Mucosal Immunology

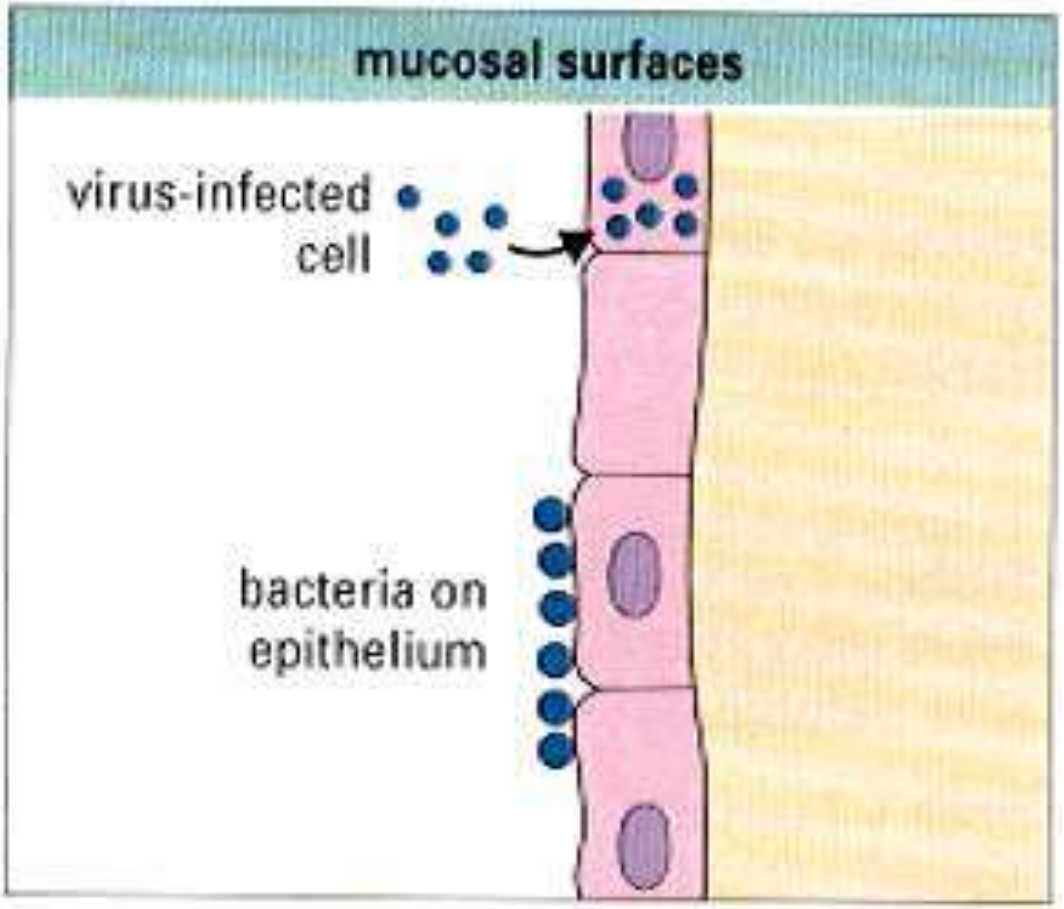
Mucosal Immunology
- Lecture Objectives -

To learn about:

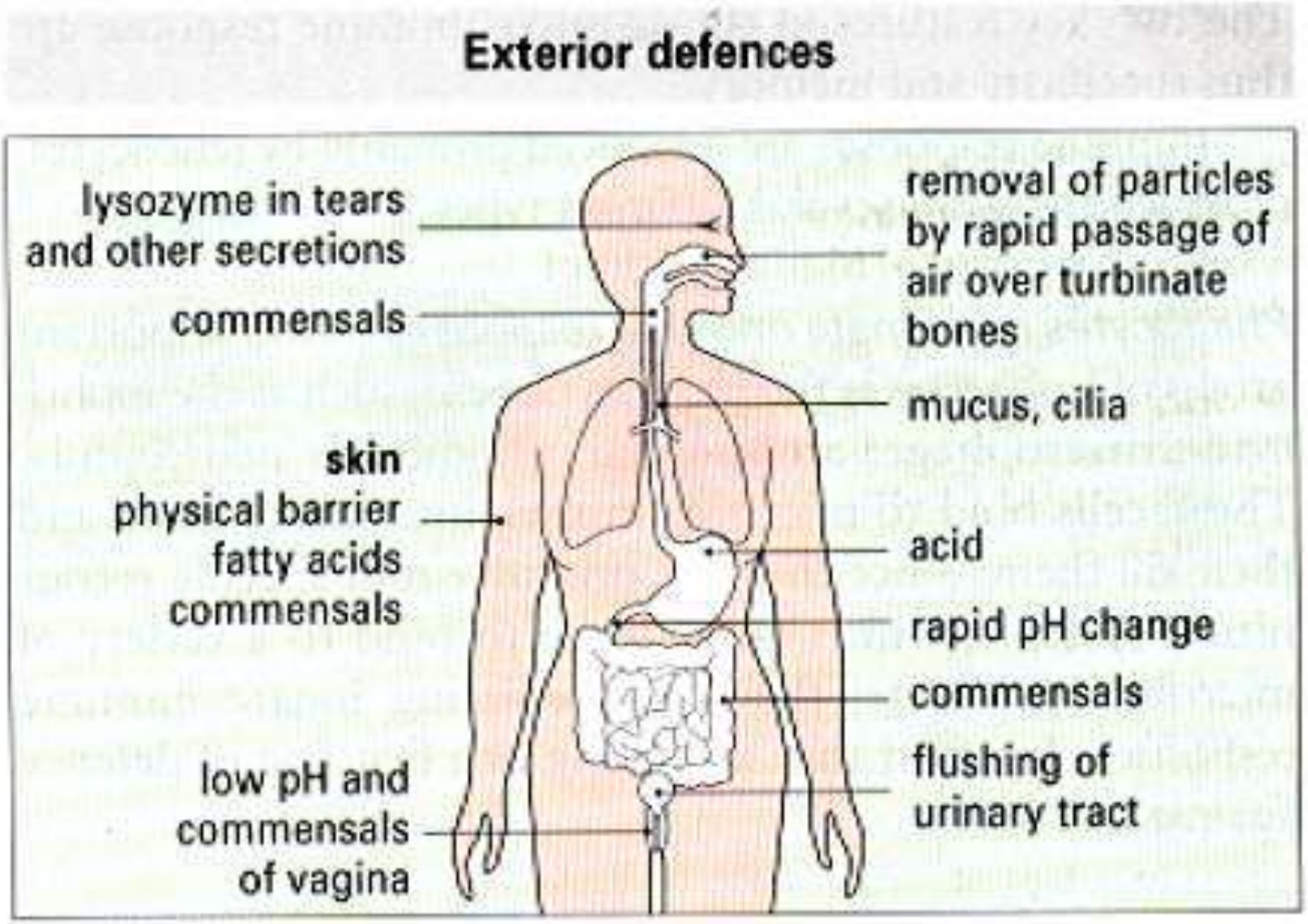
- Common mucosal immunity.
- Cells and structures important to mucosal immunity.
- How mucosal immune responses occur.
- Unique features of IgA immunity.
- Mucosal immunoregulation and oral tolerance.

Mucosal Immunology
- Lecture Outline -

I. Introduction.
II. Mucosa-associated lymphoid tissue (MALT)
III. Induction of mucosal immune responses.
IV. Lymphocyte trafficking and common mucosal immunity.
V. Unique features of IgA immunity
VI. Mucosal T cells.
VII. Oral Tolerance.
VIII. Conclusion



Mucosal surfaces such as the gut are heavily challenged by pathogens. The challenge to host defense: protect against and clear infection; do not respond to harmless antigens (food); effect host defense without damaging the mucosal surface.

