Patogênese Bacteriana – fatores de virulência

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Microbiologia Médica – Patrick Muray, Ken Rosenthal e Mechael Pfaller, 6 ed. ou posterior

Mims – Microbiologia Médica – Goering, Dockrell, Zuckerman, Roitt, Chiodini, 5 ed ou posterior

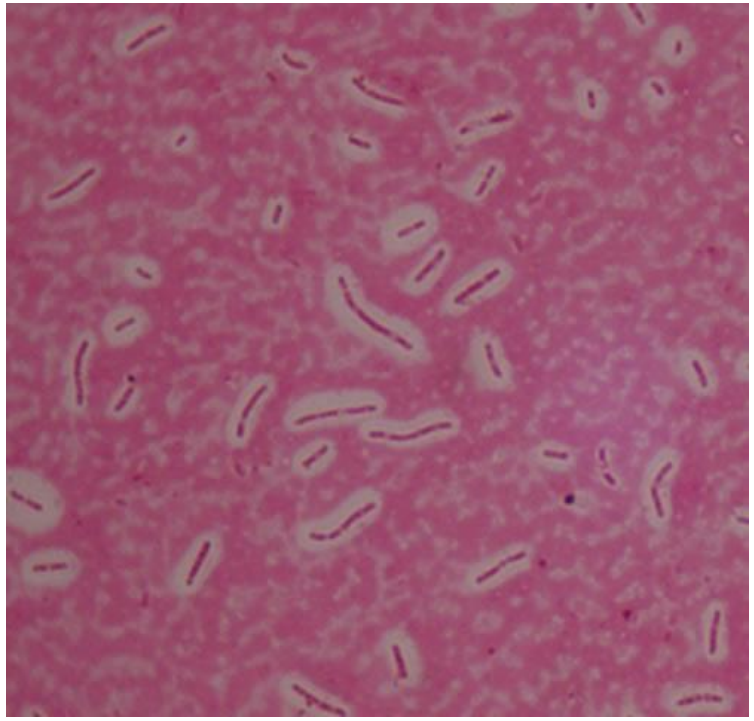
Fatores de virulência = fatores que aumentam a habilidade de uma bactéria em causar doença.

Podem estar relacionados com:

1. **Estruturas de evasão**
2. **Liberação de toxinas**
3. **agentes estimuladores de resposta imune**
4. **Destruição direta dos tecidos**
5. **produtos do metabolismo e estruturas de capitação de nutrientes**

Uma doença infecciosa bacteriana e uma combinação entre o dano causado pela bactéria e das consequências das respostas inatas e imune à infecção

-Como as bactérias podem cruzar as barreiras do corpo?

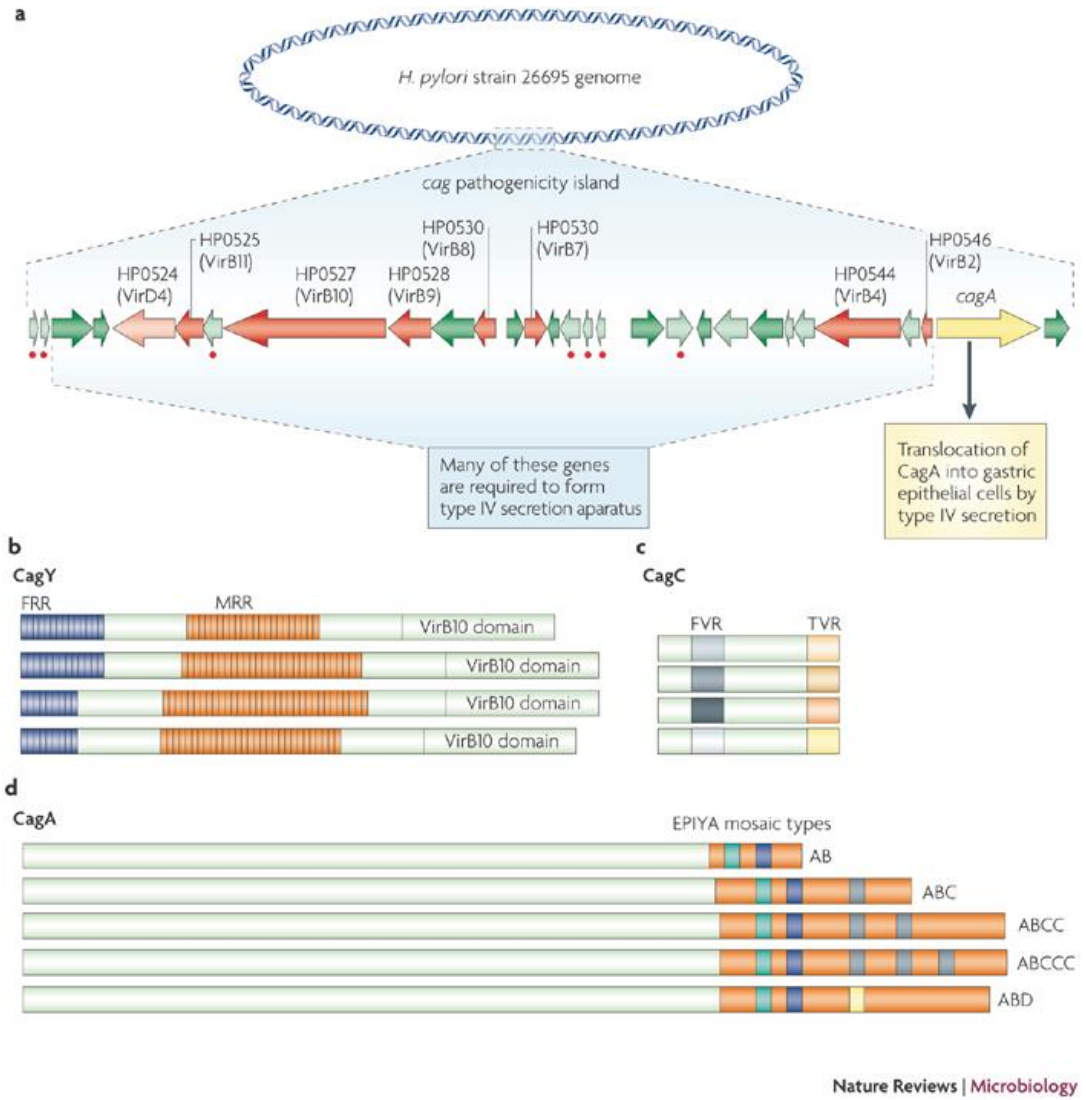


-Qual o efeito disso para nossa saúde?

Ilhas de patogenicidade = regiões cromossômicas que contêm conjunto de genes que codificam diversos fatores de virulência

Geralmente é um transposon inserido no cromossomo ou plasmídeo

Os genes são ativos por um determinado estímulo, como pH



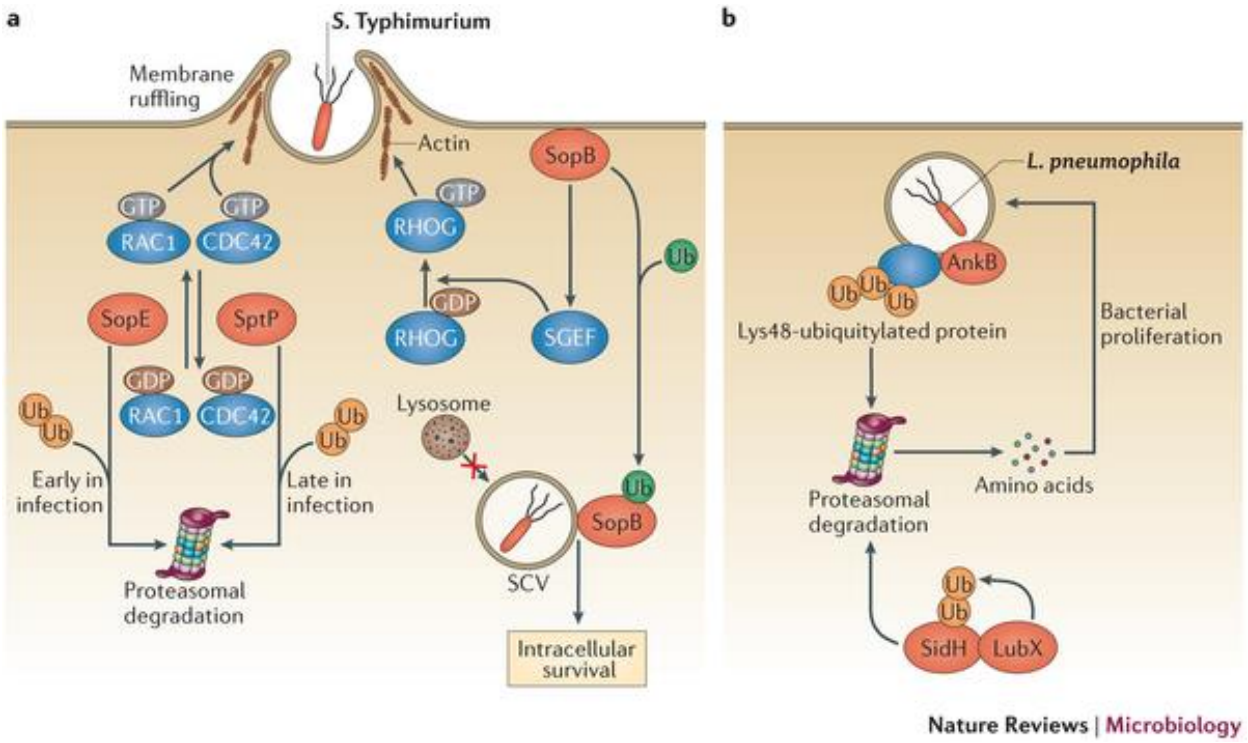
1. Mecanismos de evasão

Estratégias de evasão bacteriana das defesas naturais, não adaptativas

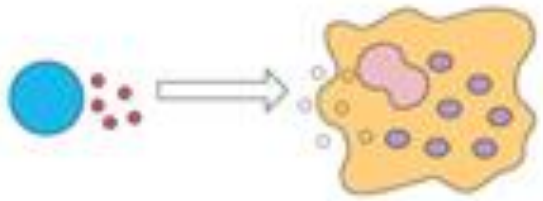
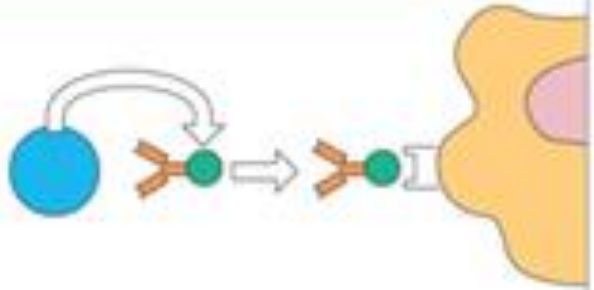
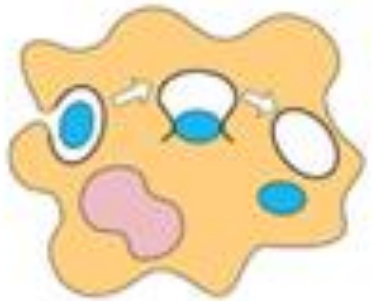
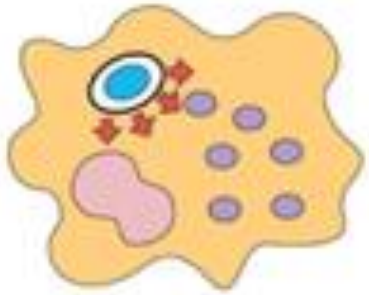
- Matar os fagócitos ou sobreviver à fagocitose
- Interferir na ação ciliar
- Interferir na ativação do complemento
- Evitar o reconhecimento do sistema imune
- Clivagem do peróxido de hidrogênio e peptídeos antimicrobianos

Alteração do citoesqueleto

Produção de proteínas chamadas de invasinas. Ex. *Salmonella typhimurium*

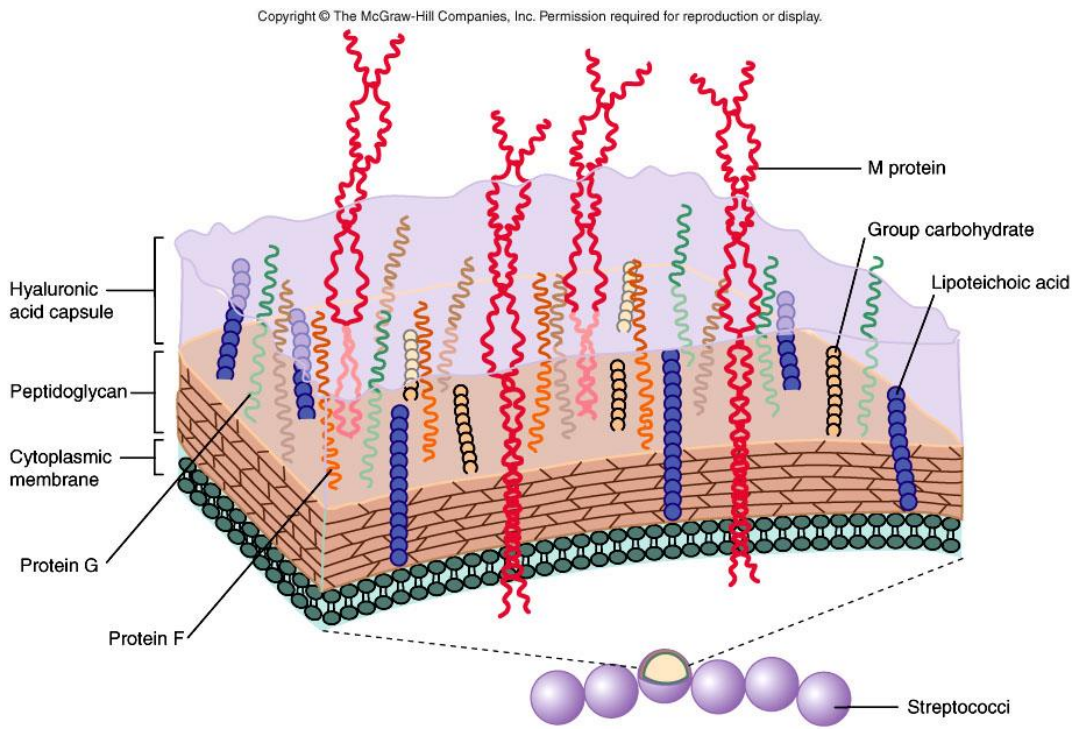


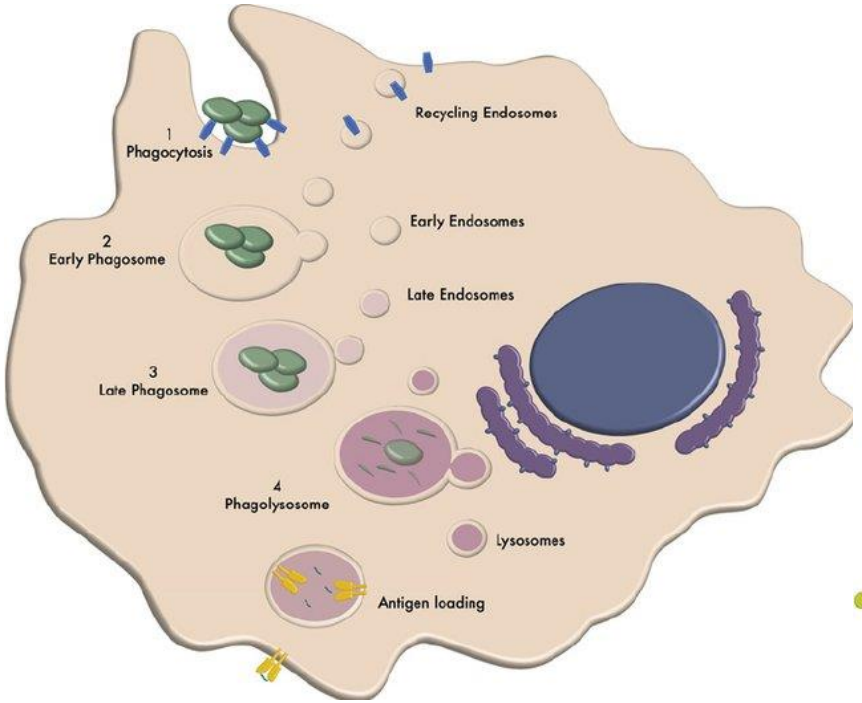
Como evitar a fagocitose?

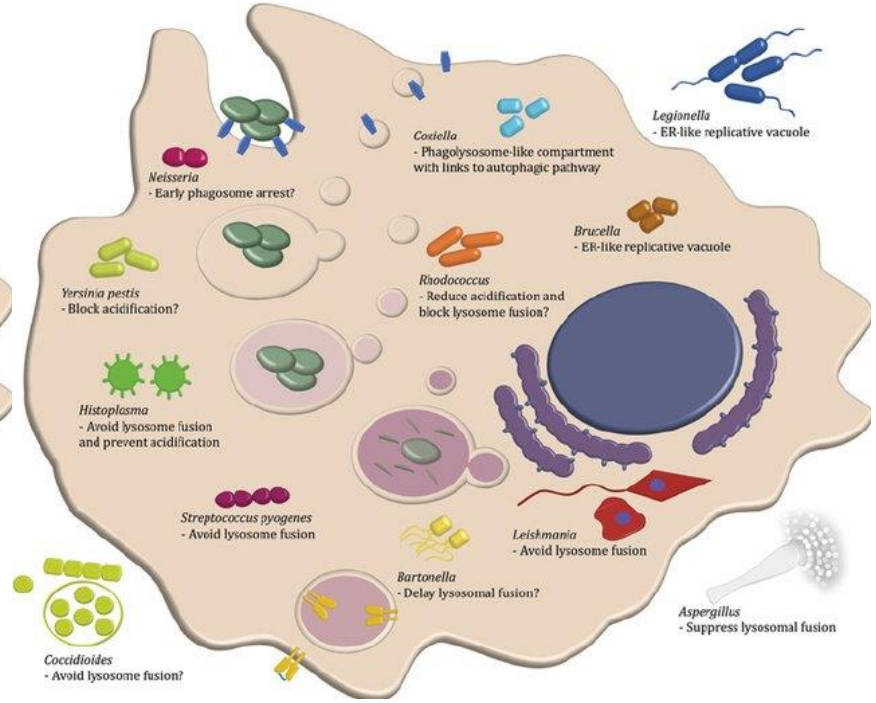
toxin release	opsonization prevented	contact with phagocyte prevented
		
organism releases toxin, e.g. staphylococci, streptococci, amoebae → phagocyte killed by toxin	organism (e.g. staphylococci) produces a protein (e.g. protein A) which prevents interaction between opsonizing antibody and phagocyte, so preventing phagocytosis	organism possesses a capsule which prevents contact with the phagocyte, e.g. *Streptococcus pneumoniae*, haemophilus, *Bacillus anthracis*
phagolysosome fusion inhibited	**escape into the cytoplasm**	**resistance to killing**
		
fusion of phagosome and lysosome inhibited by organism, e.g. *Mycobacterium tuberculosis*, toxoplasma, chlamydia	organism escapes from the phagolysosome into the cytoplasm and replicates within the phagocyte, e.g. Listeria, leishmania, *T. cruzi*. Even *M. tuberculosis* may do this!	organism resists killing by producing antioxidants, e.g. by catalase in staphylococci, or by scavenging free radicals, e.g. by phenolic glycolipid of *M. leprae*

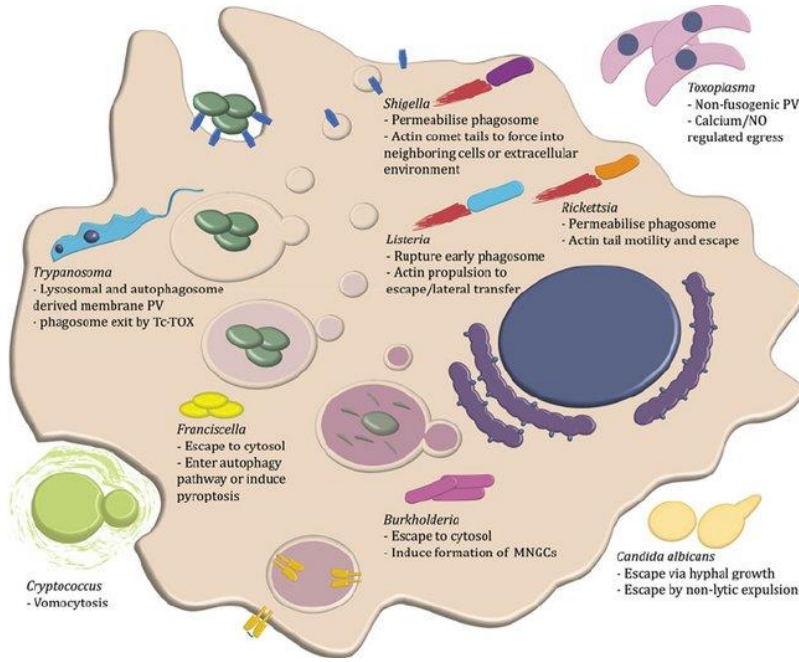
Componentes da parede celular que auxiliam a adesão e invasão

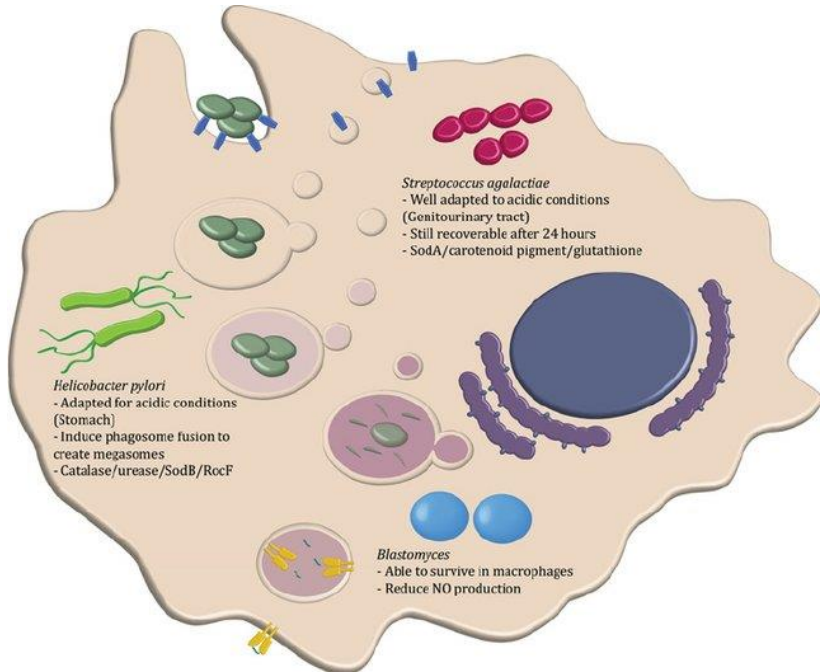
- Proteínas ou outras substâncias químicas: proteína M em *Streptococcus pyogenes* – impedem a ação da fagocitose pelos glóbulos brancos. Assim como as proteínas L auxiliam no adesão.

