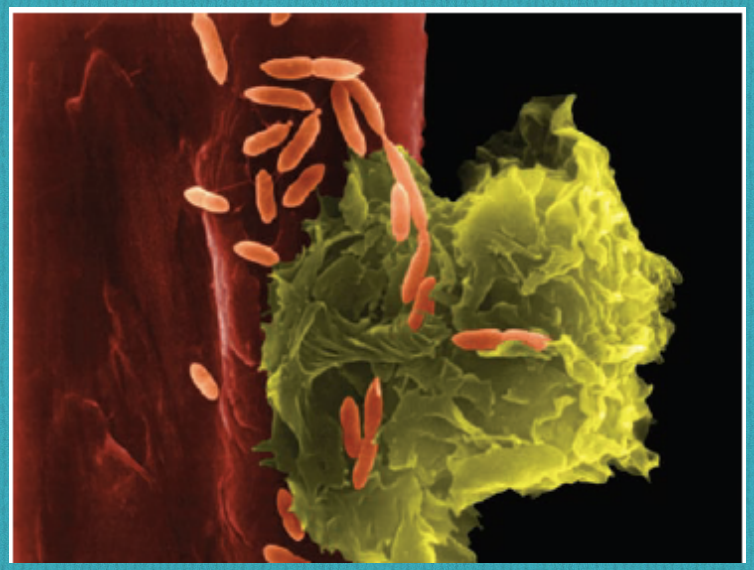
Curso de Ciencias Biologicas
Disciplina BMI-296 - Imunologia Basica



Aula 2 – Imunidade inata

Profa. Dra. Alessandra Pontillo

Lab. Imunogenetica/Dep. Imunologia/ICB/USP

Imunidade inata

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

Barreiras anatômicas (físicas, químicas)

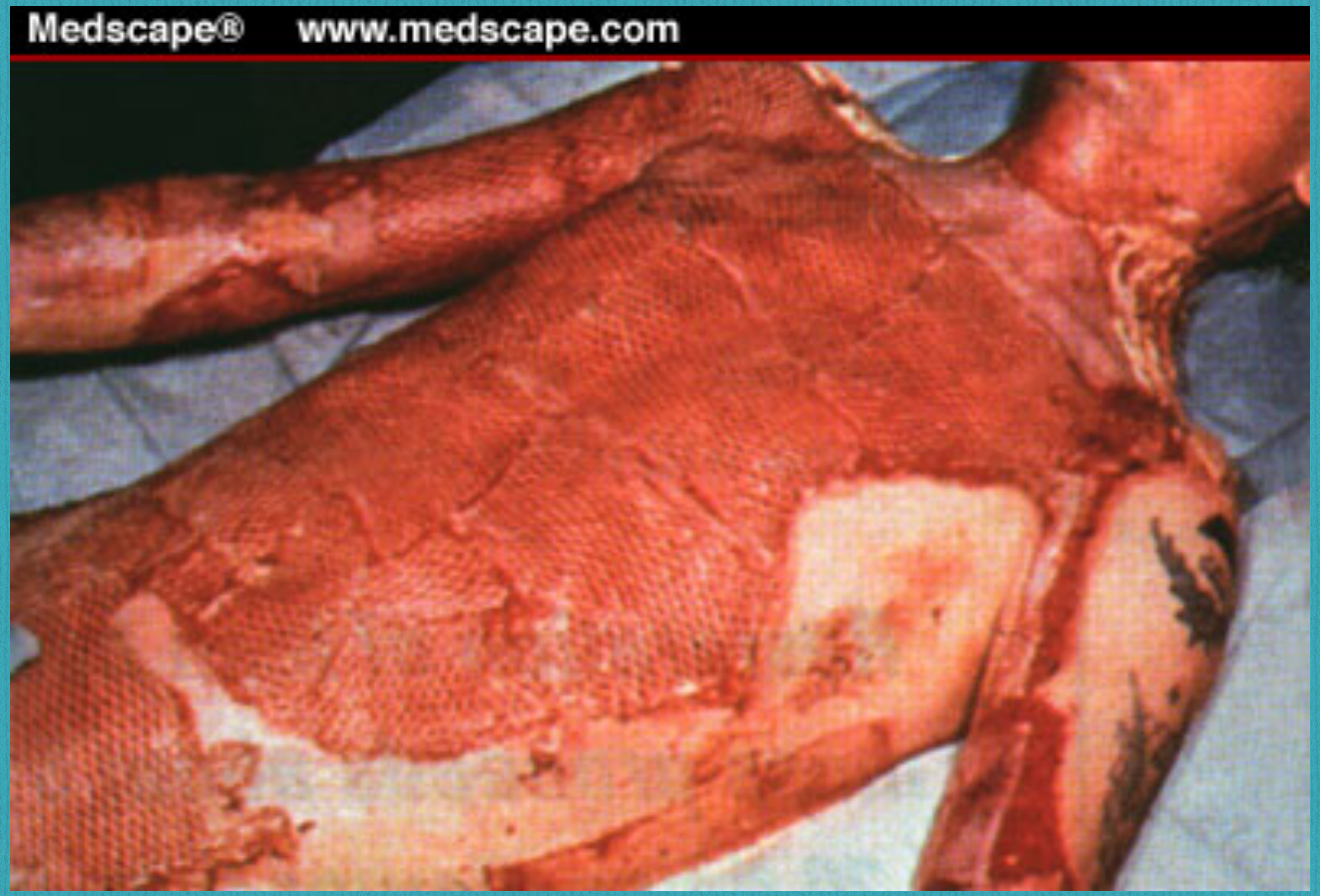
Resposta celular

Resposta humoral

Barreiras anatômicas

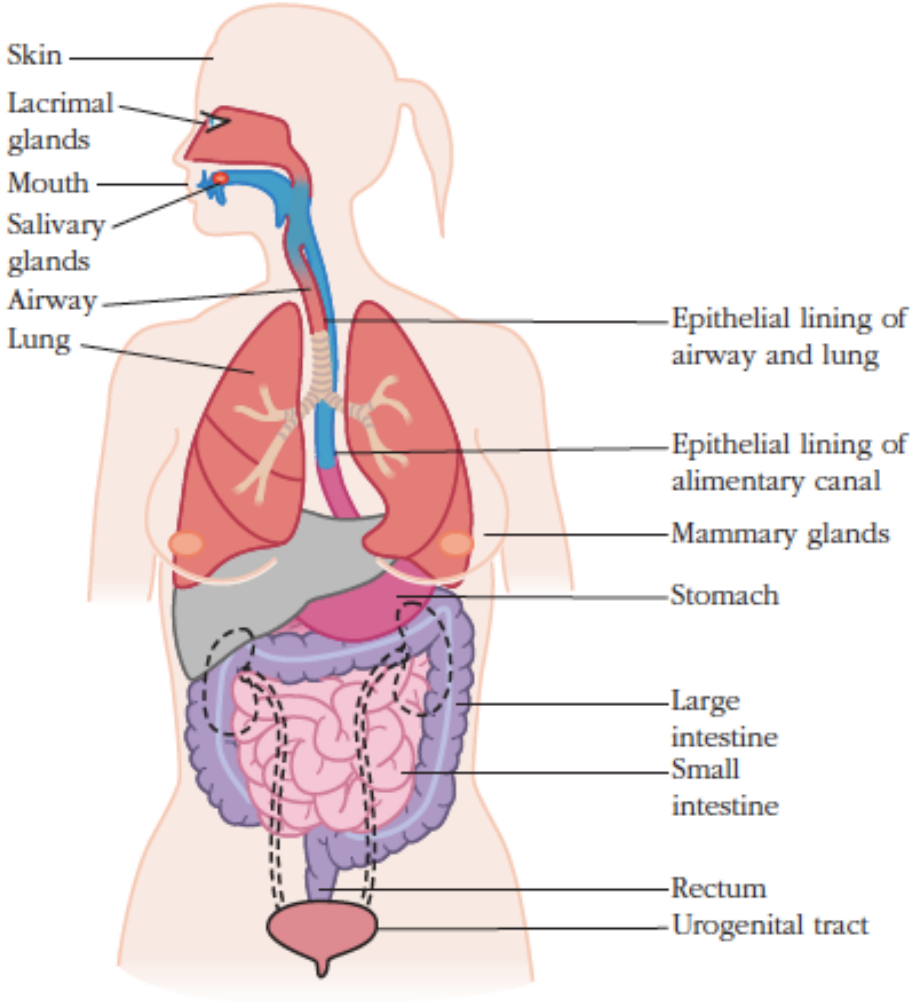
- 1 linha de defesa
- Físicas: epitélios de pele e mucosas (preveem entrada de patógenos/agentes perigosos)
- Químicas: pH (acido) & substancias solúveis com atividade microbicida

Barreiras anatômicas: protegem mesmo?



Do que more um paciente com ustiao grave?

