins, synaptobrevin (cleaved by toxin types B, D, F, and G), syntaxin (cleaved by toxin type C), and synaptosomal-associated protein (cleaved by toxin types A, C, and E). Botulinum toxin seemingly does not cross the blood-brain barrier; therefore neural-neural junction functions in the CNS remain intact. Notwithstanding the profound neurologic signs of spastic paralysis and flaccid paralysis that occur with tetanus (C. *tetani*) and botulism (C. *botulinum*), respectively, macroscopic or microscopic lesions are not observed in the nervous system.

Meningitis (*Escherichia coli* and Other Bacterial Species). The pathogenesis of meningitis shares many of the mechanisms discussed for porcine polyserositis (see section on Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Pigs) and embolic vasculopathy/vasculitis (see section on Bacterial Diseases of Organ Systems, Cardiovascular System and Lymphatic Vessels, Disorders of Domestic Animals).

Disorders of Ruminants (Cattle, Sheep, and Goats)

Listeriosis (*Listeria monocytogenes*). The mechanism of injury in listeriosis is cell lysis caused by acute inflammation and its mediators and degradative enzymes. Gross lesions are often not observed, but when present consist of nodules and linear bands of gray-yellow

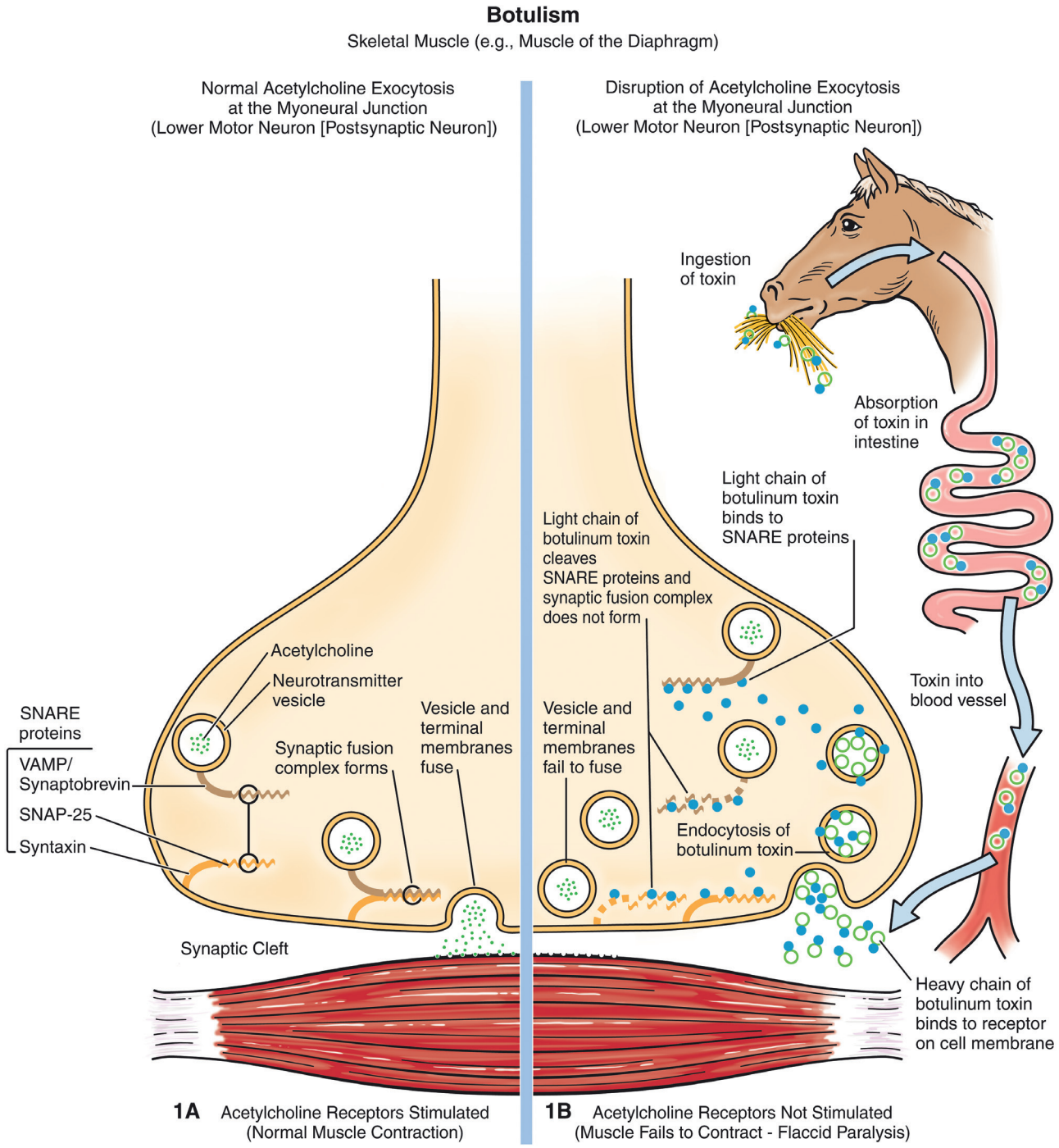


Figure 4-28 Mechanism of Myoneural Junction Dysfunction in Botulism. Note that botulinum toxin reaches the myoneural junction via the circulatory system. *SNAP,* Synaptosomal-associated protein; *SNARE,* soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; *VAMP,* vesicle-associated membrane protein. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

exudate (perivascular microabscesses formed by neutrophils) mixed with active hyperemia and/or hemorrhage commonly arranged in a perivascular pattern that are uniquely localized to the brainstem (see Fig. 14-88).

Cattle, sheep, and goats encounter *Listeria monocytogenes* in soil, animal feed, water, and feces; however, the greatest risk for contracting the disease occurs when ruminants are fed improperly stored silage in which the pH is not acidic enough to prevent overgrowth of the bacterium. Consumption of *L. monocytogenes*–contaminated silage is not sufficient to cause CNS disease, unless it occurs with a penetrating injury of the oral cavity caused by a stick or other sharp object (nail) that carries the bacterium in the silage into the submucosal connective tissue of the oral cavity or tongue. At this point the bacterium colonizes oral tissues, enters nerve endings in the oral cavity, and ascends into the CNS via retrograde axonal transport in cranial nerves. The oral cavity is primarily innervated by the trigeminal and other cranial nerves that terminate in the brainstem. Thus *L. monocytogenes* ultimately localizes to the brainstem (i.e., pons, medulla oblongata, and proximal cervical spinal cord). The mechanism of entry into nerve endings is unknown; however, it has

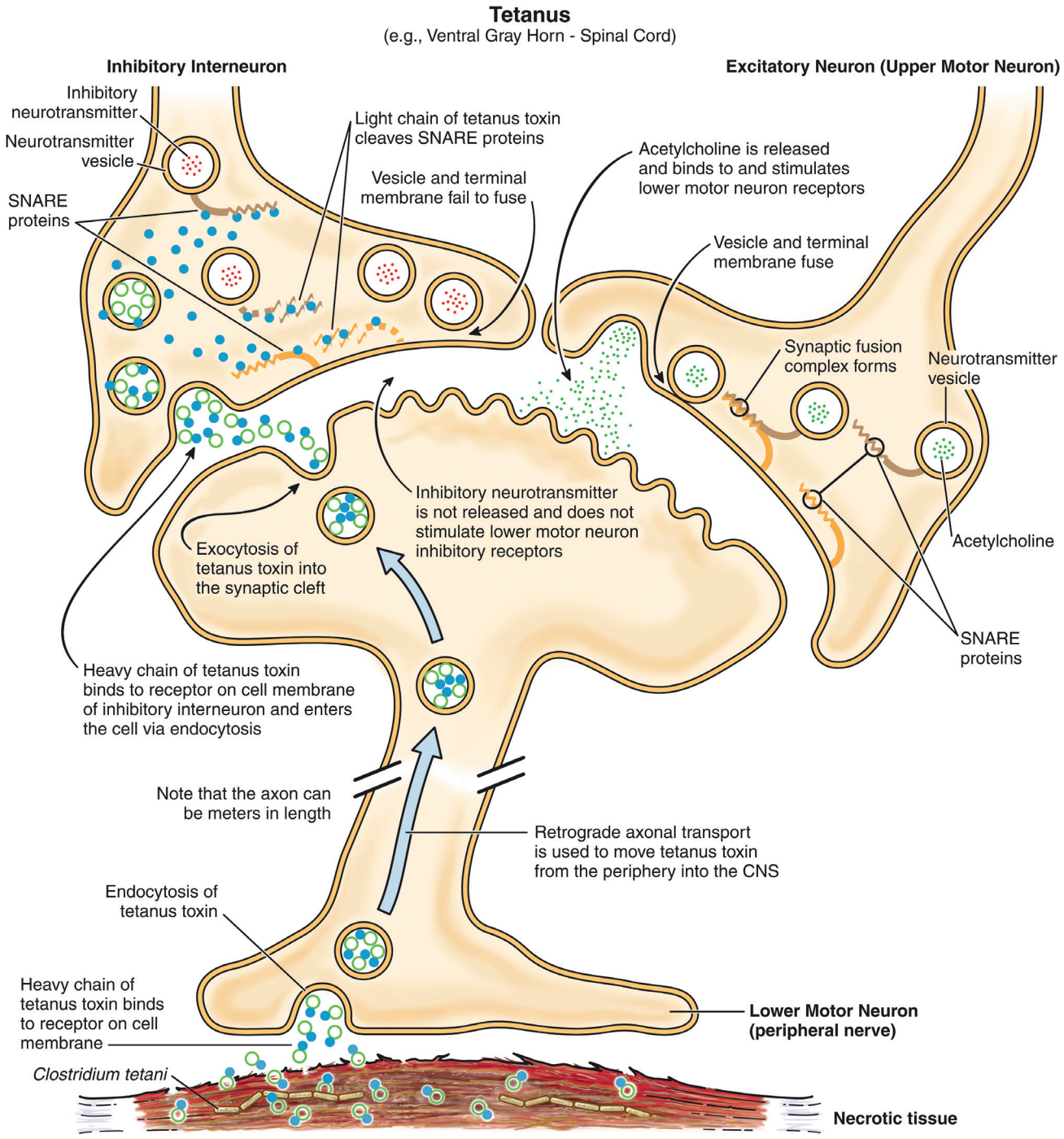


Figure 4-29 Mechanism of Neural-Neural Junction Dysfunction in Tetanus. Note that tetanus toxin reaches the neural-neural junction via retrograde axonal transport. The selectivity of tetanus toxin for inhibitory interneurons is likely mediated by the expression of different glycosylphosphatidylinositol-anchored protein(s) on different types of neurons. The B-domain of tetanus toxin appears to bind only to the type of glycosylphosphatidylinositol-anchored protein expressed on inhibitory interneurons. *CNS*, Central nervous system; *SNARE*, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

been shown experimentally in cell culture systems that *L. monocytogenes* gains entry into typically nonphagocytic cells through endocytosis and endocytic vesicles. Bacterial internalization, the entry process, is mediated by internalins (type A and B) that use target cell receptor E-cadherin, a transmembrane glycoprotein. Because *L. monocytogenes* resides in endosomes intracellularly within cell bodies of neurons when it arrives in the brainstem, it initially does not disrupt the blood-brain barrier and thus does not activate defense mechanisms provided by the innate (inflammation) and adaptive immune responses. The cytoplasm of infected neuron cell bodies appears to be permissive and allows free proliferation of the bacterium. This permissive environment also appears to be promoted by a bacterial virulence factor called *listeriolysin* O that inhibits immune responses and allows infected cells to hide from defense mechanisms.

Once free in the cytoplasm, the bacterium replicates to sufficient numbers and then begins the process of infecting other cells. In the cytoplasm the bacterium has a doubling time of approximately 1

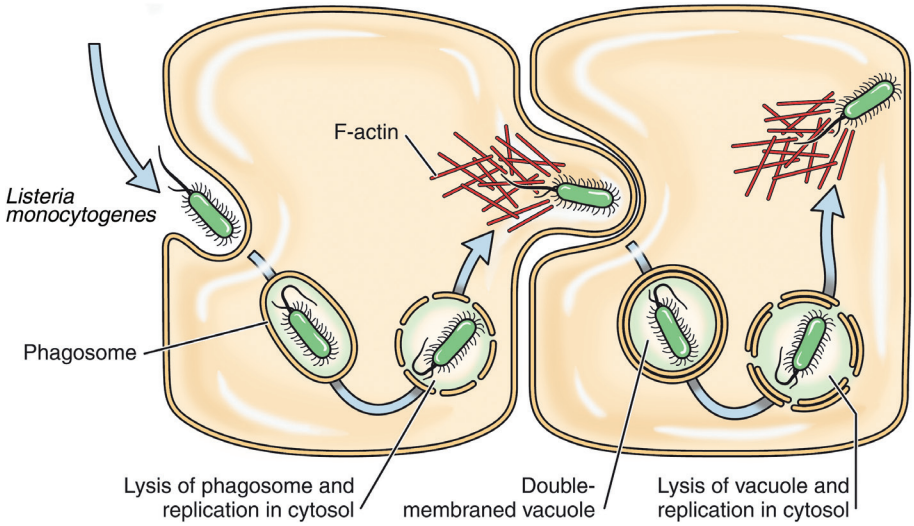


Figure 4-30 Mechanism of Infection in Listeriosis. *Listeria monocytogenes* propels itself via actin polymerization (*Listeria* actin-based motility) within a membrane pseudopod into cell membranes of adjacent neural cells forming invaginations of the membrane that ultimately result in double-membrane endocytotic phagocytic vesicles.

hour. When the number of bacterium in the cytoplasm reaches a level sufficient to facilitate infection of adjacent cells, bacteria move themselves, facilitated by a virulence factor in the cytoplasm, to the inner side of the cell membrane via polymerization and depolymerization of target cell actin filaments. Once near the cell membrane, aggregates of bacteria use a bacterial surface protein called *surface protein act*A to propel themselves via actin polymerization (listerial actin-based motility) and a pseudopod into cell membranes of adjacent cells, forming invaginations of the membrane that ultimately result in double-membrane endocytotic phagocytic vesicles (Fig. 4-30). This process is random, so it does not appear to target specific cells in the nervous system, only neighboring cells. These double-membrane endocytotic phagocytic vesicles are lysed by listeriolysin O, phospholipase C, and lecithinase, and the bacteria are released into the cytoplasm of newly infected cells. Experimentally, *L. monocytogenes* has been shown to infect neutrophils, macrophages, fibroblasts, endothelial cells, and various types of nerve cells, including neurons and microglial cells. It appears that infection of and injury to endothelial cells of capillaries initiates the inflammatory process. Once initiated, the blood-brain barrier is disrupted, and activation of the entire inflammatory cascade ensues. Neutrophils are the primary effector cells used by animal defense mechanisms to kill the bacterium. Experimentally, *L. monocytogenes*–infected endothelial cells express exuberant endothelial adhesion molecules (P- and E-selectin, intercellular adhesion molecule 1 [ICAM-1], and vascular cell-adhesion molecule 1 [VCAM-1]) resulting in activation of the neutrophil adhesion cascade and neutrophil binding, both components of the acute inflammatory response. The bacterium can also spread from macrophages to endothelial cells.

Thrombotic Meningoencephalitis (*Histophilus somni*). The pathogenesis of thrombotic meningoencephalitis (TME) begins as pulmonary histophilosis (see section on Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants [Cattle, Sheep, and Goats]). The mechanism of injury in the nervous system is infarction (cell lysis) secondary to occlusive ischemia caused by bacterial-induced arteritis (vasculitis) and subsequent thrombosis caused by acute inflammation and its mediators and degradative enzymes. Gross lesions are red hemorrhagic infarcts of varied sizes distributed at random throughout nervous tissue, especially in the cerebral cortices (see Fig. 14-89).

Cattle encounter *Histophilus somni* (formerly *Haemophilus somnus*) via inhalation of fomites or water droplets contaminated with the bacterium. It likely exists in nasal or oral biofilms as a commensal bacterium of mucosae. Environmental stressors, such as overcrowding, combined with other factors, such as poor ventilation and humidity or abrupt changes in ambient air temperature, could alter the commensal relationship, allowing the bacteria to replicate in sufficient numbers to colonize mucosae and spread the bacterium to other animals. After colonization of the respiratory mucosae, the bacterium spreads to the lung (see section on Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants [Cattle, Sheep, and Goats]; Pulmonary Histophilosis) and then gains access to the vascular system in areas of pulmonary inflammation, embolizes to the CNS (septicemia), and colonizes and infects small arterioles likely via ligand-receptor interactions. The first encounter with endothelial cells of arterioles occurs at anatomic sites in the brain in which there are abrupt changes in the laminar flow of blood resulting in turbulence, such as occurs at the interface between gray and white matter in the cerebral cortex. Turbulence appears to make the luminal surface of endothelial cell membrane more adherent to the bacterium and platelets. Experimentally, *H. somni* and its membrane LOS (a truncated form of LPS) have been shown to activate bovine platelets and increase the expression of adhesion molecules, such as ICAM-1 and E-selectin and tissue factor (factor III) on endothelial cells (surface is procoagulant). Tissue factor is a protein necessary for activation of blood coagulation cascades. Thus, because strains of *H. somni* have virulence factors that enhance the adherence of the bacterium to endothelial cells, such areas are prone to endothelial injury, exposure of collagen, platelet aggregation and activation, activation of clotting cascades, arterial thrombosis and obstruction, and infarction (the lesion of thrombotic meningoencephalitis).

Focal Symmetric Encephalomalacia (*Clostridium perfringens*). The pathogenesis of focal symmetric encephalomalacia begins as an enterotoxemia caused by *C. perfringens* in the small intestine of the alimentary system (see section on Bacterial Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Domestic Animals; Enterotoxemia (*Clostridium perfringens*). The mechanism of injury is acute coagulative necrosis of cells and tissues caused by

bacterial toxins, especially ε-toxin. Because ε-toxin is a permease that alters cell permeability, the vascular beds in affected intestinal tissues readily absorb toxins into the circulatory system. The mechanism of injury is cell lysis caused by bacterial toxins that act directly on endothelial cell membranes and neurons causing acute coagulative necrosis of affected cells (angiotoxin). Gross lesions include bilaterally symmetric malacia (acute coagulative necrosis of neuron cell bodies) and liquefactive necrosis of the basal ganglia, internal capsule, thalamus, and substantia nigra with edema and hemorrhage. Cerebral edema causes indistinct sulci and flattened gyri and in severe cases, coning of the cerebellar vermis through the foramen magnum.

It appears that the sequence of steps leading to focal symmetric encephalomalacia occurs in the first phase or early in the second phase of enterotoxemia before toxin-induced massive necrosis of the intestine occurs (see section on Bacterial Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Domestic Animals; Enterotoxemia [*Clostridium perfringens*]). Because microvascular beds of the alimentary system injured by enterotoxins are more permeable, they absorb large quantities of toxins that are carried to the brain via the blood vascular system. In the CNS they act to increase the permeability of capillary beds leading to release into the neuropil of blood plasma containing toxins, resulting in severe generalized vasogenic cerebral edema. Circulating ε-toxin likely accumulates preferentially in the brain via ligand-receptor interactions. Receptors expressed on different populations of endothelial cells in the body and within the brain may determine in part the specificity for certain neurons and nuclear groups within the nervous system. The cell membrane of cerebral endothelial cells is the probable site of toxin binding, and it appears that toxin-induced injury of endothelial cells leads to the expression of more receptors for circulating ε-toxin. Injury to the endothelium disrupts the integrity of the blood-brain barrier, leading to increased vascular permeability, vasogenic edema, and the diffusion of toxin into the neuropil, where it encounters neuron cell bodies. Acute coagulative necrosis of neuron cell bodies has been attributed to toxin-induced microthrombosis of capillaries, resulting in neuronal ischemia, and by direct cytotoxic action on neurons and other neural cells. The selective nature of neuronal lysis caused by ε-toxin may be explained by ligand-receptor interactions, selective metabolic vulnerability of specific populations of neurons, and/or the concentration of ε-toxin.

***Mannheimia* Meningoencephalitis (*Mannheimia haemolytica* A1).** The pathogenesis of *Mannheimia* meningoencephalitis shares many of the mechanisms discussed in the following sections: (1) Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants (Cattle, Sheep, and Goats); Bovine Pneumonic Pasteurellosis/Mannheimiosis (*Mannheimia [Pasteurella] haemolytica*); (2) Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Pigs; Porcine Polyserositis (*Haemophilus suis/parasuis*, *Actinobacillus suis*, *Streptococcus suis*, or *Escherichia coli*); and (3) Bacterial Diseases of Organ Systems, Cardiovascular System and Lymphatic Vessels, Disorders of Domestic Animals, Embolic Vasculopathy/Vasculitis (*Actinobacillus equuli*, *Escherichia coli*, *Staphylococcus* spp., *Streptococcus* spp., *Fusobacterium necrophorum*).

Disorders of Pigs

Edema Disease (*Escherichia coli*). See section on Bacterial Diseases of Organ Systems, Cardiovascular System and Lymphatic Vessels, Disorders of Pigs, Edema Disease (*Escherichia coli*); also see section on Bacterial Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Pigs; Edema Disease (*Escherichia coli*).

Pigs encounter *E. coli* through ingestion followed by colonization of intestinal mucosae (enterotoxemia phase). The enterotoxemia phase is discussed in the section on Bacterial Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Domestic Animals. This disease is caused by a specific strain of hemolytic *E. coli* having virulence factors for a bacterial enterotoxin called *Shiga toxin 2e* (also known as *verotoxin 2e*). It was initially called *edema disease principle*. It is absorbed in the alimentary system, circulates systemically in the blood vascular system, and is carried in the circulatory system to the brain. The mechanism of injury is lysis of endothelial and smooth muscle cells of arterioles (fibrinoid arteriopathy/arteriolopathy); thus the toxin biologically behaves as an angiotoxin. In the brain, vascular lesions are followed by secondary ischemia and necrosis of neural cells, particularly neurons in brainstem nuclei. Gross lesions include symmetric (bilateral) areas of yellow-gray malacia involving specific nuclei of the brainstem (see E-Fig. 10-27). It is unclear why lesions are symmetric; however, this observation suggests that ligand-receptor interactions or other targeting mechanisms are involved in cell specificity. Speculatively, receptors for Shiga toxin 2e could be expressed on specific populations of endothelial cells located within affected nuclei. Some endothelial and smooth muscle cells of arteritis and arterioles do express receptors for this toxin. This toxin causes vascular permeability changes and edema followed by endothelial injury and lysis leading to hemorrhage, intravascular coagulation, microthrombosis, and infarction (grossly malacia). Shiga toxin 2e acts to disrupt protein synthesis in affected cells leading to cell lysis.

Muscle

Disorders of Ruminants (Cattle, Sheep, and Goats)

Blackleg (*Clostridium chauvoei*). The mechanism of injury in blackleg is necrosis (acute gangrenous myositis) of muscle, connective tissues, and nervous tissues caused by α- and β-toxins released from vegetative forms of *C. chauvoei*. Gross lesions occur in large striated muscle groups and include dark red to black muscle that appears dry and contains gas bubbles (see Fig. 15-37). Affected muscle may have a rancid (spoiled butter) smell.

Cattle, sheep, and goats encounter spores through ingestion of plant matter and topsoil contaminated with spores often after disturbances (excavations) of the soil and pasture bed. Spores are carried by swallowing and peristalsis through the oral pharynx, esophagus, abomasum, and rumen to their final destination, the small intestine. It appears that spores can remain dormant in the small intestine or germinate into vegetative forms and become normal inhabitants of the alimentary system. How spores interact with mucosae and gain access to epithelial cells and mucosal macrophages is unknown. Other similar pathogens use M cells to enter Peyer's patches, so it is plausible that spores may gain access to M cells and use endocytosis/transcytosis to reach and infect macrophages in Peyer's patches. Although suggested but unproved, spores are likely the form of the bacterium that spread via M cells entry and then systemically by leukocyte trafficking to muscle and lie dormant in endosomes (or cytoplasm) of macrophages and dendritic cells. However, it is possible that vegetative forms of the bacterium spread to muscle as described earlier to ultimately "go dormant" and produce spores in macrophages and dendritic cells. If one or more of these mechanisms occurs, tropism for macrophages and dendritic cells of muscle is probably mediated by ligand-receptor interactions. Blackleg often follows some form of traumatic injury to muscle. It is thought that injury creates an anaerobic microenvironment with

lowered oxidation-reduction (redox) potential suitable for germination of spores. It is likely that traumatic injury and anaerobic conditions cause lysis of infected macrophages and dendritic cells with release of spores into the microenvironment. Spores germinate into vegetative forms of the bacterium and produce large quantities of a variety of toxins such as *C. chauvoei* toxin A (pore-forming α-toxin), oxygen-stable hemolysin, DNase (β-toxin), hyaluronidase (δ-toxin), oxygen-labile hemolysin, and neuraminidase. These toxins diffuse out from the site of bacterial replication and coagulate muscle tissue and its vascular supply, resulting in acute gangrenous myositis.

Malignant Edema (*Clostridium septicum*). The pathogenesis of malignant edema is similar to that of blackleg discussed in the previous section regarding bacterial replication, the production of toxins, tissue injury, and gross lesions affecting striated muscle and blood vessels. However, the mechanism of spread to muscle is different. Cattle, sheep, and goats encounter spores through wounds caused by penetrating objects, such as a wire, which carries spores into the wound. Wounds caused by castration, tail docking, unsanitary vaccination, and other management practices can also be infected with spores. The injury must be sufficient to create an anaerobic microenvironment in the wound with lowering of the oxidation-reduction (redox) potential suitable for germination of spores. Once spores germinate, vegetative forms release toxins that injure and coagulate muscle and vascular tissues resulting in necrosis and edema much like in blackleg.

Big Head and Black Disease (*Clostridium novyi*). The pathogeneses of big head and black disease are very similar to malignant edema and blackleg, respectively. In big head of sheep, penetrating wounds of the skin of the head caused by horns during fighting establish the initial anaerobic microenvironment for spores to germinate. The resulting pathogenesis is similar to that which occurs in malignant edema. Black disease of cattle and sheep occurs because of fluke migration (*F. hepatica*) through the liver (see Figs. 8-60 and 8-61) that causes hepatocellular necrosis and establishes an anaerobic microenvironment suitable for germination of spores. Kupffer cells likely contain dormant spores. As in blackleg, spores are likely ingested, enter through mucosae (M cells) of the small intestine, become phagocytosed by cells of the monocyte-macrophage system, and spread via leukocyte trafficking to Kupffer cells of the liver.

Bone, Joints, Tendons, and Ligaments

Disorders of Ruminants (Cattle, Sheep, and Goats)

Lumpy Jaw (*Actinomyces bovis*). The mechanism of injury in lumpy jaw is cell lysis attributable to pyogranulomatous inflammation and its mediators. Gross lesions include misshapen bones of the mandible and/or maxilla resulting from abscesses, fibrosis, and fistulous tracts (i.e., pyogranulomatous osteomyelitis). Cut surface of affected bone has numerous randomly distributed discrete and coalescing yellow-white granulomas surrounded by remodeled bone intermixed with bands of fibrous connective tissue (see Fig. 16-55). *Actinomyces bovis* is a commensal bacterium of mucosae of the oral cavity of cattle and sheep, likely existing in a biofilm. The bacterium can infect bone by several routes: (1) genetic or developmental defects of the tooth root and/or socket that provide access to bone, (2) injury to a tooth and its socket opening a pathway into the bone, and (3) penetrating wounds that give the bacterium access to the bone and its periosteum. During chewing, the bacterium is carried by direct extension through the mucosa into submucosal connective tissues via penetrating wounds such as those caused by sharp foreign bodies like sticks or wires. The object may penetrate the periosteum and the bone, giving the bacterium direct access to these tissues. The bacterium colonizes submucosal connective tissue, and LPS of the cell wall, in part, likely plays a key role in the pathogenesis of the granulomatous inflammatory response. Little is known about virulence factors, ligand-receptor interactions, target cells, toxins, capsule antiphagocytic molecules, or other factors that may contribute to the pathogenicity of this bacterium. *A. bovis* can spread via lymphatic vessels to regional lymph nodes and cause a similar inflammatory response in these tissues.

Integumentary System

Disorders of Pigs

Greasy Pig Disease (*Staphylococcus hyicus*). The mechanism of injury in greasy pig disease is cell lysis and exfoliation of cells of the skin secondary to inflammation and its mediators and degradative enzymes. Gross lesions include areas of patchy red skin (active hyperemia of acute inflammation) followed by thickening of the reddened skin and the formation of reddish brown macules, vesicles, and pustules first around the eyes, nose, lips, and ears and then the flanks and abdomen (see Fig. 17-69). Affected skin, mostly through inflammation, exudes large quantities of a greasy exudate consisting of serum and sebum mixed with inflammatory cells, degradative enzymes, and cell debris. This exudate is the basis for naming the disease exudative epidermitis.

Pigs encounter *Staphylococcus hyicus* through fomites and body fluids contaminated with the bacterium. This bacterium is likely a commensal organism (biofilm) that resides in the skin and hair follicles of healthy pigs. Environmental stressors, such as skin trauma caused by overcrowding combined with other factors such as poor ventilation and humidity or abrupt changes in ambient air temperature, could alter the commensal relationship, allowing bacteria to replicate in sufficient numbers to colonize the skin, spread the bacterium to other animals, and cause disease. Infected droplets are deposited on the surface of the skin, but the bacterium under most conditions is not able to infect and colonize intact skin. It appears that skin trauma is usually a prerequisite for colonization because abrasions on the feet and legs or lacerations on the body often precede the onset of the disease.

The role of virulence factors, ligand-receptor interactions, and cells of the monocyte-macrophage system are poorly understood in the pathogenesis of the disease. Exfoliation-inducing as well as epidermitis-inducing exotoxins cause separation of epithelial cells of the stratum corneum and spinosum and aid in the invasion of the bacterium into the skin. It serves to expose vascularized ECM tissues in traumatized skin. Fibronectin-binding proteins expressed on the surface of the bacteria appear to act as adhesins, allowing the bacteria to bind to the fibronectin present in collagen, fibrin, and heparin sulfate proteoglycans of traumatized skin. Fibronectin is a glycoprotein of vascularized ECM tissues and is produced by cells such as fibroblasts. Once the skin is colonized, the infection appears to spread to the hair follicles, leading to suppurative inflammation and sebaceous gland hyperplasia and hypersecretion (i.e., greasy pig). It also appears that acute inflammation and its effector cells, such as neutrophils, play a central role in the onset and progression of the skin lesions. Capsule polysaccharides and protein A in the bacterial wall appear to block phagocytosis of the bacterium by neutrophils and increase the ability of bacteria to survive and replicate in vascularized ECM tissues of the skin.

Diamond Skin Disease (*Erysipelothrix rhusiopathiae*). The mechanism of injury in diamond skin disease is cell lysis and infarction of skin secondary to cutaneous vasculitis. Gross lesions include active hyperemia and red-purple skin affecting the ears, ventral abdomen, and legs followed by thrombosis, ischemia, and infarction resulting in rhomboidal (diamond) red-purple areas of skin (cutaneous infarcts) (see Figs. 17-70 and 10-80).

Pigs encounter *Erysipelothrix rhusiopathiae* through ingestion of fomites and body fluids contaminated with the bacterium. This bacterium is likely a commensal organism that resides in a biofilm of mucosae of the pharynx and tonsillar epithelia of healthy pigs. Environmental stressors, such as overcrowding combined with other factors such as poor ventilation and humidity or abrupt changes in ambient air temperature, can alter the commensal relationship, allowing the bacteria to replicate in sufficient numbers to colonize mucosae and spread the bacterium to other animals. Infected droplets are deposited on pharyngeal mucosae, where bacteria encounter the mucus layer and mucosal epithelial cells. It is unclear how this nonmotile bacterium is able to penetrate the mucus layer and gain direct access to the luminal membrane of epithelial cells. Additionally, it is unclear if and how the bacterium colonizes the mucus layer and mucosae. It appears that neuraminidase may be a virulence factor for *E. rhusiopathiae* potentially involved in initial interactions with and invasion of the mucus layer of pharyngeal mucosae. Neuraminidase acts to remove sialic acid from glycoproteins, glycolipids, and oligosaccharides expressed on target cells, potentially exposing new receptors for the bacterium. Also, it is probably important in the disease when the bacterium attaches to, colonizes, and invades cutaneous endothelial cells leading to vasculitis, thrombosis, infarction, and disseminated intravascular coagulation.

Other virulence factors involved in mucosal colonization and systemic spread of the bacterium include capsular polysaccharides (antiphagocytic properties), surface proteins (adhesins, antiphagocytic properties, biofilm formation), invasins such as hyaluronidase (invade ECM tissues), and enzymes such as superoxide dismutase and catalase (block the effects of the respiratory burst of phagocytosis and oxygen free radicals). Transcytosis could move bacteria through mucosal cells to the basal surface of mucosal epithelial cells to encounter local macrophages and lymphoid cells in the tonsils. Alternatively, mucosal macrophages could phagocytose bacteria in the mucus layer, migrate through the mucosal barrier, and spread them via leukocyte trafficking to the same cells.

Macrophages within the tonsil are likely used by the bacterium for replication and growth and then to spread bacteria via leukocyte trafficking in lymphatic vessels to regional lymph nodes to infect additional macrophages. Ligand-receptor interactions are likely involved, and bacterial surface proteins appear to serve as adhesins to macrophage and endothelial cell membrane receptors. Specific bacterial adhesins and target cell receptors on macrophages and endothelial cells have not been identified. Once bound to cell membrane, the bacterium is phagocytosed and retained in a phagosome within the cell cytoplasm. *E. rhusiopathiae* grows and replicates intracellularly in phagosomes and phagolysosomes. Capsular polysaccharides are able to inhibit phagocytosis of the bacterium by neutrophils and to a limited extent by macrophages. However, macrophages are used by the bacterium to isolate it from host innate and adaptive immune responses. Although it appears that phagosome-lysosome fusion occurs, capsular polysaccharides appear to block the oxidative burst and prevent killing of the bacterium by molecules present in the lysosome (see Figs. 4-13 and 4-14). Although undetermined, bacteria-infected macrophages in regional lymph nodes then probably spread the bacterium systemically via leukocyte trafficking using lymphatic vessels and the thoracic duct or postcapillary venules and the venous system to the systemic circulatory system and then to capillary beds within the skin. Cutaneous infarcts (also vascular-related lesions in other organs such as the kidney) suggest these bacteria may have tropisms for vascular endothelial cells. It is unclear why this occurs, but it is likely linked to the expression of bacterial virulence factors and ligand-receptor interactions with host endothelial cells. In addition to cell lysis attributable to direct infection of endothelial cells by the bacterium, bacterial neuraminidase may also activate the alternative complement pathway and induce thrombocytopenia, producing complement-derived chemotactic factors that could contribute to injury of capillary beds in local vascularized connective tissues. These mechanisms may contribute, in part, to the development of vegetative valvular endocarditis and arthritis that occurs in the chronic septicemic form of this disease.

Disorders of Dogs

Canine Pyoderma (*Staphylococcus intermedius*). The pathogenesis of canine pyoderma appears to be similar to that of greasy pig disease (see section on Bacterial Diseases of Organ Systems, Integumentary System, Disorders of Pigs, Greasy Pig Disease [*Staphylococcus hyicus*]). Skin trauma arising from pruritus and scratching or from existing skin disease leads to the exposure of vascularized ECM tissues and its colonization by bacteria. Although incompletely characterized, it is likely that a variety of virulence factors are involved in canine pyoderma, including surface proteins (colonization of host tissues); invasins such as leukocidin, kinases, hyaluronidase (promote bacterial spread in tissues); surface factors such as capsule polysaccharides and protein A (inhibit phagocytosis); and exotoxins and exfoliative toxins such as hemolysins, leukotoxin, and leukocidin (cause cell lysis).

Female Reproductive System and Mammary Gland

Disorders of Domestic Animals

Brucellosis (*Brucella* spp.). The pathogenesis of brucellosis begins as an infection of regional and systemic lymph nodes facilitated by entry through mucosae of the respiratory and alimentary systems (see section on Bacterial Diseases of Organ Systems; Bone Marrow, Blood Cells, and Lymphatic System; Disorders of Domestic Animals; Brucellosis [*Brucella* spp.] for more detail). The mechanism of injury is cell lysis caused by pyogranulomatous inflammation and its mediators and degradative enzymes. Gross lesions include aborted fetuses; necrosis, inflammation, and fibrinoid exudation of uterine caruncles and fetal cotyledons (see Fig. 4-27); and a yellow-white uterine exudate. Bacteria spread in macrophages via leukocyte trafficking from regional lymph nodes to the caruncular side of placentomas when they likely leave the vascular system to migrate into and through these tissues. Although it is unclear what additional cells in the placentoma are infected during transplacental spread to the fetus, trophoblasts are infected with bacteria. Other cell types may be involved. Bacteria then could spread in fetal macrophage-like cells within the fetal circulatory system to the fetus via the umbilical cord or within the allantoic and amniotic membranes and infect the fetus via contact with fetal mucosae of the respiratory or alimentary systems, but if this occurs or what cells facilitate this spread is unclear.

Disorders of Ruminants (Cattle, Sheep, and Goats)

Bovine Mastitis (*Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae,* and *Escherichia coli*). The mechanism of injury in bovine mastitis is lysis of all cell populations in the mammary gland from (1) bacterial toxins, (2) inflammation and its mediators and degradative enzymes, and (3) induced reparative responses such as fibrosis. Gross lesions in acute mastitis include firm, swollen, edematous, and occasionally hemorrhagic glands and ectatic ducts and sinuses containing yellow-white exudate (see Figs. 18-49 to 18-51, 18-54, and 18-55). In chronic mastitis, tissues are firm and consist of large zones of fibrous connective tissue that have replaced and displaced remaining normal glands (see Fig. 18-52). Inflammatory exudate is difficult

to observe unless abscesses have formed. Ducts and sinuses may be ectatic.

Animals encounter these bacteria through physical contact in fomites or fluid droplets from mammary gland, uterine, or fecal origin on milking equipment and human hands. They commonly become commensal microbes that reside in biofilms of the mucous membranes of the teat canal and mammary duct and sinuses. Trauma to mucosae in the gland induced by pressure changes acting on the duct system caused by milking likely makes mucosae more suitable for colonization and alters the commensal relationship, allowing the bacteria to replicate in sufficient numbers to spread the bacterium within the gland and to other animals mechanically during milking. Mastitis is an ascending infection, and milk in canals and sinuses is a suitable culture media for initial growth of bacteria. This environment is not suitable in the long term for survival of bacteria; thus they attempt to colonize mucosae to sustain the infection. Ligand-receptor interactions are likely involved in the adherence of these bacteria to receptors on mucosal epithelial cells; however, specific bacterial adhesins and target cell receptors have not been clearly identified. Once mucosae are colonized, bacteria employ mechanisms to sustain the infection. For example, *S. aureus* produces toxins, such as superantigens, leukocidins, hemolysins, coagulase, and likely α-, β-, and δ-toxins (virulence factors) that result in cell membrane injury and cell lysis and the activation of mucosal macrophages and inflammation. The severity of this lesion and its progression to gangrenous mastitis in the peracute and acute forms are dependent on the type and quantity of toxins secreted by the bacterium as determined by its virulence factors. Additionally, activated mucosal macrophages secrete proinflammatory cytokines resulting in the recruitment of neutrophils from the systemic circulation, through the mucosa, and ultimately into the milk, thus increasing the somatic cell count. With the focus of inflammation on the mucosa, epithelial cells and subjacent basement membrane are injured, killed, and sloughed, providing bacteria with access to vascularized ECM tissues of the gland. Using bacterial surface proteins, they are able to adhere to and colonize ECM tissues, likely using receptors expressed on molecules such as fibronectin, vitronectin, laminin, and collagen in the matrix. This process allows bacteria to evade many of the harmful actions of the innate and adaptive immune responses. Additionally, capsular polysaccharides block phagocytosis by neutrophils and macrophages. As a result, acute inflammation progresses with time to chronic inflammation with fibrosis (see Chapter 3), which is a common manifestation of mastitis caused by *S. aureus*.

Chronic mastitis is often linked to formation of mucosal biofilms. In mastitis caused by *Streptococcus agalactiae* and *Streptococcus dysgalactiae*, the bacteria use most of the mechanisms employed by *S. aureus* with one important exception. They lack virulence factors that injure the mucosa and allow the bacterium to invade vascularized ECM tissues and colonize this area. Thus the bacterium is limited to colonizing the mucosa and causing inflammation at the mucosal barrier. The outcome of this process is loss of mucosal epithelial cells lining glands, collapse of the glands, and replacement of the glands with fibrous connective tissue. In mastitis caused by *E. coli* and other coliforms, the bacterium uses most of the mechanisms discussed previously. However, in the initial phases of colonizing the mucosa, endotoxins (LPS) and other toxic molecules released from Gram-negative bacteria cause tissue injury and cell lysis, affecting the mucosa, lamina propria, submucosa, and capillary beds. The concurrent acute inflammatory response with neutrophils and their degradative enzymes exacerbate the severity of the injury. This outcome leads to tissue necrosis, edema, and hemorrhage. Endotoxin is also absorbed by the capillaries and can cause endotoxic shock of the circulatory system and death of affected animals (see Chapters 2 and 3).

Male Reproductive System

Disorders of Domestic Animals

Brucellosis (*Brucella* spp.). The pathogenesis of brucellosis begins as an infection of regional and systemic lymph nodes facilitated by entry through mucosae of the respiratory and alimentary systems (see Bacterial Diseases of Organ Systems; Bone Marrow, Blood Cells, and Lymphatic System; Disorders of Domestic Animals; Brucellosis [*Brucella* spp.] for more detail). The mechanism of injury is cell lysis caused by pyogranulomatous inflammation and its mediators and degradative enzymes. *Brucella* spp. spread in macrophages via leukocyte trafficking from regional lymph nodes to the testes, epididymides, and other male reproductive tissues. Gross lesions include enlarged and deformed testes and epididymides attributable to a yellow-white pyogranulomatous exudate resulting from the inflammatory response against the bacteria in the tissues (see Fig. 4-27).

Viral Diseases

Viruses are approximately a hundred times smaller than bacteria, and viruses, like bacteria, are genetically programmed to replicate endlessly, if all growth factors, metabolic needs, and microenvironments for replication are satisfactorily met. However, viruses are unable to produce energy and contain a limited number of enzymes; therefore they are completely dependent on target cells for such resources and thus are obligate intracellular parasites. They have evolved to specifically use target cells in animals that are susceptible to and suitable for completion of their viral replication cycles.

Target Cells

The term target cell assigns specificity to which cell(s) (see Fig. 4-6) of what organ system(s) are infected by viruses. This process is based on ligand-receptor interactions (see section on Target Cells and Substances at the beginning of this chapter), in which attachment and binding occur between envelope or capsid attachment proteins on the surface of viruses and receptors on target cell membranes (Fig. 4-31). Receptors are often expressed in unique patterns on target cells, and these patterns may determine the routes used by viruses to infect target cells. For example, parvoviruses and herpesviruses use specific receptors with specific distribution patterns to attach to and enter target cells. Parvovirus (canine parvovirus enteritis) infects intestinal crypt epithelial cells through receptors expressed on the basolateral surface of the cells, thus using a circuitous route via leukocyte trafficking to Peyer's patches and M cells to gain access to this surface. Although this route is not likely the most direct route to crypt epithelial cells, it may be advantageous for survival of the virus to avoid contact with gastric acids, bile, and other potentially toxic molecules in the intestinal lumen. Bovine herpesvirus 1 (infectious bovine rhinotracheitis [IBR]) infects epithelial cells of the respiratory system through receptors expressed on the apical and lateral surfaces of the cells. These receptors are distributed above junctional complexes formed with adjacent epithelial cells; therefore virus in the lumen of the respiratory tract can encounter appropriate receptors on mucosae.

In total, experimental studies suggest there are approximately 10^4 to 10^6 potential receptors for viruses expressed on a single target cell. This total is composed of many types or categories of receptors, so the total number of a specific kind of receptor is much less than this range. Receptors on target cells include those for complement, growth factors, neurotransmitters, integrins, adhesion molecules, complement regulatory proteins, phospholipids, and carbohydrates.

