Imunologia de Mucosa

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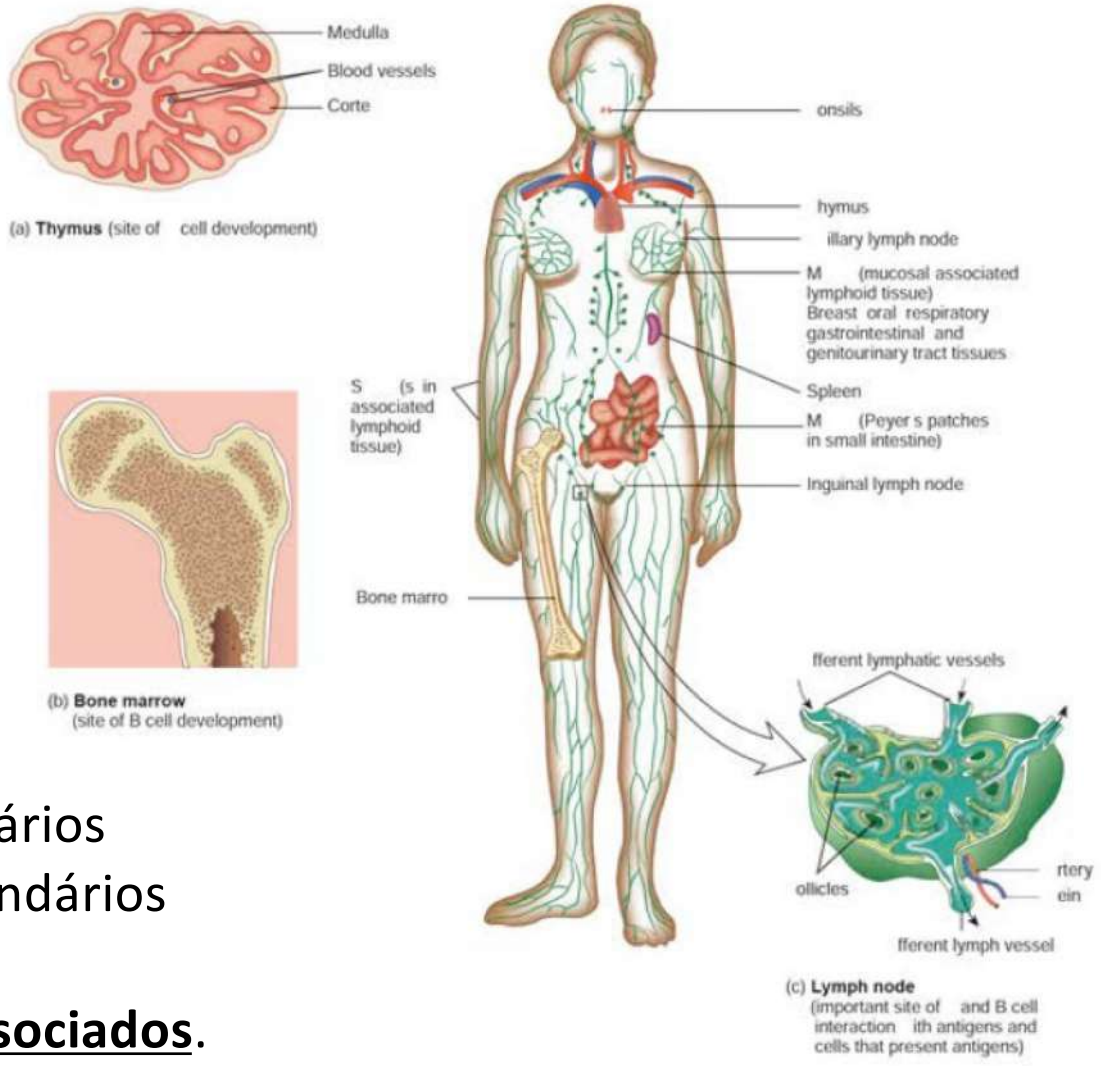
Tópicos da aula

- Componentes do sistema imune de mucosa: barreira física e barreira ativa
- Respostas canônicas: Tolerância Oral, Imunidade a vacinas, Th17/22, IgA, Linfócitos Inatos
- Particularidades de tecidos de barreira
- Interação com microbiota

Como está organizado o sistema imunológico pelo corpo ?



- Órgãos linfoides Primários
- Órgãos linfoides Secundários
- Circulação
- **Barreiras e órgãos associados**.

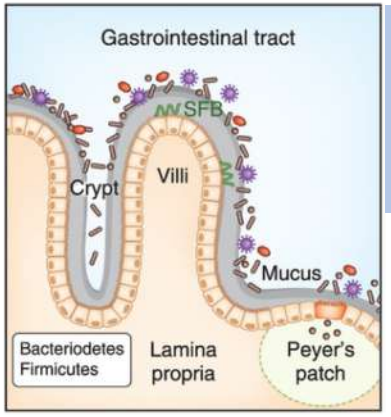
Figure 13.2: The distribution of Lymphoid tissues in the body

Porque estudar o Sistema Imune de mucosa?

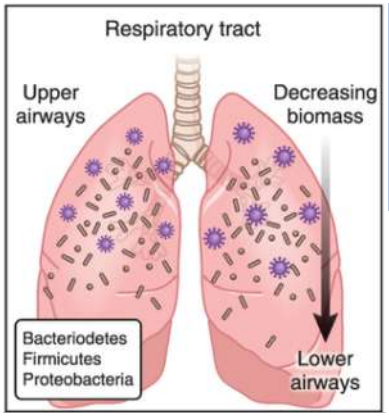
Números de Linfócitos em Diferentes Tecidos

Baço	70×10^9
Linfonodos	190×10^9
Medula óssea	50×10^9
Sangue	10×10^9
Pele	20×10^9
Intestinos	50×10^9
Fígado	10×10^9
Pulmões	30×10^9

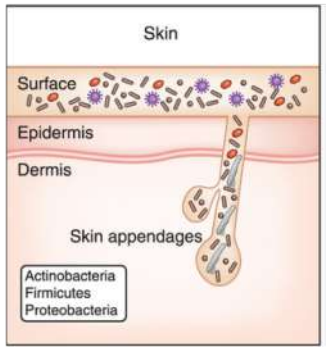
Porque estudar o Sistema Imune de mucosa/barreira?



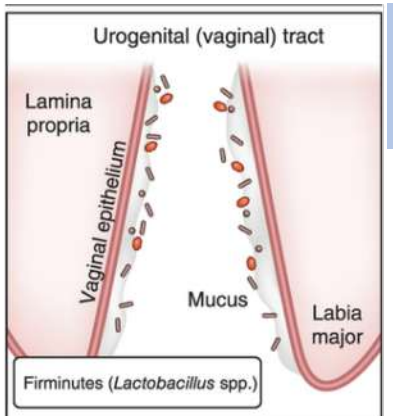
- Absorção de nutrientes
- Tolerância
- Superfície (200m^2)



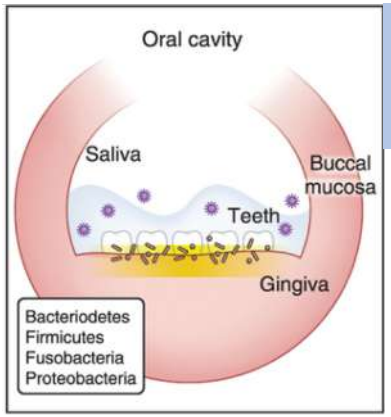
- Respiração
- Exposição a antígenos ambientais



- Superfície
- Exposição ambiental

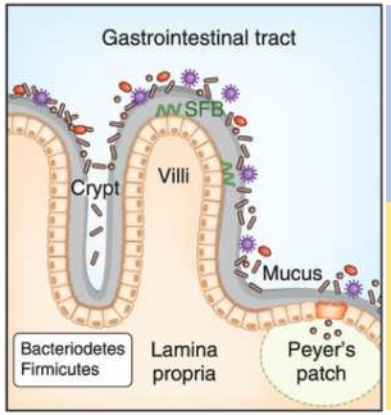


- Reprodução
- Exposição ambiental



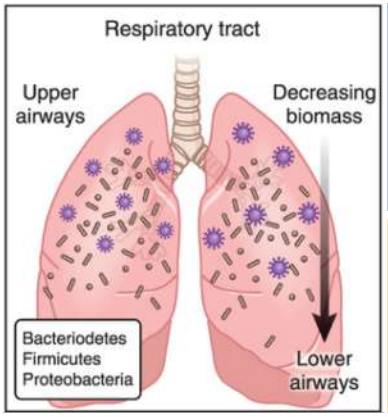
- Digestão
- Exposição ambiental

Porque estudar o Sistema Imune de mucosa/barreira?



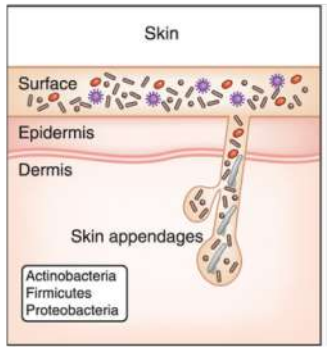
- Absorção de nutrientes
- Tolerância
- Superfície (200m^2)

- Tonsilas, linfonodos dren.
- Placas de Peyer
- Lâmina própria, GALT



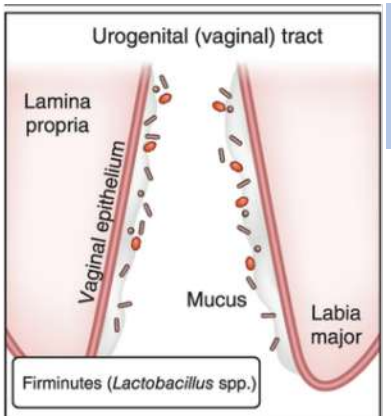
- Respiração
- Exposição a antígenos ambientais

- Tonsilas
- Adenóides
- Folículos linfóides associados

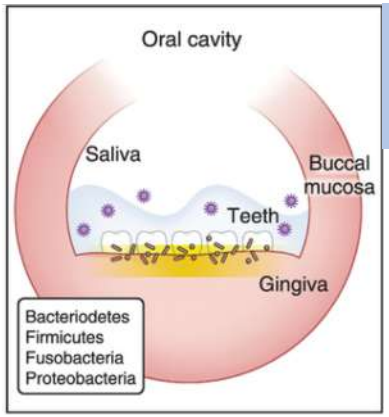


- Superfície
- Exposição ambiental

- Epitélio estratificado queratinizado
- Aglomerados celulares



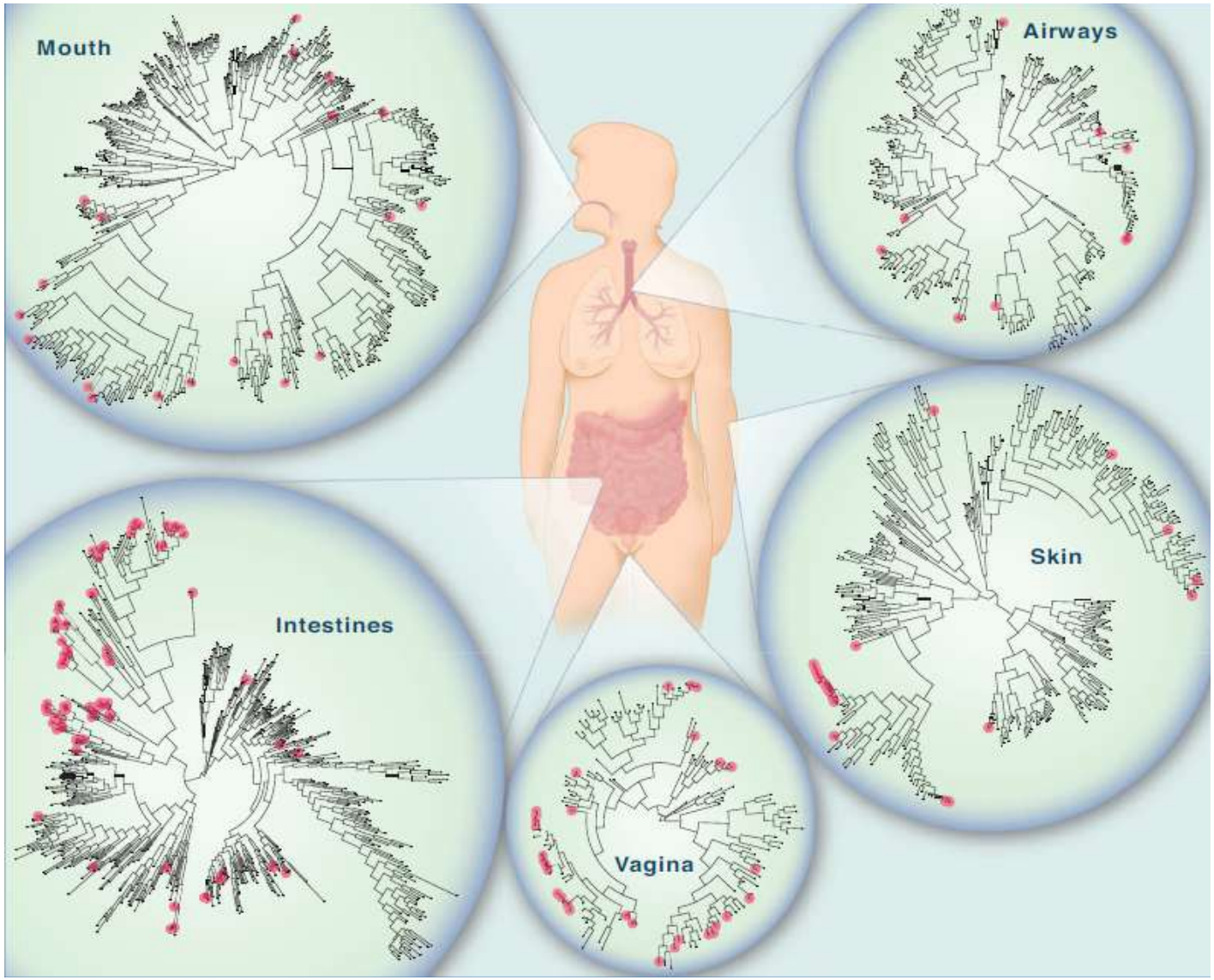
- Reprodução
- Exposição ambiental



- Digestão
- Exposição ambiental

GALT: Gut-associated lymphoid tissue
BALT: Bronchial-associated lymphoid tissue
NALT:-Nasal-associated lymphoid tissue

‘Meta’organismo humano



Fisiologia

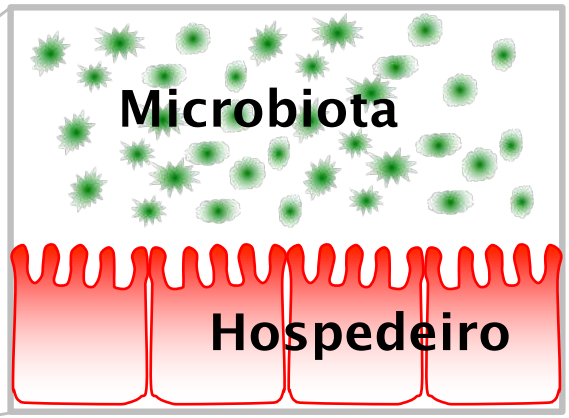
- Absorção de Nutrientes
- Síntese de Vitaminas
- Metabolismo de bile e hormônios
- Fermentação de caboidratos

- Comportamento e Cognição

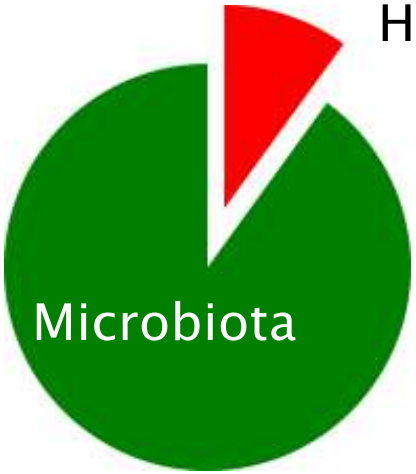
- **Sistema Imunológico**

doi: 10.1126/science.1195568

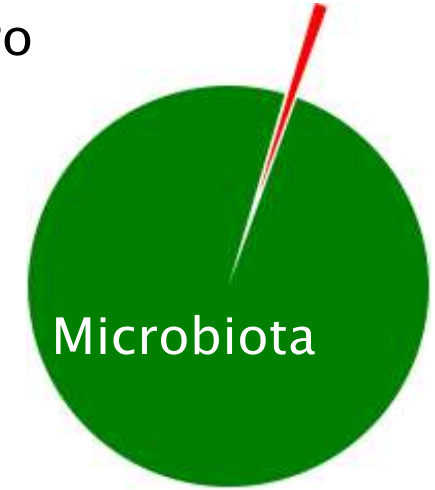
‘Meta’organismo humano



Células no seu corpo

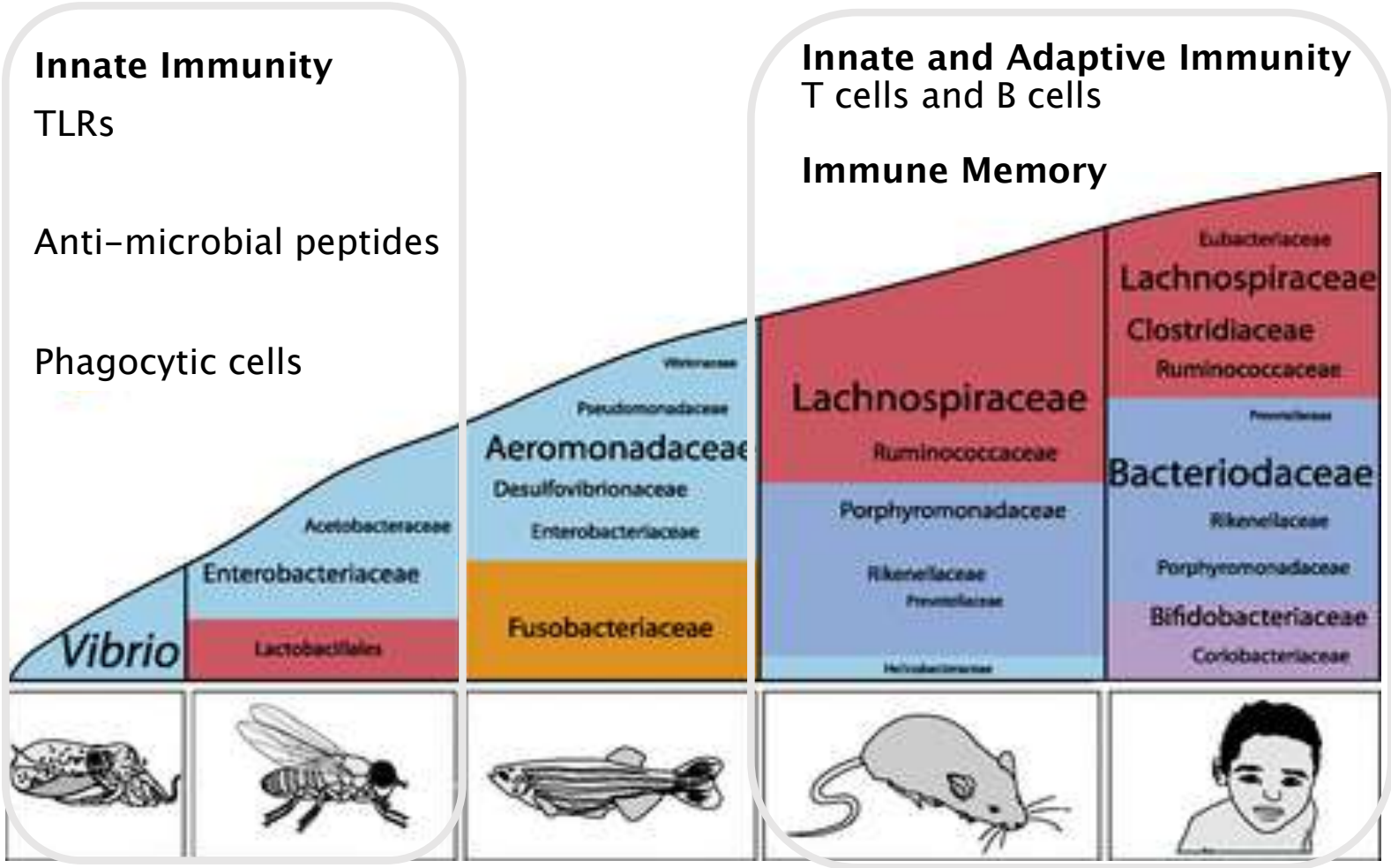


Genes no seu corpo



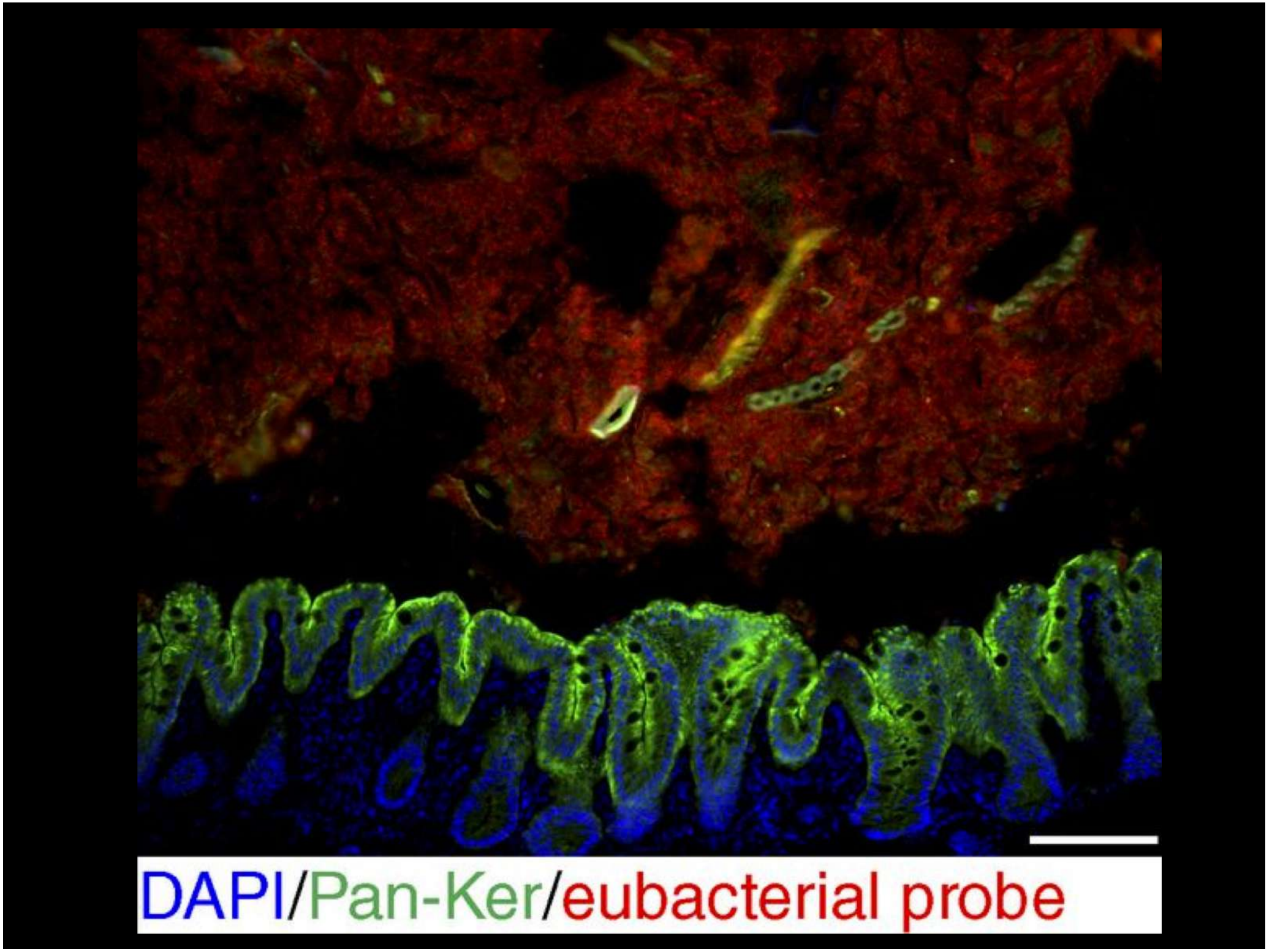
Gordon, Knight, Relman, Wang

Complexidade da microbiota aumentou à medida em que o sistema imune evoluiu

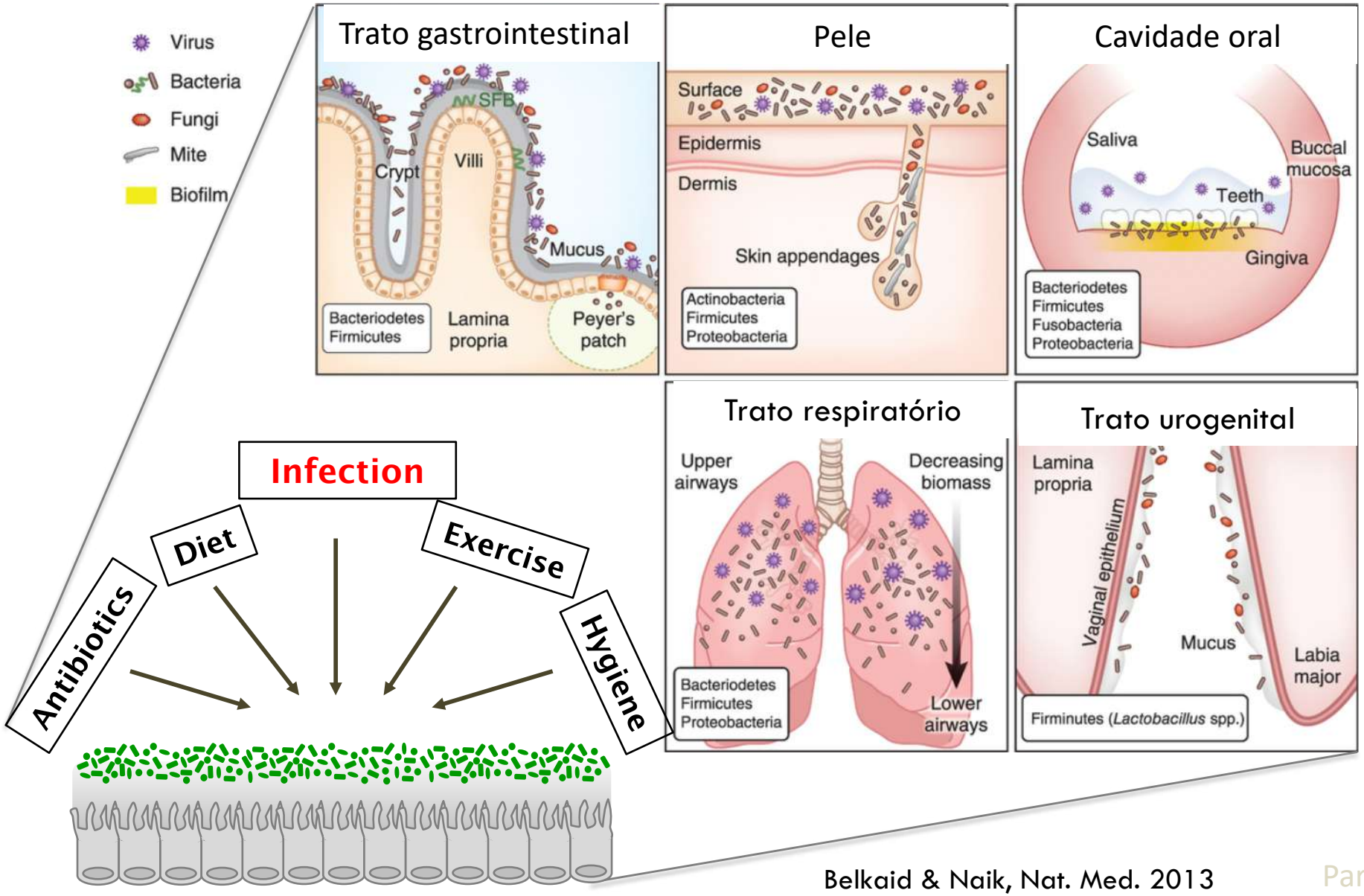


~6 OTUs ~1000 OTUs

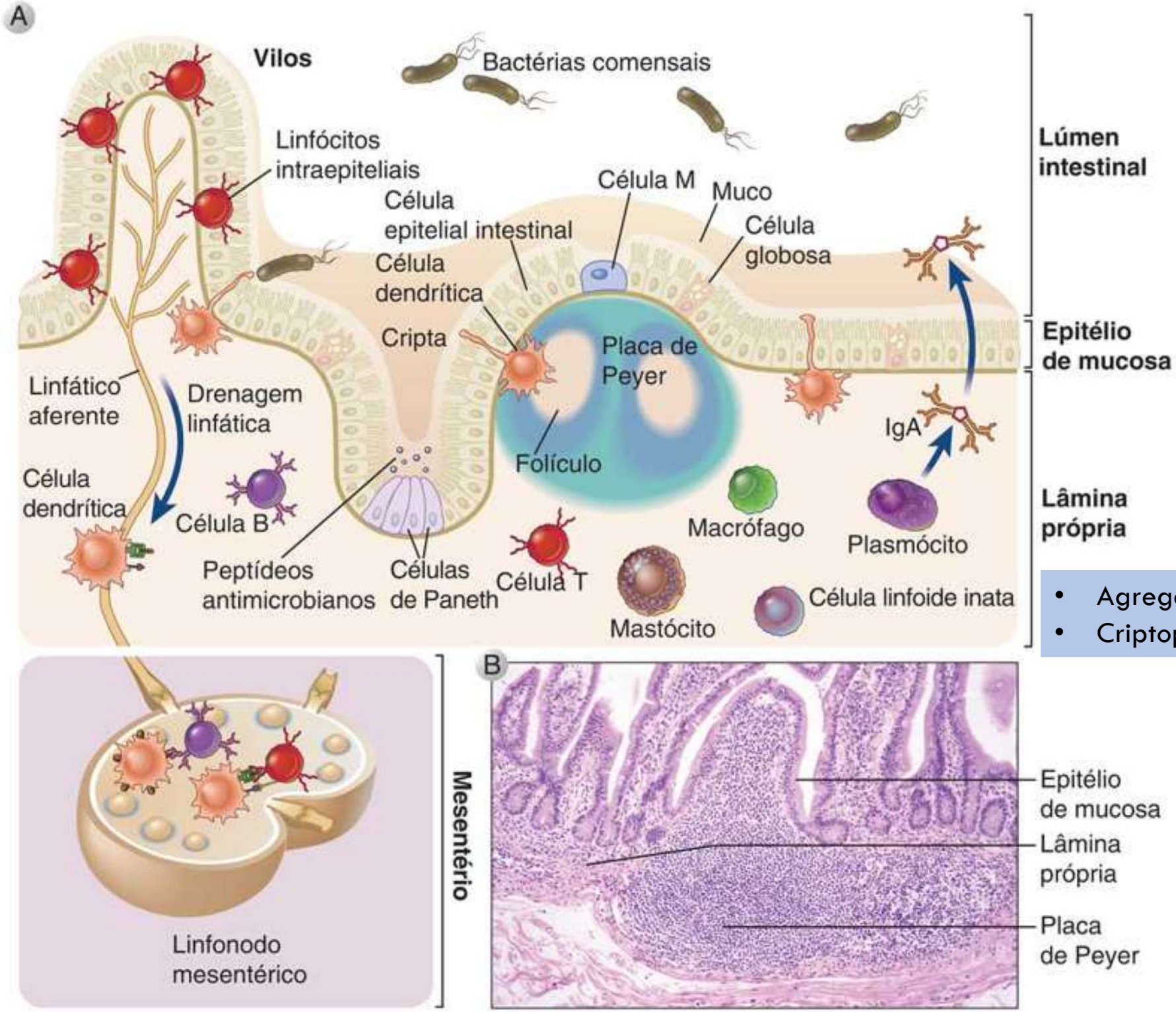
Modified from Kostic, Howitt and Garrett *Genes Dev.* 2012 and McFall-Ngai *Nature* 2007


DAPI/Pan-Ker/eubacterial probe

Tecidos de barreira



Belkaid & Naik, Nat. Med. 2013



- Agregados linfóides
- Criptoplacas

BALT: Bronchial-associated lymphoid tissue

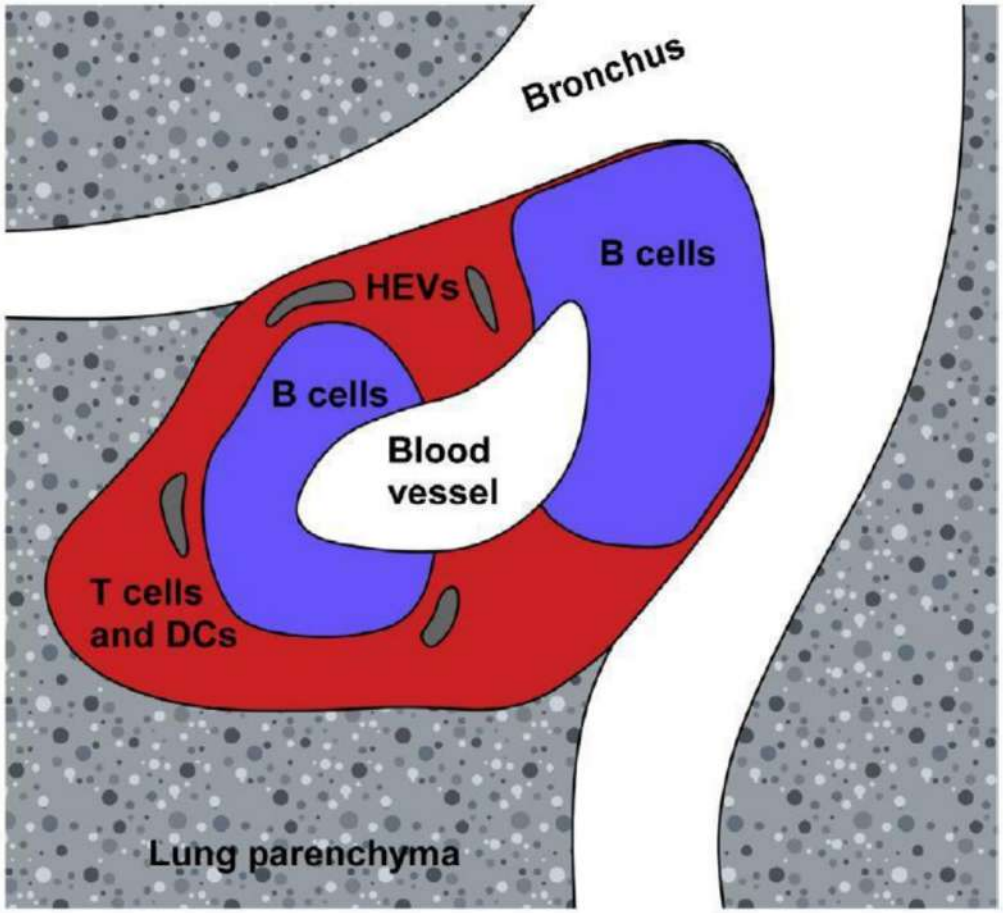
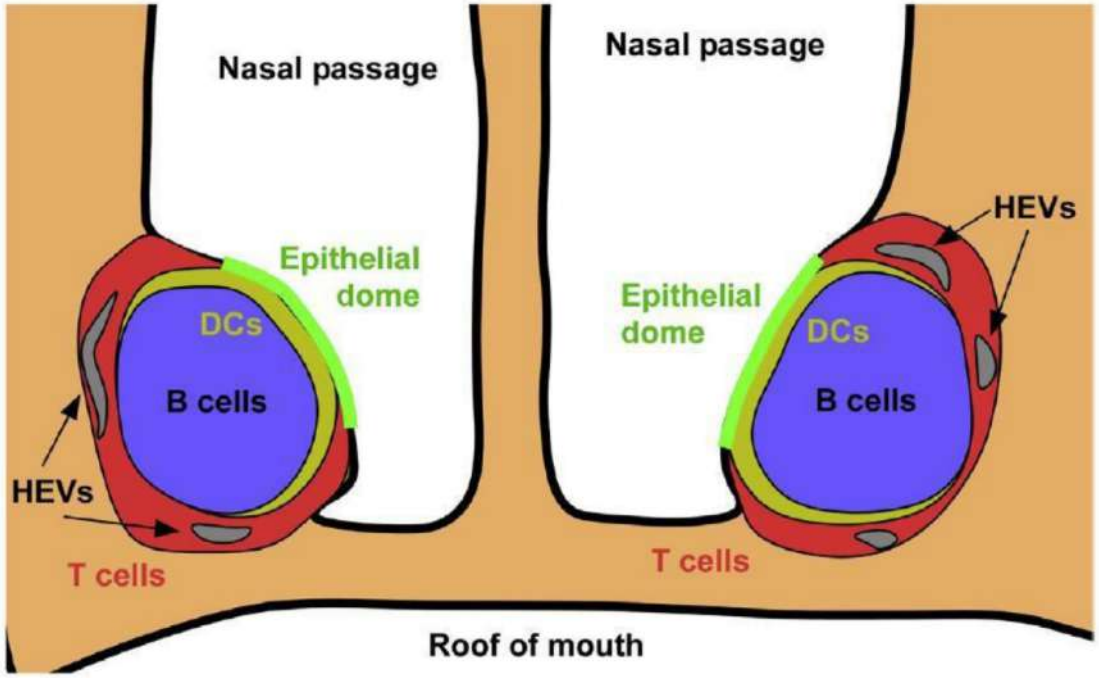


FIGURE 2 Structure of BALT. Although the structure of BALT varies widely, it is often observed along the major airways in the lung and typically fills the perivascular space surrounding small pulmonary arteries. B cell follicles (blue) are observed underneath the bronchial epithelium, but also away from the epithelium as well. T cell areas (red) surround the B cell follicles and may fill in the space between follicles in larger areas of BALT. HEVs (gray) are observed surrounding the B cell follicles in the T cell zone. (See color plate section.)