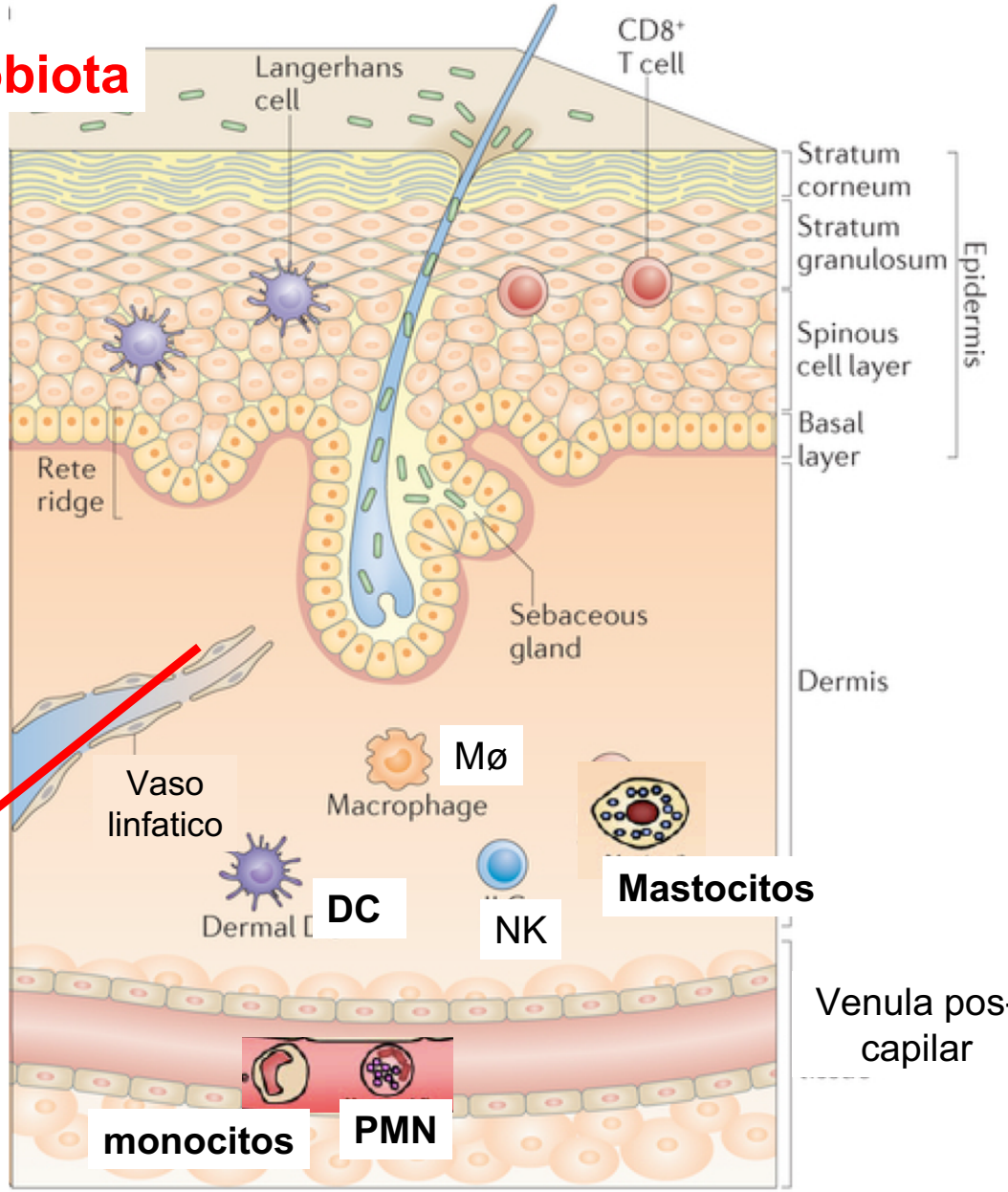
Barreiras anatômicas



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

microbiota



epidermide

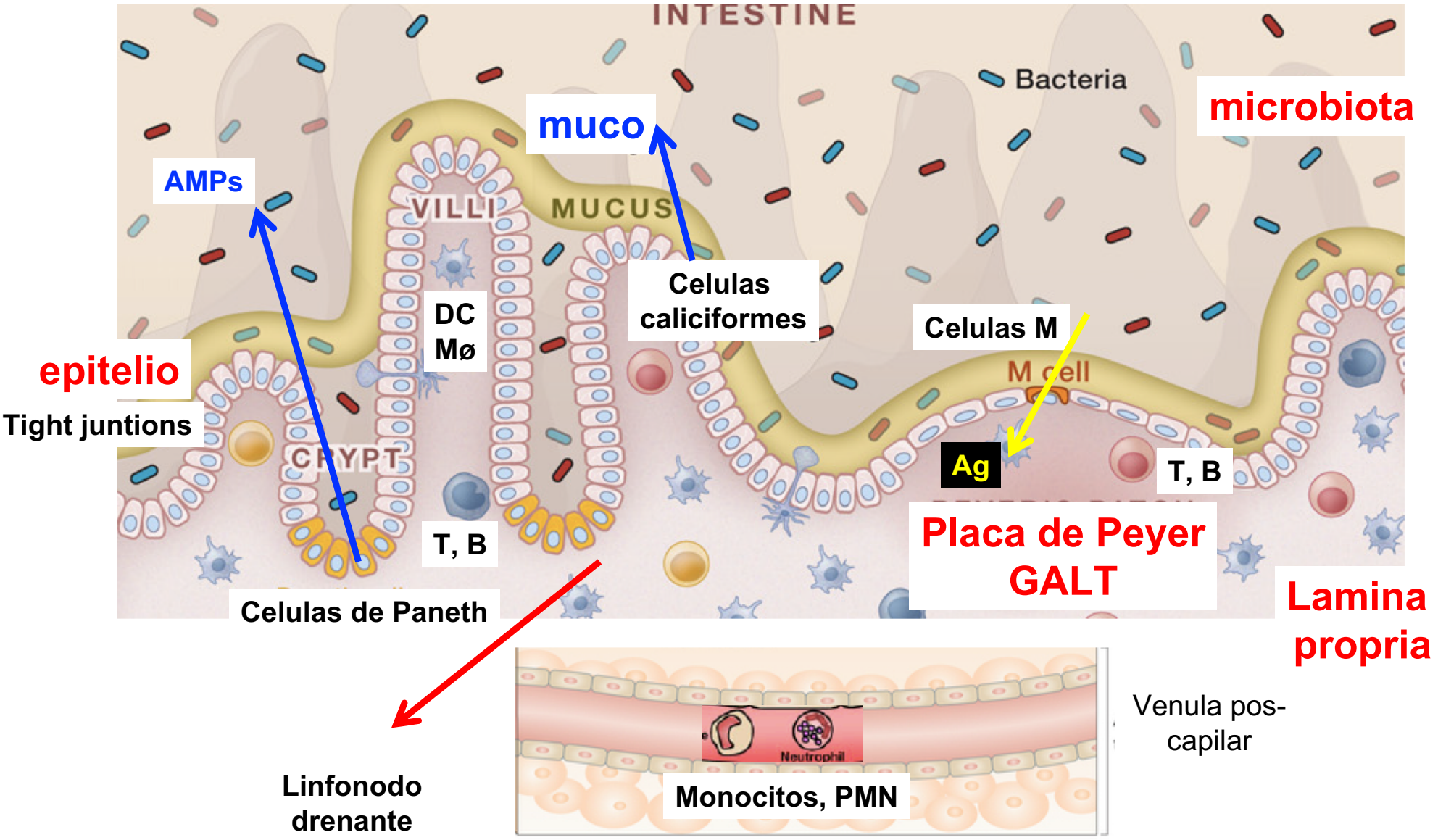
Queratinocitos

derma

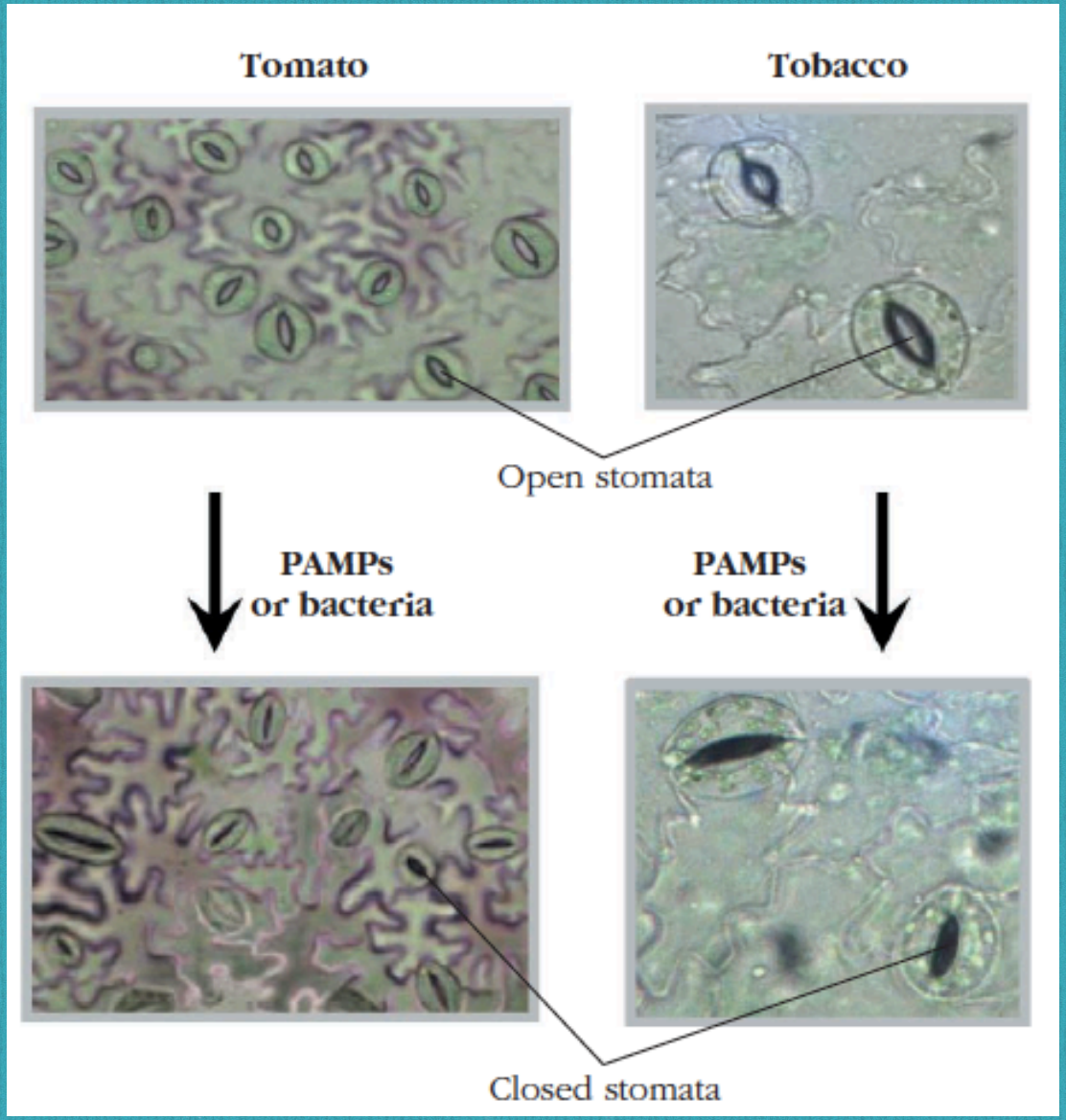
Tecido conetivo

Linfonodo drenante

Mucosa


INTESTINE
Bacteria
microbiota
muco
AMPs
VILLI
MUCUS
Celulas caliciformes
DC
Mø
epitelio
Tight juntions
Celulas M
M cell
CRYPT
Ag
T, B
T, B
Placa de Peyer
GALT
Lamina propria
Celulas de Paneth
Neutrophil
Monocitos, PMN
Venula pos-capilar
Linfonodo drenante

Barreiras fisicas nas plantas



Moleculas antimicrobianas

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

Proteins and peptides*	Location	Antimicrobial activities
Lysozyme *Enzima glicolitico*	Mucosal/glandular secretions (e.g., tears, saliva, respiratory tract)	Cleaves glycosidic bonds of peptidoglycans in cell walls of bacteria, leading to lysis
Lactoferrin *Prroteina sequestra Fe*	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** *Prroteinas baixo PM*	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) *AMPs*	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)** *AMPs*	Mucosal epithelia (e.g., respiratory tract, urogenital tract)	Disrupts membranes of bacteria; additional toxic effects intracellularly; kills cells.
Surfactant proteins SP-A, SP-D *Lectinas/receptores soluveis*	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)

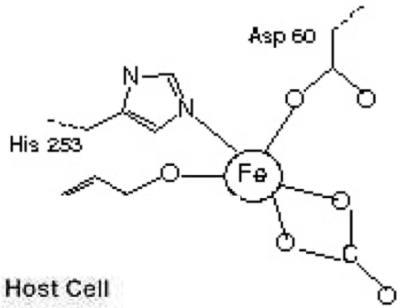
- liga a superficie de bacterios ---> facilita fagocitose

