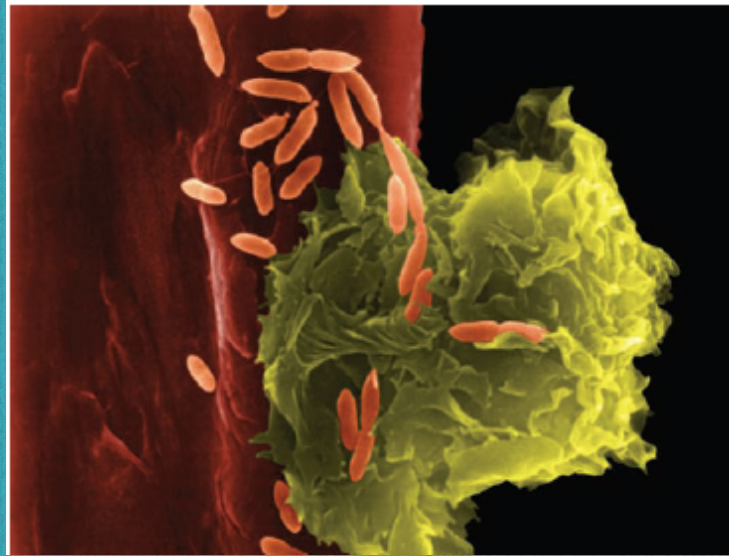


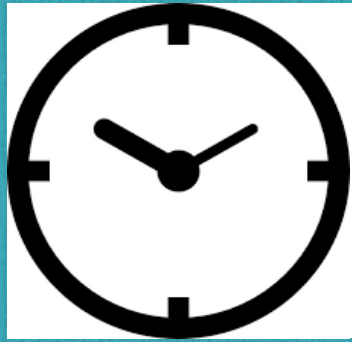
Curso de Ciências Biológicas
Disciplina BMI-296 - Imunologia Básica



Aula 2 – Imunidade inata

Profa. Dra. Alessandra Pontillo

Lab. Imunogenética/Dep. Imunologia/ICB/USP



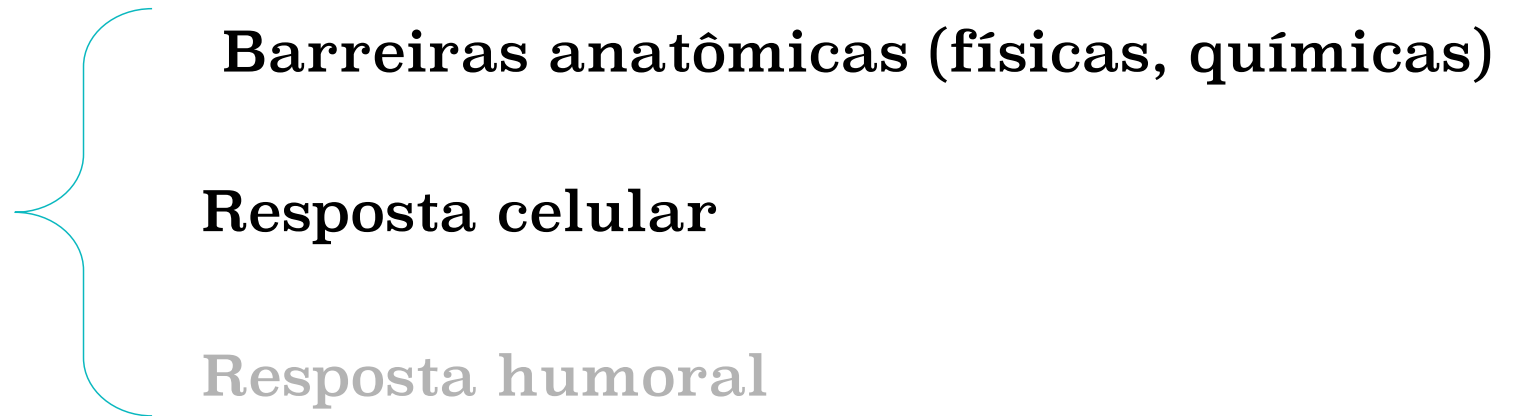
“15’ Brain storming”

Como uma célula poderia eliminar um patógeno?



Imunidade inata

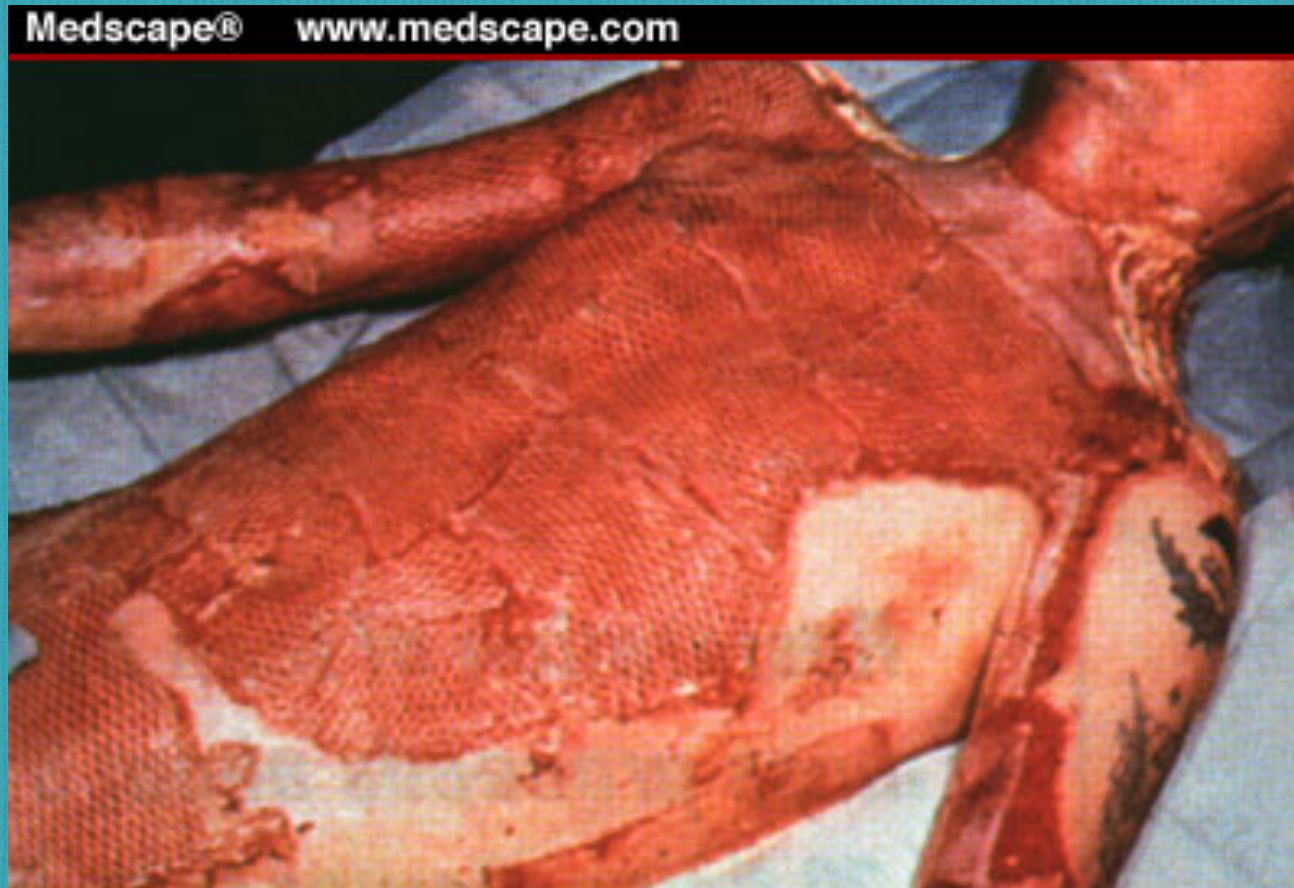
- pronta para atuar frente a um desafio
- “antiga” (plantas-invertebrados-vertebrados)



Barreiras anatômicas

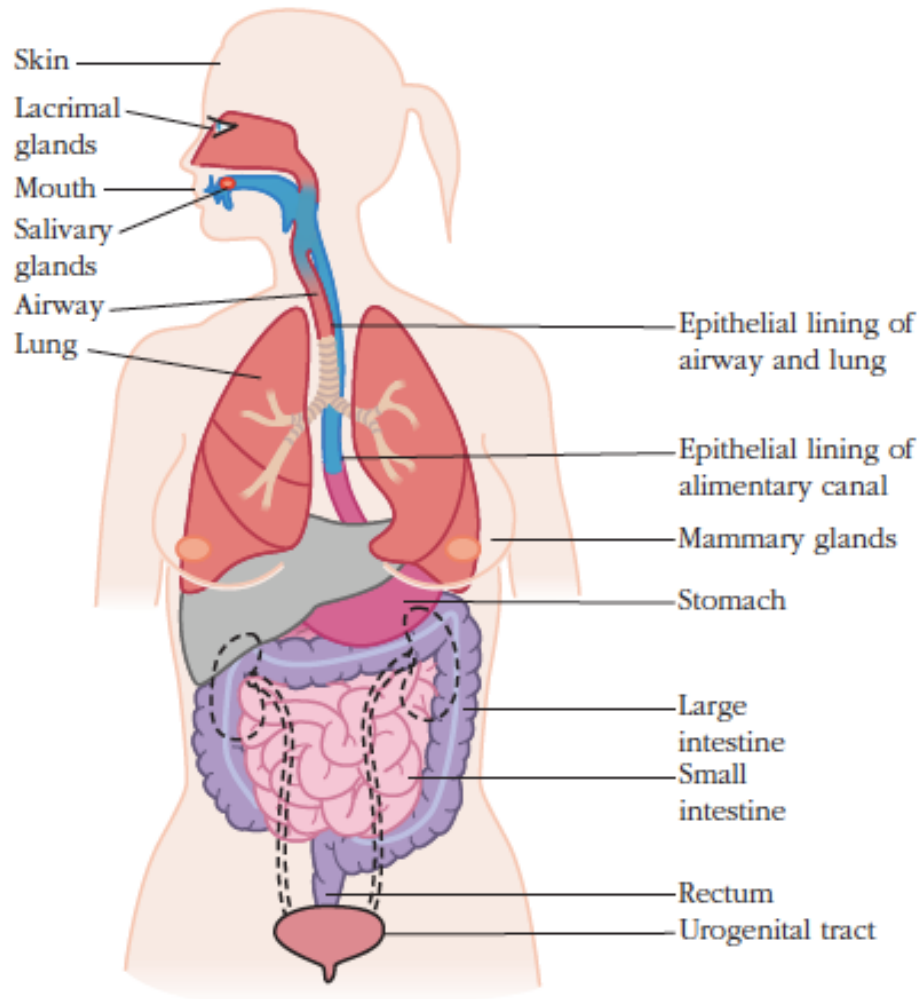
- **1 linha de defesa**
- **Físicas: epitélios de pele e mucosas (preveem entrada de patógenos/agentes perigosos)**
- **Químicas: pH (ácido) & substâncias solúveis com atividade microbicida**

Barreiras anatômicas: protegem mesmo?