NALT:-Nasal-associated lymphoid tissue

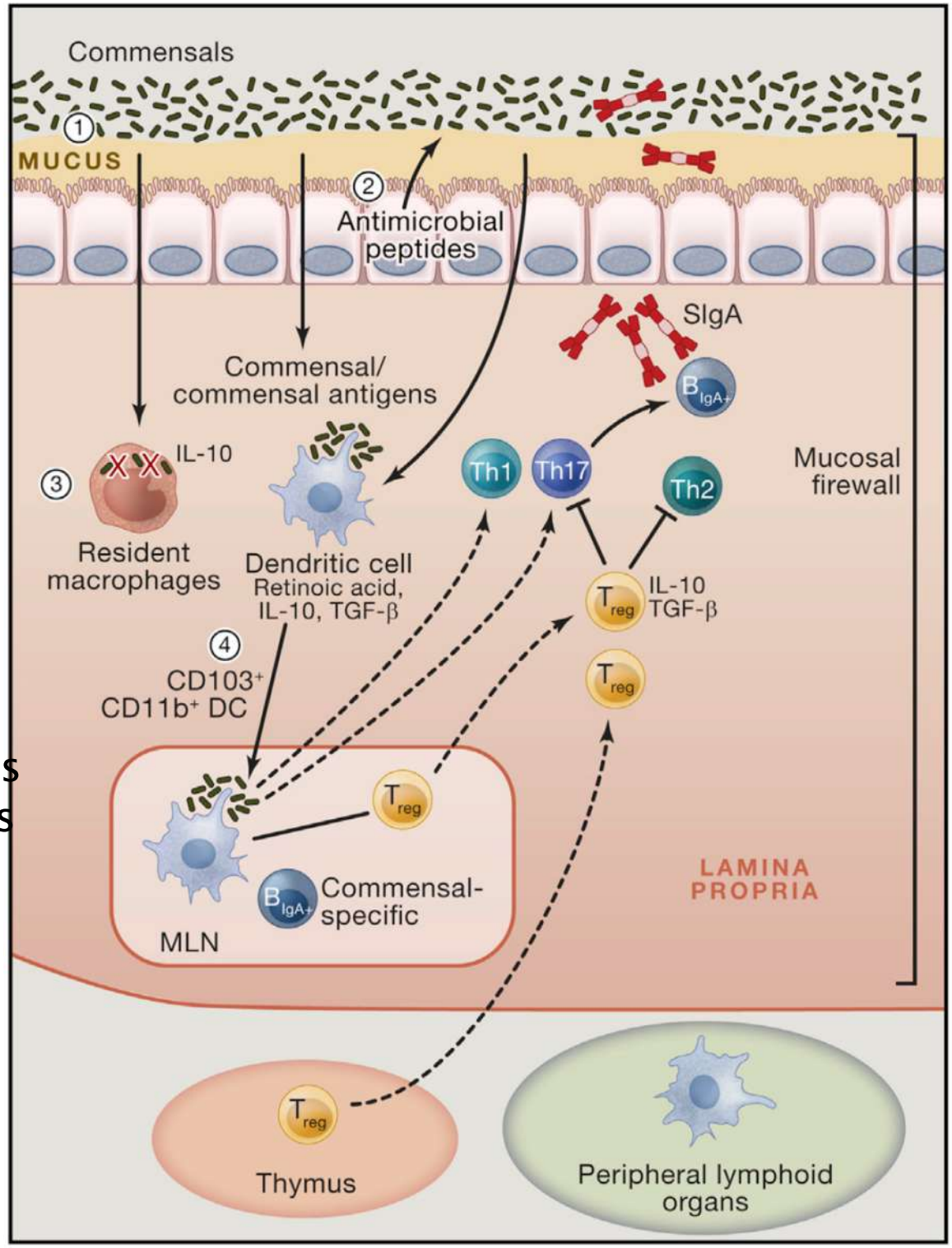
FIGURE 1 Cross-section of murine NALT. NALT is seen in cross-section as a single B cell follicle, but consists of a series of B cell follicles that run lengthwise along the nasal passages. The B cell follicle (blue) is situated underneath a dome epithelium (green) that is underlined with a thin layer of DCs (yellow) that are poised to receive antigens transported across the epithelium. The T cell area (red) surrounds the B cell follicle and may also be between B cell follicles. HEVs (gray) are found in the T cell zone and at the border of the T and B cell areas. (See color plate section.)



Componentes

Barreira Física
- Epitélio/ Junções celulares
- Muco
- Peptídeos antimicrobianos
- *Epitélio Ciliado
- *pH

Barreira ativa
- Células dendríticas especializadas
- Células T efetoras especializadas (Th17 e Treg)
- IgA
- Células Inatas (macrófagos e ILCs)
- Microbiota

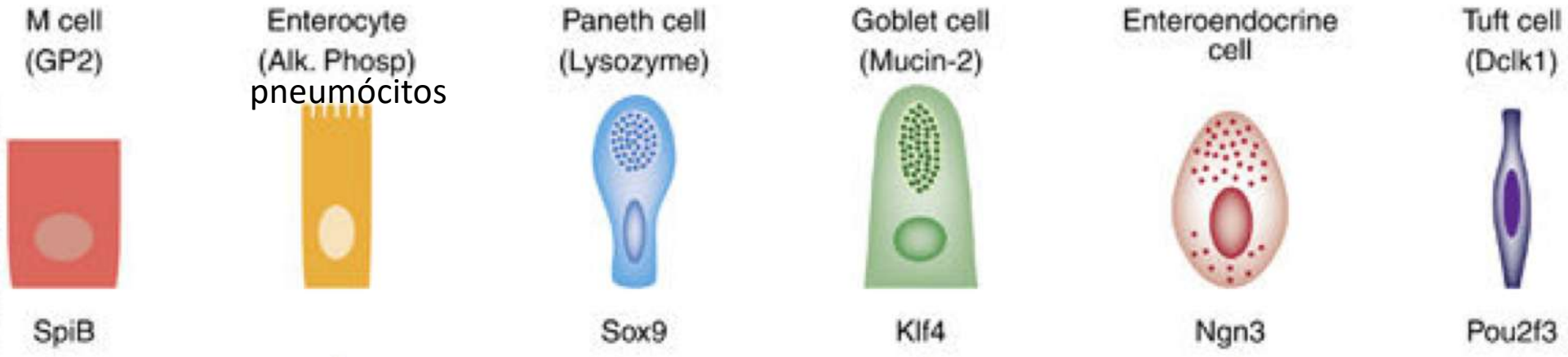


Cell 157, March 27, 2014

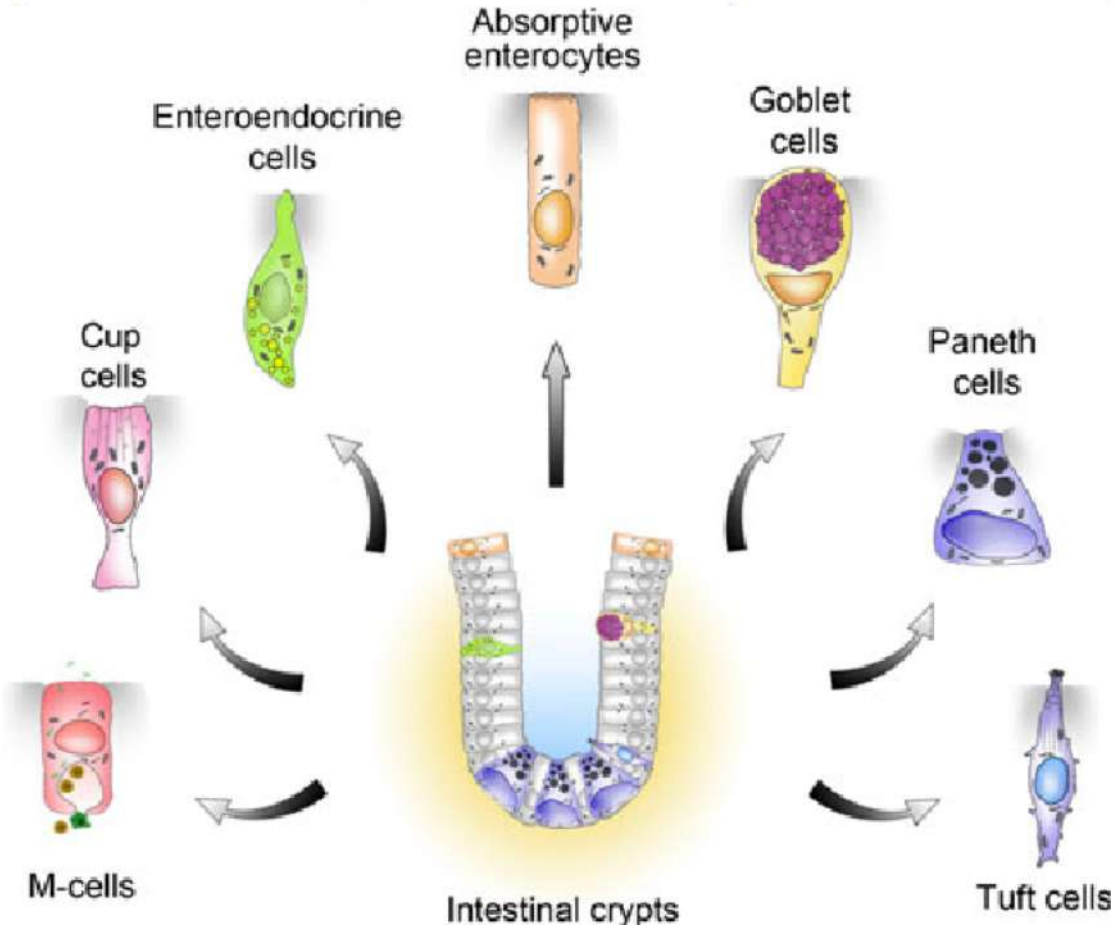
Tópicos da aula

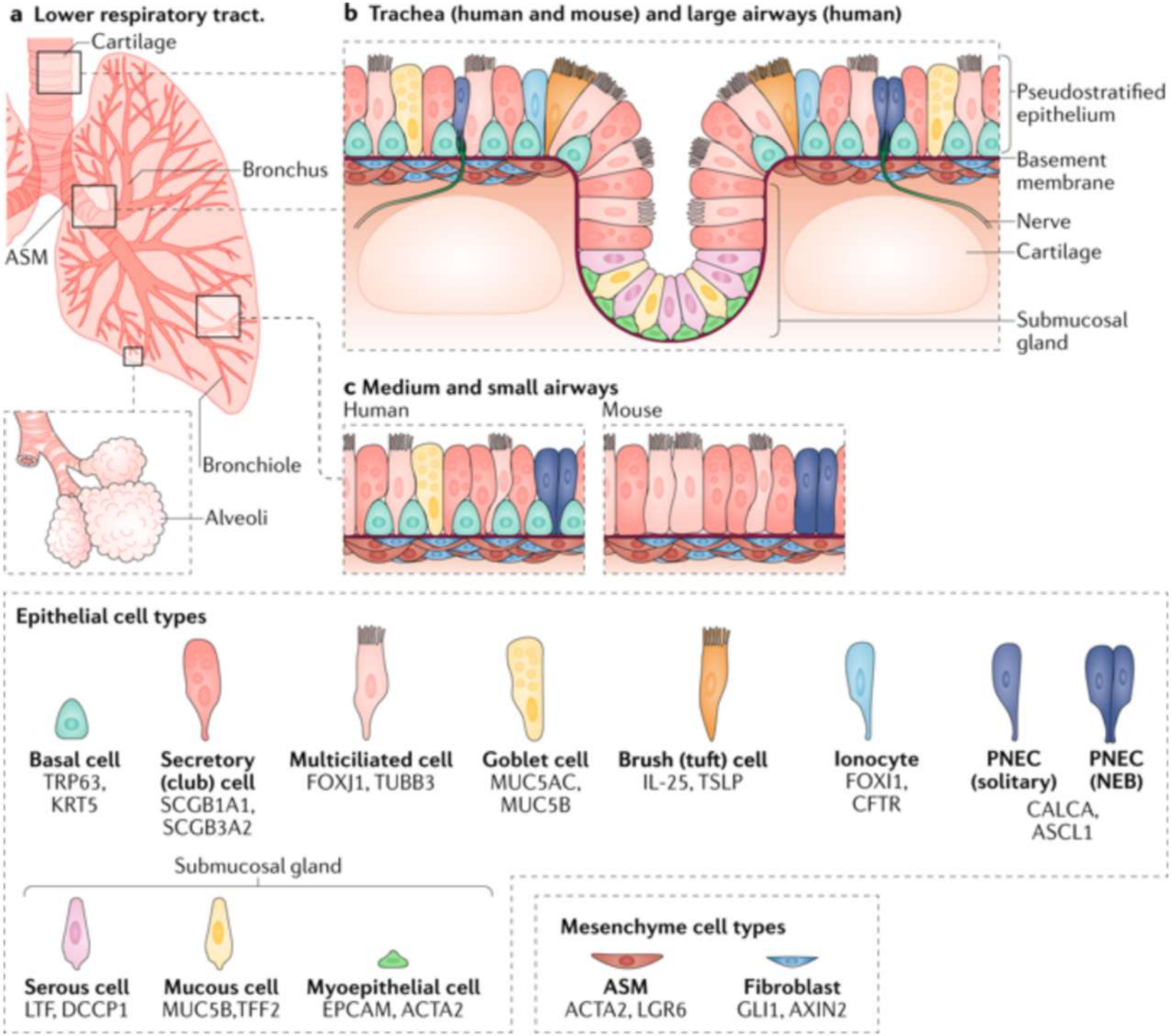
- Conceitos e Componentes: barreira física
- Componentes: barreira ativa
- Respostas canônicas: Tolerância Oral, Imunidade a vacinas, Th17/22, IgA, Linfócitos Inatos
- Particularidades de tecidos de barreira
- Interação com microbiota e doença

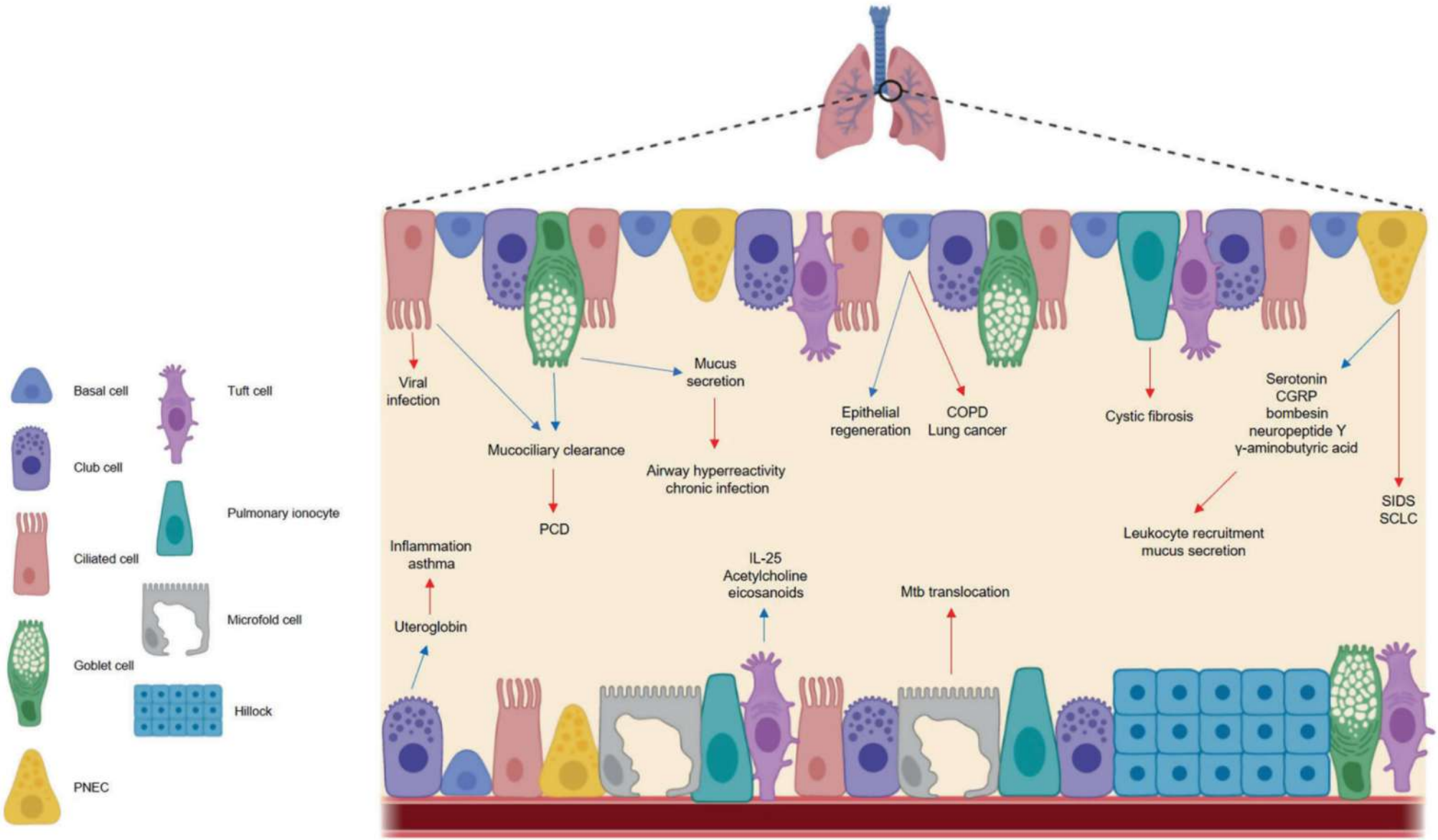
Epitélio: diferentes células com funções especializadas



pneumócitos



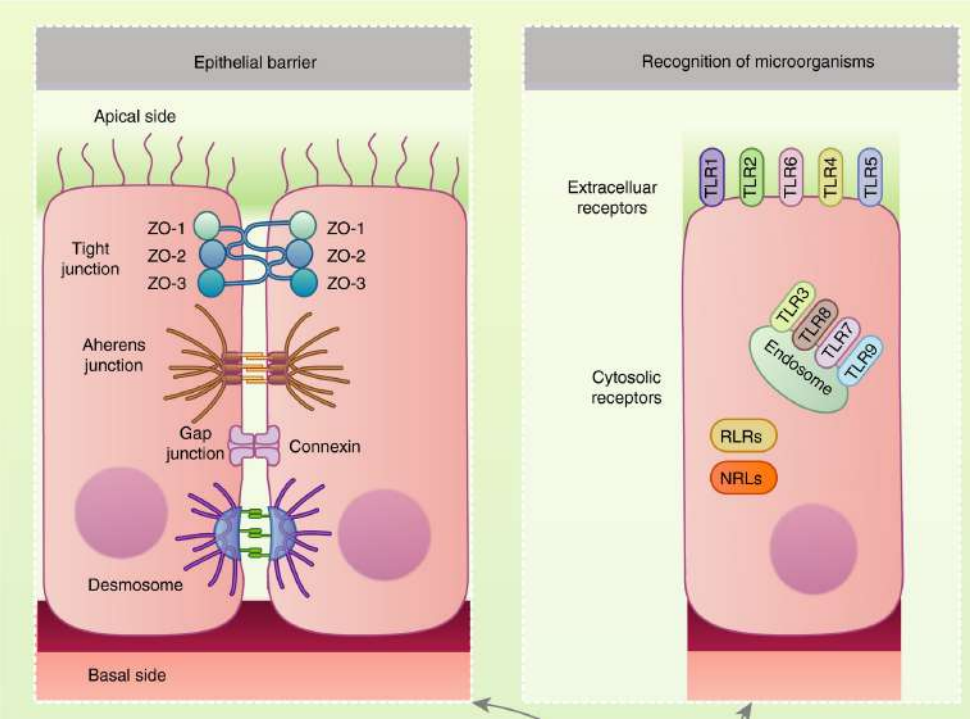

a Lower respiratory tract.
Cartilage
Bronchus
ASM
Bronchiole
Alveoli
b Trachea (human and mouse) and large airways (human)
Pseudostratified epithelium
Basement membrane
Nerve
Cartilage
Submucosal gland
c Medium and small airways
Human
Mouse
Epithelial cell types
Basal cell
TRP63, KRT5
Secretory (club) cell
SCGB1A1, SCGB3A2
Multiciliated cell
FOXJ1, TUBB3
Goblet cell
MUC5AC, MUC5B
Brush (tuft) cell
IL-25, TSLP
Ionocyte
FOXI1, CFTR
PNEC (solitary)
PNEC (NEB)
CALCA, ASCL1
Submucosal gland
Serous cell
LTF, DCCP1
Mucous cell
MUC5B,TFF2
Myoepithelial cell
EPCAM, ACTA2
Mesenchyme cell types
ASM
ACTA2, LGR6
Fibroblast
GLI1, AXIN2

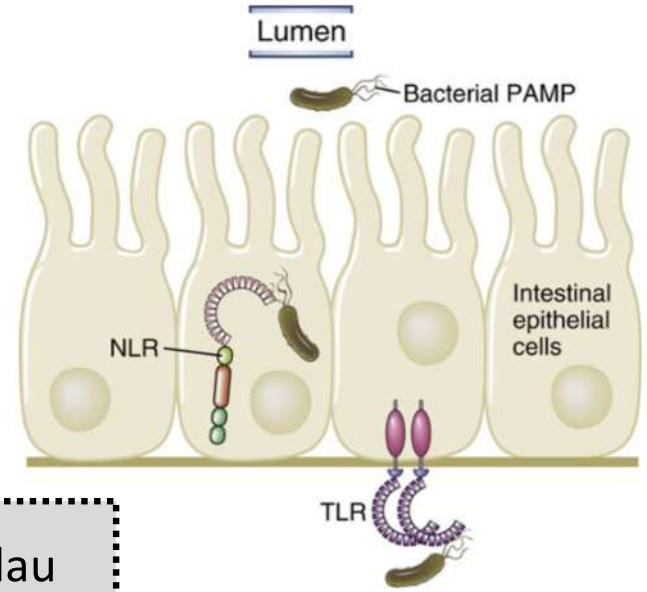


Davis & Wypych, 2021

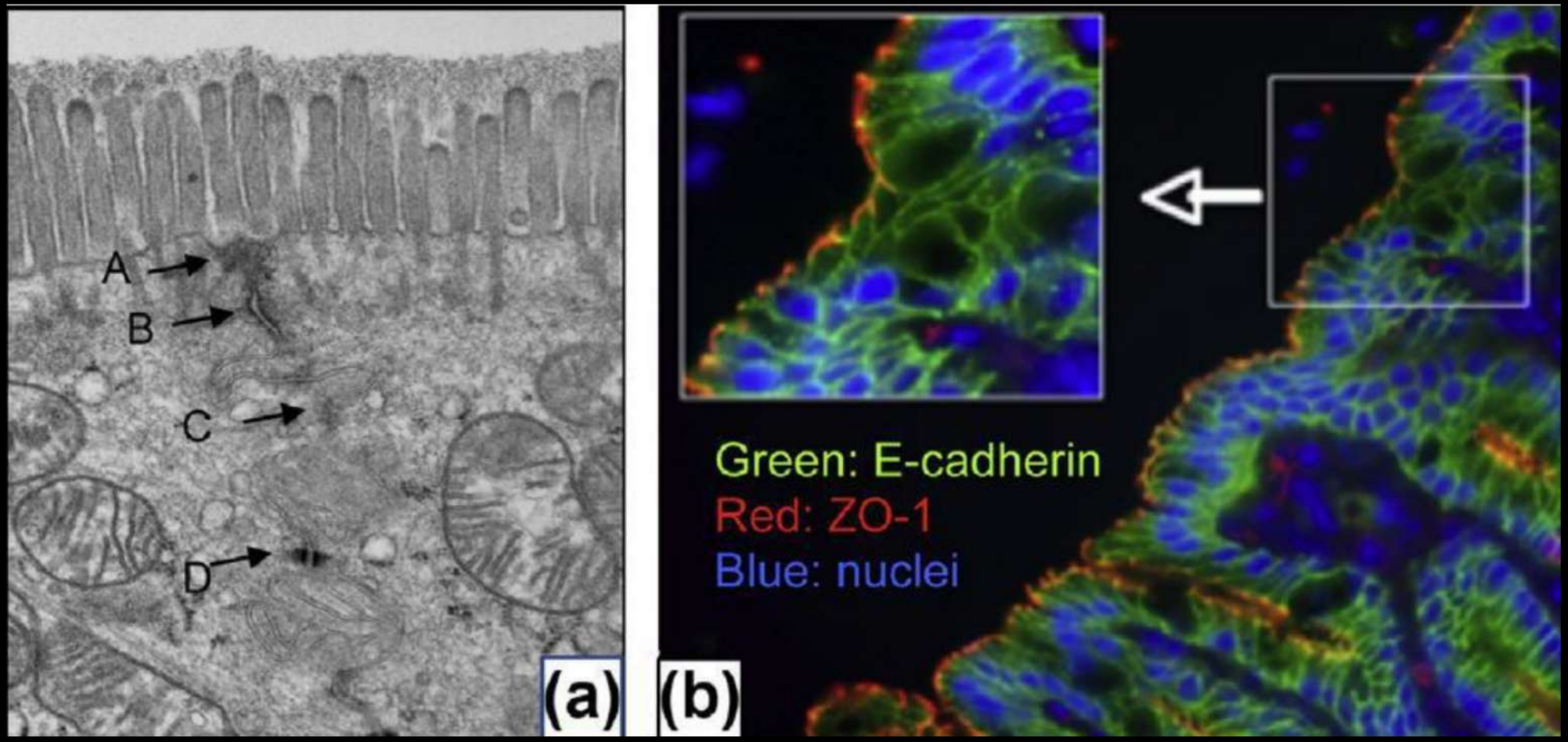
Epitélios: função imunológica

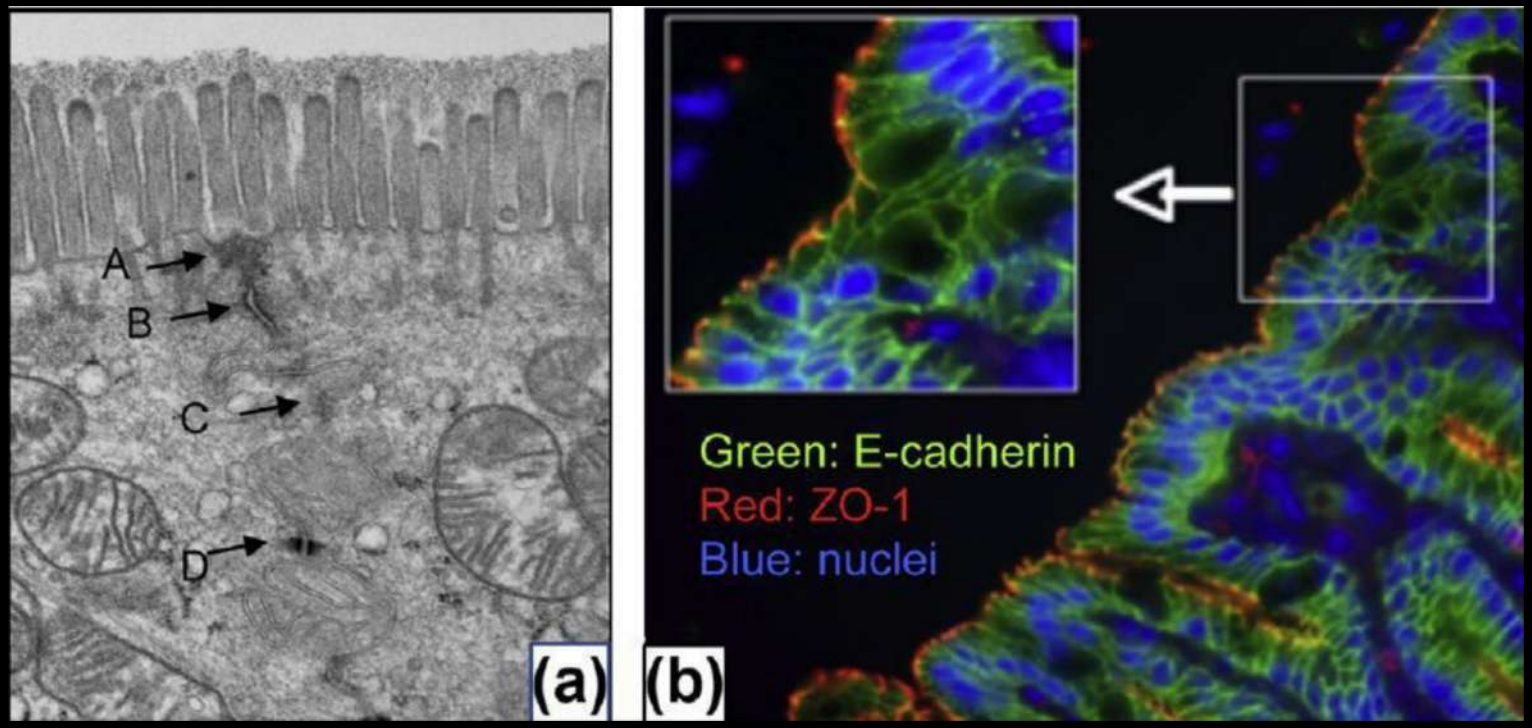
- Simples, estratificado ou pseudoestratificado
- Junções
- Microvilosidades
- Cílios
- Células especializadas: M, Paneth, globulares/caliciformes, Club, Tuft...
- Produção de fatores antimicrobianos: defensinas, catalecidinas e lectinas do tipo C, Óxido Nítrico, Inteferons e IFN I
- Surfactantes (pulmão)
- RECEPTORES para PAMP e DAMP

