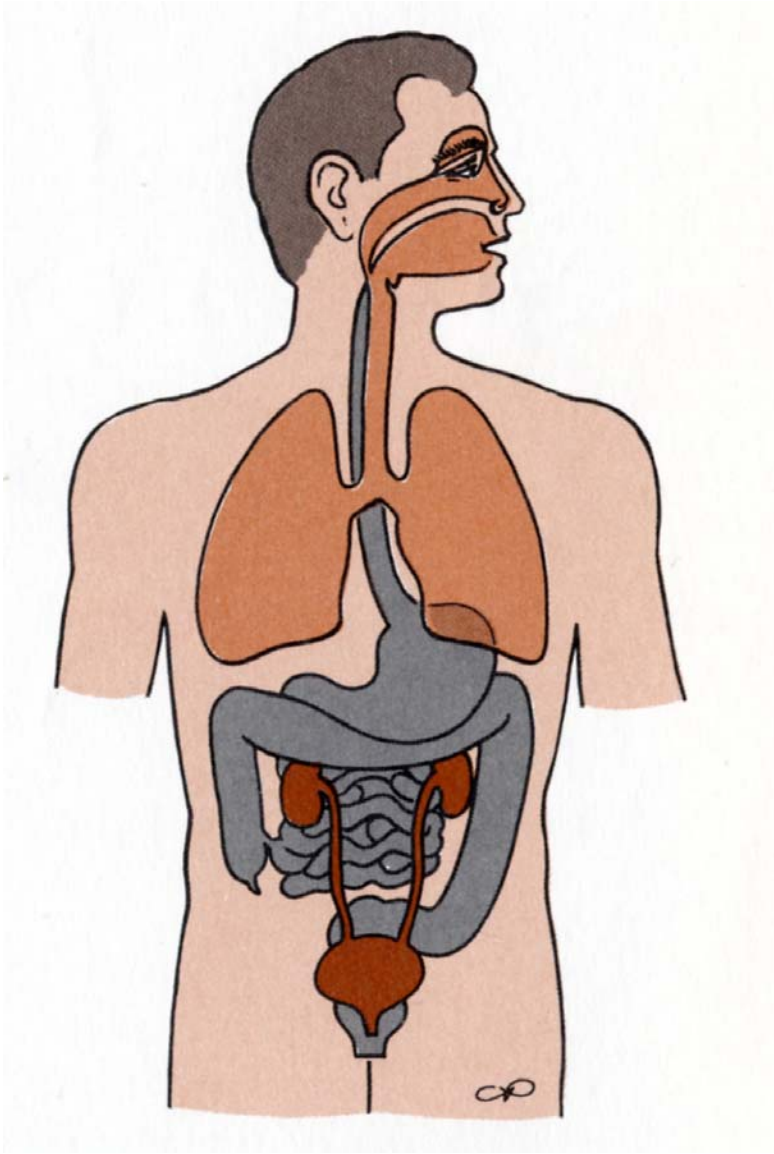
The Mucosal Immune System

April 29, 2009

The skin is exposed to the external environment but is sealed. Seal consists of dermis and substances produced by the skin.

The mucosa is an extension of the skin and lines the digestive track from mouth to anus - total surface area is 200X greater than skin surface.



Schaechter et al. *Mechanisms of Microbial Disease*, 2nd ed.

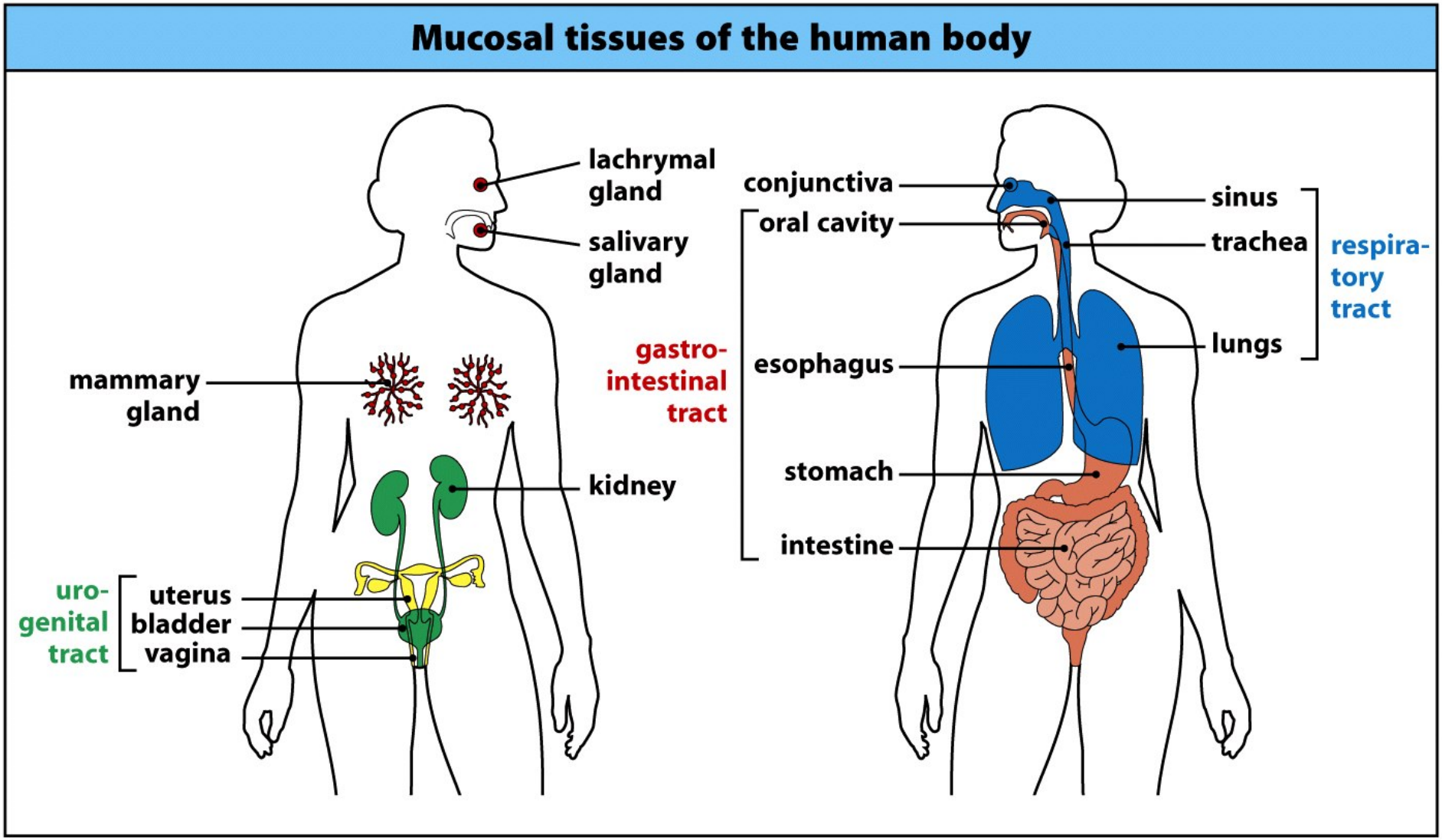


Figure 11-1 Immunobiology, 7ed. (© Garland Science 2008)

MALT (mucosa-associated lymphoid tissue) includes:

- **GALT** (gut-associated lymphoid tissue)
  - Peyer's patches (PP)
  - Mesenteric lymph nodes (MLN)
  - Appendix
  - Solitary lymphoid nodes
- **NALT** (nasopharyngeal-associated lymphoid tissue)
  - Salivary glands
  - Tonsils
- **BALT** (bronchus-associated lymphoid tissue)
- **Urogenital**

Box 1 | Advantages and disadvantages of nasal vaccination

Advantages

- Is the most effective route to elicit optimal protective immunity in both mucosal and systemic immune compartments.
- Can effectively induce antigen-specific immunity in the reproductive tract, as well as in the upper respiratory tract.
- Can generate cross-protective immunity in the gut through the common mucosal immune system.
- Can avoid degradation of vaccine antigen caused by digestive enzymes, so requires a smaller dose of antigen than oral immunization.
- Does not require injection, so is less painful.
- Does not require trained medical personnel for delivery.

Disadvantages

- Possible deposition of antigen in the central nervous system through the olfactory bulbs and olfactory nerves; this requires further investigation.
- Requires adjuvant safety to be clinically determined; clinical studies indicate that Bell's palsy is caused by influenza nasal vaccine that contains the native form of Escherichia coli heat-labile enterotoxin as a mucosal adjuvant.

Nature Immunology 4:699-710, 2004

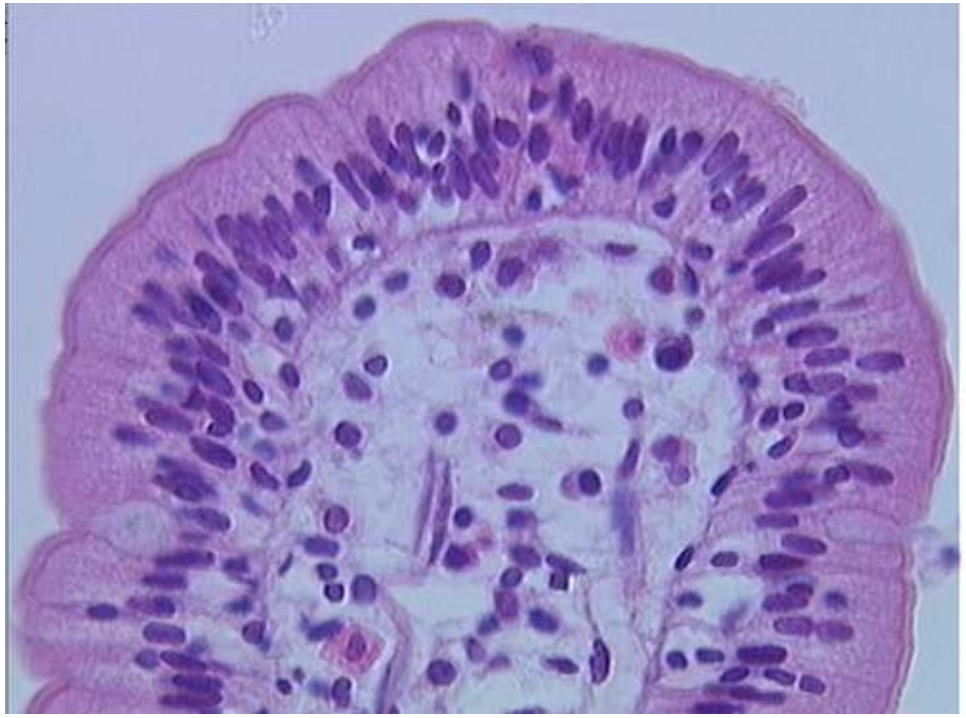
Mucosal immune responses differ from systemic immune responses:

- the major isotype in mucosal secretions is secretory, dimeric IgA
- most of the antibody-producing cells and effector T cells exist within MALT
- there are separate inductive and effector lymphoid sites

Enteric bacterial flora - major stimulus for development of mucosal immune system

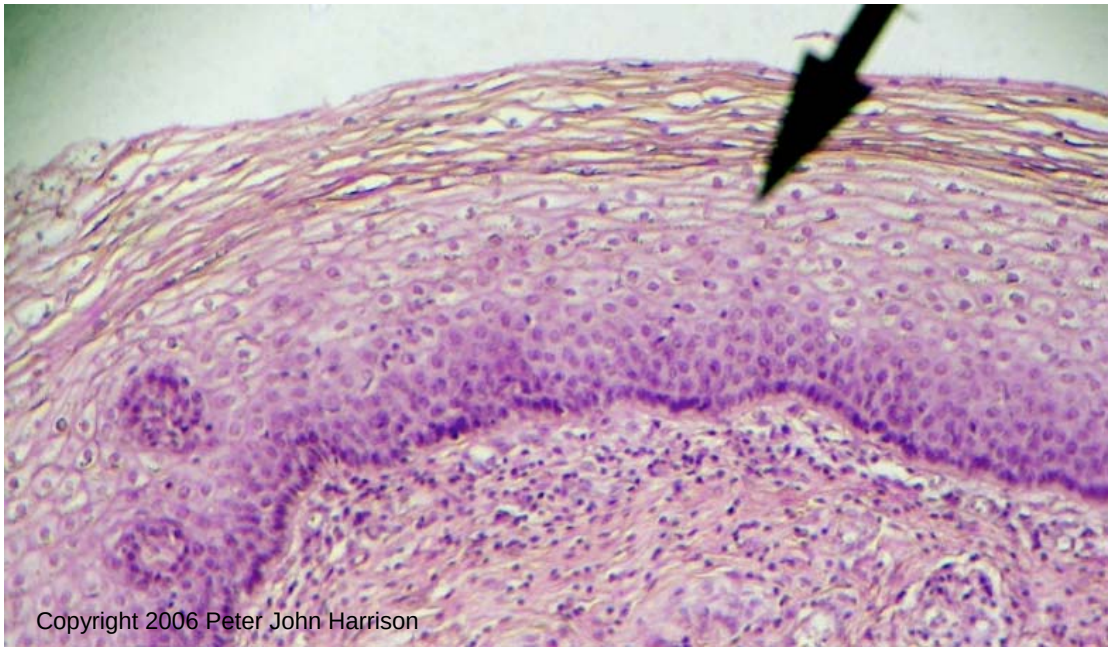
Type I mucosal surfaces are covered by simple epithelium - expresses a simple polymeric Ig receptor (pIgR) that allows dimeric IgA to access the lumen.

- Intestine
- Lungs
- Uterus



Type II mucosal surfaces are covered by stratified squamous epithelium which provides physical protective barriers for activities that are important for the host species.

- Oral cavity
- Vaginal cavity



Defense systems within the gut

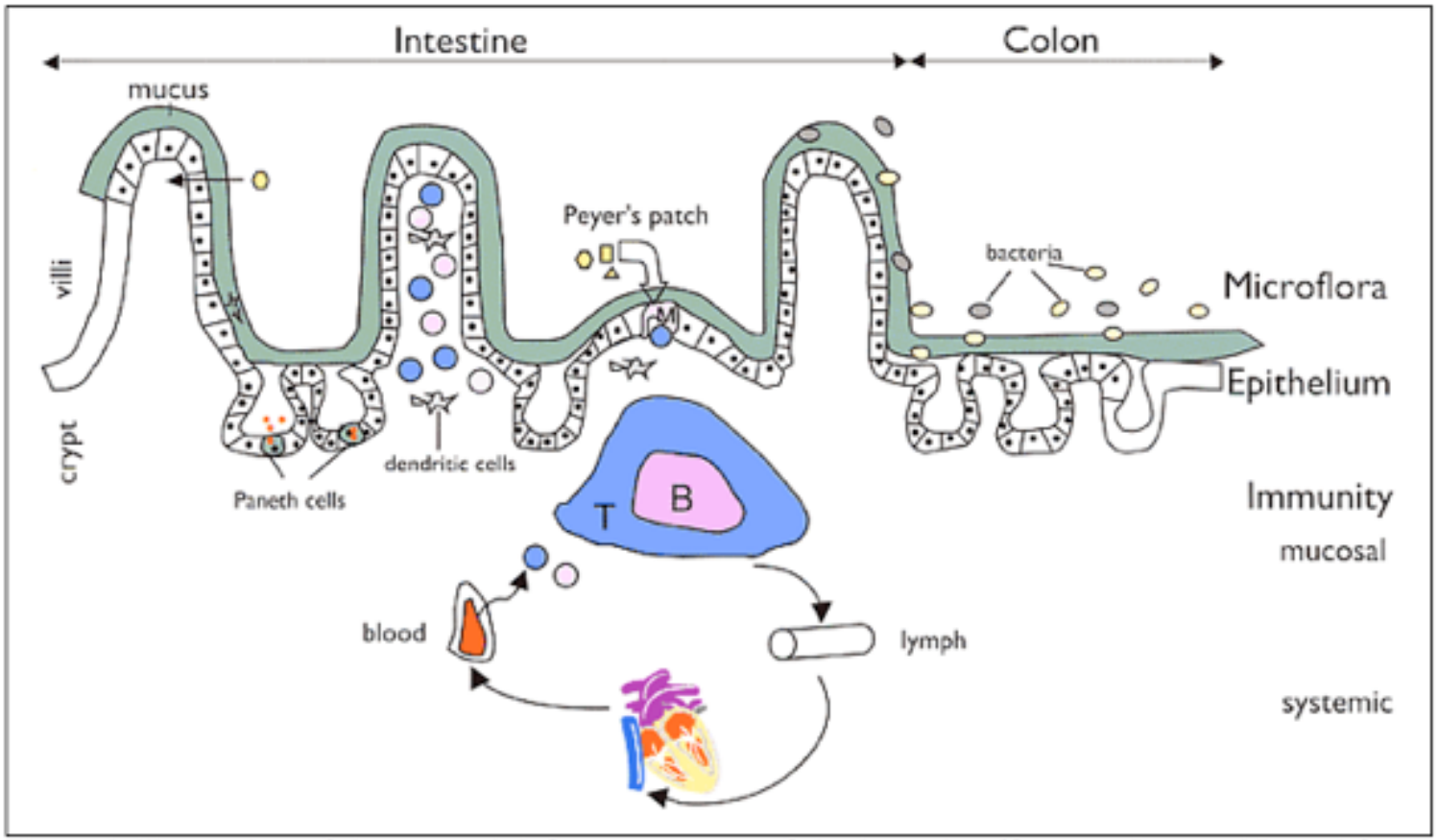


Figure I: Illustration of the natural defense systems of the intestine (Source: DanoneVitapole)

- Nonimmunologic barriers
- Immune system - innate and adaptive
- Gut flora (commensal bacteria)

Barriers to infection in the gut

- Enzymes present in saliva
- Low pH in the stomach
- Bile - stimulates peristalsis
- Intestinal mucus
- Tight junctions joining epithelial cells in the intestine.

