Virulence factors and their importance in pathology

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Current challenges in microbiology:

Major cause of mortality:
3rd frequent cause of mortality in developed countries

New pathogens:
SARS, AIDS, *Helicobacter pylori,* Q fever, *...*

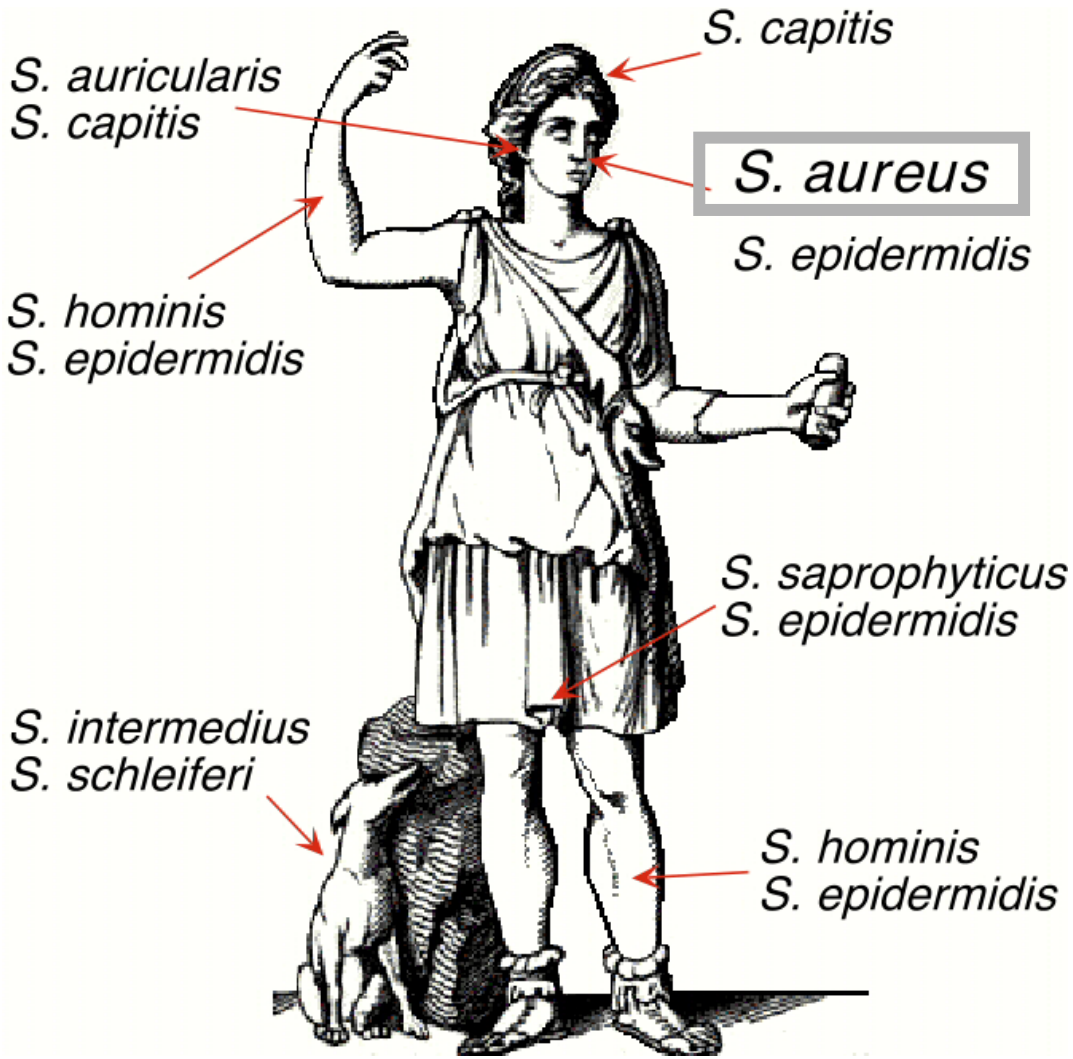
Antibiotic resistance:
Multiresistant staphylococci, enterococci, mycobacteria,...

Bioterrorism:
Anthrax, smallpox...

Sepsis patient

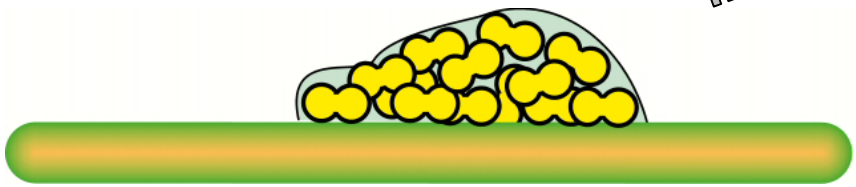
The human body surface is an ecosystem for > 500 bacterial species

Staphylococci on human skin

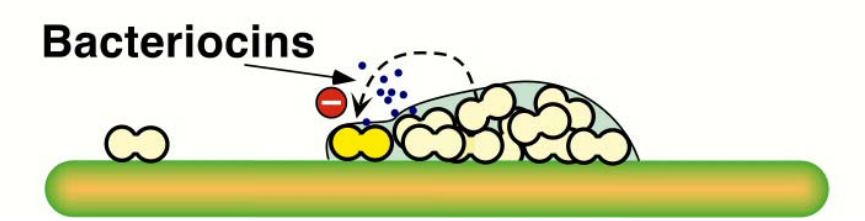


Why do certain bacteria cause disease?

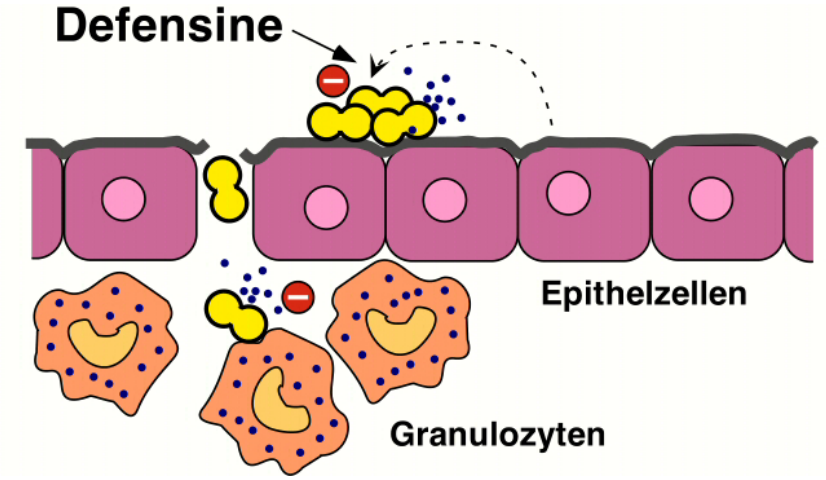
What to do bacteria do when they are starving?



1. Inhibit competitors



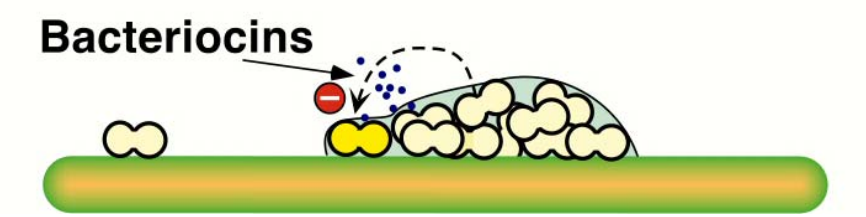
2. Colonize new habitats



Virulence factors
confer the ability to invade host tissues

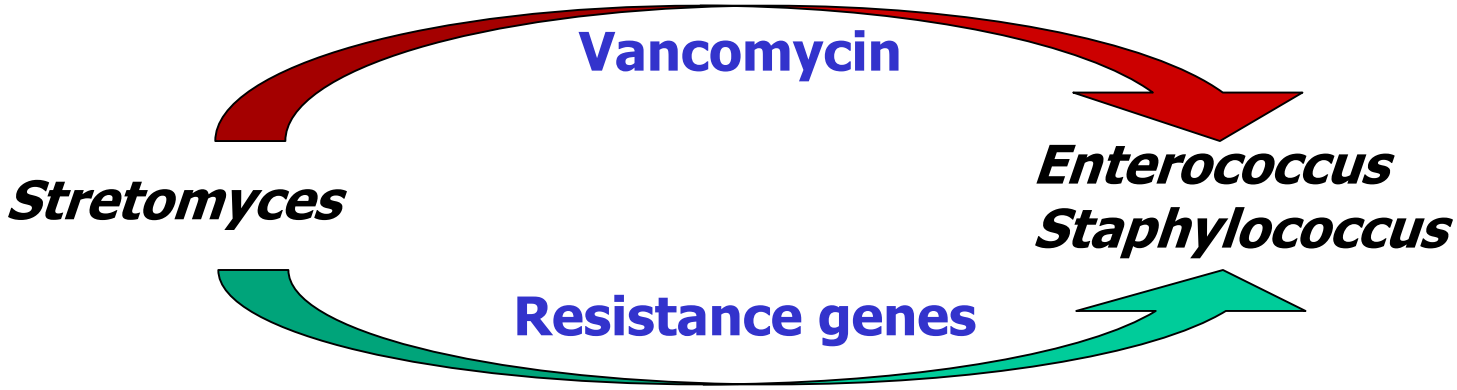
Antibiotics/bacteriocins are microbial products

Antibiotic producers bear specific resistance genes



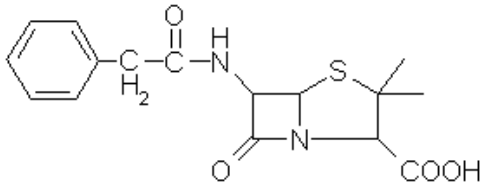
E.g. The antibiotic vancomycin:

- Produced by soil bacteria (streptomycetes)
- Lateral transfer of resistance genes!



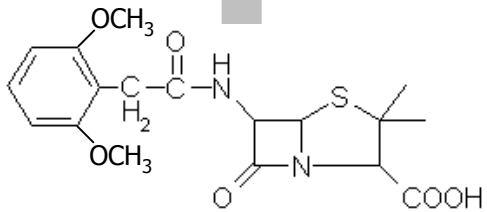
The *Staphylococcus aureus* story

1941
Penicillin



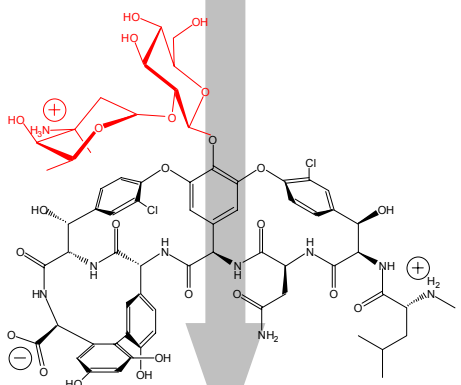
2004
90% resistance by penicillinases

1961
Penicillinase-stable penicillins (Methicillin)



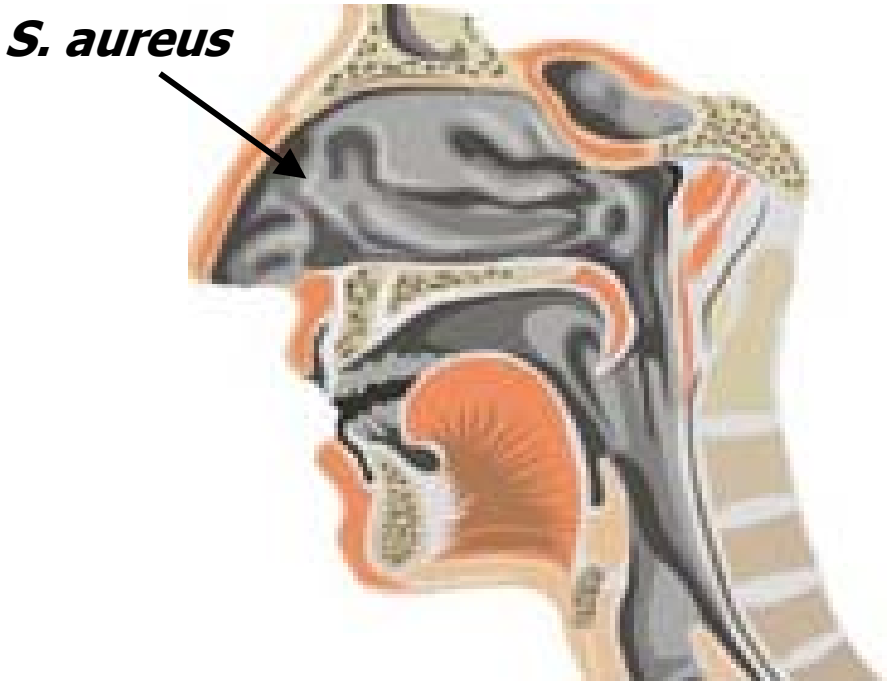
2004
Up to 60% resistance (MRSA)

