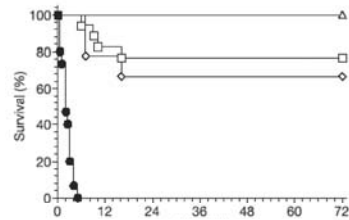


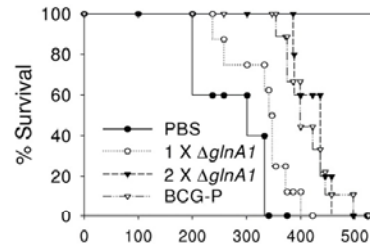
Extreme virulence

anthrax



4 hours

tuberculosis



8,400 hours

Streptococcus pyogenes:

Commensal -----flesh eating

- Tonsillopharyngeal cellulitis or abscess
- Otitis media
- Sinusitis
- Necrotizing fasciitis
- Streptococcal bacteremia
- Meningitis
- Tonsillopharyngitis
- Acute rheumatic fever
- Acute glomerulonephritis
- Streptococcal toxic shock syndrome (strep TSS)

Necrotizing fasciitis



Tonsillar pharyngitis

Copyright © 2008 Pearson Education, Inc., publishing as Benjamin Cummings.

Pathogenicity

VS

Virulence

virulence

- 1. How many bacteria are required to cause disease (infectious dose)
- 2. How much damage to host
- 3. How fast host succumbs

Infectious
Dose (humans)

scrub typhus	3	i.d.
tularemia	2-10	inhalat'n
malaria	10	i.v.
syphilis	57	i.d.
Typhoid fever	10^5	ingest
cholera	10^8	ingest
<i>E. coli</i>	10^8	ingest
shigella	10^9	ingest
Measles	0.2	inhalat'n
Polio virus	2	ingest
Coxsackie virus	18	inhalat'n
Influenza A2	790	inhalat'n

STEP	Phenomenon	Requirement
Attachment Entry into host	Infection Entry	Evade host's mechanisms of protection and cleansing
Local or general spread	Local events Spread	Evade immediate local defenses and natural barriers to spread
Multiplication	Multiplication	Many offspring will die in host or en route to fresh host
Evasion of host defenses	Microbial answers to host defense	Evade phagocytosis and immune defenses long enough to complete full cycle
Shedding/exit	Transmission	Ensure spread to new host
Cause damage in host	Pathology Disease	NOT STRICTLY NECESSARY but often occurs

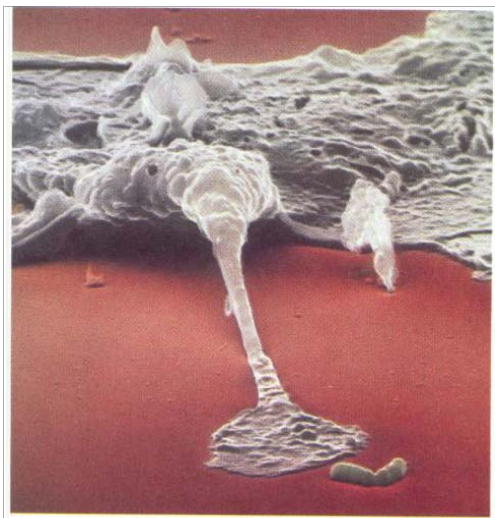
Mims 1995

“Balanced” pathogenicity

- Pathogens evolve to be balanced:
 - Too virulent is not good, indicates emerging pathogens
- Imbalance:
 - Moving from one part of the world to another (TB, yellow fever)
 - Humans are an irrelevant host (rabies, psittacosis, plague)
 - Domesticated animal “spillover” (anthrax)
 - Exotic novel areas (Marburg, Lassa)

Encounter with phagocytic cell

- Central feature of infection and pathogenesis
- Ingest, kill and digest
- Success of these processes determines the course of infection



MOVIE

Why study microbial pathogenesis?

