

Mucosal Immunity

Lloyd Mayer, MD

ABSTRACT. Food allergy is the manifestation of an abnormal immune response to antigen delivered by the oral route. Normal mucosal immune responses are generally associated with suppression of immunity. A normal mucosal immune response relies heavily on a number of factors: strong physical barriers, luminal digestion of potential antigens, selective antigen sampling sites, and unique T-cell subpopulations that effect suppression. In the newborn, several of these pathways are not matured, allowing for sensitization rather than suppression. With age, the mucosa associated lymphoid tissue matures, and in most individuals this allows for generation of the normal suppressed tone of the mucosa associated lymphoid tissue. As a consequence, food allergies are largely outgrown. This article deals with the normal facets of mucosal immune responses and postulates how the different processes may be defective in food-allergic patients. *Pediatrics* 2003;111:1595–1600; *gastrointestinal allergy, food allergy, food hypersensitivity, oral tolerance, mucosal immunology.*

ABBREVIATIONS. sIgA, secretory immunoglobulin A; GI, gastrointestinal; MALT, mucosa-associated lymphoid tissue; Ag, antigen; IgE, immunoglobulin E; IEC, intestinal epithelial cell; SC, secretory component; IgM, immunoglobulin M.

The mucosal immune system is recognized by differences from its systemic counterpart. In many ways, it is the opposite of what might be viewed as systemic immunity, suppression rather than active immune responses. It is thought that this difference reflects the distinct challenges of each system: the mucosa directly exposed to the external environment and taxed with antigenic loads consisting of commensal bacteria, dietary antigens, and viruses at far greater quantities on a daily basis than the systemic immune system sees in a lifetime. It is recognized that the mucosal immune response is also distinct, largely focused on suppressing immunity rather than promoting it.^{1–4} The mucosal immune system uses a number of mechanisms to protect the host against an aggressive immune response to luminal constituents.

These include a strong physical barrier; the presence of luminal enzymes that alter the nature of the antigen itself; the presence of specific regulatory T cells in both the organized and disorganized lymphoid tissue of the gut; and the production of an

antibody, secretory immunoglobulin A (sIgA), which is highly suited for the hostile environment of the gut (Fig 1). All of these in concert eventuate in the immunosuppressed tone of the gastrointestinal (GI) tract. Defects in any individual component may predispose to intestinal inflammation or food allergy.

MUCOSAL BARRIER

The mucosal barrier is a complex structure composed of both cellular and noncellular components.⁵ Probably the most significant barrier to antigen entry into the mucosa-associated lymphoid tissue (MALT) is the presence of enzymes starting in the mouth and extending down to the stomach, small bowel, and colon. Proteolytic enzymes in the stomach (pepsin, papain) and small bowel (trypsin, chymotrypsin, pancreatic proteases) perform a function that they were designed to perform, digestion. Breakdown of large polypeptides into small dipeptides and tripeptides accomplishes 2 tasks: it allows for the process of digestion and absorption of nutrients to occur, and it renders potentially immunogenic proteins nonimmunogenic (peptides <8–10 amino acids in length are poor immunogens). Couple the effects of these proteases with the emulsifying effect of bile salts and the effects of enzymes that break down carbohydrates and you have a potent system to alter antigen (Ag) exposure. Add on extremes of pH in the stomach and proximal small bowel and bacterial products in the colon and it seems amazing that immune responses to oral Ag can occur at all. Fortunately for the host, they do, and many of these responses provide protection against potential pathogens. The decision to respond or suppress a response may relate to the pathway used by the Ag to gain access to the host. Invasive pathogens (breaking the barrier) elicit aggressive responses, whereas luminal colonizers require a more tolerant response.

A key component of the barrier is the products of the mucin genes. Mucin glycoproteins line the surface epithelium from the nasal cavity/oropharynx to the rectum.^{6–10} Mucus-producing goblet cells continuously produce a thick barrier covering adjacent epithelium. Particles, bacteria, and viruses become trapped in the mucus layer and are expelled by the peristaltic process of the gut. This barrier prevents potential pathogens and antigens from gaining access to the underlying epithelium, a process called nonimmune exclusion. Mucins also serve as a reservoir for sIgA. This antibody traverses the epithelium and is secreted into the lumen.

sIgA present in the mucus layer binds bacteria/viruses and prevents epithelial attachment. An asso-

From the Department of Medicine and Immunobiology, Mount Sinai School of Medicine, New York, New York.