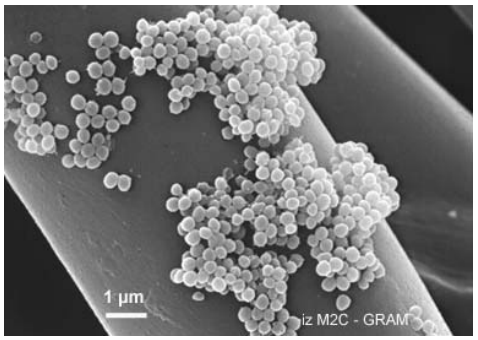
Glycopeptide (Vancomycin)



2002 - 2004
***3 cases* of vanco resistance (VRSA)!**

S. aureus infections





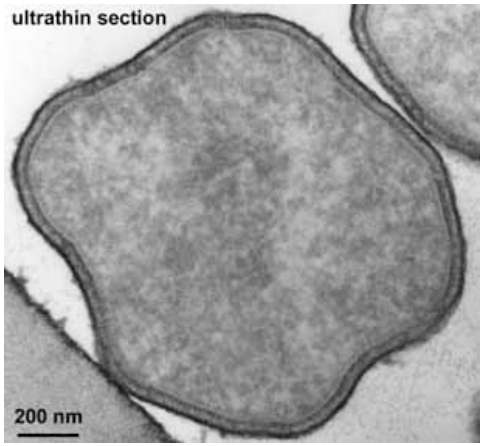
Infected implant

- Skin and **wound infections**
- Catheter and **device-related infections**
- 40% of **nosocomial infections**
- **Sepsis**, septic shock
- More than **30.000 deaths** per year (USA)
- Multiple antibiotic resistance (**MRSA, VRSA**,...)

The extremest microbial habitats:

Extremely *hot*:

E.g. *Pyrolobus*: life at 113°C

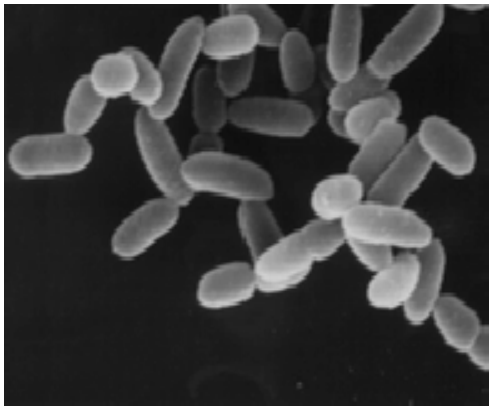


Extremely *acidic*:

E.g. *Picrophilus*: life at pH 0,0

Extremely *alcaline*:

E.g. *Natronobacterium*: life at pH 12



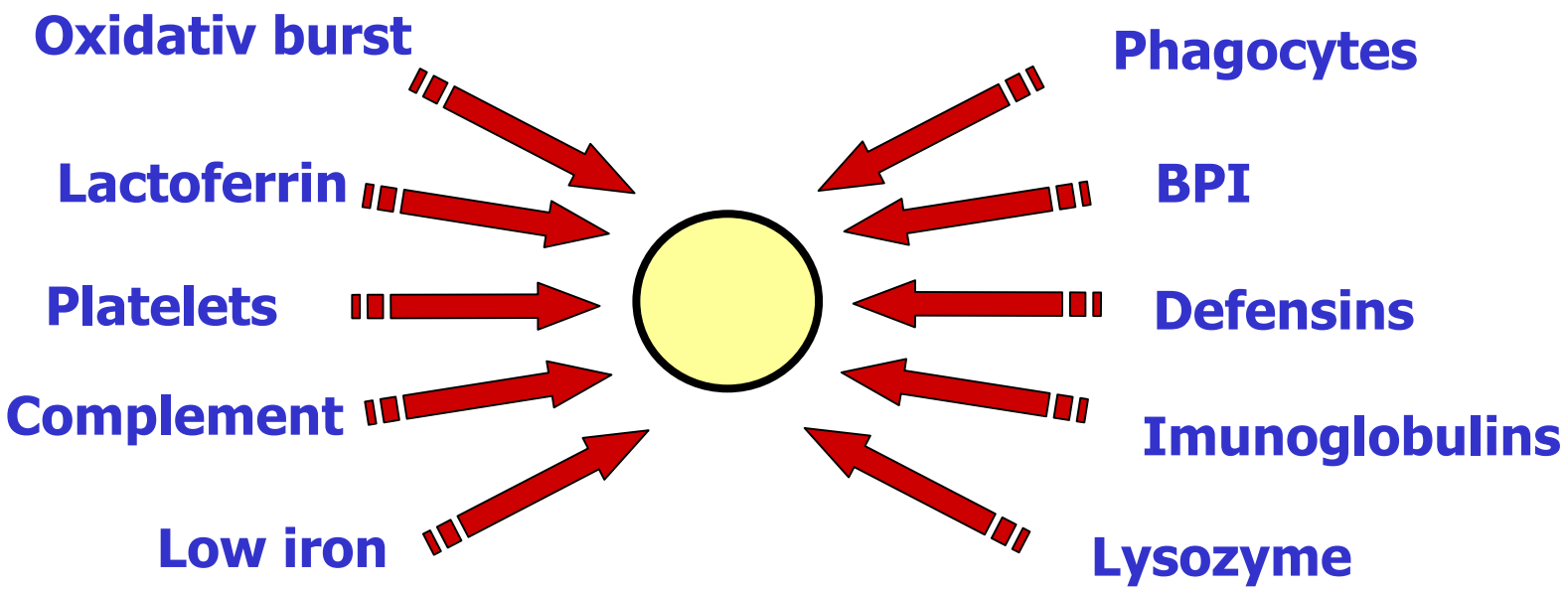
Extremely *salty*:

E.g. *Halobacterium*: life at 32% NaCl

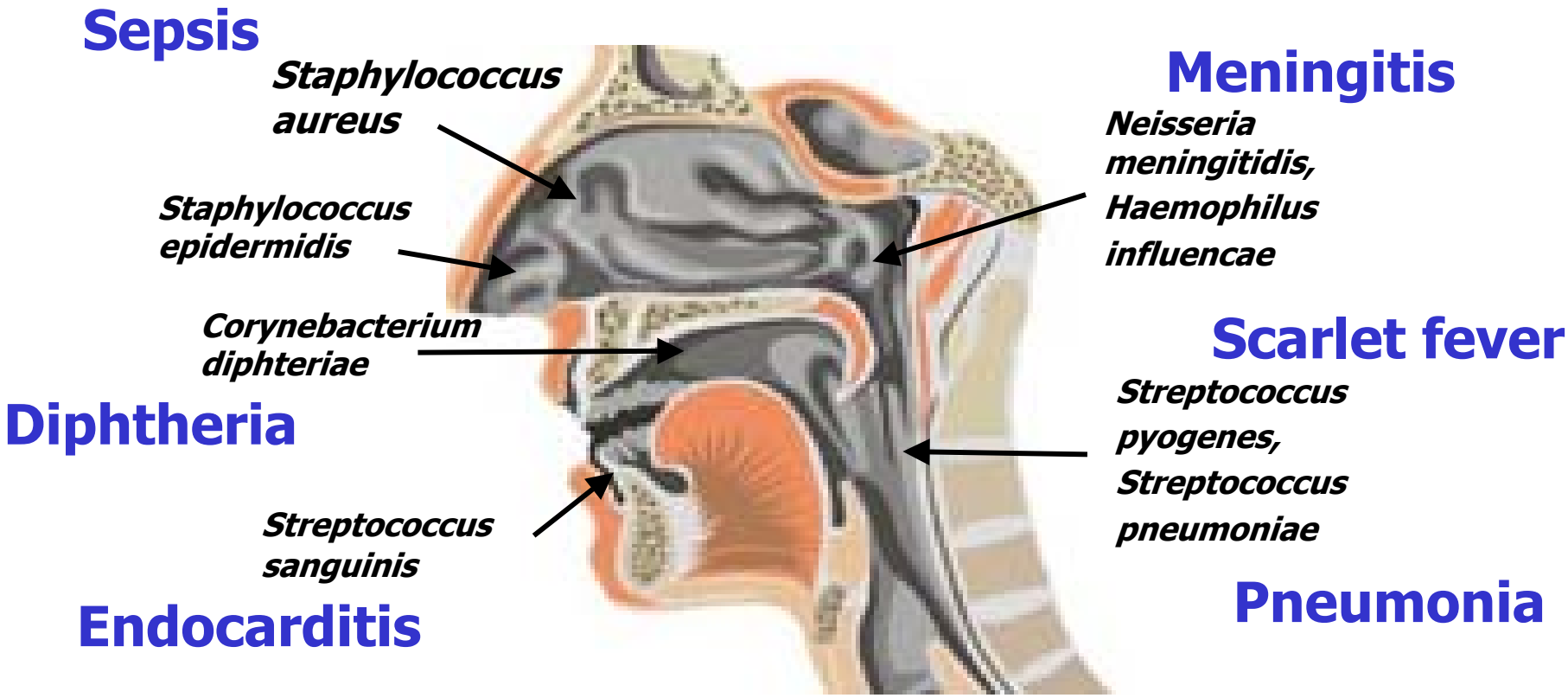
The extremest microbial habitats:

Extremely *hostile*:

E.g. *Staphylococcus aureus*: life exposed to the human immune system



Are mirobial pathogens rare?



We are constantly exposed to **virulence factors**

Conclusions I:

Virulence factors ...

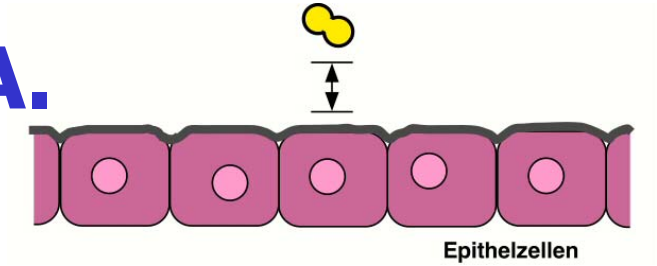
... confer the ability to invade and multiply in host tissues.

... are very diverse in origin and function.

... are frequently produced by the microbial flora.

Defense lines against microbial pathogens:

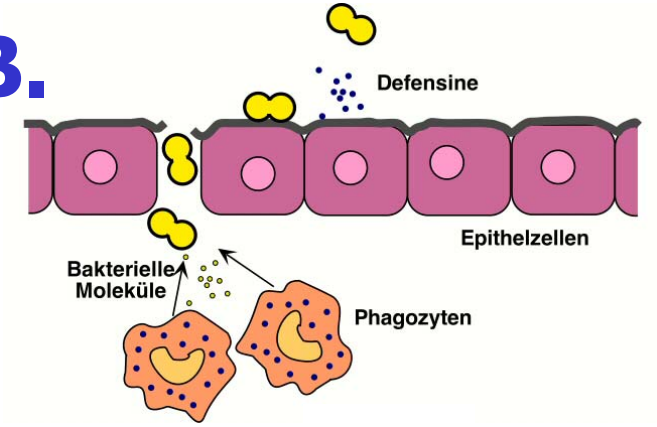
A.



