Chapter 15

Microbial Mechanisms of Pathogenicity

Are bacteria logical? No!

- Bacteria did not evolve to cause human diseases
- Bacteria evolved mechanisms to procure nutrients
- Bacteria need to find space that they can occupy and procure nutrients // we just happen to be a good target for bacteria – warm, moist, and full of nutrients
- Bacteria also need to evolve defensive mechanisms to protect themselves against other microbes and the defense mechanisms of their hosts
- This sets the stage for…….

The classic dynamic, “Spy VS Spy”!

Bacteria VS Host

Who will “out evolve” the other?

Mechanisms of Pathogenicity

- **Pathogenicity**: the ability of a microbe to cause disease in the host
- **Virulence**: the extent of bacterial pathogenicity
  - How likely to cause disease
  - Bacterial “virulence factors” increases pathogenicity
  - Status of host will also influence microbe’s pathogenicity

Portals of Entry

- Where the microbe enters the host (big three!)
- Mucous membranes (respiratory system / digestive system / urogenital system)
- Skin
- Parenteral route (direct introduction of microbe into tissue)

Portals of Entry

- Each microbe has a preferred portal of entry
- Most common portal of entry is mucous membranes of respiratory system
- Microbes use hair follicles and sweat ducts to enter skin
- If gain access via different portal // may not be able to cause disease…….
  - Salmonella typhi (typhoid fever) // swallowed preferred route – cause disease // rub on skin – no disease
  - Streptococci (pneumonia) // inhaled into lungs – cause disease // swallowed – no signs or symptoms

Portals of Entry and Organisms Typically Involved

Table 11.3 Portals of Entry and Organisms Typically Involved

Portal of Entry	Organism/Disease	How Access Is Gained
Skin	*Staphylococcus aureus, Streptococcus pyogenes,* gangrene, tetanus	Via nicks, abrasions, punctures, areas of broken skin
	Herpes simplex (type 1)	Via mucous membranes of the lips
	Helminth worms	Burrow through the skin
	Viruses, rickettsias, protozoa (i.e. malaria, West Nile virus)	Via insect bites
	Haemophilus aegyptius, Chlamydia trachomatis, Neisseria gonorrhoeae	Via the conjunctiva of the eye
Gastrointestinal tract	*Salmonella, Shigella, Vibrio, Escherichia coli,* poliovirus, hepatitis A, echovirus, rotavirus, enteric protozoans (*Giardia lamblia, Entamoeba histolytica*)	By eating/drinking contaminated foods and fluids Via fomites (inanimate objects contaminated with the infectious organism)
Respiratory tract	Meningitis, diphtheria, whooping cough, influenza, measles, mumps, rubella, chickenpox, common cold, *Streptococcus pneumoniae, Klebsiella, Mycoplasma, Cryptococcus, Pneumocystis, Mycobacterium tuberculosis, Histoplasma*	Via inhalation of offending organism
Urogenital tract	HIV, *Trichomonas, Candida albicans,* hepatitis B, syphilis, gonorrhea, chlamydia, herpes, genital warts	Enter through the skin/mucosa of penis, external genitalia, vagina/cervix, urethra; may enter through an unbroken surface or through a cut or abrasion

Numbers of Invading Microbes

- **ID_{50}**: infectious dose for 50% of the test population

- **LD_{50}**: lethal dose (of a toxin) for 50% of the test population

Bacillus anthracis

Portal of Entry	**ID_{50}**
Skin	10–50 endospores
Inhalation	10,000–20,000 endospores
Ingestion	250,000–1,000,000 endospores

Infection dose will change if bacteria use different portal of entries.

Toxins

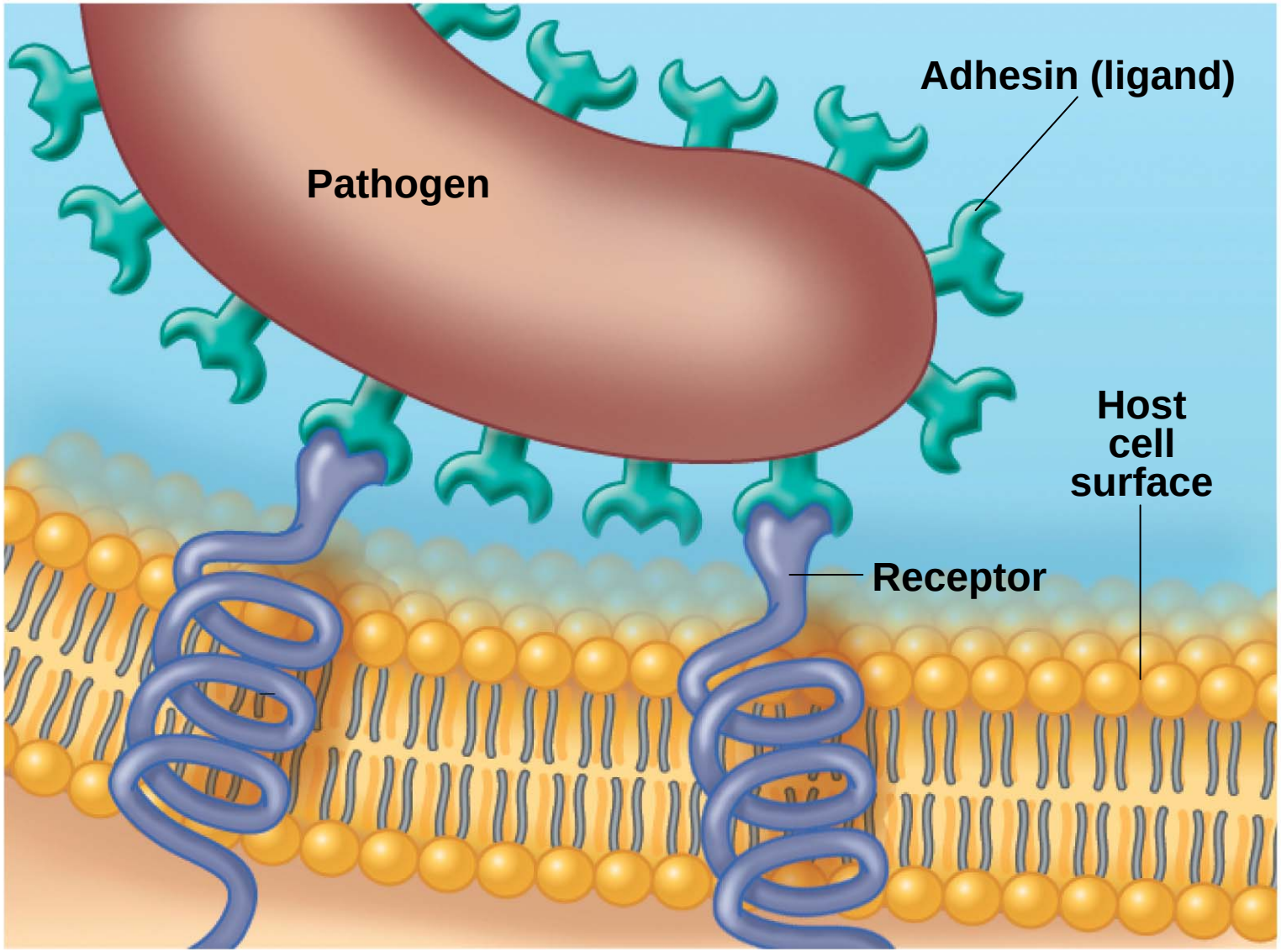
Portal of Entry	**ID_{50}**
Botulinum	0.03 ng/kg
Shiga toxin	250 ng/kg
Staphylococcal enterotoxin	1350 ng/kg

Infection dose will change with the potency of toxin.

The Importance of Adherence

- For most pathogens = necessary step
- Adhesins (also called ligands) // bind to receptors on host cells – receptor is often a sugar molecule
  - Glycocalyx: *Streptococcus mutans*
  - Fimbriae: *Escherichia coli and Neisseria gonorrhea*
  - M protein: *Streptococcus pyogenes*
- *Note: ability to form the glycocalyx is an important step for biofilm formation*

Adherence

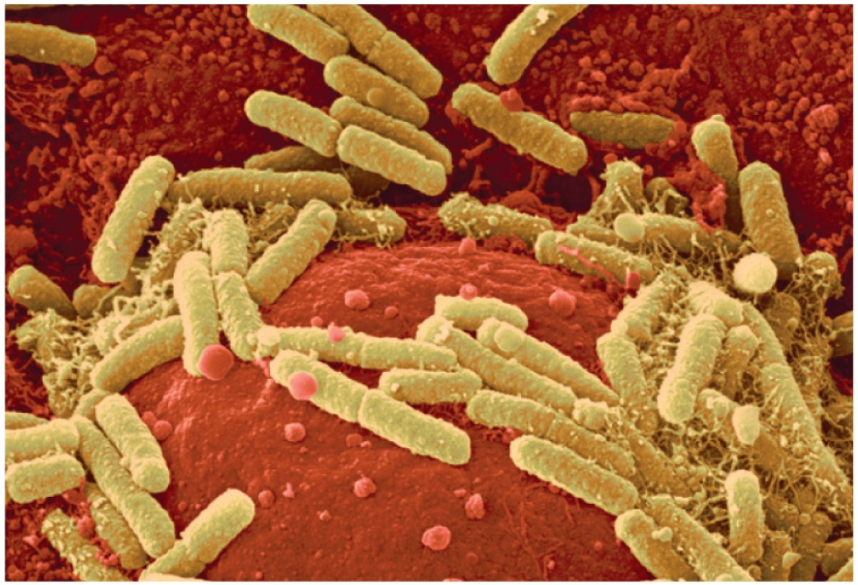


Surface molecules on a pathogen, called adhesins or ligands, bind specifically to complementary surface receptors on cells of certain host tissues.