Essential components of the intestinal innate immune mechanisms

[EC crosstalk with B cells T-independent]*

Immunoglobulins	Secretory IgA[a]
Antimicrobial peptides and proteins	Defensins, lysozyme, secretory phospolipase A2, angiogenins
Microbial	Commensal intestinal flora
Others	Gastric acid, biliary and pancreatic secretions, mucins

[a] Although by definition belonging to adaptive immunity, sIgA acts in first line mucosal defense, a key feature of innate immunity.

*Epithelial cells recognize microorganisms and communicate with and orchestrate both innate and acquired immune responses. They can produce different cytokines in response to different commensal bacteria. Uptake of bacteria by epithelial cells has been observed.

Yuan & Walker: J Pediatr Gastroenterol Nutr 38:463-473, 2004

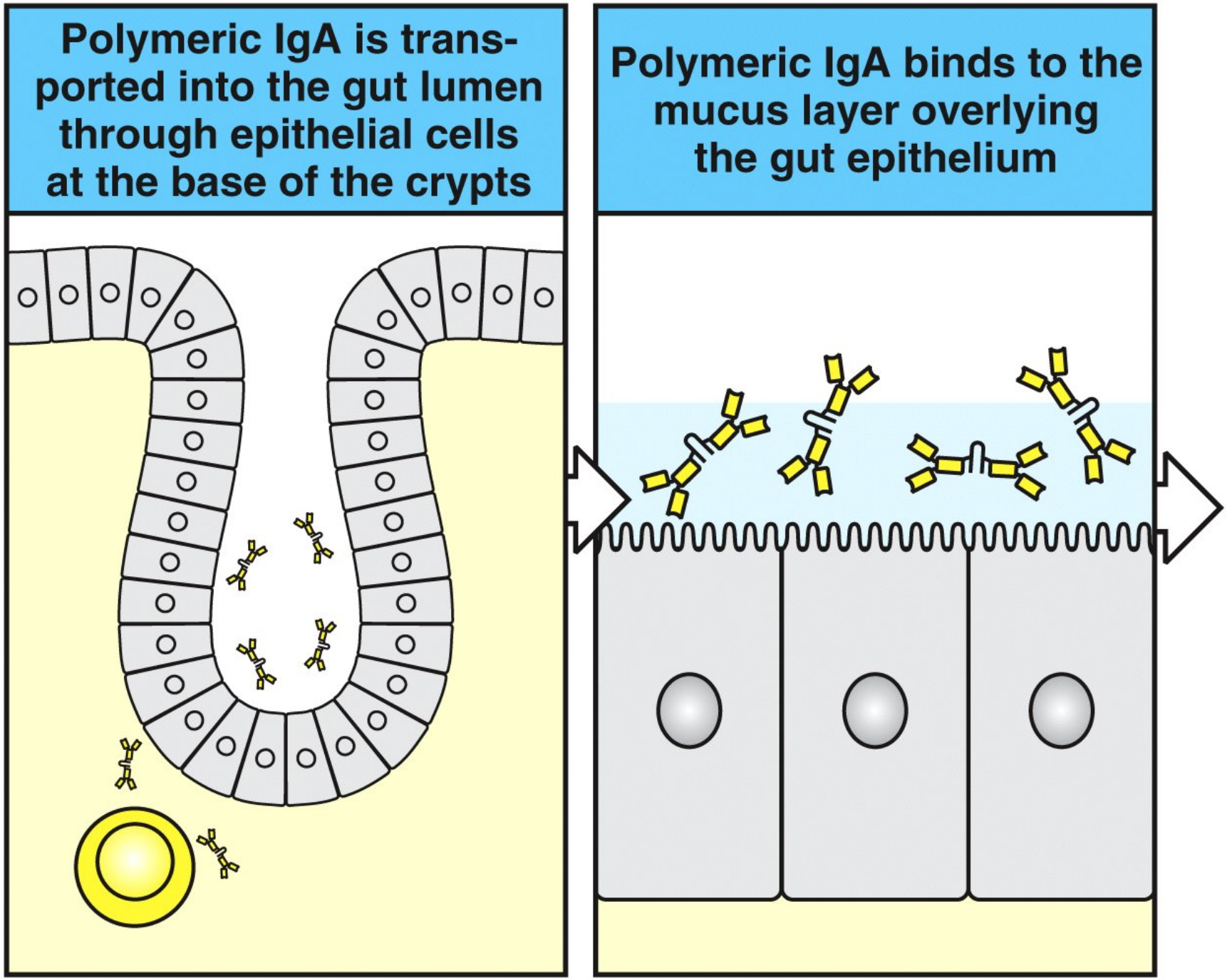
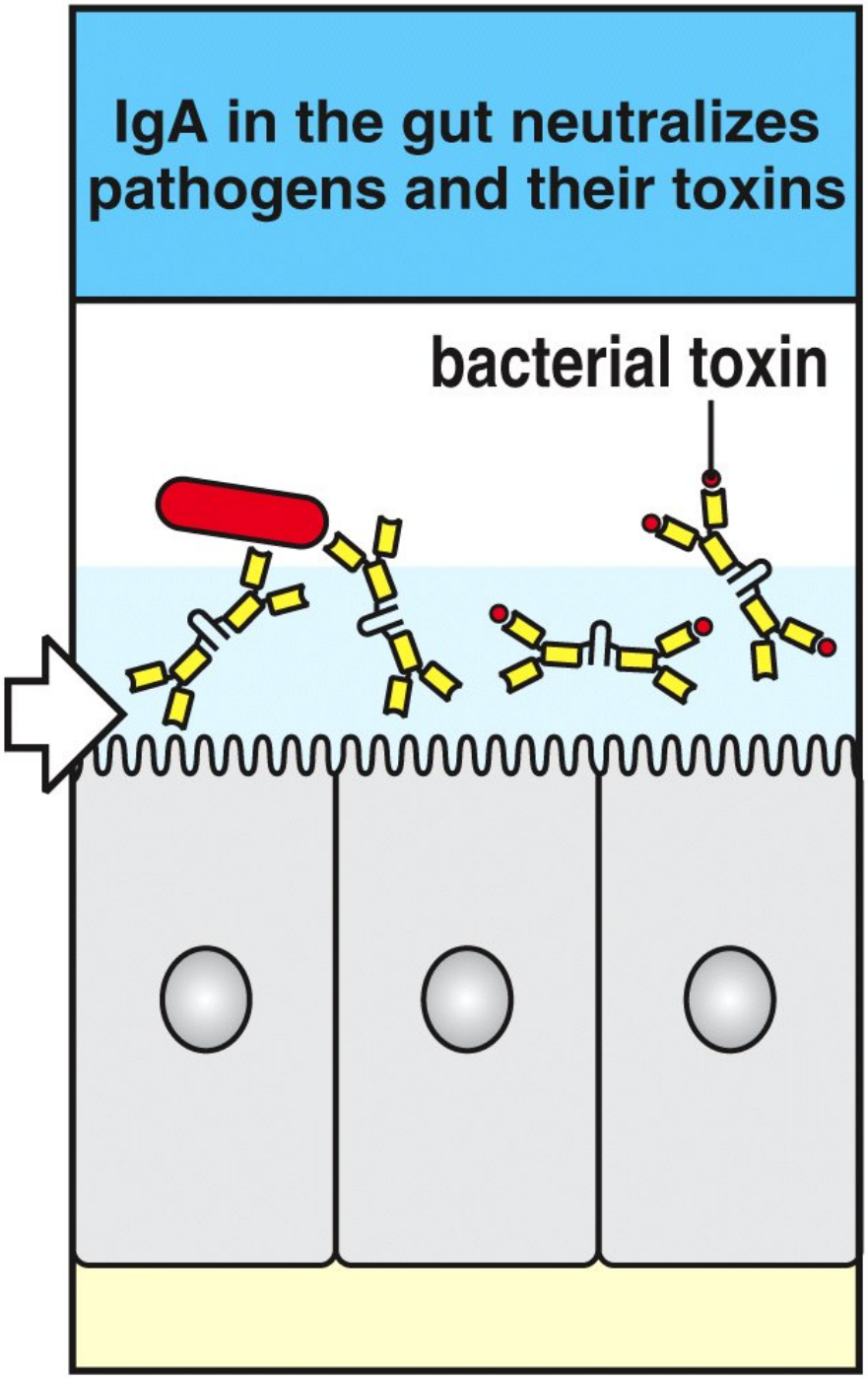


Figure 10-24 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)



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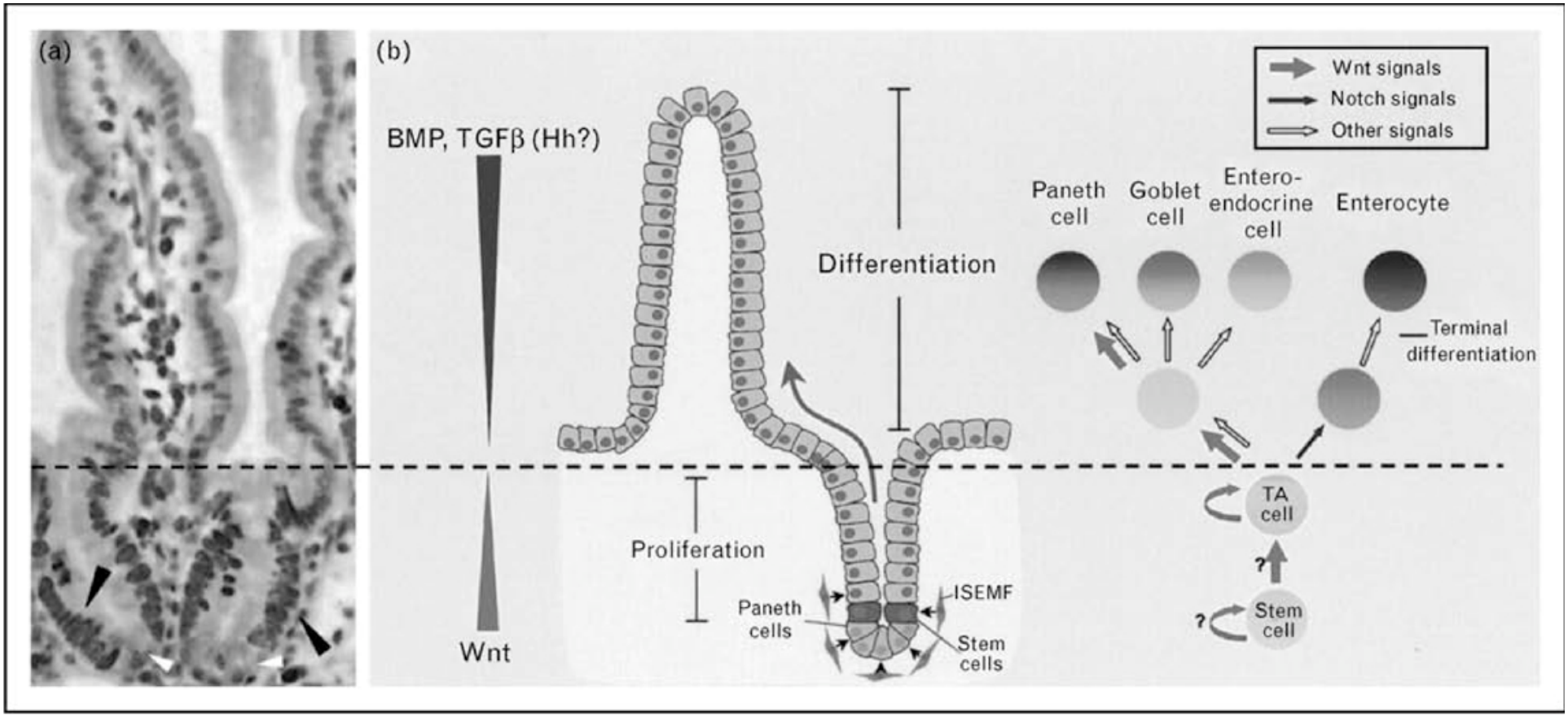
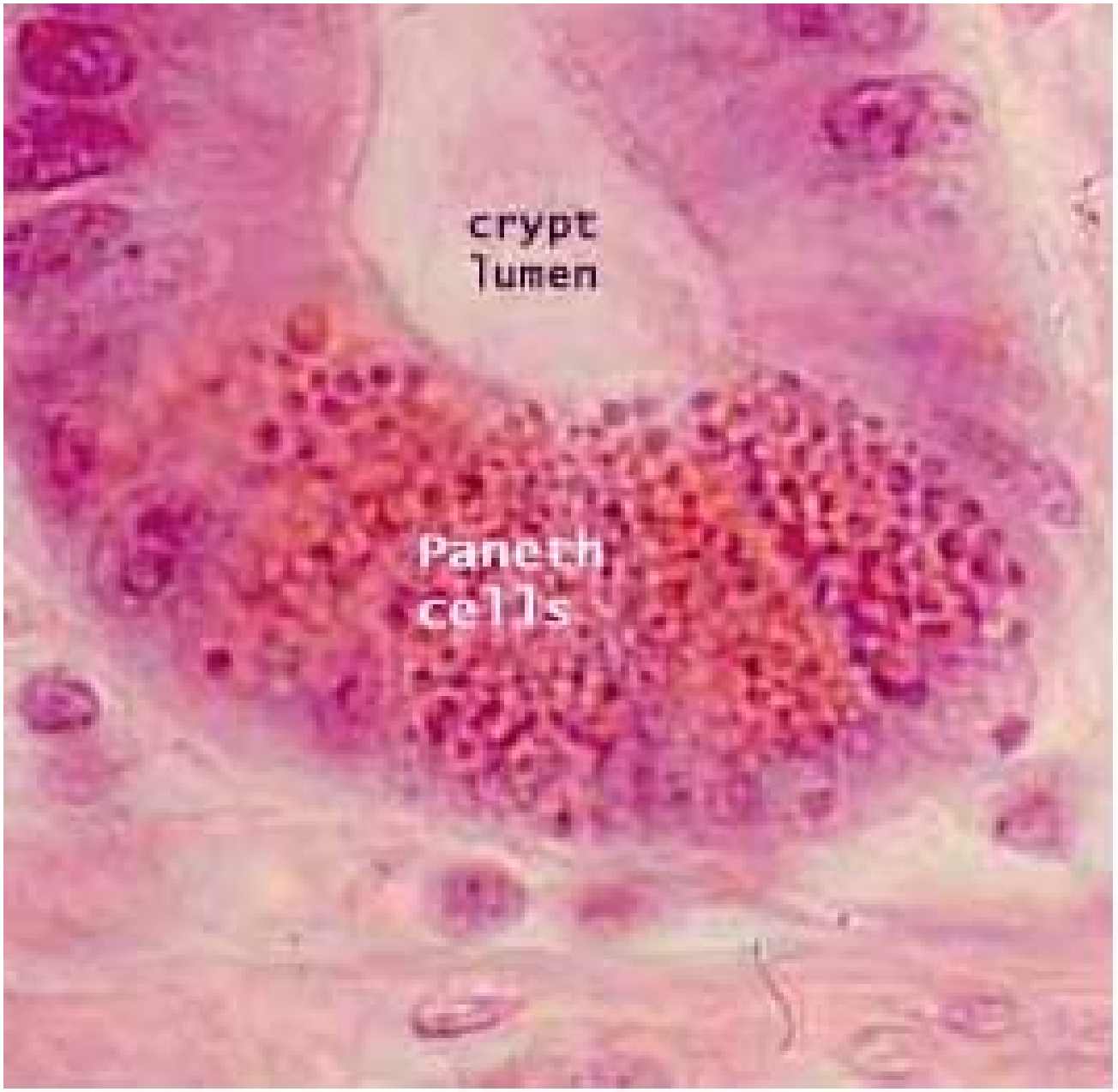
Defensin* Secretion