Physical defense

- **Passive** prevention of bacterial entry

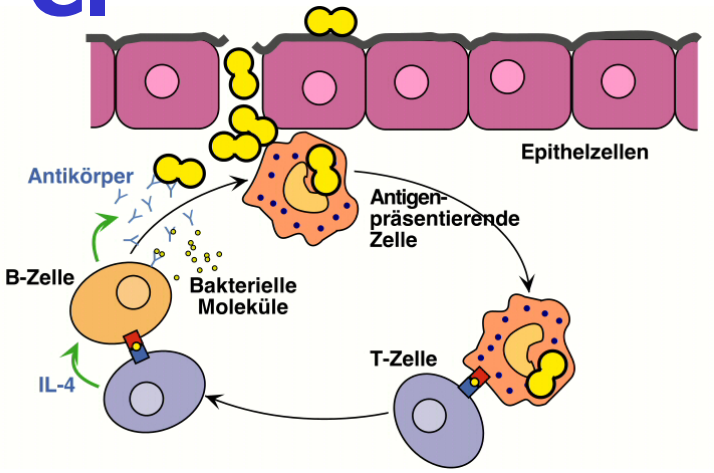
B.



Innate immune system

- In **superficial** infections
- Kills bacteria fast (**minutes/hours**)
- No lymphocytes/antibodies required

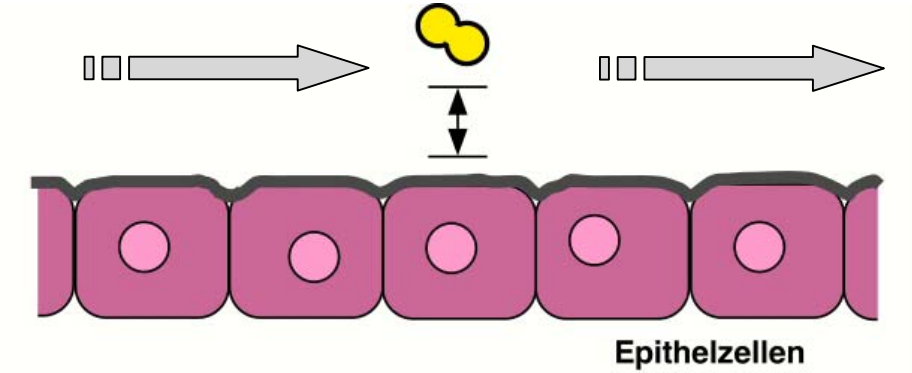
C.



Adaptive immune system

- In **severe** infections
- Takes **days/weeks** to kill bacteria
- Uses **lymphocytes** & **antibodies**

Bacterial evasion of physical defense



Defense mechanisms:

Epidermis,
tight junctions

Mucous, ciliary movement

Low pH in the stomach

Virulence factors:

Destructive enzymes,
transmigration

Specific adhesins

Acid tolerance

Shigella flexneri traverses the intestinal epithelium

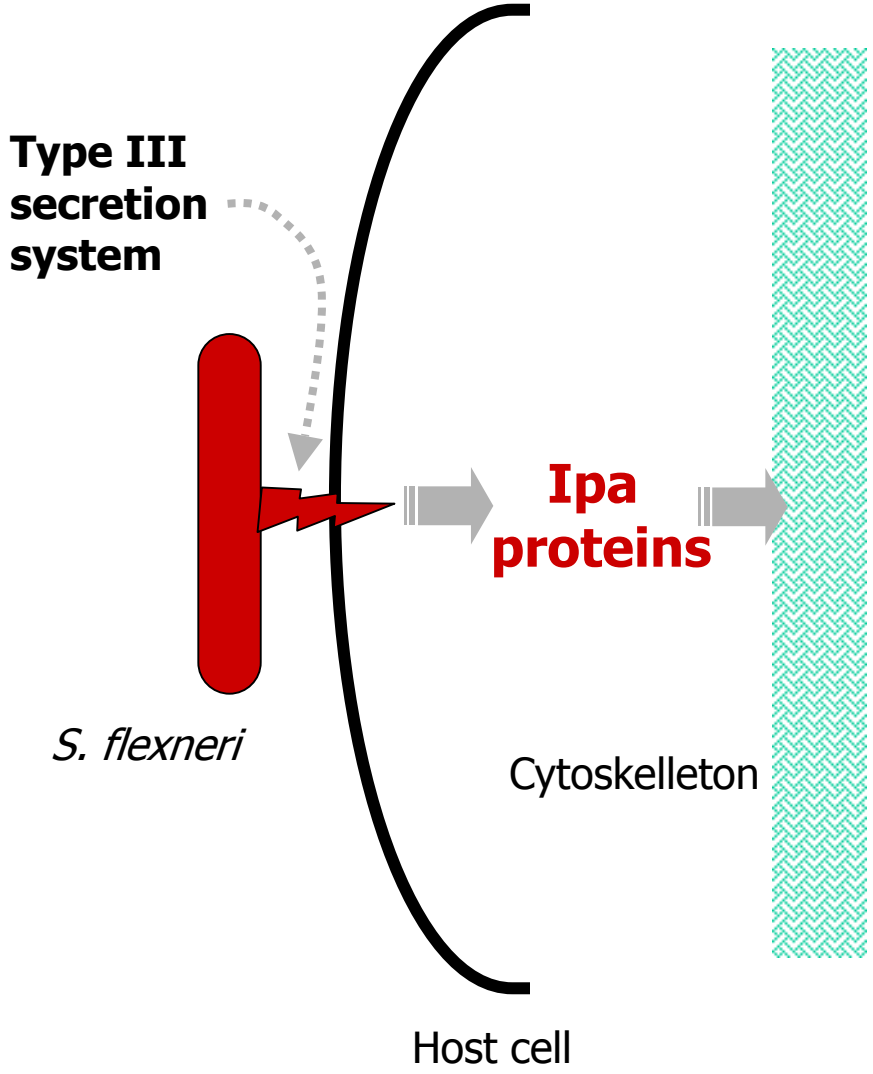
Shigella causes severe diarrhea (**dysentery**)

Intestinal epithelium

S. flexneri

1. *Shigella* induces **phagocytoses** in epitehlial cells
2. *Shigella* moves between cells by **actin polymerization**

Shigella transfers effector proteins into host cells



Ipa proteins induces phagocytosis in epithelial cells

IpaB/C are injected via a **typ III secretion system** and induce endoocytosis by rearranging the cytoskelleton

Conclusions II:

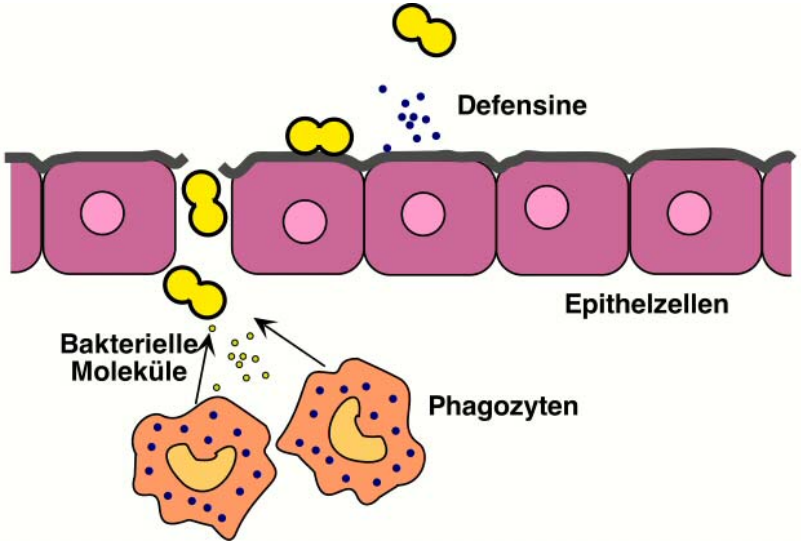
Pysical barriers are eluded by...

... destructive enzymes (*Candida albicans*...).

... transmigration through epithelial cells (*Shigella flexneri*...).

... tolerating low pH in the stomach (*Helicobacter pylori,...*).

Evasion of *innate* immunity



Defense mechanisms:

Antimicrobial peptides

Phagocytes

Recognition by preformed receptors

Virulence factors:

Peptide resistance

Evasion of phagocytosis

Disguise mechanisms, receptor antagonists

Innate human 'peptide antibiotics'

(Cationic antimicrobial peptides = CAMPs)

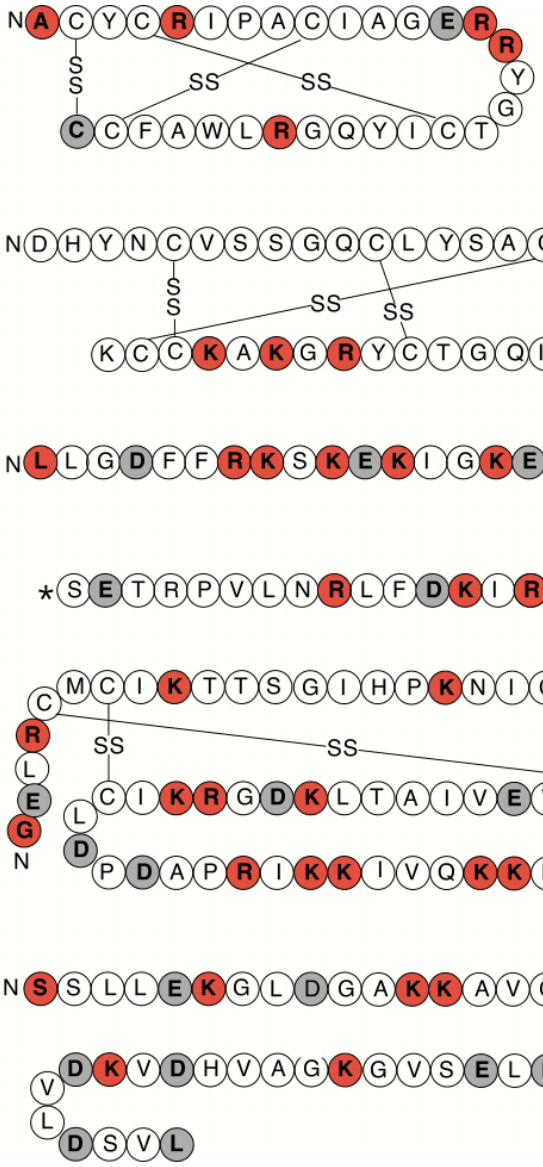
α-Defensin hNP-1
(Granulocytes,
Paneth cells, T cells)

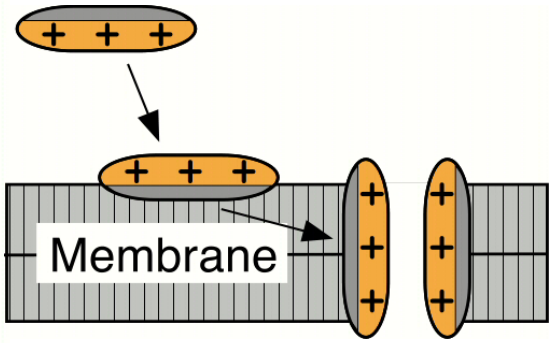
β-Defensin hBD1
(Epithelia, skin)

Cathelicidin LL-37
(Epithelia, skin,
Granulocytes)

Thrombocidin TC1
(Platelets)

Dermcidin
(Sweat glands)





CAMPs form pores
in bacterial cytoplasmic
membranes

Positive

Negative

Host defenses factors are '*positive by nature*' - Bacteria are '*negative by nature*'

Antimicrobial host factors are

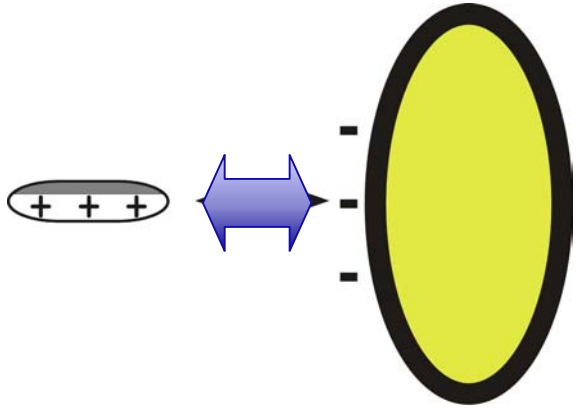
Positively charged:

- Antimicrobial peptides
- Class IIA phospholipase A2
- Lactoferrin
- Myeloperoxidase
- Lysozyme, ...