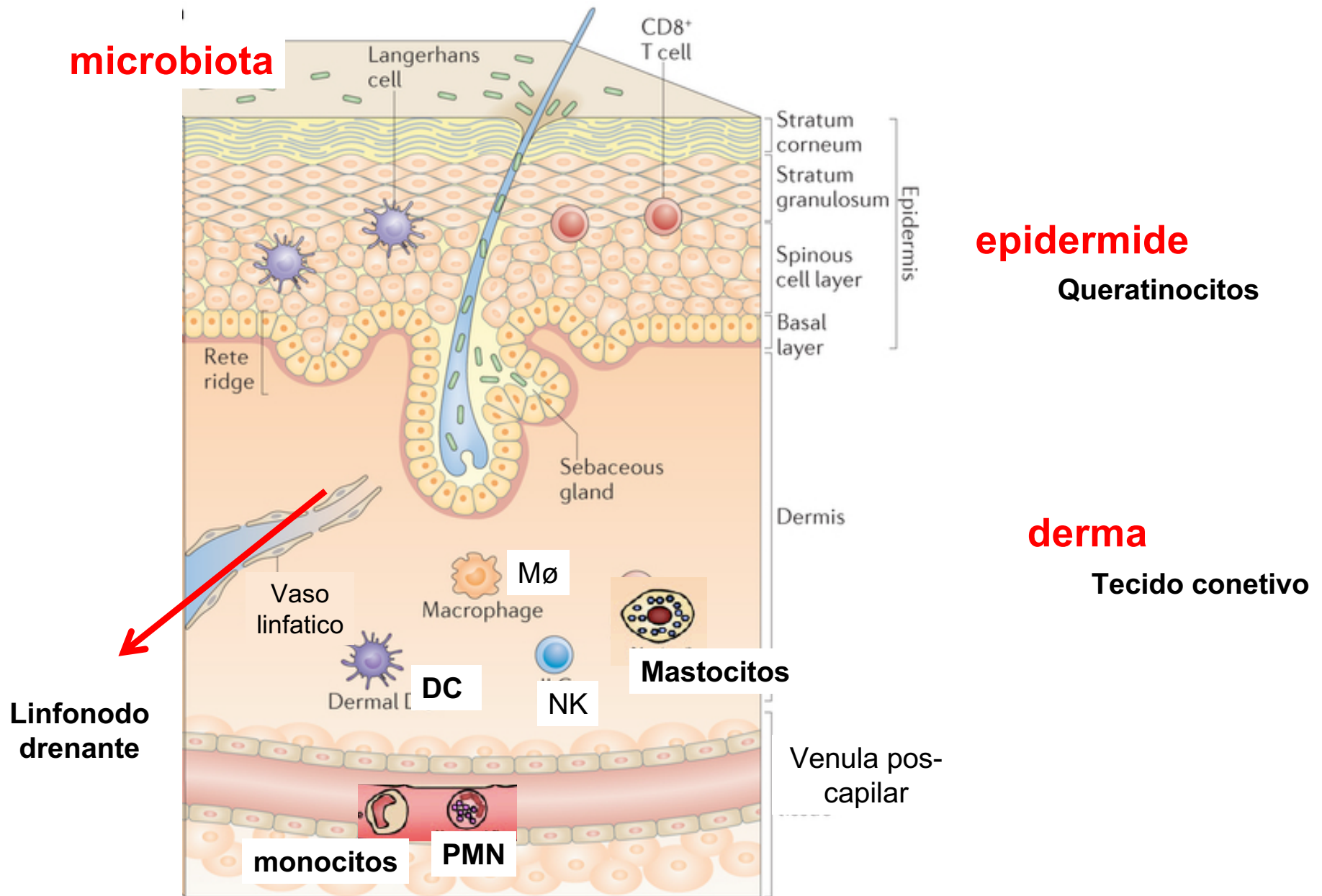
Do que more um paciente com ustiaio grave?

Barreiras anatômicas

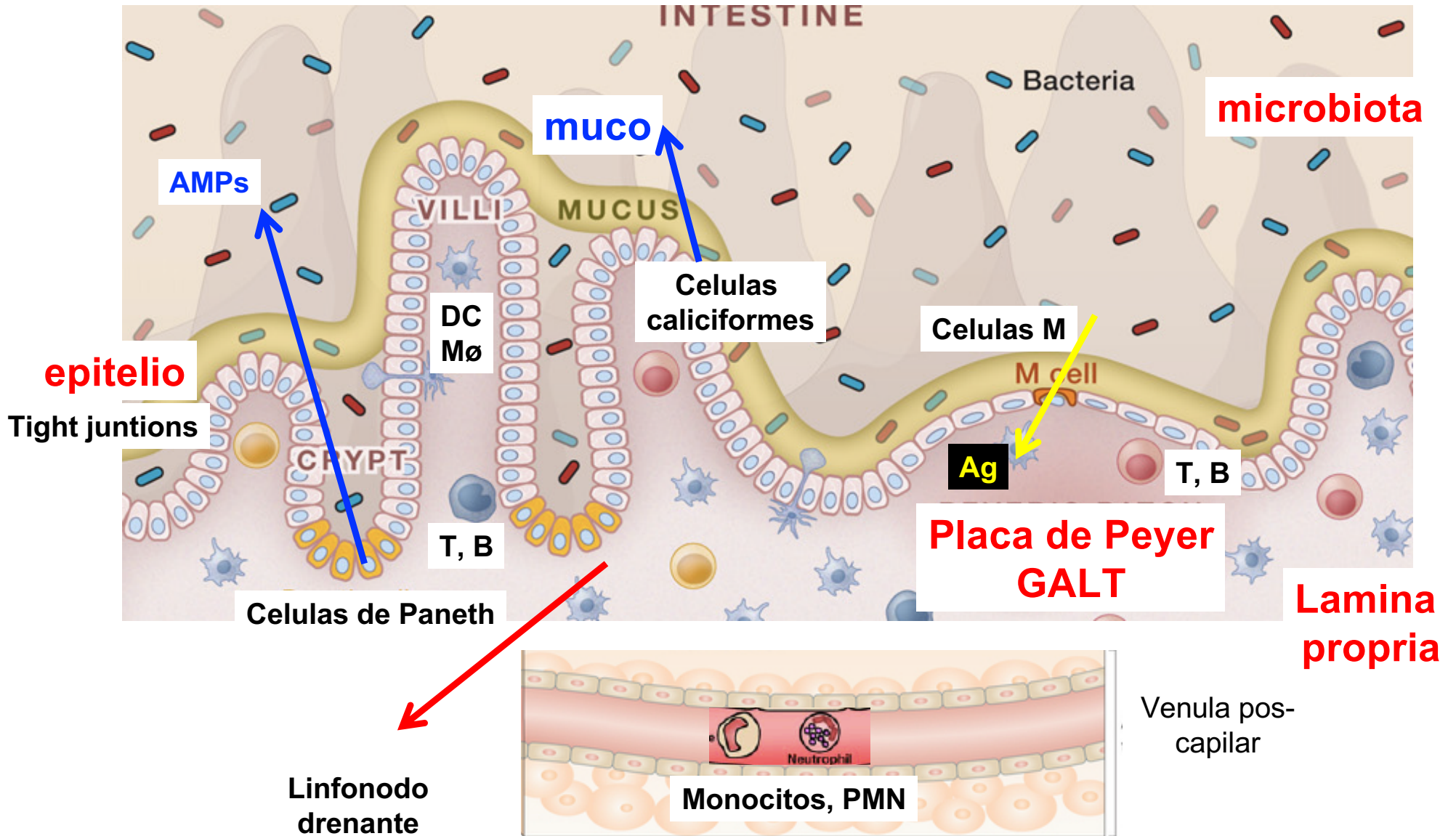


Organ or tissue	Innate mechanisms protecting skin/epithelium
Skin	Antimicrobial peptides, fatty acids in sebum
Mouth and upper alimentary canal	Enzymes, antimicrobial peptides, and sweeping of surface by directional flow of fluid toward stomach
Stomach	Low pH, digestive enzymes, antimicrobial peptides, fluid flow toward intestine
Small intestine	Digestive enzymes, antimicrobial peptides, fluid flow to large intestine
Large intestine	Normal intestinal flora compete with invading microbes, fluid/feces expelled from rectum
Airway and lungs	Cilia sweep mucus outward, coughing, sneezing expel mucus, macrophages in alveoli of lungs
Urogenital tract	Flushing by urine, aggregation by urinary mucins; low pH, anti-microbial peptides, proteins in vaginal secretions
Salivary, lacrimal, and mammary glands	Flushing by secretions; anti-microbial peptides and proteins in vaginal secretions

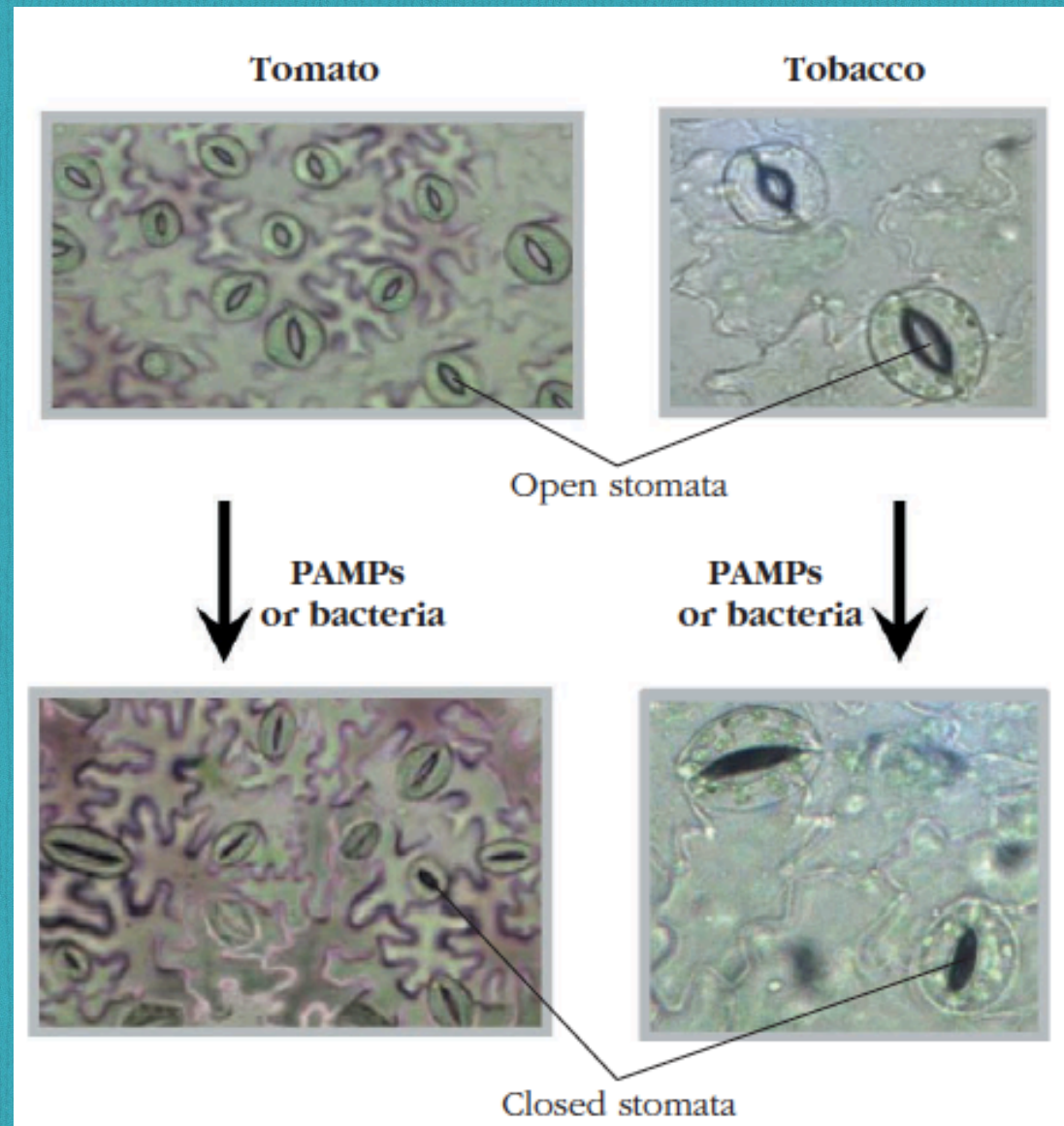
Pele



Mucosa



Barreiras físicas nas plantas



Moleculas antimicrobianas

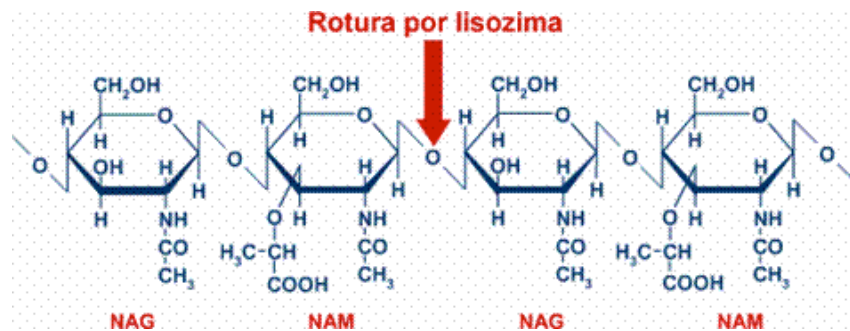
TABLE 5-2 Some human antimicrobial proteins and peptides at epithelial surfaces