Adherence and Tooth Decay

- Strptococcus mutans / plays key role / able to attach to enamel of tooth by way of its glycocalyx
- S. mutans produce an exoenzyme / glucoyltransferase / converts glucose into sticky polysaccharide called dextran, which forms the glycocalyx
- *Actinomyces (branching facultative anaerobe) uses fimbria to attach to the glycocalyx of S. mutans*
- *This is the genesis of dental plaque = biofilm // many other organisms enter into the biofilm // this biofilm may be colonized by over 400 different species (see video)*

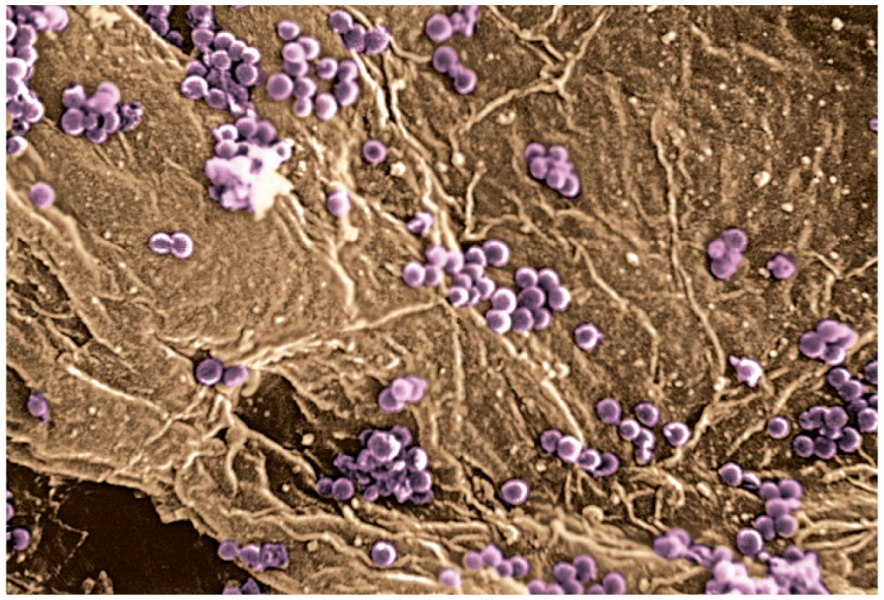
More Examples of Adherence



(b) *E. coli* bacteria (yellow-green) on human urinary bladder cells

SEM

1 μm



(c) Bacteria (purple) adhering to human skin

SEM

9 μm

Will Disease Result from an Encounter Between a (Human) Host and a Microorganism?

Microbe X			Host			Outcomes
Virulence	Percentage of optimal infectious dose	Correct portal of entry	Genetic profile that resists Microbe X (nonspecific defenses)	Previous exposure to Microbe X (specific immunity)	General level of health	
High / Low	100 / 0	Off		On		Microbe passes through unnoticed.
High / Low	100 / 0	On		On		Microbe passes through unnoticed. or Microbe becomes established without disease (colonization or infection).
High / Low	100 / 0	Off		Off		Microbe passes through unnoticed. or Microbe becomes established without disease (colonization or infection).
High / Low	100 / 0	On		Off		Microbe causes disease.

How Bacterial Pathogens Overcome Host Defenses

- Capsules
- Cell Wall Components
- Extracellular enzymes (exoenzymes)
- Antigenic variation
- Penetration of host cell cytoskeleton

Capsules

- Helps to prevent phagocytosis by host WBC
  - *E.g. // Streptococcus pneumoniae (pneumococcal pneumonia)*
  - *This microbe uses its capsule as a virulence factor*
  - *Strains with capsules are virulent – cause disease*
  - *Strains without capsules susceptible to phogocytosis – do not cause disease*
  - *How do hosts counter bacterial strategy? /// Host immune system make antibodies to molecules in bacterial capsule // antibodies attach to capsule – WBC able to "recognize and destroy" microbe*
  - *What do antibodies do? Render bacteria harmless and tag bacteria for destruction*

Cell Wall Components

- M protein resists phagocytosis
  - *Streptococcus pyogenes*
  - *On cell wall and fimbriae*
  - *M protein also help microbe attach to epithelial cells*
- Opa protein // ligand & inhibits T helper cells
  - *Neisseria gonorrhoeae*
  - *Opa = adherin & invasin / allows for microbe to move inside the host cell*
- Mycolic acid (waxy lipid)
  - *Mycobacterium tuberculosis*
  - *Resist digestion by phogocytes / live inside host cells*
  - *Also resist drying out of microbe / helps to retain water*

Exoenzymes

- Variety of **exoenzymes** / used to digest materials between cells of host or form new polymers around bacteria!

- Coagulase: coagulates fibrinogen // note: When you form a blood clot, one part of the clot is a fiber called fibrin. It exist in blood plasma as fibrinogen and is converted by the enzyme thrombin to form fibrin. Fibrin strands "stick together" forming the framework of the blood clot. Bacteria in our tissue spaces use coagulase to form fibrin. (note: in inflammation, after a respiratory burst, the host uses fibrin to contain the site of infection)

  - used to wall off bacteria from host defenses

  - Staphylococcus / walling off process in boils produced by staphylococcus

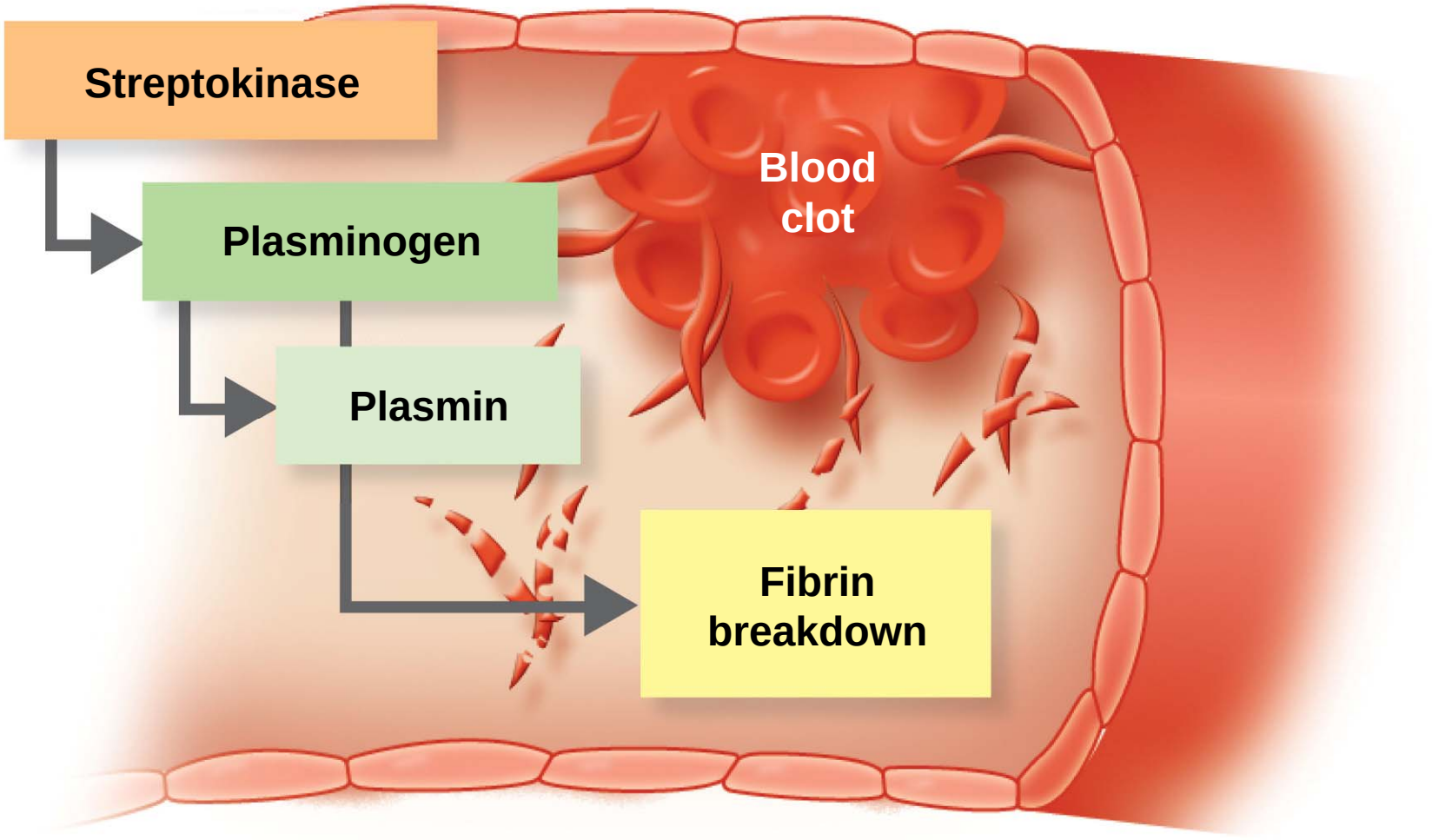
Exoenzymes

- Kinases: digest fibrin clots
  - Digest clots formed by host to isolate infections
  - Help microbes spread through tissue
  - Necrotizing fascilitis / Streptococcus pyrogenes makes Streptokinase – destruction of tissue at rate of 2cm per hour

