Introduction to Microbiology

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Lecture 9

Pathogenesis of bacterial infection

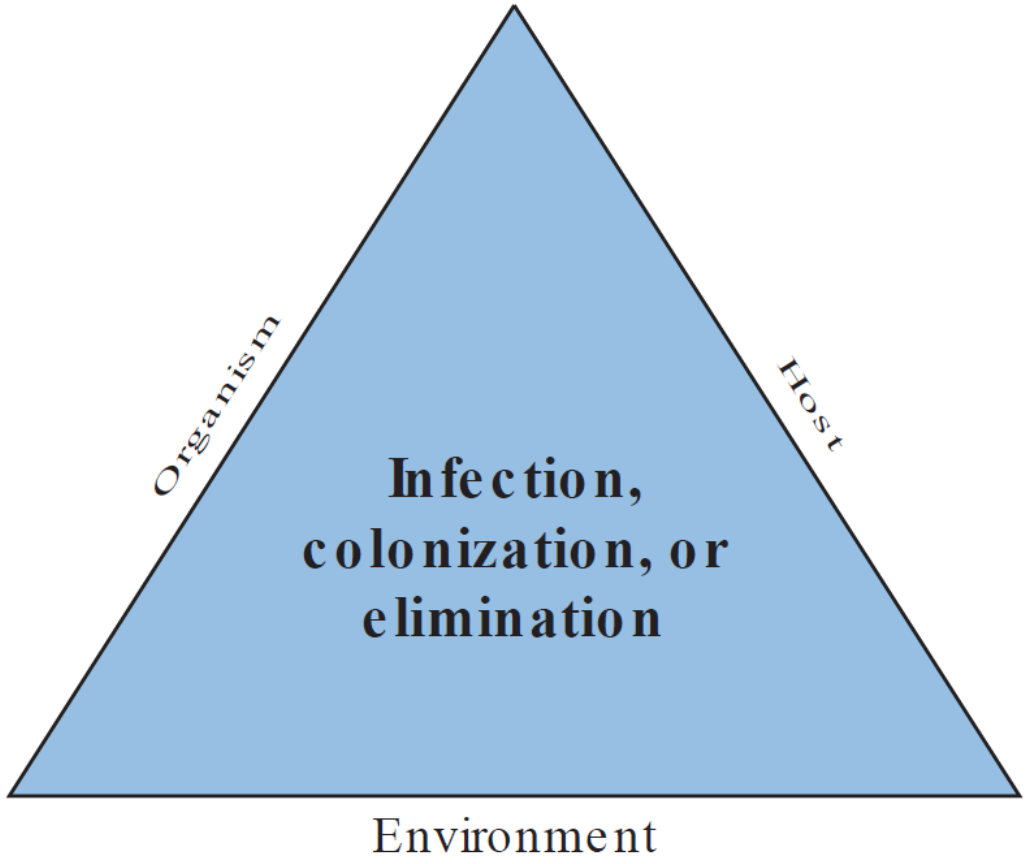
Although most bacteria are **harmless** or often **beneficial**, some are **pathogenic**, with the number of species estimated as **fewer than a hundred** that are seen to **cause infectious** diseases in humans.

By contrast, **several thousand species** exist **in the human digestive system without causing disease.**

> Pathogenicity is, in a sense, a highly skilled trade, and only a tiny minority of all the numberless tons of microbes on the earth has ever involved itself in it; most bacteria are busy with their own business, browsing and recycling the rest of life. Indeed, pathogenicity often seems to me a sort of biological accident in which signals are misdirected by the microbe or misinterpreted by the host.
>
> —Lewis Thomas, *The Medusa and the Snail*

وليس كل سبب يصل إلى البدن يفعل فيه بل قد يحتاج مع ذلك إلى أمور ثلاثة: إلى قوة من قوته الفاعلة، وقوة من قوة البدن الإستعدادية، وتمكن من ملاقاة أحدهما الآخر زماناً في مثله يصدر ذلك الفعل عنه.

The Canon of Medicine
القانون في الطب
Avicenna (Ibn Sina) in 1025



Pathogenesis of bacterial infection

For bacteria **to cause disease (to be pathogenic)** , it needs to have some attributes to help it reach the host and persist within the host and replicate, while causing harm (disease) to the host. Characteristics of bacteria that are pathogens are sometimes referred to as **virulence factors -**but can be shared with non-pathogenic bacteria- and include:

- transmissibility,
- adherence to host cells
- motility
- persistence
- invasion of host cells and tissues
- Toxigenicity
- Iron uptake mechanisms
- the ability to evade or survive the host's immune system.
- Resistance to antimicrobials and disinfectants.

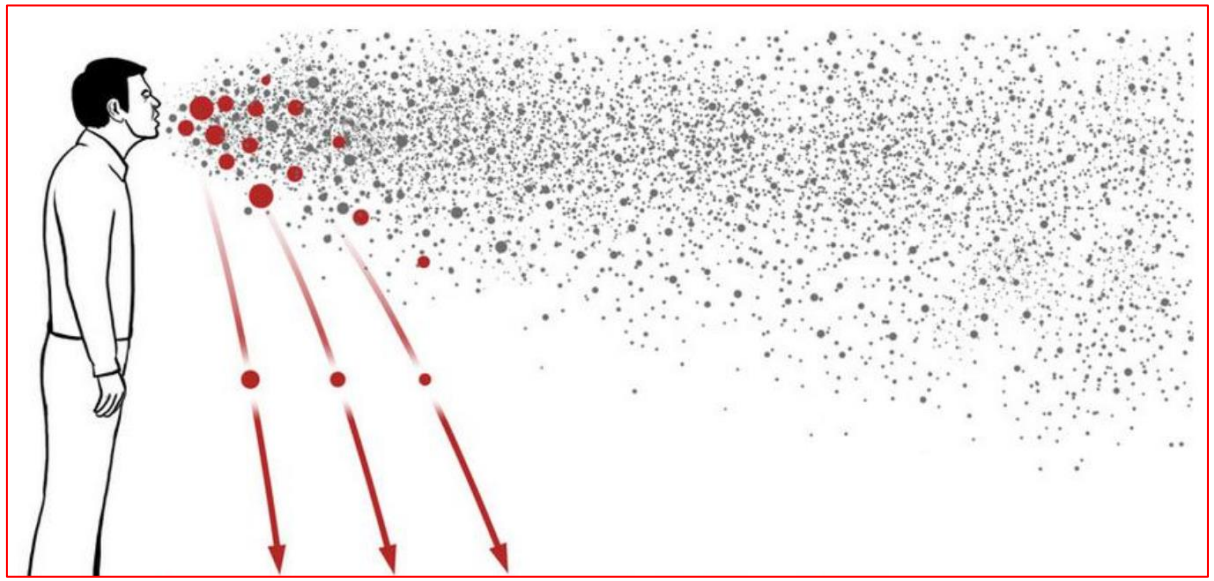
Transmission

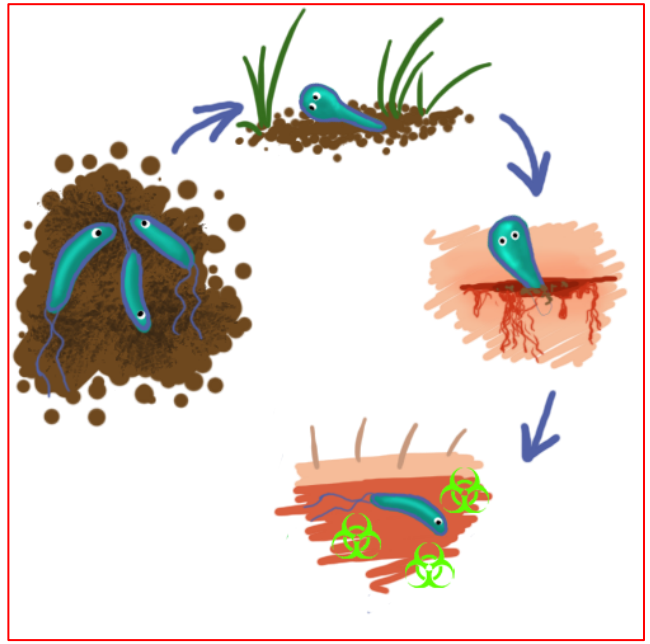
- Bacteria can adapt to a variety of environments that include external sources such as **soil, water and organic matter** or internal milieu as found **within insect vectors, animals and humans.**

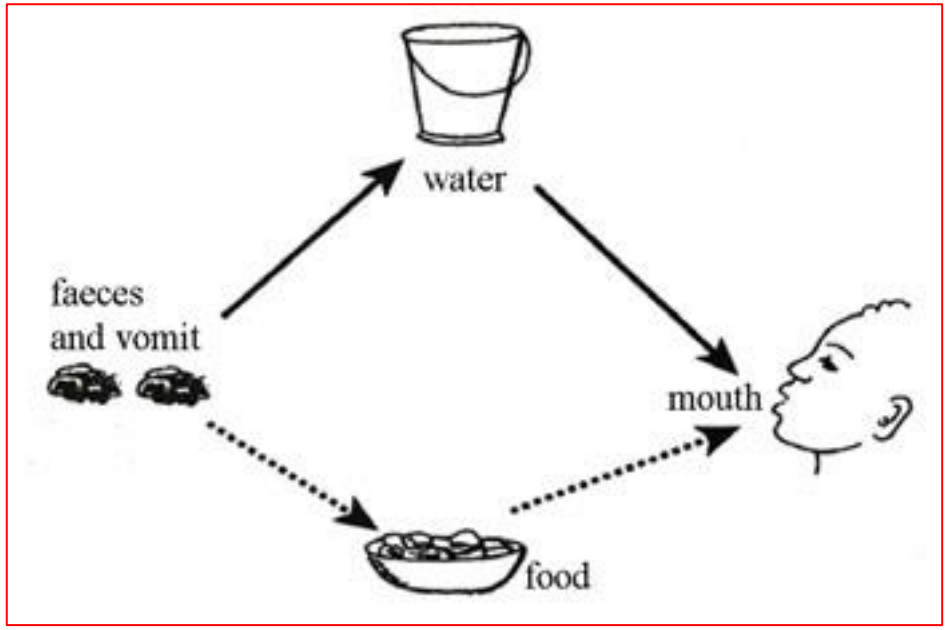
- By producing asymptomatic infection or **mild disease rather than death of the host**, microorganisms that normally live in people **enhance the possibility of transmission from** one person to another.

