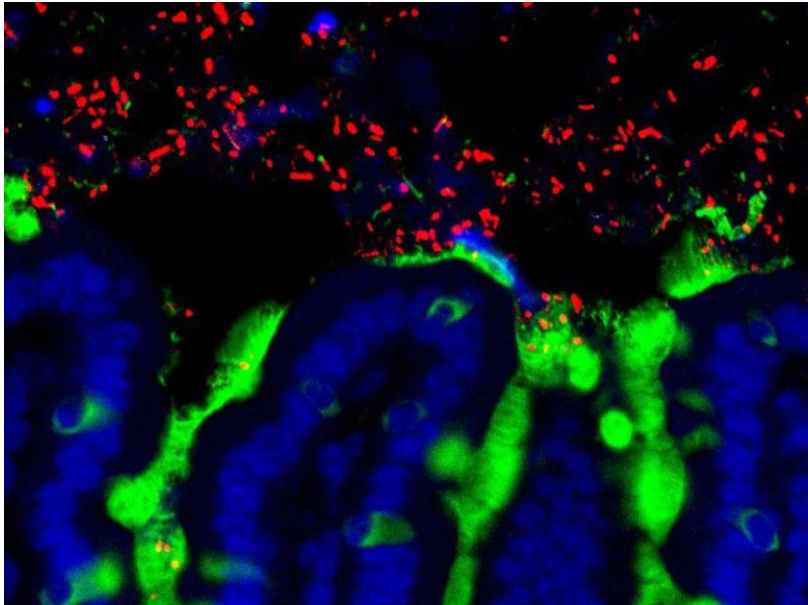


MUCOSAL IMMUNOLOGY



Cathryn Nagler, Ph.D.



PRITZKER SCHOOL OF
MOLECULAR ENGINEERING



THE UNIVERSITY OF
CHICAGO
BIOLOGICAL
SCIENCES

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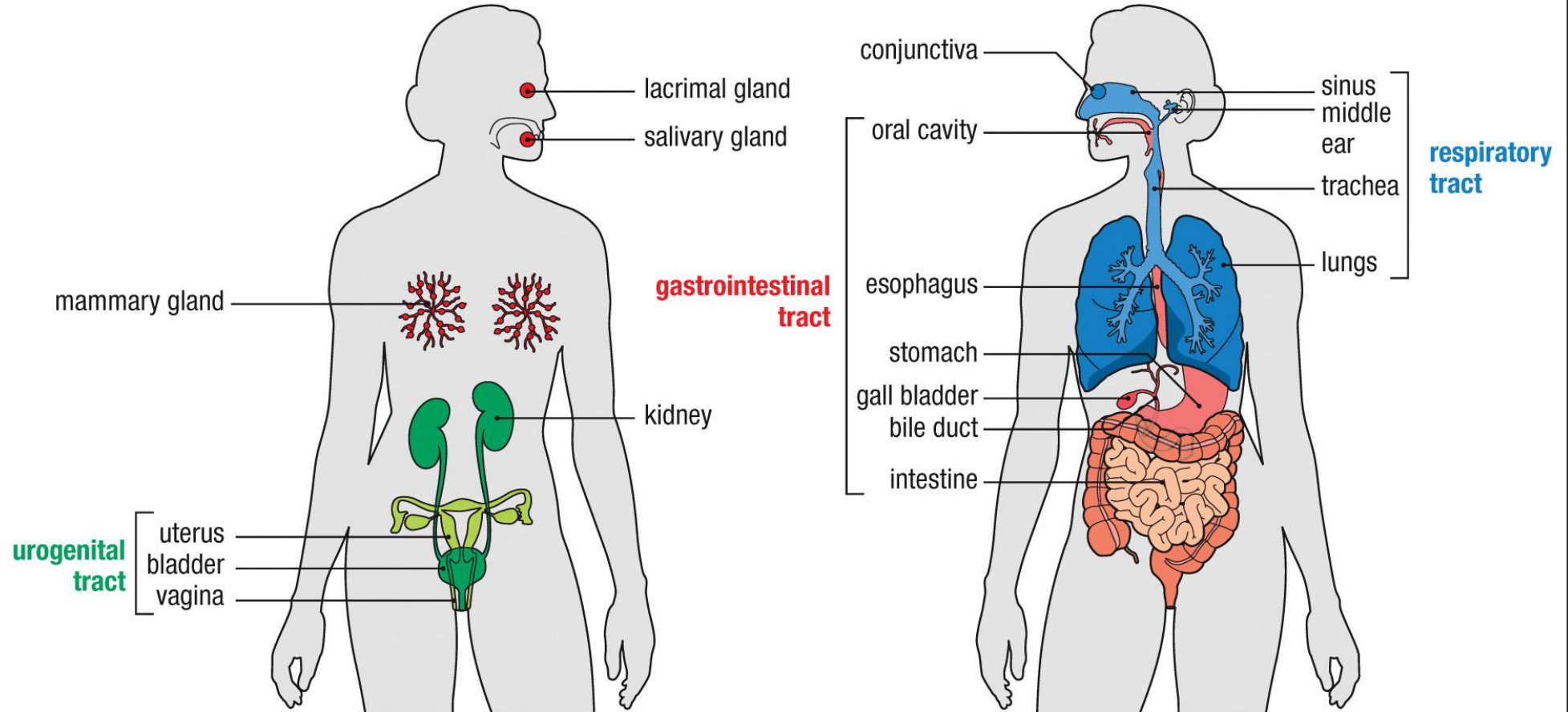
@CathyNagler

FOCIS Advanced Course in Basic and Clinical Immunology 2024

Estancia La Jolla, La Jolla, CA

February 6, 2024

Mucosal tissues of the human body



Mucosal surfaces are the major portals of entry for antigen

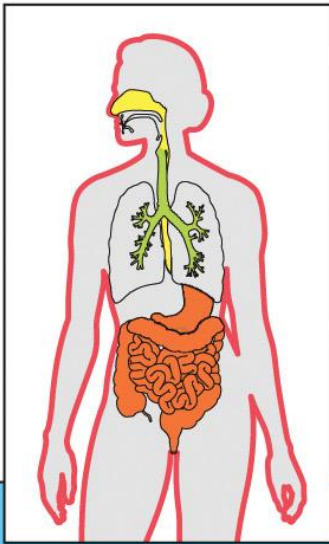
Largest area of contact of the immune system with the environment.

**Largest accumulation of lymphoid tissue in the body:
 6×10^{10} antibody forming cells in mucosal tissues vs
 2.5×10^{10} in lymphoid organs.**

The gut associated lymphoid tissue (GALT) contains more lymphocytes than all of the secondary lymphoid organs combined!

Secretory IgA is produced at a rate of 40-60 mg/kg/day.

A variety of different types of epithelium line the barrier tissues



Mucosae

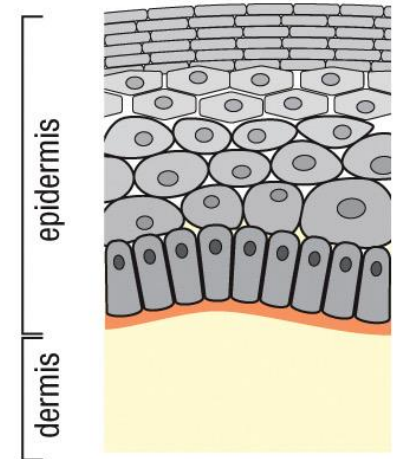
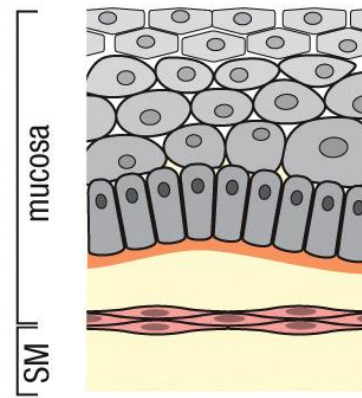
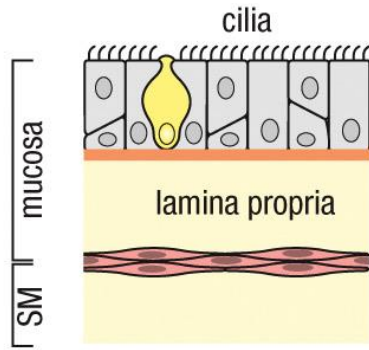
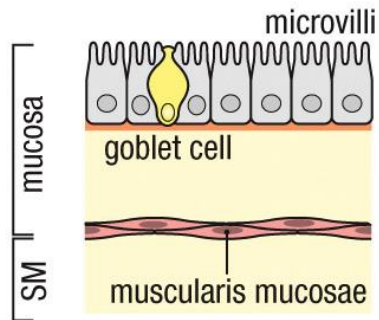
Skin

Simple columnar epithelium

Pseudostratified columnar epithelium

Nonkeratinized stratified squamous epithelium

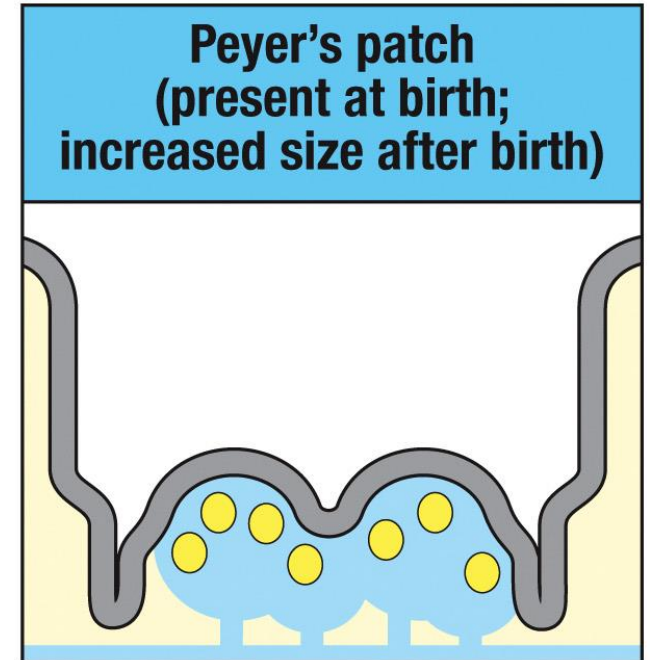
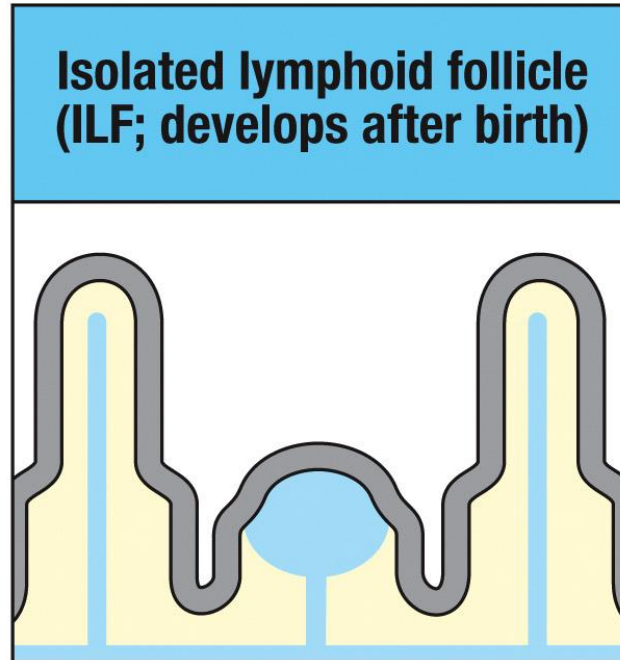
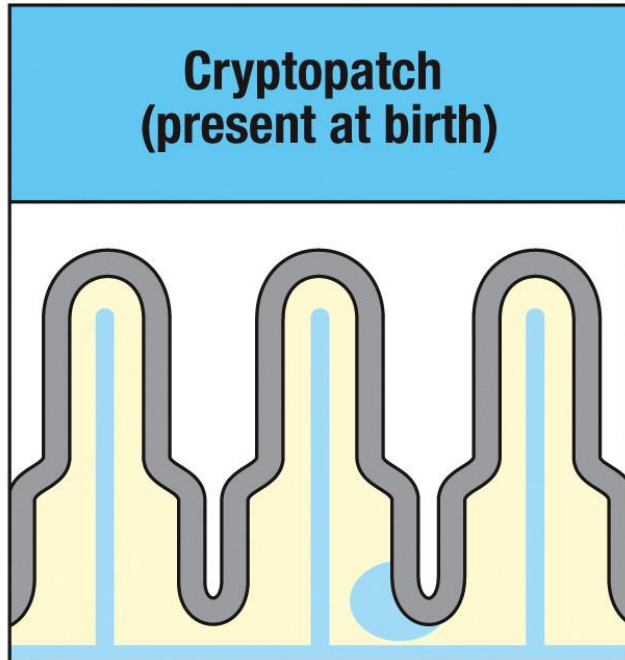
Keratinized stratified squamous epithelium