Exoenzymes

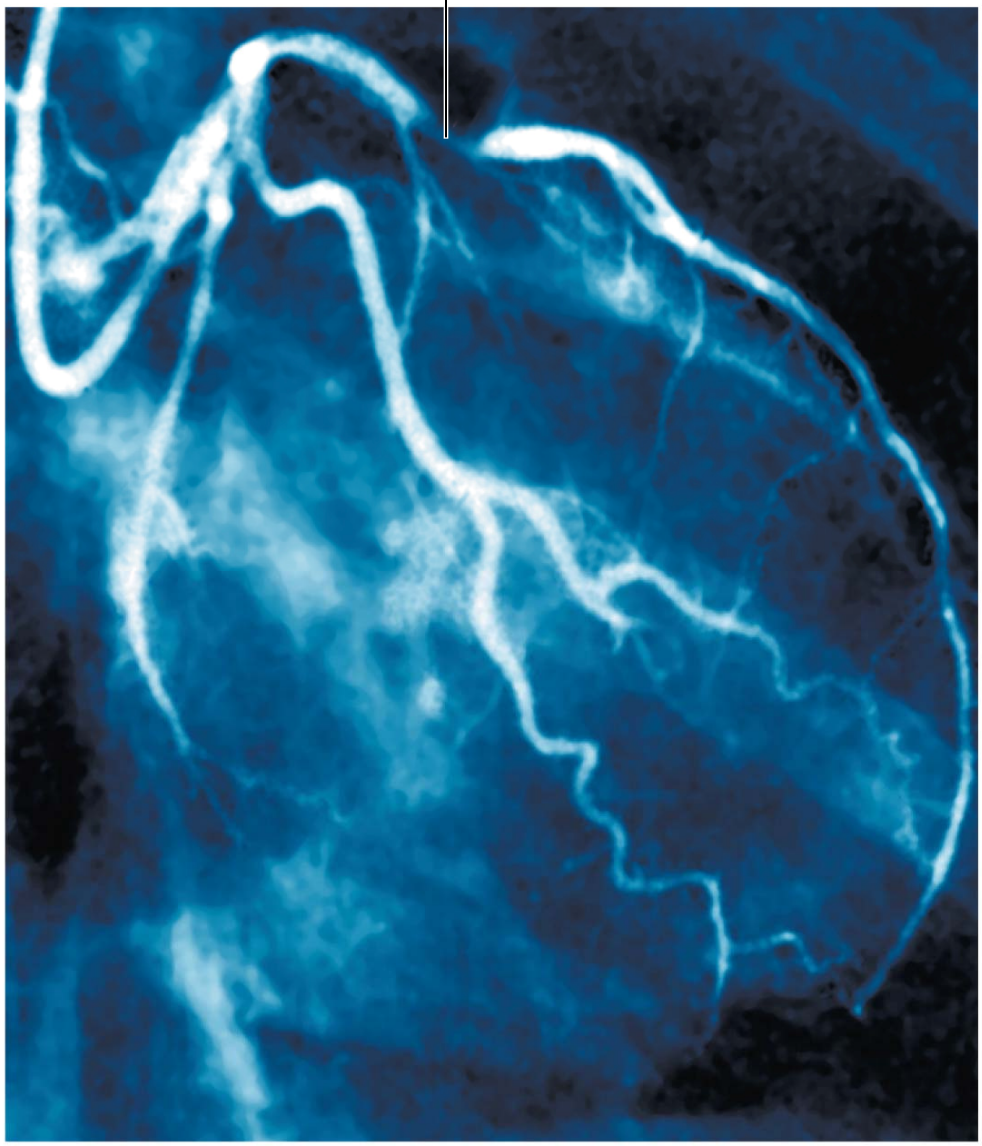
- **Hyaluronidase**: made by micobes to hydrolyzes hyaluronic acid
  - Hyaluronic acid is “glue” holding cells together in tissue
  - Host uses hyaluronic acid to slow movement of bacteria through tissue // exoenzyme breakdown of hyaluronic acid makes it easier for bacteria to move through tissue
- **Collagenase**: hydrolyzes collagen / separates “layers” of tissue // faster movement of bacteria through tissue
- **IgA proteases**: destroy IgA antibodies / prevents immune system from “tagging” microbes for destruction

Mechanism of streptokinase

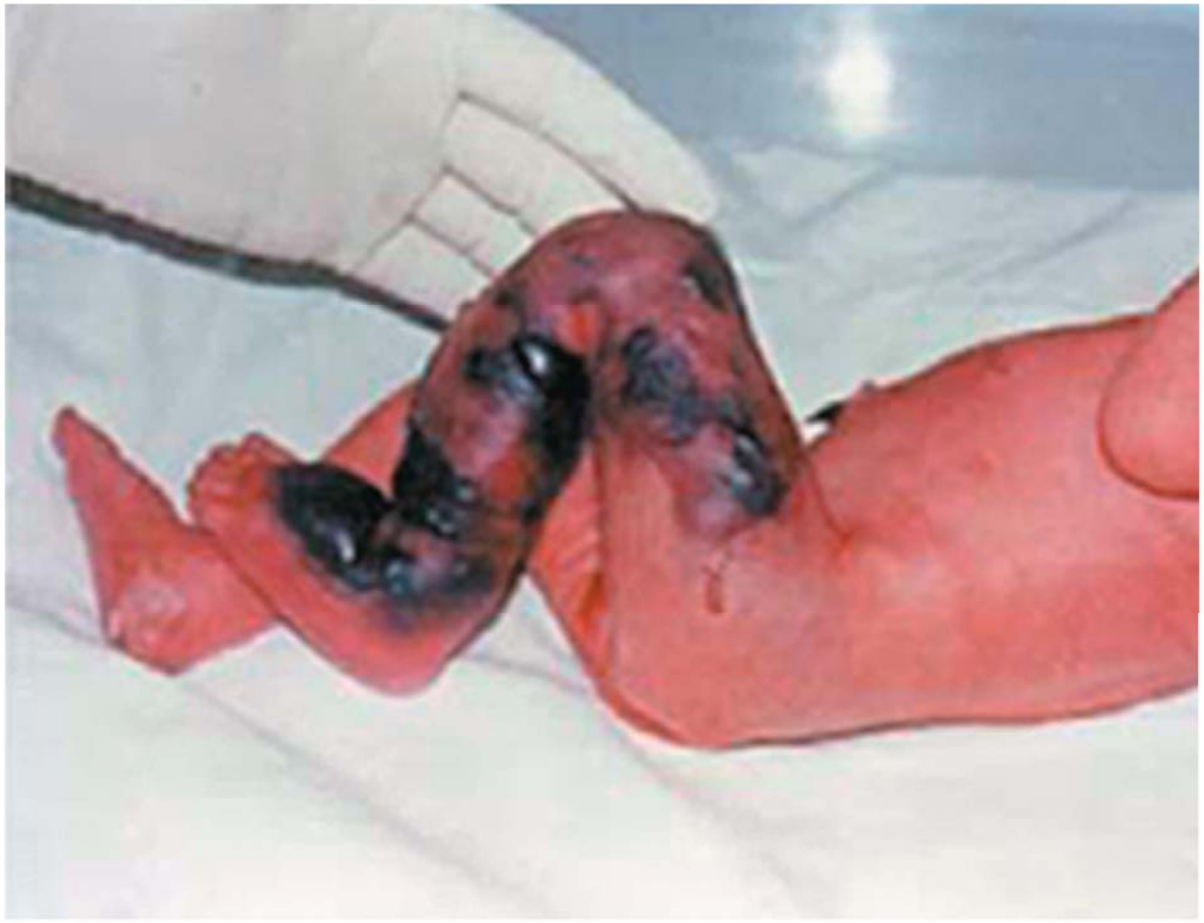


Streptokinase breaks down plasminogen (precursor to plasmin) to produce plasmin – an enzyme which breaks apart blood clots

Blocked coronary artery



Streptokinase - Used to clear blocked artery!