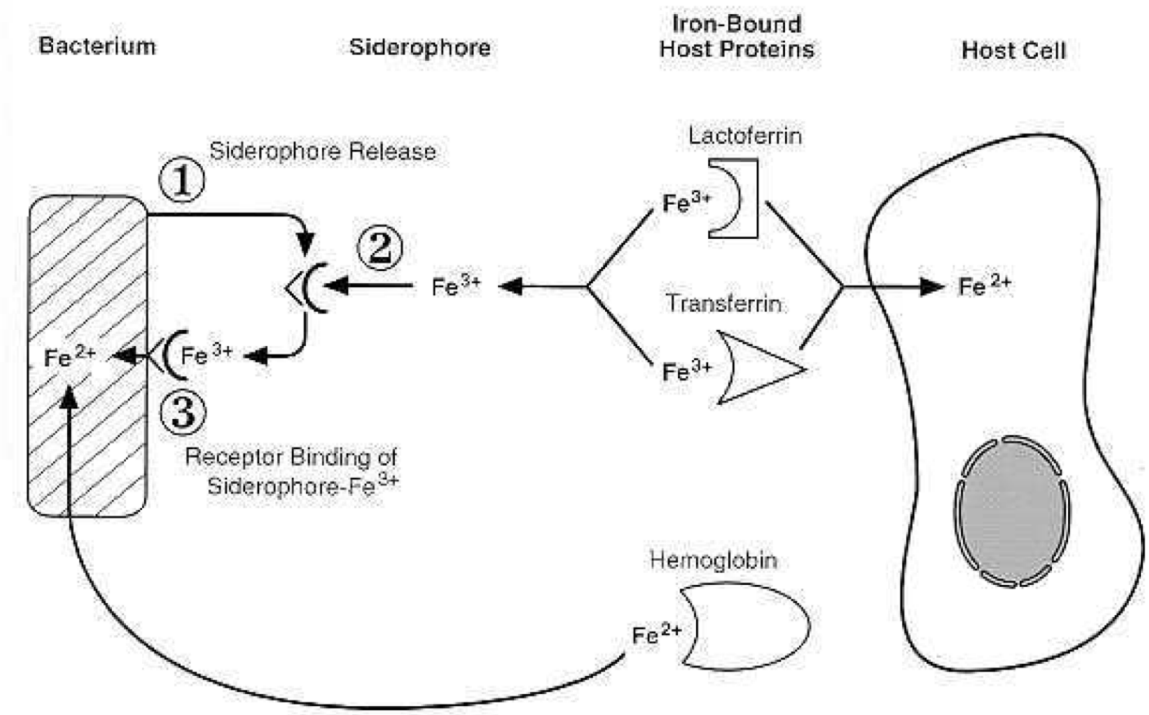
---> impede adesao

Lactoferrina (LF)

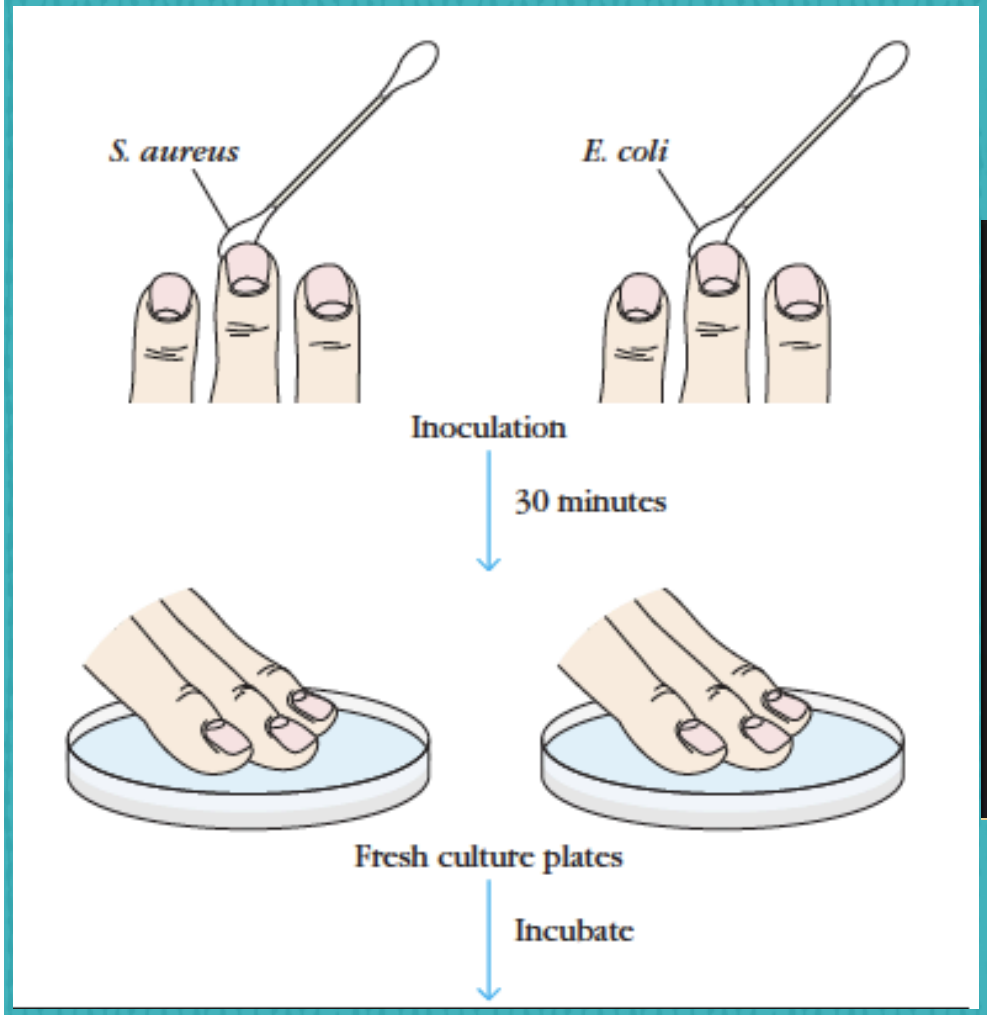
Proteína globular (80KDa) da família da transferrina

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

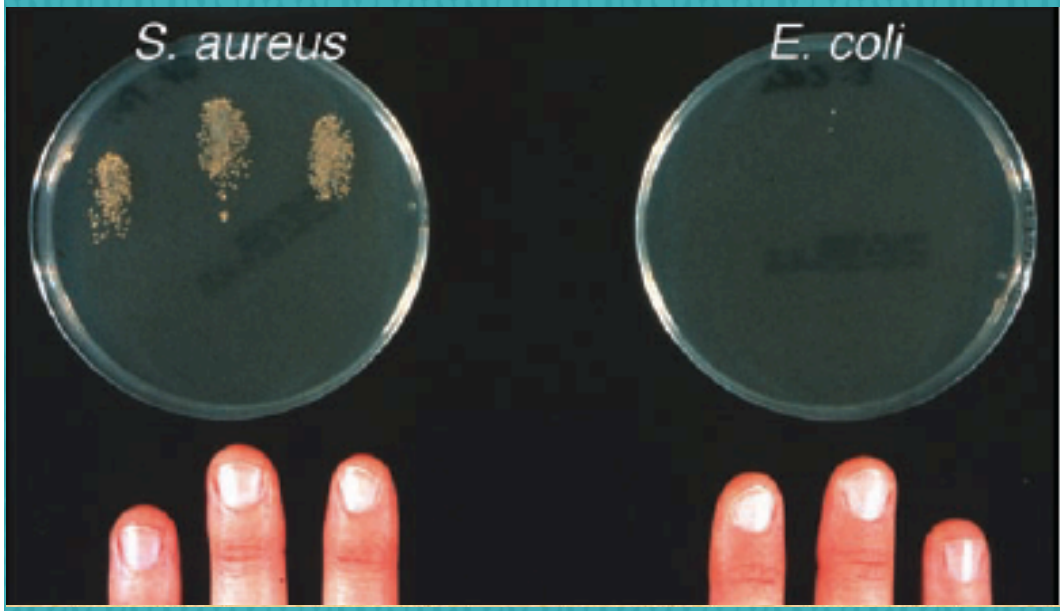




S100: protegem mesmo?

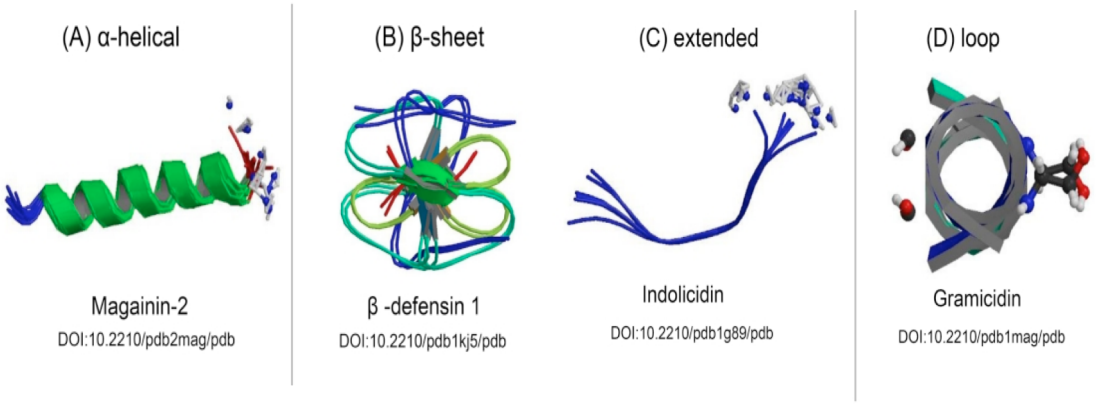


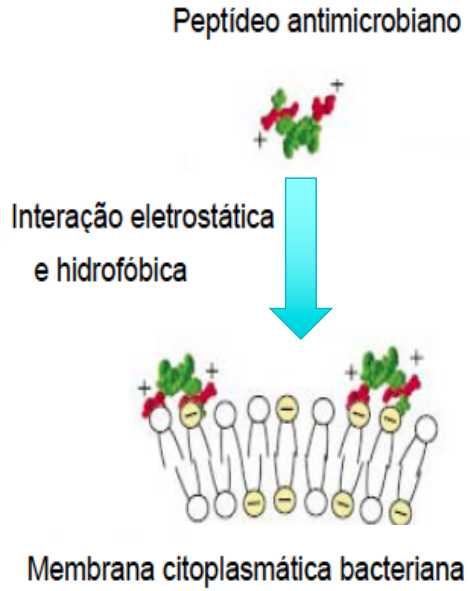
Psoriasina/S100A7 protege da colonizaçao da pele por *E coli*



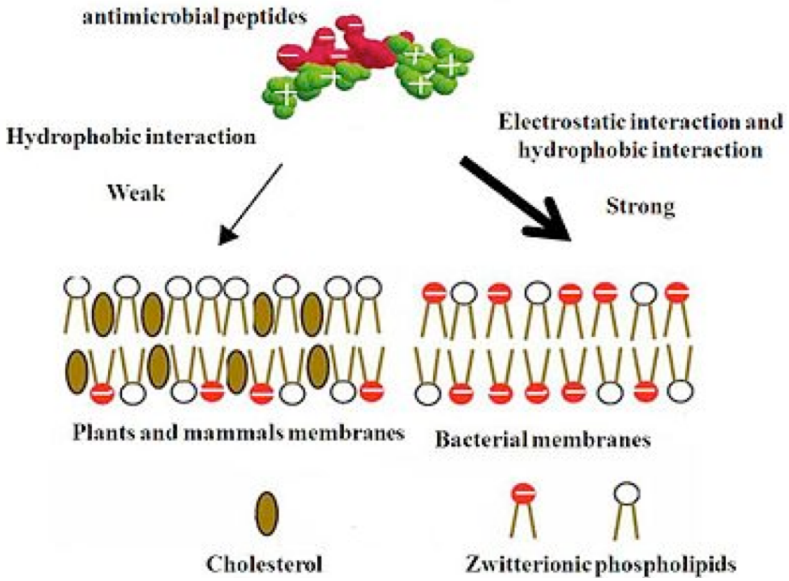
Peptideos anti-microbianos (AMPs)

AA variavel (6-100), carga positiva, anfipaticas (epitelio/secreçoes, granulos de fagocitos)