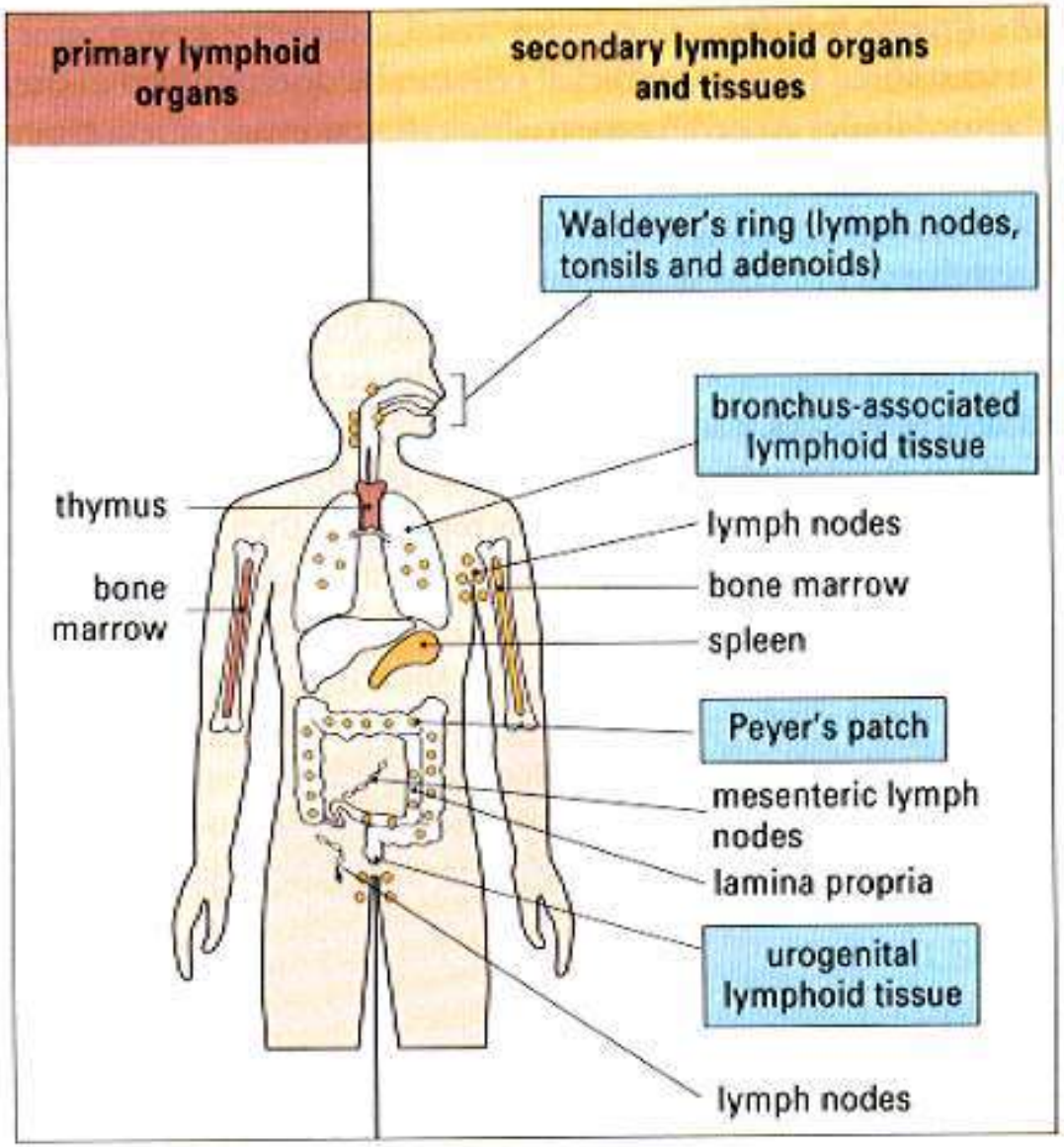


Non-antigen specific mechanisms are important but sometimes insufficient for mucosal host defense.

Mucosal Immunology - Introduction

- Mucosal immunity protects internal epithelial surfaces.
- Components of the mucosal immune system include lymphoid elements associated with internal surfaces of the body (GI, respiratory, urogenital) and exocrine secretory glands linked to these organs, such as the salivary, lachrymal, pancreas, and mammary glands.

Major lymphoid organs and tissues

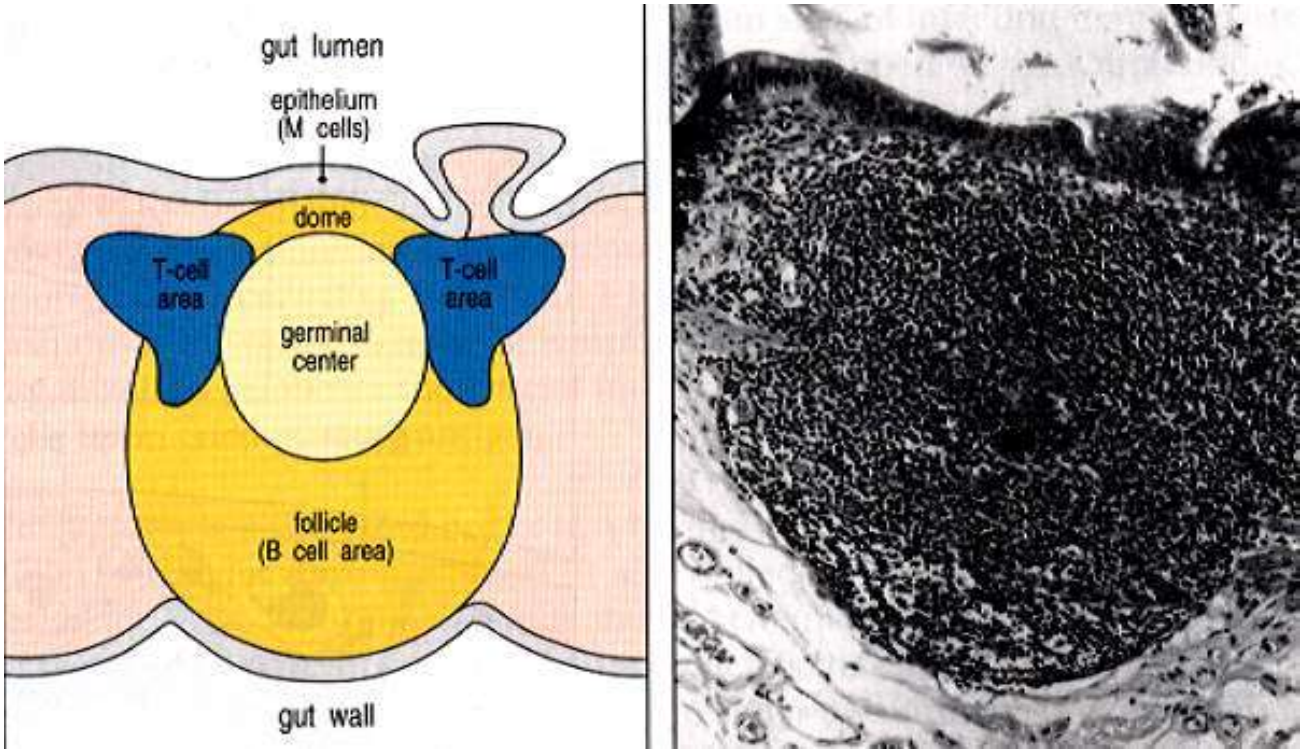


Mucosa-associated lymphoid tissue (MALT)

Examples:

- Nasal-associated lymphoid tissue (NALT).
    - tonsils, adenoids.
- Gut-associated lymphoid tissue (GALT).
    - Peyer's patches.
- Bronchus-associated lymphoid tissue (BALT)

Characteristic features of MALT



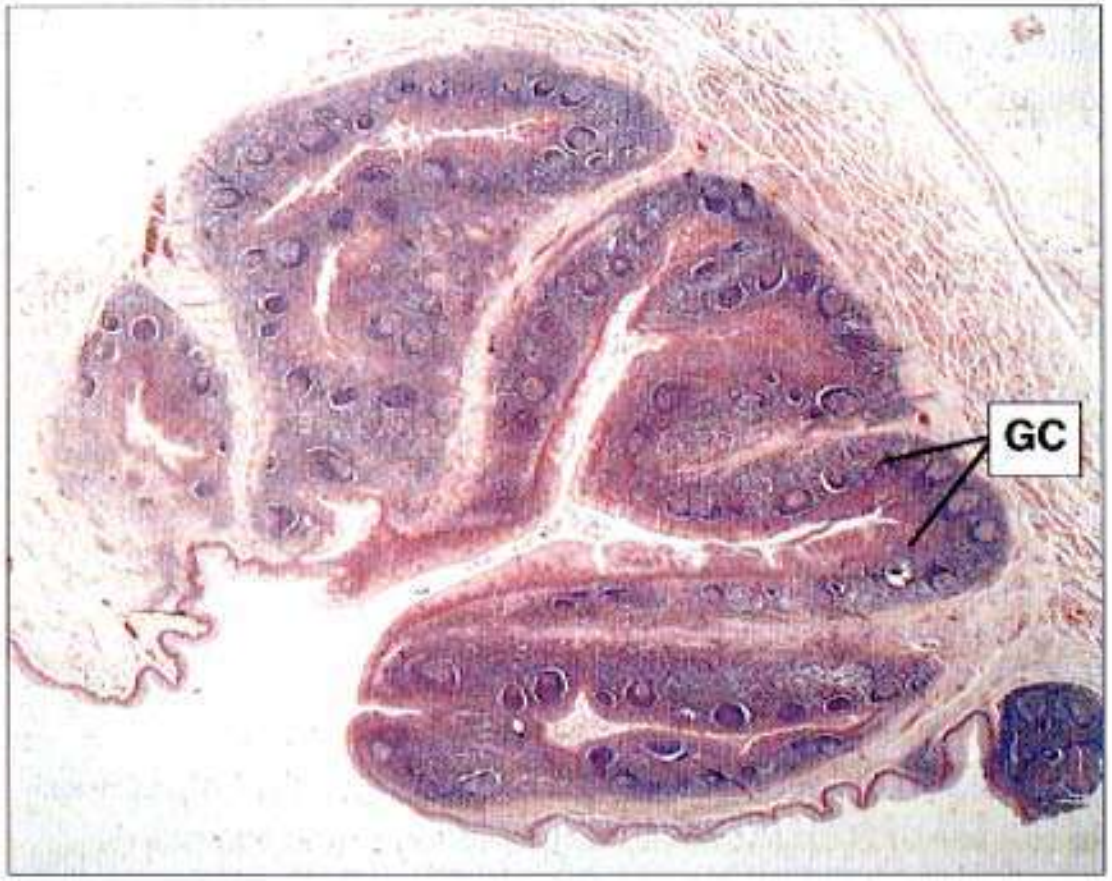


Fig. 3.20 Section of human tonsil showing MALT. This view shows the large number of germinal centres (GC) frequently found in tonsillar lymphoid tissue. H&E stain, ×4. (Courtesy of Mr C. Symes.)