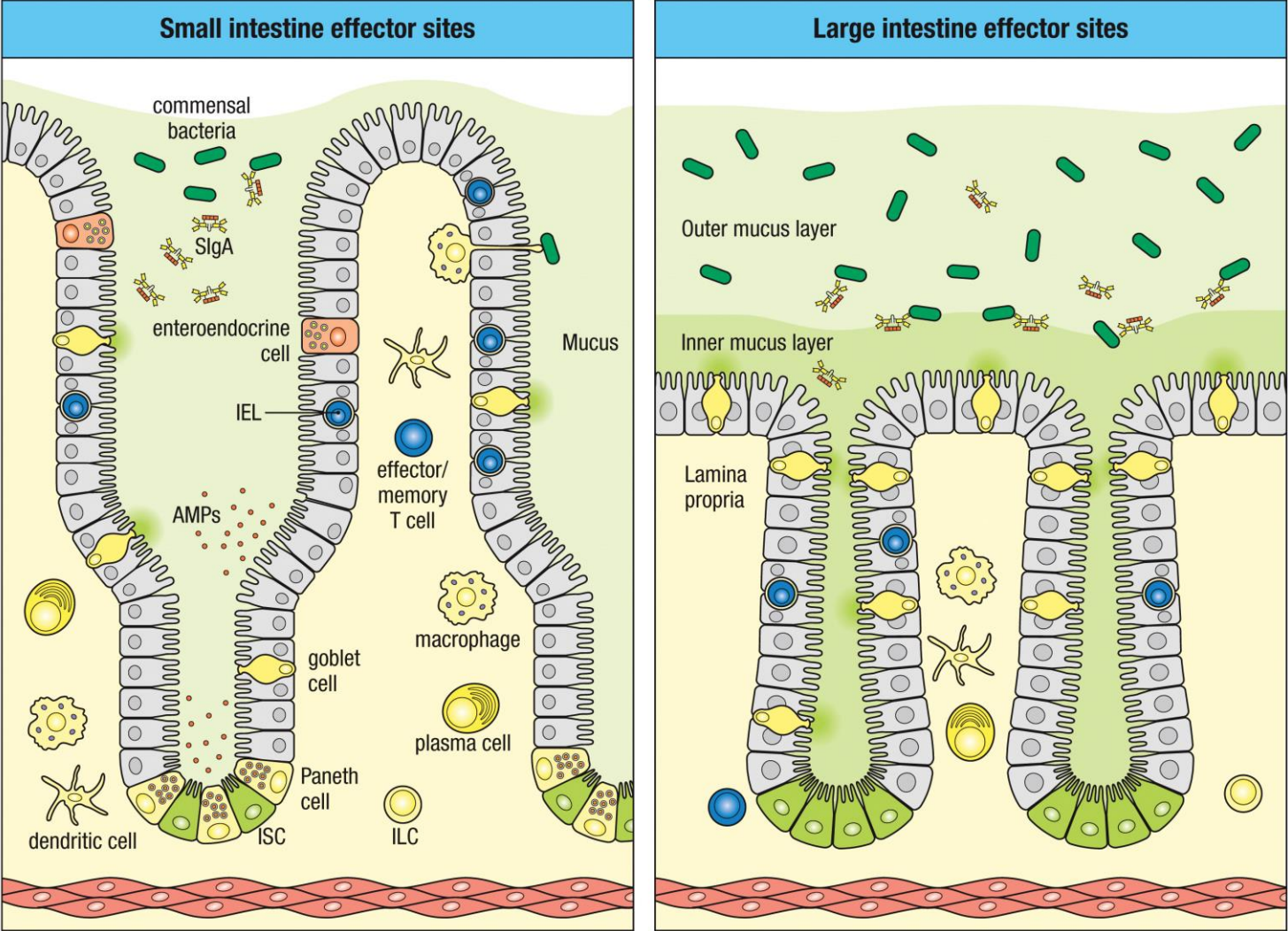
trachea, bronchi
bronchioles

oral cavity,
esophagus, rectum
upper respiratory tract

Inductive sites of the GALT

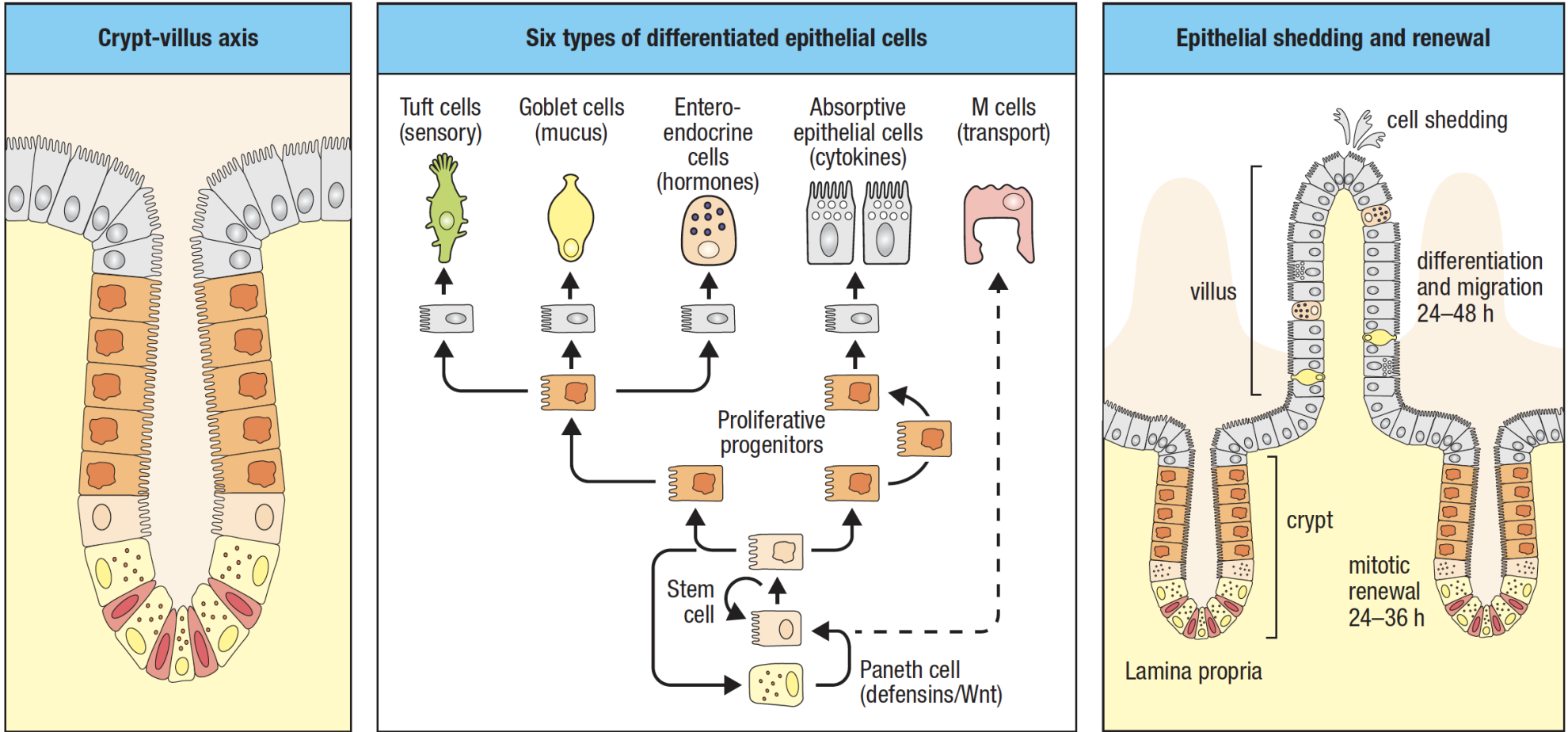


Effector sites of the GALT



Janeway's Immunobiology 10th Edition, W.W. Norton & Co. 2022

Intestinal epithelial cells arise from a common intestinal stem cell



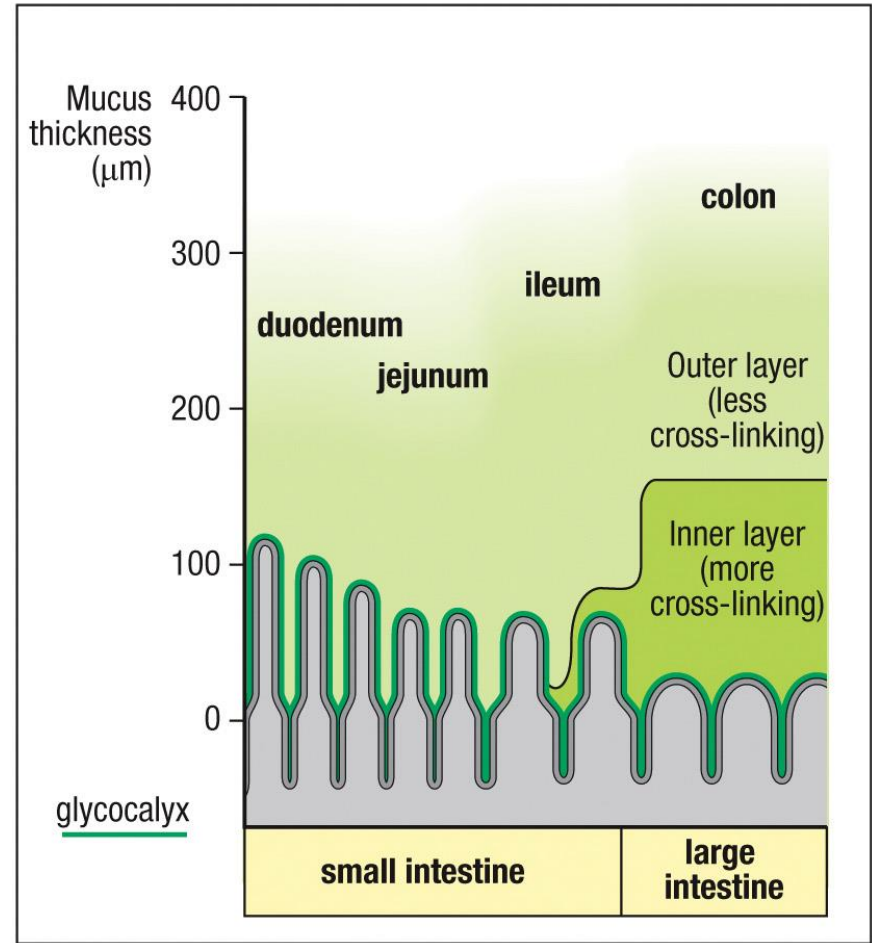
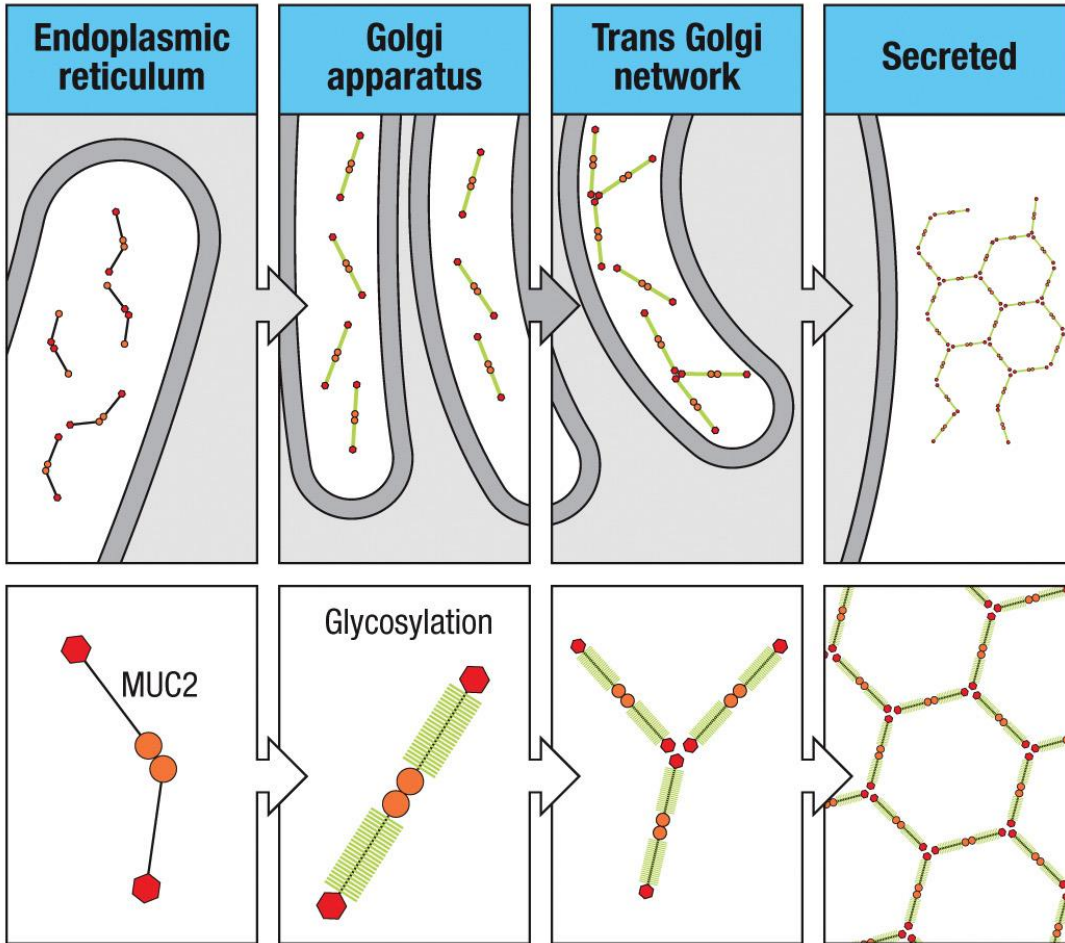
Principles of Mucosal Immunology, 2nd ed. (© CRC Press 2020)

The epithelium is self-renewing. Cells migrate from crypt to tip in 2-6 days. Highest turnover in body.

Protective adaptations of the intestinal epithelial barrier

1. **Mucus**
2. **Anti-Microbial Peptides**
3. **Specialized enterocyte cell types**
4. **Intercellular tight junctions that restrict the passage of even very small (2kD) molecules between cells**
5. **Secretory IgA**

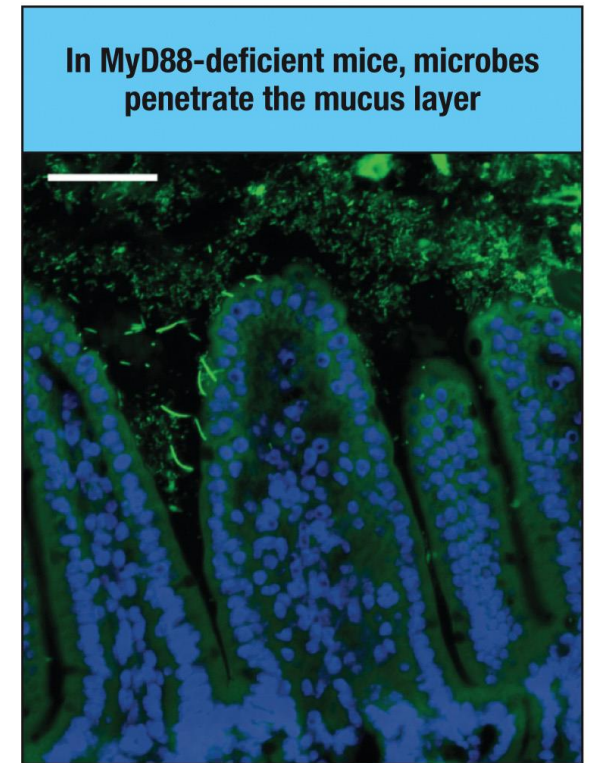
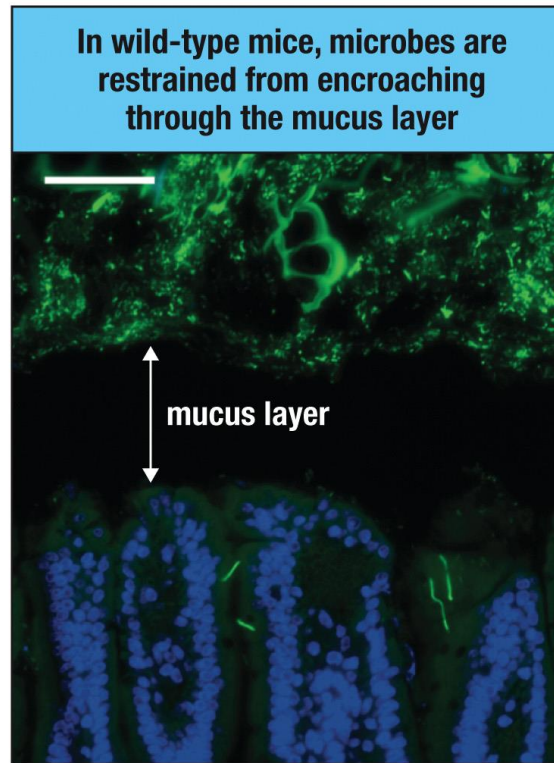
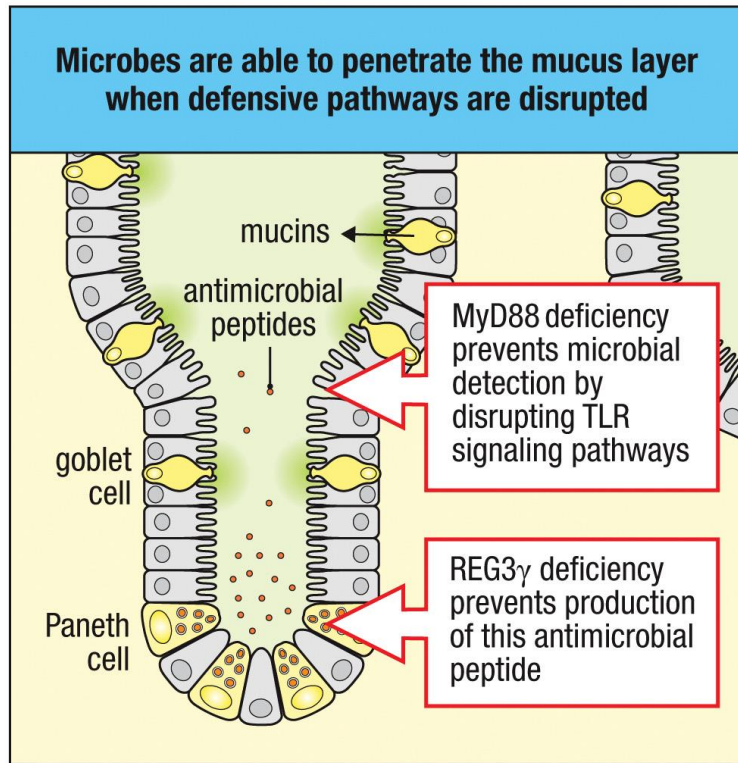
Structure and organization of mucins and **mucus**



Janeway's Immunobiology 10th Edition, W.W. Norton & Co. 2022

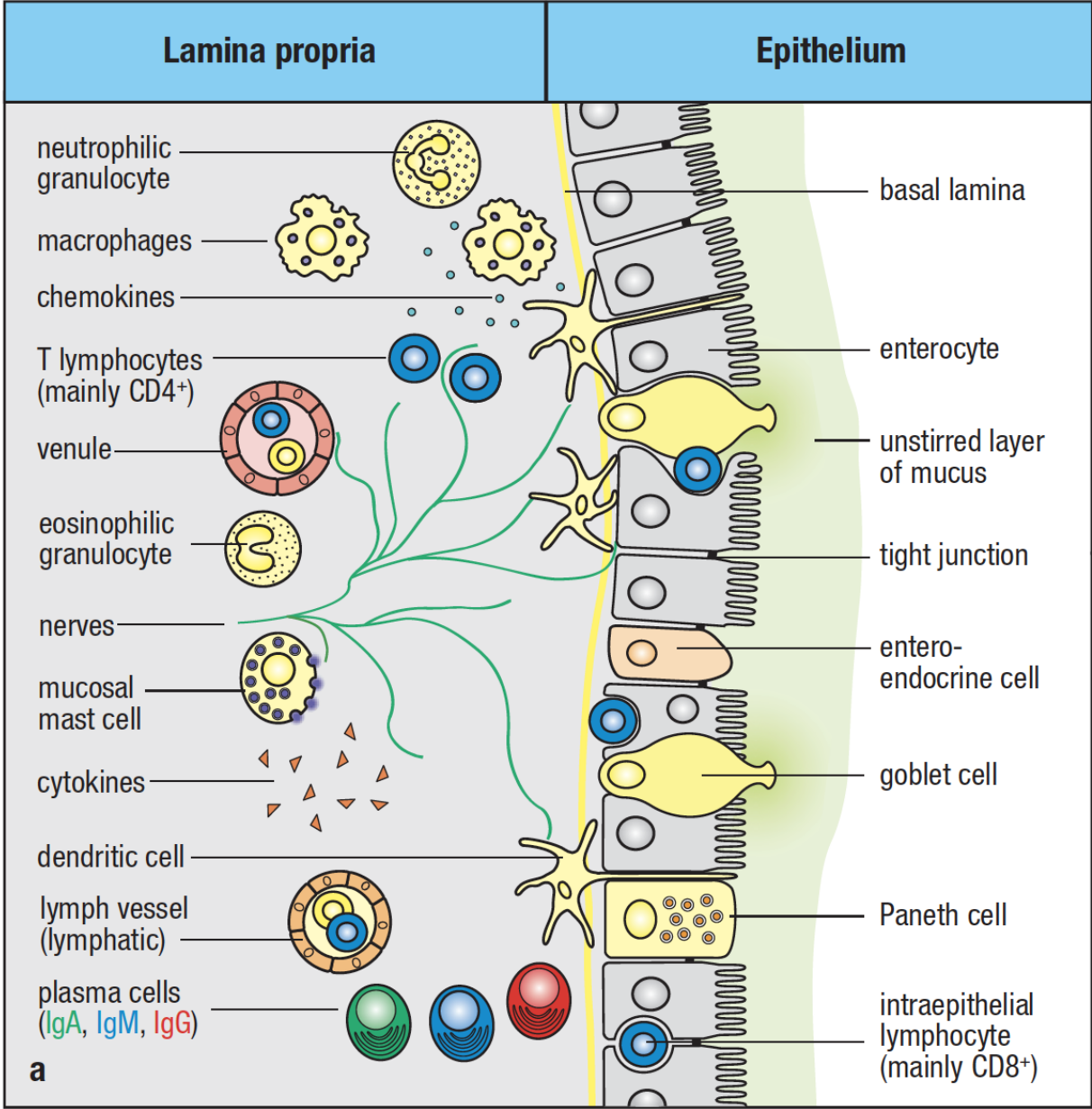
Mucins are glycoproteins with greater than 50% of their mass contributed by O-glycans. Two major types of mucins – transmembrane mucins are anchored to surface of mucosal epithelial cells and gel forming mucins (like MUC2) which are released.

Microbial signals activate the production of antimicrobial peptides that restrain bacterial encroachment of the intestinal epithelium

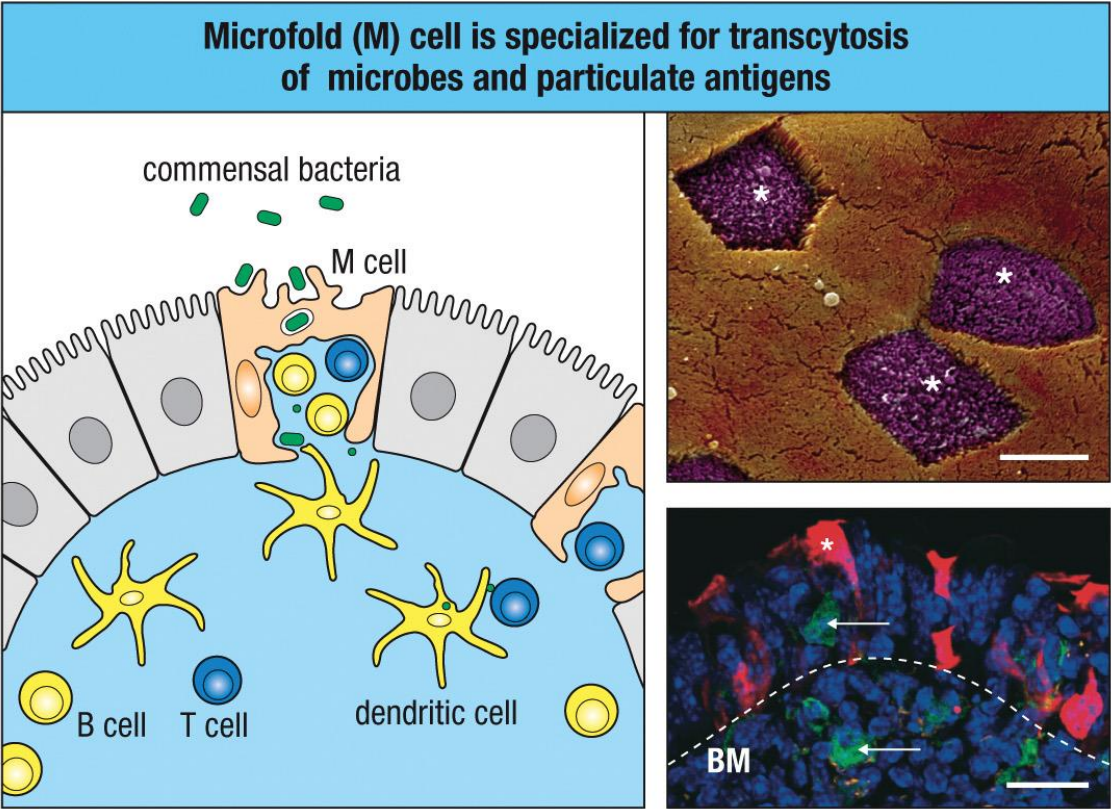
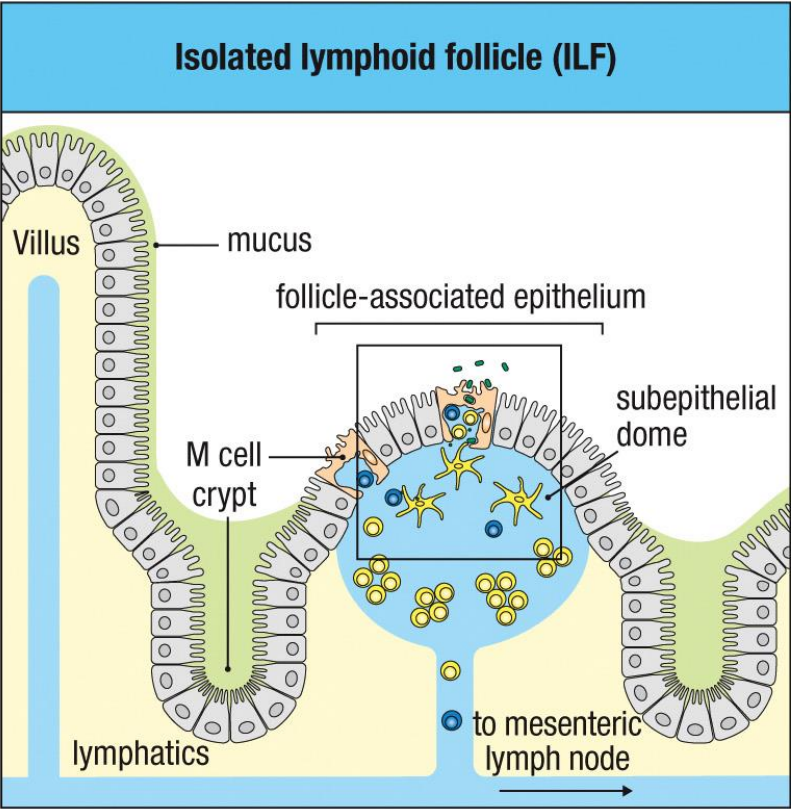


(both) Vaishnava S., et al.: *Science* 2011, 334:255–258. ©AAAS.