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Reprint requests to (L.M.) Mount Sinai Medical Center, 1425 Madison Ave, New York, NY 10029. E-mail: lloyd.mayer@mssm.edu

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Innate and Adaptive Immunity in the Gut

Innate immunity		adaptive immunity
physicochemical	cellular	
Mucus	NK cells (? some IELs)	IEL
Tight junctions	Macrophages	LPL
Epithelial membranes	Polymorphonuclear leukocytes	Regulatory cytokines
Luminal/brush border enzymes	PRRs (Toll-like receptors)	sIgA
Bile salts	Epithelial cells	Peyer's patches
pH ranges		Epithelial cells/antigen presentation
somatostatin		
trefoil factors		

Fig 1. Innate and adaptive immunity in the gut.

ciated family of factors, called trefoil factors, helps strengthen the barrier and promote restoration of the barrier if any defects occur. In the absence of mucin gene products or trefoil factors, the host is more susceptible to inflammation and less capable of repairing breaches in the barrier.^{11,12} Whether such defects exist in food allergic patients is not known but would be worth studying.

Several investigators have demonstrated that the neonate (rat and mouse) has increased intestinal permeability, allowing for passage of dietary and possibly bacterial antigens into the underlying lymphoid-rich lamina propria of the GI tract.¹³⁻¹⁷ This would bypass mechanisms involved in tolerance induction and could promote some form of active immune response. Along these lines, it has been well-documented that oral tolerance cannot be induced in the neonate. The mechanism(s) underlying this observation has not been elucidated, although the onset of tolerance seems to correlate with "gut closure." However, immaturity of the MALT is also a factor, and studies to characterize these processes have not been performed.

INTESTINAL EPITHELIAL CELLS

The next layer of the barrier is the epithelial cell. Joined together by tight junctions apically and basally, the membrane and paracellular spaces are generally impervious to large macromolecules. In fact, tight junctions prevent even the passage of di- and tripeptides. Only ions are capable of passing through. In inflamed states, as well as in the perinatal period, the tight junctions are less "tight," allowing for the passage of macromolecules into the underlying lamina propria. It is during such events that response to dietary Ags or luminal microorganisms can occur. There has been a suggestion that intestinal permeability might be abnormal in food-allergic individuals.^{18,19} Direct consistent reliable measurements have not been reported, however, so this question remains unanswered. In the setting of altered permeability, food antigens, which would normally

elicit an immunosuppressive response, might result in antigen priming. In an individual who is genetically predisposed to an allergic response (immunoglobulin E [IgE]), a food-induced allergic process may ensue.

The epithelial cell itself may play a role in this process as well. Several laboratories have shown that intestinal epithelial cells (IECs) express a number of cell surface molecules (restriction elements) involved in T cell responses. Furthermore, studies have shown that IECs can function as nonprofessional antigen-presenting cells.²⁰⁻²² A classical Ag-presenting cell possesses 3 major characteristics: 1) it expresses products of the class II major histocompatibility complex (eg, HLA-DR), 2) it takes up antigens by either receptor-mediated or fluid-phase endocytosis, and 3) antigens are processed within endosomes and loaded onto class II molecules whereupon these are expressed on the cell surface, where the complex can interact with specific T-cell receptors. Epithelial cells may act as nonprofessional Ag-presenting cells by sampling partially processed Ags from the lumen and presenting these, in the context of unique restriction elements (Fig 2), to T cells in the lamina propria. In the normal state, this interaction seems to result in the selective activation of regulatory CD8⁺ T cells. In certain disease states (eg, inflammatory bowel disease), the activation of such cells is defective, possibly explaining the persistent inflammation.²³ In food allergy, newer data suggest that additional pathways may be involved. In work by Berin et al,^{24,25} it has been shown that allergens exhibit facilitated transport across the epithelium with delivery to FcεR bearing mucosal mast cells (Fig 3).

Cross-linking IgE results in mast-cell degranulation and fluid and electrolyte secretion by the epithelial cells (IEC). This process, demonstrated in a rat model of systemic allergen exposure (ovalbumin in pertussis toxin), depends on interleukin-4-induced CD23 (low-affinity FcεR) expression on epithelial cells. IgE-antigen complexes formed in the lumen exhibit facilitated uptake by the CD23⁺ IEC, result-