→ Lise de membranas nao eukariote
→ Neutralizar toxinas
→ Sinalizaçao



H. sapiens:

- Defensinas e catelicidina/LL-37 (produzidas por celulas imunes)
- Histatina (produzida por parotide, glandulas salivares)

Peptideos anti-microbianos (AMPs)

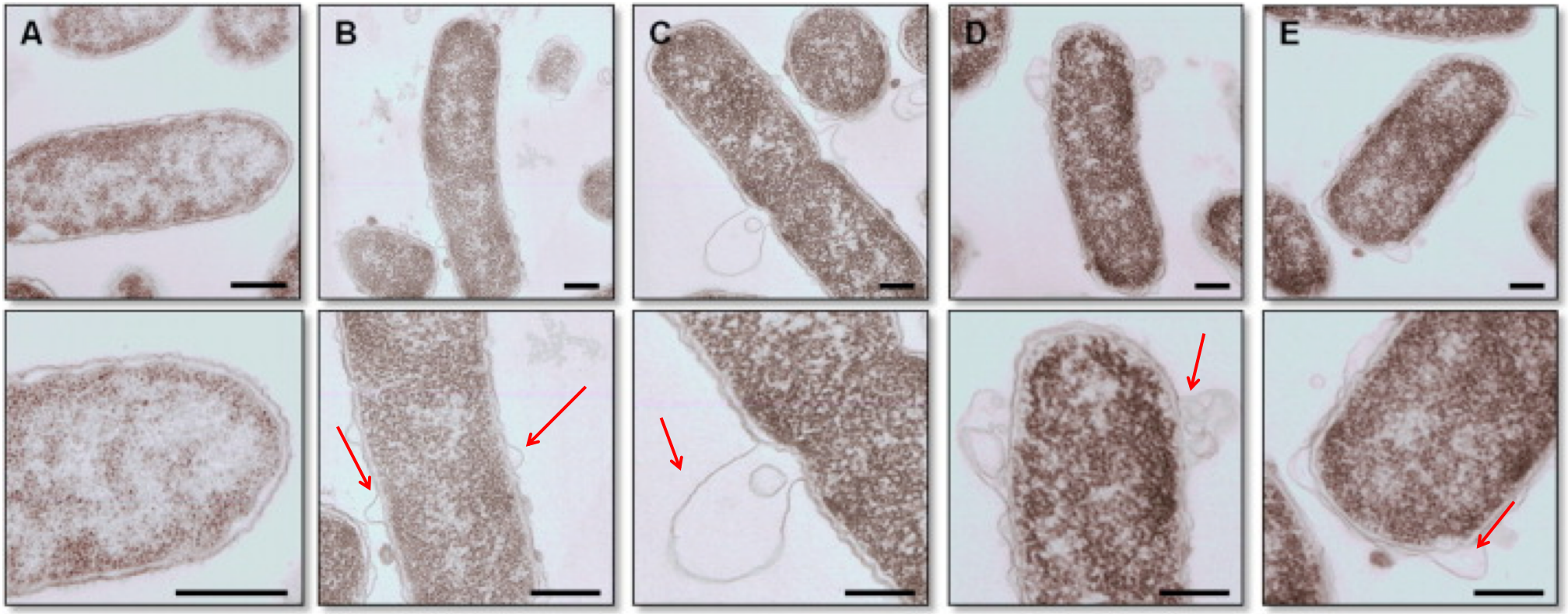
International Journal of Medical Microbiology 304 (2014) 93–99

Defensin-based anti-infective strategies

Miriam Wilmes, Hans-Georg Sahl*

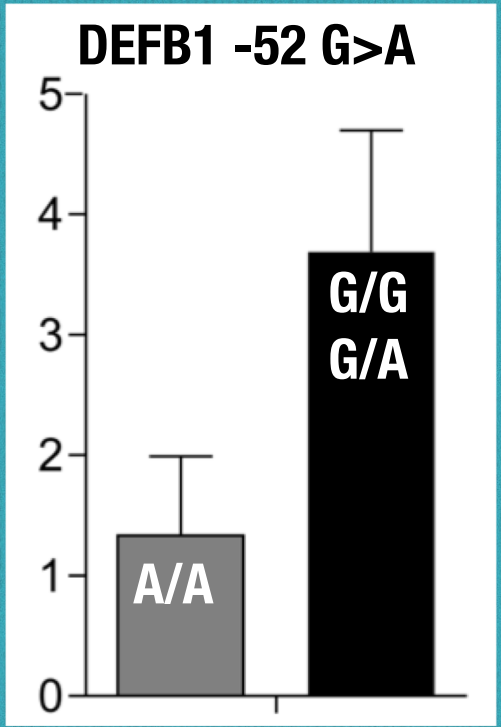
Defensin	Source	3D structure	Activity spectrum	Molecular target(s)	Immune modulation
α-Defensin HNP-1	*Homo sapiens*		Gram⁺ and Gram⁻ bacteria, fungi, viruses	Lipid II (intermediate affinity)	Yes
β-Defensin hBD3	*Homo sapiens*		Gram⁺ and Gram⁻ bacteria, fungi, viruses	Lipid II (intermediate affinity),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

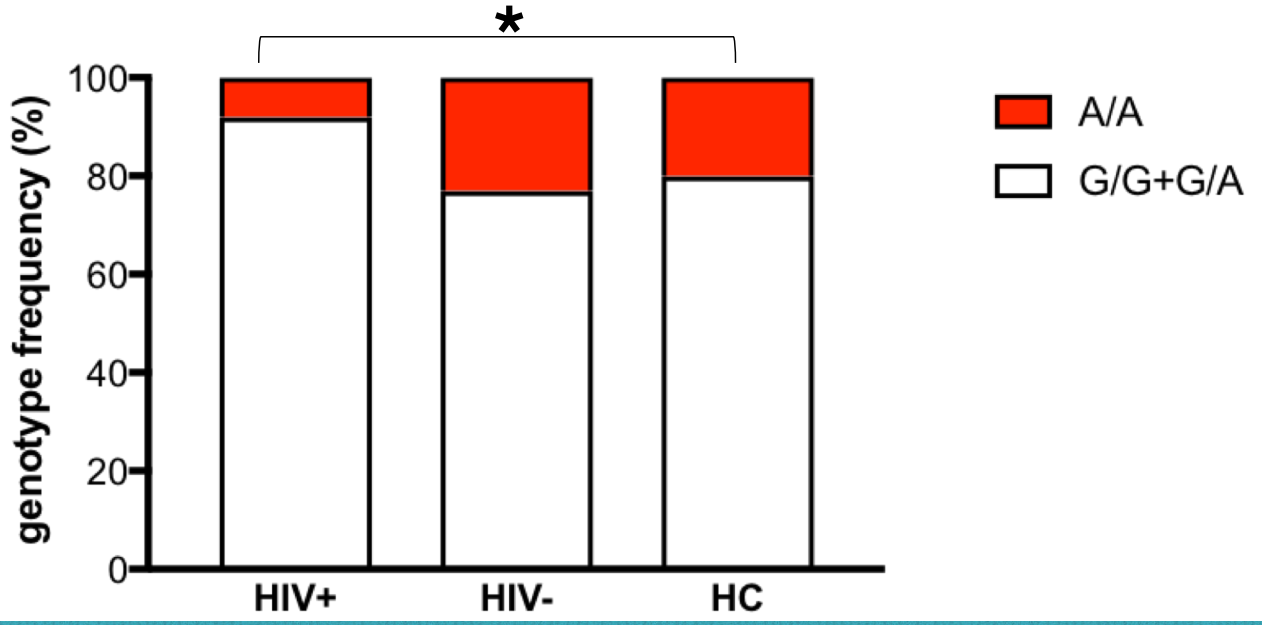
AMPs: protegem mesmo?



AIDS 2006, **20**:1669–1677

DEFB1 gene polymorphisms and increased risk of HIV-1 infection in Brazilian children

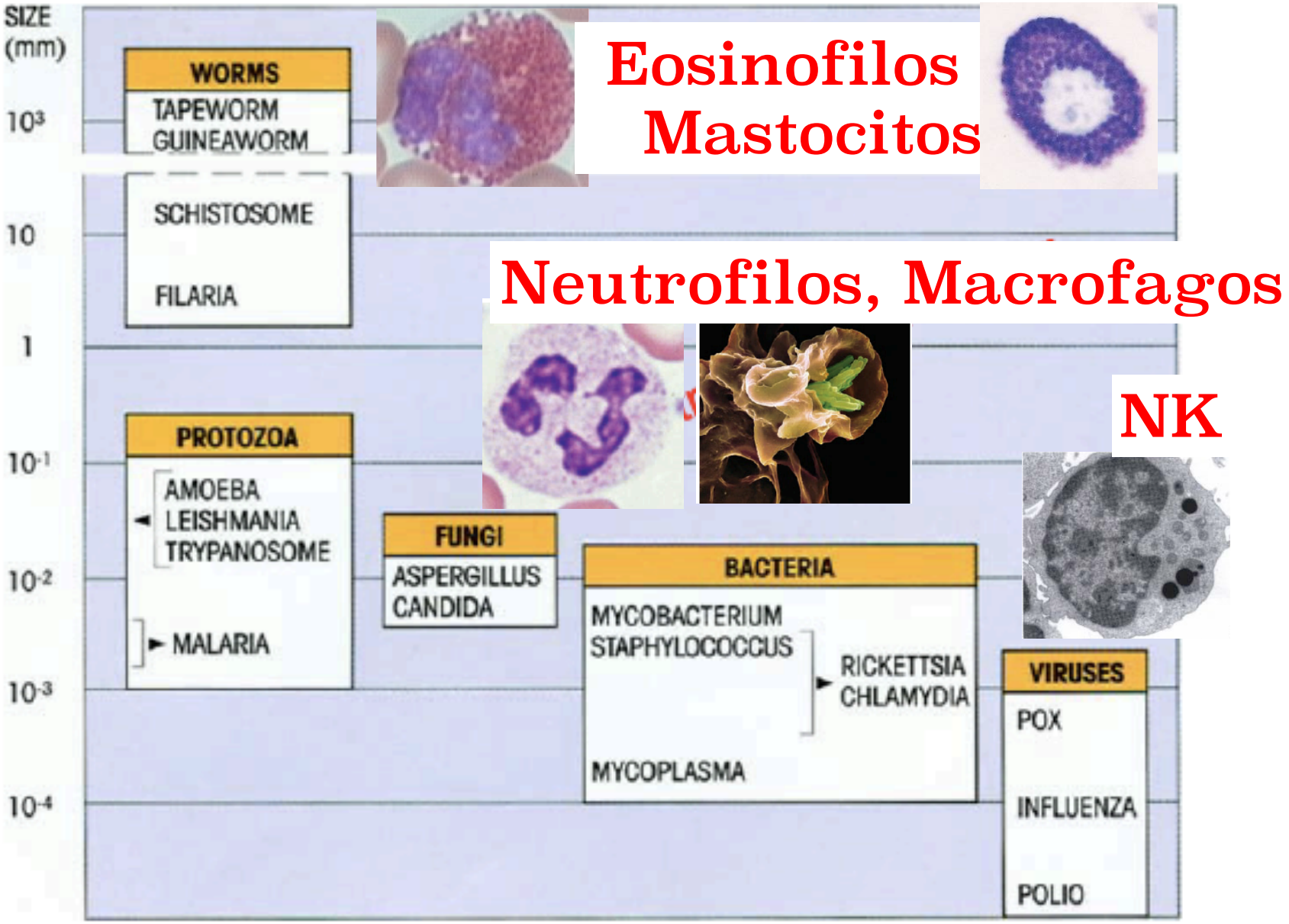
Michele Milanese[a], Ludovica Segat[a], Alessandra Pontillo[a], Luiz Claudio Arraes[b,c], José Luiz de Lima Filho[c] and Sergio Crovella[a,c]