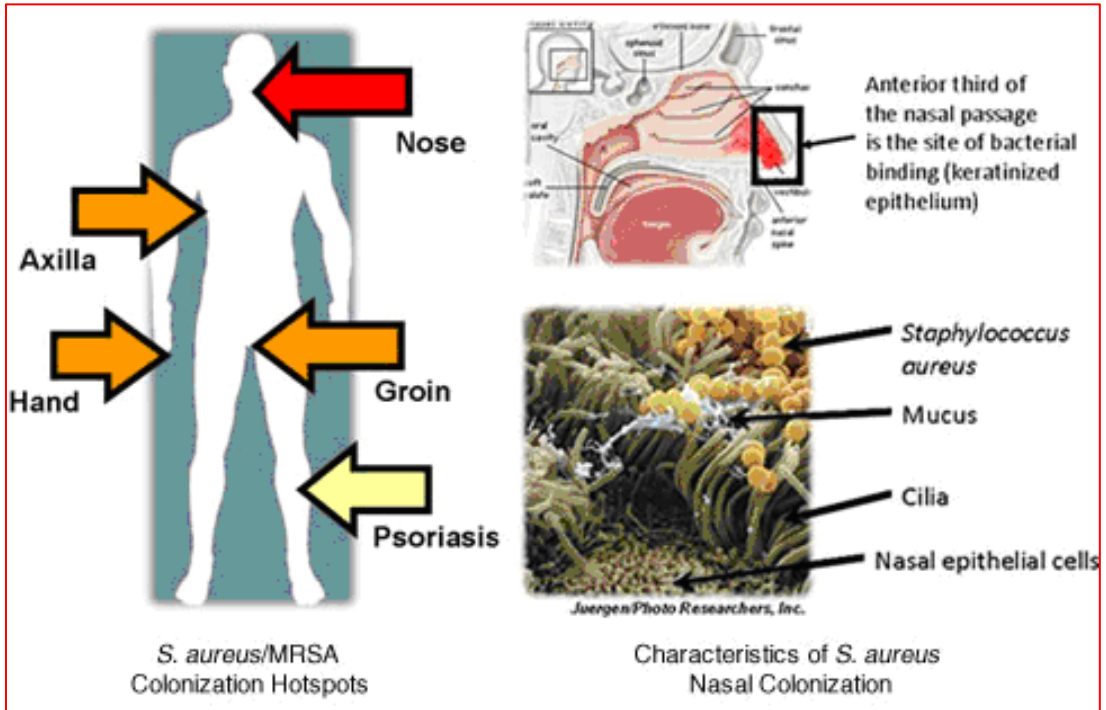
- **The clinical manifestations of diseases** (eg, diarrhea, cough, genital discharge) produced by microorganisms often **promote transmission of the agents**.

- The respiratory (upper and lower airways), gastrointestinal (primarily mouth), genital,and urinary tracts. Abnormal areas of mucous membranes and skin (eg, cuts, burns, and other injuries) are frequent **sites of entry**.






water
faeces
and vomit
mouth
food


Nose
Axilla
Hand
Groin
Psoriasis
Anterior third of the nasal passage is the site of bacterial binding (keratinized epithelium)
Staphylococcus aureus
Mucus
Cilia
Nasal epithelial cells
Juergen/Photo Researchers, Inc.
S. aureus/MRSA Colonization Hotspots
Characteristics of S. aureus Nasal Colonization

Transmission

Portal of entry

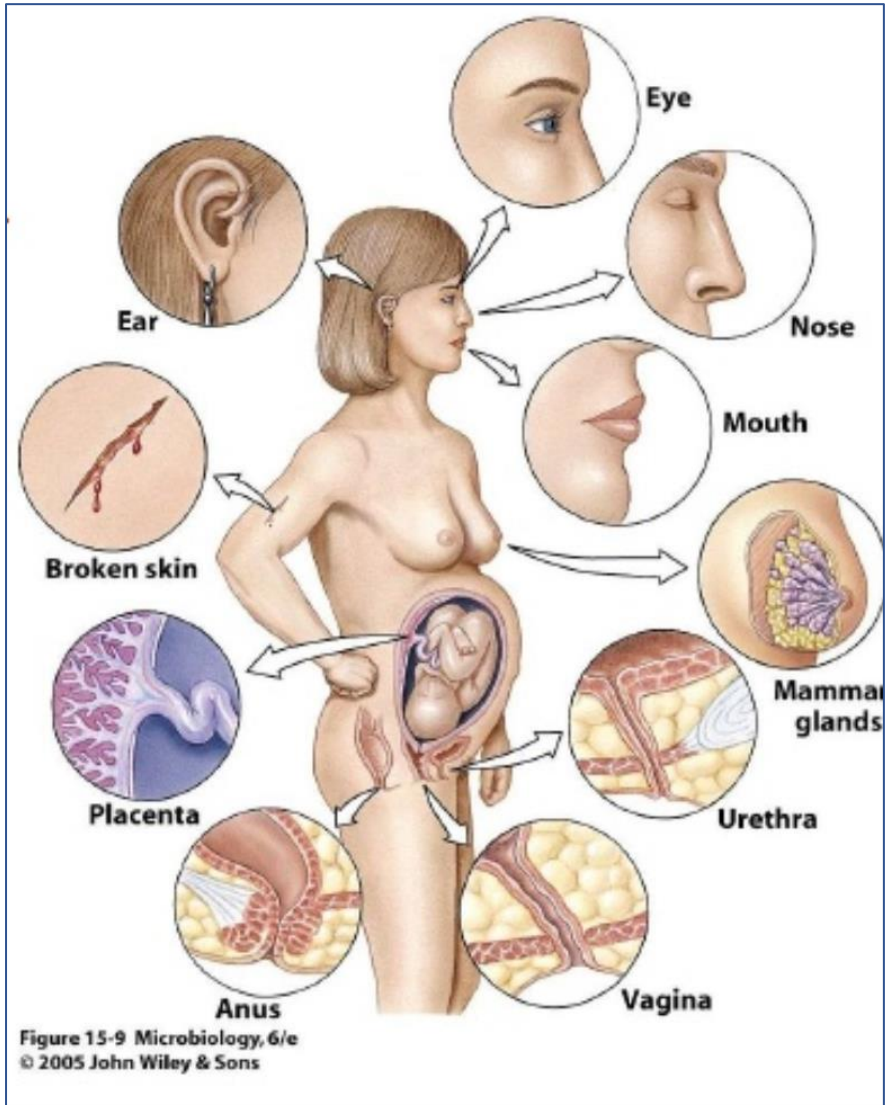


Figure 15-9 Microbiology, 6/e
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Portal of exit

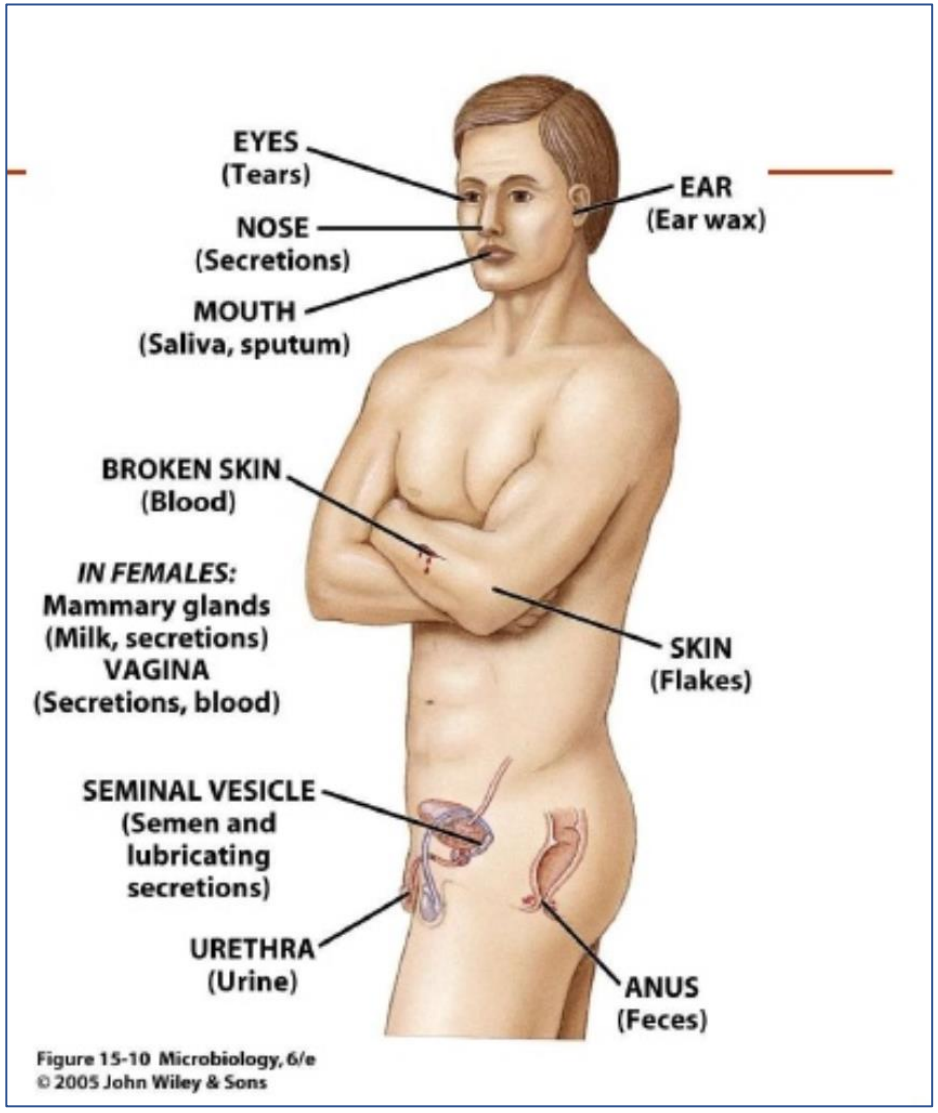

Figure 15-10 Microbiology, 6/e
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Adhesion

- Bacteria also have specific surface molecules that interact with host cells. Many bacteria have **pili**, thick rodlike appendages or **fimbriae**, shorter “hairlike” structures that extend from the bacterial cell surface and help mediate adherence of the bacteria to host cell surfaces

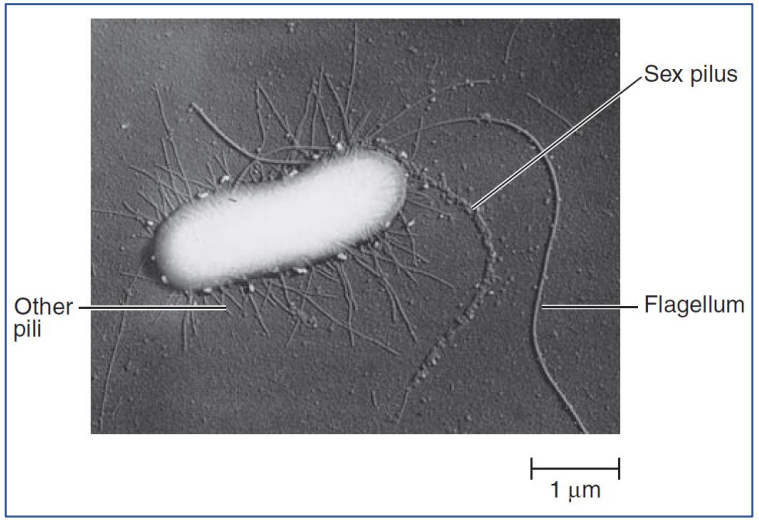
- When bacteria enter the body of the host, they must adhere to cells of a tissue surface. i**f they did not adhere, they would be swept away** by mucus and other fluids that bathe the tissue surface.