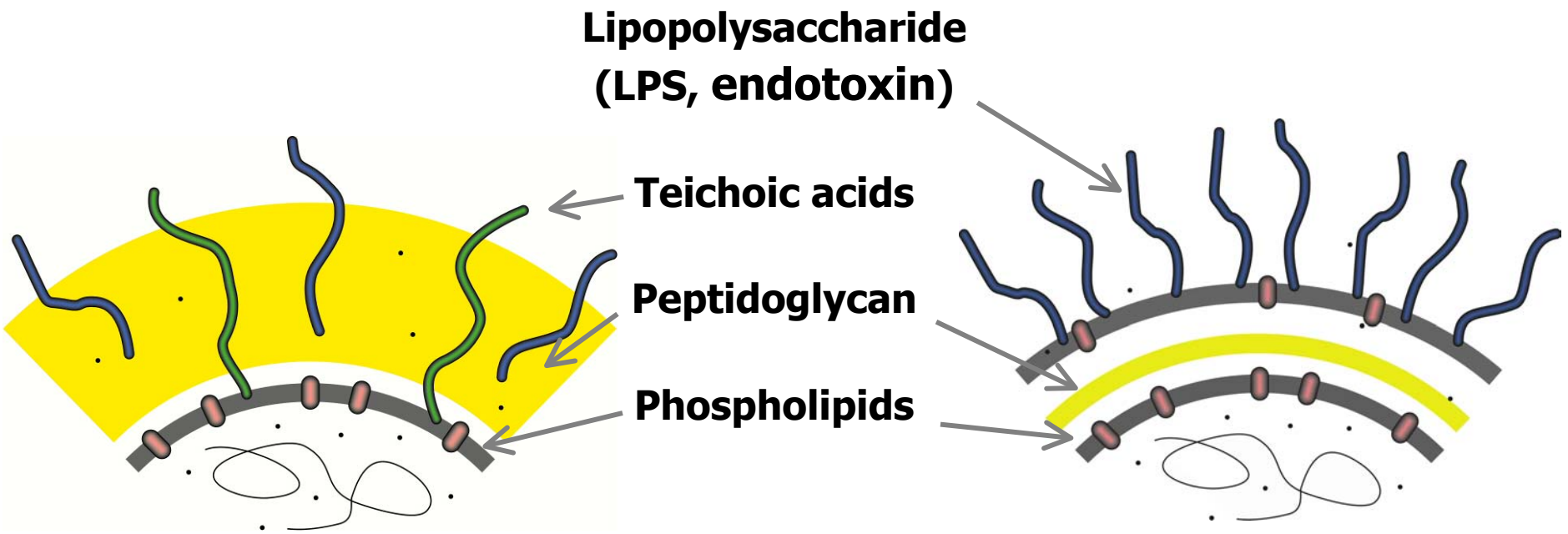
Bacterial cell envelope components are

Negatively charged:

- Peptidoglycan
- Teichoic acids
- Teichuronic acids
- Phospholipids (most)
- Lipid A, LPS,...



The negatively charged bacterial cell envelope:



Gram-*positive* bacteria
(*Staphylococcus aureus*)

Gram-*negative* bacteria
(*Shigella flexneri*)

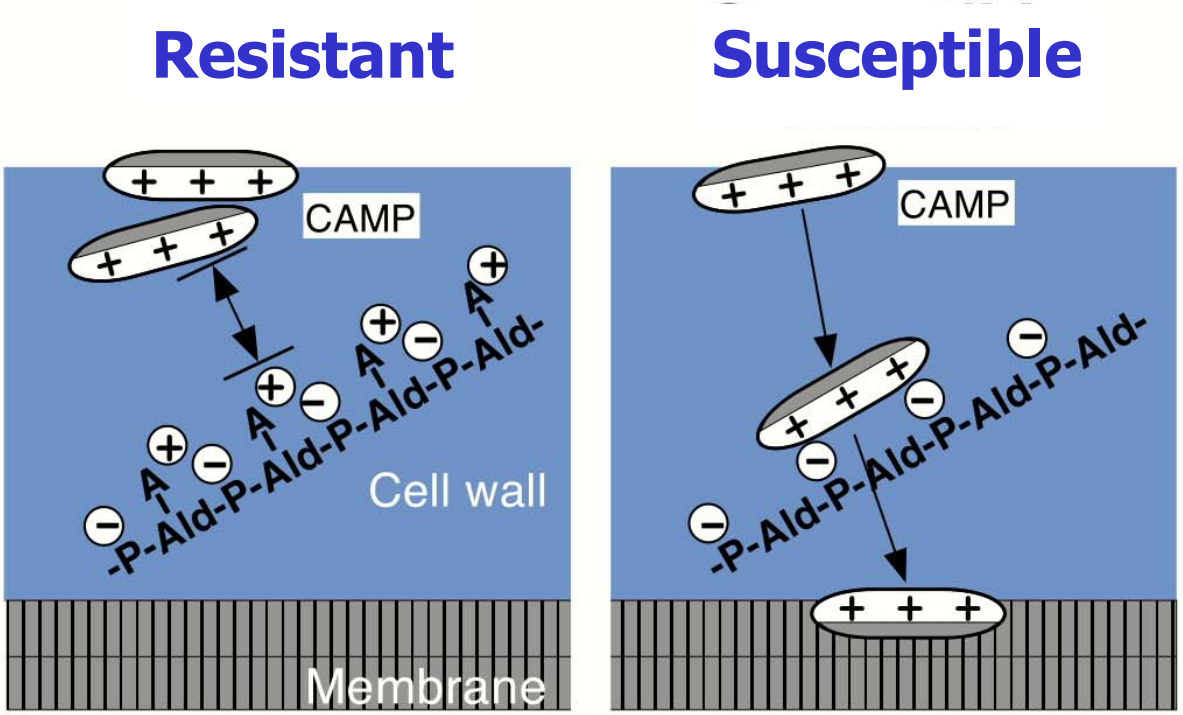
Staph. aureus is resistant to defensins

Minimal inhibitory concentration of defensin hNP1-3:

S. aureus **wild-type**: **>60** µM

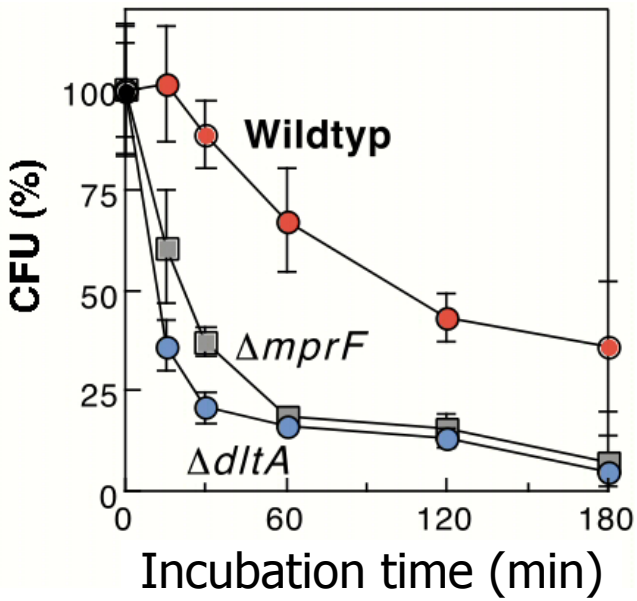
mutant Δ*dltA*: **2.9** µM

Resistance mechanism:
Introduction of positive charges into the cell wall



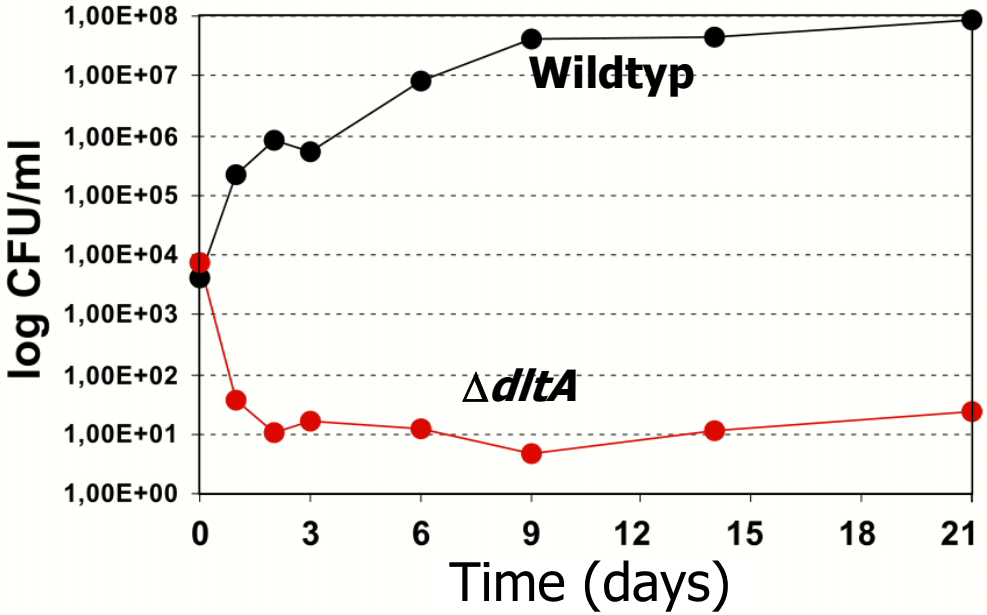
Defensin-susceptible *S. aureus* mutants are virulence attenuated

Inactivation by neutrophils



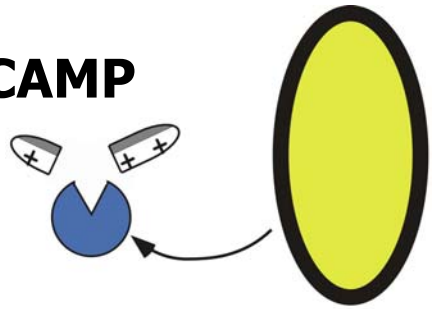
Mouse tissue cage model:

Regine Landmann et al., Basel



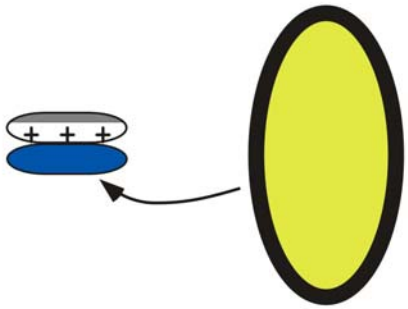
Bacterial CAMP resistance mechanisms

1. Cleavage of CAMP



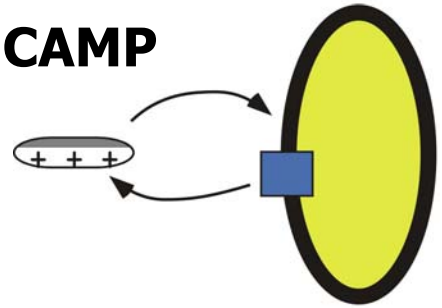
PgtE protease:
Salmonella, Escherichia, ...

2. Anti-CAMP



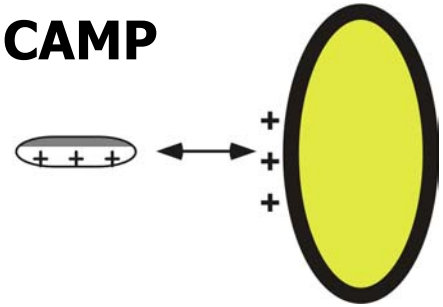
Staphylokinase:
Staphylococcus, ...