Specialized cell types in the gut epithelium and lamina propria

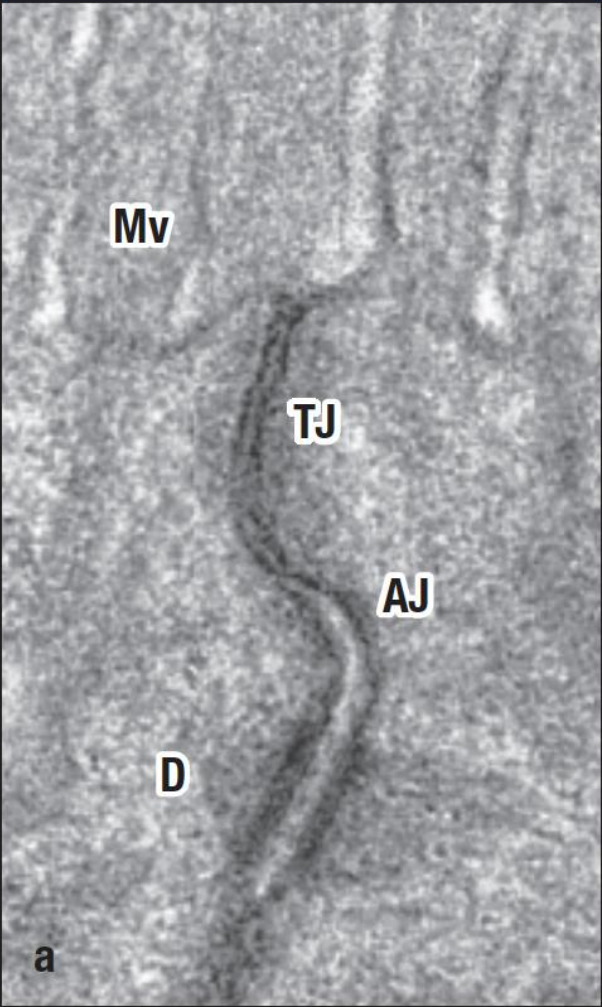


M cells are a specialized enterocyte cell type for uptake of particulate antigens



Intercellular junctional complexes between adjacent epithelial cells seal the apical surface

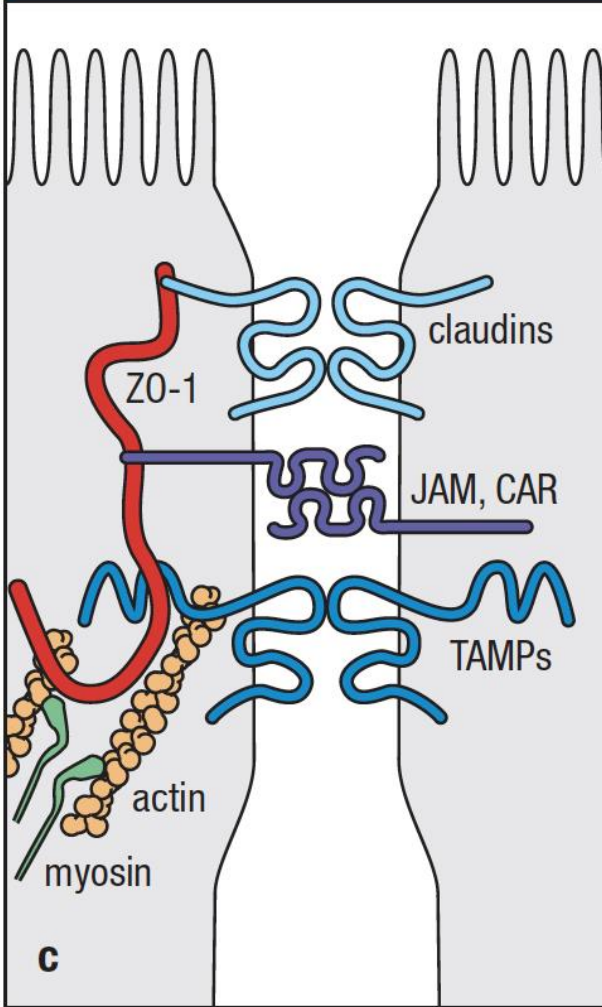
Junctional complexes between villous enterocytes



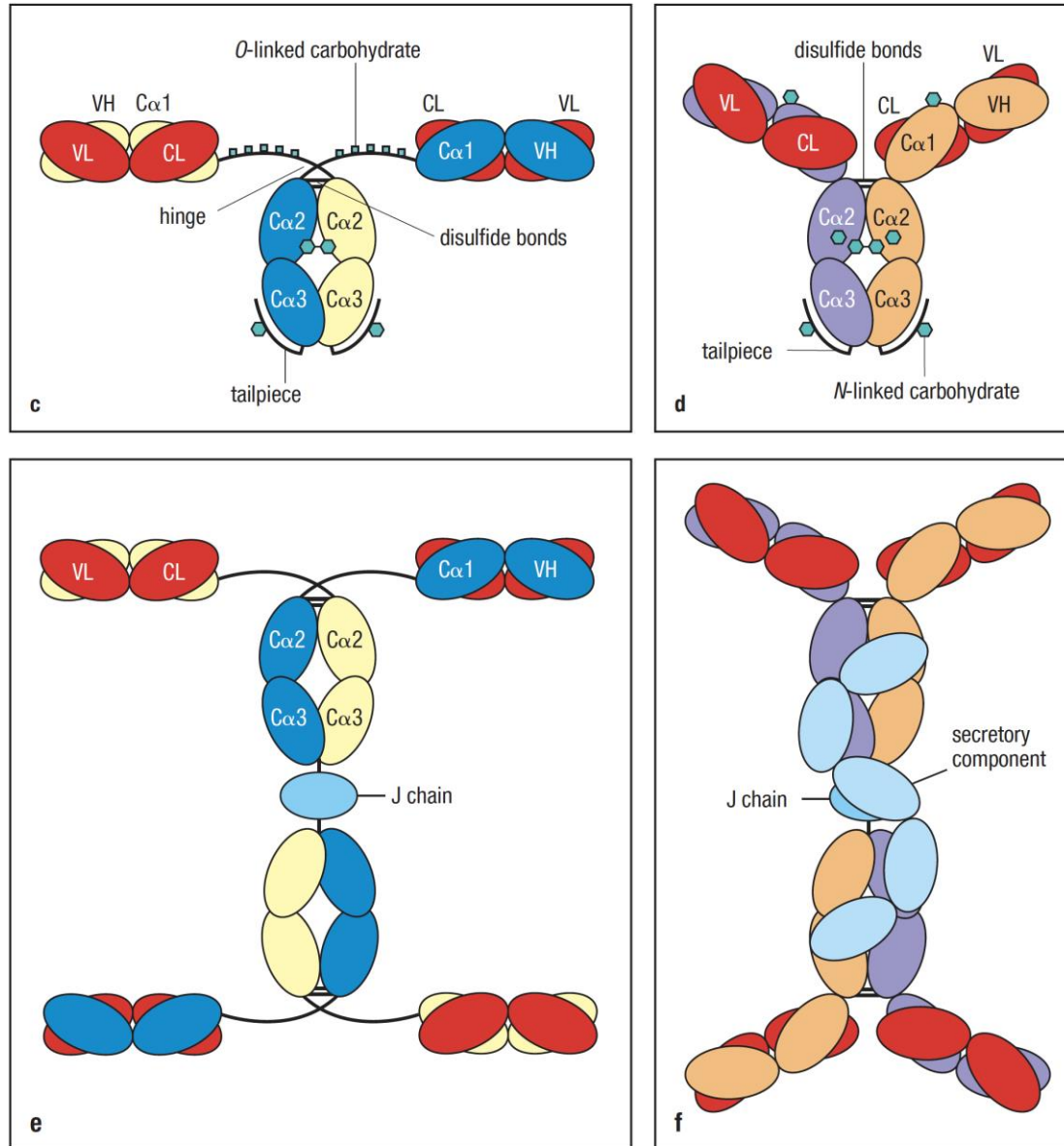
Apical microvilli and tight junction strands



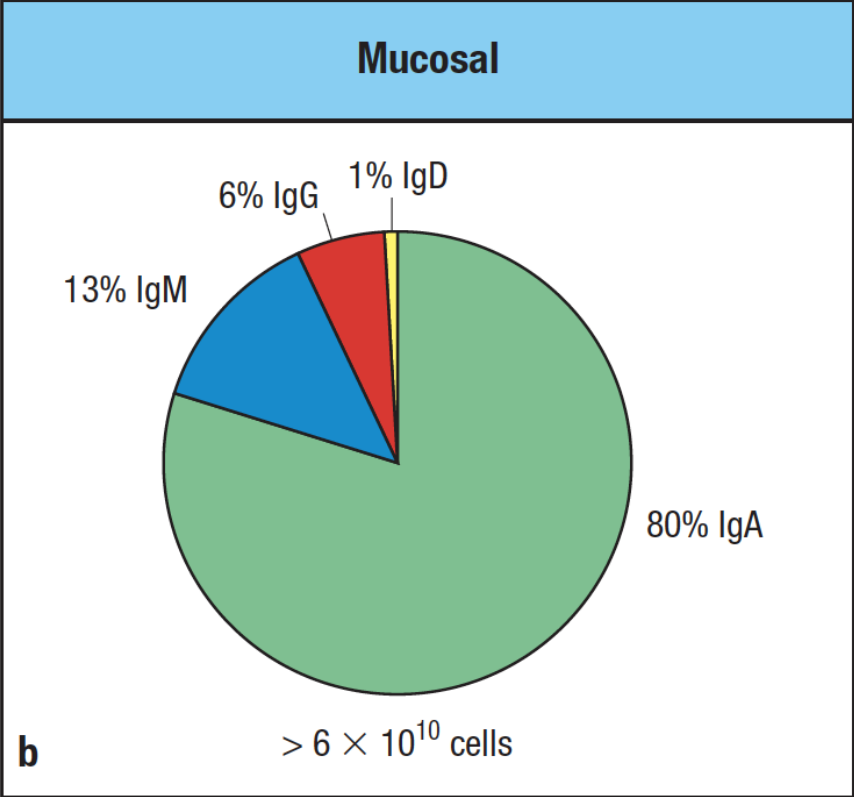
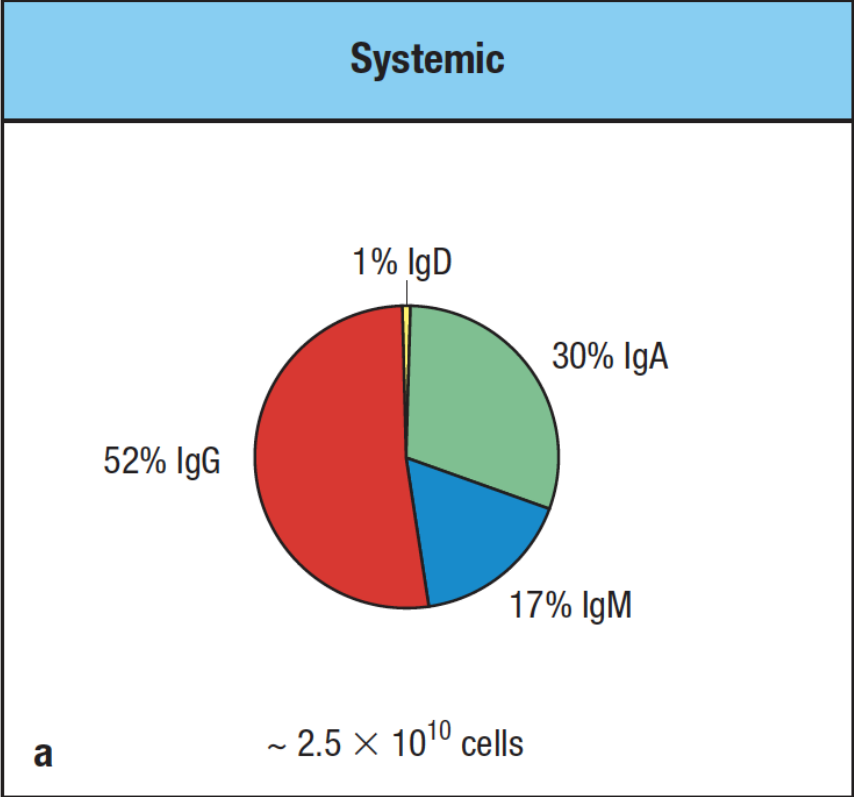
Molecular interactions



Structure of human IgA

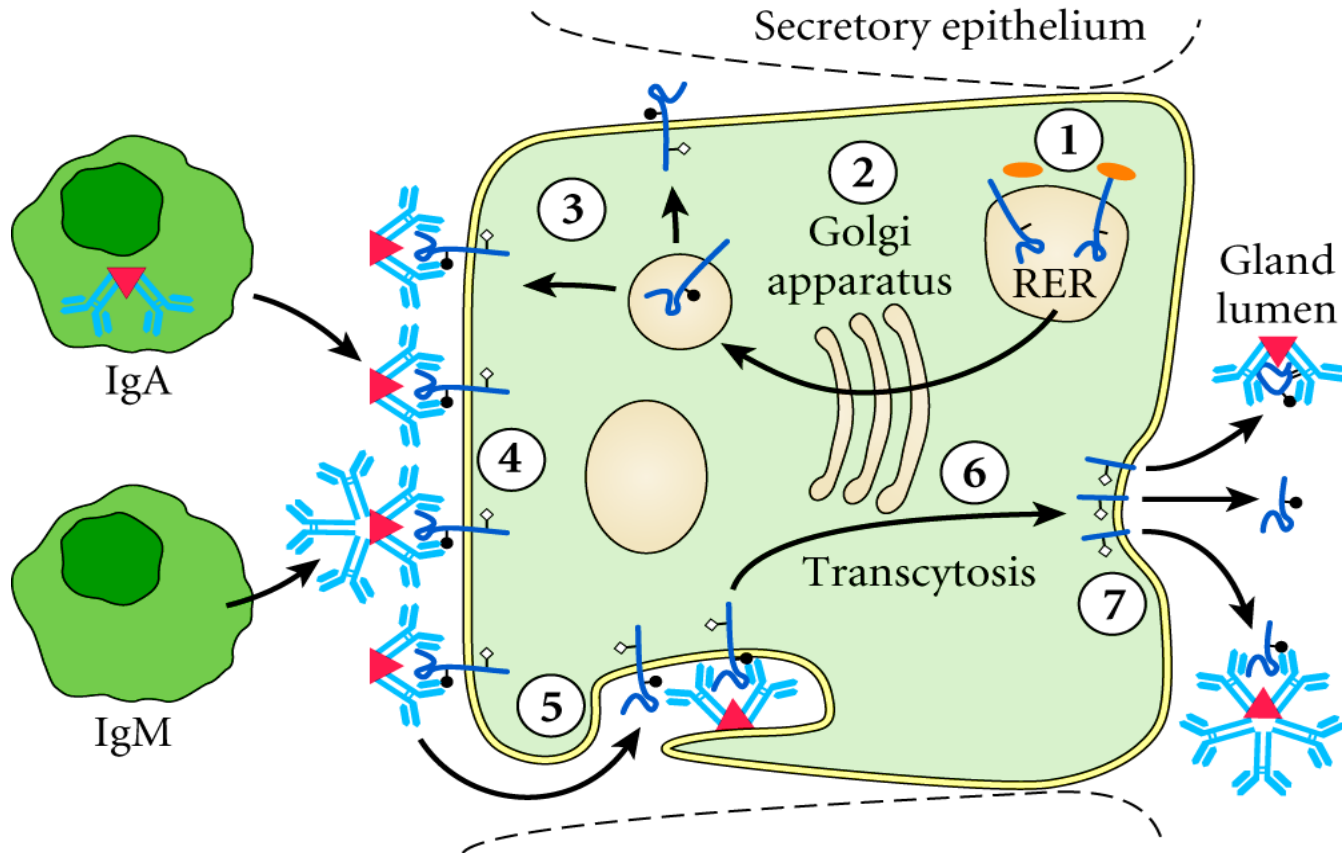


Comparative distribution of IgA-producing cells in systemic and mucosal compartments






Principles of Mucosal Immunology, 2nd ed. (© CRC Press 2020)