Proteins and peptides*	Location	Antimicrobial activities
Lysozyme <i>Enzima glicolitico</i>	Mucosal/glandular secretions (e.g., tears, saliva, respiratory tract)	Cleaves glycosidic bonds of peptidoglycans in cell walls of bacteria, leading to lysis
Lactoferrin <i>Prroteina sequestra Fe</i>	Mucosal/glandular secretions (e.g., milk, intestine mucus, nasal/respiratory and urogenital tracts)	Binds and sequesters iron, limiting growth of bacteria and fungi; disrupts microbial membranes; limits infectivity of some viruses
Secretory leukocyte protease inhibitor	Skin, mucosal/glandular secretions (e.g., intestines, respiratory, and urogenital tracts, milk)	Blocks epithelial infection by bacteria, fungi, viruses; antimicrobial
S100 proteins, e.g.: - psoriasin S100A7 - calprotectin S100A8/A9 <i>Prroteinas baixo PM</i>	Skin, mucosal/glandular secretions (e.g., tears, saliva/tongue, intestine, nasal/respiratory and urogenital tracts)	- Disrupts membranes, killing cells - Binds and sequesters divalent cations (e.g., manganese and zinc), limiting growth of bacteria and fungi
Defensins (α and β) <i>AMPs</i>	Skin, mucosal epithelia (e.g., mouth, intestine, nasal/respiratory tract, urogenital tract)	Disrupt membranes of bacteria, fungi, protozoan parasites, and viruses; additional toxic effects intracellularly; kill cells and disable viruses
Cathelicidin (LL37)** <i>AMPs</i>	Mucosal epithelia (e.g., respiratory tract, urogenital tract)	Disrupts membranes of bacteria; additional toxic effects intracellularly; kills cells.
Surfactant proteins SP-A, SP-D <i>Lectinas/receptores soluveis</i>	Secretions of respiratory tract, other mucosal epithelia	Block bacterial surface components; promotes phagocytosis

Lisozima (LYZ)

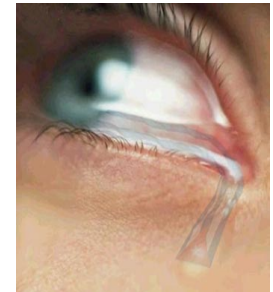
Enzima de 14 kDa, Glicosidase

- idrolise do peptidoglicano ---> lise de Gram+ (Streptococci)



- liga a superficie de bacterios ---> facilita fagocitose

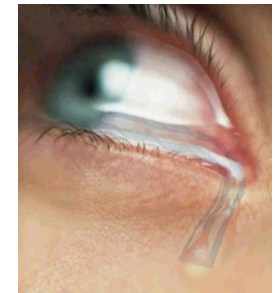
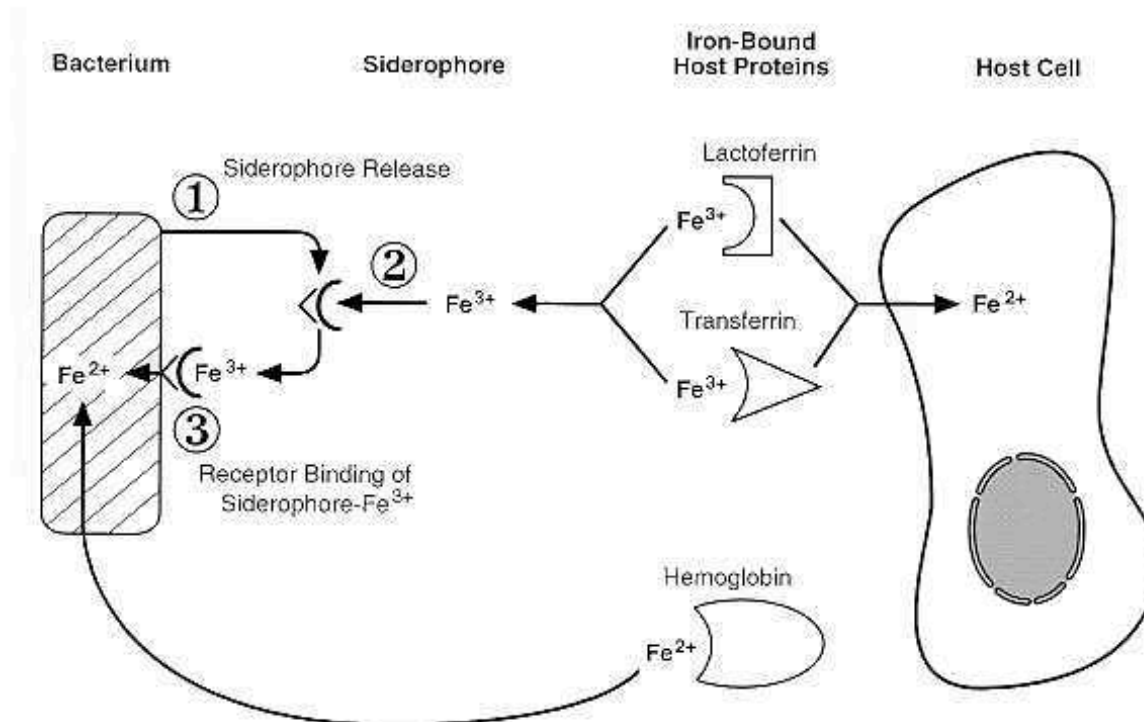
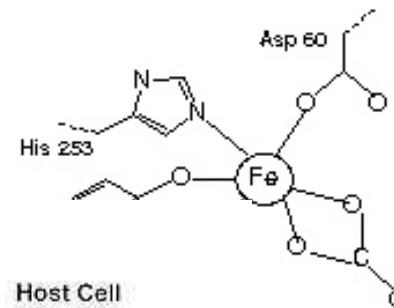
----> impede adesao



Lactoferrina (LF)

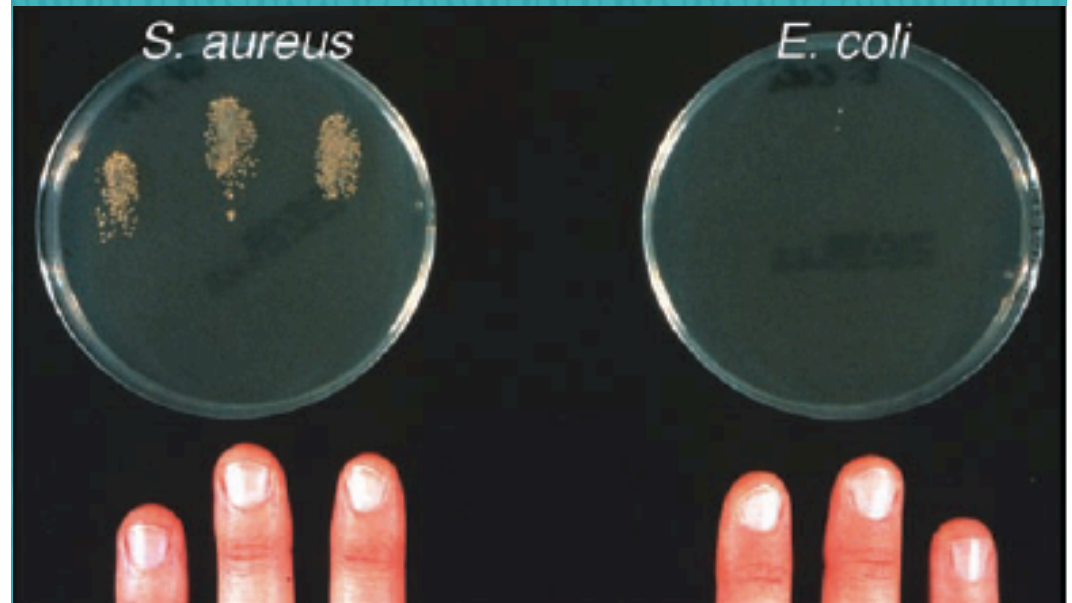
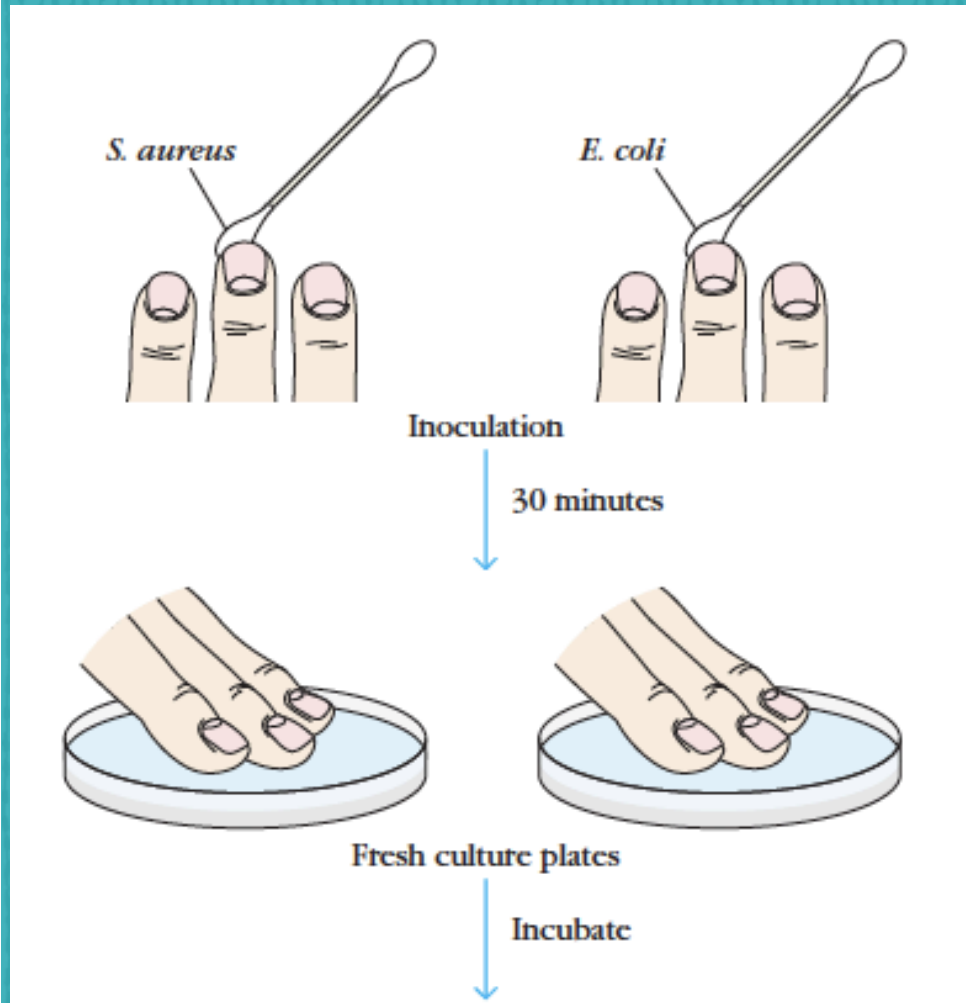
Proteína globular (80KDa) da família da transferrina

- liga o Fe ---> competição hospede/patogeno
- liga receptores microbianos e lipoperoxida---> lise
- liga receptores para vírus e vírus diretamente
- liga DNA e RNA



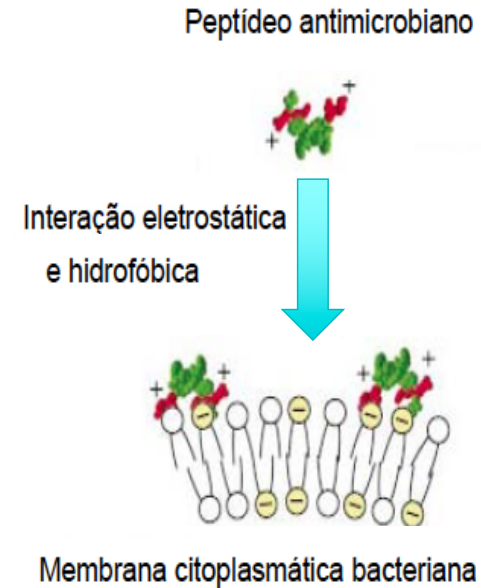
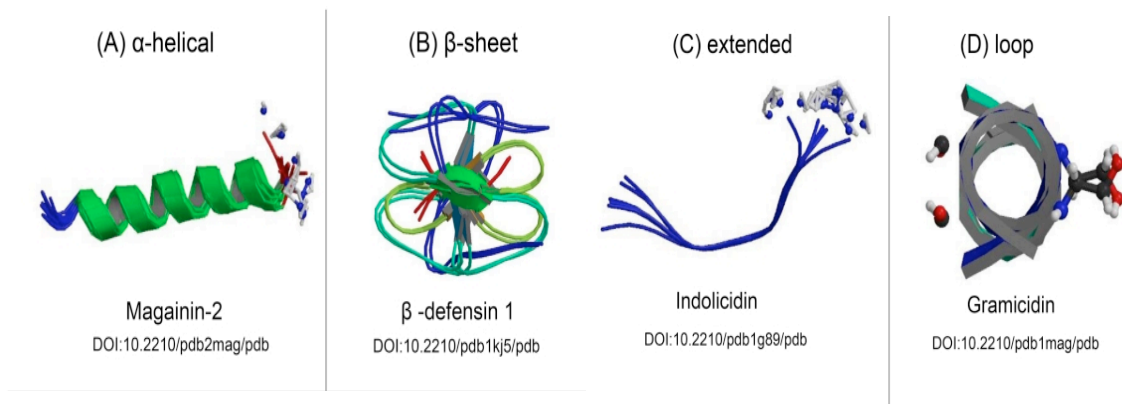
S100: protegem mesmo?