- What defenses are most important in preventing infection?
- How to identify people who lack necessary defenses?
- How to restore or replace these defenses?
- Urgent because of MDR-many diseases are no longer treatable
- Need drugs and vaccines

Host-parasite interactions

- Host defenses:
 - Body surfaces
 - Tissue and blood
- Virulence factors
 - colonization
 - damage
 - regulation of expression

Prevent contact between host and pathogen

- Clean water supplies **intestinal infections**
- Food handling and storage
- Less crowded living conditions **aerosols**
- Screens, sanitation, insecticides **insect-born**

Skin

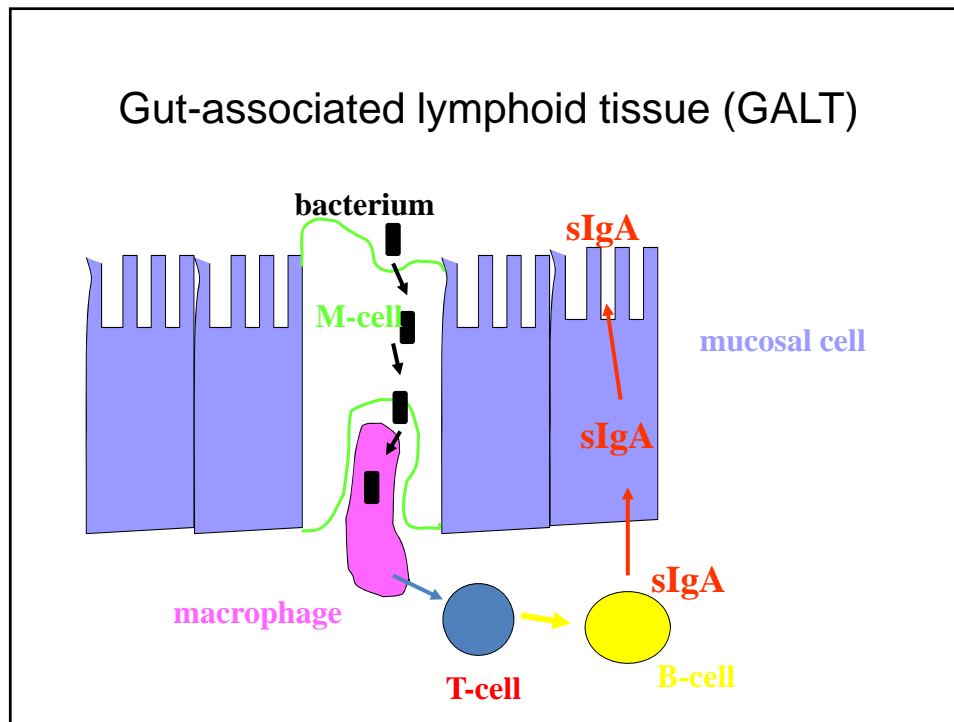
- **No example of a bacterium that can penetrate the skin unaided**
- **Dry, acidic, 37°C, layers, pores protected by lysozyme**
- **Resident microflora: mostly Gram positive**
- **Wounds or burns: SALT (skin-associated lymphoid tissue)**
- **Absence of SALT: *Pseudomonas*, *Staph aureus***

Mucous membranes

- **GI, respiratory and urogenital tract**
- **Secretion and absorption: fluid**
- **Protective cells:**
 - **goblet: produce mucus**
 - **M cells (microfold): present antigen**
 - **ciliated: (propel fragments of mucus away)**
- **MALT and GALT**

Mucus

- Polysaccharides and proteins
- Lubricant
- Physical barrier, trap bacteria
- Secretory immunoglobulin A (sIgA)
- GALT (Gut-associated lymphoid tissue)
- MALT (mucosal-associated lymphoid tissue)
- Bacteriocidal substances
 - lysozyme (degrades peptidoglycan)
 - Lactoferrin (iron competition)
 - Lactoperoxidase (superoxide radicals)



