Physiology Conference Gut Immunology, Dysentery, & IBS November 18, 2004

By: Amy Tiu, MD

Question

- The patient states, “Ever since I had that terrible bout of diarrhea, I haven’t been quite right.”
- The patient then asks, “Do you think that the food poisoning I had during my vacation caused my current condition?”

Objectives

- Overview of Innate and Adaptive immunity
- Bacillary dysentery (Shigella) and the immune response
- Is there a link to irritable bowel syndrome?
- Can an anti-inflammatory help post-infective irritable bowel syndrome?

Gut immunology: The challenge

- Discriminate between pathogens and benign organisms
- Stimulate effective protective immune response without causing excessive inflammation
- Provide a surface for absorption of nutrients while maintaining a barrier to harmful external pathogens

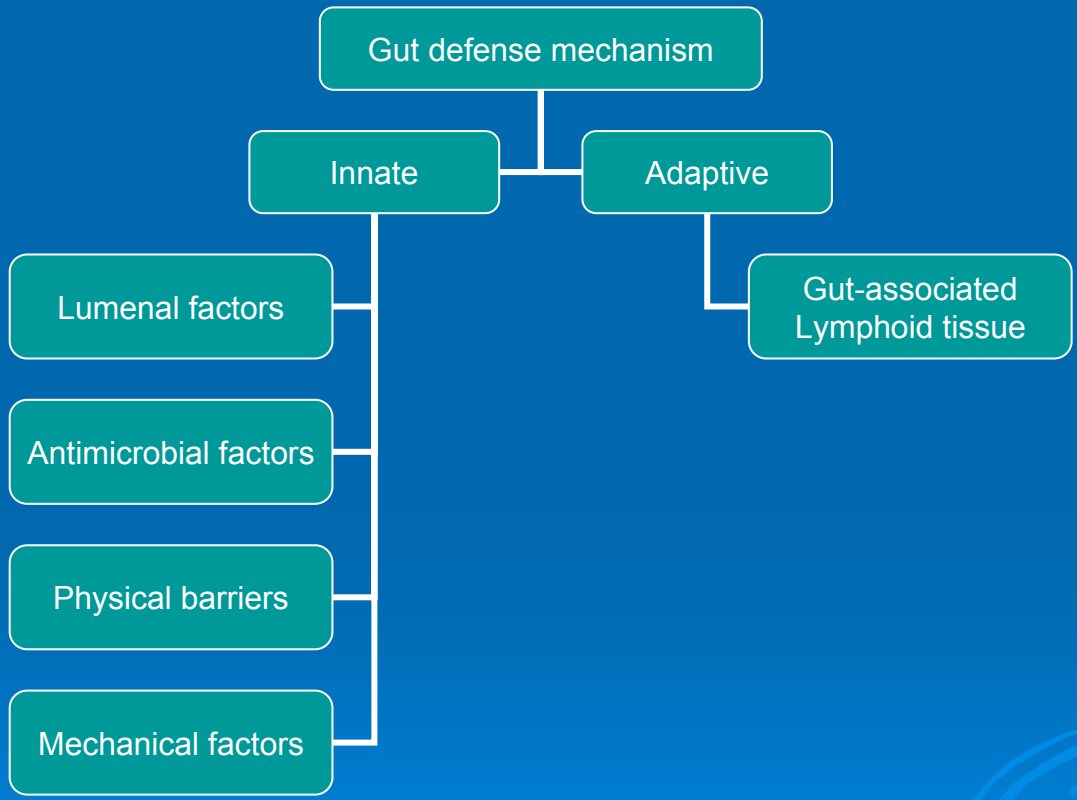
Gut immunology: Innate

- First line of defense
- Does not require prior exposure to antigen
- Acts immediately

Gut immunology: Adaptive

- Requires prior exposure
- Takes time to develop
- Highly specific response
- Provides lasting immunity
- Amplifies the initial protection provided by the innate immune response

Gut immunology



Innate: Lumenal factor

- Saliva
  - Flow 1500 cc/day
  - Specifically contains histatins (has activity against Candida albicans and Streptococcus mutans)
- Gastric Acid
  - Depends on hydrochloric acid
  - At a pH less then 4, 99% of bacteria are killed within 30 minutes

Innate: Lumenal factors

- Digestive secretions and enzymes
- Secretory IgA and IgM
- Lactoferrin from pancreas
- Lysozyme from Paneth cell
- Defensins

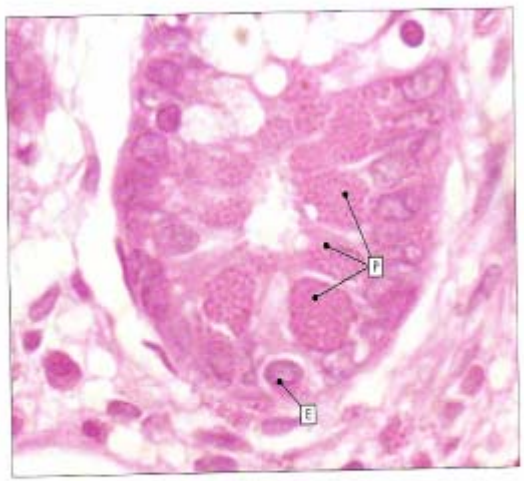


Fig. 11.40 Paneth cells.
Micrograph of the base of a small intestinal crypt from a paraffin section showing numerous Paneth cells (P) containing large numbers of bright red granules. A small endocrine cell (E) with ill-defined fine basal eosinophilic granules can also be seen.

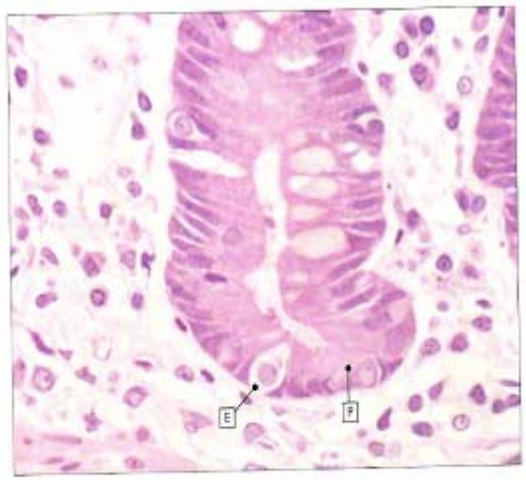


Fig. 11.41 Endocrine cell.
Micrograph of the base of a small intestinal crypt showing a typical pale-staining entero-endocrine cell (E). In this thin acrylic resin section the Paneth cell (P) granules are difficult to see.

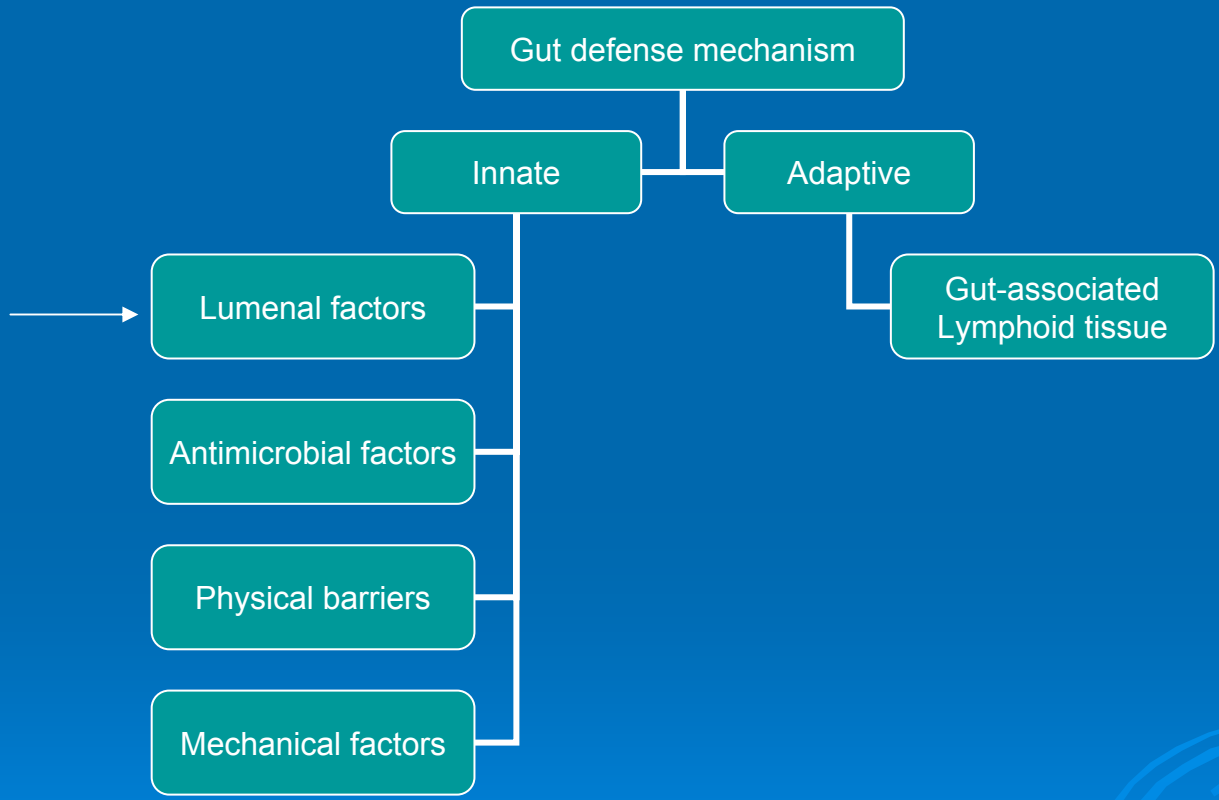
What is a defensin?

- Best characterized antimicrobial peptide
- Two classes: α and β
- Made by neutrophils and Paneth cells
- Secrete chloride ions
- Provide chemotactic factors
- An important link between the two defense systems

What is a lysozyme?