Psoriasina/S100A7 protege da colonização da pele por *E coli*



Peptídeos anti-microbianos (AMPs)

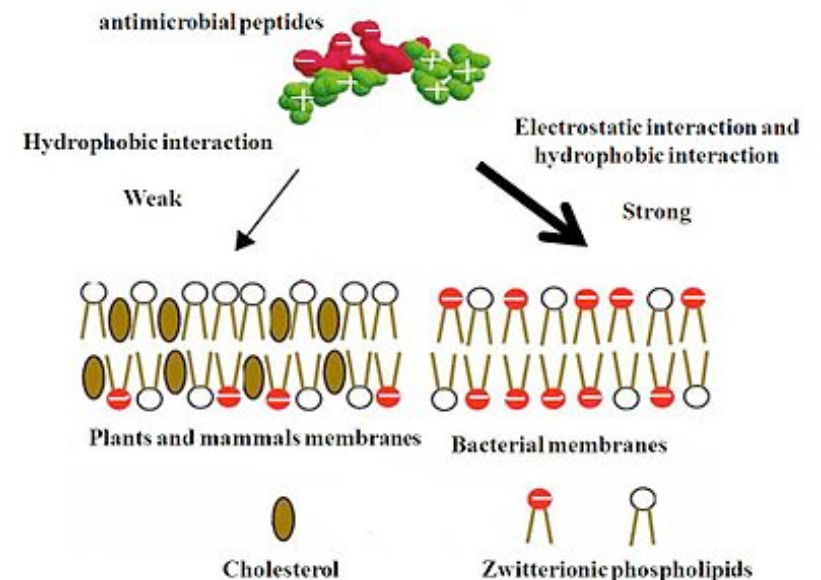
AA variável (6-100), carga positiva, anfipáticas
(epitélio/secreções, grânulos de fagócitos)



→ Lise de membranas no eucariote
→ Neutralizar toxinas
→ Sinalização

H. sapiens:

- Defensinas e catelicidina/LL-37 (produzidas por células imunes)
- Histatina (produzida por parotídeo, glândulas salivares)






Peptides anti-microbianos (AMPs)

International Journal of Medical Microbiology 304 (2014) 93–99

Defensin-based anti-infective strategies

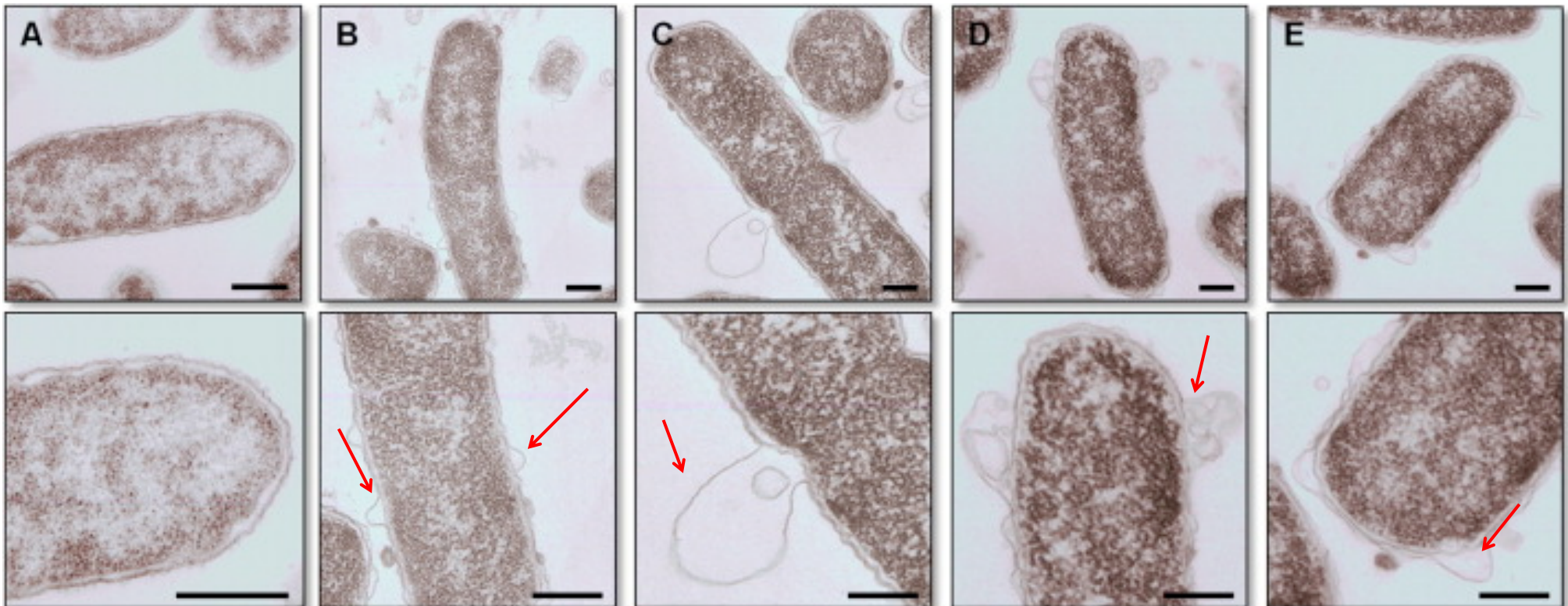
Miriam Wilmes, Hans-Georg Sahl*

Defensin	Source	3D structure	Activity spectrum	Molecular target(s)	Immune modulation
α -Defensin HNP-1	<i>Homo sapiens</i>		Gram ⁺ and Gram ⁻ bacteria, fungi, viruses	Lipid II (intermediate affinity)	Yes
β -Defensin hBD3	<i>Homo sapiens</i>		Gram ⁺ and Gram ⁻ bacteria, fungi, viruses	Lipid II (intermediate affinity), LPS	Yes
θ -Defensin RTD-1	<i>Macaca mulatta</i>		Gram ⁺ and Gram ⁻ bacteria, fungi, viruses	LTA, cytoplasmic membrane	Yes

E. coli nao tratada

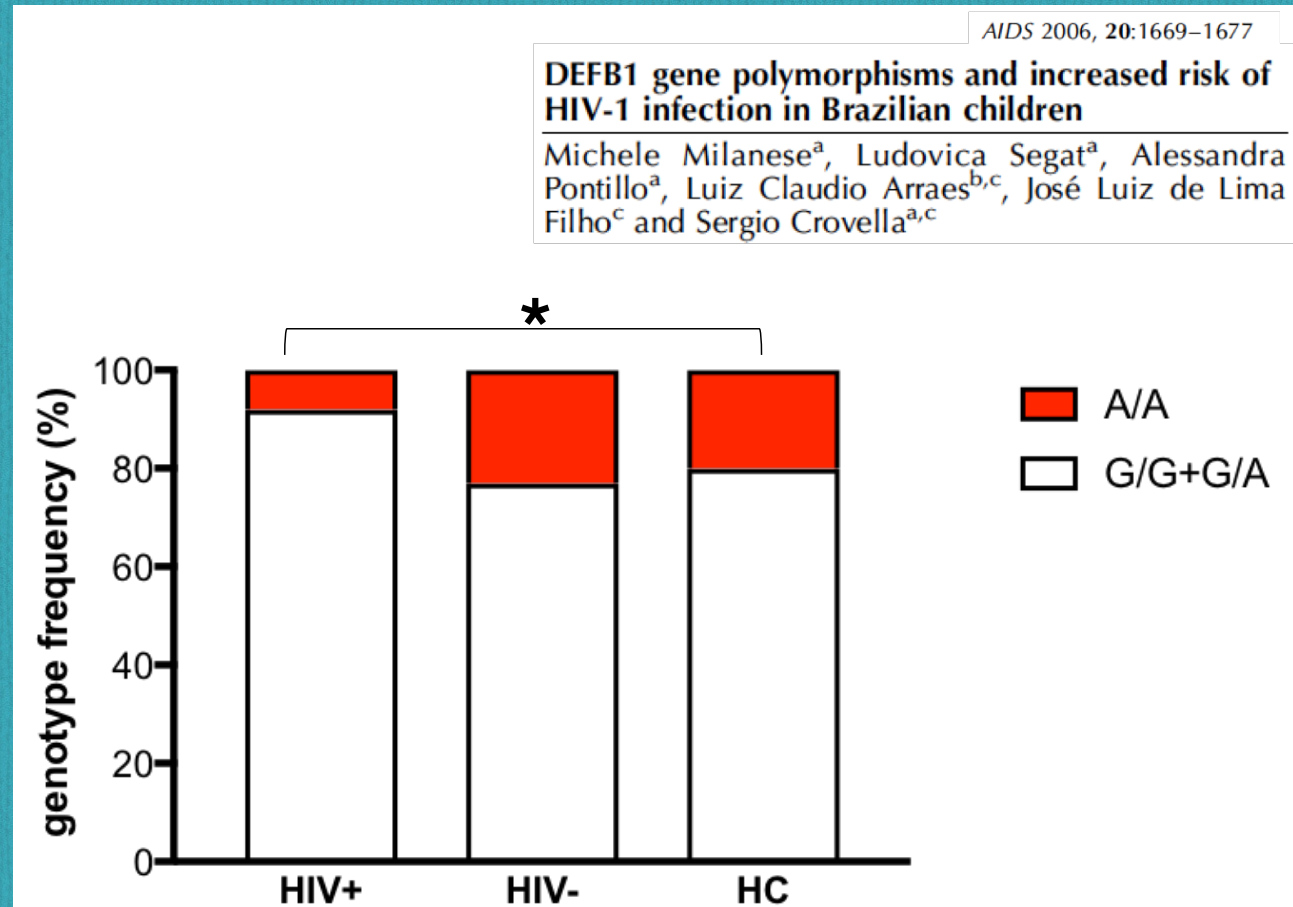
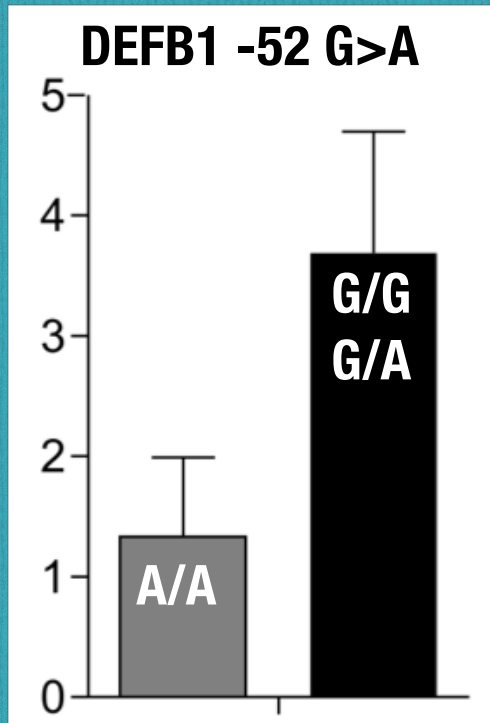
E. Coli + hBD3 30'

E. Coli + hBD3 60'



Transmission electron microscopy of hBD3-treated *E. coli* cells. A: untreated control cells. B and C: cells treated for 30 min. D and E: cells treated for 60 min. Scale bar: 0.4 μ m.