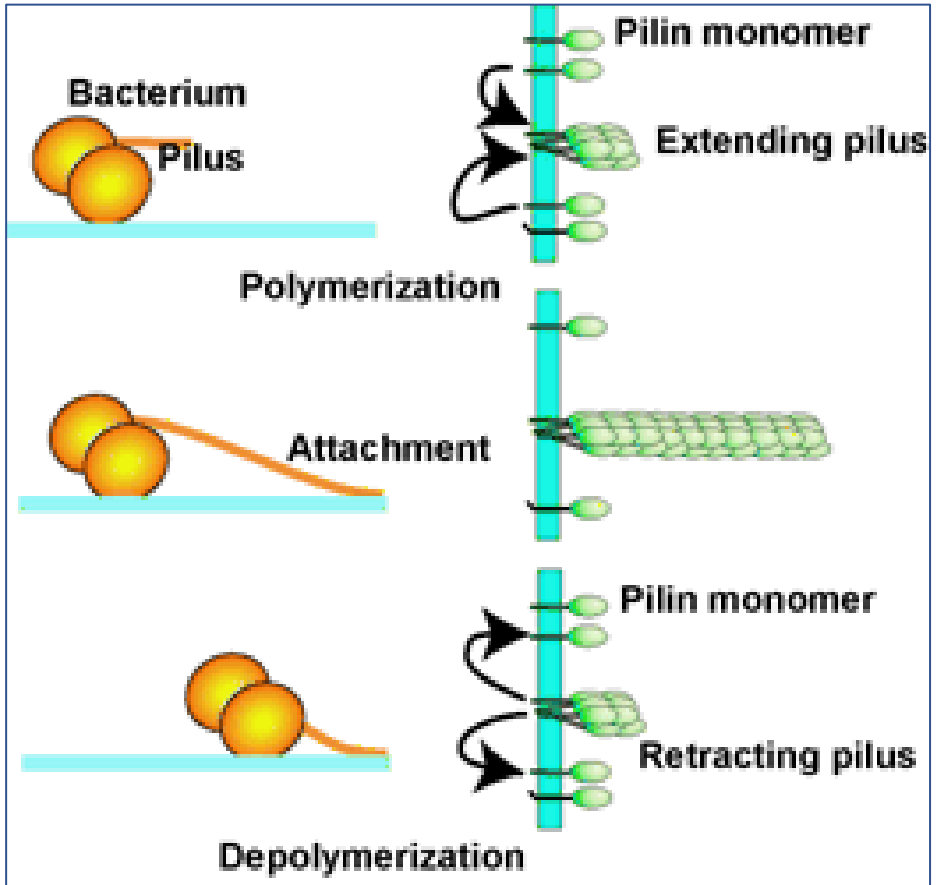
Pili (fimbria)

Composed of structural protein subunits termed **pilins**.
Minor proteins termed **adhesins** are located at the tips of pili and are responsible for the attachment properties.

Two classes can be distinguished: **ordinary pili**, which play a role in the **adherence** of symbiotic and pathogenic bacteria to host cells, and **sex pili**, which are responsible for the attachment of donor and recipient cells in bacterial **conjugation.**
Pili inhibit the phagocytic ability of leukocytes.

Their tips strongly adhere to surfaces at a distance from the cells. Pili then depolymerize from the inner end, thus retracting inside the cell. The result is that the bacterium moves in the direction of the adhering tip. This kind of surface motility is called **twitching** and is widespread among piliated bacteria. **pili grow from the inside of the cell outward**.





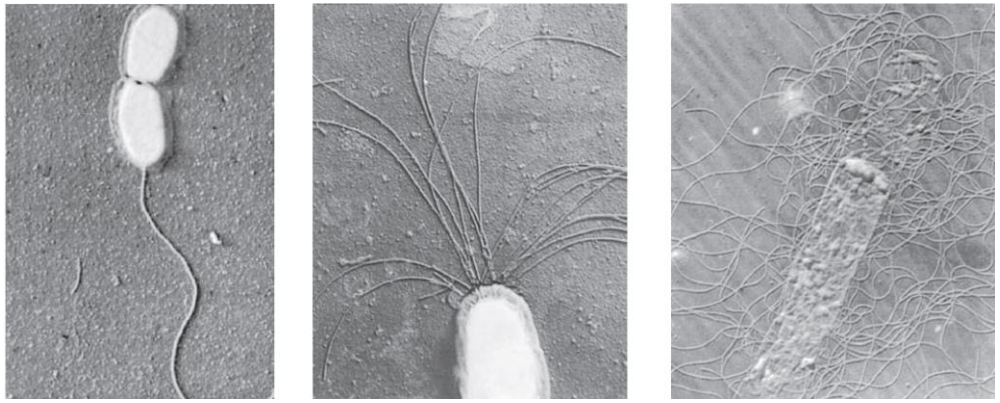
Motility

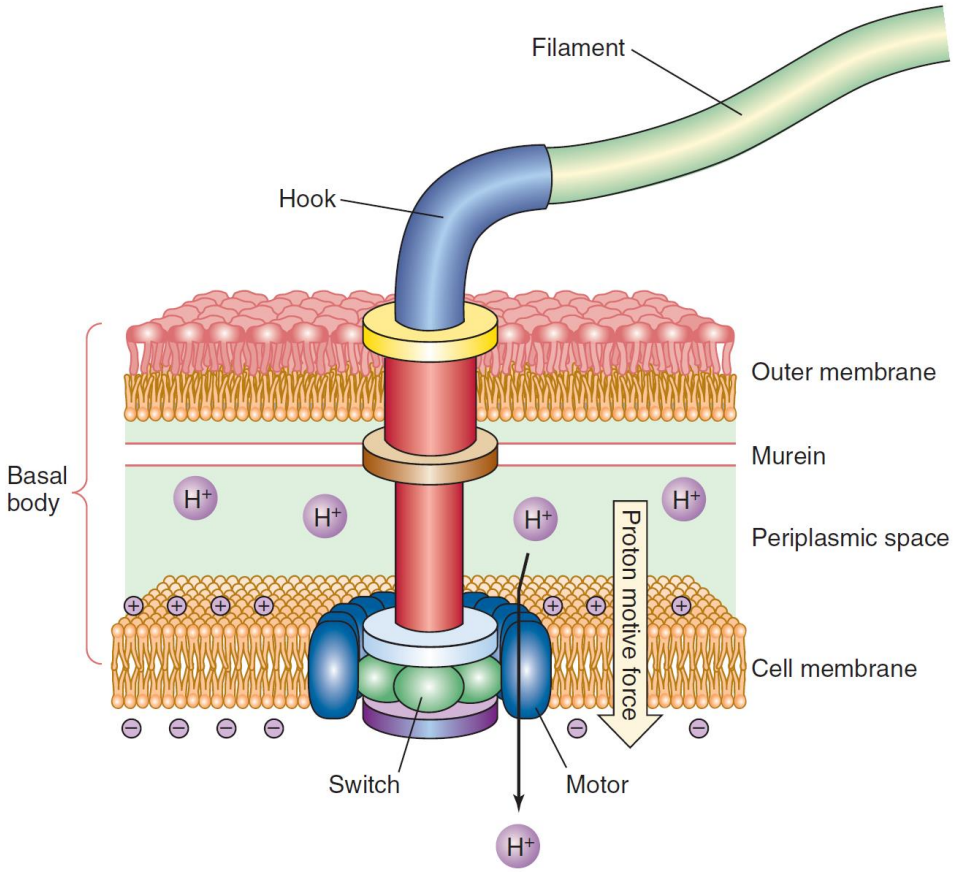
- A huge advantage for bacteria to reach the host, and manoeuvre in the host and evade the immune system is for a bacterium to be **motile** – to have the ability to direct its own movement.

- The bacterial **flagellum** is an amazingly complex molecular machine with a diversity of roles in pathogenesis including reaching the optimal host site, **colonization** or **invasion**, **maintenance** at the infection site, and post-infection **dispersal**

Flagella

- Bacterial flagella are thread-like appendages composed of a protein subunit called **flagellin**.
- Rotation is driven by the flow of protons into the cell down the gradient produced by the primary proton pump
- highly antigenic **(H antigens) (**immune responses to infection can be directed against these proteins).
- **chemotaxis**: the net movement of the cell toward the source (a sugar or an amino acid). cell behavior brought about in response to a change in the environment is called **sensory transduction.**





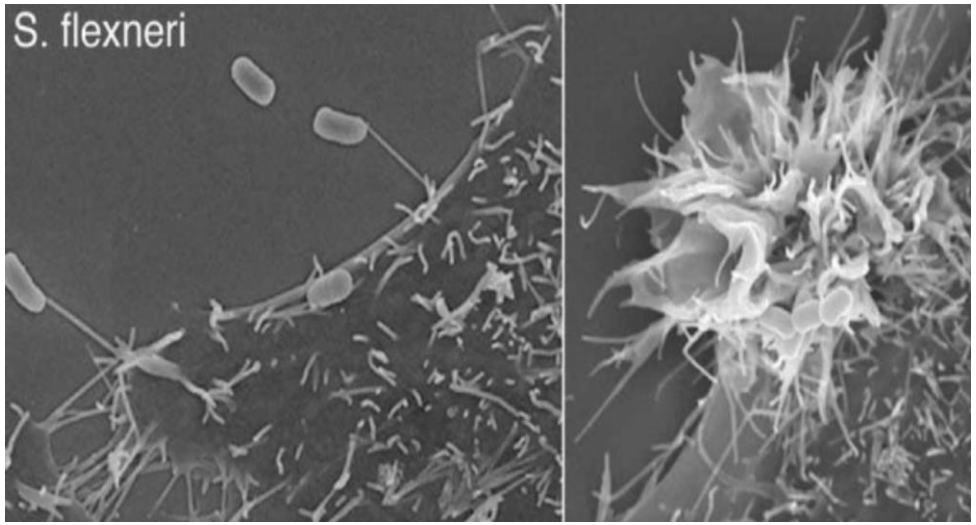
Invasion

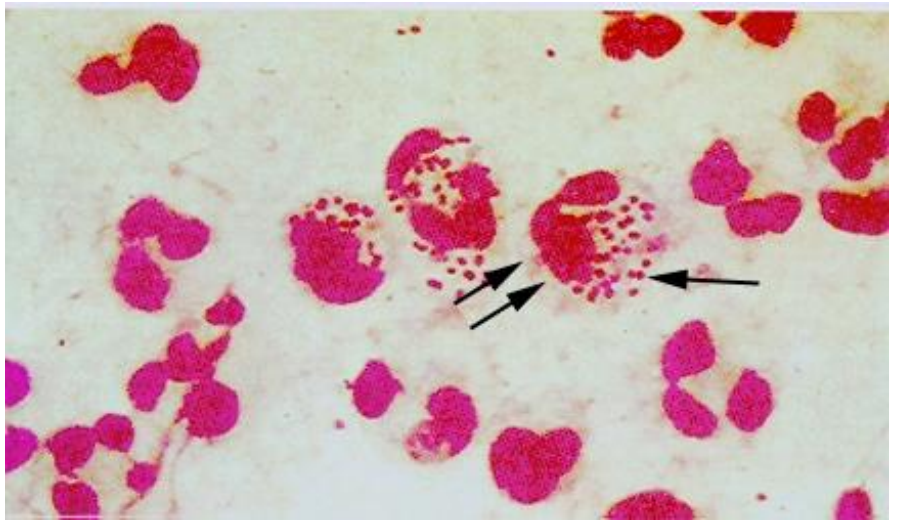
Invasion can happen **through tight junctions of epithelial surfaces**, or **through internalization** into epithelial cells.