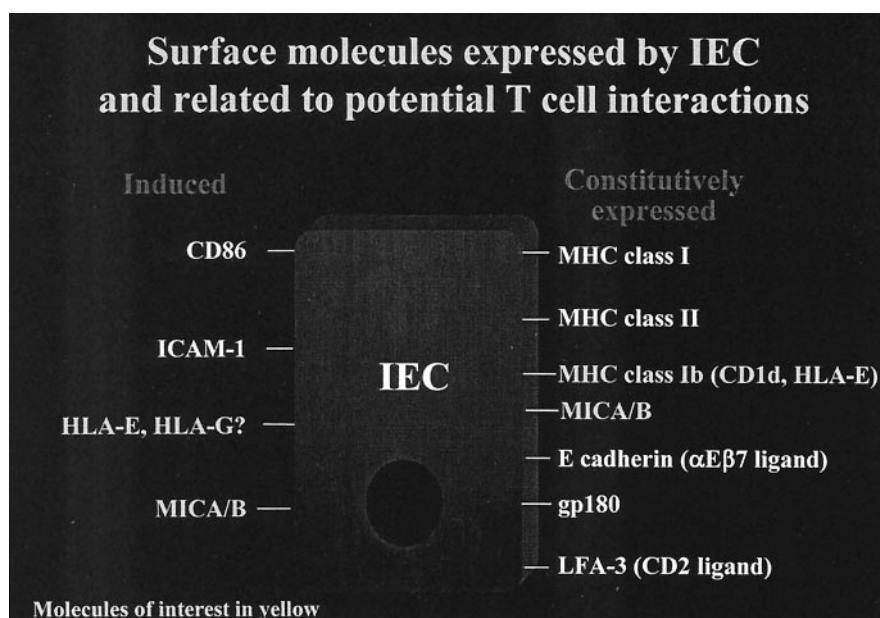


Fig 2. Surface molecules expressed by IEC and related to potential T-cell interactions.

Model proposed by Berin et al

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Intestinal epithelium in an allergic individual

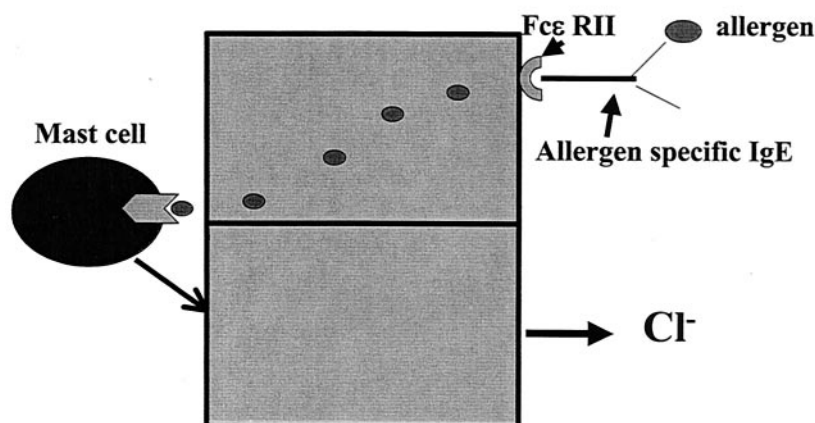


Fig 3. Model proposed by Berin et al: intestinal epithelium in an allergic individual.

ing in rapid transcytosis. How the IgE gains access to the lumen and survives the hostile environment of the gut is not known, but its occurrence in the rat model is clear. Although most food Ags do not elicit an IgE response when given orally (in this model, systemic immunization was required), a scenario in which barrier defects exist that lead to IgE priming is a testable model.

REGULATORY T CELLS

We have just mentioned a scenario in which T cells that interact with Ag presented by IECs results in the activation of regulatory (suppressor) T cells. Over the past several years, a number of regulatory T cells have been defined (Fig 4). It is interesting that all of

these were described in association with mucosal immune responses.²⁶⁻³⁰

Weiner et al^{26,27} used the term Th3 cells for cells activated in Peyer's patches after the feeding of Ag. Th3 cells secrete transforming growth factor-β, a potent immunosuppressive cytokine. It is interesting that transforming growth factor-β is also the factor that promotes isotype switching to IgA in B cells. Thus, it is well-suited for the mucosal environment. Weiner's group has suggested that Th3 cells are responsible for the phenomenon of oral tolerance (the activation of an antigen-specific nonresponse to an Ag given via the oral route).

A potentially related cell has been called TR1 for T regulatory-1 cell.³⁰ The cell was identified in human

Regulatory T cells

- CD4+ Th3 cells - TGF β
- CD4+ Tr1 cells - IL10
- CD4+/25+ T cells - cytokine vs. contact dependent inhibition (??CTLA4)
- CD8+ suppressor T cells - ?? mechanism

Fig 4. Regulatory T cells.

graft versus host disease as well as being one of the regulatory cells involved in suppressing an active Th1 response in a mouse model of inflammatory bowel disease. TR1 cells secrete interleukin-10 and can be found in the normal colon. The actual triggers and growth requirements are not clearly defined. Furthermore, it is unclear as to whether these cells represent a separate lineage or a cell that responds to specific microenvironmental stimuli. No study of such cells is available in food-allergic patients.