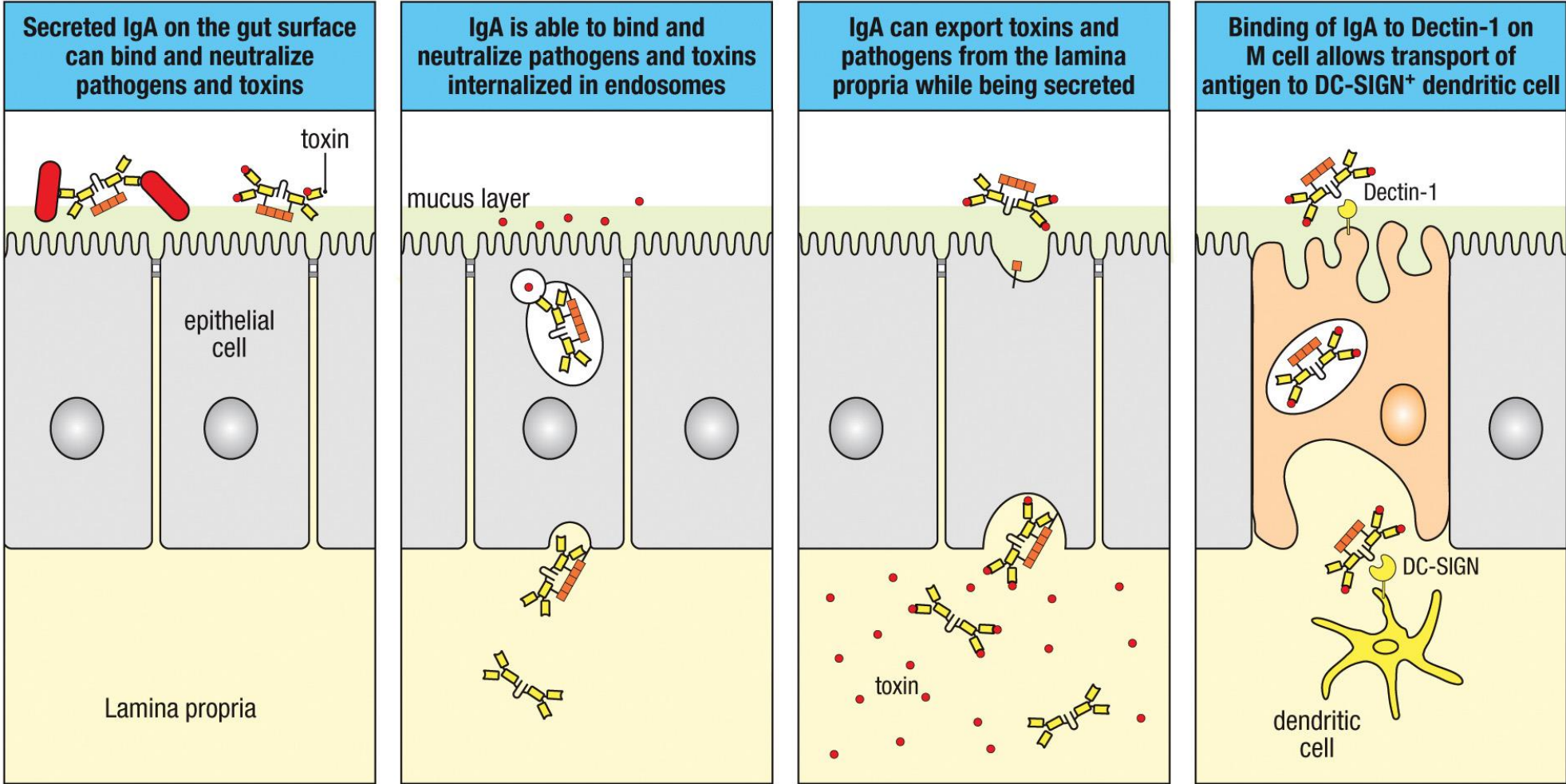
Transcytosis of secretory IgA to the luminal surface



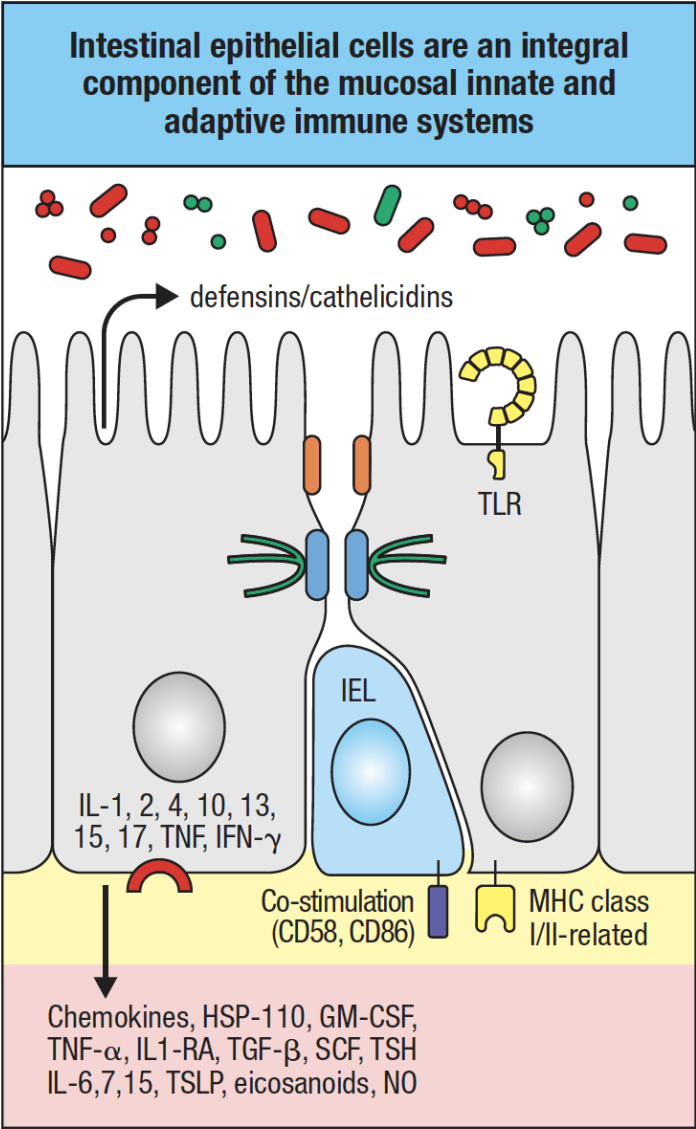
IgA binds to the polymeric Ig receptor (pIgR), also known as transmembrane secretory component (SC) on the basolateral surface and is transported to the apical surface. The portion of the pIgR attached to the Fc region of IgA is then enzymatically cleaved and stays bound to dimeric IgA as SC.

-  Transmembrane SC
-  Free or bound SC
-  J chain

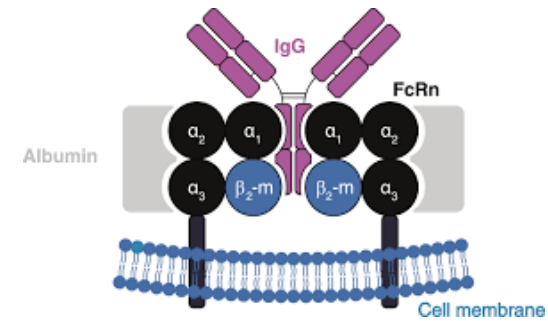
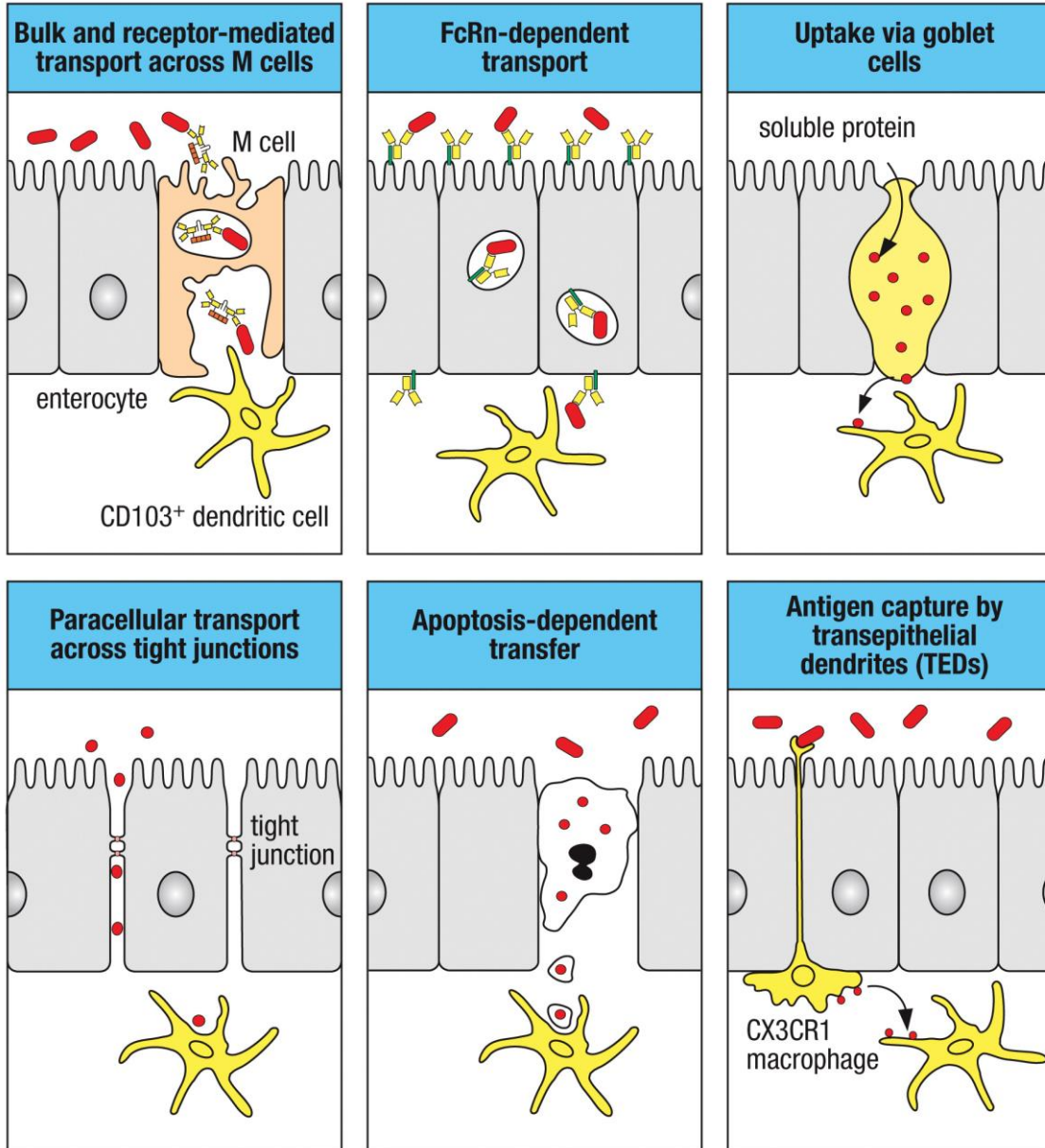
Secretory IgA has several functions at epithelial surfaces



Epithelial cells direct numerous components of the innate and adaptive immune systems at various levels

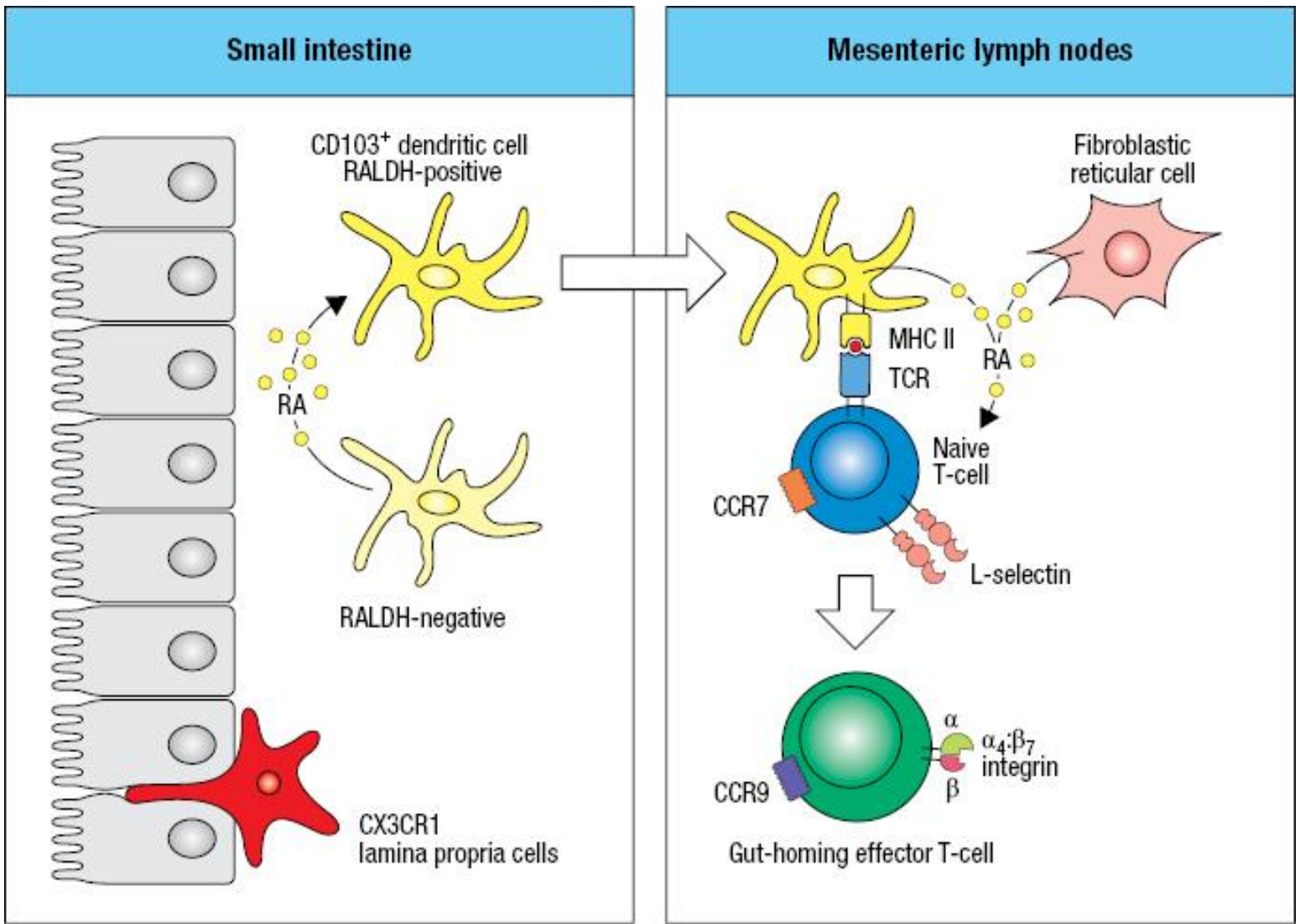


Routes of antigen uptake in the intestine

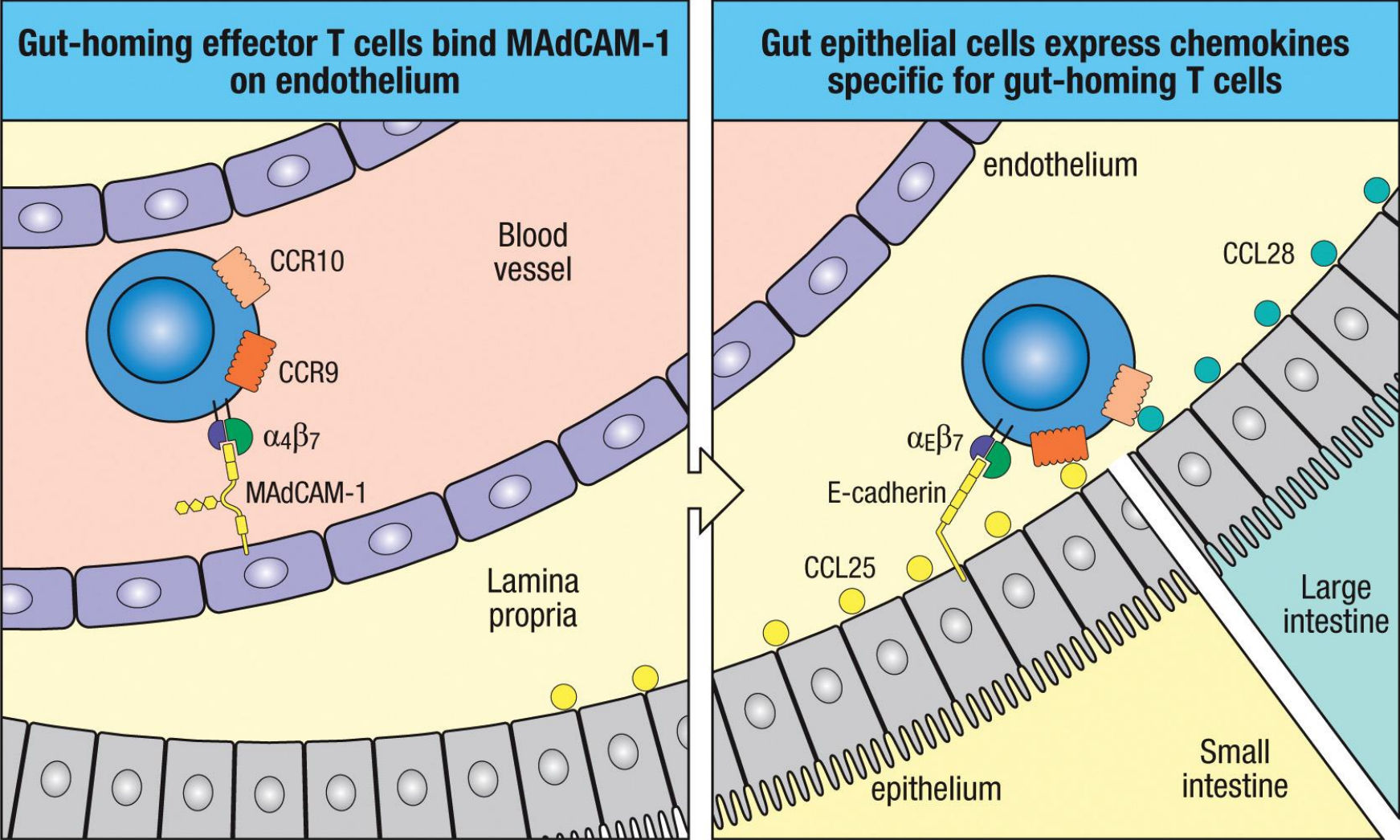


Small intestinal-derived migratory DC induce the expression of gut homing molecules

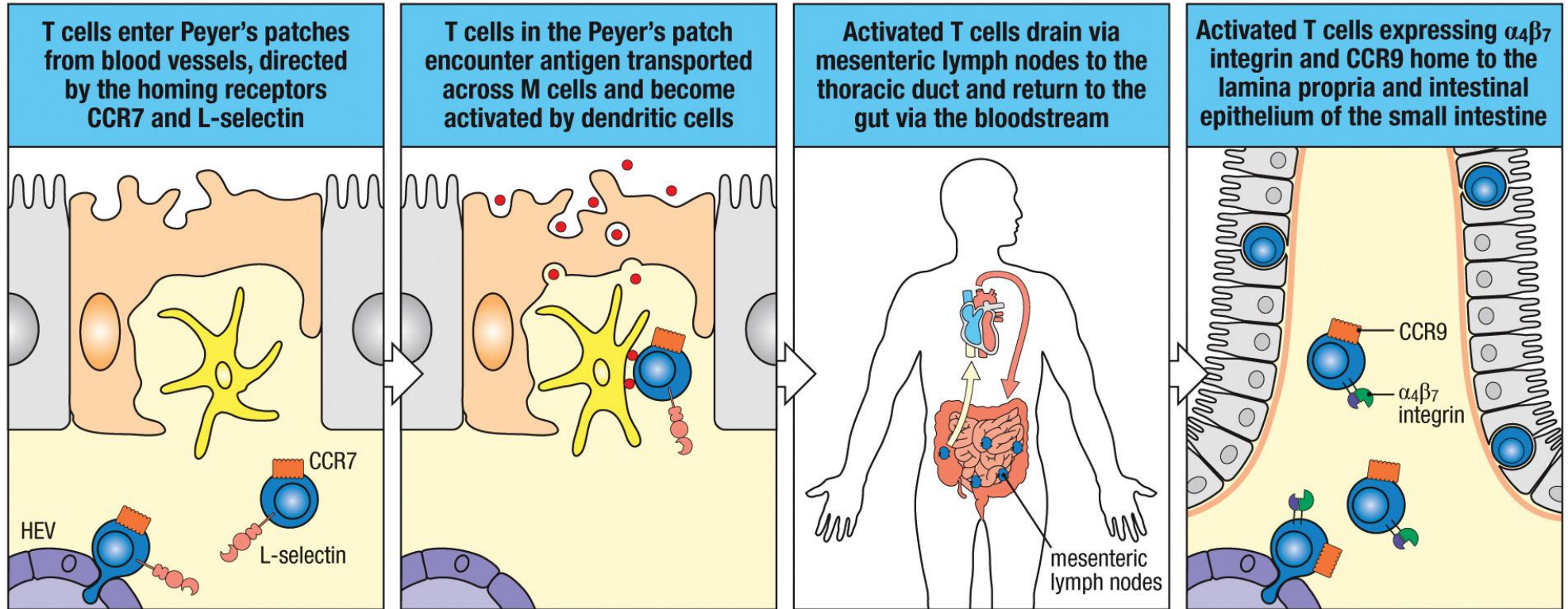
Retinoic Acid is derived from dietary vitamin A