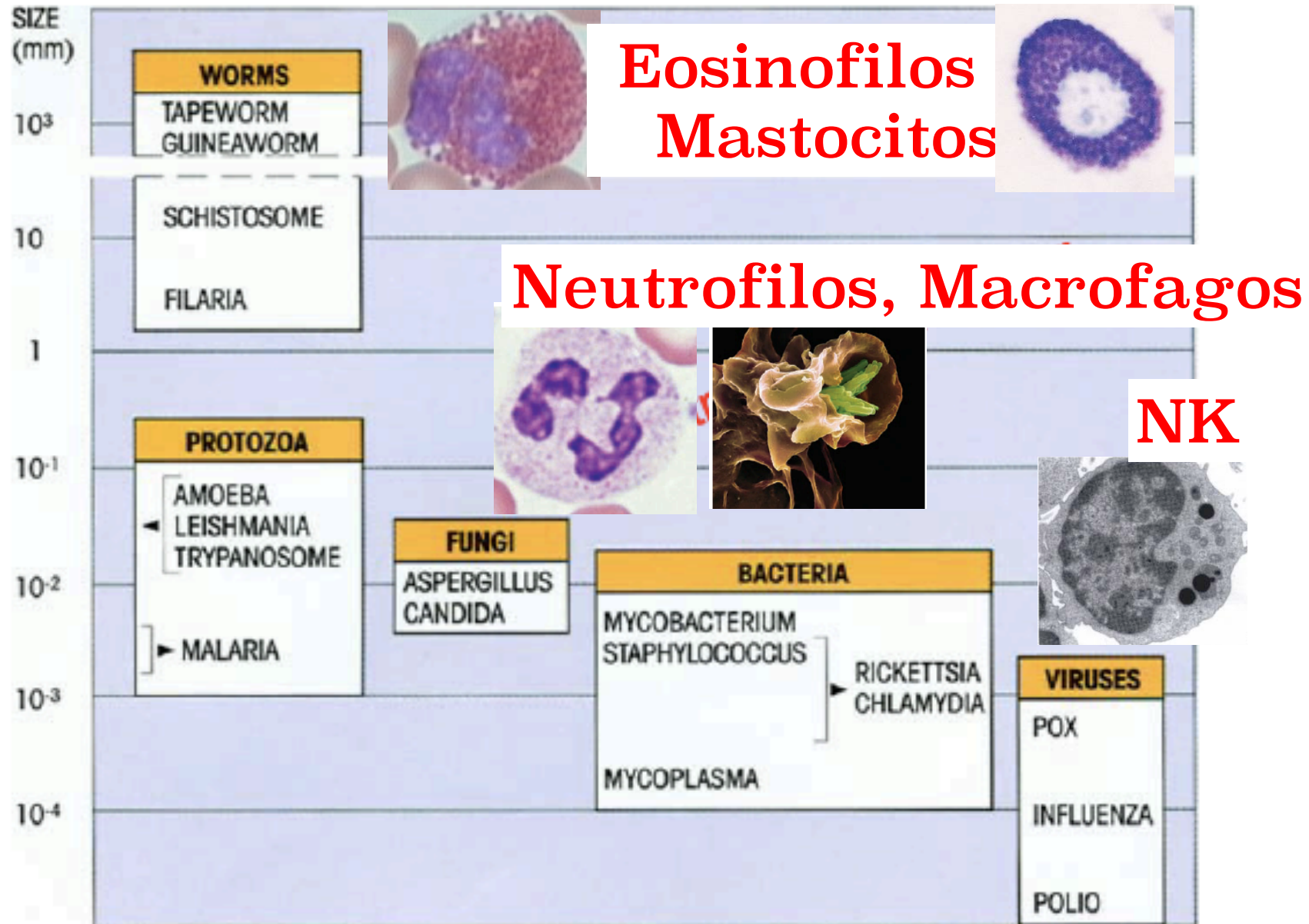
AMPs: protegem mesmo?



Imunidade inata celular

- Quando as barreiras são ultrapassadas
- **Fagocitos (macrófagos, neutrófilos)** rapidamente internalizam e digerem microbios/material extracelulares
- **Granulócitos (eosinófilos, mastócitos)** liberam conteúdo antimicrobiano e imunomodulador
- **Células NK** destroem células próprias alteradas
- Produção de moléculas microbicidas & citocinas
- Recrutamento de leucócitos (inflamação)

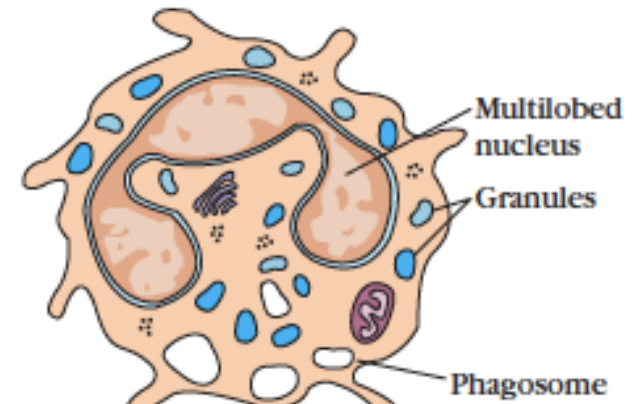
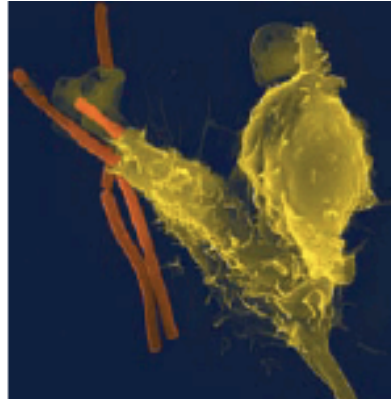
Imunidade inata celular



Fagocitos & fagocitose

- 2ª linha de defesa
- Macrófagos, Neutrófilos
- Fagocitose: “comer” partículas (patógenos extracelulares, células mortas, material) para eliminá-las
- Evolutivamente antigos (Metchinkoff)

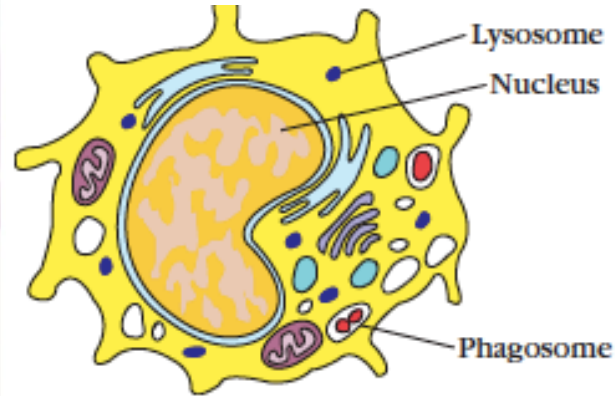
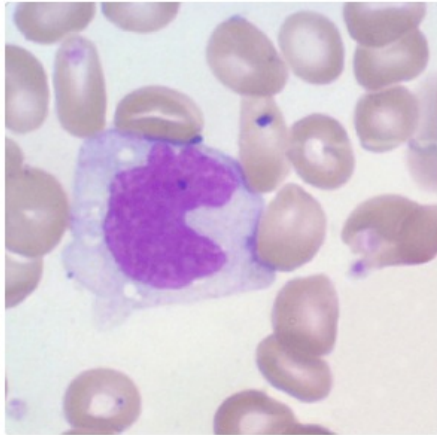
Polimorfonucleados neutrofilos



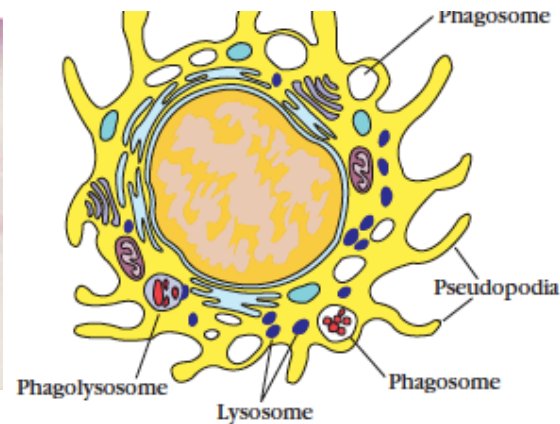
- Nucleo multilobado
- Granulos citoplasmaticos liberados após contato com patogenos
- Proteinas com função anti-microbiana direta, recrutamento leucocitos, remodelamento tecidual
- No sangue 7-10 horas; nos tecidos poucos dias (VIDA BREVE)
- 50-70% dos leucocitos circulantes; n aumenta durante infeçao
- 1 a chegar no sitio de infeçao (i.e.: pus) → **fagocitose**

Cell type	Molecule in granule	Examples	Function
Neutrophil	Proteases Antimicrobial proteins Protease inhibitors Histamine	<i>Elastase, Collagenase</i> <i>Defensins, lysozyme</i> <i>α1-anti-trypsin</i>	Tissue remodeling Direct harm to pathogens Regulation of proteases Vasodilation, inflammation

Monocitos/macrofagos



- Nucleo a U
- 5-10% dos leucocitos circulantes; n aumenta durante infeçao
- Se diferenciam em Mø nos tecidos
- **Fagocitos** e APC (M1), mecanismos batericidas, produçao mediadores inflamatorios
- Remodelamento tecidual (M2)