- Antimicrobial protein found in many human secretions
- In normal intestinal tract in gastric and pyloric glands, duodenal Brunner's glands, small intestinal Paneth cells and macrophages and granulocytes, but NOT in normal colon
- Predominantly active against Gram-positives

Gut immunology

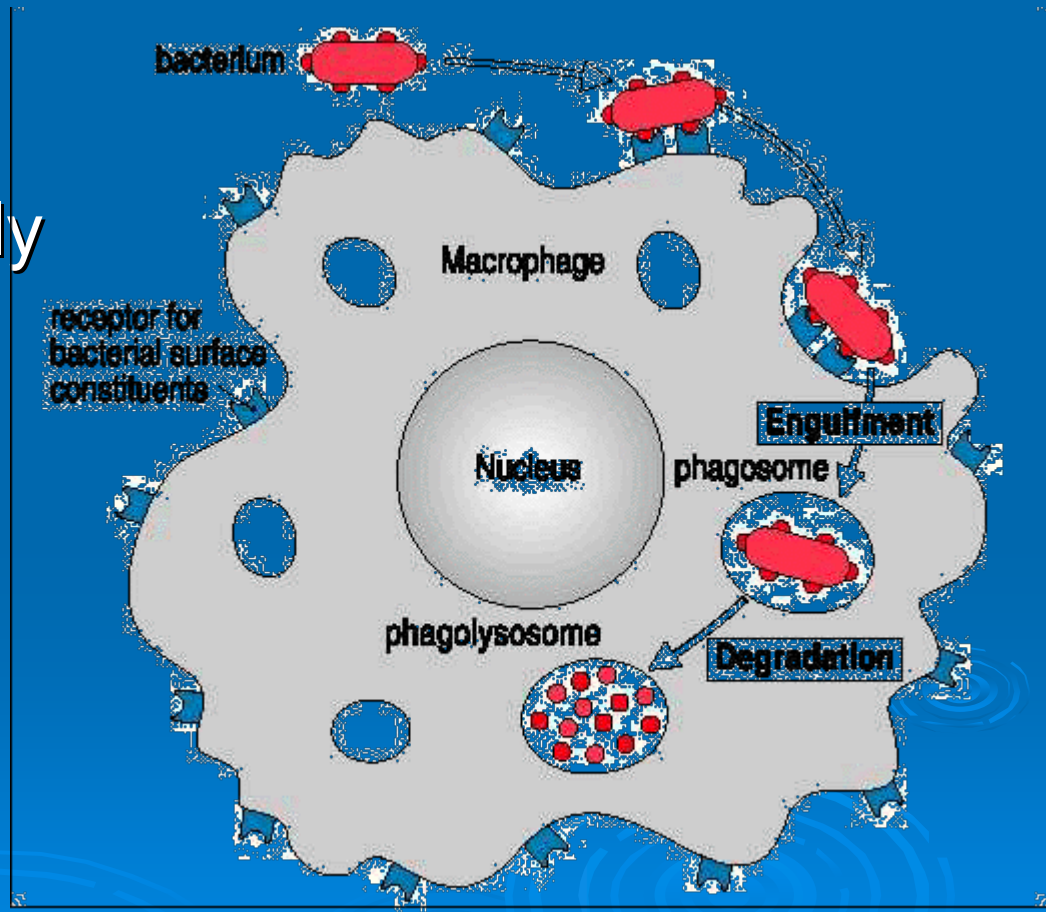


Innate: antimicrobial factors

- Preventing colonization of pathogens
  - Immunological factors (complement, phagocytes, natural killer cells)
  - Indigenous flora (estimated population 10^{14} bacteria which outnumbers the population of host cells approximately 10^{13})
- Producing antimicrobial compounds

Innate: antimicrobial factors (the immunological components)

- Complement, NK cells, phagocytes
- Available systemically but are in the activated form in the lamina propria



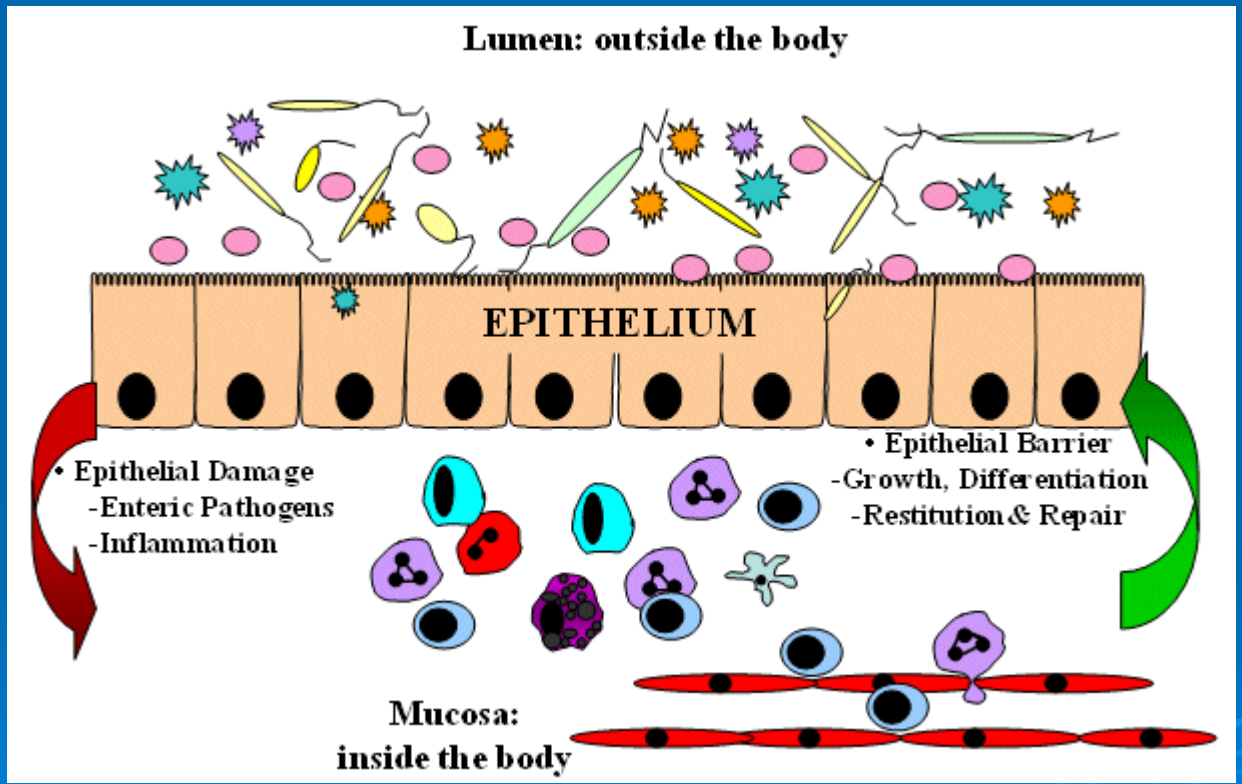
Innate: Physical Barriers

- Mucus layer
  - Goblet cells store mucin
  - Secreted constitutively or in response to a variety of secretagogues (prostaglandins, microbial products)
  - Protect the epithelial layer against invasion through the binding of their carbohydrate moeties to various microbial receptors (adhesins)
  - Probiotics such as lactobacillus may exert a protective effect by stimulating mucin production

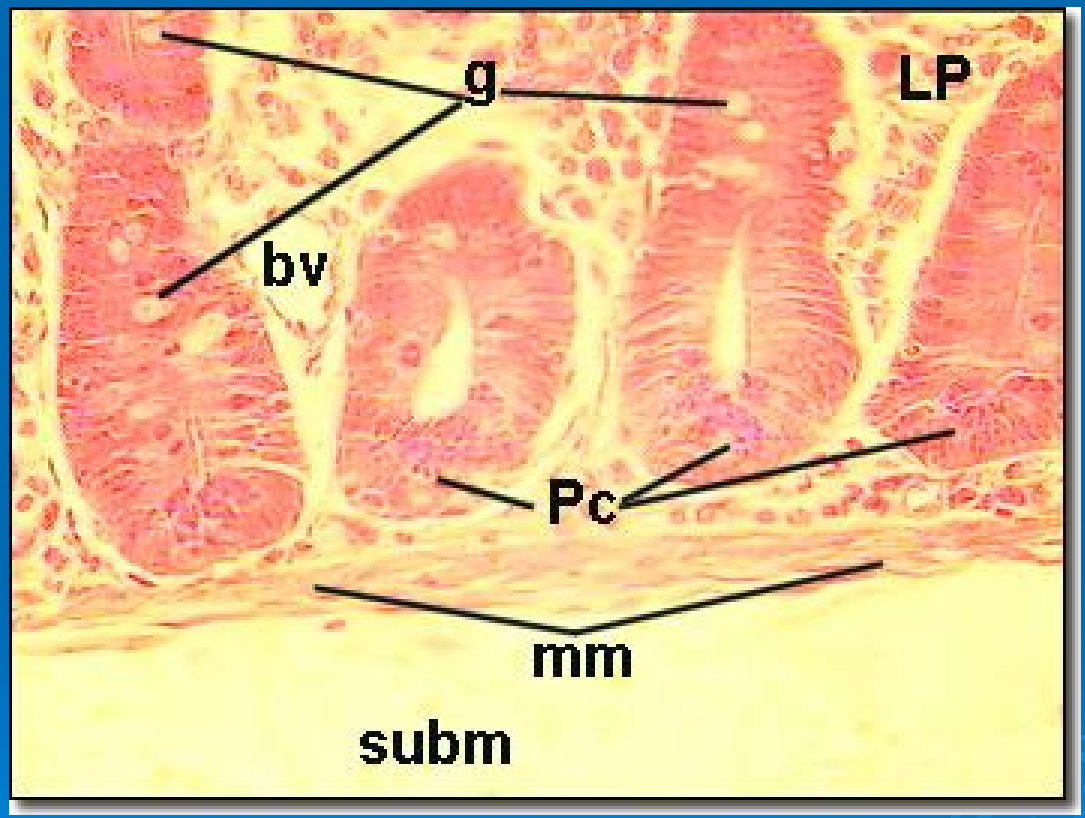
Innate: IECs

- Single columnar intestinal epithelial cell layer
- Consists of four main cell types
  - Absorptive enterocytes
  - Goblet cells
  - Enteroendocrine cells
  - Paneth cells
- Rapid turnover: 10^{11} per day in the human small intestine

