

4. Gastrointestinal mucosal immunity

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Mucosal surfaces constitute a large host-environmental interface that must be protected from pathogenic organisms. The mucosal immune system has evolved as a distinct immune organ functioning independently from its systemic counterpart. The mucosal immune system has the difficult task of mounting protective responses to invading microorganisms while maintaining a state of nonresponsiveness to commensal bacteria and food antigens. The system has unique cellular components and functional aspects that permit it to carry out this dual role. (J Allergy Clin Immunol 2008;121:S380-3.)

Key words: Mucosal immunity, oral tolerance

The mucosa-associated lymphoid tissues make up the largest immune organ in the body, acting at several host-environmental interfaces, including the gastrointestinal tract and the bronchopulmonary and genitourinary systems. There are a number of site-specific aspects to each component of mucosa-associated lymphoid tissues that are beyond the scope of this primer, and therefore our discussion will be limited to the mucosal immune system of the gastrointestinal tract or the gut-associated lymphoid tissue (GALT) system.

The human gastrointestinal tract is presented with a potentially overwhelming antigenic load each day in the form of commensal bacteria and dietary antigens. The system must be able to discriminate pathogens that require a protective immune response from normal bacterial flora or food antigens, where developing and maintaining a state of nonresponsiveness is necessary. This characteristic of the mucosal immune response, known as oral tolerance, is not only an important homeostatic process but is being used as a therapeutic approach for certain autoimmune and inflammatory diseases.

There has been a growing appreciation of the cellular and molecular aspects of GALT that make it different from the systemic immune system. There is a unique ontogeny and anatomic patterning of the GALT.¹ The cellular components of GALT are in localized microenvironments, such as Peyer's patches (PPs) and mesenteric lymph nodes (MLNs). The immune

Abbreviations used

DC:	Dendritic cell
FAE:	Follicle-associated epithelium
GALT:	Gut-associated lymphoid tissues
IEL:	Intraepithelial lymphocyte
LP:	Lamina propria
MLN:	Mesenteric lymph node
PP:	Peyer's patch
SED:	Subepithelial dome
TCR:	T-cell receptor

cells of GALT consist of T and B cells and dendritic cells (DCs) with distinctive phenotypic characteristics, and there are also unique cell types within the system, including microfold cells, Paneth cells, and intraepithelial lymphocytes (IELs). The intestinal epithelial cell, once thought to be a purely absorptive cell, has been identified as an important component of the mucosal immune system, and a greater appreciation of the innate immune system of the bowel has come to the forefront, mainly as a result of advances made in understanding the pathogenesis of inflammatory bowel disease.

A general schema for the anatomy and physiology of GALT is presented in Fig 1. The hallmarks of the system are the sampling of luminal antigens by microfold cells and probably DCs,² the antigen-driven priming and maturation of naive T and B lymphocytes starting in PPs, their egress to MLNs through lymphatics, and then their migration back to the lamina propria (LP) of the bowel.

ANATOMY OF THE GALT

Fig 1 details the exquisite structure-function relationship that is seen in the GALT. The inductive phase of the system is compartmentalized in PPs and MLNs, whereas the effector arm lies throughout the LP of the bowel.

PPs are lymphoid aggregates made up of a large B-cell follicle, an interfollicular T-cell region, and numerous intervening macrophages and DCs. This lymphoid area is found beneath a single layer of columnar cells called the follicle-associated epithelium (FAE). The formation of PPs is dependent on several factors, including the IL-7 receptor and TNF and TNF receptor family members.³ Throughout the FAE are specialized microfold cells derived from enterocytes under the influence of lymphotoxin $\alpha\beta$.⁴

Human microfold cells differ from absorptive epithelium by the absence of microvilli and membrane-associated hydrolytic enzymes and a reduced glycocalyx.⁵ They do express cathepsin E and Toll-like receptors and are capable of producing proinflammatory cytokines and chemokines.⁵ There are also regional differences in microfold cells (ie, differences in microfold cells in the colon compared with those in the small intestine). However, the functional significance of these differences is not known. Furthermore, the belief that microfold cells are localized solely