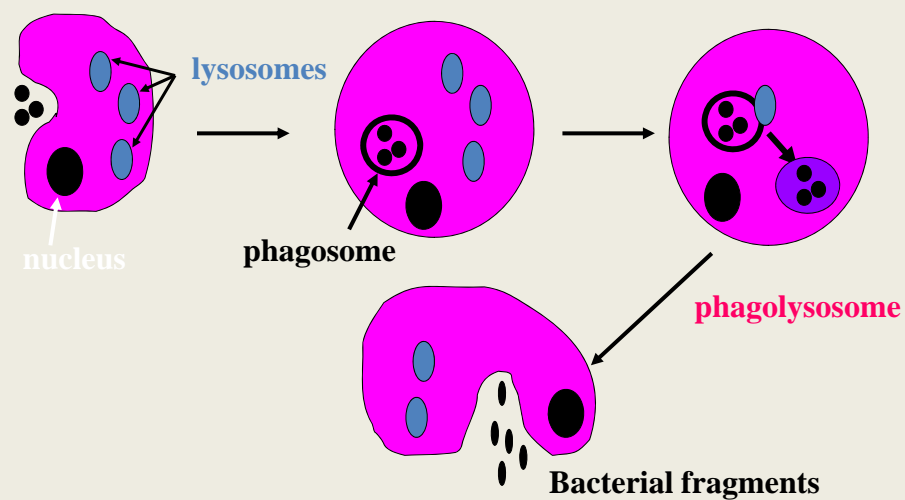
Specific mucosal surfaces

- Mouth (saliva, normal flora)
- Respiratory tract (nose: hairs; lungs: cilia, coughing, alveolar macrophages)
- Eyes (blinking, tears: most vulnerable site)
- Intestinal tract (pH, bile, washing, flora)
- Urogenital tract (pH, biofilm, flora)

Tissue and blood

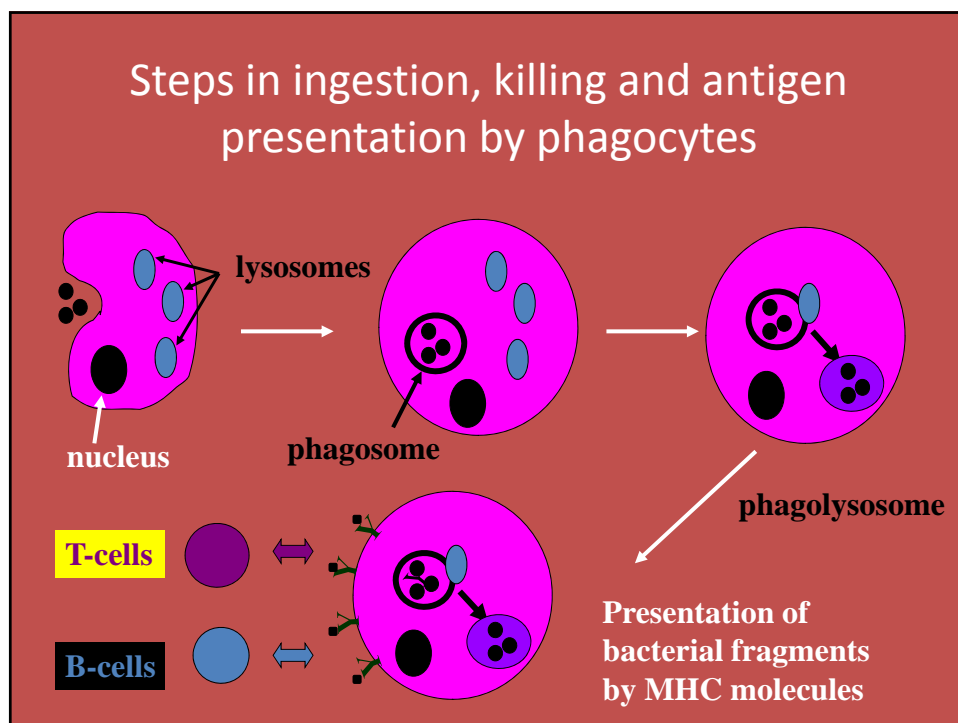
- Constitutive, non-specific defenses
 - Transferrin: iron sequestration
 - Mannose-binding protein (MBP): induced by IL-6
 - Inflammation: redness, swelling, pain
 - Complement: serum proteins that attract phagocytes
 - Macrophages/phagocytes

Steps in ingestion and killing by phagocytes



Induced defenses

- Antibodies
- Antibody production
- Activation of macrophages
- Cytotoxic T cells



Activation of macrophages

- Mediated by γ -interferon (γ -IFN)
- Increased killing capacity
 - Reactive oxygen intermediates
 - Reactive nitrogen intermediates

What is virulence?