Innate Immunity



Innate Immunity



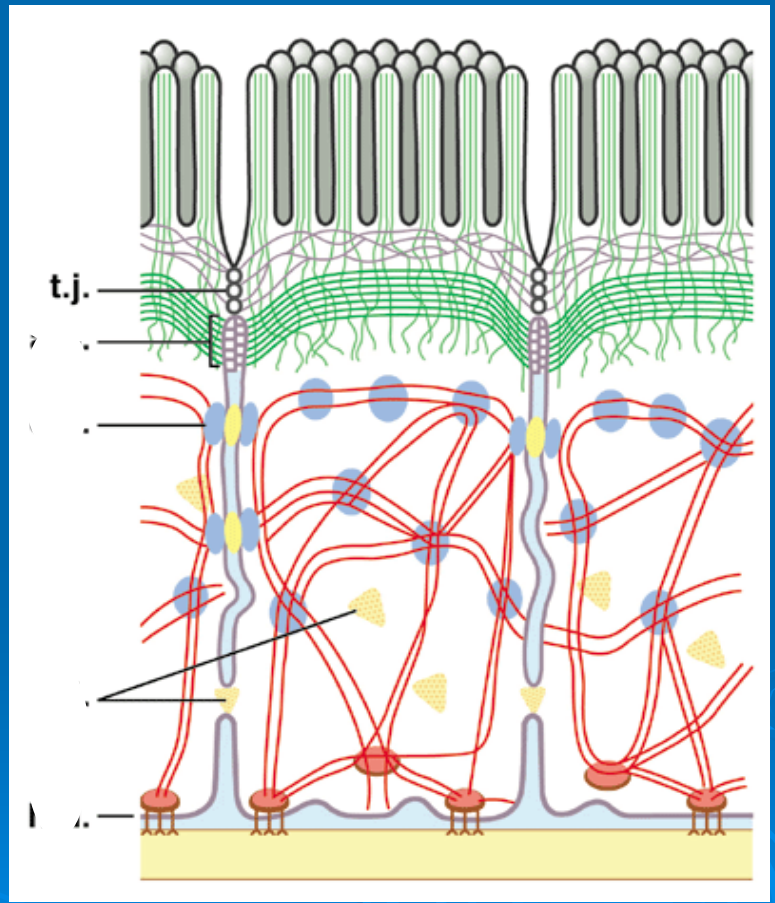
What does the IEC do?

- Mechanical barrier: can produce a mucous coat of glycocalyx that covers the surface of the epithelium
- Purge pathogens by secreting water and electrolytes
- Can act as nonprofessional APCs using MHC class I to underlying macrophages
- May signal the presence of stimuli or pathogens to effector cells (GALT)

Jabbar et al.

IECs and Tight Junctions

- Another layer of protection
- Channels only allow specific peptides and molecules to pass
- Dynamic and well-regulated



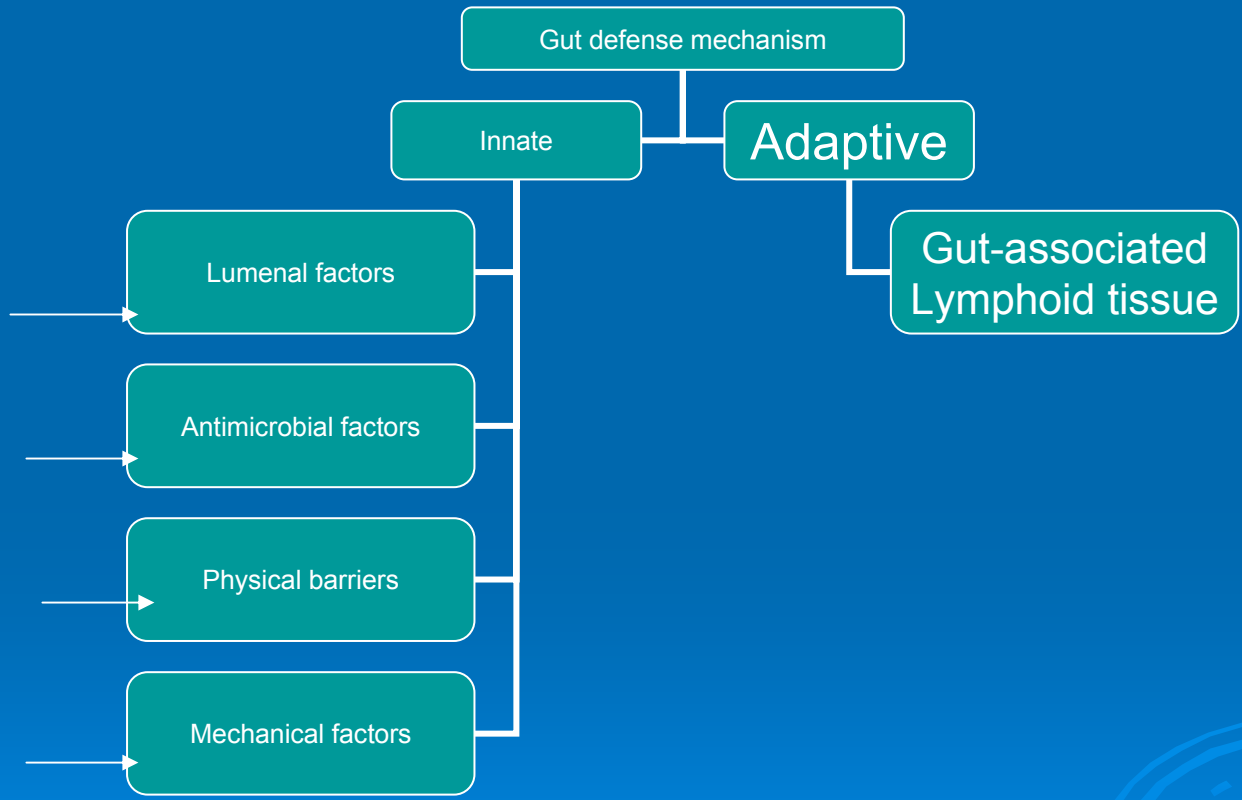
Jabbar et al.

Chandran et al.

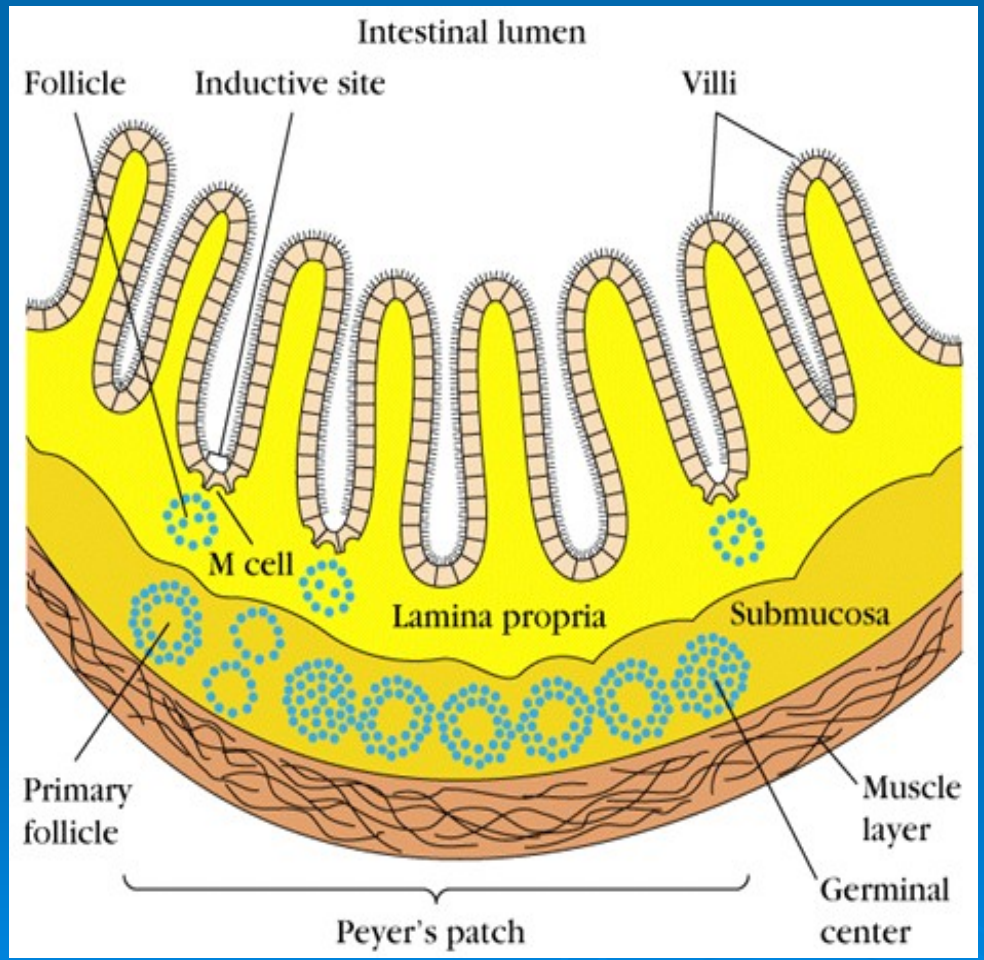
Innate: Mechanical factors

- Peristalsis
- Digestive secretions
- Desquamation

Gut immunology



GALT: Four compartments



- Intraepithelial lymphocytes (IEL)
- Lamina propria
- Peyer's patches
- Mesenteric Lymph nodes

GALT: InTRAepithelial lymphocytes (IEL)

- Heterogeneous T cell population many of which are not found in the systemic lymphoid tissues
- Most are Mature T cells of CD8+ phenotype (suppressor/cytotoxic)

GALT: IEL

- Function in homeostasis between lumen, IEC, and lamina propria
- Induces apoptosis of “old” IEC and stimulates the proliferation of IEC (keratinocyte growth factor)
- Directly eliminate damaged cells or microorganisms (perforin and granzyme)
- Downregulate inflammatory response specifically secrete TGF-β and down regulate IgA or directly act as a suppressor T cell