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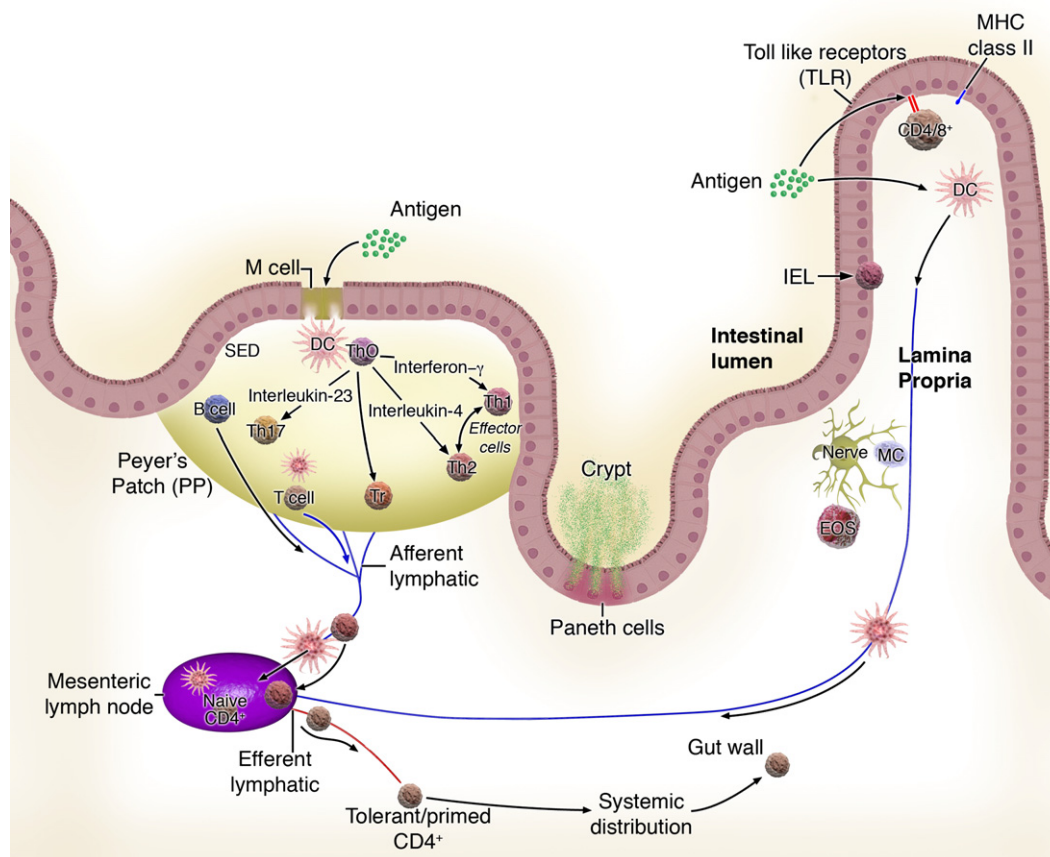


FIG 1. Anatomy of the gastrointestinal mucosa. Antigen can cross the epithelium through the microfold (M) cell or DC. The SED is occupied by a number of lymphocytes, including T_H0 cells, which, under the direction of specific cytokines, differentiate into T_H1 , T_H2 , T regulatory (Tr), or T_H17 cells. Additional lymphocyte populations include the IEL that resides juxtaposed to the intestinal epithelial cells. Other resident cells in the LP that likely participate in the immune response include mast cells (MC) and eosinophils (EOS).

to lymphoid follicles in the bowel has been challenged by the identification of the so-called intestinal villous microfold cell, found in areas devoid of organized lymphoid aggregates.⁶

A distinctive characteristic of the microfold cell is the presence of an invaginated subdomain at the basolateral membrane, forming an intraepithelial "pocket."⁷ At this site, predominantly $CD4^+$ $CD45RO$ memory T cells and both naive ($sIgD^+$) and memory ($sIgD^-$) B cells interact with the microfold cell.