*small 3-4 kD cationic peptides with a broad spectrum of antimicrobial activities

Wehkamp and Stange. Curr. Op. Gastroenterol. 22:644-650, 2006



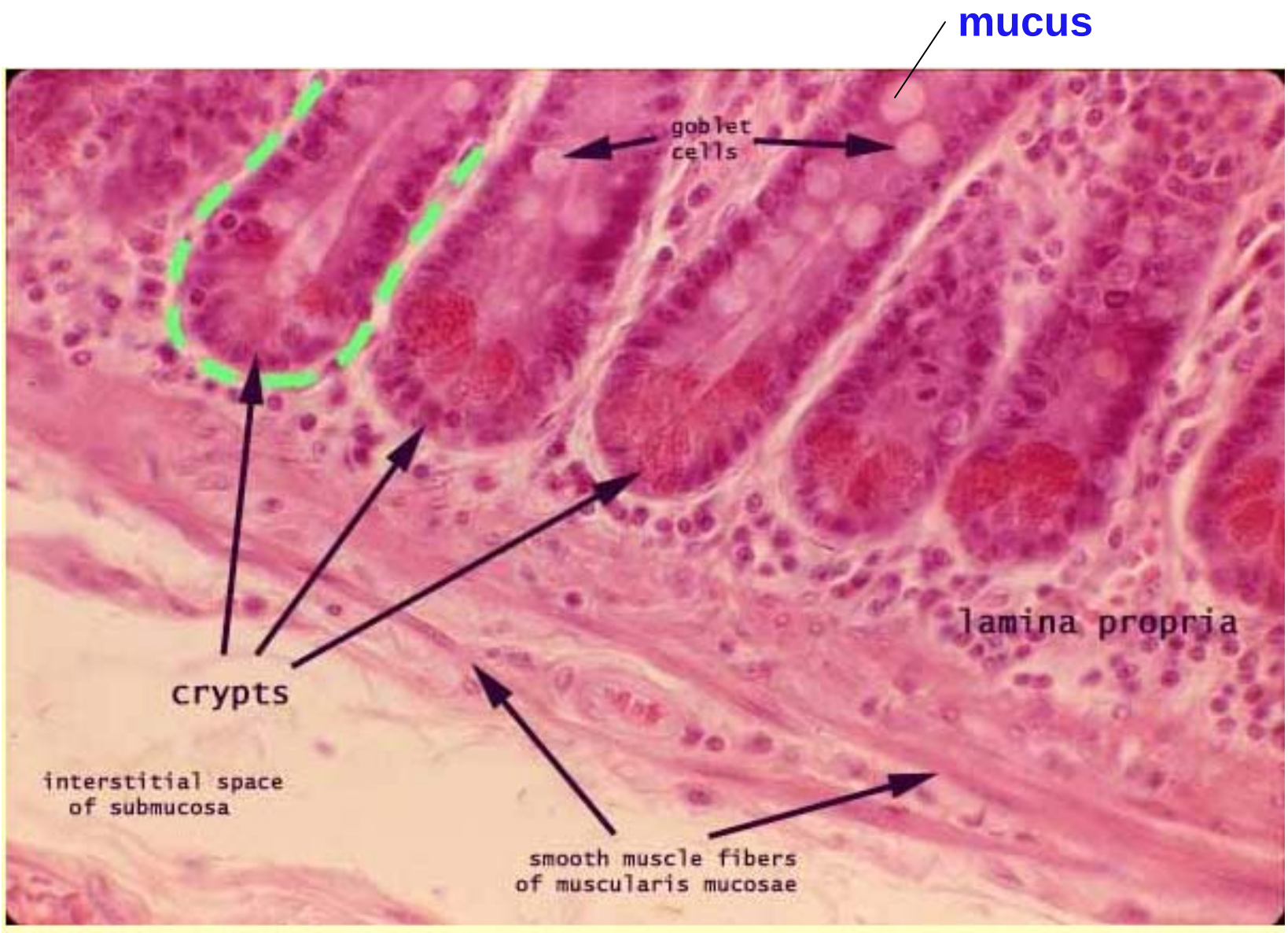
www.siumed.edu/dking2/gicells.htm

Distribution of epithelial defensins

Tissue	HD-5	HD-6	HBD-1	HBD-2
Oral and nasal mucosa			[23,50,69,70]	[23,69,71,72]
Lung, trachea			[33,48,49,73]	[22,49,53,61]
Plasma			[45]	[53]
Salivary gland	[46]	[46]	[23,46,47,74]	[23,74]
Small bowel*	[18,32,39,42,46,54,56,75]	[18,40,42,46,54,56]	[46,56]	[56]
Large bowel	[56]	[56]	[52,56]	[52,56]
Stomach	[56]	[56]	[56,68]	[56,68]
Skin			[51,76]	[13,21,76]
Eye	[77,78]	[77,78]	[77–79]	[77–80]
Kidney			[20,47,57,81]	
Mammary gland			[82]	[82]
Female genital tract	[43,44]			

HD-5, human defensin 5; HD-6, human defensin 6; HBD-1, human beta defensin 1; HBD-2, human beta defensin 2.

Fellermann: Eur J Gastroenterol Hepatol, Volume 13(7).July 2001.771-776



www.siumed.edu/dking2/gicells.htm

Immune response: antigen entry

- Follicle-associated (FAE) M cells
- Villous M cells (PP-independent IgA induction pathway) - located at a distance from PP
- Dendritic cells

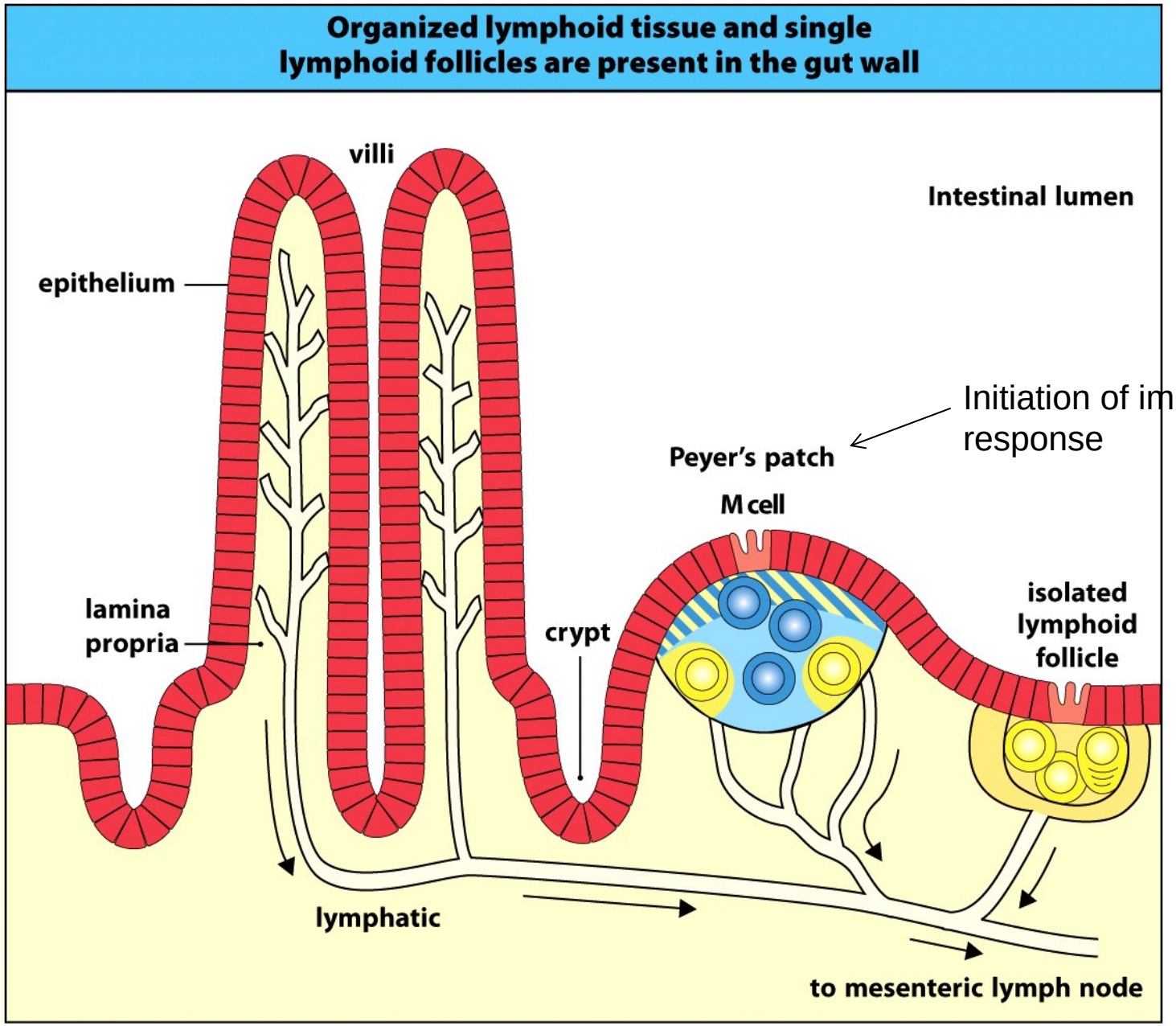


Figure 10.4 part 1 of 2 The Immune System, 3ed. (© Garland Science 2009)

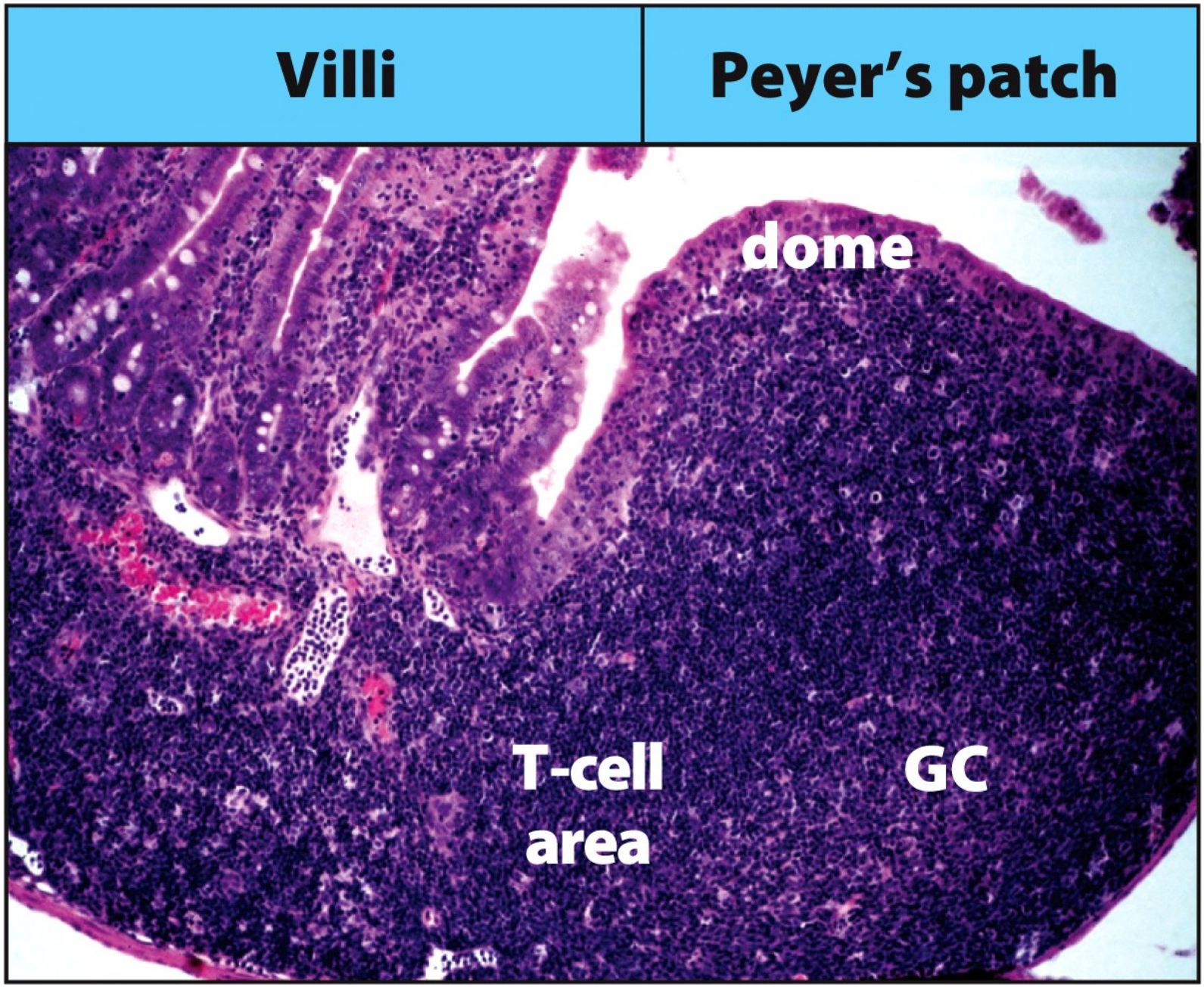


Figure 10.4 part 2 of 2 The Immune System, 3ed. (© Garland Science 2009)

M cells are specialized to transport microorganisms to gut-associated lymphoid tissue