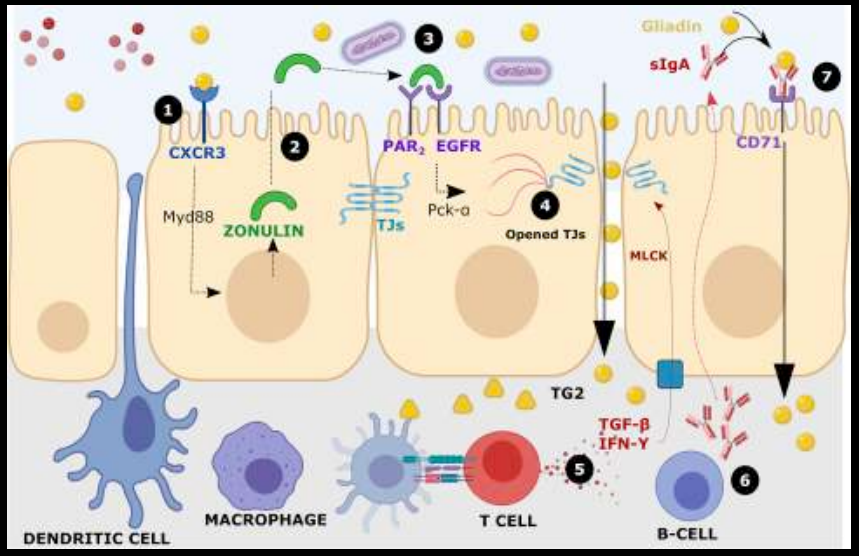


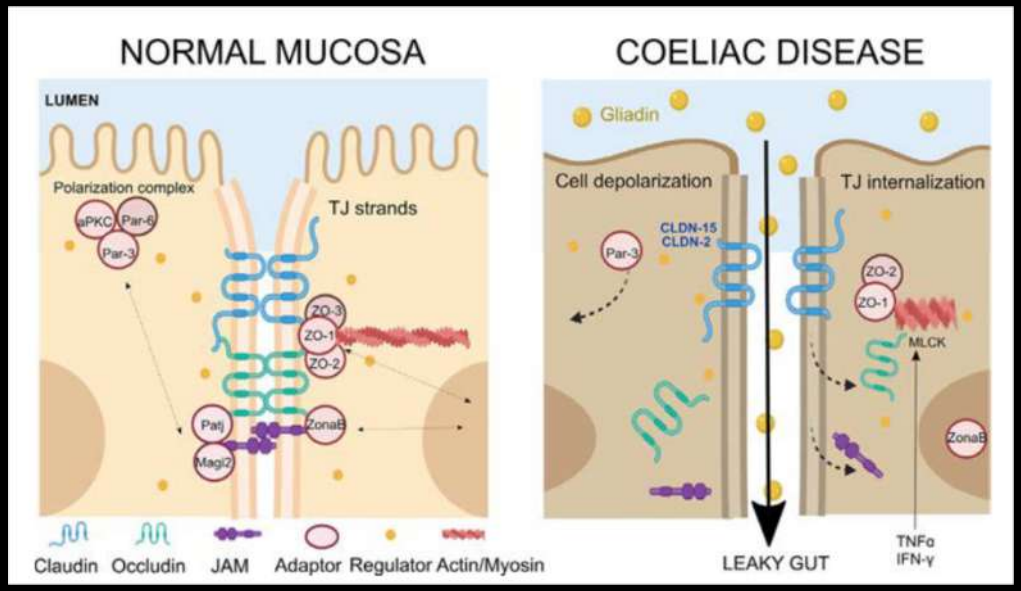


✓ Doença inflamatória intestinal / Crohn e Síndrome de Blau


A
B
C
D
(a)
(b)
Green: E-cadherin
Red: ZO-1
Blue: nuclei







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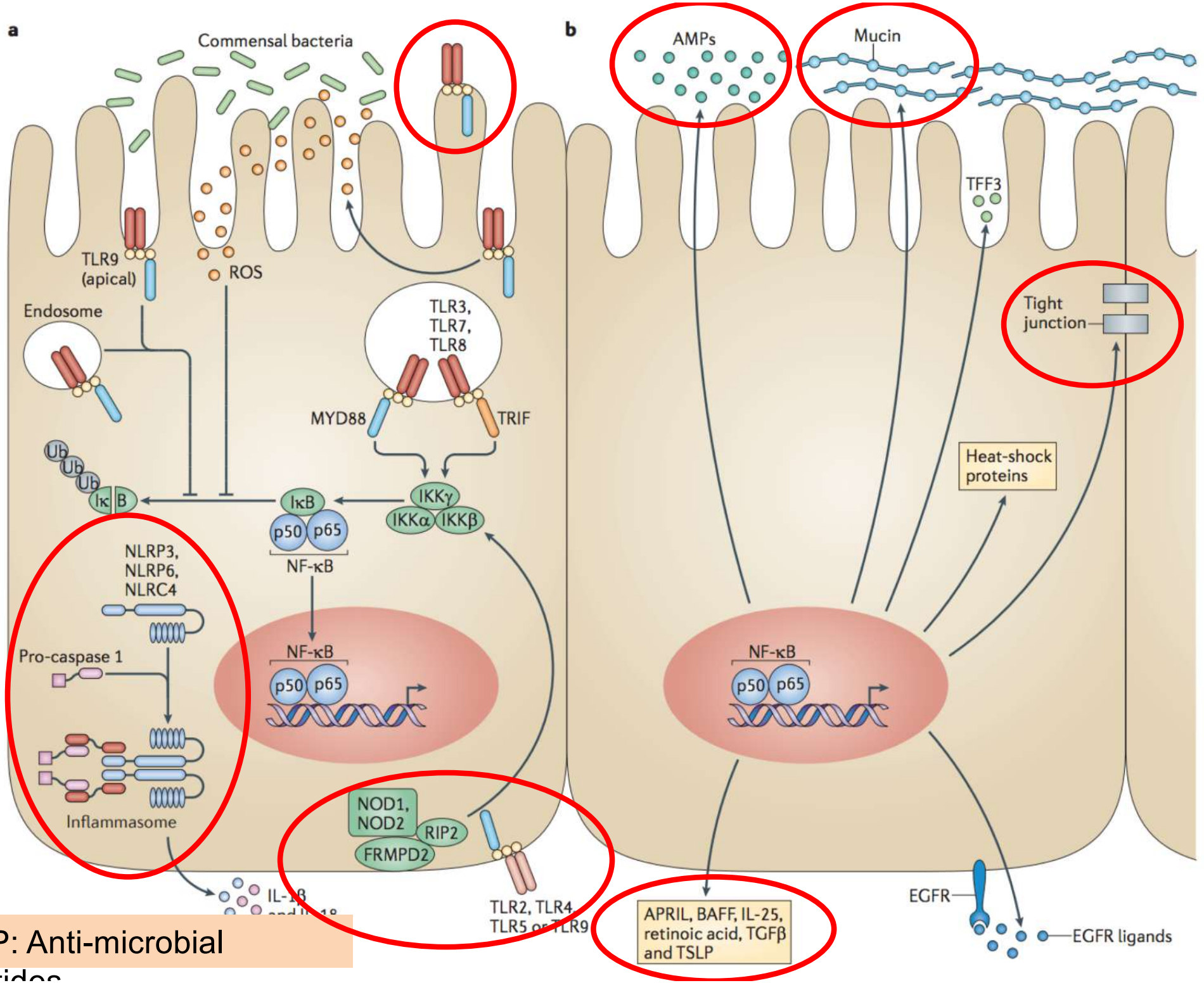
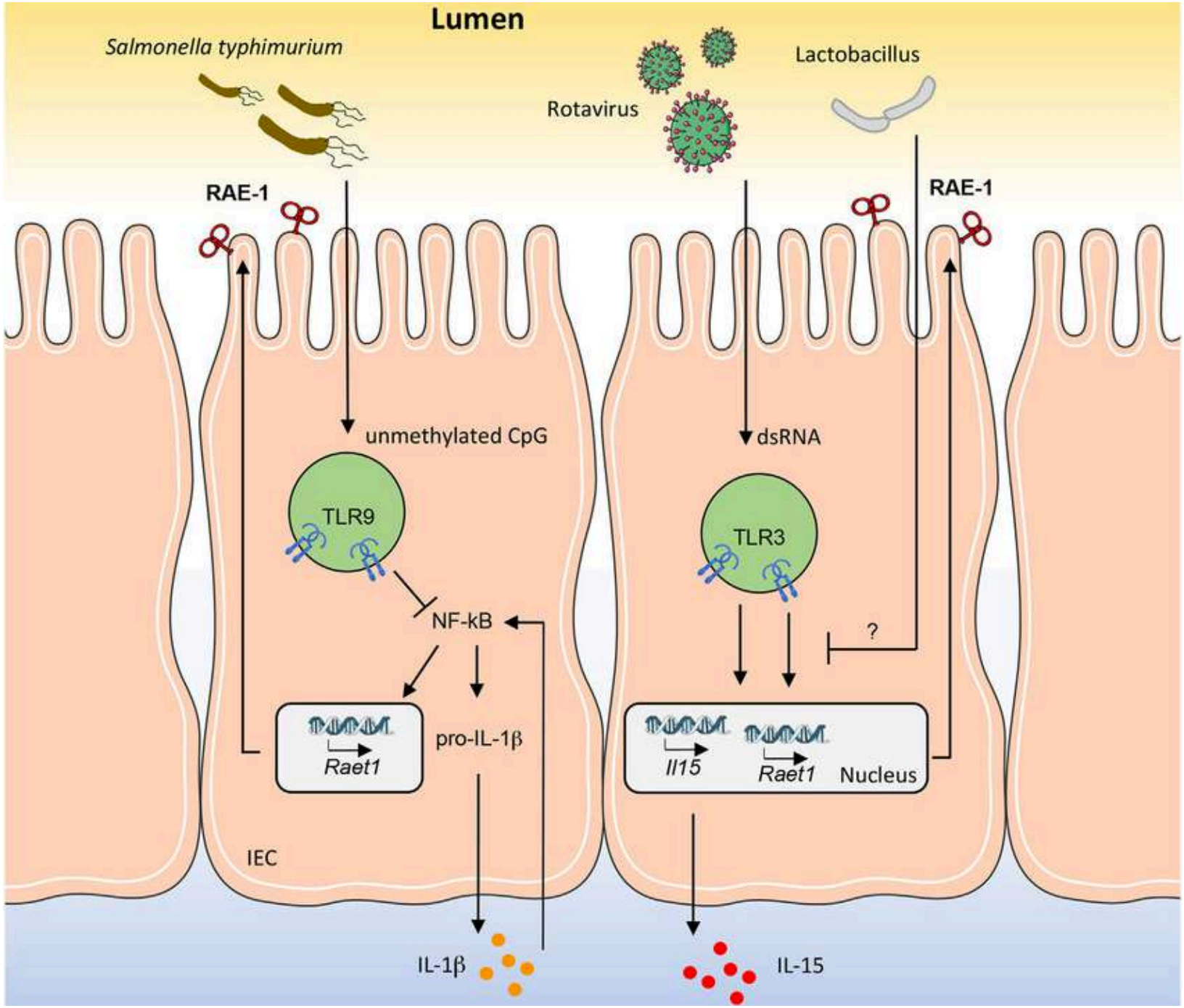
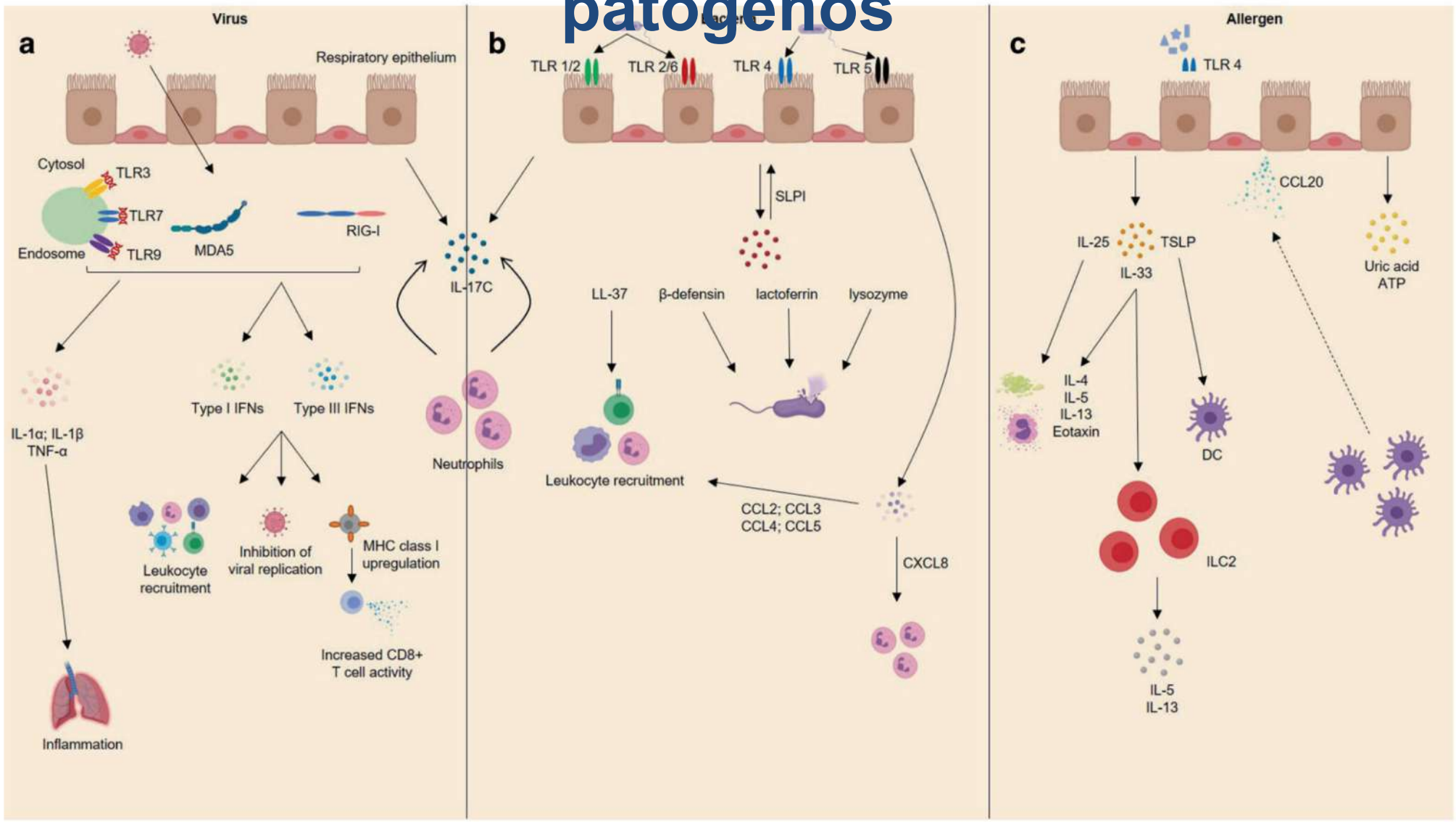


AMP: Anti-microbial peptides

Epitélio intestinal: interação com patógenos

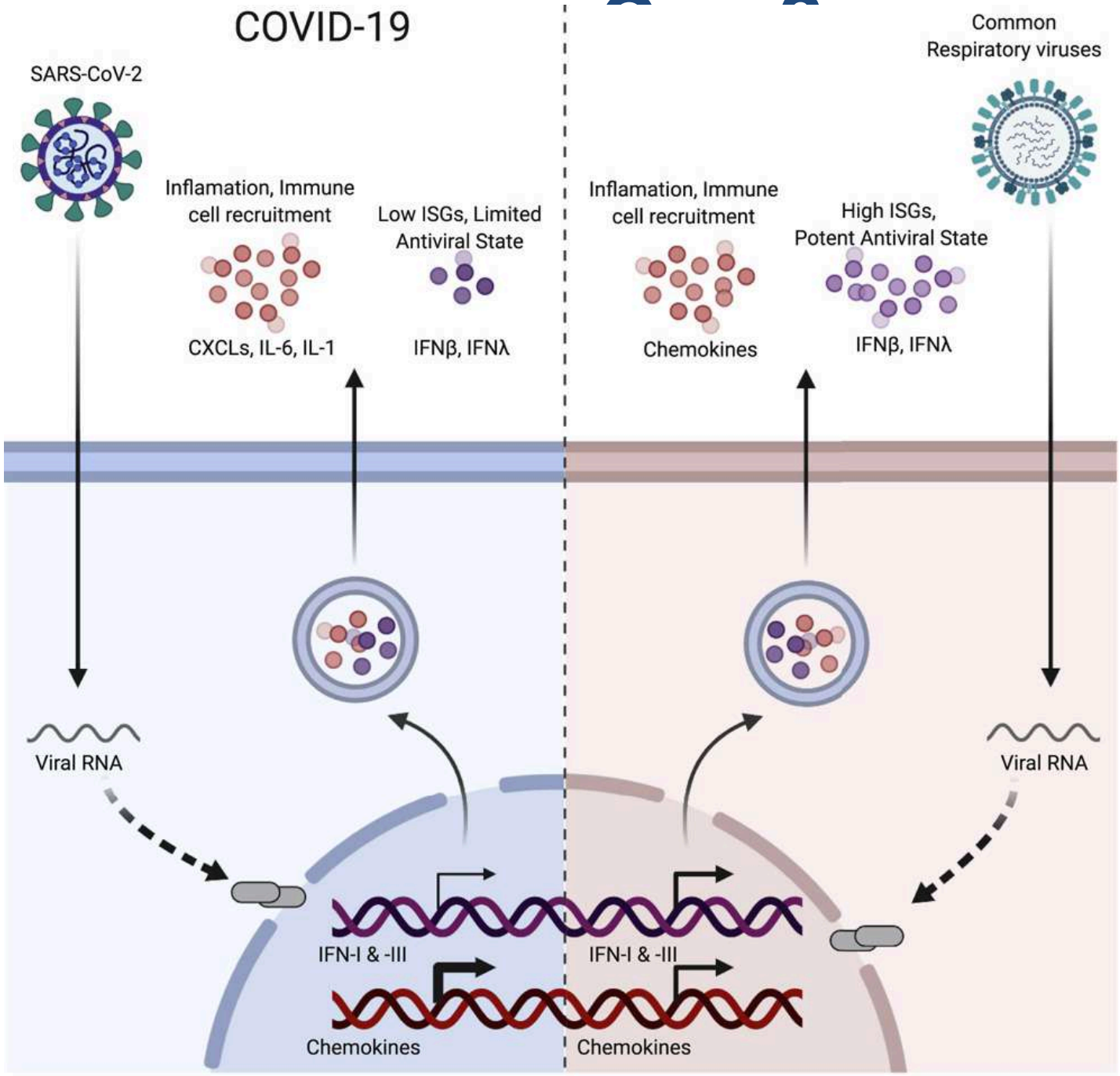


Epitélio respiratório: interação com patógenos



Davis & Wypych, 2021

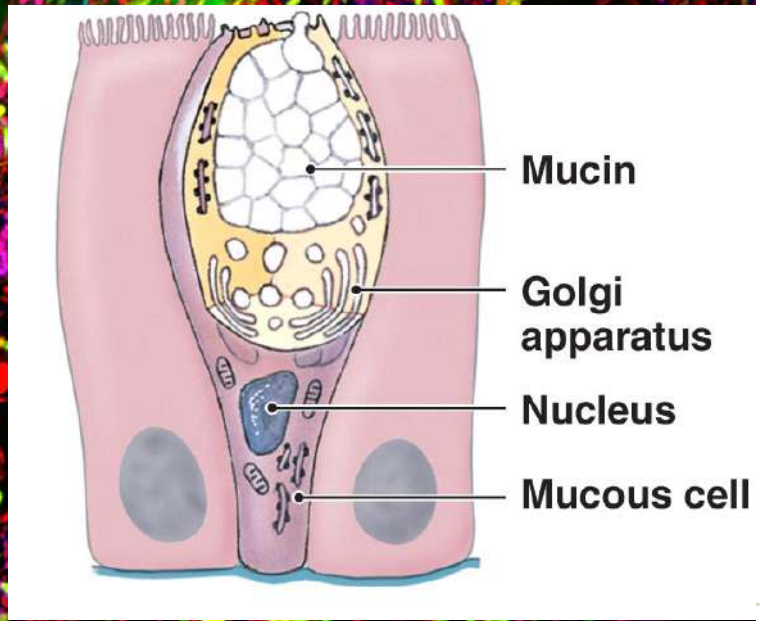
Epitélio das vias aéreas: resposta SARS-



Blanco-Mello et al, 2020

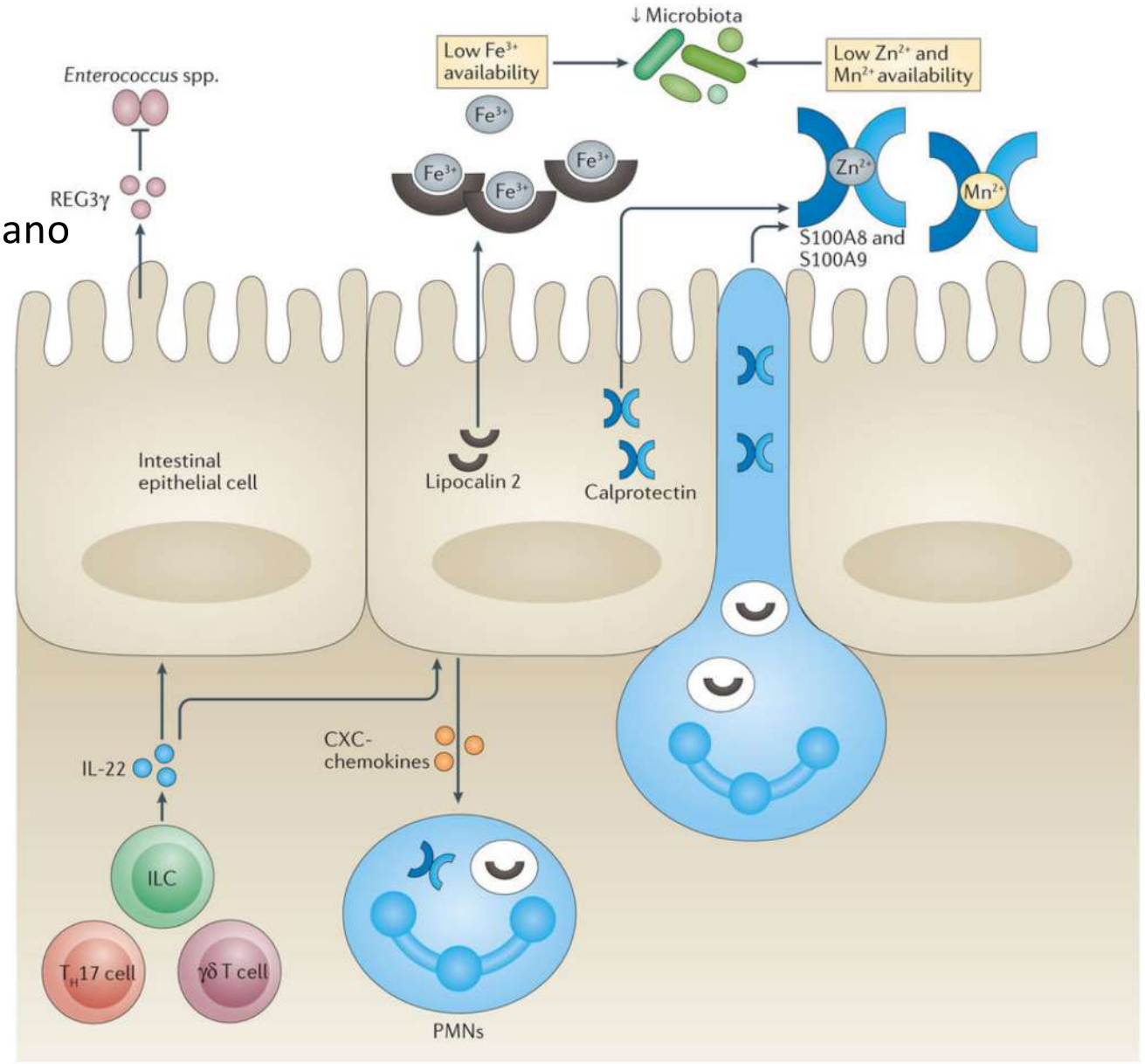
Epitélio: Muco

- ✓ Induzido classicamente por: IL-13
- ✓ outras citocinas podem interferir na sua composição (glicosilação e tispos de mucinas) :IL-1, IL-4, IL-6, IL-9, IL-13, TNF, e IFN tipo I, elastase e proteínas microbianas

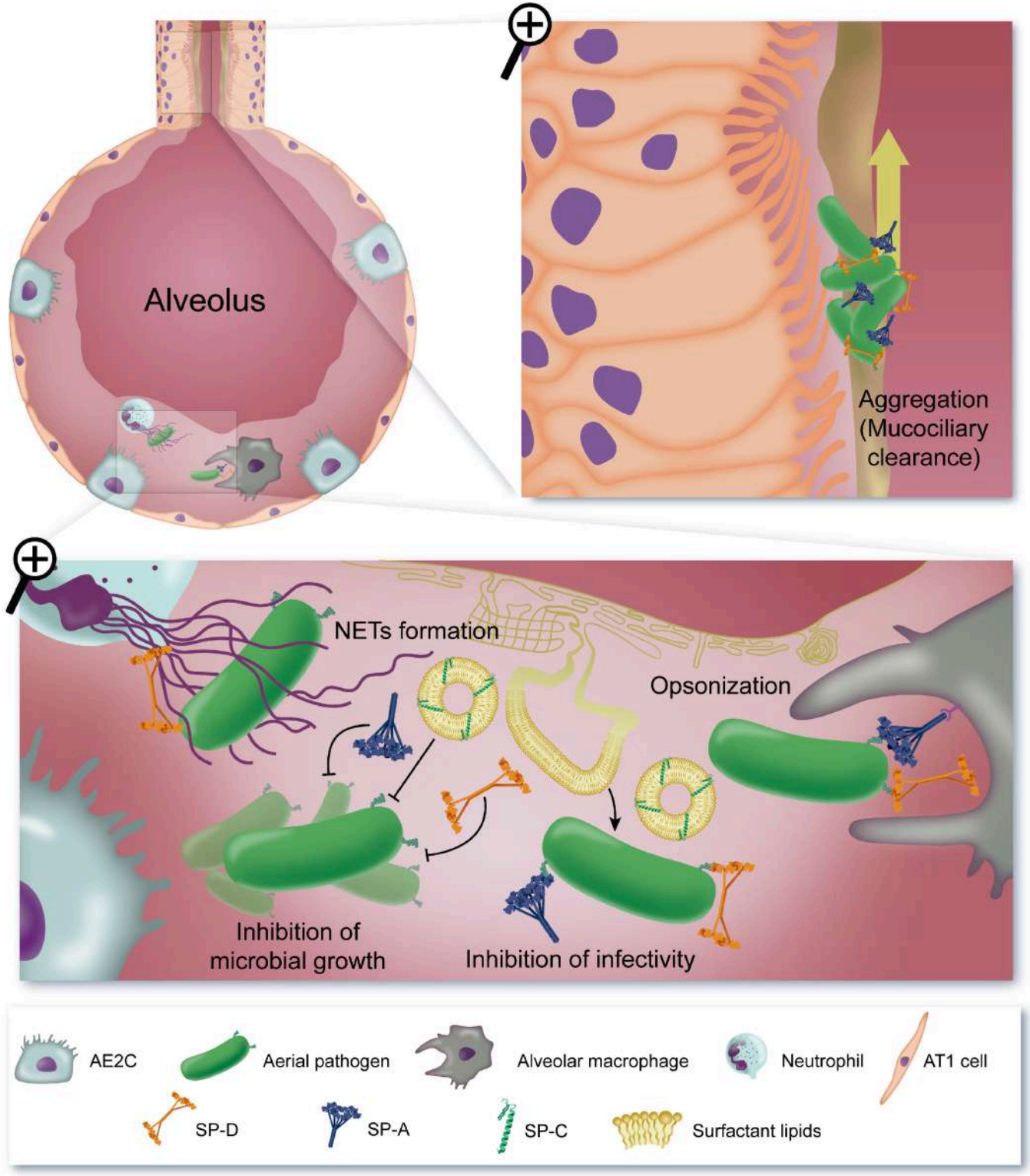


Peptídeos antimicrobianos

Liga peptideoglicano

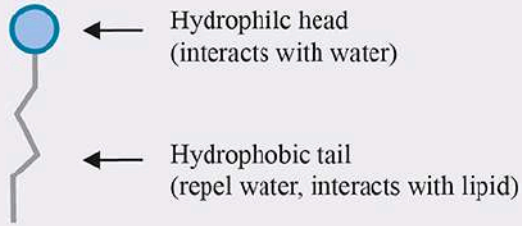


Nature Reviews | Immunology

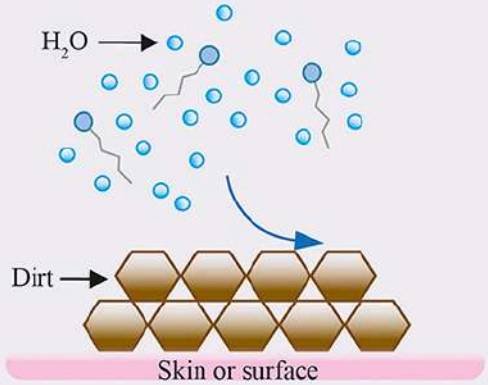

Alveolus
Aggregation
(Mucociliary
clearance)
NETs formation
Opsonization
Inhibition of
microbial growth
Inhibition of infectivity
AE2C
Aerial pathogen
Alveolar macrophage
Neutrophil
AT1 cell
SP-D
SP-A
SP-C
Surfactant lipids

A

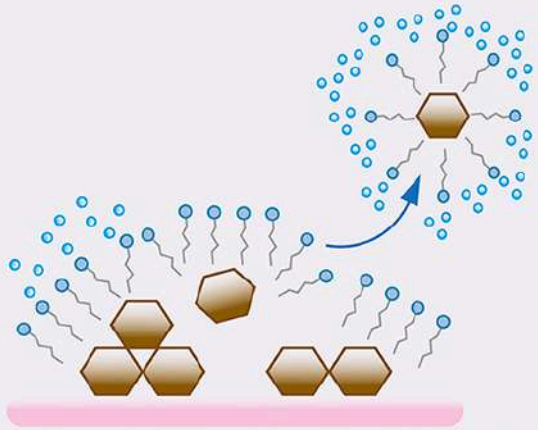
BIOSURFACTANTS MOLECULES have an amphiphilc structure with a hydrophilic head and a hydrophobic tail.



B

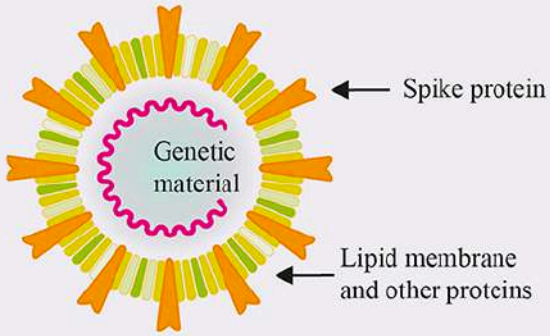


BIOSURFACTANTS hydrophobic ends surround and lift the dirt while hydrophilic heads face the water and get caught in the flow, carryng the dirt away.

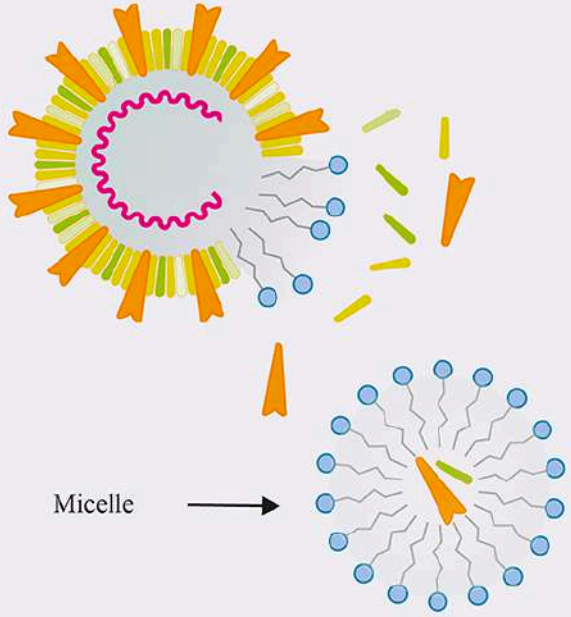


C

CORONAVIRUS has a lipidic membrane with embeded proteins that help the virus to infect the cells.

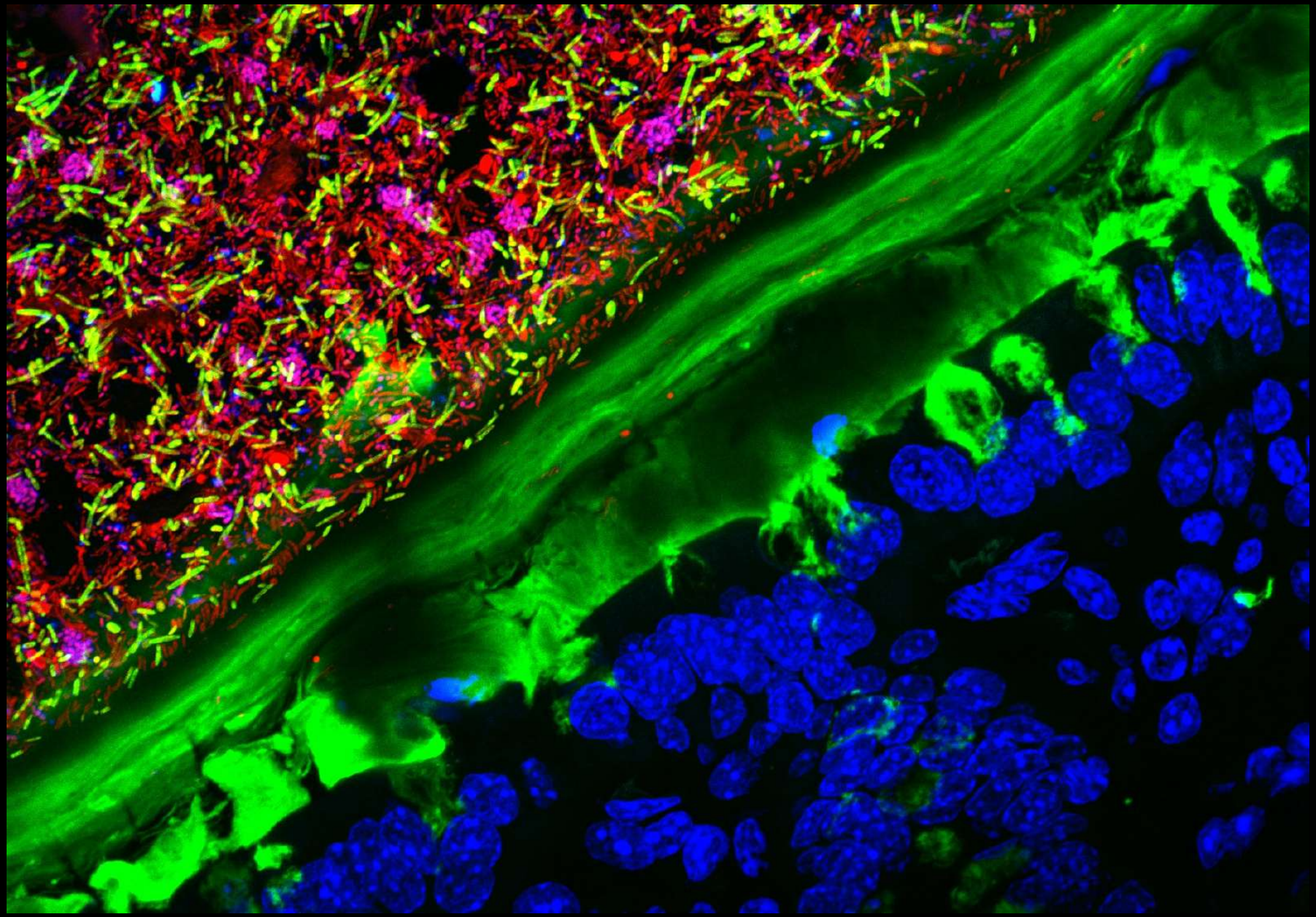


BIOSURFACTANTS destroy the virus when the lipophilic tails wedge into the lipid membrane and fall it apart. Virus fragments are encapsulated into micelles and are washed away.

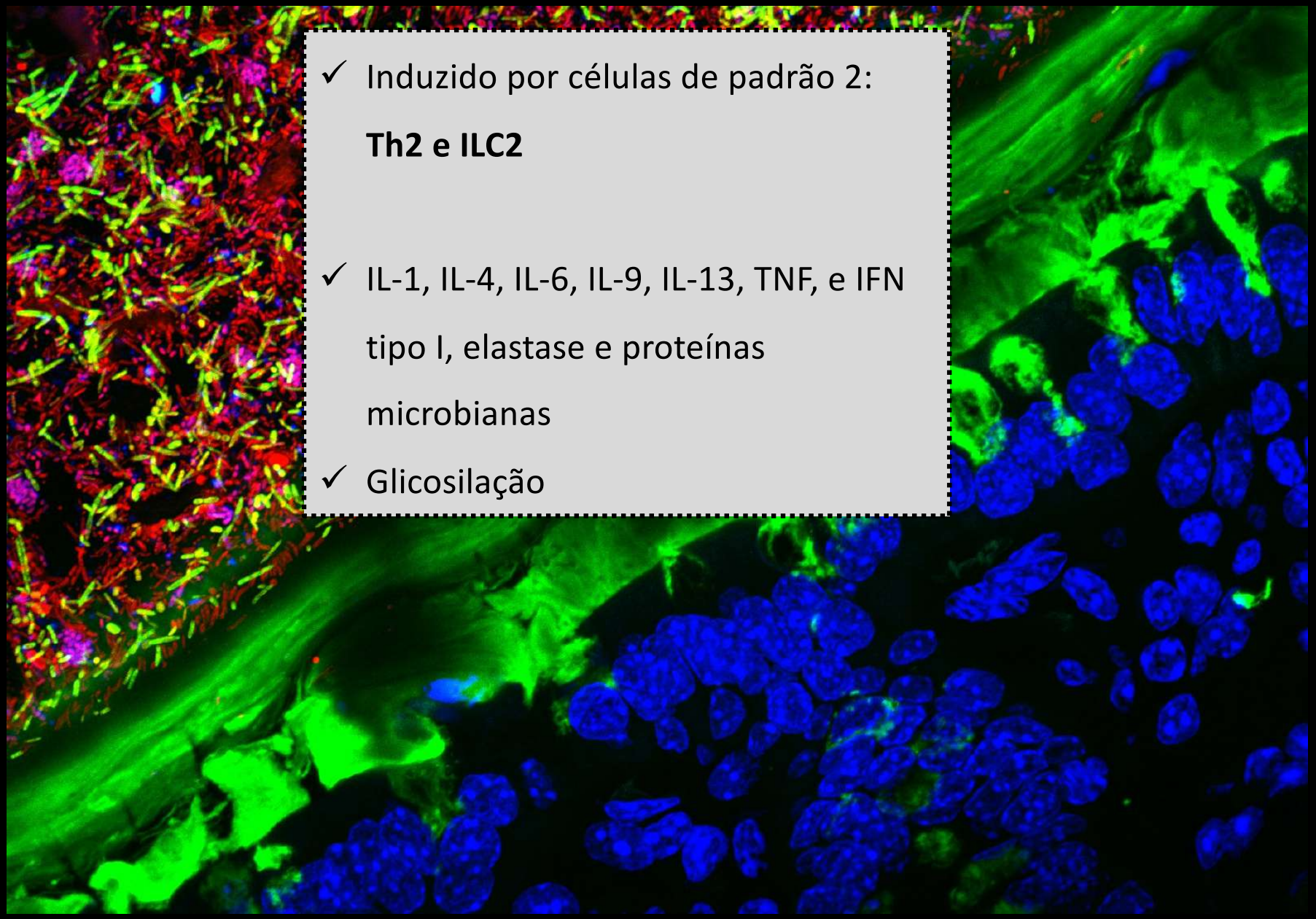


Os peptídeos antimicrobianos não se encontram “soltos” no exterior das mucosas, mas sim ancorados à camada de muco.

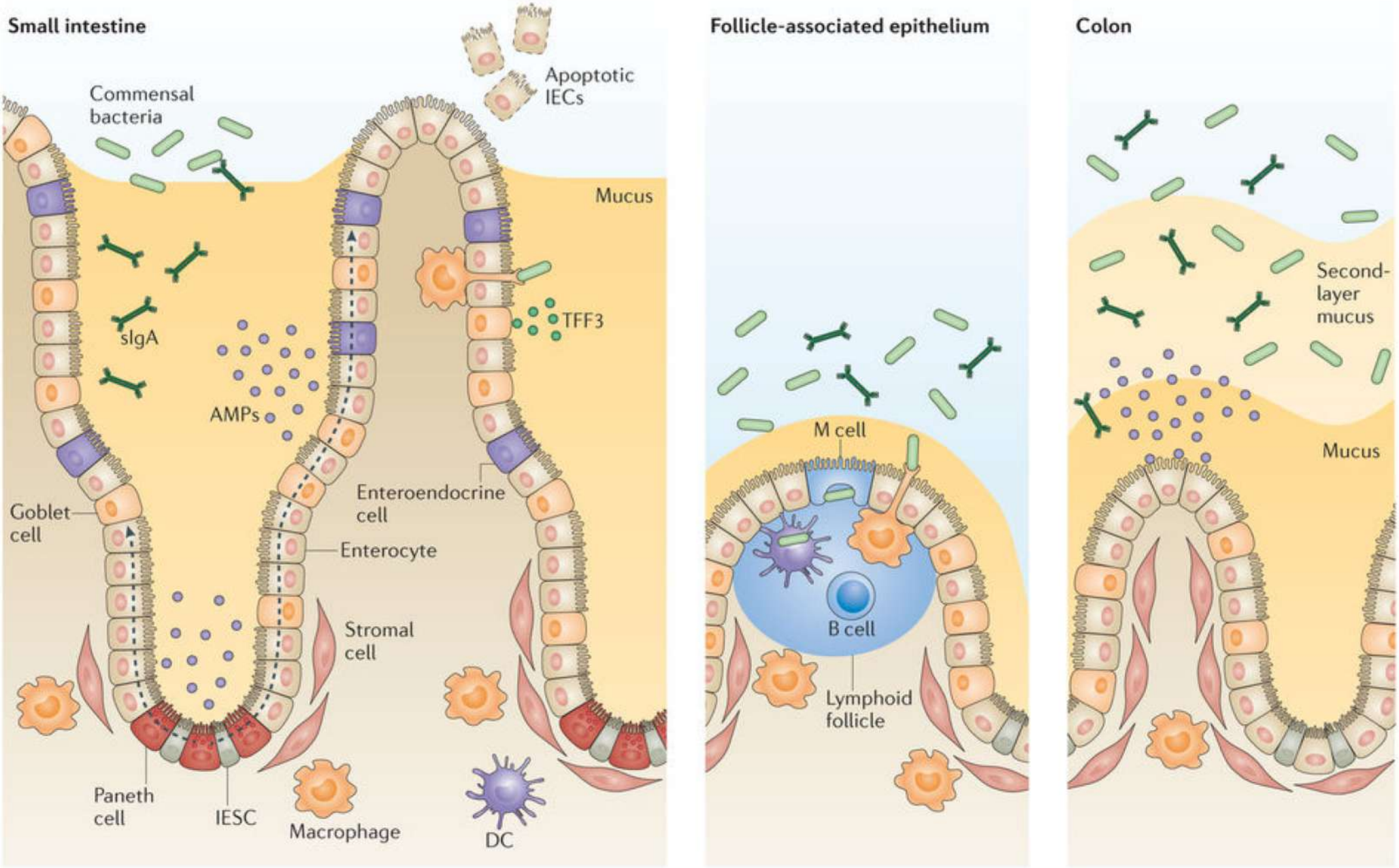
Muco



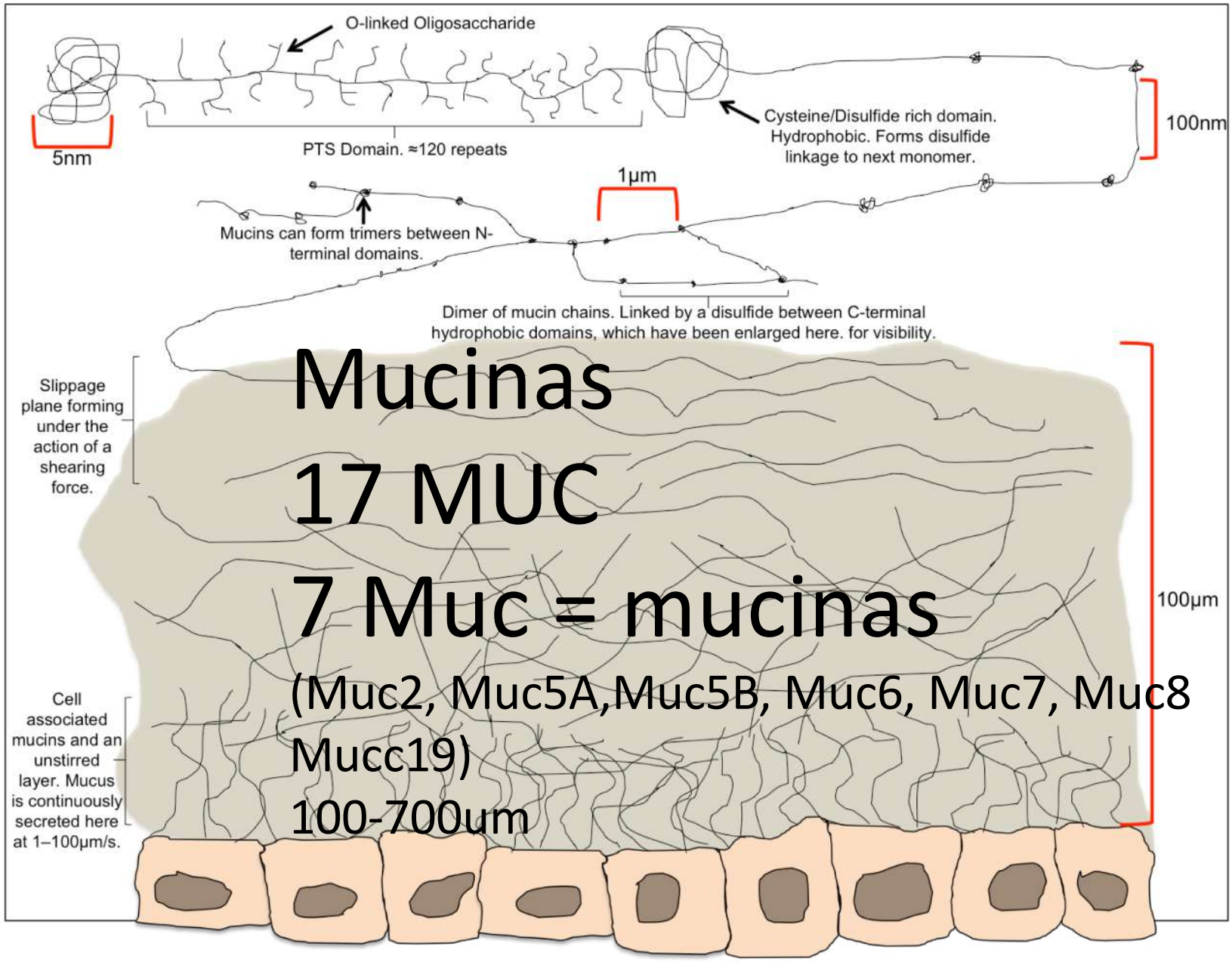
Muco



- ✓ Induzido por células de padrão 2: **Th2 e ILC2**
- ✓ IL-1, IL-4, IL-6, IL-9, IL-13, TNF, e IFN tipo I, elastase e proteínas microbianas
- ✓ Glicosilação