3. Extrusion of CAMP



MtrCDE efflux pump:
Neisseria,...

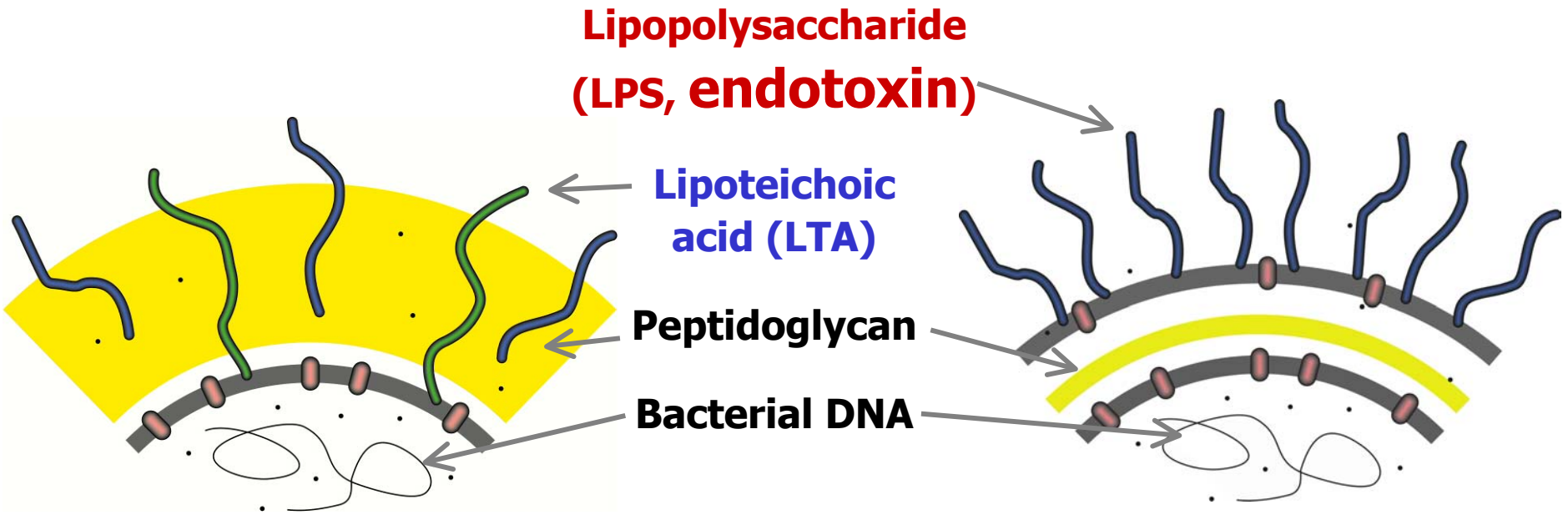
4. Repulsion of CAMP



Modification of teich. acids and lipids:
Staphylococcus, Listeria, Streptococcus, ...

Modification of lipid A:
Salmonella, Pseudomonas, Legionella,...

Bacterial molecules activate the innate immune system and cause inflammation



Gram-*positive* bacteria (*Staphylococcus aureus*)

Gram-*negative* bacteria (*Shigella flexneri*)

Host TLR receptors recognize conserved bacterial molecules

Gram-positive:

Lipoteichoic acid,

Lipopeptides

Gram-negative:

Lipopoly-saccharide

Humans have **10 different TLRs**; some ligands are still unknown

Activation of TLRs leads to inflammatory responses

TLRs → NF-κB
(transcription factor)

Epithelial cells:
- → **Defensin production**
- → **IL-8 produktion**

Endothelial cells:
- → **Adhesive for leukocytes**
- → **Permeabilisation**

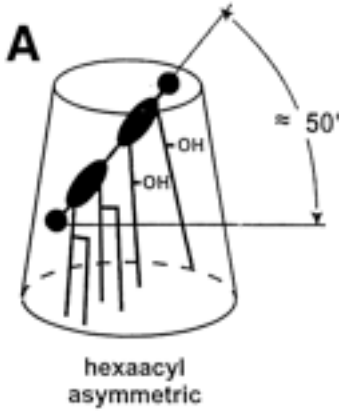
Phagocytes:
- → **Cytokine production**
- → **increased killing**

Chlamydia produce LPS with very low inflammatory activity

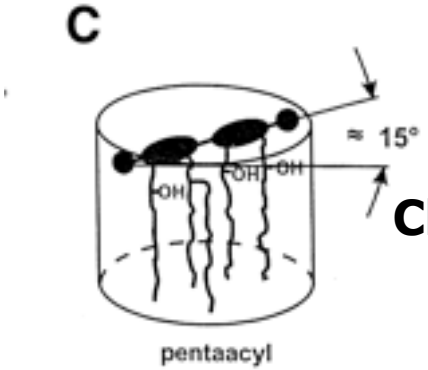
Chlamydia pneumoniae

- **Obligate intracellular pathogens**
- **Cause persistant infections**

Active LPS:
(**6** fatty acids)

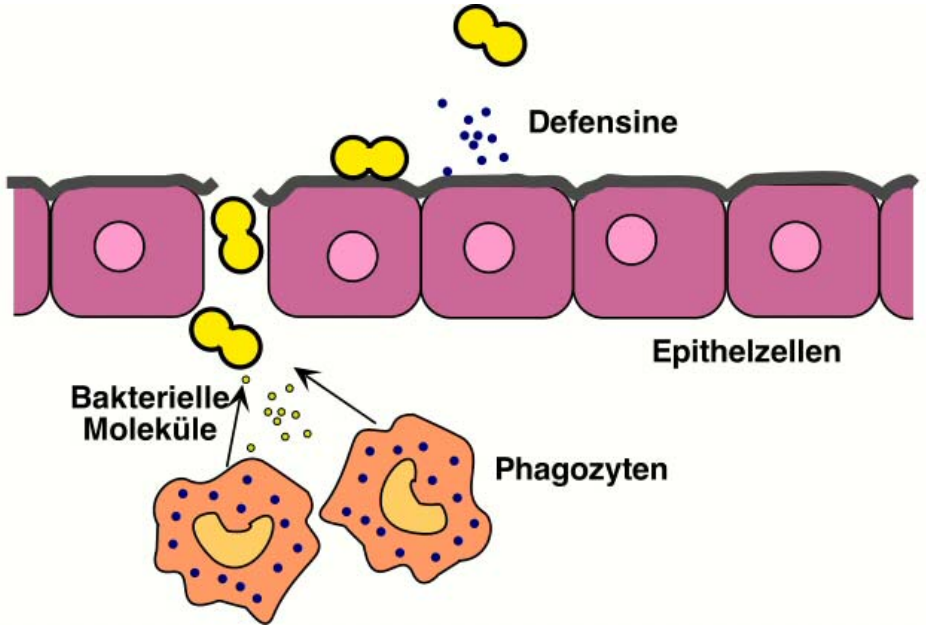


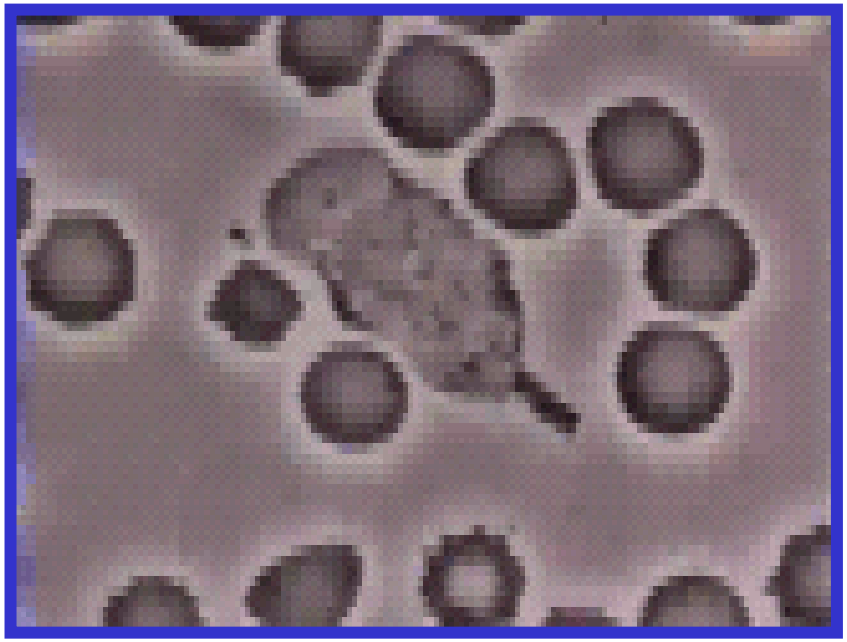
Inactive LPS:
(**4-5** fatty acids)



Chlamydial LPS

Which bacterial molecules cause leukocyte chemotaxis?





Courtesy T. Stossel

Leucocytes recognize bacterial molecules

Role of formylated peptides in chemotaxis?

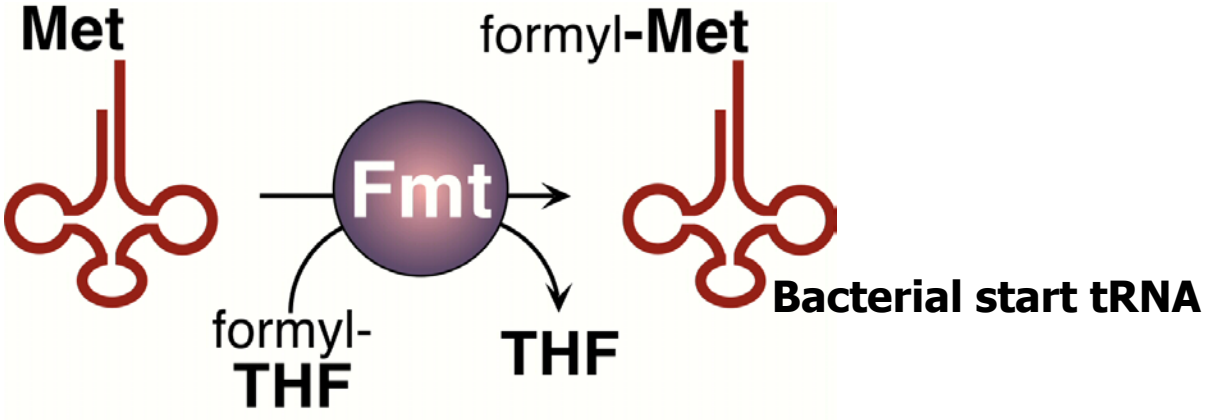
Protein synthesis:

mRNA +
Aa-tRNAs +
Ribosome

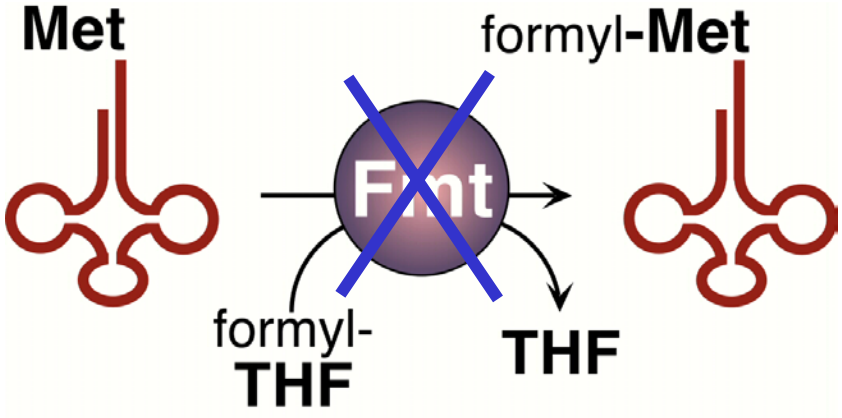
Eucaryotes:
Methionine-Proteins

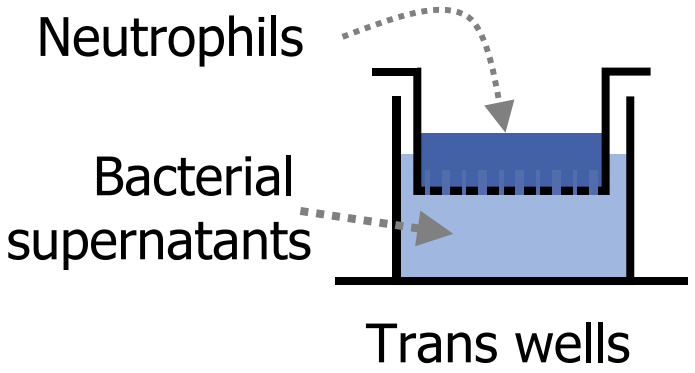
Bacteria:
***Formyl*-Methionine-Proteins**