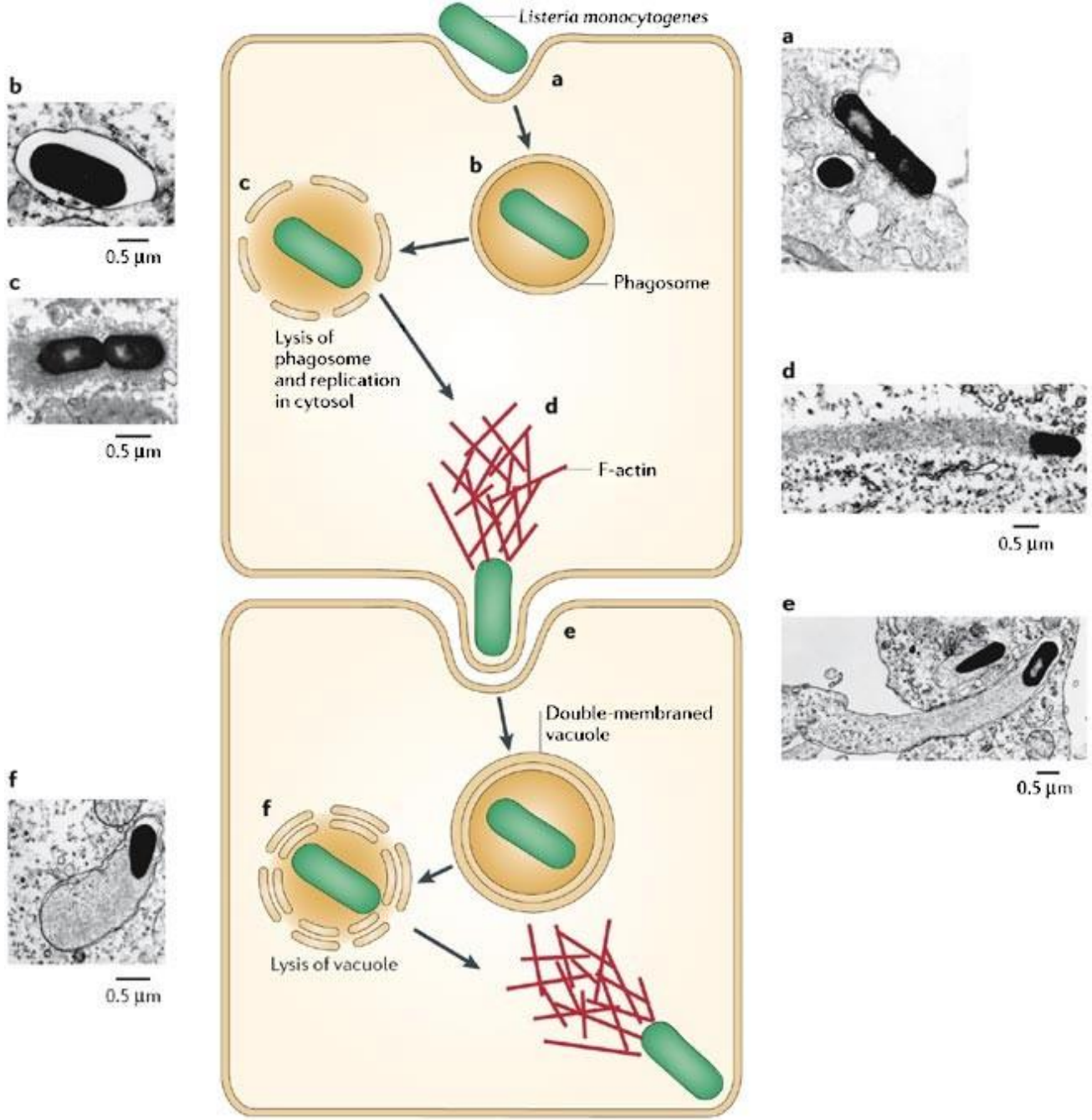



1
Phagocytosis
Recycling Endosomes
Early Endosomes
2
Early Phagosome
Late Endosomes
3
Late Phagosome
4
Phagolysosome
Lysosomes
Antigen loading

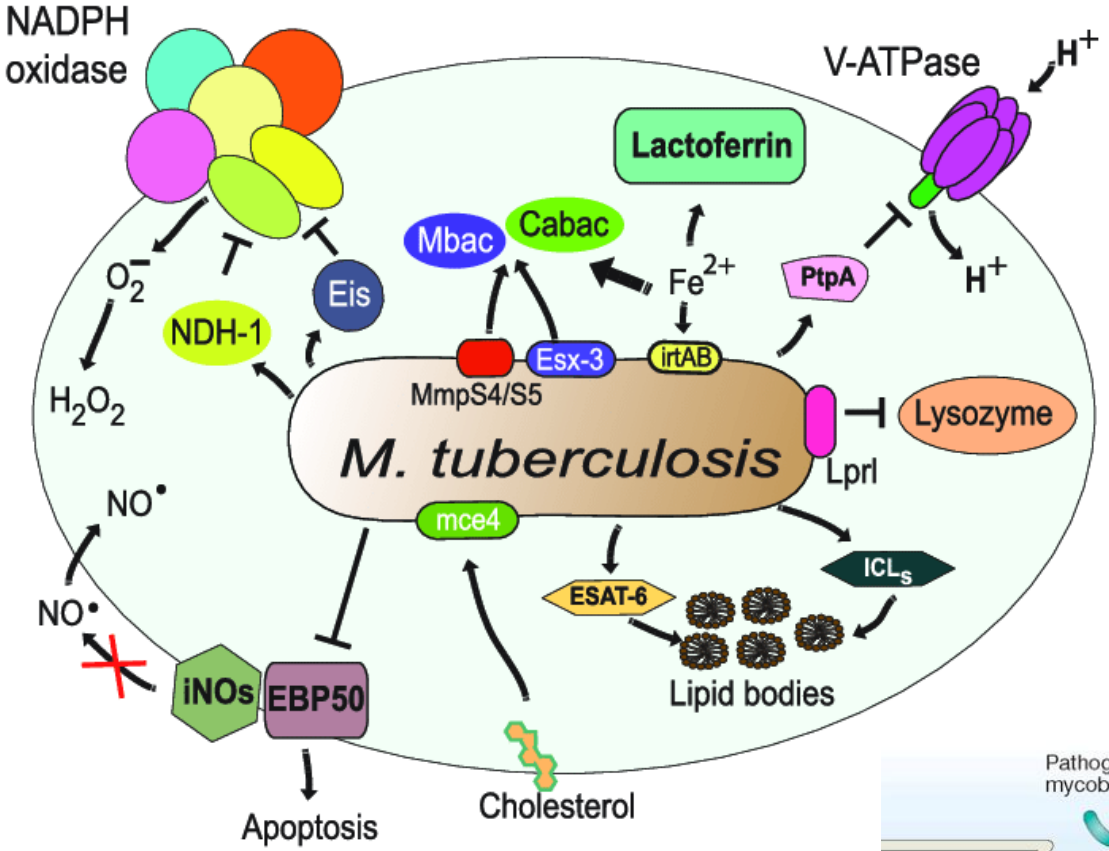

Neisseria
- Early phagosome arrest?
Coxiella
- Phagolysosome-like compartment with links to autophagic pathway
Legionella
- ER-like replicative vacuole
Yersinia pestis
- Block acidification?
Brucella
- ER-like replicative vacuole
Rhodococcus
- Reduce acidification and block lysosome fusion?
Histoplasma
- Avoid lysosome fusion and prevent acidification
Streptococcus pyogenes
- Avoid lysosome fusion
Leishmania
- Avoid lysosome fusion
Bartonella
- Delay lysosomal fusion?
Aspergillus
- Suppress lysosomal fusion
Coccidioides
- Avoid lysosome fusion?

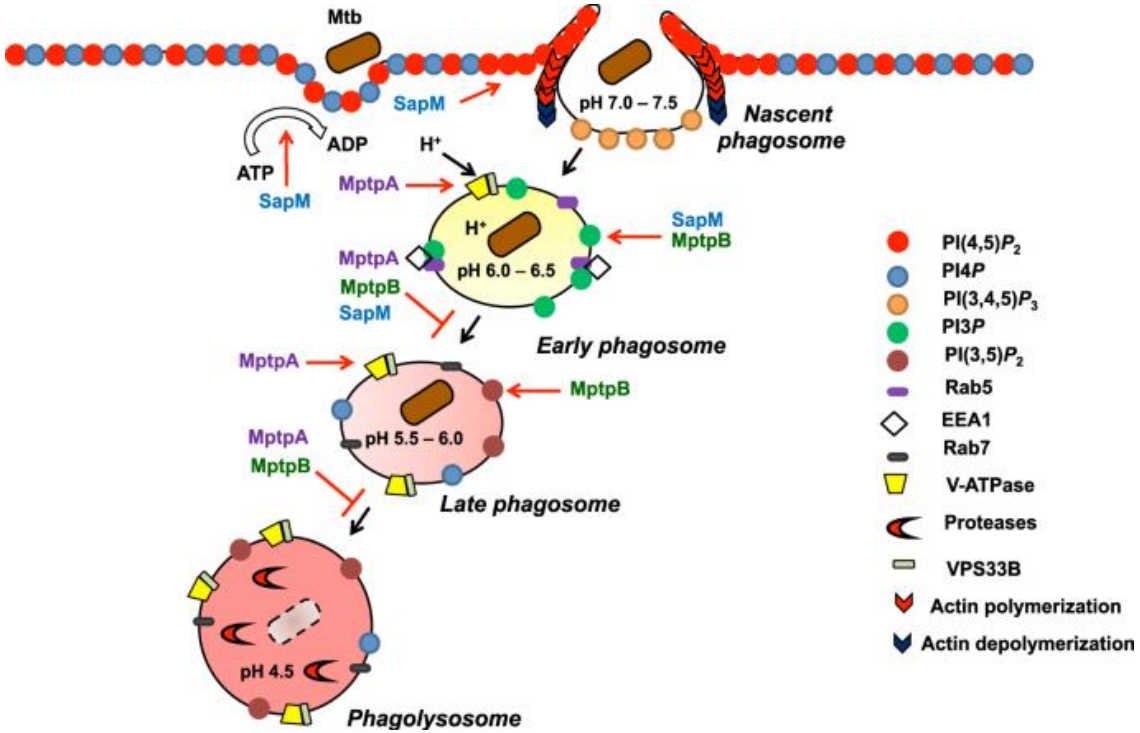

Toxoplasma
- Non-fusogenic PV
- Calcium/NO regulated egress
Shigella
- Permeabilise phagosome
- Actin comet tails to force into neighboring cells or extracellular environment
Rickettsia
- Permeabilise phagosome
- Actin tail motility and escape
Listeria
- Rupture early phagosome
- Actin propulsion to escape/lateral transfer
Trypanosoma
- Lysosomal and autophagosome derived membrane PV
- phagosome exit by Tc-TOX
Franciscella
- Escape to cytosol
- Enter autophagy pathway or induce pyroptosis
Burkholderia
- Escape to cytosol
- Induce formation of MNGCs
Candida albicans
- Escape via hyphal growth
- Escape by non-lytic expulsion
Cryptococcus
- Vomocytosis

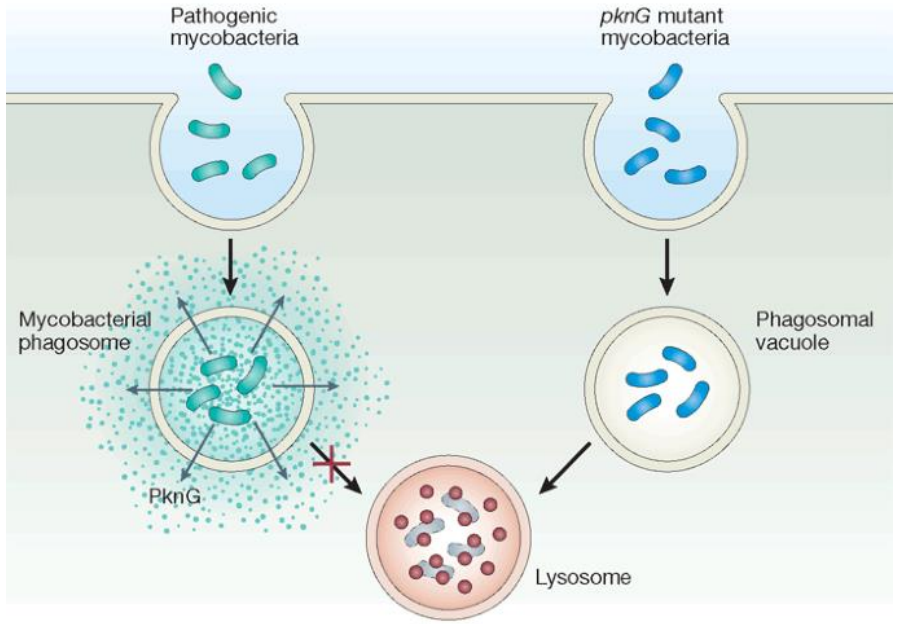

Streptococcus agalactiae
- Well adapted to acidic conditions (Genitourinary tract)
- Still recoverable after 24 hours
- SodA/carotenoid pigment/glutathione
Helicobacter pylori
- Adapted for acidic conditions (Stomach)
- Induce phagosome fusion to create megasomes
- Catalase/urease/SodB/RocF
Blastomyces
- Able to survive in macrophages
- Reduce NO production

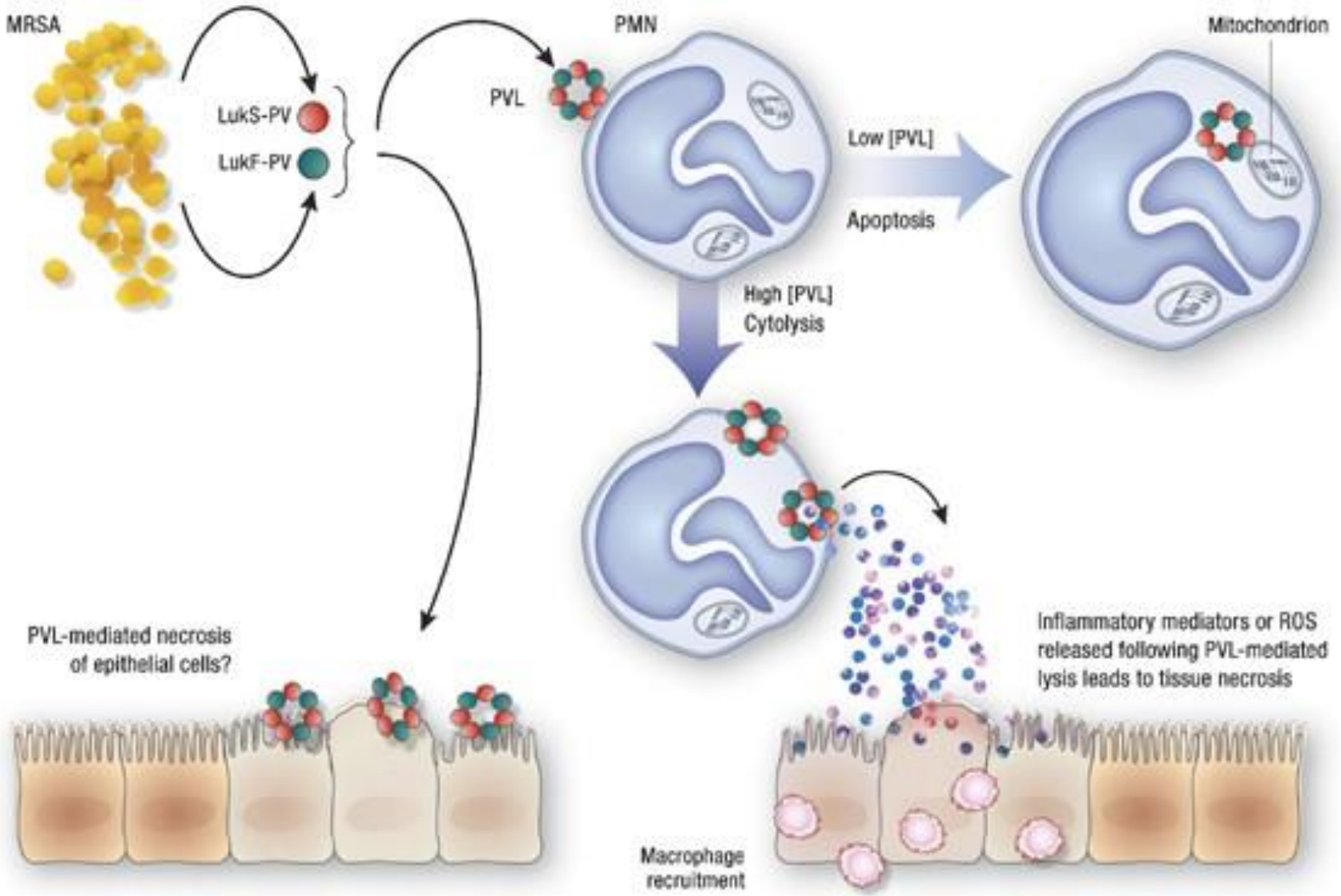


https://youtu.be/4JWpG8XPku4

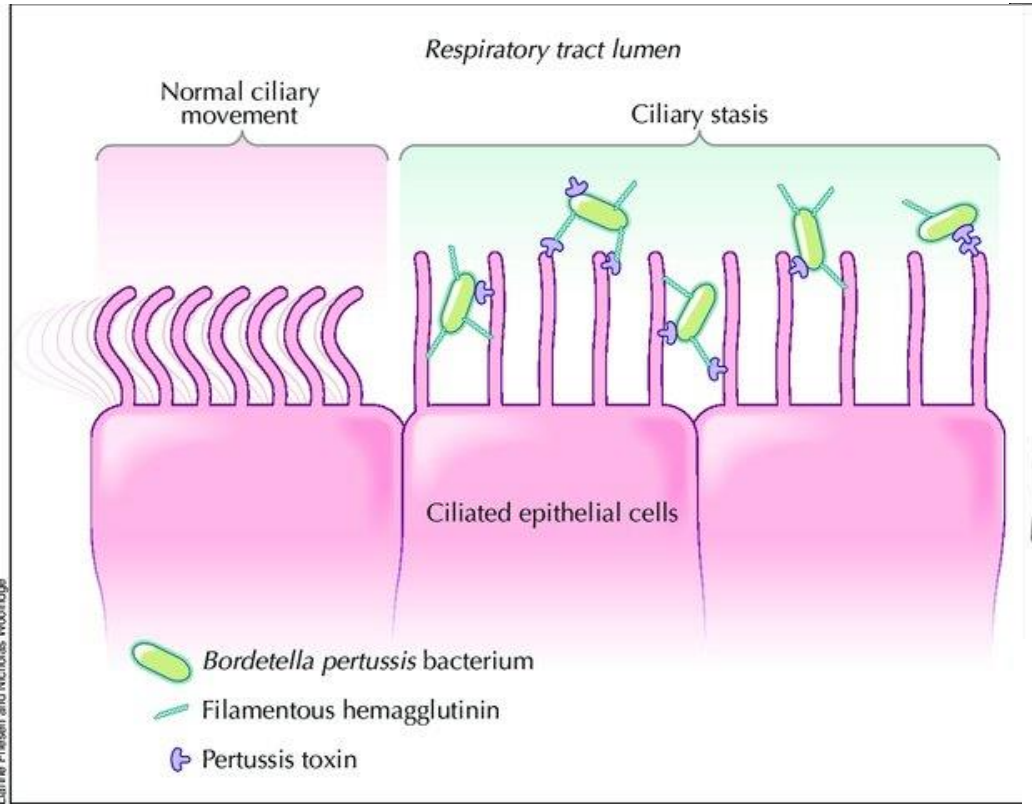

NADPH oxidase
V-ATPase
H+
Lactoferrin
Mbac
Cabac
Fe2+
PtpA
H+
O2-
Eis
NDH-1
H2O2
Esx-3
irtAB
MmpS4/S5
M. tuberculosis
Lysozyme
LprI
NO•
mce4
ICLs
ESAT-6
Lipid bodies
NO•
iNOs
EBP50
Apoptosis
Cholesterol