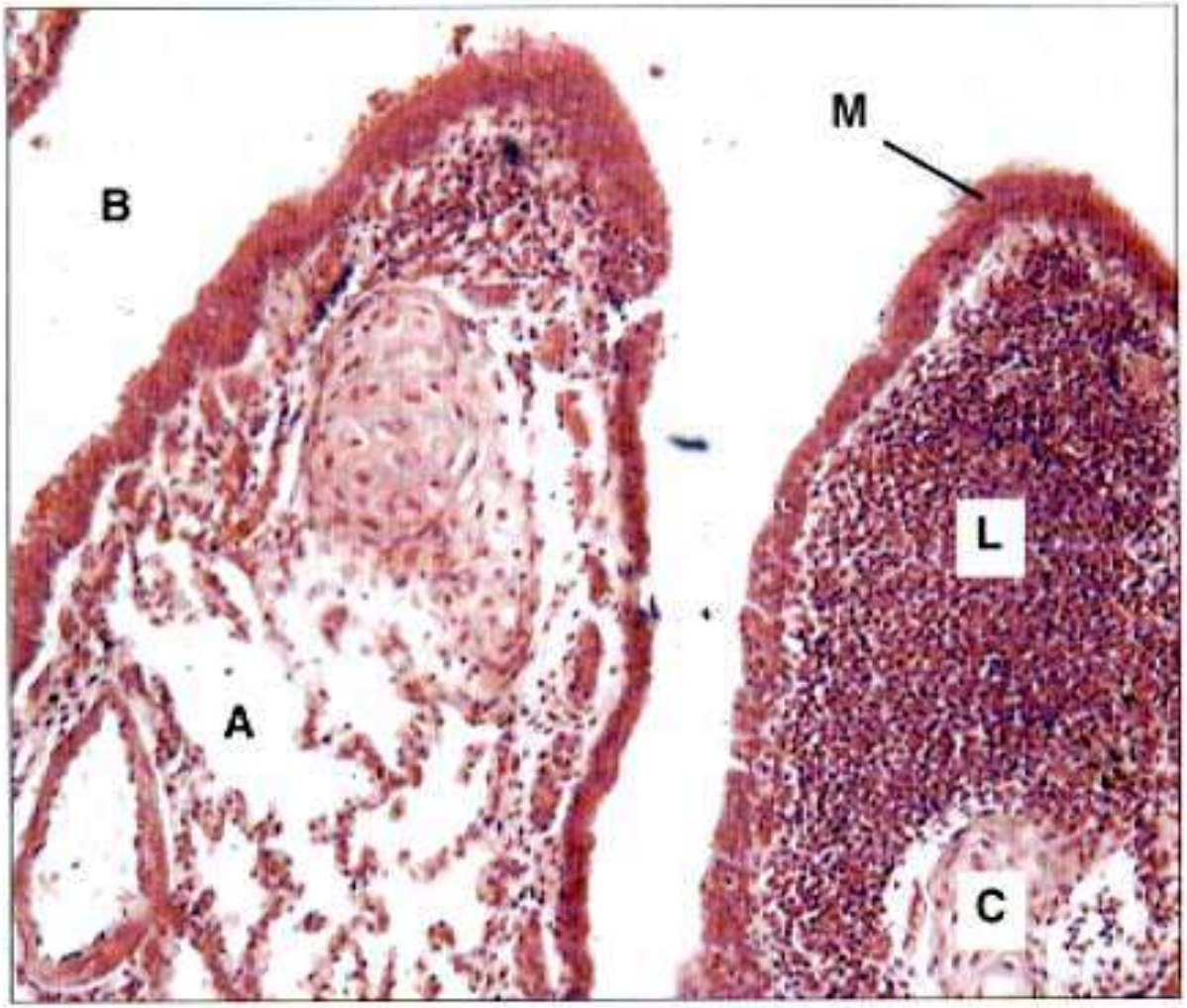
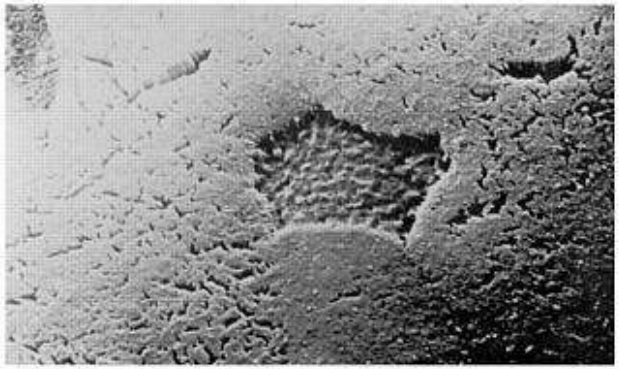


Fig. 3.21 Section of lung showing MALT. This section shows diffuse accumulation of lymphocytes in the bronchial wall. A = alveolar space; B = bronchial lumen; C = cartilage; L = lymphocytes; M = mucosal epithelium. H&E stain, ×40.

M cells facilitate antigen uptake.



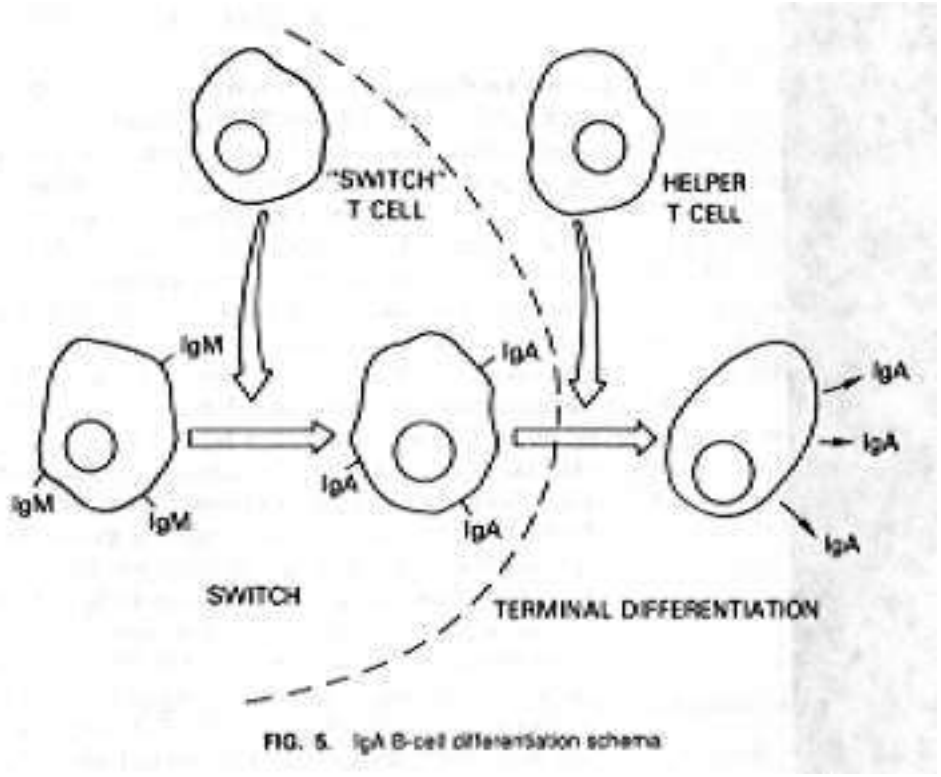


FIG. 5. IgA B-cell differentiation schema

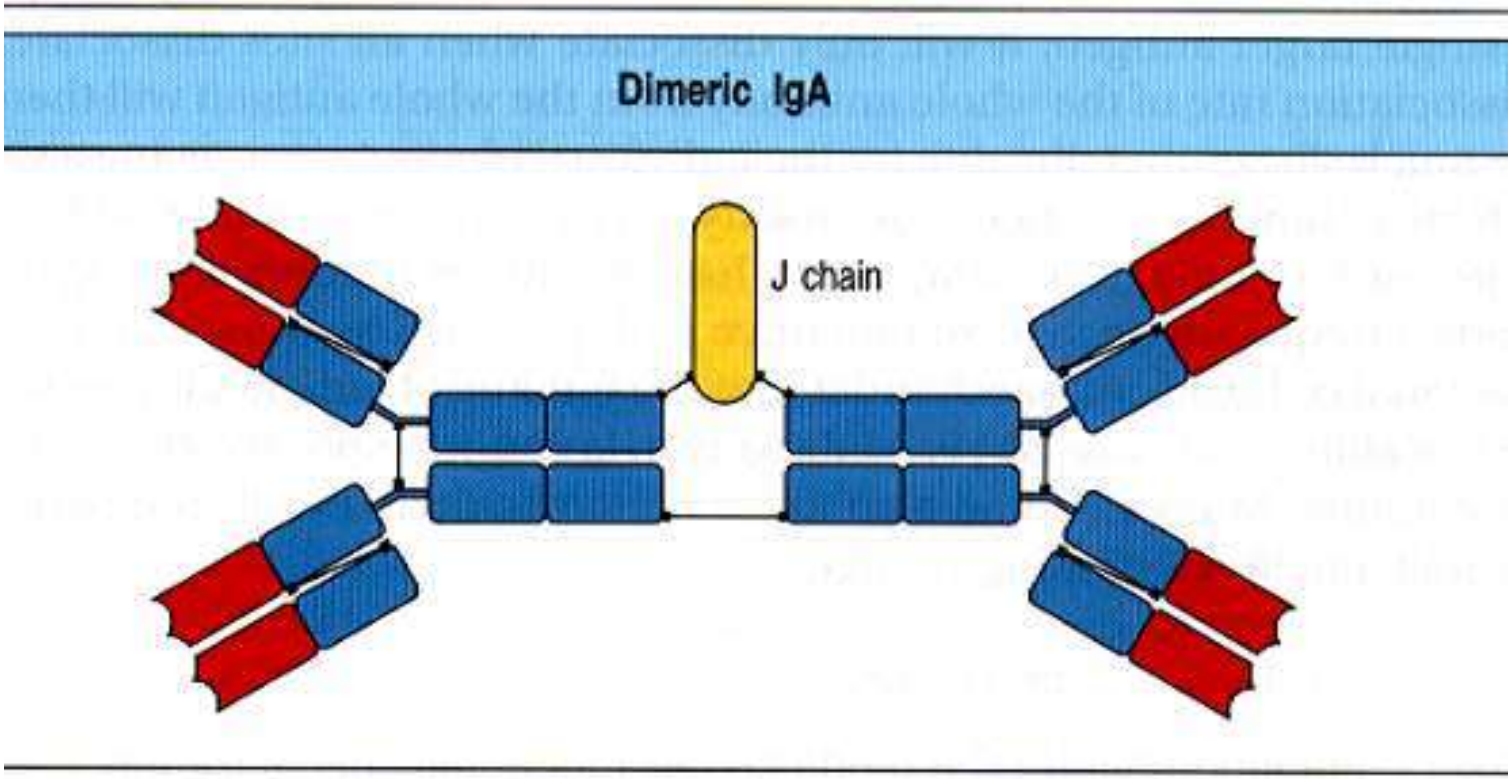
MALT is equipped with T cells preferentially supporting B cell class switch to IgA. TGF-□ and IL-5 are both important in IgA class switching.