Small intestine
Commensal bacteria
Apoptotic IECs
Mucus
sIgA
AMPs
TFF3
Goblet cell
Enteroendocrine cell
Enterocyte
Stromal cell
Paneth cell
IESC
Macrophage
DC
Follicle-associated epithelium
M cell
B cell
Lymphoid follicle
Colon
Second-layer mucus
Mucus

Muco



Mucinas

17 MUC

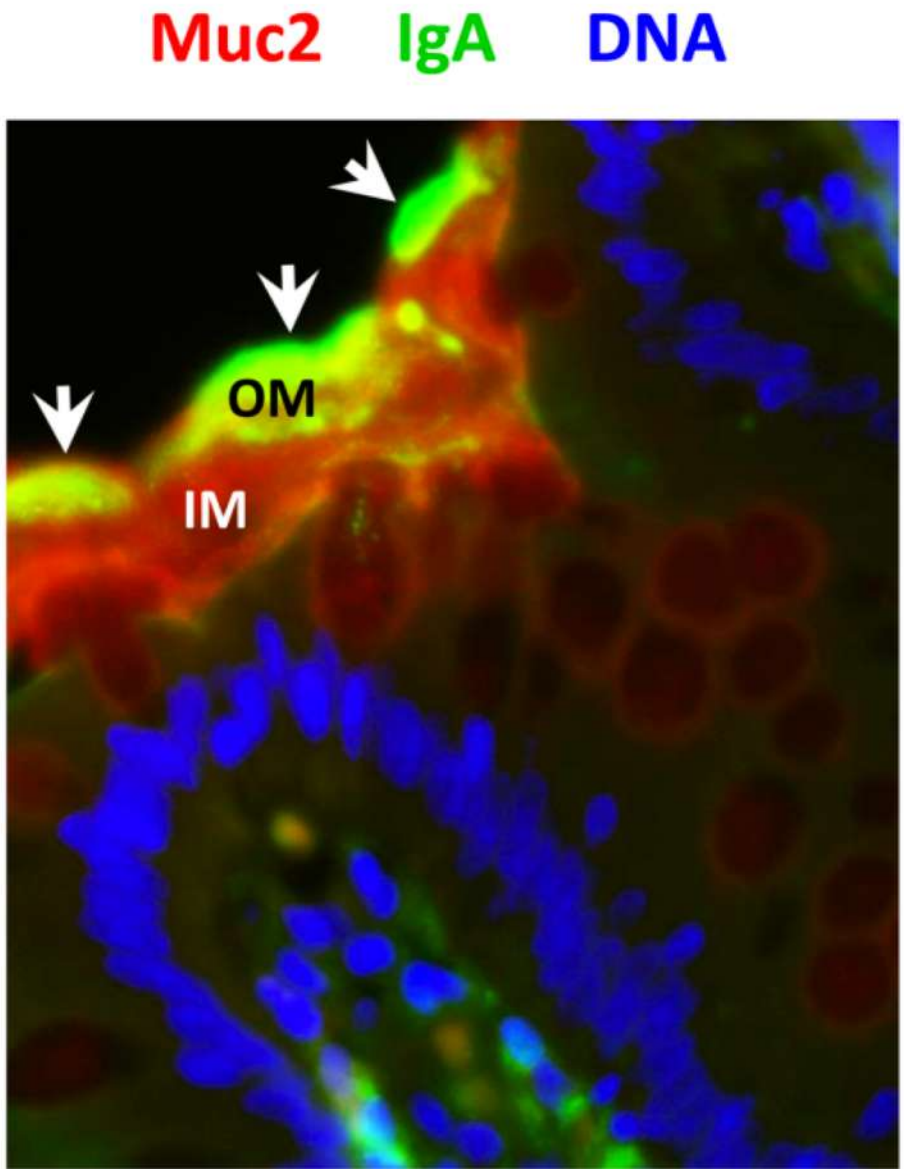
7 Muc = mucinas

(Muc2, Muc5A,Muc5B, Muc6, Muc7, Muc8 Mucc19)

100-700um

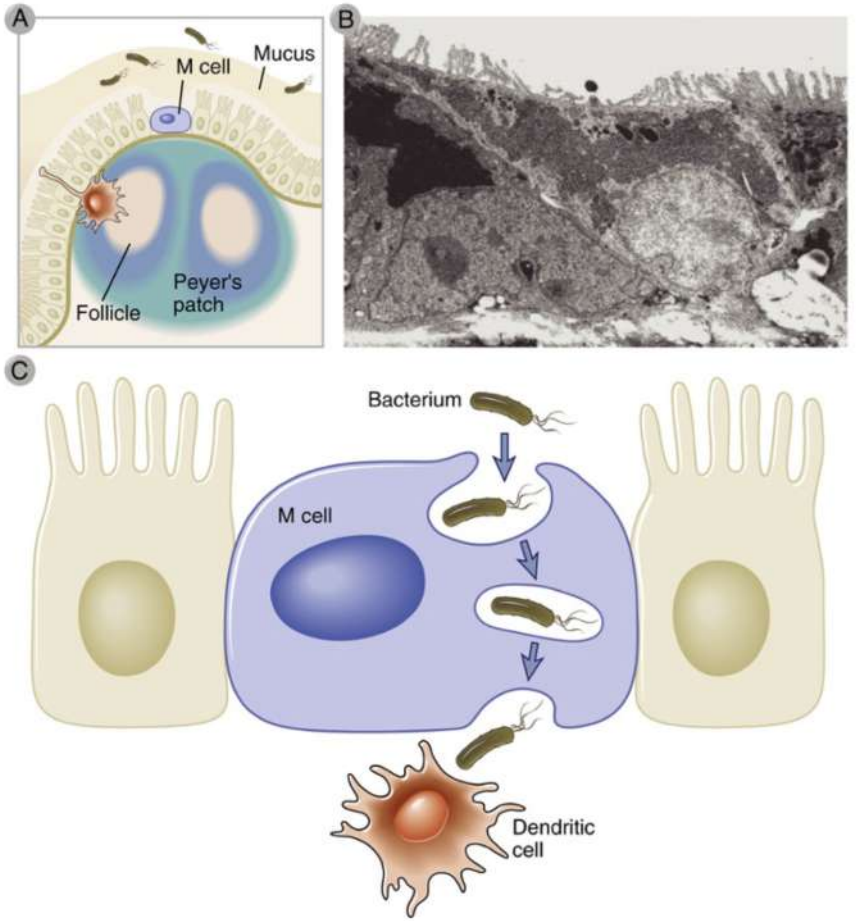
Muco

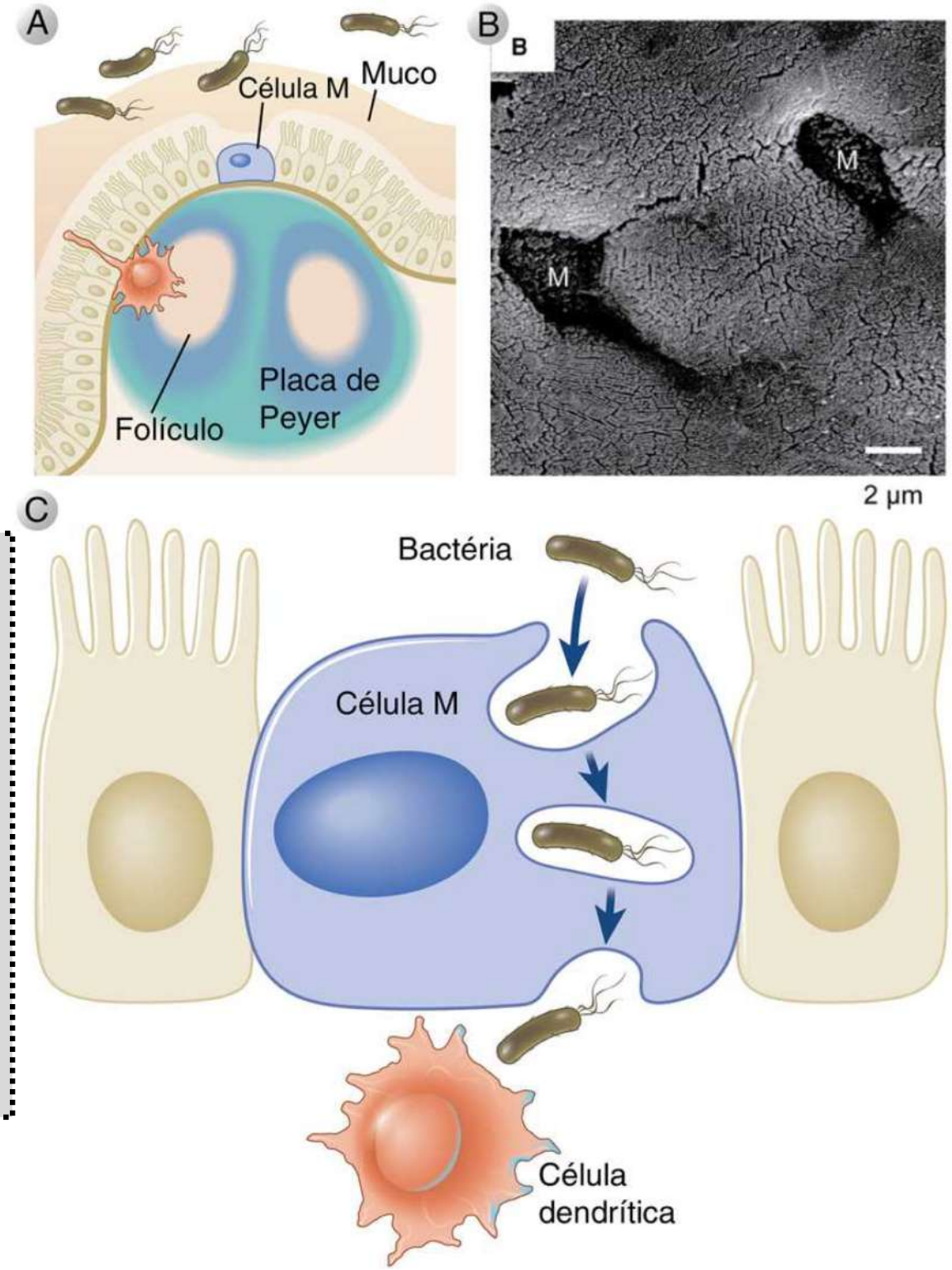
- ✓ Além de peptídeos anti-microbioanos, a IgA secretada para o lúmen do intestino fica localizada na cama de muco.



Epitélio: Células M (Microfenestradas)

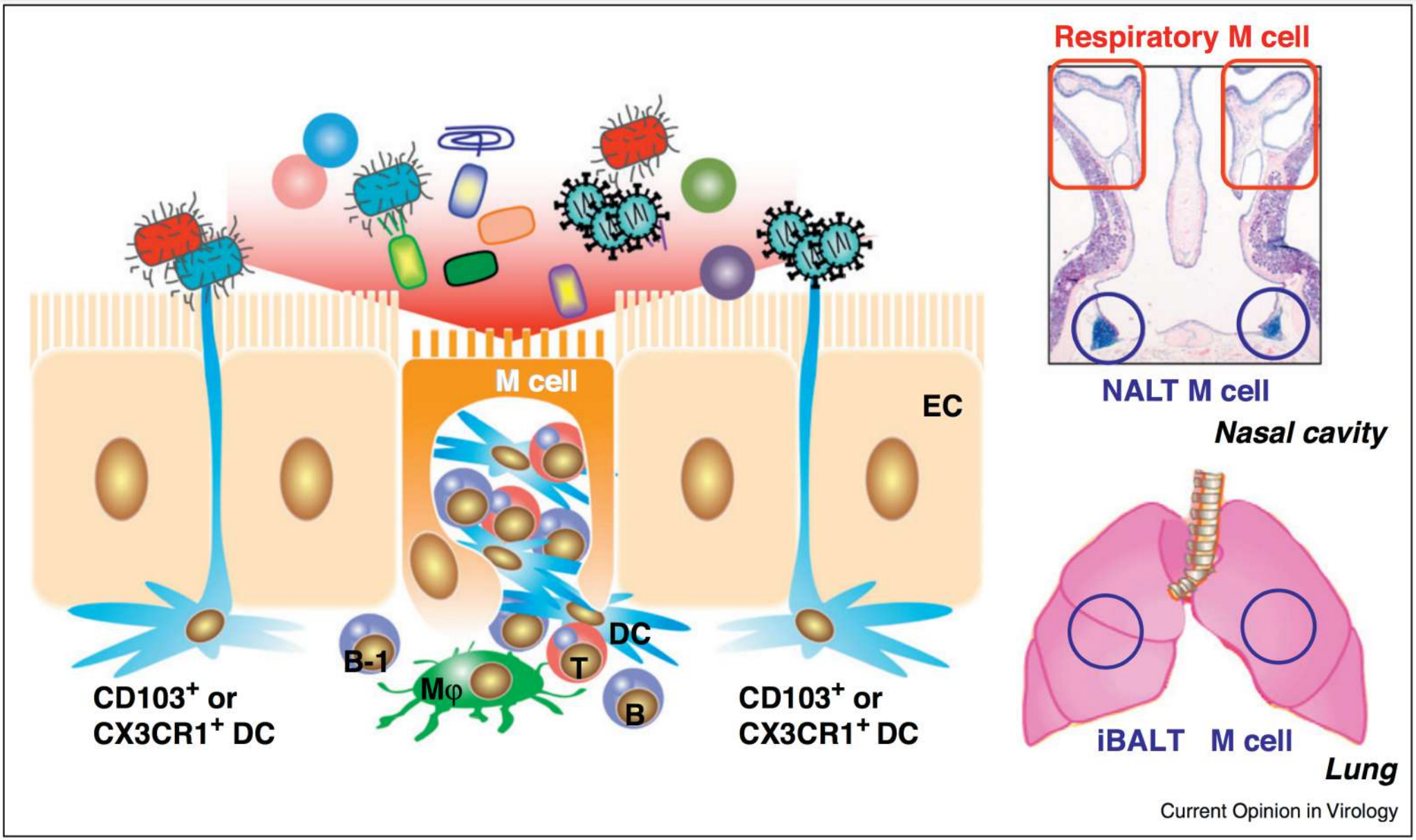
- ✓ Células M são células especializadas no transporte de antígenos, partículas e microorganismos do lúmen para o parênquima tecidual
- ✓ São encontradas no epitélio pulmonar e intestinal.
- ✓ No intestino, ficam localizadas nas Placas de Peyer





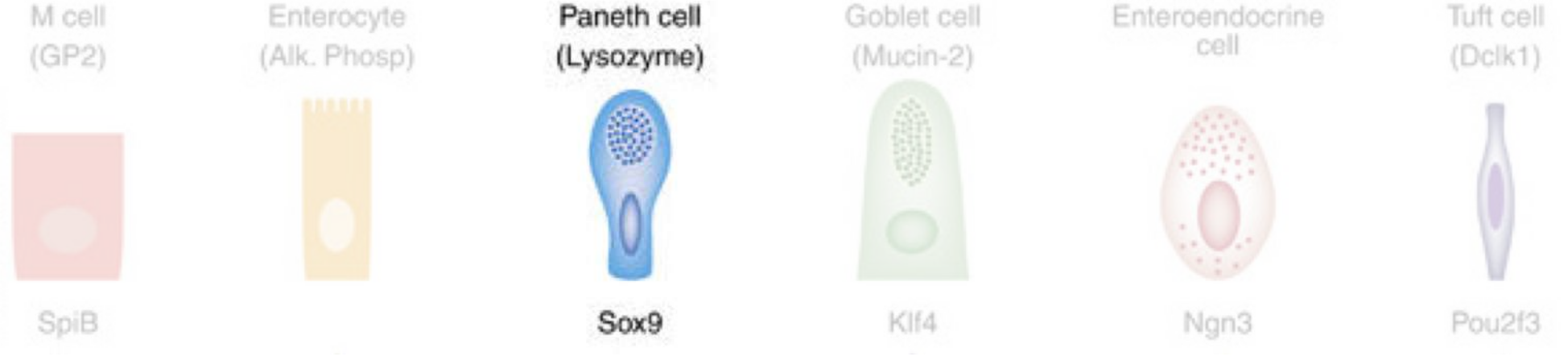
- ✓ Placas de Peyer: São aglomerados de linfócitos que se organizam em folículos de linfócitos B e zonas de linfócitos T ao longo do intestino delgado: Local importante para produção de anticorpos (IgA)
- ✓ Placa cecal: presente no intestino grosso

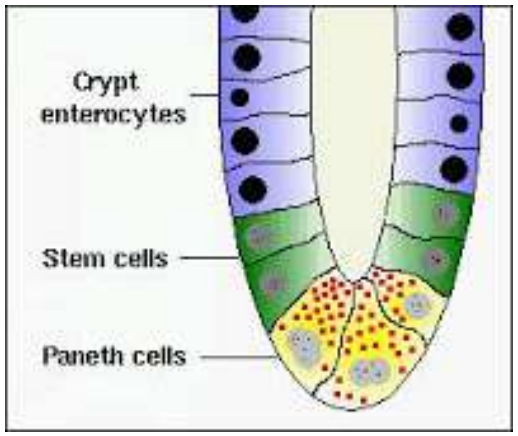
Células M (Microfold/Microfrenestrada)

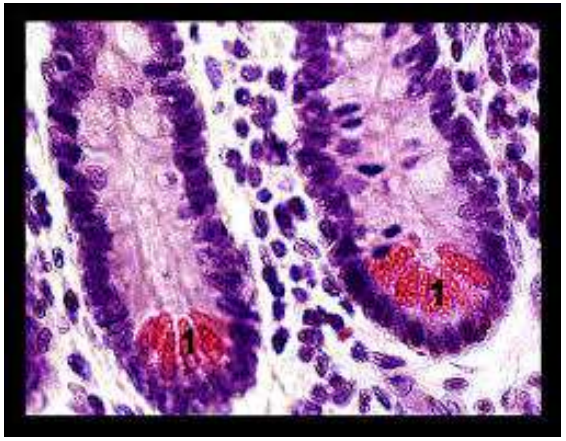


Sato & Kiyono, 2012

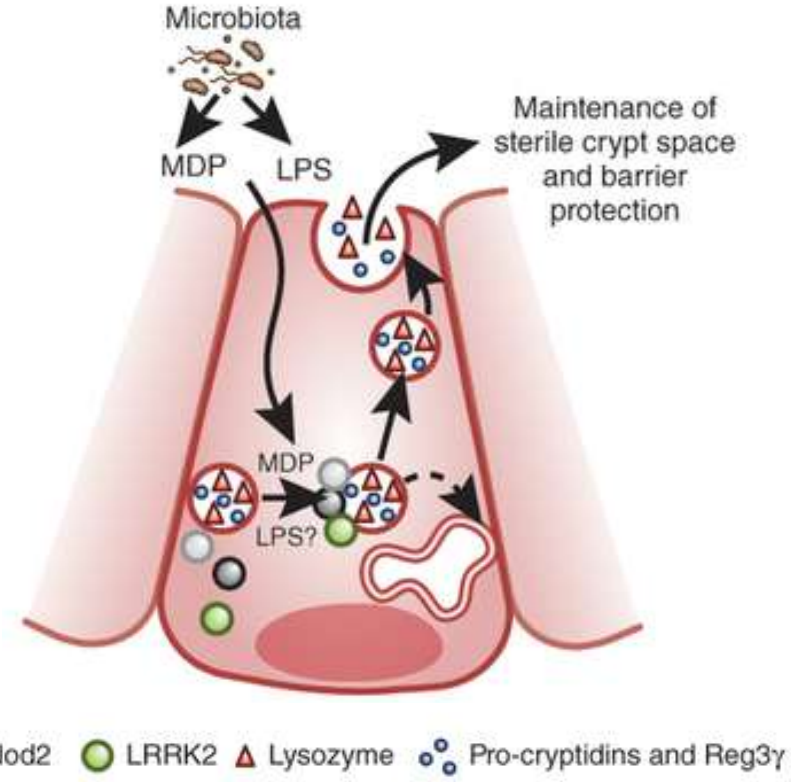
Epitélio: células de Paneth



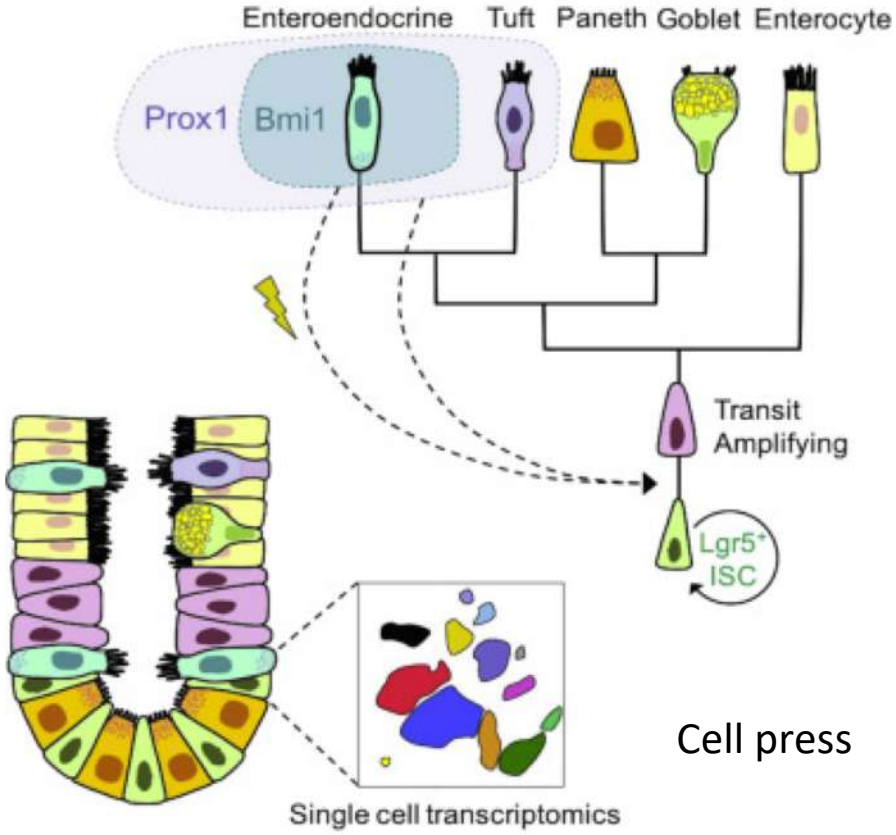




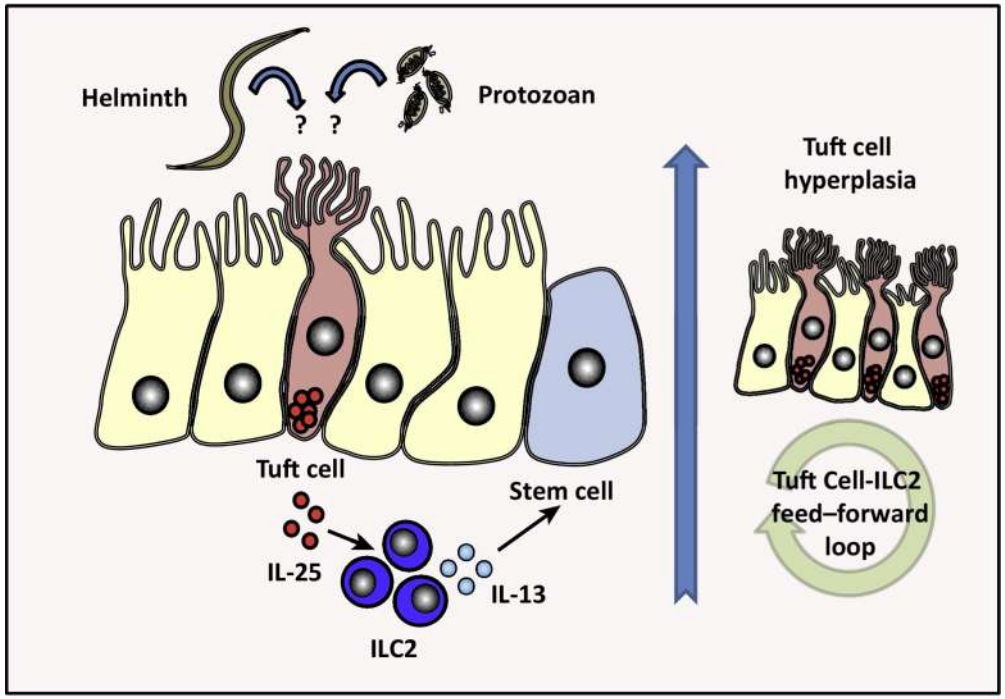
Células presentes no epitélio intestinal, principalmente na base das criptas, especializadas na produção de peptídeos animicrobioanos (defensinas, catalecidinas, ...)



Epitélio: células de Tuft

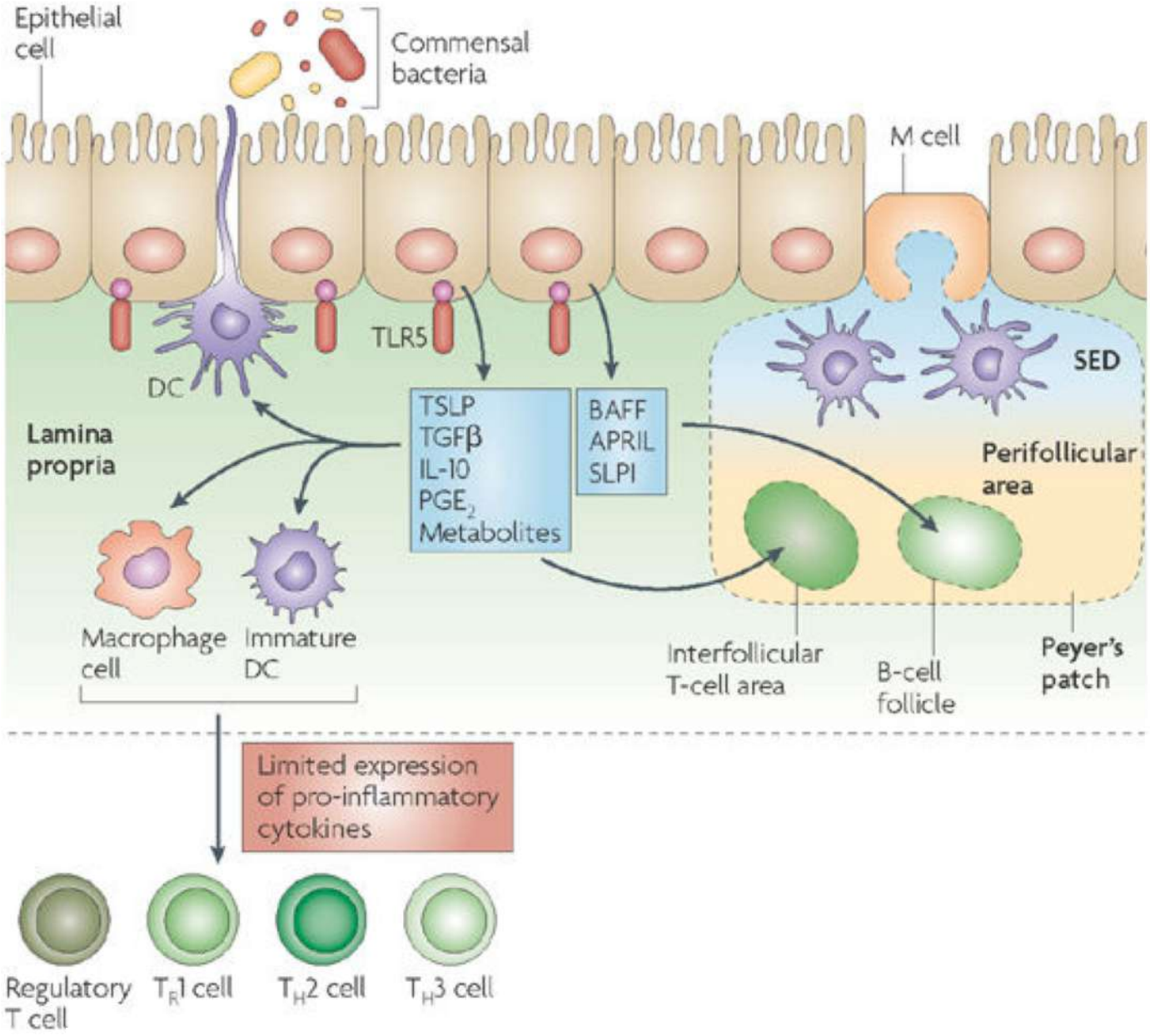


Cell press



Trends in Parasitology

A função das células epiteliais define o padrão da imunidade adaptativa na mucosa



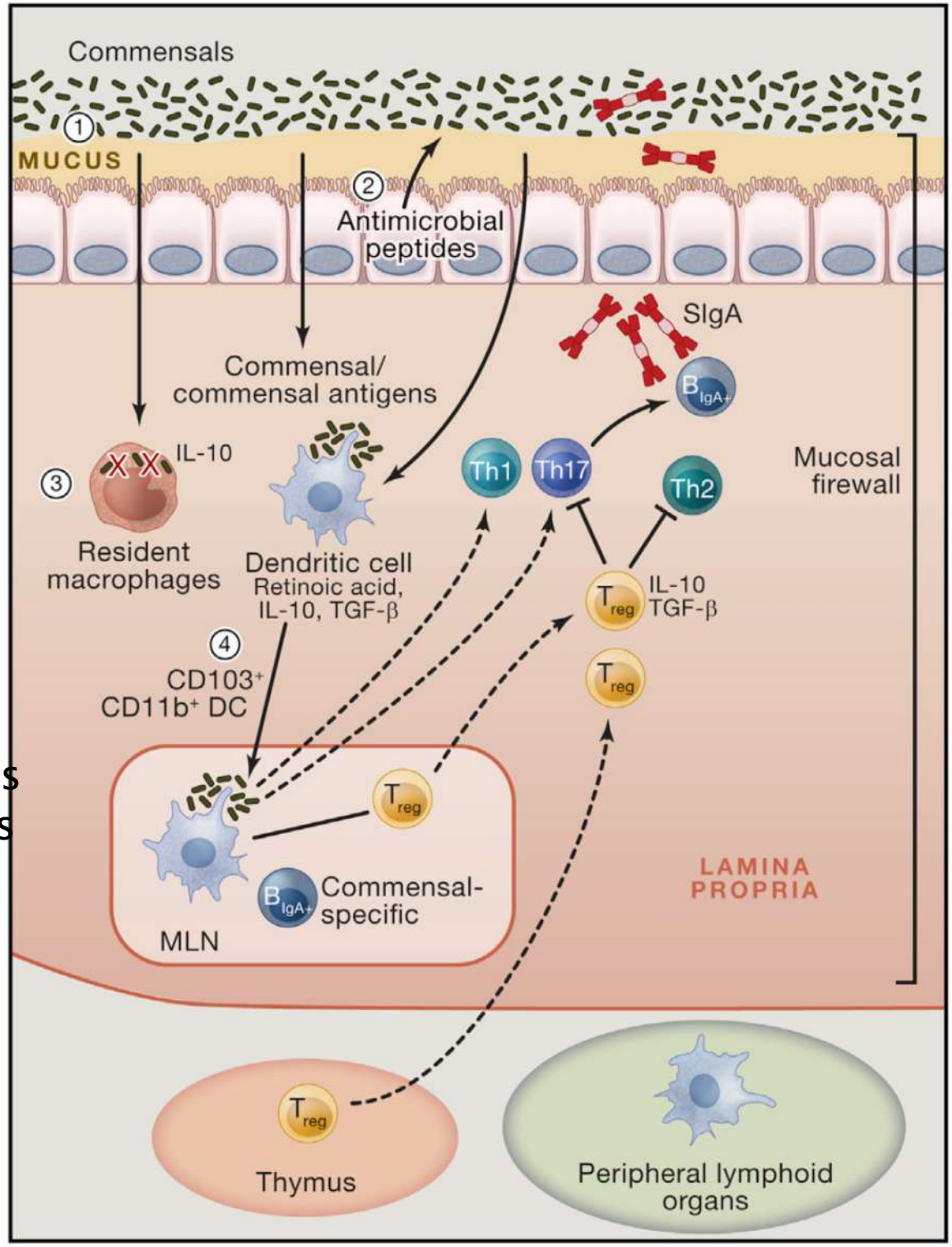
Tópicos da aula

- Conceitos e Componentes: barreira física
- Componentes: barreira ativa
- Respostas canônicas: Tolerância Oral, Imunidade a vacinas, Th17/22, IgA, Linfócitos Inatos
- Particularidades de tecidos de barreira
- Interação com microbiota e doença

Componentes

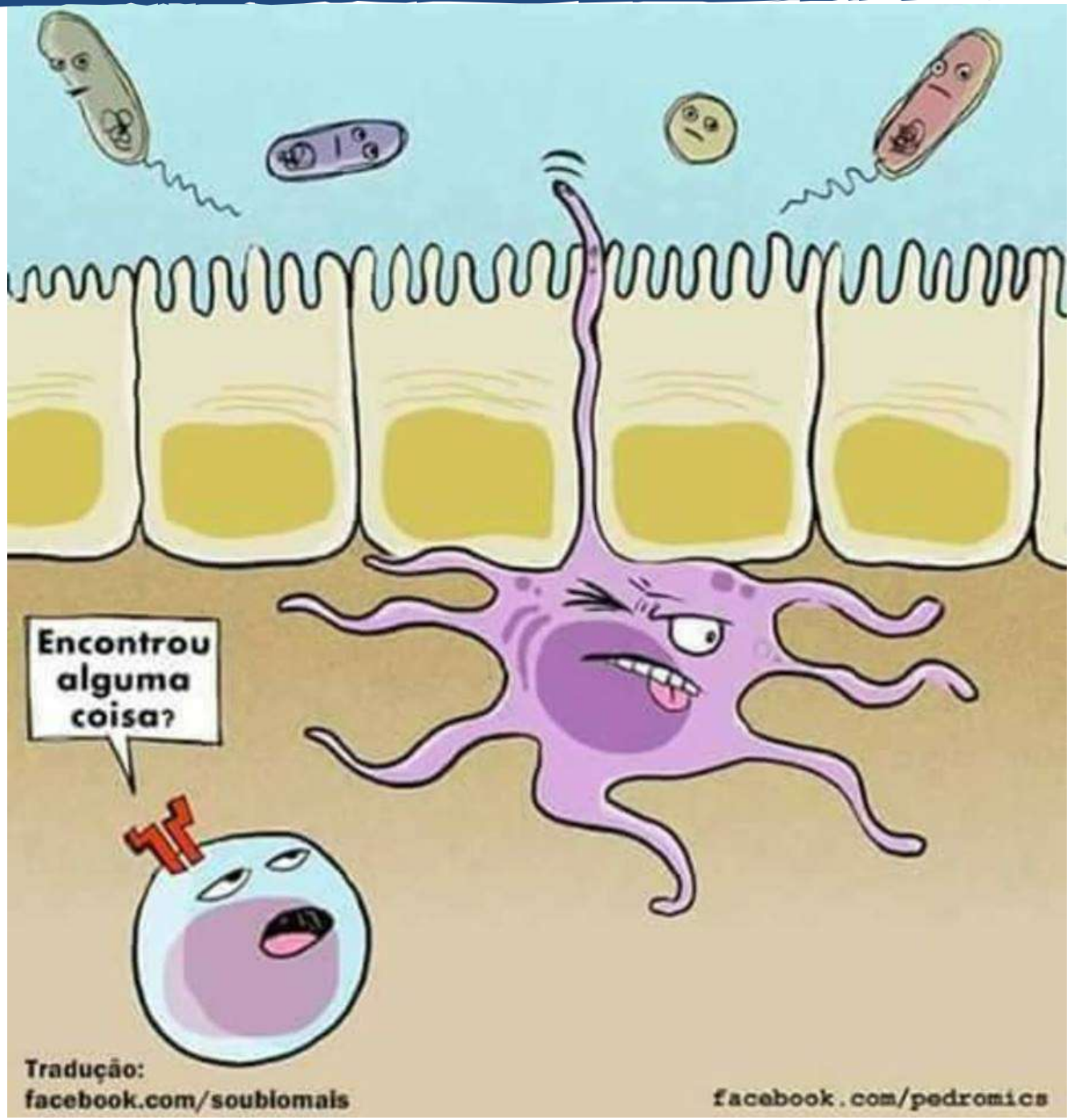
Barreira Física
- Epitélio/ Junções celulares
- Muco
- Peptídeos antimicrobianos
- *Epitélio Ciliado
- *pH

Barreira ativa
- Células dendríticas especializadas
- Células T efetoras especializadas (Th17 e Treg)
- IgA
- Células Inatas (macrófagos e ILCs)
- Microbiota