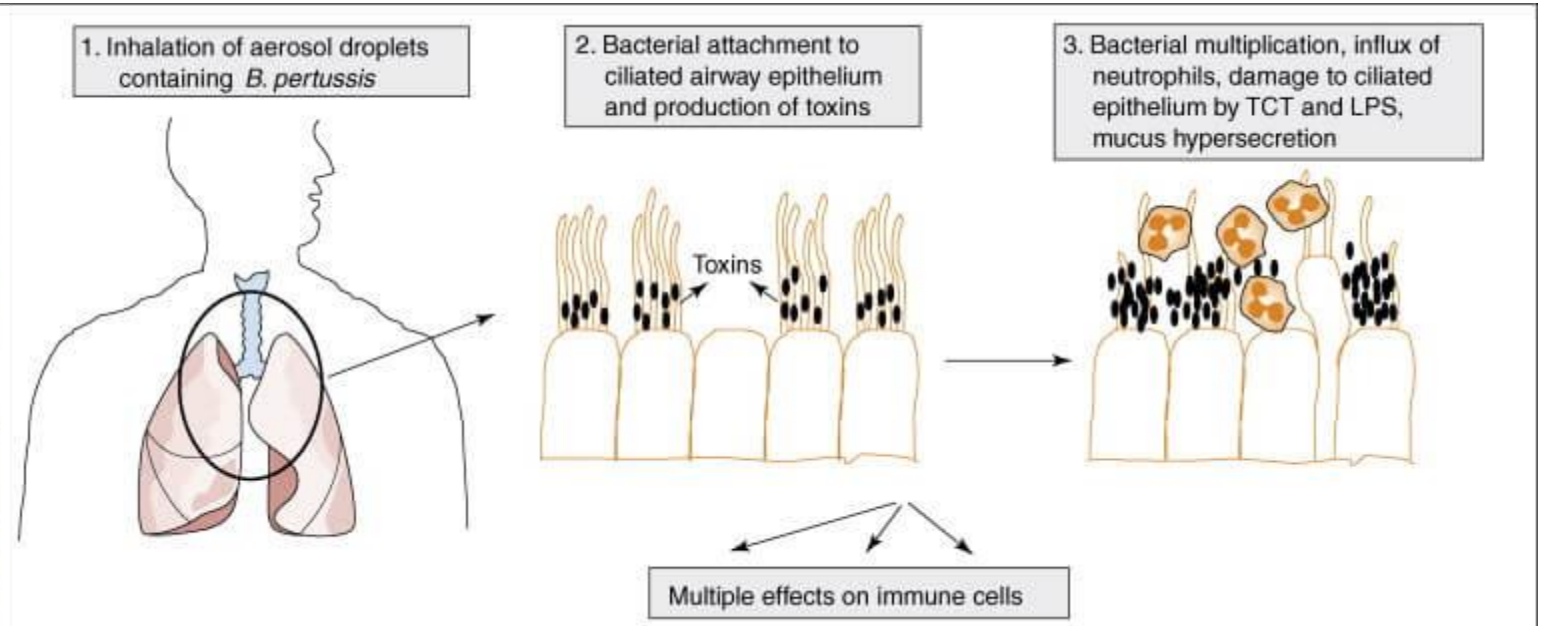

Mtb
SapM
pH 7.0 – 7.5
Nascent phagosome
ATP
ADP
SapM
H+
MptpA
H+
pH 6.0 – 6.5
SapM
MptpB
MptpA
MptpB
SapM
Early phagosome
MptpA
MptpB
pH 5.5 – 6.0
MptpA
MptpB
Late phagosome
pH 4.5
Phagolysosome
PI(4,5)P2
PI4P
PI(3,4,5)P3
PI3P
PI(3,5)P2
Rab5
EEA1
Rab7
V-ATPase
Proteases
VPS33B
Actin polymerization
Actin depolymerization


Pathogenic mycobacteria
pknG mutant mycobacteria
Mycobacterial phagosome
PknG
Phagosomal vacuole
Lysosome


MRSA
LukS-PV
LukF-PV
PVL
PMN
Mitochondrion
Low [PVL]
Apoptosis
High [PVL]
Cytolysis
PVL-mediated necrosis
of epithelial cells?
Inflammatory mediators or ROS
released following PVL-mediated
lysis leads to tissue necrosis
Macrophage
recruitment

Interferência na ação ciliar por *Bordetella*





Interferência na ativação do complemento

1 – presença de cápsula previne a ativação;

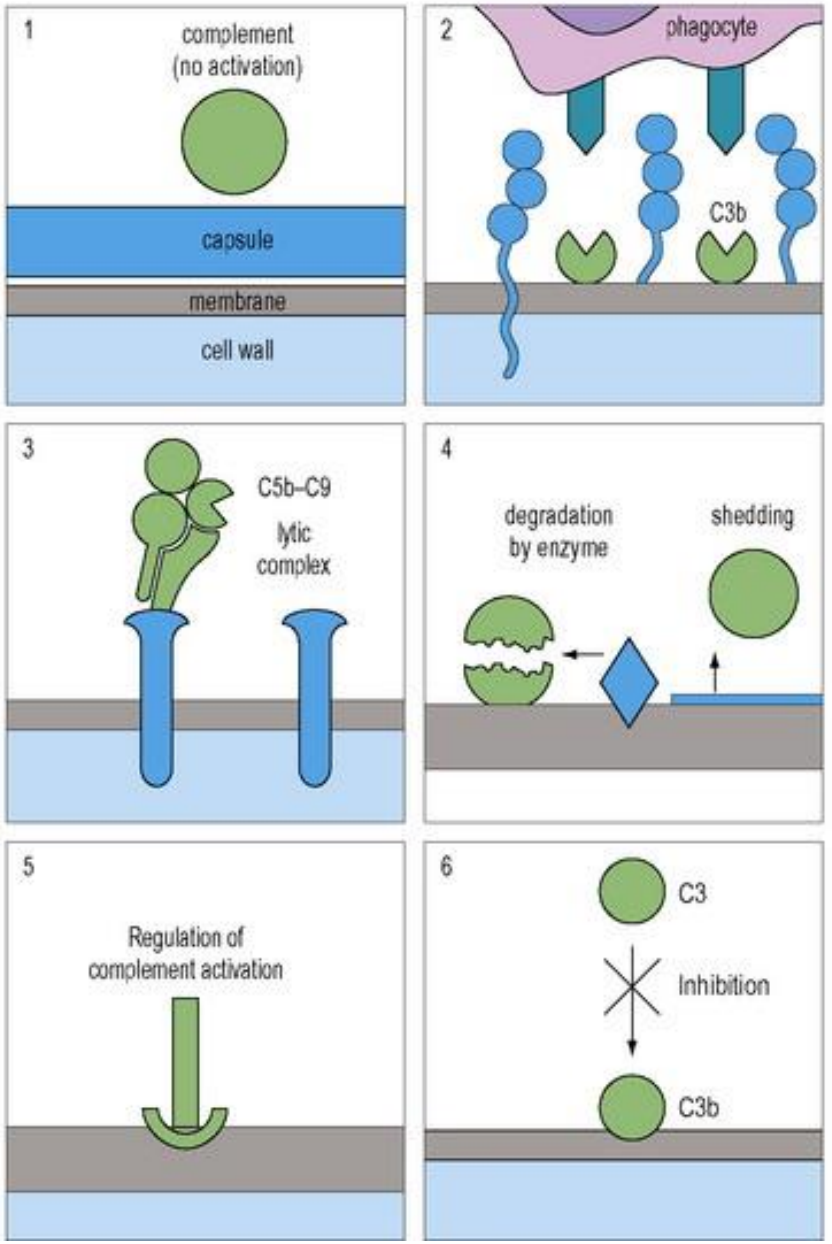
2 – configuração da superfície bacteriana evitando que receptores celulares não tenham acesso a C3b;

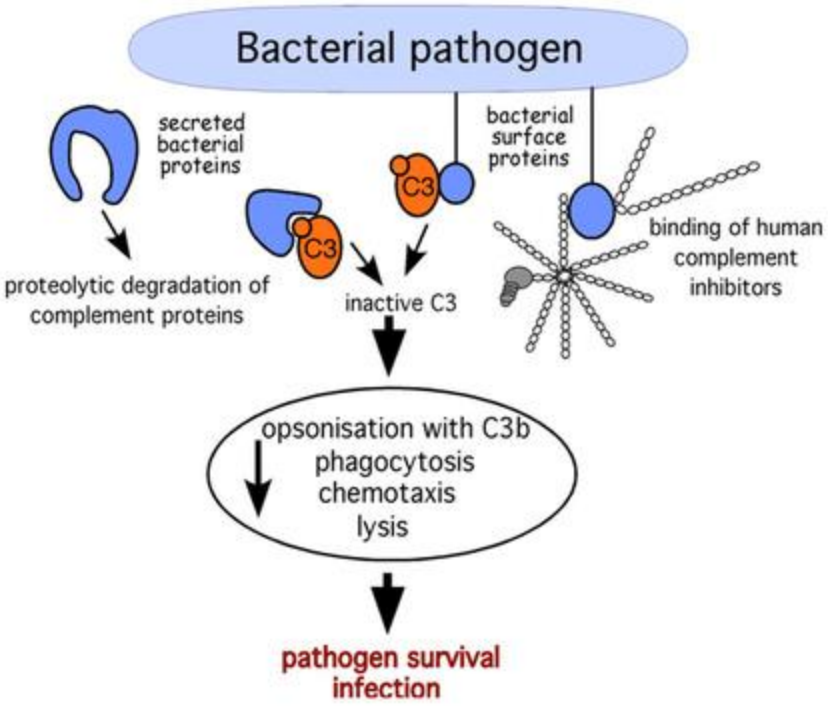
3- estruturas superficiais podem ser expressas e divergem da anexação do complexo lítico da membrana celular

4- proteases ligadas a membrana degradam o complemento

5- a superfície bacteriana pode apresentar inibidores da ativação do complemento

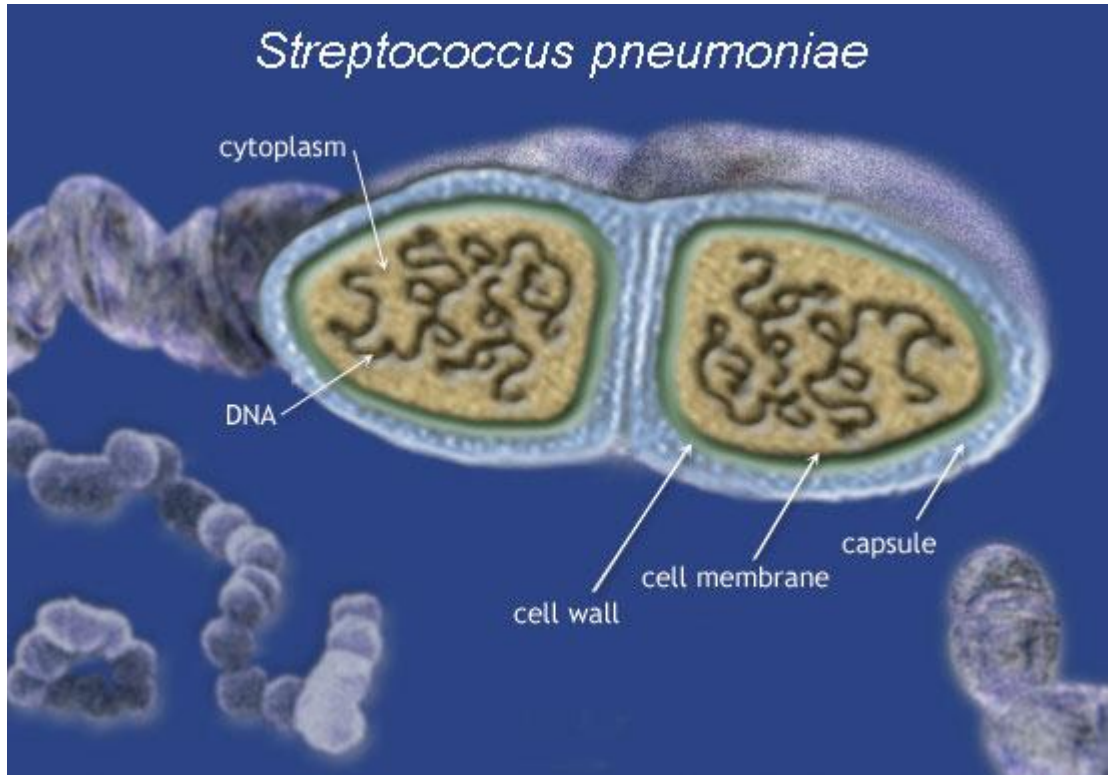
6- inibidores diretos das convertases C3 e C5 bloqueando a ativação do complemento



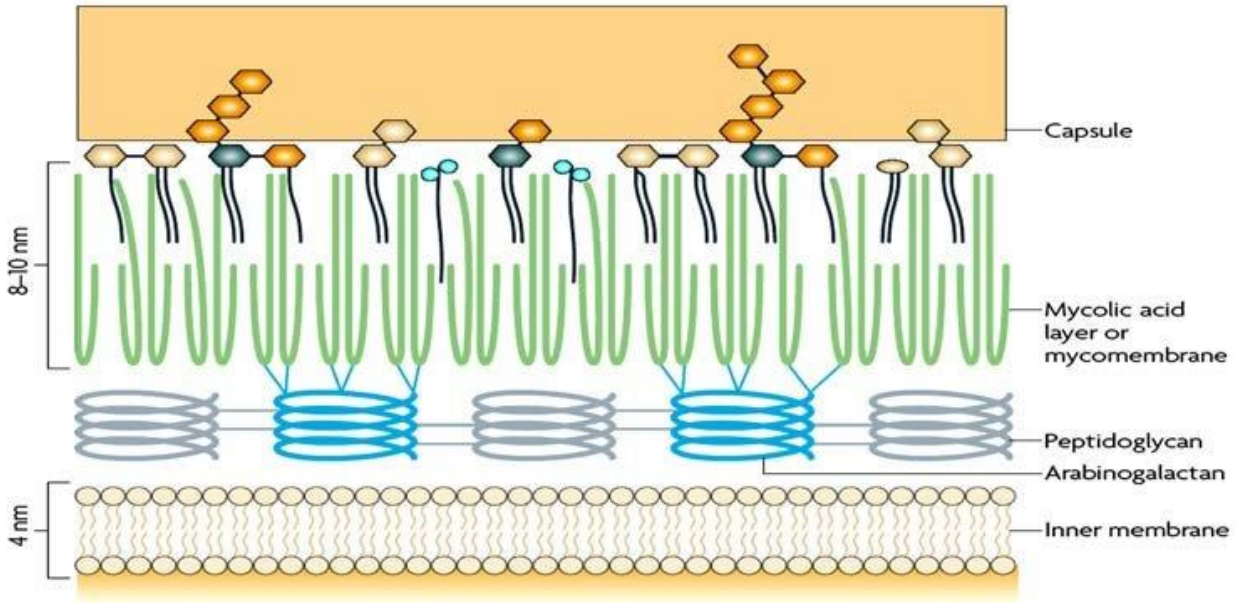


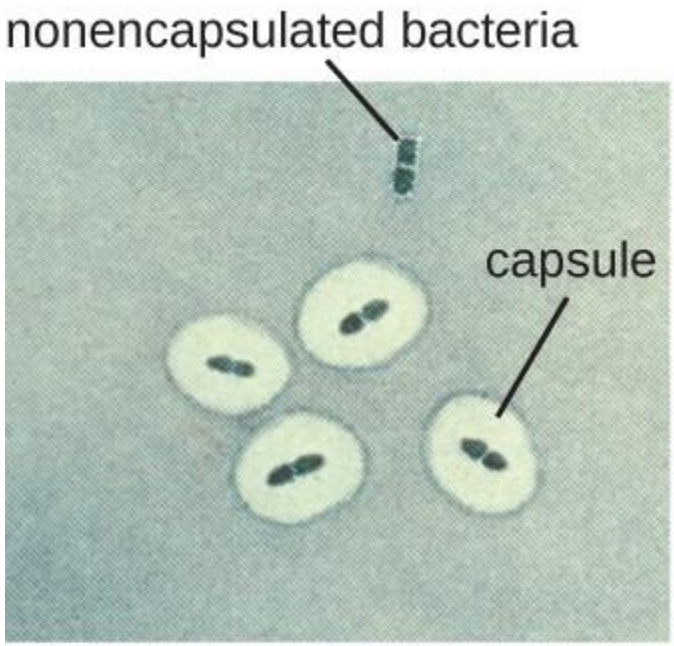
Evitar o reconhecimento pelo constituintes celulares e humorais do sistema imune

- Cápsula = impede a fagocitose

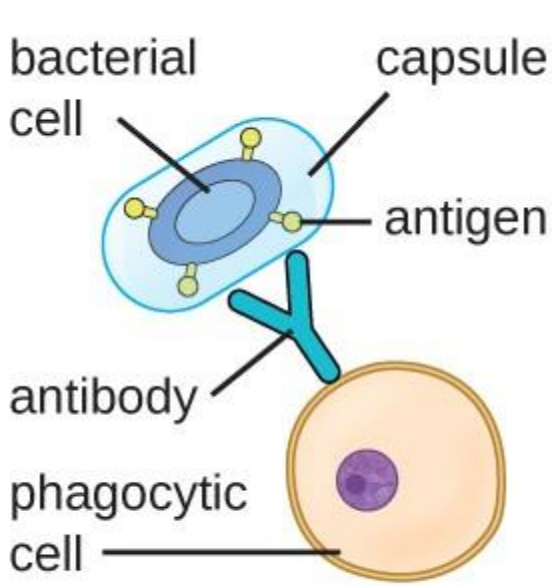


Parede celular micobacterina

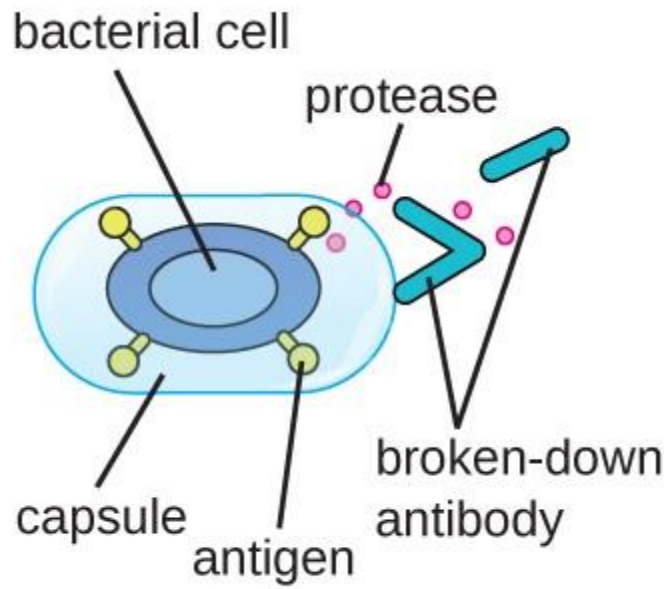



nonencapsulated bacteria
capsule

(a)


bacterial cell
capsule
antigen
antibody
phagocytic cell

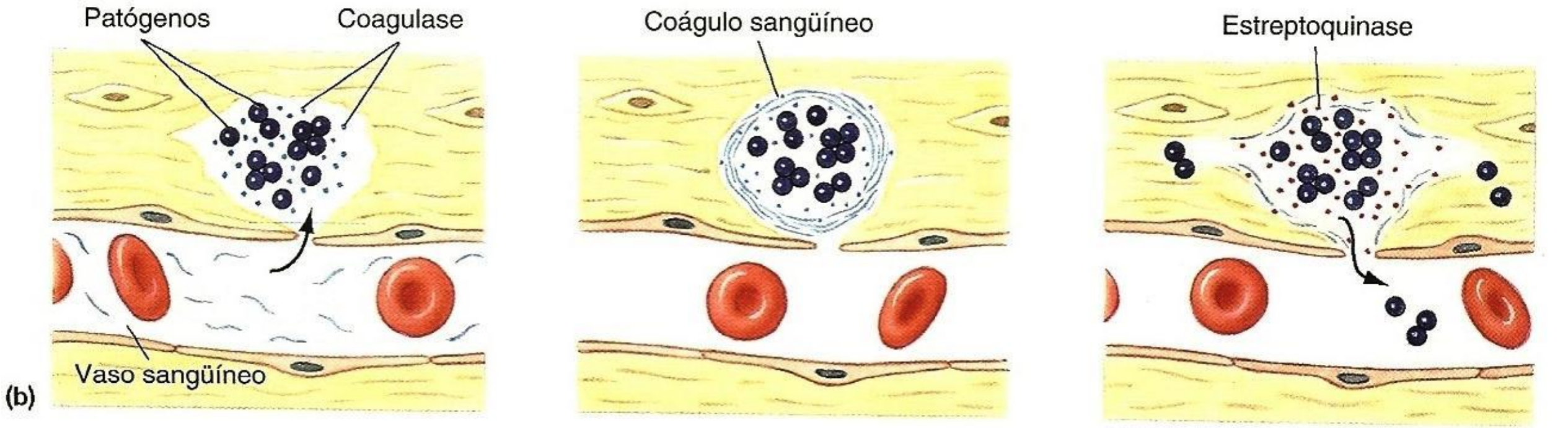
(b)


bacterial cell
protease
capsule
antigen
broken-down antibody

(c)