The major function of microfold cells is the transepithelial vesicular transport of antigens from the lumen directly to the subepithelial lymphoid tissues. Microfold cells have been shown to transport particulate proteins, bacteria, viruses, and noninfectious particles.^{5,7} This "sampling" of luminal antigens and microorganisms is thought to be important in the development of immune responses and tolerance. However, their precise role in antigen processing and presentation remains unclear. Growing evidence suggests that PPs (and, by inference, microfold cells) are not absolutely necessary for the induction of mucosal immune responses or tolerance^{1,8} and suggests that MLNs might provide an important redundancy in the system.

Various pathogenic organisms exploit the propensity for vectorial transport by microfold cells as a mechanism to gain entry for infection, but microfold cells also transport commensal

bacteria and are thought to be a mechanism to regulate immune responses to endogenous flora.⁷

INDUCTION OF A MUCOSAL IMMUNE RESPONSE

Beneath the microfold cells and FAE lies the subepithelial dome (SED), an area that is rich in T and B lymphocytes and DCs. There are at least 4 different subsets of $CD11c^+$ DCs in the PPs, such as $CD11b^+CD8\alpha^-$, $CD11\beta^-CD8\alpha^+$, $CD11\beta^-CD8\alpha^-$, and $CD11c^{int}CD8\alpha^+B220^+$,⁹ with the majority of DCs in the SED being $CD11c^+CD11b^-CD8\alpha^-$ with an "immature" phenotype.⁷ There is considerable evidence for subspecification of these DC subpopulations within PPs.¹⁰

DCs within the SED are capable of migrating within the PPs, acquiring antigen at the basolateral surface of the microfold cell and then moving to the interfollicular zone T-cell area, presumably for antigen presentation. In addition, DCs migrate to distant sites, including MLNs, and even to the LP of the intestine to orchestrate an immune response. Experimental evidence supports an immunomodulatory role for DCs, including the induction of oral tolerance and protective immune responses.⁹ Additional support was provided by a recent report demonstrating the involvement of $CD103^+$ DCs from MLNs in the conversion of naive T cells in the intestine into $Foxp3^+$ T-regulatory cells.¹¹

The PP is also the major site of IgA⁺ B-cell development. This occurs under the influence of cellular signals from DCs and T cells, and the local production of cytokines, including TGF- β and IL-10.¹² IgA⁺ B cells (and other immune cells) migrate from PPs and ultimately take residence in the intestinal tract by migrating first to the MLNs, then to the circulation through the lymphatic system, and finally into the LP of the gut. This final step is regulated by the interaction of site-specific adhesion molecules, $\alpha_4\beta_7$ on lymphocytes and mucosal addressin cell adhesion molecule 1 on the high endothelial venules in the LP. The preferential homing of IgA⁺ B cells to the gut is the result of the selective response of these cells to thymus-expressed chemokine (CCL25) expressed on small intestinal epithelial cells.¹³ Other potential signals influencing lymphocyte migration include CXCL12 and CCL28. Although PPs represent an important source of the IgA-producing B cells found in the gut (approximately 75%), they are not the only source. IgA-producing cells in the gut can be derived from peritoneal B1 lymphocytes (about 25%), are driven by commensal bacteria in a T cell-dependent manner, and are thought to be important in modulating the mucosal immune response to bacterial flora.¹⁴

The B cells that migrate into the LP mature into IgA-producing plasma cells. T-lymphocyte cells localize in the small intestine as a result of the selective expression of $\alpha_4\beta_7$ and CCR9. CD4⁺ and CD8⁺ T cells are found throughout the LP, whereas CD8⁺ T cells are preferentially found in the epithelium. The T cells in the LP have a memory phenotype, and the cytokines produced by CD4⁺ T cells in the LP include IFN- γ , IL-4, and IL-10. It is likely that these T cells represent a heterogeneous population of true effector cells, "effector memory cells," and regulatory T cells, which act to provide help for B-cell production of IgA while maintaining tolerance to commensal bacteria and possibly other antigens.¹