GALT: IEL & Homing

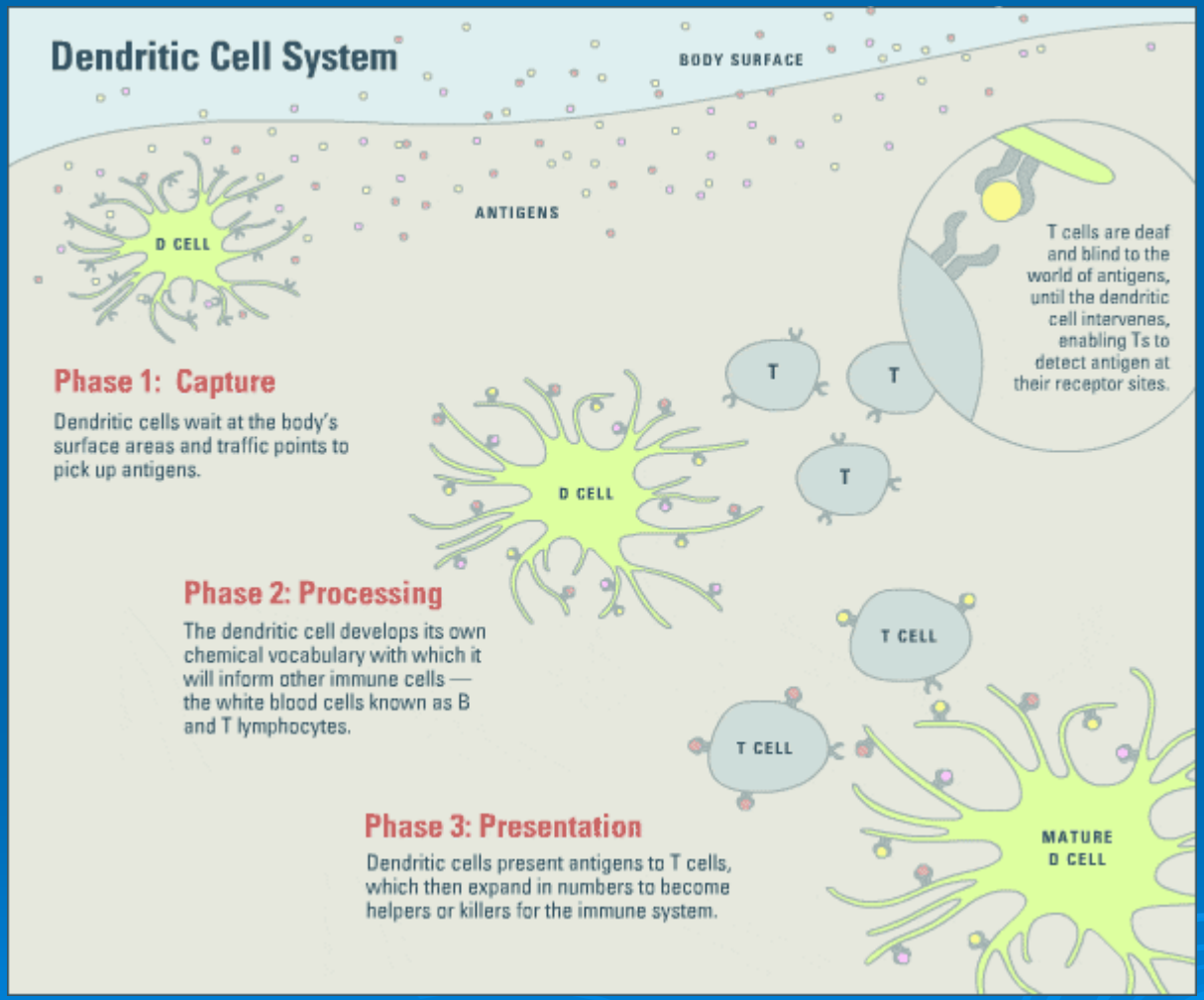
- What is homing? Process by which activated immune cells exit from original site to systemic circulation and then return which helps to increase the efficiency of immune surveillance
- IEL use E-cadherin and αEβ7 (adhesion molecules) for homing and maintaining interaction with IEC

GALT: Lamina Propria

- Main cell population
  - CD 4 + T lymphocytes (T helper cells)
  - Dendritic cells
  - Macrophages
  - B cells

Lamina Propria: A closer look

- Dendritic cells and macrophages are professional antigenic presenting cells needed in the gut to expose naïve T helper cells (Th0) to antigens
- This leads to the T helper cells to differentiate in one of three types of Th cells
  - Th1
  - Th2
  - Th3 and Regulatory T cells (Tr1)



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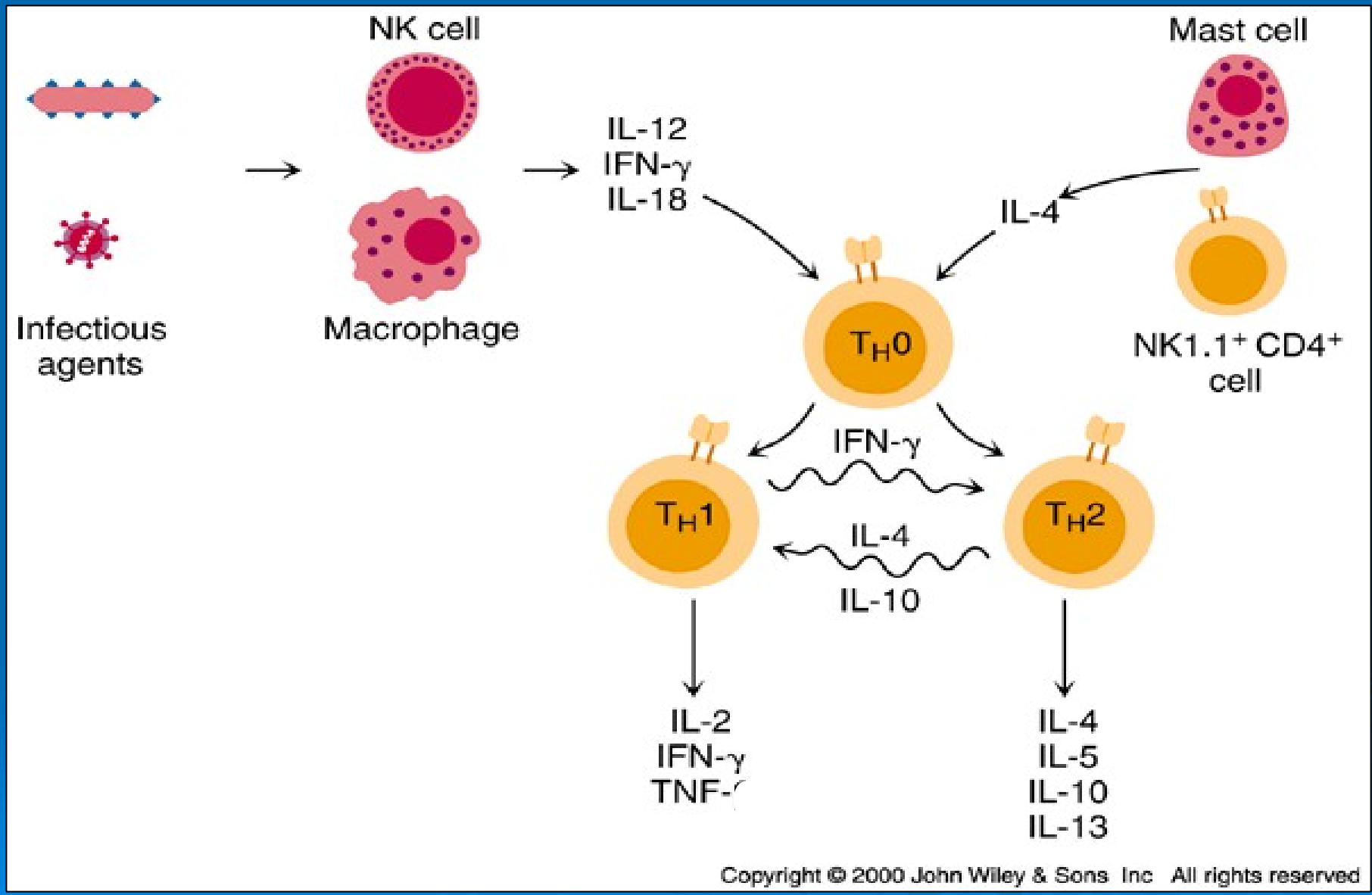
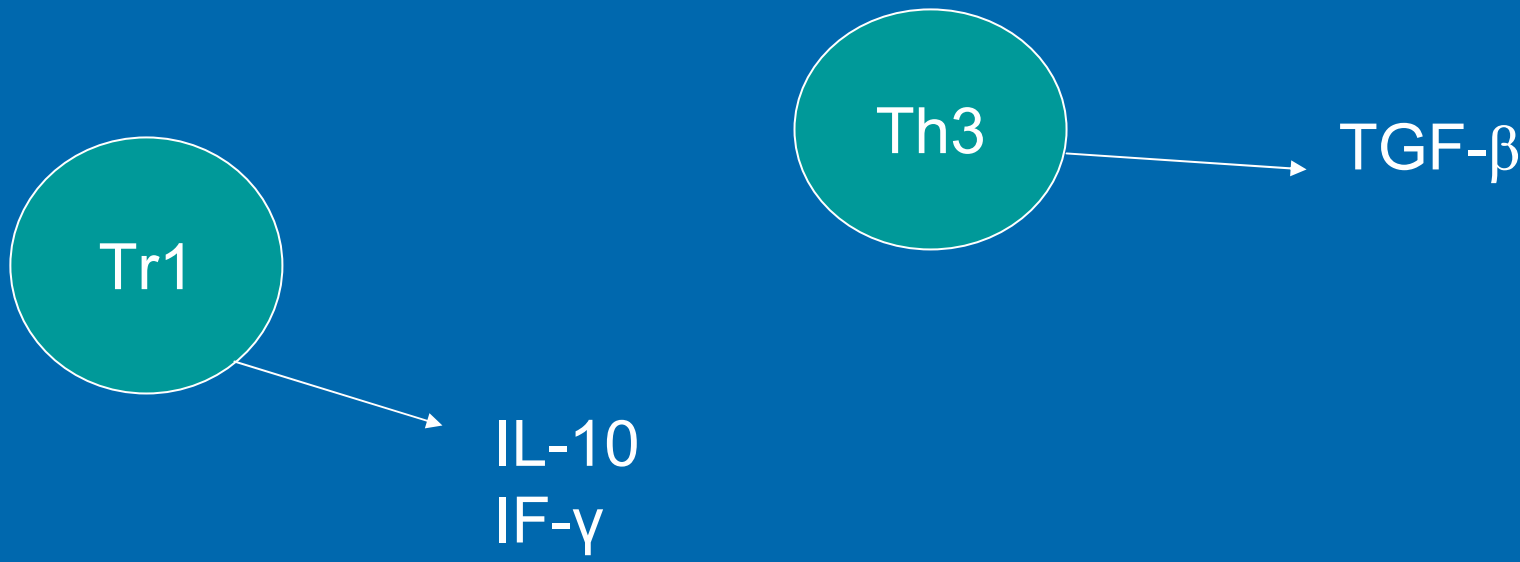
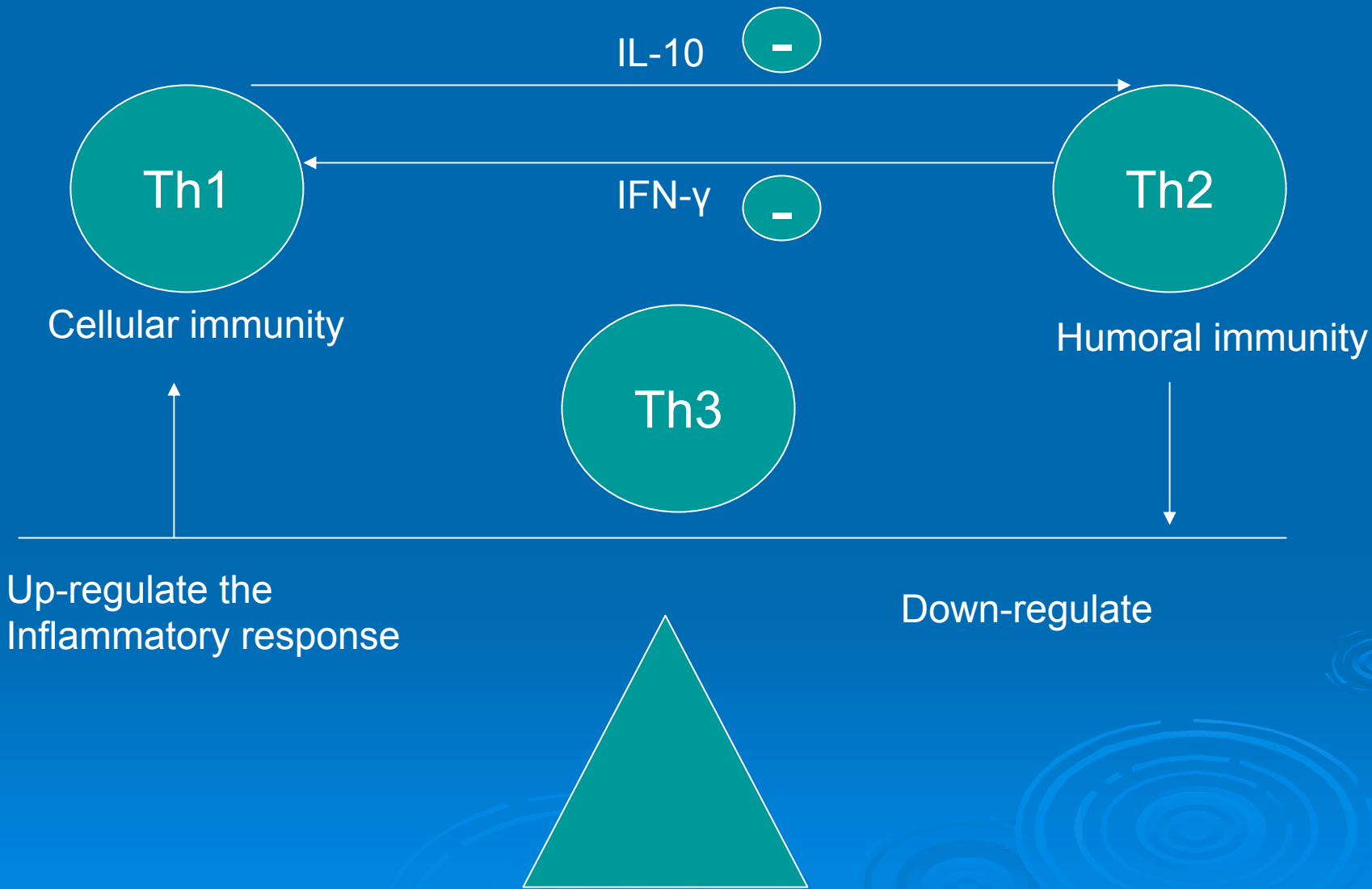