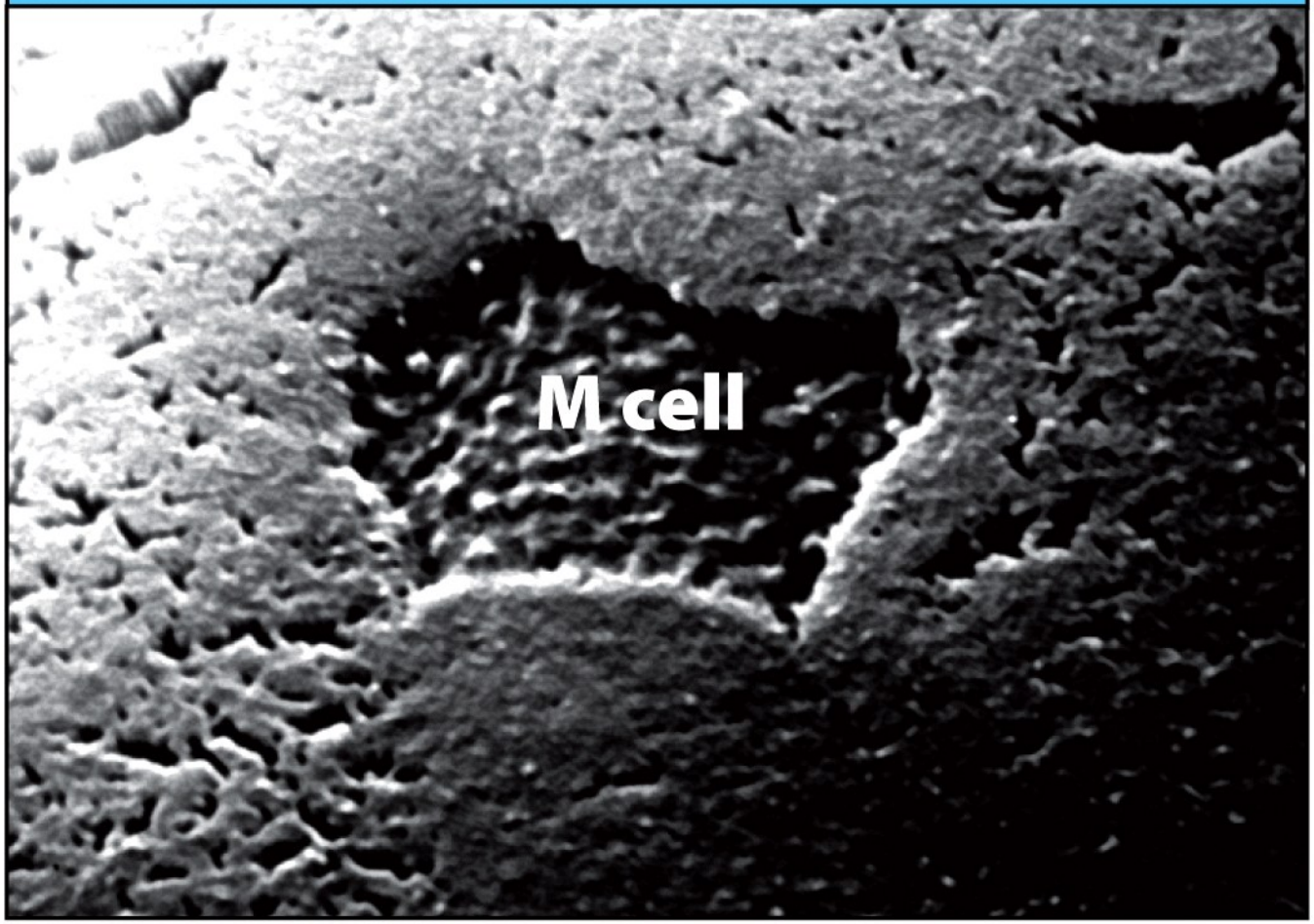
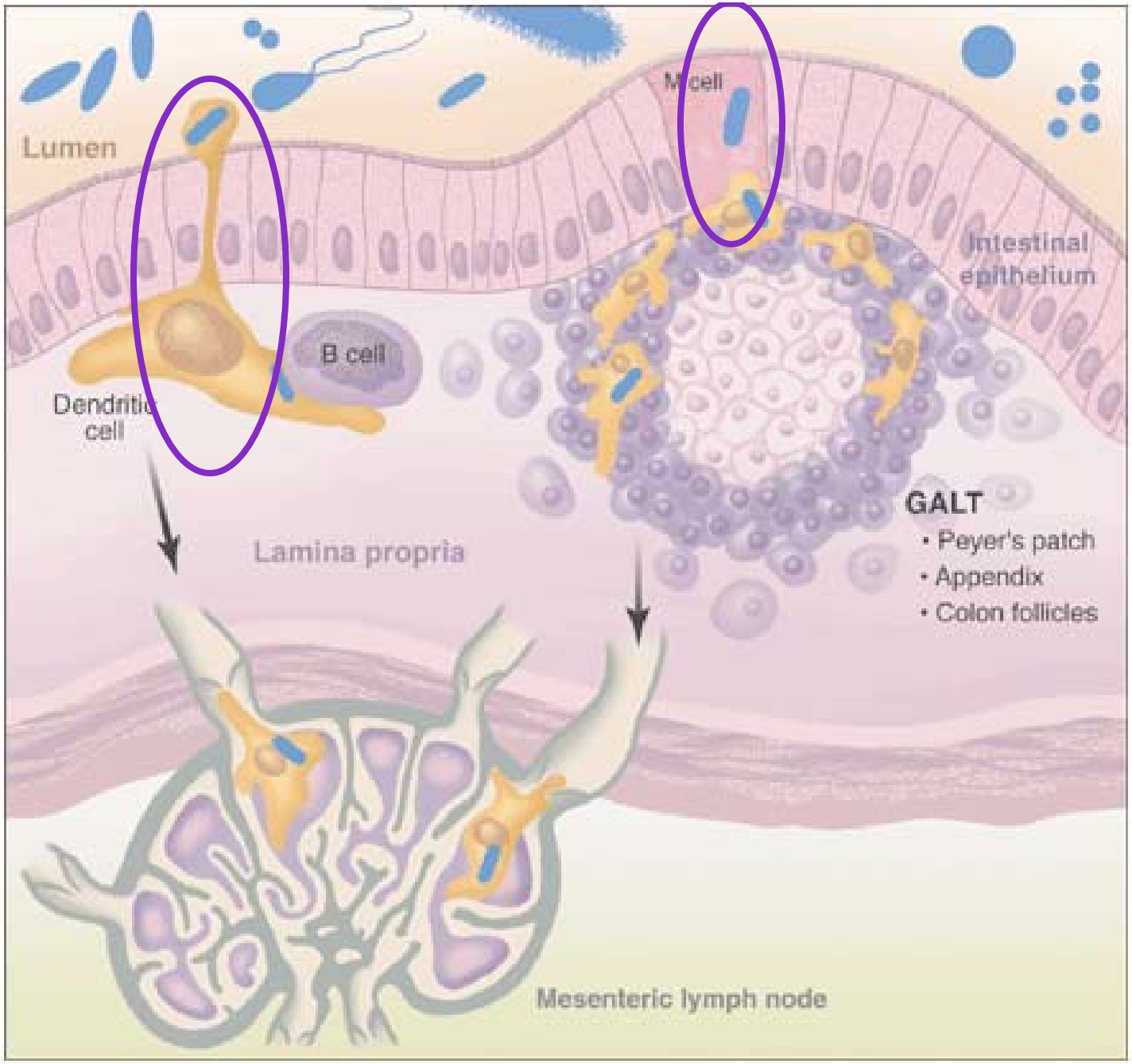


Figure 10.5 The Immune System, 3ed. (© Garland Science 2009)



Kraehenbuhl & Corbett. Science 303:1624-1625, 2004

Sampling of bacteria in lumen

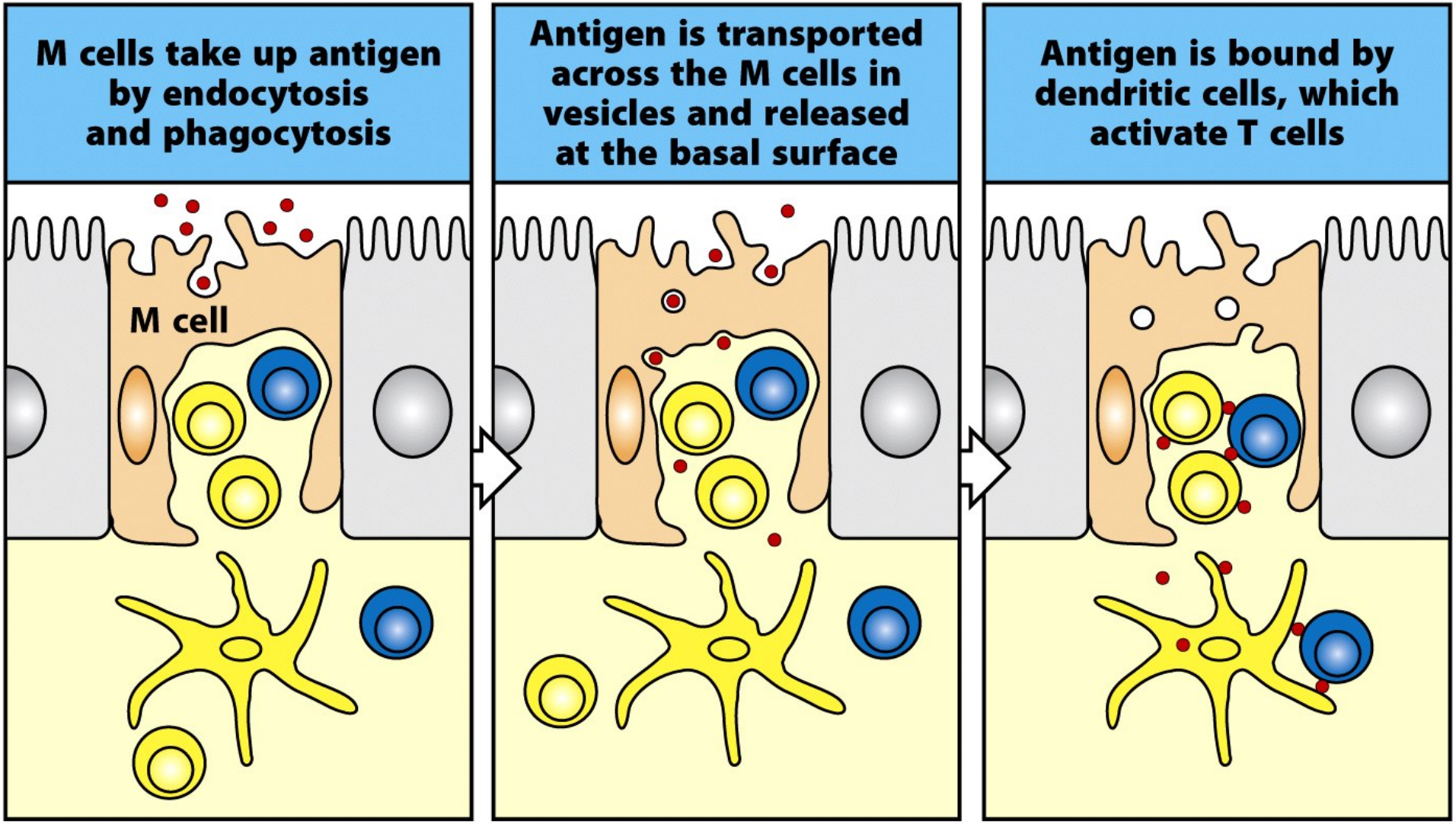
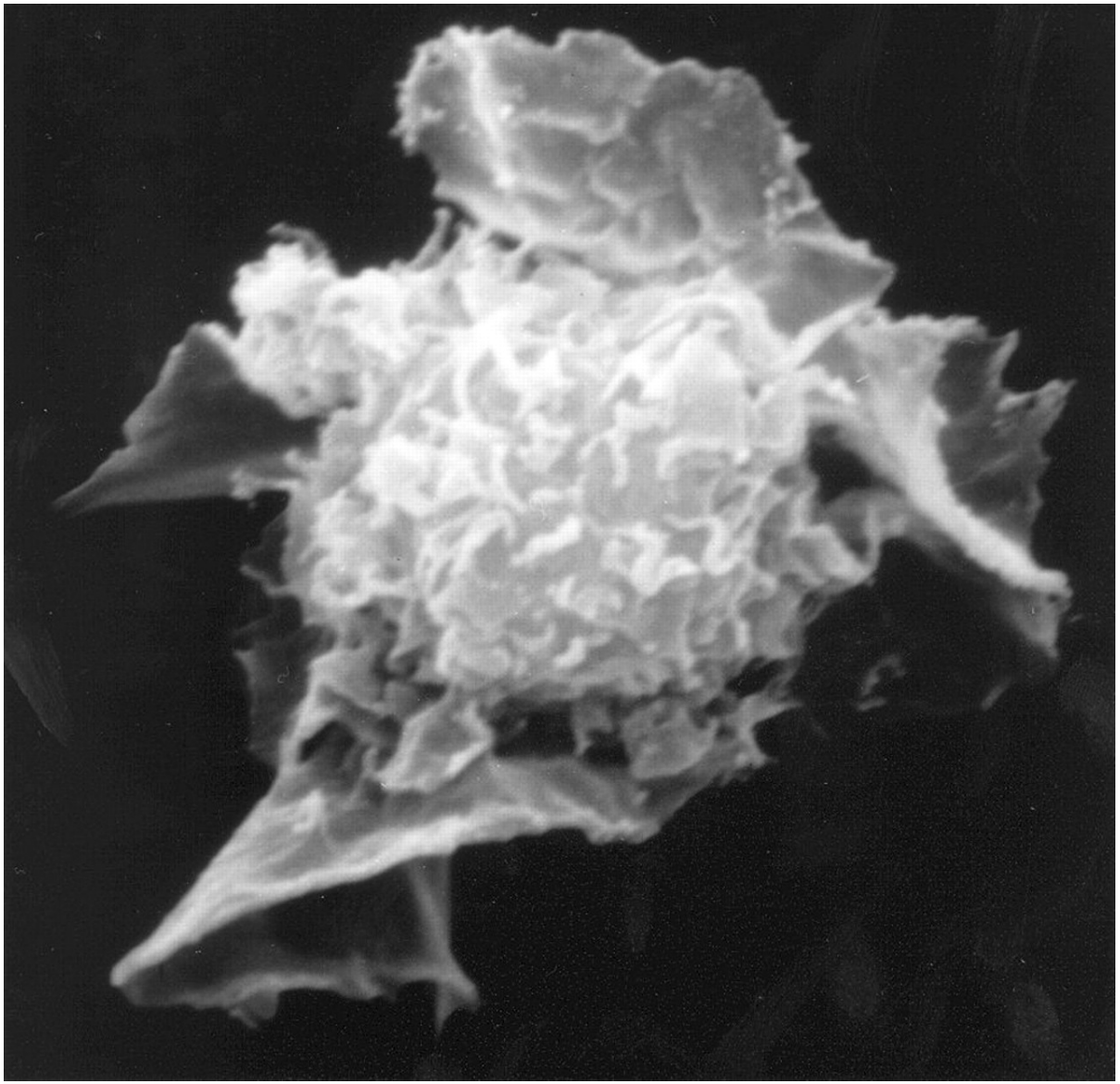


Figure 11-8 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

Dendritic Cell

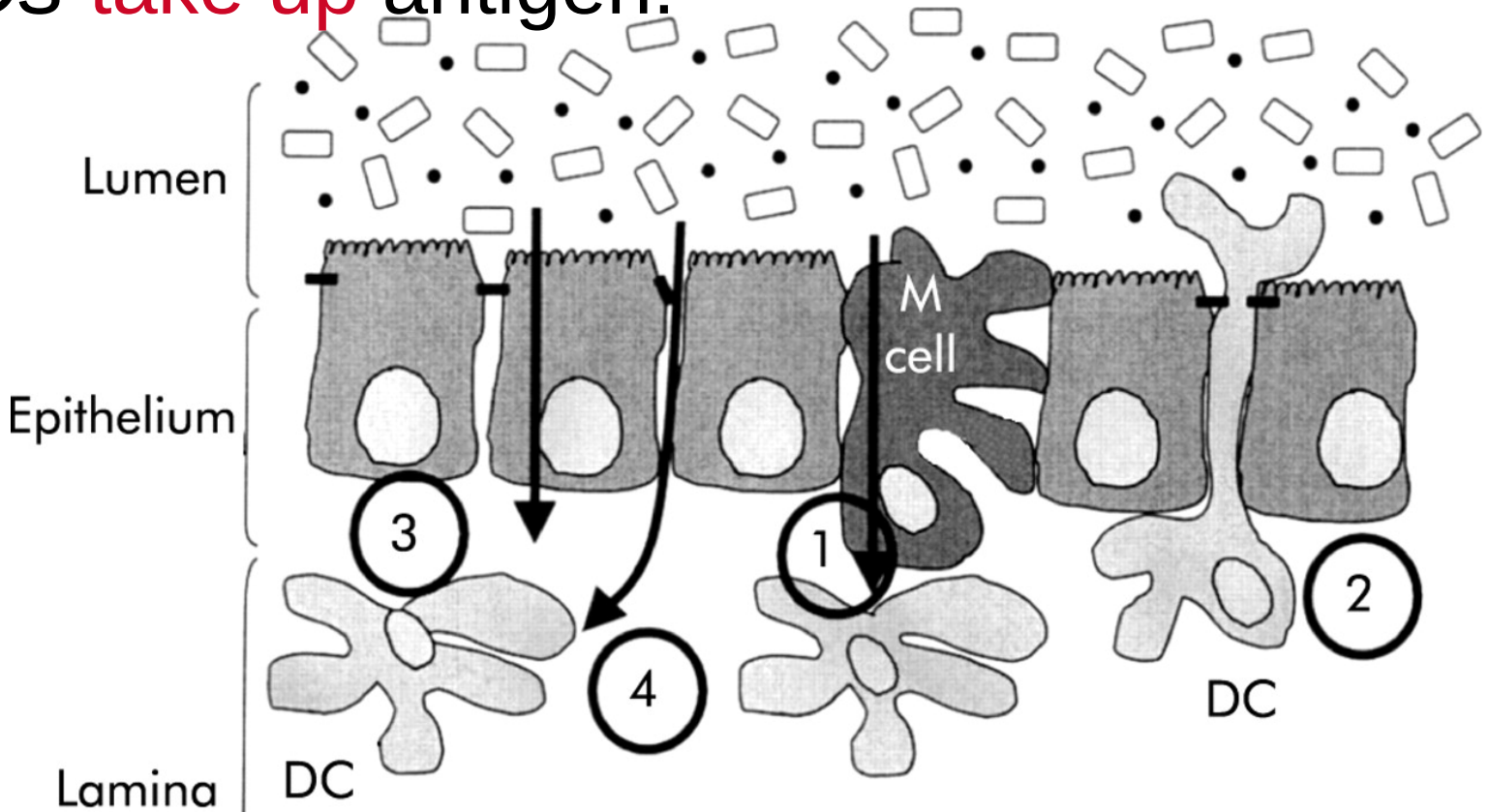


Stagg, A J et al. Gut 2003;52:1522-1529

Gut Dendritic Cells

- Found in cryptopatches, isolated lymph follicles, Peyer's patches, and mesenteric lymph nodes.
- Subsets - seem to depend on chemokine signaling.
- Can protect colonic epithelial integrity by secreting IL-22.