- **Infection vs. disease**
- **Colonization/asymptomatic carriage**
- **Virulence: the ability of a bacterium to cause infection**
- **Virulence factor: product or strategy that promotes ability to cause disease**

“Koch’s postulates”

- How could you prove that a specific organism causes a specific infection?

Koch’s postulates

- **1. Bacterium should be found in all hosts with the disease.**
- **2. Bacterium should be isolated from lesions of infected host and maintained in pure culture.**
- **3. Pure culture should produce symptoms of disease when inoculated into a susceptible host.**
- **4. Same bacterium should be reisolated in pure culture from the intentionally infected host.**

Limitations to Koch's postulates

- Assumption: that virulence is a trait entirely contributed by the bacterium, independent of the host
- Emphasis: on ability to culture organism
- Assumption: that different strains of same species are equally virulent
- Assumption: that a single species causes disease
- Requirement that organism be reinoculated into human or animal

Koch's Postulates: The Molecular Version

- Gene should be found in all strains that cause disease and not in avirulent strains
- Disruption of gene in virulent organism should reduce virulence
- Introducing gene into avirulent host should induce virulence
- Gene should be expressed in animal or human volunteer during disease

Colonization and invasion

- **Adherence**
 - Pili, or fimbriae
 - Afimbrial adhesins
 - Adhesins of Gram-positive bacteria
 - Integrins
 - Motility and chemotaxis
 - sIgA proteases
 - Iron acquisition

Surviving host offenses:

- Complement
- Phagocytosis
- Induced immune defenses (antibodies, specific cytotoxic cells, etc)

Evading complement

- Bacteria produce capsules: protect against opsonization and killing by phagocytes
- Host response against capsules: produce antibodies that binds the capsule
 - Vaccine strategy: capsular material prevents infection by encapsulated bacteria
- Bacteria can modify complement binding sites (like LPS)

Surviving phagocytosis

- **Some bacteria survive within phagocytes: impervious to host defenses**
 - Escape vacuole before fusion with lysosome
 - Prevent fusion from occurring
 - **Acquire traits that resist toxic compounds**
 - Resistance to defensins
 - Catalase and superoxide dismutase
 - Cell walls refractory to lysozymal proteases and lysozyme

Evading the host's antibody response

- Changing pilus type
- Antigenic variation
- Coat surface with host-like substances
- Subvert antibody binding:
 - **protein A binds Fc portion and inverts IgG**
 - **bind lactoferrin: dual function**

Virulence factors that damage the host

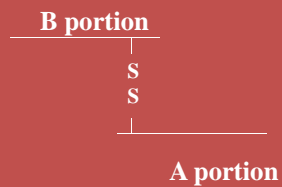
- Endotoxin and other toxic cell wall components; septic shock
- Exotoxins (cholera, anthrax)
- Hydrolytic enzymes
 - Hyaluronidases, proteases, DNases
- Bacterial products that provoke an autoimmune response

Endotoxin

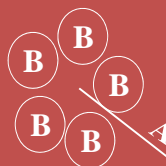
- Lipopolysaccharide
- Lipid A (toxic) + core + O antigen
- Lipid A is embedded in OM; lysis
- Activates complement, stimulates cytokine release