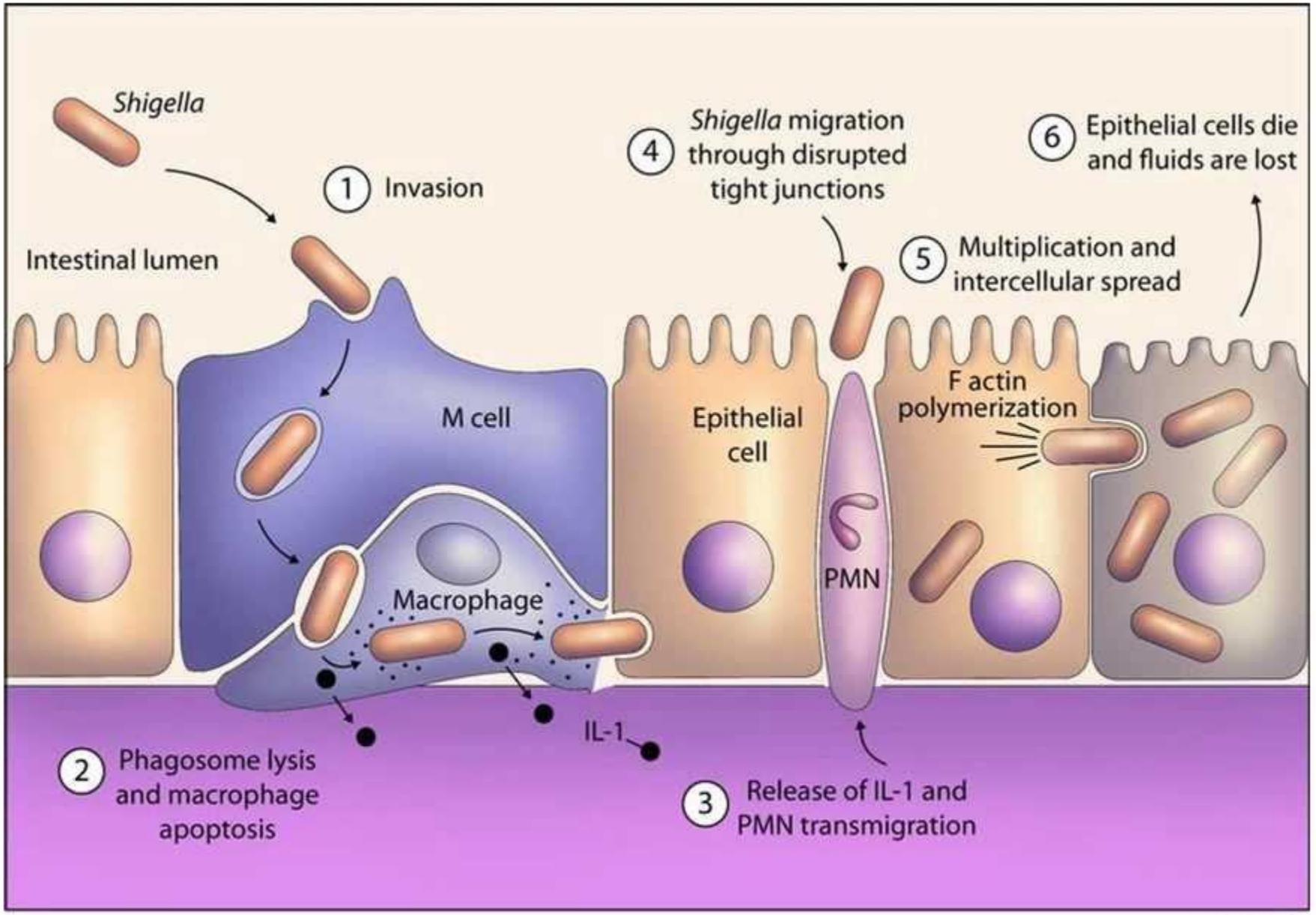
In-vitro models and knockouts are important in understanding the process of invasion**.**

Active process between cells and pathogen. Usually requires **actin polymerization**

Once inside the cells, the bacteria can be transported by **vesicles to the lysosome, or can remain or escape the vesicles to multiply in the cytoplasm, or be released to the extracellular space to invade other cells**. Bacteria can also induce **apoptosis** in cells they invade.






Shigella
1 Invasion
Intestinal lumen
M cell
Macrophage
IL-1
2 Phagosome lysis and macrophage apoptosis
4 Shigella migration through disrupted tight junctions
Epithelial cell
PMN
3 Release of IL-1 and PMN transmigration
5 Multiplication and intercellular spread
F actin polymerization
6 Epithelial cells die and fluids are lost
CTLT

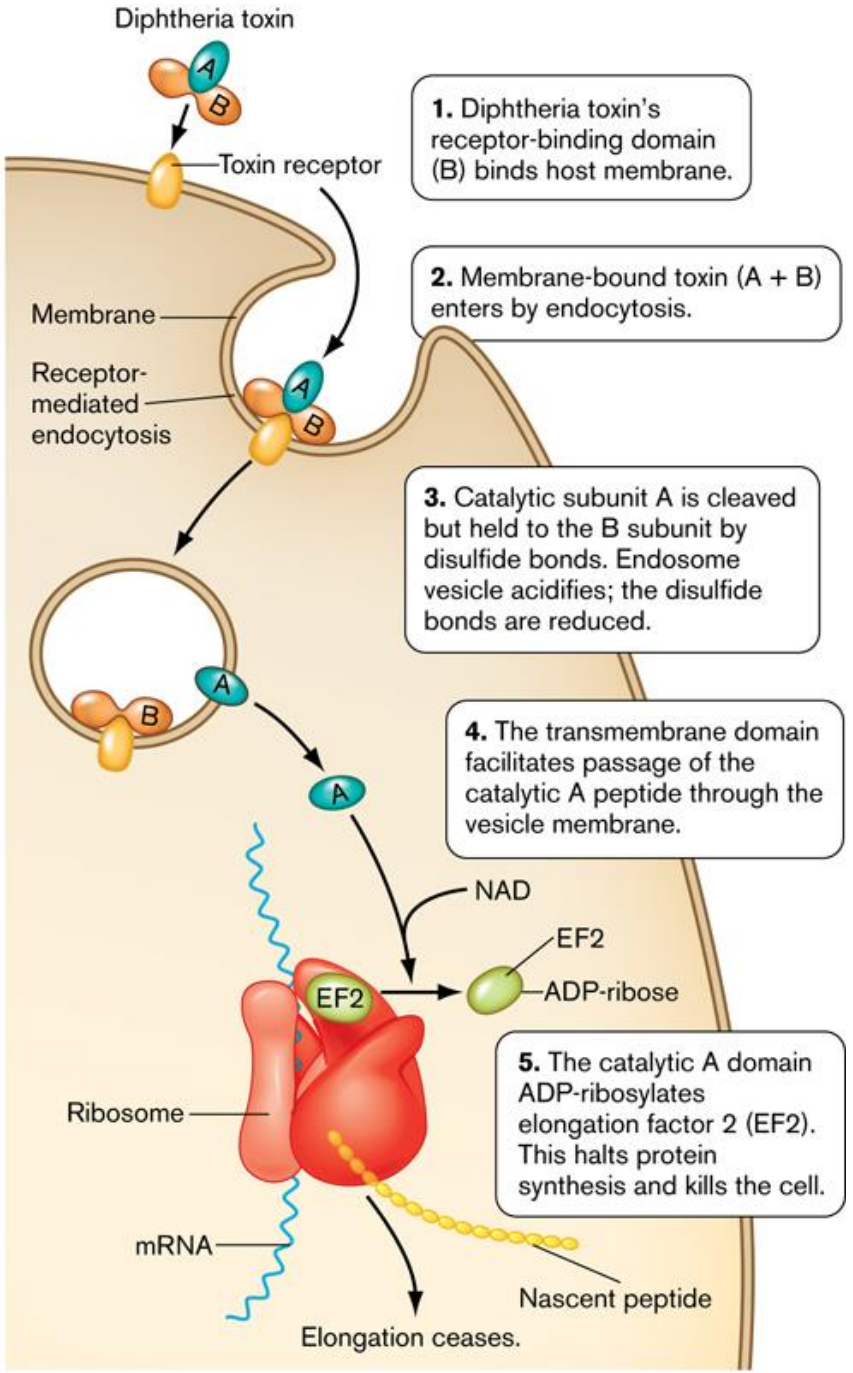
Toxins/ Exotoxins

Exotoxins (secreted actively, by contact only, or by cell death) or **endotoxins** (part of bacterial cell wall) .

Exotoxins are bases for some vaccines (toxoids)

Made of A (toxic activity) and B (helps attachment and internalization into cells) subunits.

Exotoxins associated with diarrheal diseases are frequently called **enterotoxins.**