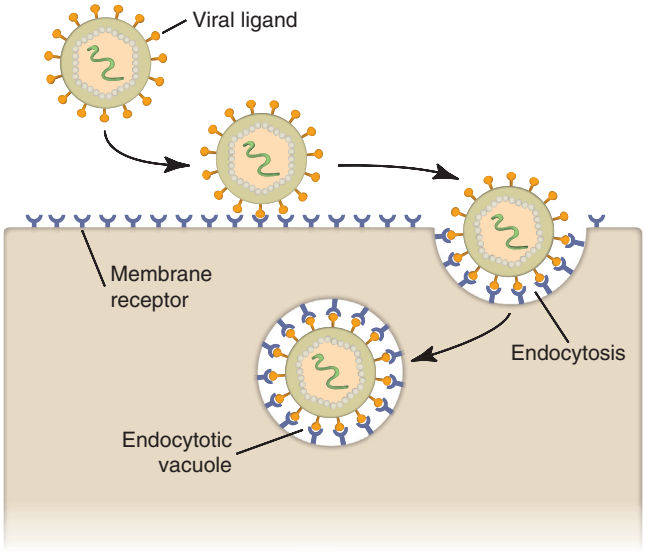


Figure 4-31 Ligand-Receptor Interactions. Ligand (viral envelope or capsid proteins)-receptor (target cell membrane proteins) interactions common to all cells are used by viruses to attach to and infect specific target cells. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

In general, specific viruses use one of these receptor types to infect a specific type of cell; however, some viruses use several receptors (coreceptors), which allows for invasion of a variety of cell types (i.e., pantropic viruses such as canine distemper virus).

In the context of diseases caused by viruses, target cells that allow replication of a virus are called *permissive cells*, whereas those that do not are called nonpermissive cells. Generally, virus-infected permissive cells are usually killed by the virus (cell lysis), whereas virus-infected nonpermissive cells are not killed. As an example, the pathogenesis of the lentiviral disease, maedi-visna, is determined, in part, by nonpermissive cells (immature progenitor monoblasts and promonocytes in bone marrow) and permissive cells (mature monocytes and macrophages in the blood vascular system and tissues). Infection of nonpermissive progenitor cells in bone marrow is used to provide a reservoir of immunologically protected virus-infected cells that become permissive when they mature into monocytes and macrophages in the vascular system and when they migrate into specific tissues and organs. These permissive macrophages are ultimately killed by virus replication, and the cell is lysed to release virus into areas with new target cells such as in the lung, brain, mammary gland, and synovia.

Viral Pathogenicity and Replication Cycle

Viral pathogenicity is a term used to express the relative severity of disease, clinical signs, and lesions caused by viruses. It is determined, in large part, by the expression of viral genes used to produce structural or functional proteins and other molecules needed to sustain or enhance replication of the virus. Similar to bacteria, viral genes and their proteins behave as virulence factors; however, the number of viral virulence factors is nowhere near the number, complexity, and diversity of those expressed by bacteria. In viruses the actions of virulence factors focus on (1) attachment to, replication in, and shedding from target cells and (2) the processes of modulating and/or evading host defense mechanisms. Thus the type, quantity, and arrangement of nucleic acid in viruses provide the bases for genomic diversity and the transfer of virulence factors among viruses (see later section on Mechanisms of Genomic Change).

During viral replication the pathogenicity of a disease and the survival of target cells are determined by (1) how the virus uses and/or alters the functions of cell organelles and the transcriptional and translational processes and (2) how it escapes from target cells such as by lysis. The phrase *virus replication cycle* is used herein to merge under a single key concept the chronologic sequence of steps that occurs when a virus encounters and enters cells, takes over the functions of cellular organelles and metabolic processes, produces new virus, and ultimately injures or lyses cells to cause disease. The outcome of encounters between viruses and target cells are often reflected in specific organ systems by clinical signs and alterations in biochemical analyses of blood samples.

The viral replication cycle has five key steps (Fig. 4-32):

- Attachment
  - Viruses have attachment proteins in their capsids or envelopes that bind to specific receptors on target cell membranes.
- Entry (Penetration)
  - Following binding, viruses enter cells via endocytosis/phagocytosis and release their contents into the cell.
- Spread (Uncoating)
  - Viral nucleic acid (RNA or DNA) and/or proteins are moved to specific locations within the cell.
- Replication
  - Viral nucleic acid (RNA or DNA) and/or proteins take over functions of normal cell processes to synthesize and aggregate additional copies of these components.
- Shedding (Release or Egress)
  - Viruses reassemble from resynthesized components and escape from cells by budding or cell lysis.

Specific groups of viruses (nonenveloped viruses) attach to target cells using a protein coat (viral coat, capsid, or capsomeres) (Fig. 4-33); others attach via a viral envelope (enveloped viruses) (see Fig. 4-33). Protein molecules, derived from viral genes and expressed in the protein capsid or envelope, are called *attachment proteins*. Attachment proteins are viral virulence factors that provided the basis for genomic diversity among viruses and play a central role in their replication and existence. However, the "infectious" advantages provided by attachment proteins for the virus are counterbalanced by specific disadvantages. Viral attachment proteins in the virus and present in infected target cells are recognized as foreign by the innate and adaptive immune systems via defense mechanisms provided by TLRs, NK cells, cytotoxic T lymphocytes, and acute inflammation, as examples. Once attachment occurs, viruses can enter target cells by one of two main mechanisms: (1) receptor-mediated endocytosis (phagocytosis) or (2) fusion. Once inside target cells, viruses initiate a variety of virus-specified processes to complete their replication cycles, such as replication of their genome, core proteins, and capsid and envelope proteins; assembly of new viruses; and release of new viruses from the cell.

When a DNA virus enters a target cell and its components are released into the cytoplasm, the DNA genome is transferred into the nucleus, where it uses target cell nuclear organelles to transcribe viral messenger RNA (mRNA) and later replicate new viral DNA (see Fig. 4-32). Viral mRNA leaves the nucleus and in the cytoplasm is translated into structural and nonstructural proteins of the virus by target cell organelles. After all viral proteins are translated in the target cell cytoplasm, new viral DNA is replicated (transcribed) and transferred into the cytoplasm, where it is assembled with structural and nonstructural proteins to form new virus.

When an RNA virus enters a cell and its components are released into the cytoplasm, the RNA genome, depending on the virus, can (1) replicate new viral RNA from the cytoplasmic viral RNA via

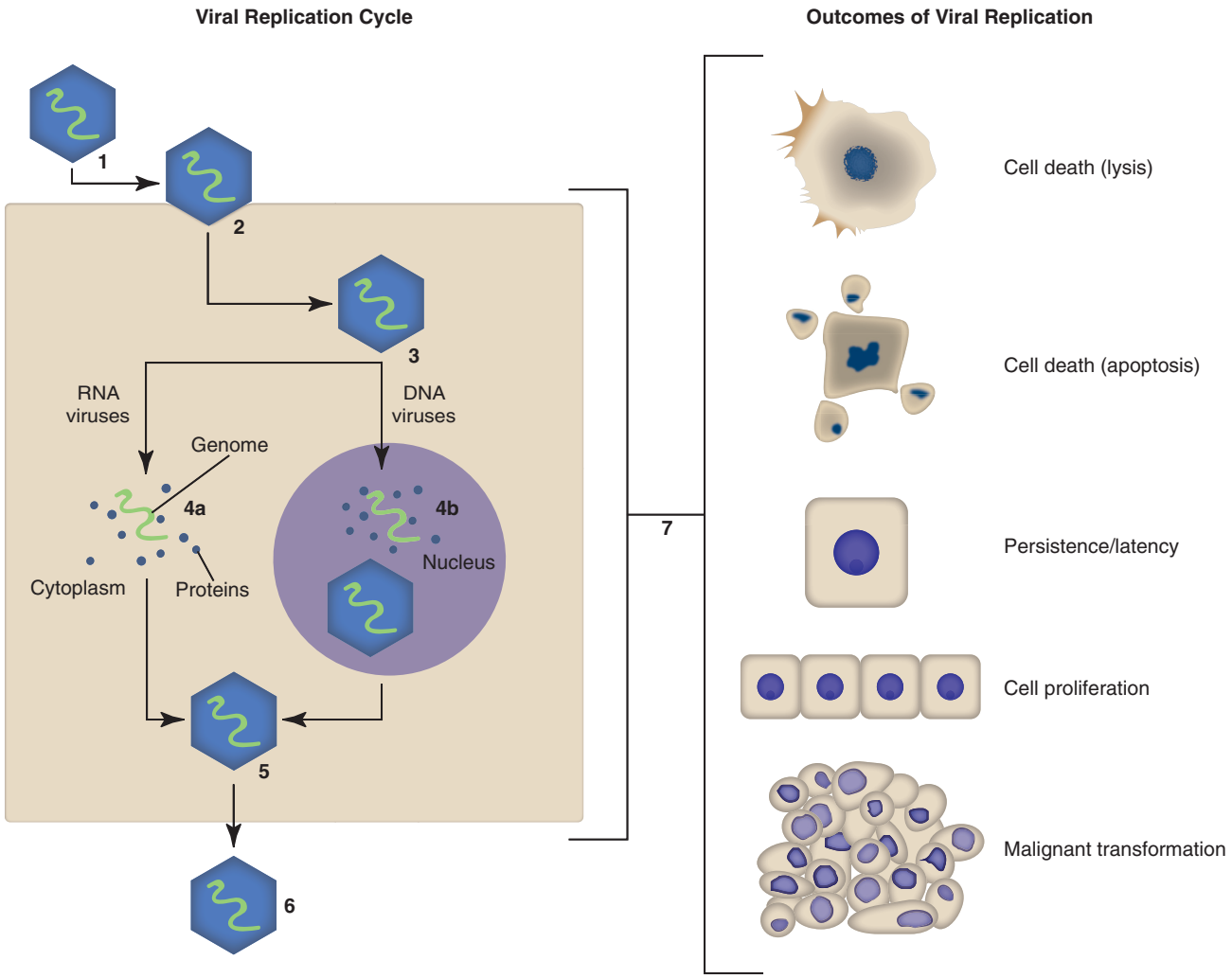


Figure 4-32 Viral Replication Cycles. Viruses do not replicate through division; in its place they use the organelles and biochemical processes of target cells to produce copies of their genome and proteins and assemble them into progeny. *1*, Interaction with and "recognition" of a target cell. *2*, Attach to the target cell membrane via ligand-receptor interactions (see Fig. 4-31). *3*, Enter the target cell via endocytosis/membrane fusion and spread genomic and protein components via "uncoating" to specific locations within the cell. *4a*, RNA viruses most commonly replicate in the cytoplasm. *4b*, DNA viruses most commonly replicate in the nucleus. *5*, RNA viruses reassemble in the cytoplasm; whereas DNA viruses are reassembled in the nucleus and transported through the cytocavitary system (see Fig. 1-3) for release at the cell membrane. *6*, Release of progeny from the target cell by budding or cell lysis. *7*, Outcomes of viral infection: cell death (lysis) (e.g., parvovirus—canine parvovirus enteritis); cell death (apoptosis) (e.g., morbillivirus—canine distemper); persistent infection (e.g., lentivirus—ovine progressive pneumonia); latent infection (e.g., herpesvirus—infectious bovine rhinotracheitis); cell proliferation (e.g., papillomavirus—sarcoids of horse skin); malignant transformation (e.g., retrovirus—feline leukemia). (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

its own viral RNA-dependent RNA polymerase or (2) make viral DNA from viral RNA via RNA-dependent DNA polymerase (viral reverse transcriptase) and then use target cell nuclear and cytoplasmic organelles to transcribe and translate new proteins and viral RNA (see Fig. 4-32). Thus the genome of RNA viruses must express genes that code for enzymes such as RNA-dependent RNA polymerase and RNA-dependent DNA polymerase. Detailed coverage of these processes is outside the scope of this chapter and can be reviewed in virology textbooks; however, these replicative processes often lead to injury and cell lysis.

In general, nonenveloped viruses (viral protein coats or capsids) are released from target cells only when cell lysis occurs, whereas enveloped viruses are released from target cells by budding from cell membrane, and the cell usually does not lyse (a viable cell remains) except for infections with herpesviruses (see later). Enveloped viruses with envelope glycoproteins must acquire an envelope by budding through cellular membranes such as the plasma membrane, membranes of the Golgi complex or rough endoplasmic reticulum, or nuclear membrane. During transcription and translation of viral genes and proteins, new viral envelope glycoproteins are inserted into target cell membranes of the ER and Golgi apparatus as examples (i.e., cytocavitary system) and then moved to specific locations in cell membrane within the cell. These are the sites where virus buds from target cell membrane and acquires envelope glycoproteins. Most viruses that bud from the cell membrane do not cause cell lysis except for those that bud from the Golgi complex or rough endoplasmic reticulum (flavivirus, coronavirus, arterivirus, and bunyavirus) or the nuclear membrane (herpesvirus).

Virus capsid proteins and envelope glycoproteins are used immunologically as a means to clinically prevent (e.g., vaccination) or control (e.g., pharmaceutical products) diseases caused by viruses by developing strategies to block one of more of the steps in the viral

attachment or replication cycle. Antibiotics have no effect on viruses; however, fortunately, viral infections (viral antigens) usually activate host innate and adaptive defense mechanisms and cause an immune response (cell-mediated), which can completely eliminate a virus or prevent an infection by a virus (vaccination). However, these defensive responses can also injure and lyse target cells, leading to disease. The list of structural and biochemical effects that viruses have on target cells is extensive. These effects are often called *cytopathic effects*, and as a general rule, many viral infections result in lysis of target cells. Depending on the virus and its replication cycle, injury to and lysis of target cells can occur at any point during the attachment, fusion, penetration, synthesis, assembly, or release phases. Generally viruses cause injury and lysis most commonly by two mechanisms: (1) as a result of taking over cell transcriptional and translational processes and (2) when they exit from infected cells. Additionally, causes of cell lysis include alterations in cell membrane structure and function, including direct damage to cell membranes (e.g., lytic phospholipids, endolysins, holins, and spanins), pore formation (viroporins), ion transport, and secondary messenger systems; alterations in metabolic processes, including activation cascades leading to altered cellular activities; alterations of target cell antigenic or immune properties, shape, and growth characteristics; inhibition of the synthesis of target cell macromolecules, including DNA, RNA, and protein; and direct (protein messenger molecules) and indirect (inflammatory mediators) activation of cell lysis and apoptosis cascades.

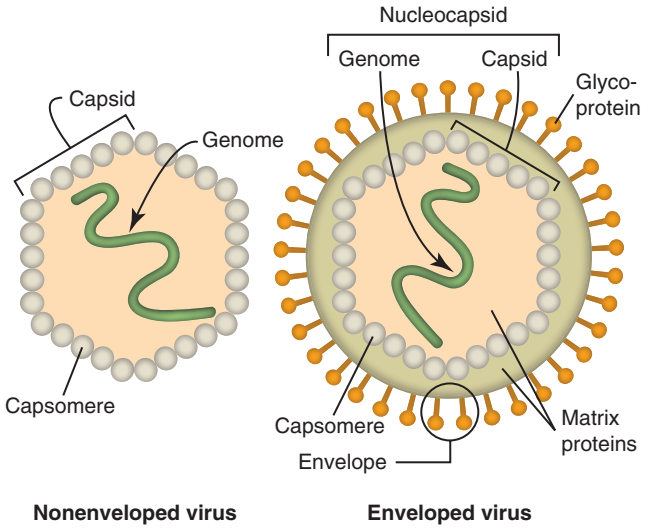


Figure 4-33 Morphologic Characteristics of Viruses. Nonenveloped viruses attach to target cells using capsomeres and capsids, whereas enveloped viruses attach using a viral envelope. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

Virulence Factors

Virulence factors also have been identified for viruses (see Table 4-1). The purpose of these factors is to improve a virus's ability to complete its replication cycle in the target cell, thus spreading and propagating the virus to naïve animals. Virulence factors control the processes involved in (1) replication, including attachment to, entering, replication in, and release of virus from target cells, and (2) evading, modulating, or suppressing the host's innate and adaptive immune responses. For example, feline immunodeficiency virus hides within the immune system and replicates and spreads within macrophages and T lymphocytes. Other viruses have evolved mechanisms to evade cytotoxic T lymphocyte and NK cell lysis of virus-infected target cells, disrupt complement activation, synthesize cytokine homologues that interfere with normal immunologic functions, and synthesize molecules that inhibit interferon responses or block the induction of apoptosis in virus-infected cells. Other viral virulence factors include viral proteins, as well as by-products of virus replication, such as caspases and caspase-like molecules, that accumulate in the cell and have toxin-like activities on target cells (Fig. 4-34; Box 4-4). As an example of a viral toxin,

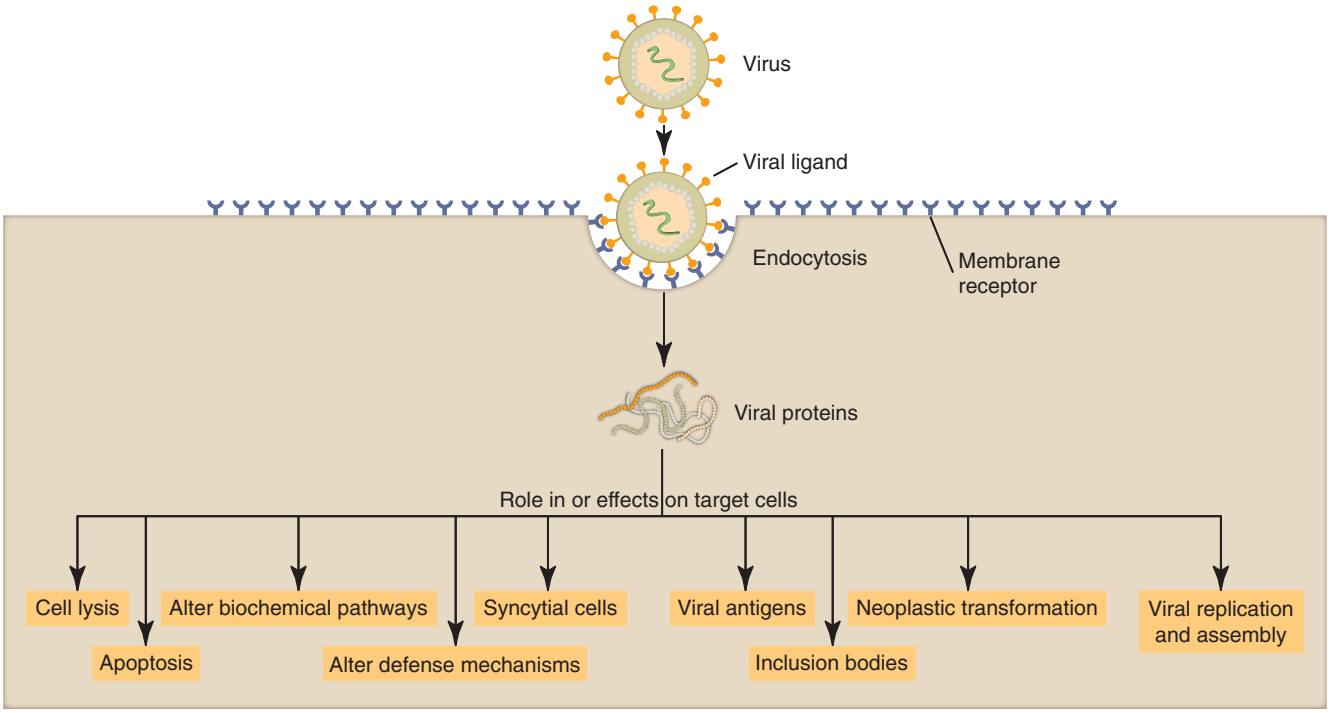


Figure 4-34 Viral Proteins—Role in or Effects on Target Cells. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

Box 4-4 Viral Proteins–Role in or Effects on Target Cells (see Fig. 4-34)

Role or effects	Proteins involved
Inclusion bodies	Aggregates of excessive production of viral proteins–cytoplasmic inclusions (RNA viruses) vs. intranuclear inclusions (DNA viruses))
Syncytial cells	Syncytia formation–viral fusion proteins (bovine respiratory syncytial virus)
Cell lysis	Viroporins, lytic phospholipids, endolysins, holins, and spanins
Apoptosis	Apoptotic proteins (inhibit or activate)–caspases
Viral replication and assembly	Replication cycle–target cell-adaptation proteins
	Structural proteins used in the construction of new progeny (membrane, capsid proteins)
	Structural proteins used to enter target cell (membrane and envelope proteins, membrane fusion proteins)
	Functional enzymes needed for viral genome transcription and replication (synthesized by the virus within the target cell) (e.g., RNA-dependent RNA polymerase, reverse transcriptase)
Viral antigens	Envelope and capsid proteins
Alter biochemical pathways	Inhibitory factors that stop host-cell DNA, RNA, and protein synthesis
Alter defense mechanisms	Inhibitory factors that block effects of interferon, phagocytosis, and acquired immune responses (immunoglobulin proteases)
Neoplastic transformation	Insert viral oncogenes directly into the target cell genome or insert viral genes that enhance the activity of existing oncogenic genes (proto-oncogenes) in the target cell genome

rotavirus-infected enterocytes secrete a viral-directed toxin called *NSP4* into the intestinal lumen. Adjacent enterocytes not infected with virus absorb this toxin, and it acts on a cytoplasmic messenger system to cause a secretory diarrhea. This diarrhea occurs before there is lysis of virus-infected enterocytes.

The number of virulence factors for viruses when compared to bacteria is extremely small and is directly related to the number of genes in the respective microbes. The number of genes in viruses ranges between 10^1 and 10^2, whereas the range in bacteria is 10^3 to 10^4 genes. Correspondingly, the number of virulence factors is low in viruses and much higher in bacteria. The introduction of a new viral virulence factor to a viral family results from genomic variation through genetic drift, reassortment, recombination, or defective interfering viruses (see section on Mechanisms of Genomic Change). Breaks in protection normally provided by commercial vaccines or the reemergence of a vaccinated/protected disease in certain regions of the country are often the result of genomic variation in the street virus and the introduction of a new viral strain such as have occurred with canine distemper and parvovirus infections.

Mechanisms of Genomic Change

Viruses are often classified as DNA or RNA viruses based on the nucleic acid used to form their genes. In general, the competitive advantages for infecting target cells favor RNA viruses because they have an extremely high mutation rate, which increases their chances of expressing virulence factors that improve their ability to complete their replication cycle. However, it is likely that this advantage is counterbalanced by their slower replication speed, allowing host defense mechanisms to intervene in the replication process and kill the virus and/or virus-infected cell. *Genomic variation* is a broad term used to categorize a group of biologic processes that allow viruses to acquire new virulence factors (genetic diversity) that favor their survival through infective and replicative mechanisms in target cells. The most common form of genomic variation is called *antigenic drift (genetic drift)*, a natural mutation in a viral genome over time. It is caused by a spontaneous point mutation of individual nucleic acid bases in viral DNA or RNA. These point mutations are usually silent and do not change the protein encoded by the affected gene; however, some mutations can result in a new protein (e.g., capsid or envelope proteins as examples), thus providing an opportunity for the virus to improve its chances of infectivity, replication, and spread during its replication cycle. As an example, a "new strain" of virus arising from antigenic drift may have a "new" attachment protein in its capsid or envelope. Because of the length of time it takes to develop an effective immune response to the new protein, the immune system is ineffective in defending the animal against viral attachment and entry into target cells. Similarly, mutations could occur in viral genes linked to biologic processes involved in spread, replication, or shedding, which could also make the virus more pathogenic.

Antigenic shift occurs when two or more different strains of the same virus or strains of two or more different viruses combine (also known as reassortment) to form a new virus that has a mixture of genes from two or more of the original virus strains. An example of antigenic shift occurs with influenza A viruses (RNA viruses) that cause influenza in horses, pigs, dogs, human beings, and other domesticated and wild animal species (see section on Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Horses; Equine Influenza [Orthomyxovirus, Enveloped RNA Virus]). When target cells are infected concurrently with two different influenza viruses, each viral strain has genes that give it a competitive advantage to infect target cells and complete its life cycle. However, when the genes of both of these parental viruses intermix under genomic reassortment in a target cell, the newly emerging virus could acquire the most pathogenic genes from both parental strains. Thus, when the virus is reassembled, it may be significantly more pathogenic (virulence factors) than either parental strain (Fig. 4-35). This increased pathogenicity may provide, as an example, the new virus with attachment proteins (envelope glycoproteins) that are immunologically unique to the farm, region, or country. As a result, animals that are naïve to this new virus and whose innate and adaptive immune systems have not interacted with these proteins have no immune memory of them. Therefore defensive immune mechanisms provide a limited effective response against the pathogenicity of this new viral strain. Similarly, reassortment could also affect genes linked to processes involved in viral entry, uncoating, spread, replication, or shedding. Depending on the virulence and effect of the reassorted genes, the new virus could be significantly more pathogenic than either of the parental strains.

Reassortment occurs only in RNA viruses because they have discrete genomic segments, much like chromosomes, that behave independently of one another. These genomic segments can undergo reassortment during viral replication, resulting in new viruses with genomes different from the original infecting virus. Segmented genomes confer evolutionary advantages to RNA viruses. Antigenic shift also occurs from a process called *recombination*. Recombination occurs in DNA viruses and results in rearrangements within the viral genome and deletion or duplication of viral genes, as well as the acquisition of unrelated genetic material. Genetic recombination comes about when a strand of DNA is broken and then rejoined to

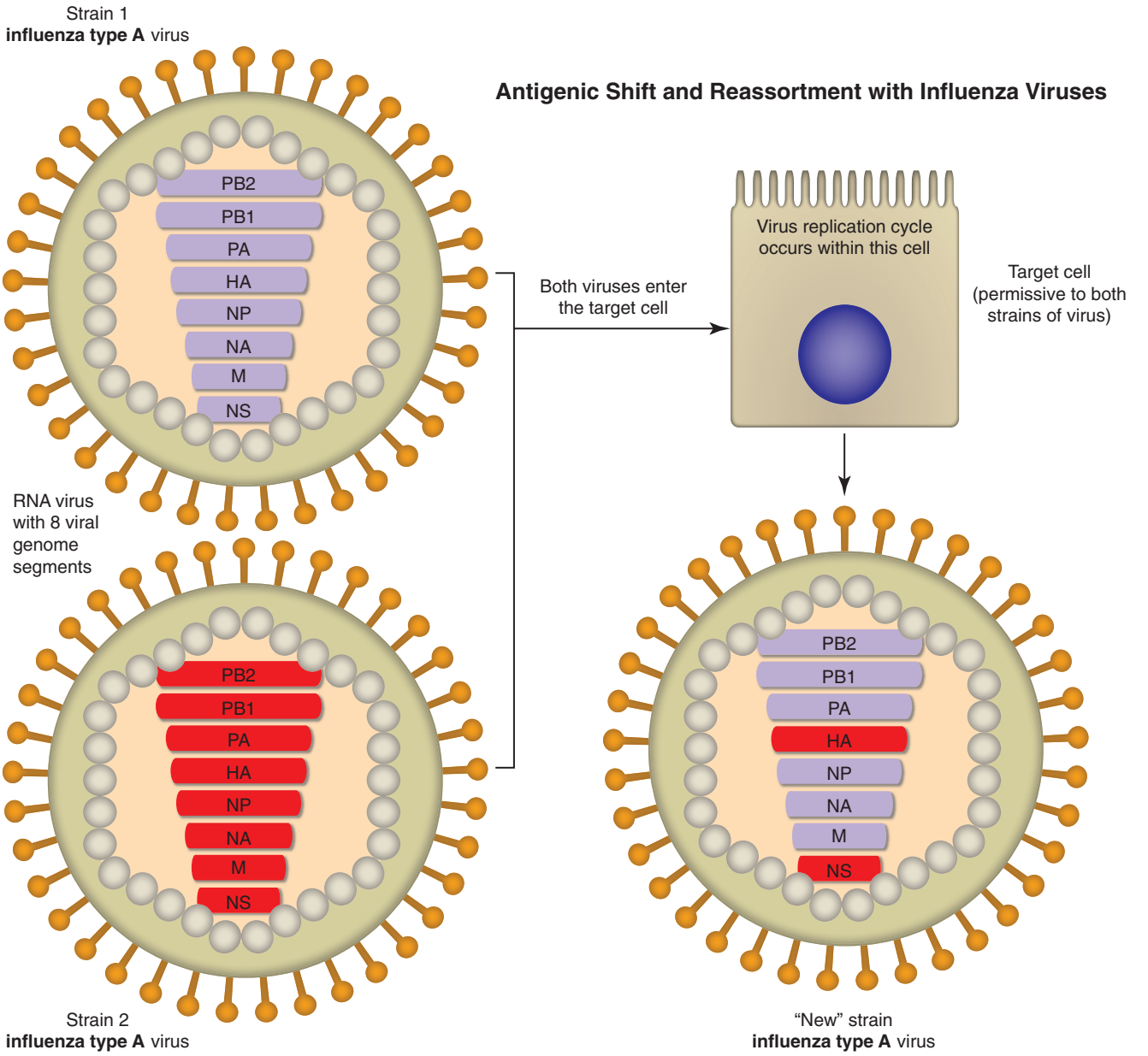


Figure 4-35 Antigenic Shift in Influenza Virus. Antigenic shift occurs when two different influenza viruses coinfect a target cell from an animal that is permissive for both viruses. When new virus is produced and released, it contains RNA strands resulting from the mixing of the strands of both infecting viruses, such that a hybrid virus is produced. In this example the hybrid virus contains new genetic information that allows it to more effectively attach and bind to target cells and to evade immunity that normally provides the animal with partial protection against infection. PB2 genome segment—viral polymerase involved in the replication cycle; PB1 genome segment—viral polymerase involved in the replication cycle; PA genome segment—viral polymerase involved in the replication cycle; HA genome segment—attachment and binding to the target cell; NP genome segment—structural protein for the virus; NA genome segment—attachment and binding to the target cell; M genome segment—regulates processes involved in the replication cycle; NS genome segment—evasion of immune defenses (blocks antiviral responses). (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

the end of a different DNA molecule. A final mechanism for genomic change occurs in RNA and DNA viruses and involves *defective interfering viruses* that cannot replicate by themselves and therefore compete with nondefective viral genomes for a limited supply of replication enzymes. They can interfere with the replication of complete viruses in target cells and significantly decrease the numbers of newly replicated virus, thus favoring success of new mutants that may arise in the viral replication process.

Defense Mechanisms

Defense mechanisms include barrier systems, immunologic and biologic processes, effector cells (especially cytotoxic NK cells and cytotoxic T lymphocytes, which kill virus-infected target cells), and other effector molecules described in the opening sections of this chapter, in the sections covering bacteria, and in Chapters 3 and 5. The genome of the animal probably determines susceptibility to some viral infections via expression of or lack of expression of viral membrane receptors or through effects on the immune system. Stress (overcrowding), nutritional status, and environmental factors, such as temperature, humidity, and ventilation, also affect the susceptibility of animals to viral infections. Innate and adaptive immune mechanisms are actively involved in protection against viruses. However, it is important to remember that actions of the immune system, especially of T lymphocytes and NK cells, against viral

infections have both beneficial and harmful outcomes. Beneficial outcomes include the return to normal structure and function of infected target cells and tissues and an animal that is free of and fully protected (vaccinated) against the virus. Harmful outcomes include the failure to return to normal structure and function of infected target cells because the cells and tissues, their stem cells, supporting stroma, basement membrane, and vascularized ECM tissues have been degraded by enzymes from neutrophils of acute inflammation or by macrophages of chronic inflammation and replaced by fibrous connective tissue. The innate immune system and TLRs, in response to viral antigens, induce inflammatory responses, cause secretion of cytokines and interferon, and activate the adaptive immune system.

Cell-mediated immunity (cytotoxic NK cells and cytotoxic T lymphocytes) and interferons are the most important adaptive defense mechanism against viral infections. The monocyte-macrophage system, through phagocytosis (endocytosis), is active in containing the spread of viruses, whereas phagocytosis by neutrophils does not play an important role. Antibody deficiencies usually do not affect the outcome of viral infections, whereas antibodies are important in preventing reinfection (autoimmunization or vaccination). Although viruses are obligate intracellular parasites, they have evolved sophisticated mechanisms to take over target cell transcriptional and translational processes. This approach to replication results in alteration of cell membranes that are now recognized as foreign by lymphocytes of the immune system. Virus replication and spread are abruptly stopped when virus-infected target cells are killed by cytotoxic NK cells and cytotoxic T lymphocytes. Interferons, a group of molecules that act on virus-infected cells to inhibit virus replication, function by inducing the synthesis of target cell proteins that inhibit translational activities of the virus (Fig. 4-36). The synthesis of interferon is induced by virus infection of target cells and by the action of proinflammatory molecules on these cells. Viral infection of target cells can also activate complement cascades independent of an antibody response. Complement components can act as opsonins (e.g., phagocytosis of viruses) and can cause lysis of viruses or virus-infected cells. Many of the viruses discussed in this chapter are able to infect cells of the lymphoid and monocyte-macrophage systems and dendritic cells. Under normal conditions such cells are migratory immunosurveillance cells, behaving as sentinel cells for the adaptive immune system and monitoring for the presence of foreign antigens expressed by microbes or microbe-infected target cells throughout the body. As part of their normal immunosurveillance functions, these cells migrate via lymphatic and blood vascular systems throughout all tissues and organs of the body, including the brain. It is through these normal migratory

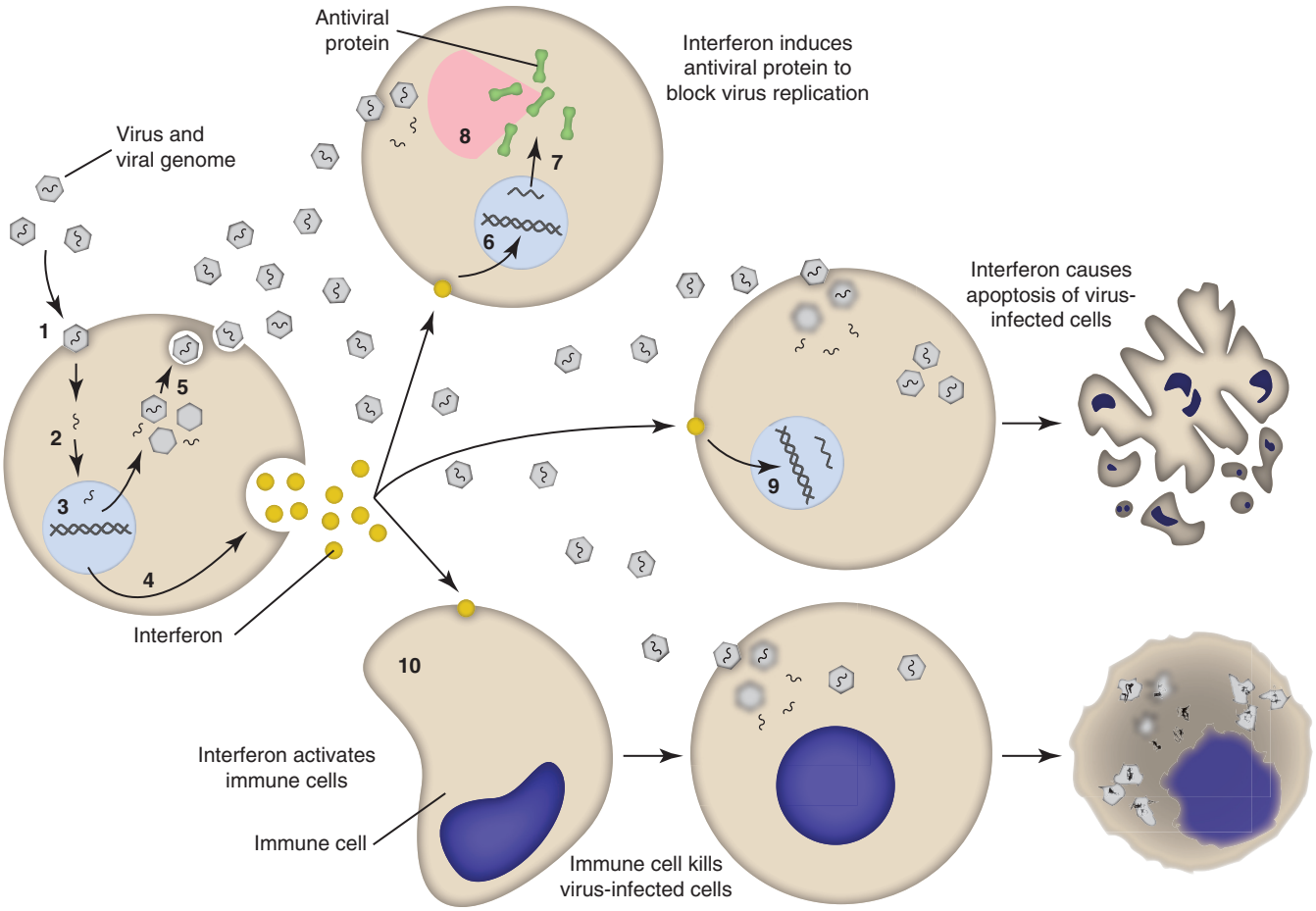


Figure 4-36 Actions of Interferon in Viral Infection of Target Cells. *1*, Virus attaches and enters the target cell. *2*, Viral genome enters nucleus for replication. *3*, Viral genome indirectly activates host genome to produce messenger RNA (mRNA) for interferon. *4*, Interferon is translated and secreted from the infected cell. *5*, Virus is reassembled and released from the cell to infect other target cells. *6*, Interferon stimulates the genome of the target cell to synthesize mRNA for an antiviral protein. *7*, Antiviral protein is translated and dispersed in the cytoplasm. *8*, Antiviral protein blocks the attachment, entry, and disassembly of the virus in the target cell. *9*, Interferon stimulates the genome of the target cell to undergo apoptosis. *10*, Interferon activates and modulates immune cells to kill virus-infected cells. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

pathways that viruses within these infected cells are able to spread to other tissues and organs. This process is termed *leukocyte trafficking* or *cell-associated* viremia. Viruses can also spread to other cells as a cell-free viremia in the blood vascular or lymphatic systems.

Viral Diseases of Organ Systems

Although viral diseases often affect several different organ systems, diseases in this section are placed into a specific organ system based on which organ system demonstrates the primary gross lesion (or lesions) that is most commonly used to initially recognize and identify the viral disease. The heading for each viral disease includes information on whether the virus is enveloped or nonenveloped (type of cell injury during viral shedding) and the type of nucleic acid (virulence factors, genomic diversity) it contains. This information is useful in understanding the mechanisms of injury in specific viral diseases. Viral diseases are identified by a primary mechanism of injury in E-Table 4-4.

Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity

Disorders of Domestic Animals

Rotavirus Enteritis (Rotavirus, Nonenveloped RNA Virus). The mechanism of injury and pathogenesis of rotavirus enteritis are similar to those of transmissible gastroenteritis in pigs (for more detail see section on Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Pigs; Transmissible Gastroenteritis [Coronavirus, Enveloped RNA Virus]). However, the clinical outcomes and pathogenicity (virulence factors) are much less severe (see Fig. 4-39). Viral capsid attachment proteins, VP4 and VP7, appear to be involved in the attachment and entry of virus into villus enterocytes through a multistage receptor-mediated process by binding to target cell membrane proteins such as sialic acids, integrins, heat shock proteins, and gangliosides located on apical surfaces. The replication of rotavirus in villus enterocytes results in the production of NSP4, an enterotoxin that (1) induces a secretory diarrhea, (2) stimulates the enteric nervous system and causes intestinal hypermotility, and (3) increases the concentration of intracellular calcium, disrupting the cytoskeletal system and tight junctions, and results in increased mucosal permeability. NSP4 also appears to cause dysfunction of cell membrane systems modulating electrolyte and water movement such as calcium ion–dependent chloride secretion, sodium-glucose transport proteins, brush-border membrane disaccharidases, and calcium ion–dependent secretion reflexes. Subsequently, rotavirus completes its replication cycle in infected enterocytes and is shed from these cells via cell lysis resulting in atrophy of intestinal villi and hyperplasia of crypt epithelial cells. Both the absorptive capacity of villi and their disaccharidase activity are impaired. Undigested and/or unabsorbed dietary disaccharides accumulate in the lumen of the intestine, thereby creating an osmotic gradient that draws fluids from the intestine into the lumen, leading to malabsorption and an osmotic diarrhea (see Chapter 7). Antigenic shift via reassortment of RNA segments of the viral genome from two or more strains of virus may be an important underlying mechanism in the emergence of new more pathogenic strains of rotavirus.

Vesicular Stomatitis (Vesiculovirus, Enveloped RNA Virus). Because the clinical signs of vesicular stomatitis are identical to those of foot-and-mouth disease (an especially dangerous microbe [see foot-and-mouth disease later]), affected animals must be carefully screened and the cause of the lesions identified. The mechanism of injury in vesicular stomatitis is cell dysfunction and lysis leading to intercellular edema with vesiculation, erosion, and ulceration of mucosae and skin. Gross lesions occur on the tongue, oral cavity, hoof coronary bands and interdigital skin, and teats. The pathogenesis has not been determined to an extent that results in an understanding of the chronologic sequence of steps leading to disease. The virus, an arbovirus, is spread to cattle, horses, and pigs primarily by sandflies and blackflies and rarely by instruments or equipment. Animals encounter the virus through the bite wounds of these insects, where the biting process injures blood vessels and capillaries, resulting in virus being deposited directly into plasma of blood vessels and/or into interstitial fluids (plasma leaked from vasopuncture) within vascularized submucosal and subcutaneous ECM (connective) tissues. It appears that vesicular lesions occur at or near the sites of insect bites, suggesting that the virus infects target cells locally and there is no systemic spread of virus to mucosae or skin through leukocyte trafficking or viremia. Squamous epithelial cells of mucosae and skin are the primary target cells for viral infection, but Langerhans cells (dendritic cells) and cells of the monocyte-macrophage system, although likely target cells for infection with virus, have not been clearly identified as target cells. In addition, it is likely but unproved that local migration of dendritic cells and cells of the monocyte-macrophage system spread virus to additional local target cells as described later. Lesions suggest that epithelial cells of the stratum basale and/or spinosum must be targets for virus infection, replication, and escape (through cell lysis). Lysis of these cells results in the formation of intercellular spaces that fill with fluid and form vesicles. Trauma likely ruptures the vesicles and leads to erosion/ulceration of the overlying mucosa or skin; however, acute inflammation may also contribute to the process. Virus appears to use envelope glycoprotein G as an attachment protein to bind with low-density lipoprotein (LDL) receptors on epithelial cells, enter the cells via endocytosis, replicate in the cytoplasm, and escape from the cell by cytolysis.

Disorders of Ruminants (Cattle, Sheep, and Goats)

Bovine Viral Diarrhea–Mucosal Disease (BVD Virus, Pestivirus, Enveloped RNA Virus). The variety of diseases caused by BVD virus is diverse and complex. Some of these diseases will be discussed in this chapter and other chapters of this book. Bovine viral diarrhea–mucosal disease as discussed herein refers to the disease that affects mucosae of the alimentary system from the oral cavity to the small intestine. The mechanism of injury in bovine viral diarrhea–mucosal disease is dysfunction and lysis of mucosal epithelial cells of the oral cavity and esophagus (stratified squamous epithelium) and of the small intestines (enterocytes [columnar epithelium]) preceded by dysfunction and lysis of submucosal lymphocytes in MALT, such as those in Peyer's patches. Gross lesions include erosion, ulceration, and hemorrhage of mucosae of oral/nasal cavities and pharynx, esophagus, and small intestine (see Figs. 7-34, 7-158, 7-159, and 7-160).