Evitar o reconhecimento pelo constituintes celulares e humorais do sistema imune

Presença de coagulase – *Staphylococcus aureus*



1. Patógenos produzem coagulase

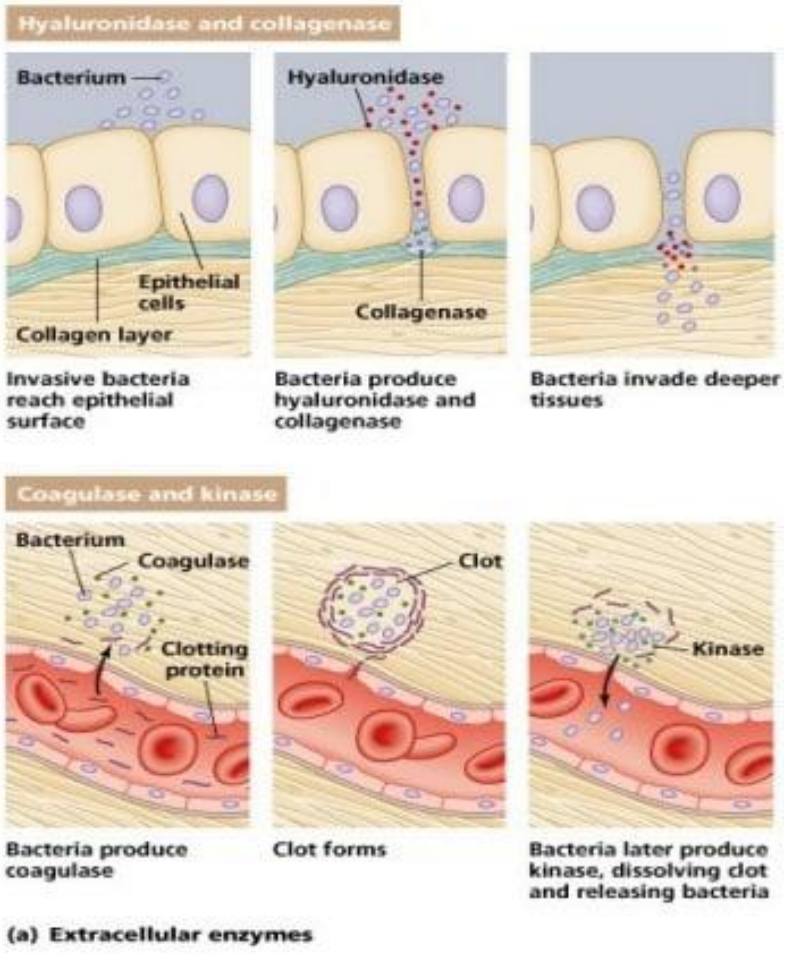
2. Forma-se um coágulo sangüíneo ao redor dos patógenos

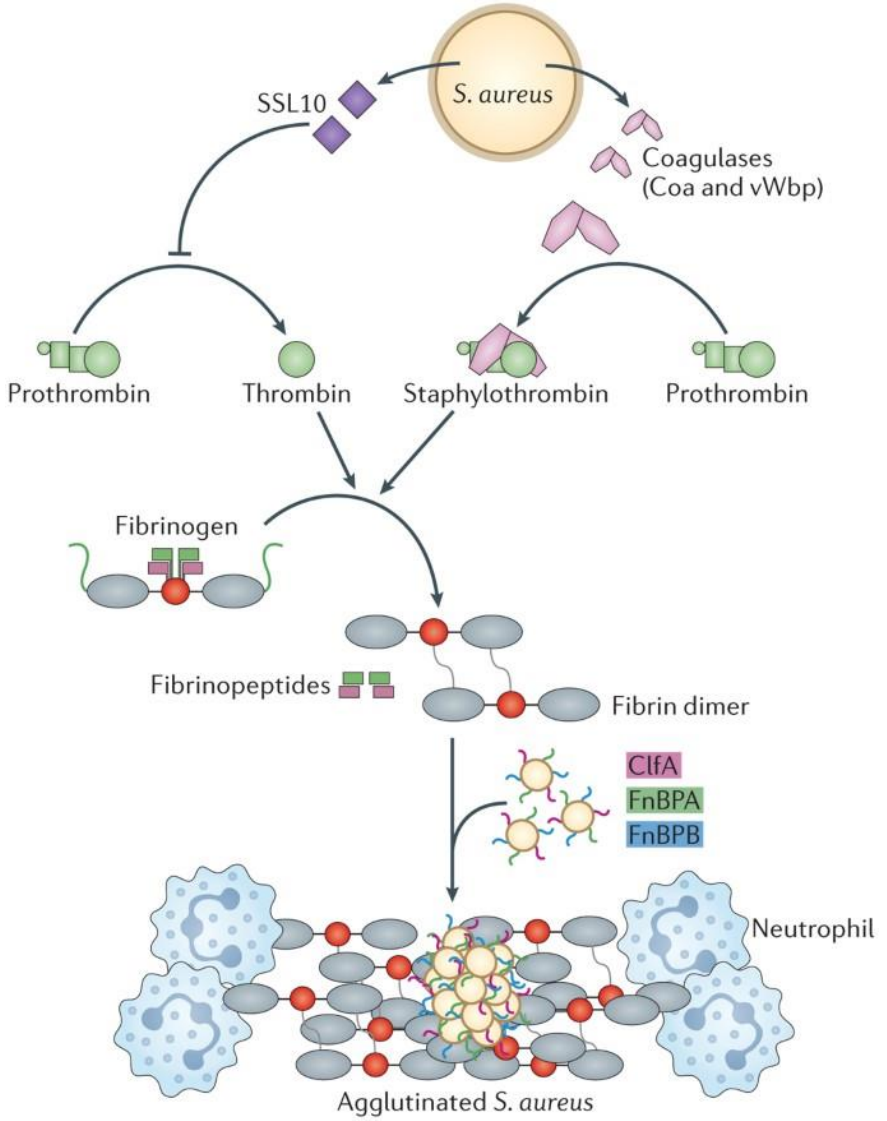
3. Patógenos produzem estreptoquinase, ocorrendo a dissolução do coágulo e liberando as bactérias

Evitar o reconhecimento pelo constituintes celulares e humorais do sistema imune

Invasiveness

- Hyaluronidase
- Coagulase
- Streptokinase (dissolves Clots)





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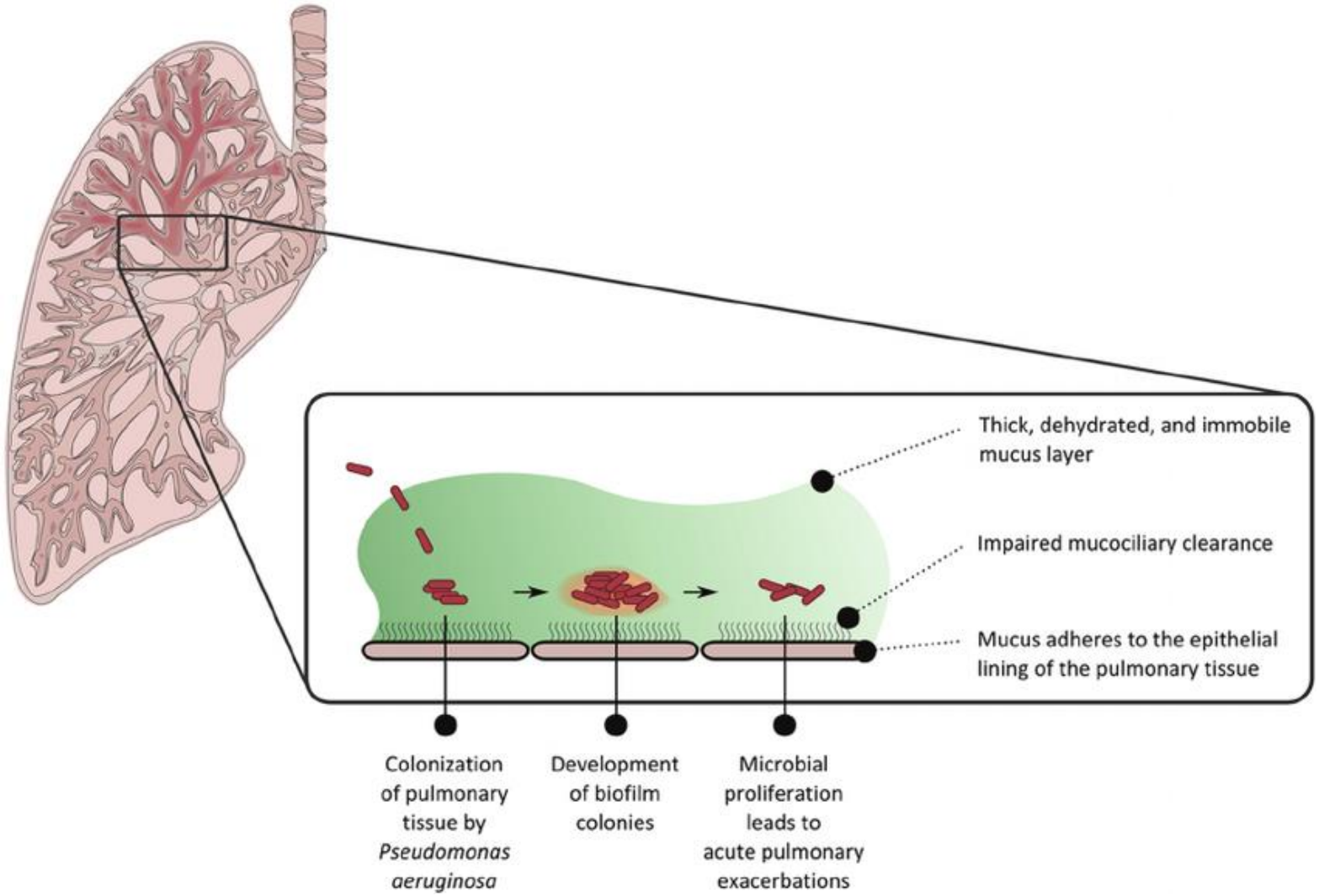
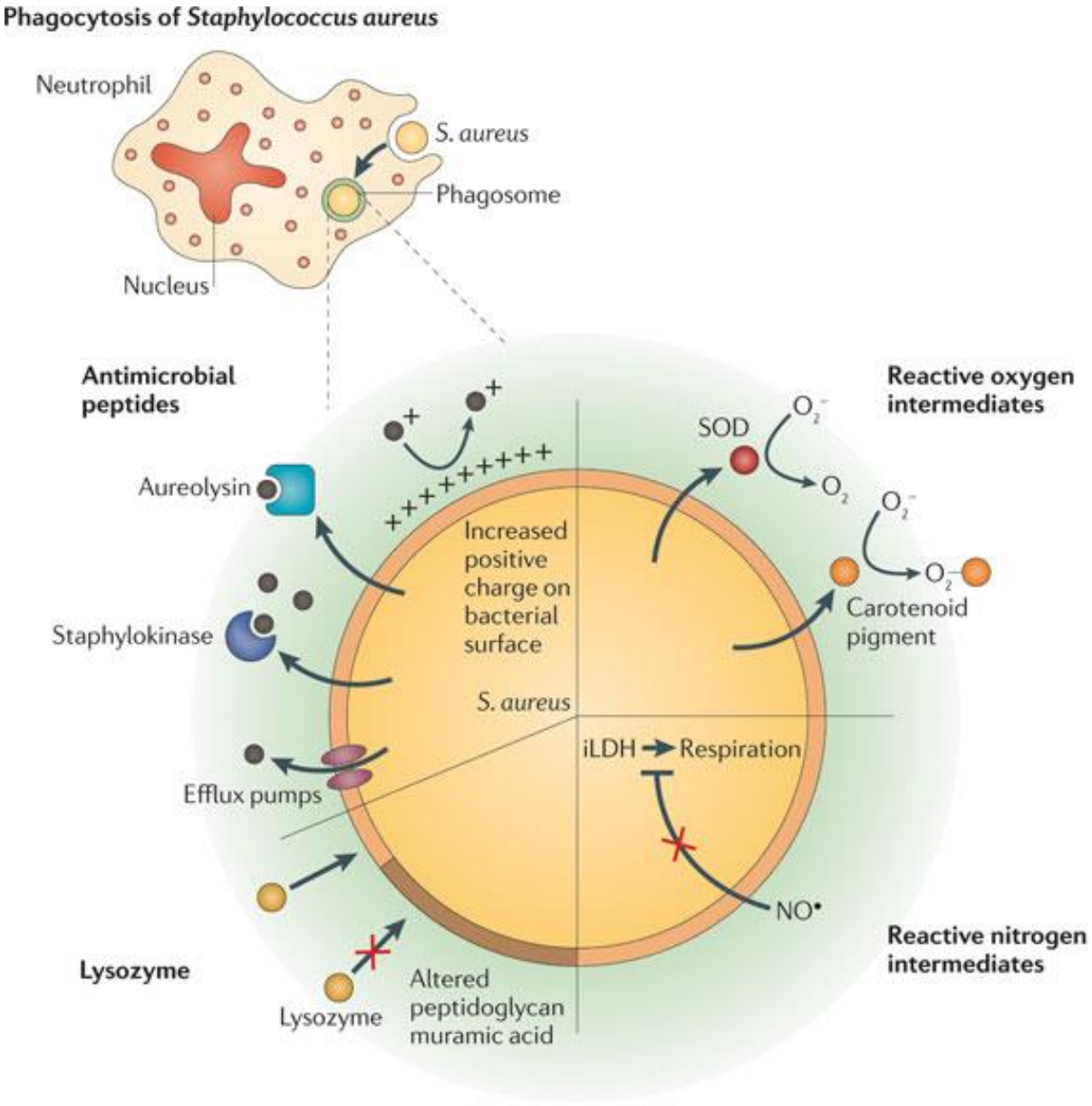
Evitar o reconhecimento pelo constituintes celulares e humorais do sistema imune



Destruição do peróxido de hidrogênio e outros componentes antimicrobianos do hospedeiro



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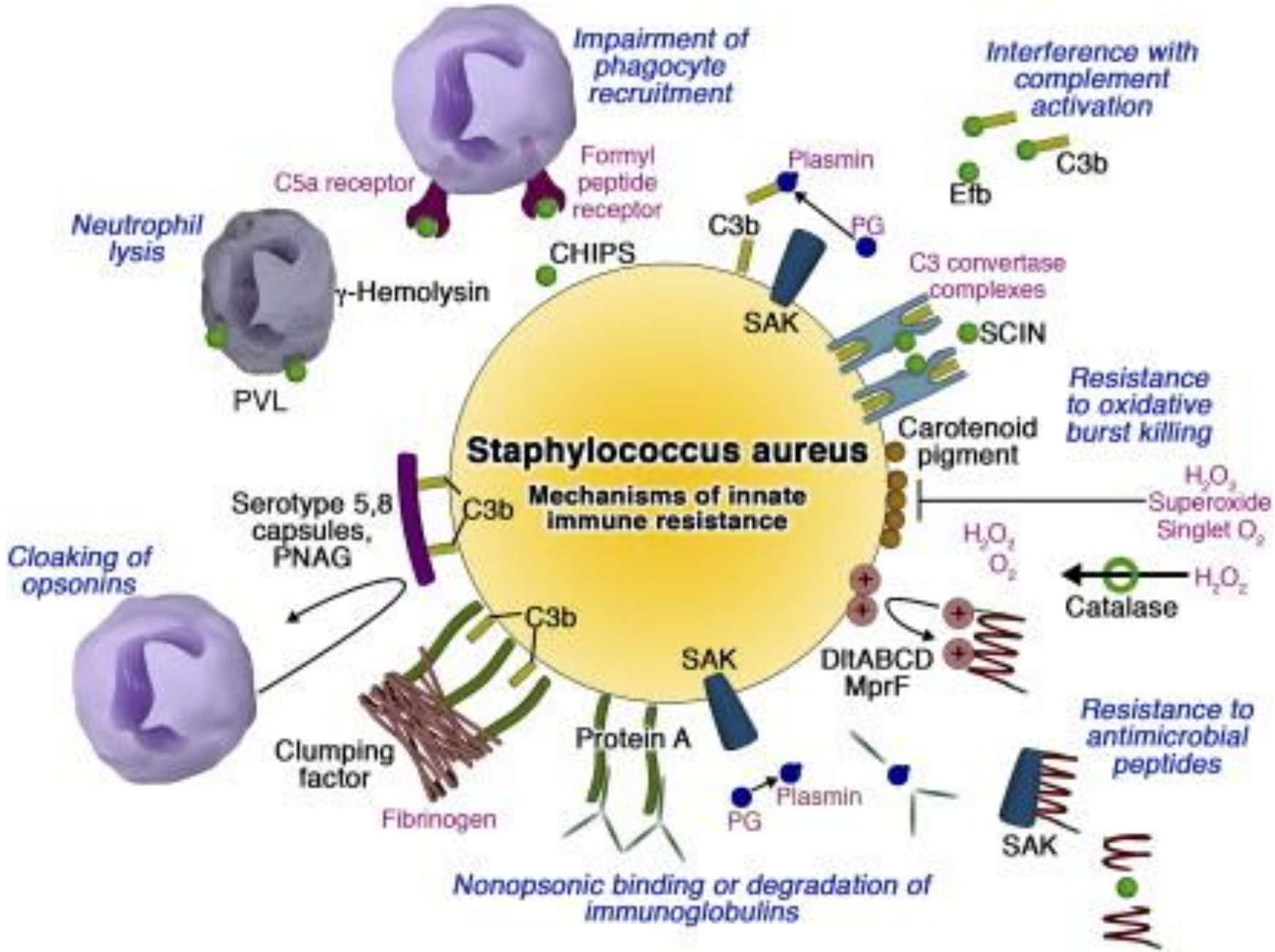
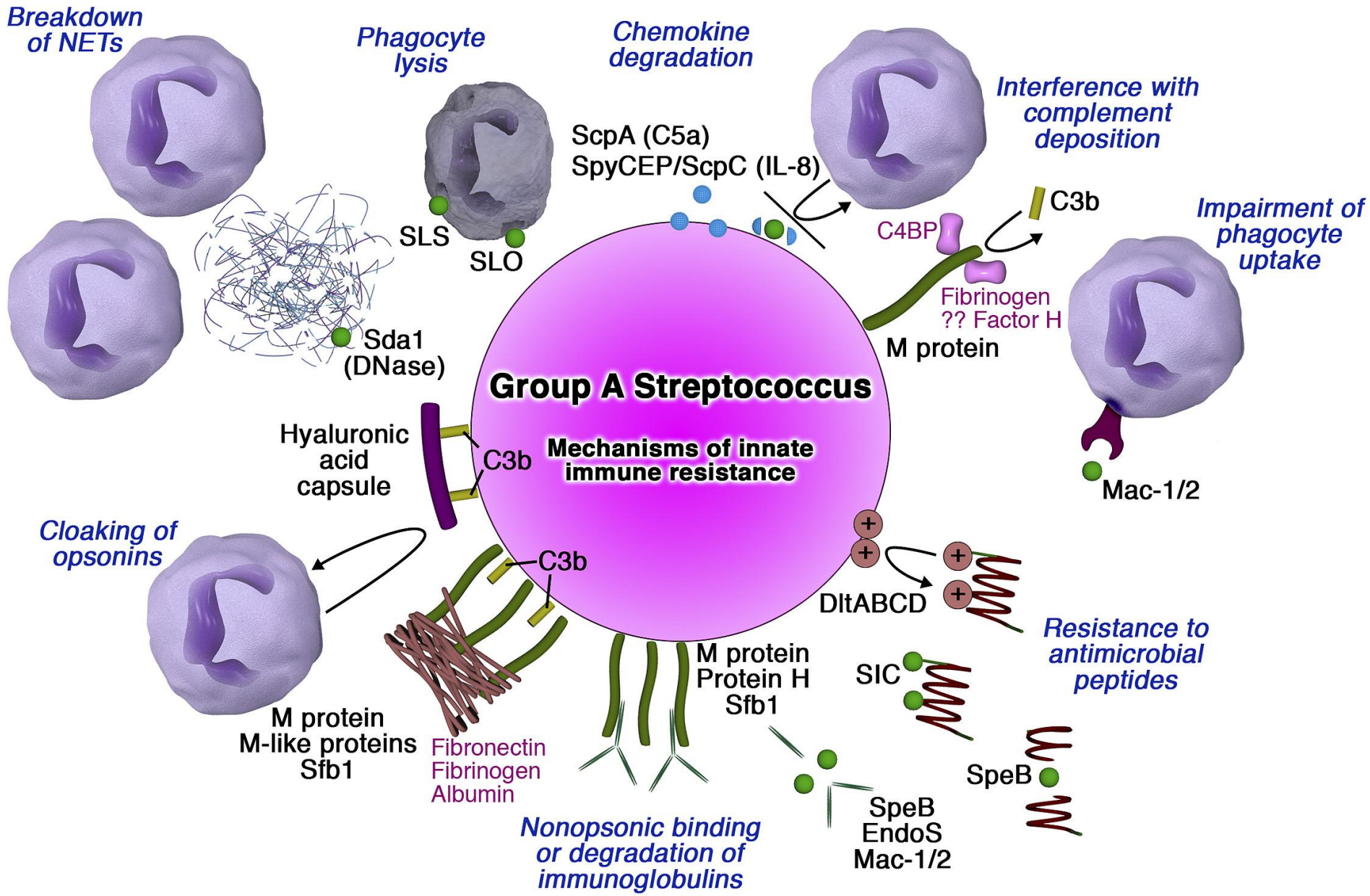