Mechanisms for preferential migration of mucosal-derived lymphoblasts to mucosal sites.

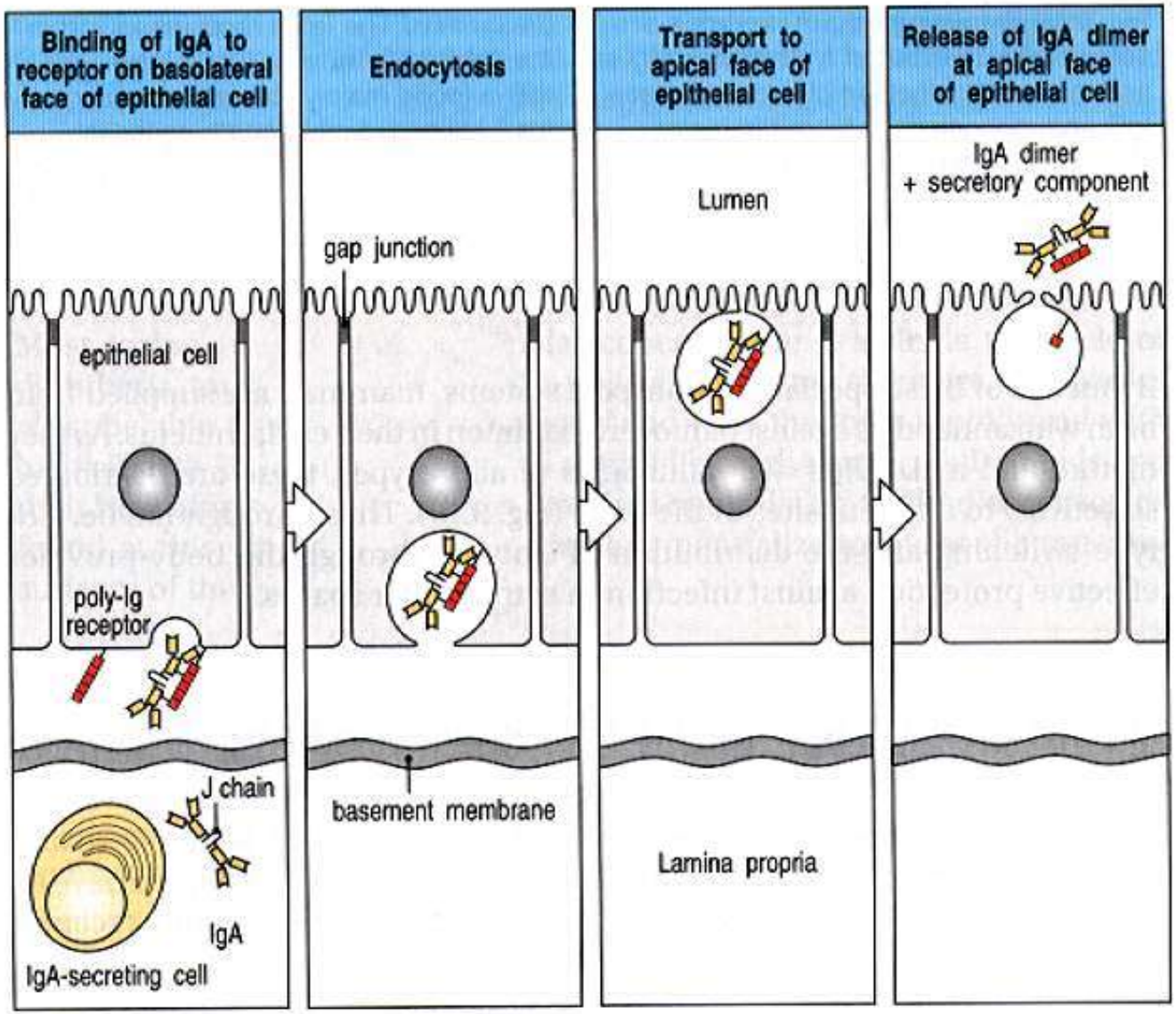
- Preferential migration is believed to result from expression of unique complementary adhesion molecules by mucosal lymphblasts and endothelial cells that target mucosal endothelium for traffic.
- Lymphoblast: $\alpha_4\beta_7$ integrin
- Mucosal endothelium: mucosal addressin cell adhesion molecule (MAdCAM-1).

Unique features of IgA immunity

- In the human, IgA is found in both monomeric and dimeric forms.
- Monomeric IgA is produced mostly in bone marrow and found mainly in blood.
- Dimeric IgA is produced mostly in lamina propria of mucosal tissues and found mainly in external secretions.
- Dimeric IgA is actively transported into external secretions via the polymeric immunoglobulin receptor (Pig-R).



Dimeric IgA consists of two IgA monomers bound by J chain. Individual B cells are committed to secretion of either monomeric or dimeric IgA.



Active transport of dIgA produces secretory IgA.

Phenotypic differences between human LPLs and IELs

cell type	TCR αβ	TCR γδ	CD4	CD8
lamina propria lymphocytes	>95%	<5%	70%	30%
intra-epithelial lymphocytes	60–90%	10–40%	<10%	70%

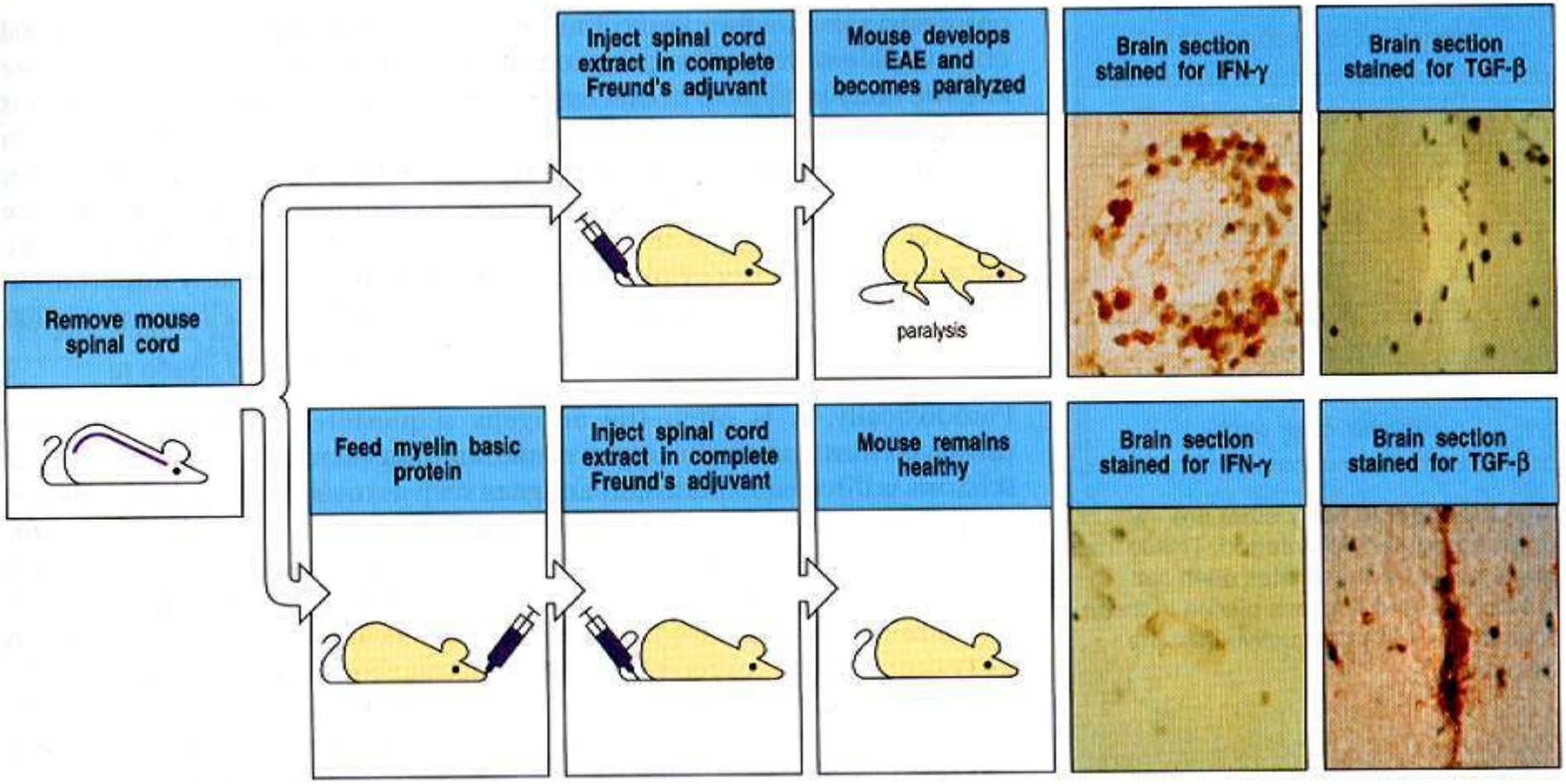
IELs are a unique population of cells with features not found elsewhere. One feature is the prominent presence of □□TCR+,CD8+ cells in the IEL compartment. These cells may play important roles in immunoregulation and epithelial renewal during infection or enteropathy.