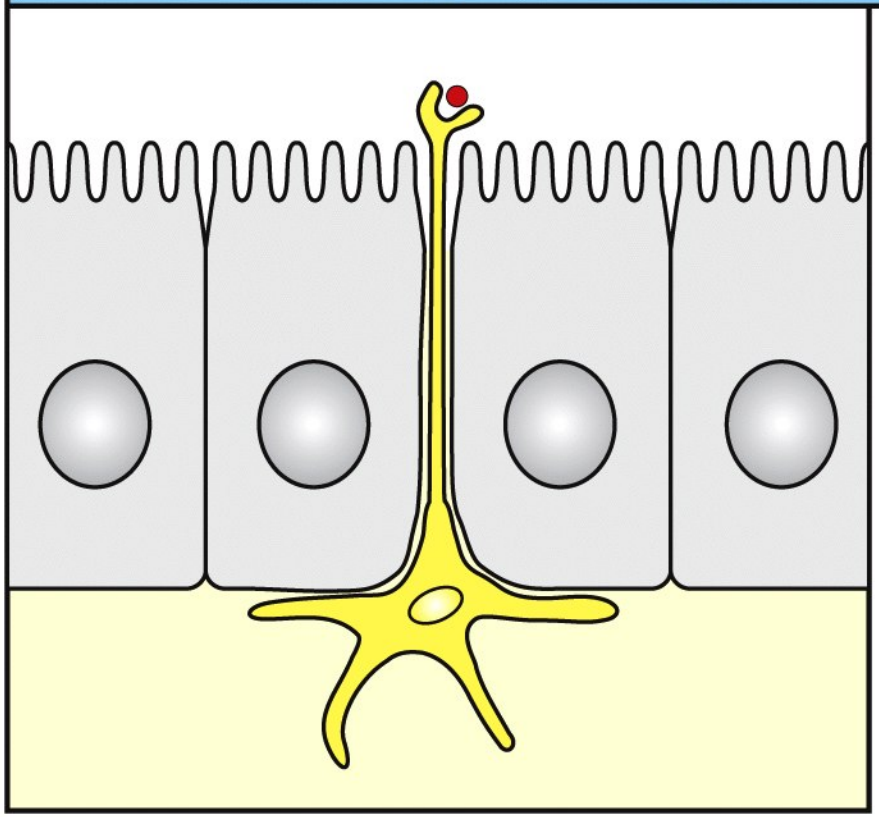
DCs take up antigen:



1 - following transport of ags by M cells
2 - reaching between epithelial cells directly into the lumen
3 - via the epithelium, either by uptake of material transported by epithelial cells or following uptake of apoptotic epithelial cells
4 - by direct access to ags as a result of breaks in epithelial integrity

Stagg, A J et al. Gut 2003;52:1522-1529

Dendritic cells can extend processes across the epithelial layer to capture antigen from the lumen of the gut



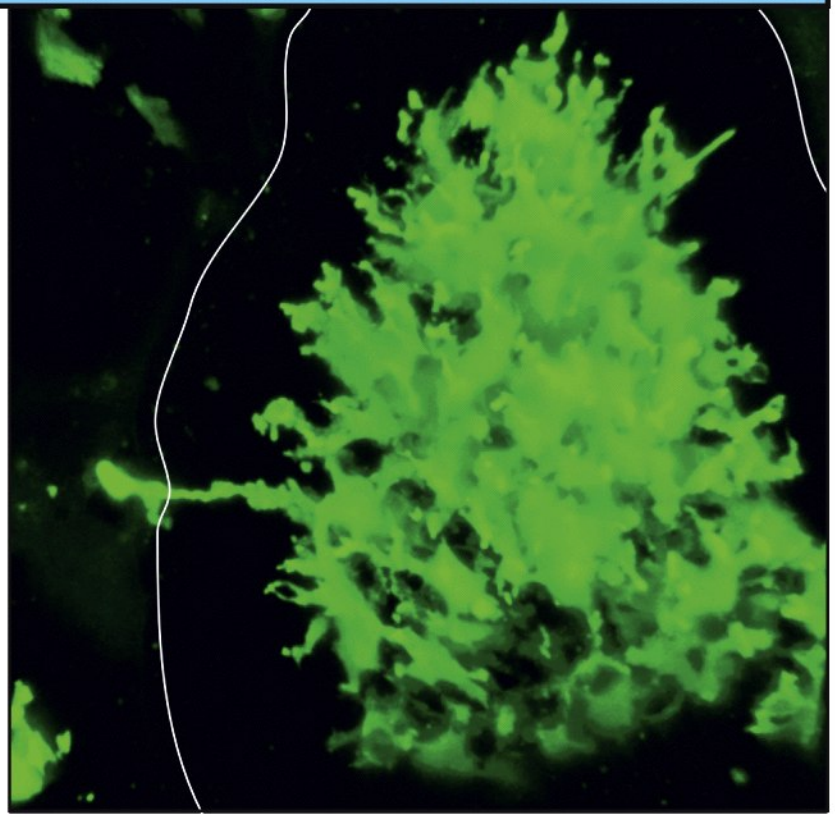


Figure 11-9 Immunobiology, 7ed. (© Garland Science 2008)

Dendritic cells recognize pathogens through pattern recognition receptors (PRRs):

- TLRs (LPS, peptidoglycan, unmethylated CpG motifs, double-stranded viral RNA)
  - TLR2 - Gram positive cell wall components
  - TLR4 - LPS from *E coli* - essential for maturation & cytokine production in LPS-stimulated murine DC
  - TLR5 - Flagellin from Gram negative bacteria
  - TLR9 - CpG motifs from bacterial DNA
- Mannose receptors
- NOD1
  - recognizes muramyl-tripeptides from Gram negative bacteria)
- NOD2
  - recognizes muramyl-dipeptides common to all peptidoglycans of all bacteria species)

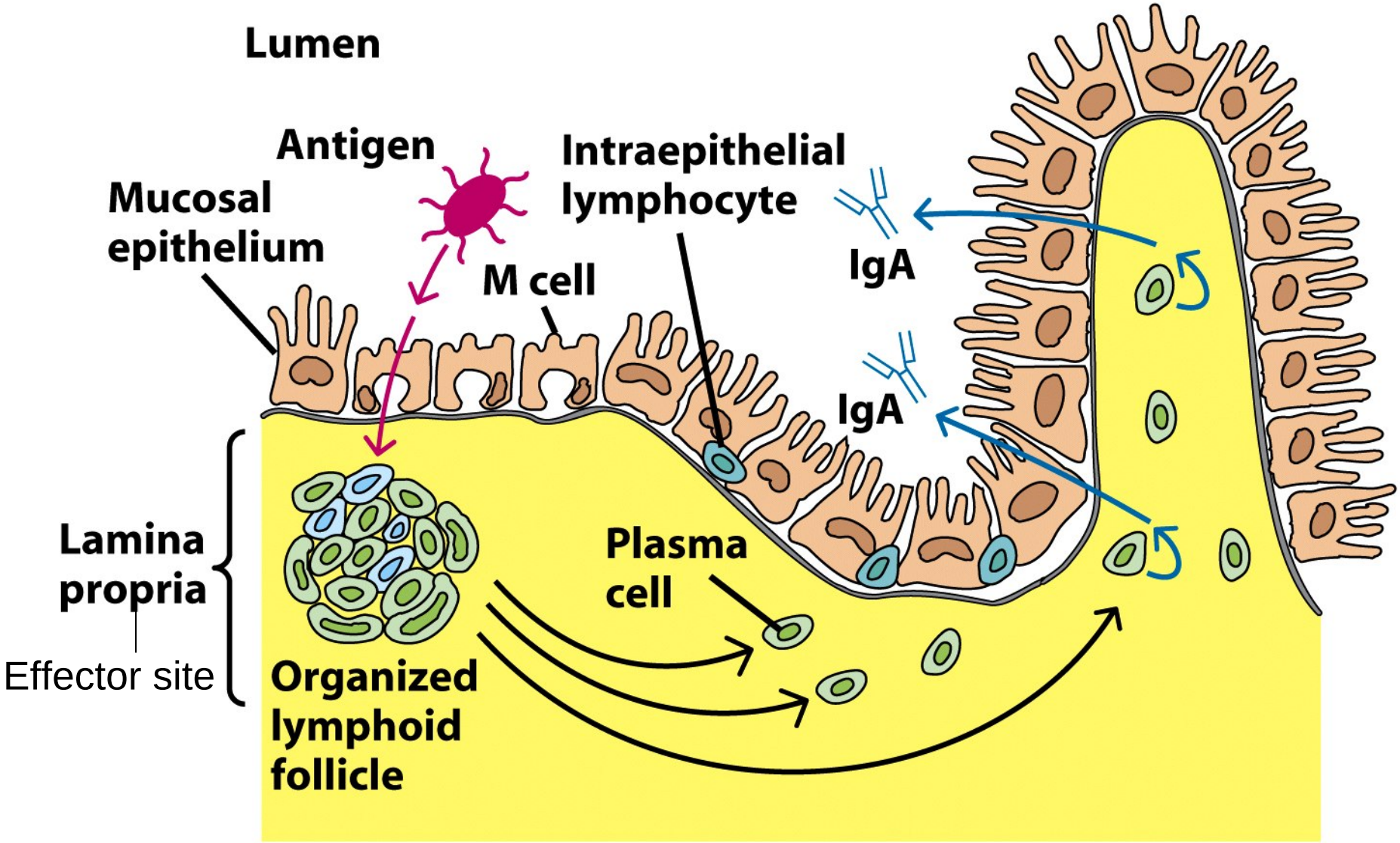


Figure 2-19b
Kuby IMMUNOLOGY, Sixth Edition

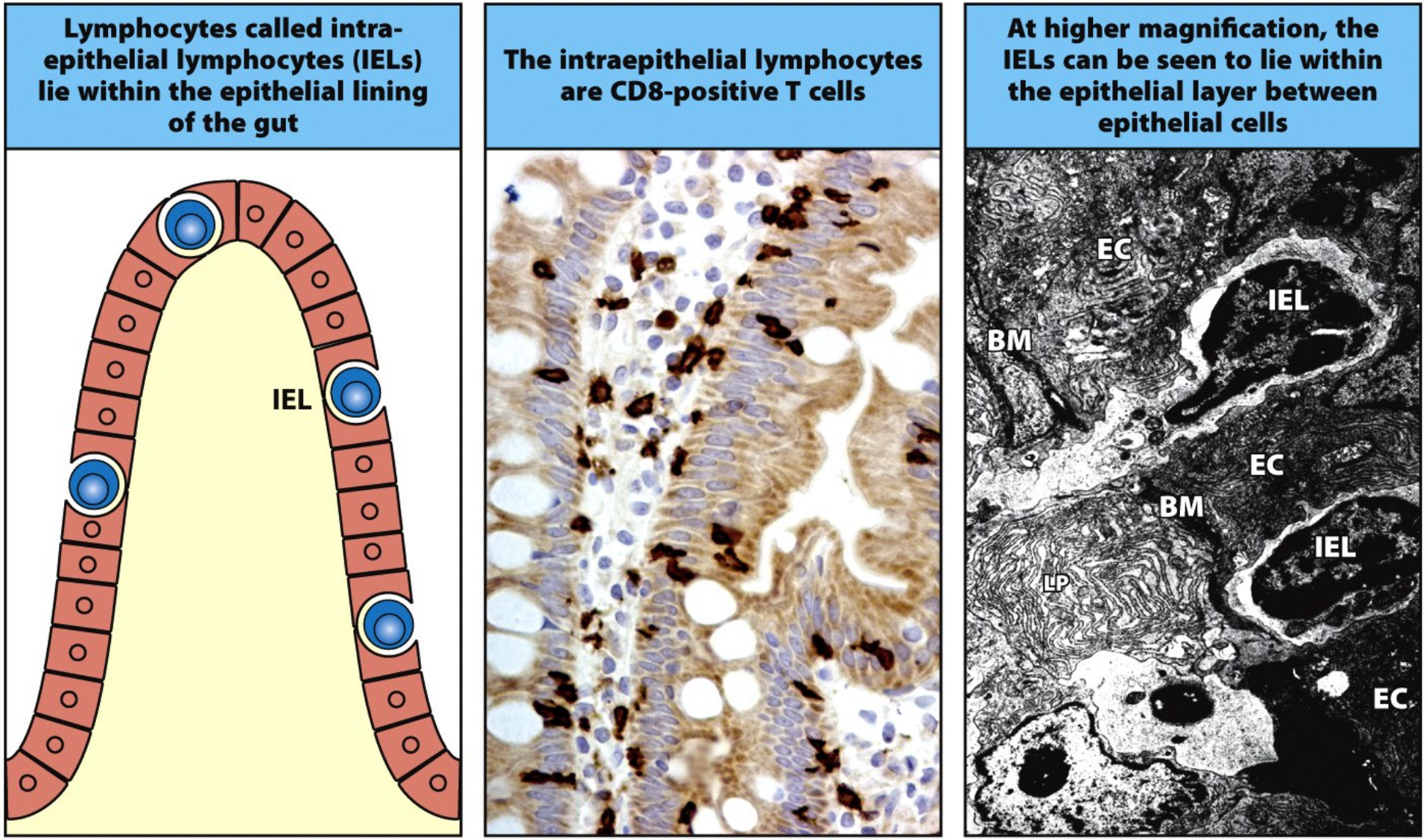


Figure 11-16 Immunobiology, 7ed. (© Garland Science 2008)

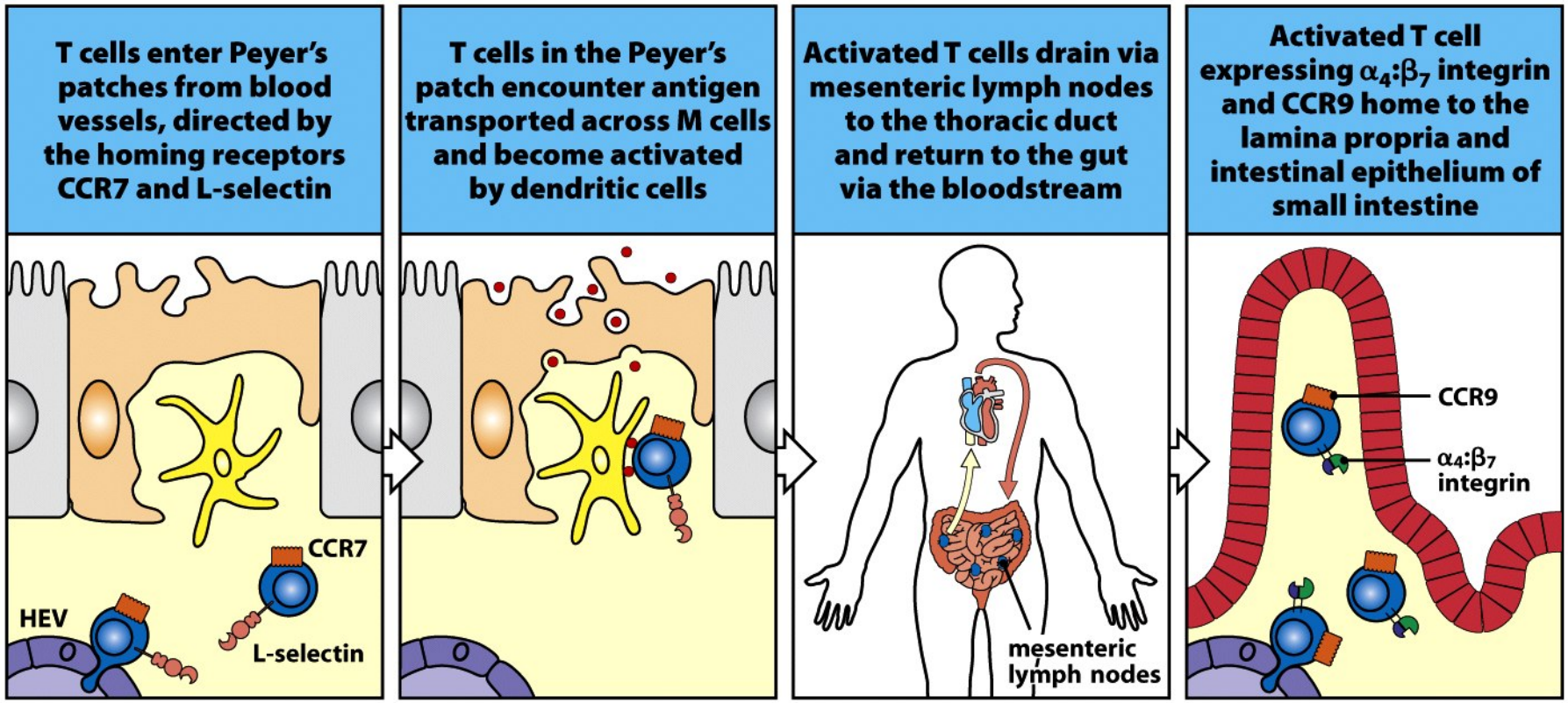


Figure 11-11 Immunobiology, 7ed. (© Garland Science 2008)