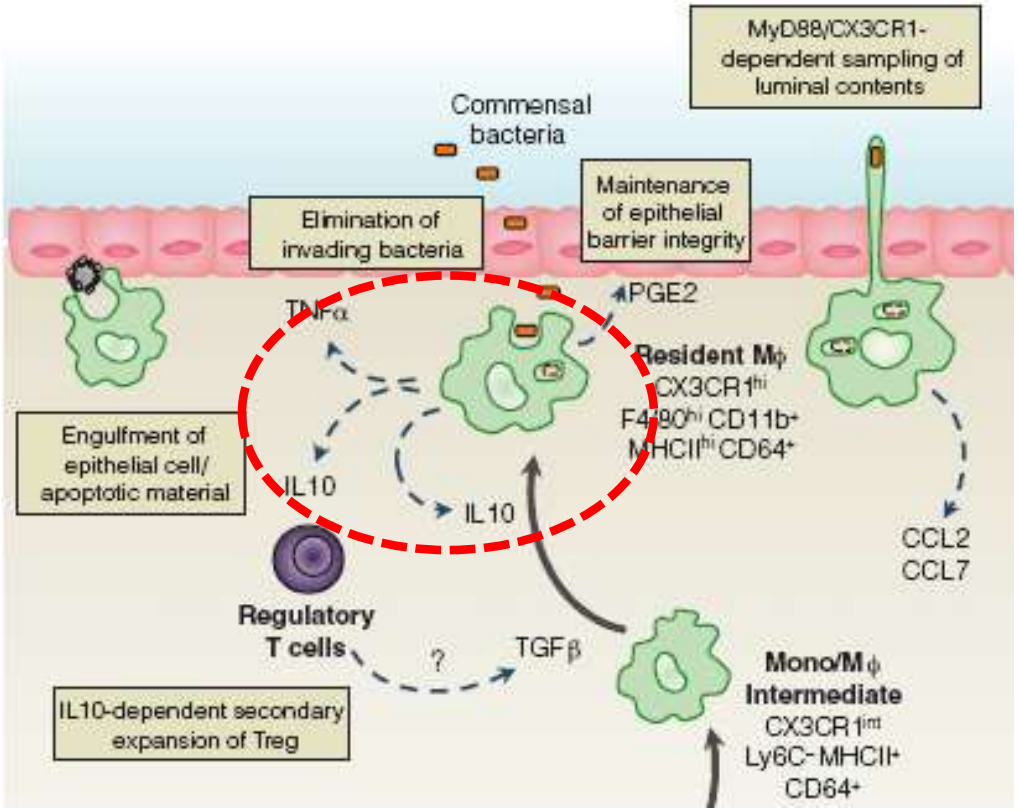


Cell 157, March 27, 2014

Sistema Fagocítico Mononuclear

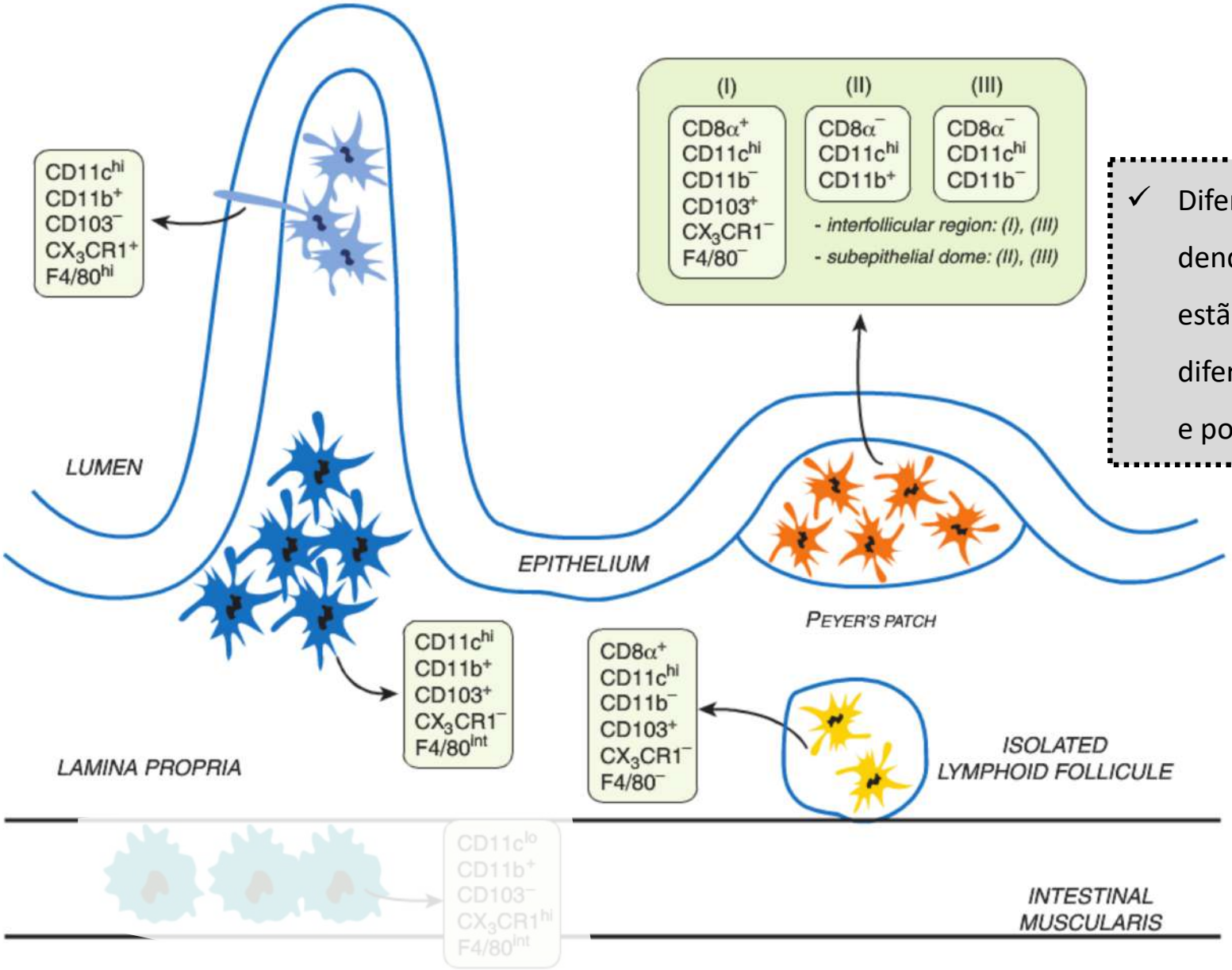


Sistema Fagocítico Mononuclear



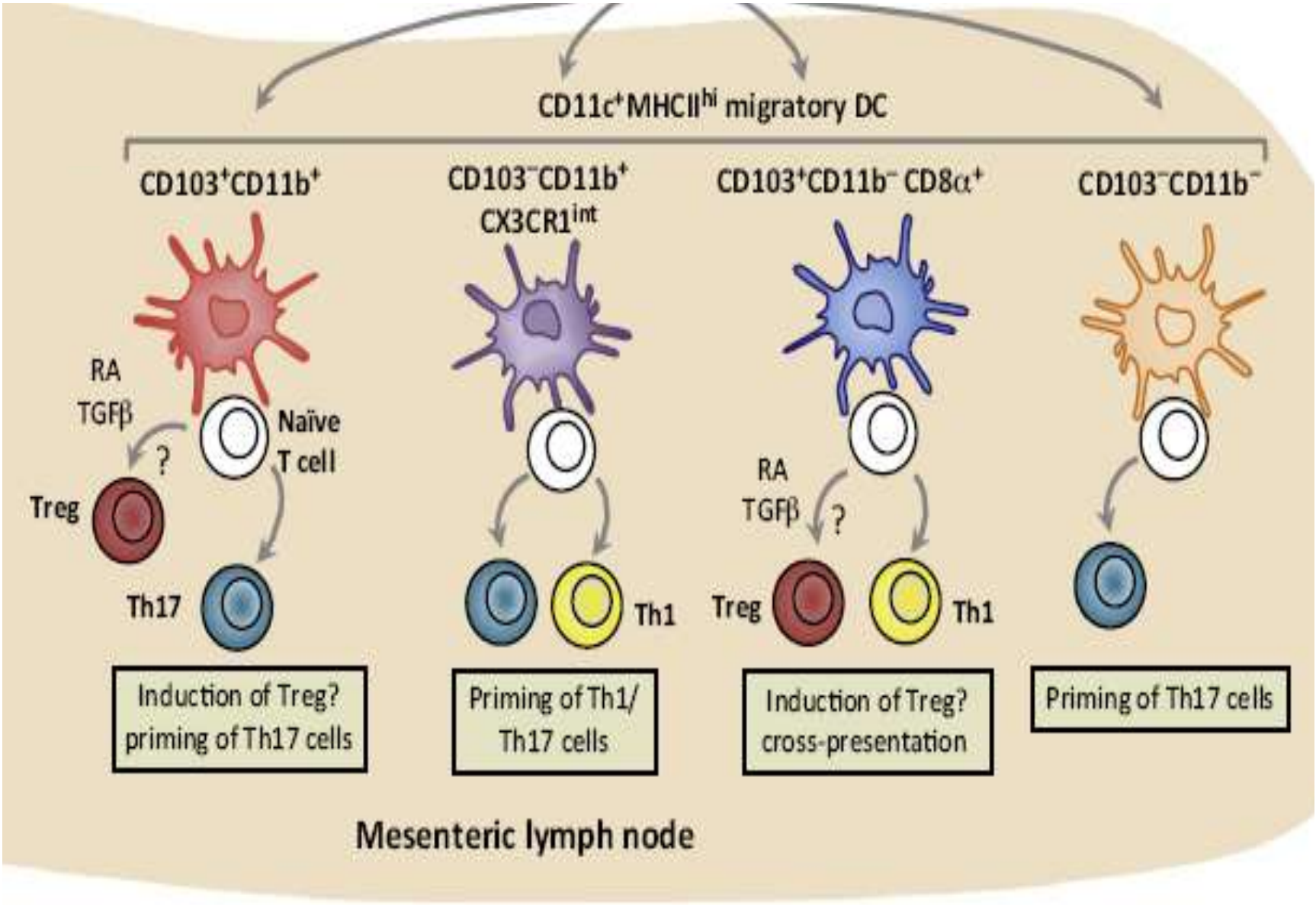
doi: 10.1111/imr.12192

Sistema Fagocítico Mononuclear



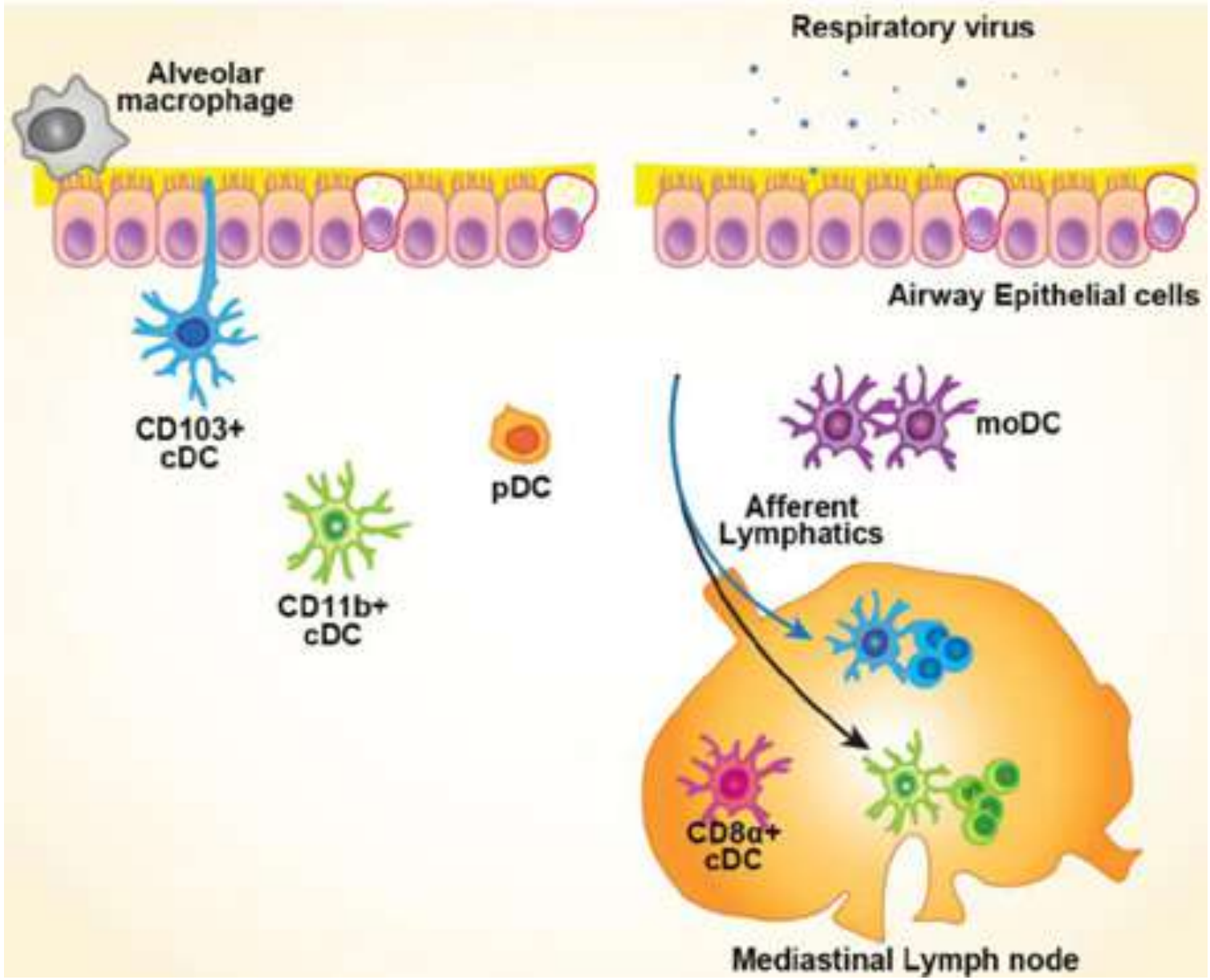
- ✓ Diferentes subtipos de células dendríticas e macrófagos estão localizados em diferentes regiões da mucosa e possuem diferentes funções

Subtipos de Células Dendríticas

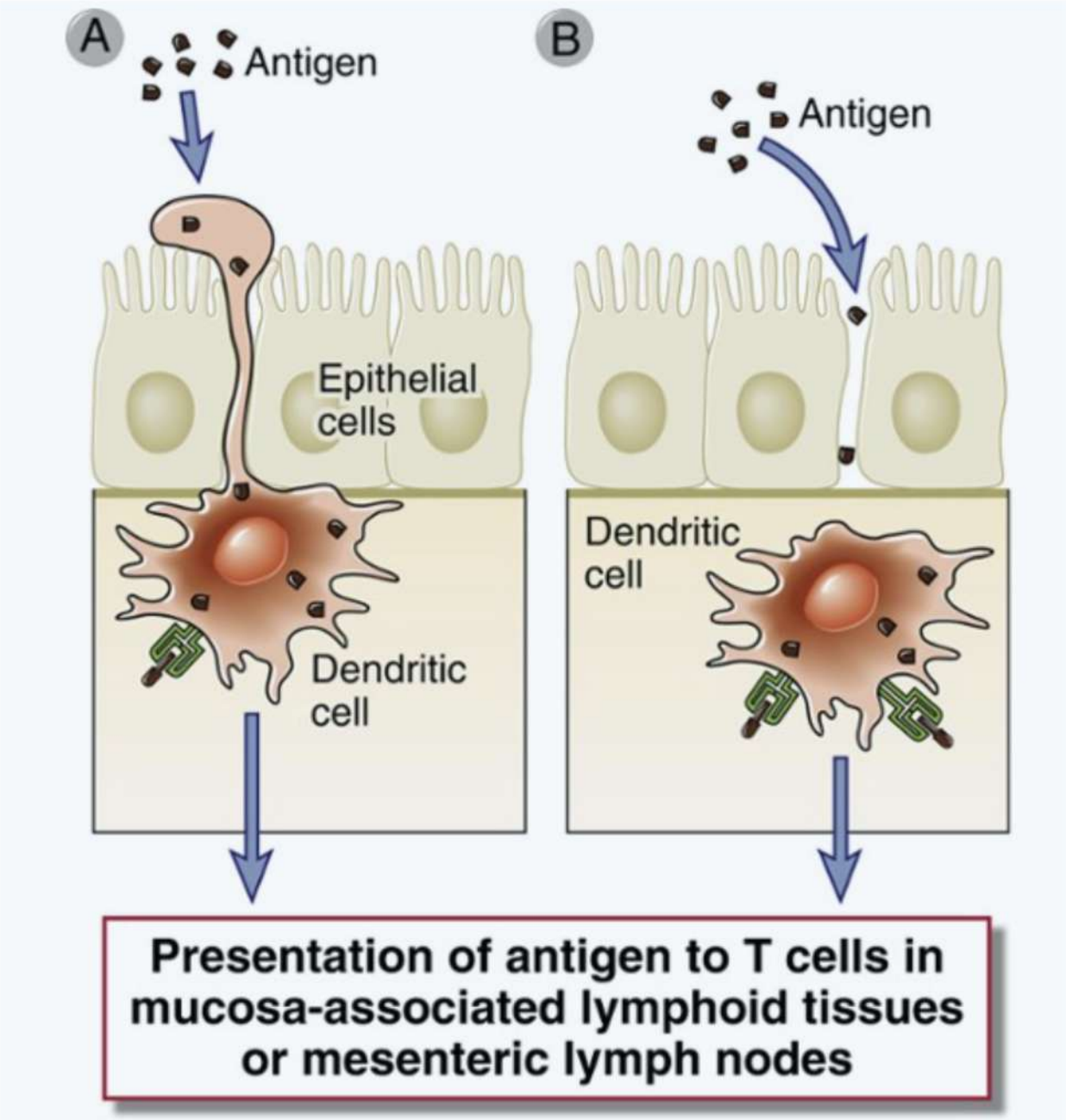


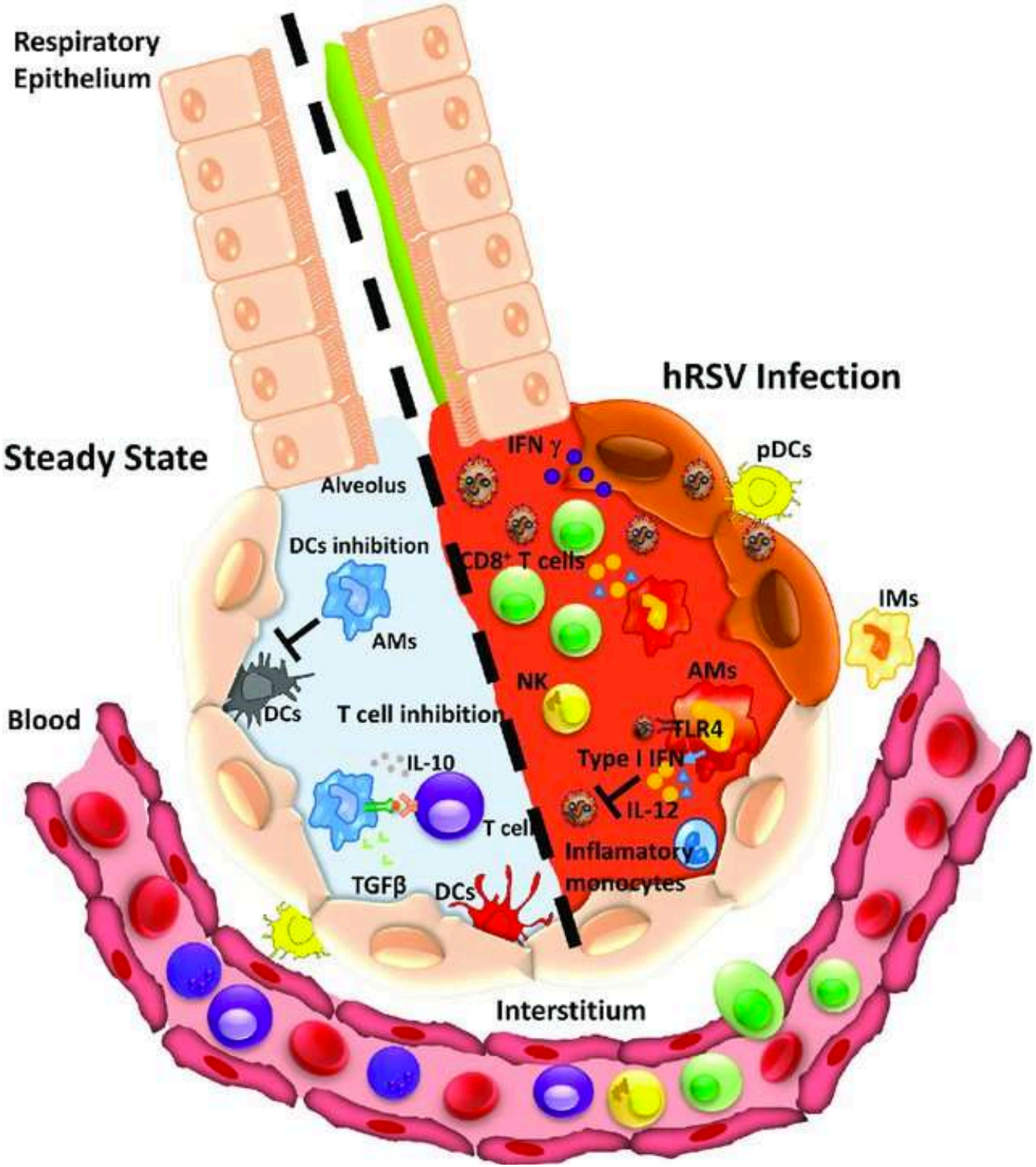
http://dx.doi.org/10.1016/j.it.2014.04.003

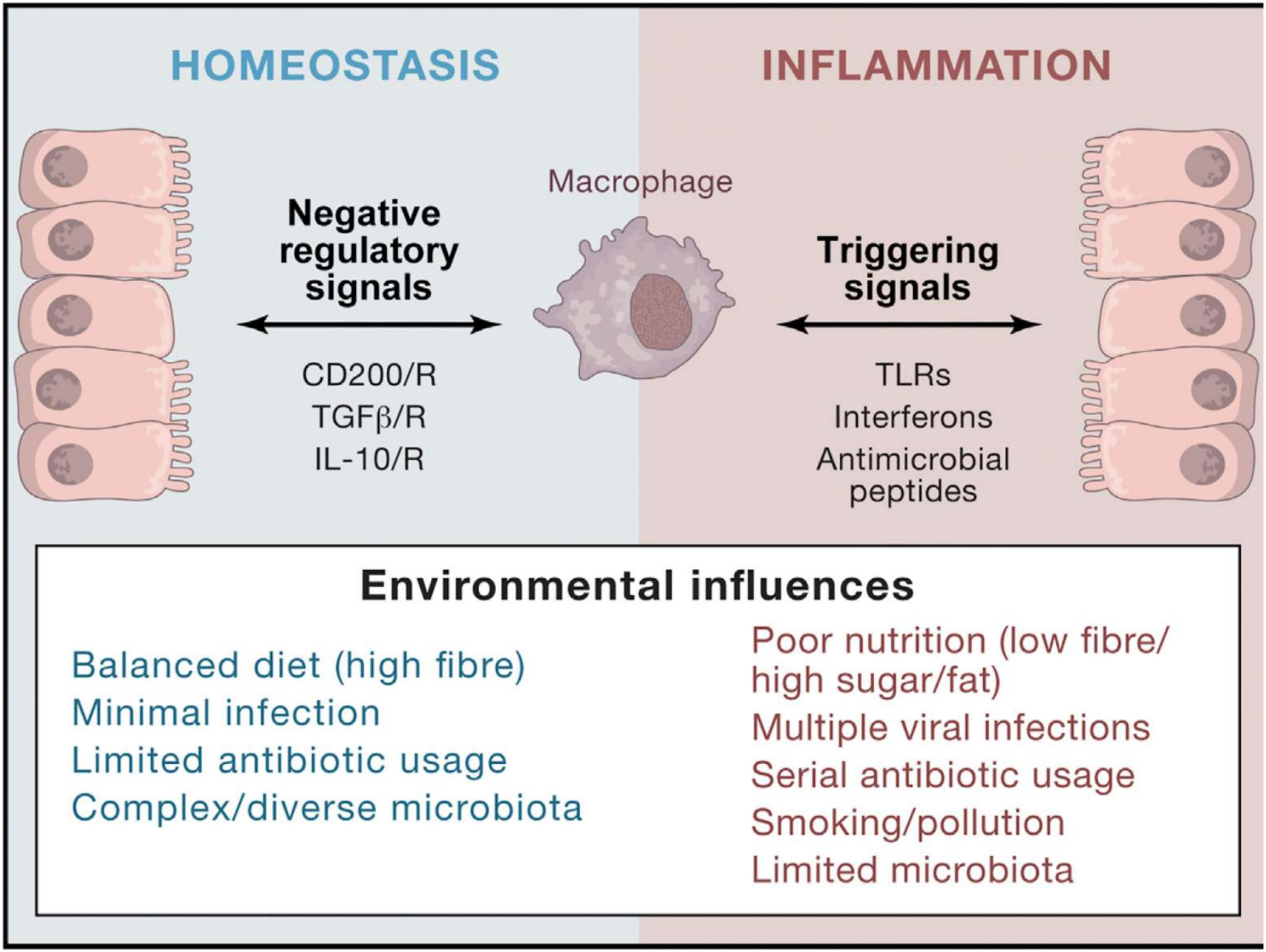
Fagócitos mononuclerares no pulmão



Antigen uptake




Respiratory Epithelium
Steady State
hRSV Infection
Alveolus
DCs inhibition
AMs
DCs
T cell inhibition
IL-10
T cell
TGFβ
DCs
Blood
IFN γ
pDCs
CD8+ T cells
IMs
AMs
NK
TLR4
Type I IFN
IL-12
Inflamatory monocytes
Interstitium


HOMEOSTASIS
INFLAMMATION
Macrophage
Negative regulatory signals
CD200/R
TGFβ/R
IL-10/R
Triggering signals
TLRs
Interferons
Antimicrobial peptides
Environmental influences
Balanced diet (high fibre)
Minimal infection
Limited antibiotic usage
Complex/diverse microbiota
Poor nutrition (low fibre/ high sugar/fat)
Multiple viral infections
Serial antibiotic usage
Smoking/pollution
Limited microbiota

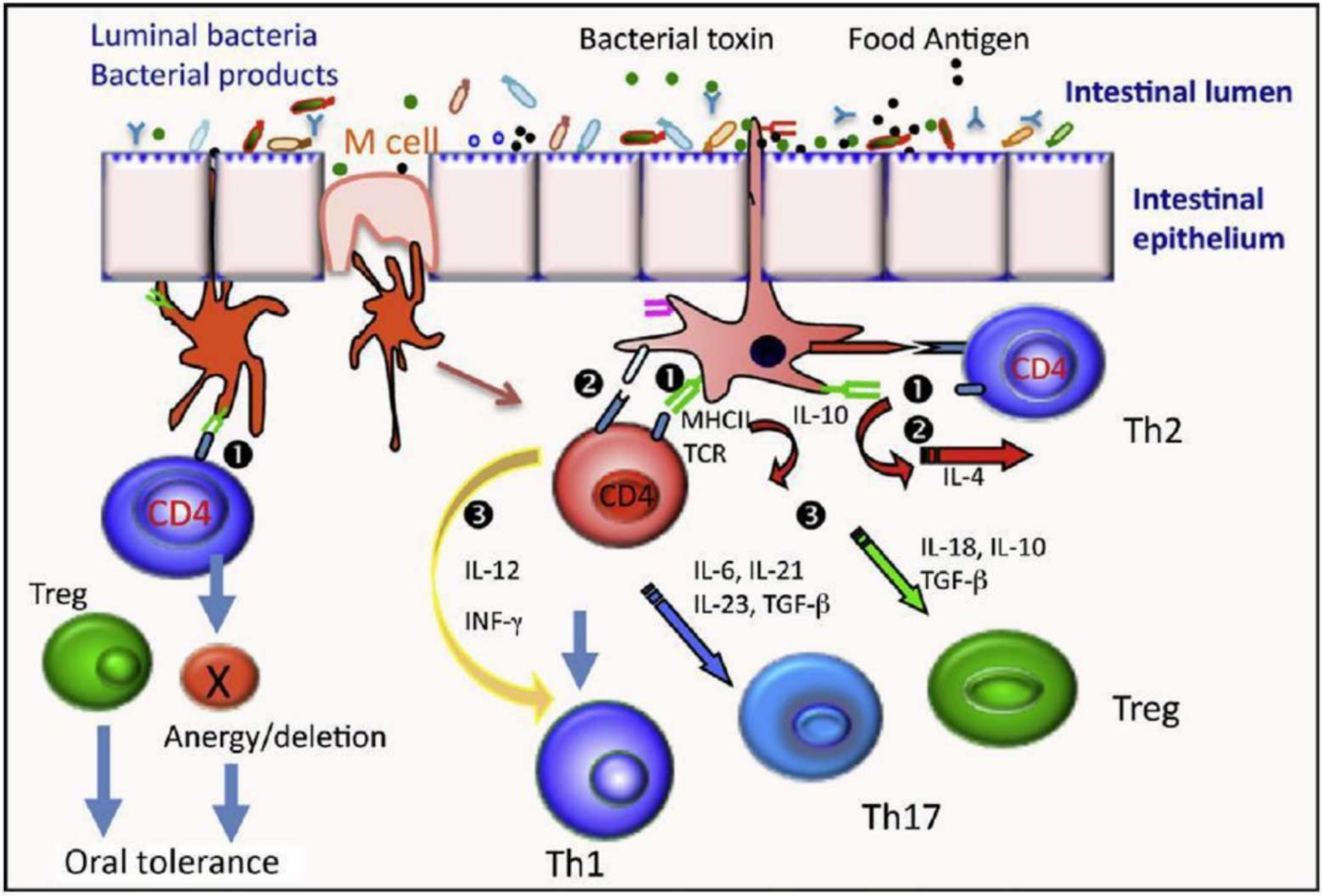
Função das células do sistema mononuclear fagocítico da mucosa

- Reparo tecidual
- Vigilância do tecido de barreira
- Ativação de linfócitos especializados com capacidade de migração para a mucosa
- Ontogenia: saco vitelínico, precursores de medula óssea, monócitos

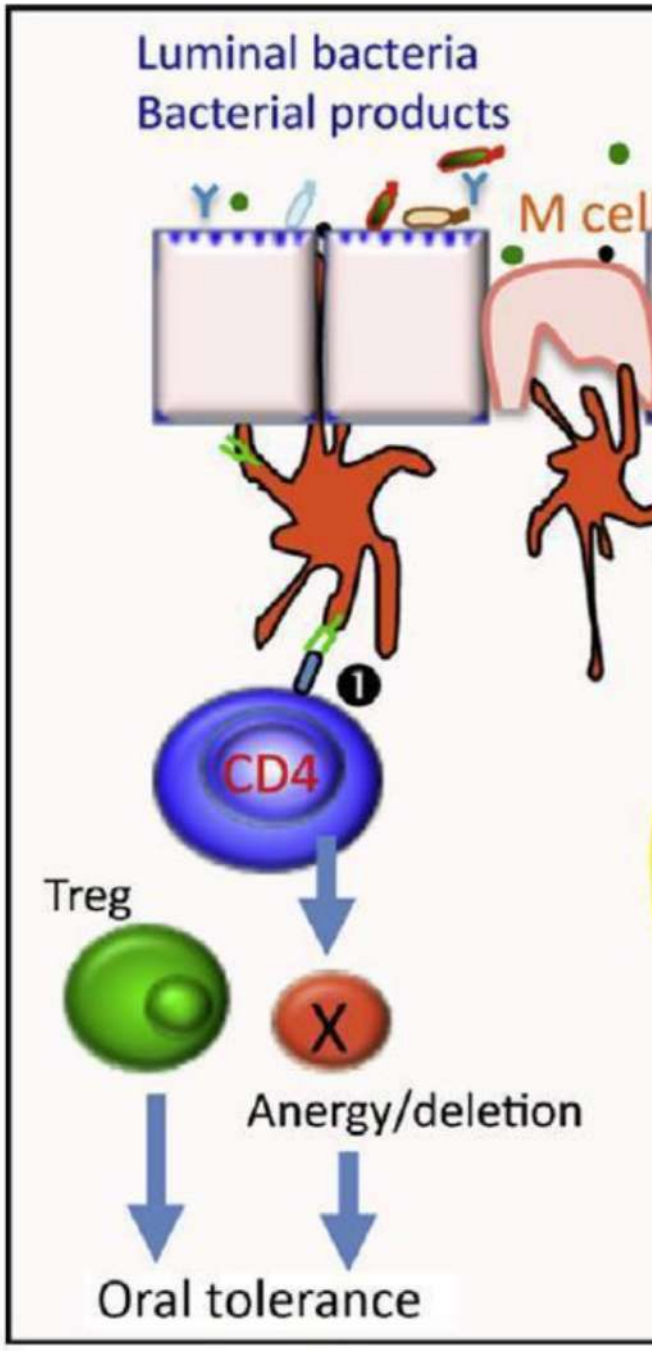
Tópicos da aula

- Conceitos e Componentes: barreira física
- Componentes: barreira ativa
- **Respostas canônicas:** Tolerância Oral, Imunidade a vacinas, Th17/22, IgA, Linfócitos Inatos
- Particularidades de tecidos de barreira
- Interação com microbiota e doença

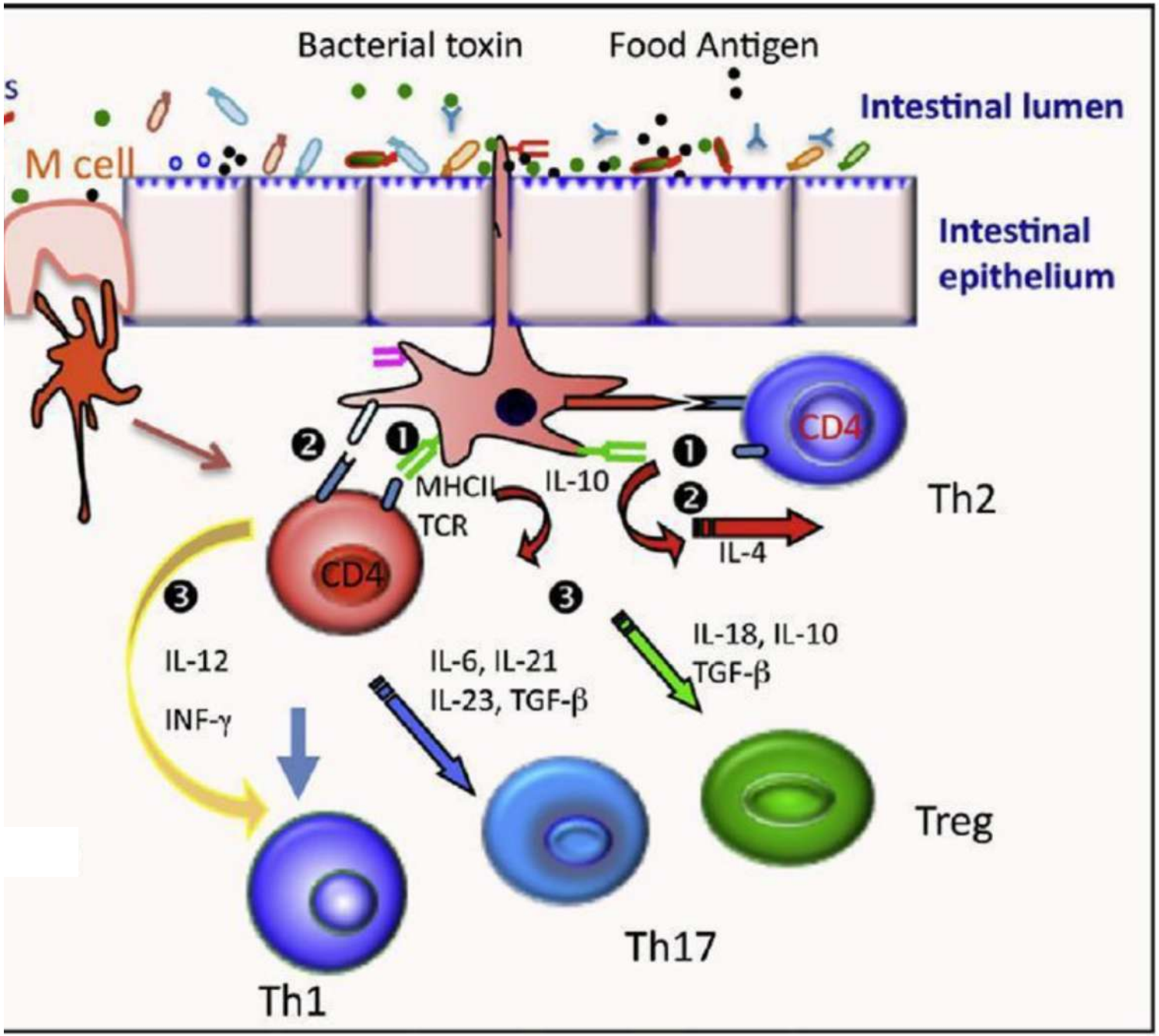
Dinâmica da resposta imune intestinal



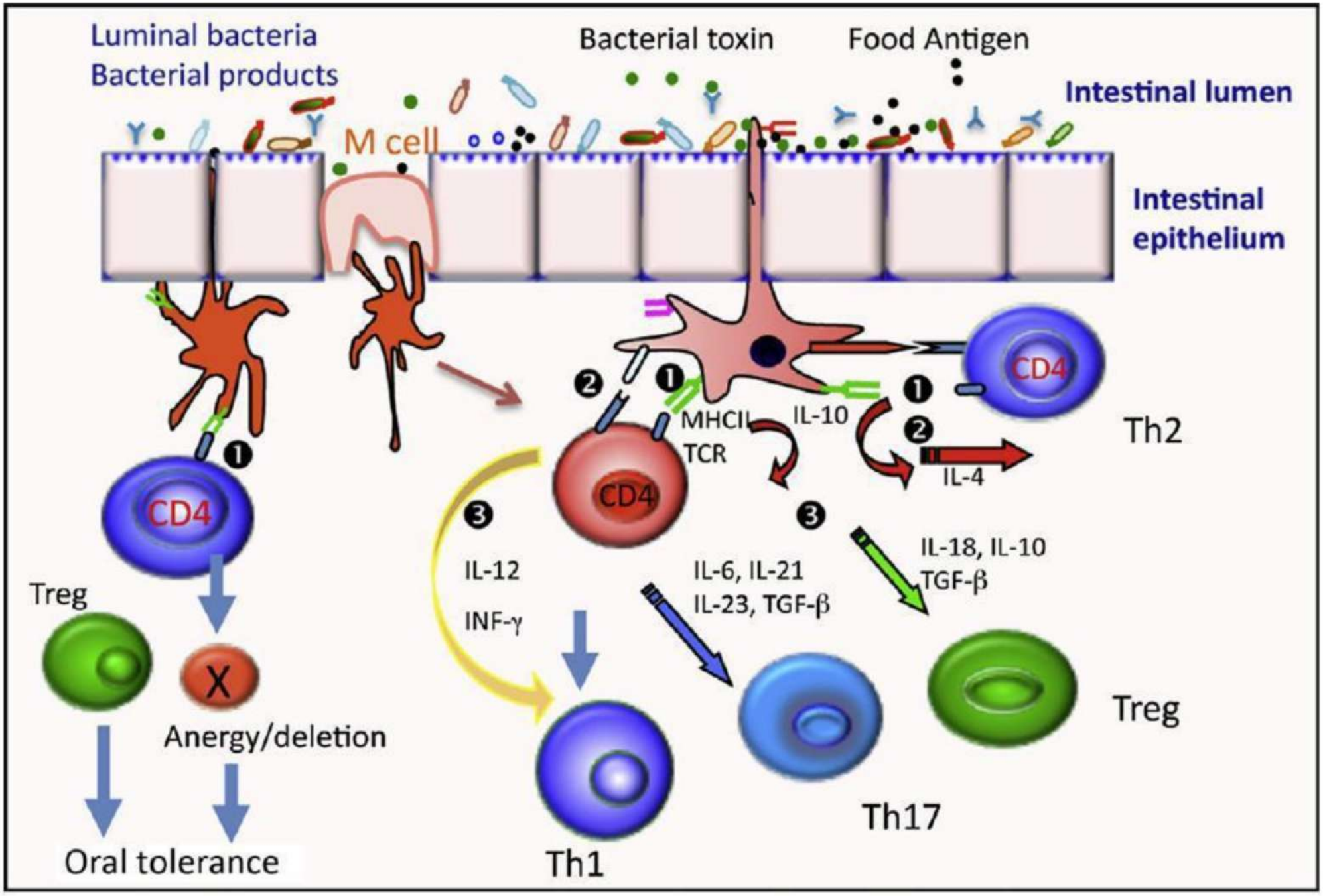
Dinâmica da resposta imune intestinal



Dinâmica da resposta imune intestinal



Dinâmica da resposta imune intestinal

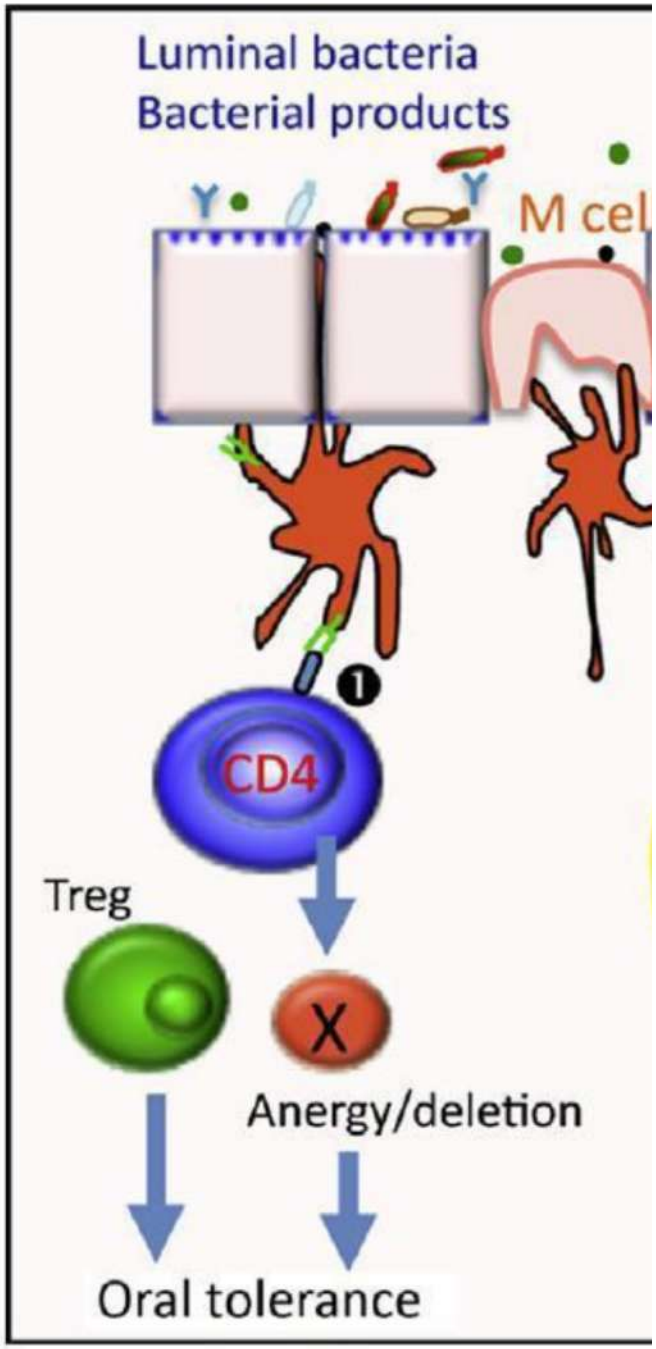


O que define qual padrão de resposta será induzido?