Streptokinase // cause of necrotizing fasciitis - destroy tissue

Tissue destruction at 2cm per hour / faster than growth
of Streptococcus pyrogen / enzyme increases rate of microbe’s spread

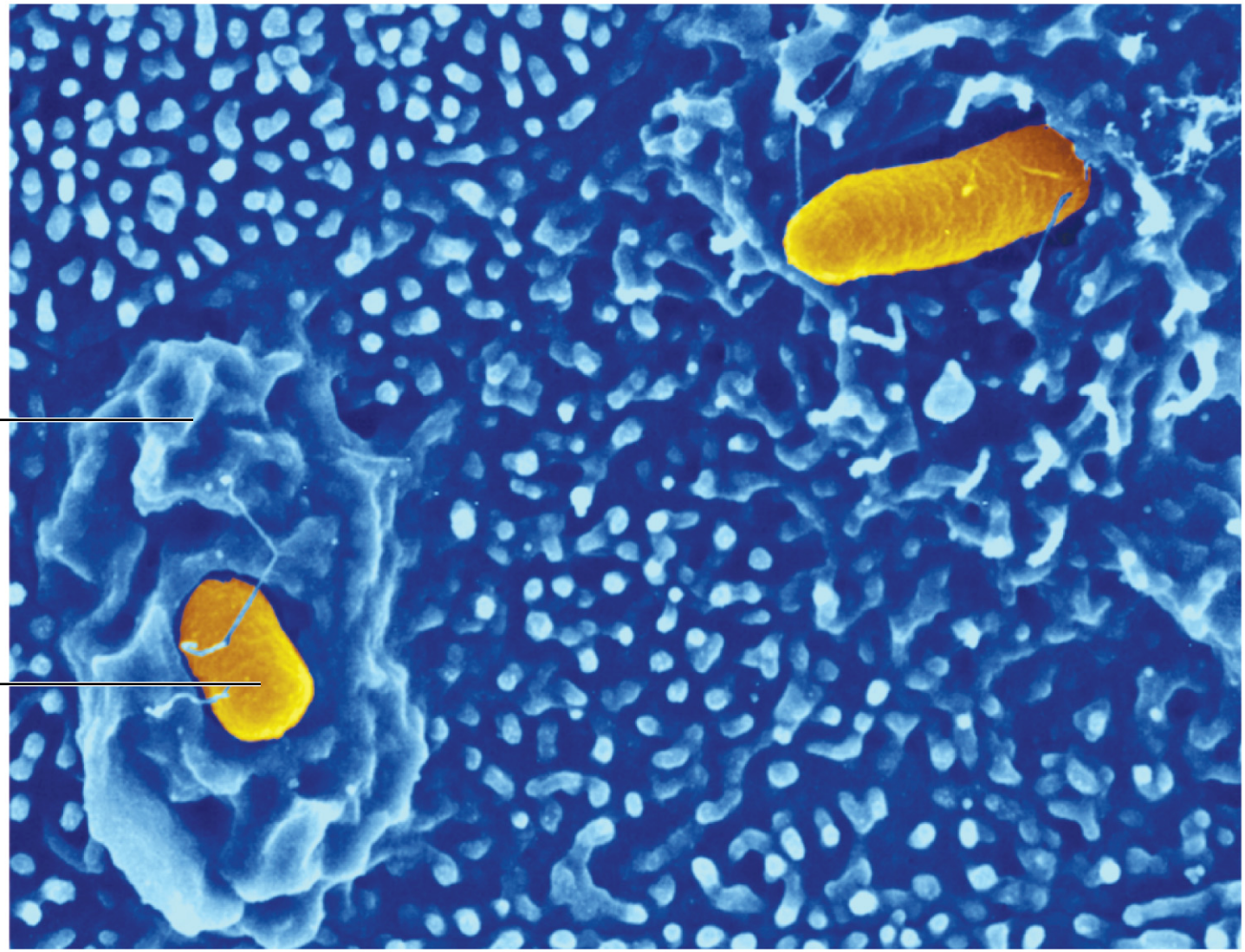
Antigenic Variation

- Host defenses respond to antigen (proteins) on the surface of the pathogen
- Host's adaptive immunity takes several days to respond to microbe on first exposure // response "should" eliminates microbe
- If microbe can "change" its "antigen identity" before host responds then microbe can evade adaptive immunity
- N. gonorrhoeae has several copies of the Opa encoding gene /// each with different antigen signatures
- *Influenza virus / exhibit both antigenic shift and drift*

Penetration into the Host Cell Cytoskeleton

- Eukaryotic cells have a “cytoskeleton” made up of different types of proteins / one protein is called actin
- Some bacteria produce invasins // interact with host cell’s actin /// allows bacteria to invade host cell
- Invasins – reacts with host cell actin to “splash” through cell membrane // called membrane ruffling
- Microbes once inside host cell use cytoskeleton to move throughout cytoplasm // also move between host cells
- *Salmonella and E. coli* - alters host actin to enter a host cell
- *Listeria* - Use actin to move from one cell to the next

Salmonella entering intestinal epithelial cells as a result of ruffling.



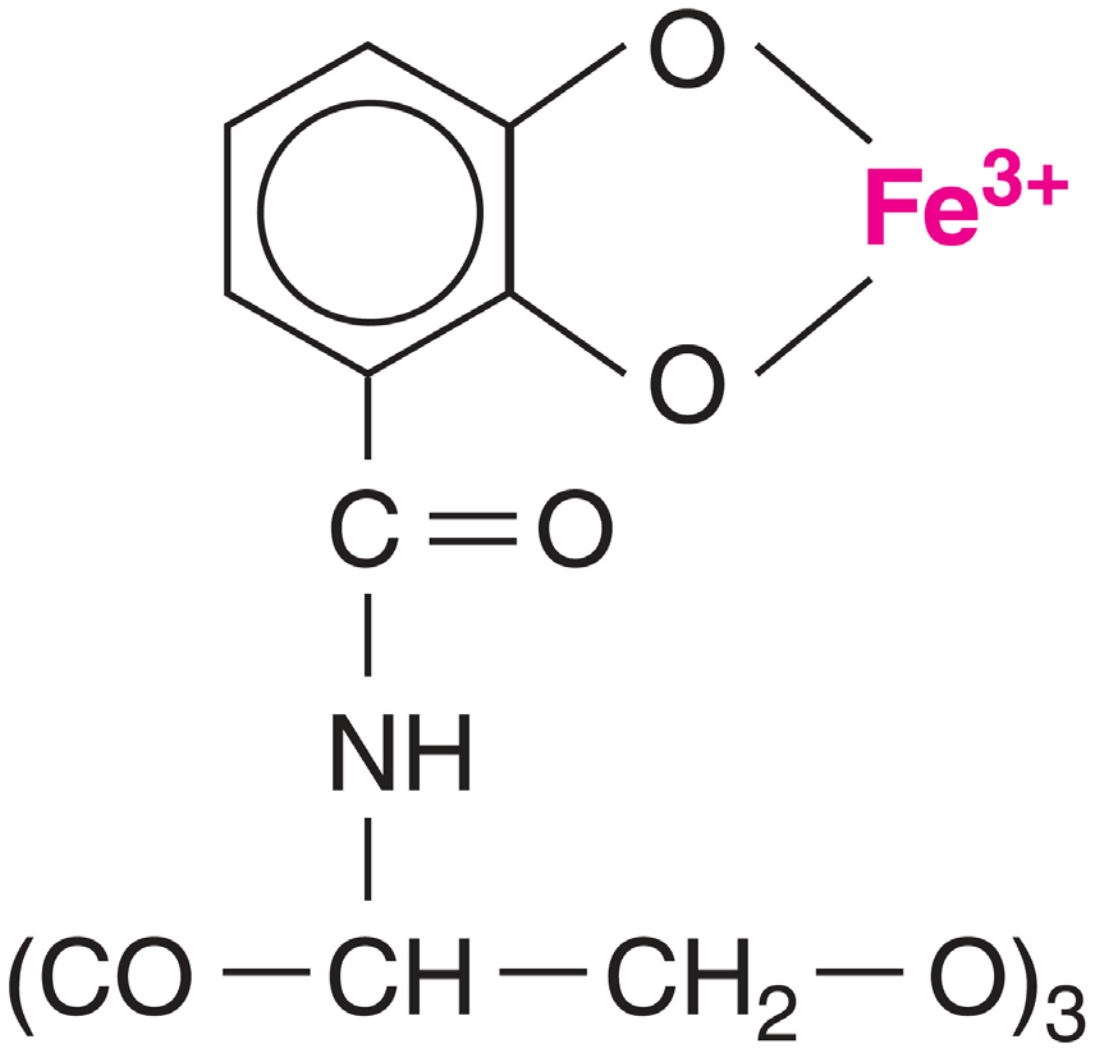
How Bacterial Pathogens Damage Host Cells

- Use host's nutrients
- Direct damage
- Produce toxins

Using Host's Nutrients: Siderrophores

- Iron is an essential nutrient (both for microbes and their host)
- Some pathogens acquire iron by secreting a protein called siderophores
- Siderophores "captures" iron from host
- Siderophores are called a "community good" / indicate microbes work in ways that benefit microbes across species

One type of bacterial siderophore.