Oral Tolerance

- Oral tolerance is the generation of systemic immune unresponsiveness by feeding of antigen. The antigen is usually soluble and without adjuvant or proinflammatory activity.

- Oral tolerance is likely a mechanism for prevention of harmful immune responses to harmless antigens such as foods.

- A number of mechanisms may underlie oral tolerance, including clonal deletion, clonal anergy, or active suppression by T cells (cytotoxic, TH2, or TGF-□ producing)



Oral tolerance as a treatment for experimental allergic encephalomyelits. Induction of oral tolerance is being studied for use clinically.

Oral Tolerance

- **State of immunological unresponsiveness to antigen induced by feeding.**

- **It is a feature of the common mucosal immune system.**

The mucosal immune system

- Consists of the gastro-intestinal tract, respiratory system, genito-urinary system, liver.
- Common lymphoid circulation
- Epithelial cells line the mucosa
- Largest area exposed to the external environment
- Heaviest antigenic load

Features of mucosal tolerance?

- Normal immune function
- Tolerance can be local or systemic
- It requires a functional immune system
- Symbiosis - in the absence of commensals, a poor immune response develops and oral tolerance cannot be induced

General properties of mucosal tolerance:

- Antigen specific.
- Often partial (eg. antibodies inhibited, but T cell responses may remain).
- Not complete (eg. may be a quantitative reduction in antibody levels).
- Wanes with time.

General properties of mucosal tolerance cont'd

- Easier to abrogate a response than reduce and established response.
- Good immunogens are better at inducing tolerance!
- Dose and route dependent.

Breakdown of oral tolerance

- Immune responses to food
  - leads to food intolerance
  - eg coeliac disease

- Immune responses to commensal bacteria
  - leads to inflammatory bowel disease (IBD)
  - eg crohn's disease, ulcerative colitis

Balance

Respond **Don’t respond**

fight and eradicate
PATHOGENS

Ignore
SELF
FOOD

Mechanism?

- Central tolerance ⇨ deletion of self-reactive T cells in the thymus
- Peripheral tolerance ⇨ an area of very active research!
  - deletion
  - immune deviation
  - anergy
  - suppression / regulation

♦ Deletion ♦

- Mechanism of ‘central’ tolerance (negative selection in the thymus)
- Apoptosis of specific T lymphocytes (eg fas-fasL)
- Shown to play a role in ‘peripheral’ tolerance in sites of immune privilege (eg stromal cells in the testes express fasL)

Peripheral deletion of antigen-reactive T cells in oral tolerance

REF: Nature 1995 Jul 13;376(6536):177-80

Chen Y, Inobe J, Marks R, Gonnella P, Kuchroo VK, Weiner HL

- oral antigen can delete antigen-reactive T cells in Peyer's patches, in mice transgenic for the ovalbumin-specific T-cell receptor genes.
- The deletion was mediated by apoptosis, and was dependent on dosage and frequency of feeding.
- At lower doses deletion was not observed; instead there was induction of antigen-specific cells that produced transforming growth factor (TGF)-beta and interleukin (IL)-4 and IL-10 cytokines.
- At higher doses, both Th1 and Th2 cells were deleted following their initial activation, whereas cells which secrete TGF-beta were resistant to deletion.
- These findings demonstrate that orally administered antigen can induce tolerance not only by active suppression and clonal anergy but by extrathymic deletion of antigen-reactive Th1 and Th2 cells

Deletion summary

- Generally observed at high doses of fed antigen:

↓Activation induced cell death (AICD) mediated by fas/fasL interactions

↓Growth factor deprival

♦Inhibitory cytokines♦

- Transforming growth factor beta (TGFβ) non-specifically inhibits the growth of lymphocytes *(Th3)*
- Specific immune responses can be inhibited by IL-4 and IL-10
- Some populations of T lymphocytes (both CD4 and CD8) can consume IL-2, the T cell growth factor. Surrounding cells therefore fail to grow

One example of many

📖 Feeding oral insulin to mice prevents virus induced insulin-dependent diabetes in a mouse model. IL-4 and IL-10 were generated which inhibited a specific immune response.

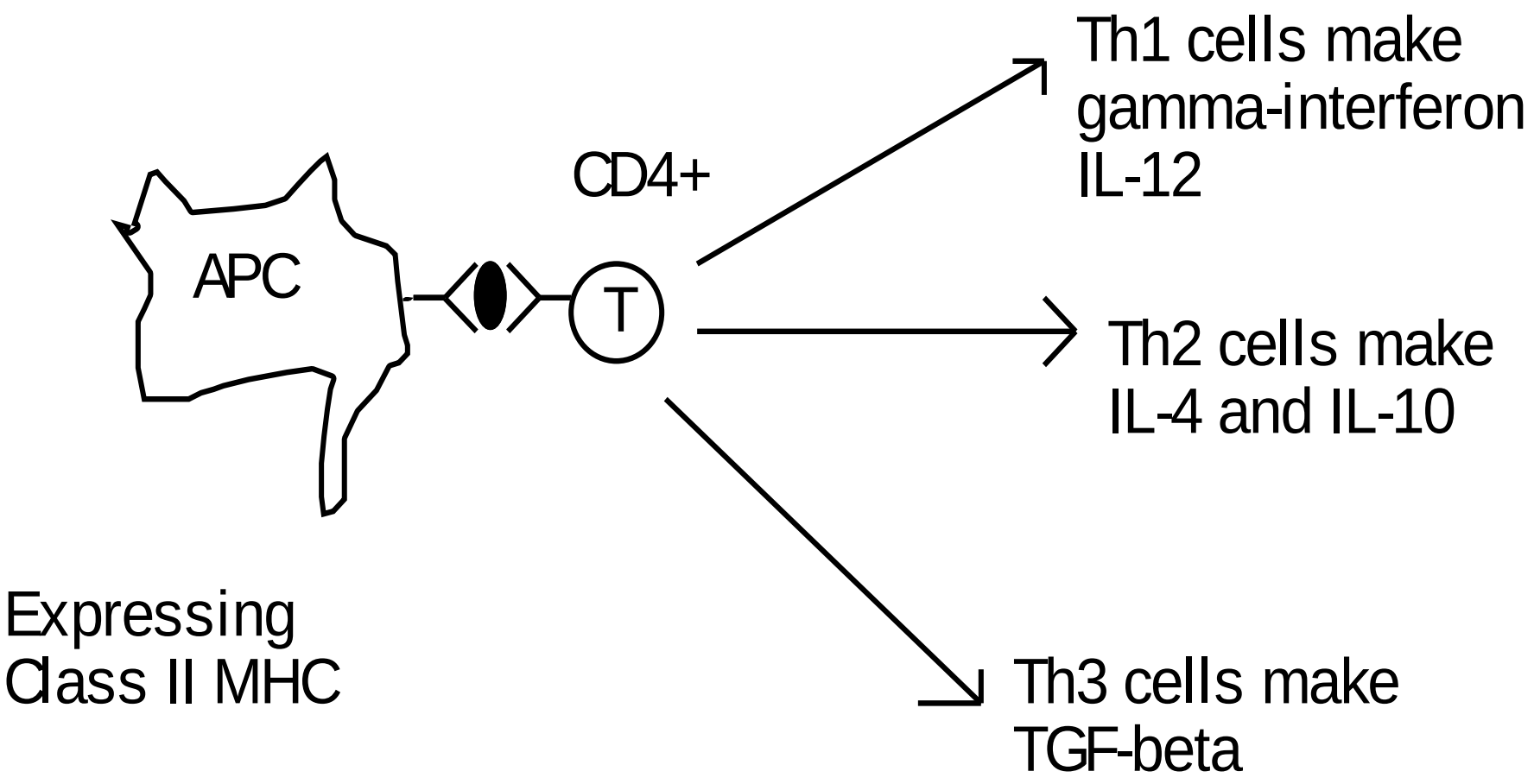
REF: Von Herrath et al., J Clin Invest 98, 1324. 1996

Immune Deviation

🔔 CD4+ T lymphocytes are activated by antigen presenting cells (APC)

- Th1 cells - important in inflammatory responses (eg delayed type hypersensitivity)
- Th2 cells - important in helping antibody responses. Suppress Th1 cells (IL-4, IL-10).

⇨Therefore Th1 immune responses may be inhibited if Th2 cells are stimulated instead.


Th1 cells make
gamma-interferon
IL-12
CD4+
APC
T
Th2 cells make
IL-4 and IL-10
Expressing
Class II MHC
Th3 cells make
TGF-beta

♦ Non-productive antigen presentation ♦

- T cells are activated by antigen presenting cells

3 signals are required to activate a T cell

↓Specific recognition - TCR ‘sees’ the right MHC-peptide complex …. signal 1

↓Costimulation - CD28 binds B7 … signal 2

↓Cytokines - local micro-environment will instruct the kind of T cell needed… signal 3

Response vs non-response

🔔 T lymphocyte activation requires 2 signals

Signal ❶ ➔ T cell proliferation

\+ Signal ❷ (IL-2 & IL-2r)