Impairment of phagocyte recruitment
C5a receptor
Formyl peptide receptor
Neutrophil lysis
γ-Hemolysin
PVL
CHIPS
Interference with complement activation
C3b
Efb
Plasmin
PG
C3b
SAK
C3 convertase complexes
SCIN
Resistance to oxidative burst killing
Carotenoid pigment
H_2O_2
Superoxide
Singlet O_2
H_2O_2
O_2
H_2O_2
Catalase
DltABCD
MprF
Staphylococcus aureus
Mechanisms of innate immune resistance
Serotype 5,8 capsules, PNAG
C3b
Cloaking of opsonins
C3b
SAK
Protein A
Clumping factor
Fibrinogen
Plasmin
PG
Resistance to antimicrobial peptides
SAK
Nonopsonic binding or degradation of immunoglobulins


Breakdown of NETs
Phagocyte lysis
Chemokine degradation
Interference with complement deposition
Impairment of phagocyte uptake
ScpA (C5a)
SpyCEP/ScpC (IL-8)
SLS
SLO
C4BP
C3b
Fibrinogen
?? Factor H
M protein
Sda1
(DNase)
Group A Streptococcus
Mechanisms of innate immune resistance
Hyaluronic acid capsule
C3b
Mac-1/2
Cloaking of opsonins
C3b
DltABCD
Resistance to antimicrobial peptides
M protein
Protein H
Sfb1
SIC
M protein
M-like proteins
Sfb1
Fibronectin
Fibrinogen
Albumin
SpeB
SpeB
EndoS
Mac-1/2
Nonopsonic binding or degradation of immunoglobulins

Estratégias para evasão de defesas adaptativas

1- Mascaramento de antígenos

2- variação antigênica

3- imunossupressão

Mascaramento de antígenos

Estratégia adotada para microrganismos intracelulares

Colonização de “locais privilegiados”, como sistema nervoso central, articulações, testículos e placenta, ou mesmo aquele gerados pelo próprio microrganismo

Mimetizar antígenos do hospedeiro

Absorção de antígenos

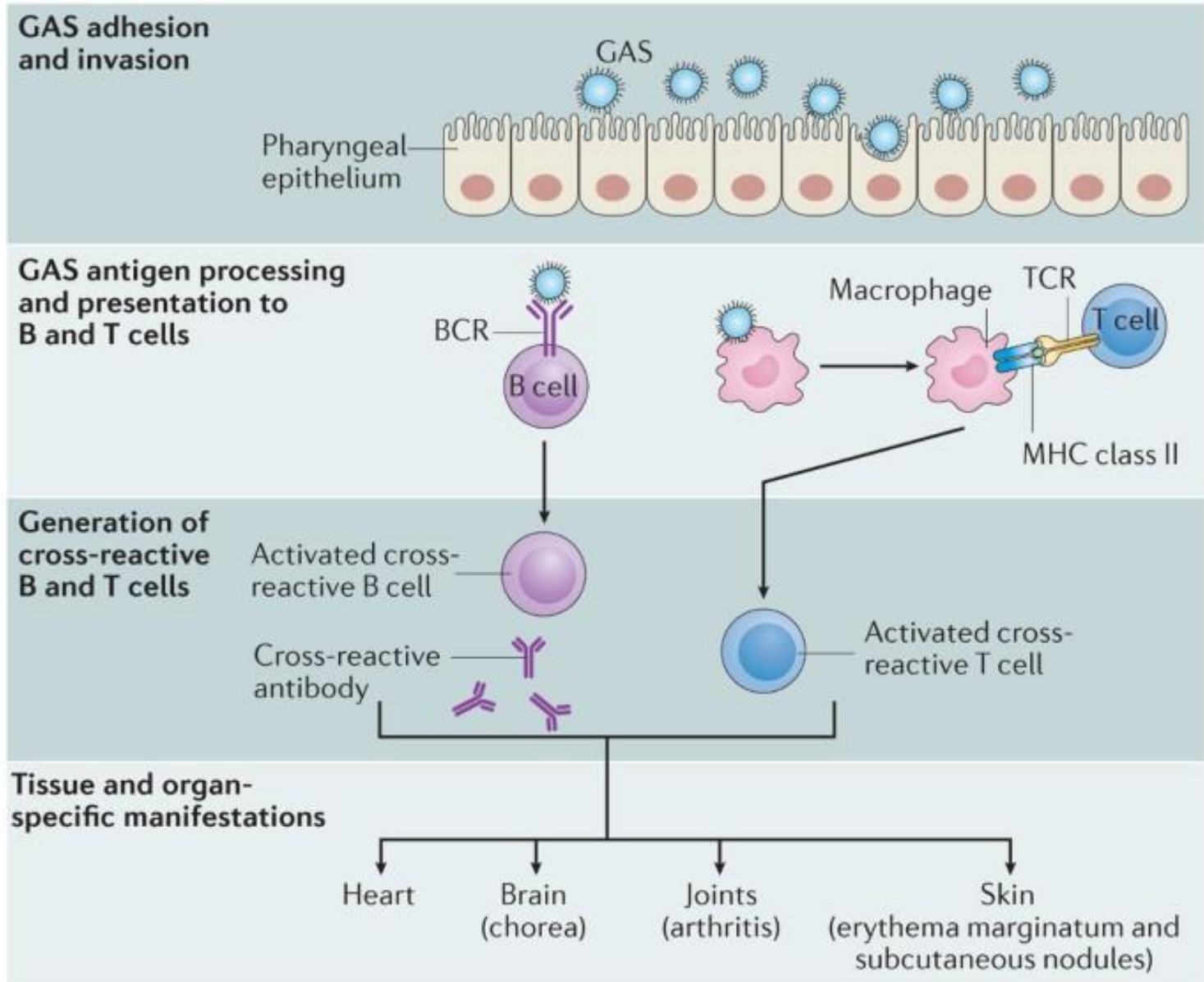
Imunomodulações: -

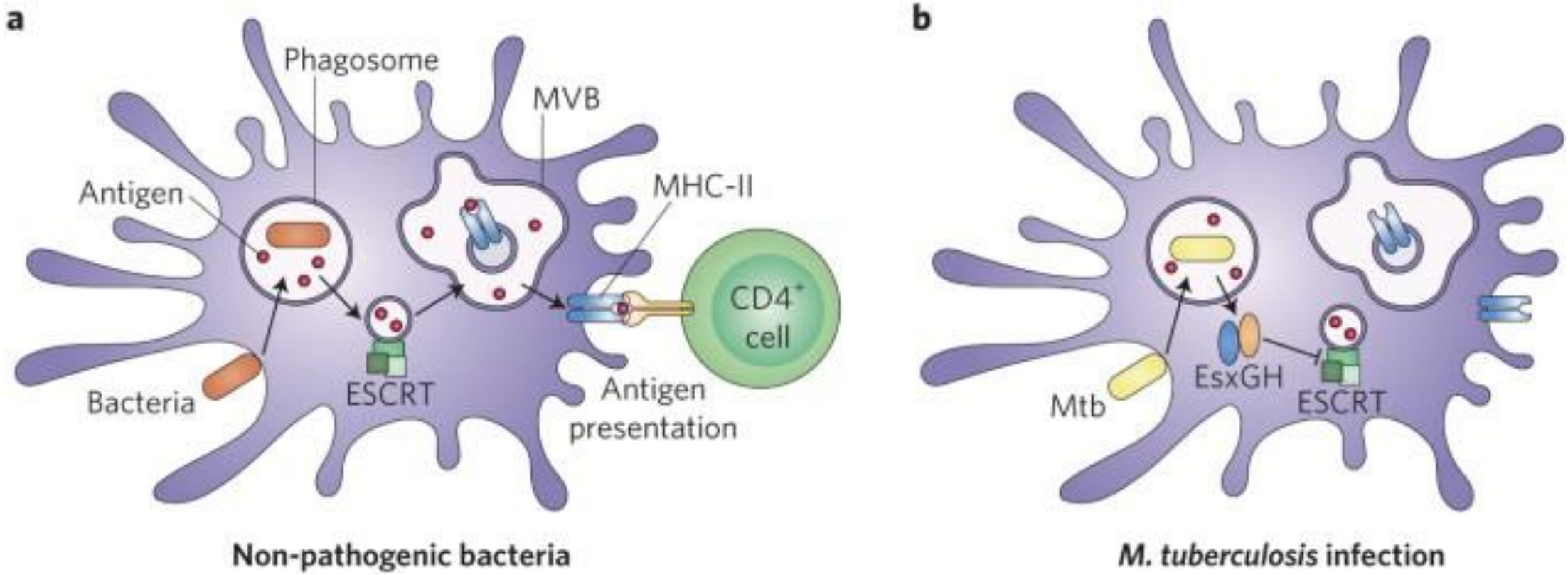
Perturbações no equilíbrio das respostas imunológicas

produção de grande quantidade de antígeno

produção de antígenos com receptores Fc (não desencadeia atividade)

indução de células T reguladoras

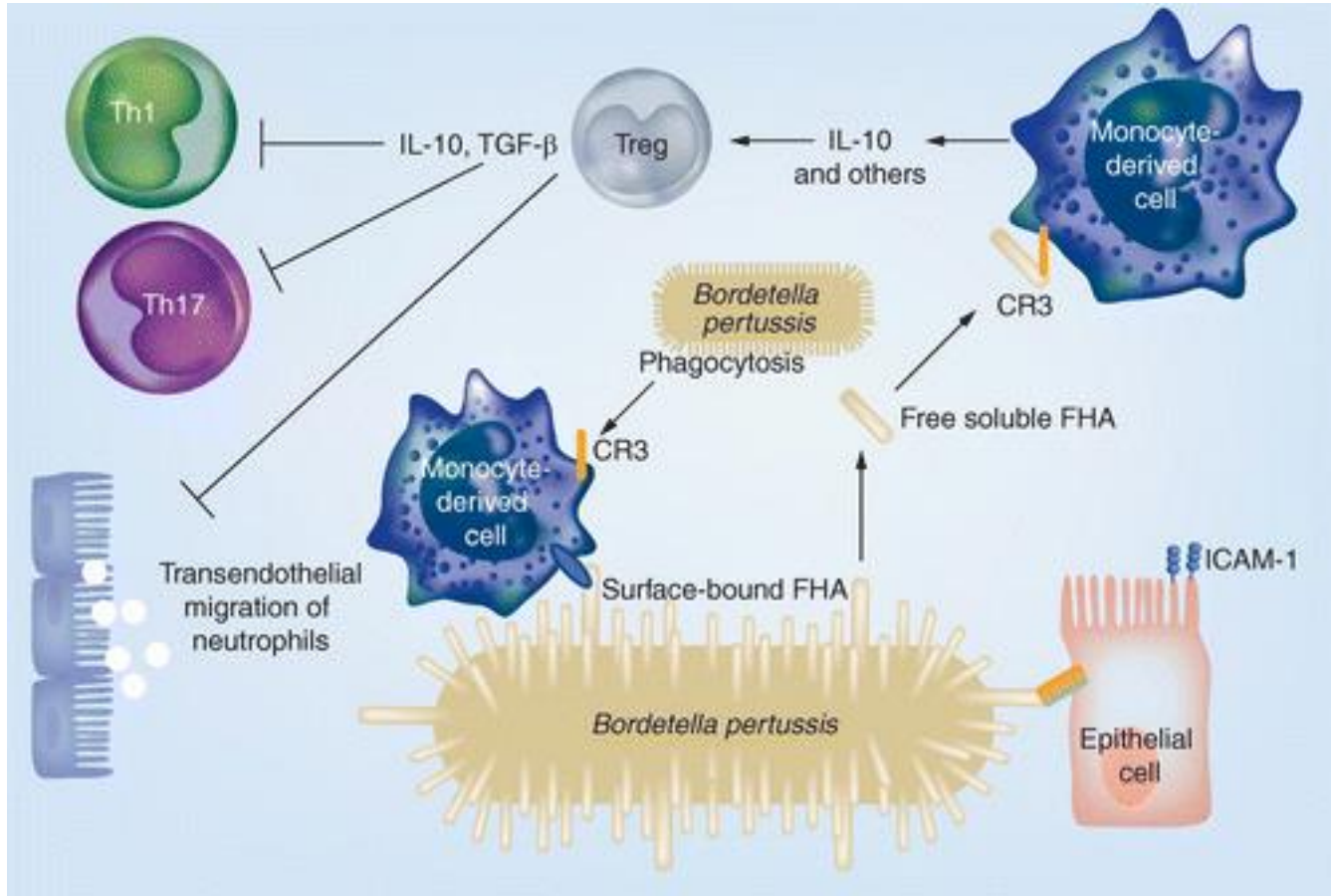

GAS adhesion and invasion
GAS
Pharyngeal epithelium
GAS antigen processing and presentation to B and T cells
BCR
B cell
Macrophage
TCR
T cell
MHC class II
Generation of cross-reactive B and T cells
Activated cross-reactive B cell
Cross-reactive antibody
Activated cross-reactive T cell
Tissue and organ-specific manifestations
Heart
Brain (chorea)
Joints (arthritis)
Skin (erythema marginatum and subcutaneous nodules)

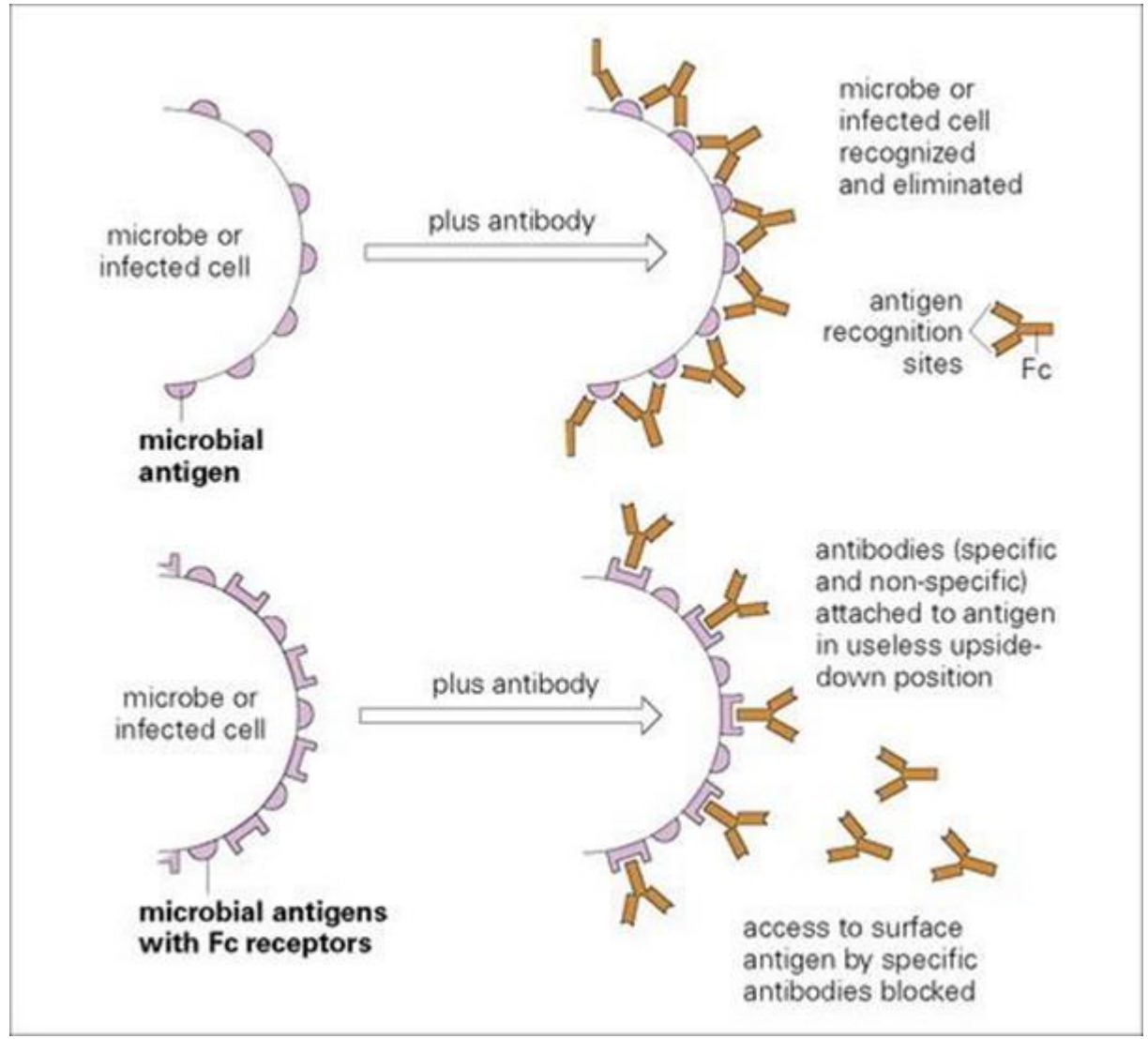


Non-pathogenic bacteria

***M. tuberculosis* infection**

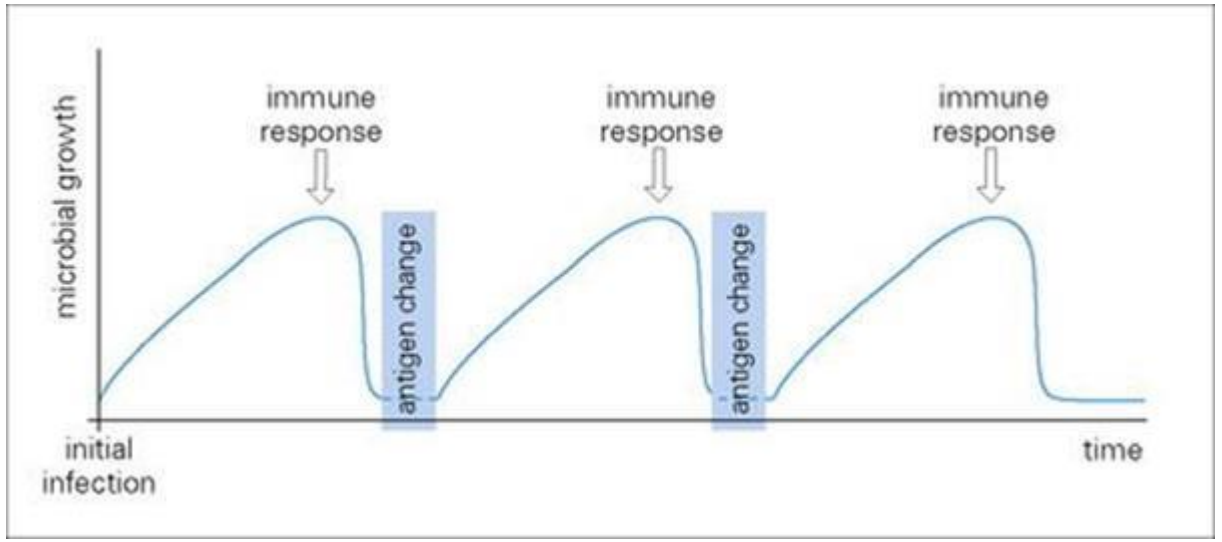
Imunomodulação: *Bordetella pertussis*




microbe or infected cell
plus antibody
microbe or infected cell recognized and eliminated
antigen recognition sites
Fc
microbial antigen
microbe or infected cell
plus antibody
antibodies (specific and non-specific) attached to antigen in useless upside-down position
microbial antigens with Fc receptors
access to surface antigen by specific antibodies blocked