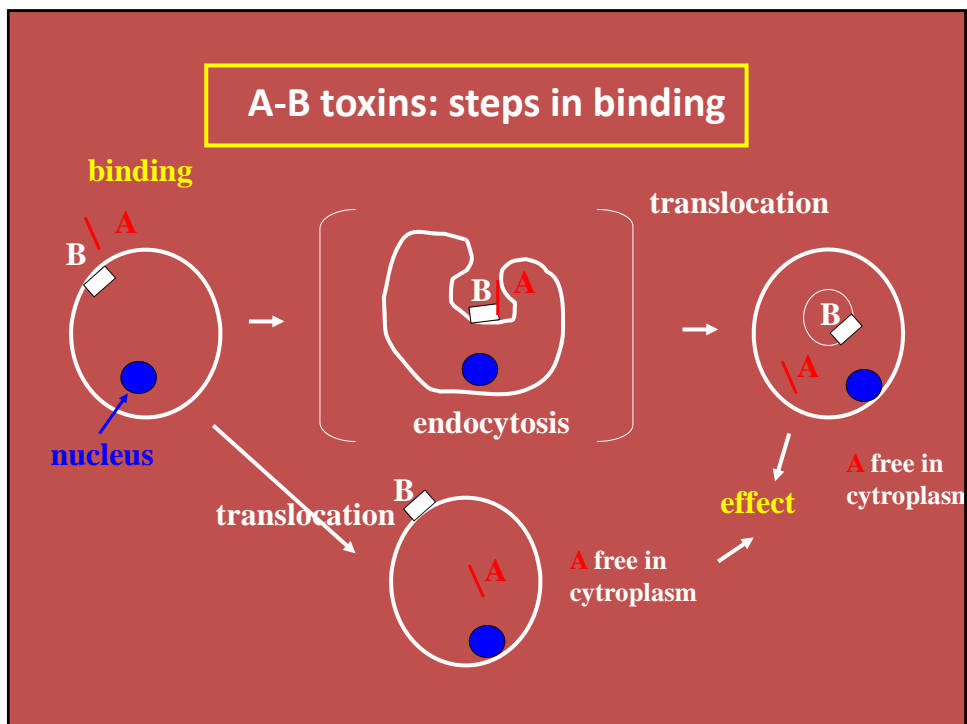
AB-type toxins

Simple A-B



Compound A-B toxin





Other toxic cell wall components

- **Gram positives:**
 - Peptidoglycan
 - Teichoic acids
 - combination

Regulation of virulence genes

- **Adaptive challenges faced by bacteria**
 - Temperature
 - Osmolarity
 - pH
 - O₂
 - Carbon/nitrogen
 - Iron
 - surfaces

CHANGE IN DNA SEQUENCE

gene amplification

gene rearrangement

CHANGE IN NUMBER OF TRANSCRIPTS

activators

**transcript
stability**

repressors

CHANGE IN AMOUNT OF GENE PRODUCT

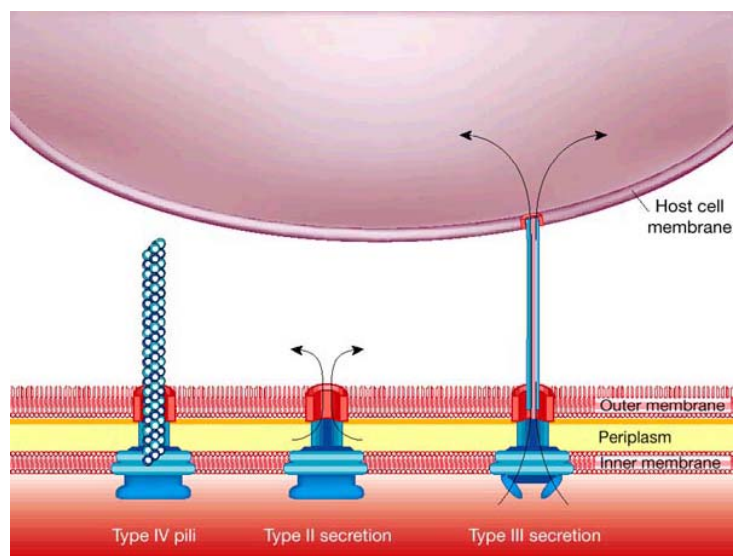
**Covalent
modification**

**Proteolytic
cleavage**

**Binding to
host cell proteins**

Type III secretion apparatus

- Export system that delivers proteins through both inner and outer membranes of Gram negative pathogens
- >50 genes required; conserved among different species
- Large cytoplasmic bulb with a protruding needle that inserts into target host cell
- Example: *Yersinia* (plague): injects proteins that block phagocytosis and induce suicide (apoptosis)



Summary

- **1. Virulence**
- **2. Constitutive host defenses**
- **3. Induced host defenses**
- **4. Bacterial virulence determinants**
- **5. Host response to virulence determinants/bacterial response to host defenses**
- **6. Regulation of expression of virulence**