Imunidade inata celular

- Quando as barreiras são ultrepassadas
- Fagocitos (macrofagos, neutrofilos) rapidamente internalizam e digerem microbios/material extracelulares
- Granulocitos (eosinofilos, mastocitos) liberam conteudo antimicrobiano e imunomodulador
- Celulas NK destroem celulas proprias alteradas
- Produçao de moleculas microbicidas & citocinas
- Recrutamento de leucocitos (inflamaçao)

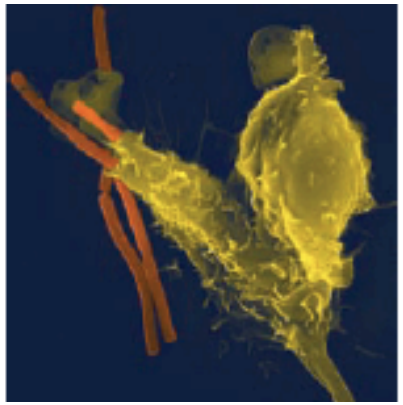
Imunidade inata celular

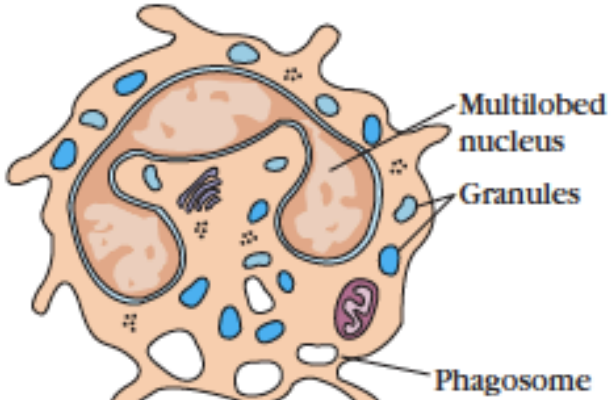


Fagocitos & fagocitose

- 2ª linha de defesa
- Macrofagos, Neutrofilos
- Fagocitose: “comer” particulas (patogenos extracelulares, celulas mortas, material) para elimina-las
- Evolutivamente antigos (Metchinkoff)

Polimorfonucleados neutrofilos

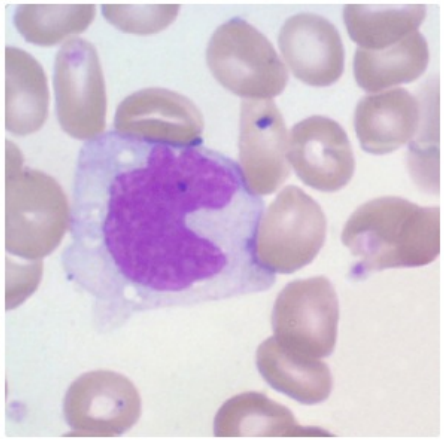


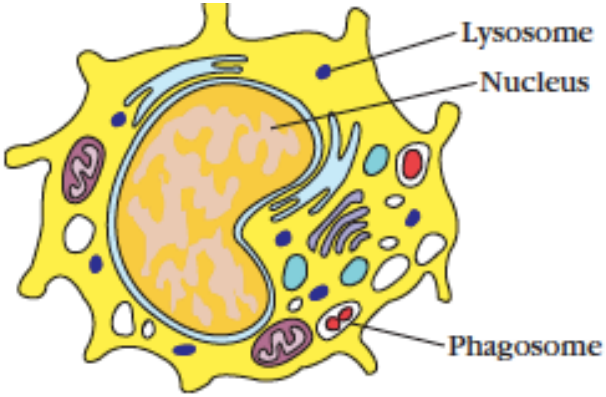


- Nucleo multilobado
- Granulos citoplasmaticos liberados após contato com patogenos
- Proteinas com funçao anti-microbiana direta, recrutamento leucocitos, remodelamento tecidual
- No sangue 7-10 horas; nos tecidos poucos dias
- 50-70% dos leucocitos circulantes; n aumenta durante infeçao
- 1 a chegar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

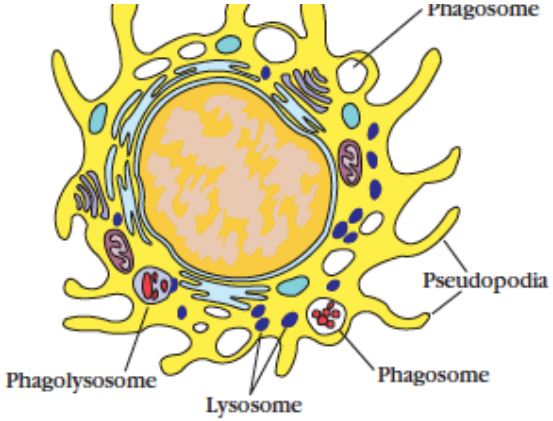
Cell type	Molecule in granule	Examples	Function
Neutrophil	Proteases	*Elastase, Collagenase*	Tissue remodeling
	Antimicrobial proteins	*Defensins, lysozyme*	Direct harm to pathogens
	Protease inhibitors	*α1-anti-trypsin*	Regulation of proteases
	Histamine		Vasodilation, inflammation

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

BACTERIA

Formil-peptideos

PAMPs

reconhecimento

“comer” particulas (patogenos extracelulares, celulas mortas, material) para elimina-las

Fagocitose

Receptor type on phagocytes	Examples	Ligands
		Microbial ligands (found on microbes)
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 direto do patogeno

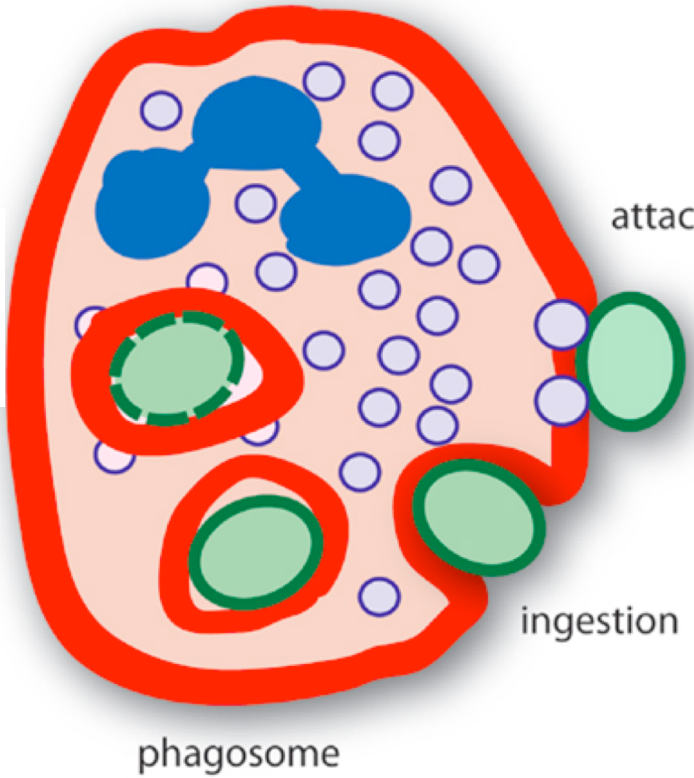
Reconhecimento indireto (atraves moleculas soluveis que ligam o patogeno/OPSONINAS)

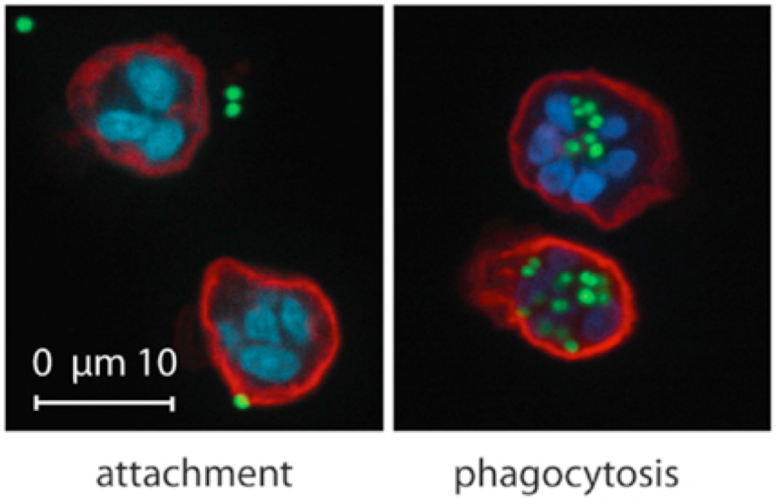
Collagen-domain receptor	CD91/calreticulin	Collectins SP-A, SP-D, MBL; L-ficolin; C1q
Complement receptors	CR1, CR3, CR4, CRIg, C1qRp	Complement components and fragments*
Immunoglobulin Fc receptors	FcαR	Specific IgA antibodies bound to antigen#
	FcγRs	Specific IgG antibodies bound to antigen;#
		C-reactive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