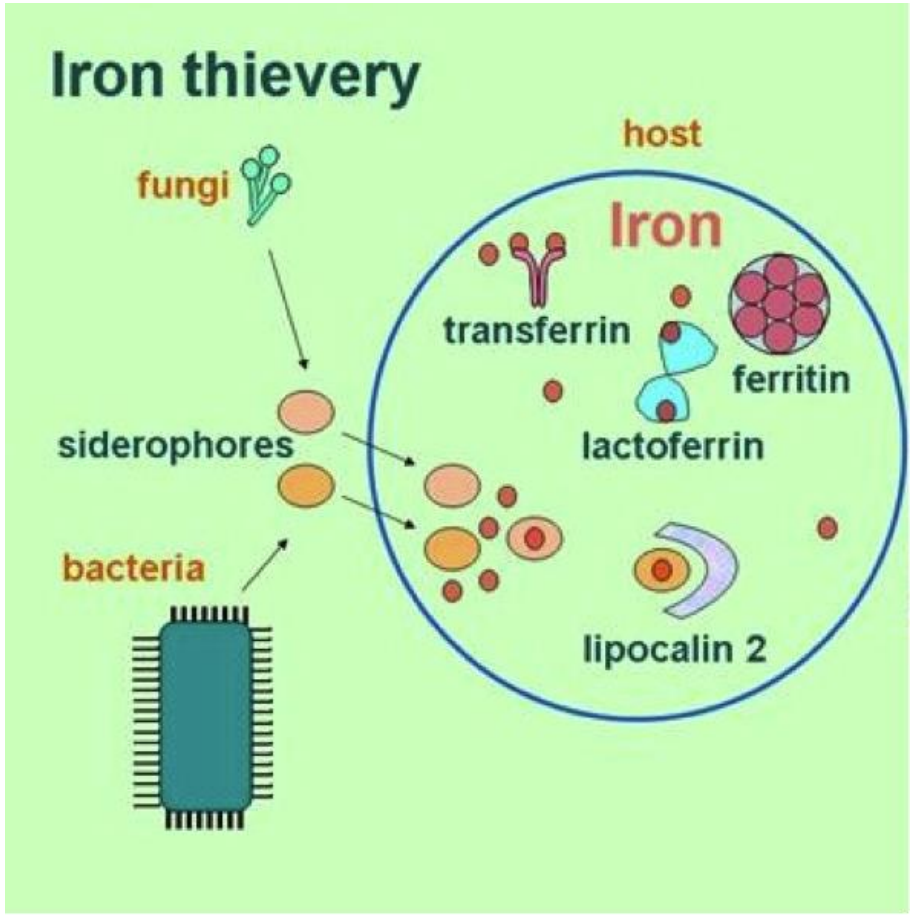
Variação antigênica

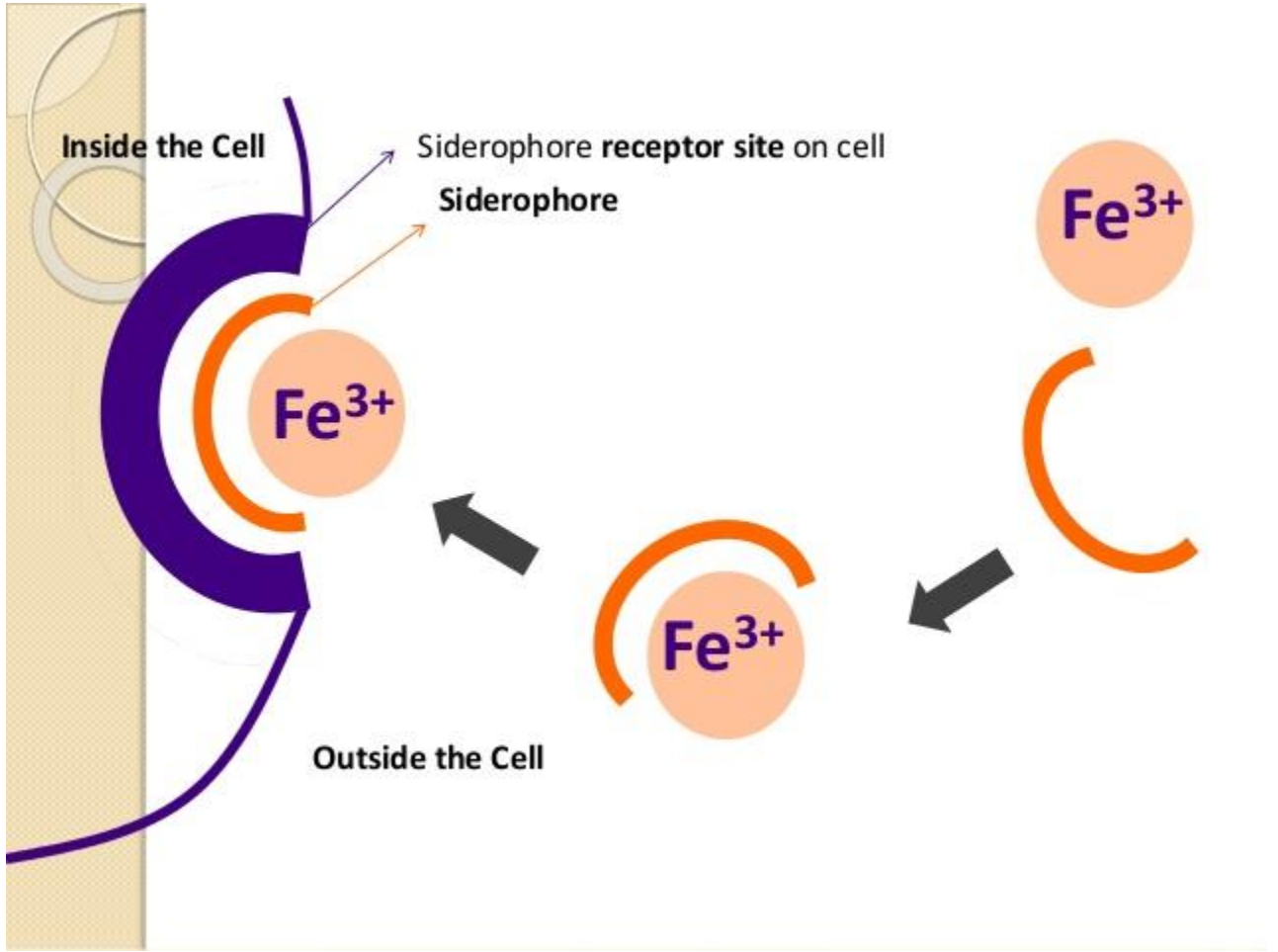
Algumas bactérias podem alterar seus antígenos de superfície escapando dos anticorpos produzidos pelo hospedeiro. *Neisseria gonorrhoeae*



Utilizando os nutrientes do hospedeiro

Captação de ferro





Toxinas e consequências patológicas das infecções

Toxinas


Paciente apresentando postura conhecida como
"opistótono", decorrente da contratura muscular
generalizada causada pela toxina tetânica
(Fonte: Biblioteca Pública de Imagem de
Saúde, Filadélfia, EUA)

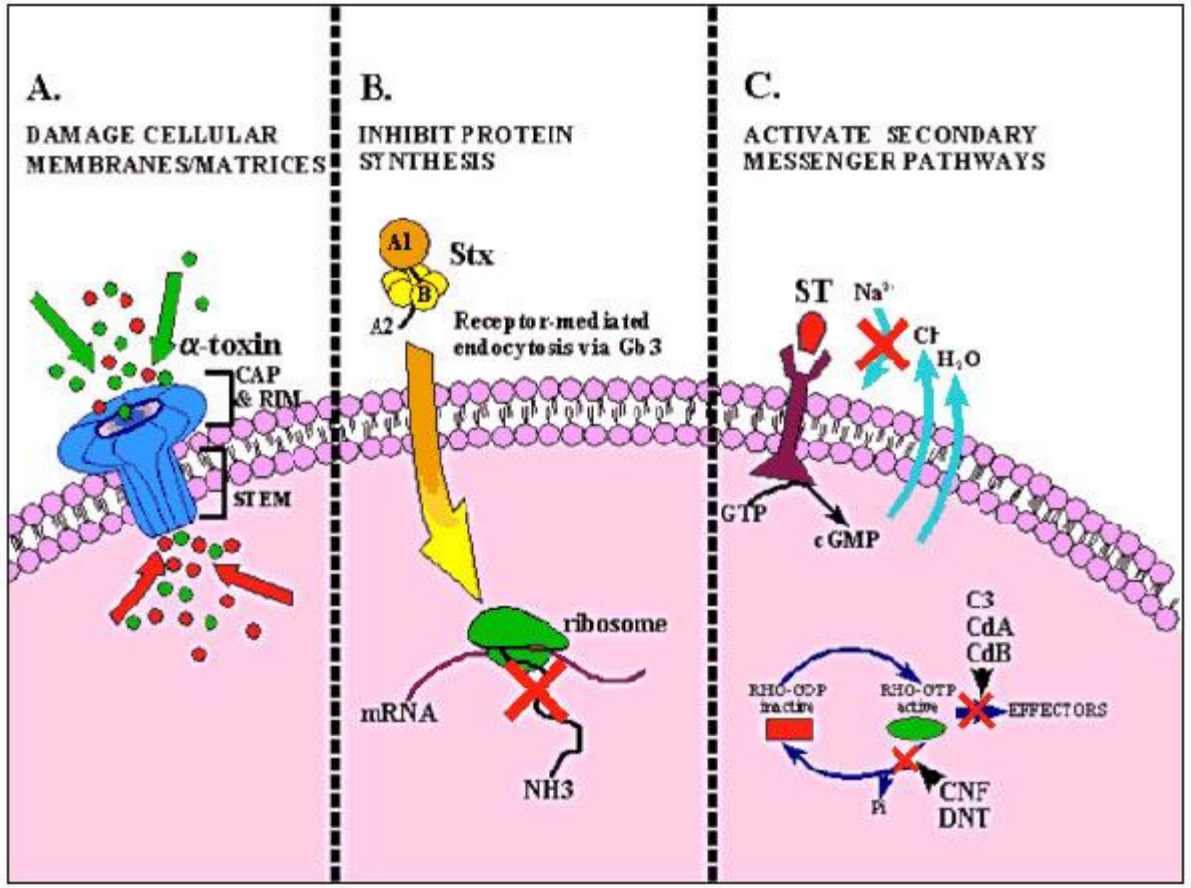
Toxinas

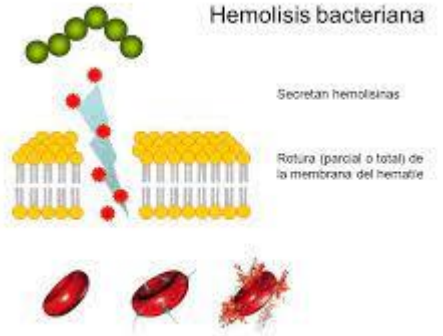
São produtos bacterianos que danificam diretamente o tecido ou promovem atividades biológicas destrutivas

Podem ser substâncias tóxicas ou enzimas degradativas que causam a lise celular ou se ligam a receptores específicos e desencadeiam uma reação tóxica em um tecido-alvo específico

Podem também ser componentes da parede celular que desencadeiam respostas sistêmicas

Muitas vezes as toxinas são as responsáveis por causar os sintomas das doenças





Enzimas como toxinas

Enzimas podem digerir o material entre as células e induzir a formação ou a degradação de coágulos

-coagulases – enzimas bacterianas que coagulam o fibrinogênio no sangue. Comum em *Staphylococcus*

-quinases – degradam fibrina e digerem coágulos. Ex. fibronolisina (estreptocinase)

- Hilaronidase – hidrolisa ácido hialurônico de tecidos conectivos. Tem papel na necrose de ferimentos.

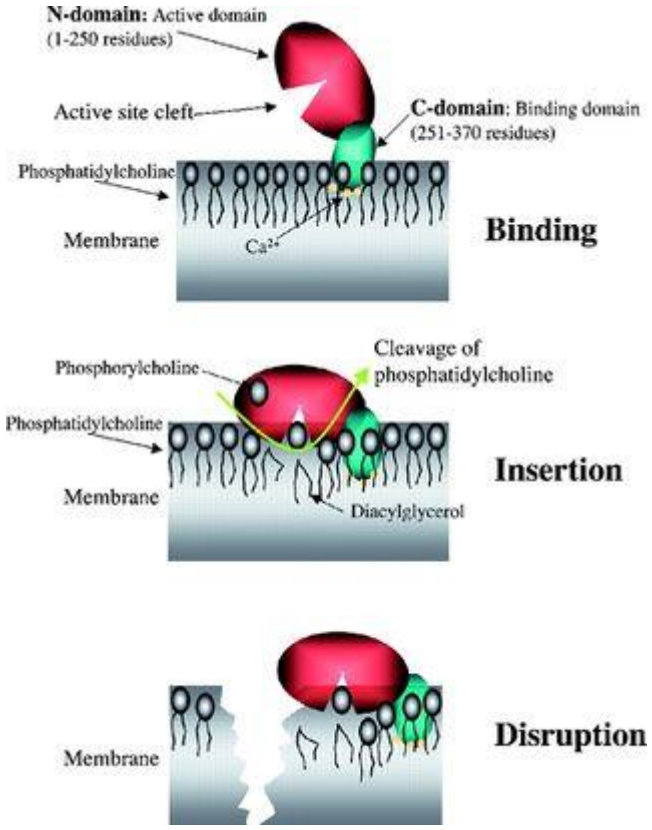
- Colagenase – hidrolisa colágeno de tecidos conectivos de músculos – ocasiona gangrena gasoso. Comum em *Chostridium*

- Proteases IgA – hidrolisam anticorpos. Enzima produzida por *N. gonorrhoeae*

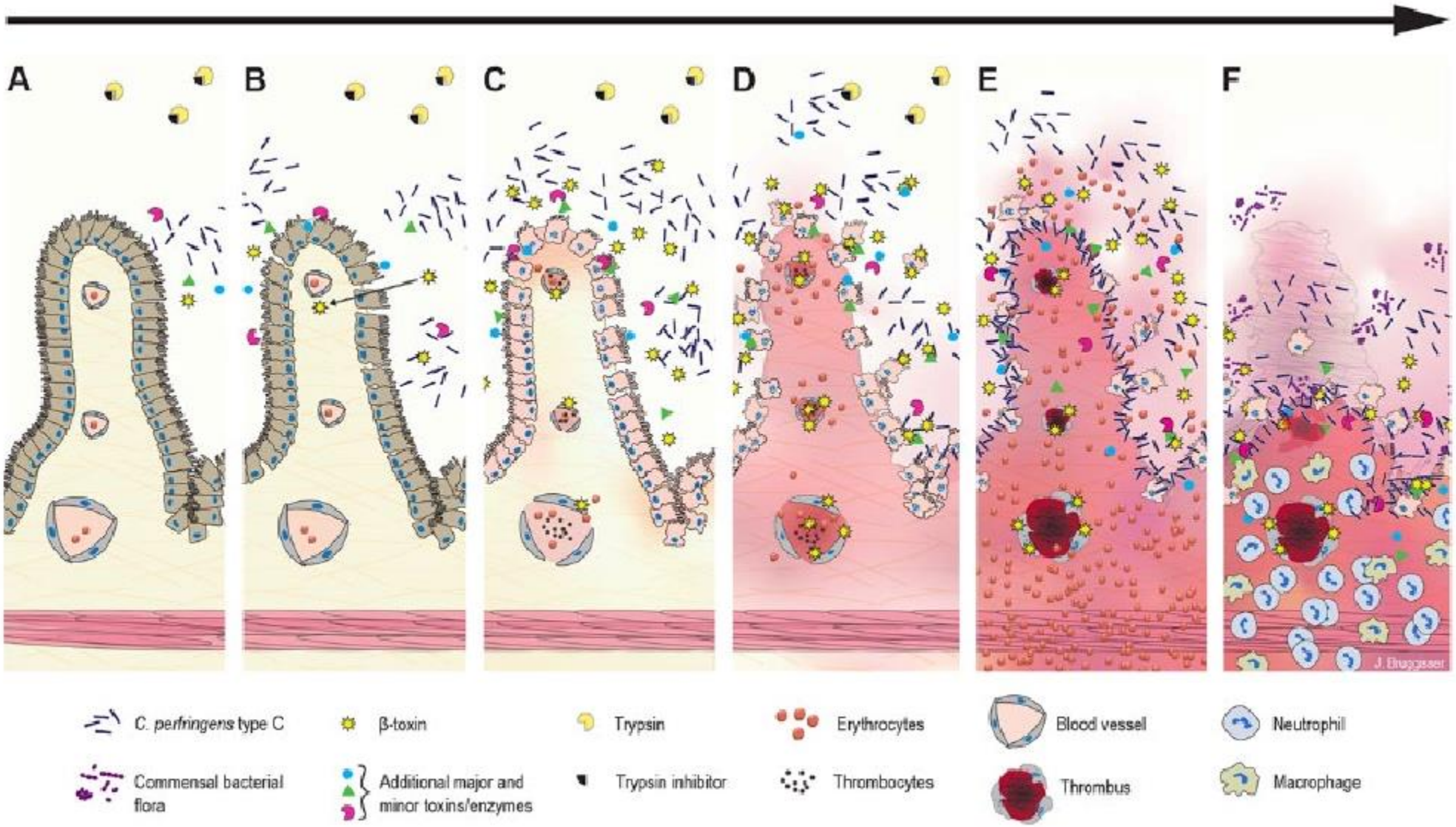
Destruição dos tecidos

Produtos provenientes do metabolismo da bactéria

Enzimas degradativas



Clostridium perfringens


A
B
C
D
E
F
C. perfringens type C
β-toxin
Trypsin
Erythrocytes
Blood vessel
Neutrophil
Commensal bacterial flora
Additional major and minor toxins/enzymes
Trypsin inhibitor
Thrombocytes
Thrombus
Macrophage

Tipos de toxinas

Toxina pré-formada – toxinas presentes nos alimentos antes da ingestão

Exotoxinas – proteínas que podem ser produzidas pelas bactérias Gram positivas e Gram negativas que matam ou alteram uma função de uma célula hospedeira.

Endotoxinas – componentes da parede celular bacteriana Gram negativa (lipopolissacarídio, LPS) que causam uma reação imune extremamente potente, podendo levar ao choque

(a) Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.

(b) Endotoxins are the lipid portions of lipopolysaccharides (LPSs) 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.

Key Concept

Toxins are of two general types: exotoxins and endotoxins.

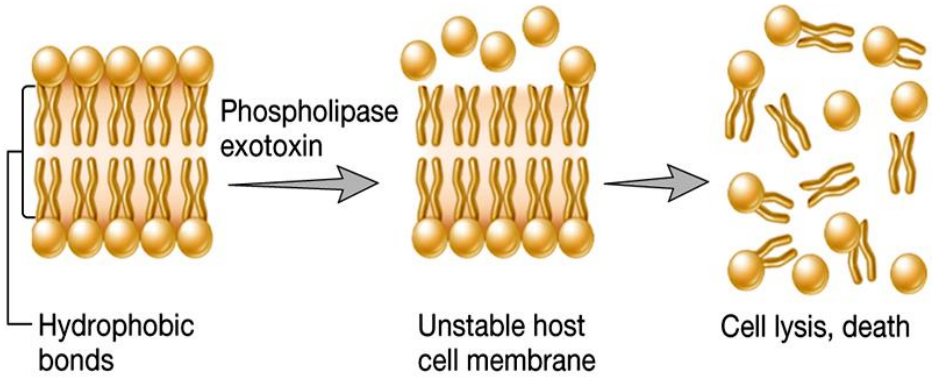
Exotoxinas

-Geralmente presentes em plasmídeos

-podem atuar localmente ou em locais distantes da infecção

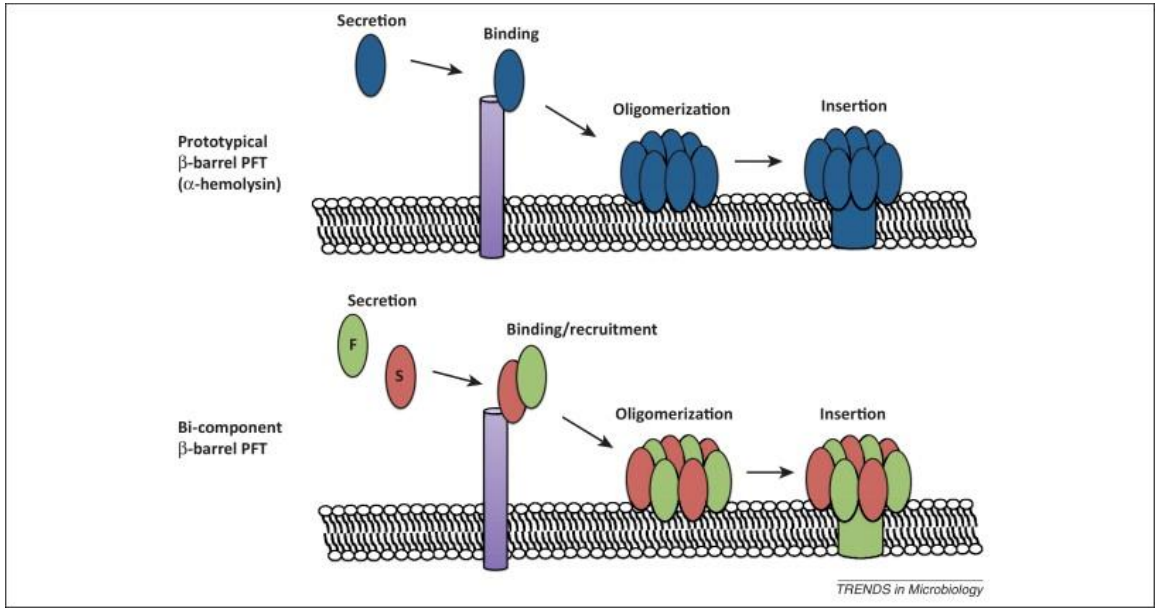
-pode ser uma toxina pré-formada

Toxinas citolíticas:
Toxina α de *Clostridium perfringes* (fosfolipase C)

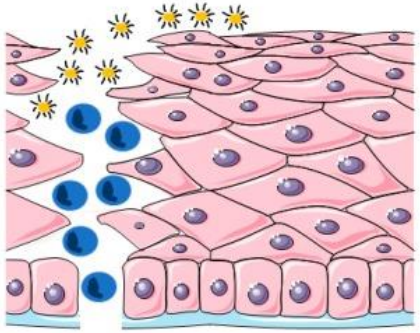


(b)