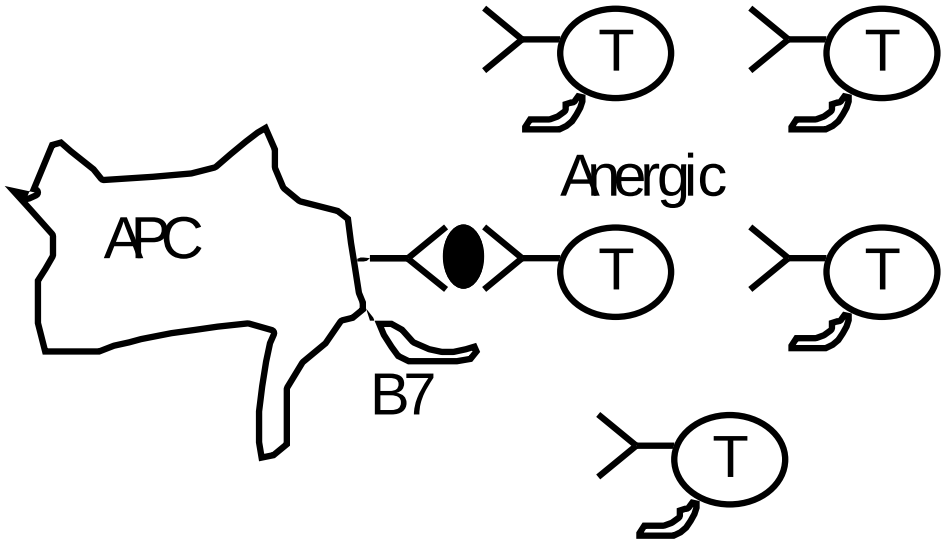
Signal ❶ alone ➔ No proliferation

Signal 2 absence / blockade

- Some epithelial cells in the gut and lung normally express class II MHC, but not costimulatory molecules and therefore cannot provide signal 2
- Reagents (eg CTLA4 Ig) have been developed to block the interaction of CD28 with B7 on APC and therefore block signal 2

☒Anergy

- Results in a specific hypresponsiveness
- Anergic cells do not respond to specific MHC+peptide plus costimulation
- Anergic cells may then block APC - and inhibit immune responses
- Anergic cells may consume IL-2
- Anergic cells are more susceptible to programmed cell death (apoptosis)



Blockade of antigen presentation by anergic T cells

Ref - Cobbold S & Waldmann H (1998) Infectious Tolerance. *Current Opinion in Immunology 10,518-524*

♦ Regulation ♦

- There has been a great deal of discussion of 'suppressor cells' *(especially in the 1980s)*
- Suppressor cells have proved difficult to clone and phenotype
- Many cells exert a suppressive effect
- A range of 'regulatory T cells (Treg)' have now been described

Regulation of self tolerance?

- Central tolerance is incomplete
- TCR bind at low affinity and can potentially recognise a number of MHC/peptide
- Auto-reactive T cells exist at high frequency in the periphery
- Auto-immunity - is it a result of defective T cell regulation?

Regulatory T cells

- A population of CD4+T cells has been implicated in the suppression of inflammatory immune responses
- Antigen specific
- Turn off specific inflammatory immune responses
- Mechanism unclear…

Evidence from different models...

- CD4 + T reg
- CD25+ (IL2r α)
- CD8
- CD4-CD8- $\alpha\beta$ T cells
- $\gamma\delta$ T cells
- NK T cells
- thymic dependent / independent

Bystander suppression

- Antigen-specific suppression is induced by feeding
- Suppression is triggered by re-encounter of antigen
- Release of inhibitory cytokines will non-specifically inhibit other cells

Models of oral tolerance

- Eat soluble antigen
- Inject antigen
- Measure immune response
  - T cell proliferation
  - antibody production
  - cytokine profile

Multiple models of oral tolerance have been proposed (Weiner, 1997)

- Animal models
- Human models
- Clinical trials

Murine model - Garside *et al.,*

- Murine model in which OVA- specific T cells could be tracked with a specific monoclonal antibody
- Adoptively transfer so that only a few T cells in the mouse were specific to OVA

Results

- PRIMING - Ova injection resulted in:
  - specific antibody production
  - proliferation of OVA specific T cells
  - DTH response
- TOLERANCE - Feeding Ova abrogated these responses

↓ demonstrated that priming and tolerance could be induced in this model.

Where did the responses take place?

PRIMING

- d3 peak of OVA specific T cells in peripheral lymph node

TOLERANCE

- d3 peak of OVA specific T cells in peripheral lymph node

T cell proliferation

PRIMING

- T cell division in peripheral lymph nodes (pln), mesenteric lymph nodes (mln) and peyers patches (pp) at 2 days

TOLERANCE

- T cell division in peripheral lymph nodes (pln), mesenteric lymph nodes (mln) and peyers patches (pp) at 2 days

T cell phenotype

PRIMING

- Ova specific T cells develop a ‘memory’ phenotype. Changes detected as early as 6h after feeding.

TOLERANCE

- Ova specific T cells develop a ‘memory’ phenotype. Changes detected as early as 6h after feeding.

Differences...

- Early systemic and local immune response in priming and tolerance was very similar
- However, later immune responses were very different (immunity vs tolerance)

☒Tolerant T cells did not move into B cell area and stimulate their expansion

Potential

- Can oral tolerance be used therapeutically?
- Do inbred animal models relate to outbred human populations?
- Can mechanisms of regulation be generated *ex vivo* or *in vivo* for clinical treatment?

Clinical trials

- A number of clinical trials for auto-immune disease are in progress:

Disease	**Antigen**
Multiple Sclerosis (MS)	Myelin Basic Protein (MPB)
Rheumatoid Arthritis (RA)	Type II collagen
Type I Diabetes	Insulin
Uveitis	S-antigen
Transplant Rejection	MHC molecules

Diabetes trials

- The NIH sponsored trial of methods to prevent type 1 diabetes (DPT-1) is still ongoing.
- The oral insulin arm of this study using a product covered by our patents is approximately 65% enrolled. It will likely be several more years before the results of this study are known.

Results to date

- The largest of these, in which positive interim results were reported for adult patients, has now been submitted for publication.
- The two smaller trials showed no benefit to the younger patient populations they enrolled.

$\Rightarrow$PROBLEMS DOSE / TIMING / ETC

ICU3 Immunology of the Gut

- Cellular organisation of the gut immune system
- Responses to antigen challenge
- GI Diseases

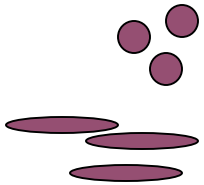
Why do we Need to Understand How the Gut Immune System Works?

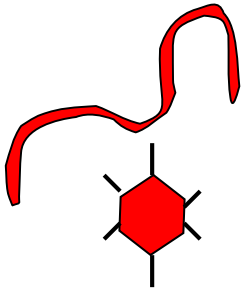
- The gut is the major site of contact in the body for foreign antigens
- Gastrointestinal diseases kill more than 2 million people every year
- Lack of effective mucosal vaccines

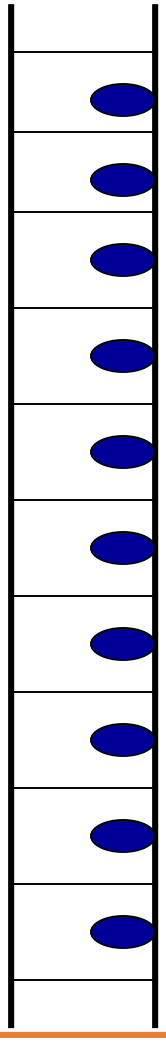
Multiple Factors protect against GI pathogens

- Saliva
- Stomach acid & enzymes
- Bile
- Water and electrolyte secretion
- Mucosal products (mucus, defensins)
- Epithelial barrier
- Peristalsis
- Bacterial flora

The Gut is Bombarded by Foreign Antigens







No Response (Tolerance)

Response (Immune Activation)

- **Eradication**
- **Containment**

- **Disease**

mucosal barrier

The Human Gut Flora

- Rapidly colonises gut after birth
- Comprises more than 10^{14} organisms
- Weighs 1-2 kg
- More than 400 species
- An individuals flora is immunologically distinct
- Symbiotic relationship with host
- Probiotics