Direct Damage

- After microbe attaches to host they “extract” nutrients from host
- Some microbes actually enter into the host’s cell via phagocytosis
- These microbes have defensives that avoid digestion by the host cell // e.g. capsules, M proteins, Opa
- Some pathogen grow inside host’s cells // then may rupture host cell or exit via exocytosis
- Bacteria may enter and exit a host cell /// this does not necessarily damage host’s plasma membrane

Direct Damage

- E. coli and Salmonella (both gram negative) enter host by phagocytosis
- Cause damage to host by using its resources
- Escape by exocytosis
- *Note - most bacterial damage done by “toxins”*
- *Toxins are poisons (exotoxins vs endotoxins)*
- *Exotoxins are proteins /// many are special type of enzyme called exoenzymes /// remember enzymes may be used over and over again! Makes them very potent!*

Direct Damage by Toxins

- Toxins = poisonous substances
- Produced by some microbes
- Toxigenicity – capacity of microorganism to produce toxins
- Toxemia – presence of toxins in the blood
- Toxoid – a weakened exotoxin used to stimulate immune system to produce antibody against the exotoxin.

Direct Damage by Toxins

- Toxins are transported by blood and lymph
- Toxins interact with host's cells to cause damage or death
- 220 known bacterial toxins
- 40% cause disease by damaging eukaryotic cell membrane damage

Direct Damage by Toxins

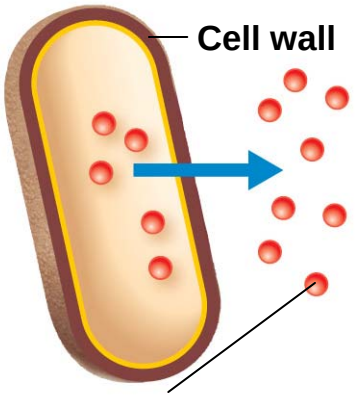
- Two types of toxins // exotoxins and endotoxins

- Toxins May Cause:
  - fever
  - chills
  - shock
  - intravascular coagulation
  - cardiovascular disturbances
  - diarrhea
  - inhibit protein synthesis
  - destroy RBC
  - disrupt nerve function

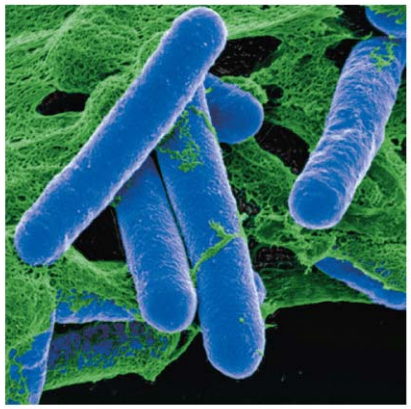
Exotoxins and Endotoxins Mechanisms of Action

exotoxins

Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted into the surrounding medium during log phase.



Exotoxin: toxic substances released outside the cell

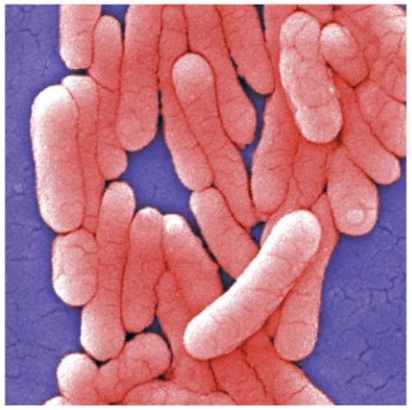


***Clostridium botulinum*, an example of a gram-positive bacterium that produces exotoxins**

endotoxins

Endotoxins are the lipid portions of lipopolysaccharides (LPS) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A; see Figure 4.13c). The endotoxins are liberated when the bacteria die and the cell wall breaks apart.

Endotoxins: toxins composed of lipids that are part of the cell membrane



***Salmonella typhimurium*, an example of a gram-negative bacterium that produces endotoxins**

Exotoxins

- Exotoxins are proteins produced “inside” the microbe
- Proteins are often enzymes
- Exotoxins are associated with specific symptoms and signs
- Produced by gram positive bacteria (but gram negative bacteria may also produce exotoxins)
- Gene for exotoxins carried by plasmids or by bacteriophages
- Therefore, exotoxins maybe “shared” with other bacteria

Exotoxins as Plasmids

- Small independent circular genetic code / independent replication
- Often carry resistance factors
- May also carry factors which increase microbe's pahogenicity
- Plasmids transferred between different bacteria using pili in conjugation
- E.g. Tetanus neurotoxin, heat liabile enterotoxin, staphylococcal enterotoxin

Exotoxins

- 1mg of botulinum exotoxin / enough to kill 1 million guinea pigs
- Toxin (not microbe) causes disease
- Host immune system produce specific antibodies (antitoxins) against these proteins!
- Toxoids / altered exotoxins used as vaccination product – stimulates host immune system

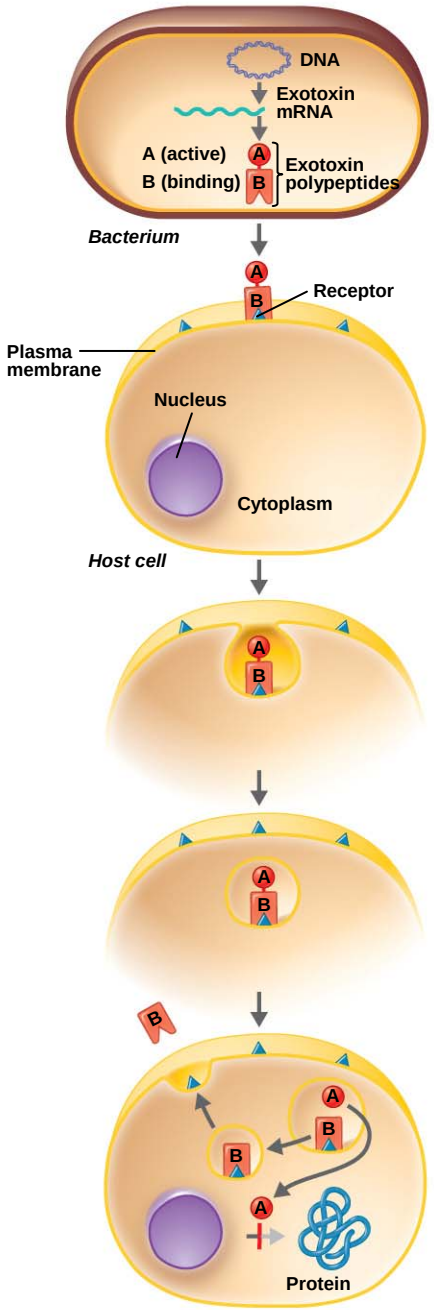
Types of Exotoxins

- A-B toxins
- Membrane-disrupting toxins
- Super-antigens

A-B Toxins (exotoxin)

- Most toxins are A-B type / two polypeptides
- “B” allows toxin to bind to host and move A-B toxin into host
- “A” is an active enzyme / alters metabolism of host
- Usually by inhibiting host protein synthesis

The action of an A-B exotoxin.



1. Bacterium produces and releases exotoxin.
2. B (binding) component of exotoxin attaches to host cell receptor.
3. A-B exotoxin enters host cell by receptor-mediated endocytosis.
4. A-B exotoxin enclosed in pinched-off portion of plasma membrane during pinocytosis.
5. A-B components of exotoxin separate. The A component alters cell function by inhibiting protein synthesis. The B component is released from the host cell.

Membrane Disrupting Toxins (exotoxins)

- Staphylococcus aureus / forms protein channels
- Clostridium perfringens / disrupts the phospholipid bilayer
- Leukocidins – microbe exotoxin which specifically disrupt the membrane of WBC
  - Staphylococci and streptococci produce most leukocidins
  - These microbes also produce hemolysins / form protein channels in RBC (releases iron!)