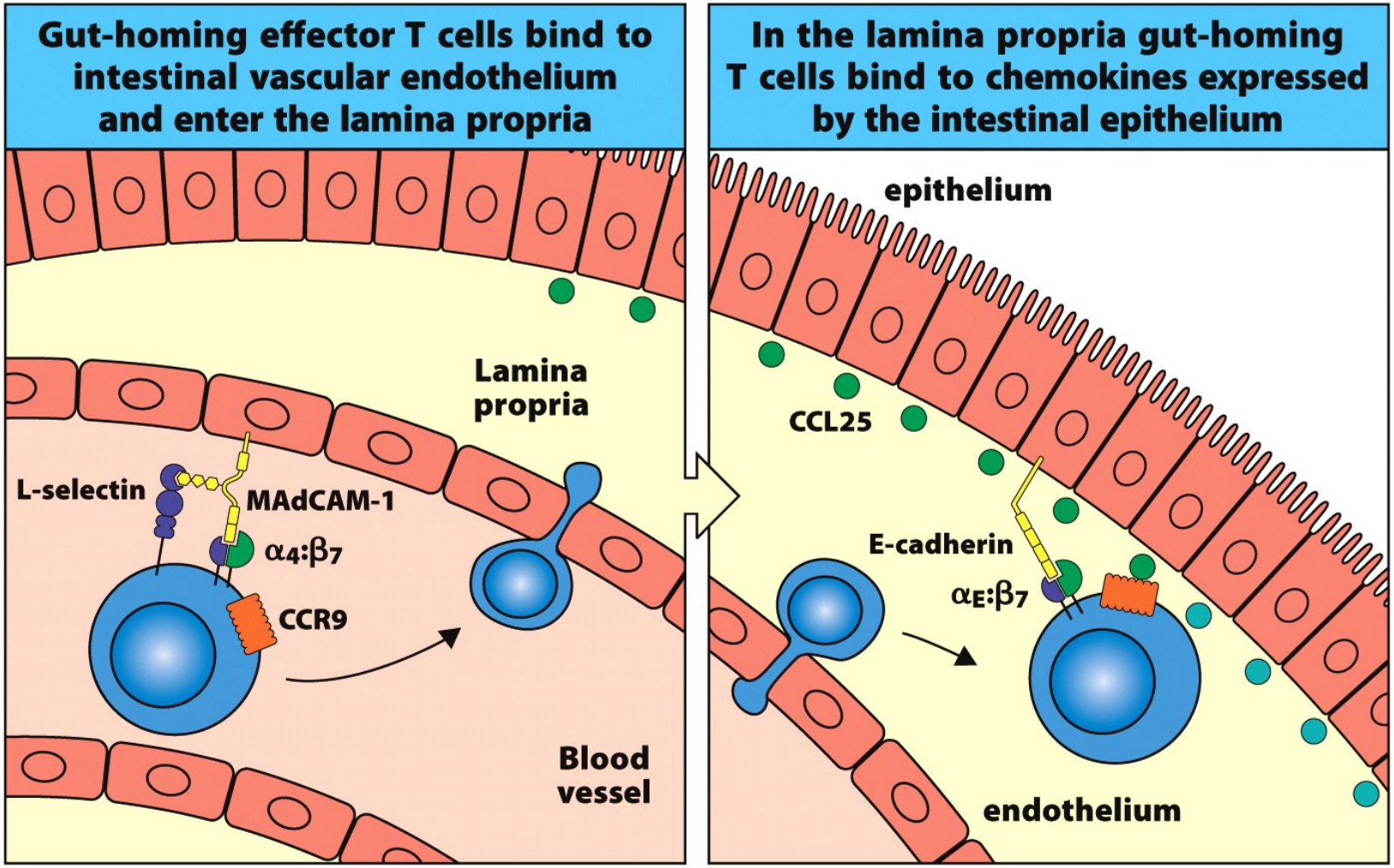


Figure 10.11 The Immune System, 3ed. (© Garland Science 2009)

CCL25 - TECK - homing of T cells to gut
CCL28 - MEC - homing of T cells to mucosal surfaces

Lymphocytes (small intestine)

- Largely effector/memory phenotype
- Conventional T cells
  - CD4+ and CD8+
  - Transient residents
- Regulatory T cells - promote oral tolerance; prevent unwanted inflammation
  - Tr1 - secrete IL-10
  - Th3 - secrete TGFß (enables class switching to IgA)
  - nTreg - high levels of Foxp3
- Intraepithelial lymphocytes (IEL) - markers are those of chronically activated T cells - primarily CD8+
  - CD8αβ+TCRαβ+ (dominant population)
  - **CD8β-CD8αα+ expressing either TCRαβ+ or TCRγδ+**
- Dendritic cells (CD103+ DCs) "train/educate" T cells to home to gut
  - Generation of retinoic acid from retinol by gut dendritic cells induces gut-homing molecules CCR9 and α4ß7 on T cells
  - In MLN, CD103+ DCs present ag to CD4+ & CD8+ T cells

Features of mucosal B lymphocytes

- During their resting stages B cells can traffic through mucosal lymphoid follicles.
- As plasmablasts they can migrate to the lamina propria.
- They tend to become committed to IgA production. However IgM and IgG are also produced.
- There is some evidence that mucosal epithelial cells can condition mucosal DCs to present ag directly to mucosal B cells to produce immunoglobulins.

IgA has multiple properties that are adapted for host defense in the GI tract

- IgA relatively resistant to proteolysis (IgA2 > IgA1)
- Poor activator of complement
- Inhibits
  - Bacterial adhesion
  - Macromolecule absorption
  - Inflammatory effects of other immunoglobulins
- Neutralizes viruses, toxins
- Enhances nonspecific defense mechanisms
  - Lactoperoxidase
  - Lactoferrin
- Mediates antibody dependent cytotoxicity

IgA

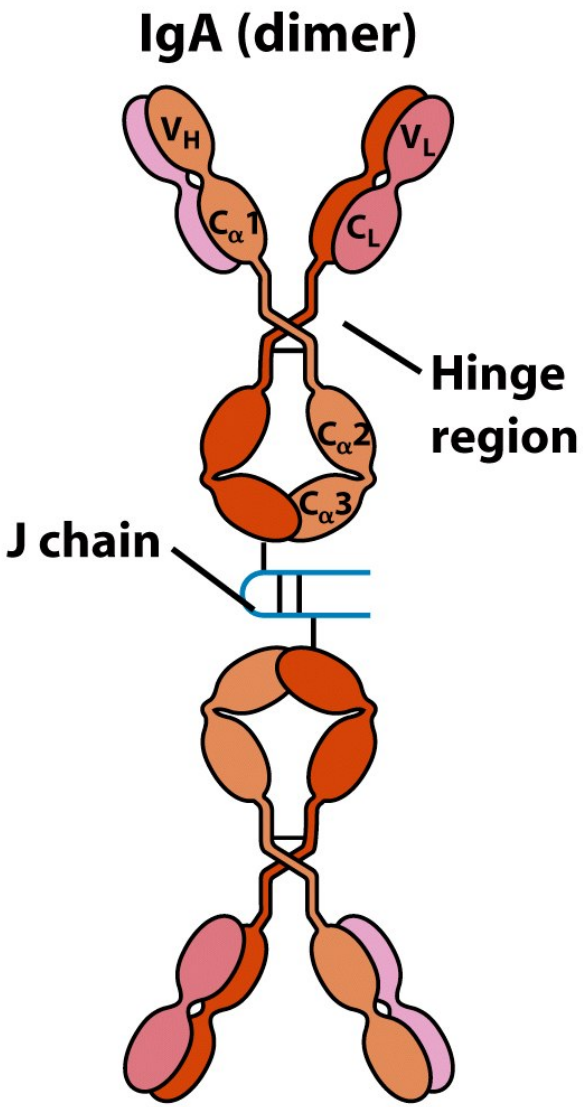


Figure 4-17d
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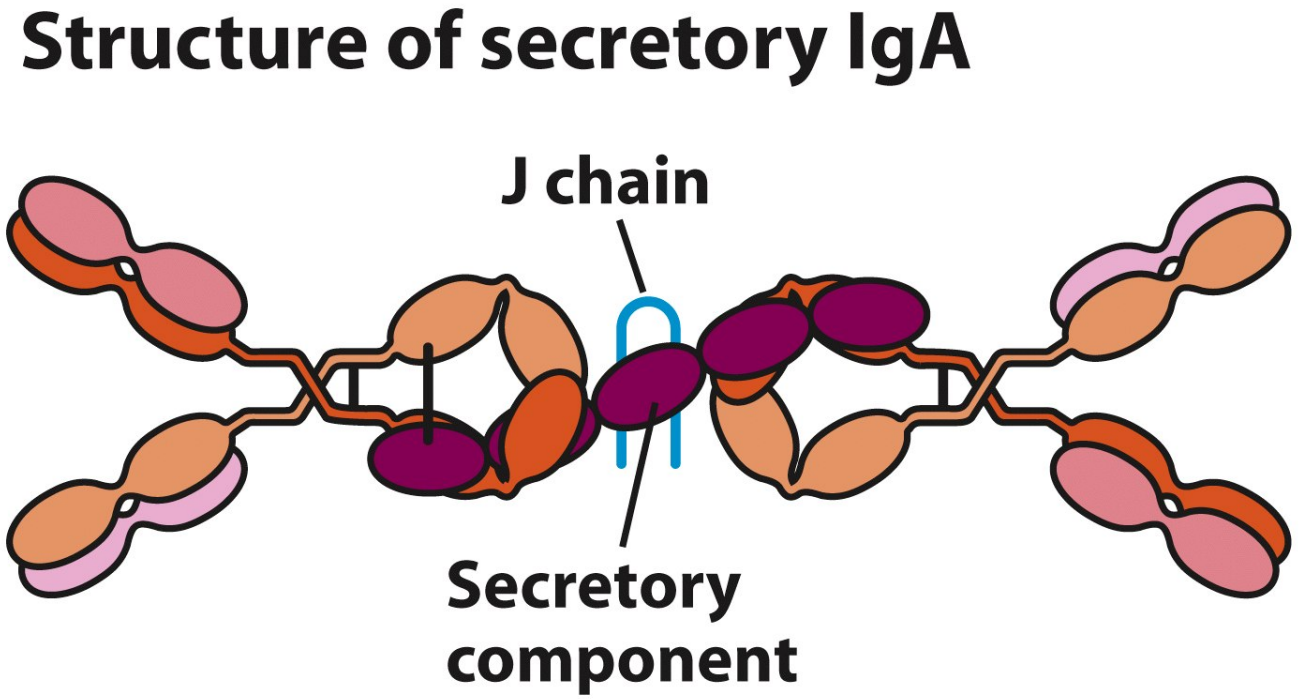

Figure 4-19a
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Serum - monomer