The typical pathogenesis of mucosal disease involves two forms of BVD virus, a *noncytopathic form* and a *cytopathic form*, acting synergistically to cause lesions. The noncytopathic form of the virus can be introduced into the herd in new stock, through commingling of cattle, semen, or other management practices that allow contact with carrier animals. When naïve pregnant cows (normal immune responses, unvaccinated, no prior exposure) have contact with carrier animals, they may become infected with the noncytopathic form of the virus. These cows are asymptomatic, but they functionally serve as a means for the virus to infect the fetus and establish "persistently infected (PI)" calves. These PI calves, present in small numbers, usually die before a year of age but serve as farm reservoirs for noncytopathic virus as they constantly shed virus in body secretions (saliva, tears) and feces into the environment. The cytopathic form of the virus commonly arises from a noncytopathic form that

E-Table 4-4 Mechanisms of Injury in Diseases Caused by Viruses

Cell Lysis	Cell Proliferation	Inflammation	Neoplastic Transformation	Primary Cell Dysfunction
ALIMENTARY SYSTEM AND THE PERITONEUM, OMENTUM, MESENTERY, AND PERITONEAL CAVITY				
Disorders of Domestic Animals				
• Rotavirus enteritis • Vesicular stomatitis				• Rotavirus enteritis
Disorders of Ruminants (Cattle, Sheep, and Goats)				
• Bovine viral diarrhea-mucosal disease • Rinderpest • Contagious ecthyma • Bovine papular stomatitis • Foot-and-mouth disease	• Contagious ecthyma • Bovine papular stomatitis			
Disorders of Pigs				
• Transmissible gastroenteritis • Porcine epidemic diarrhea • Swine vesicular disease • Vesicular exanthema of pigs • Foot-and-mouth disease				
Disorders of Dogs				
• Parvovirus enteritis • Canine enteric coronavirus • Canine distemper				
Disorders of Cats				
• Feline infectious peritonitis • Parvovirus enteritis		• Feline infectious peritonitis		
HEPATOBILIARY SYSTEM AND EXOCRINE PANCREAS				
Disorders of Ruminants (Cattle, Sheep, and Goats)				
• Wesselsbron's disease • Rift Valley fever				
Disorders of Dogs				
• Infectious canine hepatitis				
RESPIRATORY SYSTEM, MEDIASTINUM, AND PLEURAE				
Disorders of Horses				
• Equine influenza • Equine viral rhinopneumonitis • Equine viral arteritis				
Disorders of Ruminants (Cattle, Sheep, and Goats)				
• Infectious bovine rhinotracheitis • Bovine respiratory syncytial virus pneumonia • Bovine influenza • Maedi • Caprine pneumonia		• Maedi • Caprine pneumonia		
Disorders of Pigs				
• Porcine reproductive and respiratory syndrome • Swine influenza • Inclusion body rhinitis		• Porcine reproductive and respiratory syndrome • Inclusion body rhinitis		
Disorders of Dogs				
• Canine infectious tracheobronchitis • Canine distemper • Canine influenza		• Canine infectious tracheobronchitis		
Disorders of Cats				
• Feline viral rhinotracheitis • Feline calicivirus				

Continued

E-Table 4-4 Mechanisms of Injury in Diseases Caused by Viruses–cont'd

Cell Lysis	Cell Proliferation	Inflammation	Neoplastic Transformation	Primary Cell Dysfunction
CARDIOVASCULAR SYSTEM AND LYMPHATIC VESSELS				
Disorders of Horses				
• Equine Herpesvirus Myeloencephalopathy • Equine viral arteritis • African horse sickness				
Disorders of Ruminants (Cattle, Sheep, and Goats)				
• Bluetongue • Bovine malignant catarrhal fever				
Disorders of Pigs				
• Classic swine fever • African swine fever				
Disorders of Dogs				
• Parvovirus myocarditis • Canine herpesvirus infection • Canine circovirus				
Disorders of Cats				
• Feline infectious peritonitis		• Feline infectious peritonitis		
BONE MARROW, BLOOD CELLS, AND LYMPHATIC SYSTEM				
Disorders of Horses				
	• Equine infectious anemia	• Equine infectious anemia		
Disorders of Ruminants (Cattle, Sheep, and Goats)				
	• Enzootic bovine lymphoma		• Enzootic bovine lymphoma	
Disorders of Pigs				
• Postweaning multisystemic wasting syndrome	• Postweaning multisystemic wasting syndrome			
Disorders of Dogs				
• Canine distemper				
Disorders of Cats				
• Feline leukemia • Feline acquired immunodeficiency syndrome			• Feline leukemia	
NERVOUS SYSTEM				
Disorders of Domestic Animals				
				• Rabies
Disorders of Horses				
• Equine polioencephalitis-polioencephalomyelitis • West Nile virus polioencephalitis-polioencephalomyelitis • Equine herpesvirus myeloencephalopathy				
Disorders of Ruminants (Cattle, Sheep, and Goats)				
• Bovine cerebellar hypoplasia • Bovine herpesvirus meningoencephalitis		• Visna • Caprine encephalitis		

E-Table 4-4 Mechanisms of Injury in Diseases Caused by Viruses–cont'd

Cell Lysis	Cell Proliferation	Inflammation	Neoplastic Transformation	Primary Cell Dysfunction
Disorders of Pigs				
• Pseudorabies				
Disorders of Dogs				
• Canine distemper				
Disorders of Cats				
• Parvovirus-induced cerebellar hypoplasia				
BONE, JOINTS, TENDONS, AND LIGAMENTS				
Disorders of Ruminants (Cattle, Sheep, and Goats)				
		• Caprine arthritis		
INTEGUMENTARY SYSTEM				
Disorders of Domestic Animals				
• Vesicular stomatitis	• Viral papillomas			
• Foot-and-mouth disease				
Disorders of Ruminants (Cattle, Sheep, and Goats)				
• Pox	• Pox			
• Contagious ecthyma	• Contagious ecthyma			
• Bovine papular stomatitis	• Bovine papular stomatitis			
Disorders of Pigs				
• Swine vesicular disease				
• Vesicular exanthema of pigs				
FEMALE REPRODUCTIVE SYSTEM AND MAMMARY GLAND				
Disorders of Horses				
• Equine herpesvirus abortion				
• Coital exanthema				
• Equine viral arteritis				
Disorders of Ruminants (Cattle, Sheep, and Goats)				
• Bovine herpesvirus abortion				
• Infectious pustular vulvovaginitis				
Disorders of Pigs				
• Porcine reproductive and respiratory syndrome				
• Porcine parvovirus abortion				
• Porcine cytomegalovirus abortion				
MALE REPRODUCTIVE SYSTEM				
Disorders of Horses				
• Coital exanthema				
Disorders of Ruminants (Cattle, Sheep, and Goats)				
• Infectious pustular balanoposthitis				
EYE				
Disorders of Cats				
• Feline herpetic keratitis				

exists in the herd through mutations (antigenic drift or shift) of its viral genome, or it is introduced into the herd as a new virus via a carrier animal. The noncytopathic form makes cattle immunotolerant to cytopathic forms of BVD virus, and mucosal disease occurs when immunotolerant cattle are exposed to a cytopathic form. Cattle that have not been exposed to a noncytopathic form of the virus are not immunotolerant and develop a normal adaptive immune response to the cytopathic form. Thus they are usually able to prevent or limit the severity of mucosal disease that occurs unless the viral strain has several highly pathogenic virulence factors.

For convenience, let's begin the sequence of the steps that ultimately lead to mucosal disease with the exposure of pregnant cows to the noncytopathic form. Cows encounter the noncytopathic form in fomites from contaminated body fluids or wastes through direct contact with PI calves or carrier animals. The noncytopathic form is inhaled or ingested and deposited on mucosae of the oral, nasal, and pharyngeal cavities; especially favored are mucosae overlying the tonsil. It has not been determined if and how virus penetrates the mucus layer to gain access to mucosal epithelial cells or submucosal macrophages, lymphocytes, and/or dendritic cells, but this process could be facilitated via phagocytosis in the mucus layer by mucosa-associated macrophages, lymphocytes, and/or dendritic cells migrating through the mucosa. Noncytopathic virus probably infects and replicates in monocytes, macrophages, lymphocytes, and dendritic cells and is spread via leukocyte trafficking in lymphatic vessels from tonsil and submucosal lymphoid nodules to regional lymph nodes and then systemically to the caruncular side of placentomas. Noncytopathic virus can infect trophoblasts in the placentoma, and the virus likely completes a replication cycle in these cells. It is unclear how virus exits from trophoblasts and spreads to the fetus; however, fetal macrophage-like cells are likely involved. Virus probably infects these cells as they migrate into and through the caruncles and/or cotyledons and then enter the fetal vascular system and spread to the fetus. Additionally, noncytopathic virus can infect and spread within the allantoic and amniotic membranes and then infect the fetus, but it is unclear which cells facilitate this spread.

Bovine fetuses infected in utero become immunotolerant (see Chapter 5) to the noncytopathic form of the virus. They also do not recognize antigens from cytopathic forms of the virus as foreign, and as a result they fail to develop an effective adaptive immune response. Therefore, when exposed to cytopathic virus, mucosal disease ensues in these calves. Mechanistically, the sequence of steps that ultimately leads to mucosal disease begins when these immunotolerant calves inhale or ingest cytopathic virus and it is deposited on mucosa of the oral, nasal, and pharyngeal cavities and tonsil. The mechanism of infection and spread of the virus from the mucus layer systemically to MALT of the alimentary system, especially Peyer's patches (MALT), is similar to that described earlier for the noncytopathic virus. The cytopathic virus infects follicular dendritic cells and B lymphocytes in MALT and then spreads probably via leukocyte trafficking to infect and kill overlying stratified squamous epithelial cells and/or crypt enterocytes, resulting in mucosal erosions, ulcerations, and hemorrhage. In the small intestine, because of the lysis of crypt enterocytes, there is a failure of replacement of sloughed villus enterocytes after normal enterocyte turnover at the villus tip. This outcome may in part explain the mucosal lesions and initiate the formation of ulcers. Hemorrhage occurring with the ulcers could be the result of exposure of capillary beds to endotoxins or other toxic molecules absorbed through an open intestinal barrier system (cell junctions). Diarrhea could also occur secondary to the absorption of large quantities of endotoxins into the lamina propria and deeper supporting stroma that contains the enteric nervous system, resulting in an acquired dysautonomia (see Chapter 14). It has been recently reported that certain molecules released from lymphocytes and/or monocytes infected with cytopathic virus can initiate apoptosis in bystander lymphocytes and monocytes not infected with virus. The role of apoptosis in ulceration of mucosae has not been determined. Additionally, a vasculopathy involving arterioles and small arteries in submucosal tissue of Peyer's patches has been reported and is characterized by segmental necrosis of vascular walls and lymphohistiocytic perivasculitis. Potentially, such lesions could cause endothelial injury and occlusive thrombi, resulting in infarction of the mucosal enterocytes overlying Peyer's patches. Lymphoid cells in Peyer's patches initially proliferate when infected, but infection is followed by massive lysis of lymphocytes as part of the viral replication cycle, likely caused by a virus-induced apoptotic mechanism.

Ligand-receptor interactions are involved in encounters with both forms of the virus and with all types of its target cells. Studies suggest that glycoproteins (E1 and E2) present in the outer membrane of the virus may act as attachment proteins. Clathrin, lysosomal-associated membrane protein-2, and mannose receptors may be involved in entry into target cells via receptor-mediated endocytosis.

Rinderpest (Cattle Plague, Morbillivirus, Enveloped RNA Virus). Because of similarities in clinical presentations, lesions, causative viruses, and mechanisms of infection and spread between rinderpest and other viral diseases, the following materials should be reviewed: (1) morbilliviruses—local, regional, and systemic infection and spread and their target cells in the section on canine distemper; (2) bovine viral diarrhea–mucosal disease—clinical presentation and lesions; and (3) parvoviruses—mechanisms used to infect and spread between cells.

The mechanism of injury in rinderpest is dysfunction and lysis of mucosal epithelial cells, dendritic cells (Langerhans cells [oral cavity]), M cells, lymphocytes, and macrophages of the alimentary system from the oral cavity to the small intestine. Gross lesions include erosions, ulcerations, and hemorrhages of the oral cavity, including the gums, lips, hard and soft palate, cheeks, and base of the tongue, the esophagus, and the small intestine over Peyer's patches (Fig. 4-37). Lymph nodes, especially mesenteric nodes, are enlarged, hemorrhagic, and edematous.

Cattle (and likely sheep and goats) encounter the virus in fomites from body fluids and wastes, such as nasal-ocular fluids, salvia, urine, and feces, through direct contact with virus-infected cattle. Virus is inhaled, deposited on, and trapped in mucosae of the conductive and exchange components of the respiratory system through centrifugal and inertial turbulence. It has not been determined if and how virus penetrates the mucus layer to gain access to mucosal epithelial cells, mucosal macrophages, and/or dendritic cells. Virus probably infects and replicates in mucosal macrophages and dendritic cells as they migrate through the mucus layer and mucosae and then is spread by these cells locally through leukocyte trafficking to the submucosa, where they infect and replicate in tissue macrophages, lymphocytes, and dendritic cells. These cells then spread virus via leukocyte trafficking through afferent lymphatic vessels to regional lymph nodes. Similar cells are infected and used to spread the virus systemically via lymphatic vessels, the thoracic duct, and the blood vascular system systemically to lymph nodes and other organ systems, including the alimentary and respiratory systems. Systemically, primary target cells for infection include those cells in Peyer's patches of the small intestine and in lymphoid nodules, including Langerhans cells of the malpighian layer of stratified squamous epithelium of the oral cavity and esophagus.

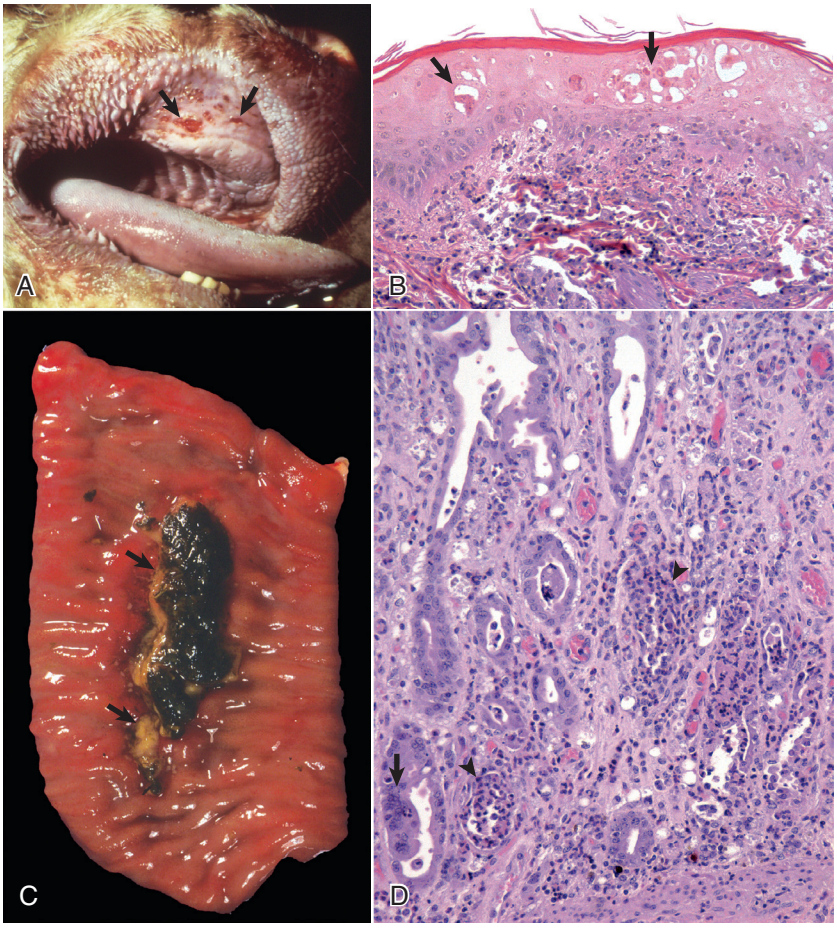


Figure 4-37 Rinderpest. A, Oral mucosa, dental pad. Note the erosions and ulcers *(arrows)* adjacent to the dental pad caused by rinderpest virus. **B,** Oral mucosa. Focal aggregates of epithelial cells in the mucosa are swollen, necrotic, and some are detached *(arrows)*. When abraded by ingesta or other trauma, the mechanical force applied to the lesion in **A** can separate the epithelium overlying the lesion, and it will grow and lead to ulcers or abrasions, depending on depth of the epithelial loss. Note the acute inflammatory response in the lamina propria. H&E stain. **C,** Ileum. The mucosa overlying Peyer's patches is ulcerated and covered with fibrin mixed with hemorrhage *(arrows)*. This lesion appears to result from spread of the virus from underlying lymphocytes in Peyer's patches to epithelial cells of the crypts. **D,** Epithelial cells of the crypts are hyperplastic and form syncytia *(arrow)*. In other areas, crypt enterocytes and cells in the adjacent lamina propria are necrotic *(arrowheads)* and accompanied by acute inflammation. This process leads to ulceration of the intestinal mucosa. H&E stain. (**A** and **C** courtesy Dr. C. Brown, College of Veterinary Medicine, The University of Georgia. **B** and **D** courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

Erosive lesions in the oral-pharyngeal-lingual mucosae begin in the malpighian layer (stratum basale [germinativum], stratum spinosum, and stratum granulosum). Langerhans cells (dendritic cells) are located in the malpighian layer and are sentinel cells that migrate in and out monitoring for foreign antigens. Although unproved, Langerhans cells are likely infected with rinderpest virus via encounters with virus-infected macrophages migrating through these mucosae. Infected oral Langerhans cells also spread virus to contiguous squamous epithelial cells. Here the virus replication cycle results in lysis of infected squamous epithelial cells (oral-pharyngeal-lingual mucosal ulceration) and release of virus into the alimentary system. Erosive lesions in intestinal mucosae likely occur via a similar mechanism facilitated by the infection and migration of macrophages, monocytes, and dendritic cells systemically and into and through Peyer's patches and then to contiguous enterocytes. The entry of rinderpest virus into mucosal enterocytes has a polarized pattern restricted to their basolateral areas, the areas nearest Peyer's patches and M cells. The virus replication cycle results in lysis of infected enterocytes (small intestine mucosal ulceration) and release of virus into the alimentary system.

Similar to distemper virus, the rinderpest virus has envelope and hemagglutinin/fusion surface glycoproteins for attachment and fusion, respectively, to target cell membrane glycoprotein receptor CD150 (signaling lymphocyte activation molecule [SLAM]). SLAM has been demonstrated in membranes of lymphocytes, monocytes, and macrophages and of epithelial cells of the respiratory, alimentary, and integumentary systems.

Contagious Ecthyma (Orf, Sore Mouth, Pustular Dermatitis: Parapoxvirus; Enveloped DNA Virus). The mechanism of injury in contagious ecthyma is (1) dysfunction and lysis of squamous epithelial cells of the oral mucosa (squamous epithelium) and/or skin caused by viral replication and cytolysis and (2) exuberant hyperplasia (proliferation) of squamous epithelial cells of oral mucosa and/or skin through modulation of regulatory activities in the cell-division cycle by virulence factors expressed in the viral genome. Gross lesions include (1) macules, papules, vesicles, pustules, scabs, and scars and in cases having extensive injury resulting from vesicles and pustules (2) a reparative response with proliferation of mucosal squamous epithelial cells resulting in a thickened and granulation tissue–like appearance of affected mucosa (see Figs. 7-150 and

17-65). Lesions are most easily observed on wool-free or hair-free areas such as the muzzle (lips and mouth) and udder (teats) but also can occur in the skin of the perineum, groin, prepuce, scrotum, axilla, and vulva. This disease is zoonotic.

Sheep and goats encounter the virus in fomites of fluids from ruptured macules, vesicles, and pustules and from skin debris and scabs through direct contact with virus-infected animals. The virus can also be spread through mechanical contact with contaminated clothing, instruments, and clippers. Virus gains access to the malpighian layer of the squamous epithelium through traumatic abrasions, lacerations, or burns and infects Langerhans cells (dendritic cells) and capillary endothelial cells. Infection of additional Langerhans cells occurs when virus-infected dendritic cells migrate through the dermis and subcutis of the malpighian layer. Infection of endothelial cells may be facilitated by the migration of virus-infected dendritic cells through the capillary wall. Virus appears to use F1L envelope protein as an attachment protein to bind to glycosaminoglycan heparin sulfate receptor proteins on the surface of target cells. Endothelial cells are injured and lysed (killed) by virus, and injury is accompanied by vascular dilation, leakage (edema), and active hyperemia, likely contributing to formation of macules, vesicles, and papules in the skin. Reparative and regenerative responses contribute to proliferative lesions (hyperplasia) of mucosae and skin. Hyperplasia is apparently caused by (1) synthesis of vascular endothelial growth factor molecules from virus-infected capillary endothelial cells, (2) proliferation of new capillaries as occurs in angiogenesis, and (3) the concurrent proliferation of mucosal epithelial cells much like in the formation of granulation tissue. Virus also infects cells of the stratum basale (germinativum) that are regenerating (actively dividing [mitotic]) as a reparative response to the initial injury of mucosae; however, the relationship between infection of these epithelial cells and the proliferative response that ensues is unclear.

Bovine Papular Stomatitis (Parapoxvirus, Enveloped DNA Virus). The pathogenesis and mechanism of injury in bovine papular stomatitis are similar to those of contagious ecthyma discussed in the preceding section. The disease occurs primarily in cattle and also in sheep and goats (see Figs. 7-148 and 7-149).

Foot-and-Mouth Disease (Aphthovirus, Nonenveloped RNA Virus). The pathogenesis and mechanisms of injury in foot-and-mouth disease in cattle and pigs (less common in sheep and goats) are likely similar to that of swine vesicular disease and vesicular exanthema of pigs (see Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Pigs). In summary, virus encounters target cells through inhalation or ingestion and establishes a local infection in the oronasal-pharyngeal mucosae especially of the tonsil and subsequently in submucosal lymphoid cells, macrophages, and dendritic cells. It then spreads via leukocyte trafficking or cell-free viremia in afferent lymphatic vessels to regional lymph nodes to sustain and amplify the infection and then systemically via leukocyte trafficking or cell-free viremia to infect, replicate in, and lyse epithelial cells of the stratum spongiosum of stratified squamous mucosa and skin, resulting in vesicles (Fig. 4-38). Capsid proteins used by the virus to attach and bind to target cells appear to include VP1-4 attachment proteins, whereas α integrins (Vβ1, Vβ3, and Vβ6) expressed on target cells are used are receptors.

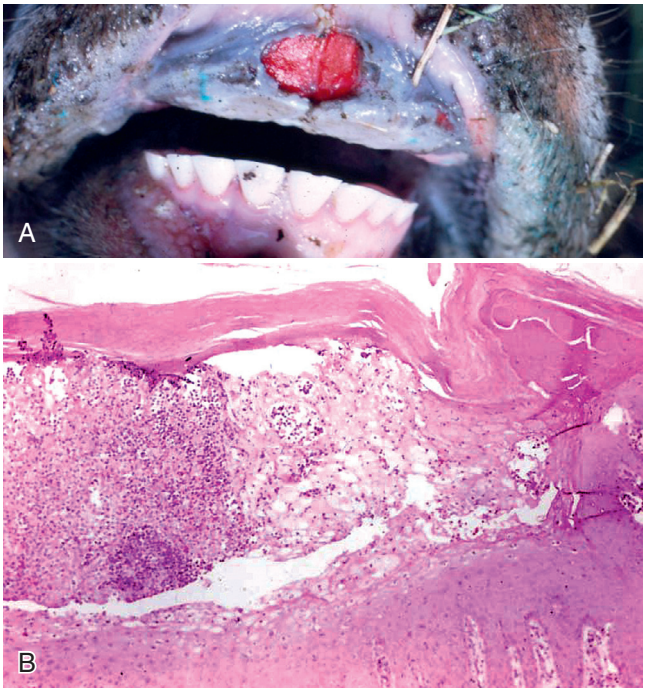
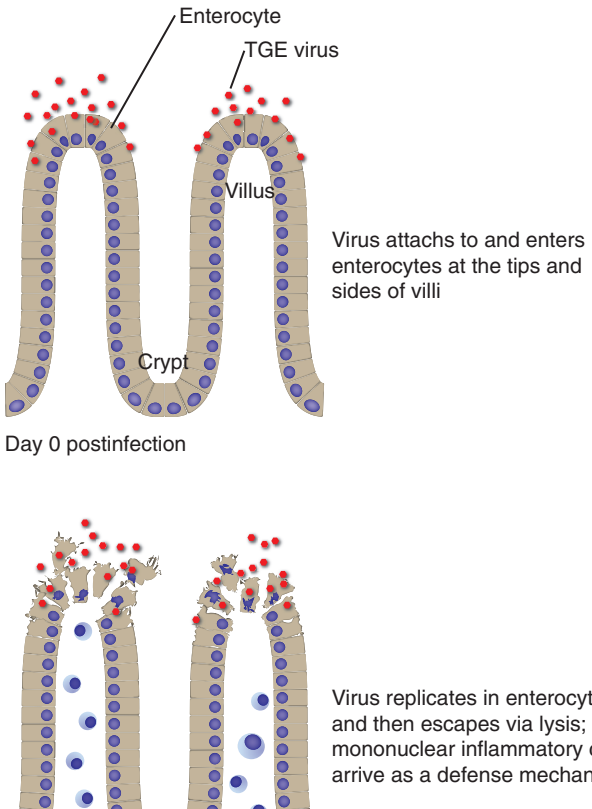


Figure 4-38 Foot-and-Mouth Disease. A, Ox. Note the ulcer on the mucosa of the upper dental pad. Such ulcers begin as fluid-filled vesicles that rupture, usually from the trauma of chewing or prehension. Vesicles and ulcers that result from their rupture may occur on all mucosae of the body, including the dental pad, tongue, gingiva, coronary bands, and teats, as examples. **B,** The mucosa has a large focus of a previous vesicle, which is now partially filled with edema fluid, fibrin, cellular debris, and acute inflammatory cells, forming a pustule. H&E stain. (**A** courtesy Dr. M. Adsit, College of Veterinary Medicine, The University of Georgia; and Noah's Arkive, College of Veterinary Medicine, The University of Georgia. **B** courtesy Dr. C. Brown, College of Veterinary Medicine, The University of Georgia.)

Disorders of Pigs

Transmissible Gastroenteritis (Coronavirus, Enveloped RNA Virus). The mechanism of injury in transmissible gastroenteritis (TGE) is dysfunction and lysis of epithelial cells (villus enterocytes) covering tips and sides of intestinal villi (Fig. 4-39, *A*). Gross lesions include congestion and thinning of the wall of the small intestine and shortening (atrophy) of villi (see Fig. 4-39, *B*; also see Figs. 7-166, 7-167, and 7-168).

Piglets encounter virus in fomites contaminated with feces through direct contact with virus-infected pigs. Virus is ingested, and swallowing and peristasis carry it through the oral pharynx, esophagus, and stomach to the small intestine, where it is trapped in the mucus layer. It is unclear how the virus is able to evade the actions of digestive enzymes, bile acids, and other microbial-lytic molecules. The mucus layer has mucins and mucin-like glycoproteins that contain sialic acid. The viral envelope contains S protein, an attachment protein, which binds to sialic acid in the mucus layer. It has not been determined how the virus penetrates the mucus layer to gain access to enterocytes. When in contact with cell membrane of enterocytes, S protein binds to a glycoprotein receptor, aminopeptidase N, which is expressed on apical surfaces of enterocytes located on the tips and sides of villi. E2 protein, also present in the envelope of the virus, is thought to facilitate entry of virus into the cytoplasm of the enterocyte. These interactions enable the attachment and entry of virus into the cytoplasm of villus enterocytes where the virus replicates. Virus then lyses enterocytes on the tips and sides of villi and escapes into the lumen of the small intestine to be passed in the feces. Injured and killed villus enterocytes are sloughed, resulting in collapse (atrophy) of the villus. Basement membranes are not injured, and crypt enterocytes divide and migrate up the denuded villus to cover exposed basement membrane (see



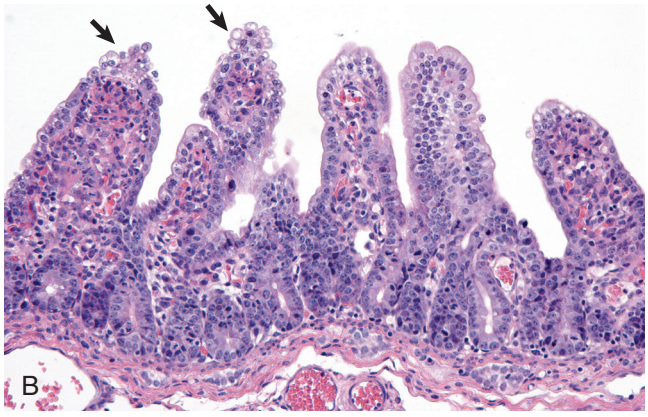


Figure 4-39 Mechanism of Viral Infections that Target Villus Absorptive Enterocytes. A, Transmissible gastroenteritis (TGE) virus and rotavirus use similar mechanisms to infect villus enterocytes and cause disease. **B,** Small intestine, villus atrophy. Following the initial loss of tip enterocytes (*arrows*), the villi contract, reducing the surface area to be reepithelialized. Note the crypt epithelium becomes hyperplastic with numerous mitoses, and the villi are covered by a less specialized, usually low cuboidal epithelium. Acute inflammatory cells infiltrate the villus lamina propria. H&E stain. (**A** and **B** courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

Fig. 4-39, A). Early in the reparative process, these migrating cells are flattened squamous-like cells stretched over the basement membrane. As the cells increase in density and maturity, they regain a more columnar structure. Additionally, the loss of enterocytes and exposure of basement membrane allows endotoxins and other potentially harmful molecules in digesta to cross into the lamina propria, enter capillary and lymphatic vessels, and through absorption cause systemic cardiovascular and hemodynamic effects. Finally, a malabsorption osmotic diarrhea also occurs because of the loss of enterocytes and the failure to digest carbohydrates (impaired hydrolysis) and other molecules in the digesta. The glycocalyx of the microvillus border (see Chapter 7) formed by affected enterocytes contains enzymes that are used to digest sugars. This activity is lost when enterocytes are lysed and sloughed into the intestinal lumen and leads to fermentation of substrates like glucose by resident bacterial flora (maldigestion). By-products of fermentation create on osmotic gradient that draws fluids across intestinal mucosa into the lumen to dilute the fermentation by-products. This process results in an osmotic diarrhea.

Porcine Epidemic Diarrhea (Coronavirus, Enveloped RNA Virus). Porcine epidemic diarrhea (PED) is an "especially dangerous and contagious disease" caused by porcine epidemic diarrhea virus (PEDV). Porcine epidemic diarrhea was diagnosed in the United States in May 2013, and in Canada in the winter of 2014. Additionally, a new viral strain, likely arising via antigenic drift and/or shift (see section on Viral Diseases, Mechanisms of Genomic Change), was detected in January 2014 in the state of Ohio. Economic losses to pork producers in the United States and Canada, as examples, at the time of this writing were undetermined but estimated to be substantial (≈ $900 million to $1.8 billion dollars). Virus affects "preweaning" piglets most severely; death losses of 75% to 100% are commonly reported in infected herds. The pathogenesis and mechanism of injury are very similar to those of transmissible gastroenteritis discussed in the previous section. Lesions in porcine epidemic diarrhea and transmissible gastroenteritis are similar (see Fig. 4-39); however, death losses are much more severe with porcine epidemic diarrhea infections because all animals exposed to the virus are "naïve" and have not acquired immune defense mechanisms via colostral antibodies (passive immunity) or through immunization (adaptive immunity) following natural exposure or vaccination (currently available only in South Korea, Japan, and China) as exist for transmissible gastroenteritis.

Swine Vesicular Disease (Enterovirus, Nonenveloped RNA Virus). The mechanism of injury in swine vesicular disease is cell dysfunction and lysis leading to intercellular edema (vesiculation), rupture of vesicles, and subsequent erosion and ulceration of mucosae and skin having vesicles. Gross lesions include vesicles, erosions, and ulcers on mucosae and skin of the snout, mouth, tongue, hoof coronary bands and interdigital skin, and teats. Pigs encounter virus through (1) contact with infected vesicular fluid, (2) contact with contaminated clothing or instruments, or (3) ingestion of contaminated pig offal, by-products, or meat products. It appears that the virus can enter the body through inhalation, ingestion, or contact with abraded skin.

Through inhalation or ingestion the virus encounters oronasal-pharyngeal mucosae, especially of the tonsil. It has not been determined if and how the virus penetrates the mucus layer to gain access to mucosal epithelial cells, mucosal macrophages, and/or dendritic cells. The role of mucosal epithelial cells in infection is unclear. The virus probably infects and replicates in mucosal macrophages, lymphocytes, and/or dendritic cells as they migrate through the mucus layer and mucosae and then is spread by these cells locally through leukocyte trafficking to the lamina propria and submucosa, where they infect and replicate in macrophages, lymphocytes, and dendritic cells of lymphoid nodules and aggregates. From here the virus spreads via lymphatic vessels to regional lymph nodes and infects similar cells, spreads systemically in these cells to other organ systems, including mucosae and skin via lymphatic vessels, the thoracic duct, and the blood vascular system.

Through ingestion the virus encounters mucosa of the small intestine, most likely overlying Peyer's patches. Although unproved, the virus likely infects M cells, which spread virus to tissue macrophages, dendritic cells, and other cells in Peyer's patches. Here similar cells are infected, and they migrate via leukocyte trafficking to spread virus via lymphatic vessels to regional lymph nodes and then systemically to other organ systems, including mucosa and skin.

Finally, it has been suggested that virus can infect Langerhans (dendritic cells) or other cells of the malpighian layer if the skin of the coronary band of the hooves is traumatized and epithelial cells of the stratum basale and/or spinosum are exposed to the environment. Virus can replicate in these squamous epithelial cells and also likely in Langerhans cells. Thus they may serve as a site of local infection, followed by spread via leukocyte trafficking to regional lymph nodes via lymphatic vessels, and systemic spread to other organ systems, including mucosa and skin.

No matter which route is used to establish, sustain, and amplify the systemic infection, it appears that virus can infect, injure, and lyse squamous epithelial (mucosae) and dendritic cells of the skin, resulting in vesicle formation. The mechanisms involved in vesicle formation have not been identified but could be similar to those used in poxvirus infections or vesicular stomatitis. It is unclear whether spread is via a cell-free viremia or leukocyte trafficking; both mechanisms of virus spread have been demonstrated in enterovirus infections. Virus appears to use capsid proteins, VP1-4, as attachment proteins to bind to glycoprotein receptors, such as ICAM, expressed on the surface of target cells. When interacting with cell receptors, viral capsid proteins undergo conformational changes leading to fusion of virus to cell membrane and internalization of virus within the target cell. The diversity of ICAM receptors expressed on a variety of cell membranes probably determines target cell specificity. Additionally, coxsackievirus-adenovirus receptor and sulfated glycosaminoglycans, such as heparin sulfate, may also be used as receptors on target cells.

Vesicular Exanthema of Pigs (Calicivirus, Nonenveloped RNA Virus). The pathogenesis and mechanisms of injury in vesicular exanthema of pigs are likely similar to those of swine vesicular disease discussed in the previous section. Capsid proteins used by the virus to attach and bind to target cells and receptors for virus on target cells have not been clearly identified. Vesicles are shown in Figures 7-33.

Foot-and-Mouth Disease (Aphthovirus, Nonenveloped RNA Virus). See Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Ruminants (Cattle, Sheep, and Goats); Foot-and-Mouth Disease (Aphthovirus, Nonenveloped RNA Virus).

Disorders of Dogs

Parvovirus Enteritis (Parvovirus, Nonenveloped DNA Virus). Parvovirus enteritis is a general name used to group two closely related strains of parvovirus that cause canine parvovirus enteritis and feline panleukopenia (feline parvovirus enteritis). The mechanism of injury is lysis of crypt epithelial cells and lymphocytes, including lymphocytes in the bone marrow. Specificity for these mitotically active cells occurs because parvoviruses require a target cell–derived duplex transcription template, which is available only when cells divide during the S phase of the cell cycle. Parvoviruses are unable

to turn on DNA synthesis in target cells, so they must wait for target cells to enter the S phase of the cell cycle (see E-Fig. 1-18) before infecting these cells. Gross lesions include segmental areas of the mucosa that are rough and granular (enterocyte necrosis, villus atrophy) with areas of hemorrhage, acute inflammation, and fibrin exudation (see Fig. 7-180).

Dogs and cats encounter parvoviruses in fomites from body fluids contaminated with fecal matter through direct contact with infected animals. The virus is inhaled or ingested; deposited on mucosae of the oral, nasal, and pharyngeal cavities; and trapped in the mucus layer. It has not been determined if and how virus penetrates the mucus layer to gain access to mucosal epithelial cells, mucosal macrophages, lymphocytes, and/or dendritic cells. Virus probably infects macrophages or dendritic cells migrating in the mucus layer and on the surface of mucosae. Virus replicates in these cells and is then spread via leukocyte trafficking to the lamina propria of the tonsils. Here additional macrophages and lymphocytes are infected and spread the virus via leukocyte trafficking in lymphatic and blood vascular systems to regional lymph nodes and systemically to the spleen, thymus, lymph nodes, bone marrow, and mucosa-associated lymphoid nodules such as Peyer's patches of the small intestine. Virus may also be spread as a cell-free viremia in lymph via lymphatic vessels to regional lymph nodes.

In these diseases the majority of virus-infected intestinal epithelial cells are found in crypts neighboring Peyer's patches in the small intestine. Experimental studies demonstrate that virus arrives at Peyer's patches before it reaches contiguous crypt enterocytes. Although not yet shown with canine or feline parvovirus, other similar viruses spread from Peyer's patches to M cells contiguous with Peyer's patches. Morphologically, M-cell processes extend into the mucosa and are contiguous with crypt enterocytes forming intestinal crypts. Additionally, virus entry into intestinal epithelial cells has a polarized pattern in which entry is restricted to the basolateral areas of crypt enterocytes, the areas nearest Peyer's patches and M cells. Collectively these findings suggest that virus initially spreads to the intestine and crypt cells via the blood vascular system and not in ingesta via peristalsis. It is unclear whether virus arrives as a cell-free viremia or within cells of the monocyte-macrophage and/or lymphoid systems; however, (1) virus infects such cells in the oral, nasal, pharyngeal mucosa, and tonsil and regional lymph nodes and (2) leukocyte trafficking is commonly used by other viruses to spread virus systemically to lymphoid and other organ systems, suggesting that parvovirus is spread to the intestine via leukocyte trafficking.

Infection is initiated through capsid-mediated attachment proteins to one or more glycosylated receptors on target cell membranes and is followed by entry via receptor-mediated endocytosis. It appears that canine transferrin receptor may need to present on target cells in canids. Parvoviruses also appear to use coreceptors for the attachment and entry processes. Attachment receptors may assist in aggregating virus near the cell membrane, and entry receptors may assist the virus in penetrating the cell membrane. In the dog this process requires capsid proteins to bind to transferrin receptors, whereas in the cat the process requires capsid proteins to bind to neuraminic acid and transferrin receptors. These receptors appear to determine which cells and which species of animal are infected by parvovirus strains. Parvoviruses are released from infected crypt enterocytes when the cell is lysed after the replication cycle is completed. Because of this outcome, parvovirus enteritis causes an osmotic malabsorption-maldigestion diarrhea. Diarrhea occurs because of the failure of replacement of absorptive enterocytes covering villi that are lost through normal turnover (≈48-hour life span). As a result, affected villi collapse, are atrophic, and all absorptive and digestive surfaces are lost; thus dietary carbohydrates are available for fermentation by intestinal bacteria. Under normal conditions, enterocytes covering villi are replaced by dividing crypt epithelial cells that move up and cover the villus. The loss of enterocytes covering villi also functionally opens a barrier system that normally prevents endotoxins from being absorbed by capillary beds in the lamina propria of the villi. Endotoxic shock and disseminated intravascular coagulation can result and kill the affected animal. Panleukopenia also occurs because of virus-induced cytolysis of rapidly dividing stem cells in the bone marrow. The effects of parvovirus on organs of the lymphatic system are discussed in the section on Viral Diseases of Organ Systems; Bone Marrow, Blood Cells, and Lymphatic System; Disorders of Dogs, and the effects of parvovirus on the heart are discussed in the section on Viral Diseases of Organ Systems, Cardiovascular System and Lymphatic Vessels, Disorders of Dogs.

Canine Enteric Coronavirus (Canine Coronavirus, Enveloped RNA Virus). The pathogenesis and mechanism of injury in canine enteric coronavirus are likely similar to but much less severe than those of canine parvovirus enteritis discussed in the previous section. Lysis of villus enterocytes may occur as the result of virus-induced apoptosis. Additionally, canine coronavirus may make villus enterocytes more susceptible to parvovirus infection. Thus coinfection may result in a disease more severe than that caused by either virus independently.

Canine Distemper (Morbillivirus, Enveloped RNA Virus). The pathogenesis and mechanisms of injury in canine distemper as related to the small intestine are discussed in the section on Viral Diseases of Organ Systems, Nervous System, Disorders of Dogs, Canine Distemper (Morbillivirus, Enveloped RNA Virus).

Disorders of Cats

Feline Infectious Peritonitis (Feline Infectious Peritonitis Virus, Nonenveloped RNA Virus). See the section on Viral Diseases of Organ Systems, Cardiovascular System and Lymphatic Vessels, Disorders of Cats, Feline Infectious Peritonitis (Feline Enteric Coronavirus/Feline Infectious Peritonitis Virus, Enveloped RNA Virus).

Parvovirus Enteritis (Parvovirus, Nonenveloped DNA Virus). The disease caused by parvovirus in cats is called feline panleukopenia or feline parvovirus enteritis. The pathogenesis and mechanisms of injury in feline parvovirus enteritis are likely very similar to those of canine parvovirus enteritis (see the section on Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Dogs; Parvovirus Enteritis [Parvovirus, Nonenveloped DNA Virus]).

Hepatobiliary System and Exocrine Pancreas

Disorders of Ruminants (Cattle, Sheep, and Goats)

Wesselsbron's Disease (Flavivirus, Enveloped RNA Virus). The mechanism of injury in Wesselsbron's disease is disruption and lysis of hepatocytes affecting young to very young sheep, cattle, and goats (ruminants). Gross lesions include an enlarged yellow to orange-brown liver (hepatomegaly) with randomly distributed white-gray foci (≈1 mm in diameter) of miliary necrosis of hepatocytes. Ruminants encounter this arbovirus through bite wounds from virus-infected mosquitoes; domestic herbivores likely serve as the animal reservoir. Seasonal variations in temperature and precipitation influence the population density of mosquitoes and thus the occurrence of disease. Virus can enter the circulatory system through direct penetration of a blood vessel during a bite wound, in which virus infects monocytes. It can also be deposited in vascularized ECM (connective) tissues, in which virus gains access to cutaneous blood

and fluids, as well as Langerhans cells (dendritic cells) and trafficking tissue macrophages.

Through either route, virus-infected monocytes, macrophages, and/or dendritic cells spread virus via lymphatic vessels to regional lymph nodes, where similar cells are infected. These cells then spread virus systemically via lymphatic vessels and the thoracic duct or postcapillary venules to the blood vascular system and other lymphoid tissues, such as the spleen, and other organ systems, such as the liver. This virus uses hepatocytes and Kupffer cells (part of the monocyte-macrophage system) as target cells. Hypertrophy and hyperplasia of Kupffer cells has been reported experimentally; however, their role in the pathogenesis of Wesselsbron's disease has not been determined. The virus may also possibly spread via cell-free viremia. Although unidentified at this time, viral envelope glycoproteins probably serve as attachment proteins for receptors expressed on specific populations of target cells, thus probably determining cellular tropism for the virus.

Rift Valley Fever (Phlebovirus, Enveloped RNA Virus). The pathogenesis and mechanisms of injury in Rift Valley fever are similar to those of Wesselsbron's disease discussed in the previous section.

Disorders of Dogs

Infectious Canine Hepatitis (Canine Adenovirus Infection, Canine Adenovirus Type 1, Nonenveloped DNA Virus). The mechanism of injury in infectious canine hepatitis is cell lysis (cytolysis) affecting epithelial cells of the liver and kidney and endothelial cells of all organ systems. Gross lesions include randomly distributed white-gray foci (≈1 mm in diameter) of miliary necrosis, as well as mucosal and serosal hyperemia and hemorrhage, and edema of multiple organ systems, including the liver, kidney, lymph nodes, thymus, gastric serosa, pancreas, and subcutaneous tissues (see Fig. 8-79). Edema of the gallbladder wall is prominent and is likely the result of injury of vascular endothelial cells leading to changes in permeability. Tonsillar enlargement, characteristic of the disease, probably results from proliferation of lymphocytes as part of the innate and/or adaptive immune responses against virus-infected cells through hyperplasia of uninfected lymphocytes in response to inflammatory mediators or through recruitment of lymphocytes from other lymphoid tissues and organs.

Dogs encounter the virus in fomites from body fluids such as saliva, urine, or feces. Virus enters the body through ingestion and likely inhalation, and it is trapped in the mucus layer of oral and pharyngeal mucosae, especially of the tonsils. It has not been determined if and how virus penetrates the mucus layer to gain access to mucosal epithelial cells, mucosal macrophages, and/or dendritic cells. Virus probably infects and replicates in mucosal macrophages and dendritic cells as they migrate through the mucus layer and mucosae. It is then spread by these cells locally through leukocyte trafficking to the lamina propria and submucosa and tonsil, where they infect and replicate in additional tissue macrophages, lymphocytes, and dendritic cells and then spread via afferent lymphatic vessels to regional lymph nodes and infect similar cells. A cell-free viremia has also been proposed as a mechanism of spread.

Although undetermined, virus may also be swallowed and through peristalsis encounter and infect M cells and spread to and infect macrophages, dendritic cells, and lymphocytes in Peyer's patches and then be spread to regional mesenteric lymph nodes. It is unclear how the virus is able to evade the actions of digestive enzymes, bile acids, and other microbial-lytic molecules. A virus capsid protein called *fiber protein* has been identified and may serve as an attachment protein that binds to target cell receptors such as coxsackievirus-adenovirus or integrin receptors.

Using either the inhalation or ingestion route of infection and spread, virus spreads either within virus-infected macrophages or as a cell-free viremia from regional lymph nodes, systemically to infect endothelial cells and their contiguous epithelial cells in many organ systems, including liver, kidneys, spleen, and lungs. Infection of, replication in, and release of virus from endothelial and epithelial cells cause their lysis and subsequent necrosis. The attachment of virus or virus-infected macrophages to endothelial cells is likely facilitated by molecules of the leukocyte adhesion cascade (see Chapter 3). Virus infects and replicates in endothelial cells, leading to endothelial cell injury and lysis (necrosis-vasculitis). Depending on the severity of endothelial cell injury, vasculitis can be followed by hemorrhage and edema (increased vascular permeability) and disseminated intravascular coagulation. Infection of epithelial cells of the liver and kidney are likely facilitated by ligand-receptor interactions, although none have been identified. Infection and lysis of lymphocytes in lymphoid tissues and likely bone marrow may account for the leukopenia occurring early in the infection.