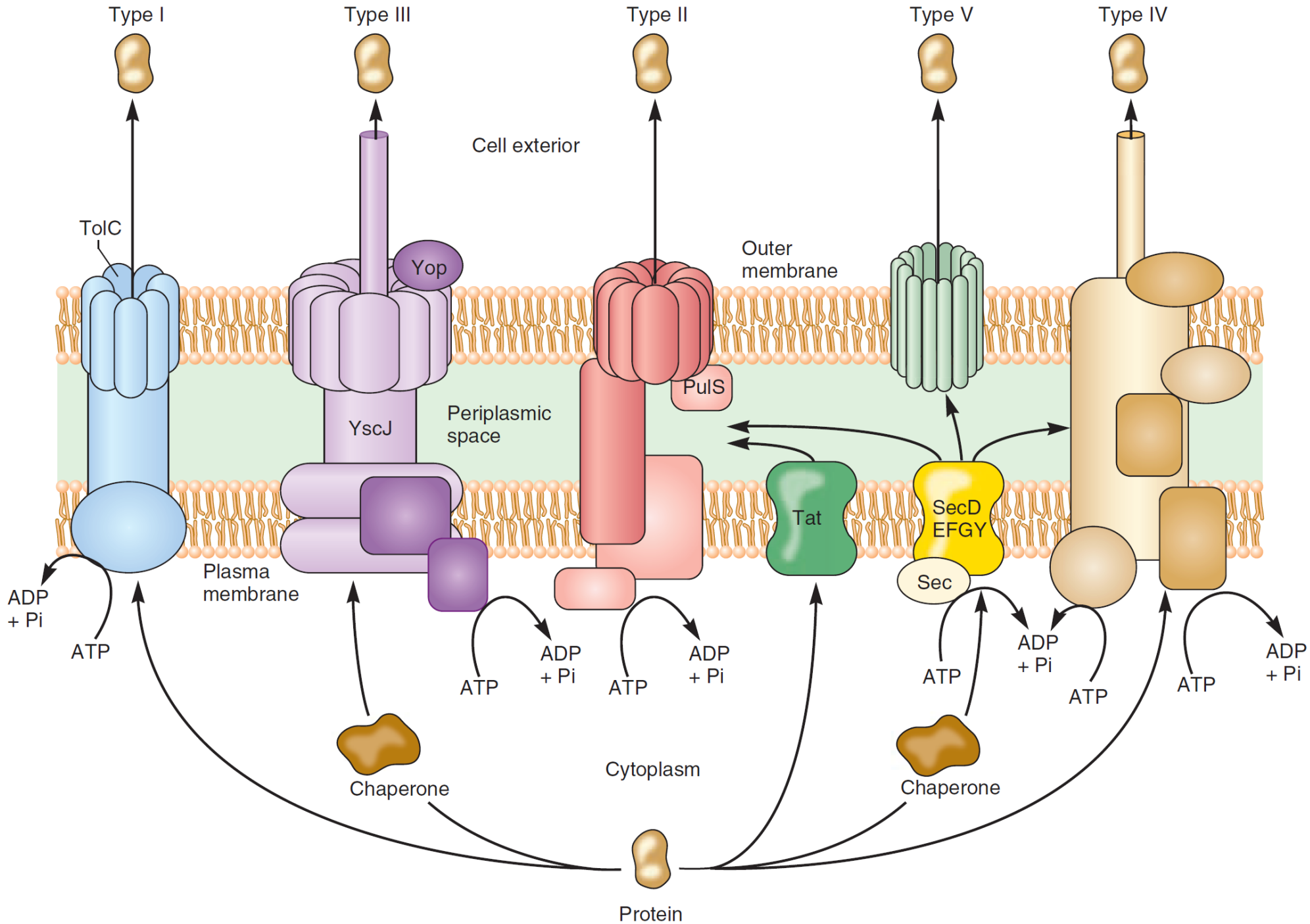
Toxin	Organism	Gene Location	Subunit Structure	Target Cell Receptor	Biological Effects
Anthrax toxins	*Bacillus anthracis*	Plasmid	Three separate proteins (EF, LF, PA)	Tumor endothelial marker-8 (TEM-8); capillary morphogenesis protein 2 (CMG2)	EF + PA: increase in target cell cAMP level, localized edema; LF + PA: death of target cells and experimental animals
Bordetella	*Bordetella* spp.	Chromosomal	A-B	Unknown, probably glycolipid	Adenylate cyclase toxin. Increase in target cell cAMP level, modified cell function, or cell death
Botulinum toxin	*Clostridium botulinum*	Phage	A-B	Polysialogangliosides plus synaptotagmin (co-receptors)	Decrease in peripheral presynaptic acetylcholine release, flaccid paralysis
Cholera toxin	*Vibrio cholerae*	Chromosomal	A-B_5	Ganglioside (GM_1)	Activation of adenylate cyclase, increase in cAMP level, secretory diarrhea
Diphtheria toxin	*Corynebacterium diphtheriae*	Phage	A-B	Growth factor receptor precursor	Inhibition of protein synthesis, cell death
Heat-labile enterotoxins	*Escherichia coli*	Plasmid	Similar or identical to cholera toxin		
Pertussis toxin	*Bordetella pertussis*	Chromosomal	A-B_5	Surface glycoproteins with terminal sialic acid residues	Block of signal transduction mediated by target G proteins
Pseudomonas exotoxin A	*Pseudomonas aeruginosa*	Chromosomal	A-B	α_2-Macroglobulin receptor (α_2-MR)	Similar or identical to diphtheria toxin
Shiga toxin	*Shigella dysenteriae*	Chromosomal	A-B_5	Globotriaosylceramide (Gb3)	Inhibition of protein synthesis, cell death
Shiga-like toxins	*Shigella* spp., *E. coli*	Phage	Similar or identical to Shiga toxin		
Tetanus toxin	*Clostridium tetani*	Plasmid	A-B	Polysialogangliosides plus 15-kDa glycoprotein (co-receptors)	Decrease in neurotransmitter release from inhibitory neurons, spastic paralysis

Secretion systems

Bacterial secretion systems are **protein complexes present on the cell membranes** of bacteria **for secretion of substances**. Specifically, they are the cellular devices used by **pathogenic bacteria** to **secrete their virulence factors** (mainly of proteins) to invade the host cells. They can be classified into different types based on their specific structure, composition and activity.

Type III secretion pathway is a contact-dependent system. It is activated by contact with a host cell, and then injects a toxin protein into the host cell directly.

The type **I and IV secretion systems** have been described in both gram-negative and gram-positive bacteria, but the type **II, III, V, and VI secretion systems have been found only in gram-negative bacteria**.


Type I
Type III
Type II
Type V
Type IV
Cell exterior
TolC
Yop
Outer membrane
PulS
YscJ
Periplasmic space
Tat
SecD EFGY
Sec
Plasma membrane
ADP + Pi
ATP
ATP
ADP + Pi
ATP
ADP + Pi
ATP
ADP + Pi
ATP
ADP + Pi
Chaperone
Cytoplasm
Chaperone
Protein

Secretion systems

Secretion System	Genus Species	Substrate and Role in Pathogenesis
Type 1 (*Sec*-independent)	*Escherichia coli*	α Hemolysin makes holes in cell membranes
	Proteus vulgaris	Hemolysin
	Morganella morganii	Hemolysin
	Bordetella pertussis	Adenylate cyclase which catalyzes synthesis of cAMP
	Pseudomonas aeruginosa	Alkaline protease
	Serratia marcescens	Zn protease yields host cell damage
Type 2 (*Sec* dependent)	*Pseudomonas aeruginosa*	Elastase, exotoxin A, phospholipase C, others
	Legionella pneumophila	Acid phosphatase, lipase, phospholipase, protease, RNAse
	Vibrio cholera	Cholera toxin
	Serratia marcescens	Hemolysin
Type 3 (*Sec*-independent; contact-dependent)	*Yersinia* species	Ysc-Yop system; toxins that block phagocytosis and induce apoptosis
	Pseudomonas aeruginosa	Cytotoxin
	Shigella species	Controls host cell signaling, invasion, and death
	Salmonella enterica subspecies *enterica* serotypes Choleraesuis, Dublin, Paratyphi, Typhi, Typhimurium, and so on	Effectors from *Salmonella* pathogenicity islands I and II (SPI1 and SPI2), which promote attachment to and invasion of host cells
	Escherichia coli	Enterohemorrhagic (EHEC) and enteropathogenic (EPEC); disruption of epithelial barriers and tight junctions
	Vibrio parahaemolyticus	Direct cytotoxicity

Toxins/ Endotoxins

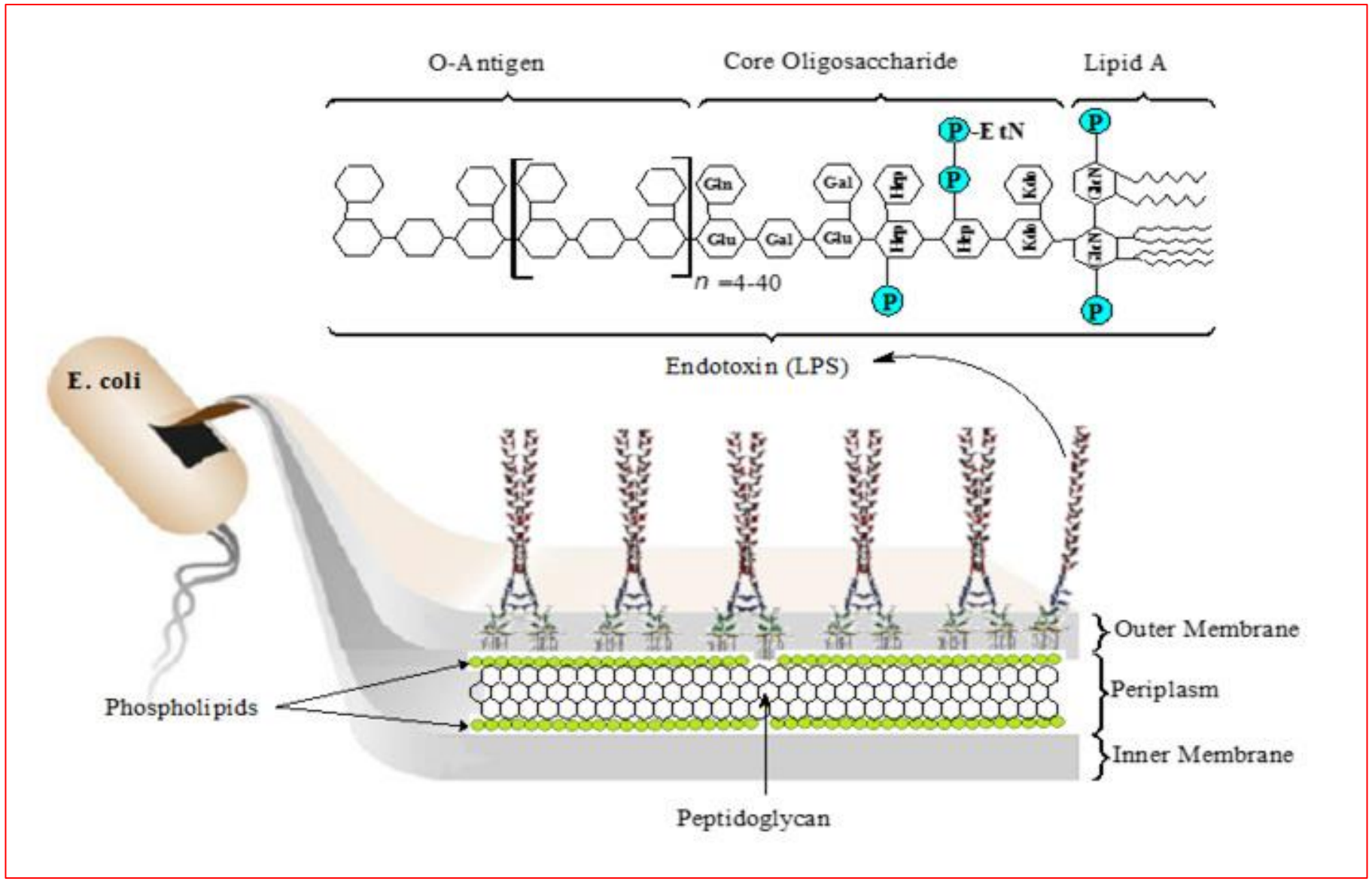
The **LPS** (endotoxin) of gram-negative bacteria are **bacterial cell wall components** that are often liberated when the bacteria lyse.

The substances are **heat-stable**.

In response to LPS**, proinflammatory cytokines** such as IL-1, TNF-α **are released**, and the **complement and coagulation cascades are activated**.

The following can be observed clinically or experimentally:
fever, leukopenia, and hypoglycemia; hypotension and shock resulting in impaired perfusion of essential organs (eg, brain, heart, kidney); intravascular coagulation; and death from massive organ dysfunction.

On the other hand **peptidoglycan** released from gram-positive bacteria can cause similar immune responses, but much **less potent than endotoxin** (LPS).