What about Th3 and Tr1?

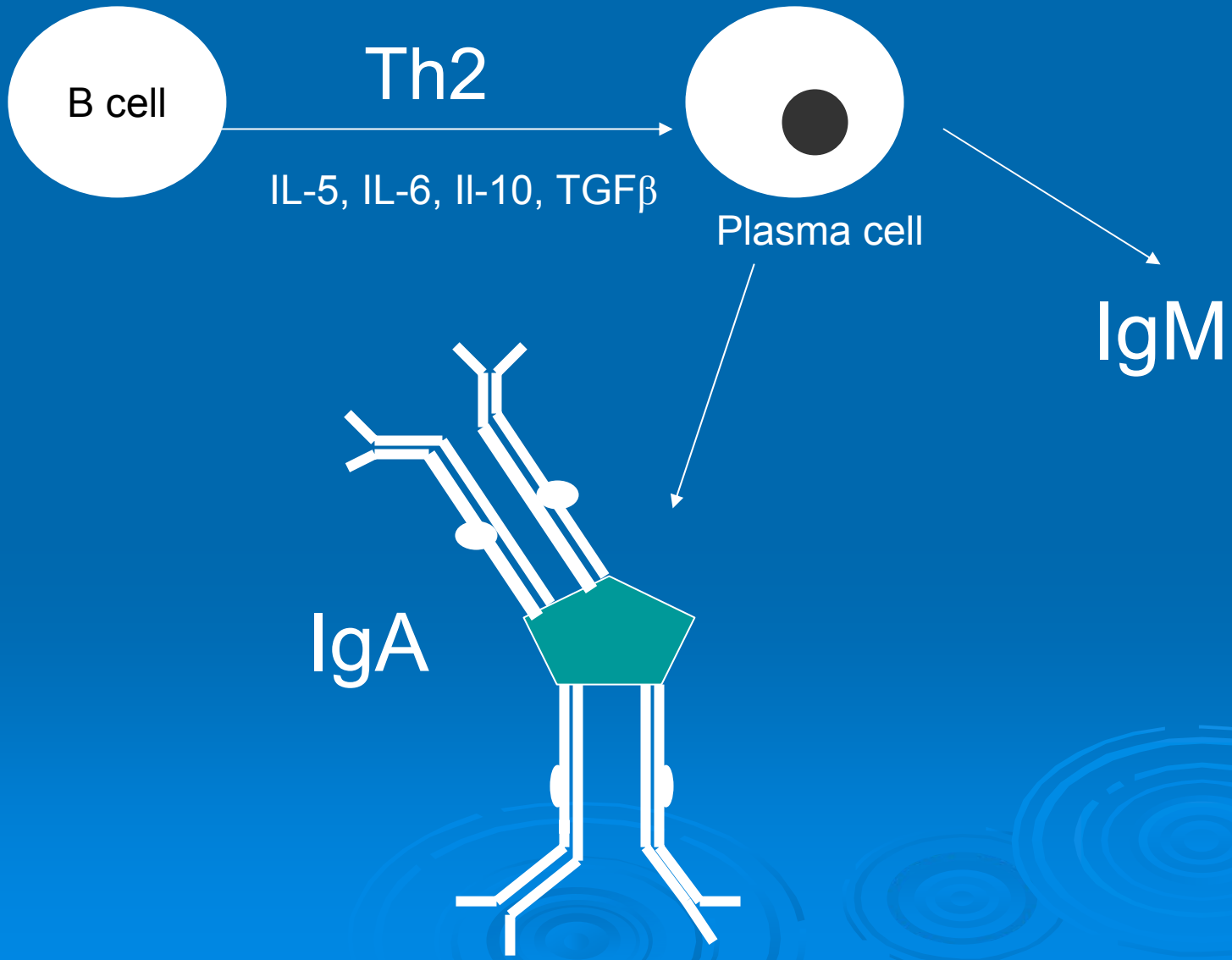


These cells produce suppressive cytokines

Balance of Th1/Th2



Role of B Cells, Th2, and IgA



Role of IgA

- Does not activate complement or an inflammatory response
- Directed against surface molecules
- Has its own enterohepatic circulation