Respiratory System, Mediastinum, and Pleurae

Disorders of Horses

Equine Influenza (Orthomyxovirus, Enveloped RNA Virus). The mechanism of injury in equine influenza is lysis of epithelial cells of the oral, nasal, pharyngeal, and respiratory mucosae. Gross lesions include active hyperemia, hemorrhage, edema, and necrosis leading to mucosal erosions and ulcers often covered with a mucofibrinous membrane.

Horses encounter virus in fomites from body fluids contaminated with virus through direct contact with virus-infected animals. Virus is inhaled and deposited on and/or trapped in the mucus layer of mucosae of the nasal and pharyngeal cavities and of the conductive component of the respiratory system through centrifugal and inertial turbulence. Virus must penetrate the mucus layer to gain access to ciliated epithelial cells (mucociliary apparatus); however, mucus contains glycoprotein receptor molecules that bind to virus and prevent it from attaching to these cells. This defense mechanism allows virus to be removed via the mucociliary apparatus and phagocytosis and killing by mucosal macrophages. To counteract this defense mechanism, the virus has a neuraminidase (virulence factor) that destroys receptors that mimic viral glycoprotein receptors in the mucus. However, it has not been determined how virus penetrates the mucus layer to gain access to mucosal epithelial cells. When virus encounters these cells, hemagglutinin and neuraminidase glycoproteins in its viral envelope bind to target cell membrane receptors composed of sialyloligosaccharides. This ligand-receptor binding allows virus to attach to and enter ciliated epithelial cells. The overall structure of sialyloligosaccharide receptors, in part, determines the target specificity at the cellular and the species levels. Early in the encounter before lysis affects cell function, the mucociliary apparatus spreads virus to additional target cells. This mechanism of spread becomes less effective as virus lyses ciliated and nonciliated mucosal epithelial cells and the physiologic continuity of the mucociliary apparatus is disrupted. It also appears that virus can spread in lymphatic vessels to regional lymph nodes via cell-free viremia or leukocyte trafficking and infect lymphocytes and macrophages. It is likely that lysis of lymphoid cells (immunosuppression) and ciliated epithelial cells and disruption of the mucociliary apparatus makes horses more susceptible to secondary bacterial diseases of the respiratory system. Hemagglutinin (HA) and neuraminidase (NA) are surface glycoproteins of the RNA virus that are very important in its pathogenesis in all animal species. Reassortment (antigenic shift) of the segmented RNA genome usually involves genes for HA and NA, which are the two virulence factors

most commonly associated with influenza epidemics and pandemics in human beings. Hemagglutinin is involved in ligand-receptor interactions that enable attachment to and entry of the virus into specific populations of target cells, whereas neuraminidase is involved in shedding of virus from infected cells.

A serious outbreak of equine influenza occurred in 2007 in Australia. A group of horses that entered the country for an exhibition carried a strain of influenza virus that was genetically different (antigenic drift and/or shift) than strains present in horses within the country; therefore resident horses had no or limited innate or adaptive immunity to this strain of virus. Additionally, antigenic drift and/or shift involving heterologous influenza viruses, including equine influenza virus, appear to be the underlying mechanism behind outbreaks of severe respiratory disease in racing greyhounds and English foxhounds. Experimentally it has been shown that canine and equine respiratory epithelium express similar sialyloligosaccharides. This finding suggests that receptors recognized by equine influenza virus are expressed on canine respiratory epithelial cells; nevertheless, subtle differences in receptor specificity may exist.

Equine Viral Rhinopneumonitis (Equine Herpesvirus, Alphaherpesvirus, Enveloped DNA Virus). The pathogenesis and mechanisms of injury in equine viral rhinopneumonitis are similar to those of infectious bovine rhinotracheitis; see Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants (Cattle, Sheep, and Goats); Infectious Bovine Rhinotracheitis (Bovine Herpesvirus, Alphaherpesvirus, Enveloped DNA Virus).

Equine Viral Arteritis (Arterivirus, Enveloped RNA Virus). Because some clinical signs of disease caused by equine viral arteritis virus arise from alterations in the respiratory system, it has been commonly considered a respiratory disease. However, the primary target cells in the lung are endothelial cells and thus this disease is discussed in the section on Viral Diseases of Organ Systems, Cardiovascular System and Lymphatic Vessels, Disorders of Horses, Equine Viral Arteritis (Arterivirus, Enveloped RNA Virus).

Disorders of Ruminants (Cattle, Sheep, and Goats)

Infectious Bovine Rhinotracheitis (Bovine Herpesvirus, Alphaherpesvirus, Enveloped DNA Virus). Bovine herpesvirus targets cells of the respiratory system but can also infect cells of the nervous system (see Viral Diseases of Organ Systems, Nervous System, Disorders of Ruminants (Cattle, Sheep, and Goats), Bovine Herpesvirus Meningoencephalitis [Bovine Herpesvirus 5: Alphaherpesvirus, Enveloped DNA Virus]). The mechanism of injury in infectious bovine rhinotracheitis in the respiratory system is lysis of nonciliated and ciliated (mucociliary apparatus) epithelial cells of the oral, nasal, pharyngeal, and respiratory mucosae. Gross lesions include active hyperemia, hemorrhage, edema, and necrosis leading to large areas of mucosal erosions and ulcers often covered with a fibrinous membrane (see Fig. 9-22).

Cattle encounter bovine herpesvirus in fomites of body fluids contaminated with virus through direct contact with virus-infected animals. Virus can be (1) inhaled or ingested and deposited on and trapped in mucus layers of oral, nasal, and pharyngeal mucosae; (2) inhaled and deposited and trapped in the mucus layer of the mucosa of the conductive component of the respiratory system through centrifugal and inertial turbulence; or (3) deposited on conjunctival mucosa. It has not been determined if and how virus penetrates mucus layers of these mucosae to gain access to epithelial cells or if mucosal macrophages and/or dendritic cells are involved in trafficking virus to target cells. Viral envelope glycoproteins B, C, and D are used to attach to and enter a variety of target cells by binding to an array of glycosaminoglycan receptors, such as herpesvirus entry mediator A, nectin-1 and nectin-2 (herpesvirus entry proteins C and B), and 3-*O*-sulfated heparin sulfate, most commonly expressed on mucosal epithelial cells and also on sensory nerve endings that innervate the mucosa. These receptors have a polarized pattern of expression and are present only on apical and lateral surfaces of mucosal epithelial cells above junctional complexes; therefore inhalation of virus provides it with optimal opportunities for interactions with appropriate receptors. Virus infects nonciliated and ciliated mucosal epithelial cells, completes its replication cycle in these cells, buds from nuclear and cell membranes, and through cell lysis or budding from membranes is released from infected cells back onto mucosae to infect additional cells or to be spread in fomites into the environment. The mechanism of cell lysis is unclear; however, (1) budding from nuclear and cell membranes may be sufficient to eventually cause lysis of the cell and (2) the products of "lytic" viral genes transcribed and translated late in the viral replication cycle may also cause cell lysis.

Bovine herpesvirus can also attach to and enter sensory nerve endings of the trigeminal and olfactory nerves in the respiratory mucosae. It then spreads via retrograde axonal transport in these nerves to other neurons in the CNS. Neurons serve as reservoir cells in which virus establishes a latent infection. Additionally, because neurons express no MHC class II molecules and low concentrations of MHC class I molecules, they are less likely, even when infected with virus, to be recognized by and acted on by cytotoxic and helper T lymphocytes, macrophages trafficking through the nervous system, and by resident microglial cells. During latency, viral genomes are present in the nucleus of infected neurons, but no viral proteins (antigens) are synthesized. With activation, the virus reestablishes its replication cycle and through axonal transport mechanisms spreads back to nerve endings in mucous membranes to be released and infect adjacent mucosal epithelial cells and transmit the disease. Bovine herpesvirus produces proteins that (1) disrupt the synthesis of interferon, (2) block the recognition of virus-infected cells by cytotoxic T lymphocytes, and (3) block the homing of T lymphocytes to virus-infected cells. Virus can also infect and induce high levels of apoptosis in T helper lymphocytes, thus suppressing the adaptive immune response to the virus. It is likely that a combination of these immunosuppressive mechanisms and disruption of the mucociliary apparatus through lysis of virus-infected ciliated mucosal epithelial cells make affected animals more susceptible to many secondary bacterial diseases of the respiratory system, such as pasteurellosis or mannheimiosis, that follow an outbreak of infectious bovine rhinotracheitis.

Bovine Respiratory Syncytial Virus Pneumonia (Pneumovirus, Enveloped RNA Virus). The mechanism of injury in bovine respiratory syncytial virus pneumonia is dysfunction and lysis of cells of the respiratory mucosae, including ciliated cells of the conductive system and alveolar type II pneumocytes of the O_2-CO_2 exchange component from infection by virus and from acute inflammation and its mediators and degradative enzymes. Gross lesions include active hyperemia, interstitial edema and inflammation (proliferative and exudative bronchiolitis), and subpleural and interstitial emphysema. Syncytial cells with intracytoplasmic inclusion bodies are observed in microscopic lesions (see Fig. 9-83).

Cattle encounter bovine respiratory syncytial virus in fomites from body fluids contaminated with virus through direct contact with virus-infected animals. It is inhaled, deposited on, and trapped in the mucus layer of the mucosa of the conductive component of the respiratory system through centrifugal and inertial turbulence, but it has not been determined if and how virus penetrates mucus layers to gain access to epithelial cells or if mucosal

macrophages and/or dendritic cells are involved. Virus infects and replicates in all epithelial cells; however, ciliated cells are the primary target cells. When virus encounters ciliated cells, it attaches and binds to membrane glycosaminoglycan receptors via heparin-binding domains on envelope glycoprotein G (attachment protein) and enters via envelope glycoprotein F (fusion protein). Fusion protein also appears to induce the formation of syncytial cells, a means by which cells infected with virus are able to interact with noninfected cells and spread the virus between cells. It has also been shown that virus can infect and replicate in lung dendritic cells and alveolar macrophages and cause the synthesis of interferons and interleukins. Infection of all target cell types appears to induce a cascade of proinflammatory chemokines and cytokines that recruit neutrophils, lymphocytes, and macrophages to the site, resulting in cell and tissue injury (inflammation). Additionally, TLR3 and TLR4 may initiate this cascade. In tissue culture experiments, virus appears to cause little or no injury to ciliated epithelial cells, suggesting that lesions may result in part from host defense mechanisms such as those modulated by the innate and adaptive immune responses. Bovine respiratory syncytial virus is part of bovine respiratory disease complex (shipping fever) (see section on Bacterial Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants (Cattle, Sheep, and Goats); Bovine Respiratory Disease Complex). This complex is characterized chronologically by (1) environmental or management stressors that suppress protective mechanisms in the respiratory system such as the production of protective mucus, (2) a primary viral infection that injures structural protective mechanisms such as the mucociliary apparatus, and (3) a secondary bacterial infection that causes severe inflammation often with fibrin exudation.

Bovine Influenza (Orthomyxovirus, Enveloped RNA Virus). The pathogenesis and mechanisms of injury in bovine influenza are similar to those of equine influenza (see section on Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Horses; Equine Influenza [Orthomyxovirus, Enveloped RNA Virus]).

Ovine Progressive Pneumonia, (Maedi; Maedi-Visna Virus [Ovine Lentivirus], Enveloped RNA Virus). The mechanism of injury in ovine progressive pneumonia is dysfunction and lysis of cells of the respiratory system from infection with virus and from chronic-active granulomatous interstitial inflammation and its mediators and degradative enzymes. Ovine lentivirus persistently infects monocyte precursor cells, systemic monocytes, alveolar and tissue macrophages, and dendritic cells. Gross lesions include dense, rubbery, and enlarged lung lobes that are uniformly affected and grayish-yellow to grayish-blue (see Figs. 9-75 and 9-92). Cut surfaces bulge and are rubbery and are not edematous or exudative, but excessive mucus may be present in airways.

Sheep most likely encounter virus in fomites from respiratory fluids through direct contact with virus-infected animals. In the fluid, virus may be free or in alveolar macrophages. However, any condition that facilitates mechanical transfer of infected blood to the circulatory system or mucosae of uninfected animals can also serve to spread virus. It is inhaled, deposited on, and trapped in the mucus layer of the mucosa of the conductive component of the respiratory system through centrifugal and inertial turbulence. Inhaled free virus is probably phagocytized by alveolar macrophages in the mucus layer, whereas virus within inhaled macrophages is released by exocytosis or through lysis of the macrophage by the virus or the animal's immune cells. Following either of these processes, free virus is phagocytized by alveolar macrophages in the mucus layer. They then migrate to local lymphoid tissues (BALT) and release virus via exocytosis or cell lysis, and virus infects naïve macrophages. These macrophages then spread via leukocyte trafficking in afferent lymphatic vessels to regional lymph nodes, where additional macrophages are infected, and then systemically to all organ systems, including bone marrow.

In the bone marrow, virus infects immature monocyte precursor cells (monoblasts or promonocytes and likely similar cells in spleen and lymph nodes), where small numbers of infected precursor cells serve as biologic reservoirs for the distribution (via the circulatory and lymphatic systems) of virus-infected monocytes back into the blood. Infected monocytes migrate in the circulatory system to tissues where they enter ECM, mature into tissue macrophages, and release virus to infect resident tissue macrophages such as alveolar macrophages. Virus likely uses envelope glycoproteins to attach and bind to, and fuse with alveolar macrophages and other target cells that express small ruminant lentivirus receptors A or B or some other membrane receptor. All cells infected with virus are permanently infected (persistent infection) because virus inserts its genome into chromosomal DNA of target cells.

The ability of virus to replicate in a cell is directly related to the maturity of the permanently infected cell. Once in bone marrow, virus integrates into precursor monocytes (monoblasts or promonocytes) and persistently infects a very small number of these cells. However, it is not able to replicate in these precursor monocytes. As these cells differentiate into monocytes, they migrate to tissues and organ systems that use the services of the monocyte-macrophage system and in these tissues differentiate into macrophages. Virus-infected monocytes in the peripheral blood are not able to produce virus. However, when infected monocytes mature and differentiate into macrophages in tissues, virus is able to replicate in these cells and produce viral proteins and proinflammatory chemokines and cytokines that initiate and sustain inflammation as well as release virus into ECM. This virus can now infect and activate other susceptible types of tissue macrophages, such as alveolar macrophages, and this interaction initiates and sustains the chronic active granulomatous inflammatory process.

The chronic active granulomatous inflammatory response so characteristic of ovine progressive pneumonia is a recurring process linked to the life span of tissue macrophages, which normally ranges from 6 to 16 days but can be shortened because of the viral replication cycle. When tissue macrophages die from viral infection, virus and viral proteins are released into the ECM, initiate inflammation, and recruit additional macrophages into the site to phagocytize free virus. This cyclic process creates and sustains an extensive inflammatory response in affected tissues. As naïve tissue macrophages are recruited into the ECM, new monocytes infected with virus arrive from bone marrow, differentiate into tissue macrophages, replicate virus, and release virus into the ECM to infect these naïve tissue macrophages, consequently sustaining the virus and the inflammatory response against it that ensues. Additionally, virus within these newly arriving monocytes may have undergone genetic variation (antigenic drift/shift [reassortment]) in the bone marrow. Because affected animals have no or limited innate or adaptive immunity to this "new" strain of virus, the inflammatory process begins anew. Virus-infected monocytes travel throughout all tissues and organ systems of the body; however, chronic-active inflammation occurs only in specific tissues. It appears that selectivity and specificity of lung, brain, mammary gland, and synovia occur in tissues where tissue macrophages are permissive to genome integration. Kupffer cells in the liver are not permissive and do not allow transcription of viral RNA, and the liver does not develop lesions. Based on this mechanism, involvement of the lung (maedi) and brain (visna) should occur in the same sheep at the same time; however, this

outcome is not common. The mechanism for this outcome is unknown.

Interstitial pneumonia results from virus-infected alveolar macrophages expressing high concentrations of proinflammatory chemokine, IL-8, that recruit inflammatory cells (not infected with virus) into the lung. These uninfected and recruited lymphocytes, plasma cells, macrophages, and neutrophils produce additional proinflammatory cytokines capable of sustaining inflammation and propagating the interstitial pneumonia. Thus a small number of virus-infected alveolar macrophages, responding to molecules through specific cell-membrane receptors, use a cascade of membrane, cytoplasmic, and nuclear messenger systems to control and sustain a large inflammatory response. Additionally, several studies suggest that lesions in ovine progressive pneumonia are, in part, immune mediated and that cytotoxic T lymphocytes may be important effector cells. Virus-infected macrophages present viral antigens to T lymphocytes, and activated T lymphocytes in turn release cytokines that lead to differentiation of monocytes to macrophages and recruitment of additional inflammatory cells. Host defense mechanisms are ineffective in ending virus infection because (1) the viral genome becomes part of the target cell genome, (2) viral infection of cells of the monocyte-macrophage system results in dysfunction of this system and an ineffective adaptive immune response (see Chapters 3 and 5), and (3) the parental virus can modify its progeny through repeated cycles of gene reassortment (genetic variation) so that these progeny are able to escape an effective adaptive immune response (cyclical [recurring] infection).

Caprine Pneumonia (Caprine Arthritis-Encephalitis Virus, Enveloped RNA Virus). The pathogenesis and mechanism of injury in caprine pneumonia are similar to those of ovine progressive pneumonia (see sections on Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants [Cattle, Sheep, and Goats], Ovine Progressive Pneumonia [Maedi; Maedi-Visna Virus (Ovine Lentivirus), Enveloped RNA Virus]) and caprine encephalitis of goats (see Viral Diseases of Organ Systems, Nervous System, Disorders of Ruminants [Cattle, Sheep, and Goats], Caprine Encephalitis [Caprine Arthritis-Encephalitis Virus, Enveloped RNA Virus]).

Disorders of Pigs

Porcine Reproductive and Respiratory Syndrome (Mystery Swine Disease; PRRS Virus, Arterivirus, Enveloped RNA Virus). The mechanism of injury in porcine reproductive and respiratory syndrome (PRRS) is lysis of all cell populations in the lung and associated regional lymph nodes secondary to acute inflammation (interstitial pneumonia) and its mediators and degradative enzymes. Gross lesions include lung lobules distributed at random throughout all lung lobes that are firm (consolidation) and red-tan to beige with septal edema. Lymph nodes, especially those draining the lungs, are enlarged, firm, and edematous and have a beige-white cut surface that bulges. These lesions may, in part, be attributable to secondary infection with a bacterium like *P. multocida*.

Porcine reproductive and respiratory syndrome occurs in two sequential stages: an acute stage followed by a persistent stage (i.e., persistent infection). In the acute stage, pigs encounter virus in fomites from body fluids through direct contact with virus-infected pigs. It is inhaled, deposited on, and trapped in the mucus layer of the mucosa of the conductive and O_2-CO_2 exchange components of the respiratory system through centrifugal and inertial turbulence. Pulmonary alveolar macrophages probably phagocytize the virus in the mucus layer and then spread it via leukocyte trafficking to BALT, alveolar macrophages of pulmonary septa, pneumocytes of alveoli, and epithelial cells of bronchioles, where infection and replication occur and acute inflammation (acute interstitial pneumonia and alveolitis) ensues. In this context, virus appears to be able to escape being killed in these cells. Potential mechanisms are discussed later. Concurrently, virus-infected macrophages migrate via afferent lymphatic vessels to regional lymph nodes (tracheobronchial) and infect macrophages and lymphocytes. It has also been suggested, but unproved, that dendritic cells may be infected and spread the virus. Viral infection causes (1) hypertrophy and hyperplasia of macrophages and lymphocytes leading to enlarged lymph nodes; (2) production of proinflammatory cytokines resulting in lysis of infected cells, releasing virus into surrounding ECM to maintain the infection, acute inflammation, and edema; and (3) the initiation of an adaptive immune response. Spread to other systemic organ systems (potentially the reproductive system) occurs at this stage, but it is unclear if spread is cell-free or cell-associated in macrophages (the latter being the most likely). In these organ systems, virus also infects cells of the monocyte-macrophage system.

In the persistent stage, virus via leukocyte trafficking establishes reservoirs in tissues, such as the tonsil, spleen, lymph nodes, and lung and in cells of the monocyte-macrophage system, such as alveolar macrophages. In part, infection of cells of the monocyte-macrophage system is likely determined by ligand-receptor interactions and may be related to the presence of sialoadhesin, a glycoprotein macrophage-specific receptor, expressed on cells of monocyte-macrophage lineage as well as scavenger receptor CD163 and heparan sulfate receptors. As an enveloped virus, PRRSV escapes from cells without causing cell lysis, but infected alveolar macrophages release proinflammatory cytokines, leading to acute inflammation and the recruitment of additional inflammatory cells, followed by cell lysis attributable to mediators and degradative enzymes of the inflammatory response. As a result, alveoli become filled with neutrophils, necrotic cell debris arising from killing of cells by the degradative activities of inflammatory cell enzymes, and edema fluid. Acute inflammation may also cause limited injury of the mucociliary apparatus, leading to increased opportunities for secondary bacterial pneumonia caused by bacteria such as *Pasteurella* or *Mannheimia*.

It appears that PRRSV, possibly mediated via nucleocapsid proteins, has both suppressive and stimulatory activities on cells it infects in the immune system. On the one hand, it is able to alter functions of the innate and adaptive immune systems, specifically of cells of the monocyte-macrophage system, by suppressing the ability of these cells to (1) kill virus-infected cells, (2) phagocytose, kill, and present antigens to effector cells, (3) stimulate other effector cells, and (4) secrete cytokines such as IFN-α, and TNF-α that are necessary to implement an effective immune response. The virus also appears to be able to modulate and/or minimize the effects of interferon in activating immune cells to act against virus and virus-infected cells. On the other hand, during the acute phase of infection virus is able to stimulate cells to significantly increase the production of IL-10 from infected cells. This cytokine is immunosuppressive and interacts with a wide array of immune cells, including the cells of the monocyte-macrophage system and lymphocytes, resulting in inhibition of innate and adaptive immunity, particularly the cell-mediated immune responses.

Swine Influenza (Orthomyxovirus, Enveloped RNA Virus). The pathogenesis and mechanisms of injury in swine influenza are similar to those of equine influenza (see section on Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Horses; Equine Influenza [Orthomyxovirus, Enveloped RNA Virus]).

Inclusion Body Rhinitis–Porcine Cytomegalovirus Infection (Herpesvirus-Cytomegalovirus, Enveloped DNA Virus). The

pathogenesis of porcine cytomegalovirus infection has not been studied in sufficient detail to provide an evidence-based discussion of the chronologic sequence of steps characteristic of the disease. The mechanism of injury is probably dysfunction and lysis of epithelial cells of the nasal and respiratory mucosa via infection with virus, especially the epithelial cells that form the mucous glands of the nasal cavity and from acute inflammation and its mediators and degradative enzymes. Gross lesions can include active hyperemia and hemorrhage, congestion, and mucopurulent exudate covering mucosal surfaces of the nasal septum and turbinates.

Pigs encounter porcine cytomegalovirus in fomites from body fluids contaminated with virus through direct contact with virus-infected animals. It is inhaled, deposited on, and trapped in the mucus layer of the mucosa of the conductive component of the respiratory system, especially of the nasal cavity, through centrifugal and inertial turbulence, but it has not been determined if and how virus penetrates mucus layers of these mucosae to gain access to epithelial cells or if mucosal macrophages and/or dendritic cells are involved. Therefore it appears that virus encounters epithelial cells that form the mucous glands by direct contact of the virus with apical surfaces of these cells or via mucosa-associated macrophages (or dendritic cells) that carry the virus to these cells. The conjunctiva may also be a source of infectious virus that later infects the mucosa of the nasal turbinates and septum via the lacrimal duct. Envelope attachment and fusion glycoproteins of the virus and target cell membrane receptor proteins are likely involved in infection, virus replication, and spread of virus to other cells and tissues. It is unclear how virus spreads systemically from the nasal cavity to other organ systems; however, in other animal models and human beings, leukocyte trafficking and cells of the monocyte-macrophage system are involved in the spread of similar viruses. Also, virus appears to be able to persist in cells of the monocyte-macrophage system; target and infect endothelial cells, causing lysis and hemorrhage; and infect and injure erythroid precursor cells in bone marrow, resulting in neonatal anemia.

Disorders of Dogs

Canine Infectious Respiratory Disease Complex. Canine infectious respiratory disease complex is a disease in which there is primary injury of the conductive component of the respiratory system caused by a virus, leading to increased susceptibility to infection with other bacteria, such as *B. bronchiseptica*. See the next section, Canine Infectious Tracheobronchitis, for greater detail.

Canine Infectious Tracheobronchitis (Canine Cough, Kennel Cough; Canine Parainfluenza Virus, Enveloped RNA Virus). Canine infectious tracheobronchitis is a disease in which there is primary injury caused by canine parainfluenza virus, leading to increased susceptibility secondarily to infection with *B. bronchiseptica* (or other bacteria). Other viruses (canine adenovirus type 2, canine respiratory coronavirus, reovirus, canine herpesvirus, canine distemper virus) and other bacteria (*Mycoplasma* spp., *Streptococcus equi* subsp. *zooepidemicus*) have been implicated in canine infectious tracheobronchitis, thus the phrase *canine infectious respiratory disease complex* has been used to categorize this multifactorial pathogenesis. The mechanism of injury is dysfunction and lysis of ciliated epithelial cells of the mucociliary apparatus primarily from virus-induced cytolysis and secondarily from acute inflammation (bronchitis/bronchiolitis) and its mediators and degradative enzymes. Gross lesions include active hyperemia and granularity (necrosis) of the respiratory mucosae and concurrent inflammation of mucosae and submucosae (see Fig. 9-101).

Dogs encounter parainfluenza virus in fomites of oronasal-pharyngeal fluids through direct contact with virus-infected dogs. It is inhaled, deposited on, and trapped in the mucus layer of mucosae of the conductive component of the respiratory system through centrifugal and inertial turbulence, but it has not been determined if and how virus penetrates mucus layers of these mucosae to gain access to epithelial cells or if mucosal macrophages and/or dendritic cells are involved. Virus infects and replicates in all epithelial cells; however, ciliated mucosal cells are the primary target cells. It appears that virus attaches to and enters cells via viral attachment glycoproteins (HN and F glycoproteins) that bind to sialic acid receptors located on the apical and lateral surface of ciliated epithelia cells. Viral infection disrupts the normal function of the mucociliary apparatus. Therefore normal and injured ciliated and nonciliated epithelial cells have a greater opportunity to encounter and interact with secondary bacteria, especially *B. bronchiseptica*, because the bacterium is not successfully removed by mucociliary clearance from the conductive component of the respiratory system.

B. bronchiseptica is inhaled, deposited on, and trapped in the mucus layer of the mucosa of the conductive component of the respiratory system through centrifugal and inertial turbulence. The bacterium colonizes ciliated epithelium via fimbrial and nonfimbrial adhesins such as filamentous hemagglutinin and pertactin. It has not been determined if and how it penetrates mucus layers to gain access to epithelial cells or if mucosal macrophages and/or dendritic cells are involved. Once ciliated cells are colonized, *B. bronchiseptica* releases exotoxins, such as adenylate cyclase-hemolysin and DNT and endotoxins, that further impair function of the mucociliary apparatus, allowing for additional colonization of mucosae by the bacterium at new sites. These outcomes, especially dysfunction of the mucociliary apparatus, contribute to "dependent settling" via gravity of bacteria into bronchi of dependent lung lobes, resulting in secondary bronchopneumonia. This damage results in an acute inflammatory response that further injures mucosae throughout the lung. *B. bronchiseptica* toxins may also disrupt phagocytosis and/or killing of bacteria by alveolar macrophages and neutrophils and suppress cellular and humoral immune responses. The bacterium can also invade epithelial cells, evade immunologic defense mechanisms, and establish a persistent infection.

Canine Distemper (Morbillivirus, Enveloped RNA Virus). The pathogenesis and mechanisms of injury in canine distemper as related to the respiratory system are discussed in the section on Viral Diseases of Organ Systems, Nervous System, Disorders of Dogs, Canine Distemper (Morbillivirus, Enveloped RNA Virus).

Canine Influenza (Orthomyxovirus, Enveloped RNA virus). The pathogenesis and mechanisms of injury in canine influenza are similar to those of equine influenza (see section on Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Horses; Equine Influenza [Orthomyxovirus, Enveloped RNA Virus]).

Canine influenza virus (H3N8) was originally diagnosed in racing greyhounds in January 2004 at a track in Florida, and the virus subsequently spread to 11 other states with dog racing tracks. Canine influenza virus (H3N8) appears to have resulted from mutation of the viral genome (antigenic drift/shift) of equine influenza virus (H3N8). Recently an outbreak of canine influenza occurred in the winter/spring 2015 in northern Illinois and midwestern states. The virus subsequently spread to New England and other areas. This outbreak was caused by canine influenza virus H3N2, which was originally limited within Korea, China, and Thailand.

When a new viral strain such as canine influenza virus H3N8 arises through recombination, natural selection, or antigenic drift/shift of viral genes such as those from equine influenza virus H3N8, infected dogs are exposed to a variety of new viral proteins. Many of these proteins behave as "new" virulence factors providing the

virus with increased pathogenicity and thus the ability to cause disease. Other proteins serve only as immunogens unique to influenza viruses. When a new strain of virus occurs, dogs have limited innate and no acquired immune responses to use as defense mechanisms. However, if the dog was vaccinated with a vaccine based the H3N8 viral strain, the immune response may provide some cross-protection to newly introduced viral strains such as canine influenza virus H3N2.

Disorders of Cats

Feline Upper Respiratory Disease Complex. Feline upper respiratory disease complex is a syndrome caused by feline viral rhinotracheitis virus and feline calicivirus acting concurrently and synergistically to cause disease. The mechanism of injury is dysfunction and lysis of ciliated epithelial cells of the mucociliary apparatus primarily from virus-induced cytolysis and secondarily from acute inflammation (bronchitis/bronchiolitis) and its mediators and degradative enzymes. The pathogenesis and mechanisms of injury for each virus are discussed in the next two sections.

Feline Viral Rhinotracheitis (Feline Herpesvirus, Alphaherpesvirus, Enveloped DNA Virus). The pathogenesis and mechanisms of injury in feline viral rhinotracheitis are similar to those of infectious bovine rhinotracheitis and equine viral rhinopneumonitis discussed earlier. Additionally, virus also infects mucosal macrophages and spreads to and infects similar cells in regional lymphoid nodes and then systemically via leukocyte trafficking or cell-free viremia to infect bone, eye, and lung, resulting in lysis of osteoblasts and osteocytes in the turbinates, necrosis of conjunctival and corneal epithelial cells, and necrosis of alveolar macrophages, respectively. Viral envelope glycoprotein G has been shown to attach and bind to chemokine receptors on target cells.

Feline Calicivirus (Calicivirus, Nonenveloped RNA Virus). The pathogenesis and mechanisms of injury in feline calicivirus infection are similar to those of feline viral rhinotracheitis, infectious bovine rhinotracheitis, and equine viral rhinopneumonitis discussed earlier. Although the mechanism of injury is probably necrosis and cell lysis, experimental studies have suggested that synthesis of caspases can be induced in virus-infected cells, resulting in apoptosis of these cells. Virus infects and replicates in mucosal epithelial cells and likely mucosal macrophages and then spreads in lymphatic vessels to regional lymph nodes via leukocyte trafficking (or cell-free viremia) to infect additional lymphocytes and macrophages. These cells then spread virus systemically to infect synovial macrophages and pulmonary alveolar macrophages, leading to synovitis and probably interstitial pneumonia, respectively. Ligand-receptor interactions are likely involved in tropism for specific cell types. It is unclear whether interstitial pneumonia (1) results from inhalation and infection of apical membranes of mucosal epithelial cells and alveolar macrophages of mucosae of the conductive and O_2-CO_2 exchange components of the respiratory system, (2) is the result of leukocyte trafficking of virus-infected lymphocytes, macrophages, and monocytes back to the lung after infecting and being amplified in regional and systemic lymph nodes and lymphoid organs, or (3) is attributable to a combination of both mechanisms.

A syndrome termed *virulent systemic feline calicivirus infection* has been characterized clinically. In addition to epithelial cell tropism, this virulent strain has acquired tropism for endothelial cells. It causes systemic vascular injury, lysis of endothelial cells, microthrombosis, and disseminated intravascular coagulation, resulting in multiple–organ system failure. This change in viral pathogenicity likely occurred through reassortment of viral capsid genes (antigenic shift) leading to enhanced virulence factors that modulate attachment and entry and likely replication in endothelial cells. Furthermore, an additional virulence factor appears to contribute to an exuberant target cell cytokine response as a defense mechanism against virus-infected epithelial and endothelial cells. Thus vascular lesions may in part be immune mediated and worsened by the actions of cytokines.

Cardiovascular System and Lymphatic Vessels

Disorders of Horses

Equine Viral Arteritis (Arterivirus, Enveloped RNA Virus). The mechanism of injury in equine viral arteritis is lysis of endothelial cells, myocytes, and pericytes of small muscular arterioles and venules of multiple organ systems, especially of the lungs. Gross lesions include (1) congestion, edema, and hemorrhage in subcutaneous tissues of the limbs and abdomen; (2) hydroperitoneum, hydropericardium, and hydroabdomen; and (3) edema and hemorrhage in lymph nodes and intestines.

Horses inhale virus in fomites of body fluids, most commonly urine, through direct contact with virus-infected animals. It is deposited on mucosae of the conductive and O_2-CO_2 exchange systems through centrifugal and inertial turbulence and trapped in the mucus layer. Virus is likely phagocytosed by bronchiolar and alveolar macrophages as they migrate through the mucus layer and mucosae and spread locally through leukocyte trafficking to the submucosa (BALT), where they infect additional tissue macrophages. From here macrophages spread virus to regional lymph nodes via afferent lymphatic vessels, where additional macrophages are infected. Ligand-receptor interactions are probably involved in tropism for specific cell types. Virus expresses envelope glycoproteins and a nucleocapsid protein; however, their role in binding to target cells has not been clearly defined. Additionally, membrane receptors for the virus have not been identified. Macrophages leave regional lymph nodes and enter the circulatory system via postcapillary venules or lymphatic vessels and the thoracic duct. During vascular migration, infected macrophages encounter endothelial cells, myocytes, and pericytes of small arterioles (and venules), and their interactions are facilitated by molecules of the leukocyte adhesion cascade (see Chapter 3). Monocytes containing viral antigens have been observed adhering to endothelial cells, and virus appears to spread from monocytes to endothelial cells, myocytes, and pericytes of these vessels.

Migrating monocytes also encounter and infect hepatocytes, adrenal cortical cells, seminiferous tubular cells, and thyroid follicular cells. Virus-induced vascular injury leads to cell lysis characterized by endothelial swelling, degeneration, and necrosis, acute and chronic (lymphomonocytic) inflammation, necrosis of myocytes, and thrombus formation, leading to edema and hemorrhage in many tissues and organs. Virus replication occurs in endothelial cells, and the expression of envelope glycoproteins in endothelial cells likely activates acute inflammation, fibrinogenesis, the complement cascade, and the recruitment of neutrophils into vascular intima and tunica media and in severe cases results in fibrinoid necrosis and vasculitis. The role of proinflammatory chemokines and cytokines in vascular injury has not been defined. A lymphomonocytic inflammatory cell population is also commonly found in the tunica media and adventitia of blood vessels, suggesting that cytolytic T lymphocytes could induce cytolysis of virus-infected endothelial cells. Why lysis of endothelial cells and myocytes dominates over lysis of epithelial cells, such as those in renal tubules, is unknown. However, infection and lysis of renal tubular epithelial cells with release of virus into urine appear to be the mechanism by which virus is spread to naïve horses.

African Horse Sickness (Orbivirus, Nonenveloped RNA Virus). The pathogenesis and mechanism of injury in African horse sickness

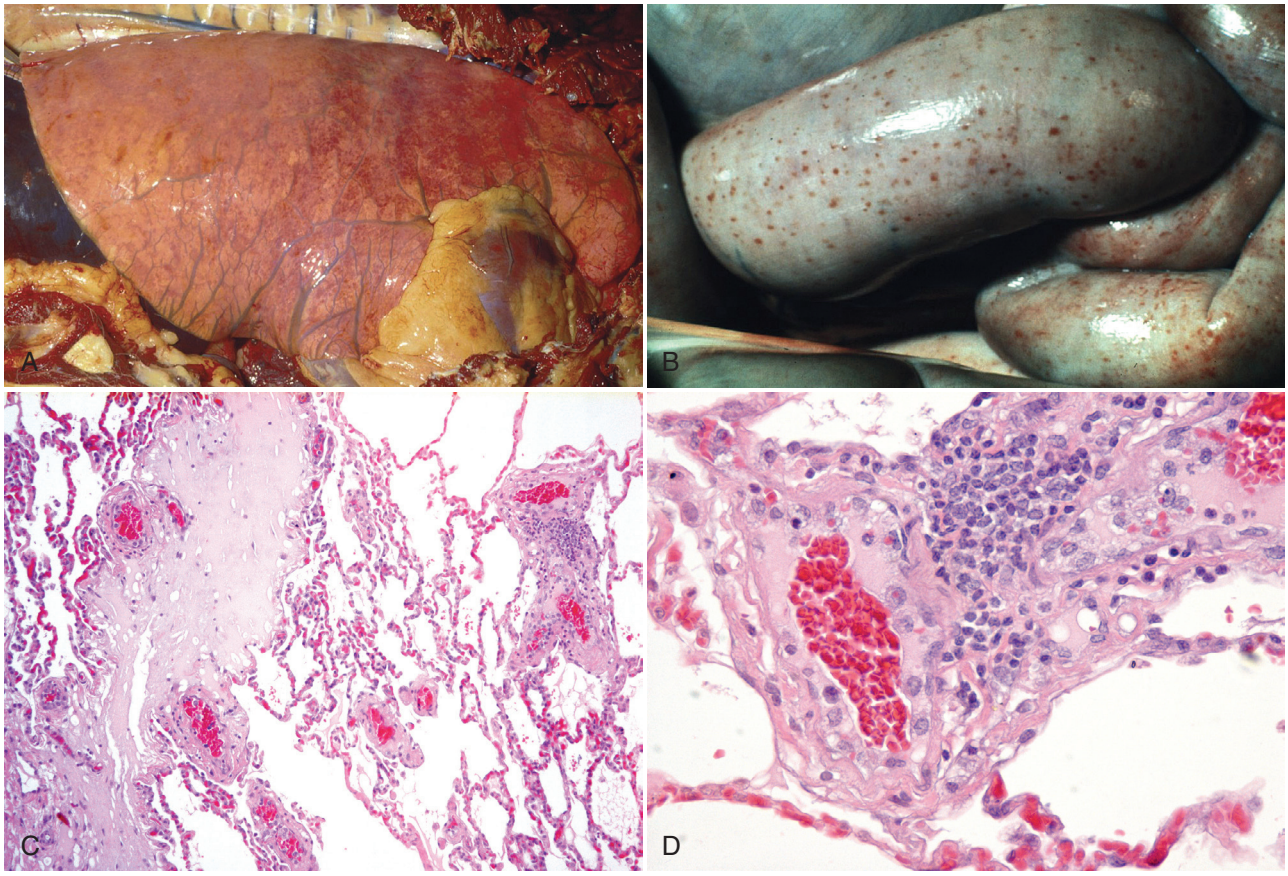


Figure 4-40 African Horse Sickness. A, Pulmonary edema. The interlobular septa are widely separated and distended with edema fluid. Edema fluid is also present in alveoli and alveolar septa. Also note the suffusive hemorrhage of the visceral pleura. These lesions are caused by infection of endothelial cells of the capillaries of the interlobular and alveolar septa by African horse sickness virus, resulting in endothelial cell barrier malfunction and lysis of endothelial cells. **B,** Colonic serosa, petechial and ecchymotic hemorrhages. These lesions are also caused by infection of and damage to endothelial cells. **C,** Lung, interlobular edema. The interlobular septum and alveoli contain edema fluid. Capillaries and venules are surrounded by bronchial-associated lymphoid tissue (BALT). H&E stain. **D,** Higher magnification of **C.** The endothelial cells of venules are swollen, have vacuolated and reticulated cytoplasm, and have large reactive nuclei consistent with responses to injury caused by infection of these cells by African horse sickness virus. Note the BALT. H&E stain. (**A** courtesy Dr. D. Gregg, Plum Island Animal Disease Center; and Noah's Arkive, College of Veterinary Medicine, The University of Georgia. **B** courtesy Dr. R. Breeze, Plum Island Animal Disease Center; and Noah's Arkive, College of Veterinary Medicine, The University of Georgia. **C** and **D** courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

are similar to those of bluetongue disease discussed later. The mechanism of injury is endothelial cell barrier dysfunction and virus-induced dysfunction and lysis of endothelial cells. There are four clinical forms of African horse sickness; however, in each form the gross lesions are characteristic of vascular (endothelial cell) injury and include edema (pulmonary, systemic, subcutaneous, intramuscular, supraorbital fossae, eyelids, lips, cheeks, tongue, intermandibular space, and larynx), active hyperemia, petechial and ecchymotic hemorrhages (serosal [epicardial, endocardial], subcapsular [spleen], cortical [kidney], and mucosal [intestines]), hydrothorax, hydropericardium, ascites, and rhabdomyocytic necrosis (Fig. 4-40). The expression of these forms may be related to differences in viral tropism for different types of vascular endothelial cells within organ systems of the body or the permissiveness of different types of endothelial cells in allowing the virus to replicate efficiently or in large numbers. African horse sickness virus also infects cells of the dendritic, lymphoid, and monocyte-macrophage systems.

African horse sickness is a noncontagious disease of horses, donkeys, and mules. Animals encounter virus in bite wounds from midges. After skin penetration, virus can enter the circulatory system or be deposited in vascularized ECM (connective) tissues. If a blood vessel is penetrated, virus can enter the circulatory system and infect macrophages and lymphocytes or be carried cell-free to systemic lymphoid tissues. If deposited in connective tissue, virus gains access to cutaneous blood and fluids, as well as cutaneous dendritic cells (Langerhans cells) and tissue macrophages. Although unproved, it is likely that virus infects these cells, and virus-infected macrophages or dendritic cells spread the virus via leukocyte trafficking and lymphatic vessels to regional lymph nodes. Here virus infects lymphocytes and additional dendritic cells and macrophages. African horse sickness virus has two attachment proteins, capsid structural proteins (VP2 and VP5). These proteins bind to glycosaminoglycans on target cell membranes and facilitate attachment and entry of virus.

From regional lymph nodes, virus spreads systemically in macrophages via leukocyte trafficking to the circulatory system, through postcapillary venules and/or lymphatic vessels and the thoracic duct, to infect, injure, and kill vascular endothelial cells in the lungs, heart, spleen, lymph nodes, liver, and kidney. Infected macrophages likely interact with endothelial cells of these organs by adhering to and migrating through the endothelium, likely by activating the leukocyte adhesion cascade (see Chapter 3). Virus spreads from macrophages and infects and replicates in endothelial cells, resulting in direct injury and inducing an acute inflammatory response.

Vascular lesions are probably lytic and characterized by endothelial swelling, degeneration, and necrosis, and depending on the severity of injury, vasculitis can be followed by hemorrhage and edema (increased vascular permeability) affecting the lung and vascular thrombosis leading to tissue infarction. Necrosis of rhabdomyocytes in the heart has been attributed to the release of endogenous catecholamines, but experimental findings suggest that necrosis is caused by microthrombosis of myocardial capillaries, likely resulting in myocyte ischemia. Rarely, disseminated intravascular coagulation has been reported in African horse sickness. Additionally, NS3, a protein inserted in the target cell membrane by the virus, may be cytotoxic (acting as a viroporin that alters target cell membrane permeability) and involved in membrane damage and the release of virus from infected endothelial cells.

Disorders of Ruminants (Cattle, Sheep, and Goats)

Bluetongue (Orbivirus, Nonenveloped RNA Virus). The mechanism of injury in bluetongue is dysfunction and lysis of endothelial cells. Gross lesions include systemic hemorrhage, edema, and vasculitis. Such lesions are more severe in sheep when compared to cattle, apparently because there are species differences in the susceptibility of endothelial cells to infection and the severity of endothelial injury. Bluetongue is a noncontagious disease of sheep, cattle, and other ruminants (deer). The virus is encountered in fluids from hematophagous *Culicoides* (biting midges), which is the insect vector for the virus. After skin penetration, virus gains access to cutaneous blood and fluids, as well as cutaneous dendritic cells (Langerhans cells), monocytes, and tissue macrophages. Although unproved, virus probably infects these cells, and virus-infected monocytes and macrophages migrate to local lymph nodules and/or lymphoid aggregates, then to regional lymph nodes via afferent lymphatic vessels. Here virus infects lymphocytes and additional dendritic cells, monocytes, and macrophages. Macrophages then enter the blood vascular and lymphatic systems (blood vascular via the thoracic duct) and migrate in the vascular system to all organ systems. There they adhere to, migrate through, and reside in the walls of blood vessels and thus are in direct contact with endothelial cells. Virus lyses and escapes from these macrophages and binds to receptors on endothelial cells.