→ ***Produçao de mediadores***

- ***Recrutar e ativar leucocitos***
- ***Inflamaçao***

Fagocitose





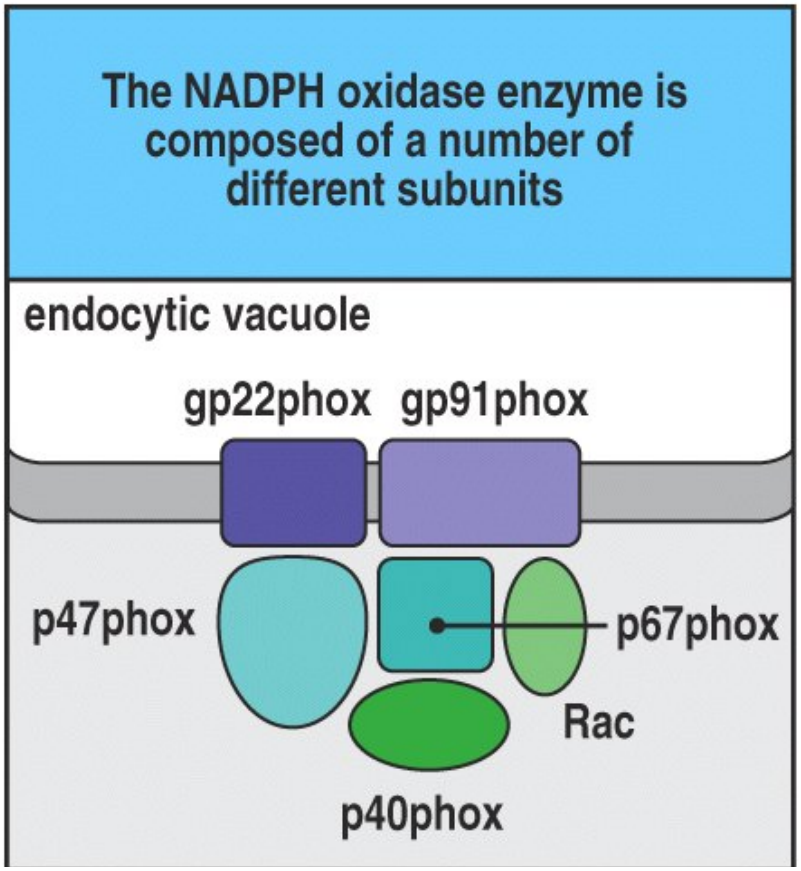
Fagolisossoma: killing/digestao

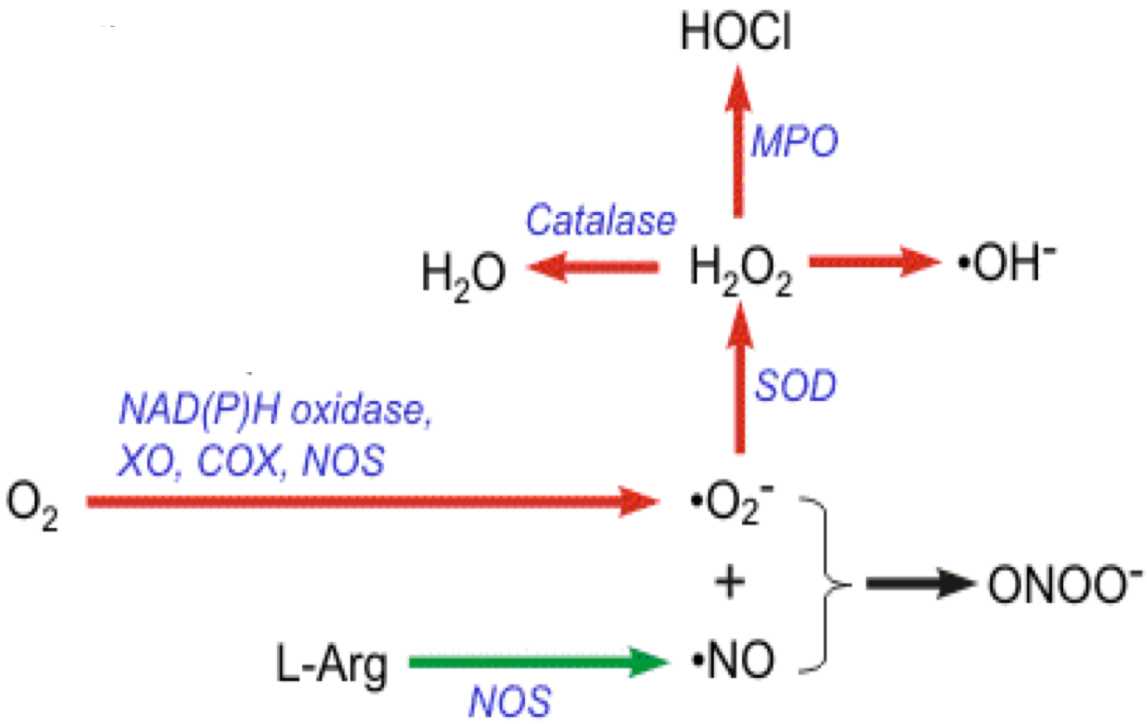
Agentes anti-microbianos

- 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)



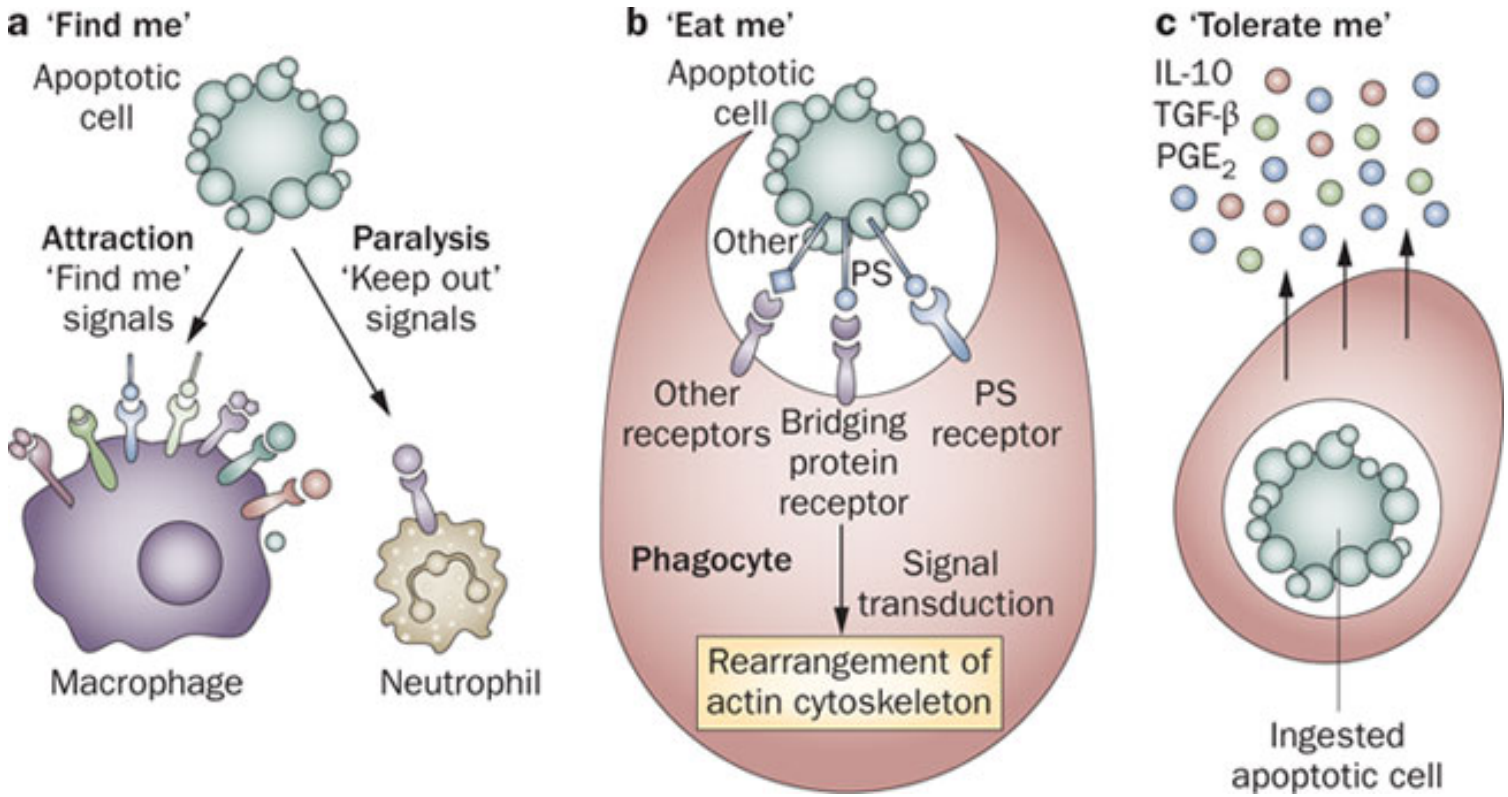


Mutaçoes nos genes da NADPH oxidase causam a DOENCA CRONICA GRANULOMATOSA

Fagocitose “fisiologica”