ADAPTIVE IMMUNITY IN THE GASTROINTESTINAL TRACT

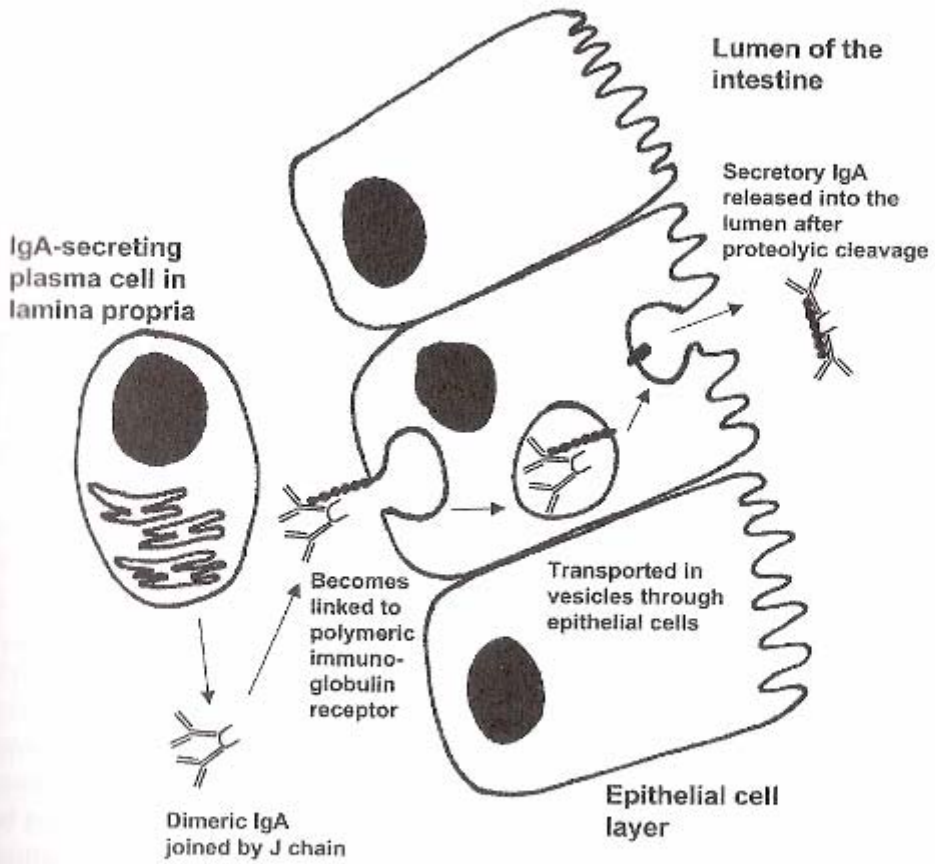
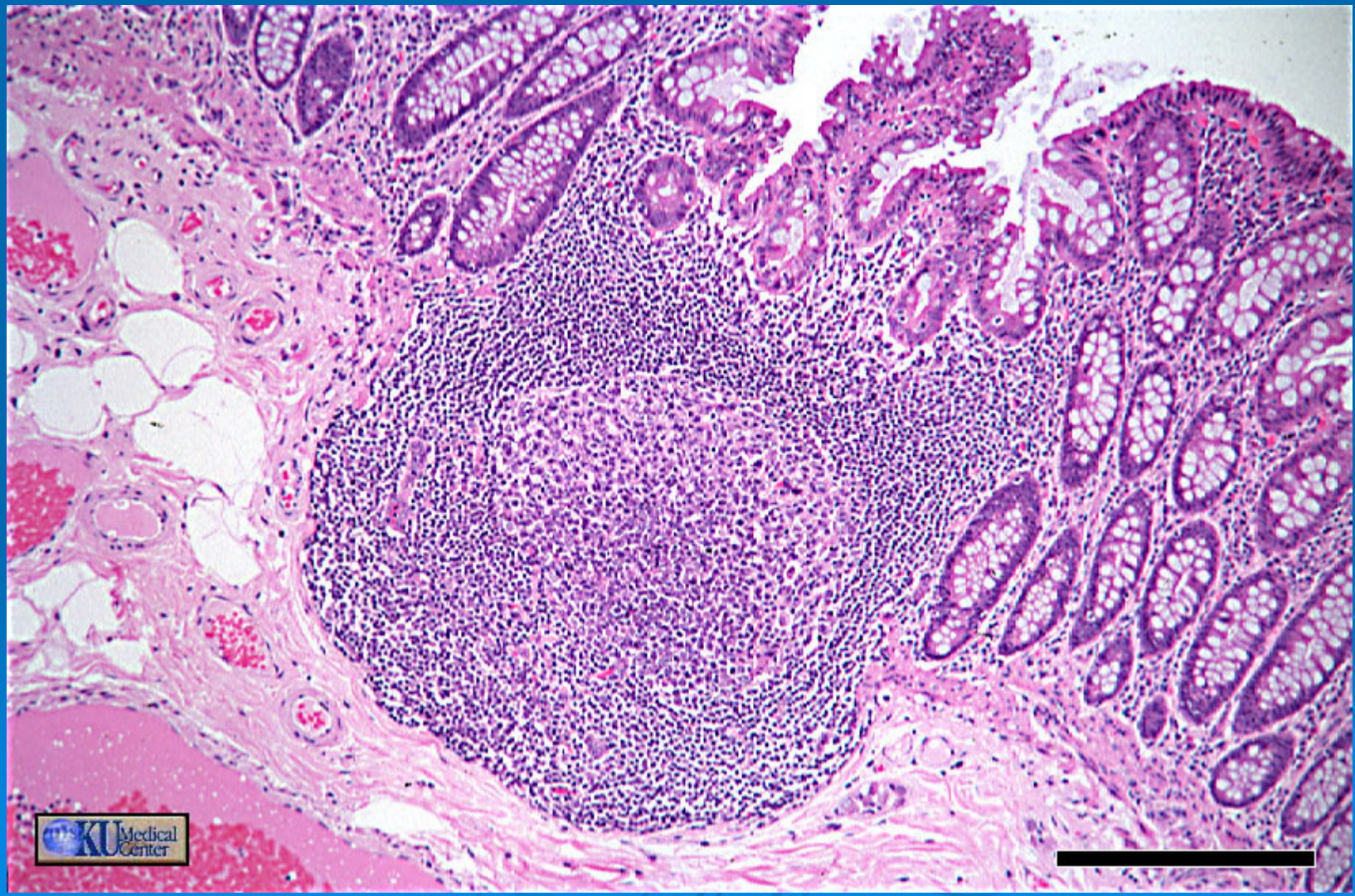


Figure 2.2. Diagram of secretory mechanism of intestinal epithelial cells which delivers dimeric IgA to the intestinal lumen.

Mahida 2001

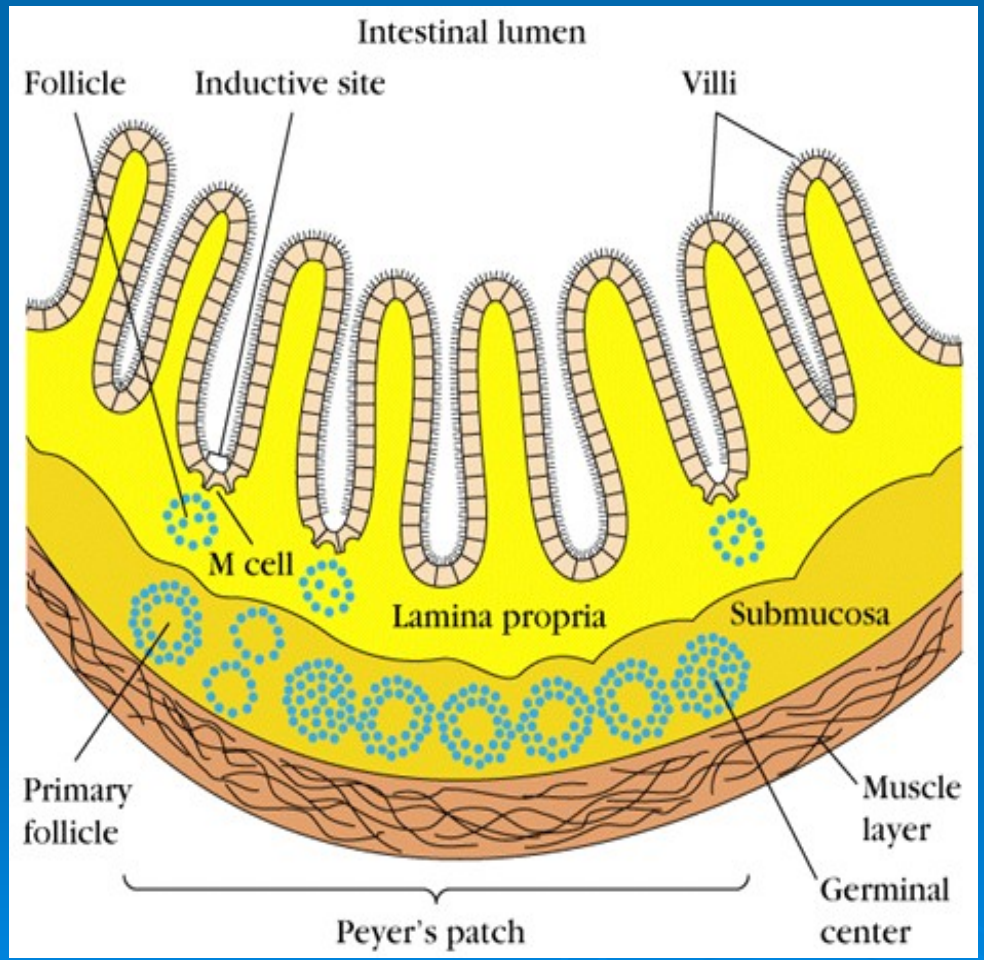
GALT: Peyer's Patch



Peyer's Patch: Contents

- Both CD4+ and CD8+ T cells
- B cells
- Macrophages
- Dendritic cells
- M cells (membranous cells)

GALT: Four compartments



- Intraepithelial lymphocytes (IEL)
- Lamina propria
- Peyer's patches
- Mesenteric Lymph nodes

What is an M cell?