Bluetongue virus has two attachment proteins, capsid structural proteins (VP2 and VP5). These proteins bind to glycosaminoglycans in target cell membranes and facilitate attachment and penetration of virus into macrophages and likely into endothelial cells. Systemically the attachment of virus-infected macrophages to endothelial cells is likely facilitated by molecules of the leukocyte adhesion cascade (see Chapter 3). Virus in infected monocytes/macrophages that attach to endothelial cells escape (via cell lysis) from these monocytes/macrophages and adhere to, infect, and replicate in endothelial cells, leading to increased permeability of the endothelium as well as to endothelial cell injury and lysis (necrosis-vasculitis). Changes in permeability may also be attributable, in part, to the production of vasoactive cytokines such as TNF-α by virus-infected monocytes/macrophages, leading to increased permeability of the vascular endothelium. With excessive leakage of fluids, hypovolemic (circulatory) shock may occur. Finally, depending on the severity of endothelial cell injury, vasculitis can be followed by hemorrhage and edema affecting the lung and by vascular thrombosis leading to oral mucosal ulcerations, tissue infarction, and disseminated intravascular coagulation, which can kill the affected animal.

Bovine Malignant Catarrhal Fever (Ovine Herpesvirus 2 and Alcelaphine Herpesvirus 1 [γ-Herpesviruses], Enveloped DNA Virus). The mechanism of injury in bovine malignant catarrhal fever is dysfunction and lysis of vascular endothelial cells and hyperplasia, dysfunction, and lysis of lymphocytes in lymphoid tissues. Gross lesions include (1) erosive, ulcerative, and hemorrhagic lesions of mucosae of gingiva, tongue, oral papillae, hard and soft palate, oral pharynx, esophagus, turbinates, trachea, rumen, reticulum, and omasum (see Fig. 7-35); (2) enlargement of lymphoid organs and tissues followed by atrophy; and (3) increased size of visceral organs and tissues resulting from perivascular accumulations of lymphocytes (lymphoproliferative vasculitis).

Worldwide, sheep are the reservoir animal for sheep-associated ovine herpesvirus 2 (OvHV-2), which causes malignant catarrhal fever in cattle, bison, pigs, and deer. In Africa, blue wildebeest are the reservoir animal for wildebeest-associated alcelaphine herpesvirus 1 (AlHV-1), which causes malignant catarrhal fever in cattle. These viruses persist in carrier animals without ill effects.

The chronologic sequence of steps leading to malignant catarrhal fever has not been clearly determined. Animals probably encounter these viruses through inhalation and ingestion of fomites from oronasal-pharyngeal-ocular fluids (also seminal fluid) from reservoir animals that are actively shedding virus. In reservoir animals, OvHV-2 is shed predominantly through nasal secretions derived from turbinates, and shedding episodes are stress induced and occur more frequently in lambs than adult sheep. Virus is deposited on mucosae of the oral, nasal, and pharyngeal cavities; the conjunctiva; or the conductive component of the respiratory system through centrifugal and inertial turbulence. It is trapped in the mucus layer and apparently phagocytosed by mucosal macrophages and spread to submucosae and BALT via leukocyte trafficking. It has not been determined if dendritic cells are involved. In submucosae, virus infects lymphocytes (possibly B lymphocytes), macrophages, and monocytes and spreads in $CD8^+$ T lymphocytes via leukocyte trafficking to regional lymph nodes and then systemically to other organ systems and lymphoid tissues. Ligand-receptor interactions are probably involved in tropism for specific cell types, but viral envelope glycoproteins or cell receptors have not been identified.

Virus-infected $CD8^+$ T lymphocytes are distributed in intimal, medial, and adventitial tissues of blood vessels (vascular and perivascular pattern) in organ systems. This tropism may be determined by (1) ligand-receptor interactions or (2) permissiveness of specific vascular cells to viral infection and replication. As part of this tropism, virus-infected $CD8^+$ T lymphocytes produce proinflammatory cytokines and express viral glycoproteins in their cell membranes. Proinflammatory cytokines can act as cytotoxic molecules and may injure and kill bystander cells, such as those in the vasculature, and viral glycoproteins may recruit lymphocytes, macrophages, and monocytes and lesser numbers of neutrophils and plasma cells into perivascular and vascular tissues, leading to lymphoproliferative necrotizing vasculitis and vascular wall necrosis. It is not known if virus can infect, injure, and kill endothelial cells directly. The cause of the erosive, ulcerative, and hemorrhagic lesions has not been determined; however, infarction of mucosal blood vessels secondary to thrombosis induced by necrotizing vasculitis could potentially lead to these outcomes. Additionally, virus-infected large granular lymphocytes and other recruited cytotoxic lymphocytes and macrophages may play roles in vascular injury because they have been shown to be cytotoxic for vascular endothelial cells.

Atrophy of lymphoid tissues after viral infection is not likely caused by virus-induced cell lysis. Because lymphocytes are short-lived effector cells, atrophy is likely the result of normal cell aging and turnover that follows massive proliferation.

Apparently there is a lack of spread of virus between susceptible animals because they are dead-end hosts for these viruses. Virus spread appears to require cell-free virus in body fluids, and in

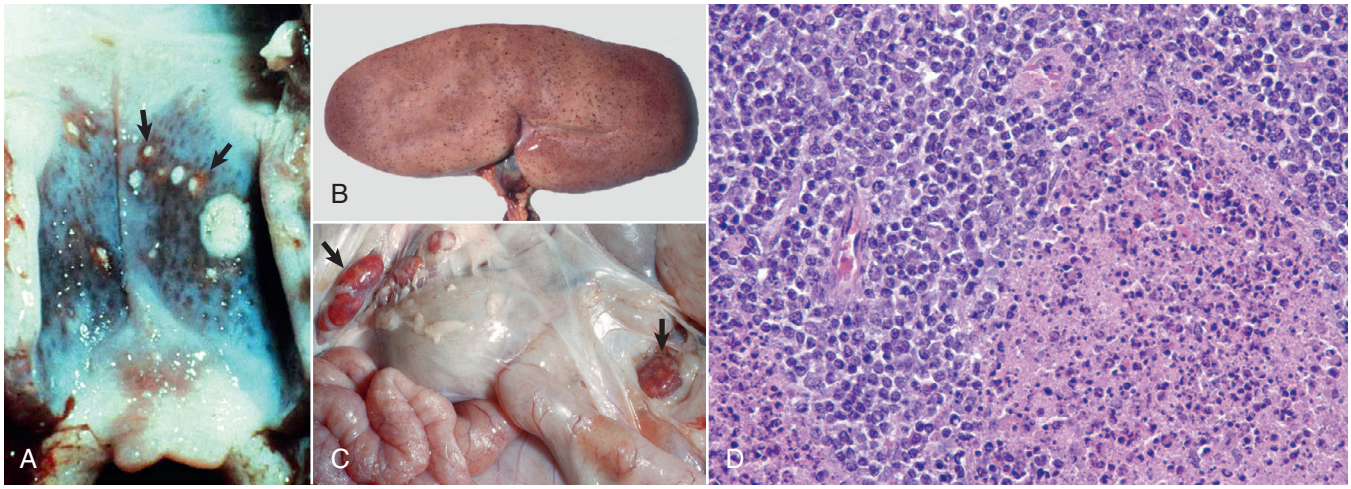


Figure 4-41 Classic Swine Fever (Hog Cholera). Lesions in classic swine fever are similar to those observed in African swine fever, but usually less severe. See Figure 4-42 for lesions of African swine fever. **A,** The tonsil (of the soft palate), a tissue of choice for isolation and identification of the virus, contains foci of hemorrhage and necrosis *(arrows)*, the result of necrosis of mucosal epithelial cells in tonsillar crypts and necrosis of the adjacent endothelial cells and lymphocytes in the lamina propria from infection with virus. **B,** Kidney. The cortical surface has numerous randomly distributed petechia caused by injury to and subsequent necrosis of endothelial cells following their infection with classic swine fever virus. **C,** Mesenteric lymph nodes *(arrows)* are enlarged and congested due to vascular injury caused by virus, resulting in blood in the subcapsular sinuses. **D,** Tonsillar crypt lymphoid nodules. Note the focal necrosis of lymphocytes *(right lower half of image)* in the nodules caused by infection with virus. H&E stain. (**A** courtesy Dr. R. Breeze, Plum Island Animal Disease Center; and Noah's Arkive, College of Veterinary Medicine, The University of Georgia. **B** courtesy Dr. D. Gregg, Plum Island Animal Disease Center and Noah's Arkive, College of Veterinary Medicine, The University of Georgia. **C** courtesy Dr. M.D. McGavin, College of Veterinary Medicine, University of Tennessee. **D** courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

susceptible animals the virus replicates in a cell-associated manner (lymphocytes, macrophages, monocytes) and cell-free virus is not produced. Because virus-infected target cells do not produce infectious virus during the virus replication cycle, these species are unable to transmit virus to other animals (see carrier animals earlier).

Disorders of Pigs

Classic Swine Fever (Hog Cholera, Pestivirus, Enveloped RNA Virus). The mechanism of injury in classic swine fever is probably cytokine-induced dysfunction and lysis of endothelial cells of multiple organ systems, of macrophages and monocytes, and of hemopoietic cells in the bone marrow. Gross lesions include a red-blue discoloration of the skin; hydropericardium, hydrothorax, and hydroperitoneum; hemorrhage and necrosis of the palatine tonsil; and petechial and ecchymotic hemorrhages in most organs of the body, especially the kidney (Fig. 4-41).

Pigs encounter virus through (1) ingestion and likely inhalation of fomites from body fluids, body waste, or offal or other virus-contaminated pork products and (2) mechanical transfer from virus-contaminated vehicles, clothes/boots, instruments, and needles. Virus is deposited on mucosae of the oral and nasal pharynx, especially of the tonsil, where it infects and replicates in epithelial cells of tonsillar crypts. It has not been determined how virus penetrates the mucus layer to gain access to mucosal epithelial cells or if mucosal macrophages or dendritic cells phagocytose virus in the mucus layer and spread it through leukocyte trafficking to the lamina propria and submucosa. E^{rns} and E2 envelope glycoproteins and other viral envelope glycoproteins appear to be involved in binding to and entering mucosal epithelial cells and macrophages via cell surface glycosaminoglycan receptors such as heparin sulfate. Although unknown, virus likely buds from basal surfaces of tonsillar epithelial cells and infects subjacent mucosal macrophages in lymphoid aggregates (MALT). Infected macrophages migrate via leukocyte trafficking in afferent lymphatic vessels to regional lymph nodes such as the submandibular and pharyngeal. Here, through release of proinflammatory chemokines and cytokines, they likely recruit additional macrophages (i.e., monocytes) from the systemic circulation and also cause lymphoid hyperplasia in affected lymph nodes and lymphoid tissues. Virus-infected macrophages provide virus to infect these additional macrophages and lymphocytes. In addition to enlargement from hyperplasia, lymph nodes become edematous and hemorrhagic because of endothelial cell injury within capillaries caused by the actions of cytokines and mediators of acute inflammation (see later) initiated and modulated by virus-infected tissue macrophages.

Subsequently, virus-infected macrophages leave regional lymph nodes and enter the circulatory system via postcapillary venules or lymphatic vessels and the thoracic duct to migrate systemically to all organ systems. Infected macrophages probably interact with endothelial cells of all organs by adhering to and migrating into and through the endothelium, likely via cytokines (TNF-α and IL-1) and activation of the leukocyte adhesion cascade (see Chapter 3). Initially, lysis of endothelial cells was thought to be caused by viral replication in these cells; however, it is now thought that lysis is attributable to the effects of cytokines released from macrophages. Viral replication in macrophages (and lymphocytes) appears to result in the production and release of large amounts of proinflammatory cytokines into the ECM subjacent to endothelial cells from intact or lysed macrophages. This outcome results in injury to and lysis of endothelial cells by these cytokines and/or by mediators and degradative enzymes of the acute inflammatory response that ensues. Additionally, vascular lesions are characterized by endothelial swelling, degeneration, and necrosis (cytolysis); acute and chronic (lymphomonocytic) inflammation; necrosis of myocytes; and thrombus formation, leading to edema and hemorrhage in many tissues and organs. This pattern of injury serves as the basis for hemorrhage observed grossly and microscopically in lesions of the kidney.

Macrophages also spread virus to lymphoid tissues (spleen and lymph nodes) and bone marrow, where it either infects and lyses these cells or causes lysis via an apoptotic mechanism or a cytokine,

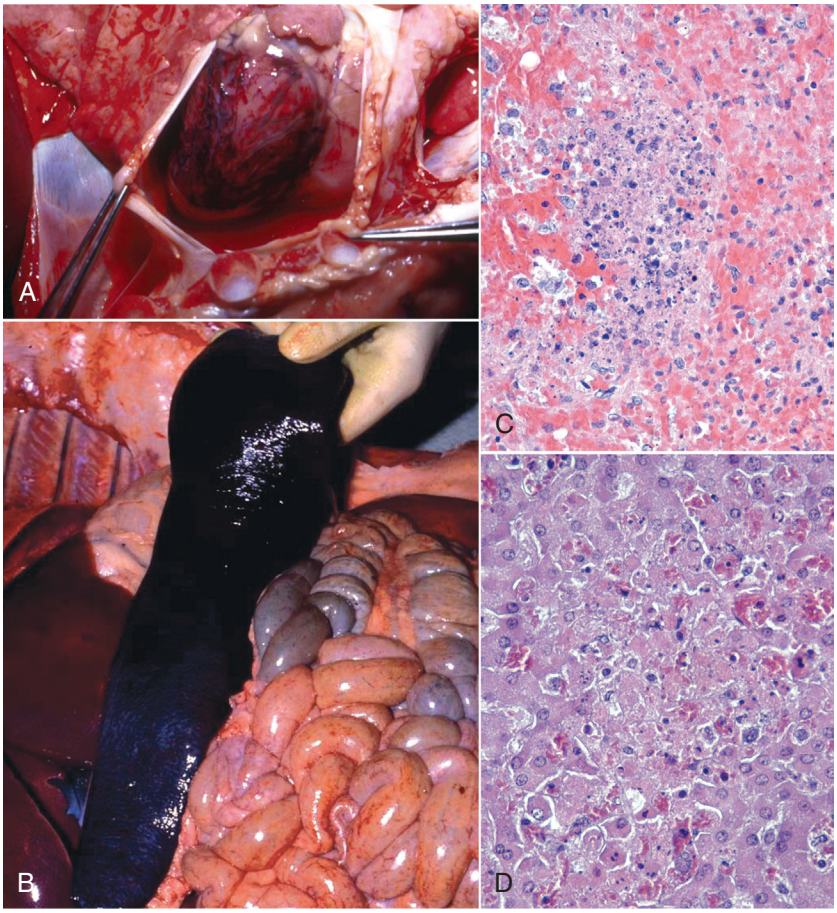


Figure 4-42 African Swine Fever. Lesions in African swine fever are similar to those observed in classic swine fever, but usually much more severe. See Figure 4-41 for lesions of classic swine fever. **A,** Epicardium and pericardial cavity. The epicardium and subjacent myocardium have numerous randomly distributed ecchymoses caused by injury to and subsequent necrosis of endothelial cells from infection with African swine fever virus. Note the accumulation of a fibrinous effusion in the pericardial cavity. **B,** Splenomegaly, bloody spleen. The spleen is congested with blood and friable as a result of vascular damage caused by the virus. Lymph nodes (not shown here) are also congested and edematous (see classic swine fever). **C,** Endothelial cells and lymphoid cells of white pulp of the spleen are necrotic (e.g., pyknosis, karyolysis). H&E stain. **D,** Endothelial cells lining sinusoids of the liver are necrotic (e.g., pyknosis, karyolysis). Also note the necrosis of some hepatocytes. H&E stain. (**A** courtesy Dr. C. Brown, College of Veterinary Medicine, The University of Georgia. **B** courtesy Dr. D. Gregg, Plum Island Animal Disease Center; and Noah's Arkive, College of Veterinary Medicine, The University of Georgia. **C** and **D** courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

resulting in severely impaired adaptive immune responses with decreased neutralizing antibody production, decreased numbers of phagocytes, decreased cell-mediated immune responses, and decreased numbers of platelets. Virus also probably causes severe loss of monocytes, macrophages, and lymphocytes in all organ systems via cell lysis and apoptosis induced by proinflammatory cytokines. The impairment and loss of these defense mechanisms makes pigs more susceptible to other infectious diseases.

African Swine Fever (Asfivirus, Enveloped DNA Virus). The pathogenesis, mechanisms of injury, and clinical outcomes of African swine fever are very similar to those of classic swine fever discussed in the previous section. In summary, the pathogenicity (virulence factors) of lesions and disease caused by African swine fever virus are much more severe (Fig. 4-42). Additionally, African swine fever virus can also gain access to the blood vascular system and directly infect macrophages through bites of ticks. Virus envelope glycoproteins p12, p54, and p30 appear to be involved in attaching and binding to and entering target cells via cell receptors. Target cell receptors have not been clearly identified. Cytokines released from macrophages are probably the cause of lysis of endothelial cells in all organ systems, resulting in vasculitis, systemic petechial and ecchymotic hemorrhages, disseminated intravascular coagulation, collapse of the circulatory system, shock, and death of virus-infected pigs.

Disorders of Dogs

Parvovirus Myocarditis (Parvovirus, Nonenveloped DNA Virus). See the section on Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Dogs; Parvovirus Enteritis (Parvovirus, Nonenveloped DNA Virus) for information on the pathogenesis of viral spread and replication before spreading to the heart. The mechanism of injury in parvovirus myocarditis is cell lysis (necrosis of rhabdomyocytes) attributable to infection with virus. Gross lesions include gray-white areas of varied sizes distributed in the myocardium (see Fig. 10-81). It is likely that virus spreads via leukocyte trafficking or cell-free viremia in lymphatic or blood vessels from Peyer's patches to regional lymph nodes and then systemically in the circulatory system to capillary endothelial cells and rhabdomyocytes in the heart. Endothelial cells are dividing cells, and studies suggest that in the heart, virus initially infects and replicates in these cells and then spreads to infect contiguous cardiac rhabdomyocytes.

Rhabdomyocytes are also actively dividing cells in dogs under 15 days of age; therefore they can be infected with virus and are lysed with release of virus. This outcome results in necrosis of rhabdomyocytes and ectopic irritable foci, cardiac arrhythmias, and unexpected death of puppies. If puppies survive this stage, healing mechanisms cause cardiac fibrosis that can contribute clinically to dysfunction of the cardiac conduction system and contraction of the cardiac musculature later in life. Specificity for these mitotically active cells occurs because parvoviruses require a target cell–derived duplex transcription template, which is available only when cells divide during the S phase of the cell cycle (see the section on parvovirus enteritis for more detail). Ligand-receptor interactions are also probably involved, but unknown. It appears that canine transferrin receptor may need to present on target cells.

Canine Herpesvirus Infection (Canine Herpesvirus Type 1, Enveloped DNA Virus). The mechanism of injury in canine herpesvirus infection is lysis of endothelial cells of all organ systems and of epithelial cells of multiple organ systems (pantropic). Gross lesions include mucosal and serosal hemorrhage and randomly distributed white-gray foci (≈1 mm in diameter) of miliary necrosis within organ systems, especially the kidneys (see Fig. 11-67). Miliary necrosis can also be observed in spleen, lymph nodes, lung, and liver.

Puppies ingest and inhale virus in fomites from body fluids of the birth canal or nasal-oral cavity of bitches through grooming. It is deposited on mucosae of the nasal and oral pharynx, especially those of the tonsil, and is thought to infect mucosal epithelial cells. It has not been determined if and how virus penetrates the mucus layer to gain access to mucosal epithelial cells. Although it appears that virus infects lymphocytes in the tonsil, it has not been determined if and how virus spreads from mucosal epithelial cells to lymphocytes or if leukocyte trafficking via mucosa-associated macrophages or dendritic cells is involved. It is also unclear if or how virus migrates to regional or systemic lymphoid nodes, thymus, or spleen before it spreads systemically to infect endothelial and epithelial cells of other organ systems; however, virus appears to be spread systemically in lymphocytes via leukocyte trafficking. It has not been satisfactorily determined (1) how virus-infected lymphocytes interact with endothelial and epithelial cells or if this interaction is facilitated by molecules of the leukocyte adhesion cascade (see Chapter 3); (2) how virus infects and replicates in endothelial cells, leading to injury and cell lysis; and (3) if injury results in vasculitis and vascular thrombosis, leading to tissue infarction and disseminated intravascular coagulation. Additionally, the potential role of cytokines, derived from virus-infected lymphocytes or macrophages, in causing or contributing to endothelial cell injury and lysis has not been determined. Canine herpesvirus expresses envelope glycoproteins B, C, and D; however, their role in attaching and binding to target cells has not been clearly defined. Heparin sulfate may serve as a target cell receptor for canine herpesvirus. The disease is most severe in puppies less than 5 weeks of age; it is thought that low body temperatures of puppies increases the pathogenicity of the virus by improving its ability to enter cells, replicate, and spread.

Canine Circovirus Infection (Circovirus, Nonenveloped DNA Virus). This disorder of dogs was first recognized in the fall of 2013 in Ohio. Its mechanism of injury and pathogenesis are unknown; however, gross lesions characterized by a fibrinonecrotic vasculitis suggest that vascular endothelial cells are one of its target cells. Review of the earlier discussion of canine herpesvirus infection may be useful. It is unclear if this virus is the primary cause of this disorder or if it is the result of coinfection with other pathogens such as canine enteric coronavirus or one of six other pathogens.

Disorders of Cats

Feline Infectious Peritonitis (Feline Enteric Coronavirus/Feline Infectious Peritonitis Virus, Enveloped RNA Virus). A presumed mutation (antigenic drift/shift) of the 3c gene of feline enteric coronavirus genome appears to result in a "new" virulence form (biotype) virus, feline infectious peritonitis virus that causes feline infectious peritonitis (FIP). This mutation, which is thought to occur within mucosal macrophages and/or blood monocytes, seems to inhibit or block function(s) of the 3c gene, allowing the mutated virus to assume greater pathogenicity by enhancing cell tropism for and internalization and replication in macrophages. The mechanism of injury is chronic-active pyogranulomatous inflammation (vasculitis and perivasculitis) and its mediators and degradative enzymes. Gross lesions include gray-white nodules of varied sizes that have a perivascular pattern of distribution and in some cases a linear pattern following blood vessels in serosa and mesenteries (see Figs. 7-16, 11-68, and 14-105). Body cavities may contain a thick yellow exudate containing fibrin and pyogranulomatous inflammatory cells (see Fig. 7-16).

Cats encounter feline enteric coronavirus by ingestion of virus-contaminated fomites through two routes: (1) contact with virus-contaminated feces in litter boxes and (2) contact with carrier cats, usually queens. Fomites from saliva or respiratory droplets probably serve as a source of the virus to infect naïve cats via ingestion; therefore grooming behavior increases the likelihood that virus will enter the oral cavity. Feline enteric coronavirus is swallowed and moved via intestinal peristalsis to the alimentary system, where it gains access to mucosae. The replication of enteric coronavirus is primarily restricted to mature intestinal epithelial cells (terminally differentiated cells with a life span of 3 to 8 days); however, the virus can enter a carrier state and persist in unidentified cells of the intestinal mucosa. These cells are likely progenitor cells (i.e., crypt stem cells) with infinite life spans, so cell lysis through normal enterocyte turnover does not affect the carrier state of the virus. Feline enteric coronavirus spreads from enterocytes and carrier cells into the lamina propria and then to macrophages in Peyer's patches. It has not been determined if and how virus penetrates the mucus layer to gain access to mucosal epithelial cells or if mucosal macrophages, dendritic cells, or M cells are involved. Leukocyte trafficking to the submucosa by these cells would explain how virus spreads to macrophages in Peyer's patches.

Feline enteric coronavirus likely uses proteins, such as S1 protein, and potentially other glycoproteins such as S2, M, and E, to attach and bind to feline aminopeptidase-*N*, a cell membrane receptor on monocytes and macrophages. Other less well characterized attachment proteins and target cell receptors have been described in other strains of mutated virus. In mucosal macrophages of Peyer's patches and in blood monocytes, feline enteric coronavirus mutates into feline infectious peritonitis virus. Thus the genome of each new feline infectious peritonitis virus variant is unique to an individual cat. When feline enteric coronavirus mutates to feline infectious peritonitis virus, feline infectious peritonitis virus acquires virulence factors that allow it to infect and replicate in cells of the monocyte-macrophage system, resulting in rapid dissemination of the virus throughout the body.

Monocytes and macrophages infected with feline infectious peritonitis virus spread from Peyer's patches to regional lymph nodes via leukocyte trafficking in lymphatic vessels and infect additional macrophages. They then migrate in efferent lymphatic vessels through the thoracic duct into the circulatory system and to all tissues of the body and infect additional populations of free and fixed tissue macrophages. Virus-infected macrophages appear to target small and medium-sized veins of serosal membranes and tissues, cause damage

to endothelial cells, and are recognized as foreign by the cat's innate (inflammation) and adaptive (cell-mediated and humoral) defense mechanisms (see Chapters 3 and 5). This process likely involves activation of the leukocyte adhesion cascade and binding of macrophages and monocytes to endothelial cells facilitated by ligand-receptor interactions and the activation of acute inflammation via proinflammatory cytokines released from activated macrophages and monocytes. All of these processes result in injury of vascular and perivascular tissues (vasculitis).

Cats with a strong cell-mediated response do not develop feline infectious peritonitis. Cats with a weak cell-mediated response have the dry (noneffusive) form; cats with no cell-mediated response have the wet form (effusive). An effective humoral response appears to increase the severity of disease. Tissue macrophages provide a source of viral antigens in and around venules, and if adequate antibody is present, antigen-antibody complexes form and a type III hypersensitivity response ensues. Where immune complexes are formed (i.e., basement membrane of endothelial cells) or whether they are free or cell associated is not clearly understood. These complexes activate complement, resulting in chemotaxis and accumulation of neutrophils via the leukocyte adhesion cascade. Additionally, they also activate tissue macrophages, leading to the secretion of a variety of proinflammatory cytokines that act on endothelial cells to increase neutrophil and mononuclear cell chemotaxis into the area and open tight junctions of endothelial cells (increased permeability), thereby allowing leakage of plasma and fibrin into body cavities. These mechanisms result in the vasocentric pyogranulomas and pyogranulomatous inflammation, fibrinous effusions, and fibrinous polyserositis (feline coronaviral polyserositis) so characteristic of feline infectious peritonitis. A type IV hypersensitivity reaction may be involved in the pathogenesis of some pyogranulomas. It appears that the commonly used categories of wet and dry forms and type III and type IV hypersensitivities are based more on clinical characteristics and immunologic tests, respectively, than on any morphologic criteria. Experimental studies have shown that there are no distinct histopathologic lesions that distinguish wet from dry cases, type III from type IV hypersensitivities, or acute/subacute cases from chronic cases.

Bone Marrow, Blood Cells, and Lymphatic System

Disorders of Horses

Equine Infectious Anemia (Equine Infectious Anemia Virus, Enveloped RNA Virus). The mechanism of injury in equine infectious anemia (EIA) is inflammation (and proliferation [hypertrophy and hyperplasia]) of the monocyte-macrophage and lymphoid systems, particularly in the spleen and lymph nodes, resulting in chronic-active splenitis and lymphadenitis. Virus does not cause cell lysis. Gross lesions include an enlarged spleen (splenomegaly) and lymph nodes (lymphadenomegaly) with abundant white-gray lymphoid tissue arranged in follicles and solid sheets of cells that often bulge from cut surfaces.

Equine infectious anemia is a bloodborne infection. Horses initially encounter virus through penetrating wounds of the blood vascular system in the skin, either from fly (horseflies, deerflies) or mosquito bites or from contaminated needles. Once in the bloodstream, free virus infects monocytes (nonpermissive cells), but because they are not fully differentiated macrophages, virus cannot fully replicate. Thus monocytes spread virus in the circulatory system via leukocyte trafficking to all organ systems. Infected monocytes then migrate through blood vessel walls and enter ECM of tissues, where they differentiate into tissue macrophages (permissive cells). In these cells, virus can replicate and serve to infect other macrophages and lymphocytes, especially in lymphoid tissues such as spleen and lymph nodes. It appears the virus has several envelope glycoproteins such as surface envelope protein (gp120) and gp90 (and likely others) that attach and bind to equine lentivirus receptor-1 present in cell membranes of monocytes and macrophages. Infected macrophages produce proinflammatory chemokines and cytokines that recruit additional monocytes and lymphocytes into organs; thus splenomegaly and lymphadenomegaly ensue. Virus does not cause cell lysis.

The two clinical phases of equine infectious anemia are acute and chronic. In the acute phase, horses have recurring fever, anemia, thrombocytopenia, and petechia with interspersed periods of quiescence. Fever is likely attributable to release of proinflammatory cytokines and endogenous pyrogens from activated macrophages during the leukocyte trafficking phases of the disease. Anemia occurs from phagocytosis and complement-mediated lysis of erythrocytes that have had their cell membranes altered by virus, antibody, complement, and/or fibrinogen. Pulmonary intravascular macrophages, Kupffer cells, and fixed macrophages lining vascular sinusoids in the spleen and lymph nodes are reservoirs for virus and release it continuously into the bloodstream. Cell-free virus adsorbs onto the surface of red blood cells (and likely platelets) in the circulatory system. Adsorbed viral proteins act as haptens that are recognized as foreign by cells of the monocyte-macrophage system and are phagocytosed. Additionally, the hapten is processed and presented to lymphocytes, leading to a humoral immune response and the generation of plasma cells that secrete antibody against the hapten and other antigens on the red blood cell membrane (type II hypersensitivity response). If the hapten-antibody complex fixes complement, red blood cells are lysed intravascularly. If complement is not fixed, red blood cells are phagocytosed by cells of the monocyte-macrophage system and lysed extravascularly. Both of these mechanisms result in severe anemia. The cause of thrombocytopenia is less clear and is thought to occur because of activation of platelets and concurrent binding of fibrinogen to the surface of platelets during acute viremic phases of the disease. It is likely that activated platelets are quickly phagocytosed by the monocyte-macrophage system, leading to thrombocytopenia. Petechial hemorrhages may be attributable to vascular injury caused by direct infection of endothelial cells by virus or more likely by secondary responses to injury induced by innate and adaptive immune defense mechanisms.

In chronic equine infectious anemia, recurrence of disease is caused by antigenic variation (antigenic drift/shift) of surface glycoproteins of the virus. This genetic variation results in "new" virus that expresses new surface glycoproteins, thus beginning anew the process of developing effective cell-mediated and humoral responses. Long-lasting "immunity" to equine infectious anemia appears to require that an adaptive immune response control the disease before antigenic variation occurs. Large quantities of virus are replicated in cells of the monocyte-macrophage system, and virus is not eliminated from these cells during the acute phase of the disease. As adaptive immune responses develop, cytotoxic T lymphocytes are thought to control, to a limited extent, viremia and virus replication in infected monocytes and macrophages. However, it appears that control of the disease (no or minimal anemia and thrombocytopenia) is linked to an effective antibody response against the virus that takes 6 to 8 months to develop.

Disorders of Ruminants (Cattle, Sheep, and Goats)

Enzootic Bovine Lymphoma (Lymphosarcoma, Bovine Leukosis Virus–Associated Malignant Lymphoma, Deltaretrovirus: Bovine Leukemia Virus, Enveloped RNA Virus). The mechanism of injury in enzootic bovine lymphoma is provirus-induced malignant transformation of B lymphocytes. Gross lesions include proliferation of

neoplastic cells and their infiltration into perivascular spaces in organ systems resulting in (1) generalized enlargement of affected organs with increased pallor or (2) the formation of one or more solid white nodules distributed at random in the affected tissue (see Figs. 7-90, 10-46, 10-47, 13-60, 13-87, and 13-88). Additionally, cells can occupy and proliferate in confined spaces, causing compressive atrophy of tissue in these spaces such as axons in the spinal cord, hemopoietic cells in the bone marrow, and the retina in the eye. Organ systems commonly having lesions include superficial and visceral lymph nodes and thymus, skin, abomasum, heart, spleen, kidneys, uterus (caruncles), spinal meninges, retrobulbar lymphatic tissue, bone, and bone marrow. Malignant transformation is a sequence of steps in which normal cells acquire the biologic behaviors of neoplastic cells such as uncontrolled growth, tissue invasion, and metastasis. In cattle it takes several years for this transformation to occur and be manifested in overt lymphoma. This long prodromal period is likely caused by the complexity and interplay of injurious and reparative processes induced by the provirus that eventually result in dysfunction or mutation of regulatory cell cycle genes. Bovine leukemia virus infects B lymphocytes and thus is not free as a virus in blood or body fluids but is a provirus, cell associated, and integrated into the target cell's genome. When its replication cycle is completed, new virus is released from provirus-infected B lymphocytes. New virus serves to sustain and amplify the infection by infecting naïve B lymphocytes and cells of the monocyte-macrophage system.

Cattle and calves encounter provirus-infected B lymphocytes in blood, inflammatory exudates, and colostrum or milk. Provirus-infected B lymphocytes must gain access to the blood vascular and/or lymphatic systems and eventually to tissues and target cells suitable for infection. When they gain access to tissues, it is unclear whether B lymphocytes (1) behave as trafficking leukocytes and migrate into the vascular and/or lymphatic systems to spread new virus to other cells and tissues or (2) undergo cytolysis and release virus into tissues to infect local tissue macrophages, lymphocytes, or dendritic cells such as Langerhans cells. The use of needles or surgical instruments contaminated with blood (provirus-infected B lymphocytes) can transfer these cells directly into the vascular system or place them in vascularized subcutaneous tissues or muscle in close proximity to capillary and lymphatic vascular beds. Such exposure may require traumatic injury to skin or mucous membranes. Insect bites can apparently result in the same outcome. In either case, provirus-infected B lymphocytes, deposited in these locations, encounter cells of the monocyte-macrophage and lymphoid systems and dendritic cells. It has been shown that virus can infect these cells, but whether these cells spread virus or provirus to regional lymph nodes and then systemically via leukocyte trafficking in these cells or in B lymphocytes is unknown.

Transplacental spread of bovine leukemia virus from cows to calves also occurs through the blood. Provirus-infected B lymphocytes can also be present in inflammatory exudates, such as those occurring with postpartum metritis or vaginitis, and must gain access to capillary and lymphatic beds in host animals as previously described. Finally, provirus-infected B lymphocytes can be present in colostrum or milk, and it has been suggested that enzootic bovine lymphoma can result from virus entering the body via the alimentary system and gaining access to the blood vascular system. However, the role, as examples, of alimentary peristalsis, gastric acidity, mucosal mucous barrier systems, mucosal epithelial barrier systems, mucosal immunity, and M cells has not been adequately addressed in experimental studies. Although hypothetical, provirus-infected B lymphocytes in colostrum or milk could behave as typical trafficking leukocytes and thereby attach to and migrate through mucosae of the oral and nasal pharynx and gain access to local MALTs, lymphatic vessels, regional lymph nodes, and systemic lymphoid tissues.

It appears that whatever route is used by provirus-infected B lymphocytes to enter the body, they must gain access to the blood vascular system to establish, sustain, and amplify an infection regionally and systemically. Virus uses bovine leukemia virus envelope glycoproteins (gp51, gp30) to attach to and enter naïve host B lymphocytes that express a novel membrane protein called *bovine leukemia virus–binding receptor*. Other studies have shown that B lymphocytes expressing surface immunoglobulin M and cell surface markers CD5 and CD11b are more susceptible to infection with virus; however, the role of these molecules as receptors is unclear.

The mechanism of B lymphocyte transformation has not been established. Transformation may be linked to a mechanism called *gene transactivation*. When the genome of bovine leukemia virus (provirus) is integrated into the genome of a B lymphocyte, the provirus asserts control over the transcriptional and translational organelles and processes of the target cell. Genes of bovine leukemia provirus express a protein called bovine leukemia virus Tax protein (p34tax) that appears to stimulate the proliferation (increased mitoses) of B lymphocytes and increases viral replication in target cells. Tax protein also interacts with target cell genes and appears to transactivate genes that express proteins modulating target cell growth, such as cell division and differentiation, and are involved in regulatory steps of cell proliferation and longevity. Experimentally, Tax protein has been shown to be able to immortalize rat embryo fibroblasts in tissue culture and to cooperate with an oncogene to transform tissue culture cells that can then be grown as tumors in live animals. Collectively these findings suggest that transformation of B lymphocytes, leading to bovine lymphoma, is linked to the likely long-term actions of p34tax on target cell regulatory genes, but the chronologic stages of transformation are uncertain. Studies suggest that transformation may also result from Tax protein forming complexes with proteins expressed by tumor-suppressor genes such as p53, whereas other studies suggest that point mutations in the p53 gene may be one of the critical steps leading to lymphoma. The proteins translated from tumor-suppressor genes have an inhibitory effect on the regulation of the cell cycle and function to inhibit cell division, inhibit division of cells with damaged DNA, initiate apoptosis of cells with damaged DNA, and amplify cell adhesion (metastasis suppressors). When the activities of the p53 gene and its protein gene products are perturbed or inhibited, transformation of affected cells could occur.

Disorders of Pigs

Postweaning Multisystemic Wasting Syndrome (Porcine Circovirus Type 2, Nonenveloped DNA Virus). The mechanism of injury in postweaning multisystemic wasting syndrome (PMWS) is virus-induced dysfunction and lysis of lymphocytes leading to lymphocyte depletion and immunosuppression. Virus appears to require dividing cells, like lymphocytes, in the S phase of the cell cycle for infection and replication. Gross lesions include systemic enlargement of lymph nodes, normal-sized lymph nodes, and small atrophic lymph nodes, which are a continuum of changes in the response of lymphocytes to viral infection, replication, and release. Initial infection is likely correlated with viral replication and intense hyperplasia (lymphadenomegaly). Hyperplasia is followed by release of virus from infected lymphocytes, a process that kills lymphocytes and results in atrophy. Microscopic lesions are unique in the fact that inflammation is granulomatous with macrophage-derived syncytial giant cells.

Pigs encounter virus in fomites from oronasal-pharyngeal body fluids, feces, and urine from infected animals. Virus is inhaled or

ingested and deposited on mucosae. In the respiratory system, virus is deposited on and trapped in the mucus layer by centrifugal and inertial turbulence and encounters mucosa of the tonsils. It has not been determined if and how virus penetrates the mucus layer to gain access to mucosal epithelial cells, mucosal macrophages, and/or dendritic cells. In the alimentary system, it is swallowed, gains access to the small intestine through peristalsis, and encounters M cells overlying Peyer's patches. M cells lack a mucus layer, and virus has direct access to cell membranes.

It appears that virus establishes an infection in lymphoid tissues of the tonsil and Peyer's patches by infecting mucosal dendritic cells, macrophages, and lymphocytes. Except for M cells, it is not clear how virus spreads through the mucosal epithelium to reach cells in lamina propria and submucosae (MALT), but leukocyte trafficking is likely involved. Spread through the mucosal epithelium could also occur through ligand-receptor interactions, followed by viral transcytosis to the basal surfaces with release on the abluminal side. Once macrophages, dendritic cells, and lymphocytes are infected locally, virus spreads by leukocyte trafficking in macrophages and dendritic cells via afferent lymphatic vessels to regional lymph nodes and then systemically through postcapillary venules or lymphatic vessels and the thoracic duct to the circulatory system to lymphocytes in the spleen, lymph nodes, and other lymphoid tissues.

Virus uses a viral capsid attachment protein to attach to heparin sulfate and chondroitin sulfate B (glycosaminoglycan [sialic acids] receptors) on macrophages, dendritic cells, and lymphocytes to enter and infect these cells. Macrophages are nonpermissive to virus and appear to serve primarily as trafficking cells to spread virus to other locations, whereas lymphocytes are permissive to virus and allow viral replication. Therefore lymphocytes are injured and killed during replication and release. Although virus-induced necrosis has been suggested as the mechanism for cell lysis, apoptosis may actually be the main cause through a viral protein that activates caspase pathways and apoptosis. Other studies suggest that lymphoid loss may result from reduced production of lymphoid cells in the bone marrow or reduced proliferation in secondary lymphoid tissues, resulting in depletion of all types of T and B lymphocytes, immunosuppression, and increased susceptibility to secondary opportunistic infections. Although there is no proof that it is the causal agent, porcine circovirus type 2 (PCV2) has also been linked to several other conditions, including PCV2 pneumonia, PCV2 enteritis, PCV2 reproductive failure, and PCV2 porcine dermatitis and nephropathy syndrome. Many of these conditions have concurrent infections caused by other microbes. These conditions have been grouped under the term PCV2-associated diseases and will not be covered in this chapter because of limited information.

Disorders of Dogs

Canine Distemper (Morbillivirus, Enveloped RNA Virus). See Viral Diseases of Organ Systems, Nervous System, Disorders of Dogs, Canine Distemper Canine Distemper (Morbillivirus, Enveloped RNA Virus) for discussion on pathogenesis and mechanisms of spread and injury. In summary, thymic atrophy and lymphoid depletion (lymphopenia) are manifestations of infection with canine distemper virus. Viral lymphotropism can lead to loss (lysis) of lymphoid cells (and macrophages), including specific types of T and B lymphocytes, resulting in severe immunosuppression. Additionally, loss of SLAM-positive lymphoid cells and specific cells that enable cell-mediated and humoral immunity likely contribute to this outcome. Loss of lymphocytes (and macrophages) appears to be caused by virus-induced cytolysis/apoptosis (not likely via the effects of cytokines) of infected lymphoid cells.

Disorders of Cats

Feline Leukemia (Feline Leukemia Virus, Retrovirus, Enveloped RNA Virus). The mechanism of injury in feline leukemia is virus-induced dysfunction, lysis, and/or neoplastic transformation of lymphoid cells leading to (1) lymphoma (lymphosarcoma) and leukemia, (2) malfunction of visceral organ systems, lymphoid tissues, or bone marrow, usually through compressive atrophy of parenchymal cells, in which the neoplastic cells proliferate, and (3) immunosuppression, resulting in increased susceptibility to other microbial diseases. Gross lesions include proliferation of neoplastic cells and their infiltration into perivascular spaces in organ systems resulting in (1) generalized enlargement of affected organs with increased pallor or (2) the formation of one or more solid white nodules distributed at random in the affected tissue (see Figs. 7-88, 13-83, and 13-94). Additionally, cells can occupy and proliferate in confined spaces, causing compressive atrophy of tissues such as axons in the spinal cord, hemopoietic cells in the bone marrow, and the retina in the eye. Discussion of the syndromes and lesions caused by feline leukemia virus (FeLV) is outside the scope of this chapter; however, they include (1) lymphoma (lymphosarcoma) and all of its forms (alimentary, thymic, anterior mediastinal, multicentric, atypical) based on anatomic distribution, (2) leukemia, (3) myeloproliferative disorders, (4) nonregenerative anemia, (5) panleukopenia-like syndrome, and (6) glomerulonephritis.

Cats encounter FeLV-A (see later) in fomites from body fluids, such as salivary and nasal secretions, through direct contact with virus-infected cats. Thus grooming behaviors are important in the transmission of the disease. Virus is ingested or inhaled and is deposited on mucous membranes of the oral and nasal pharynx, and it attaches to, infects, and replicates locally in mucosal epithelial cells and mucosa-associated macrophages and lymphocytes, especially in areas with tonsils. Virus spreads via leukocyte trafficking in lymphocytes and macrophages through afferent lymphatic vessels to pharyngeal lymph nodes (regional nodes), where it infects and replicates in additional lymphocytes and macrophages. It has not been determined how virus penetrates the mucus layer to gain access to and cross the mucosal epithelium or if dendritic cells are involved in infection or spread.

B lymphocytes appear to be the primary cells used to spread virus via leukocyte trafficking, whereas T lymphocytes appear to be the primary target cell for infection. Therefore T lymphocyte dysfunction is the principal cause of clinical signs of the disease. From regional lymph nodes, virus spreads systemically in B lymphocytes via leukocyte trafficking to the circulatory system, through postcapillary venules or lymphatic vessels and the thoracic duct to systemic lymph nodes and lymphoid organs, such as the spleen and Peyer's patches, and then to bone marrow and mucosa of the salivary glands. As stated earlier, salivary gland secretions and grooming behaviors spread the virus to naïve cats.