Secretions - dimer predominates

Formation of secretory IgA

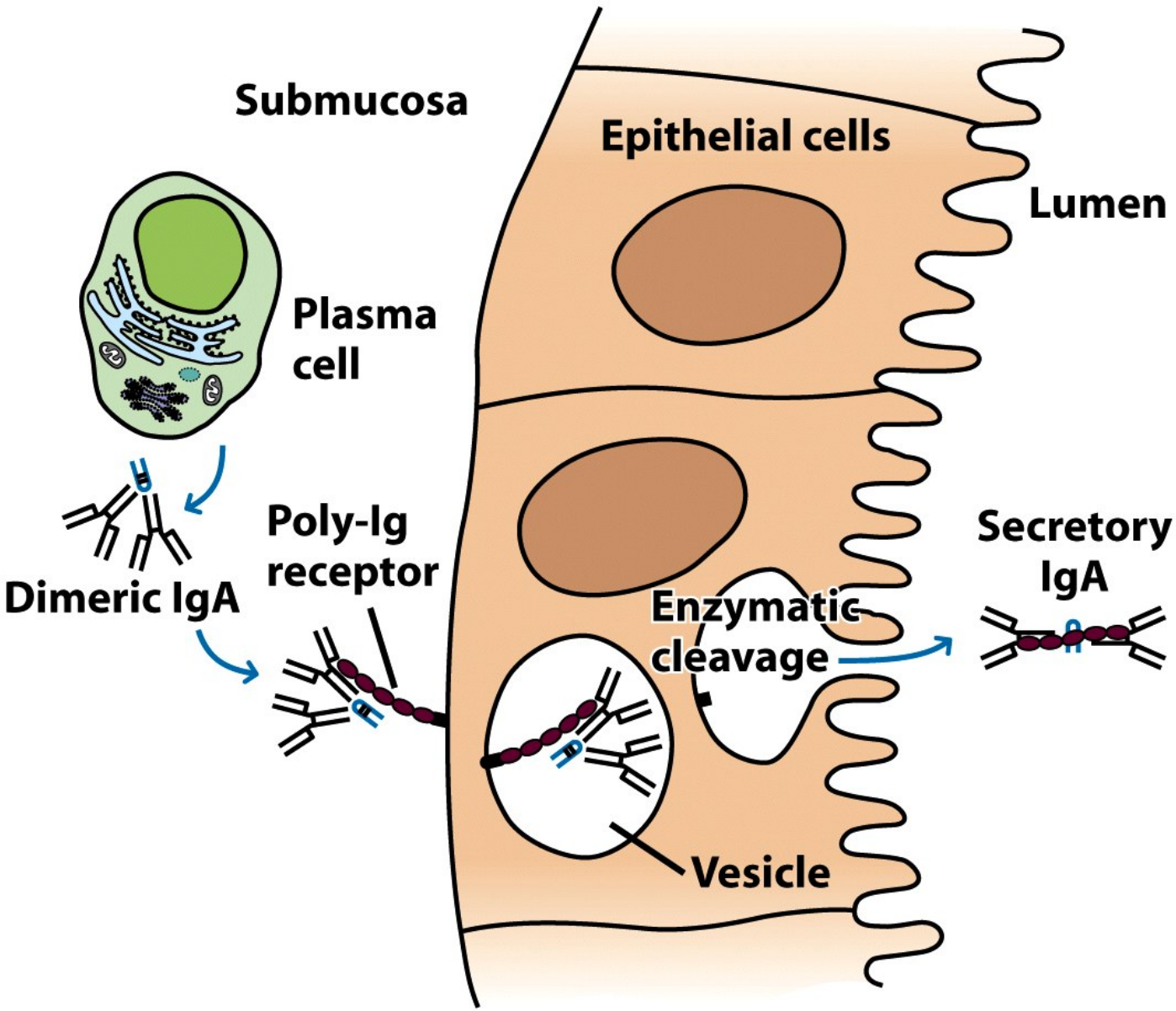

Figure 4-19b
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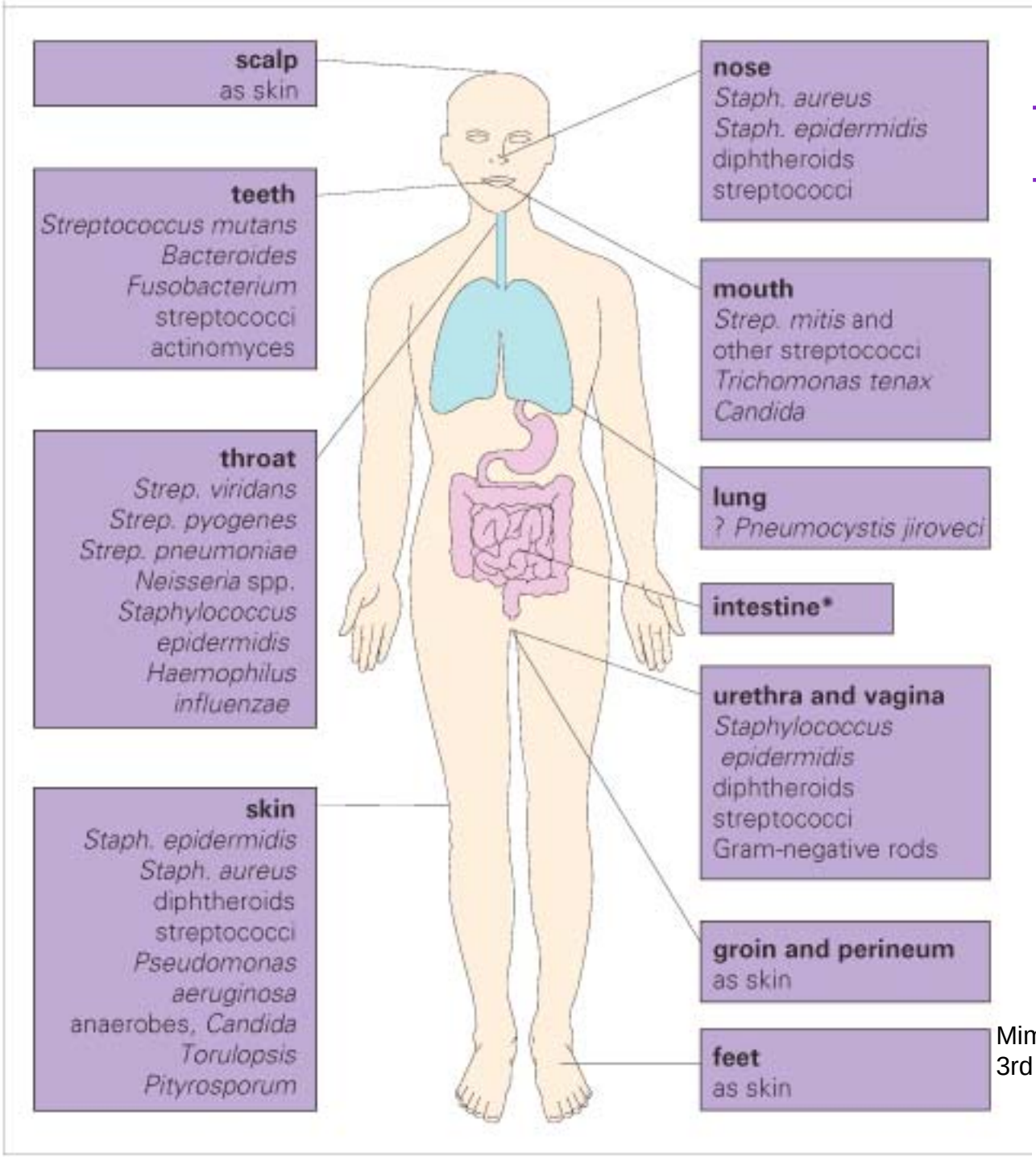
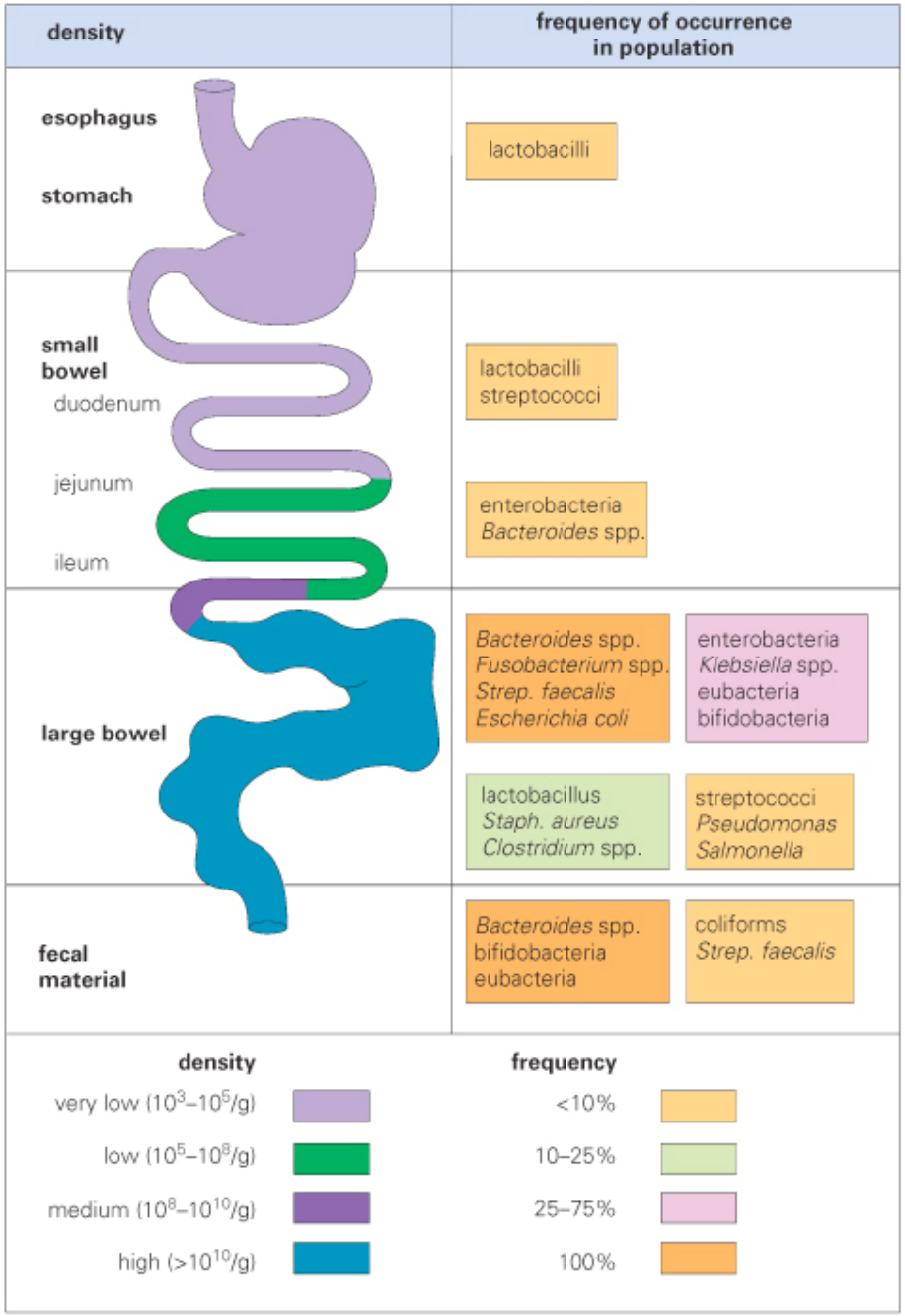
Normal flora and their location



Mims et al. Medical Microbiology 3rd ed. Elsevier.



The longitudinal distribution, frequency of occurrence and densities of the bacteria making up the normal flora of the human gastrointestinal tract.

Commensal bacteria (Latin = “at the table together”)

- Prevent colonization by more pathogenic species
- Produce metabolites that are used by the host
- Colonization of the gut begins immediately after birth
  - 10^{13}-10^{14} microorganisms
  - 400 to 500 different species
  - Majority are obligate anaerobes
- Negative effects on normal bacterial flora may explain the rise of immune disorders (allergies and IBD)

Beneficial effects of indigenous GI microflora

- Formation of anatomical structures (Peyer's patches)
- Expansion of germinal center reactions involving B and T cells
- Increased IgA production by intestinal B cells
- Expansion of IEL populations
- Bacterial antagonism
- Maintain GI tract peristalsis and intestinal mucosal integrity
- Convert dietary precarcinogens and carcinogens to noncarcinogens
- Synthesis of vitamin K and vitamin B complexes

- However, translocating bacteria can cause infections in debilitated patients

Why does the immune system ignore commensals?

- Mesenteric lymph nodes form a barrier that prevents commensals from reaching the systemic compartment of the host immune system and from eliciting a damaging immune response.
- DCs present ag directly to B cells resulting in IgA production that prevents the bacteria from straying beyond the gut mucosa.

Macpherson & Uhr. Science 303:1662-1665, 2004.

Why does the immune system ignore commensals?

- Sequestration of indigenous microflora by surface epithelia
- Regulation of magnitude and duration of TLR signaling
- Proinflammatory bacteria may be controlled by anti-inflammatory effects of commensals
- Blocking of NFκB activation by inhibiting IκB-α ubiquitination

Why does the immune system ignore commensals?

- Commensal bacteria may use type III or type IV secretion systems - might be able to deliver bacterial effector molecules to host cells which modify the outcome of infection with pathogenic bacteria.

Why does the immune system ignore commensals?

- Treg cells - tolerance, primarily local but probably systemic as well
- IL-10-producing dendritic cells
- Inhibition of the generation of Th1 cells

Commensals as therapeutics

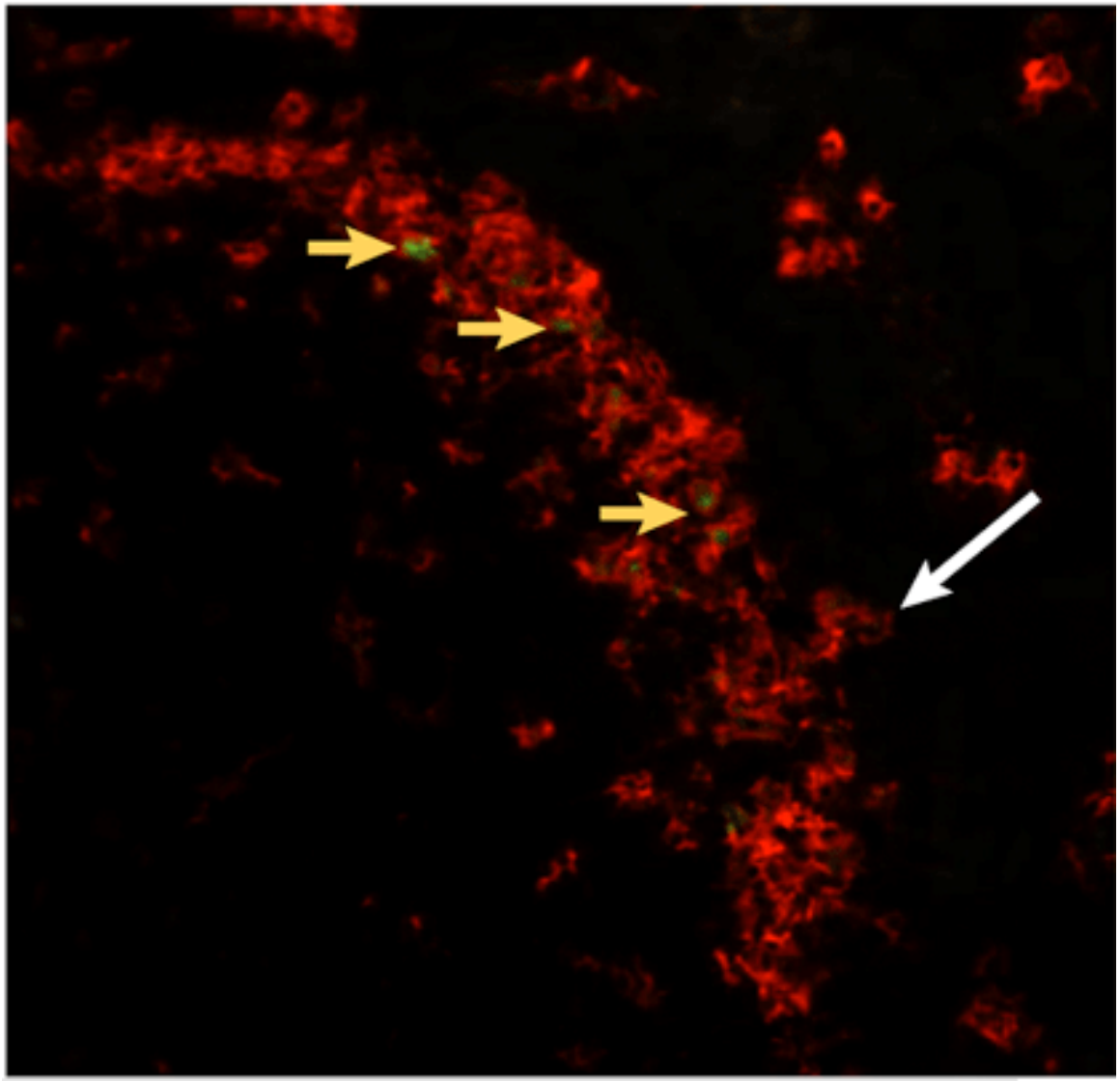
- Probiotics - dietary supplements containing potentially beneficial bacteria (primarily Lactobacillus sp, Bifidobacterium sp) and yeasts (*Saccharomyces boulardii*)
- Bacterial products

Target Disorders

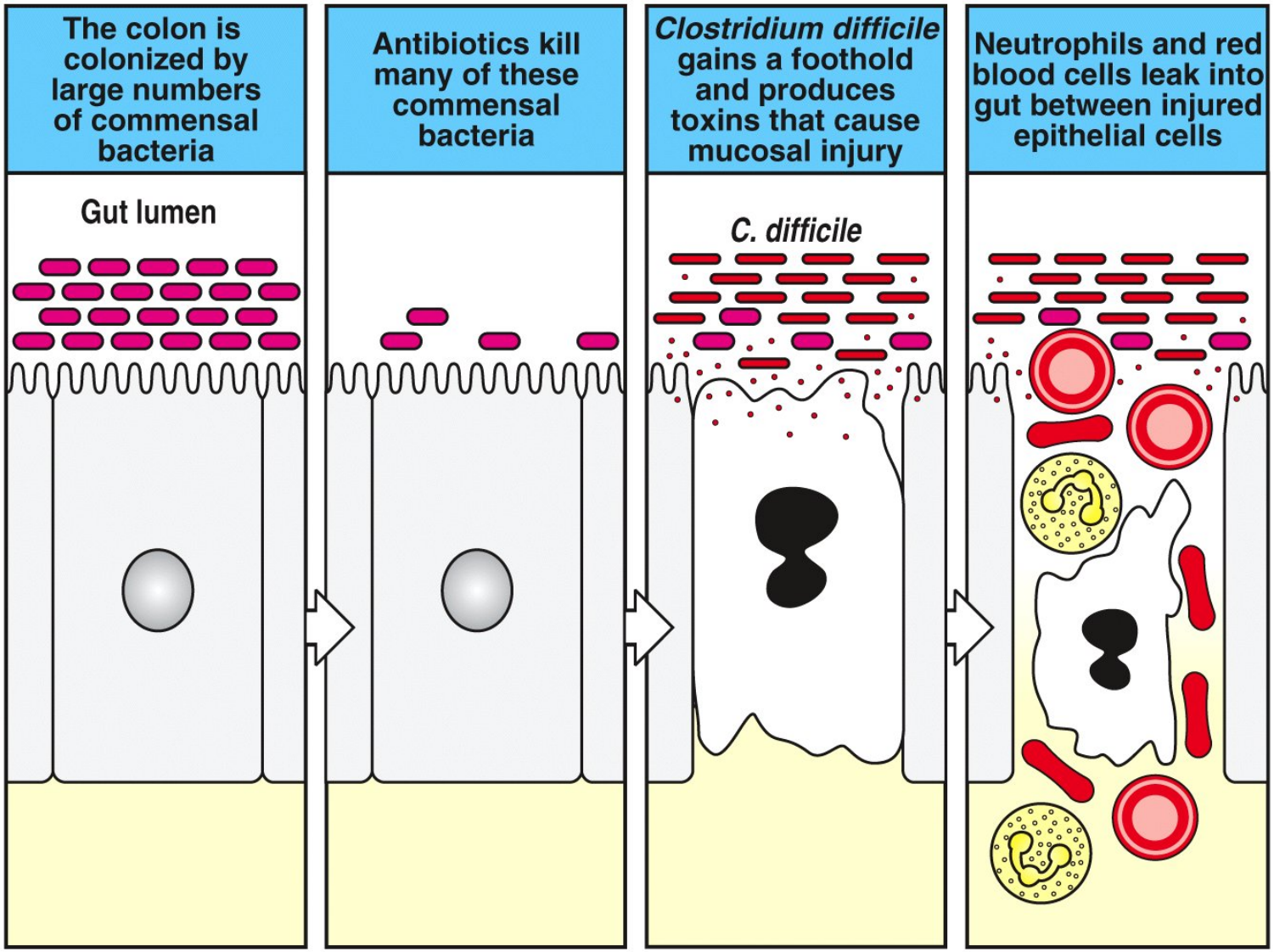
Probiotic Microbes: A Report from the Academy of Microbiology based on a colloquium convened November 5-7, 2005, in Baltimore, Maryland

- Diarrhea
- Pouchitis
- Irritable bowel syndrome
- Bladder cancer
- Urogenital infections
- *Clostridium difficile* infection
- Atopic Eczema