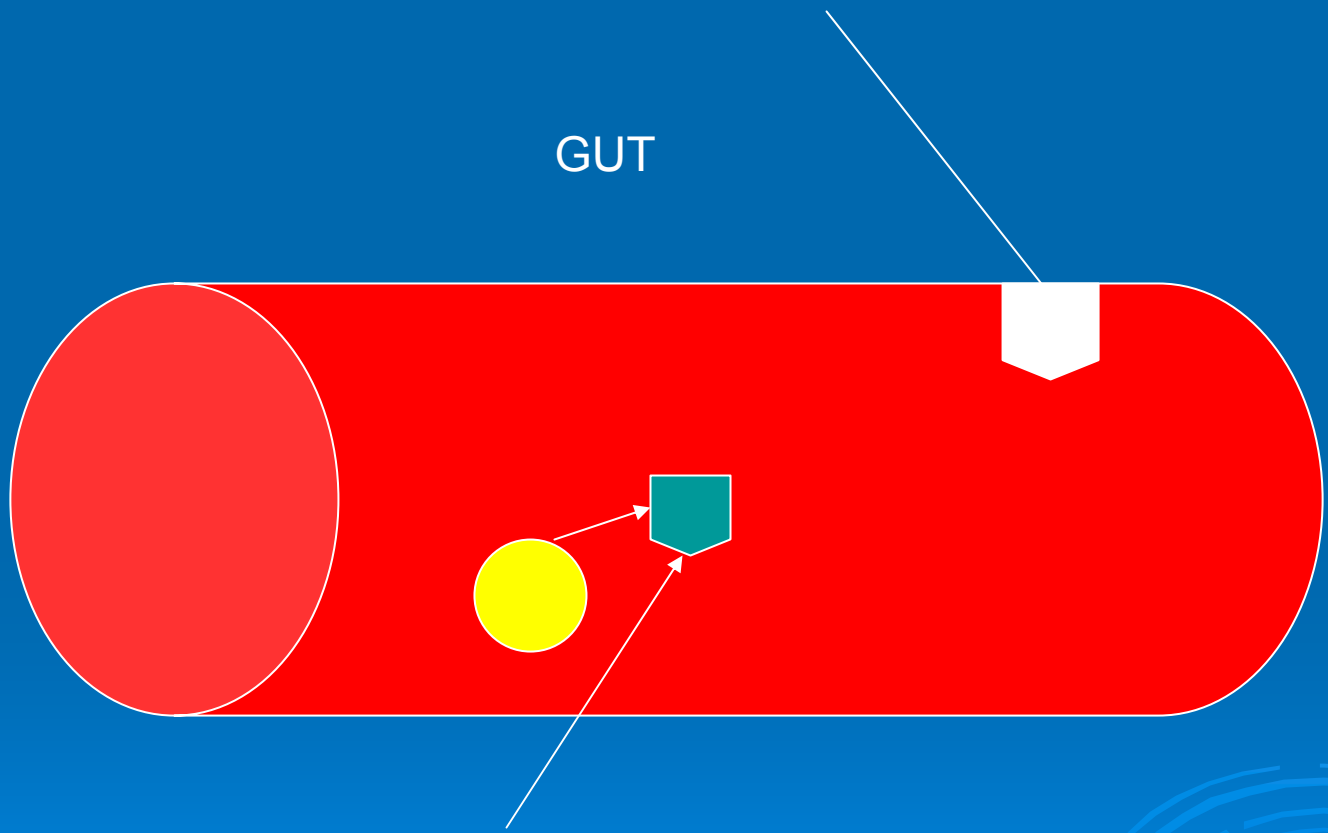
- Derived from stem cells in the crypts of lymphoid tissue
- Covers the Peyer's patches
- Specialized cells which can efficiently take up antigens which are then taken up by APCs in the Peyer's patch
- Susceptible to invasion and destruction

Peyer's Patch: Homing

- Relies on homing to traffic undifferentiated immune cells and activated immune cells to and from systemic circulation and gut immune system

- What are the components required for homing?
  - (LOCK) MAdCAM 1 on vascular endothelium
  - (KEYS) L-selectin and α4/β7 on immune cells

MAdCAM-1
(mucosal adressin cellular adhesion molecule -1)

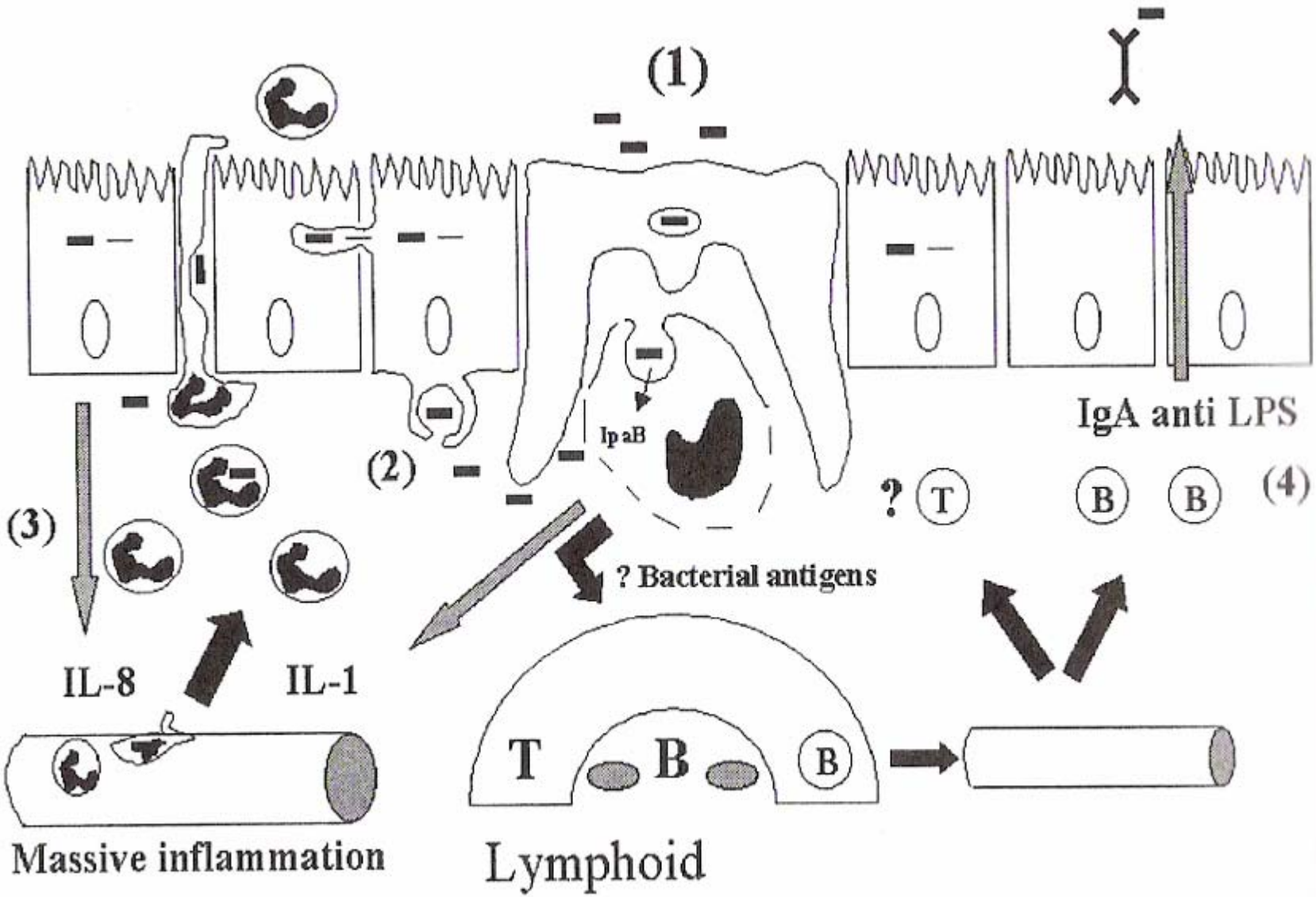


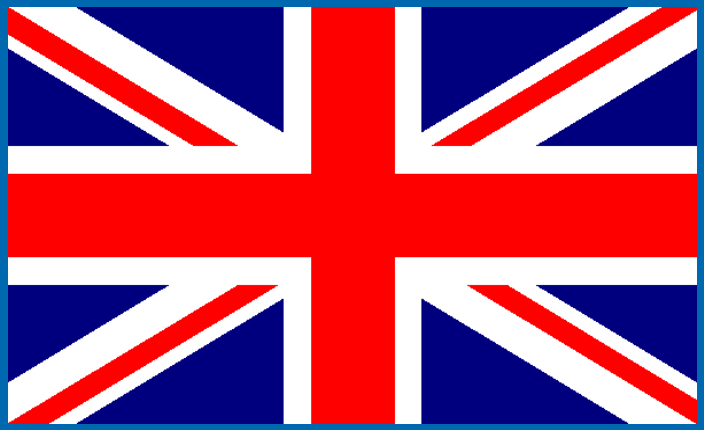
GALT: Mesenteric Lymph Nodes (MLN)

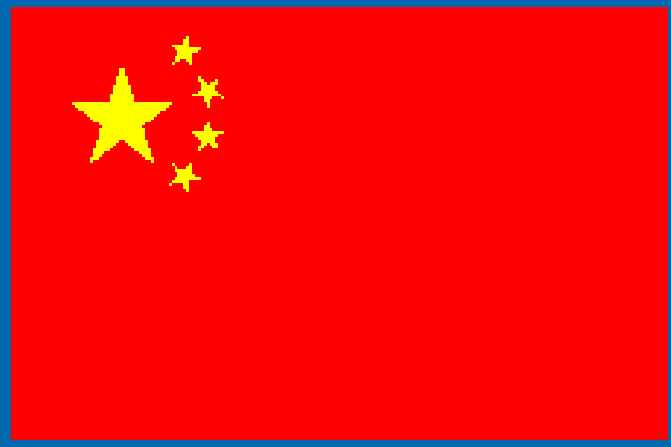
- T and B cells, Macrophages, interdigitating cells, and Langerhans cells
- Function: cell population expansion
- Mechanisms: trapping and presenting antigen to lymphocytes; expansion of activated B and T lymphocytes

Jabbar et al

Shigella (Bacillary dysentery)
The immune system in action


(1)
IpaB
(2)
(3)
IL-8
IL-1
Massive inflammation
? Bacterial antigens
T
B
B
Lymphoid
? T
B
B
(4)
IgA anti LPS





Two places with infectious diarrhea
Two places with IBS
The question:

Does infection play a role in the pathogenesis of IBS?

Brief Study Highlights

- In 1997, the prevalance of functional bowel disorders six months after infectious diarrhea was 25%

Neal KR, Hebden J, Spiller R. Prevalence of gastrointestinal symptoms six months after gastroenteritis and risk factors for development of the irritable bowel syndrome: postsurvey of patients. BMJ 1997;314:779-81

- A cohort study in 1999, indicated that during a one year follow up, the diagnostic rate of irritable bowel syndrome was 4.4% in patients after an episode of bacterial gastroenteritis compared with a rate of 0.3% in the general population cohort.

Rodriguez LAG, Ruigomez A. Increased risk of irritable bowel syndrome after bacterial gastroenteritis: cohort study. BMJ 1999;318:565-6

Brief Study Highlights

- A UK study has shown that >50% of IBS patients remain symptomatic six years post-gastroenteritis. Also Post-infectious IBS (PI-IBS) had more diarreahal features than non-infectious-IBS.

Neal KR, Barker L, Spiller R.C. Prognosis in post-infective irritable bowel syndrome: six year follow up study. Gut 2002;51:410-13

Immunological aspects in PI-IBS

- Dunlop S, Jenkins D, Spiller, R. Distinctive Clinical, Psychological, and Histological Features of Postinfective Irritable Bowel Syndrome
- Subjects: 75 consecutive IBS outpatients (ROME II) and 36 healthy control subjects
- Workup included symptom questionnaire and rectal biopsy, which included staining and quantification of lamina propria (intraepithelial T lymphocytes, IEL), serotonin-containing enterochromaffin cells (EC) and mast cells.