There are four known subgroups of FeLV, designated FeLV-A, FeLV-B, FeLV-C, and FeLV-T. For successful replication, virus requires rapidly dividing cells such as lymphocytes and the opportunity to establish a persistent infection in these cells. Clinical syndromes caused by FeLV arise from persistent infection of T lymphocytes in bone marrow. Persistent infections result from modulation of virus and cellular gene expression and modification of the cat's immune response by the virus. Persistence lasts for long periods, often the life of the cat, and occurs when the virus is not eliminated by the adaptive immune response because of dysfunction of cytotoxic T lymphocytes.

FeLV-A is the only subgroup that can be transmitted between cats. This occurs primarily through saliva. Once cats are infected with subgroup FeLV-A, the virus establishes a persistent infection of

bone marrow cells, which are most likely T lymphocytes or their precursor cells. However, the virus is not integrated in the cat's genome. Because viral replication occurs continually in these cells, there is greater opportunity for genomic variation (antigenic drift/shift) to occur and new viral virulence factors to be introduced. Subgroup FeLV-B appears to have arisen through recombination (antigenic shift) of endogenous genes of subgroup FeLV-A, whereas subgroup FeLV-C appears to have arisen through point mutations (antigenic drift) of endogenous genes of subgroup FeLV-A. Cats may be infected with only subgroup FeLV-A or a combination of subgroup FeLV-A with FeLV-B and/or FeLV-C. In general, subgroup FeLV-A causes immunosuppression and is found in approximately 100% of virus-infected cats; subgroup FeLV-B causes neoplastic transformation and is found in approximately 50% of virus-infected cats; and subgroup FeLV-C causes anemia and is found in approximately 1% to 2% of virus-infected cats. FeLV-T arises through genomic variation of FeLV-A, infects T lymphocytes, and causes an immunodeficiency syndrome.

All subgroups of virus use envelope glycoproteins, probably surface glycoprotein (SU) and the transmembrane protein (TM), to attach to receptors on and enter T lymphocytes, other lymphocytes, and mucosal epithelial cells. Receptors for viral glycoproteins on target cells include (1) feline thiamine transport protein (FeTHTR1) as a receptor for FeLV-A; (2) feline phosphate transporter proteins 1 and/or 2 (FePit1 or FePit2) as receptors for FeLV-B; and (3) FeLV-C cellular receptor (FeLVCR), a heme transporter protein, as a receptor for FeLV-C. FeLV-T uses two proteins to attach to, enter, and infect cells. FePit1 is used as a receptor, whereas FeLIX, a protein secreted primarily by T lymphocytes, is used to restrict tropism to T lymphocytes. Retroviruses also have envelope glycoproteins that form membrane-spanning glycoprotein systems that attach to and bind with membrane-spanning receptors on lymphocytes. To infect T lymphocytes, FeLV-T expresses in its viral envelope a membrane-spanning glycoprotein that attaches to and binds with a target cell membrane-spanning receptor molecule (FePit1). It also appears that expression of a specific target cell receptor, the total number of receptors expressed on the cell, and the use of soluble cofactors play roles in determining which target cells are infected by FeLVs. Additionally, persistent infection of bone marrow cells by FeLV-A provides many opportunities for mutations in the envelope gene that result in the expression of new viral subgroups through mutations in viral envelope glycoproteins that recognize new target cell membrane receptors. It is likely that the clinical syndromes and lesions caused by these virus subgroups are related to genomic variation through the expression of envelope surface glycoproteins that determine and restrict attachment, entry, infection, and replication in target cells.

Immunosuppression and lymphopenia coincide with systemic involvement of lymphoid tissues, specifically T lymphocytes. Cats persistently infected with FeLV-A commonly die of secondary bacterial and viral opportunistic infections. Immunosuppression, targeted primarily to the cell-mediated immune system, appears to result from (1) a reduction in the number of lymphocytes, especially cytotoxic and helper cell T lymphocytes, through virus-induced cell lysis; (2) suppression of lymphokines (interferon-δ and interleukin) secreted by activated T lymphocytes that could eliminate virus and virus-infected cells; (3) production of FeLV protein p15, which suppresses lymphocyte function (controversial); (4) dysfunction of lymphokine-induced activation of macrophages; and (5) dysfunction of neutrophil phagocytosis. Estimates suggest that approximately 50% of cats with certain bacterial infections and hemobartonellosis (*Mycoplasma haemofelis*) and 75% of cats with toxoplasmosis (*Toxoplasma gondii*) are infected with and have their immune systems suppressed by FeLV. Additionally, virus-induced immunosuppression has also been associated with feline infectious peritonitis, chronic oral and gingival diseases, poor reparative responses in inflammation, recurrent abscesses and skin infections, respiratory diseases, acute enteritides, otitis, and virus-induced malignancies such as sarcomas.

Neoplastic transformation follows persistent infection of T lymphocytes, usually in bone marrow. Virus produces reverse transcriptase (a retrovirus) that transcribes viral RNA into proviral DNA and facilitates the insertion of proviral DNA into chromosomal DNA of T lymphocytes or other bone marrow cells. When virus has integrated its genome into the target cell DNA, the viral genome is passed to all new generations of cells when the cell is mitotic. Reverse transcriptase is carried by the virus and is released into target cell cytoplasm along with its viral RNA genome after attachment and entry. Neoplastic transformation of T lymphocytes or other bone marrow cells occurs when DNA provirus integrates into chromosomal DNA at critical regions that (1) contain oncogenes such as the cellular gene c-myc or (2) are near genes influencing the expression of c-myc genes. Activation of these genes and the expression of their gene products result in a series of alterations to the cell regulatory environment that leads to irreversible changes in cell behavior characteristic of neoplastic transformation (see Chapter 6). Feline oncornavirus-associated cell membrane antigen (FOCMA) is expressed on the cell membranes of transformed cells and is not found on normal (nontransformed) cells, even if they are infected with virus.

Feline Acquired Immunodeficiency Syndrome (Feline Immunodeficiency Virus, Lentivirus, Enveloped RNA Virus). The mechanism of injury in feline acquired immunodeficiency syndrome is provirus-induced dysfunction and lysis of $CD4^+$ T lymphocytes leading to immunosuppression. Gross lesions include transient lymph node enlargement (lymphadenomegaly) followed by the occurrence of secondary opportunistic microbial infections. Feline immunodeficiency virus causes persistent and gradual depletion of $CD4^+$ T lymphocytes (T helper [T_H] lymphocytes, effector T lymphocytes), resulting in an immunodeficiency syndrome characterized by chronic stomatitis and gingivitis, wasting syndrome (malnutrition), neurologic manifestations, and an increased incidence of lymphoma. The cause of $CD4^+$ T lymphocyte depletion is unknown. It may have a multifactorial basis, including lysis of cells caused directly by viral infection, lysis (turnover) after massive and rapid replication of virus-infected and noninfected cells stimulated by viral antigen and/or proinflammatory cytokines or other molecules, provirus-induced suppression of cell proliferation, lysis of provirus-infected $CD4^+$ T lymphocyte by adaptive immune responses, or apoptosis of provirus-infected cells.

Cats encounter the virus in blood, most commonly as a provirus in infected $CD4^+$ T lymphocytes, and much less commonly as free virus in fomites from saliva. During fights that result in bite wounds that bleed, blood contaminated with provirus-infected $CD4^+$ T lymphocytes encounters (1) oral mucosae (macrophages and dendritic cells), especially of the tonsils, through surface contamination and (2) macrophages and dendritic cells (Langerhans cells) of the skin through penetrating wounds. It appears that virus is able to establish a local infection in mucosal dendritic cells, macrophages, and lymphocytes; however, it is not clear how virus penetrates the mucus layer to gain access to mucosal epithelial cells, mucosal macrophages, and/or dendritic cells and migrates through the mucosal epithelium to reach cells in the lamina propria and submucosa (MALT). In mucosae, several proposed mechanisms of spread could be involved: (1) migration (leukocyte trafficking) of provirus-infected $CD4^+$ T lymphocytes through the epithelium into the submucosa, (2) infection of mucosal epithelial cells via a ligand-cell

receptor endocytotic mechanism through virus released from provirus-infected $CD4^+$ T lymphocytes, (3) infection of mucosal epithelial cells via a ligand–target cell receptor mechanism by cell-free virus, or (4) transfer of cell-free virus via viral transcytosis, the process by which virus is transported across the interior of a cell in vesicles to be released from the basal surface on the abluminal side. In skin, free-virus or provirus-infected $CD4^+$ T lymphocytes could be carried via blood or saliva into penetrating wounds of the dermis and subcutis, where, through cell lysis, exocytosis, or direct extension, the virus or provirus could gain access to Langerhans cells and tissue macrophages.

It appears that by whatever route used by virus or provirus to enter the body, it must gain access to local mucosal (MALT) or skin-associated lymphoid tissues (MALT-like) and $CD4^+$ T lymphocytes, macrophages, and dendritic cells to establish an infection. Once these cells are infected, virus is then spread by leukocyte trafficking via afferent lymphatic vessels to regional lymph nodes and then systemically via leukocyte trafficking to the spleen and other lymphoid tissues through postcapillary venules or lymphatic vessels and the thoracic duct. Some studies suggest that virus may also spread to the oral cavity and tonsillar mucosa via saliva either by provirus-infected $CD4^+$ T lymphocytes or a cell-free viremia, especially if cats with chronic stomatitis and gingivitis are involved in grooming behavior or cat fights. Target cells for infection include $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, B lymphocytes, cells of the monocyte-macrophage system, dendritic cells, megakaryocytes, and astrocytes. Virus envelope glycoproteins, probably surface glycoprotein (SU) and the transmembrane protein (TM), bind to target cell membrane receptors and serve to facilitate infection through virus attachment and entry into target cells. Different strains of virus appear to express different envelope glycoproteins (and other proteins); thus these molecules likely contribute to viral pathogenicity. Target cells express feline CD134 receptor and CXCR4 cofactor (chemokine receptor) in their membranes, both of which act as coreceptors and are needed for virus attachment, binding, and entry into target cells.

Nervous System

Disorders of Domestic Animals

Rabies (Lyssavirus, Enveloped RNA Virus). The mechanism of injury in rabies is neuronal dysfunction possibly caused by one of several mechanisms, including viral takeover of RNA transcription and translation in neurons, disruption of neurotransmitter functions, dysfunction of ion channels, and/or induction of the synthesis of NO. Rabies virus infects neurons of all mammalian species. Gross lesions are not present in nervous tissue; however, inclusion bodies (Negri bodies) and chronic lymphomonocytic perivascular inflammation characteristic of viral infections are observed (see Fig. 14-45). In addition to neurons, the virus infects glial cells in the nervous system and epithelial cells such as those in the salivary glands.

Animals encounter virus in fomites from saliva through a skin-penetrating bite wound from a rabid animal. Virus gains access to interstitial (extracellular) body fluids and plasma (bite wound hemorrhage); diffuses at random in this fluid; and encounters, attaches to, and enters striated muscles cells via binding of rabies virus envelope G protein to neurotransmitter receptors, such as acetylcholine receptors, located in muscle cell membranes. Envelope G protein is an important determinant of rabies neurovirulence and which neuron pathways are infected with virus in the nervous system. Virus then replicates in muscle, buds from cell membranes, enters interstitial fluids of myoneural (neuromuscular junction) junctions, and randomly encounters and binds to acetylcholine receptors, neuronal cell-adhesion molecule receptors, neurotrophin receptors, or other types of gangliosides in cell membranes of unmyelinated axon terminals (nerve endings) of lower motor neurons of peripheral nerves. Similar processes are also used to spread and replicate virus in cranial nerves after bite wounds to the face. Once bound, virus enters the cytoplasm of nerve endings through pinocytosis via clathrin-coated pits and the formation of vesicles. Virus in vesicles spreads centripetally from myoneural junctions to the cell body of the nerve via retrograde fast axonal transport, likely using the dynein light chain microtubule-based transport system (see Chapter 14; see E-Fig. 14-3). Virus replicates in the cell body of neurons and travels to dendrites via axonal transport where it buds from cell membrane of dendritic processes into synaptic clefts of neural-neural junctions. It randomly encounters receptors on motor nerve endings within the gray matter of the brain and ventral gray horns of the spinal cord. Mechanistically, viral replication and spread in motor neurons within the spinal cord and brain are identical to that in peripheral spinal nerves. The exact mechanism that facilitates transsynaptic spread of rabies virus is unknown. It may be linked in part to viral assembly where M protein encapsulates the virus and assists in moving the virus to cell membranes such as those in synapses that contain glycoproteins essential for formation of the viral envelope and viral budding. Envelope G protein is also required for attachment to cell membrane and transsynaptic spread of the virus to the next neuron in the neural pathway.

Virus uses axonal transport mechanisms to spread throughout the body via afferent and efferent neural pathways to infect epithelial cells of the salivary glands (see Fig. 14-44). Rabies virus, through these neural pathways, can also infect other cells such as those in taste buds, nasal cavity, skin and hair follicles, adrenal gland, pancreas, kidney, heart muscle, and the retina and cornea. In fact, the "furious" and "dumb" forms of rabies in domestic animals (see Chapter 14) are likely attributable to infection of specific neuronal populations and pathways such as those in the hippocampal formation or cerebellum, respectively. Virus spreads to salivary glands through axonal transport using parasympathetic nerves present in the facial (VII) and glossopharyngeal (IX) cranial nerves and sympathetic nerves in the thoracic segments of T1 to T3 spinal cord segments. In addition to spreading virus to the salivary glands, viral infection of parasympathetic and sympathetic nerves also results in increased salivary gland secretions: (1) directly through stimulating β-adrenergic receptors on the salivary acinar and ductal cells, leading to an increase in cAMP concentrations and the corresponding increase of saliva secretion, and (2) indirectly through stimulating nerves innervating blood vessels that supply the salivary glands. Virus buds from the cell membrane of these nerve terminals, infects salivary acinar epithelial cells through the envelope G protein–specific cell surface receptor mechanism, and replicates in and is amplified to large quantities in these cells. Virus then buds from apical (luminal) surfaces of salivary cell membranes, mixes with saliva, and can be transmitted in a bite wound. The apical specificity of viral budding is established during the assembly stage of viral replication. Viral genome and proteins form "envelope" complexes in the salivary epithelial cell cytoplasm that congregate at areas of the cell membrane that contain matching glycoprotein receptors and then bud from this membrane into the salivary gland lumen to eventually enter the duct system and saliva.

Disorders of Horses

Equine Polioencephalitis-Polioencephalomyelitis (Alphavirus, Enveloped RNA Virus). The mechanism of injury in equine polioencephalitis-polioencephalomyelitis is disruption and lysis of

neurons in the CNS. Gross lesions include active hyperemia, vasculitis, hemorrhage, and yellow-white-gray areas of necrosis in gray matter of the nervous system, especially the spinal cord (see Fig. 14-79). Because neurons are the primary target cell, lesions are most commonly observed in gray matter (i.e., polio-), areas in which neuron cell bodies are located, and thus these diseases are classified as polioencephalitides or polioencephalomyelitides. Equine polioencephalitis-polioencephalomyelitis is used herein to group three closely related strains of alphaviruses that cause eastern equine encephalomyelitis, western equine encephalomyelitis, and Venezuelan equine encephalomyelitis. St. Louis encephalomyelitis is the human counterpart of these diseases in horses. Such diseases have also been called *arbovirus polioencephalitis-polioencephalomyelitis*. The term *arbovirus* is derived from the fact that these viruses are *a*rthropod-*b*orne; this term was shortened to *arbo* and is used as a disease acronym.

Horses encounter viruses through skin-penetrating bite wounds from virus-infected mosquitoes. Mosquitoes are infected when they bite and consume infected blood from birds, the reservoir for the virus. Seasonal variations in temperature and precipitation greatly influence the population density of mosquitoes and thus the occurrence of disease. Following penetration of horse skin with their proboscis, virus-infected mosquitoes deposit virus directly into the circulatory system, where it infects blood monocytes, or into vascularized ECM (connective) tissue, where it infects dendritic cells (Langerhans cells) and tissue macrophages. In these cells, virus is spread via leukocyte trafficking to regional lymph nodes either by the circulatory system or afferent lymphatic vessels where it infects additional lymphocytes. It may also spread to regional lymph nodes via cell-free viremia in lymphatic vessels.

Viral envelope contains three membrane glycoproteins, E1, E2, and E3. Attachment protein E2 is used to attach to a target cell receptor, whereas viral envelope fusion protein E1 is used to enter target cells via endocytosis. Receptors for E1 and E2 proteins occur on a variety of cell types and probably determine which cells, such as lymphocytes, are used for leukocyte trafficking and ultimately which organ systems, such as the nervous system, are targeted for infection by virus. Virus then spreads systemically via leukocyte trafficking in lymphocytes and macrophages through postcapillary venules or lymphatic vessels and the thoracic duct to the circulatory system to systemic lymph nodes, spleen, thymus, bone marrow, Peyer's patches, pancreas, and skeletal muscle. Infection results in necrosis of myeloid cells in bone marrow and lymphocytes in lymph nodes and spleen. Proinflammatory cytokines, such as IFN-γ, and antiinflammatory cytokines, such IL-10 produced by infected lymphocytes, may cause cell lysis. Cytokines released into the blood vascular system may also act on the blood-brain barrier, making it more susceptible to viral infection via changes in its barrier functions and permeability. In eastern equine encephalomyelitis, osteoblasts appear to be the target cell used to amplify virus so it can spread to the nervous system. In this specific disease, dendritic cells, lymphoid cells, and cells of the monocyte-macrophage system are not as susceptible to infection, and thus systemic lymph nodes and spleen are infected to a limited degree with minimal injury and lysis. Although it is unclear how virus spreads to and enters the CNS, leukocyte trafficking by lymphocytes and macrophages (monocytes) appears to be the probable mechanism. Cell-free viremia may also occur.

West Nile Virus Polioencephalitis-Polioencephalomyelitis (Flavivirus, Enveloped RNA Virus). The pathogenesis and mechanism of injury in West Nile virus polioencephalitis-polioencephalomyelitis are similar to those of equine polioencephalitis-polioencephalomyelitis discussed earlier.

Equine Herpesvirus Myeloencephalopathy (Equine Herpesvirus 1: Alphaherpesvirus, Enveloped DNA Virus). The mechanism of injury in equine herpesvirus myeloencephalopathy is dysfunction and lysis of endothelial cells in small arterioles of the brain and spinal cord; however, the mechanism is uncertain but most likely caused by virus replication. Immune complexes (type III hypersensitivity reaction) and the fixation of complement (immune complex–induced vasculitis) have also been suggested. Gross lesions in the brain and spinal cord include randomly distributed foci of edema, hemorrhage, and vasocentric malacia (yellow-white-gray areas) consistent with vascular occlusion, resulting in infarction (see Fig. 14-80).

Horses encounter virus in fomites from body fluids through direct contact with virus-infected animals. It is inhaled or ingested and deposited on mucosae of the oral, nasal, and pharyngeal cavities or inhaled and deposited on mucosae of the conductive component of the respiratory system through centrifugal and inertial turbulence. Virus infects and replicates in mucosal epithelial and endothelial cells, next in contiguous mucosal and submucosal lymphocytes and likely macrophages, monocytes, and dendritic cells (MALT), and then spreads via leukocyte trafficking in afferent lymphatic vessels to regional lymph nodes. It has not been determined if and how virus penetrates the mucus layer to gain access to mucosal epithelial and endothelial cells or if or how mucosal macrophages and/or dendritic cells are involved, though their participation is very likely. Although specific ligands and receptors have not been identified, viral envelope glycoproteins likely attach to glycosaminoglycan receptors on target cell membranes and use this binding to enter the cells listed earlier. Infection appears to be sustained and amplified in lymphocytes and likely macrophages and monocytes of regional lymph nodes. Virus is then spread systemically through blood and lymphatic vessels in infected leukocytes via trafficking in the circulatory system. Infected lymphocytes and macrophages probably use envelope adhesion molecules to bind to receptors on vascular endothelium during migration via leukocyte trafficking through the wall of blood vessels and interact with their cell membranes. These interactions appear to lead to virus infecting and replicating in endothelial cells, myocytes, and pericytes of small arterioles in the brain and spinal cord, causing vasculitis and thrombosis. It is not known why these cells, especially endothelial cells, are targets for virus infection; however, ligand-receptor interactions or permissiveness of these cells is likely involved. Activation of endothelial and leukocyte adhesion molecules is an important step in spreading virus to endothelial cells and thus may contribute to endothelial cell tropism for viral infection.

Disorders of Ruminants (Cattle, Sheep, and Goats)

Bovine Cerebellar Hypoplasia (BVD Virus, Pestivirus, Enveloped RNA Virus). A syndrome, probably involving mechanisms similar to those that occur in animals infected in utero with parvovirus, occurs in calves infected in utero with bovine viral diarrhea–mucosal disease virus (see Fig. 14-36). For more detail, see the following sections:

- Viral Diseases of Organ Systems, Nervous System, Disorders of Cats, Parvovirus-Induced Cerebellar Hypoplasia
- Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Ruminants (Cattle, Sheep, and Goats); Bovine Viral Diarrhea–Mucosal Disease (BVD Virus, Pestivirus, Enveloped RNA Virus)
- Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity;

Disorders of Dogs; Parvovirus Enteritis (Parvovirus, Nonenveloped DNA Virus).

Bovine Herpesvirus Meningoencephalitis (Bovine Herpesvirus 5: Alphaherpesvirus, Enveloped DNA Virus). Mechanistically, bovine herpesvirus 5 behaves much like bovine herpesvirus 1. It infects, spreads, and replicates in the same target cells but is more neurovirulent and induces severe and often fatal encephalitis. See the discussion of infectious bovine rhinotracheitis (bovine herpesvirus 1) in Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants (Cattle, Sheep, and Goats). The mechanism of injury in bovine herpesvirus meningoencephalitis is dysfunction and lysis of neurons and astrocytes caused by viral replication and chemical mediators of inflammation. The latter consist of proinflammatory chemokines and cytokines arising from cytotoxic T lymphocytes as part of a lymphomonocytic inflammatory response (innate and adaptive immune responses). Gross lesions include randomly distributed areas of cerebral edema, active hyperemia, hemorrhage, and malacia.

Cattle encounter virus in fomites from body fluids through direct contact with virus-infected animals. It is inhaled or ingested and deposited on mucosae of the oral, nasal, and pharyngeal cavities and of the conjunctiva or inhaled and deposited on mucosae of the conductive component of the respiratory system through centrifugal and inertial turbulence. Viral envelope glycoproteins B, C, D, and E likely attach to receptors on sensory nerve endings that innervate these mucosae. They can also probably attach to receptors on a variety of other target cells. These receptors include glycosaminoglycan receptors such as herpesvirus entry mediator A, nectin-1 and nectin-2 (herpesvirus entry proteins C and B), and 3-*O*-sulfated heparin sulfate. It has not been determined how virus penetrates the mucus layer to gain access to mucosal sensory nerve endings. Through these nerve endings, virus enters neurons within the trigeminal and olfactory cranial nerves and spreads via retrograde axonal transport to other neurons and glial cells within the CNS. It appears that envelope glycoprotein E and 3-O-sulfated heparin sulfate receptors may amplify viral attachment, entry, and spread within the CNS. The mechanism of malacia remains unknown but does not appear to be caused by obvious vascular injury. Neuronal lesions are consistent with necrosis, likely caused by virus-induced injury and cell lysis or by chemical mediators of inflammation. In addition, overproduction of NO in virus-infected neurons and astrocytes could result in their dysfunction and lysis and that of contiguous noninfected cells. Bovine herpesvirus 5 can enter latency in the nervous system, through mechanisms likely identical for bovine herpesvirus 1.

Visna (Maedi-Visna Virus [Ovine Lentivirus], Enveloped RNA Virus). The chronologic sequence of steps that characterizes the pathogenesis of injury in visna is similar to that which occurs in ovine progressive pneumonia (maedi) of sheep. See the discussion of maedi in Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants (Cattle, Sheep, and Goats). In the CNS the mechanism of injury is chronic-active (granulomatous) inflammation resulting in demyelinating encephalitis. Gross lesions include foci of yellow-white malacia distributed at random in the CNS. Virus is spread to the CNS by leukocyte trafficking of virus-infected monocytes and macrophages arising in the lung and bone marrow. Ovine lentivirus persistently infects cells of the monocyte-macrophage system, including microglial cells (local tissue macrophages in the CNS), and all of these cell types are central to the genesis of the inflammatory response in the CNS.

Caprine Encephalitis (Caprine Arthritis-Encephalitis Virus, Enveloped RNA Virus). The pathogenesis and mechanism of injury in caprine encephalitis are similar to those that occur in ovine progressive pneumonia (maedi) of sheep (see section on the Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants [Cattle, Sheep, and Goats]); however, the initial route of exposure is by ingestion of virus-infected milk or colostrum. The mechanism of injury is chronic-active (granulomatous) inflammation of the CNS resulting in demyelinating myelitis. Gross lesions include foci of yellow-white malacia distributed at random in the CNS, especially the spinal cord (see Fig. 14-90). Caprine arthritis-encephalitis virus persistently infects cells of the monocyte-macrophage system; thus microglial cells (local tissue macrophages) and trafficking monocytes serve as the cell type central to the genesis of the inflammatory response. Kid goats are primarily exposed to virus through the ingestion of virus-infected milk or colostrum. Although not proven, virus likely infects M cells overlying Peyer's patches. Once the cells are infected, virus is transferred to and released from basilar surfaces of M cells to gain access to macrophages and lymphocytes within Peyer's patches. It is here that macrophages are infected with virus and then serve to spread virus to monocyte precursor cells in the bone marrow and ultimately to the CNS.

Disorders of Pigs

Pseudorabies (Aujeszky's Disease) (Alphaherpesviruses, Enveloped DNA Virus). The mechanism of injury in pseudorabies is disruption and lysis of neurons likely caused by the actions of immune cells, such as cytolytic T lymphocytes, interacting with virus-infected neurons. Because neurons (within neuron cell bodies) are the primary target of viral infection, lesions are most commonly observed in gray matter (polio-) and as a result, this disease is a polioencephalitis or polioencephalomyelitis. Gross lesions characteristic of injury are usually not observed but in severe cases could include randomly distributed areas of active hyperemia and hemorrhage.

Pigs encounter virus in fomites from oronasal-pharyngeal body fluids most commonly through inhalation and potentially through contamination of skin-penetrating bite wounds. When inhaled, virus is deposited on mucosae of the oral, nasal, and pharyngeal cavities, especially of the tonsil or on mucosae of the conductive component of the respiratory system through centrifugal and inertial turbulence. In the tonsil, virus may infect and replicate in mucosal epithelial cells, mucosal and submucosal macrophages, and dendritic cells (MALT). In the lung, virus also infects and replicates in similar cells (BALT), including alveolar macrophages, which it kills, resulting in a secondary bronchopneumonia. Virus attachment and entry is likely mediated by binding of viral envelope glycoproteins to target cell membrane receptors. In the nasal and pharyngeal mucosae and submucosae, especially of the tonsil, virus encounters and infects sensory nerve endings of the olfactory, glossopharyngeal, and trigeminal cranial nerves and uses retrograde axonal transport to enter the brain. Virus can spread transsynaptically throughout the CNS by using mechanisms similar to those described in rabies and infect and replicate in many types of neurons. Viral envelope glycoproteins C, B, D, H, and L are used to attach to, fuse with, and enter membranes of nerve endings. These glycoproteins are also involved in transsynaptic spread to other neurons in the CNS and to other neural cells, such as astrocytes, microglial cells, ependymal cells, and trafficking monocytes/macrophages, as well as in the formation of syncytial cells and the modulation of innate and adaptive immune responses.

Virus cannot replicate in neural cells; thus they are incapable of spreading infection to other cells in the CNS. This outcome may represent a local intrinsic and/or innate immune defense mechanism that isolates through phagocytosis the virus in astrocytes,

monocytes-macrophages, and microglial cells and restricts spread of virus to other cells. Latent infections involve the trigeminal nerves and ganglia, but tonsillar lymph nodules may also be involved. Potentially, peripheral nerve endings in the skin, subcutis, and muscle may be exposed to infection via bite wounds and can be used by virus to gain access to and enter the CNS by mechanisms similar to those described in rabies.

Viral envelope glycoproteins in membranes of infected neurons are targets for neutralizing antibodies, cytotoxic T lymphocytes, and lymphokine-activated killer cells and are part of the chronic perivascular lymphomonocytic inflammatory response characteristic of viral infections. These cells appear to contribute in a large manner to neuronal injury and lysis in pseudorabies. Hypertrophy and hyperplasia of astrocytes, microglia, and monocytes-macrophages occur spatially and temporally with the severity of neuronal injury; however, the potential role of biologically active molecules, such as cytokines (e.g., TNF-α), from these cells is unclear.

Disorders of Dogs

Canine Distemper (Morbillivirus, Enveloped RNA Virus). The mechanism of injury in canine distemper, a pantropic virus,[8] is dysfunction and lysis of neuronal, epithelial, mesenchymal, neuroendocrine, and hematopoietic cells in many different tissues and organ systems. The nervous system is the primary organ system affected by distemper virus; however, the virus must infect and replicate in target cells of the respiratory and/or alimentary systems before spreading to the nervous system. As a result, canine distemper virus may also cause diseases of these organ systems. Gross lesions are not observed in the nervous system. Lymph nodes (and spleen) are initially enlarged, hemorrhagic, and edematous but then undergo cell lysis, resulting in loss of T and B lymphocytes in the spleen, lymph nodes, MALT, tonsil, and thymus (immunosuppression). Alterations in bone marrow are minimal; but thrombocytopenia may occur. Anterior-ventral regions of the lung may be firm (consolidation) and have yellow-tan-gray appearance (secondary bronchopneumonia); cut surfaces have discrete and coalescing areas of yellow-tan-gray exudate infiltrating and compressing contiguous lung parenchyma. Airways are hyperemic and often covered with mucopurulent exudate. Pleural surfaces may or may not be affected. The small intestine may be congested and have thin walls and shortened villi (atrophy).

Dogs encounter distemper virus in fomites from body fluids of the nasal and oral cavities, through direct contact with infected dogs. To reach the CNS the virus needs to infect mucosal lymphocytes, macrophages, and/or likely dendritic cells in one or more portals of entry and then spread via leukocyte trafficking to encounter target cells in the CNS (see later) and also in many other organ systems. The portals of entry, encounters with target cells, and pathways of spread include the oronasal pharynx, lung, and small intestine and are discussed at the end of this section. In summary, virus is inhaled and deposited in mucus and on mucosae of the pharynx and of the conductive (bronchi/bronchioles) and O_2-CO_2 exchange (alveoli) systems through centrifugal and inertial turbulence. It is also probably ingested and via swallowing and peristalsis encounters enterocytes (and mucus) and M cells (no mucus) of the small intestine.

Virus-infected lymphocytes and macrophages spread distemper virus via the blood vascular system to the CNS through leukocyte trafficking and cell-free viremia. At the blood-brain barrier, infected cells and virus likely interact with endothelial cells via the leukocyte adhesion cascade (see Chapter 3) and adhere to and migrate through the endothelium. Virus also infects and replicates in endothelial cells, resulting in a perivascular lymphomonocytic inflammatory response characteristic of viral infections in the CNS. Virus then infects and replicates in vascular pericytes, microglial cells, and perivascular astrocytic foot processes, as well as in choroid plexus epithelial cells. At this point, depending on how it is able to spread within the CNS, virus can cause disease in gray matter (neurons: polioencephalomyelitis), white matter (oligodendroglial cells: demyelinating leukoencephalomyelitis), or both. The location (i.e., gray matter versus white matter) of viral infection appears to be determined by the status of vaccination and adaptive immunity (degree of immunosuppression) in the infected dog and by the pathogenicity (virulence factors) of the strain of distemper virus. Additionally, the clinical signs accompanying infection of the CNS by distemper virus are most likely related, in part, to the degree of injury involving neurons and oligodendroglial cells, or a combination of both cell types.

- **Infection of neurons—**Neuronal infection likely arises after spread of virus to neurons from virus-infected pericytes and perivascular astrocytic foot processes. Virus-infected astrocytes may also serve as a reservoir for spreading virus within the CNS (see astrocytes in Chapter 14). Viral infection of neurons results in neuronal necrosis and subsequent neuronophagia via resident microglial cells and trafficking monocytes, macrophages, and lymphocytes.
- **Infection of oligodendroglial cells—**Spread of virus to oligodendroglial cells probably arises from infection of ependymal cells. Virus escapes from choroid plexus epithelium via cell lysis and is carried in cerebral spinal fluid (CSF) to infect ependymal cells via their apical surfaces and then spread via transcytosis to contiguous oligodendroglial cells in the subependymal white matter. However, infection through the blood vascular system, capillaries and postcapillary venules, and virus-infected pericytes and perivascular astrocytic foot processes has not been excluded as a potential infective mechanism. Involvement of oligodendroglial cells results in demyelinating leukoencephalomyelitis, which has an acute phase and a chronic phase. Two mechanisms have been proposed for the acute phase of demyelinating leukoencephalomyelitis: (1) lysis of oligodendroglial cells from infection or (2) a type II hypersensitivity reaction against proteins such as myelin basic protein and myelin-associated glycoprotein.
  - For a cell lysis mechanism, there is no evidence of virus-induced apoptosis or necrosis of oligodendroglial cells, and although virus can infect oligodendroglial cells, no viral proteins are present in these cells. Astrocytes and microglial cells can be infected and show activation such as hypertrophy and hyperplasia. It has been hypothesized that toxic molecules, such as proinflammatory cytokines produced by these glial cells, act to disrupt the function of oligodendroglial cells and kill the cells.
  - For a hypersensitivity reaction mechanism, microscopic lesions of vacuolization (intramyelinic edema) of myelin lamellae surrounding axons in white matter accompanied by reactive astrocytes, macrophages (monocytes), resident microglial cells, and occasional multinucleated giant cells are consistent with this type of immune-mediated injury. As this injury progresses, the inflammatory response becomes more intense and is characterized by perivascular mononuclear infiltrations. Myelin is phagocytosed by macrophages (monocytes) and microglial cells and the lesion is repaired by proliferation of astrocytic processes, thus forming dense plaques (astrocytic scars).

[8]The ability to infect many kinds of cells and tissues.

The chronic phase of demyelinating leukoencephalomyelitis appears to be a bystander mechanism involving inflammation and virus-induced immune responses, such as antibody-dependent cell-mediated reactions (cytotoxic T lymphocytes) against viral proteins expressed in oligodendroglial cell membranes, leading to macrophage-mediated separation, damage, and phagocytosis of myelin lamellae. Myelin damage is likely the result of proteolytic enzymes, oxygen free radicals, and cytokines from activated macrophages, monocytes, and resident microglia. Lipids from damaged lamellae stimulate an intense phagocytic response and likely initiate recruitment of additional monocytes and macrophages into the lesions. Disruption of the blood-brain barrier by proteolytic enzymes appears to play a role in the influx of inflammatory cells probably mediated by viral infection of astrocytes through their foot process involved in the structure and function of the blood-brain barrier.

Distemper virus can encounter and enter macrophages, lymphocytes, and/or dendritic cells through the respiratory and alimentary systems, especially at the oronasal pharynx; the bronchi, bronchioles, and lung; and the small intestine.

- **Oronasal pharynx—**Infection of the oronasal pharynx begins in the mucus layer, where virus is likely phagocytized by mucosa-associated lymphocytes and macrophages and likely dendritic cells and spread via leukocyte trafficking across the tonsillar mucosa to MALT of the tonsils. Here, naïve lymphocytes and macrophages are infected with virus and migrate in afferent lymphatic vessels to regional lymph nodes, where they infect new cells and then migrate systemically through postcapillary venules or efferent lymphatic vessels and the thoracic duct to the circulatory system. These infected cells leave the circulatory system and enter the spleen, thymus, lymph nodes, bone marrow, mucosa-associated lymphoid nodules and Peyer's patches, and liver (Kupffer cells) to infect additional naïve lymphocytes and macrophages. Infection of cells may also occur via a cell-free viremia and through virus-infected platelets. After infection of systemic lymphoid tissue, infected cells (leukocyte trafficking) or cell-free virus spreads to parenchymal organs, including the nervous, respiratory, alimentary, and urinary systems. Concurrently, massive lysis of lymphocytes may also occur, resulting in immunosuppression and dysfunction of immune responses to the virus. In these systems, virus infects a wide variety of epithelial and mesenchymal cells (pantropic virus) and lyses these cells as it replicates and escapes from them. Virus also gains access to ameloblasts during the embryonic development of adult teeth, infects and lyses these cells, and causes a condition known as *enamel hypoplasia* (see Fig. 7-45).
- **Lung—**Infection of the lung begins in the mucus layer of the conductive component of the respiratory system and in the O_2-CO_2 exchange component. Primary target cells are ciliated and nonciliated mucosal epithelial cells of bronchi and bronchioles. It has not been determined how virus penetrates the mucus layer to gain access to mucosal epithelial cells or whether this occurs via direct contact of virus with these cells or through contact with trafficking leukocytes. In bronchi and bronchioles, virus is able to infect mucosa-associated lymphocytes, alveolar macrophages, dendritic cells, and ciliated and nonciliated mucosal epithelial cells. Following replication, these cells are lysed, thereby disrupting the function of the mucociliary apparatus and the removal of particulate debris and secondary bacteria. This outcome contributes to a secondary suppurative bronchopneumonia. Furthermore, in the O_2-CO_2 exchange component, primary target cells are pneumocytes and alveolar macrophages and with replication and lysis of these cells, the function of the air-blood barrier and oxygenation of blood are also disrupted. Loss of the mucociliary apparatus, lysis of macrophages and lymphocytes leading to reduction in phagocytosis and antigen presentation by macrophages, and disruption of innate and adaptive immune responses contribute to secondary suppurative bronchopneumonia in dogs with distemper infections. Cell debris from lysis, viral antigens, and activation of T lymphocytes may also contribute to acute inflammation and the release of proinflammatory cytokines into ECM, perpetuating the inflammatory response. These mechanisms also cause substantial tissue injury and cell lysis.
- **Small intestine—**Infection of the small intestine and its spread of virus to the CNS is not well defined or characterized, if it occurs at all. However, the virus can cause disease of the alimentary system alone. Virus is thought to be ingested, and swallowing and peristasis carry it through the oral pharynx, esophagus, and stomach to the small intestine, where it is trapped in the mucus layer. It is unclear how the virus is able to evade the actions of digestive enzymes, bile acids, and other microbial-lytic molecules and then penetrate the mucus layer and encounter enterocytes. Virus is likely phagocytized by mucosa-associated lymphocytes and macrophages and likely dendritic cells and spread via leukocyte trafficking to and across enterocytes. Virus is probably able to replicate in enterocytes. However, they are lysed when virus replicates in them, leading to a malabsorption/osmotic diarrhea, which occurs because of the loss of enterocytes and the failure to digest carbohydrates (impaired hydrolysis) and other molecules in the digesta. Virus that crosses mucosa through leukocyte trafficking likely reaches MALT. Here similar cells are infected, and they migrate via leukocyte trafficking to spread virus via lymphatic vessels to regional lymph nodes and then systemically to other organ systems. Although unproved, the virus also likely infects M cells, which spread virus to tissue macrophages, dendritic cells, and other cells in Peyer's patches and then systemically to other organ systems by leukocyte trafficking as described earlier.

Virus uses two viral envelope proteins: an attachment protein called *viral H protein* and a fusion protein called *viral F protein* bind to cell membrane glycoprotein receptors. Viral fusion proteins are involved in the penetration of virus into uninfected lymphocytes, spread of virus from cell to cell, and formation of syncytial cells (e.g., CD9 transmembrane protein) characteristically seen in the lungs. It has been shown experimentally that when virus-infected lymphocytes encounter uninfected lymphocytes and other cell types, they are induced to express new and/or increased numbers of SLAM cellular receptors. Molecules secreted by virus-infected lymphocytes likely mediate this process and thus may serve as a means to amplify virus infection in dogs. Glycoprotein receptor CD150 (SLAM) occurs in membranes of lymphocytes, monocytes, macrophages, transitional epithelial cells, endothelial cells, and unspecified cells in the stomach, small intestine, and lung.

Disorders of Cats

Parvovirus-Induced Cerebellar Hypoplasia (Parvovirus, Nonenveloped DNA Virus). See parvovirus enteritis in the section on Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Dogs for information about mechanisms of viral spread and replication before involvement of the CNS.

In pregnant cats, parvovirus is able to cross the placenta and infect dividing cells in the developing cerebellum of kittens, resulting in cerebellar hypoplasia (see Fig. 14-35). Whether by leukocyte trafficking or cell-free viremia, parvoviruses are able to gain access to cells in the placenta. Virus infects and replicates in placental

trophoblasts and spreads to, infects, and replicates in cytotrophoblasts and cells of the mesenchymal stroma of the fetal placenta. From these cells, virus then gains access to the fetal vascular system and spreads to, infects, and replicates in hematopoietic cells and other dividing cells. It has also been suggested that placental macrophages (or macrophage-like cells) and fetal endothelial cells are likely involved in the replication and spread of the virus to the developing CNS in the fetus. Although virus can infect a large number of different cells in the fetus, it is unclear why fetal infection is clinically dominated by injury to cells of the cerebellum, specifically cells of the external granular layer and Purkinje cells. Ligand-receptor interactions could contribute to this specificity; however, the ability of specific cells to divide and other unknown mechanisms are likely involved.

Parvoviruses infect and replicate in dividing cells. In the fetus, cells of the external granular layer of the cerebellum are dividing cells, whereas Purkinje cells are nondividing cells. However, cell lysis is observed in both of these cell types, although only one of them is a dividing cell population. Granule precursor cells of the cerebellar external granular layer are the major target cells for parvovirus replication during the perinatal period because they are able enter the S phase of the mitotic cycle. Purkinje cells are also infected, but they are nondividing postmitotic cells. It appears that virus infects Purkinje cells via a target cell membrane transferrin receptor that is commonly used by parvovirus for entering other types of target cells. Virus is unable to replicate in postmitotic Purkinje cells, but transcription of viral proteins does occur. It has been suggested that a nonstructural parvovirus protein NS1 is produced at low concentrations during the G_0 and G_1 phases of the cell cycle (see E-Fig. 1-18). Because NS1 is known to be highly cytotoxic and able to induce alterations of the cytoskeleton, this effect could result in injury and cytolysis of Purkinje cells during in utero infection with virus.

Embryologically, granule cells of the external granular layer are stem cells that contribute to formation of the cerebellum, especially the fully differentiated granule cell layer. This process is complicated and involves migration and differentiation of granule cells from the external layer into the cerebellar cortex, thus, in part, determining its "normal" size, shape, and structure. Infection and lysis of these cells by parvoviruses can severely alter the development of the cerebellum, resulting in cerebellar hypoplasia. The extent and severity of hypoplasia depends on how early in the process of migration and differentiation these cells are infected and lysed.

Although cerebellar hypoplasia is not commonly thought to occur from in utero infection of female dogs by canine parvovirus, a recent study has identified parvovirus DNA in brain tissue from puppies with the disease. However, the significance of this information remains unclear because parvovirus structural proteins were not identified in the same tissues. A similar syndrome, probably involving similar mechanisms, occurs in calves infected in utero with bovine viral diarrhea–mucosal disease virus (see Fig. 14-36).

Bone, Joints, Ligaments, and Tendons

Disorders of Ruminants (Cattle, Sheep, and Goats)

Caprine Arthritis (Caprine Arthritis-Encephalitis Syndrome, Enveloped RNA Virus). The mechanism of injury in caprine arthritis is chronic-active (granulomatous) inflammation of the synovium, resulting in proliferative synovitis. The chronologic sequence of steps that characterizes the pathogenesis of injury in caprine arthritis is similar to those that which occurs in ovine progressive pneumonia (maedi) of sheep (see section on Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants [Cattle, Sheep, and Goats]; Ovine Progressive Pneumonia [Maedi; Maedi-Visna Virus (Ovine Lentivirus); Enveloped RNA Virus]).

Integumentary System

Disorders of Domestic Animals

Vesicular Stomatitis (Vesiculovirus, Enveloped RNA Virus). See section on vesicular stomatitis in the section on Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Domestic Animals; Vesicular Stomatitis (Vesiculovirus, Enveloped RNA Virus).

Foot-and-Mouth Disease (Aphthovirus, Nonenveloped RNA Virus). See foot-and-mouth disease in the section on Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Ruminants (Cattle, Sheep, and Goats); Foot-and-Mouth Disease (Aphthovirus, Nonenveloped RNA Virus).

Viral Papillomas (Warts, Sarcoids, Papillomaviruses, Nonenveloped DNA Virus). The mechanism of injury in viral papillomas is dysfunction of genes that regulate cell proliferation, differentiation, and adhesion, resulting in benign neoplastic transformation of virus-infected epithelial cells. Cells of stratum basale (germinativum) play a central role in the pathogenesis of viral papillomas. Gross lesions include the formation of exophytic and occasionally endophytic papillomatous fronds that arise from mucosae or skin (see Fig. 17-43). Papillomaviruses are species specific and cause (1) warts of the skin and papillomas of mucosae of the alimentary system, teats and udder, and penis in cattle; (2) sarcoids of the skin in horses, donkeys, and mules; and (3) papillomas of the mucosal epithelium of the oral cavity and reproductive system in dogs.