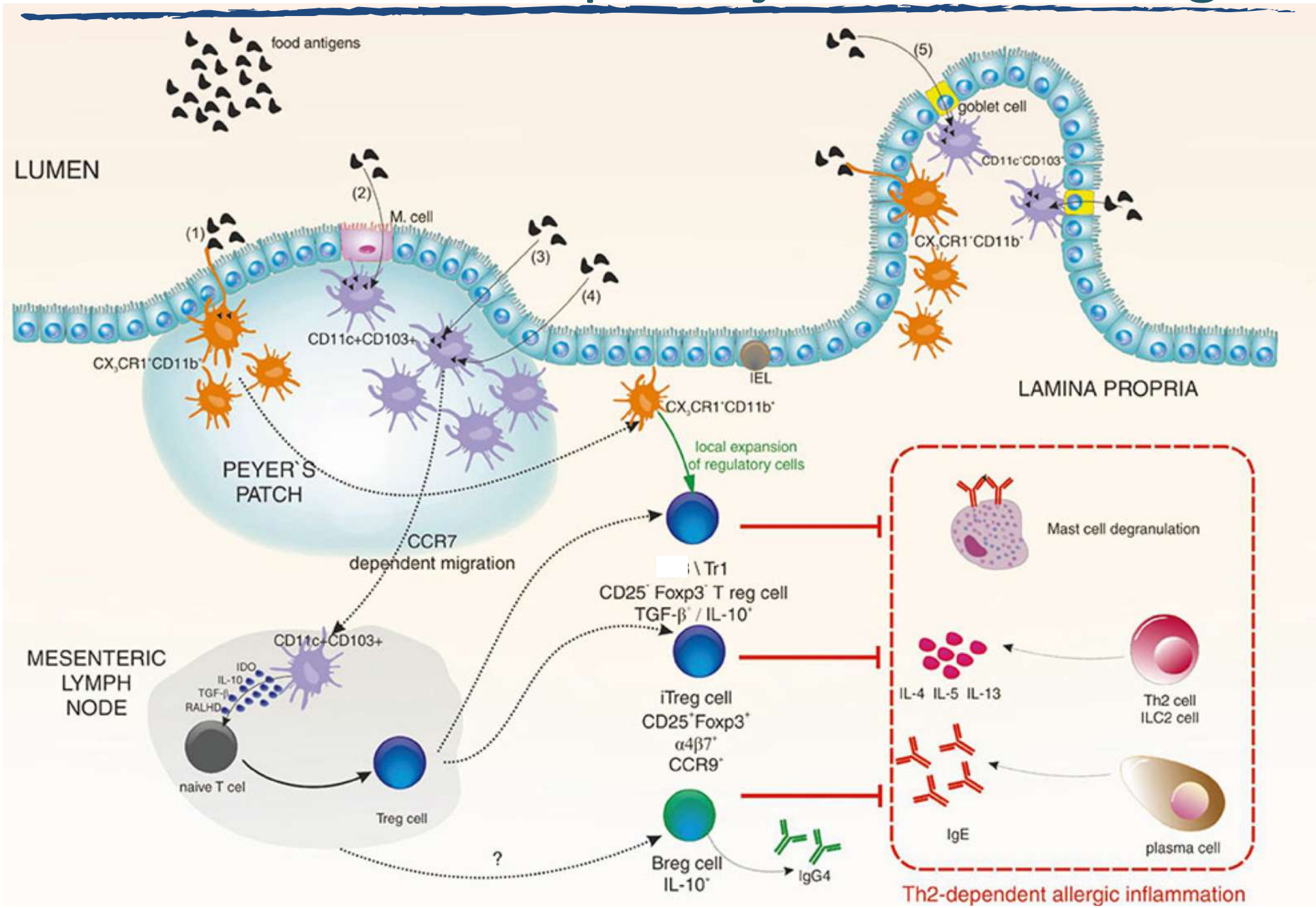
- Localização do Antígeno
- Subtipo de Célula Dendrítica
- Quantidade de Antígeno
- Presença de PAMPs e **DAMPs**
- Tropismo para tecidos de mucosa

Tolerância Oral

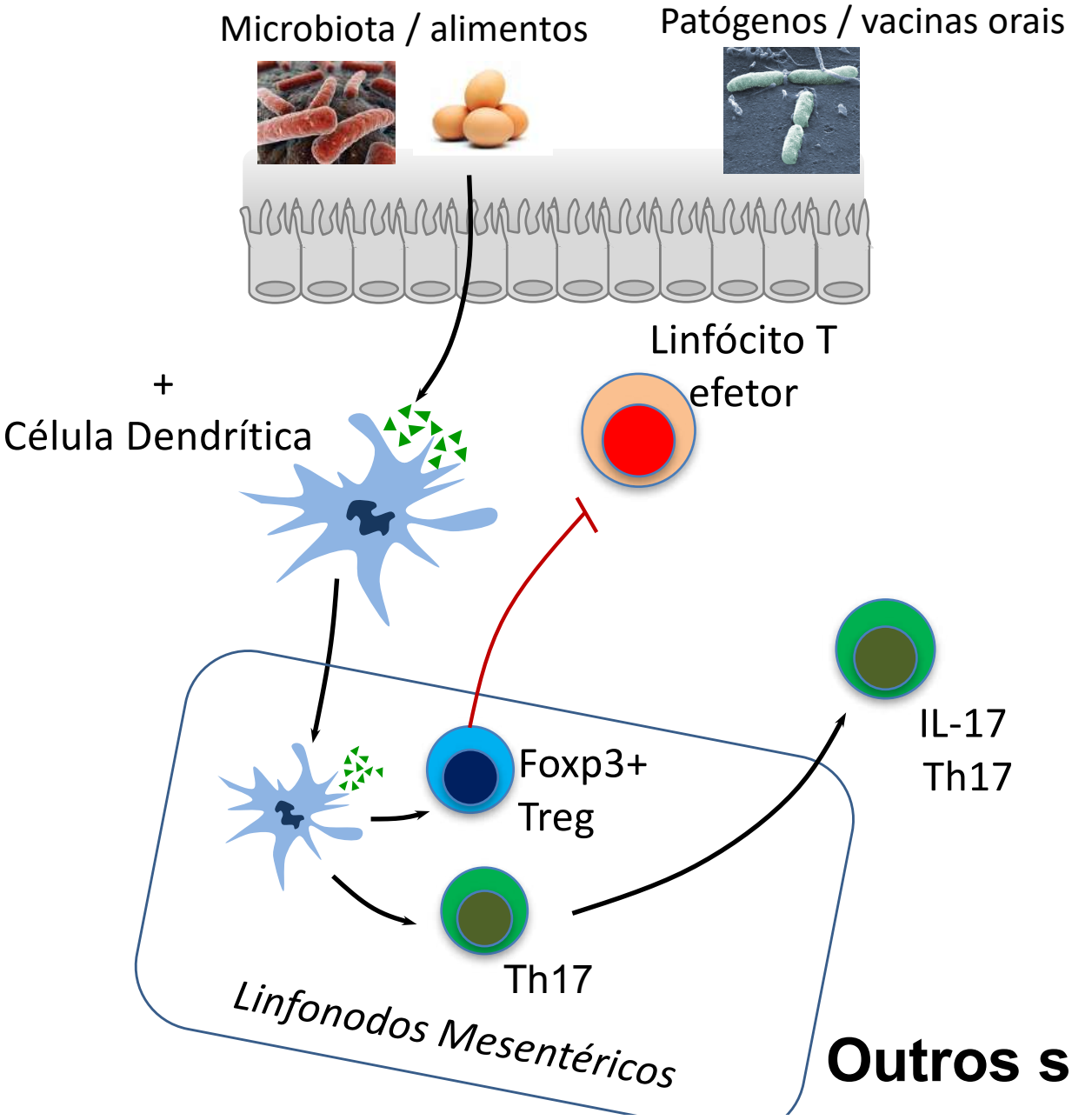
- ✓ Processo por meio do qual ocorre a indução de células T reguladoras em resposta à administração de antígenos pela via mucosa.
- ✓ Ocorre principalmente no intestino em resposta a antígenos alimentares
- ✓ Pode ser utilizado para a dessensibilização alérgica


Luminal bacteria
Bacterial products
M cel
1
CD4
Treg
X
Anergy/deletion
Oral tolerance

Tolerância Oral na proteção contra alergias



Ativação de linfócitos especializados



Th1
Th2
IgA

Outros sítios de mucosa

Vacinas Orais/nasais

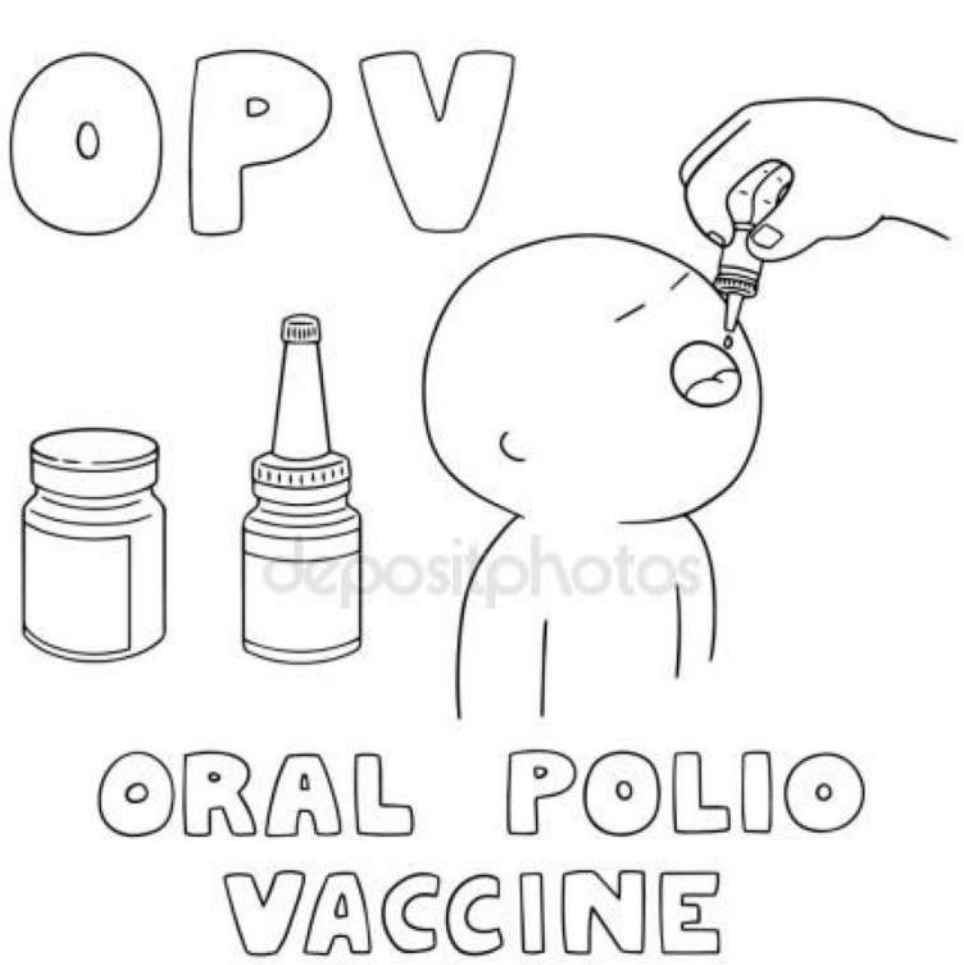


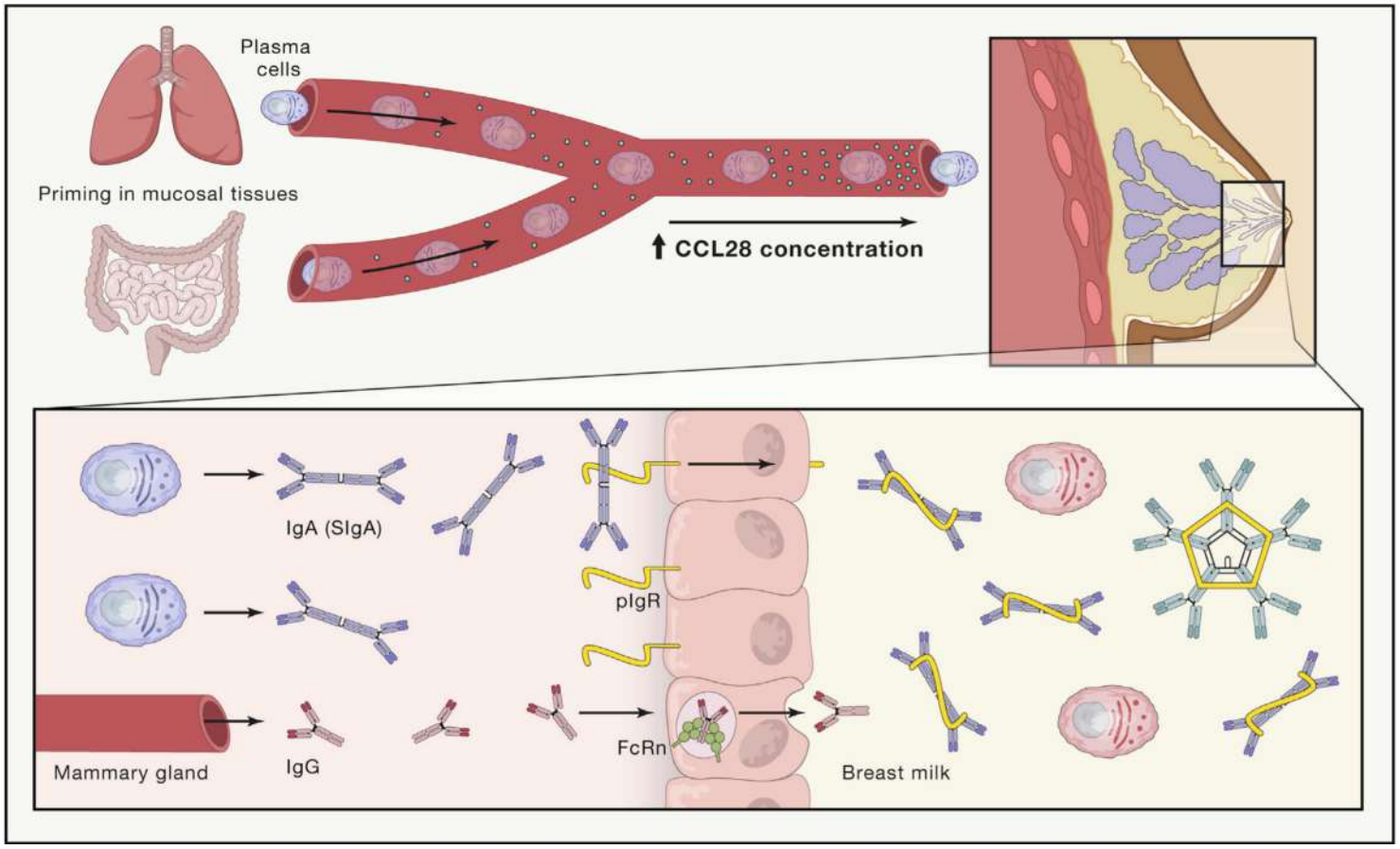
Table 1 Selected List of Bacterial and Food Antigens Used in Mucosal Immunization Studies in Humans and Animals

Antigen	Results and Comments	Author
Yersinia multocida (chicken cholera)	Oral immunization; protection induced	Pasteur (1880)
Vibrio cholerae	Oral immunization, moderate protection	Klemperer (1892) and Metchnikoff (1903)[a]
Mycobacterium tuberculosis	Serum antibodies induced by oral immunization	Calmette and Guérin (1906–1923)[a]
Yersinia pestis		
Corynebacterium diphtheria		Dserzgowdky (1910) and Enlows (1925)
Shigella dysenteriae	Limited protection	Besredka (1919, 1927)
Salmonella typhi	Oral immunization preferable to systemic	Vaillant (1922)[a], Besredka (1919, 1927) and Combiesco et al., (1923)
Streptococcus pneumoniae	Protection achieved by nasal immunization	Bull and McKee, 1929
Staphylococcus pyogenes	Protection achieved by oral immunization	Ross (1930)
Abrin, ricin, robin	Partial protection Oral immunization results in systemic and mucosal protection	Combiesco and Calab (1924) and Ehrlich (1891a, 1891b)
Cow's milk and whey	Prevention of anaphylaxis by feeding	Besredka (1909)
Cow's milk, ox blood, egg white, zein, oats	Decrease in systemic reactivity after prolonged but not short ingestion of these antigens	Wells and Osborne (1911)
Dinitrochlorobenzene	Inhibition of systemic (skin) reactivity after hapten feeding; inability to suppress skin sensitivity by oral immunization in previously sensitized animals[b]	Chase (1946)
Poison ivy	Oral ingestion results in decreased skin reactivity in a few studies; discouraged for lack of efficacy	Stevens (1945)
Horse serum and meat	Sensitization for anaphylaxis	Rosenau and Anderson (1907)[a]
Proteins from rice, corn, and oat flour	Precipitins in serum	Magnus, 1906[a]

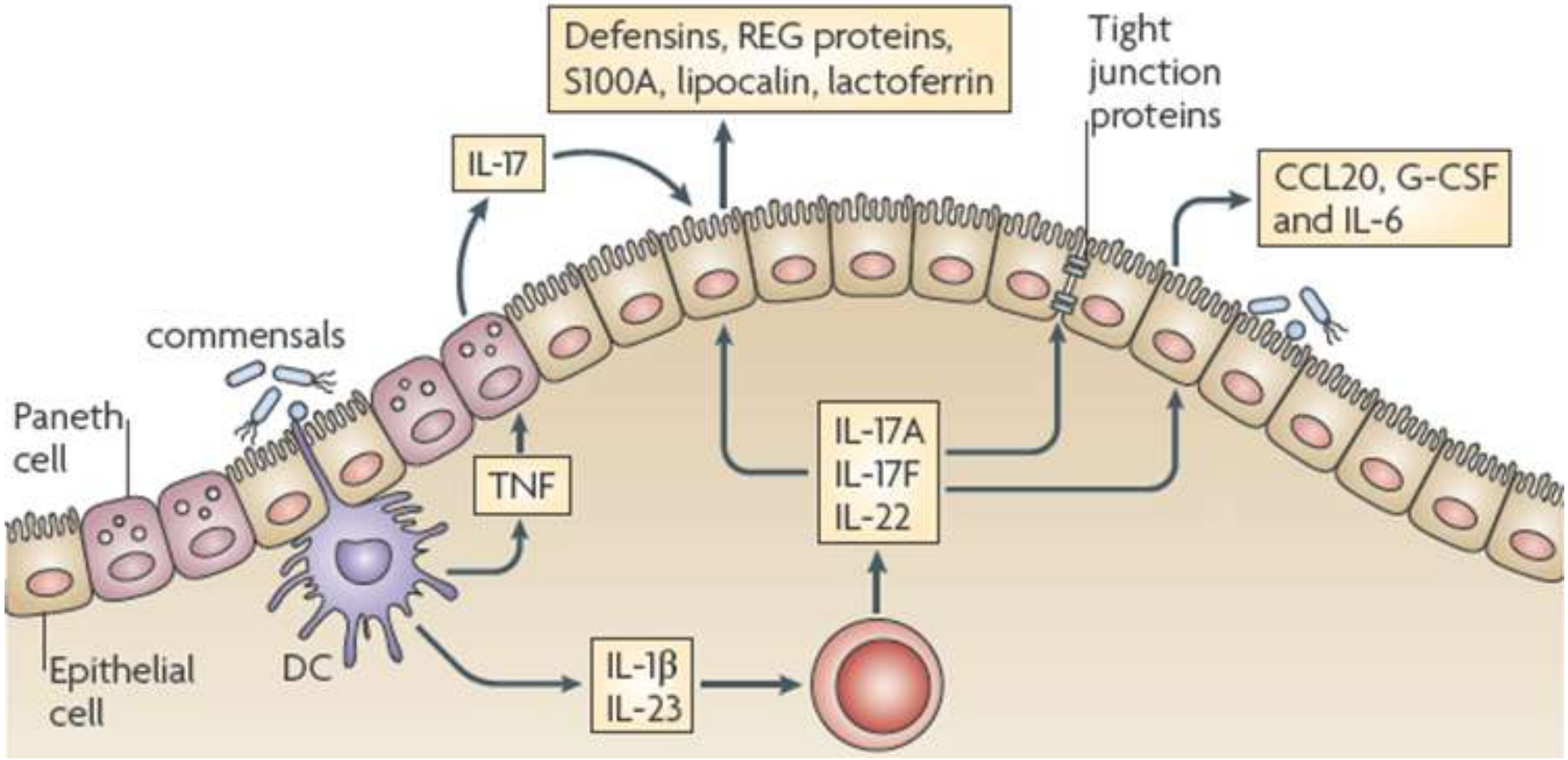
[a]*Data from Bull and McKee (1929), Chase (1946), Gay (1924), Stevens (1945), Klingman (1958), Wells and Osborne (1911), and Calmette (1923).*
[b]*See Table 3.*

Circulação de linfócitos especializados entre as diferentes mucosas

- ✓ Imunização por via oral ou nasal pode ser empregada para indução de imunidade em outros tecidos de mucosa, como por explo no trato urogenital.



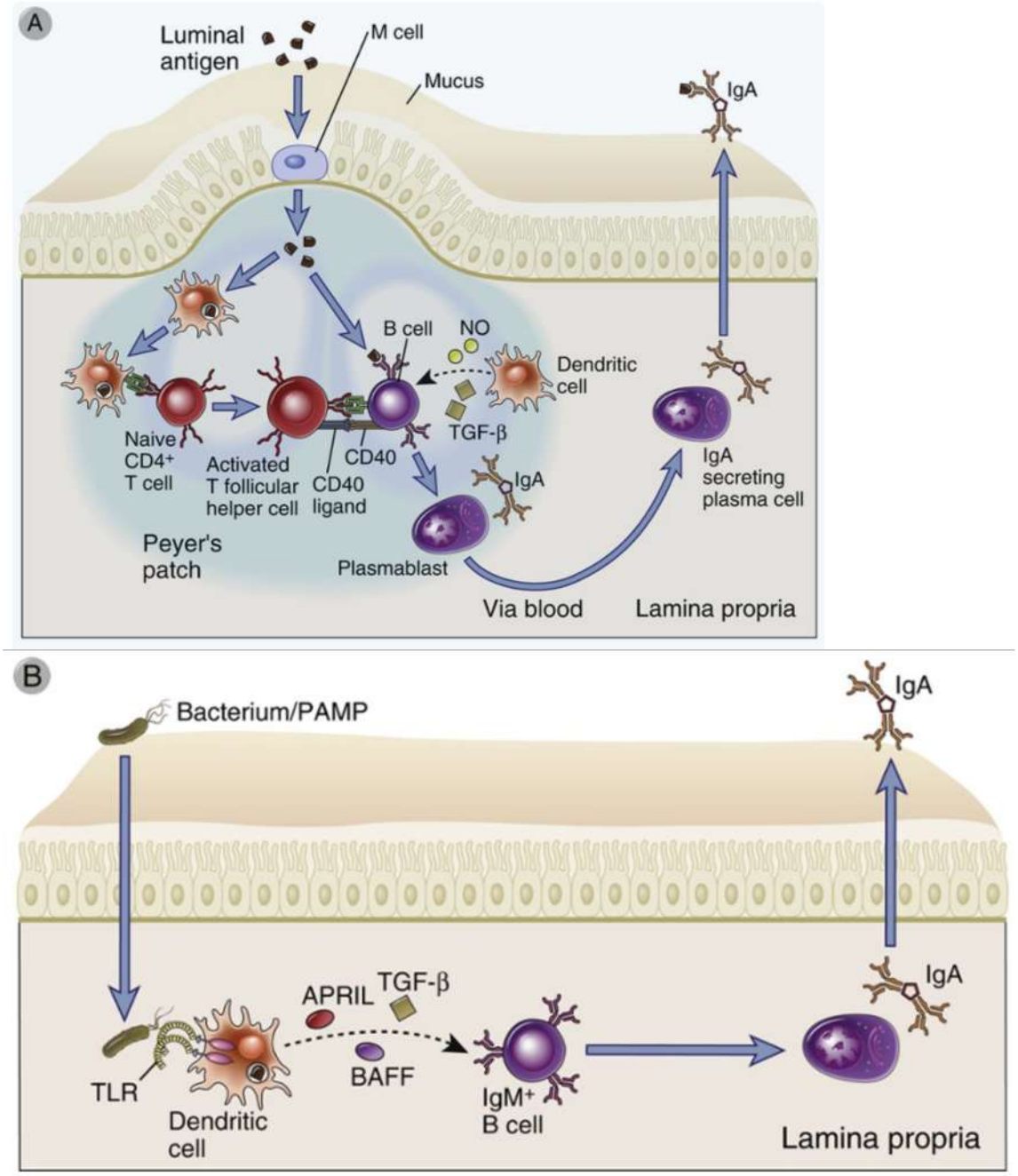
Th17/22



Patógenos extracelulares
Vacinas

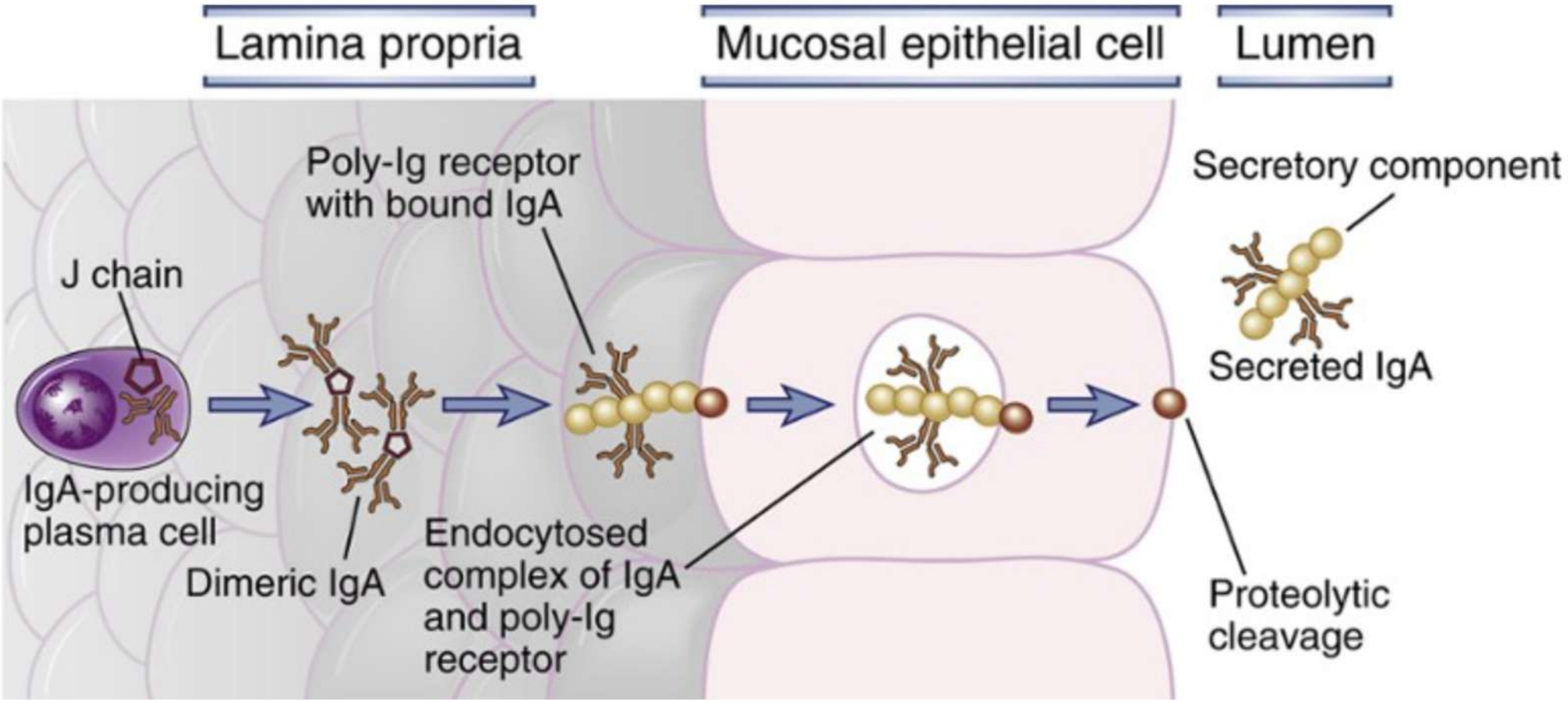
doi:10.1038/nri2800

Produção de IgA



- Pulmão, troca de classe acontece nas tonsilas
- Trato urogenital: IgG

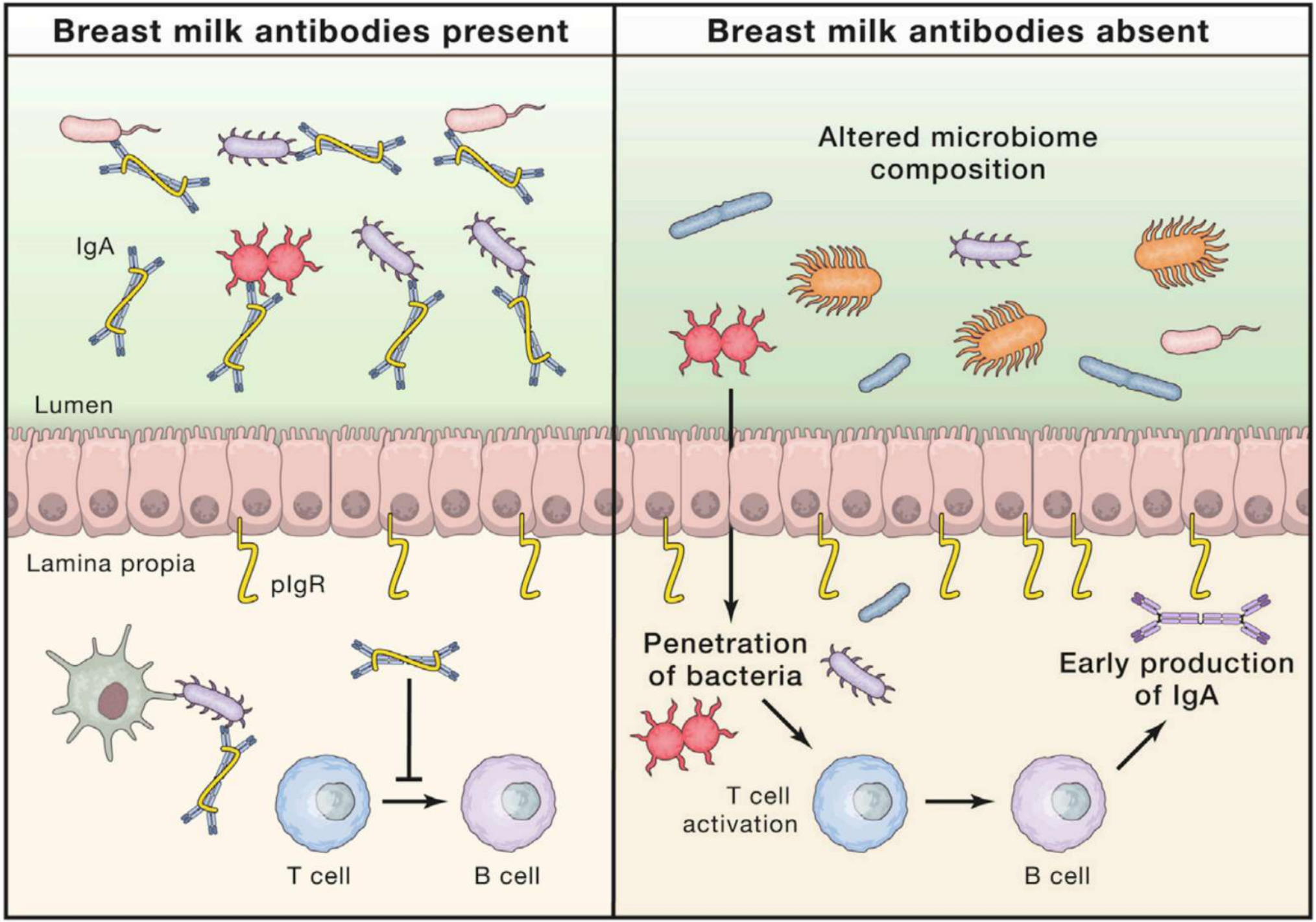
Secreção de IgA

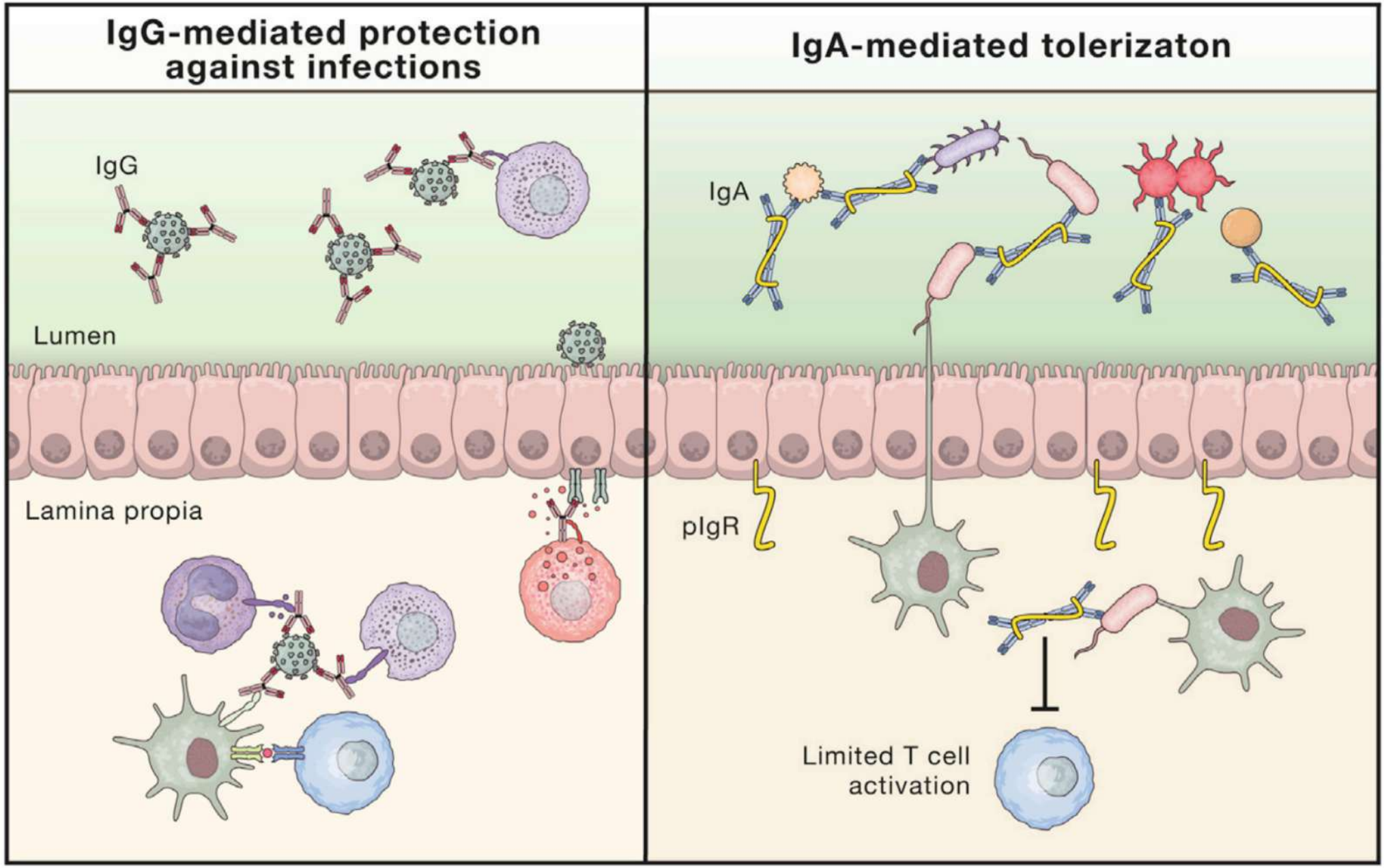


*Leite Materno

* Pele???