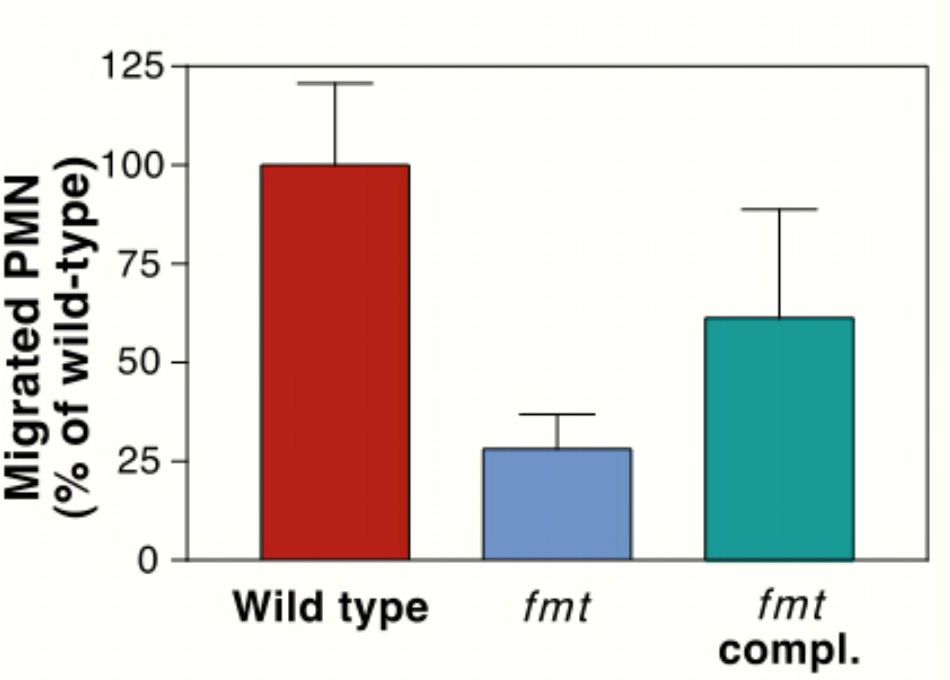
Bacterial protein synthesis starts with fMet-tRNA



S. aureus formylated peptides cause chemotaxis



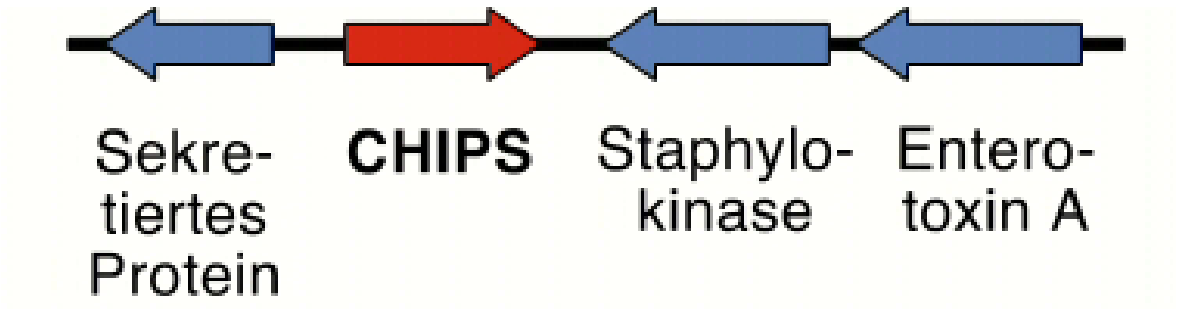




***S. aureus fmt* mutant causes reduced chemotaxis**

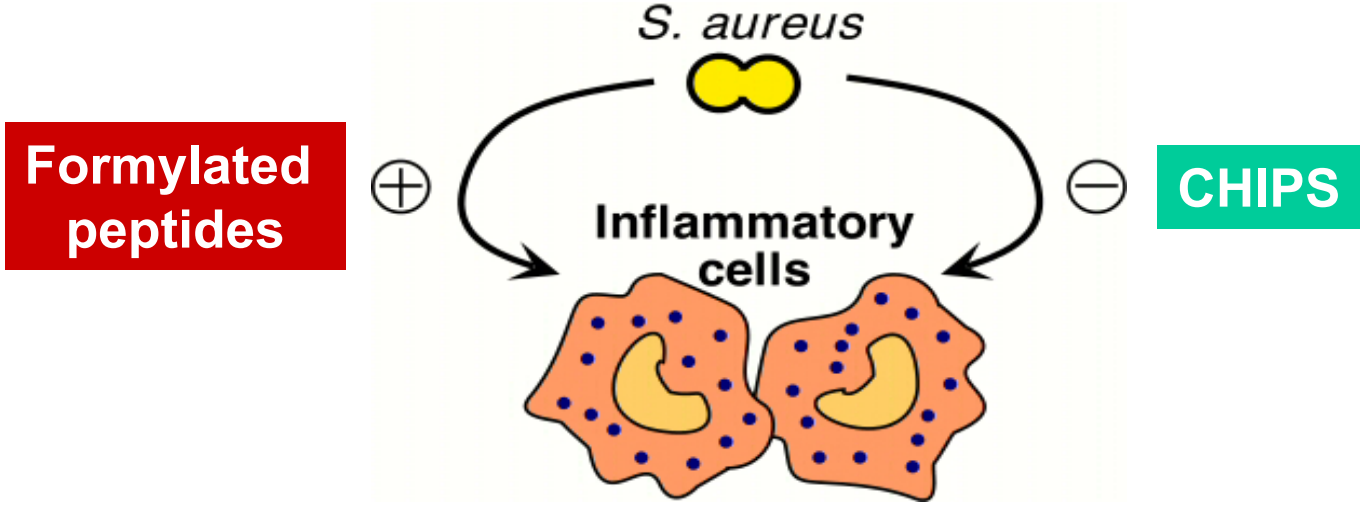
S. aureus inhibits leukocyte chemotaxis by the CHIPS protein

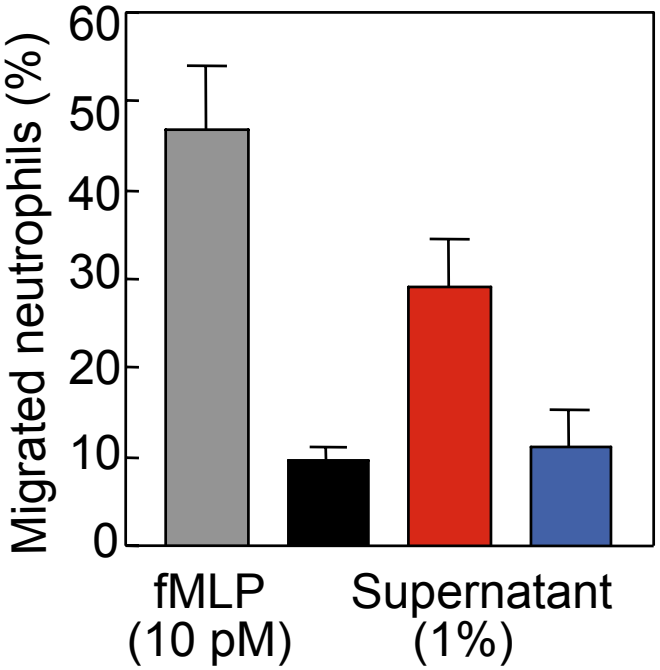
Chemotaxis-inhibitory protein of *S. aureus* CHIPS



- **CHIPS is produced by 80% of the *S. aureus* strains**
- **CHIPS blocks chemotaxis receptors on leukocytes**

CHIPS inhibits leukocyte recruitment





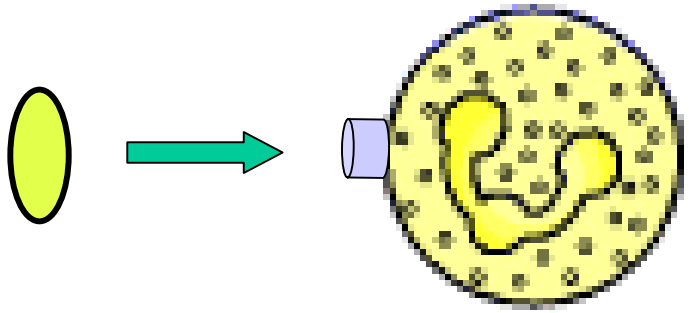
Quantification of chemotaxis:

- Wild-type
- CHIPS mutant
- CHIPS mutant, complemented

How do phagocytes recognize their pray?

1. Non-opsonic phagocytosis

Direct recognition and uptake by phagocytes

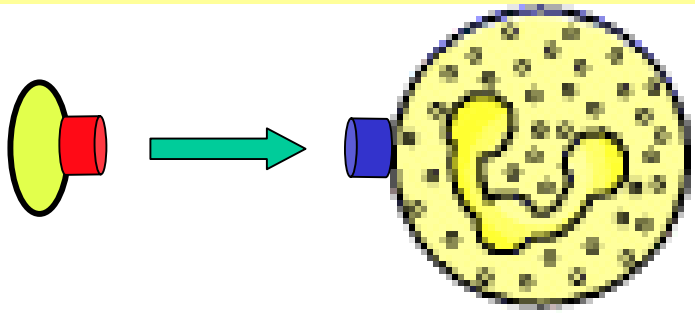


< 10% efficincy

2. Opsonic phagocytosis

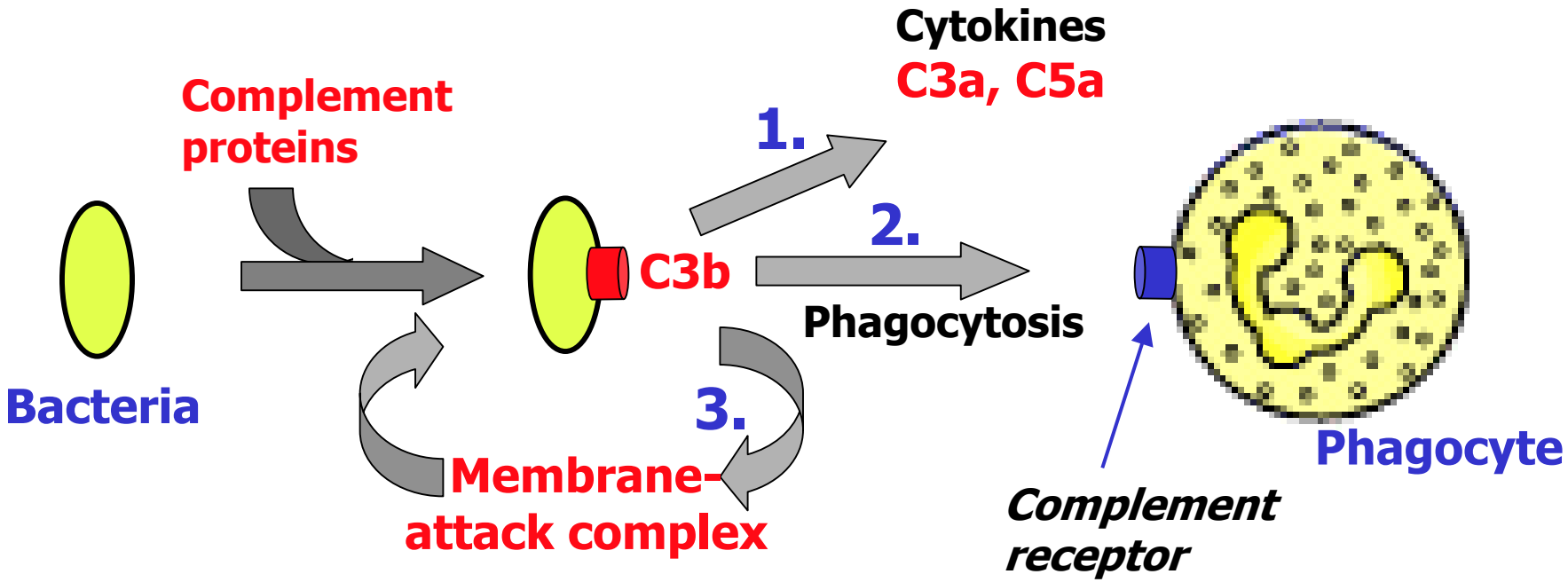
Phagocytosis of particles labeled with antibodies/complement