Fagocitose

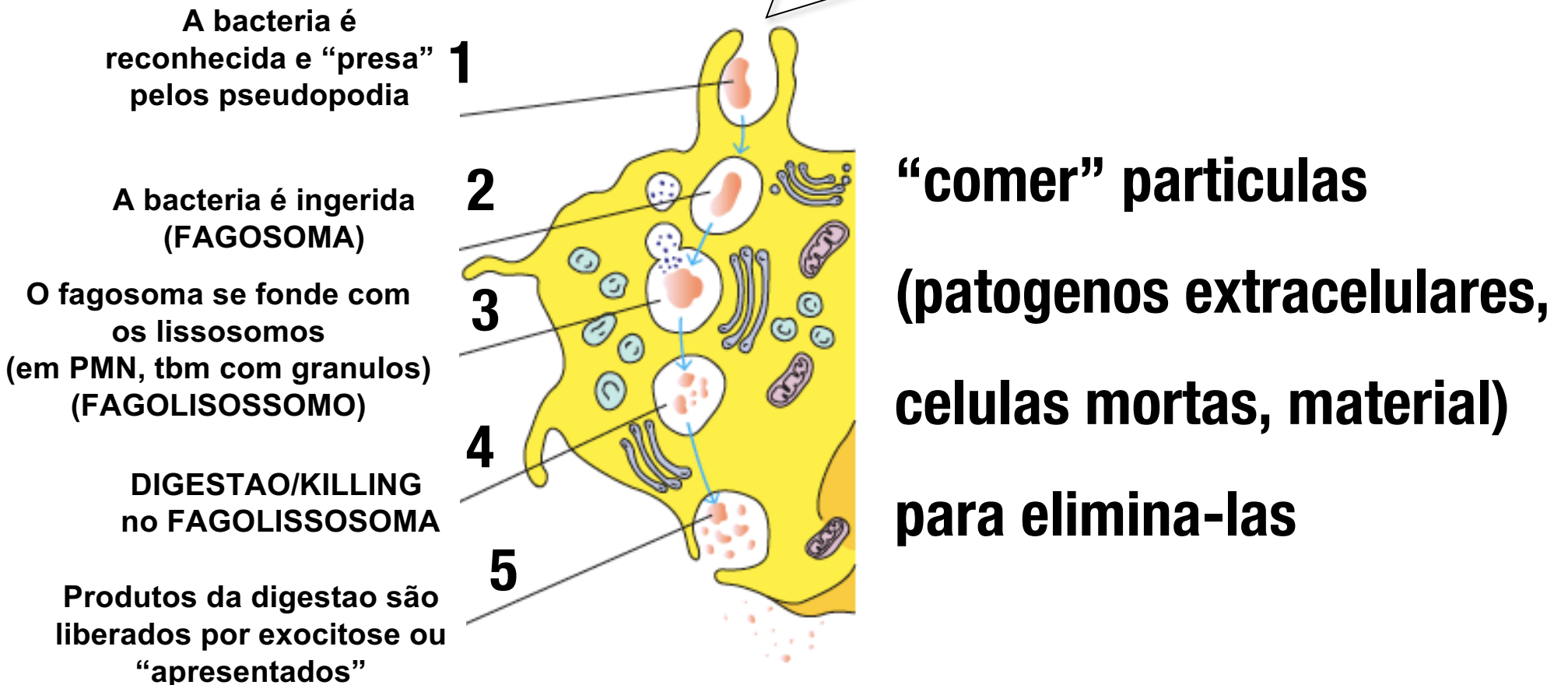
quimiotatico

Formil-peptídeos

PAMPs

reconhecimento

BACTERIA



Fagocitose

Receptor type on phagocytes	Examples	Ligands
Reconhecimento direto do patogeno		
C-type lectin receptors (CLRs)	Mannose receptor	Mannans (bacteria, fungi, parasites)
	Dectin 1	β -glucans (fungi, some bacteria)
	DC-SIGN	Mannans (bacteria, fungi, parasites)
Scavenger receptors	SR-A	Lipopolysaccharide (LPS), lipoteichoic acid (LTA) (bacteria)
	SR-B	LTA, lipopeptides, diacylglycerides (bacteria), β -glucans (fungi)

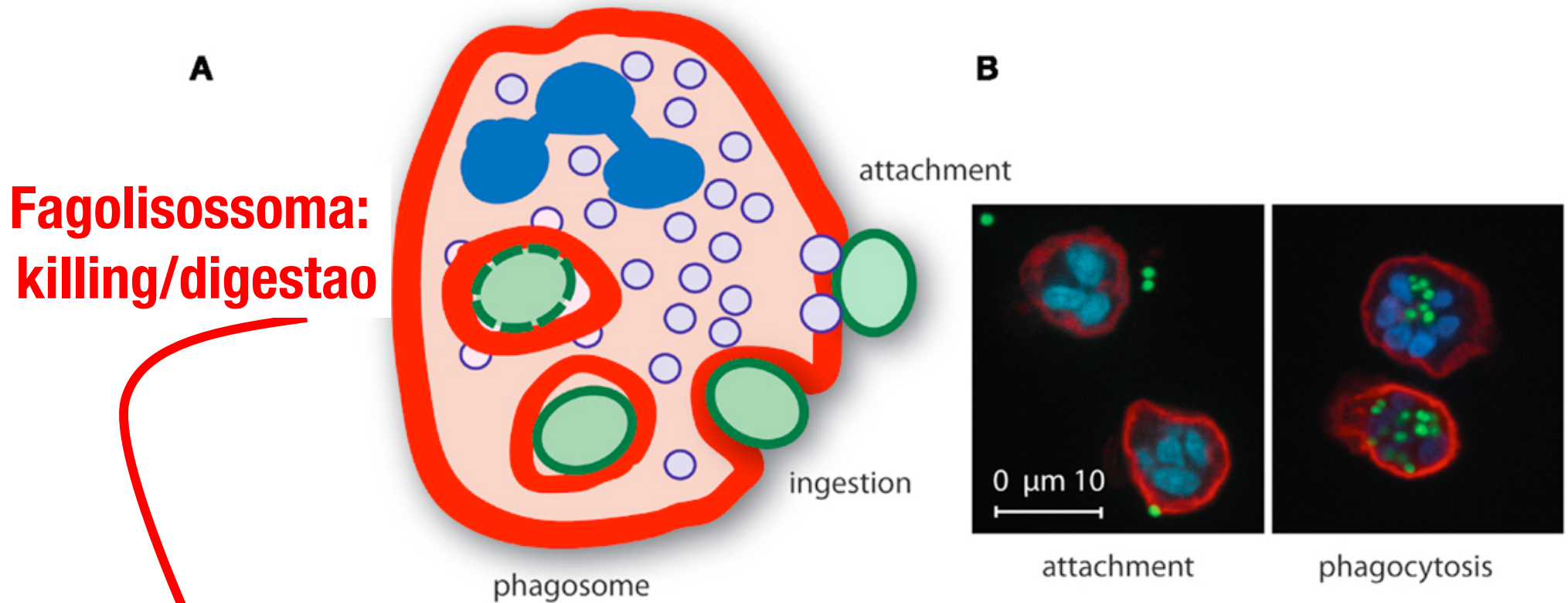
Reconhecimento indireto (atraves moleculas soluveis que ligam o patogeno/OPSONINAS)

Collagen-domain receptor	CD91/calreticulin	Collectins SP-A, SP-D, MBL; L-ficolin; C1q
Complement receptors	CR1, CR3, CR4, CR1g, C1qRp	Complement components and fragments*
Immunoglobulin Fc receptors	Fc α R	Specific IgA antibodies bound to antigen [#]
	Fc γ Rs	Specific IgG antibodies bound to antigen; [#] C-reactive protein

- **Produção de mediadores**
- **Recrutar e ativar leucocitos**
 - **Inflamação**



Fagocitose



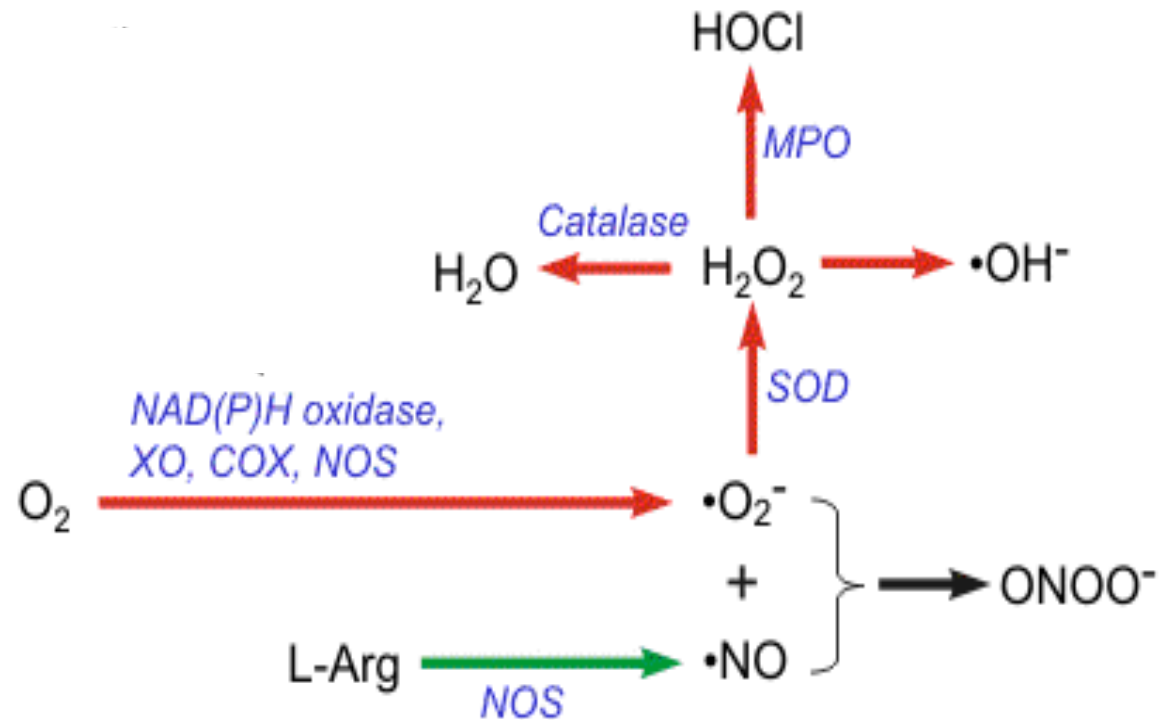
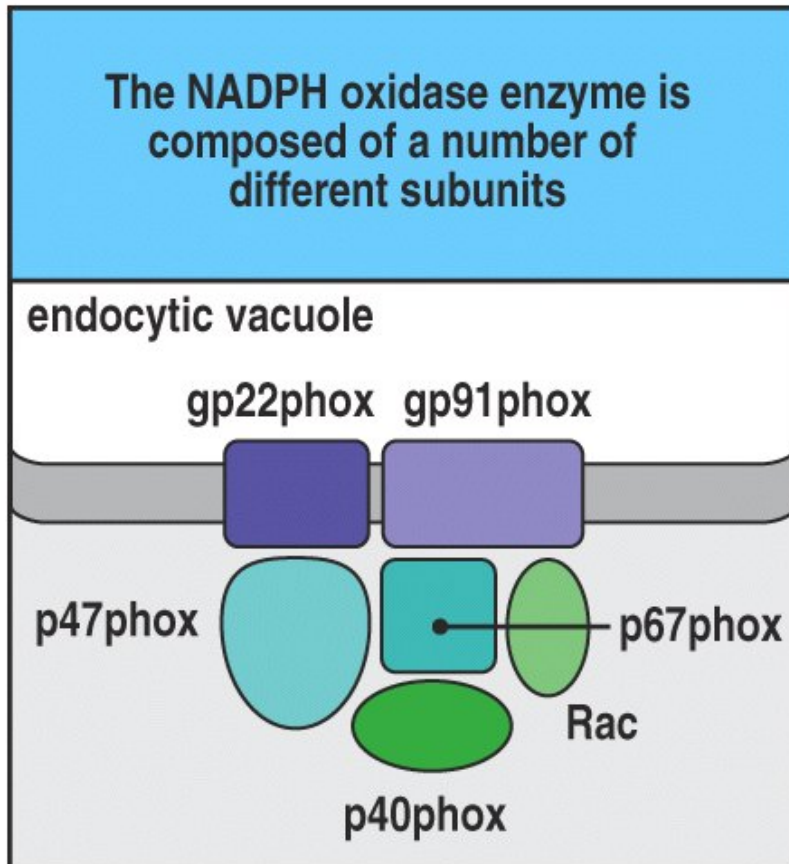
**Fagolisossoma:
killing/digestao**

Agentes anti-microbianos

- Proteinas e peptideos antimicrobianos
- pH acido
- Enzimas idroliticos ativados a pH acido (lisozima, protease)
- ROS produzidos via NADPH-oxidase
- ROS+NO (produzido via iNOS) → RNS

Fagocitose

Produção de espécies reativas de oxigênio (ROS) e de nitrogênio (RNS)