IgA ANTIBODY PRODUCTION IN THE GUT

IgA antibodies are divided into 2 subclasses, IgA1 and IgA2, with IgA2 representing the predominant form at mucosal surfaces. The IgA produced in the gut is polymeric, existing almost exclusively as dimers joined by a polypeptide termed the J-chain. Dimeric IgA is secreted into the LP. It binds to and forms a covalent complex with the polymeric immunoglobulin receptor expressed on the basolateral aspect of the intestinal epithelial cell. This complex is then actively transported across the epithelial cell to the apical surface. The bound IgA is released from the polymeric immunoglobulin receptor by means of proteolytic cleavage, generating secretory component, which remains associated with the dimeric IgA that enters the intestinal lumen and is thought to protect the antibody from degradation.

Secretory IgA acts to prevent luminal antigens, microorganisms, and other foreign proteins from penetrating the intestinal surface, and can neutralize toxins and infectious organisms.¹⁵ Newer observations suggest that IgA can also regulate the composition of the microbial environment of the gut and limit local inflammation induced by pathogen-associated molecular patterns, such as LPS.¹⁶

OTHER ASPECTS OF THE LP

Additional aspects of the microenvironment within the LP of the small intestine warrant consideration. As mentioned, the LP

has a rich network of DCs, and these DCs actually breach the intestinal epithelium to reach out into the luminal environment, raising the possibility that sampling of luminal antigen occurs outside of PPs.¹⁰

Mast cells are abundant in the human gastrointestinal tract, and although important in the host response to parasitic infection, they can also participate in innate immune responses to bacteria that breach the epithelial barrier.¹⁷ Mast cells and lymphocytes in the LP interface with the enteric nervous system, providing another pathway that can influence mucosal immune responses.¹⁸

Eosinophils are also a normal resident of the LP of the stomach and small and large bowel, at least in part because of the constitutive expression of eotaxin-1 at these sites, and can serve important effector functions in parasitic infection and allergic responses but can also contribute to normal gut homeostasis.¹⁹

MUCOSAL IMMUNITY AT THE LEVEL OF THE EPITHELIUM

IELs

IELs are a notable subset of mucosal lymphocytes. These lymphocytes reside in the intestinal epithelium, above the basement membrane and between adjacent epithelial cells. Their migration to this site is influenced by the interactions of CCR9/CCL25 (thymus-expressed chemokine) and $\alpha_E\beta_7$ (CD103)/E-cadherin.

IELs are a heterogeneous population of lymphocytes that are predominantly effector/effector memory cells made up of $\gamma\delta$ T-cell receptor (TCR) CD8⁺ T cells and 2 distinct subsets of $\alpha\beta$ TCR cells, $\alpha\beta$ TCR CD4⁺ or CD8⁺, and those that lack coreceptor expression and are therefore called double-negative cells. A common feature of all IEL subsets is the unique capacity to express CD8 $\alpha\alpha$, a characteristic of activated mucosal T cells adapted to the gut.²⁰

The pattern of expression of these TCRs by intestinal IELs is both species and site specific. In human subjects about 70% of small intestinal IELs are CD8⁺ cells. $\alpha\beta$ TCR CD8⁺ IELs are the predominant IEL in the small bowel, with $\gamma\delta$ TCR CD8⁺ IELs making up about 10 of the total population.²⁰ Unlike other T-cell populations, the development of IELs is independent of antigen presentation by either class I or class II MHCs, and IELs have a limited (oligoclonal) TCR repertoire.