Molecular control of intestine specific homing of lymphocytes



The mucosal immune system contains large numbers of effector lymphocytes even in the absence of disease

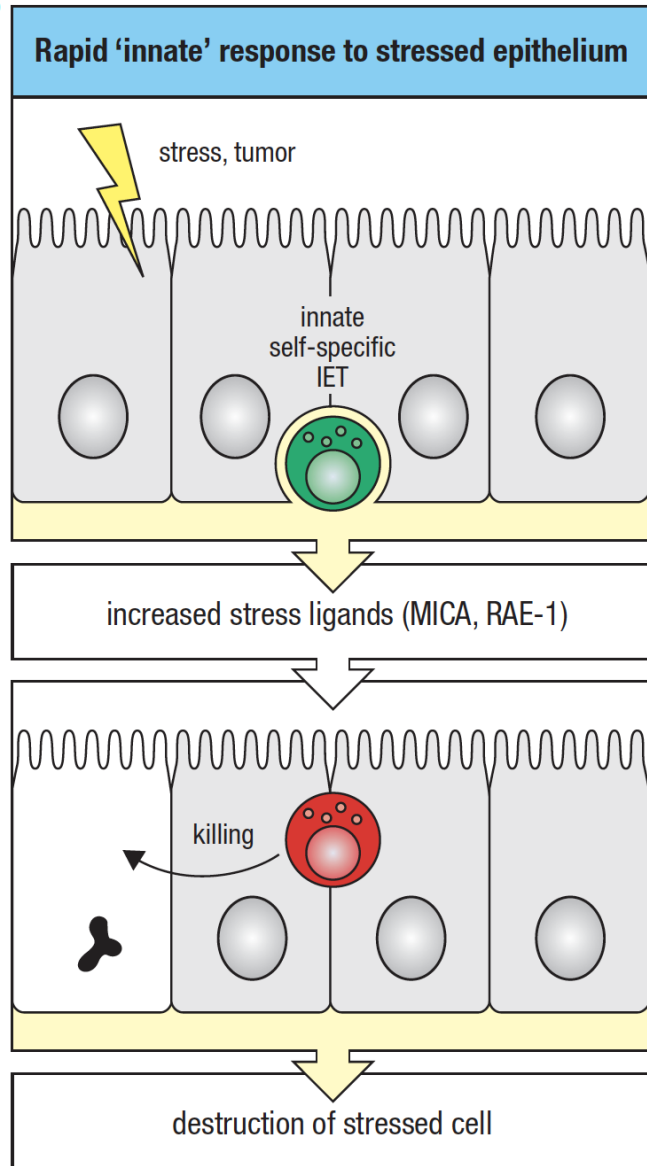


Janeway's Immunobiology 10th Edition, W.W. Norton & Co. 2022

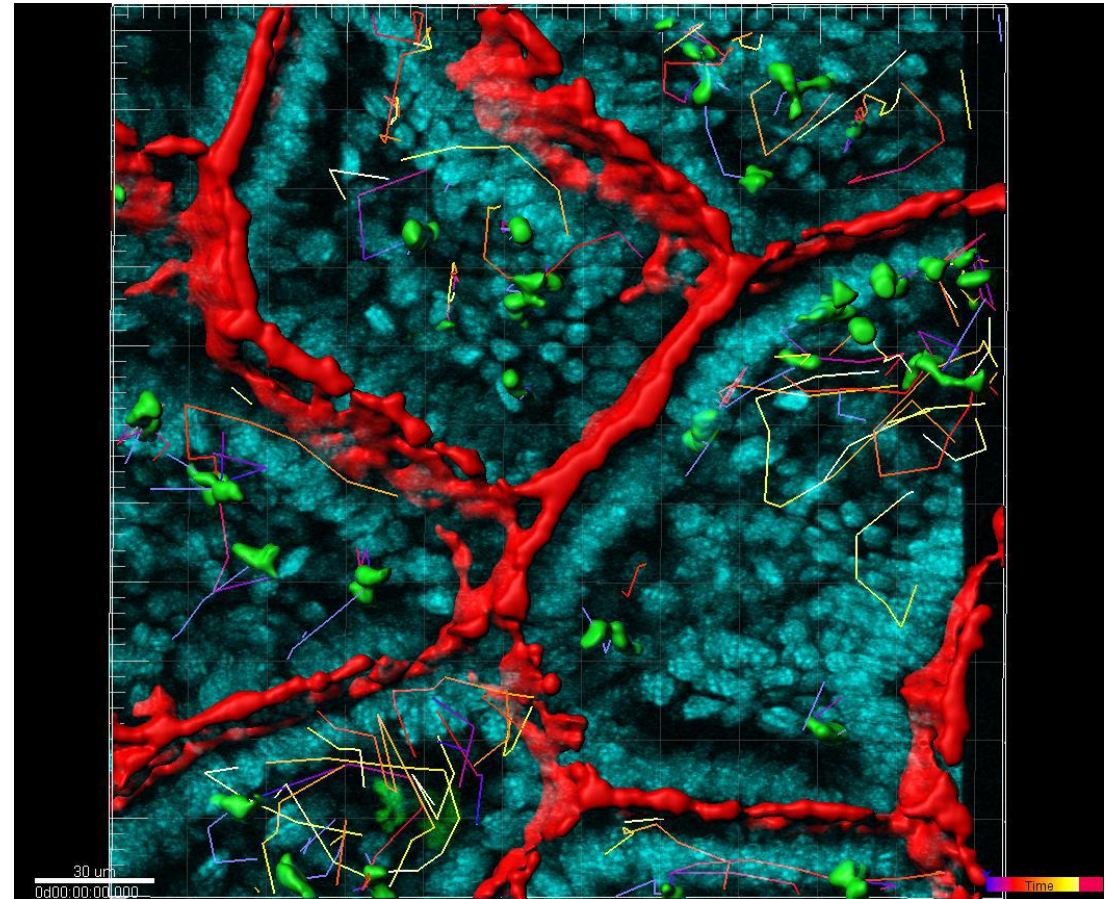
Memory T effector cells accumulate in the intestinal lamina propria, enabling the GALT to respond quickly and effectively to challenge with enteric pathogens.

Antigen challenge redistributes memory T effector cells to “man the barrier” for strategic mucosal defense.

IEL act as sentinels to detect and repair damaged epithelium



lumen $\gamma\delta$ T cell nuclei

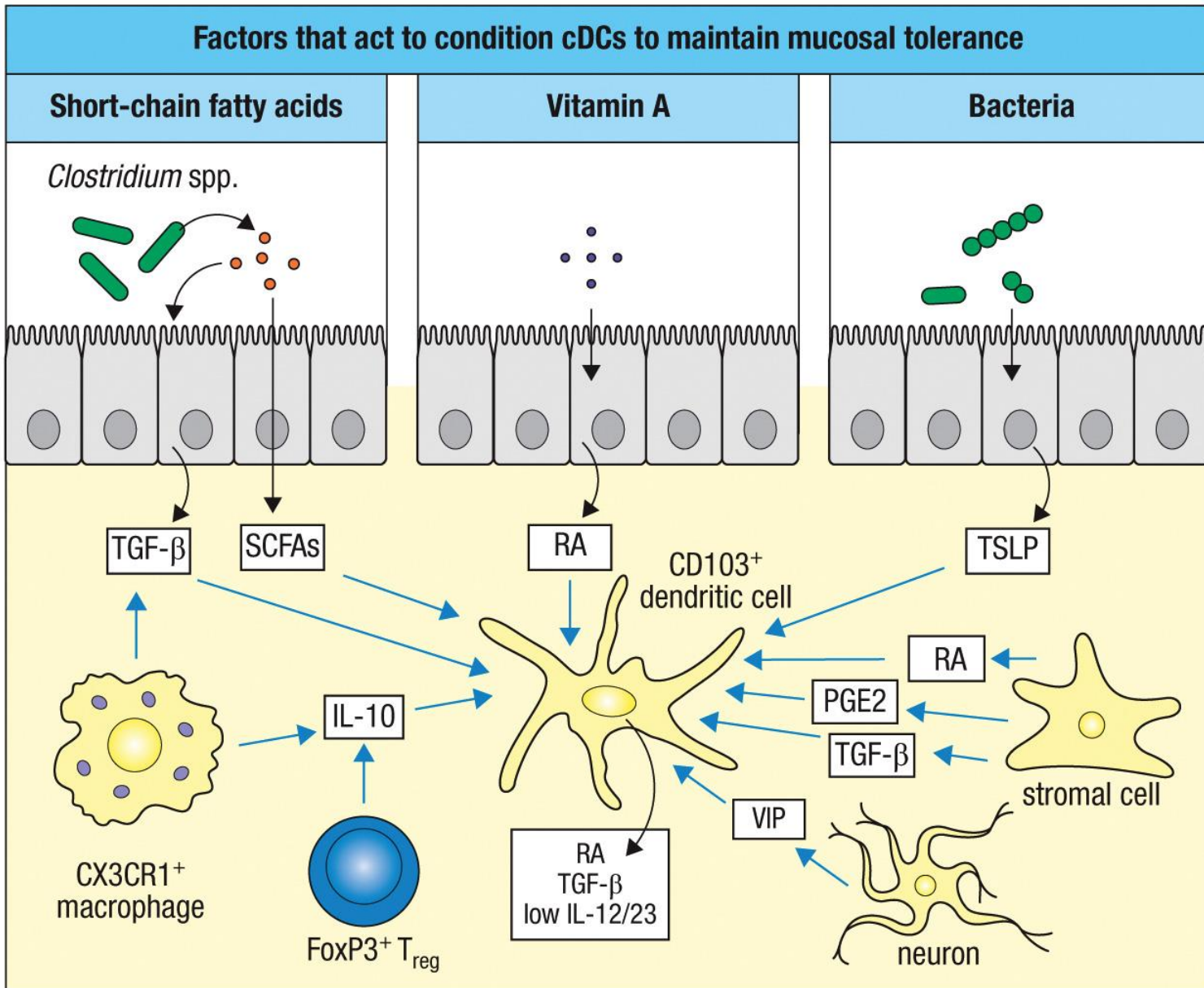


Edelblum et al PNAS 2012, 109: 7097

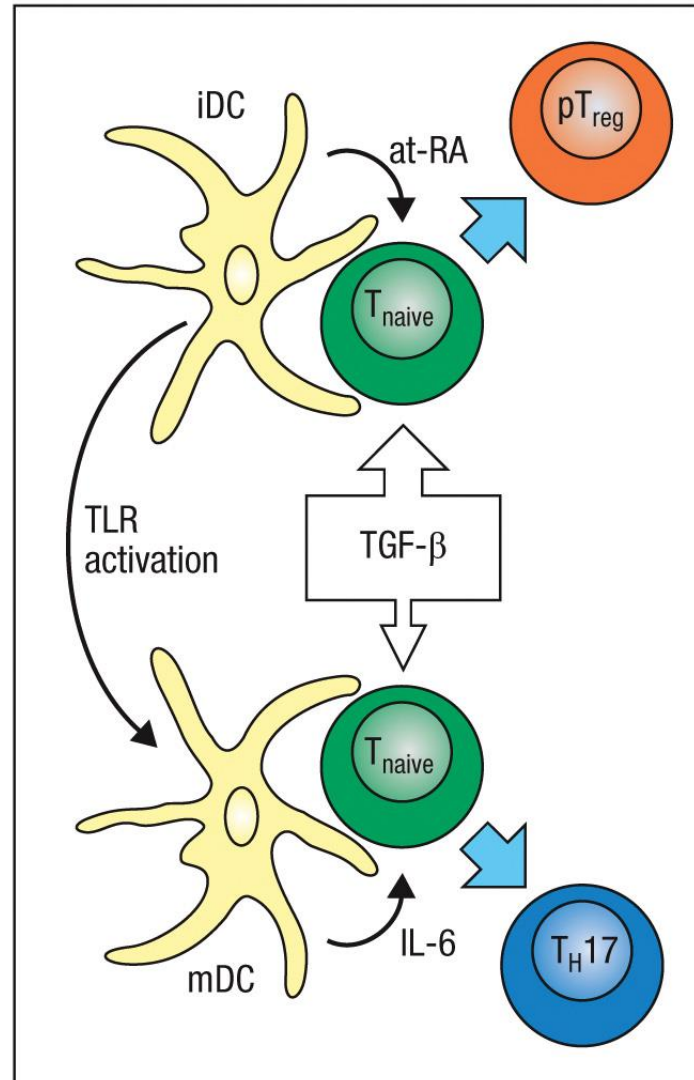
**How does the gut-associated
lymphoid tissue distinguish innocuous dietary
antigens and commensal bacteria from pathogenic
microbes**

....and mount an appropriate response to each?

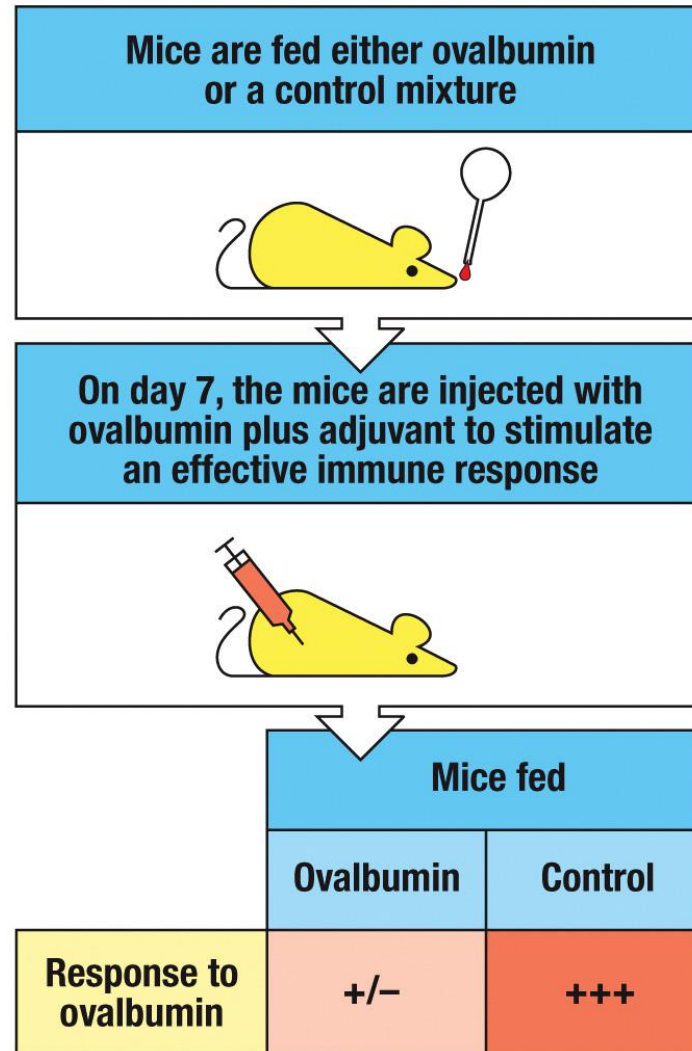
	Protective immunity	Mucosal tolerance
Antigen	Invasive bacteria, viruses, toxins	Food proteins; commensal bacteria
Primary Ig production	Intestinal IgA and IgG Specific Ab present in serum	Some local IgA Low or no Ab in serum
Primary T-cell response	Local and systemic effector and memory T cells	pT _{reg} cell induction; no local effector T-cell response
Response to antigen reexposure	Enhanced (memory) response	Low or no response or systemic response



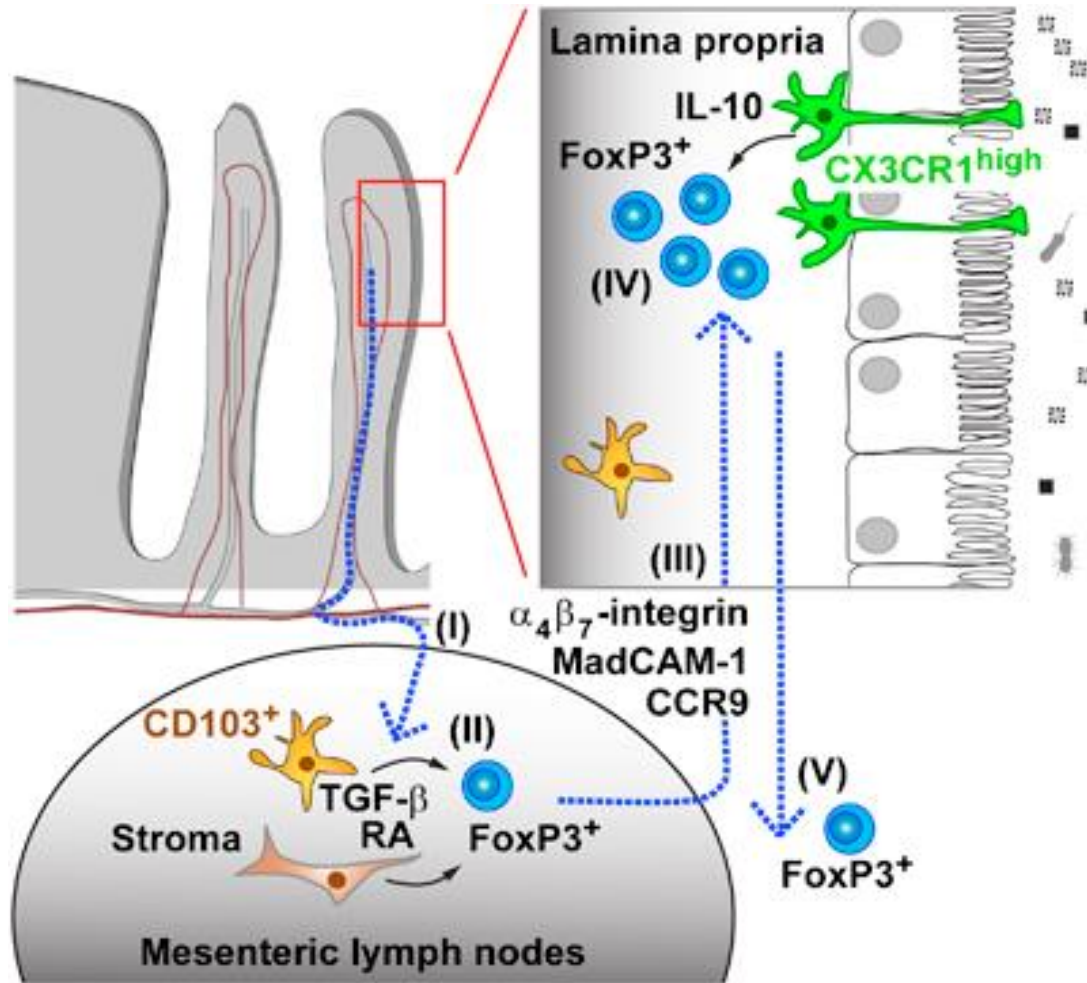
A shared requirement for TGF- β in the differentiation of pTreg and Th17 provides a development link that reflects their complementary roles in promoting mutualism with the microbiota