Last, the most recent regulatory T cell described is one that has been defined by its phenotype: CD4⁺ CD25⁺ CD45RA⁺. These cells were defined in a number of models but initially in a model of autoimmune gastritis.³¹⁻³³ These CD25⁺ cells most likely mediate their inhibition by cell-cell contact. They arise in the thymus as neonatal thymectomy predisposes to the autoimmune disorders that these cells

naturally inhibit. However, like Th3 and TR1 cells, the full description of these cells, phenotype, and function remain to be elucidated.

sIgA

This article has mentioned sIgA several times. sIgA is viewed as a benign antibody in that it fails to bind complement (which would elicit an inflammatory response) and functions mainly as an inhibitor of bacterial/viral attachment to the underlying epithelium.^{34,35} sIgA can also agglutinate antigens, trapping them in the mucus layer and facilitating their removal from the host. sIgA is protected from luminal proteases by an epithelial cell produced glycoprotein, secretory component (SC; Fig 5). This molecule envelops the Fc portion of the dimeric antibody and hides potential proteolytic cleavage sites. Immunoglobulin M (IgM) is the only other antibody capable of binding SC. Thus, in the absence of IgA (IgA deficiency with an incidence of between 1/300 and 1/600), IgM can compensate. Neither IgG nor IgE bind SC but manage to gain access to the lumen. The mechanism governing non-SC-mediated transport is unknown. It is interesting that in IgA deficiency there is a greater incidence of serum antibody to food antigens.³⁵ Whether this predisposes to food allergy is not known. The sIgA system does not fully mature until 4 years of age. Along with neonatal defects in intestinal permeability, this natural immunodeficiency may allow for priming of immune responses