Our Gut Flora Helps Prevent Colonisation by Pathogens

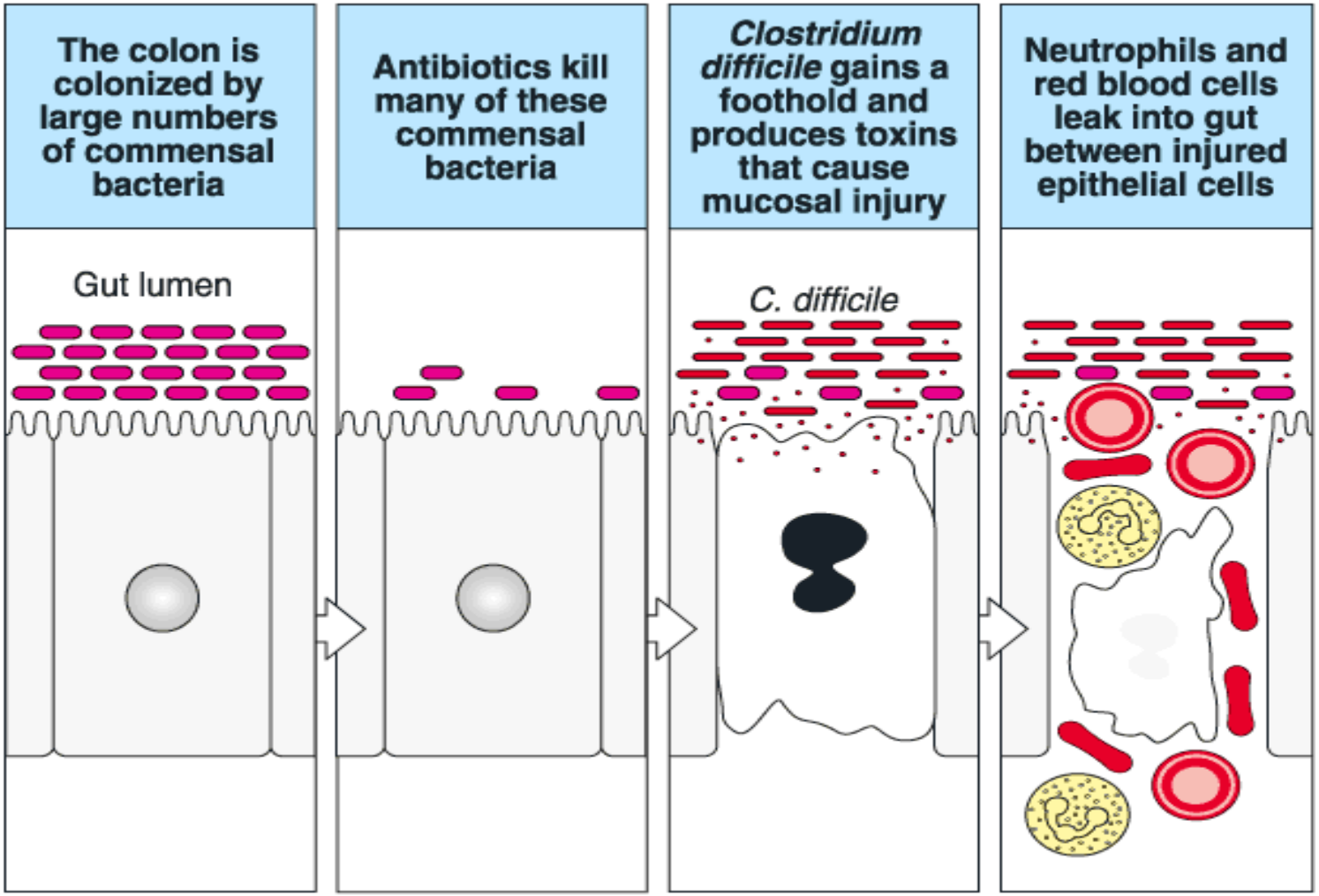


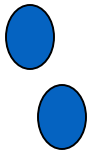
Fig 10.21 © 2001 Garland Science

Immune Responses in the Gut

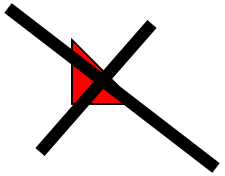
Initiation
Infection
Foreign Ag

Immune Activation
APC Activation

T Cells Switched on



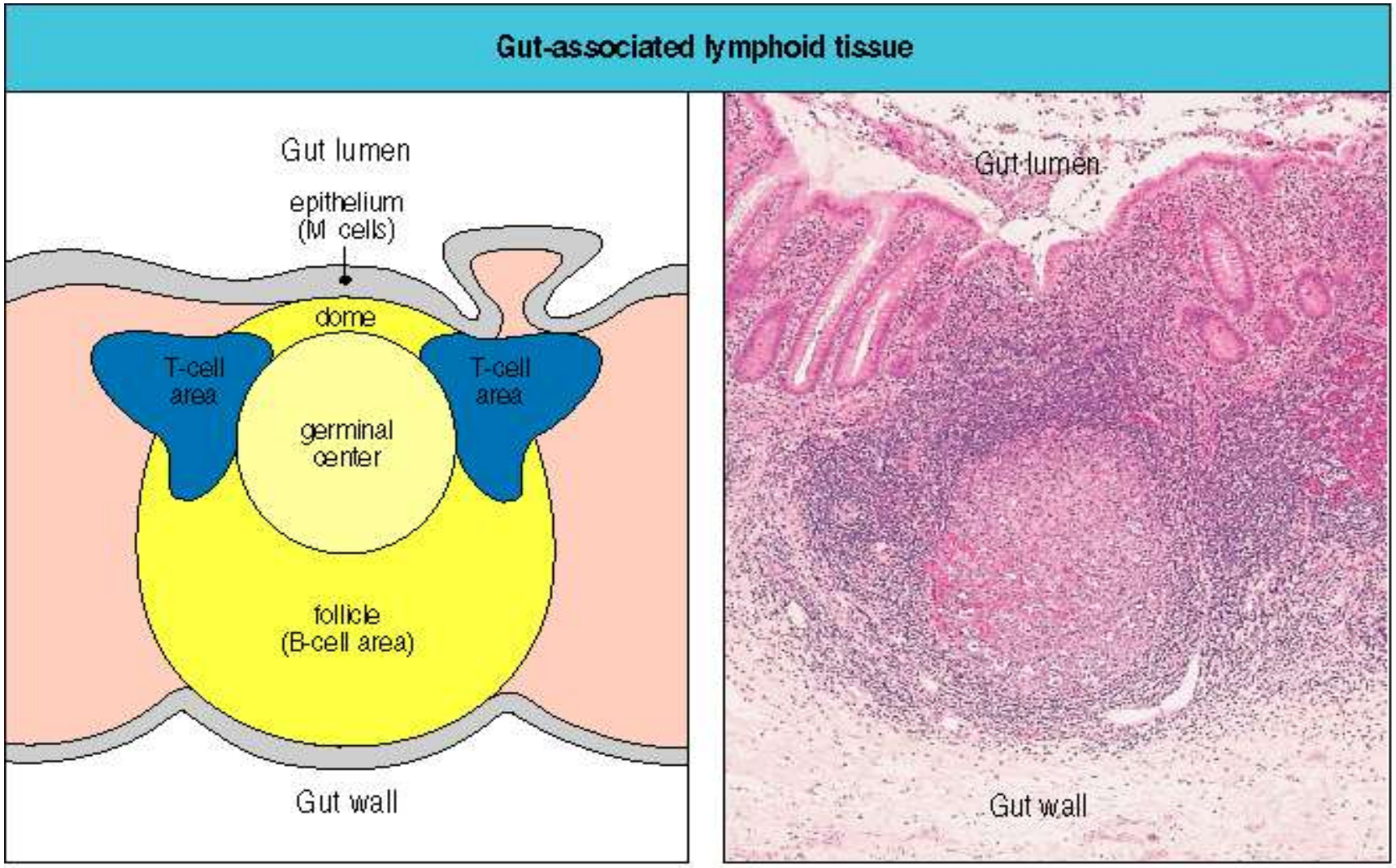
Inflammation
Pathogen erradicated



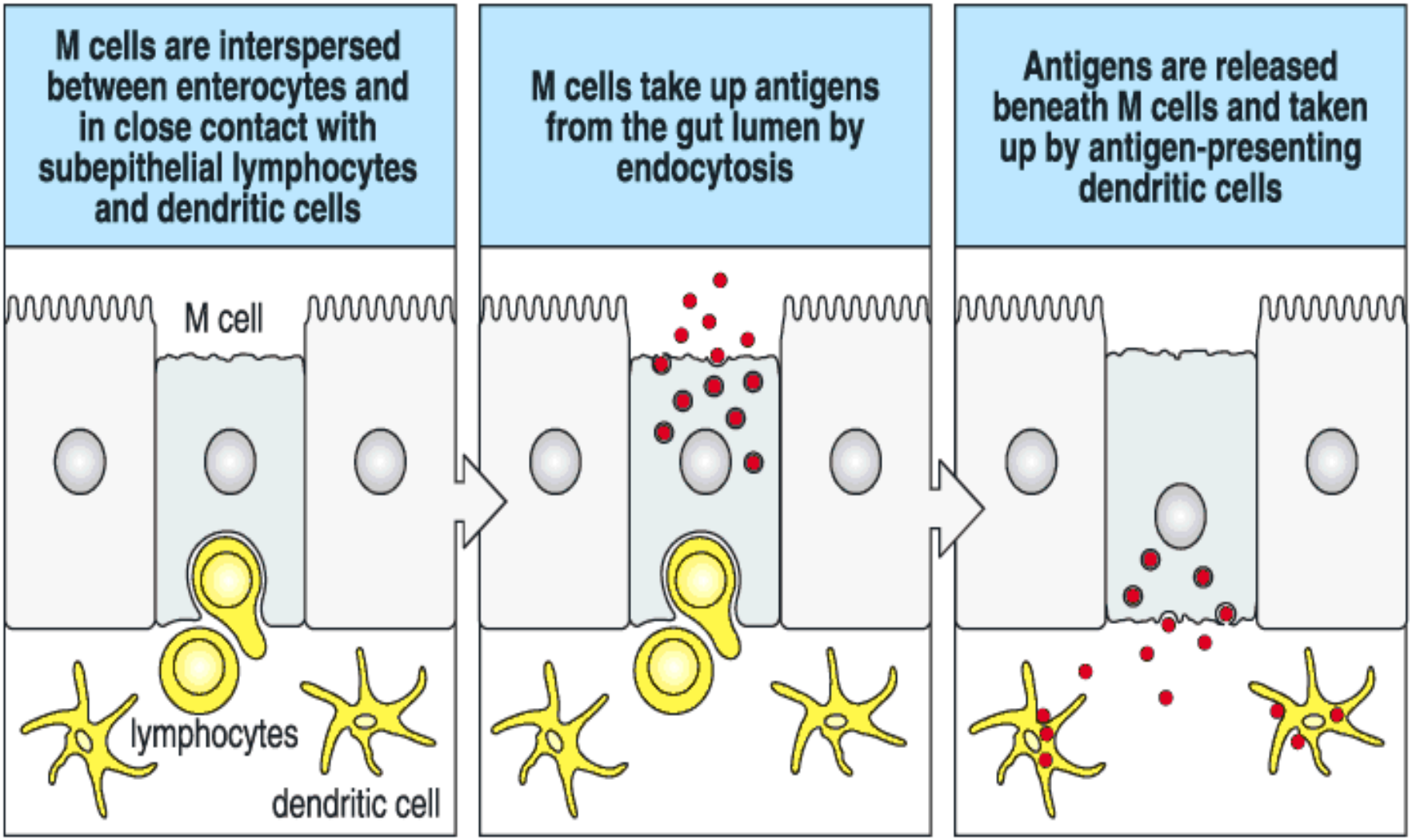
Organisation of the Mucosal Immune system

- Gut associated lymphoid tissue (GALT)
  - Tonsils
  - Adenoids
  - Peyer's patches
  - Appendix
- Intraepithelial lymphocytes
- Lamina propria lymphocytes