Oral tolerance to dietary antigens can be modeled experimentally

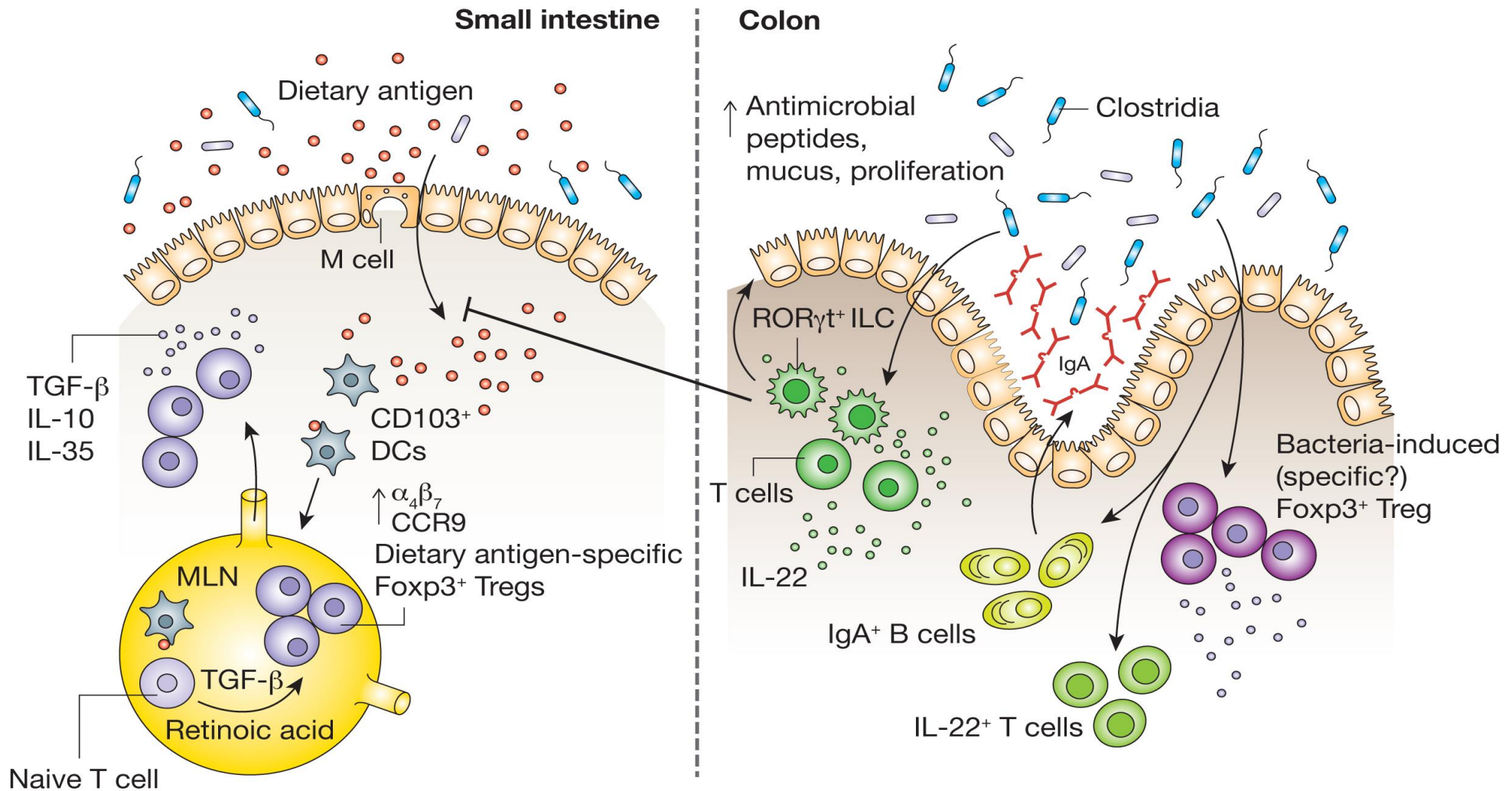


A multistep model of oral tolerance to dietary antigens

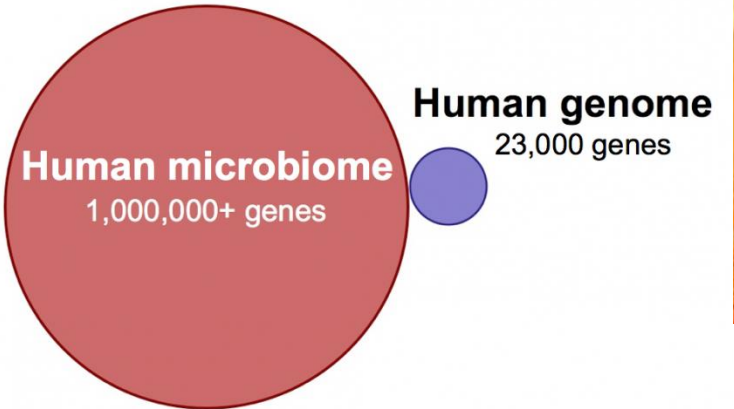
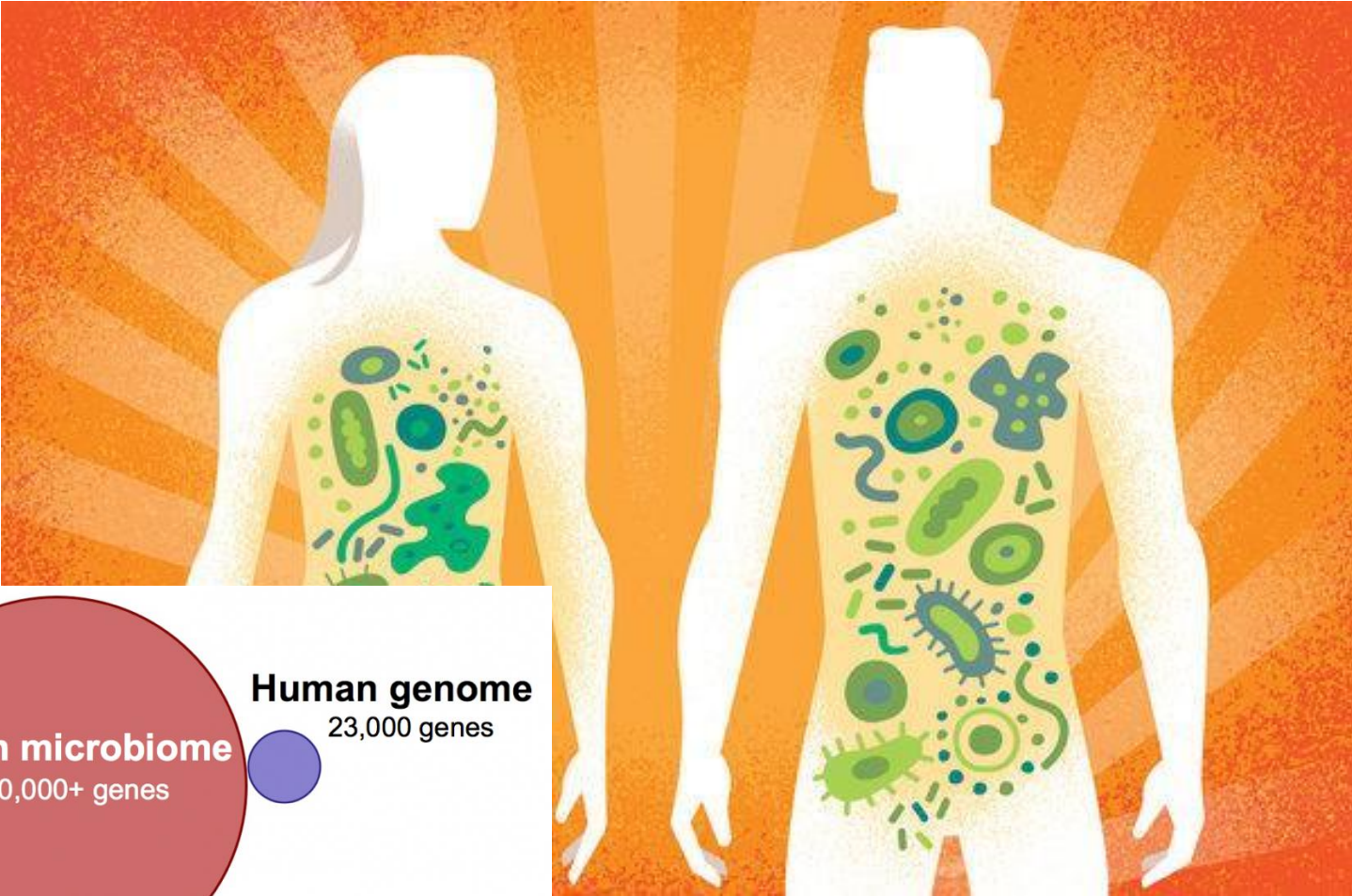


- I. Antigen loaded **CD103⁺ DC** migrate to MLN
- II. RA produced by DC and stromal cells in MLN induce homing receptors and favor **TGF- β** dependent conversion of **Foxp3⁺ Tregs**
- III. Committed **Tregs** home back to LP
- IV. Tregs expand under the influence of IL-10 produced by **CX3CR1^{hi}** macrophages
- V. Some **Tregs** exit mucosa via lymph or blood-stream to promote systemic tolerance

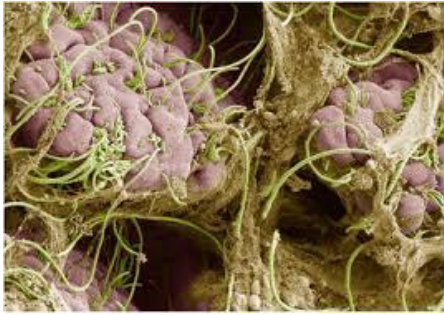
Tolerance to dietary antigen requires the induction of a bacteria-induced barrier protective response



Microbes populate our skin and mucosal surfaces and profoundly influence our health



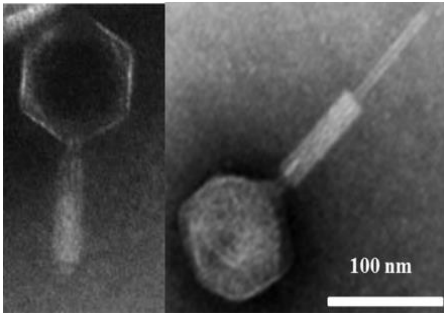
The microbiome is defined as the collective genomes of the microbes that live on the skin and mucosal surfaces.



bacteria



virus



bacteriophage



protozoa

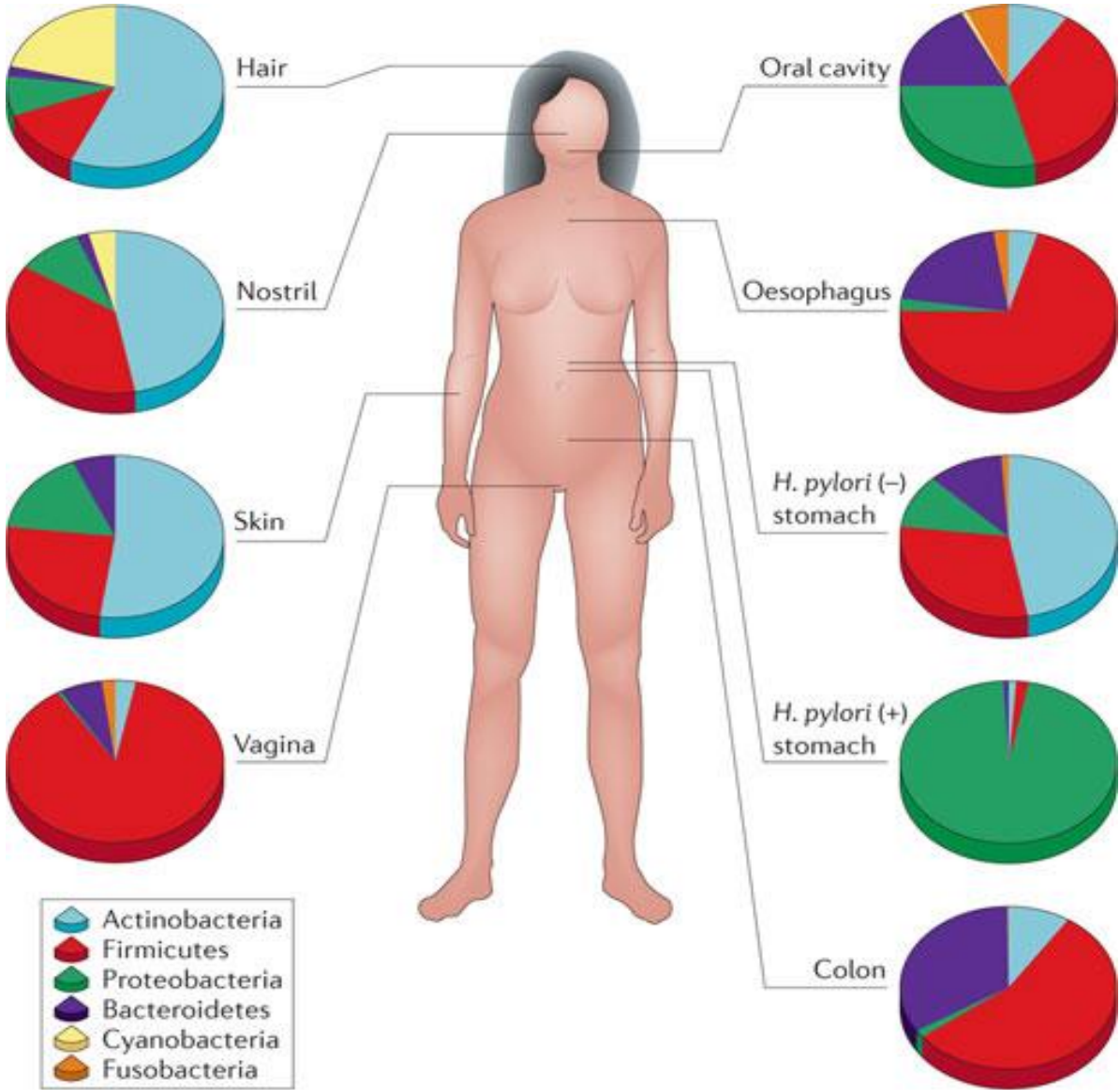


fungus



helminths

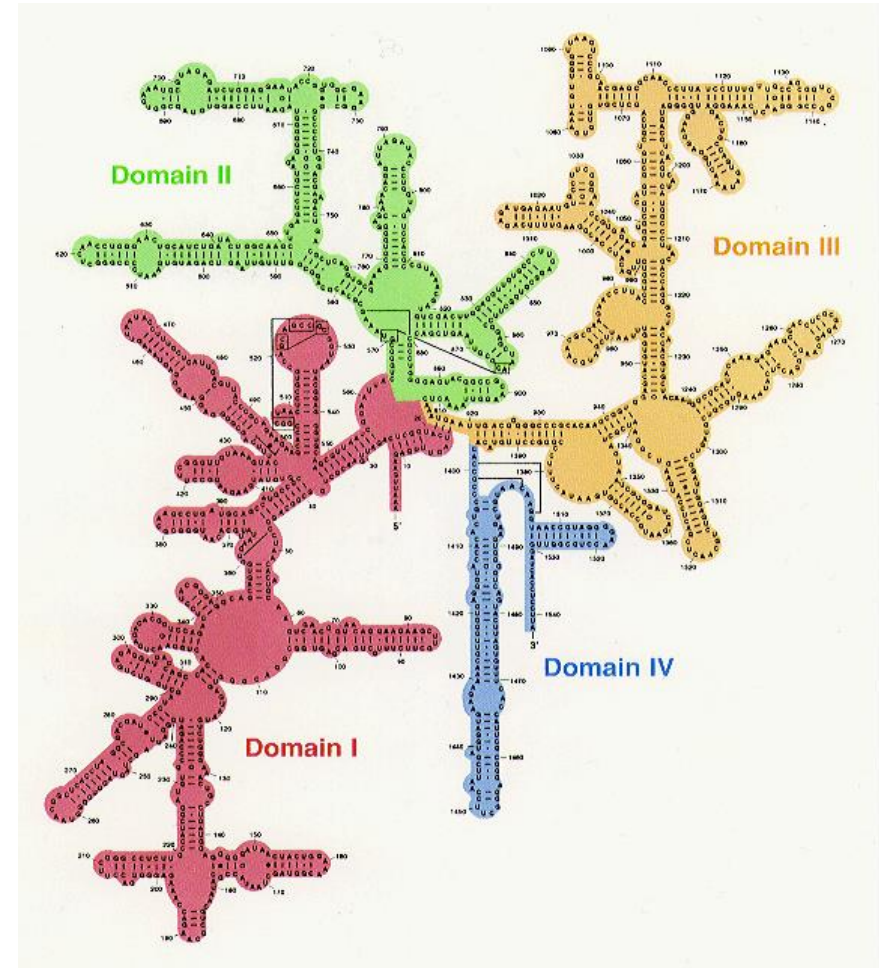
The composition of the microbiota varies by anatomical site



Culture independent methods of analysis have transformed our understanding of the composition of the microbiome

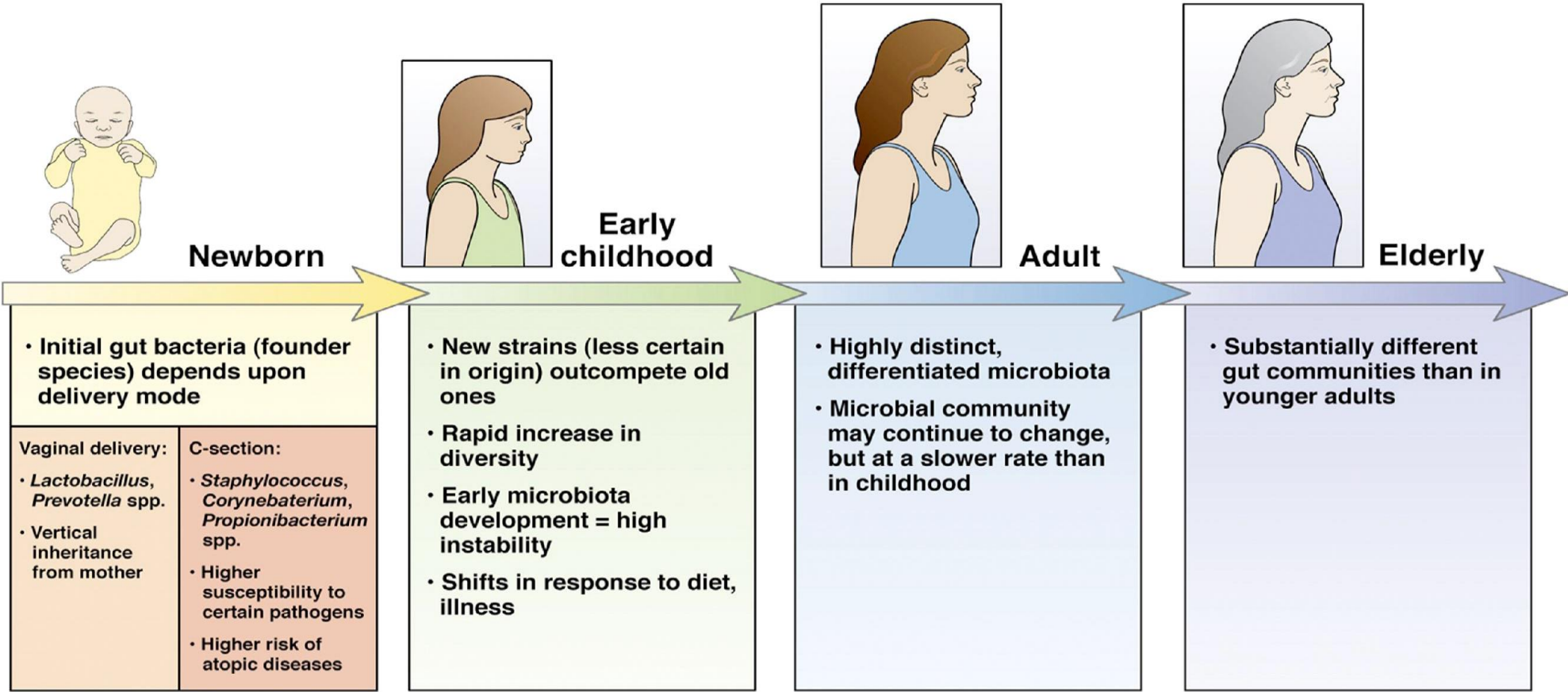
The 16S rRNA gene is highly conserved among bacterial species.