O-Antigen
Core Oligosaccharide
Lipid A
P-EtN
Glc
Gal
Hep
Kdo
Glu
Gal
Glu
Hep
Hep
Kdo
Kdo
GlcN
GlcN
P
P
P
P
n =4-40
Endotoxin (LPS)
E. coli
Phospholipids
Outer Membrane
Periplasm
Inner Membrane
Peptidoglycan

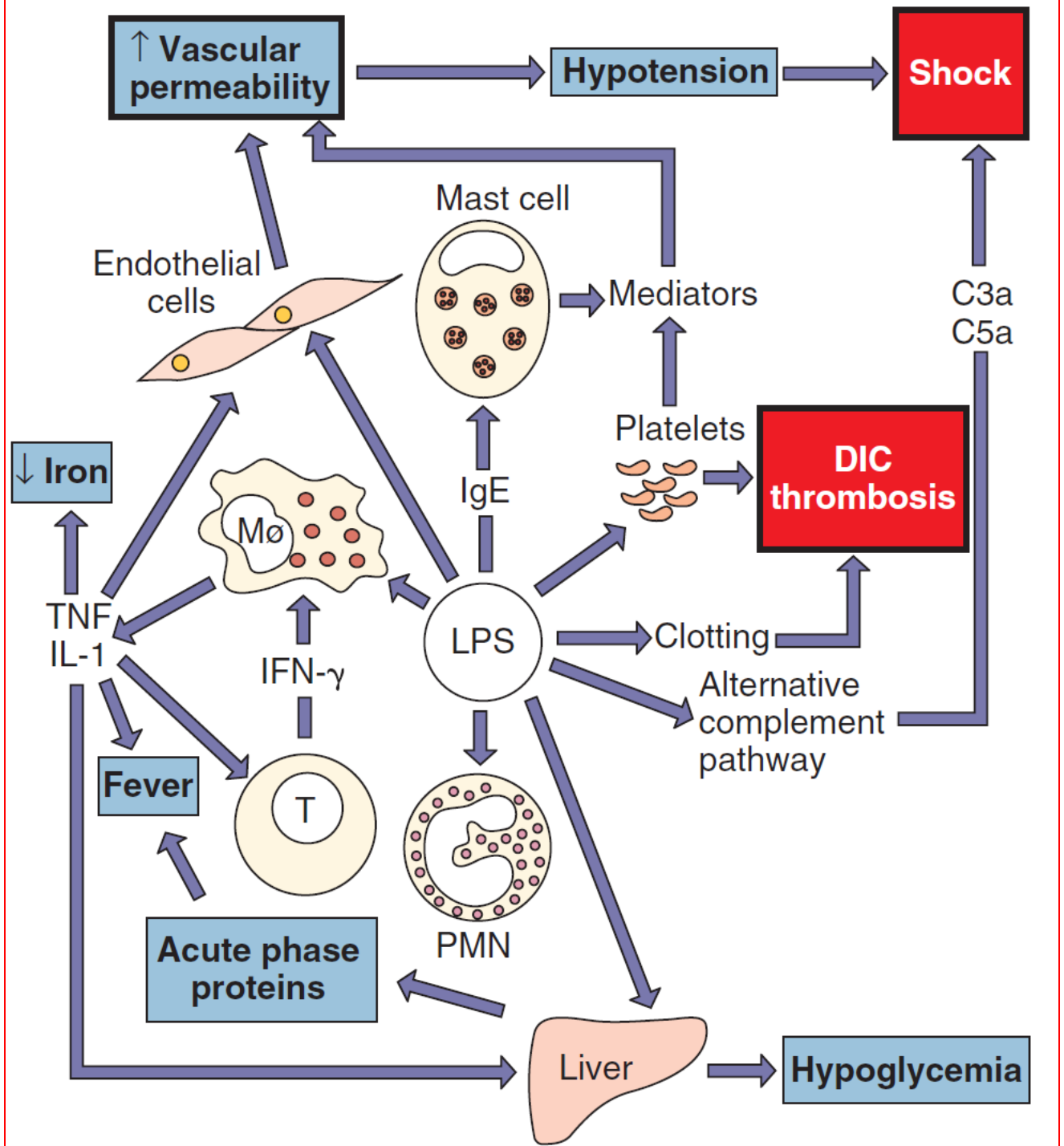

↑ Vascular permeability
Hypotension
Shock
Mast cell
Endothelial cells
Mediators
C3a
C5a
↓ Iron
IgE
Platelets
DIC thrombosis
Mø
TNF
IL-1
IFN-γ
LPS
Clotting
Alternative complement pathway
Fever
T
PMN
Acute phase proteins
Liver
Hypoglycemia

TABLE 9-4 Characteristics of Exotoxins and Endotoxins (Lipopolysaccharides)

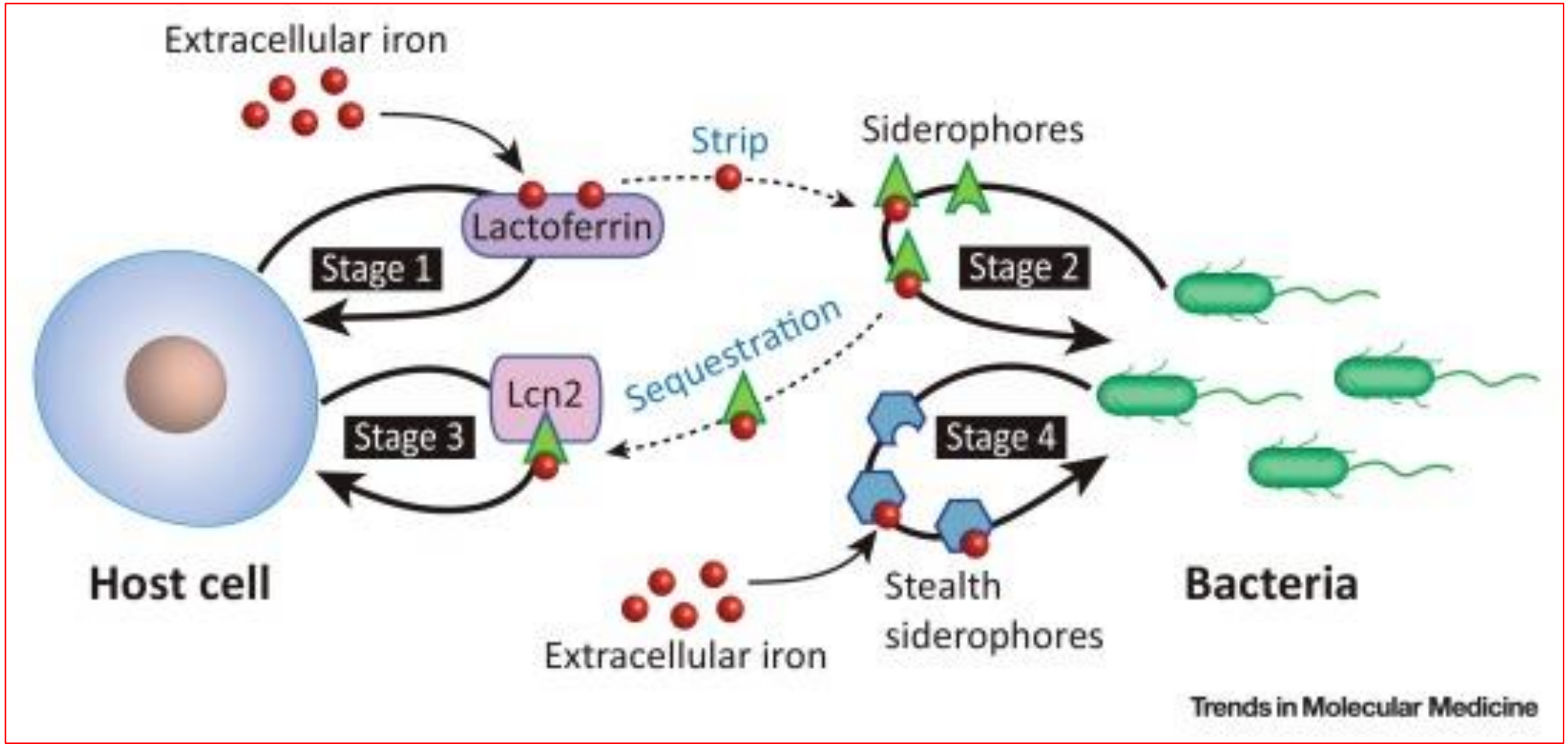
Exotoxins	Endotoxins
Excreted by living cell; high concentrations in liquid medium	Integral part of the cell wall of gram-negative bacteria; released on bacterial death and in part during growth; may not need to be released to have biologic activity
Produced by both gram-positive and gram-negative bacteria	Found only in gram-negative bacteria
Polypeptides with a molecular weight of 10,000–900,000	Lipopolysaccharide complexes; lipid A portion probably responsible for toxicity
Relatively unstable; toxicity often destroyed rapidly by heating at temperatures above 60°C	Relatively stable; withstand heating at temperatures above 60°C for hours without loss of toxicity
Highly antigenic; stimulate formation of high-titer antitoxin; antitoxin neutralizes toxin	Weakly immunogenic; antibodies are antitoxic and protective; relationship between antibody titers and protection from disease is less clear than with exotoxins
Converted to antigenic, nontoxic toxoids by formalin, acid, heat, and so on; toxoids are used to immunize (eg, tetanus toxoid)	Not converted to toxoids
Highly toxic; fatal to animals in microgram quantities or less	Moderately toxic; fatal for animals in tens to hundreds of micrograms
Usually bind to specific receptors on cells	Specific receptors not found on cells
Usually do not produce fever in the host	Usually produce fever in the host by release of interleukin-1 and other mediators
Frequently controlled by extrachromosomal genes (eg, plasmids)	Synthesis directed by chromosomal genes

Iron uptake mechanisms

- Most of the iron in a mammalian body is complexed with various proteins. Moreover, **in response to infection, iron availability is reduced** in both extracellular and intracellular compartments.

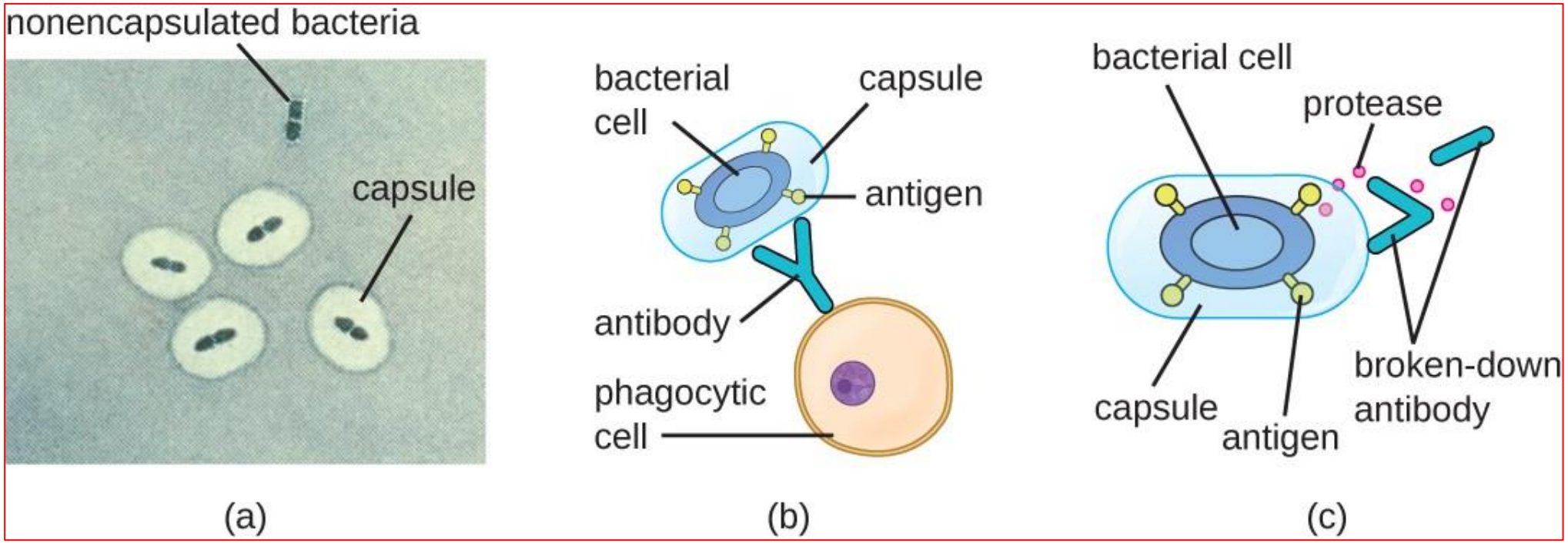
- **Bacteria need iron for growth** and successful bacterial pathogens have therefore evolved to compete successfully for iron in the highly iron-stressed environment of the host's tissues and body fluids, for example, through production of **siderophores**.