Superantigens (exotoxin)

- Damage host tissue by causing a very intense cellular immune response by the host
- The response is a nonspecific T cell activation
- Results in T cells releasing enormous amounts of cytokines / mediate cell to cell communication between cells which cause inflammation
- Cause fever, nausea, vomiting, diarrhea, shock, and even death
- E.g. staphylococci toxins / also causes food intoxication

Exotoxins / Examples

- Diptheria toxin // Corynebacterium diphteriae – diptheria toxin transferred to bacteria via lysogenic phage which carries the “tox gene” / toxin inhibits protein synthesis using the A-B toxin mechanisms

- Erythrogenic toxin / streptococcus pyogenes / superantigens damage endothelial cells under the skin – red skin rash / scarlet fever

- Botulinum toxin / Clostridium botulinum / A-B toxin / neuromuscular junction – prevents release of acetylcholine / causes flaccid paralysis

Exotoxins / Examples

- Tetanus toxin / Clostridium tetani / A-B toxin = neurotoxin / blocks relaxation pathway to anterior horn in spinal cord / results in spastic paralysis

- Vibrio enterotoxin / Vibrio cholerae / A-B toxin called cholera toxin / toxin enters epithelial cells and turns on transmembrane pump which moves ions out of cell - intestinal cells secrete large volume of ions and water molecules follow ions /// similar gene also shared with a pathogenic E. coli

- Staphylococcal enterotoxins / Staphylococcus aureu superantigen /// affects intestines similar to vibrio enterotoxin

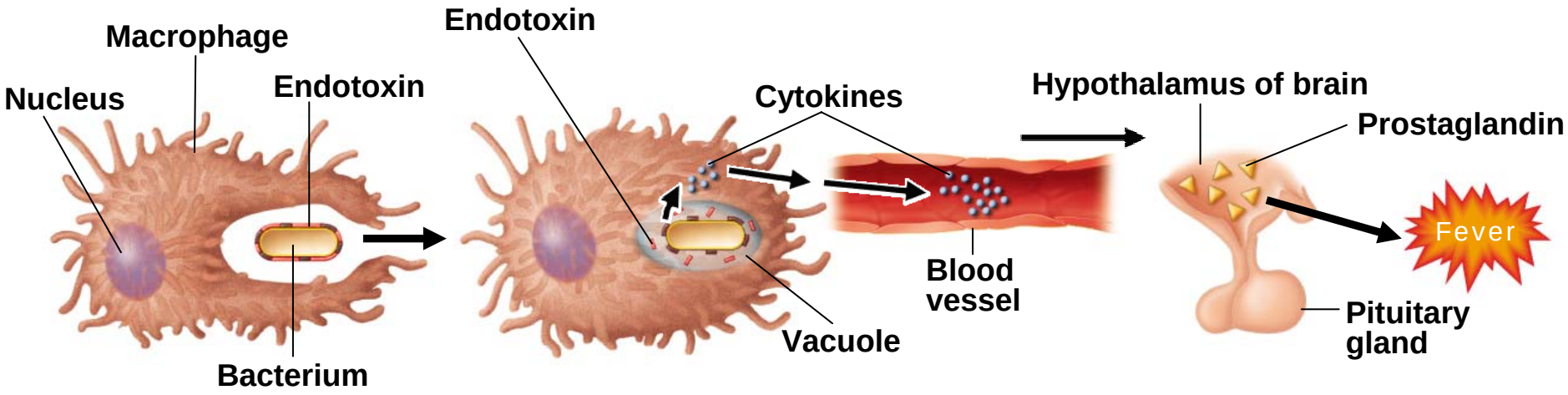
Endotoxins

- Lipopolysaccharide (not protein)
- Lipopolysaccharide “shed” from outer-membrane of gram negative bacteria
- Lipopolysaccharide released when bacteria rapidly growing or as bacteria die
- Endotoxins cause macrophage to release large amounts of cytokines
- At high concentrations, cytokines produced by host’s macrophage are toxic! (see next slide)
- Endotoxins may contaminate drugs /// may result in septic shock and death!

Endotoxins

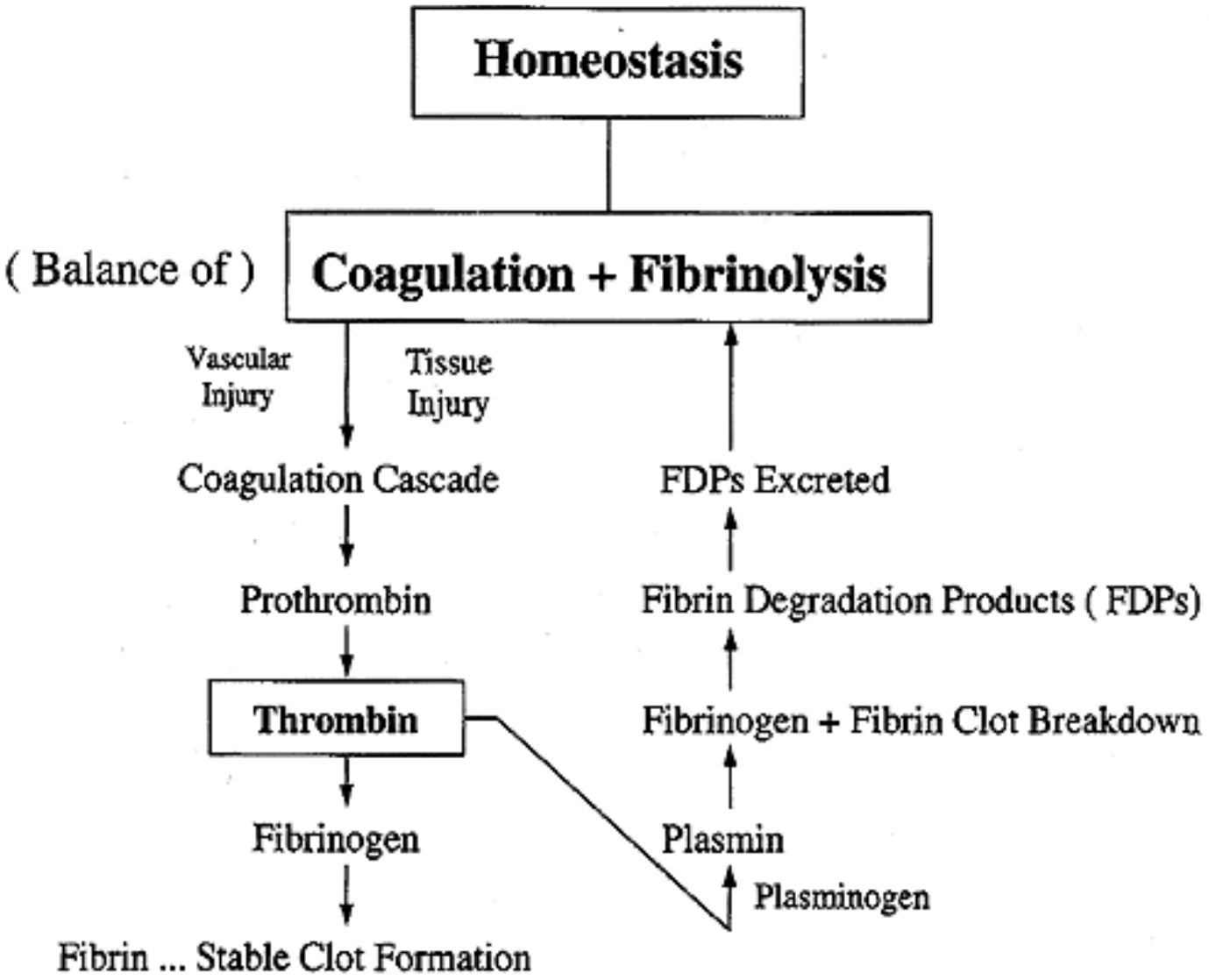
- All endotoxins from different microbes have similar outcomes (unlike exotoxins!)
- Syndrome: "the big three"
  - Fever
  - Shock (septic shock) / hypotension
  - Disseminated intravascular coagulation (followed by hemorrhage)
- 750,00 cases of septic shock in U.S per year / 50% die within 6 months