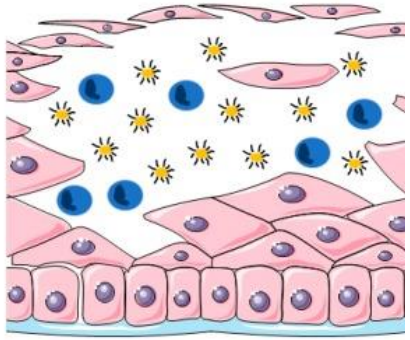
Hemolisina de *Streptococcus*



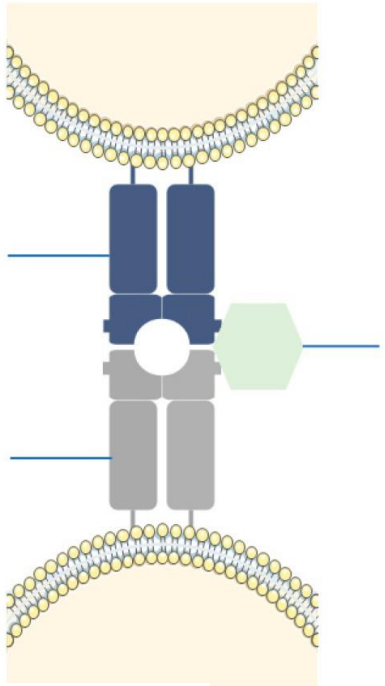
Toxinas - *Staphylococcus aureus*

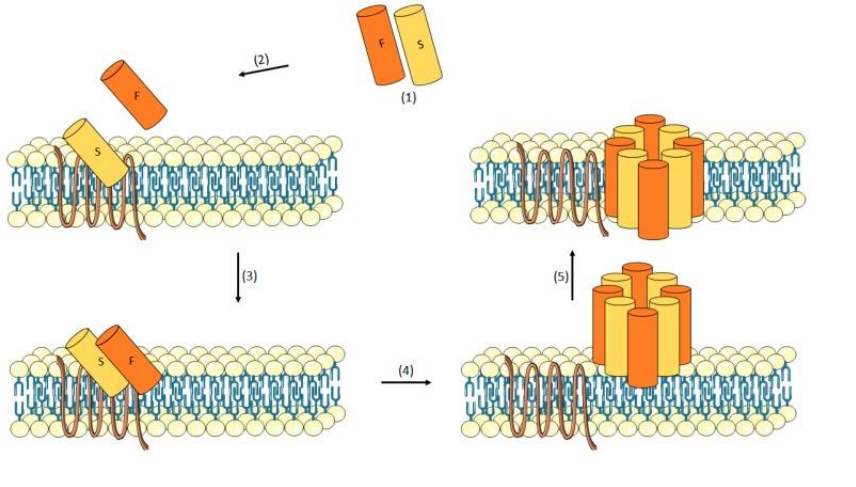


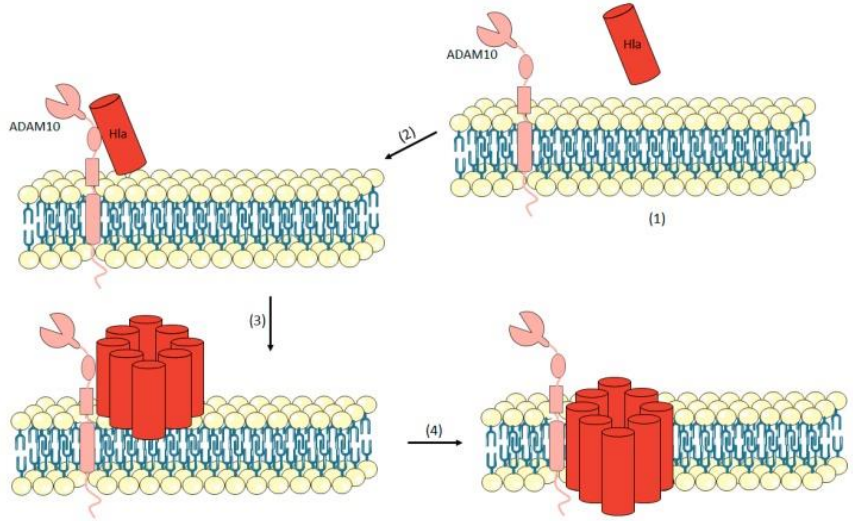
S. aureus adhere to the epidermis and then penetrate through neutrophil-created intercellular gap between superficial keratinocytes.

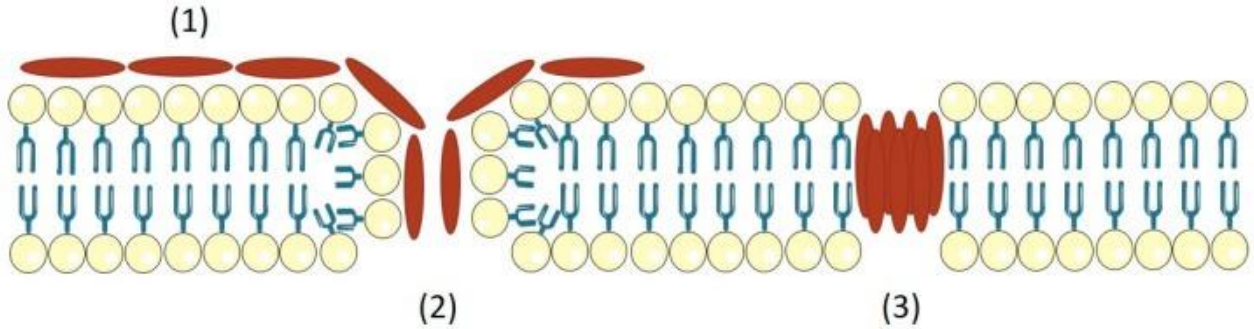


Expansion of blisters by ETs.

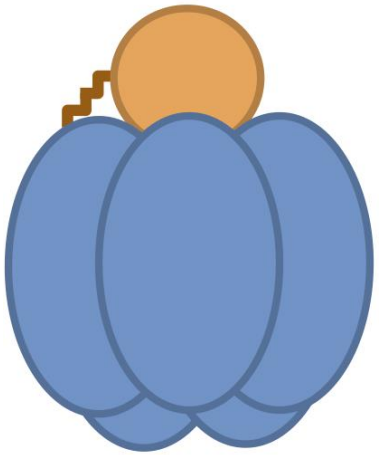






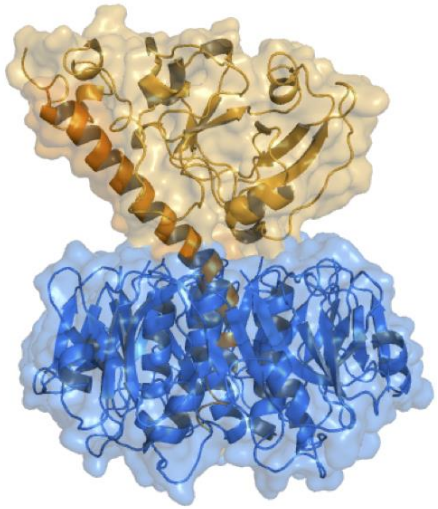


Toxinas diméricas com subunidades A-B

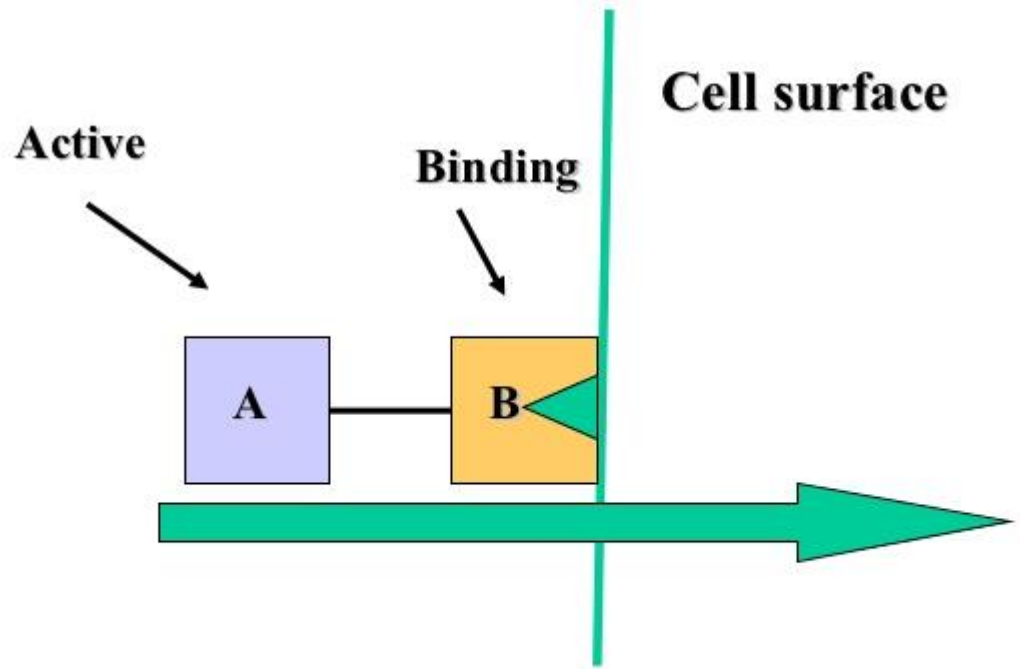


A subunit
enzymatic toxicity

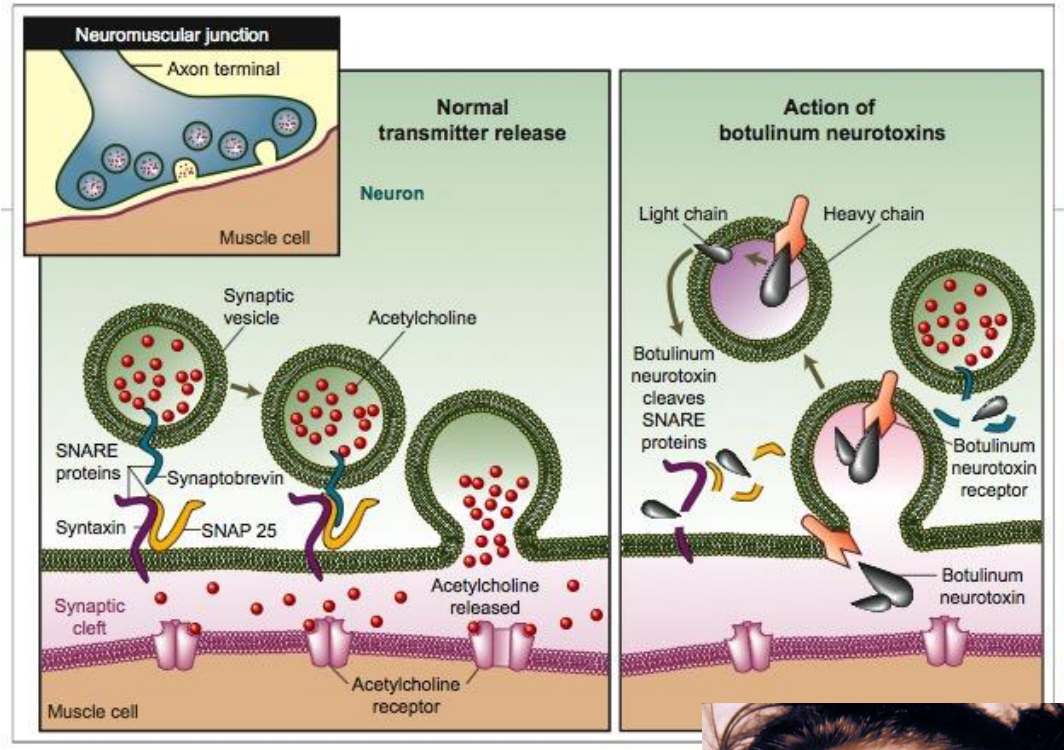
B_5 subunit
cell binding



A-B toxins



Toxina botulínica e tetânica



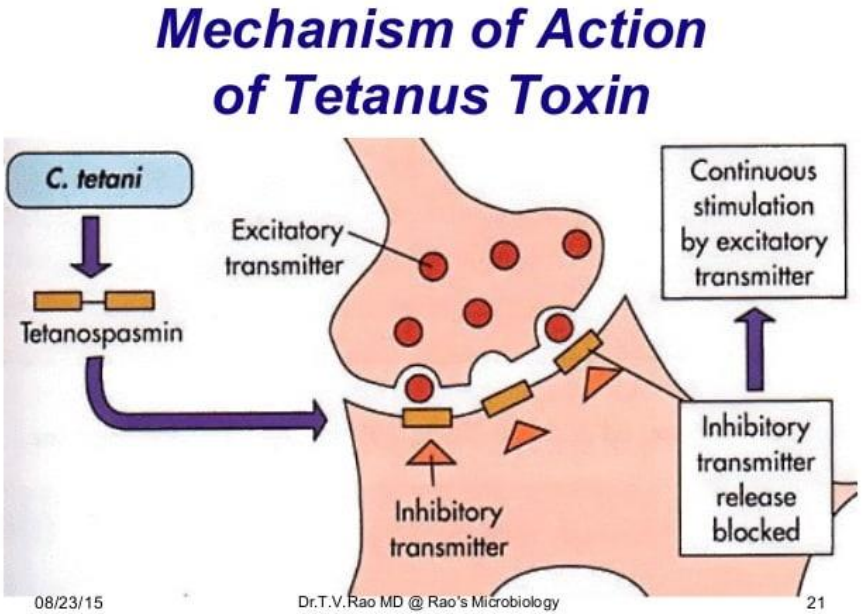
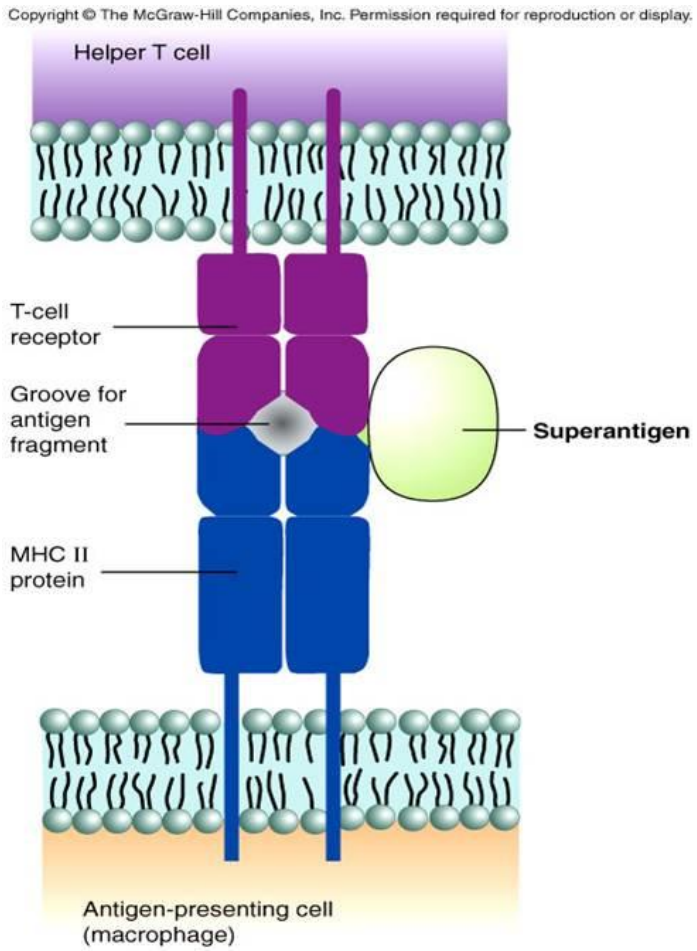


FIGURE 1
Opisthotonus

Superantígenos

São moléculas que ativam as células T por ligarem simultaneamente a um receptor de célula T e a uma molécula do MHC II



Desencadeando a liberação de uma grande quantidade de interleucinas, principalmente IL-1

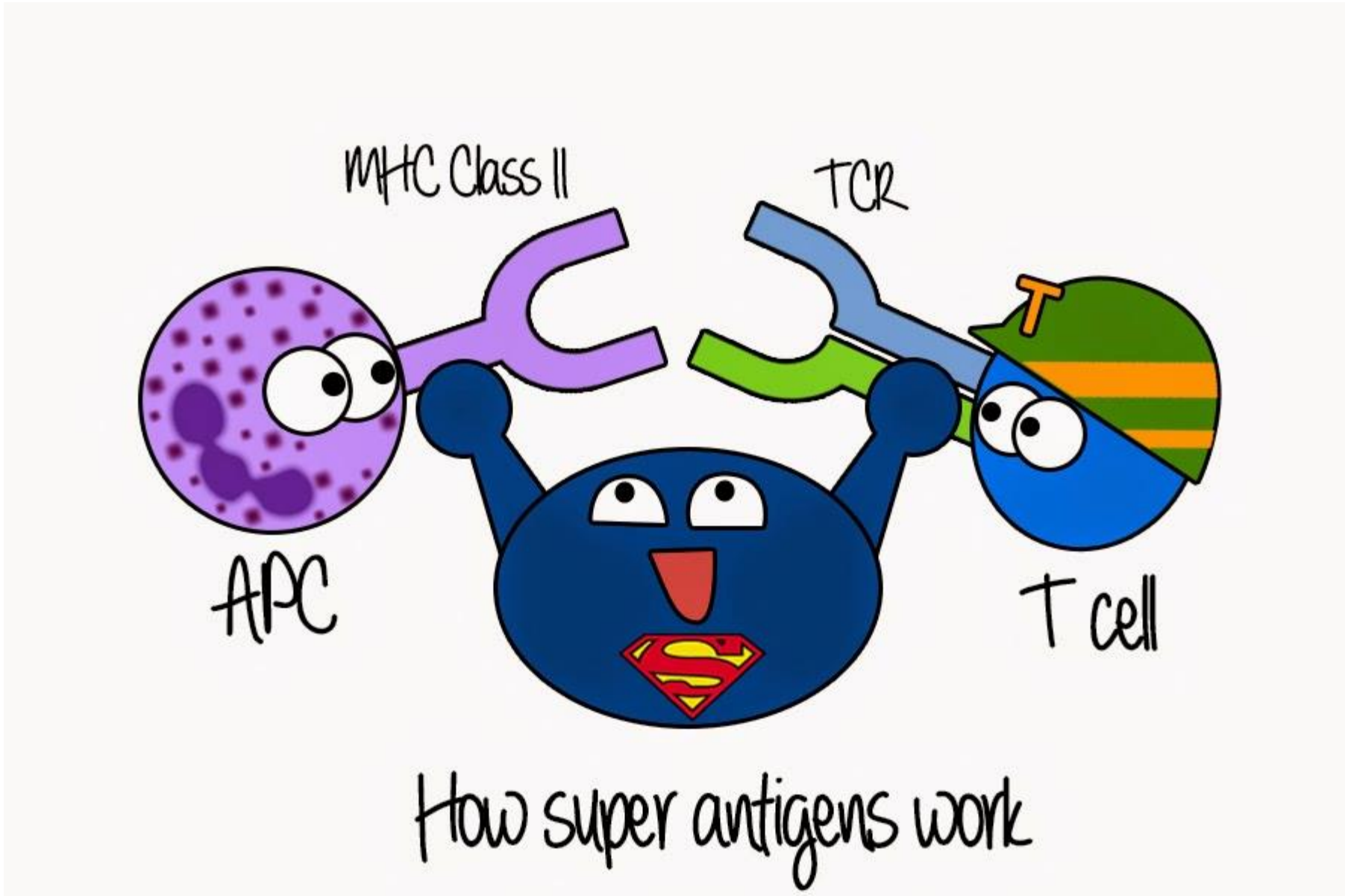
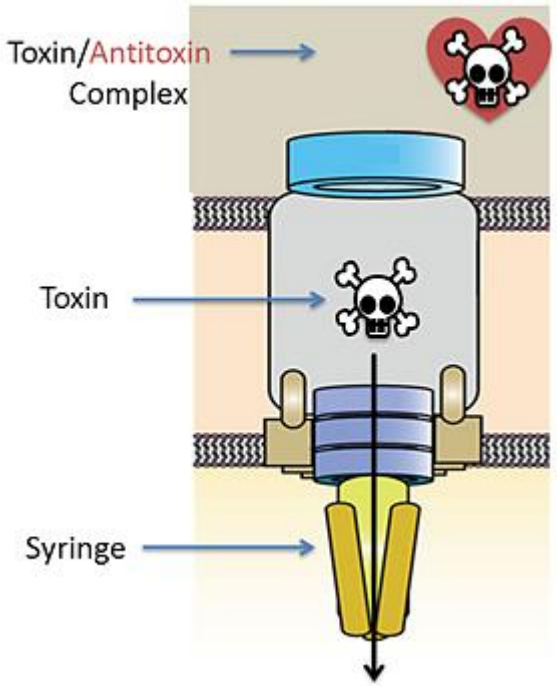
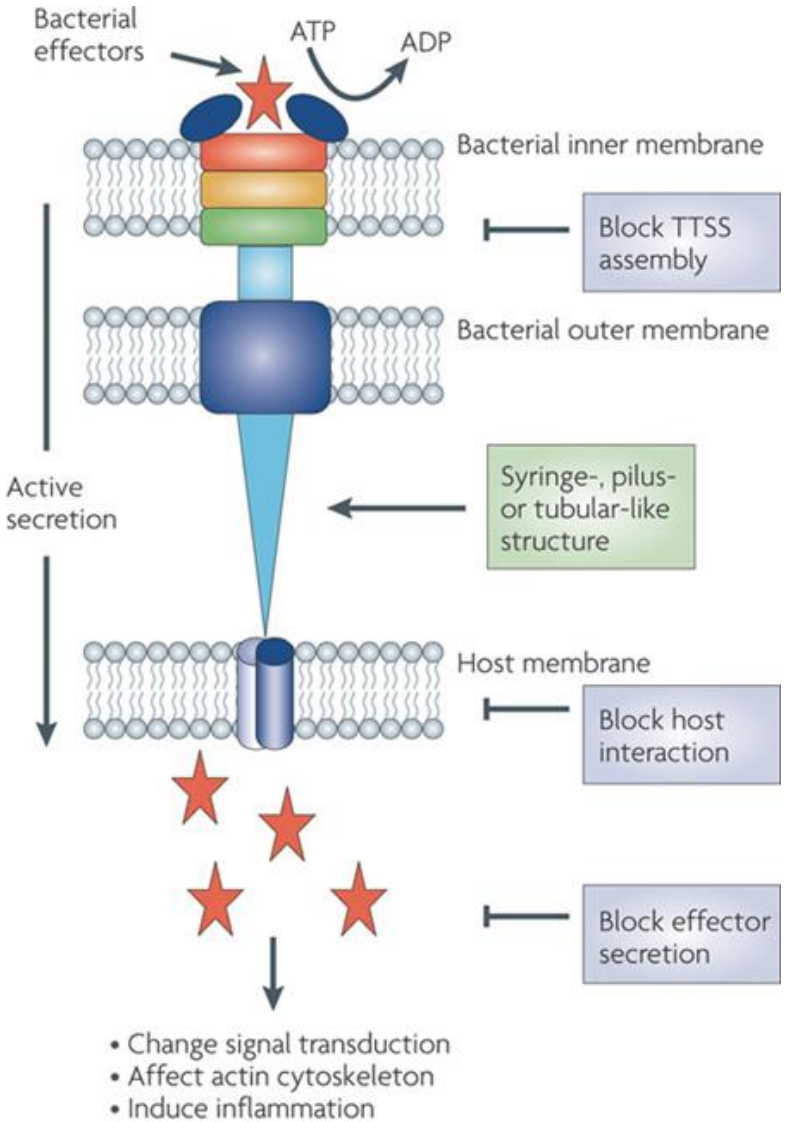