GALT Structure



Initiation of Gut Responses



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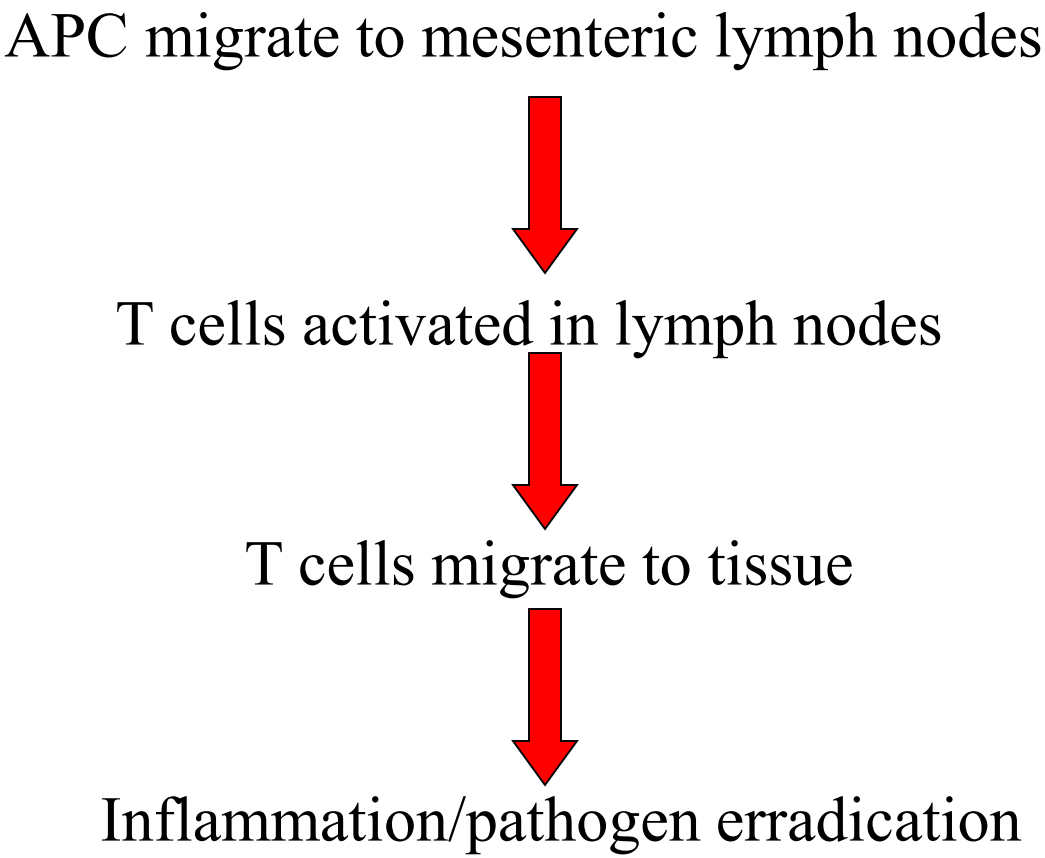
Gut Immune Responses



Lamina Propria Lymphocytes

- Found under the epithelium in the stroma
- Mostly CD4+ (T Helper Cells)
  - TH1 cells: cell mediated responses (intracellular pathogens)
  - TH2 cells:antibody mediated responses (allergens, parasites)

Intraepithelial Lymphocytes

- Found between intestinal epithelial cells
- Large granular lymphocytes
- $CD8^+$ cells
- Many are $TcR\gamma\delta^+$
- May have alternative pathway of activation
- IL2 and IFNγ
- Cytotoxic
- Immunoregulatory?

IgA

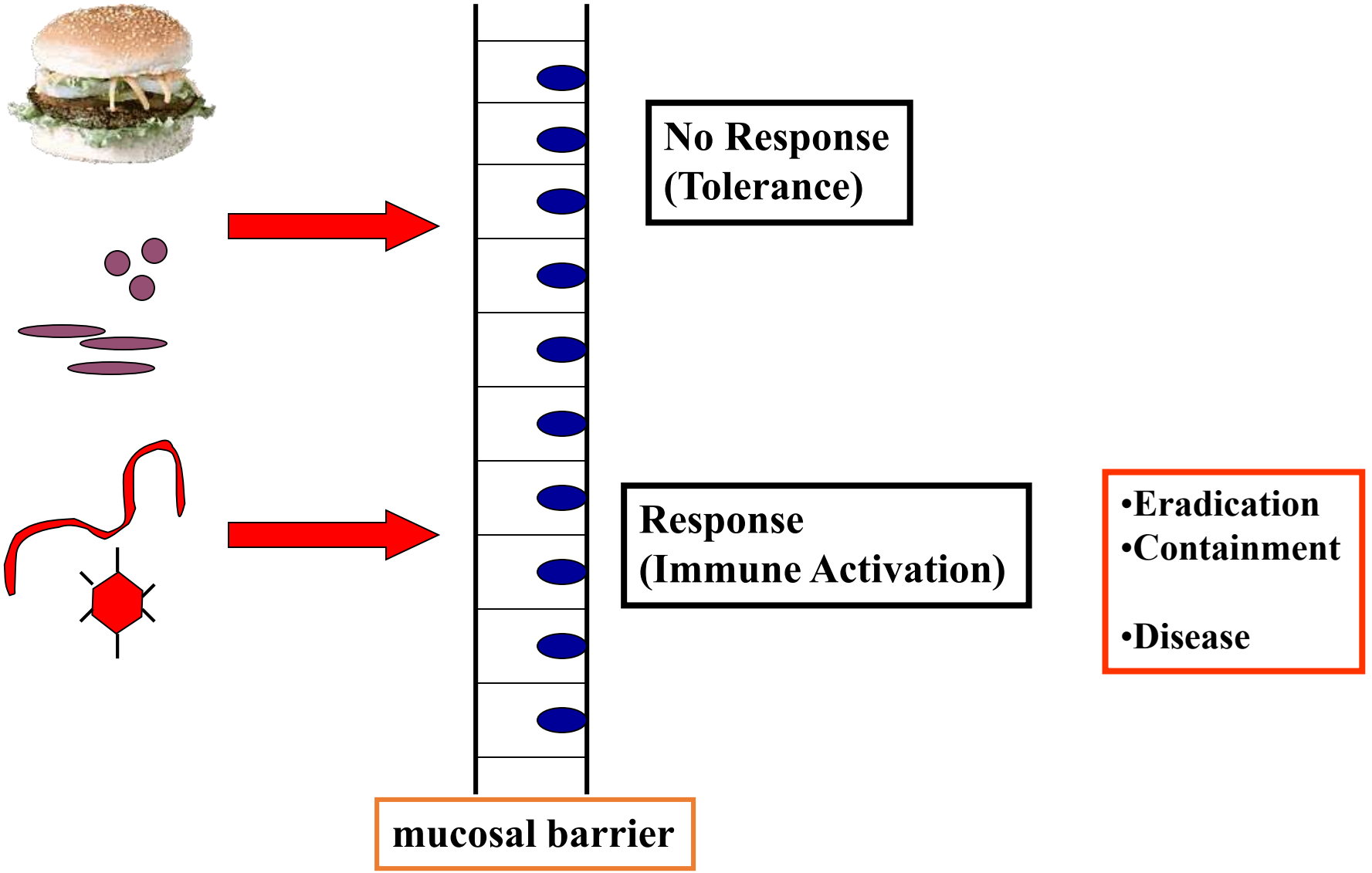
- The major Immunoglobin in the body
- The GI tract is major source
- Synthesized by plasma cells in lamina propria
- Transported via epithelium by SC1
- Protects against infectious agents
- Prevents attachment of bacteria or toxins to epithelia

Diseases of the Intestinal Immune System

Caused by:

- Failure to establish oral tolerance
- Failure to maintain oral tolerance

The Gut is Bombarded by Foreign Antigens



Oral Tolerance

- Prevents response to normal flora and food antigens
- Cause of poor or absent immune response to most orally administered antigens?

Food Allergies

- Failure to establish tolerance
- Production of IgE to an antigen (allergen) which is then encountered again
- 2-4% of children and fewer adults suffer
- Sensitive patients are usually atopic
- Treatment is simple; avoidance and replacement

Common Food Allergies

Allergen	Source
Antigen M	Codfish
Tropomysin	Shrimp
Peanut I	Peanuts
Trypsin inhibitor	Soybean

Allergic Responses

- Crosslinking of IgE on cells by food Ag
- Activation of mucosal mast cells
- Release of inflammatory mediators
  - Transepithelial fluid loss
  - Smooth muscle contraction
  - Vomiting and diarrhoea
  - Anaphylaxis

Coeliac Disease
(Gluten-Sensitive Enteropathy)

- Hypersensitivity to cereal grain, especially gliadin of wheat gluten
- 1 to 35 people affected per 10,000
- Geographical differences
- Genetic predisposition (HLA DQ2 allele in >95% of patients)
- Villous atrophy in small intestine
- Malabsorption
- Treatment is modified diet and avoidance

Inflammatory Bowel Disease

- Breakdown of oral tolerance
- Chronic relapsing and remitting inflammatory disorders of unknown etiology
  - ulcerative colitis
  - Crohn's disease
- Incidence of 1 in 600 and increasing
- >8,000 new cases of IBD /year
- >130,000 affected people in UK.
- Age range 15-35
- Symptoms include pain, bloody diarrhoea, ulcers
- No cure for CD

Interactive elements contribute to the pathogenesis of IBD

- Genetic predisposition
- Exogenous triggers
- Endogenous factors

Immunopathogenesis of IBD

- Autoimmune disorder, uncontrolled inflammatory response
- Mechanisms of epithelial cell injury unknown
- $CD4^{+}$T cell-mediated
- Commensal gut flora are an initiating stimulus

Immune Interventional Therapy for IBD