Pathogenic microbes can cross the epithelial barrier



Nature Reviews Immunology 1:59-67, 2001



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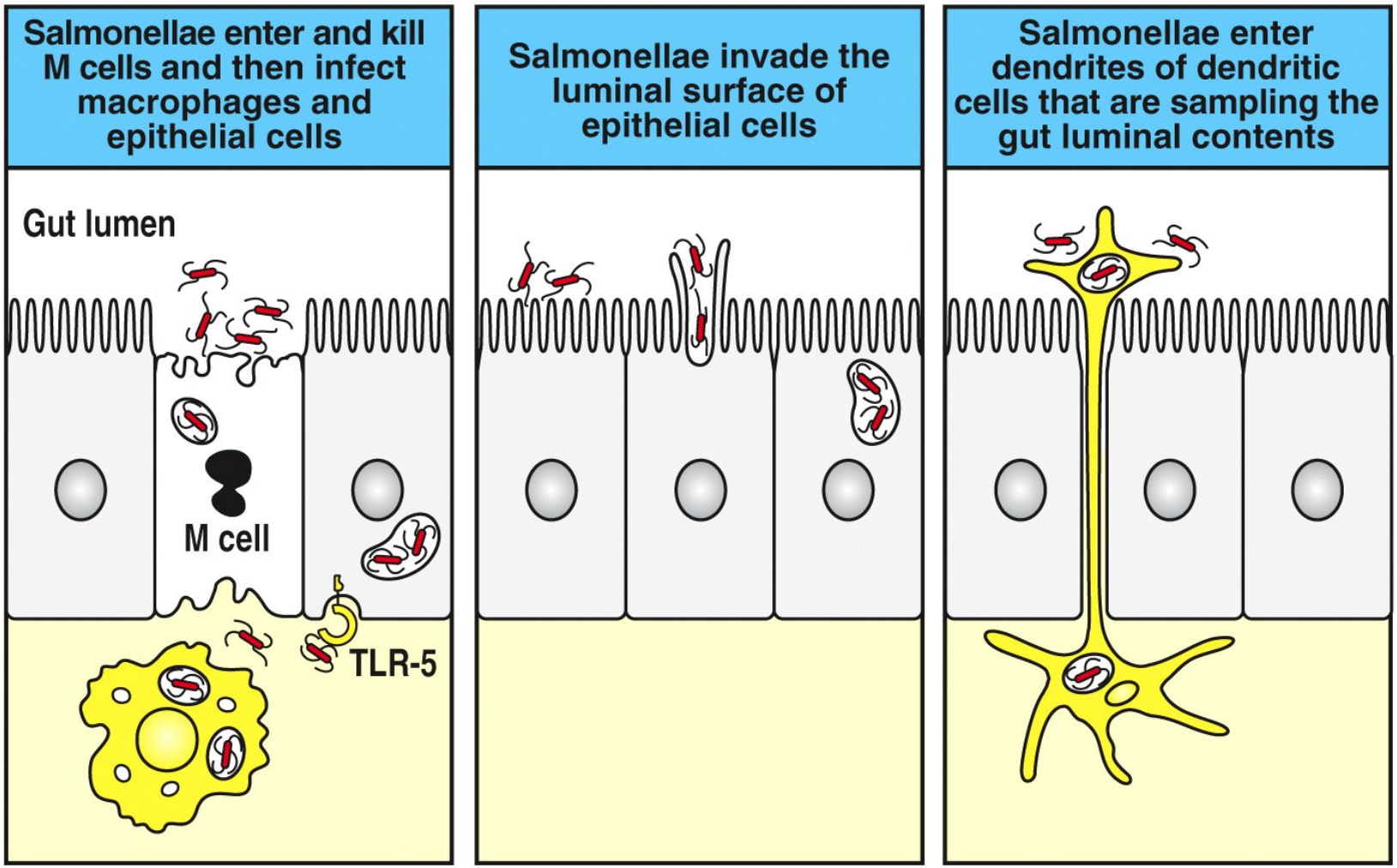
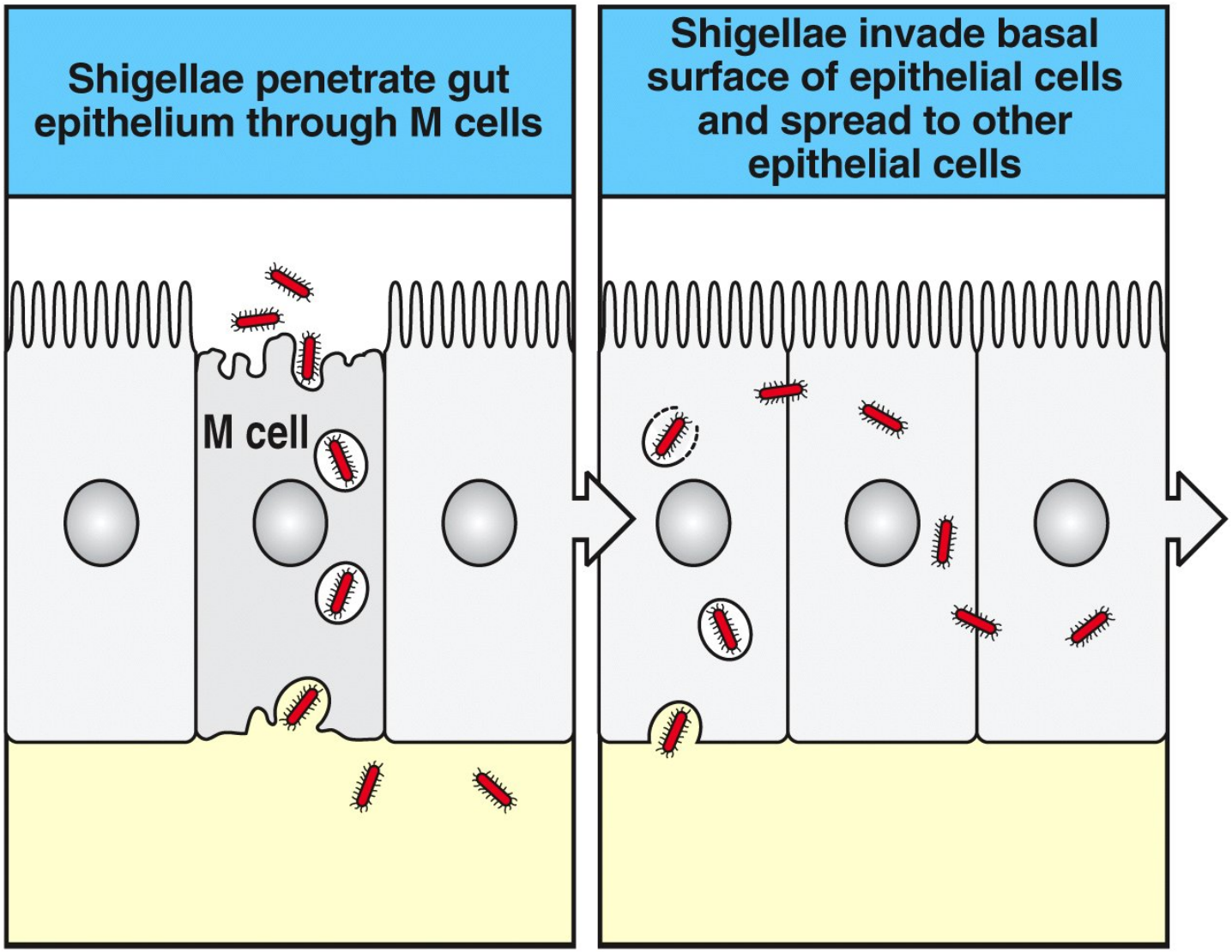


Figure 10-26 Immunobiology, 6/e. (© Garland Science 2005)



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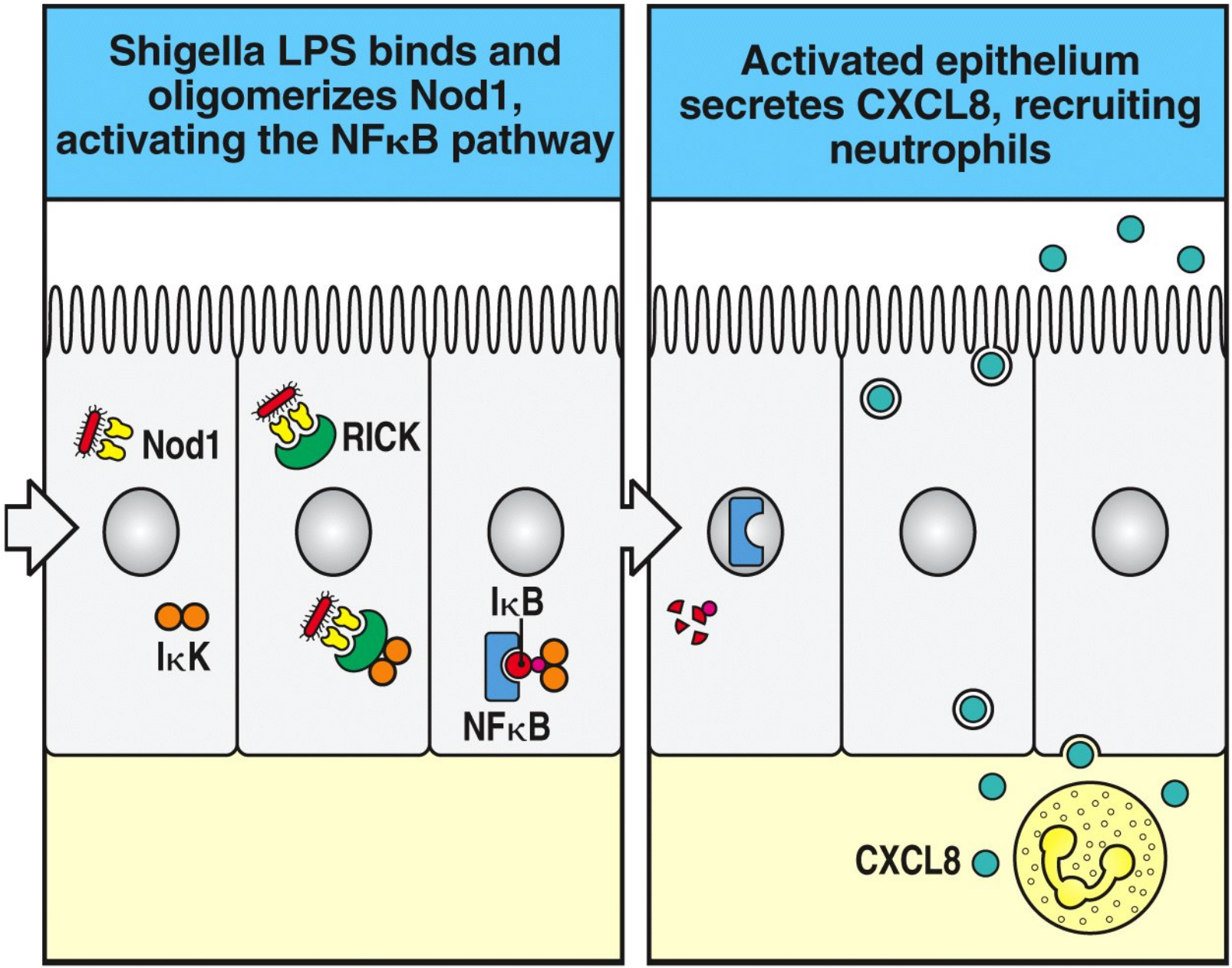

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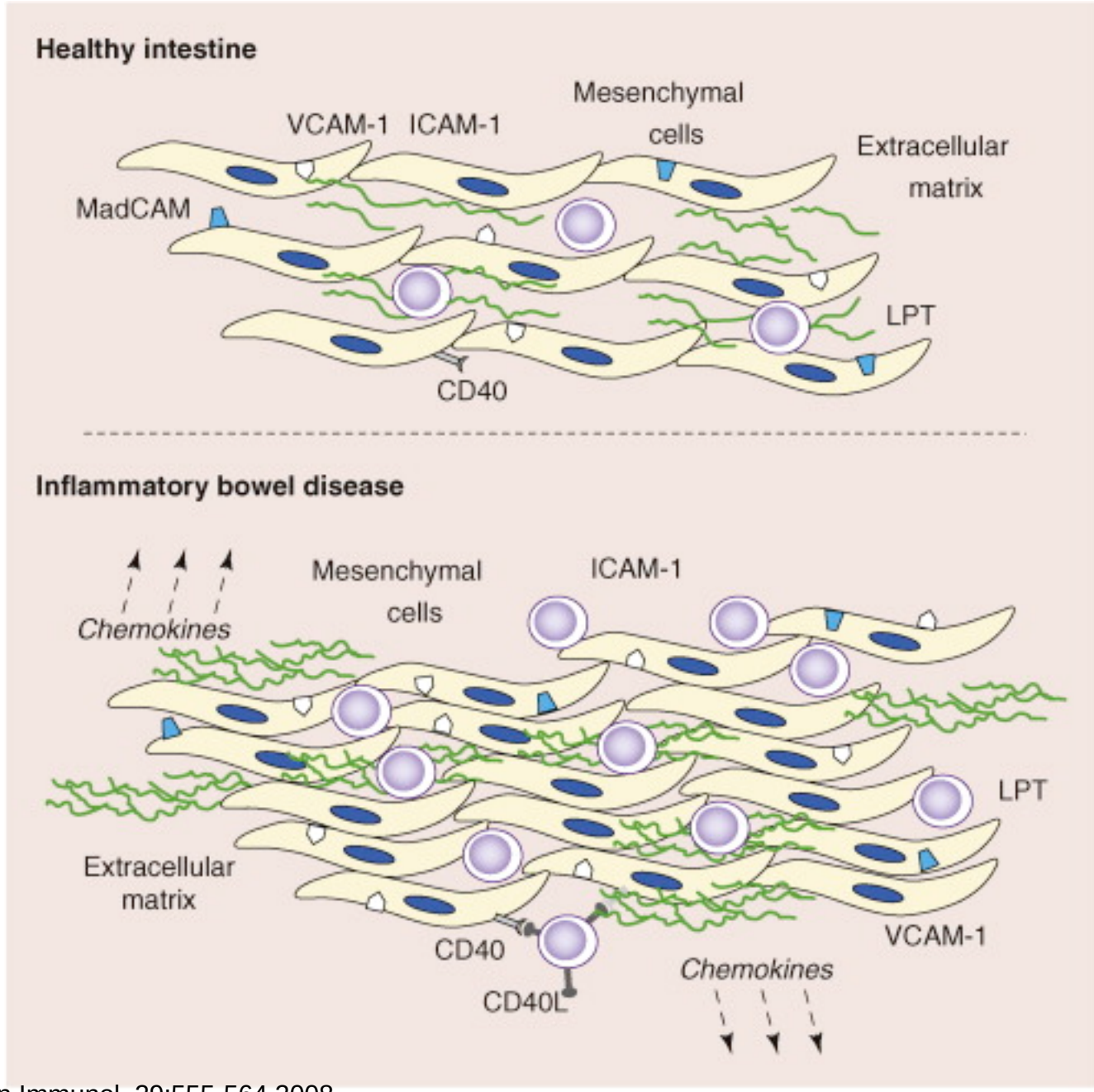

Danese, Trends in Immunol. 29:555-564,2008

Crohn's Disease

- Chronic inflammatory disease with epithelial cell damage. PMNs are present.
- Occurs primarily in Western developed countries.
- May involve any part of the gi tract - damage can be discontinuous.
- Granuloma formation, aphthous ulcers - suggests infectious agent involvement although none has been identified.
- Question: autoimmune disease?

- Th1 T cell-mediated response
  - Production of IFN-γ, TNF-α by T cells
  - Production of IL-12, IL-18 by mø
  - Increase in GM-CSF production
- Enhanced IL-12 production and Th1 activation may be due to failure of NOD2 to inhibit TLR2 signaling.
- Elevated levels of nonspecific inflammatory mediators: eicosanoids, leukotrienes, other proinflammatory cytokines and chemokines.

Treatment depends on location and extent of damage

- Steroid treatment can be given briefly
- Antidiarrheal medication
- Aminosalicylates
- Antibiotics (ciprofloxacin or metronidazole)
- Infliximab, an antibody to TNF-α, also reduces GM-CSF production
- Immunosuppressives
- Possible in very severe cases: total parenteral nutrition or surgery

Distinctive features of the mucosal immune system	
Anatomical features	Intimate interactions between mucosal epithelia and lymphoid tissues
	Discrete compartments of diffuse lymphoid tissue and more organized structures such as Peyer's patches, isolated lymphoid follicles, and tonsils
	Specialized antigen-uptake mechanisms provided by M cells in Peyer's patches, adenoids, and tonsils
Effector mechanisms	Activated effector T cells predominate even in the absence of infection
	Plasma cells are in the tissues where antibodies are needed
Immunoregulatory environment	Dominant and active downregulation of inflammatory immune responses to food and other innocuous environmental antigens
	Inhibitory macrophages and tolerance-inducing dendritic cells

Figure 10.17 The Immune System, 3ed. (© Garland Science 2009)