Results

Symptom	PI-IBS	Non-PI-IBS	P-value
Diarrhea	70%	42%	0.03
Previous tx for anxiety or depression	26%	54%	0.02

Dunlop et al

Cell quantification results

Cell	PI-IBS	Non-PI IBS	Controls	P
EC/hpf	39.4 ± 2.9	31.1 ± 1.5	31.8 ± 1.6	0.012
LP T cells/hpf	120.5 ± 6.8	118.5 ± 4.6	101.6 ± 5.9	0.042
Mast cells/hpf	41.9 ± 3.0	53.0 ± 2.4	45.9 ± 2.8	0.017

Dunlop et al

T Lymphocyte counts and frequency of diarrhea

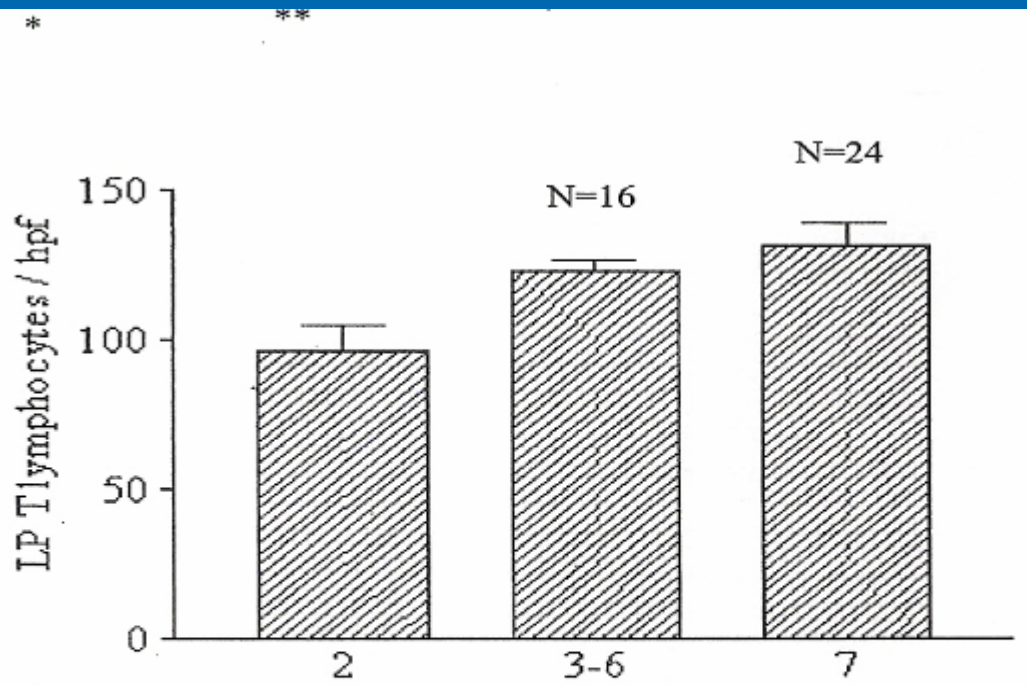


Figure 3. Lamina propria (LP) T lymphocyte counts per high power field (hpf) in 52 IBS patients with diarrheal symptoms. Lymphocyte scores increased with increasing frequency of diarrhea. *p = 0.04 *vs* 2 days/wk of loose stools. **p = 0.012 *vs* 2 days/wk of loose stools.

Dunlop et al

PI-IBS and interleukin 1:

- Gwee et al. examined sequential rectal biopsy samples from patients with PI-IBS and infectious control group during and after infection
- Measured
  - Expressions of interleukin 1 beta (IL-1β)
  - Expression of receptor antagonist (IL-1ra)

Changes in Interleukin 1(beta)mRNA expression

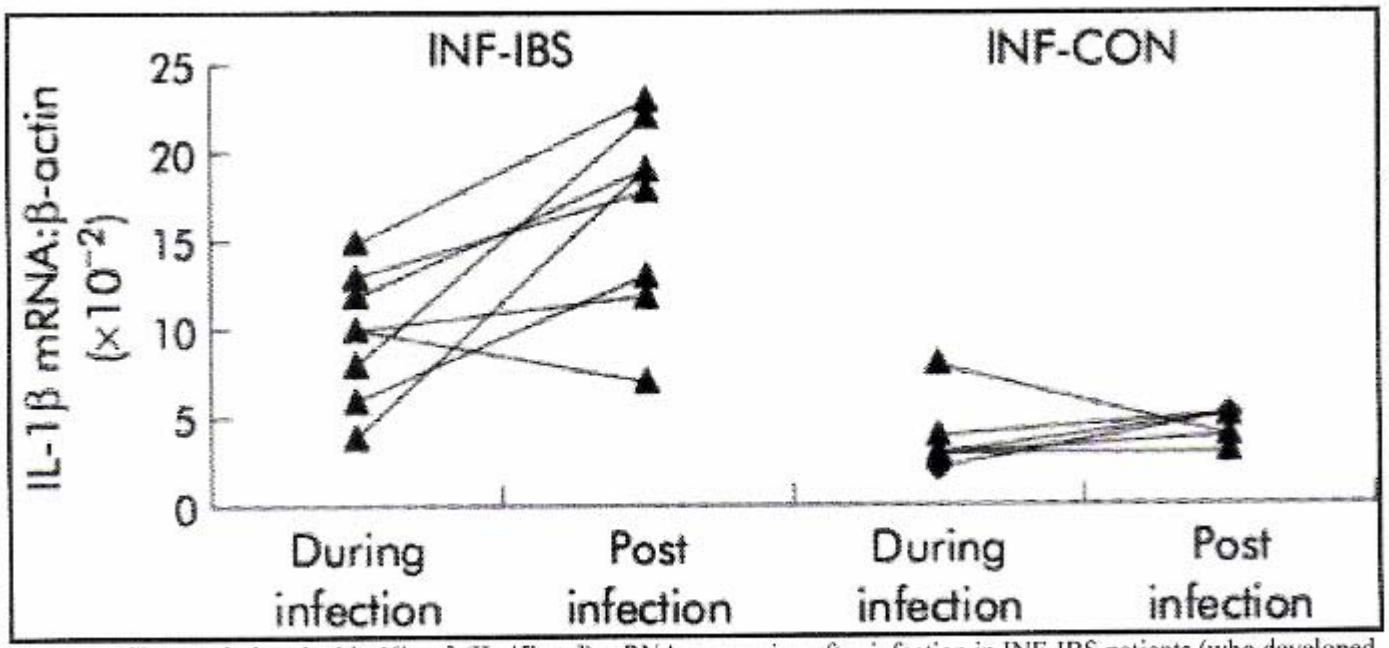


Figure 2. Changes in interleukin 1[beta] (IL-1[beta]) mRNA expression after infection in INF-IBS patients (who developed irritable bowel syndrome after acute gastroenteritis) and in INF-CON patients (who returned to normal bowel habits after acute gastroenteritis).

Similar findings in the East: Wang L-H, Fang X-C, Pan G-Z Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. Gut 2004;53:1096-1101

- Wang et al performed a cohort study in 295 patients recovering from acute gastroenteritis (controls were 243 siblings or spouses who had not been infected with Shigella) in Beijing, China

Wang L-H, Fang X-C, Pan G-Z Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. Gut 2004;53:1096-1101

- During a 1-2 year follow-up, 8.1% incidence of IBS (Rome II) among all patients compared with 0.8% of controls, and an incidence of 10.2% of IBS in those patients with documented Shigella infection
- 22.4% of patients (versus 7.4% of controls) exhibited functional gastrointestinal symptoms that did not meet the Rome II criteria

Shigella and Immune response

- Wang et al also found that expression of IL-1β mRNA in the terminal ileum and rectosigmoid mucosa was higher in PI-IBS ($p<0.01$)
- Mast cell numbers in the terminal ileum were higher in PI-IBS and non-PI-IBS patients compared with control subjects($p<0.01$)

Shigella and Immune Response

- Lastly, an increased density of 5-hydroxytryptatimine and substance P immunoreactive nerves surrounding mast cells in the ileum and colon of IBS patients. (48% with infection hx and 52% without infection hx)
- Confirms another study done by Barbara et al demonstrating a correlation between nerve to mast cell interactions with the severity of abdominal pain

Limitations

- The studies varied on the infectious agent (Campylobacter, salmonella, shigella)
- Definition of IBS
- Differences in patient recruitment
- Tissue sampling
- Use of antibiotics

Can an anti-inflammatory help?

Dunlop SP, Jenkins D, Neal KR, Naesdal M, Borgaonker, Collins M, and Spiller RC. Randomized, double-blind, placebo-controlled trial of prednisolone in post-infectious irritable bowel syndrome

Study characteristics

Subjects	Intervention	Outcomes measured
PI-IBS: new bowel symptoms developing in a previously asx individual immediately after an acute illness characterized by two or more of the following: D, V, F or positive stool culture	Placebo or prednisolone 30mg/day for 3 weeks	Mucosal enterochromaffin cells, T lymphocytes and mast cells in rectal biopsies before and after treatment and bowel symptoms

Results

- No signficiant change in enterochromaffin cell counts
- T lymphocyte counts decreased significantly after prednisolone ($p=0.003$), BUT not associated with improvement in symptoms

Conclusions

- Gut immunology has multiple components to carefully maintain a balance between protective inflammatory states and non-inflammatory absorptive function.
- Studies from very different regions of the globe provide evidence for increasing recognition that infection may contribute to the pathogenesis of diarrhea predominant IBS
- Low grade inflammation may contribute to diarrhea predominant IBS. The severity of the acute infection before PI-IBS is a strong risk factor
- Currently, there is no role for prednisolone for treatment, but other medications may need to be explored

Special Thanks

- Karen Canlas, MD copy editor and sample audience, black bag carrier fellow
- Wardrobe consultants: Ann Taylor, Benetton, Banana Republic (shoes), Kate Spade (black bag)
- Ivy Tiu, Pharm D who drew the IgA and allowed me to use her scanner