- **Siderophores** (Greek: "iron carrier") are small, high-affinity **iron-chelating compounds** secreted by microorganisms such as bacteria and fungi .


Extracellular iron
Strip
Siderophores
Lactoferrin
Stage 1
Stage 2
Sequestration
Lcn2
Stage 3
Stage 4
Host cell
Stealth
siderophores
Bacteria
Extracellular iron
Trends in Molecular Medicine

Evasion of the host immune system

Pathogenic bacteria can evade phagocytosis in many ways, examples include **capsule production**, **Protein A in Staph aurues binds antibodies in an inactive manner.** Some bacteria produce proteins that **inhibit complement activation,** therby decresing immune signaling and opsonization* of bacteria**.** Intracellulaly some bacteria **inhibit phagolysosome fusion.**

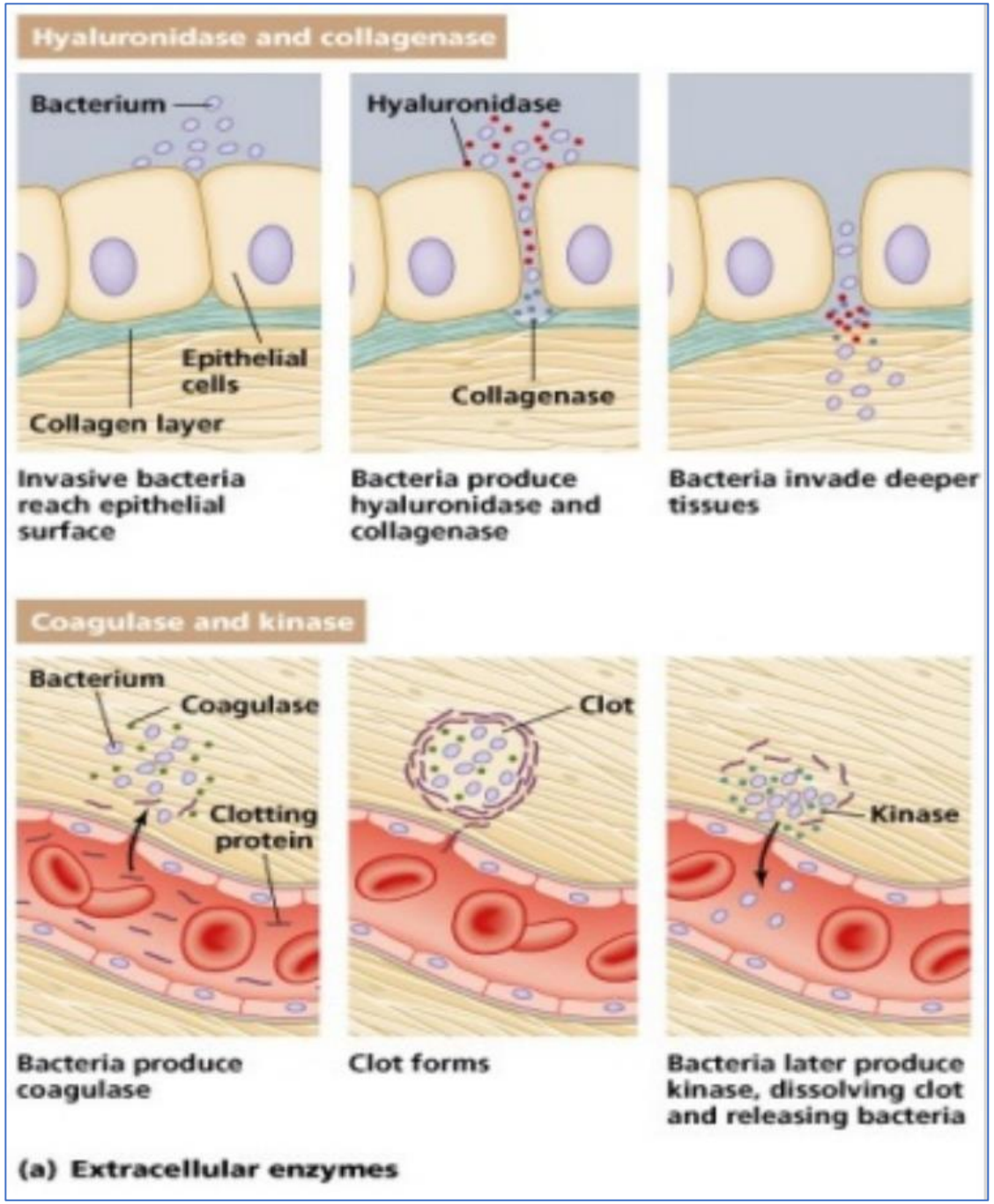


* **Opsonization** is the process in which bacteria is covered by substances to enhance phagocytosis. For example, antibodies bound on bacterial surface, as well as activated complement components depositing on bacterial surfaces are considered ”opsonins” since the make the bacteria easier to phagocytose.

Enzyme production

Pathogenic bacteria produce enzymes to degrade tissues and spread infection. E.g **Hyaluronidase and collagenase** are enzymes that hydrolyze hyaluronic acid and collagen respectively, constituents of the ground substance of connective tissue.

Bacteria produce **cytolysins which directly kill cells** usually by forming pores in their membranes **(e.g. hemolysins, leukocidins)**.



Pathogenicity islands

Chromosomal or extra chromosomal discrete genetic units that encode genes that aid in the virulence of a bacteria by coding for **adhesins, secretion systems (like type III secretion system), toxins, invasins, capsule synthesis, iron uptake systems** .

Absent in non-pathogenic bacteria. Virulence genes are **usually activated by environmental cues (e.g. Temperature change).**

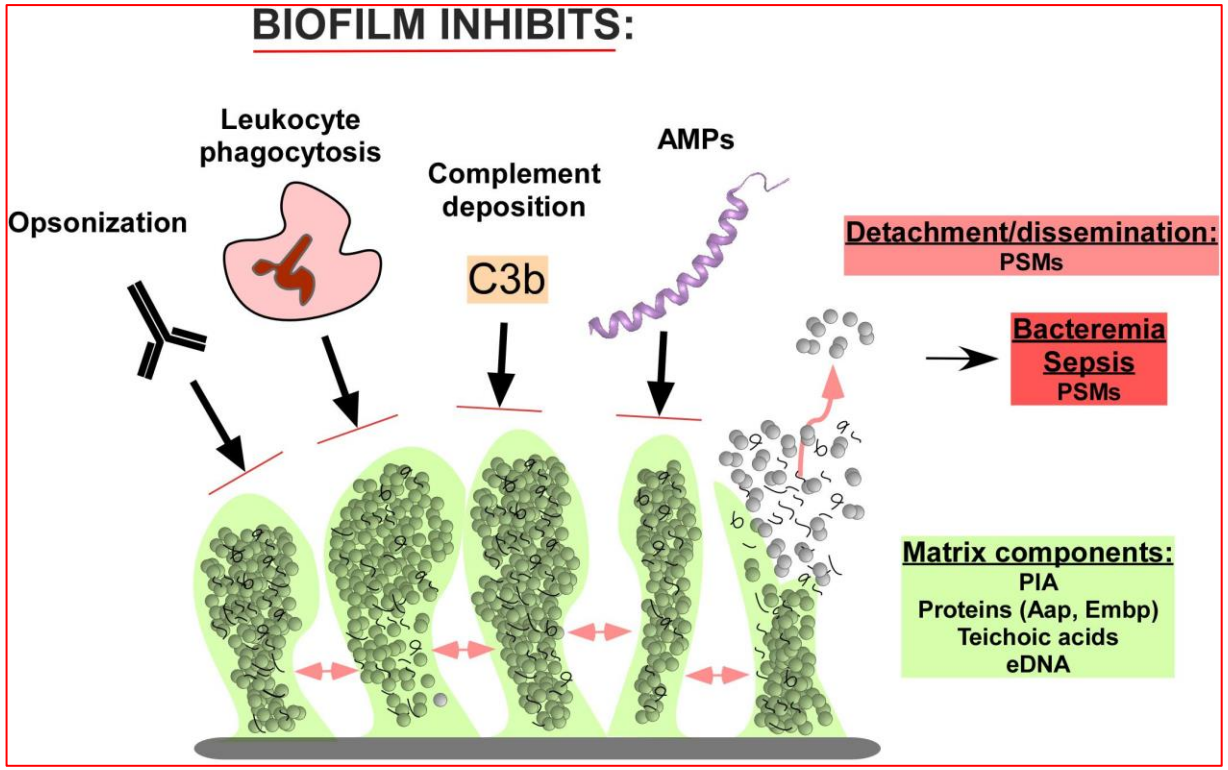
Commonly found on mobile genetic elements **(passed through plasmids, transformation, transduction, transposons**), the **G-C content** of pathogenicity islands is usually different from the rest of the genome.