Animals encounter virus through direct contact with animals of the same species having warts, papillomas, or sarcoids. From these masses, virus is released into the environment via shedding and lysis of aged and virus-infected cells of the stratum lucidum and stratum corneum. Virus must then encounter cells of the stratum basale in naïve animals; therefore viral infection must be preceded by injury of the superficial layers of the stratified epithelium of mucosae or skin, resulting in the physical exposure of target cells in the stratum basale. Because of the short life span of cells in skin and mucosa, stem cells of the stratum basale are continuously dividing to replace cells in the suprabasilar layers. Maturation of these cells begins with the least differentiated layer, the stratum basale, and progresses outwardly through the suprabasilar layers: the strata spinosum, granulosum, lucidum, and corneum. Cells of the suprabasilar layers do not divide and therefore cannot be infected by virus. Virus likely uses capsid proteins, such as bovine L1 major capsid protein and L2 minor capsid protein, to attach and bind to and enter cells of the stratum basale. Viral receptors on cells of the stratum basale have not been clearly identified; however, an integrin ($\alpha_6\,\beta_4$) and potentially heparin sulfate proteoglycans mediate the attachment and entry of virions into target cells.

Dividing cells of the stratum basale are target cells for viral infection; however, they are nonpermissive cells. Because these cells have a life span for the duration of the animal's life, they serve as a reservoir for virus (i.e., persistent infection), and it replicates its genome to a limited extent within the nucleus of these stem cells. However, because these cells are nonpermissive, virus is unable to produce infective virions. Maturation of the virus occurs as cells of the stratum basale differentiate into cells of the strata spinosum, granulosum, lucidum, and corneum (suprabasilar layers). These differentiated cells are permissive and allow virus to complete its replication cycle and produce infective virions. Virus is released from cells of the stratum lucidum and stratum corneum into the environment to spread the disease, likely when these cells age and break

down. A similar process probably occurs in infected mucosae of the alimentary system.

Neoplastic transformation of epithelial cells by papillomaviruses can result in the formation of benign tumors, such as papillomas, warts, and sarcoids, and malignant tumors, such as carcinomas. When virus infects stems cells of the stratum basale, the expression of viral genes is maintained at low numbers (approximately 20 to 100 extrachromosomal copies of viral DNA per cell) where it replicates in synchrony with the cell cycle as the cell divides. Normally, as epithelial cells leave the stratum basale and mature (differentiate), they turn off endogenous genes and the synthesis of proteins required for cell division. When virus-infected stem cells of the stratum basale divide, viral genomes are carried in cells that differentiate into cells of the suprabasilar layers. Viral proteins prevent these differentiated cells from stopping the cell cycle, thus cells of suprabasilar layers, especially the strata spinosum and granulosum, are now capable of division. Because cells of the suprabasilar layer are permissive and allow virus to complete its replication cycle and produce infective virions, large quantities of viral genes and regulatory proteins are present within these dividing target cells.

As a general rule, neoplastic transformation of virus-infected epithelial cells appears to be linked to the quantitative and qualitative expression of viral genes and gene products, such as oncoproteins, and how these molecules interact with target cell genes and gene products regulating cell proliferation, differentiation, and adhesion. It appears that strains of papillomavirus that are unable to integrate into target cell genes are most likely to cause benign transformation (papillomas, warts, and sarcoids) of virus-infected epithelial cells, whereas strains that are able to integrate into target cell genes are most likely to cause malignant transformation (carcinomas) of virus-infected epithelial cells. Therefore benign transformation involves nonpermissive cells of the stratum basale, whereas malignant transformation involves permissive cells of the suprabasilar layers. In nonpermissive cells, virus does not integrate into target cell genes and viral genes, and gene products like oncoproteins are expressed in low amounts. Thus the likelihood of papillomavirus (1) activating growth-promoting genes (oncogenes) in target cell DNA, (2) inactivating suppressor genes that would inhibit cell proliferation, and (3) altering the functional expression of genes that regulate apoptosis is very low. Malignant transformation is most likely to occur in suprabasilar cells where virus integrates into target cell genes and viral genes and gene products, such as oncoproteins, are expressed in high numbers. Thus the likelihood of virus (1) activating growth-promoting genes (oncogenes) in target cell DNA, (2) inactivating suppressor genes that would inhibit cell proliferation, and (3) altering the functional expression of genes that regulate apoptosis is very high. A similar process probably occurs in infected mucosae of the alimentary system.

Disorders of Ruminants (Cattle, Sheep, and Goats)

Pox (Cowpox [Orthopoxvirus], Sheeppox and Goatpox [Capripoxvirus], Swinepox [Suipoxvirus], Enveloped DNA Virus). The term pox is used herein to group diseases, such as bovine cowpox, sheeppox, goatpox, swinepox, and lumpy skin disease, that are caused by closely related strains of poxviruses. The mechanism of injury is dysfunction and lysis of dendritic and epithelial cells of the skin. Gross lesions include macules, papules, vesicles, pustules, scabs, and scars (see Figs. 17-64 and 17-68). Lesions are most easily observed on wool-free or hair-free areas (Fig. 4-43). In general, sheeppox and goatpox are more virulent and cause systemic disease, whereas bovine cowpox and swinepox usually do not cause systemic disease. In these latter species, spread of virus is the result of animal-to-animal contact or contact with clothing or tools/instruments

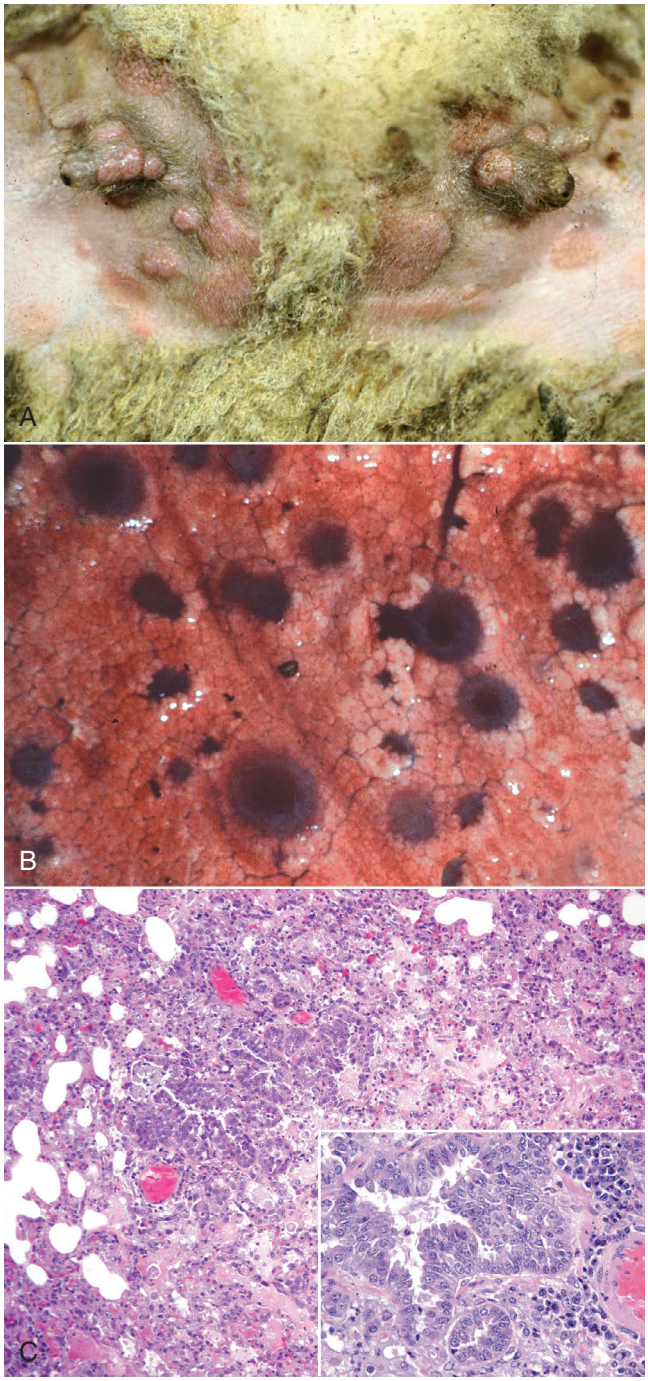


Figure 4-43 Sheeppox and Goatpox. A, Skin, teats, inguinal area. Macules, papules, vesicles, crusts (scabs), and papillomas (epidermal hyperplasia) are present on the skin of the inguinal area and teats. Additional information about the development and progression of poxvirus-induced lesions is schematically illustrated in Figure 17-32 and macroscopically and microscopically shown in Figures 17-64 (sheeppox) and 17-68 (swinepox). **B,** Lung, pox lesions. These circumferentially expanding dark red to plum-colored lesions of varied sizes are areas of proliferating bronchial and bronchiolar mucosal epithelial cells, necrotic epithelial cells, cell debris, and inflammation demonstrated in **C. C,** Lung, bronchiole. There is proliferation of mucosal epithelial cells of the lung's conductive system that are infected with poxvirus. Note the mononuclear inflammatory likely bronchial-associated lymphoid tissue (BALT) in adjacent supporting stroma. *Inset,* Higher magnification of **C.** H&E stain. (**A** courtesy Dr. D. Gregg, Plum Island Animal Disease Center; and Noah's Arkive, College of Veterinary Medicine, The University of Georgia. **B** courtesy Dr. R. Breeze, Plum Island Animal Disease Center; and Noah's Arkive, College of Veterinary Medicine, The University of Georgia. **C** courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

contaminated with virus-infected skin, scabs, or other skin debris. It appears that skin must be injured (traumatic abrasions) so that capillary endothelial cells, trafficking leukocytes, or Langerhans cells (dendritic cells) are exposed, can encounter virus, and can be infected.

As examples, bovine cowpox most commonly occurs on the teats of dairy cows, the areas most commonly injured by milking trauma in a dairy herd. Insect bites result in penetrating skin wounds that can also carry virus into contact with susceptible target cells. However, in sheeppox and goatpox, animals encounter virus through inhalation or ingestion. It is deposited on mucosae of the oronasal pharynx, especially of the tonsil, and infects and replicates in epithelial cells, mucosal lymphocytes and macrophages, and dendritic cells (MALT). It has not been determined how virus penetrates the mucus layer to gain access to mucosal epithelial cells, macrophages, and/or dendritic cells, but it is likely virus is phagocytosed by leukocytes trafficking in the mucus layer when during migration these cells encounter virus. Macrophages of the lamina propria and submucosa are infected, and virus spreads in them via leukocyte trafficking and afferent lymphatic vessels to regional lymph nodes, such as the submandibular and pharyngeal. Here proinflammatory chemokines and cytokines are released from virus-infected macrophages, and they act to recruit naïve lymphocytes and macrophages, which are infected with virus. Virus then spreads systemically via leukocyte trafficking in these lymphocytes and macrophages through postcapillary venules or lymphatic vessels and the thoracic duct to the circulatory system and then to systemic lymph nodes, spleen, and bone marrow and infects and replicates in similar cells using mechanisms as described earlier. Virus then spreads from systemic lymphoid tissues via leukocyte trafficking to the skin, lung, liver, and other organ systems.

In the skin, virus spreads from migrating macrophages and lymphocytes and infects and replicates in endothelial cells, resulting in direct injury to the vasculature and an acute inflammatory response. Endothelial cell injury accompanied by vascular dilation, active hyperemia, and acute inflammation, in part, likely account for macules and papules observed in early skin lesions. Langerhans cells (dendritic cells) are in close contact with endothelial cells in the malpighian layer of the skin. It appears that virus from capillary endothelial cells and trafficking leukocytes is able to infect Langerhans cells and then spread virus to contiguous skin epithelial cells of the stratum basale and spinosum. All of these cells allow virus to replicate; thus when epithelial cells of the stratum basale and spinosum are killed, the space formerly occupied by these cells coalesces and is filled with cell debris and intercellular edema, forming vesicles. With injury, acute inflammation ensues, as does the pustular stage. Through adaptive immune responses, viral infection is resolved and pustular lesions heal as scabs over granulation tissue that becomes scars.

It is likely that both humoral and cell-mediated immunity are important in protecting against and resolving pox diseases; however, these responses can cause injury and lysis of virus-infected target cells. Similar lesions and lesion progression may affect oral mucous membranes. Pneumonia has been reported in systemic poxvirus-induced disease. Affected lungs have variable-sized and randomly distributed pock lesions in the form of large, irregularly shaped lobular areas of consolidation (see Fig. 4-43). This pattern is consistent with hematogenous spread of the virus via leukocyte trafficking in virus-infected macrophages to pulmonary endothelial cells and then to bronchiolar and alveolar epithelial cells followed by cell lysis and acute inflammation. Although reservoir hosts for poxvirus are wild rodents, cats are now the most commonly recognized reservoir. Cats are infected with virus through their skin by an indirect mechanism when hunting virus-infected rodents; however, infection, as previously described, via a direct mechanism (inhalation) and systemic spread in monocytes and macrophages has been reported.

Poxviruses use attachment proteins to bind to glycosaminoglycan receptor proteins on the surface of target cells. Because of the volume of information related to attachment proteins and receptors in poxvirus diseases, discussion of these protein molecules is outside the scope of this chapter.

Contagious Ecthyma (Orf Virus: Parapoxvirus, Enveloped DNA Virus). See contagious ecthyma in the section on Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Ruminants (Cattle, Sheep, and Goats).

Bovine Papular Stomatitis (Parapoxvirus, Enveloped DNA Virus). See bovine papular stomatitis in the section on Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Ruminants (Cattle, Sheep, and Goats).

Disorders of Pigs

Swine Vesicular Disease (Enterovirus, Nonenveloped RNA Virus). See swine vesicular disease in the section on Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Pigs.

Vesicular Exanthema of Pigs (Calicivirus, Nonenveloped RNA Virus). See vesicular exanthema of pigs in the section on Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Pigs.

Female Reproductive System

Disorders of Horses

Equine Herpesvirus Abortion (Equine Herpesvirus 1 and 4: Alphaherpesvirus, Enveloped DNA Virus). See equine viral rhinopneumonitis in the section on Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Horses. In summary, gross lesions include abortions (born weak) and fetal lysis (mummification, stillbirths). Following inhalation, virus infects mucosal macrophages, lymphocytes, and/or dendritic cells, and these cells probably migrate in lymphatic vessels via leukocyte trafficking and spread virus to regional lymph nodes such as the tracheobronchial. Here it infects macrophages and lymphocytes and is spread systemically, via cell-free viremia or leukocyte trafficking through postcapillary venules or lymphatic vessels and the thoracic duct. Additionally, infection of vascular and lymphatic endothelial cells appears to occur. Within the circulatory system, virus ultimately reaches the uterus and placenta. It is not clear how virus spreads from the uterus, to the placenta, and then to the fetus, but some form of a fetal macrophage-like cell probably intervenes in the placentome. It is thought that abortions (also mummification and stillbirths) may result from (1) infection and lysis of cells within the fetus, (2) virus-induced vasculitis and thrombosis of the placental vasculature (infection of endometrial endothelium) resulting in placental separation, or (3) a combination of both mechanisms. Cell types involved and mechanism(s) of injury are undetermined, as are attachment proteins and target cell receptors in the fetus or endothelium of the uterus.

Coital Exanthema (Equine Herpesvirus 3: Alphaherpesvirus, Enveloped DNA Virus). The mechanism of injury in coital exanthema is dysfunction and lysis of skin and/or mucosal epithelial cells (mucocutaneous junctions) of the male and female reproductive systems. Gross lesions include active hyperemia; hemorrhage; and papules, vesicles, and pustules, resulting in erosions and ulceration

of affected mucosae and an acute inflammatory response (see Fig. 18-31). Horses encounter virus through direct contact (venereal disease) with infected horses during breeding. Mucosae do not need to be injured for virus to infect cells. Virus can also spread mechanically via hands, gloves, instruments, palpation sleeves, and sponges, if contaminated with virus. Insect bites, especially fly bites, may also be a means of spreading the virus. Although unidentified, equine herpesvirus 3 probably expresses attachment proteins in its envelope that attach and bind to specific receptors on cells of reproductive mucosae.

Equine Viral Arteritis (Arterivirus, Enveloped RNA Virus). See equine viral arteritis in the section on Viral Diseases of Organ Systems, Cardiovascular System and Lymphatic Vessels, Disorders of Horses for information on portals of entry and initial encounters of virus with target cells. In summary, gross lesions include abortions (born weak) and fetal lysis (mummification, stillbirths). Virus-infected macrophages, arising at portals of entry, spread virus via leukocyte trafficking to the endometrium, where they encounter endothelial cells, lymphocytes, and macrophages. These cells are infected with virus. It is unclear how virus spreads from the uterus, to the placenta, and then to the fetus, but some form of a fetal macrophage-like cell probably intervenes in the placentome and spreads virus in the fetus. Furthermore, in the placentome, virus-infected endothelial cells and their supportive myocytes are lysed by virus, leading to a necrotizing vasculitis. Trophoblasts can also be infected with virus. It is thought that abortions (also mummification and stillbirths) may result from (1) infection and lysis of cells within the fetus, (2) virus-induced vasculitis and thrombosis of the placental vasculature (infection of endometrial endothelium), resulting in placental separation, or (3) a combination of both mechanisms. Cell types involved and mechanism(s) of injury are undetermined, as are attachment proteins and target cell receptors in the fetus or endothelium of the uterus.

Stallion semen is also a likely source of virus (accessory sex glands). It is deposited on mucosae, and virus likely infects and replicates in mucosal macrophages and as they migrate through the mucosa and then is spread by these cells locally through leukocyte trafficking to lamina propria and submucosae, where they infect and replicate in tissue macrophages and lymphocytes. These cells then migrate to blood vessels and injure endothelial cells of the endometrium/placentome as described earlier. Attachment proteins and target cell receptors in reproductive mucosae are unknown.

Disorders of Ruminants (Cattle, Sheep, and Goats)

Bovine Herpesvirus Abortion (Bovine Herpesvirus 1: Alphaherpesvirus, Enveloped DNA Virus). See infectious bovine rhinotracheitis in the section on Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants (Cattle, Sheep, and Goats) for information on portals of entry and initial encounters of virus with target cells. In summary, gross lesions include abortions (born weak) and fetal lysis (mummification, stillbirths). From portals of entry, virus-infected mucosal macrophages, lymphocytes, or dendritic cells migrate in lymphatic vessels via leukocyte trafficking and spread virus to regional lymph nodes such as the tracheobronchial. Here it infects macrophages and lymphocytes, which spread it to the circulatory system and placenta via cell-free viremia or leukocyte trafficking through postcapillary venules or lymphatic vessels and the thoracic duct. It is not clear how virus spreads from the uterus, to the placenta, and then to the fetus, but some form of a fetal macrophage-like cell probably intervenes in the placentome. Experimental studies have shown that cells in the fetal liver are primary targets for viral infection. Additionally, but too a much lesser extent, endothelial cells of the heart, brain, and placenta are also infected. The role of virus-induced vasculitis and thrombosis of the placental vasculature (infection of endometrial endothelium) resulting in placental separation, if it occurs, has not been determined.

Infectious Pustular Vulvovaginitis/Balanoposthitis (Bovine Herpesvirus 1: Alphaherpesvirus, Enveloped DNA Virus). See infectious bovine rhinotracheitis in the section on Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants (Cattle, Sheep, and Goats) for information on portals of entry and initial encounters of virus with target cells. In summary, gross lesions include erosion and ulcerations with hemorrhage of reproductive mucosae (see Fig. 18-29). From portals of entry, virus-infected mucosal macrophages, lymphocytes, or dendritic cells migrate in lymphatic vessels via leukocyte trafficking and spread virus to regional lymph nodes such as the tracheobronchial. Here, it infects macrophages and lymphocytes, which spread it to the circulatory system and placenta via cell-free viremia or leukocyte trafficking through postcapillary venules or lymphatic vessels and the thoracic duct. Virus then spreads to epithelial cells of the mucous membranes of the penis, prepuce, vulva, or vagina via cell-free viremia or leukocyte trafficking. Because virus causes lysis of infected cells and thus erosions and ulcerations of mucosae, it may also be spread via direct contact (venereal disease) of virus-infected mucosae from the penis or prepuce with mucosae of the vulva or vagina, or vice versa, during breeding.

Disorders of Pigs

Porcine Reproductive and Respiratory Syndrome (PRRS Virus, Enveloped RNA Virus). See porcine reproductive and respiratory syndrome in the section on Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Pigs for information on portals of entry and initial encounters of virus with target cells. Although unknown, the mechanism and type of injury that occurs in the lung also probably affects a wide variety of cells in the placenta, fetal membranes, and fetus. Injury can be observed in fetal myocytes; however, it is unclear as to whether loss of myocytes is attributable to necrosis, apoptosis, or atrophy. Gross lesions include abortions (born weak) and fetal lysis (mummification, stillbirths). Virus probably spreads to the placenta in virus-infected macrophages within the circulatory system via leukocytic trafficking from an initial site of virus replication in another organ system such as the lung or uterus. It is likely that virus-infected macrophages transfer virus to fetal macrophage-like cells in the placentome, which then spread virus to all organ systems in the fetus. Although all fetuses in a litter may not be infected, it has been shown that pig fetuses in all stages of gestation can be infected with and support replication of virus resulting in normal, born weak, stillborn, and mummified fetuses.

Porcine Parvovirus Abortion (Parvovirus, Nonenveloped DNA Virus). See the following Viral Diseases sections for information on portals of entry and initial encounters of virus with target cells:

- Viral Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Dogs; Parvovirus Enteritis (Parvovirus, Nonenveloped DNA Virus)
- Viral Diseases of Organ Systems, Cardiovascular System and Lymphatic Vessels, Disorders of Dogs, Parvovirus Myocarditis (Parvovirus, Nonenveloped DNA Virus)
- Viral Diseases of Organ Systems, Nervous System, Disorders of Cats, Parvovirus-Induced Cerebellar Hypoplasia (Parvovirus, Nonenveloped DNA Virus)

The mechanism of injury is dysfunction and potentially lysis of placental and fetal cells. Gross lesions include reproductive failure,

embryonic lysis, fetal resorption, stillbirths, and mummified fetuses (see Fig. 18-56).

Pigs encounter virus through direct contact with fomites from fluids or tissues of the reproductive system, placenta, or aborted fetuses. Virus can also be transferred mechanically via hands, gloves, and instruments, if they are contaminated with virus-infected body fluids. It is ingested or inhaled and deposited on mucosae of the oral, nasal, and pharyngeal cavities, especially of the tonsil. It has not been determined if and how virus penetrates the mucus layer to gain access to tonsillar mucosal epithelial cells. Virus likely infects and replicates in mucosal macrophages and dendritic cells as they migrate through the mucus layer and mucosae and then is spread by these cells locally through leukocyte trafficking to mucosal epithelial cells and their lamina propria and submucosa, where they infect and replicate in tissue macrophages, lymphocytes, and dendritic cells of the tonsil (MALT). A cell-free viremia may also occur. These cells spread virus in afferent lymphatic vessels via leukocyte trafficking to regional lymph nodes, where they infect and replicate in similar cells. Then it spreads to the circulatory system and systemically to lymph nodes through postcapillary venules or lymphatic vessels and the thoracic duct.

From the circulatory system, it is unclear how virus interacts with and spreads from the uterus, to the placenta, and then to the fetus; however, studies suggest the spread of virus to the fetus occurs via leukocyte trafficking by fetal macrophage-like cells. Although unidentified, porcine parvovirus probably has capsid attachment proteins that bind to glycosylated cell membrane receptors (likely sialic acid–bearing cell surface receptors) of target cells in the uterus, placenta, and fetus. Virus has been identified in placental and fetal endothelial cells and in most tissues of virus-infected fetuses. Parvoviruses only infect and replicate in dividing cells because they require a target cell–derived duplex transcription template, which is available when cells divide during S phase of the cell cycle (see E-Fig. 1-18). Parvoviruses are unable to turn on DNA synthesis in target cells, so they must wait for target cells to enter the S phase of the cell cycle before they can infect cells. It is likely that the high mitotic rate of developing and growing fetal tissues is conducive to infection by virus. Virus-induced lysis of fetal cells during the first 35 days of gestation causes embryonic lysis (death) and fetal resorption, whereas infection between 35 and 70 days of gestation causes fetal death (stillbirths) and mummified fetuses.

Porcine Cytomegalovirus Abortion (Herpesvirus-Cytomegalovirus, Enveloped DNA Virus). See inclusion body rhinitis–porcine cytomegalovirus infection in the section on Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Pigs for information on portals of entry and initial encounters of porcine cytomegalovirus with target cells. The pathways of spread and mechanisms and types of injury in the placenta, fetal membranes, and fetus are probably similar to those discussed earlier for porcine parvovirus abortion and porcine reproductive and respiratory syndrome.

Male Reproductive System

See the section on the Female Reproductive System.

Eye

Disorders of Cats

Feline Herpetic Keratitis (Feline Herpesvirus 1: Alphaherpesvirus, Enveloped DNA Virus). See the following Viral Diseases sections for information on portals of entry and initial encounters of herpes viruses with target cells:

- Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Horses; Equine Viral Rhinopneumonitis (Equine Herpesvirus, Alphaherpesvirus, Enveloped DNA Virus)
- Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Ruminants (Cattle, Sheep, and Goats); Infectious Bovine Rhinotracheitis (Bovine Herpesvirus, Alphaherpesvirus, Enveloped DNA Virus)
- Viral Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Cats; Feline Viral Rhinotracheitis (Feline Herpesvirus, Alphaherpesvirus, Enveloped DNA Virus)

The mechanism of injury in feline herpetic keratitis is lysis of epithelial cells of the cornea. Gross lesions include corneal ulcerations; however, with severe injury, involvement of the underlying corneal stroma can occur, leading to edema, neovascularization, collagenization, and inflammation. These secondary lesions are attributable to inflammation and its mediators, especially those derived from cytotoxic T lymphocytes. Cats encounter virus in fomites from body fluids, such as saliva and eye and nasal secretions, contaminated through direct contact (grooming behaviors) with virus-infected cats. Virus is deposited on mucosae of conjunctiva, where it infects and replicates in the epithelium. Viral envelope glycoproteins are used to attach to and enter these cells via glycosaminoglycan receptors on conjunctival epithelial cells. Virus replicates in these cells, and with cell lysis it is released to spread in conjunctival fluids. It is carried in these fluids and encounters additional conjunctival epithelial cells and corneal epithelial cells, where it replicates and escapes via cell lysis. In the latter cell type, if severely affected, the cornea can become inflamed, edematous, and ulcerated.

Fungal Diseases (Mycoses)

Portals of entry; target cells and substances; pathways of spread; virulence factors; mechanisms of adhesion, colonization, invasion, and replication; toxins; and defense mechanisms for fungal diseases are similar to those discussed in the opening sections of this chapter and in the sections on bacterial and viral diseases.

Fungi, microbes common in the environment and as microflora of mucosae, exist as yeasts or as branched filamentous pseudohyphal or hyphal forms (molds). Most fungi discussed in this section have both forms in their life cycles and are known as *dimorphic fungi* (Fig. 4-44). They have also be classified as superficial mycoses (candidiasis, aspergillosis) and systemic or deep mycoses (histoplasmosis, coccidioidomycosis, blastomycosis, angioinvasive fungi, and cryptococcosis) based on their relative "depth" of involvement in disease affecting one or more organ systems.

Fungi contain a variety of complex molecules that are arranged to form cell walls and capsules that aid in colonization of tissues and to protect the microbe against phagocytosis and other defense mechanisms. Because of this complexity, these molecules and cell walls cannot be completely degraded and removed by acute inflammation, and the response rapidly progresses to granulomatous inflammation. Specific molecules and their roles in disease mechanisms will be discussed in sections covering individual diseases. In summary, substances, such as glucans and glycoproteins, often act to block phagocytosis, and when they are phagocytized, they are often difficult to degrade to inert materials within macrophages and neutrophils because of their physical structure and biologic constituents. Because macrophages have short life spans (6 to 16 days), these nondegraded materials are released from dead macrophages into tissues and lead to the recruitment of additional macrophages into tissues to remove the debris. Furthermore, macrophages that phagocytize this debris are "activated," resulting in the synthesis and secretion of chemokines and cytokines that recruit additional macrophages from the

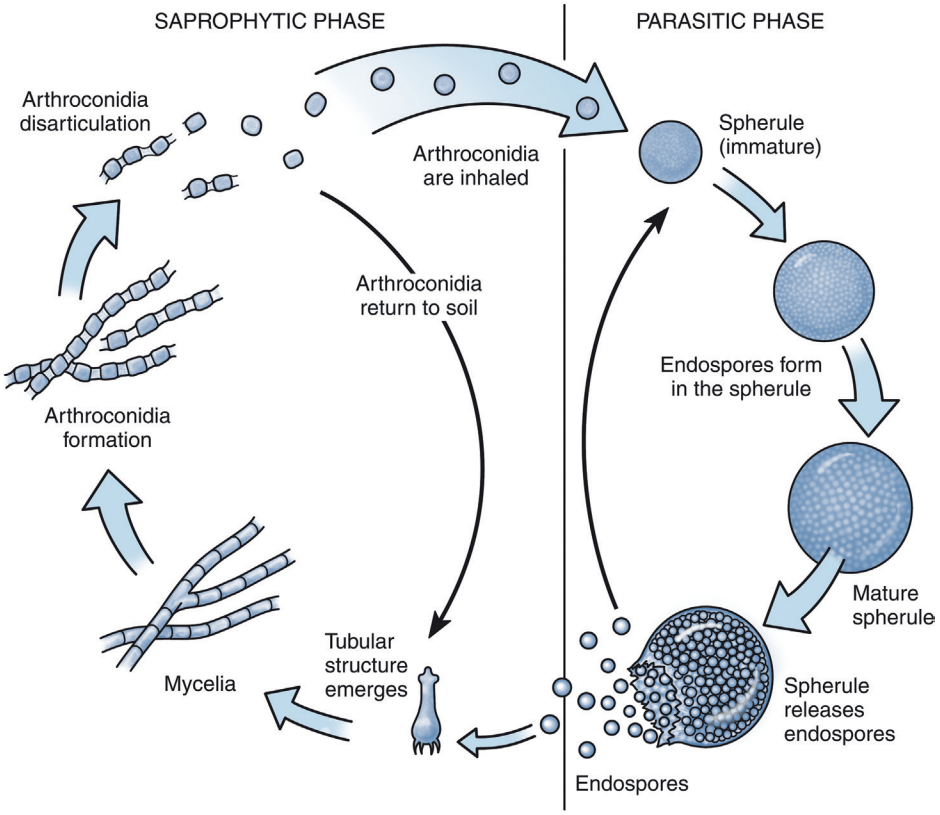


Figure 4-44 Life Cycle of *Coccidioides immitis* and Other Dimorphic Fungi.

vascular system into the site of inflammation. Because this process is repetitive with recurring cycles of replication in macrophages, death of macrophages and release of fungi and fungal debris and antigens, and phagocytosis of these materials by newly recruited and naïve macrophages, a granulomatous inflammatory response characteristic of fungal diseases occurs in affected tissues.

Fungal Diseases of Organ Systems

Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity

Disorders of Domestic Animals

Candida Glossitis—Oropharyngeal Candidiasis (Thrush) (*Candida albicans*). The mechanism of injury in candida glossitis is (1) proliferation and invasion of filamentous pseudohyphae and hyphae into lingual mucosa and (2) disruption and lysis of mucosa caused by inflammation and its chemical mediators and degradative enzymes. Gross lesions include acute pseudomembranous glossitis with an extensive white to yellow pseudomembrane consisting of desquamated epithelial cells, fibrin, and fungal hyphae covering the dorsal surface of the tongue (see Figs. 7-7 and 7-8).

Candida albicans can occur in two forms: (1) yeast that are commensal and nonpathogenic and (2) filamentous pseudohyphae and hyphae that are pathogenic. Animals encounter yeast through ingestion (and likely inhalation) where it persists as a commensal microbe that colonizes mucosa without causing injury or disease and becomes part of the normal microbial flora associated with mucosal surfaces (i.e., biofilm). The balance between commensalism and disease is tenuous, and perturbations of mucosae and/or changes in the physiologic status of the animal may shift this balance in favor of disease (i.e., the filamentous pseudohyphal and/or hyphal forms). Through a process called morphologic or phenotypic switching, the yeast form switches to the invasive and pathogenic filamentous pseudohyphal and/or hyphal form. Switching appears to occur through inducible chromosomal rearrangements in the genome of the yeast in response to changes in the mucosal environment. Switching appears to be reversible. Under normal conditions the temperature of mucosa in the oral cavity is near room temperature (25° C). This temperature favors the growth of yeast, whereas pseudohyphae and/or hyphae prefer to grow at 37° C. The yeast form is able to switch this temperature dependence for growth via chromosomal rearrangements, so that its pseudohyphae and/or hyphae forms can grow at 25° C. Switching is attributable to virulence factors selectively expressed under suitable predisposing conditions in the yeast (antigenic drift/shift) combined with the breakdown of mucosa, excessive use of broad-spectrum antibiotics and corticosteroids, hyperglycemia, tissue damage secondary to chemotherapy or radiation, or immunosuppression. Additionally, if innate (phagocytosis by neutrophils and macrophages) and adaptive (cell-mediated) immunity, important defense mechanisms in controlling candidiasis, are disrupted, switching is also favored.

A large group of virulence factors are involved in the processes of switching and infection and invasion, but no single factor accounts for virulence, and not all expressed virulence factors may be necessary for a particular stage of infection. The yeast form appears to have its own group of virulence factors; as does the pseudohyphal and hyphal form. Yeast persists in the oropharyngeal cavity by adhering to and colonizing mucosa via ligand-receptor and/or hydrophobic interactions. Yeast and pseudohyphal and hyphal forms have ligands in their cell walls such as those in the agglutinin-like (AL) family and hyphal wall protein (Hwp) family that allow these forms to adhere to epithelial cells and invade mucosa. Mannose and mannoproteins may also act as adhesins. Receptors expressed on mucosal epithelial cells can include E-cadherin, fibrinogen, fibronectin, thrombin, collagen, laminin,

and vitronectin-binding proteins. The pseudohyphal and hyphal form adheres to and invades mucosa (epithelial cells) using virulence factors, such as fungal "invasin" proteins as examples. Pseudohyphae and hyphae also express new adhesin ligands and hydrolytic aspartyl proteinases that injure the mucosa and allow them to encounter new types of adhesin receptors and entry receptors in mucosa and submucosa and invade the layer. Although uncharacterized, epithelial damage, in addition to that produced by inflammation, may also be caused by apoptosis initiated by virulence factors in pseudohyphal and/or hyphal forms.

Disorders of Dogs

Histoplasmosis (*Histoplasma capsulatum*). The mechanism of injury in histoplasmosis is cell lysis via chronic granulomatous to pyogranulomatous inflammation and its effector molecules and degradative enzymes. *Histoplasma capsulatum* has a dimorphic life cycle; the mycelial (microconidia) phase occurs in extracellular environments (25° C), whereas the yeast phase occurs intracellularly within cells of the monocyte-macrophage system (37° C). Gross lesions include thickened walls of the small intestine and enlargement of the liver, lung, spleen, and mesenteric lymph nodes (see Figs. 7-182, 8-56, 13-91, and 14-48, C). Lesions are caused by the accumulation of granulomatous inflammatory cells in perivascular spaces, resulting in (1) generalized enlargement of affected organs with increased pallor or (2) the formation of one or more solid white-yellow nodules distributed at random in the affected tissue. Lesions are most prominent in the small intestine, where inflammatory cells accumulate in the lamina propria of villi and the submucosa, resulting in thickened walls and ulcerated mucosae. Systemic lymph nodes, bone marrow, and eyes may also become infected with fungus via leukocyte trafficking and acquire a granulomatous inflammatory response.

Dogs (and cats) encounter fungus through inhalation of microconidia (2- to 5-μm in diameter spores), which can reach the lower respiratory tract (i.e., bronchi and bronchioles). They are present in soil-derived aerosols from moist and humid environments. Microconidia are deposited on mucosae of the nasopharyngeal cavity and the conductive components of the respiratory system through centrifugal and inertial turbulence. Neutrophils and alveolar macrophages phagocytize microconidia trapped in the mucus layer of mucosae. Because microconidia can be killed by macrophages and neutrophils, there is a rapid transition to the yeast form because it provides protection against innate and adaptive immune responses. Recognition, attachment, and internalization of microconidia by phagocytes are likely mediated by ligand-receptor interactions, but specific molecules have not been identified. After phagocytosis and if not killed, microconidia germinate in phagosomes into the yeast form. Transitioning from microconidia to yeast is a requirement for fungal pathogenicity. Phagosomes attempt to kill the yeast by fusing with cellular lysosomes to form phagolysosomes. Lysosomes have an acidic pH and acid hydrolases that kill or restrict the growth of yeast. The yeast is able to prevent its lysis by synthesizing proteins that inhibit acidification of the phagolysosome and the activities of lysosomal proteases. The yeast form is protected against host defenses as long as it is hidden in phagosomes of viable macrophages. Yeast is spread in alveolar macrophages via leukocyte trafficking to local lymphoid tissues like BALT, where additional macrophages are infected. From here, infected macrophages spread via leukocyte trafficking in afferent lymphatic vessels to regional lymph nodes and then systemically via the lymphatic and vascular systems to mesenteric lymph nodes and Peyer's patches. It is likely that macrophages containing yeast spread from Peyer's patches into contiguous lamina propria and submucosa of the small intestine and via lymphatic vessels to mesenteric lymph nodes.

The ligand-receptor interactions that determine location specificity have not been identified. The innate immune system identifies fungi, in part, by recognition of PAMPs (see Chapters 3 and 5) formed by α- and β-glucan surface polysaccharides in yeast cell walls. Macrophages recognize these patterns through TLRs and other PRRs expressed on macrophages and use the information to develop an appropriate immune response. Fungi have developed mechanisms to evade and/or neutralize detection by PRRs on macrophages, neutrophils, and dendritic cells such as those leading to modifications in surface polysaccharides through genomic variation. Additionally, an array of suspected virulence factors have been identified, and they include adhesins and invasins, molecules involved in iron homeostasis, and molecules that may disrupt phagosome-lysosome fusion.

Because macrophages have short life spans (6 to 16 days), yeast and yeast-derived surface polysaccharide antigens are released from dead macrophages into the lamina propria of the small intestine. These polysaccharides and the chemokines and cytokines secreted by infected macrophages lead to recruitment of additional macrophages and pyogranulomatous inflammatory cells into the lamina propria. This process is repetitive; lesions characteristic of histoplasmosis ensue because of recurring cycles of replication in macrophages, death of macrophages and release of yeast, and phagocytosis of yeast by newly recruited and naïve macrophages. Thus the volume of inflammatory exudate grows with time, resulting in a thickened intestinal wall, disruption of lymphatic vascular drainage, and disturbances of junctional complexes of villus epithelial cells, all resulting in the protein-losing enteropathy characteristic of histoplasmosis clinically.

Disorders of Cats

Histoplasmosis (*Histoplasma capsulatum*). See earlier section on Histoplasmosis.

Respiratory System, Mediastinum, and Pleurae

Disorders of Domestic Animals

Aspergillosis (*Aspergillus fumigatus*). The pathogenesis of aspergillosis has similarities to those of other fungal diseases discussed in this section. The mechanism of injury is disruption and lysis of mucosae in the nasal cavity and respiratory system caused by inflammation, its mediators and degradative enzymes, and by the concurrent proliferation and invasion of fungal hyphae. Immunosuppression, impairment of phagocytosis, chemotherapy, or prolonged corticosteroid therapy may increase an animal's susceptibility to this fungus. Gross lesions include acute pseudomembranous rhinitis and sinusitis. An extensive gray-black pseudomembrane consisting of desquamated epithelial cells, fibrin, and fungal hyphae may cover the mucosal surfaces of turbinates, sinuses, and airways (see Fig. 9-34, A). The underlying bone and cartilage may also become necrotic as hyphae invade these tissues (see Fig. 9-34, *B*). In the lungs, yellow-white granulomas of varied sizes are distributed at random in lung parenchyma or may be oriented around airways.

Animals, especially dogs, encounter fungus through inhalation of conidia (≈2 to 3 μm in diameter) that are deposited on mucosae of the nasopharynx and the conductive component of the respiratory system through centrifugal and inertial turbulence. Fungus is a saprophyte of dead or decaying matter. With suitable growing conditions, the fungus produces conidia, which are inhaled and trapped in the mucus layer of mucosae. They interact with the mucociliary apparatus and defensive molecules (see Chapter 3) released from mucosal epithelial cells and are ultimately phagocytosed by neutrophils and alveolar and mucosal macrophages. Recognition, attachment, and internalization by phagocytes are likely mediated by

ligand-receptor interactions such as sialic acid residues and molecular patterns (PAMPs) on conidia and by PRRs (TLRs) expressed on alveolar macrophages and neutrophils. In healthy animals this process results in the release of proinflammatory cytokines, which, in part, recruit additional neutrophils that phagocytize and kill conidia and hyphae that are trapped in the mucus. If an animal's phagocytes are unable to phagocytize and kill conidia (i.e., defective neutrophil functions), conidia germinate in the mucus layer and mucosae and begin the processes of colonizing mucosae of the nasal cavities and sinuses. Conidia and hyphae secrete proteases, gliotoxin, fumagillin, verruculogen, and helvolic acid that slow the beat of cilia in the mucociliary apparatus and injure ciliated mucosal epithelial cells. These outcomes lead to detachment and loss of ciliated epithelial cells and exposure and damage of underlying basement membrane (laminin). Thus when conidia germinate into hyphae, a denuded and disrupted mucosa and basement membrane provide a favorable environment for hyphae to invade mucosae. Fibrinogen, fibronectin, and complement C3 fragments from inflammation that cover exposed basement membrane as a reparative response, as well as exposed laminin and collagen, can serve as receptors for sialic acid and other conidial and hyphal glycoproteins, thus contributing to fungal pathogenicity by enhancing adhesion to and colonization and invasion of injured mucosae and basement membrane. Through these mechanisms, fungus is able to invade and spread in affected tissue and extensively damage normal tissues. Dendritic cells are also able to phagocytize conidia and hyphae and process antigens for effective innate and adaptive immune responses via proinflammatory cytokines. However, pathogenic strains of the fungus have virulence factors that appear to impair these immune responses by altering the functions of effector cells.

In dogs, aspergillosis occurs in the nasal cavities, paranasal sinuses, and/or the respiratory system. In other animal species, aspergillosis begins as an infection of the respiratory system, often asymptomatic, and then spreads to other sites likely via leukocyte trafficking of conidia-infected macrophages. These sites include the lung, mammary gland, and placenta in cattle; guttural pouches in horses; and lungs in cats. *Aspergillus* biofilms of mucosae of the respiratory tract may be involved in the pathogenesis of pulmonary disease. The ability to disseminate and spread systemically to other organs is modulated in part by the ability of conidia and hyphae to block immune responses and evade killing by phagocytes. Alveolar macrophages phagocytose conidia and hyphae through a process mediated by the recognition of PAMPs by PRRs (TLRs) expressed on alveolar macrophages and other phagocytic cells as described in the discussion of histoplasmosis. *Aspergillus fumigatus* uses β-glucans, melanin, and other molecules to block killing by reactive oxygen species, phagolysosomal acidification, and other mechanisms in macrophages and neutrophils.

Conidia and hyphae can spread systemically to other organ systems in macrophages via leukocyte trafficking. Hyphae can also disseminate via the circulatory system to other organ systems through a process called angioinvasion. They may invade endothelial cells lining capillaries, gain access to the circulatory system, break off into the bloodstream, circulate, and attach to and invade endothelium at other sites. Ligand-receptor interactions are probably involved in this process, determining which organ system and tissue types are targeted by the fungus.

Coccidioidomycosis (*Coccidioides immitis*). The pathogenesis of coccidioidomycosis is similar to that of histoplasmosis discussed earlier. The mechanism of injury is cell lysis via chronic granulomatous to pyogranulomatous inflammation and its effector molecules and degradative enzymes. It has a dimorphic life cycle (see Fig. 4-44). Gross lesions include pyogranulomatous interstitial pneumonia with yellow-white granulomas of varied sizes distributed at random in the lungs and similar appearing expansile granulomas in lymph nodes (see Fig. 3-22, *B* and E-Fig. 3-8). Bone marrow and eyes may also have granulomatous inflammation resulting from infection via leukocyte trafficking of infected macrophages (see E-Fig. 21-52). Fungus is present in the soil and with suitable growing conditions produces arthroconidia (≈3 to 6 μm in diameter) that are carried into the air by disruption of the soil, such as during construction or farming.