“Universal” primers target conserved regions of this gene and allow for amplification and sequencing of species-specific hypervariable regions for bacterial classification.

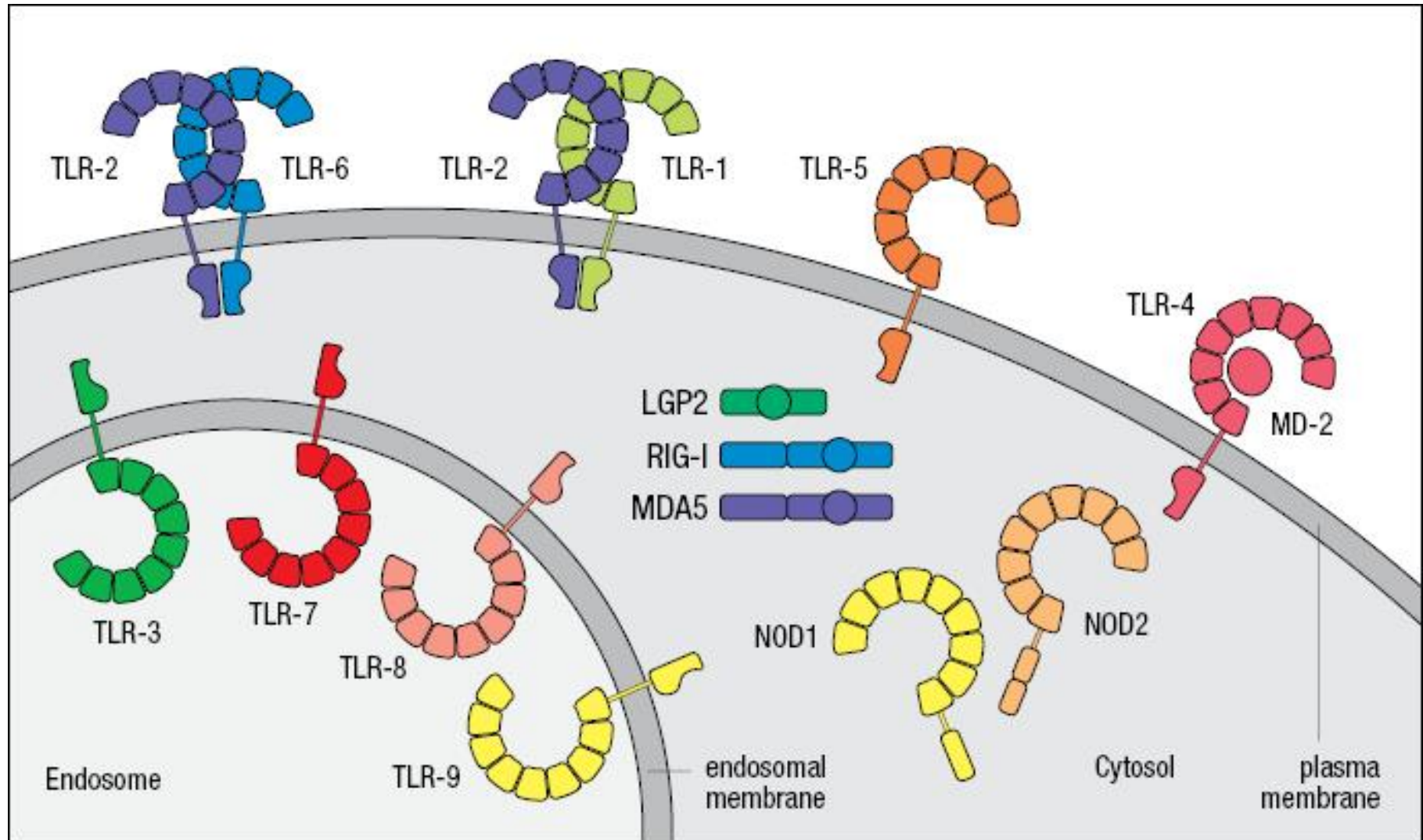


Structure of 16S ribosomal RNA

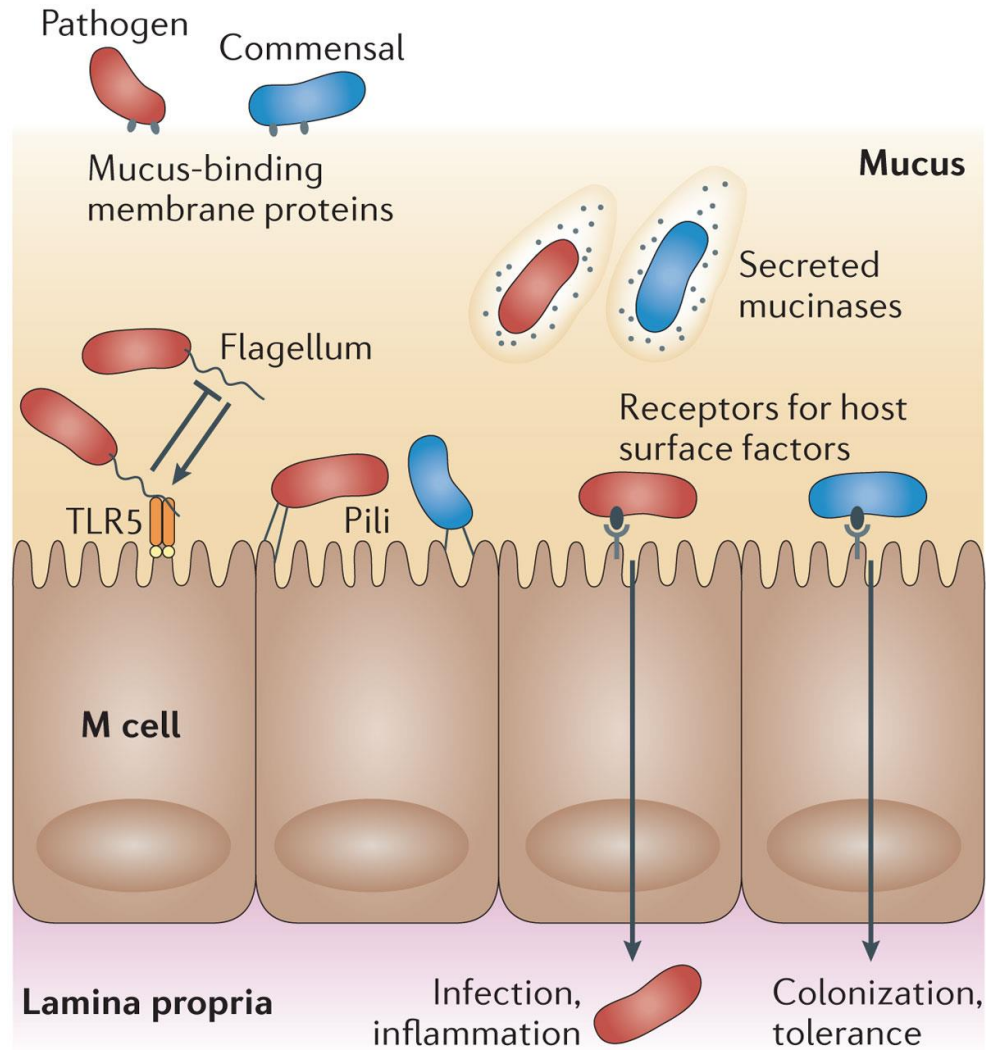
The gastrointestinal microbiota changes throughout life



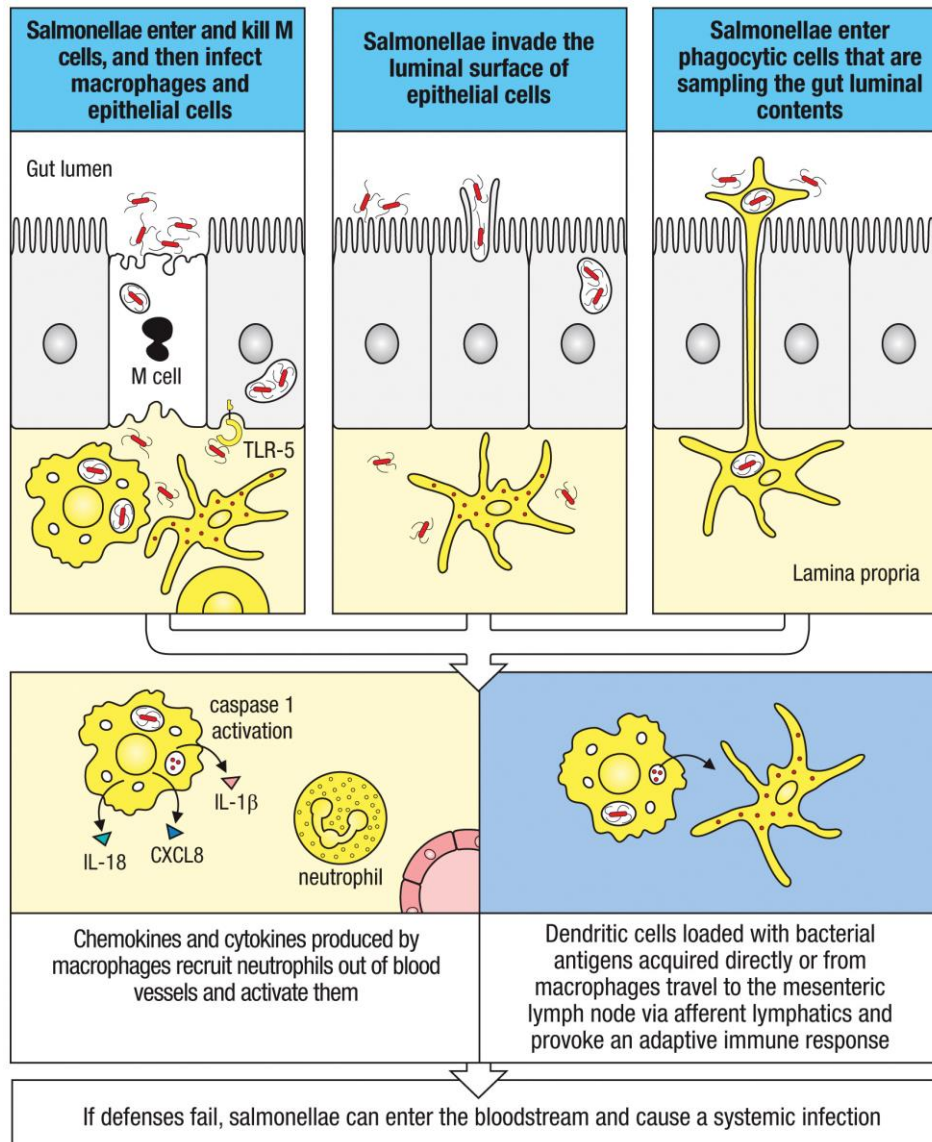
Pathogenic and commensal microbes share the same PAMPS (pathogen associated molecular patterns)



Both pathogens and commensals have access to the gut epithelium



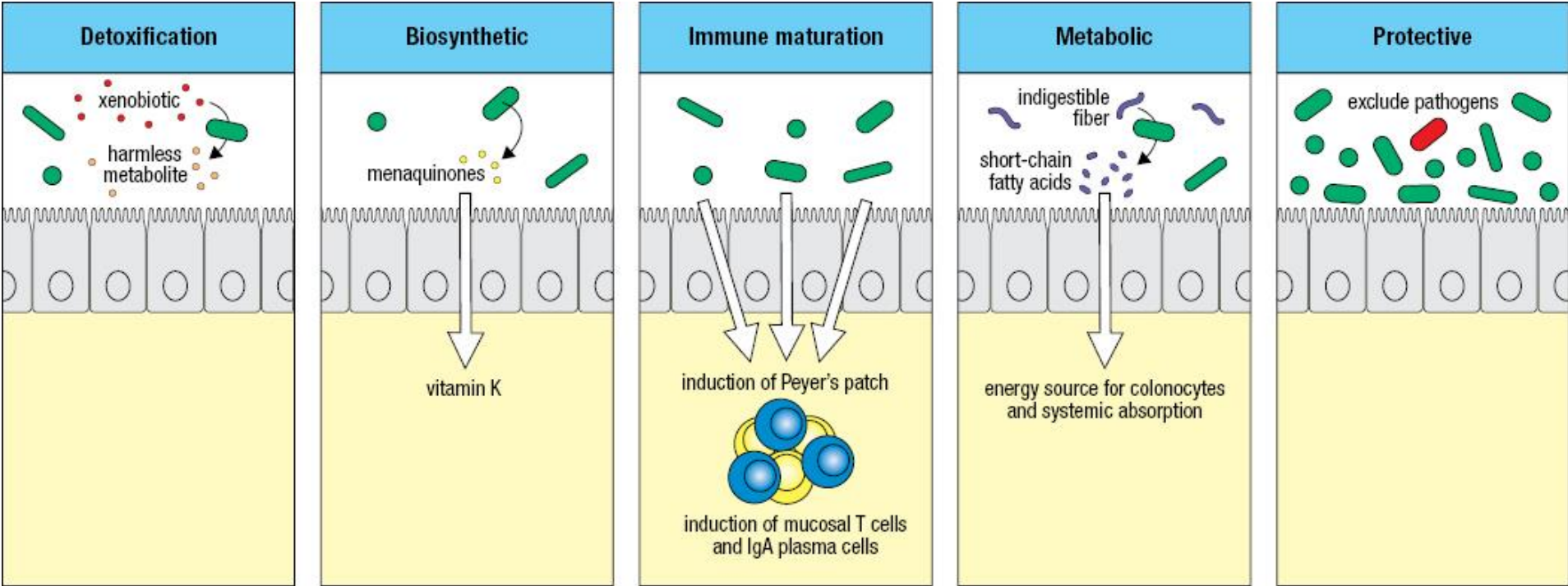
Enteric pathogens cause a local inflammatory response and the development of protective immunity



We exist in a dynamic interrelationship with our commensal microbiome!

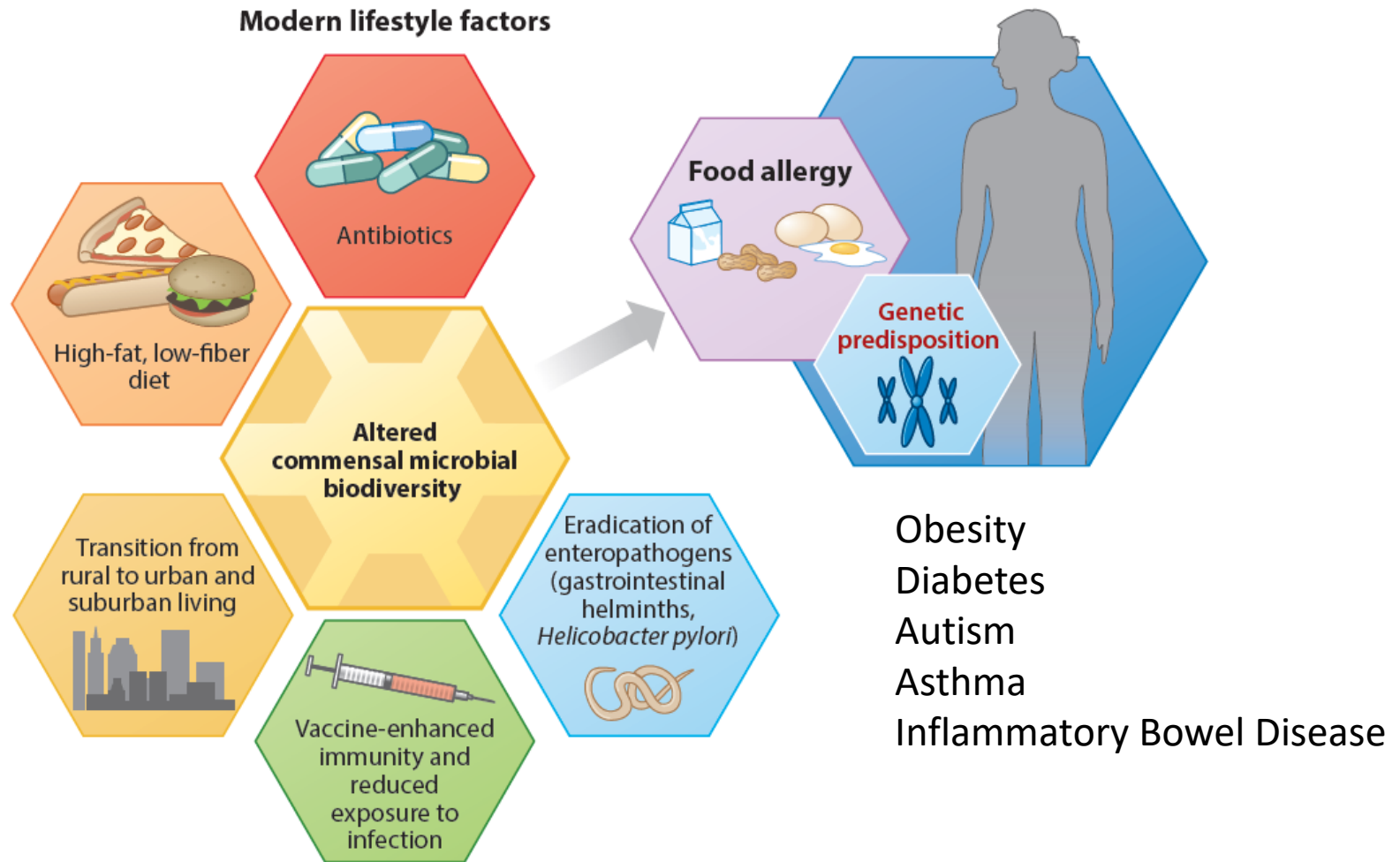
Healthy individuals “tolerate” their intestinal microbiota but are also constantly receiving signals from the microbiome that have a profound impact on both systemic and mucosal immunity.