* Grande parte da microbiota associada às mucosas está recoberta por IgA


Breast milk antibodies present
Breast milk antibodies absent
IgA
Lumen
Lamina propia
pIgR
T cell
B cell
Altered microbiome composition
Penetration of bacteria
T cell activation
B cell
Early production of IgA

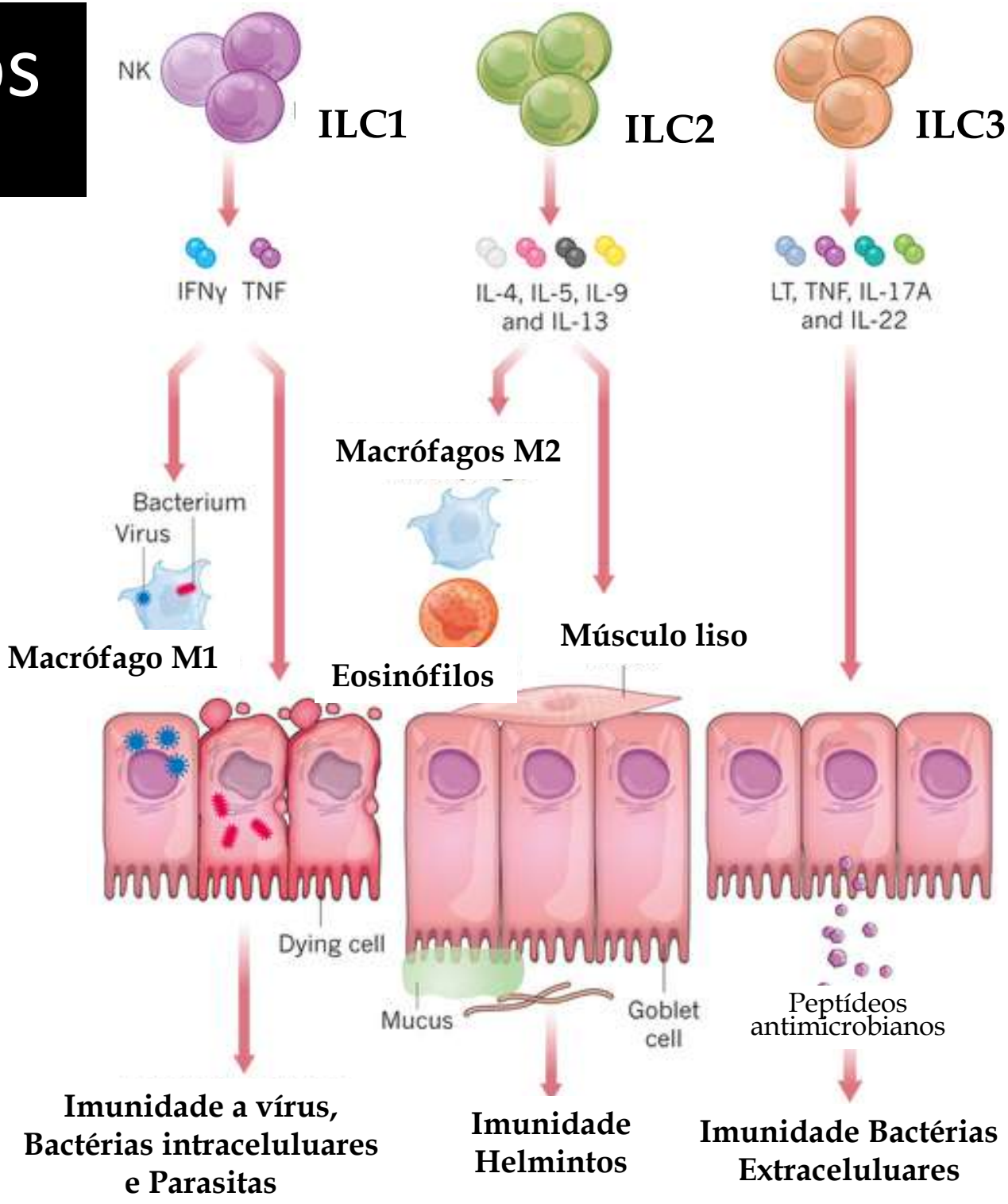

IgG-mediated protection against infections
IgA-mediated tolerizaton
IgG
Lumen
Lamina propia
IgA
pIgR
Limited T cell activation

Linfócitos Inatos
ILCs

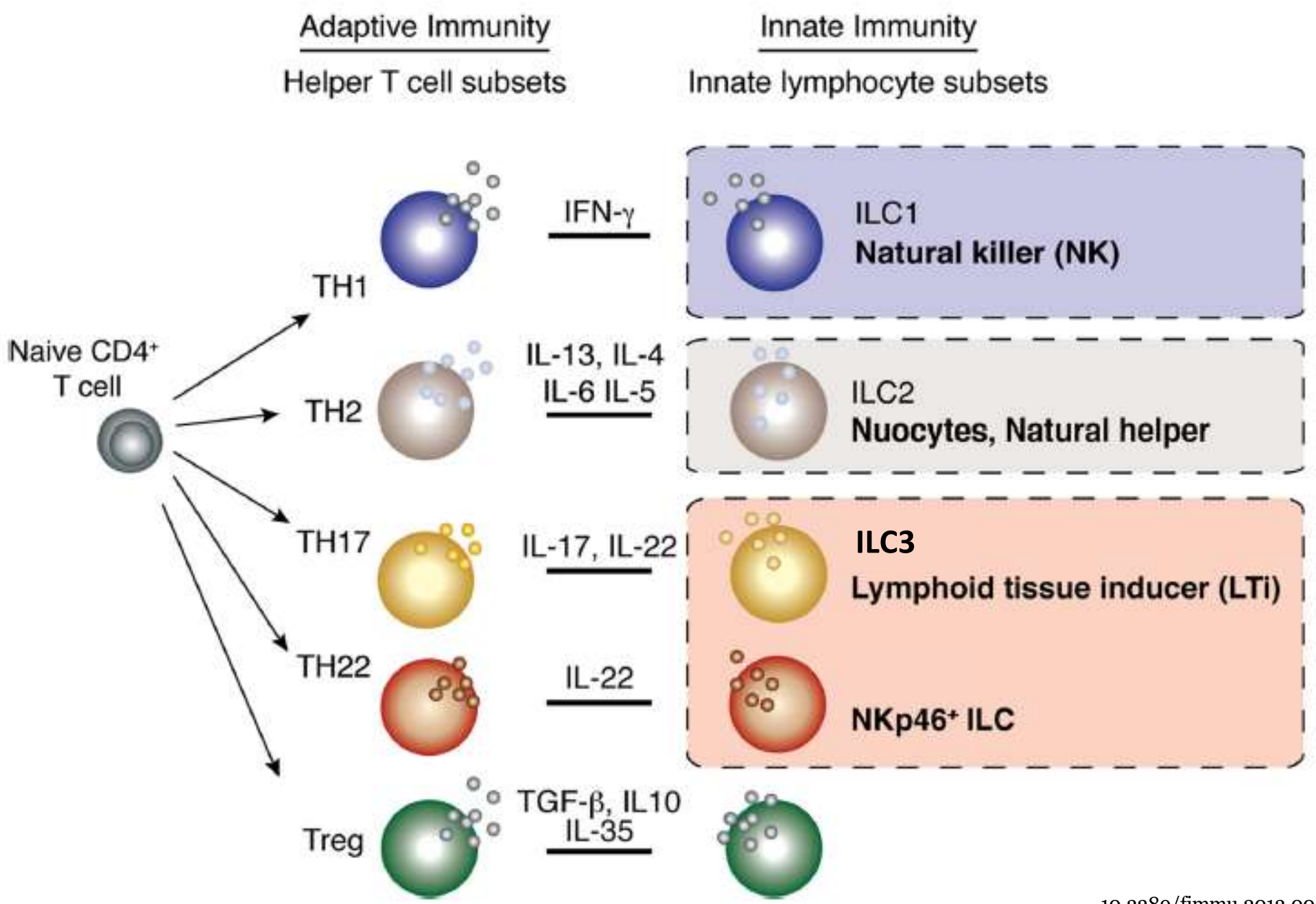
Células Linfóides Inatas

(Inate lympohoid cells - ILCs)

- ✓ Não expressam marcadores de Linhagem ou Receptores de antígeno
- ✓ Ativados por citocinas e metabólitos
- ✓ Expressam fatores de transcrição correspondentes ao expressos por subtipos de linfócitos T
- ✓ "Versão inata" dos subtipos de linfócitos T

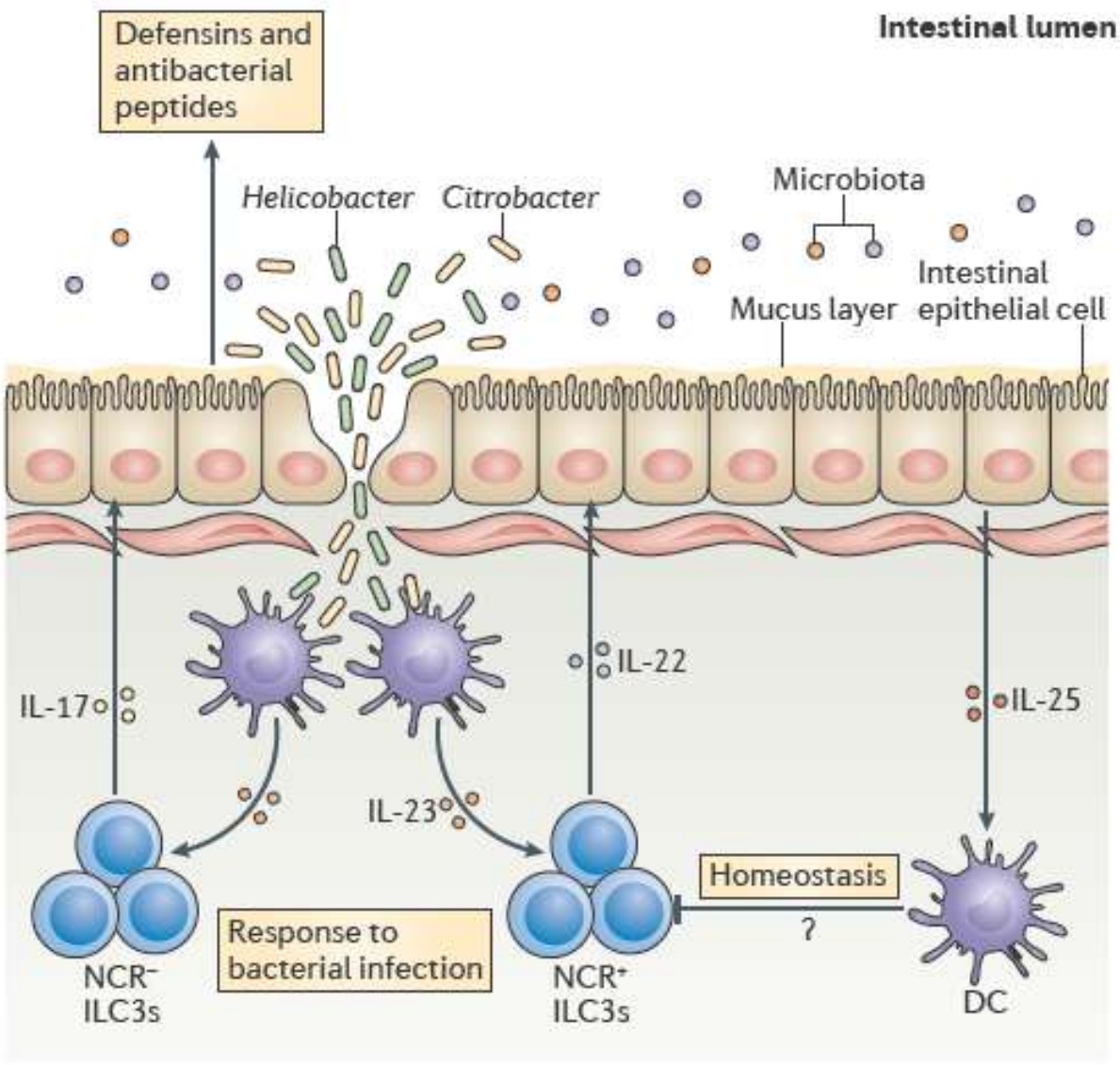


Innate Lymphoid Cells (ILCs)



10.3389/fimmu.2013.00022

ILCs – Função no intestino e pulmão



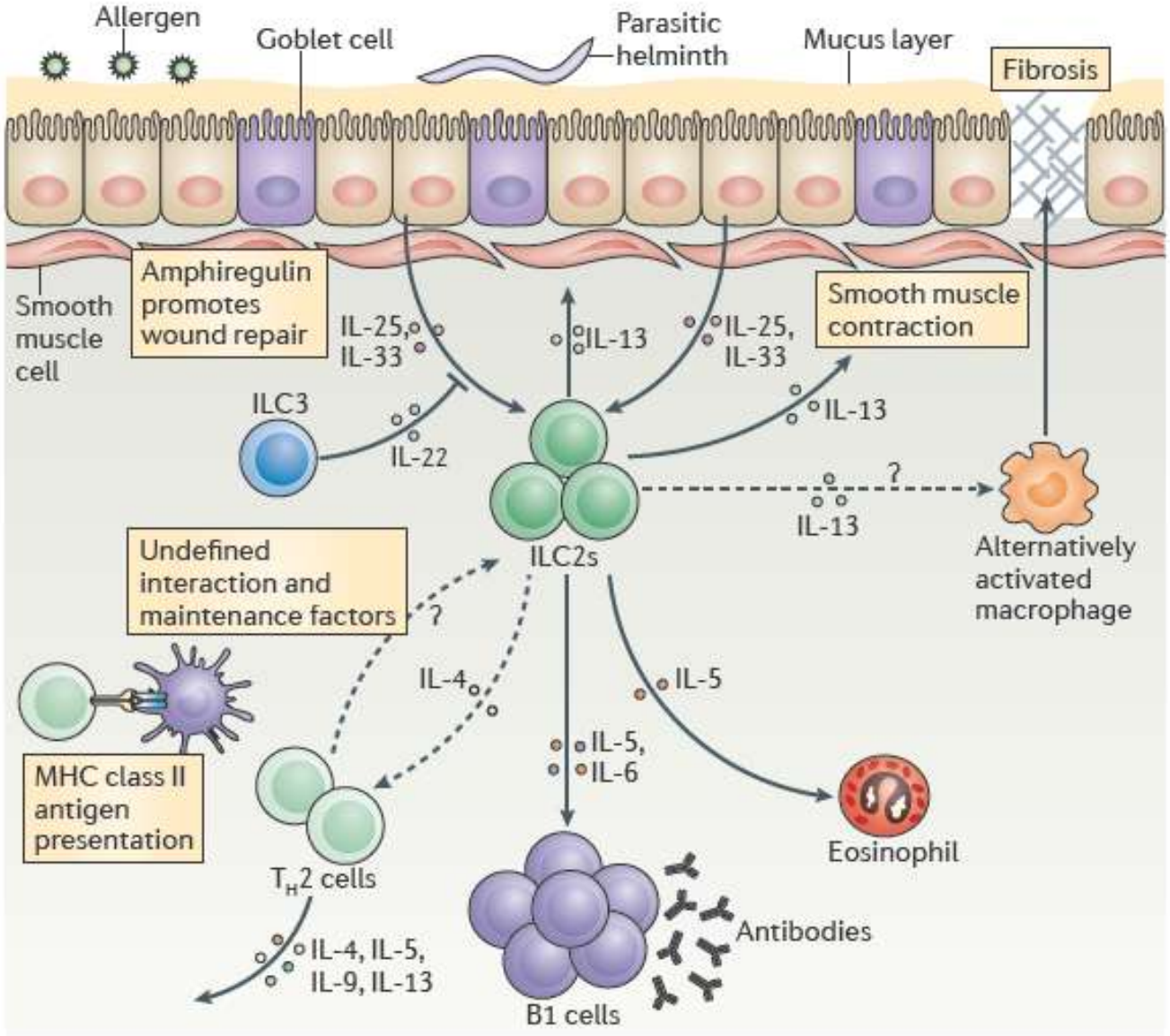
ILC1: imunidade contra bactérias intracelulares

ILC2: indução da produção de muco, recrutamento de eosinófilos

ILC3: Produção de peptídeos antimicrobianos, Reforço da barreira

doi:10.1038/nri3349

ILCs – Função no intestino e pulmão



ILC1: imunidade contra bactérias intracelulares

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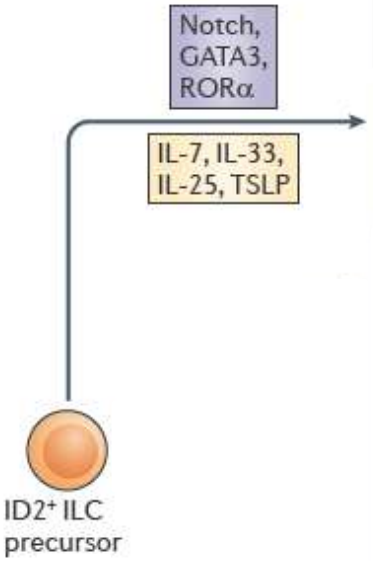
ILC3: Produção de peptídeos antimicrobianos, Reforço da barreira

doi:10.1038/nri3349

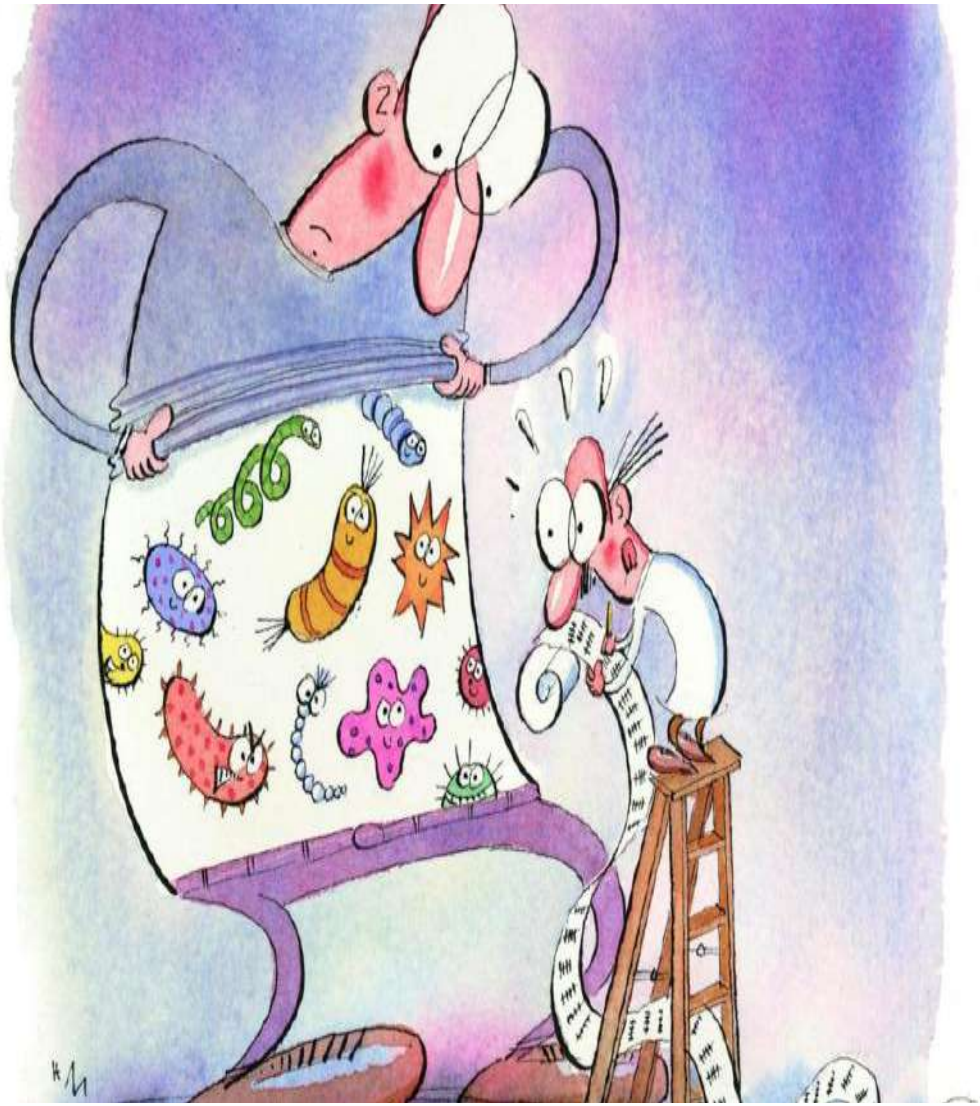
Função de linfócitos inatos e adaptativos e associação com doença



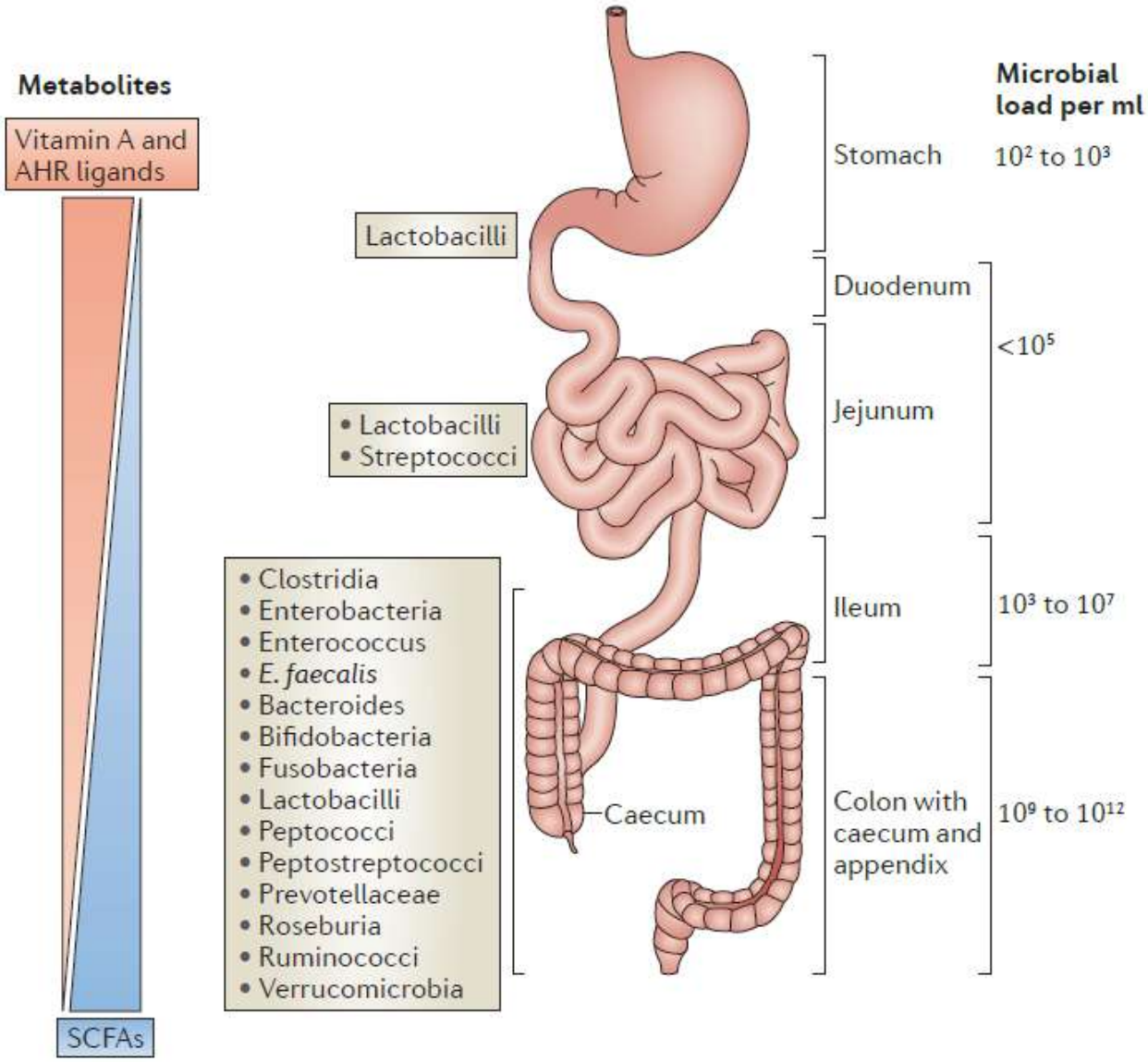
	New nomenclature	New group nomenclature	Mediators produced	Function	Disease association
Nuocyte, NHC, I_H2 cell	ILC2	Group 2 ILCs	IL-5, IL-9, IL-13, amphiregulin	• Immunity to helminths • Wound healing	Allergy and asthma

doi:10.1038/nri3349

Microbiota e imunidade de mucosa

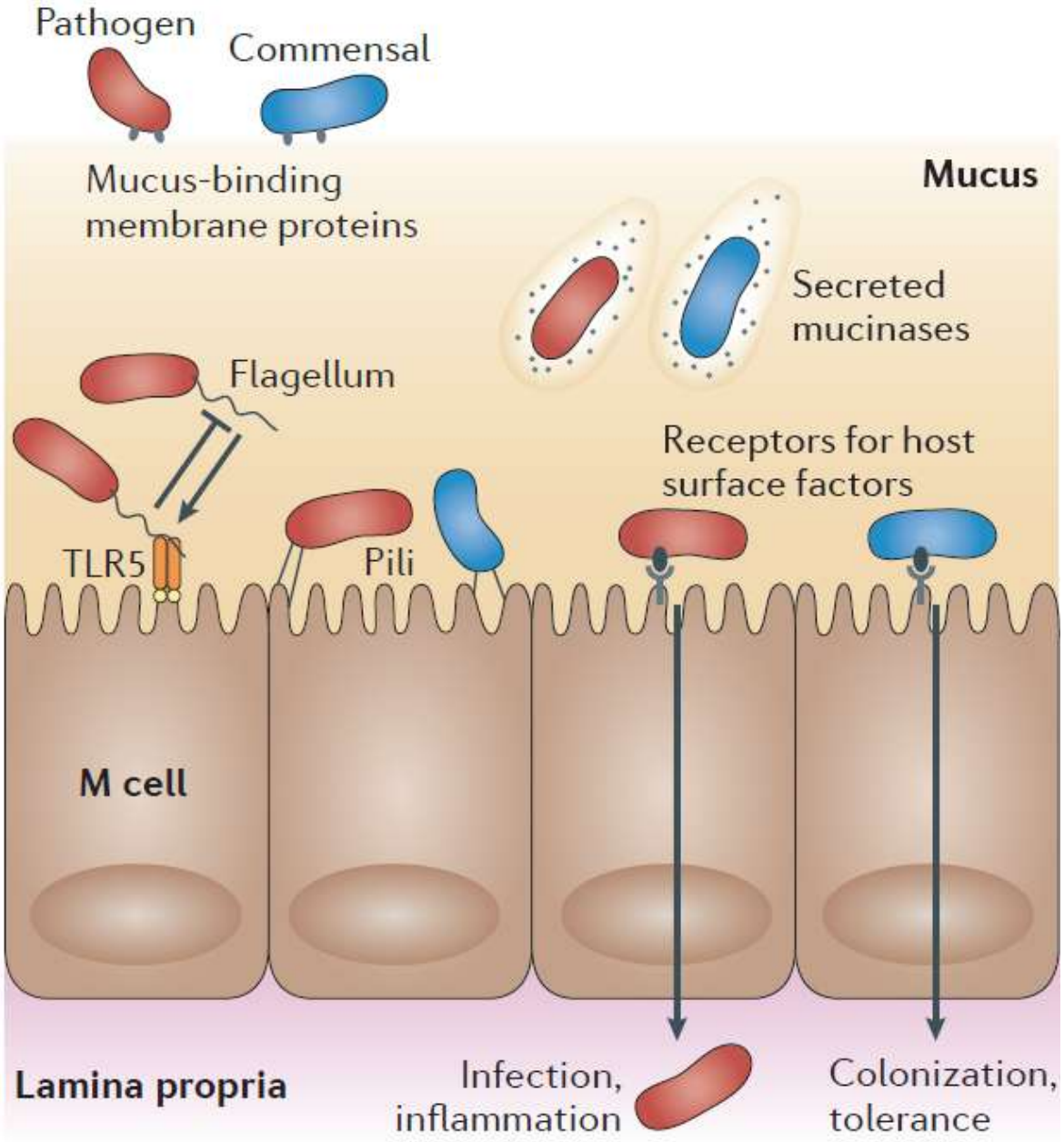


Distribuição das comunidades microbianas ao longo do intestino



doi:10.1038/nri3738

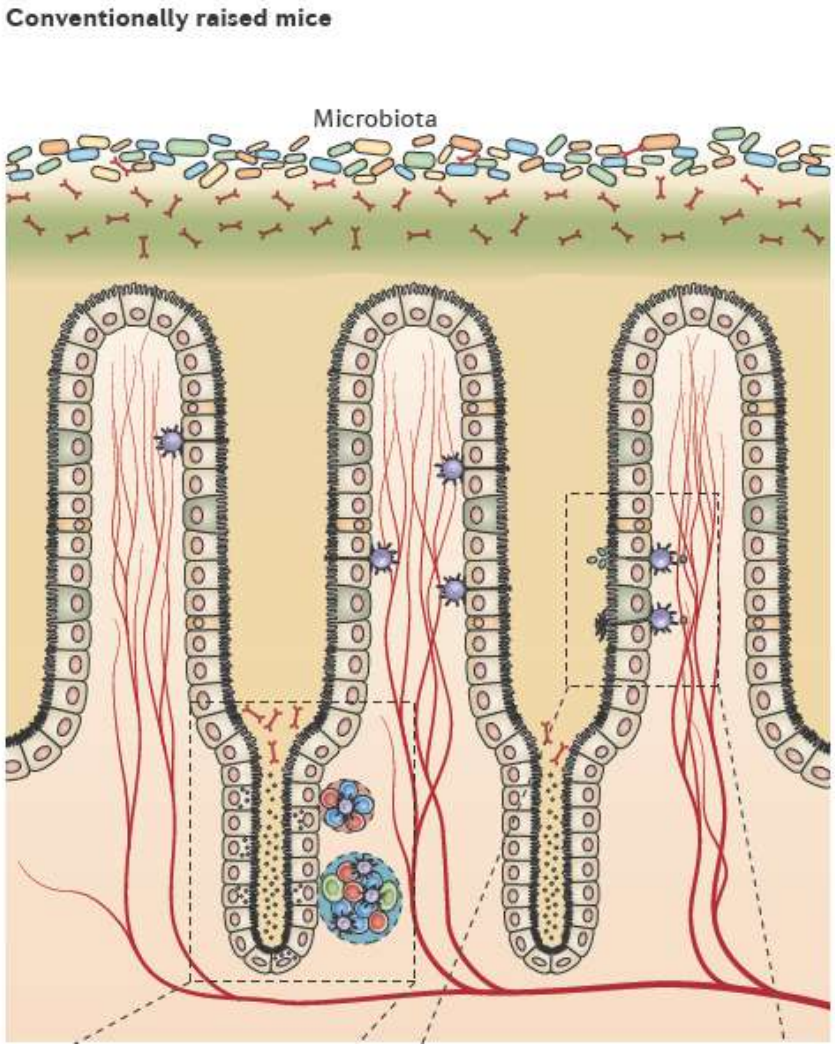
Microbiota

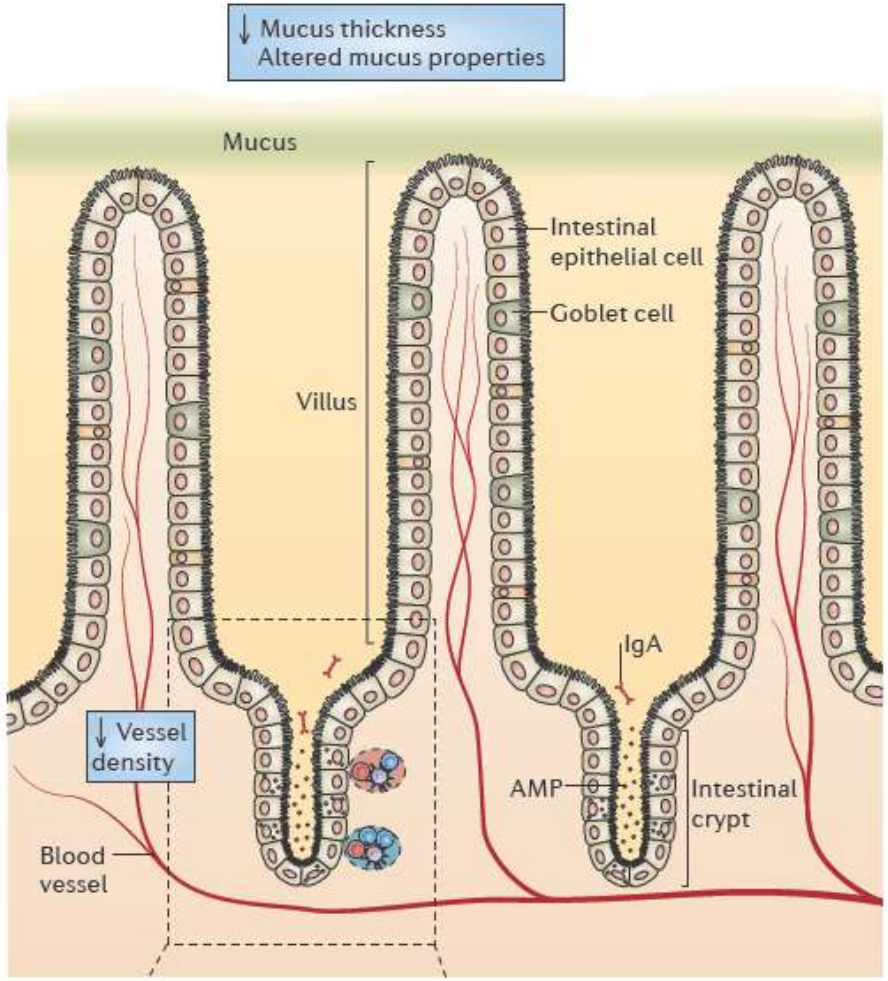


- ✓ Competem com patógenos
- ✓ Metabólitos utilizáveis pelo hospedeiro
- ✓ Degradação de produtos tóxicos
- ✓ Modulação do sistema imune
- ✓ Síntese de vitaminas (B12, K)