- **Complement (C3b)**
- **Collectins (SP-A, SP-D, ...)**
- **Antibodies (IgG1, IgG3, IgA, IgE, ...)**



> 90% efficincy

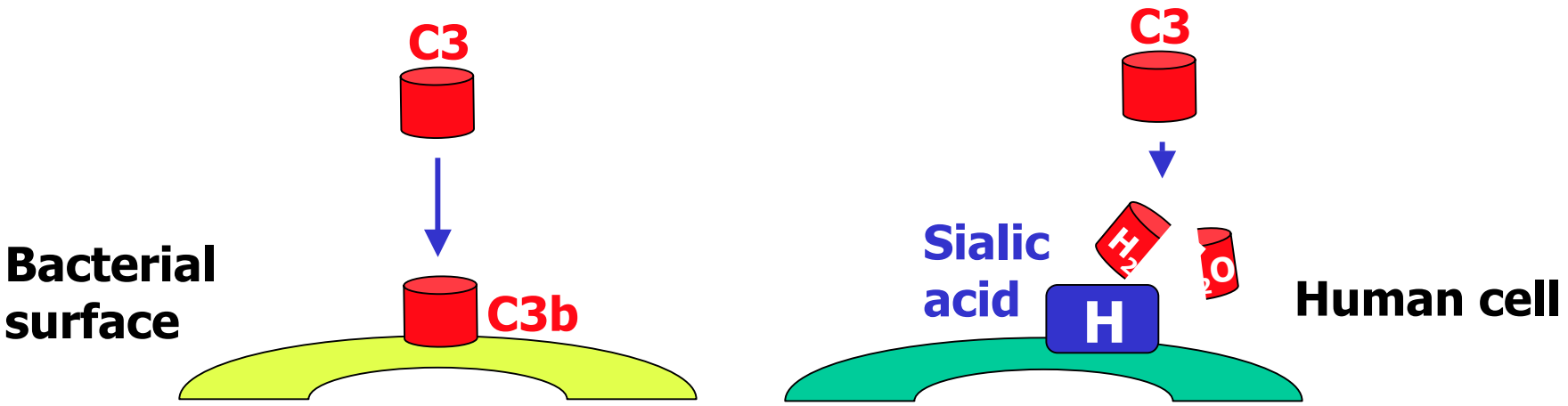
Opsonization by the complement system



Deposition of C3b causes:

- Inflammation
- Phagocytosis
- Bacterial killing

Human cells are not opsonized because of sialic acid on their surface



Factor H prevents opsonization of sialic acid-containing surfaces

Neisseria modifies its LPS with sialic acid

N. meningitidis
causes **meningitis**

Sialic acid

Many neisserial strains are
‚serum resistant'
→ No inactivation by complement

Streptococcus pyogenes destroys leukocytes by leukocidins

- **Subunits oligomerize within the leukocyte membrane**
- **Pore formation kills leukocytes**

Conclusions III:

Innate immune mechanisms are eluded by...

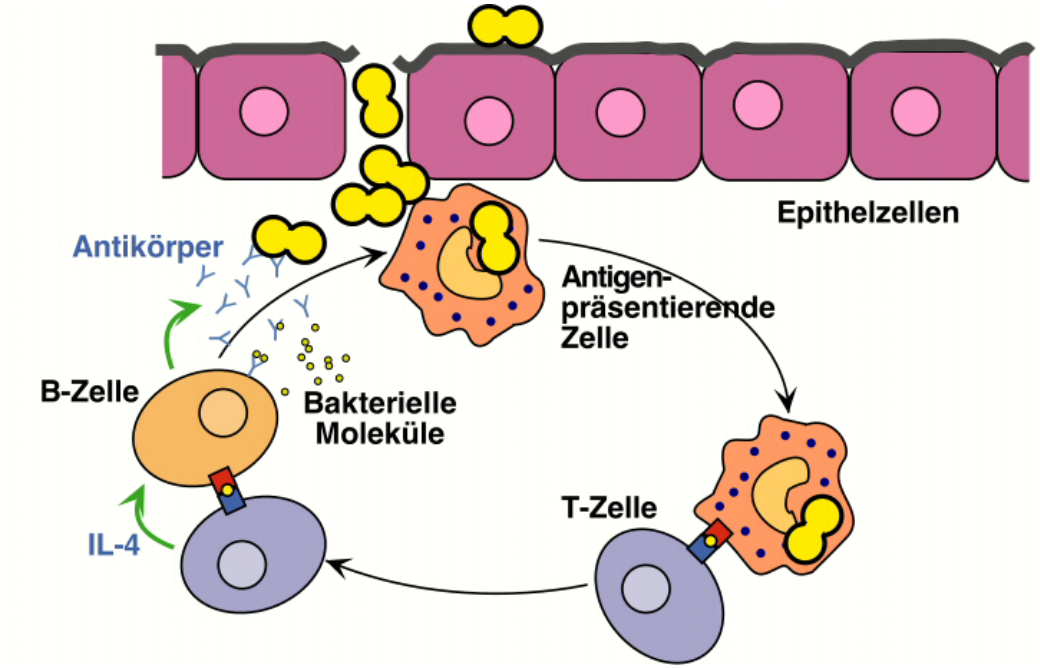
... resistance to antimicrobial host factors (*S. aureus*...).

... preventing recognition (*Chlamydia pneumoniae*,...).

... preventing opsonization (*Neisseria meningitidis*...).

... destroying leukocytes (*Streptococcus pyogenes*,...).

Evasion of *adaptive* immunity



Defense mechanisms:

Antigen-presenting cells

Immunoglobulins

T cells

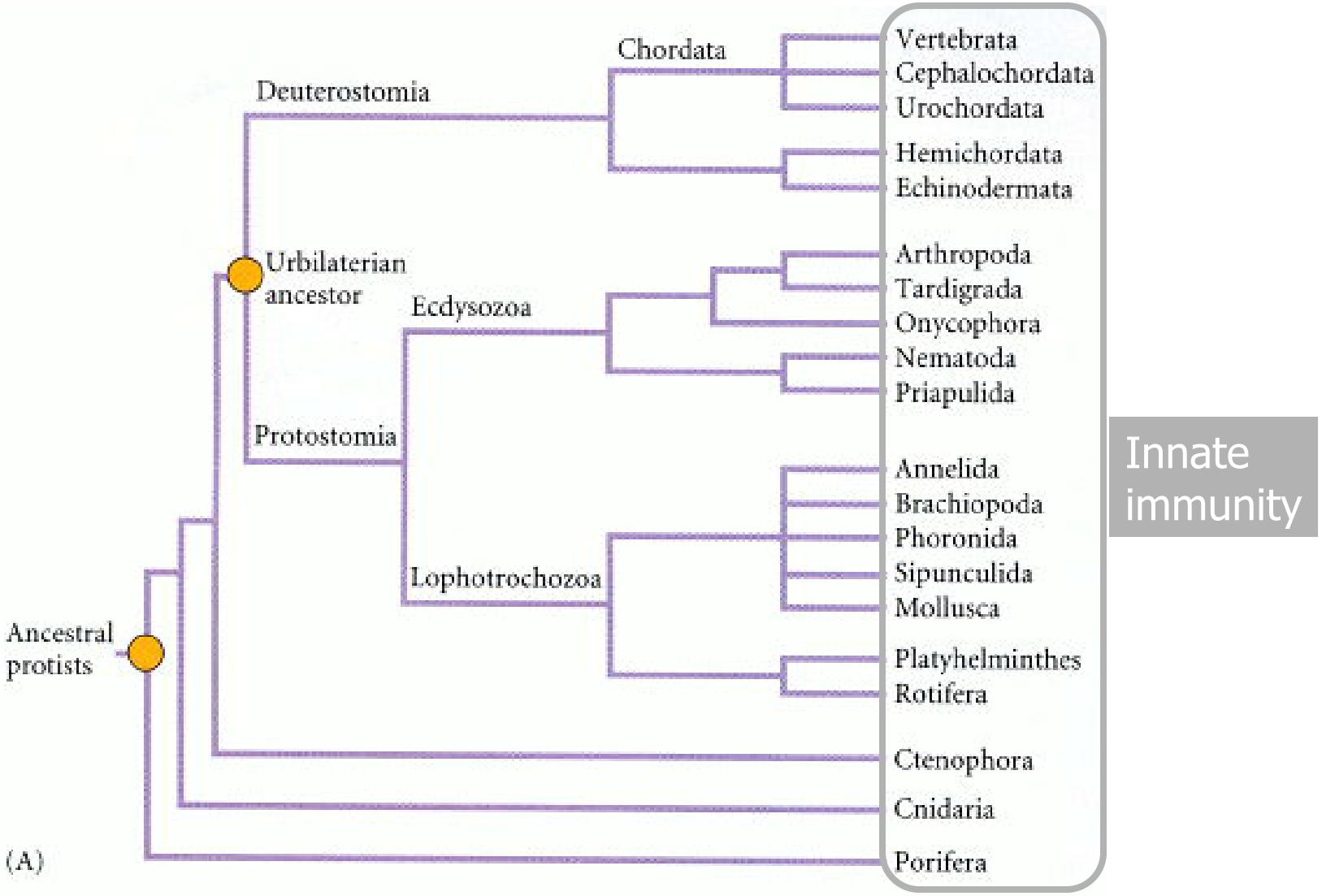
Virulence factors:

Leukocidins

Ig proteases, capsules

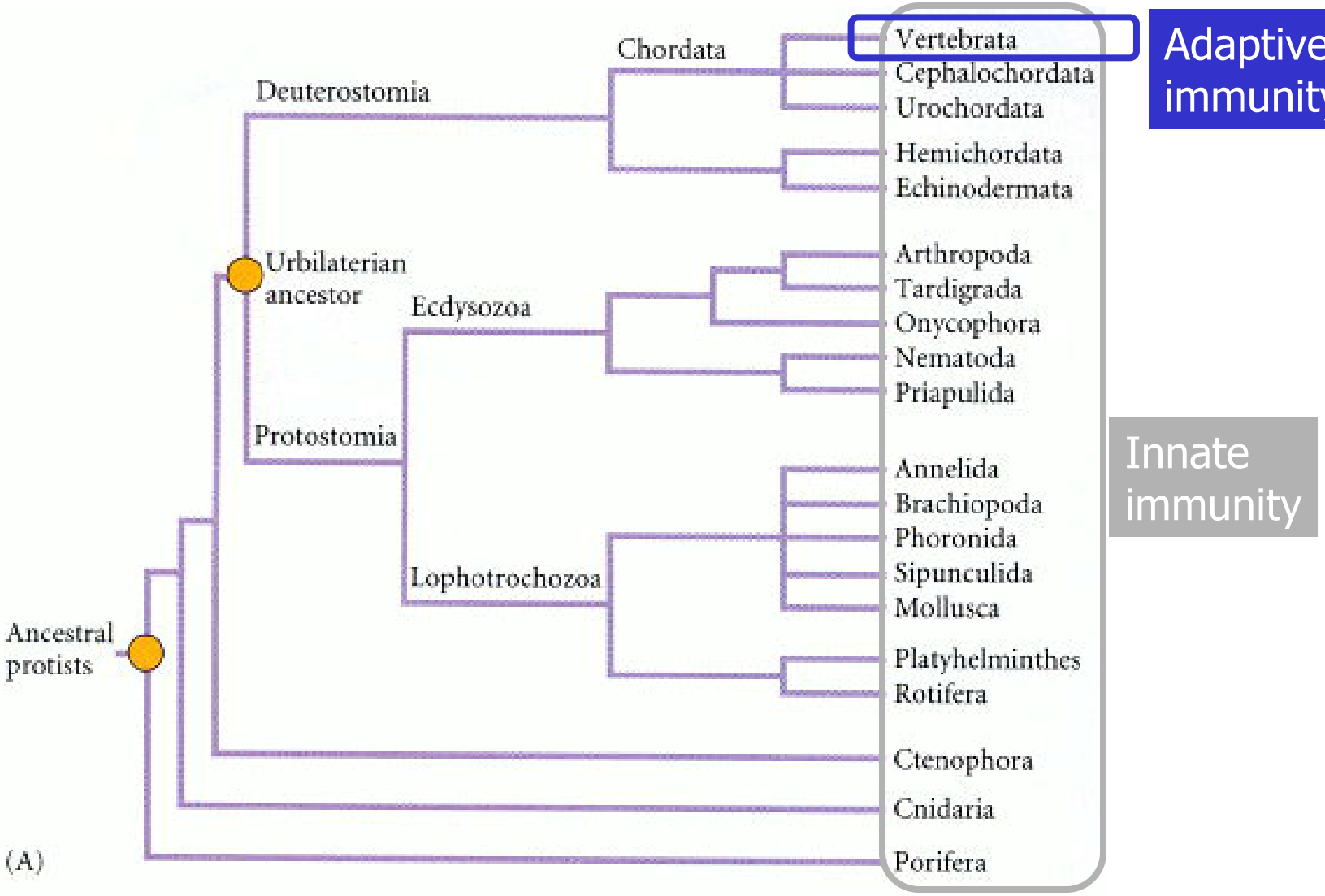
Antigen variation

Prevalence of the *innate* immune system:



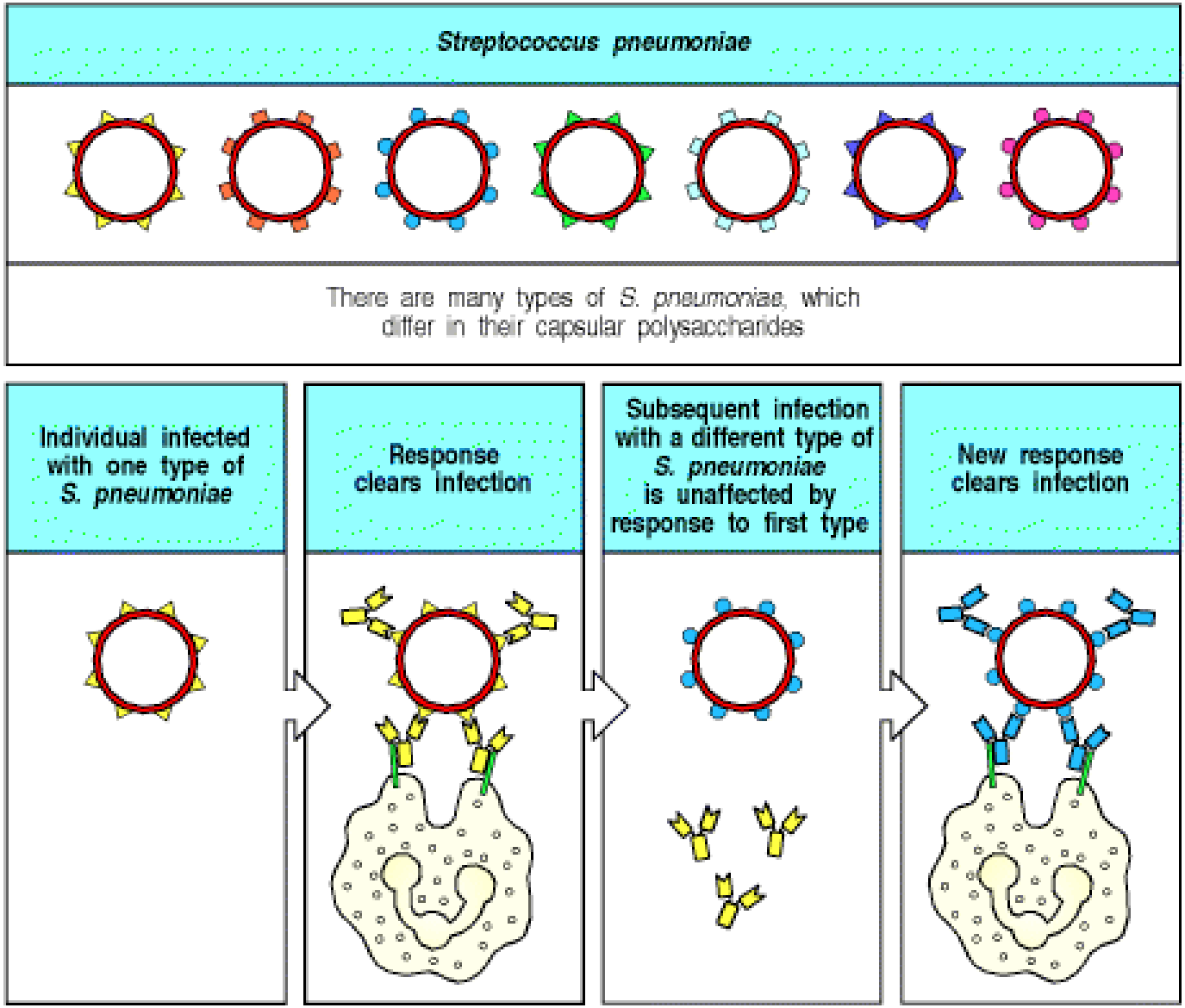
Most higher organisms have an innate immune system

Prevalence of the *adaptive* immune system:



Only vertebrates have an adaptive immune system

Streptococcus pneumoniae produces >100 types of capsular polysaccharides

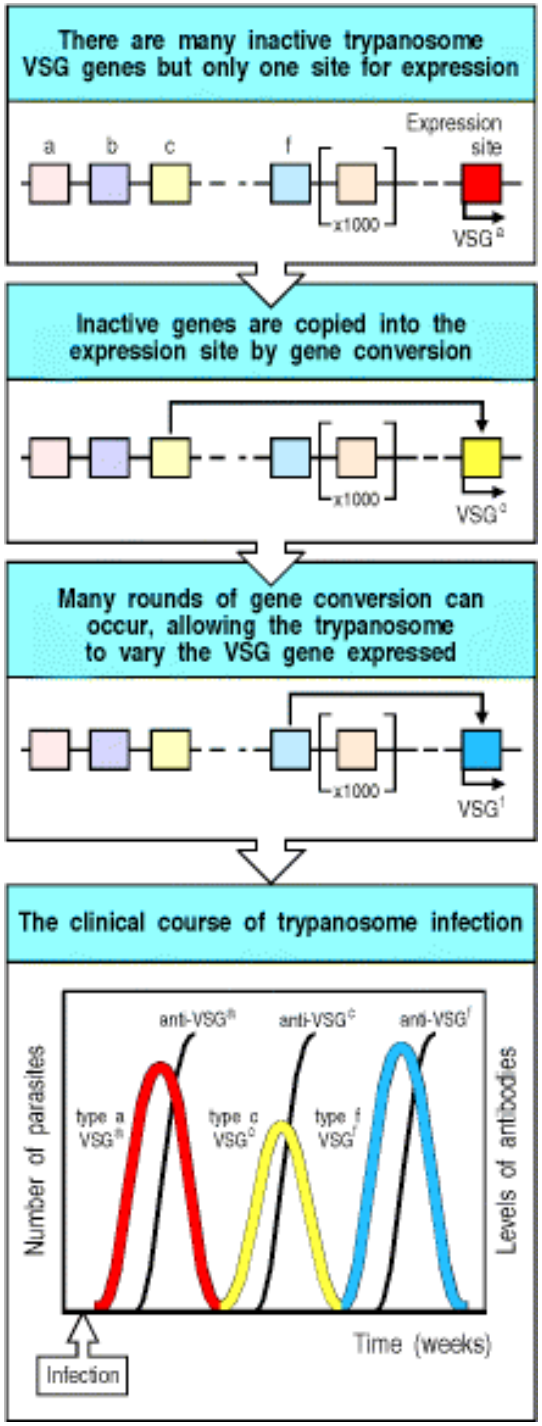


Antigenic variation causes relapsing infections

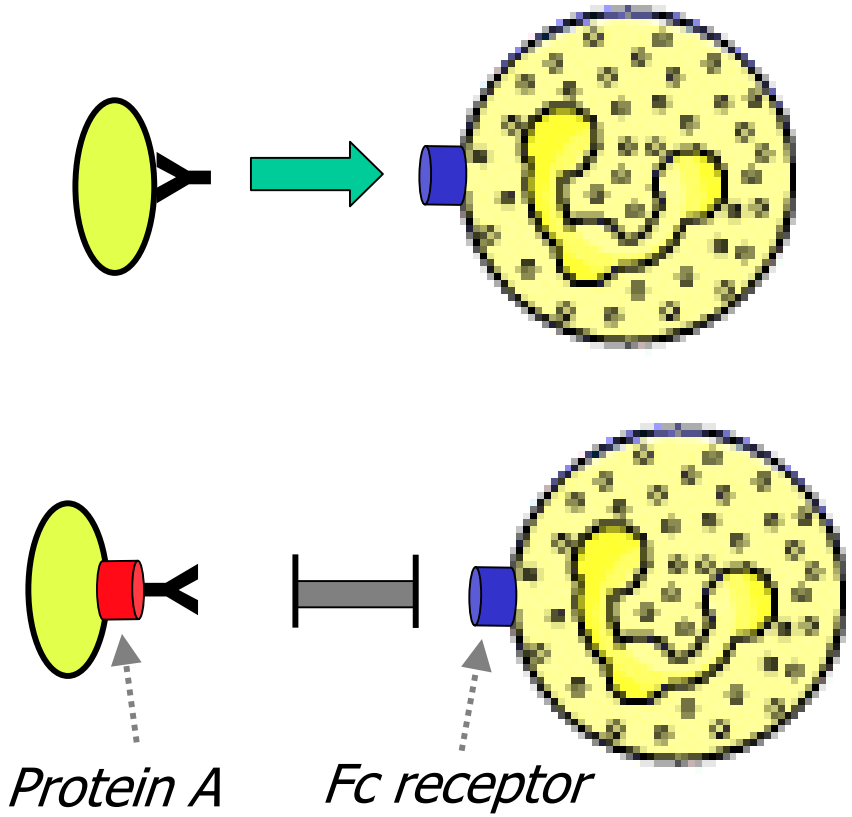
Alternating on- and offswitching of surface antigens distracts the adaptive immune system

Neisseria	**Meningitis**
	Sexually transmitted dis.
Borrelia	**Relapsing fever**
	Lyme borreliosis
Trypanosoma	**Sleeping disease**

(Protist)



Protein A of *S. aureus* prevents correct opsonization with antibodies



Protein A binds the Fc part of IgG

→ No recognition by Fc rezeptoren possible

Conclusions IV:

Adaptive immune mechanisms are eluded by...

... antiopsonic capsules (*Streptococcus pneumoniae*...).

... immunoglobulin proteases (*Neisseria*,...).

... antigenic variation (*Trypanosoma*,...).

... preventing correct opsonization (*S. aureus*,...).

Virulence factors are far more than toxins!

Adhesins,

Evasins,

Modulins,

Agressins,……