There are probably multiple functions played by IELs, but there are still large gaps in our knowledge about these cells. Several lines of evidence suggest that IELs play a role in maintaining the normal homeostasis of the intestinal epithelium.²¹ IELs also are involved in protective responses to certain pathogens and can even play a role in cancer surveillance.²⁰

Paneth cells

Paneth cells represent a unique epithelial cell type found in the intestinal tract, which participate in innate immunity. Located at the base of the crypts of Lieberkuhn in the small intestine, Paneth cells contain a variety of antimicrobial peptides, predominantly α -defensins but also lysozyme and secretory phospholipase A₂. Six human α -defensins have been identified and studied,²² demonstrating selective activity against gram-positive and gram-negative bacteria and possible viruses. The importance of these cells in innate immunity was demonstrated in mice deficient in a Paneth cell-processing enzyme, rendering them unable to produce mature

α -defensins. As a result, these mice were more susceptible to orally administered *Salmonella typhimurium* than wild-type mice.

The intestinal epithelial cell

The primary responsibility of the intestinal epithelial cell is nutrient absorption, with its role in mucosal immunity having been relegated to barrier function and the transport of secretory IgA. However, it is now apparent that the epithelial cell has the ability to actively participate in mucosal immune responses.²³ Intestinal epithelial cells can act as nonprofessional antigen-presenting cells, can recognize and respond to bacterial and viral motifs by virtue of the expression of nucleotide-binding oligomerization domain and Toll-like receptors, and can produce a variety of cytokines and chemokines that can influence immune responses.²³ In addition, intestinal epithelial cells can influence T-regulatory cell expansion in the intestine.²⁴

The precise role of intestinal epithelial cells in adaptive and innate mucosal immune responses remains to be determined, but it appears that the epithelial cell might help to maintain homeostasis by modulating lymphocyte activation and controlling local inflammation through a variety of different mechanisms and secreted products.

Specific oral tolerance induction is a potential form of treatment for food allergy-induced diseases.²⁵ The novel approach deserves careful monitoring and further study.

Summary

The mucosal immune system has a unique anatomy and physiology aimed at providing an immune system that is tolerant of food antigens and commensal bacteria but still armed to respond to pathogenic microbes. The 1-cell-layered epithelium provides the first level of protection, forming not only a barrier but also a sensor providing bidirectional communication with other resident mucosal lymphoid cells. The lymphocytes, DCs, mast cells, and eosinophils in the LP form a pluripotent network that can orchestrate an innate and adaptive immune response to potential pathogens. Further delineation of the mechanisms governing the normal responses of the mucosal immune system will provide insight into diseases states, such as food allergies, inflammatory bowel disease, chronic infections, and potentially even certain malignancies.

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Gastrointestinal mucosal immunity

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Learning objectives: "Gastrointestinal mucosal immunity"

1. To recognize the anatomy of the gastrointestinal immune mucosa.
 2. To be able to track an antigen from the luminal surface to the mesenteric lymph node.
 3. To understand the general differences between lymphocyte population in the lamina propria.
-

CME items

Question 1. The ability of the human gastrointestinal tract to protect against pathogens but not respond to commensal bacteria and food antigens is known as —

- A. immune compensation.
- B. immune regulation.
- C. naive/memory cell homeostasis.
- D. oral tolerance.

Question 2. The main function of mucosal M cells is to —

- A. produce IgA to neutralize pathogens.
- B. produce hydrolytic enzymes to digest complex antigens for ingestion by dendritic cells.
- C. transport luminal antigens to lymphoid tissue.
- D. interact with naive CD8⁺ cells at the intraepithelial pocket.

Question 3. The major site of IgA⁺ B cell development is —

- A. Peyer patches.
- B. mesenteric lymph nodes.
- C. subepithelial dome.
- D. intraepithelium.

Question 4. Intraepithelial lymphocytes are —

- A. predominantly CD8⁺ T cells.
- B. dependent on antigen presentation.
- C. a homogenous population of effector cells.
- D. composed of a broad T-cell receptor repertoire.