MHC Class II
TCR
APC
T cell
How super antigens work
Immense Immunology Insight

Table 15.2 Diseases Caused by Exotoxins

Disease	Bacterium	Type of Exotoxin	Mechanism
Botulism	*Clostridium botulinum*	A-B	Neurotoxin prevents the transmission of nerve impulses; flaccid paralysis results.
Tetanus	*Clostridium tetani*	A-B	Neurotoxin blocks nerve impulses to muscle relaxation pathway; results in uncontrollable muscle contractions.
Diphtheria	*Corynebacterium diphtheriae*	A-B	Cytotoxin inhibits protein synthesis, especially in nerve, heart, and kidney cells.
Scalded skin syndrome	*Staphylococcus aureus*	A-B	One exotoxin causes skin layers to separate and slough off (scalded skin).
Cholera	*Vibrio cholerae*	A-B	Enterotoxin causes secretion of large amounts of fluids and electrolytes that result in diarrhea.
Traveler's diarrhea	Enterotoxigenic *Escherichia coli* and *Shigella* spp.	A-B	Enterotoxin causes secretion of large amounts of fluids and electrolytes that result in diarrhea.
Anthrax	*Bacillus anthracis*	A-B	Two A components enter the cell via the same B. The A proteins cause shock and reduce the immune response.
Gas gangrene and food poisoning	*Clostridium perfringens* and other species of *Clostridium*	Membrane-disrupting	One exotoxin (cytotoxin) causes massive red blood cell destruction (hemolysis); another exotoxin (enterotoxin) is related to food poisoning and causes diarrhea.
Antibiotic-associated diarrhea	*Clostridium difficile*	Membrane-disrupting	Enterotoxin causes secretion of fluids and electrolytes that results in diarrhea; cytotoxin disrupts host cytoskeleton.
Food poisoning	*Staphylococcus aureus*	Superantigen	Enterotoxin causes secretion of fluids and electrolytes that results in diarrhea.
Toxic shock syndrome (TSS)	*Staphylococcus aureus*	Superantigen	Toxin causes secretion of fluids and electrolytes from capillaries that decreases blood volume and lowers blood pressure.

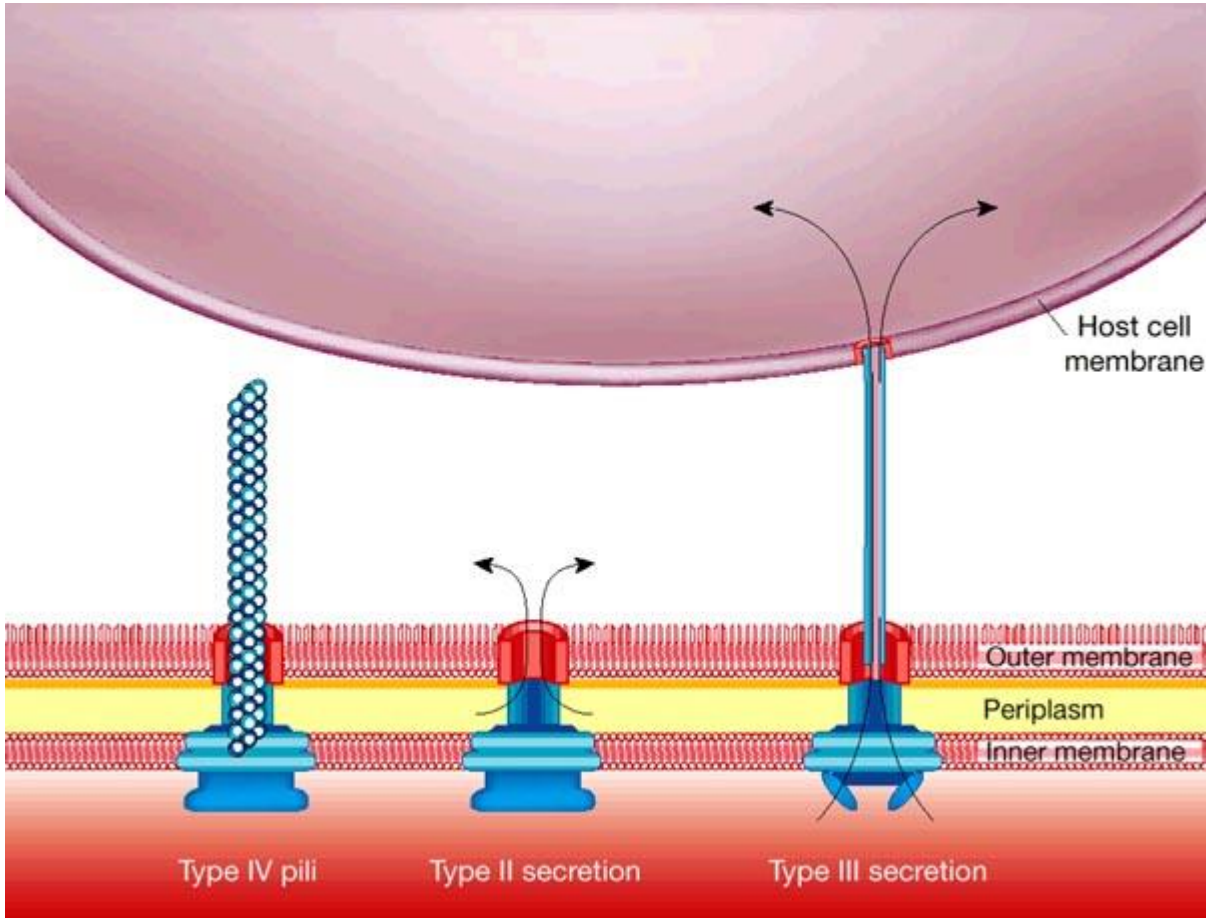
As proteínas secretadas pelas bactérias podem ser toxinas ou realizar a modulação do sistema imune





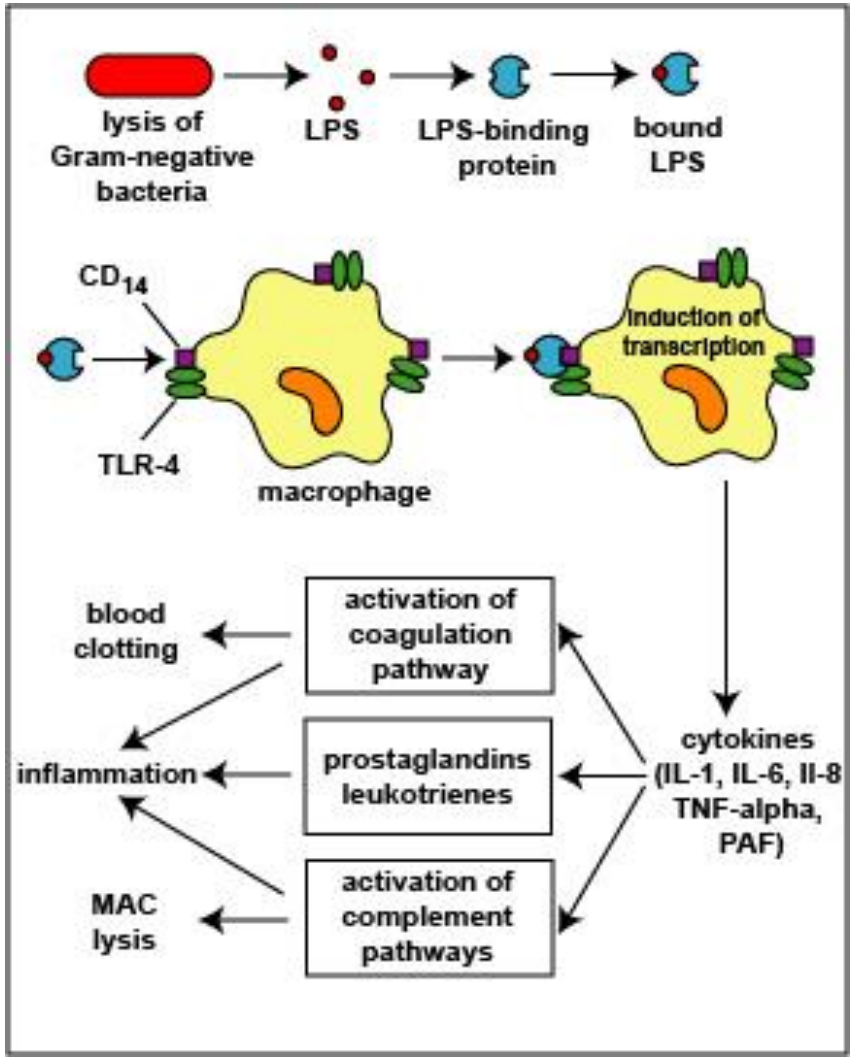
Nature Reviews | Drug Discovery

Sistema de secreção do tipo III



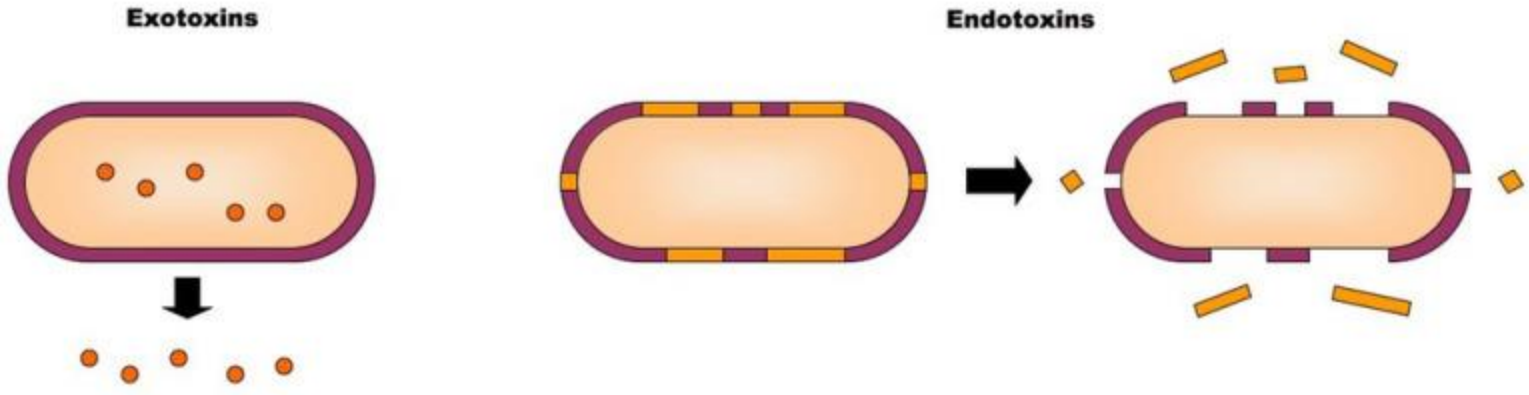
Endotoxinas

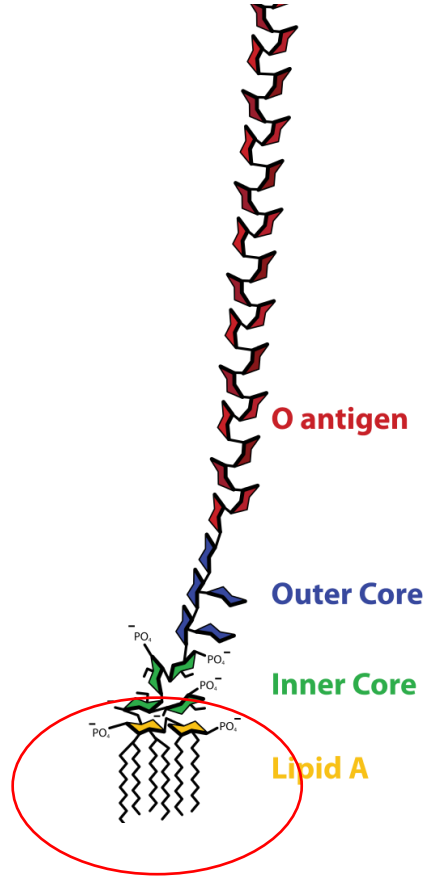
Endotoxinas são referentes a componentes do LPS e são somente presentes em bactérias Gram negativas



Endotoxinas e outros componentes da parede celular bacteriana

-Endotoxinas - componentes da parede celular de bactérias Gram negativas que atuam como sinal para ativar o sistema de defesa





Bactérias gram negativas liberam endotoxinas durante a infecção

↓

Endotoxinas ligam-se a receptores específicos (CD14 e TLR4)

↓

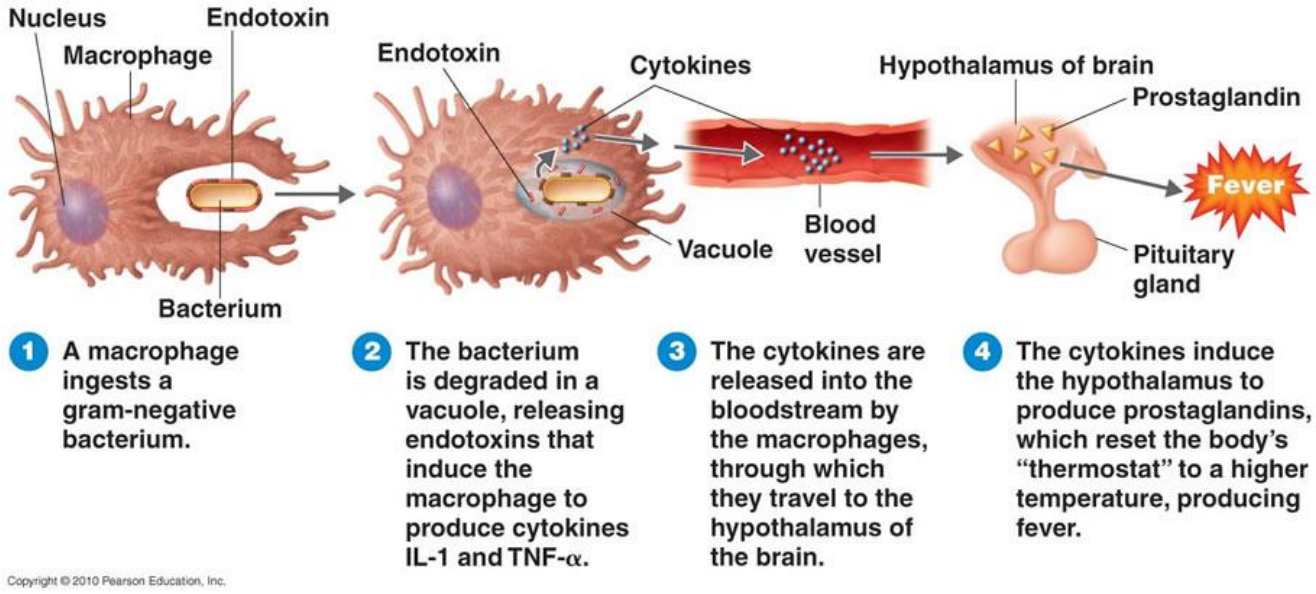
Liberão de de citocinas de fase aguda

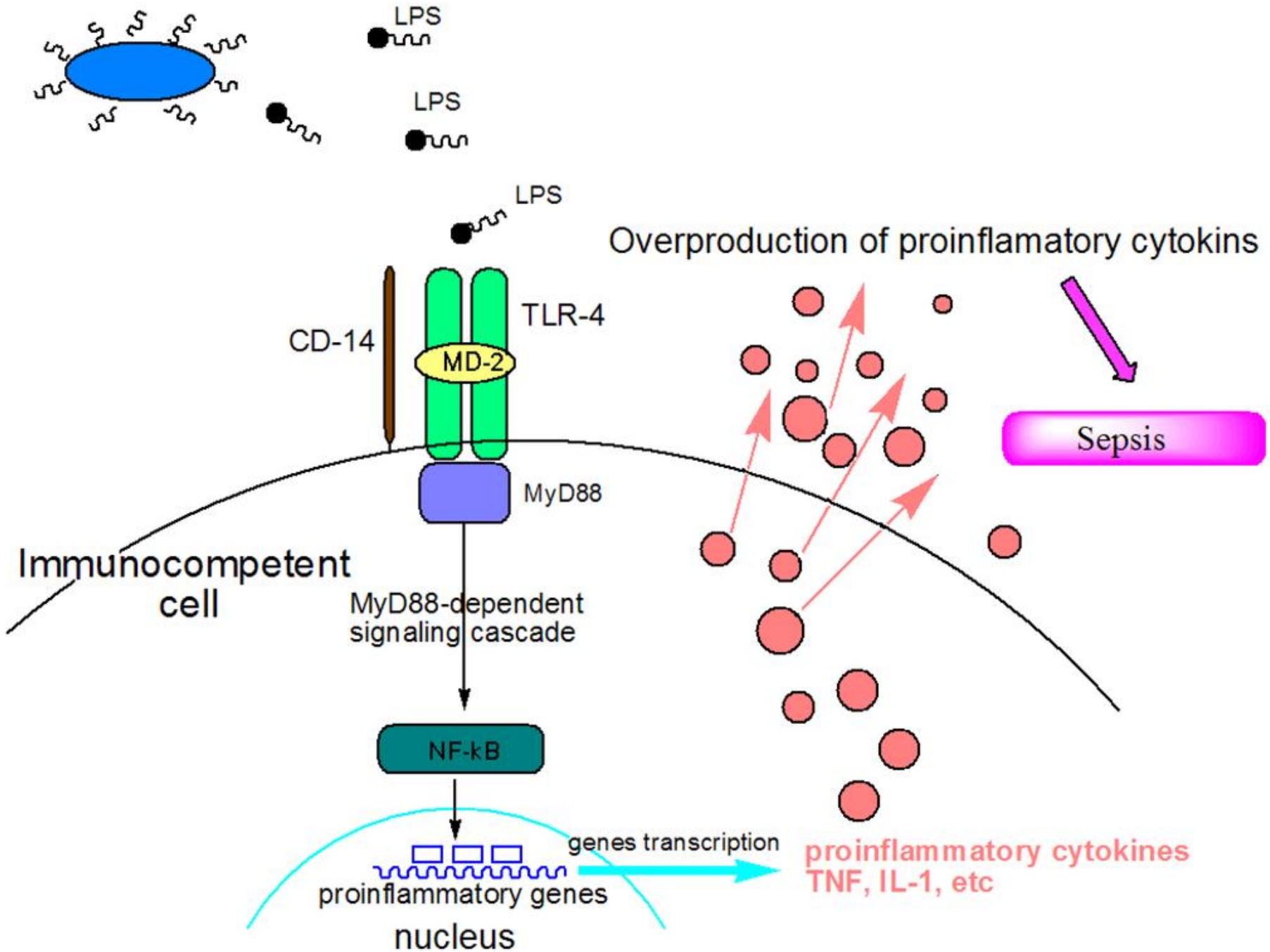
↓

Crescimento de células B

↓

Febre e vasodilatação




LPS
LPS
LPS
Overproduction of proinflamatory cytokins
CD-14
TLR-4
MD-2
MyD88
Sepsis
Immunocompetent cell
MyD88-dependent signaling cascade
NF-kB
genes transcription
proinflammatory genes
proinflammatory cytokines
TNF, IL-1, etc
nucleus


BACTERIA
TOXIN