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

Silence please



https://youtu.be/rsHyQ6A6PcI

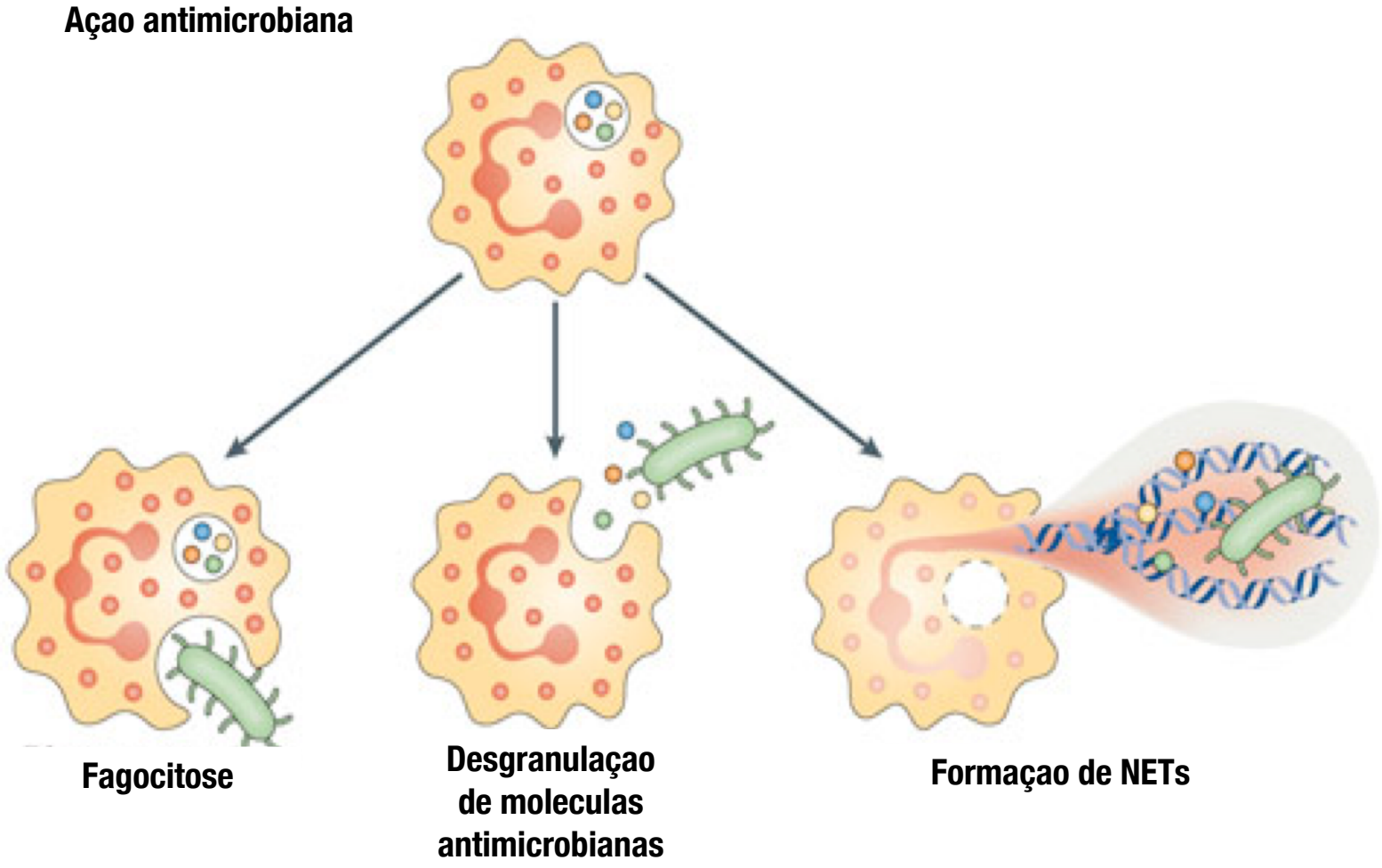
https://youtu.be/Z_mXDvZQ6dU

Evoluçao

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

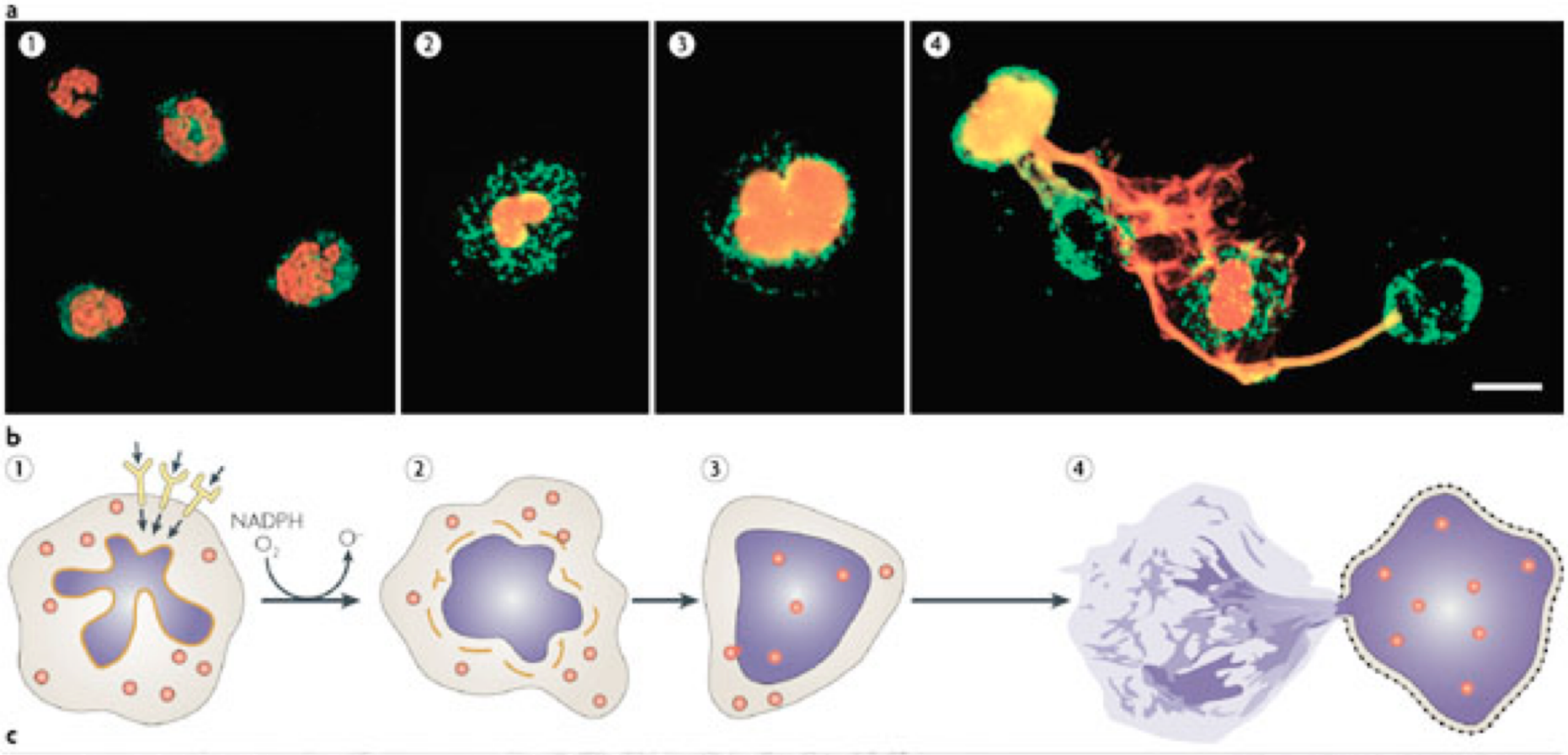
Outras acoes microbicidas

- Neutrofilos - NETs
- Neutrofilos - DESGRANULACAO

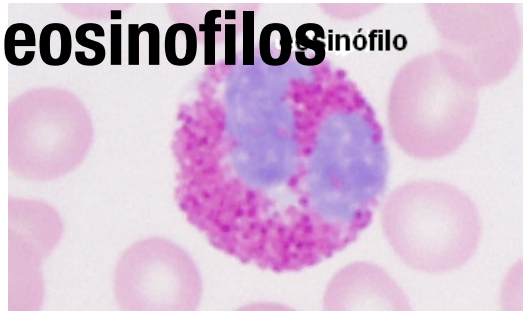


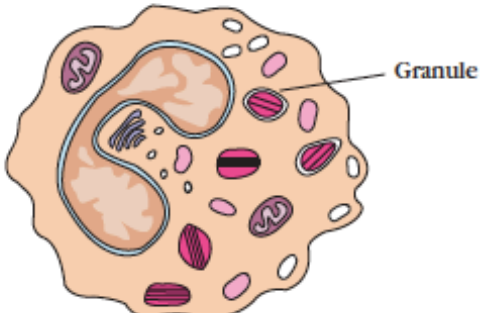
Formaçao de NETs

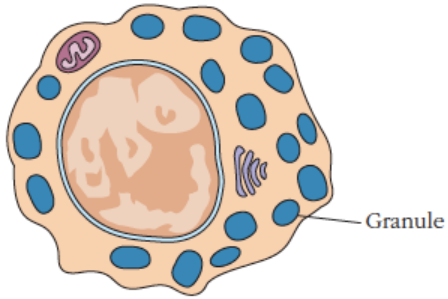
- Funçao de eliminaçao de patogeno recentemente descrita (2004).
- Os neutrofilos liberam conteudo nuclear e granular formando uma rede que intrappola os patogenos e facilita a açao das moleculas antimicrobianas para distruiçao dos patogenos (killing extracelular).



Polimorfonucleados/Granulocitos





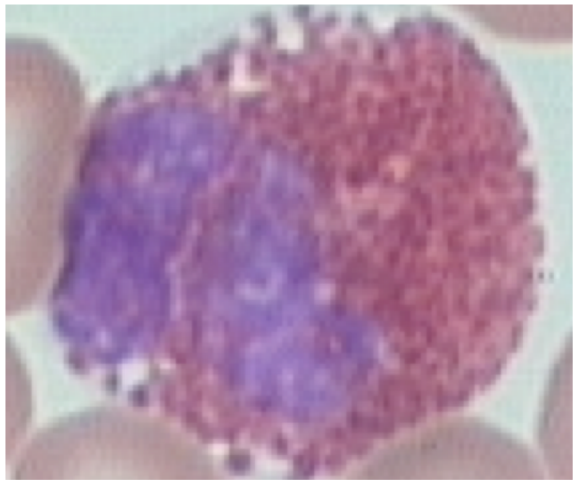


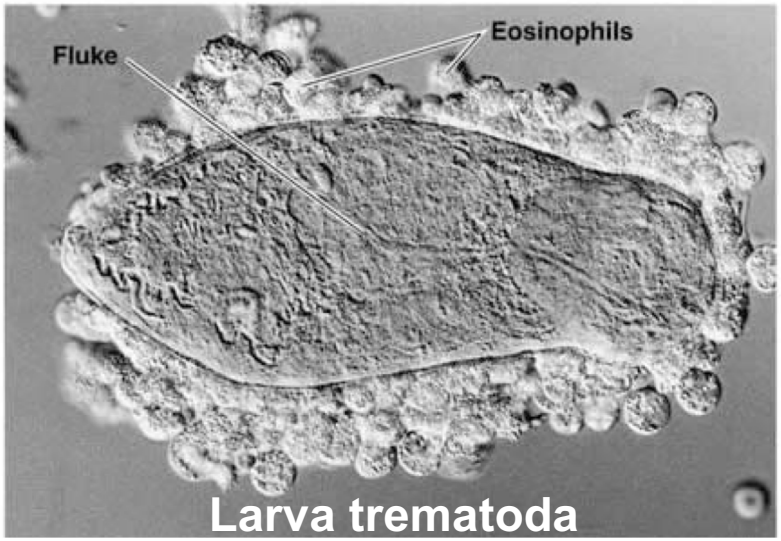
- Nucleo multilobado
- Granulos citoplasmaticos liberados após contato com patogenos/dano
- Proteinas com funçao anti-microbiana direta, recrutamento leucocitos, vasodilataçao
- Eosinofilos no sangue; mastocitos nos tecidos (vasos)

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

Eosinofilos

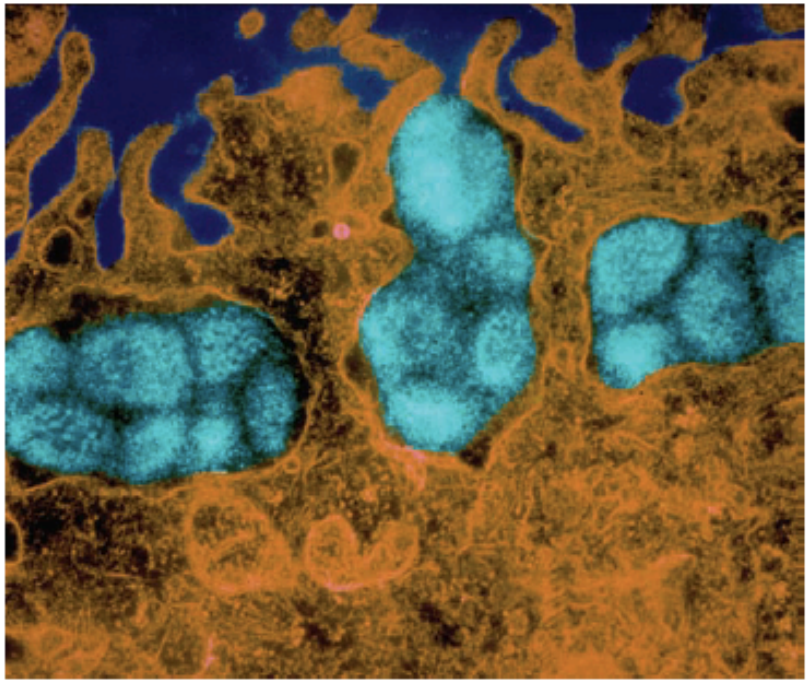
- Circulante (12 hs)/teciduais (8-12 d)
- Importantes na defesa contra protozoarios
- Elevado poder microbicida dos granulos
- Rec para IgE responsavel para alergias

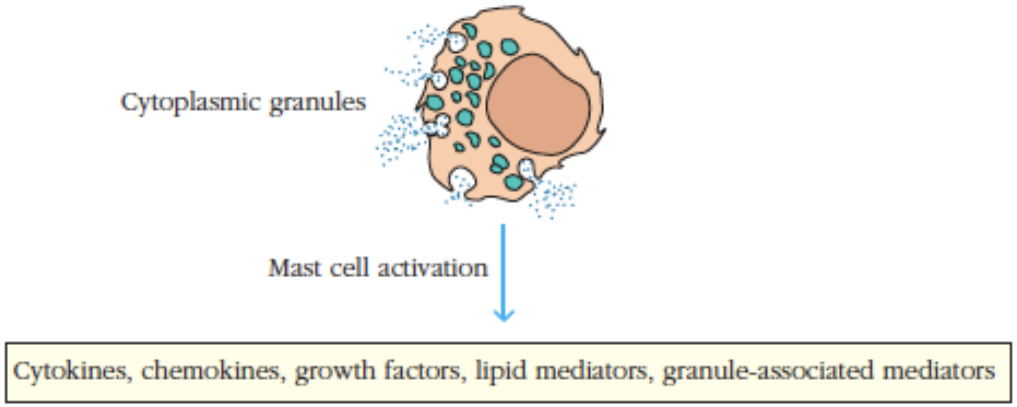




- Açao anti-microbiana/ induz ROS (proteinas cationicas, EPO)
- Modulaçao da resposta adaptativa (IL-4, IL10, TNF)
- Recrutamento leucocitos (RANTES, MIP1a)
- Vasodilataçao (ribonuclease, MBP, leucotrieno B4)