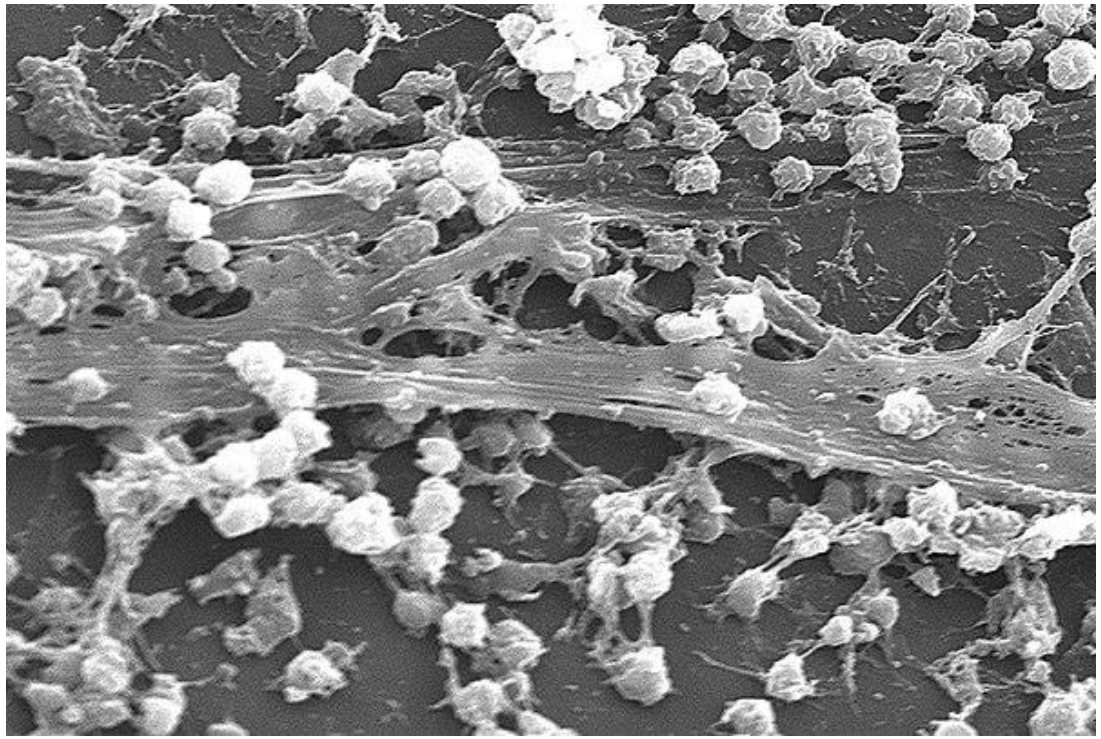
TABLE 9-2 Examples of Virulence Factors Encoded by Genes on Mobile Genetic Elements

Genus and Species	Virulence Factor and Disease
Plasmid encoded	
Escherichia coli	Heat-labile and heat-stable enterotoxins that cause diarrhea
Escherichia coli	Hemolysin (cytotoxin) of invasive disease and urinary tract infections
Escherichia coli and *Shigella* species	Adherence factors and gene products involved in mucosal invasion
Bacillus anthracis	Capsule essential for virulence (on one plasmid)
	Edema factor, lethal factor, and protective antigen are all essential for virulence (on other plasmids)
Phage encoded	
Clostridium botulinum	Botulinum toxin that causes paralysis
Corynebacterium diphtheriae	Diphtheria toxin that inhibits human protein synthesis
Vibrio cholerae	Cholera toxin that can cause a severe watery diarrhea

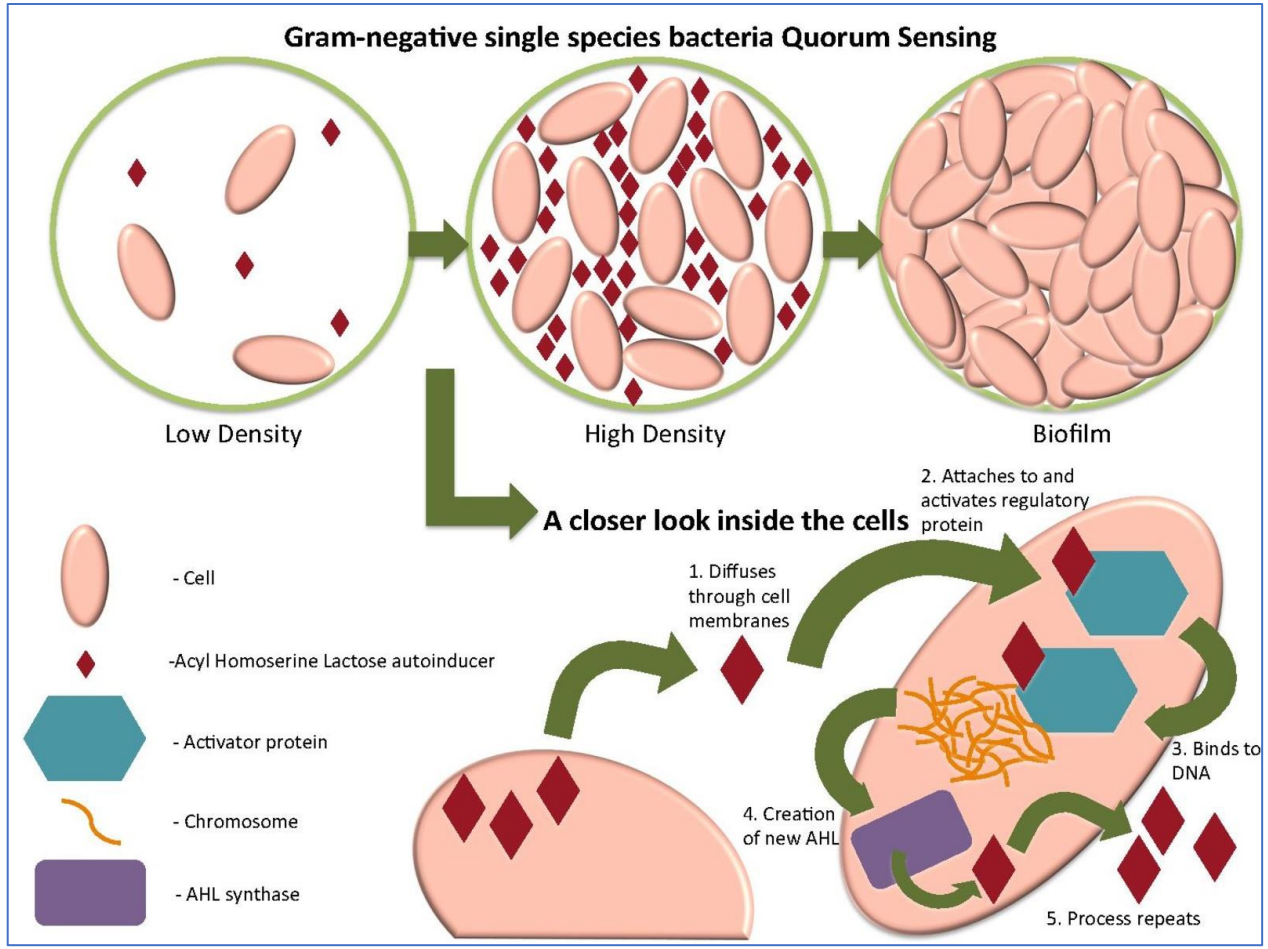
Bacterial communities / Biofilm and pathogenesis

A biofilm is an aggregate of interactive bacteria attached to a solid surface or to each other and encased in **EPS**.
The cells within the biofilm produce the EPS (extracellular polymeric substances) components, which are typically a polymeric conglomeration of **extracellular polysaccharides, proteins, lipids and DNA**
Biofilms may form on living or **non-living surfaces** and can be prevalent in natural, industrial **and hospital settings**.
Helps in persistence on surfaces, evasion of the immune response and antimicrobial resistance and dissemination.





Bacterial communities / Quorum sensing and pathogenesis



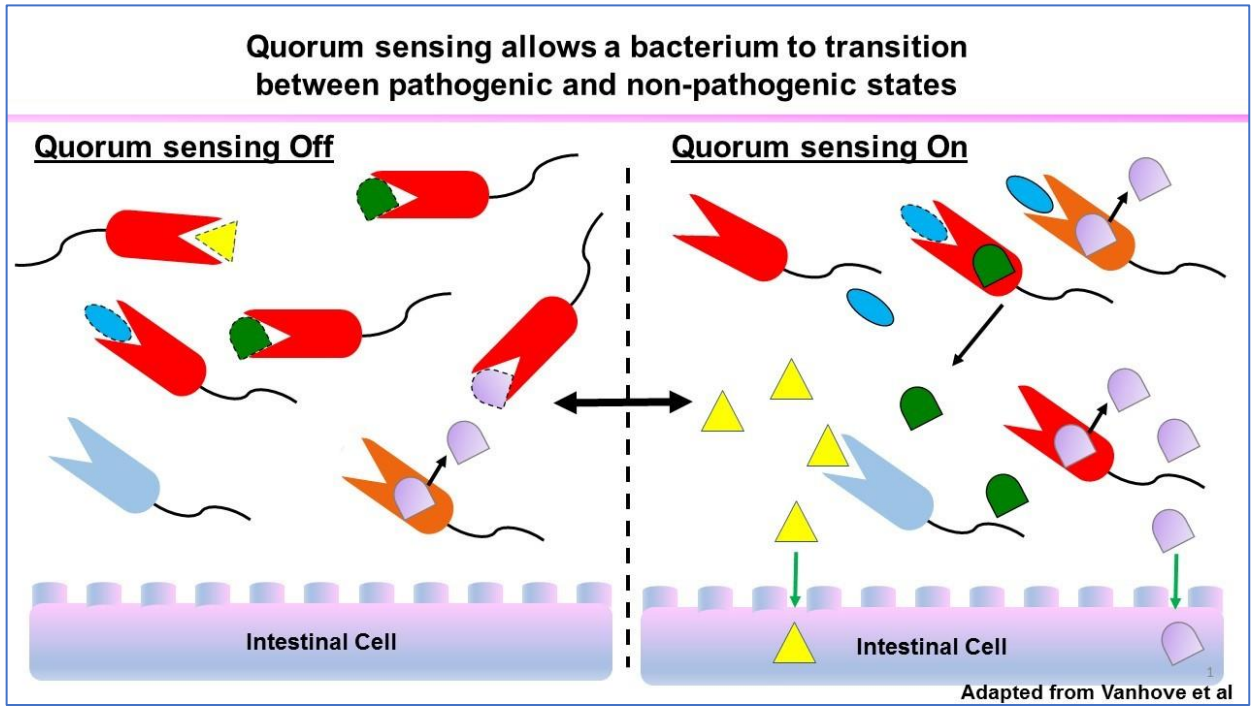


TABLE 9-1 Guidelines for Establishing the Causes of Infectious Diseases

Koch's Postulates	Molecular Koch's Postulates	Molecular Guidelines for Establishing Microbial Disease Causation
1. The microorganism should be found in all cases of the disease in question, and its distribution in the body should be in accordance with the lesions observed. 2. The microorganism should be grown in pure culture in vitro (or outside the body of the host) for several generations. 3. When such a pure culture is inoculated into susceptible animal species, the typical disease must result. 4. The microorganism must again be isolated from the lesions of such experimentally produced disease.	1. The phenotype or property under investigation should be significantly associated with pathogenic strains of a species and not with nonpathogenic strains. 2. Specific inactivation of the gene or genes associated with the suspected virulence trait should lead to a measurable decrease in pathogenicity or virulence. 3. Reversion or replacement of the mutated gene with the wild-type gene should lead to restoration of pathogenicity or virulence.	1. The nucleic acid sequence of a putative pathogen should be present in most cases of an infectious disease and preferentially in anatomic sites where pathology is evident. 2. The nucleic acid sequence of a putative pathogen should be absent from most healthy control participants. If the sequence is detected in healthy control participants, it should be present with a lower prevalence as compared with patients with disease and in lower copy numbers. 3. The copy number of a pathogen-associated nucleic acid sequence should decrease or become undetectable with resolution of the disease (eg, with effective treatment) and should increase with relapse or recurrence of disease. 4. The presence of a pathogen-associated nucleic acid sequence in healthy subjects should help predict the subsequent development of disease. 5. The nature of the pathogen inferred from analysis of its nucleic acid sequence should be consistent with the known biologic characteristics of closely related organisms and the nature of the disease. The significance of a detected microbial sequence is increased when microbial genotype predicts microbial morphology, pathology, clinical features of disease, and host response

Further reading and material:

- Jawetz, Melnick & Adelberg's Medical Microbiology, 26th edition-
  Section 3: Bacteriology
    Chapter 9: Pathogenesis of bacterial infections