Animals encounter arthroconidia through inhalation, and they are deposited on and trapped in the mucus layer of mucosae of airways through centrifugal and inertial turbulence. In the mucus layer, arthroconidia can be phagocytosed and killed by neutrophils and alveolar macrophages. However, there is a rapid transition of arthroconidia to spherules because this latter form of the fungus provides protection against phagocytosis (virulence factor). Furthermore, transformation into spherules leads to the expression of additional virulence factors that cause acute inflammation resulting in mucosal injury and colonization. Spherules, trapped in the mucus layer, grow to approximately 20 to 60 μm in diameter (occasionally up to 100 μm) and form a small number of intraspherular endospores (≈1 to 5 μm in diameter) through a process called *endosporulation*. Spherules appear to escape phagocytosis because they are too large to be phagocytized by neutrophils, macrophages, and dendritic cells. When spherules are mature or are damaged by inflammatory cells, chemical mediators, and/or degradative enzymes, they release new endospores onto intact mucosa or into injured and denuded mucosa and its ECM. Endospores are approximately 1 to 5 μm in diameter and are capable of being phagocytosed by alveolar macrophages, mucosal macrophages, and dendritic cells. These endospores then grow into second-generation spherules protected within the cytoplasm of these cells. These spherules are now capable of producing an average of 200 to 300 endospores that are released onto and into mucosa when infected cells are lysed. This process rapidly amplifies the number of endospores and spherules in respiratory mucosae and the opportunity for successful colonization of the airways and lung.

Because endosporulation and cell lysis is a repetitive process, proinflammatory cytokines released from "activated" macrophages assist in recruiting additional macrophages and neutrophils into the lung (i.e., granulomatous inflammation). In addition, macrophages infected with endospores likely spread via leukocyte trafficking in lymphatic vessels and the circulatory system locally to lymphoid tissues, regionally to lymph nodes, and systemically to other tissues such as the skin, bone, muscle, lymph nodes, adrenal glands, and CNS. A primary skin infection can also occur by direct infection of damaged skin, but rarely. Each form of the fungus (arthroconidia, spherules, and endospores) has virulence factors that provide it with the opportunity to evade defense mechanisms and barrier systems, complete its portion of the fungal life cycle, and transition to the next form of the fungus. Virulence factors include the (1) production of a spherule outer wall glycoprotein that modulates the immune response and compromises cell-mediated immunity, (2) depletion of spherule outer wall glycoprotein on the surface of endospores, which prevents their phagocytosis, and (3) production of host tissue arginase I and coccidioidal urease, which contribute to tissue damage at sites of infection. Additionally, exposed laminin and collagen may serve as receptors for fungal ligands enhancing adhesion to and colonization and invasion of injured mucosae and basement membrane.

Blastomycosis (*Blastomyces dermatitidis*). The pathogenesis and mechanism of injury in blastomycosis are similar to those of histoplasmosis and coccidioidomycosis discussed earlier. Gross lesions include pyogranulomatous interstitial pneumonia with

yellow-white granulomas of varied sizes distributed at random in the lungs (see Fig. 9-103) and similar-appearing expansile granulomas in lymph nodes. In the disseminated form, lymph nodes, skin, subcutaneous tissues, eyes, brain, and bone may also become infected by spread of yeast-infected macrophages via leukocyte trafficking and develop a pyogranulomatous inflammatory response. A primary skin infection can also occur by direct infection of damaged skin, but rarely.

Fungus is present in the soil and with suitable growing conditions, produces conidia (≈ 2 to 10 µm in diameter) that are carried into the air by disruption of the soil. Animals inhale conidia, which are deposited on and trapped in the mucus layer of mucosa of airways through centrifugal and inertial turbulence. In the mucus layer, conidia bind to alveolar macrophages via surface adhesins, such as BAD1 (*Blastomyces* adhesion factor 1). Several concurrent events may now occur, including (1) phagocytosis and killing of conidia by mucosal macrophages and neutrophils initiated by surface binding, (2) spread of conidia via leukocyte trafficking into and through mucosae, and (3) acute inflammation with tissue damage facilitated by proinflammatory cytokines released from macrophages. Inflammation with degradation of mucosa and ECM assist the fungus in encountering cells and tissues throughout lung parenchyma and spreading into lamina propria, submucosa, and deeper ECM tissues.

Because conidia are quickly killed by mucosal macrophages, they rapidly transition to the yeast form (≈12 to 15 µm in diameter) because it is more resistant to phagocytosis and killing by macrophages and neutrophils. Yeast accomplish this outcome by shedding their surface adhesins and/or produce masking capsules that allow them to avoid recognition by macrophages and neutrophils and evade phagocytosis and killing. However, because macrophages have short life spans (6 to 16 days), yeast and yeast-derived debris such as polysaccharides are released from dead macrophages into lung tissues. These materials act as chemokines and cytokines and help recruit additional macrophages and neutrophils into affected lung tissues. This process is repetitive, and lesions characteristic of blastomycosis ensue as the volume of pyogranulomatous inflammatory exudate grows within the lamina propria, submucosa, and deeper ECM tissues.

In the yeast phase the fungus has immune-modulating virulence factors (e.g., glucans) in its cell wall and other virulence factors (e.g., melanin) that provide resistance to phagocytosis and killing. Yeast evades the adaptive immune system by changing surface polysaccharides and by hiding in phagosomes. Additionally, yeast has the adhesion-promoting protein BAD1,which mediates adherence to CR3 and CD14 receptors on cell membranes of alveolar macrophages.

Disorders of Dogs

Histoplasmosis (*Histoplasma capsulatum*). See Fungal Diseases of Organ Systems; Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity; Disorders of Dogs; Histoplasmosis (*Histoplasma capsulatum*).

Blastomycosis (*Blastomyces dermatitidis*). See Fungal Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Domestic Animals; Blastomycosis (*Blastomyces dermatitidis*).

Cardiovascular System and Lymphatic Vessels

Disorders of Domestic Animals

Angioinvasive Fungi. Angioinvasive fungi include a group of microbes that have the ability to colonize and invade barrier systems such as mucosae and skin, invade the vascular system within these barrier systems, spread to other organ systems, and cause disease. Fungi in this group include *Aspergillus* spp., *Candida* spp., *Fusarium* spp., *Absidia* spp., *Rhizopus* spp., and *Mucor* spp. The spores or conidia of these fungi are common microflora of the skin, body fluids, mucosal surfaces, and intestinal content. To gain access to the vascular system, the barrier provided by skin or mucosa must be injured. Processes that result in abrasions, penetrating wounds, and cell lysis are commonly involved. Injury results in the loss of epithelial cells and the exposure of basement membrane and subjacent vascularized ECM connective tissue. Similar to what occurs with *Aspergillus fumigatus* (see Fungal Diseases of Organ Systems; Respiratory System, Mediastinum, and Pleurae; Disorders of Domestic Animals; Aspergillosis [*Aspergillus fumigatus*]), hyphae invade vascularized tissues and gain access to the circulatory system by growing in and invading through vessel walls and endothelial cells, gain access to the circulatory system, break off into the bloodstream, circulate, and attach to and invade endothelium and ECM of other organ systems. Leukocyte trafficking may also be used to spread the fungus systemically. Ligand-receptor interactions are likely involved in this process, and these interactions probably determine in certain diseases which organ systems and tissue types are targeted by fungi. In affected organ systems, the mechanism of injury is cell lysis via chronic granulomatous to pyogranulomatous inflammation and its effector molecules and degradative enzymes.

As an example, mycotic rumenitis (followed by mycotic hepatitis) of cattle is caused by several of the fungal species listed earlier. The disease is often initiated by farm management practices such as (1) diets high in grains such as corn or (2) the long-term use of antibiotics in feeds. In the first case, feedlot cattle are fed increasing quantities of corn that serve as a carbohydrate source for ruminal microflora, which convert it, in part, to lactic acid. Excessive grain in the diet (grain overload) increases the quantity of lactic acid (lactic acidosis) in the rumen, and if such animals are deprived of water, lactic acid can accumulate and result in a drop of the pH of fluids covering ruminal mucosa. This outcome results in acid burns followed by loss of the epithelium and exposure of the basement membrane and subjacent vascularized connective tissue of the lamina propria and ECM tissues. Additionally, the long-term use of antibiotics in feeds can disrupt the protective environment created by normal microbial flora and lead to colonization and invasion of the mucosa by these fungi. Angioinvasive fungi (i.e., fungal hyphae or other elements) are able to invade and colonize the injured mucosa (mycotic rumenitis and/or abomasitis) and invade the vasculature of the ECM, and then spread regionally to other organ systems such as the liver (granulomatous fungal hepatitis) (see Figs. 8-51 and 14-51). The biologic materials in cells walls of fungi characteristically elicit a granulomatous inflammatory response because they are difficult for phagocytes to degrade. Thus in this example, granulomatous fungal hepatitis ensues.

Nervous System

Disorders of Domestic Animals

Cryptococcosis (*Cryptococcus neoformans*). The pathogenesis of cryptococcosis has many similarities to those of histoplasmosis, coccidioidomycosis, and blastomycosis discussed earlier. The mechanism of injury is cell lysis likely caused by atrophy secondary to tissue distortion and compression from expanding cryptococcal cysts in brain neuropil. There is little or no inflammation in this disease. *C. neoformans* has a dimorphic life cycle. The mycelial (basidiospores) phase occurs in extracellular environments (25° C), whereas the yeast phase occurs intracellularly within cells of the monocyte-macrophage system (37° C). Gross lesions include the formation of expansile cystic spaces filled with a gelatinous matrix (the capsule)

within the brain and spinal cord, leading to compression and distortion of the tissue (see Figs. 14-49 and 14-50).

Animals encounter *C. neoformans* (dimorphic fungus) through inhalation of blastoconidia, basidiospores, or poorly encapsulated yeast cells (≈1.8 to 3.0 μm in diameter), which can reach the lower respiratory tract and alveoli. They are present in soil-derived aerosols from moist and humid environments and from bird droppings and nests. Basidiospores are deposited on the surface of mucosae of the nasopharyngeal cavity and of the conductive component of the respiratory system through centrifugal and inertial turbulence. They are readily phagocytized and killed by neutrophils and alveolar macrophages. For survival, basidiospores quickly germinate to yeast in mucosae or within phagosomes of alveolar macrophages. Yeast-derived glucosylceramide synthase is essential for survival of the yeast in mucosae, but after phagocytosis by mucosa-associated alveolar macrophages, it is not needed because the yeast uses other means of evading killing (discussed later). Yeast cells also produce phospholipases that injure alveolar type II epithelial cells and hinder the production and function of surfactant, thereby enhancing their adhesion to pneumocytes and improving chances of being phagocytosed by alveolar macrophages.

Recognition, attachment, and internalization by macrophages are likely mediated by ligand-receptor interactions, but specific molecules have not been identified. The polysaccharide capsule of yeast has antiphagocytic properties and may be immunosuppressive. The degree of encapsulation provides resistance to phagocytosis and killing by macrophages. In mucosae, unencapsulated or poorly encapsulated yeast cells are readily phagocytosed and killed, whereas encapsulated yeast is more resistant to phagocytosis and killing. The capsule's negative charge inhibits phagocytosis and killing by neutrophils and macrophages and causes complement depletion, antibody unresponsiveness, and dysregulation of cytokine secretion by monocytes and macrophages. The capsule can also inhibit recognition of yeast by macrophages and neutrophils and inhibit chemotaxis of leukocytes from the bloodstream into areas of inflammation. This latter response may account for the lack of inflammation in cysts. After phagocytosis and phagosome-lysosome fusion, yeast synthesizes additional polysaccharide capsule within the phagolysosome of the macrophage. Capsule dilutes lysosomal hydrolases and other toxic contents and provides a physical separation between the yeast and the membrane of the phagosome in which microbicidal compounds are located. This process continues until macrophages are grossly distended with capsule (>30 μm in diameter) and is the underlying mechanism of the formation of expansile cysts filled with a gelatinous matrix observed grossly in the brain. The capsule is composed primarily of two polysaccharides, glucuronoxylomannan and galactoxylomannan, and a smaller quantity of mannoprotein. These molecules also suppress the immune response.

Yeast cells appear to spread from the nasopharyngeal cavity to the CNS by direct extension into the meninges and neuropil, following compressive remodeling and osteolysis of the cribriform plate from a local infection of the nasal sinuses. However, leukocyte trafficking from the respiratory system via yeast-infected macrophages in the circulatory system with spread into the neuropil may also occur. This mechanism is hypothetical but probable based on what is known about the biology of the fungus. It is likely that yeast-infected macrophages interact through ligand-receptor interactions with endothelial cells of capillaries in the CNS. Capsule polysaccharides are also likely used to adhere and bind to brain endothelial cells and mediate endocytosis across the blood-brain barrier into the neuropil.

Once in nervous tissue, macrophages migrate through the neuropil. Because macrophages have short life spans, yeast, yeast-derived antigens, and polysaccharide capsule are released from dead macrophages into the neuropil. This outcome and its associated chemokines and cytokines recruit additional macrophages into the nervous system. This process is repetitive; thus the volume of polysaccharide capsule increases, and expansile cystic spaces filled with a gelatinous matrix are observed grossly in the brain. Additionally, melanin is an important cryptococcal virulence factor, which facilitates yeast survival during infection of the CNS. It acts as an antioxidant and eliminates reactive oxygen species that could kill the yeast. In the nervous system, yeast may use neurotransmitters, such as dopamine, norepinephrine, and epinephrine, as substrates for melanin production.

Protozoan Diseases

Portals of entry; target cells and substances; pathways of spread; virulence factors; mechanisms of adhesion, colonization, invasion, and replication; toxins; and defense mechanisms for protozoan diseases are similar to those discussed in the opening sections of this chapter and in the sections on bacterial and viral diseases.

Protozoan Diseases of Organ Systems

Alimentary System and the Peritoneum, Omentum, Mesentery, and Peritoneal Cavity

Disorders of Domestic Animals

Cryptosporidiosis (*Cryptosporidium parvum*). The mechanism of injury in cryptosporidiosis is dysfunction and lysis of epithelial cells covering tips and sides of intestinal villi, resulting from (1) dysfunction of microvilli of the brush border, (2) cytolysis of villus enterocytes after the microbe is released from infected cells, and (3) degradative effects of inflammation and its chemical mediators. Gross lesions are not observed; however, microscopic lesions include necrosis of epithelial cells, atrophy of villi, and mucosal inflammation (see E-Fig. 7-20).

Animals encounter *Cryptosporidium parvum* through direct contact with oocysts in water and food contaminated with feces from infected animals. Oocysts are ingested and carried through the oral pharynx, esophagus, stomach, and small intestine by normal peristaltic activities where they interact with gastric acids, pancreatic enzymes, and bile salts. One or more of these substances may trigger a process called *excystation*, where sporozoites are released from oocysts and randomly encounter the apical brush borders (microvilli with glycocalyx) of villus enterocytes covering tips and sides of intestinal villi. Sporozoites have a tropism for villus enterocytes of the jejunum and ileum that is apparently mediated by ligand-receptor interactions involved in attachment, invasion, and intracellular development of the protozoan. Apical complex and surface proteins expressed by sporozoites act as ligands, whereas *C. parvum* sporozoite ligand (CSL [circumsporozoite-like glycoprotein]) and probably other cell membrane proteins expressed on the apical surfaces of villus enterocytes act as receptors. Furthermore, the apical ends of sporozoites also adhere to microvilli of brush borders of villus enterocytes via sporozoite-specific lectin adherence factors such as glycoprotein 900 (GP900). Sporozoites and merozoites also express other surface glycoproteins (e.g., sporozoite and merozoite cell surface protein gp15/40/60 complex, P23, TRAP-C1 [thrombospondin-related adhesive protein *Cryptosporidium* 1]), which are virulence factors that increase the pathogenicity of the organism.

Once bound to cell membrane, sporozoites infect villus enterocytes by a mechanism dependent on parasite motility ("gliding motility"), activities of its apical complex, and secretion of enzymes from its apical organelles. Sporozoites become surrounded by cell

membranes of microvilli to form parasitophorous vacuoles. Such vacuoles are retained in the microvillus layer and do not enter, but directly communicate with, the cytoplasm of the villus enterocyte through a feeding organelle. Once in parasitophorous vacuoles, sporozoites differentiate into trophozoites and then undergo asexual multiplication to form schizonts that contain six to eight merozoites. Schizonts rupture their vacuoles to release merozoites, and thus infected villus enterocytes are lysed, resulting in cell death, disruption of cell junctions, and loss of barrier functions. Merozoites spread distally via alimentary peristalsis in the small intestine to infect additional villus enterocytes through ligand-receptor interactions. It is likely that such ligand-receptor interactions determine which "new" populations of epithelial cells and what segment of the small intestine are infected. Once infected, new schizonts are formed in villus enterocytes through (1) asexual multiplication to form schizonts and (2) sexual reproduction (gametogony) by differentiating into male microgamonts or female macrogamonts. Microgamonts release microgametes that fertilize macrogametes inside macrogamonts, resulting in the formation of oocysts with sporozoites that can reinfect additional "new" villus enterocytes or are passed in the feces to spread the infection to other animals. These multiplicative and reproductive processes cause additional cell lysis and villus atrophy and consequently amplify the severity of mucosal injury. It has also been suggested that lysis of cells and villus atrophy may be caused by cell dysfunction and damage induced by cytokines and inflammatory molecules released from T lymphocytes and macrophages in inflammation. This latter mechanism causes increased intercellular permeability and may alter secretory functions and impair absorption of villus enterocytes. Infection, injury, and loss of villus enterocytes result in diarrhea likely caused by a combination of mechanisms, including osmotic diarrhea (malabsorption), secretory diarrhea, and increased intercellular permeability from inflammation. Enterotoxins may be involved in the secretory diarrhea, but none have been identified.

Malabsorption likely occurs from dysfunction of digestive enzymes present in the brush border of villus enterocytes infected by sporozoites and the subsequent lysis of these cells, both leading to failure to digest carbohydrates (impaired hydrolysis) and other molecules in the ingesta. This outcome leads to bacterial fermentation of substrates and an osmotic diarrhea. Sporozoite-injured villus enterocytes are sloughed from the villi, resulting in collapse (atrophy) of the structure of the villus, whereas the basement membrane from under the sloughed villus enterocytes is usually unaffected and functionally normal. Because the basement membrane is not injured and remains structurally intact, villus enterocytes derived from regenerative crypt enterocytes can divide and replace sloughed cells. These regenerative cells migrate up the villus from the crypts to initially cover exposed basement membranes; thus they are recognized early in the reparative process as flattened squamous-like cells stretched over the basement membrane. As the cells increase in density and maturity, they regain a more normal columnar structure. Moreover, the loss of enterocytes allows endotoxins and other potentially harmful molecules to gain access to the capillary and lymphatic vessels in the lamina propria of the villi and through absorption cause systemic cardiovascular and hemodynamic effects.

***Coccidiosis* (Eimeria *spp.*, Isospora *spp.*).** The pathogenesis of coccidiosis is similar in many ways to that of cryptosporidiosis discussed earlier. The mechanism of injury is adenomatous proliferation (hypertrophy and hyperplasia) of infected villus enterocytes covering tips and sides of small intestinal villi, followed by lysis resulting from release of protozoans from infected cells. Gross lesions include mucosae that are initially elevated from a focal adenomatous to cerebriform epithelial response subsequently followed by active hyperemia, hemorrhage, and necrosis often with fibrinous and/or fibrinohemorrhagic cylindrical casts formed within the lumen of the intestine (see Figs. 7-125 to 7-129).

Animals (cattle, sheep, goats, and pigs) encounter the protozoan in grass, soil, and/or floors or surfaces contaminated with unsporulated oocysts from feces of infected animals. Coccidian oocysts are not infective (unsporulated) and therefore survive in pastures and other holding areas. Under the proper conditions (oxygen concentrations, humidity, and temperature), oocysts sporulate and become infective. Sporulated oocysts are ingested and carried through the oral pharynx, esophagus, stomach, and small intestine by normal peristaltic activities where they excyst and sporozoites are released. In proximity to intestinal villi, sporozoites randomly encounter mucosal villus enterocytes covering tips and sides of intestinal villi. Sporozoites have tropisms for specific animal species and for specific populations of villus enterocytes in specific segments of the small and large intestine. This tropism is determined by surface microneme proteins (MICs) that appear to be unique to each species of the protozoan. Sialic acid, galactose, and many forms of these molecules act as receptors and are arrayed on enterocyte membranes. Patterns of these receptors likely determine the specificity for microneme proteins unique to individual species of coccidia. Other ligand-receptor interactions are probably involved in invasion and intracellular development of the protozoan. Sporozoites go through one or more asexual generations and a (single) sexual generation in different segments of the small intestine (see section on Cryptosporidiosis). The replication and release of generations of these protozoans in and from mucosal villus enterocytes, respectively, account for the lesions observed grossly.

***Giardiasis* (Giardia spp.).** The pathogenesis of giardiasis is similar in many ways to those of cryptosporidiosis and coccidiosis discussed earlier. The mechanisms of injury in giardiasis are (1) dysfunction of microvilli and the glycocalyx of brush borders and (2) cell death via apoptosis of epithelial cells covering tips and sides of small intestinal villi. These outcomes result in increased permeability of the mucosal barrier system, chloride ion shifts probably via enterotoxin-mediated hypersecretion, dysfunction of digestive enzymes present in the brush border resulting in malabsorption diarrhea, and acute inflammation. Gross lesions are usually not observed; however, microscopic lesions may include loss of epithelial cells, atrophy of villi, and mucosal inflammation.

Animals (horses, cattle, sheep, goats, pigs, dogs, and cats) encounter *Giardia* spp. through direct contact with parasite cysts in water and food contaminated with feces from infected animals. Following ingestion, excystation occurs in the small intestine via the actions of gastric acid and pancreatic enzymes. An excyzoite is released into the intestinal lumen, where it matures into two trophozoites that subsequently attach to the brush border of villus epithelial cells by a cytoskeletal organelle called an *adhesive disk*. Trophozoites move via flagella but do not invade epithelial cells. The anatomic segment of intestine (duodenum, jejunum, or ileum) used for attachment is species specific, suggesting that colonization is facilitated by unique ligand-receptor interactions, especially by ligands in the outer membrane of the parasite. Trophozoites replicate attached to epithelial cells, encyst, and are then passed in feces.

Virulence factors for *Giardia* spp. have not been well characterized; however, colonization of specific segments of the small intestine involve genes for the adhesive disk, flagella, and variable small proteins (VSP) in the outer membrane of the parasite. Additionally, trophozoites appear to be able to cause apoptosis of colonized epithelial cells covering tips and sides of small intestinal villi through

both the intrinsic and extrinsic apoptotic pathways and thus contribute to the outcomes discussed in the initial paragraph. Infection, injury, and loss of villus enterocytes result in (1) glucose malabsorption and bacterial fermentation with osmotic diarrhea; (2) Cl^- hypersecretion, and (3) systemic changes in other electrolyte (Na^+) and solute concentrations, leading to alterations in hydration and systemic vascular osmolarity. The cumulative effect of injury and giardia antigens also initiates an acute inflammatory response mediated by an array of chemical mediators, which contribute to dysfunction of cellular junctions and increased intercellular permeability and thus mucosal and intestinal edema.

Nervous System

Disorders of Horses

Protozoan Encephalomyelitis (*Sarcocystis neurona*). The mechanism of injury in protozoan encephalomyelitis is disruption and lysis of neurons and neural cells from replication and release of the protozoan and from inflammation and its chemical mediators and degradative enzymes. Gross lesions include yellow-white areas of malacia mixed with hemorrhage in gray and white matter of the brain and spinal cord (see Figs. 14-81 and 14-82).

The discussion that follows is provisional and is based, in part, on known mechanisms used by *T. gondii*, *C. parvum*, and other protozoans. Horses and other animals encounter sporocysts in feed, water, grass, or soil, and/or on floors or surfaces contaminated with feces from infected opossums. They are ingested and swallowed and through peristalsis gain access to mucosae of the intestines. Here sporozoites are released from sporocysts, which then must gain access to endothelial cells to mature to merozoites, the form that spreads to the CNS. Infection and involvement of endothelial cells appears to be a step central to the pathogenesis of the disease. However, little is known about the mechanism of interaction with mucosa or spread to the brain and spinal cord. Sporozoites could (1) be phagocytized by mucosa-associated macrophages (leukocyte trafficking) and subsequently spread locally to encounter endothelial cells within capillaries of the lamina propria supporting enterocytes or (2) interact with and penetrate the mucosa to gain direct access to endothelial cells in the lamina propria. It appears, but is unproven, that leukocyte trafficking via mucosa-associated macrophages is the mechanism most likely involved in spread to endothelial cells. Once in contact with endothelial cells, sporozoites enter them and mature to form schizonts containing merozoites, which eventually rupture and release merozoites into the blood and adjacent vascular ECM. They then infect adjacent endothelial cells and repeat the replicative process. Injury to endothelial cells likely causes focal vascular inflammation that recruits macrophages into the site of injury, where they may phagocytize merozoites.

At this local site there are three potential mechanisms for systemic spread to the CNS. First, cell-free merozoites may enter the venous system, navigate the heart, and be carried via the arterial system to the CNS to interact with endothelial cells. Second, cell-free merozoites may enter the lymphatic system, be carried to the thoracic duct and enter the venous system, navigate the heart, and be carried via the arterial system to the CNS to interact with endothelial cells. Third, merozoites could be phagocytized by macrophages and carried via leukocyte trafficking to encounter and enter capillaries in the lamina propria and spread systemically via the circulatory system to the CNS or be carried to Peyer's patches or by lymphatic vessels to regional lymph nodes, and then migrate via lymphatic vessels and the thoracic duct to the circulatory system and spread systemically to the CNS. Based on known mechanisms used by *T. gondii*, *C. parvum*, and other protozoans and the need to evade innate and adaptive immune responses, it seems more likely that sporozoites would attempt to gain access to intracellular locations in mucosal epithelial cells or mucosal-associated macrophages as early as possible in the disease. Thus the latter of the three mechanisms of spread appears most probable.

It is not known how merozoites enter the CNS; however, cell-free merozoites or merozoite-infected macrophages likely interact with endothelial cells in the CNS and are subsequently infected. Ligand-receptor interactions may determine tropism in the CNS and which areas of the vasculature are infected. Schizonts containing merozoites likely develop in endothelial cells of the CNS, and when these cells are lysed, merozoites are spread into the neuropil. This process undoubtedly injures the blood-brain barrier and causes inflammation. Additionally, macrophages via leukocyte trafficking may carry merozoites into the neuropil, where they could infect neurons, microglial cells, or other neuronal cells. In all of these infected cells, schizonts form containing merozoites that lyse the infected cell when merozoites are released. This process injures endothelium and neuropil, leading to inflammation, vasculitis and thrombosis, hemorrhage, and malacia and the recruitment of additional macrophages (from circulating monocytes) and activation of resident microglial cells, resulting in the lesions characteristic of protozoan encephalomyelitis.

Reproductive System

Disorders of Ruminants (Cattle, Sheep, and Goats)

Toxoplasmosis (*Toxoplasma gondii*). The mechanism of injury in toxoplasmosis is dysfunction and lysis of epithelial cells of the placenta and the fetus resulting in abortion, neonatal mortality, and fetal malformation predominantly in sheep and goats and less commonly in cattle. Toxoplasmosis in cats is usually inconsequential and will not be discussed here. Gross lesions include active hyperemia, rough and granular mucosae consistent with necrosis, and mineralization of caruncles of the uterus and cotyledons of fetal membranes (see Fig. 18-47). A component of this lesion includes acute inflammation. Intercaruncular and intercotyledonary tissues are unaffected.

Animals, other than cats, and human beings are intermediate hosts for *T. gondii* and encounter oocysts in contaminated soil. Cats are the definitive host; thus cat feces are the source of oocysts. Oocysts are resistant to degradation and may survive in the environment for years. Under the proper conditions (oxygen concentrations, humidity, and temperature), oocysts sporulate and become infective. Sporulated oocysts are ingested and carried by normal peristaltic activities through the oral pharynx, esophagus, and stomach to the small intestine, where they excyst and release sporozoites into the intestinal lumen in close proximity to intestinal mucosal villus enterocytes. Infections are characterized by the ability of the organism to cross barrier systems such as intestinal mucosae and the blood-brain barrier, blood-retina barrier, and placenta. This process appears to involve parasite motility (linear myosin, F-actin filaments, and gliding-associated proteins) and interactions between parasite adhesins and target cell receptors that facilitate transfer of the organism through mucosae. Mounting evidence also suggests that *T. gondii* uses migrating leukocytes to disseminate (leukocyte trafficking) throughout the animal while avoiding adaptive immune responses. Sporozoites infect villus enterocytes and complete an asexual replication in parasitophorous vacuoles forming tachyzoites. In parasitophorous vacuoles the protozoan initiates the production of the antiinflammatory cytokines IL-10 and transforming growth factor-β (TGF-β), which inhibit the production of proinflammatory cytokines IL-12 and TNF-α. Tachyzoites are released via cell lysis and infect and replicate in additional enterocytes and then invade subjacent lamina propria, infect cells of the monocyte-macrophage system, and enter lymphatic vessels. They spread locally to lymphoid tissues (likely Peyer's patches) and regionally to mesenteric lymph

nodes via lymphatic vessels and then systemically via lymphatic vessels and the thoracic duct (or capillaries or postcapillary venules) to the circulatory system and then systemically to caruncular epithelial cells and cotyledonary trophoblasts.

T. gondii requires an intracellular site for growth and replication. Tachyzoite and likely sporozoite tropism for target cells appears to be mediated by ligand-receptor interactions. Infection of intestinal villus enterocytes by sporozoites and tachyzoites is a well-studied process that involves six steps that begin with the recognition of target cells and end with the formation of a parasitophorous vacuole within the same cell. Parasitophorous vacuoles are a mechanism used to modulate target cell functions in support of parasite replication and infection. Tachyzoites (and likely sporozoites) express glycosylphosphatidylinositol-linked surface proteins (SAGs) that serve as ligands, whereas intestinal villus enterocyte membrane receptors appear to include laminin, lectin, and SAG receptor proteins. Proteins, such as SAG1 and SAG3, are abundant on tachyzoites and function in target cell attachment and immune modulation and may also cause direct injury to intestinal epithelium.

When tachyzoites spread systemically from lymphoid tissues to other tissues such as the placenta, they encounter cells of the uterine caruncle and probably use ligand-receptor interactions and the six-step process described previously to infect cells of the caruncles and then spread to adjacent trophoblasts of the cotyledons. Tachyzoites replicate in these placental cells, eventually causing their lysis. Lysis leads to alterations in placental structure (necrosis and mineralization of caruncles and cotyledons), disturbances of vascular flow, and placental dysfunction that injures developing fetuses. Lesions caused by tachyzoites have also been described in the brain (inflammation and congenital malformations) and other tissues of the fetus. How and by what mechanism they spread from the placenta to the fetus is unknown (likely fetal macrophage-like cells); however, they appear to infect diverse populations of fetal cells, resulting in injury and lysis. If infection occurs early in gestation, fetal lysis and resorption occur. Infection in midgestation causes fetal lysis, leading to mummification mixed with live but weak fetuses. Infection in late gestation does not usually injure the fetus because of a good adaptive immune response.

Neosporosis (*Neospora caninum*). The pathogenesis and mechanisms of injury in neosporosis are similar to those of toxoplasmosis discussed earlier. The dog is the definitive host for *Neospora caninum*; all other animals are intermediate hosts. Much like in toxoplasmosis, abortion is the primary disease caused by *N. caninum* in cattle, sheep, goat, and pigs.

Prion Diseases

Portals of entry; target cells and substances; pathways of spread; virulence factors; mechanisms of adhesion, colonization, invasion, and replication; toxins; and defense mechanisms for prion diseases are similar to those discussed in the opening sections of this chapter and in the sections on bacterial and viral diseases.

Prion Diseases of Organ Systems

Nervous System

Disorders of Domestic Animals

Transmissible Spongiform Encephalopathies (Prion Diseases). The mechanism of injury in transmissible spongiform encephalopathies (TSEs) is metabolic dysfunction of neurons and neural cells caused by the conversion of normal cellular prion protein (PrP^{C}) to an abnormal form (PrP^{Sc}) and the accumulation of PrP^{Sc} in neurons, neural cells, and extracellularly within the neuropil (Fig. 4-45). Currently there is some research data that suggests a "potential" role for *Spiroplasma* spp., a group of small bacteria without cell walls,

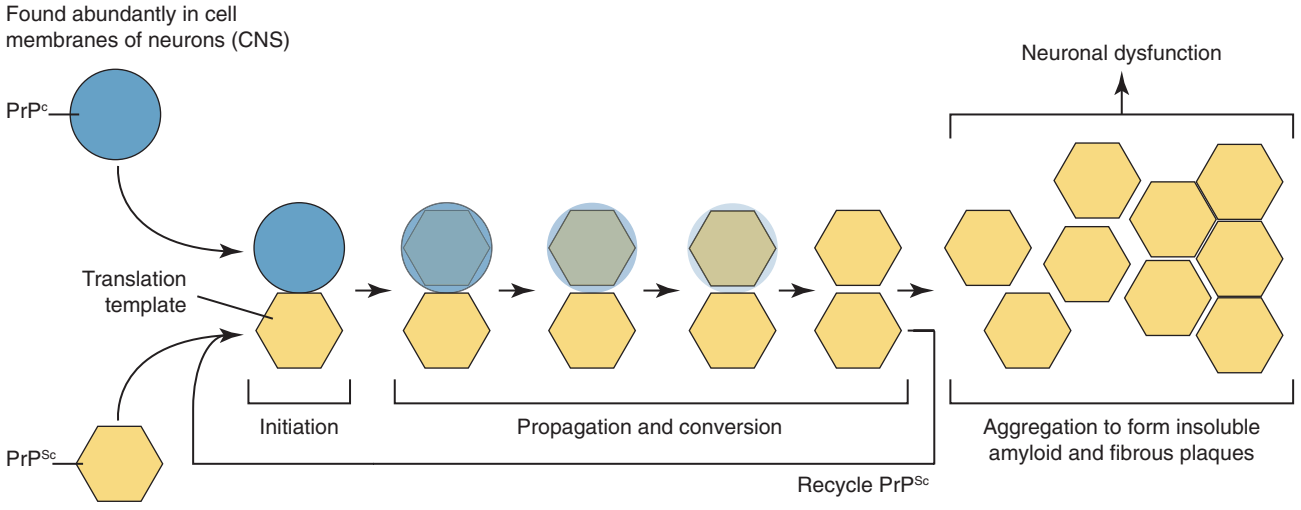


Figure 4-45 The Stepwise Process of Converting Prion Proteins to Amyloid and Fibrous Plaques. When the infectious form of prion protein (PrP^{Sc}) reaches the central nervous system (CNS) (see Fig. 4-46), it behaves as a translation template that converts normal PrP^{C} to PrP^{Sc}, a misfolded and aggregated β-sheet–rich isoform of PrP^{C}. Because neurons have large concentrations of PrP^{C} located in their cell membranes when compared to other cells of the body, PrP^{Sc} aggregation and accumulation affects the nervous system to a much greater extent. This outcome results in degeneration of neurons and the neuropil. Translation follows a stepwise process of initiation, propagation, conversion, and aggregation to end with the accumulation of large quantities of insoluble amyloid and fibrous plaques in neurons and the neuropil. Additionally, PrP^{Sc} that is not aggregated into the β-sheet–rich isoform is "recycled" to interact with PrP^{C} in a self-amplifying process resulting in the accumulation of high concentrations of PrP^{Sc}. The shapes of the prion proteins are used for illustrative purposes only and do not represent their molecular structure. *ANS*, Autonomic nervous system; *GALT*, gut-associated lymphoid tissue; *M cell*, microfold cell; *PNS*, peripheral nervous system. (Courtesy Dr. J.F. Zachary, College of Veterinary Medicine, University of Illinois.)

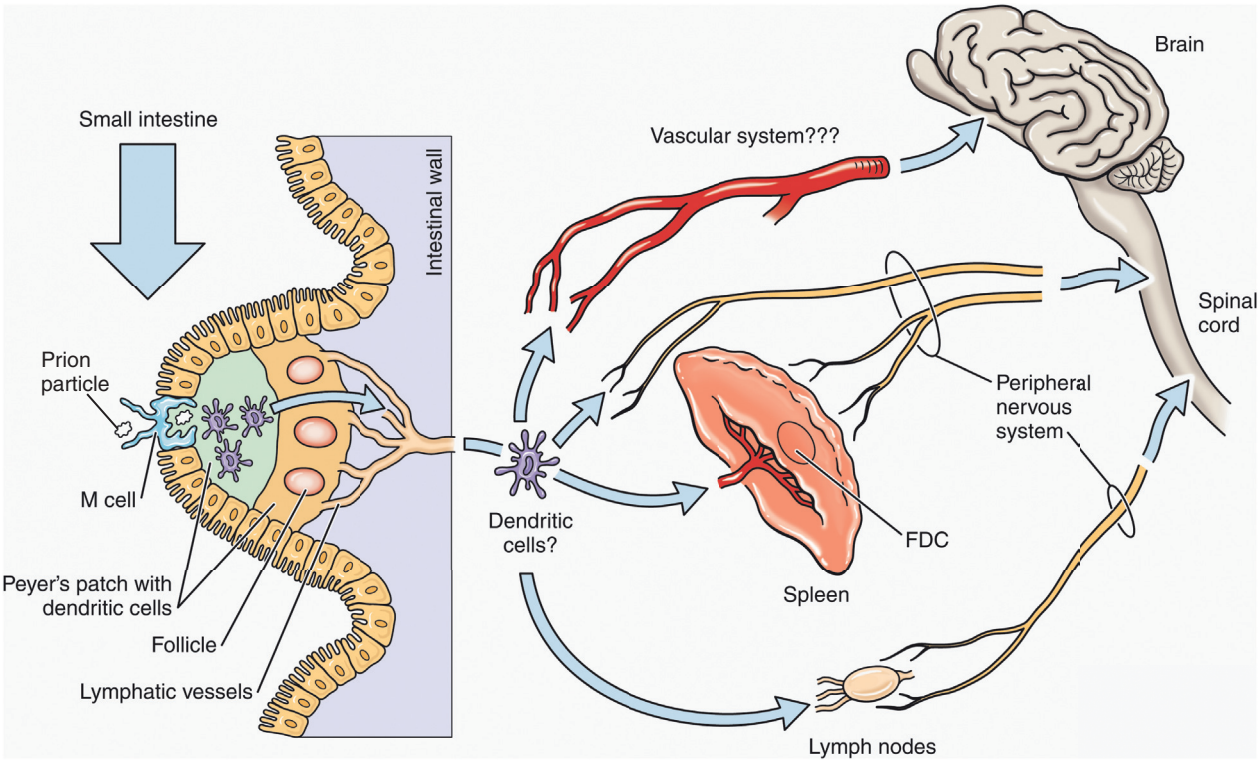


Figure 4-46 Pathogenesis of Transmissible Spongiform Encephalopathies. Prions appear to use microfold cell (M cells) (also macrophages) to enter Peyer's patches and infect dendritic cells as well as macrophages and lymphocytes. Dendritic cells (and likely macrophages) then spread prions through leukocyte trafficking in lymphatic vessels to local, regional, and systemic lymphoid nodules, lymph nodes, and/or spleen where infection is sustained and amplified, especially in follicular dendritic cells (FDCs) of the spleen and B lymphocytes. Prions released from dendritic cells are able to enter nerve endings in lymphoid tissues, and by retrograde and anterograde nerve transport they spread throughout the central nervous system (CNS). It has been hypothesized that prions may also spread to the CNS hematogenously, but the existence of this route is uncertain.

instead of prions in the pathogenesis of TSEs. These data are in dispute and will be sorted out over the next decade. Gross lesions are not observed except in chronic cases, in which atrophy of the brain may occur. Microscopic lesions characteristic of injury include intracytoplasmic vacuoles in neurons and neuropil (spongiform change), neuronal loss, gliosis, and an absence of leukocytic inflammation. These diseases in animals include scrapie (sheep and goats), bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) in deer and elk, transmissible mink encephalopathy, feline spongiform encephalopathy, and ungulate spongiform encephalopathy. The source of prions that spread transmissible spongiform encephalopathies among animals and the natural routes of transmission of prions between animals have not been determined. Soil may serve as a prion reservoir.

Animals probably encounter prions most commonly by ingestion. Alternatively, inhalation or direct contact (conjunctival mucous membranes) may also be important routes of spread in specific prion diseases such as scrapie and chronic wasting disease. Prions can be found in a tissue-free environment (urine, saliva, blood, and body waste) as a direct source of the microbe or in a tissue-associated environment (offal [i.e., entrails and internal organs of butchered animals], placentas, or decaying carcasses) as an indirect source of the microbe. This latter source appears to be the route that occurs primarily in cattle and mink. In the United Kingdom, cattle were infected with prions by ingesting offal derived from prion-infected sheep or cattle that had not been properly treated to kill the microbe. Mucosae of the oral pharynx, especially the tonsil, small intestine, nasal pharynx, and conjunctiva, are thought to be the probable locations of initial encounters with prions. It is unclear if and how prions are trapped in the mucus layer and, if so, how they gain access to mucosal epithelial cells, macrophages, and/or dendritic cells in the mucus layer. It appears that prions are able to attach to apical surfaces of mucosal epithelial cells, M cells, and possibly dendritic cells in tonsillar, alimentary, and respiratory mucosae, respectively. Transcytosis or dendritic cell migration (also potentially via macrophage migration) is probably used by prions to pass through mucosal epithelial cells and M cells to their basolateral surfaces and to gain access to and infect B and T lymphocytes, macrophages, and dendritic cells in Peyer's patches (GALT) or lymphoid nodules and aggregates like BALT. Prions are then probably spread systemically via leukocyte trafficking in lymphocytes, monocytes, and dendritic cells to other lymphoid organs such as the spleen and systemic lymph nodes. In lymphoid tissue, follicular dendritic cells and B lymphocytes are essential for prion replication, amplification, and accumulation in large numbers before spread to the nervous system.

Although in TSEs the final targets are neurons and the neuropil, the mechanisms used by specific TSEs to colonize and spread prions to reach these targets may vary based on animal species. In cattle, BSE prions encounter and colonize cells of MALT in the palatine tonsils and Peyer's patches of the distal ileum. They replicate in numbers sufficient to enter nerve endings in MALT. They then spread via retrograde axonal transport to the CNS and brain mainly following the parasympathetic and sympathetic nerve fibers of the autonomic nervous system. BSE prions amplify in large numbers almost exclusively in the CNS and PNS. In contrast in sheep, scrapie prions appear to amplify in large numbers in cells of the lymphoid and monocyte-macrophage systems before entering nerve

endings and spreading to the brain via the autonomic nervous system.

Prions are able to infect nerve endings of the vagus nerve, sympathetic nerves, and sensory nerves that innervate lymphoid tissues and organs and then use retrograde axonal transport to gain access to the CNS and spread in the nervous system via synaptically linked neurons (Fig. 4-46). It is thought that prion-infected macrophages and dendritic cells deliver prions to these nerve endings; however, it is not known if endocytosis is involved in the entry of prions into nerve endings or in their spread between cell membranes in synapses. In macrophages and dendritic cells, prions are located in multivesicular endosomes (i.e., membrane-bound vesicles inside cells) and may be transferred between cells in exosomes (i.e., cell-derived extracellular vesicles). Such a mechanism may be involved in interneuronal spread within the nervous system. Attachment proteins of prions or membrane receptors on target cells have not been identified. However, Toll-like PRRs may serve as receptors for prion entry into cells. Prions have tropisms for different animal species that are likely determined by the tertiary and quaternary structure of prions, resulting in their binding to or interaction with different molecules (receptors) and thus different target cells. Cellular tropism may also be restricted to those cells that express a cofactor compatible with the respective prion strain.

Most cells in the body have PrP^{C}; however, the highest concentrations are present in the nervous system, especially in synaptic membranes as a neuronal membrane glycoprotein. PrP^{C} is also expressed in cells of the immune system (see Figs. 4-45 and 4-46). The function of PrP^{C} is unknown, but its physiologic function may include immunoregulation, signal transduction, copper binding, synaptic transmission, induction of apoptosis, or protection against apoptotic stimuli. In neurons, PrP^{Sc} serves as a translation template that converts (conformational change) normal PrP^{C} to PrP^{Sc}, a misfolded and aggregated β-sheet–rich isoform of PrP^{C}. This folding pattern makes PrP^{Sc} resistant to the action of proteases and causes it to aggregate and accumulate as an insoluble amyloid in neurons and neuropil in the form of large amyloid and fibrous plaques. It is not known how PrP^{Sc} causes neuronal degeneration; however, reduced antioxidant protection, increased oxidative stress, loss of function of normal PrP^{C}, or toxicity caused by PrP^{Sc}, all related to the accumulation of amyloid plaque, have been proposed. The activation (hypertrophy and hyperplasia) of microglial cells may also suggest that their biologic activities and effector molecules are involved in neuronal degeneration. There are no specific virulence factors for prions.

Suggested Readings

Suggested Readings are available at www.expertconsult.com.

Suggested Readings

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