Mastocitos

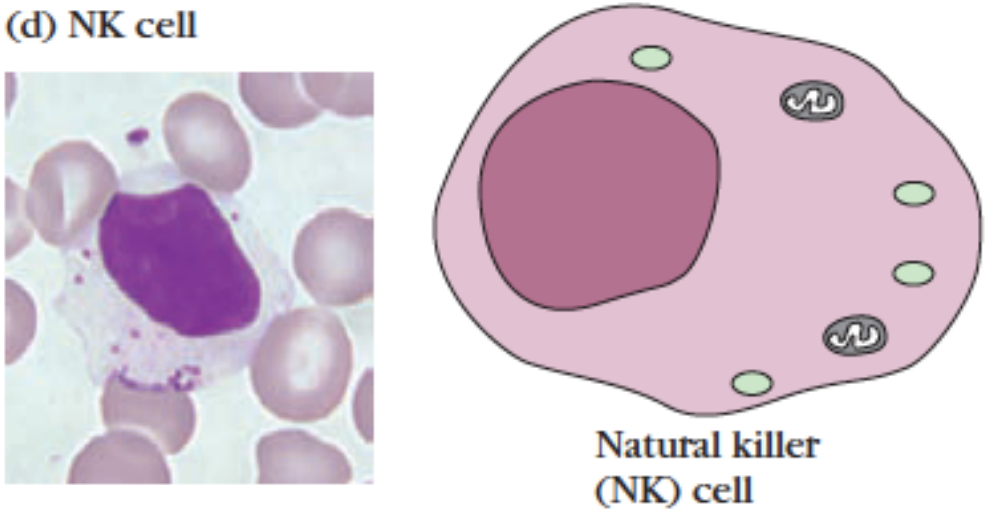




- Teciduais (pele, mucosa)
- Proximos aos vasos
- Granulos citoplasmaticos abundantes (citocinas, aminas vasoativas e outros mediadores)

- Regulaçao da permeabilidade vascular (histamina)
- Regulaçao da inflamaçao (leucotrienos)
- Modulaçao da resposta adaptativa (IL-4)
- Açao anti-microbiana
- Rec para IgE responsavel para alergias

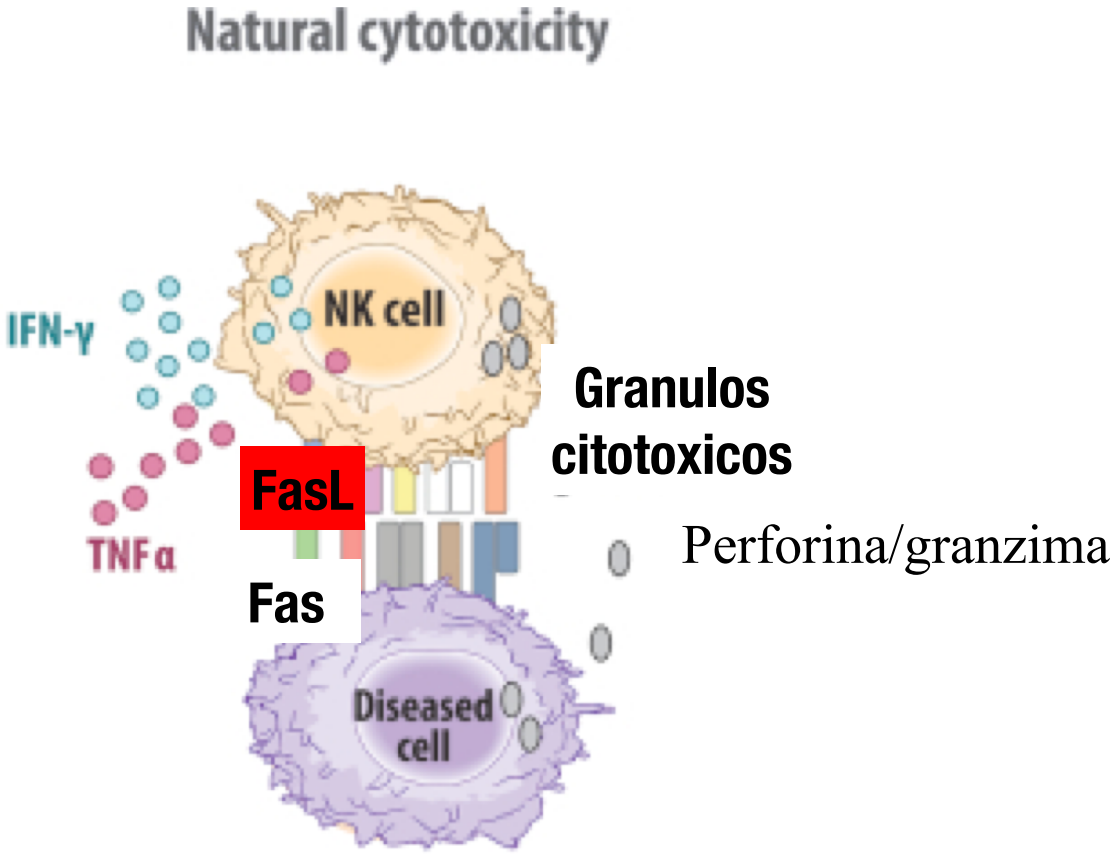
Celulas NK

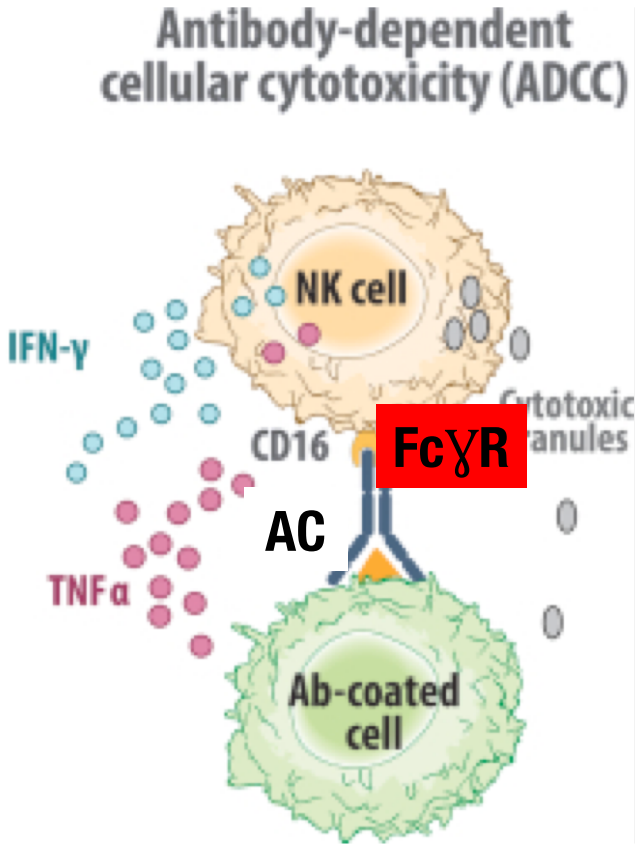


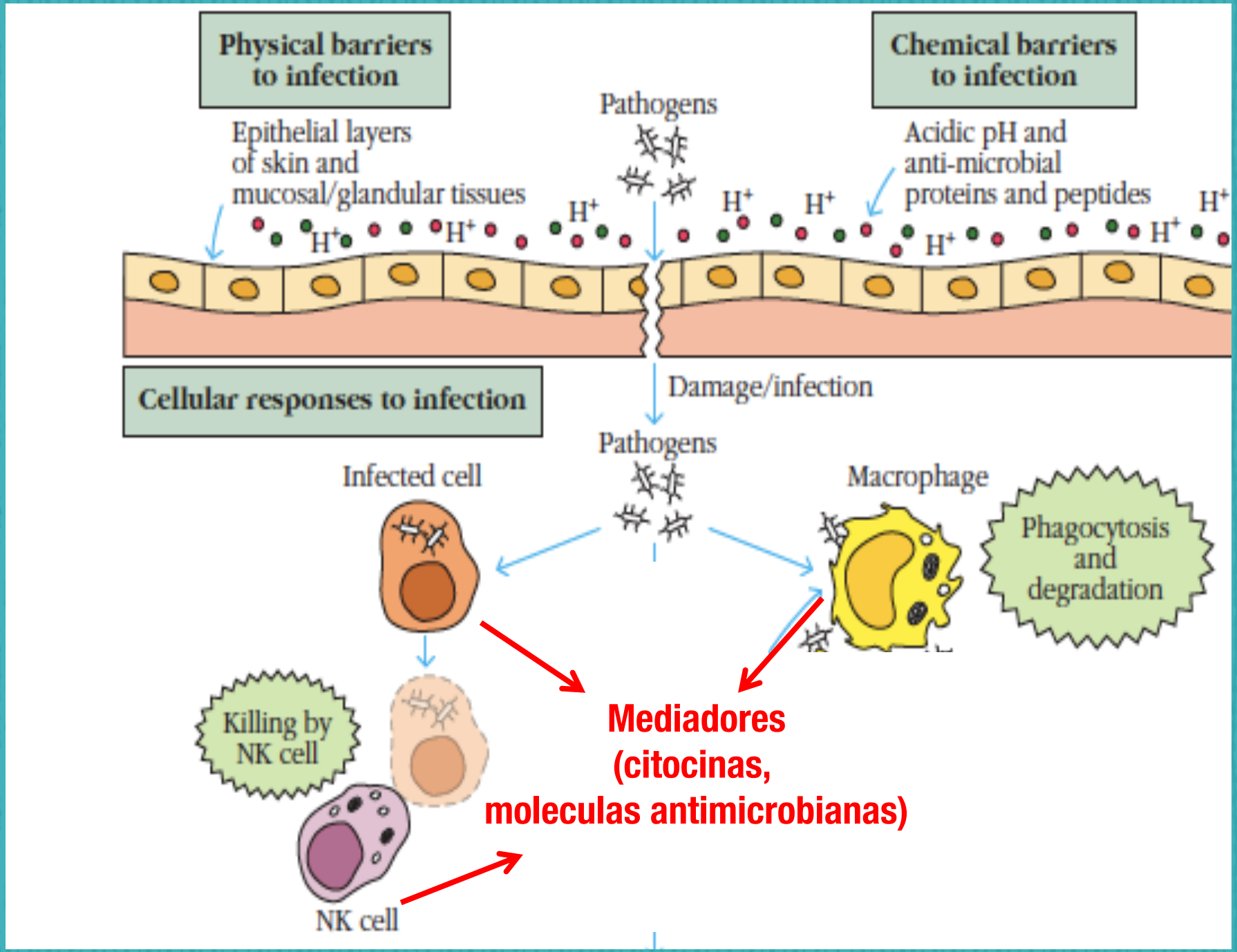
- Progenitor linfoide – linfocitos grandes e granulados
- Circulantes (5-10% dos linfocitos) (+ baço, figado)
- Granulos contendo proteinas citotoxicas (perforina, granzima)
- Secreçao de citocinas (IFN-γ, TNF)
- Eliminar celulas “anormais” (infetadas/alteradas) induzindo apoptose
- Importantes na defesa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

Celulas NK

- Atividade citotoxica contra celulas alteradas (**granulos citotoxicos, FasL/Fas**)
- Ativar Mø para potenciar a resposta microbicida (TNF, IFN-Ɣ)
- Atividade citotoxica induzida por anticorpos/ADCC






Physical barriers to infection
Chemical barriers to infection
Pathogens
Epithelial layers of skin and mucosal/glandular tissues
Acidic pH and anti-microbial proteins and peptides
H+
Cellular responses to infection
Damage/infection
Pathogens
Infected cell
Macrophage
Phagocytosis and degradation
Killing by NK cell
NK cell
Mediadores (citocinas, moleculas antimicrobianas)