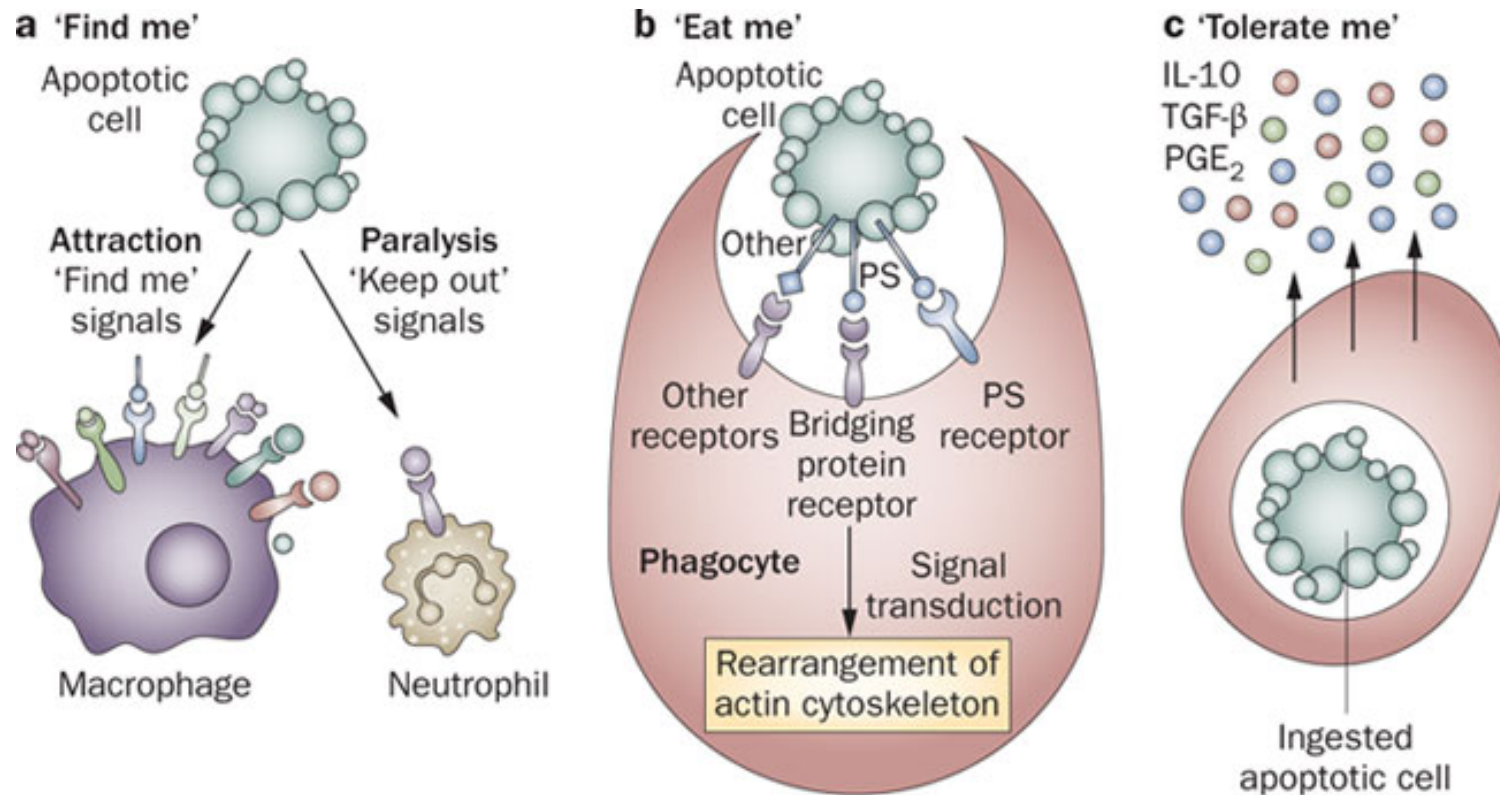


Mutações nos genes da NADPH oxidase causam a DOENÇA CRONICA GRANULOMATOSA

Fagocitose “fisiologica”

- Celulas mortas (necrose, apoptose)
- Debris celulares
- Emacias velhas (figado/Kupffer c.; baço/Mø)
- Complexos Ag/AC

Silence please



<https://youtu.be/rsHyQ6A6Pcl>

https://youtu.be/Z_mXDvZQ6dU

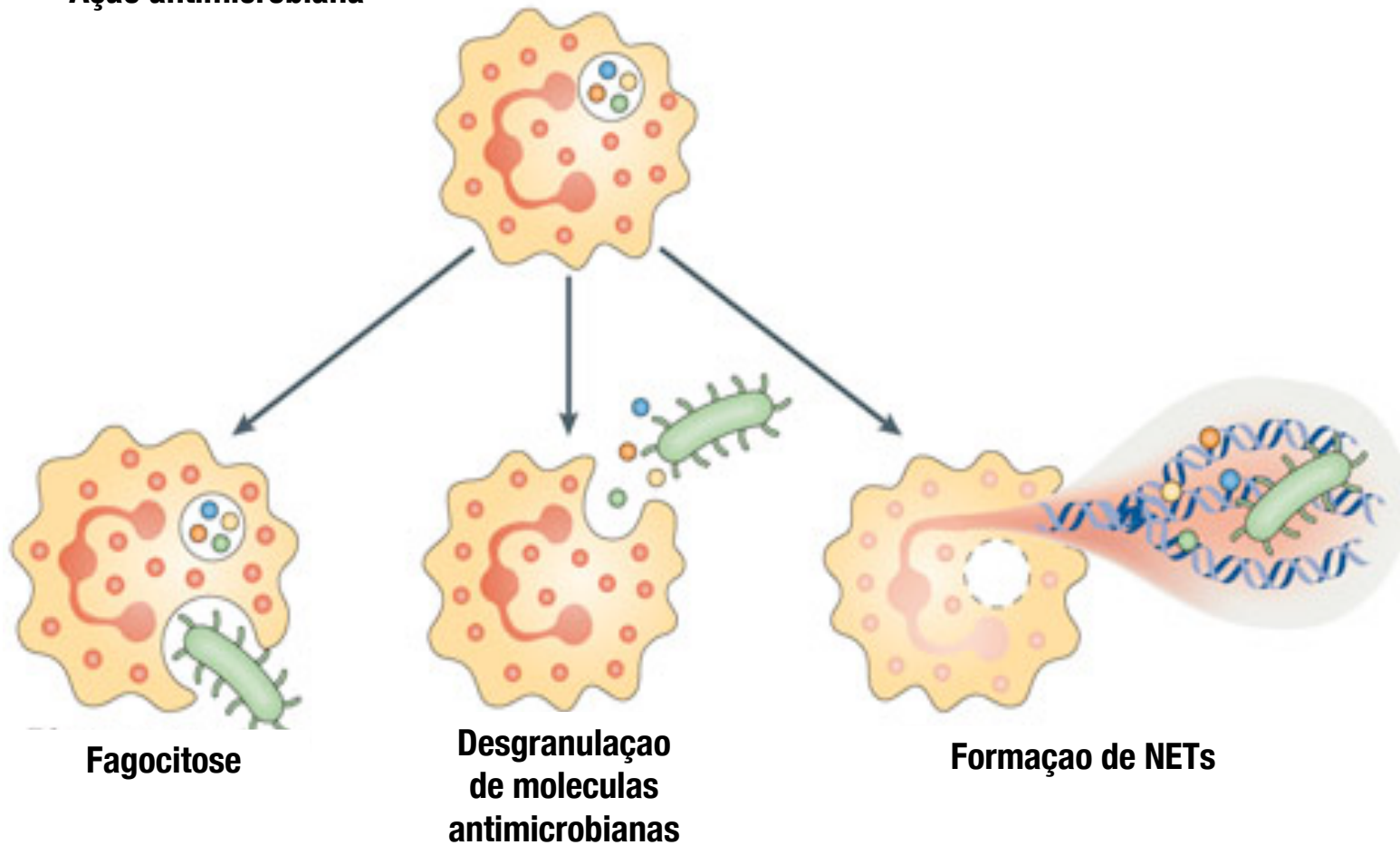
Evolução

Taxonomic group	Innate immunity (nonspecific)	Phagocytosis	Anti microbial peptides
<i>Higher plants</i>	+	-	+
<i>Invertebrate animals</i>			
Porifera (sponges)	+	+	+
Annelids (earthworms)	+	+	+
Arthropods (insects, crustaceans)	+	+	+
<i>Vertebrate animals</i>			
Jawless fish (hagfish, lamprey)	+	+	+
Elasmobranchs (cartilaginous fish; e.g., sharks, rays)	+	+	+
Bony fish (e.g., salmon, tuna)	+	+	+
Amphibians	+	+	+
Reptiles	+	+	+
Birds	+	+	+
Mammals	+	+	+

Outras acoes microbicidas

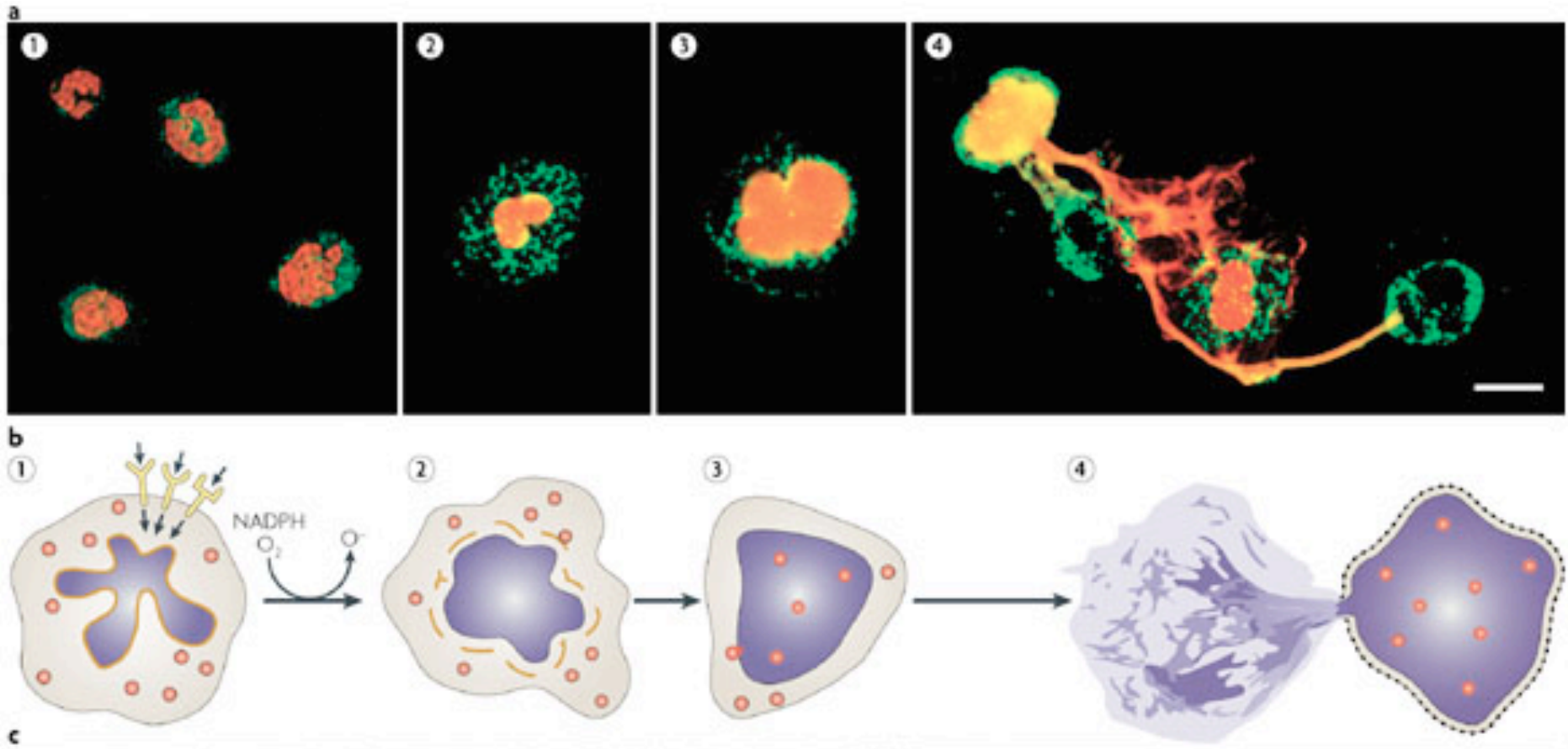
- Neutrofilos - NETs
- Neutrofilos - DESGRANULACAO

Ação antimicrobiana

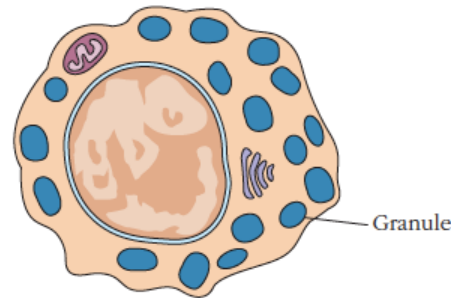
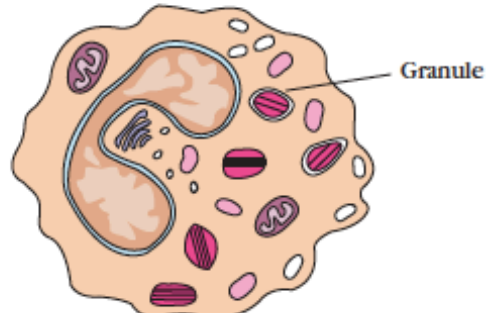
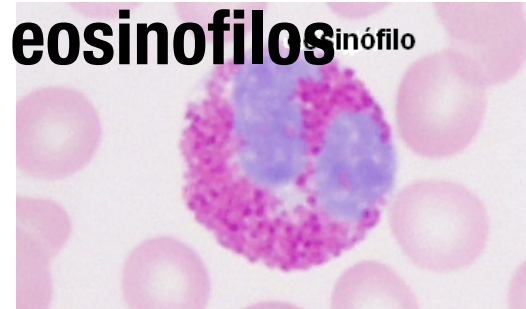


Formação de NETs

- Função de eliminação de patógeno recentemente descrita (2004).
- Os neutrófilos liberam conteúdo nuclear e granular formando uma rede que intrappola os patógenos e facilita a ação das moléculas antimicrobianas para destruição dos patógenos (killing extracelular).