doi:10.1038/nrmicro3552

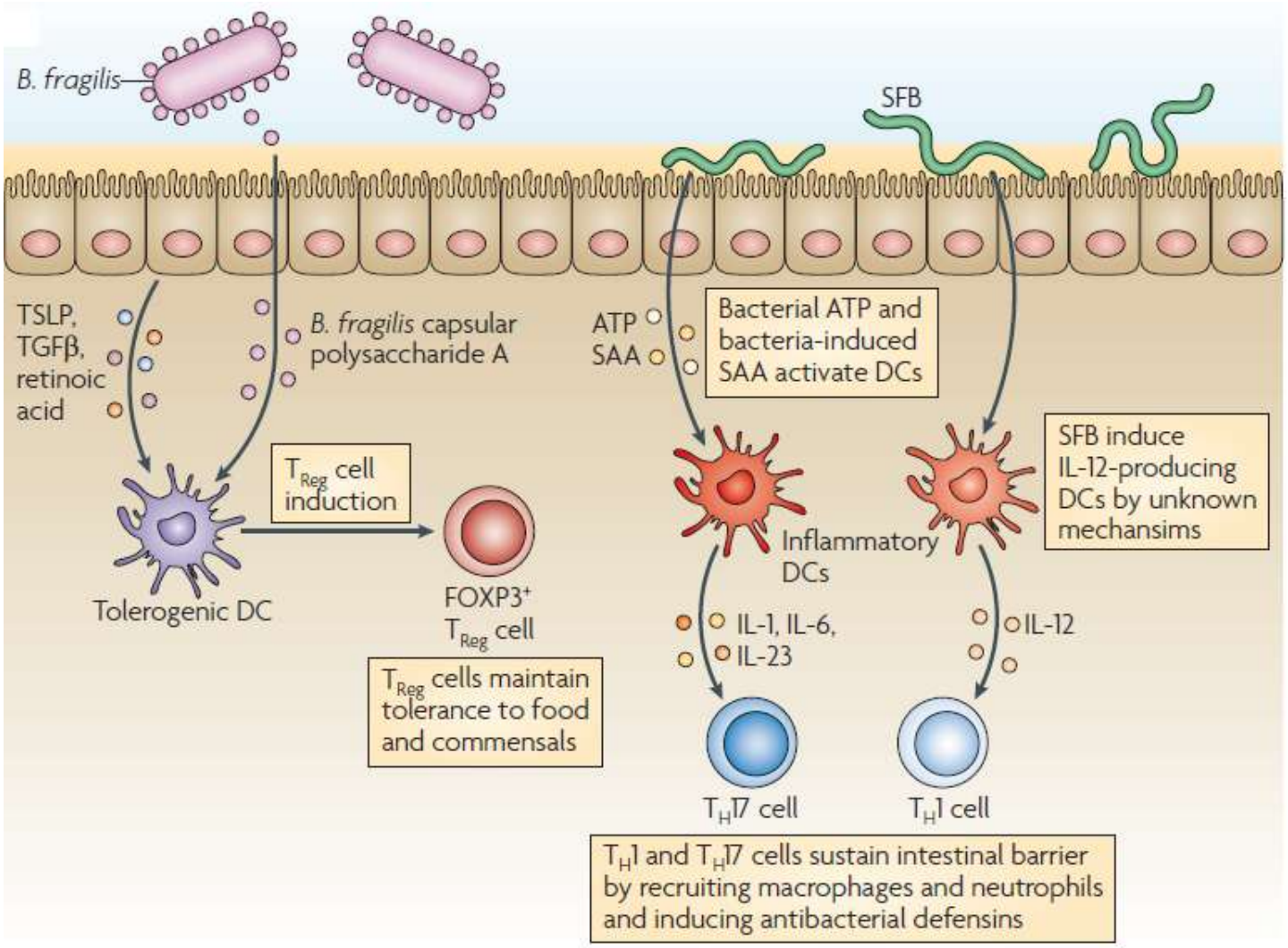
Microbiota





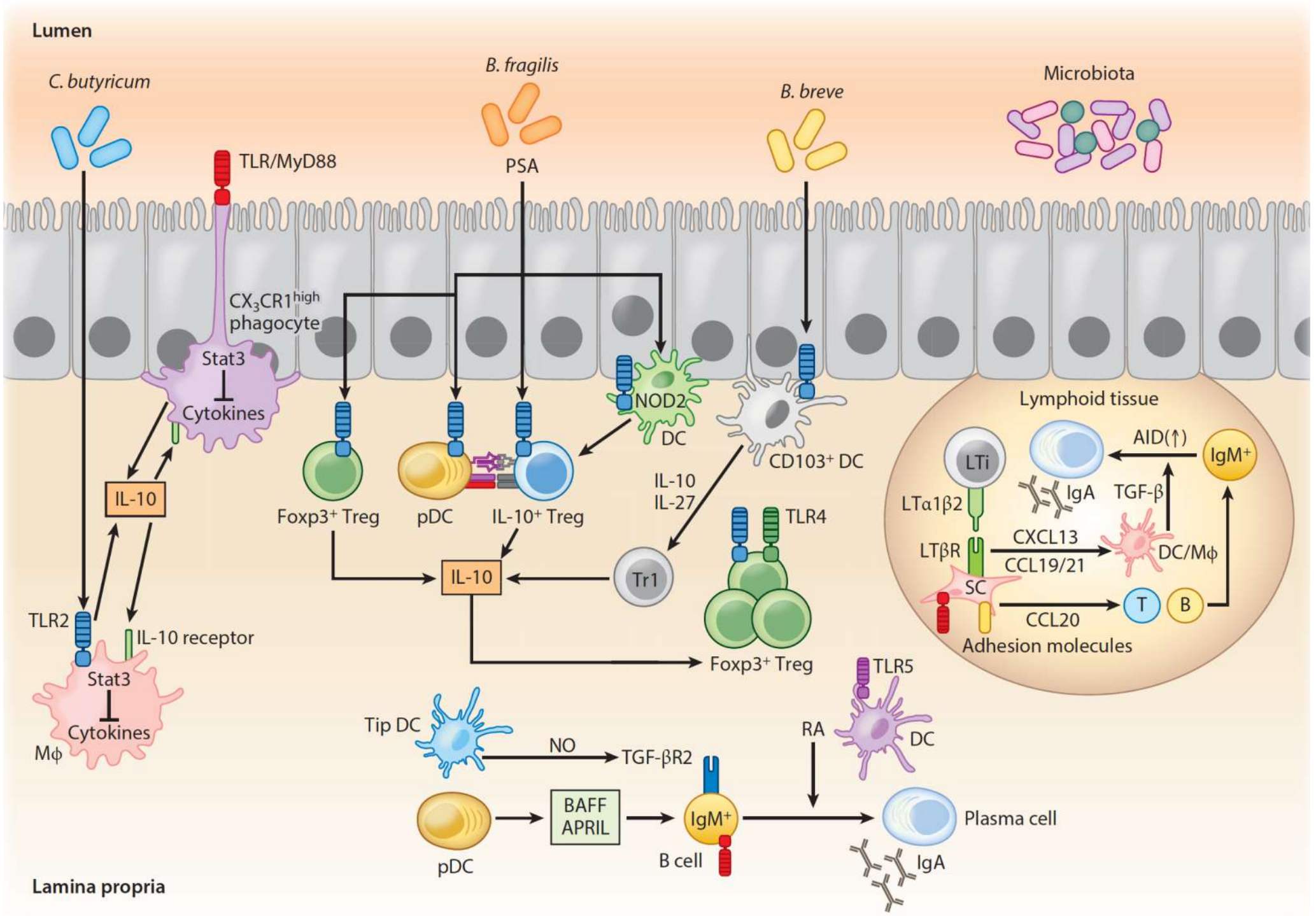
doi:10.1038/nrmicro2974

Microbiota na saúde

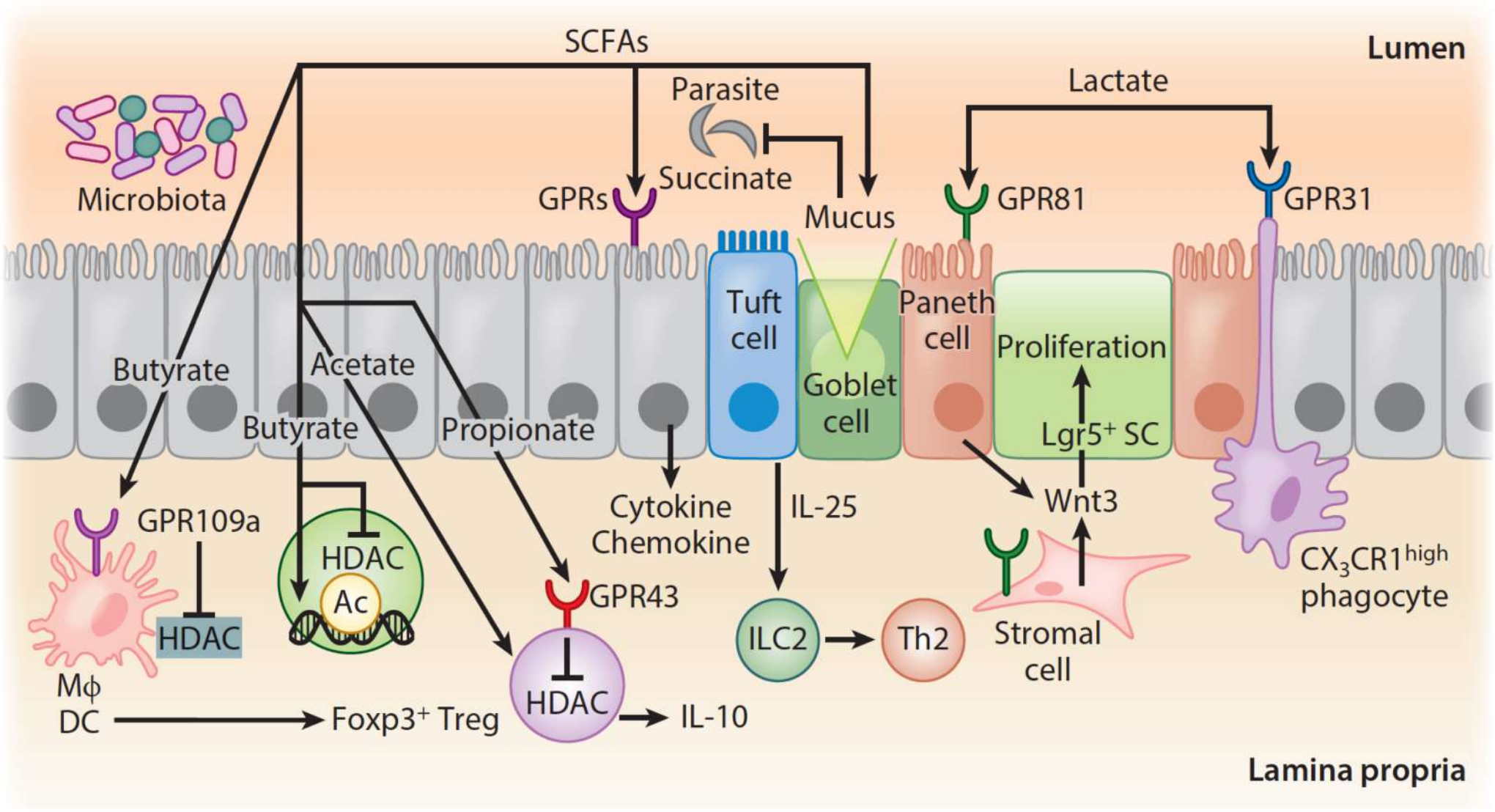


- **Indução de Treg**
- **Vacina oral**
- **Th17**
- **Troca de isotipo IgA**

doi: 10.1038/nri2850

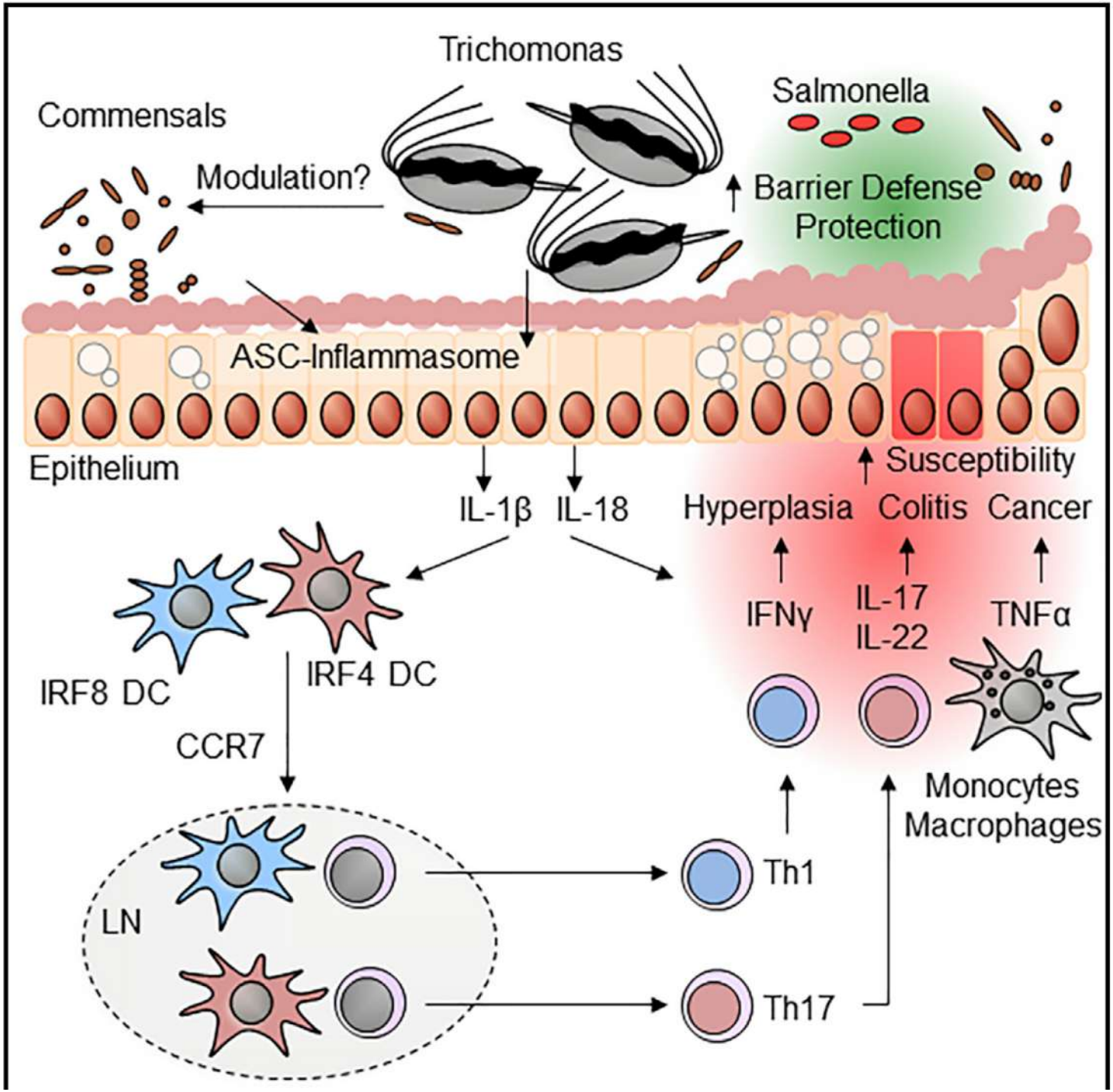


Kayama et al, 2020



Kayama et al, 2020

Microbiota na doença



Intestino:
- *Crohn's Disease*
- *Colite Ulcerativa*
- *Câncer*

Estômago
- *Úlcera, câncer colorretal*

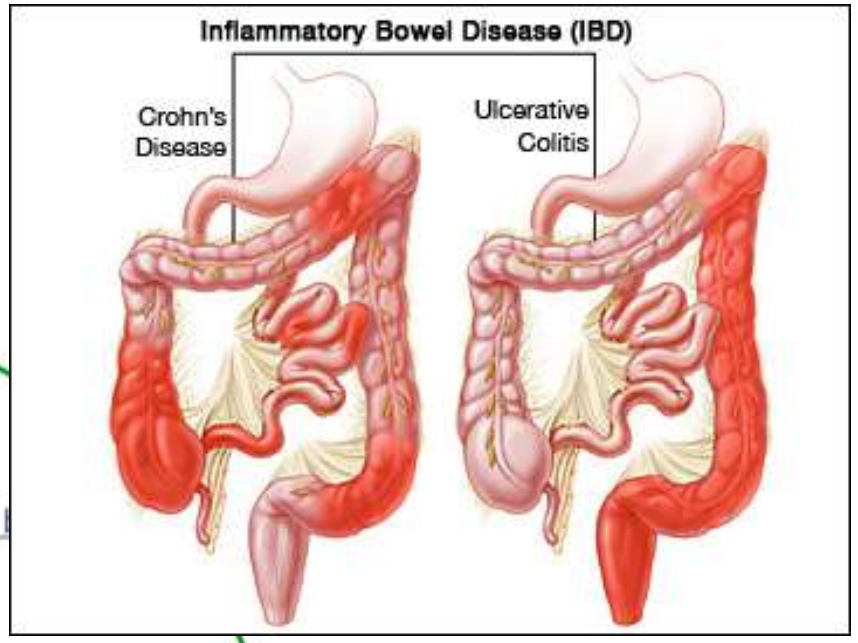
Trato Urogenital
- *Câncer*

Pulmão
- *Fibrose cística*

Pele:
- *Psoríase*

Chudnovskiy et al., 2016, Cell 167, 444–456

Doenças inflamatórias intestinais



Crohn's

Cellular innate immunity
NOD2
ATG16
IRGM
LRRK2

Immune-mediated
PTPN22
CCR6
IL2RA
IL18RAP
IL27
ERAP2
ITLN1
CCL2/CCL7
TNFSF11
BACH2
TAGAP
VAMP3

Other
DENNDIB
DNMT3A
GCKR
THADA
SP140
PRDX5
ZPF36L1
ZMIZ1
MUC1/SCAMP3
CPEB4
FADS1
5q31 (IBD5)

IBD

Th17
*IL23R**
*IL12B**
*STAT3*J*
*AK2**
TYK2

HLA
*DRB*103*

Other
*NKX2-3**
*CREM**
*C11Orf30**
*ORMDL3**
*RTEL1**
*PTGER4**
*KIF21B**
CDKAL1
ZNF365

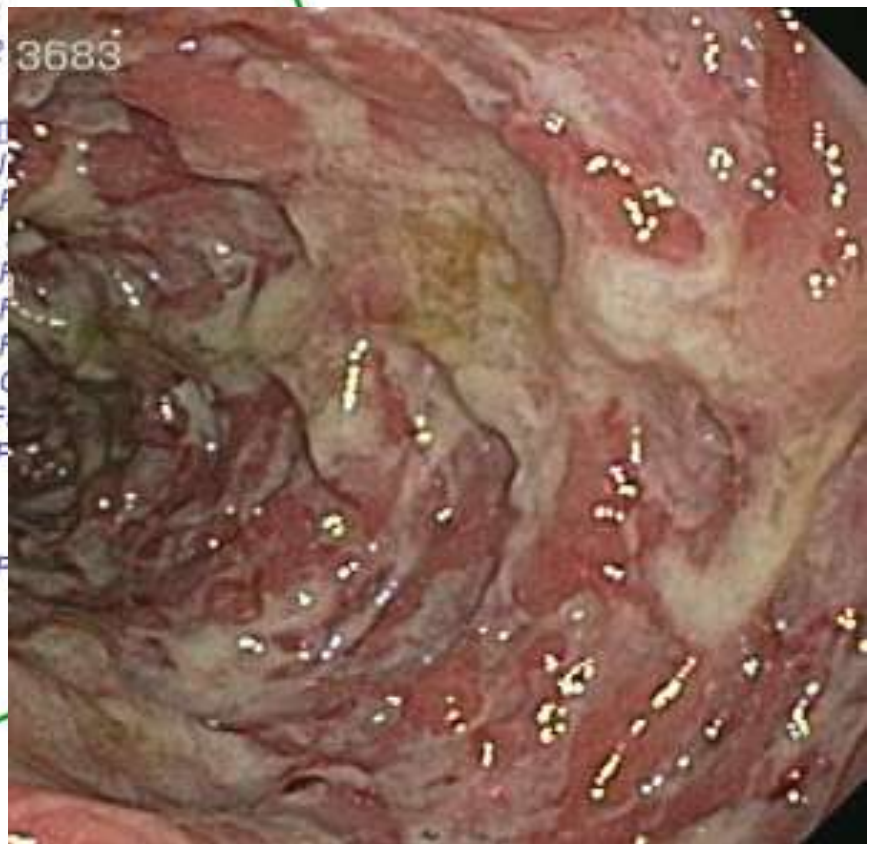
Immune-mediated
*MST1**
*IL10**
*CARD9**
*REL**
*PRDM1**
*TNFSF15**
*ICOSLG**
*IL1R2**
YDJC
SMAD3
PTPN2

UC

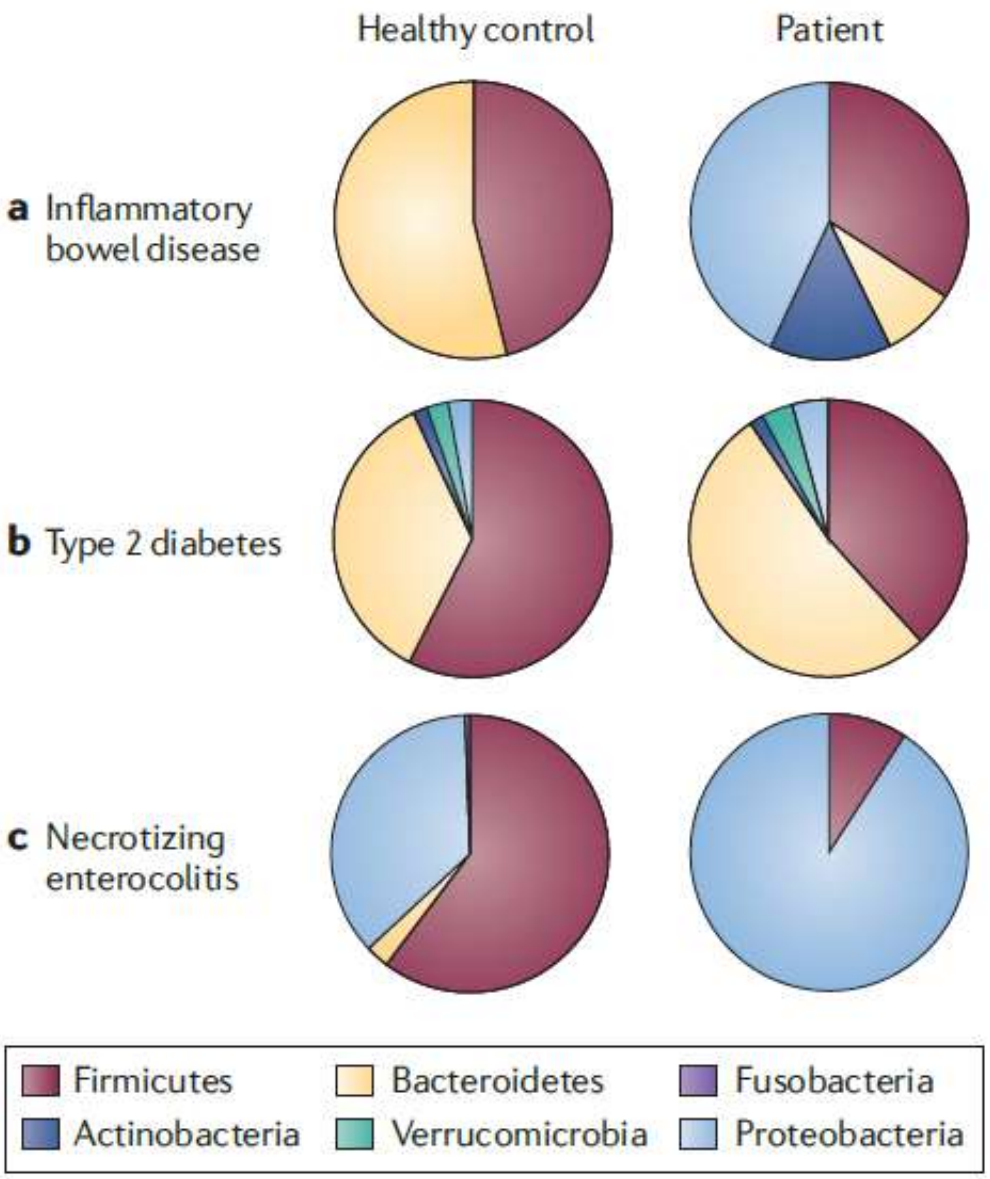
Epithelial
ECM1
HNF4A
CDH1
LAMB1
GNA12

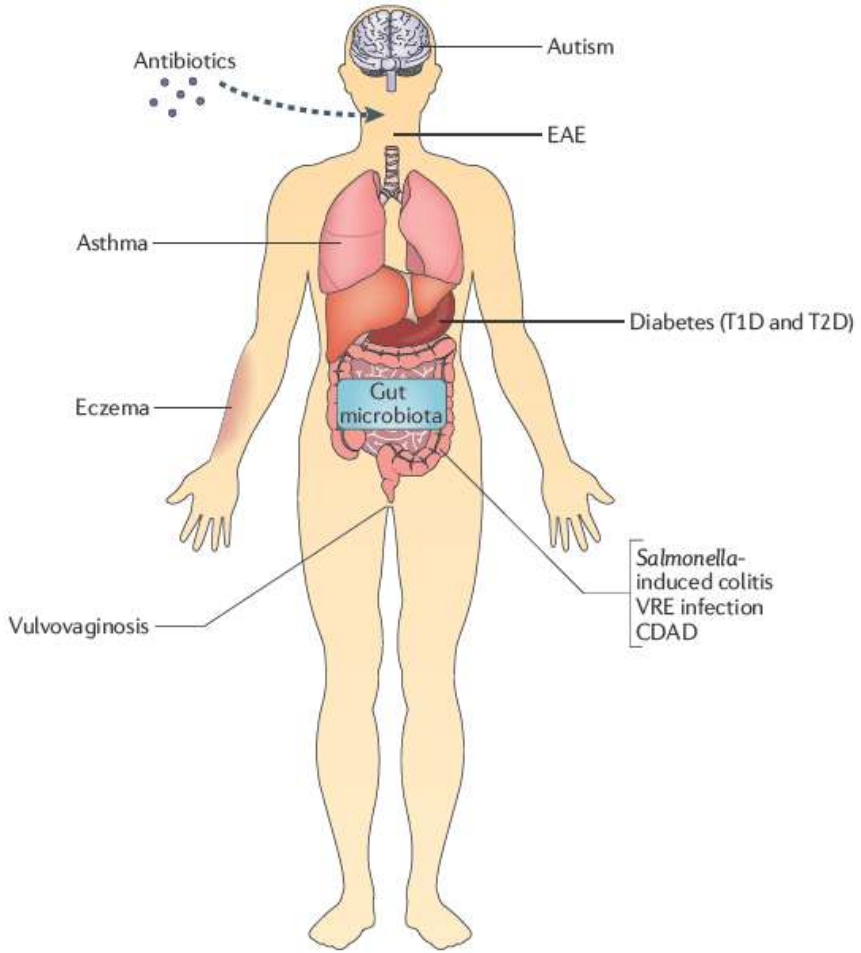
Imm
IFN
IL8
IL2
IL7
TNF
TNF
FC
IRF
LSP

Other
OTUD3 / P
DAP
PIM3
CAPN10

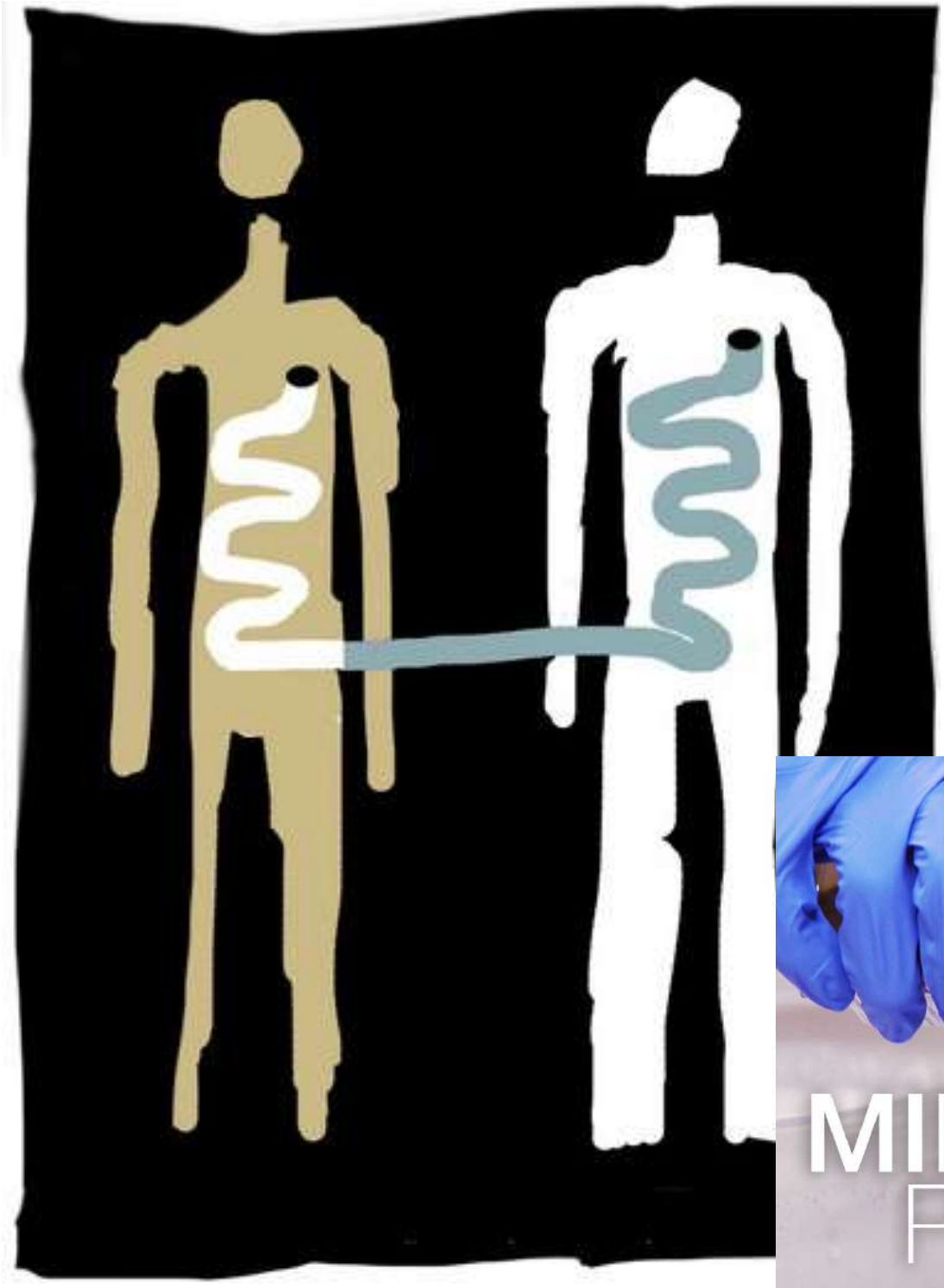


Disbiose x Doença



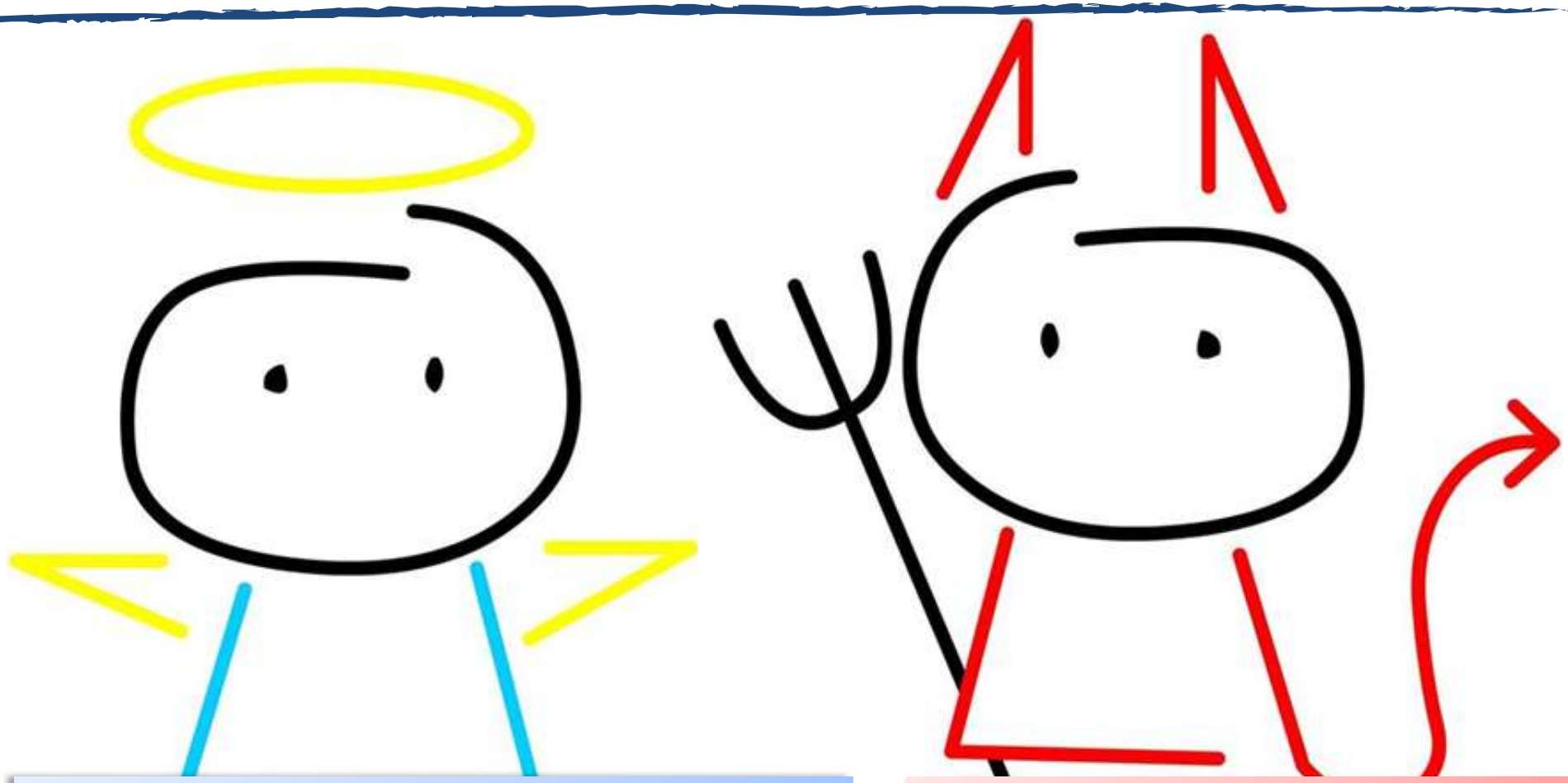


doi:10.1038/nrmicro2540




MIRACLE
POOP

Microbiota



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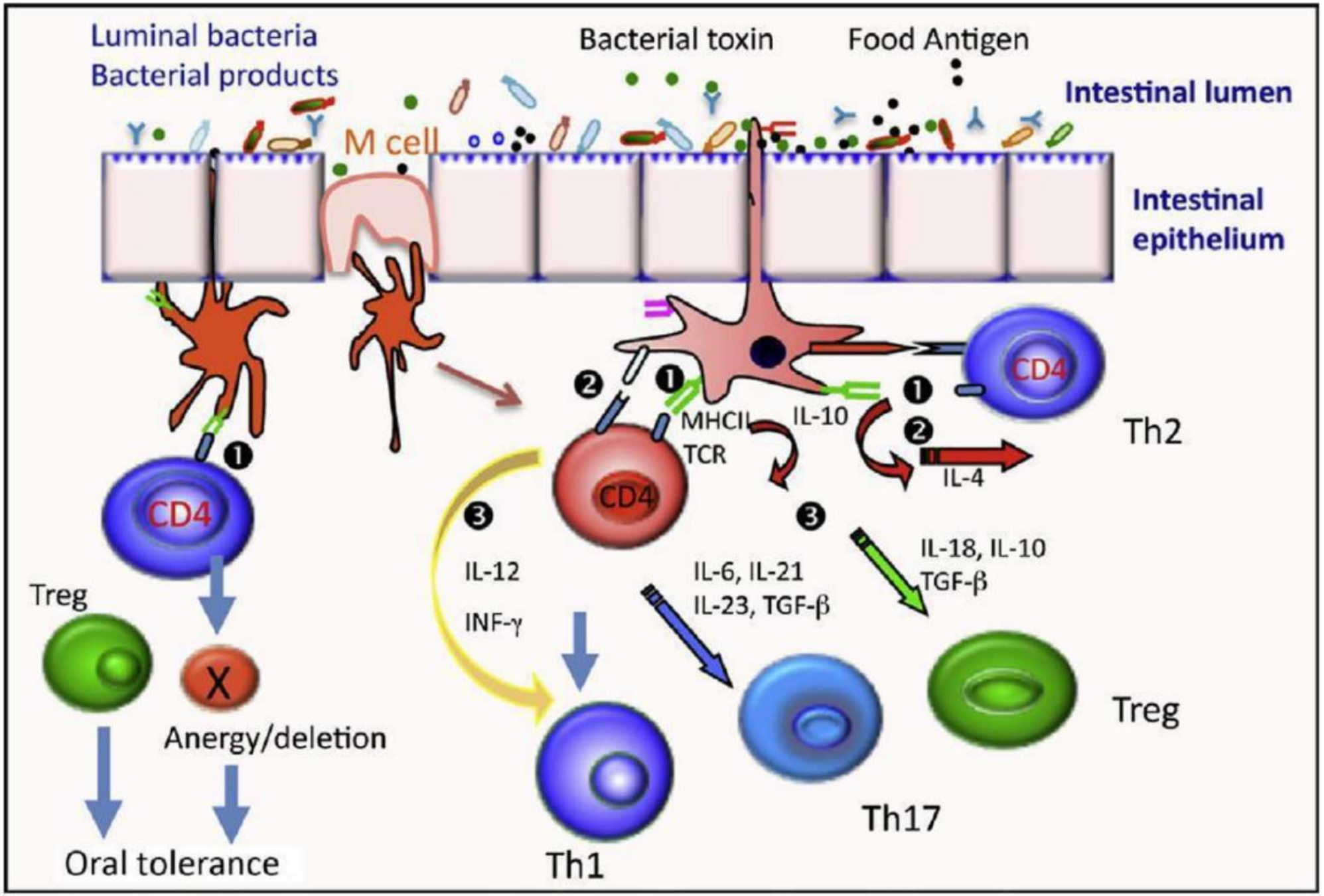
- ✓ Câncer
- ✓ Obesidade
- ✓ Doenças neurológicas

Resumindo...


Luminal bacteria
Bacterial products
Bacterial toxin
Food Antigen
Intestinal lumen
M cell
Intestinal epithelium
CD4
MHCII
TCR
IL-10
IL-4
Th2
IL-12
INF-γ
IL-6, IL-21
IL-23, TGF-β
IL-18, IL-10
TGF-β
Treg
X
Anergy/deletion
Oral tolerance
Th1
Th17
Treg