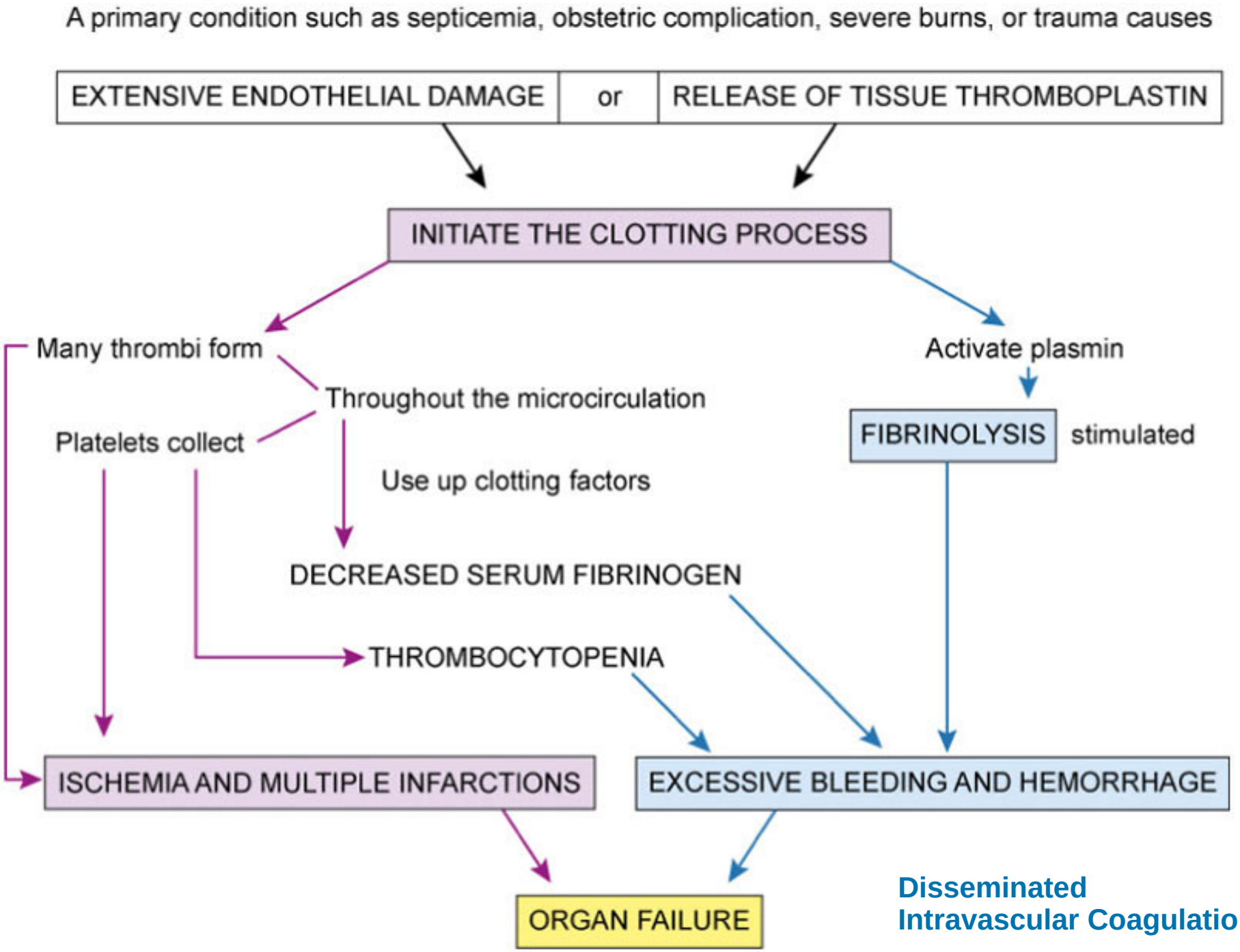
Endotoxins and the Pyrogenic Response



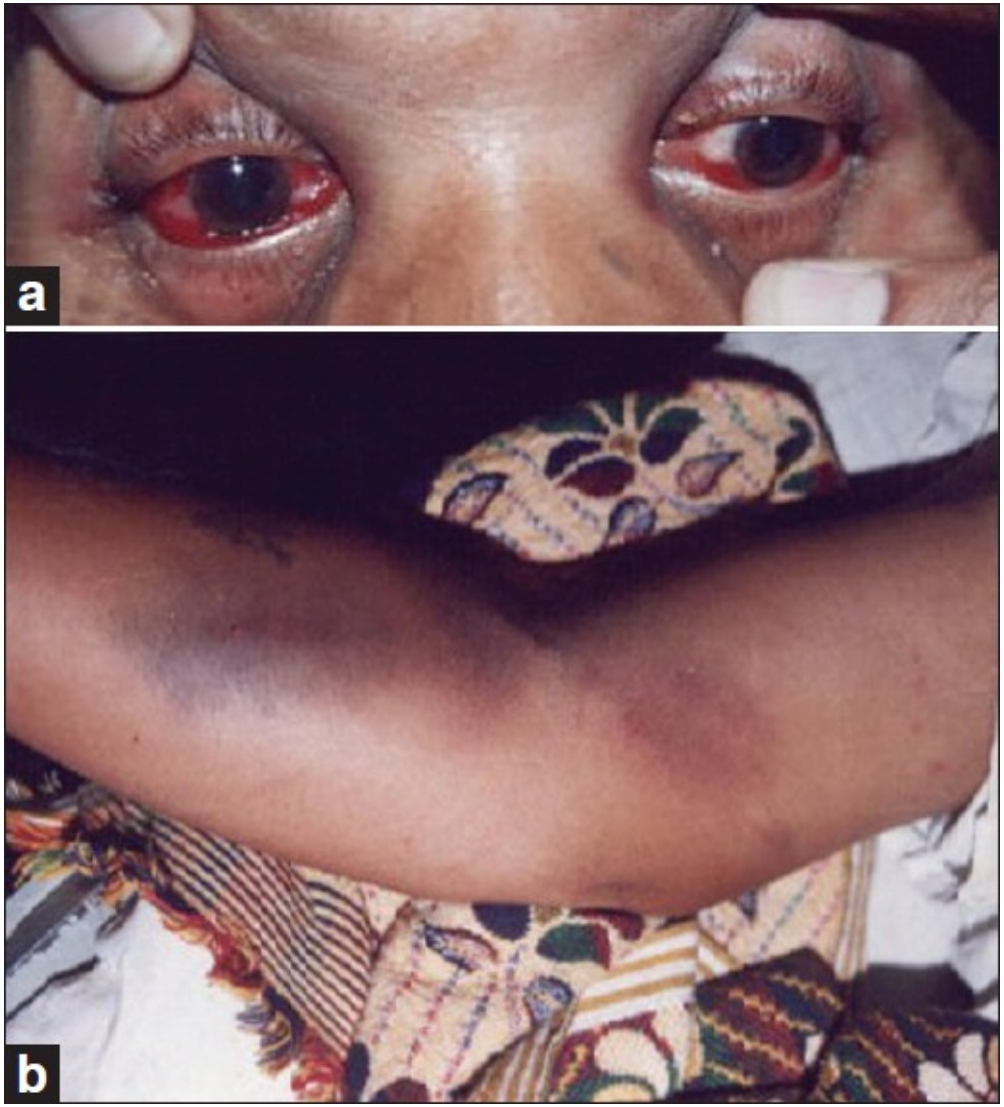
1. A macrophage ingests a gram-negative bacterium.
2. The bacterium is degraded in a vacuole, releasing endotoxins that induce the macrophage to produce cytokines IL-1 and TNF-α.
3. The cytokines are released into the bloodstream by the macrophages, through which they travel to the hypothalamus of the brain.
4. The cytokines induce the hypothalamus to produce prostaglandins, which reset the body's "thermostat" to a higher temperature, producing fever.