Polimorfonucleados/Granulocitos

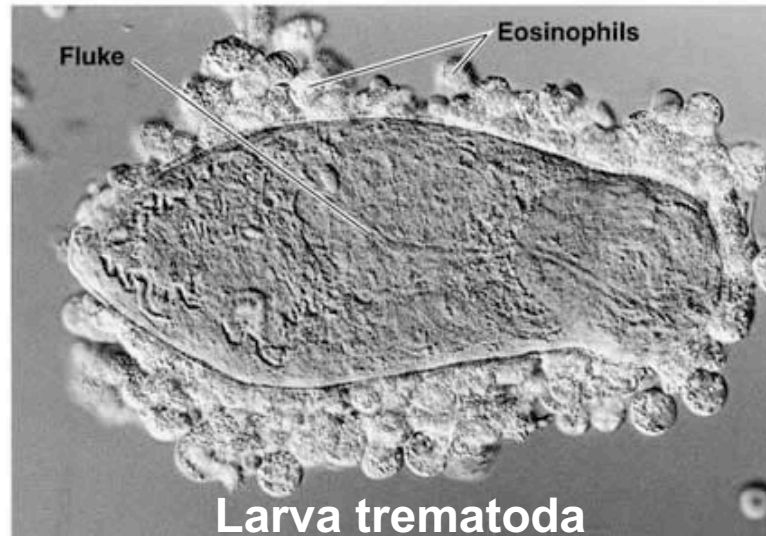
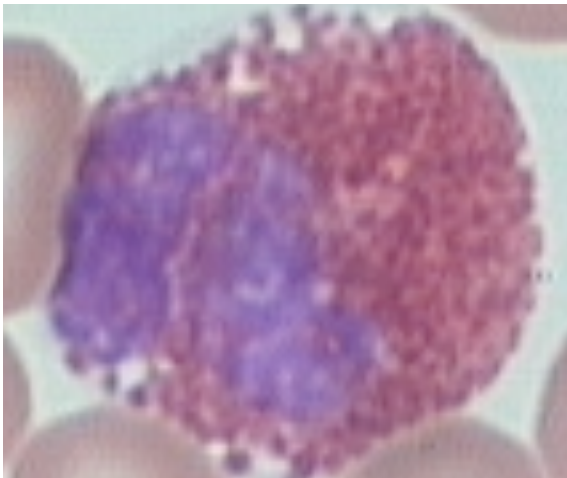


- Nucleo multilobado
- Granulos citoplasmaticos liberados após contato com patogenos/dano
- Proteinas com função anti-microbiana direta, recrutamento leucocitos, vasodilatação
- Eosinofilos no sangue; mastocitos nos tecidos (vasos)

- **DESGRANULACAO**
- **Producao de mediadores**

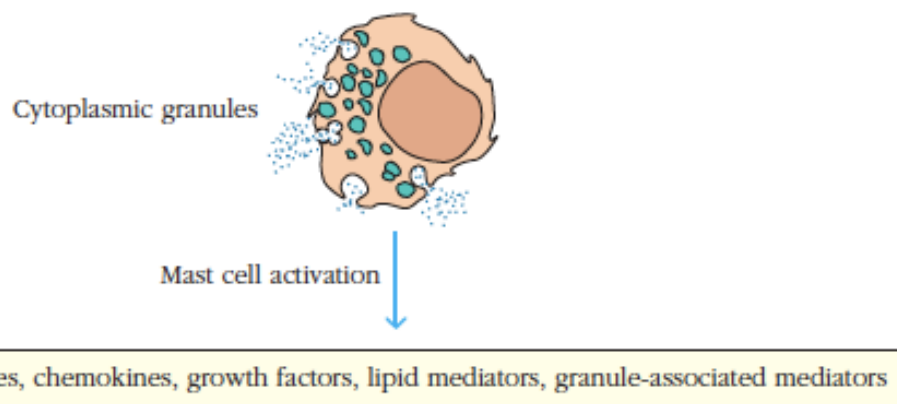
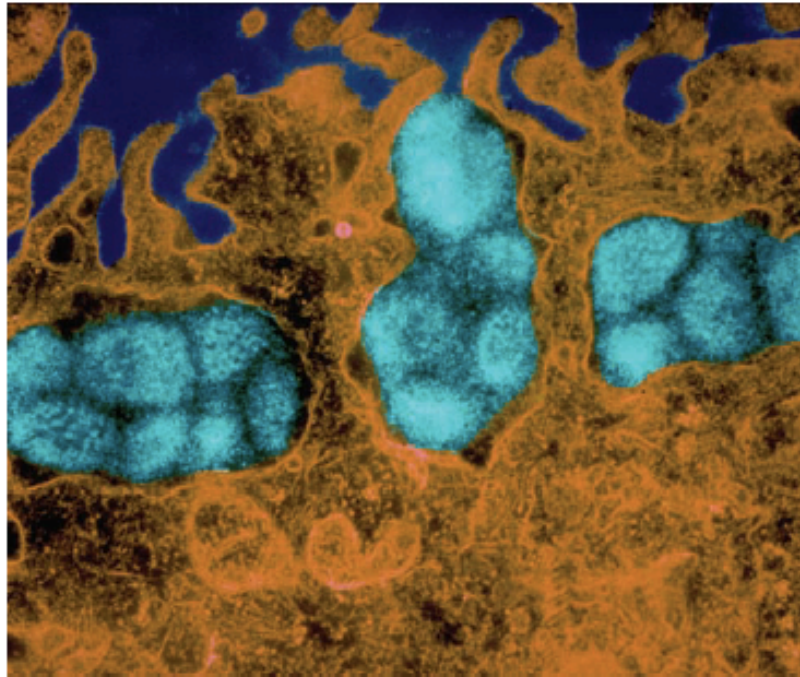
Eosinofilos

- Circulante (12 hs)/teciduals (8-12 d)
- Importantes na defesa contra protozoários
- Elevado poder microbicida dos grânulos
- Rec para IgE responsável para alergias



- Ação anti-microbiana/ induz ROS (proteínas cationicas, EPO)
- Modulação da resposta adaptativa (IL-4, IL10, TNF)
- Recrutamento leucocitos (RANTES, MIP1a)
- Vasodilatação (ribonuclease, MBP)

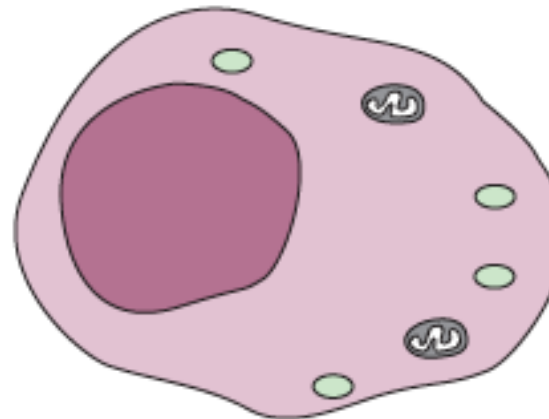
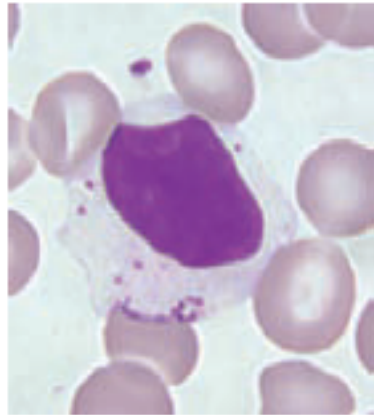
Mastocitos



- Teciduais (pele, mucosa)
- Proximos aos vasos
- Granulos citoplasmaticos abundantes (citocinas, aminas vasoativas e outros mediadores)
- Regulação da permeabilidade vascular (istamina)
- Regulação da inflamação (leucotrienos)
- Modulação da resposta adaptativa (IL-4)
- Ação anti-microbiana
- Rec para IgE responsavel para alergias

Celulas NK

(d) NK cell



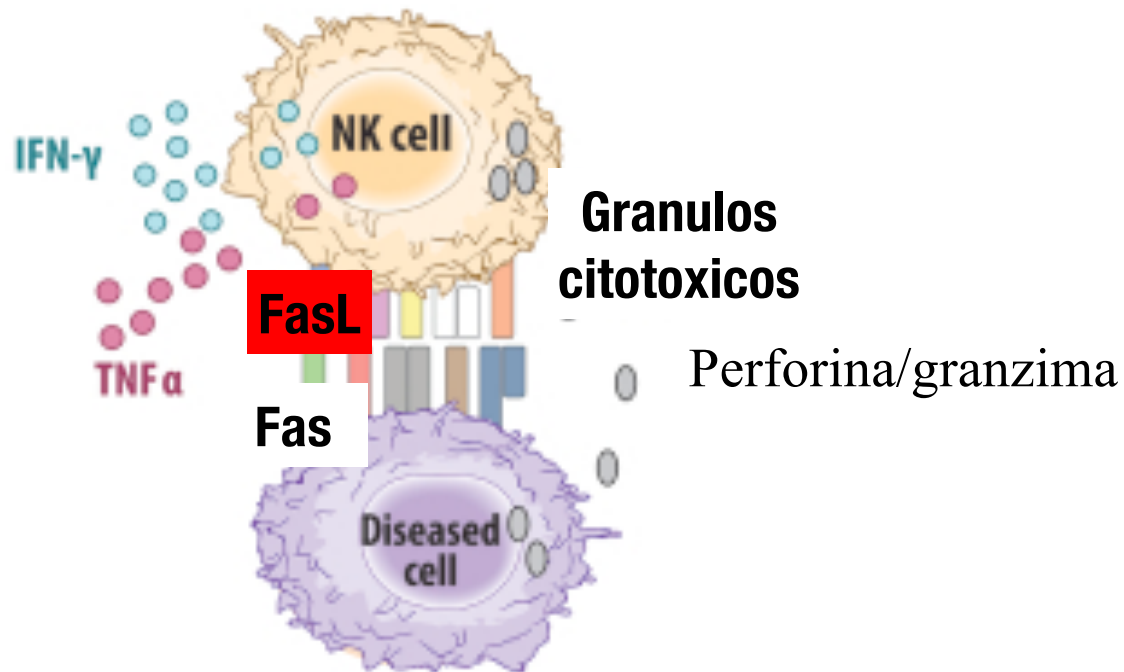
Natural killer
(NK) cell

- Progenitor linfoide – linfocitos grandes e granulados
- Circulantes (5-10% dos linfocitos) (+ baço, fígado)
- Granulos contendo proteínas citotóxicas (perforina, granzima)
- Secreção de citocinas (IFN- γ , TNF)
- Eliminar células “anormais” (infetadas/alteradas) induzindo apoptose
- Importantes na defesa anti-viral e contra os tumores

Celulas NK

- Atividade citotóxica contra células alteradas (**granulos citotóxicos, FasL/Fas**)
- Ativar Mø para potenciar a resposta microbiana (TNF, IFN- γ)
- Atividade citotóxica induzida por anticorpos/ADCC

Natural cytotoxicity



Antibody-dependent cellular cytotoxicity (ADCC)