The commensal microbiota confers many health benefits to the host

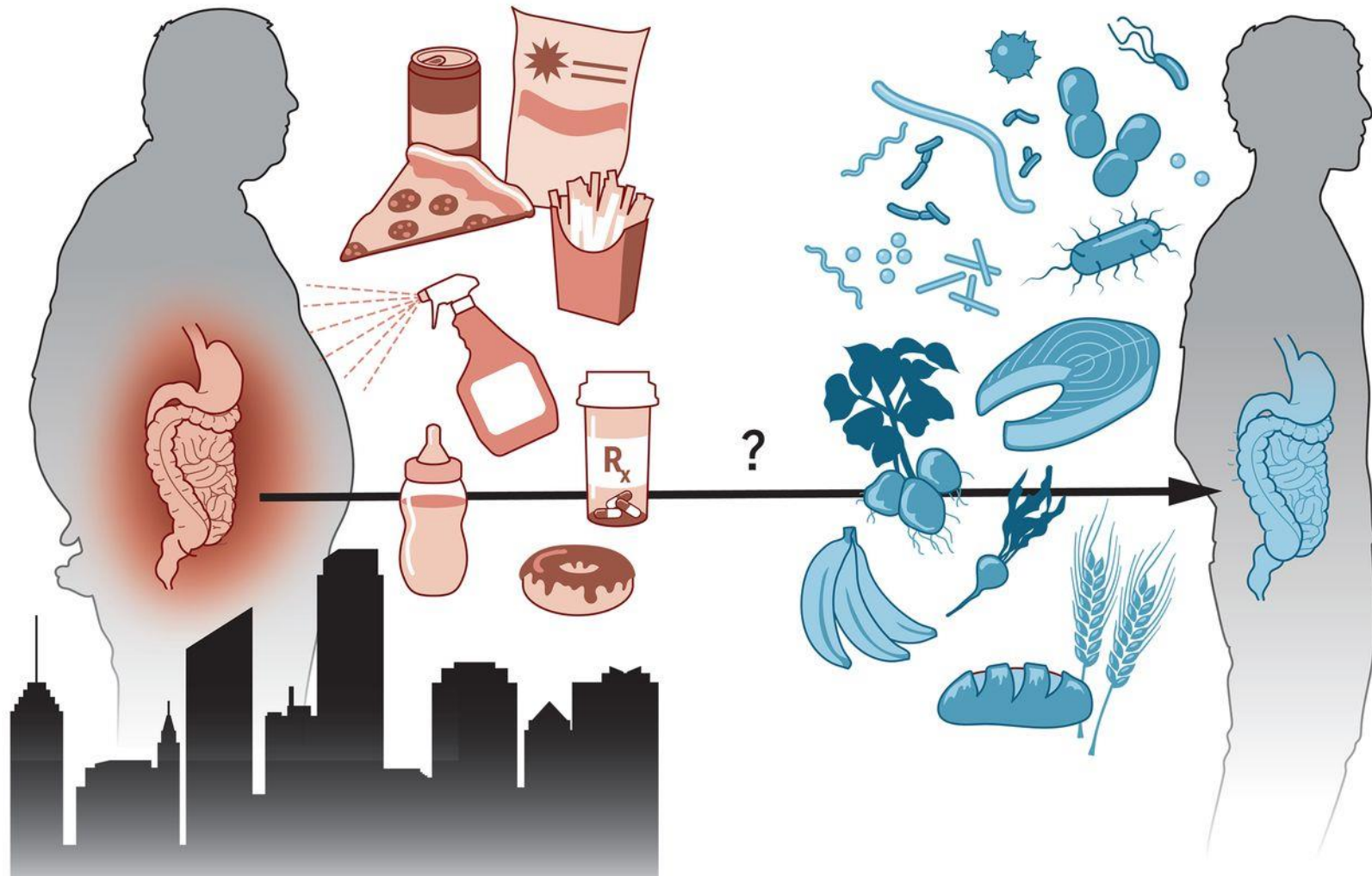


Principles of Mucosal Immunology, 2nd ed. (© CRC Press 2020)

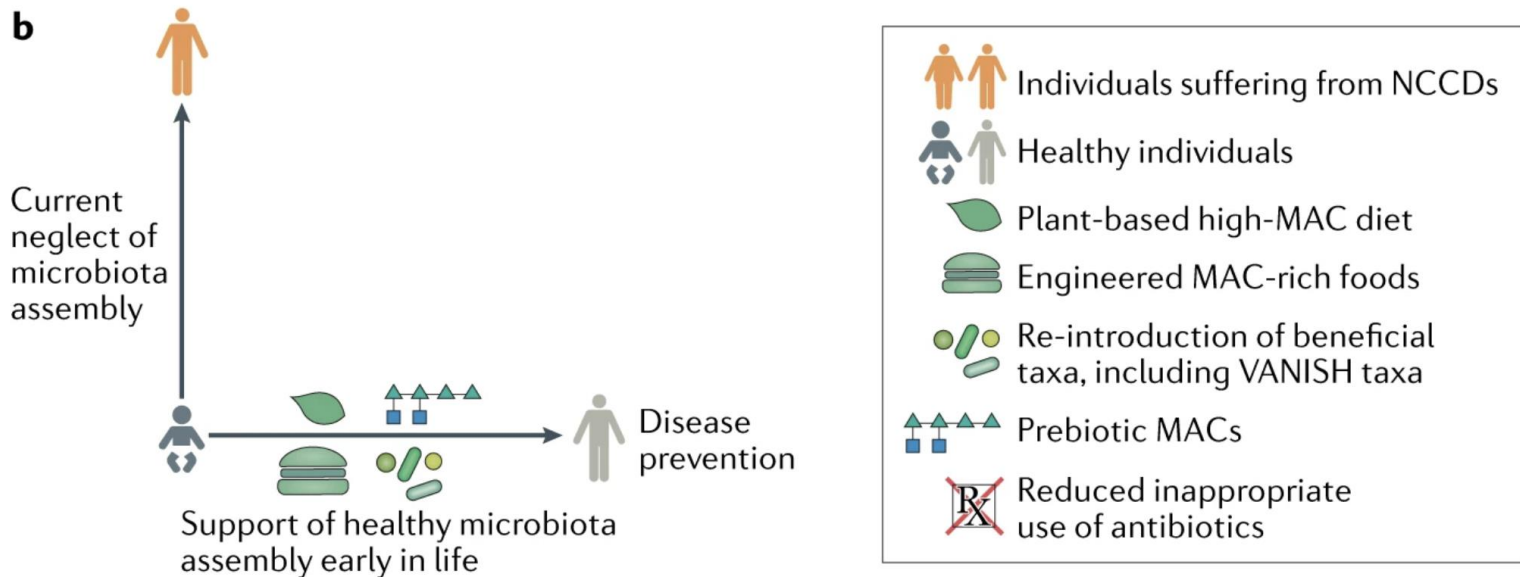
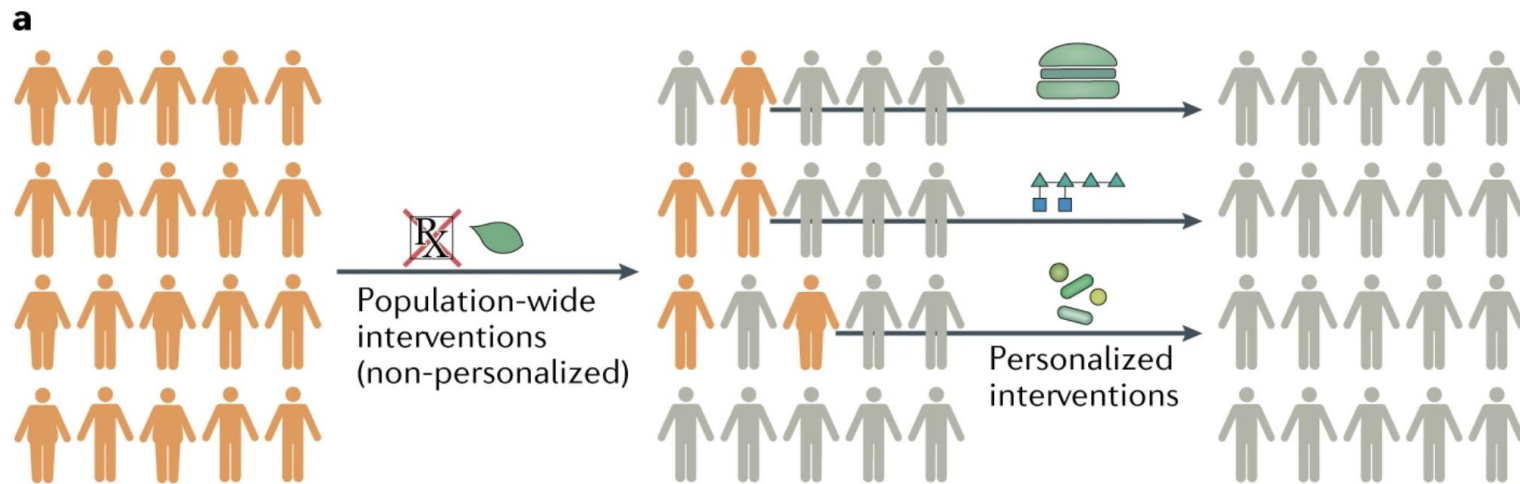
21st century lifestyle factors alter commensal microbial diversity and are driving a dramatic increase in non-communicable chronic disease (NCCD)



Most humans on earth consumed largely plant material for greater than 200,000 years; industrialization has changed the human gut microbiota



Both population-wide and personalized strategies are needed to manipulate the gut microbiota to improve health



Mucosal Vaccines

Mucosal (oral/nasal) vaccines are the preferred method for vaccination in the developing world; many don't require cold chain.

Mucosal vaccines are easily administered (needle-free), non-invasive and cost-effective.

Only mucosal vaccination elicits a protective secretory IgA response.



unicef 

Eradicating polio

We are closer than ever to ending polio.

Burden of mucosal diseases with unmet vaccine needs

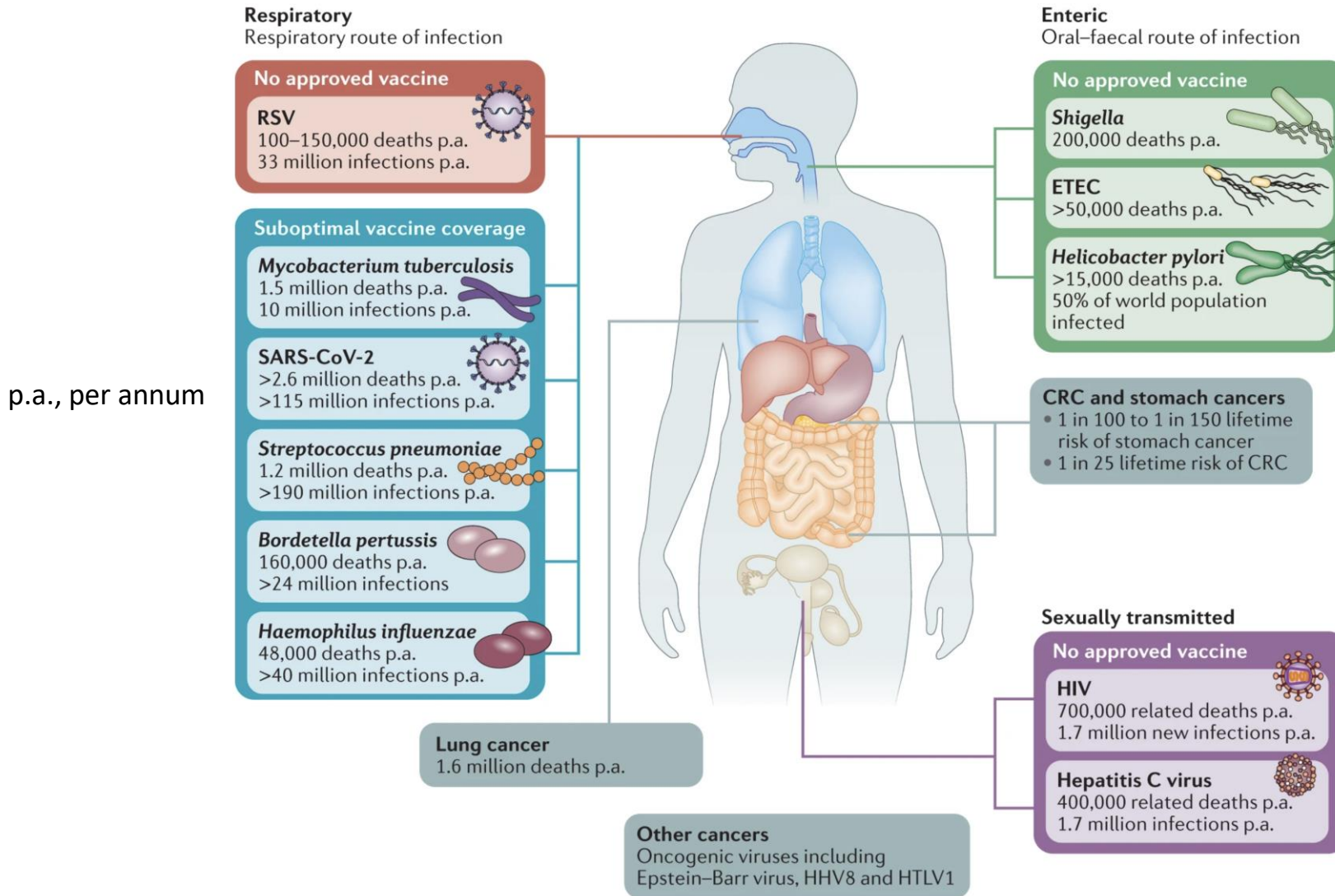


Table 1

Recommended Child and Adolescent Immunization Schedule for Ages 18 Years or Younger, United States, 2024

These recommendations must be read with the notes that follow. For those who fall behind or start late, provide catch-up vaccination at the earliest opportunity as indicated by the green bars. To determine minimum intervals between doses, see the catch-up schedule (Table 2).

Vaccine and other immunizing agents	Birth	1 mo	2 mos	4 mos	6 mos	9 mos	12 mos	15 mos	18 mos	19–23 mos	2–3 yrs	4–6 yrs	7–10 yrs	11–12 yrs	13–15 yrs	16 yrs	17–18 yrs	
Respiratory syncytial virus (RSV-mAb [Nirsevimab])	1 dose depending on maternal RSV vaccination status, See Notes					1 dose (8 through 19 months), See Notes												
Hepatitis B (HepB)	1 st dose	← 2 nd dose →		← 3 rd dose →														
Rotavirus (RV): RV1 (2-dose series), RV5 (3-dose series)	1 st dose		2 nd dose	See Notes														
Diphtheria, tetanus, acellular pertussis (DTaP <7 yrs)	1 st dose		2 nd dose	3 rd dose	← 4 th dose →			5 th dose										
Haemophilus influenzae type b (Hib)	1 st dose		2 nd dose	See Notes		← 3 rd or 4 th dose, See Notes →												
Pneumococcal conjugate (PCV15, PCV20)	1 st dose		2 nd dose	3 rd dose	← 4 th dose →													
Inactivated poliovirus (IPV <18 yrs)	1 st dose		2 nd dose	← 3 rd dose →				4 th dose	See Notes									
COVID-19 (1vCOV-mRNA, 1vCOV-aPS)	1 or more doses of updated (2023–2024 Formula) vaccine (See Notes)																	
Influenza (IIV4)	Annual vaccination 1 or 2 doses												Annual vaccination 1 dose only					
OR											Annual vaccination 1 or 2 doses		OR Annual vaccination 1 dose only					
Influenza (LAIV4)											Annual vaccination 1 or 2 doses		Annual vaccination 1 dose only					
Measles, mumps, rubella (MMR)	See Notes				← 1 st dose →			2 nd dose										
Varicella (VAR)	← 1 st dose →						2 nd dose											
Hepatitis A (HepA)	See Notes				2-dose series, See Notes													
Tetanus, diphtheria, acellular pertussis (Tdap ≥7 yrs)													1 dose					
Human papillomavirus (HPV)														See Notes				
Meningococcal (MenACWY-CRM ≥2 mos, MenACWY-TT ≥2years)	See Notes														1 st dose	2 nd dose		
Meningococcal B (MenB-4C, MenB-FHbp)														See Notes				
Respiratory syncytial virus vaccine (RSV [Abrysvo])														Seasonal administration during pregnancy, See Notes				
Dengue (DEN4CYD; 9–16 yrs)														Seropositive in endemic dengue areas (See Notes)				
Mpox																		

 Range of recommended ages for all children
 Range of recommended ages for catch-up vaccination
 Range of recommended ages for certain high-risk groups
 Recommended vaccination can begin in this age group
 Recommended vaccination based on shared clinical decision-making
 No recommendation/not applicable

Table 1 Recommended Child and Adolescent Immunization Schedule for Ages 18 Years or Younger, United States, 2024

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Diphtheria, tetanus, acellular pertussis (DTaP <7 yrs)			1 st dose	2 nd dose	3 rd dose			← 4 th dose →			5 th dose							
Haemophilus influenzae type b (Hib)			1 st dose	2 nd dose	See Notes		← 3 rd or 4 th dose, See Notes →											
Pneumococcal conjugate (PCV15, PCV20)			1 st dose	2 nd dose	3 rd dose			← 4 th dose →										
Inactivated poliovirus (IPV <18 yrs)			1 st dose	2 nd dose	← 3 rd dose →						4 th dose						See Notes	
COVID-19 (1vCOV-mRNA, 1vCOV-aPS)	1 or more doses of updated (2023–2024 Formula) vaccine (See Notes)																	
Influenza (IIV4)	Annual vaccination 1 or 2 doses																	Annual vaccination 1 dose only
Influenza (LAIV4)												Annual vaccination 1 or 2 doses		Annual vaccination 1 dose only				
Measles, mumps, rubella (MMR)					See Notes		← 1 st dose →					2 nd dose						
Varicella (VAR)						← 1 st dose →					2 nd dose							
Hepatitis A (HepA)					See Notes		2-dose series, See Notes											
Tetanus, diphtheria, acellular pertussis (Tdap ≥7 yrs)													1 dose					
Human papillomavirus (HPV)														See Notes				
Meningococcal (MenACWY-CRM ≥2 mos, MenACWY-TT ≥2years)			See Notes												1 st dose		2 nd dose	
Meningococcal B (MenB-4C, MenB-FHbp)															See Notes			
Respiratory syncytial virus vaccine (RSV [Abrysvo])															Seasonal administration during pregnancy, See Notes			
Dengue (DEN4CYD; 9-16 yrs)														Seropositive in endemic dengue areas (See Notes)				
Mpox																		

Range of recommended ages for all children
 Range of recommended ages for catch-up vaccination
 Range of recommended ages for certain high-risk groups
 Recommended vaccination can begin in this age group
 Recommended vaccination based on shared clinical decision-making
 No recommendation/ not applicable

Licensed Mucosal Vaccines

Vibrio cholerae

Inactivated

Dukoral Oral — aqueous
 Composition: heat and formaldehyde-inactivated O1 serogroups (Inaba + Ogawa) + CTB

Euvichol, Shanchol Oral — aqueous
 Composition: heat and formaldehyde-inactivated O1 serogroups (Inaba + Ogawa) + 0139

Live attenuated

Vaxchora Oral — aqueous
 Composition: live attenuated O1 serogroup (Inaba) - ctxA attenuation

Influenza A and influenza B viruses

Live attenuated/reassortant

FluMist/Fluenz Nasal — spray
 Composition: quadrivalent antigens from circulating strains incorporated into live attenuated, cold adapted donor influenza vector

Salmonella typhimurium

Live attenuated/reassortant

Typhi Vivotif Oral — capsule
 Composition:
 • Live attenuated Ty21a strain
 • Mutagenesis in LPS synthesis and Vi polysaccharide genes

Poliovirus

Live attenuated

Biopolio (bOPV) Oral — aqueous
 Composition: culture passage attenuated polioviruses 1 and 3 serotypes (5' non-coding region attenuation)

mOPV and tOPV Oral — aqueous
 Composition: culture passage attenuated polioviruses 1, 2 and 3 serotypes (5' non-coding region attenuation)

Rotavirus

Live reassortant

Rotateq Oral — aqueous
 Composition: pentavalent — five human-bovine reassortant rotaviruses (expression of G1, G2, G3, G4, G5 with P7 and G6 with P1A)

Live attenuated

Rotarix Oral — aqueous
 Composition: monovalent — culture passage attenuated (G1 with P1A expression)

All are live-attenuated or inactivated whole cell preparations

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Mucosal adjuvants

Adjuvants that are effective parenterally are generally toxic or unstable when given orally.

The tendency of the GALT to induce tolerance to soluble antigens has made identification of effective mucosal adjuvants difficult.

Microbial products such as cholera toxin, *E. coli* heat-labile toxin and oligodeoxynucleotides containing a bacterial CpG motif can act as effective mucosal adjuvants and induce both mucosal and systemic immune responses to co-administered protein antigens.

Questions?

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