Normal Physiologic Condition

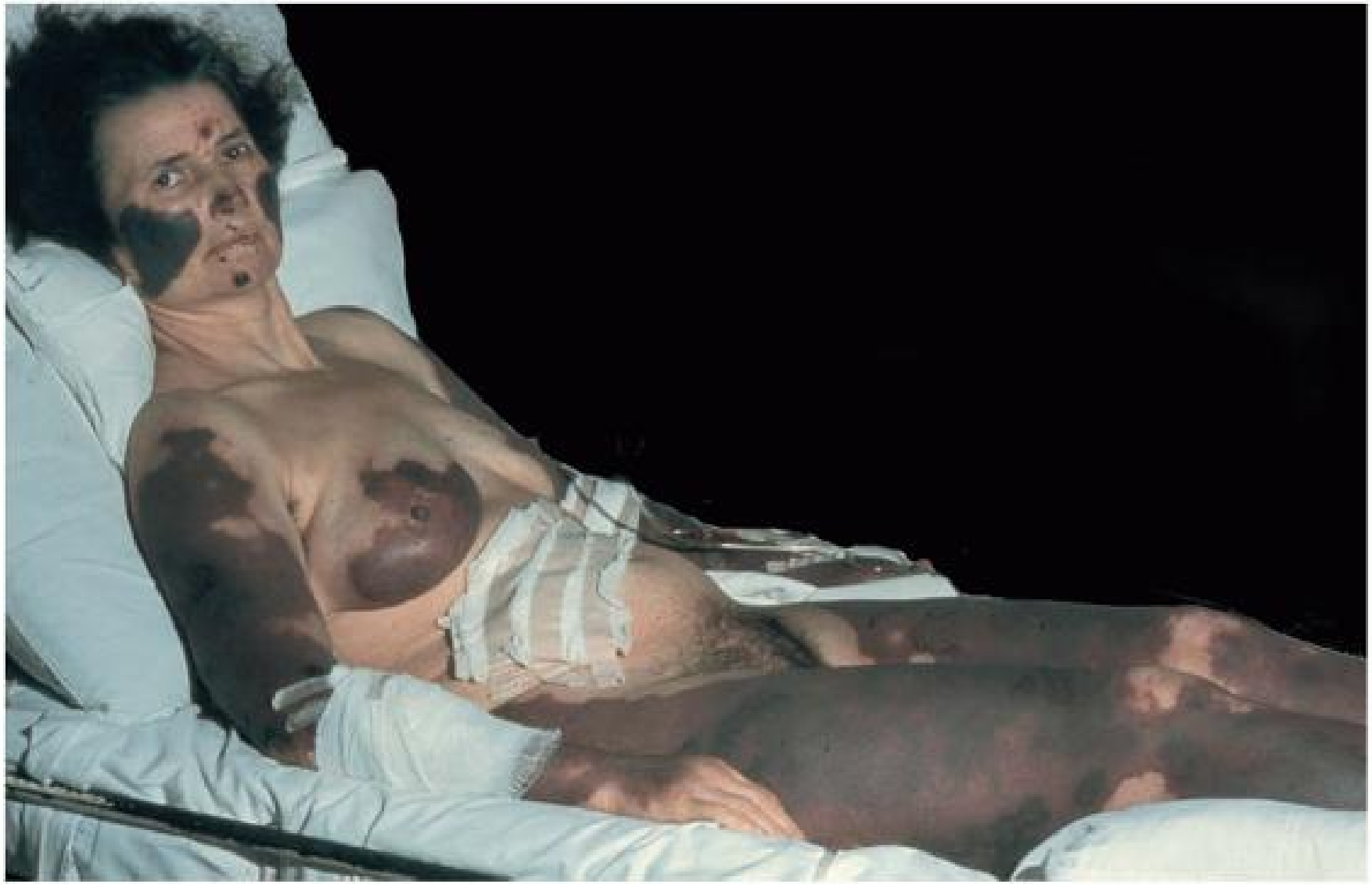




DIC – Clinical Presentation



DIC – Clinical Presentation



DIC - Spleen

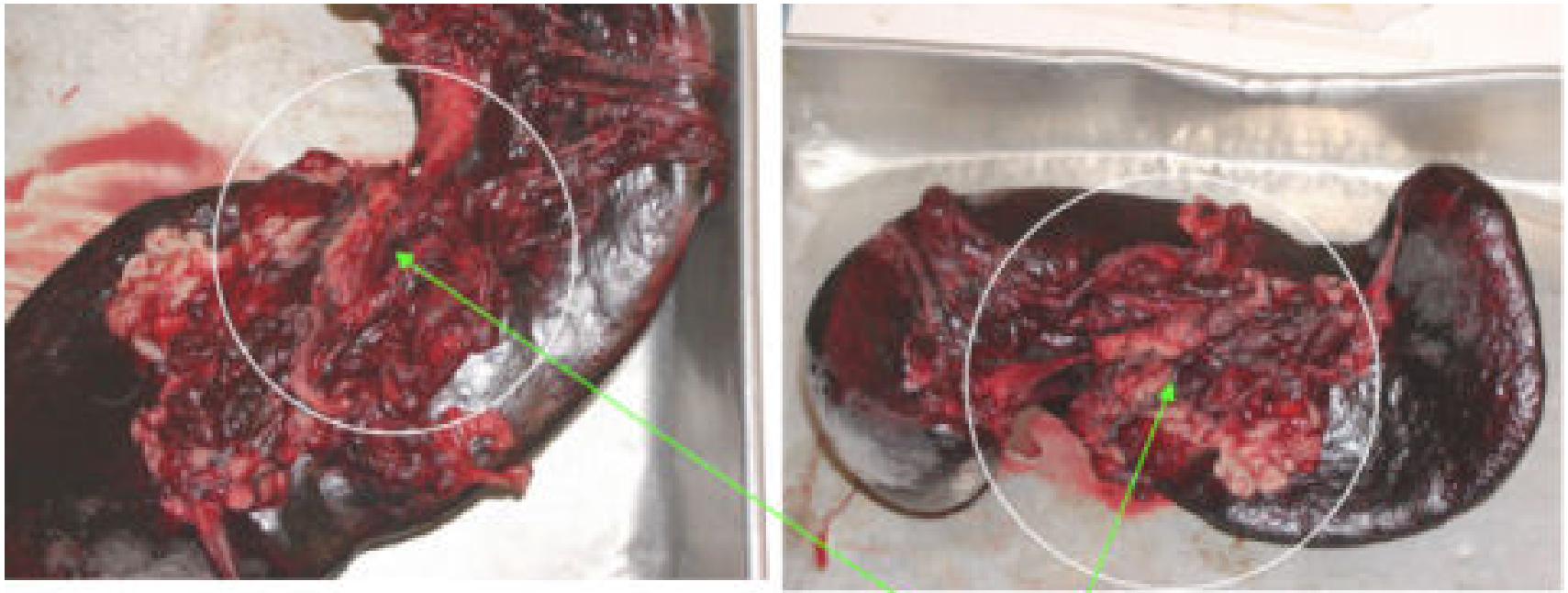


Fig. 2 — This pictures show the spleen after it has been removed due to splenic infarction. The consistency of the spleen normally is fairly spongy while in splenic infarction the spleen feels very "wooden".

Area of splenic infarction at the pedicle of the spleen and greatly enlarged spleen.

Host Cells and Virus Pathogenicity

- Virus are obligate intracellular parasites
- Virus enter host cell and are either lysogenic or lytic.
- Lysogenic virus insert gene into host chromosome and become latent.
- Lytic virus take over metabolism of host cell, reproduce, and kills host cell
- This is known as a cytopathic effect.

Host Cells and Virus Pathogenicity

- Virus generally use same portals of entry
- Mucous membranes // Skin // Parenteral route
- Adjacent cells may fuse as result of viral infection = syncytium // form giant cells // seen in measles virus, smallpox virus, herpesvirus, adenoviruses.
- Exit infected cells either by budding or lytic event

Pathogenic Properties of Fungi, Protozoa, Helminths, and Algae

- Fungal Pathogenicity
  - Do not have well defined virulence factors
  - Secrete exoenzymes (e.g. proteases) to digest external macromolecules
  - Obtain nutrients from diffusion of molecules into fungi
  - Slow growing // often chronic disease
  - Ergotism – toxin produced by fungus // common Eurpope Middle Ages disease // toxin = ergot = natural source of LSD (hallucinogenic chemical)
  - Aflatoxin – toxin produced by fungus // grows on peanuts // carcinogenic properties

Pathogenic Properties of Fungi, Protozoa, Helminths, and Algae

- Protozoa Pathogenicity
  - Disease symtoms caused by presence or waste products of protozoa (three examples)
  - Plasmodium – protozoa that causes malaria // reproduces in liver and RBC // different life stages which results in periodic rupture of RBC
  - Toxoplasma – protozoa attaches to macrophage, enters cytoplasm, fuse with lysosome but resists digestion // able to live and reproduce inside macrophage
  - Giardia lamblia (giardiasis) – attaches to epithelial cells lining digestive tract with sucking disc // use host's nutrients // avoid host immune system by antigenic variation

Pathogenic Properties of Fungi, Protozoa, Helminths, and Algae

- Helminths Pathogenicity
  - Their presence produce disease symptoms
  - Attach to host cells and use host tissue to grow and reproduce
  - Produce huge parasitic masses // worm load
  - Elephantiasis – worms blocking lymph flow

Pathogenic Properties of Fungi, Protozoa, Helminths, and Algae

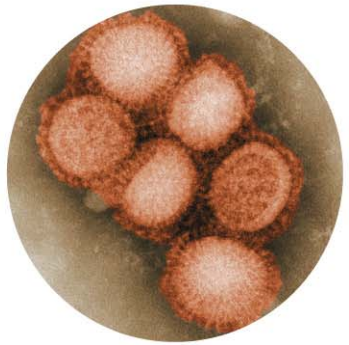
- Algae Pathogenicity
  - A few species produce a neurotoxin
  - Dinoflagellates produce saxitoxin (neurotoxin) // mollusks feed on dinoflagellates but not affected by neurotoxin, however….
    - People who eat mollusks may develop paralytic shellfish poisoning
    - Condition associated with the “red tide”

Portal of Exit

- How microbe “leaves” host
  - Generally, same as the portals of entry
  - Mucous membranes
  - Skin
  - Parenteral route

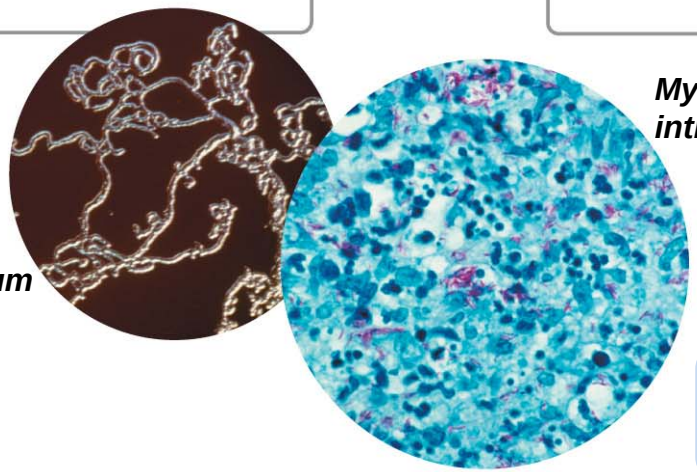
Microbial Mechanisms of Pathogenicity.

When the balance between host and microbe is tipped in favor of the microbe, an infection or disease results. Learning these mechanisms of microbial pathogenicity is fundamental to understanding how pathogens are able to overcome the host's defenses.



H1N1 flu virus

portals of entry	Number of invading microbes	penetration or evasion of host defenses	damage to host cells	portals of exit
Mucous membranes • Respiratory tract • Gastrointestinal tract • Genitourinary tract • Conjunctiva Skin Parenteral route	Adherence	Capsules Cell wall components Enzymes Antigenic variation Invasins Intracellular growth	Siderophores Direct damage Toxins • Exotoxins • Endotoxins Lysogenic conversion Cytopathic effects	Generally the same as the portals of entry for a given microbe: • Mucous membranes • Skin • Parenteral route



Clostridium tetani

Mycobacterium intracellulare

Micrographs are not shown to scale.