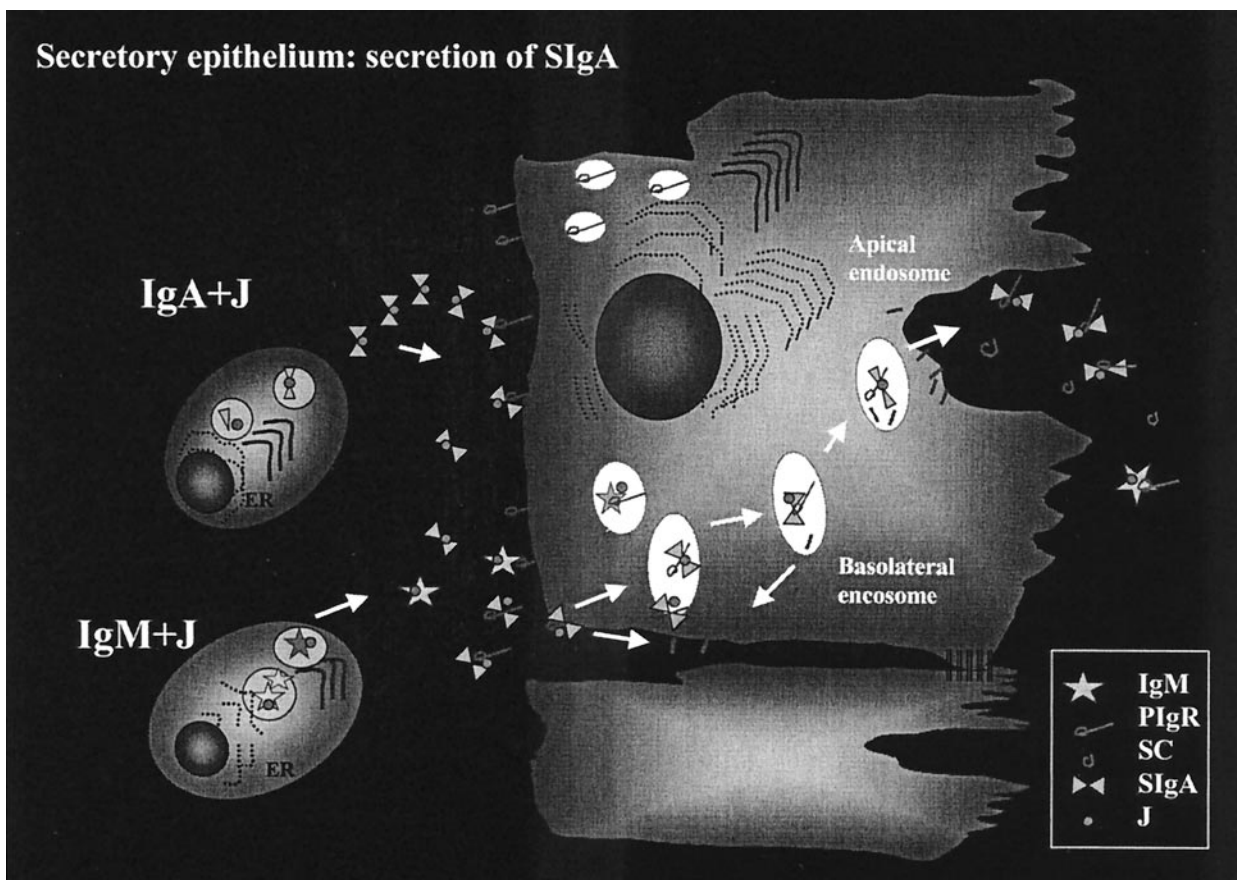


Fig 5. Secretory epithelium: secretion of sIgA.

to food antigens that do not exist in later childhood or early adulthood.

Because the sIgA system is not fully mature until 4 years of age, it has been postulated that the barrier is in itself not fully mature until this time. sIgA derived from breast milk provides passive immunity against pathogens and may provide some form of barrier function in the newborn. IgE is not a well-recognized antibody in the GI tract. It is heavily glycosylated like IgA but can easily be degraded in the stomach and upper small intestine by proteases. Clearly in food allergy, IgE must be present in the GI tract. The studies described above by Berin et al²⁴ support the presence of IgE in GI secretions. In this setting, it is used to facilitate uptake of antigens across the mucosal barrier and transfer of antigen to mucosal mast cells. sIgA and IgM antibodies are more likely to be involved in immune exclusion. There is limited evidence for a bidirectional transport pathway for IgA or IgM immune complexes in the gut.

COMMENSAL FLORA

One last component of the MALT to be considered is the role that the commensal flora plays in shaping the immunologic repertoire of the gut mucosal immune system. It has been estimated that there are between 10^{12} and 10^{14} bacteria per gram of colonic tissue.³⁶⁻³⁸ There are more bacteria in the colon than there are cells in the body. Within 24 hours of birth, we establish a flora, defined in part by maternal flora, partly by genetics (blood group substances expressed on IECs used by specific bacteria to attach to their surfaces), and last by the local environment. It is difficult to change flora even with aggressive antibiotic exposure. Similar flora grow after intestinal cleansing with broad-spectrum antibiotics. It is interesting that in the absence of bacterial flora (gnotobiotic animals), there are few if any lamina propria lymphocytes. Restoration of normal flora results in a rapid recruitment of lymphocytes to this site. Thus, controlled inflammation is the result of bacterial colonization. There is the recognition that these flora serve a useful purpose, aiding in digestion, promoting epithelial cell growth and differentiation, producing needed vitamins, etc. Importantly, the intestinal flora help to shape the immune repertoire of the host. It has been noted that germ-free animals are relatively immunodeficient.³⁹ Colonization of such animals with normal flora establishes both the mucosal and the systemic immune systems. In disease, the flora can be altered and allow for the outgrowth of less well-tolerated strains. This is the case with pseudomembranous colitis induced by the outgrowth of *Clostridium difficile*. Commensal flora normally keep this bacterial species in check. Alteration of the flora with antibiotics or inflammation can allow for these pathogenic bacteria to thrive and secrete their toxin. In such cases, the normal flora can be restored by elimination of the offending bacteria with either antibiotics or the provision of "normal flora," so-called probiotics. There is increasing evidence that probiotics aid in the therapy of inflammatory diseases as well as in *C difficile*-induced diarrhea.⁴⁰⁻⁴² There are no trials using these benign

approaches in food allergy, and there is no evidence that flora is altered in these patients. However, given the magnitude of the bacterial load in the colon (less in the small intestine by several logs), it would be extremely difficult to define the normal flora by conventional approaches (eg, culture). Recent studies have used a polymerase chain reaction-based approach using 16S ribosomal RNA unique to bacterial species.^{43,44} These advances may allow for novel insights into the role of the flora in food allergy and inflammatory disease.

CONCLUSIONS

As our understanding of normal mucosal immune responses evolves, we should gain better insights into the nature of food allergy. Clearly, this latter event reflects an abnormal mucosal immune response. Several factors may predispose to Ag priming in the neonate: high pH in the stomach, a leaky intestinal barrier, and an immature sIgA system. As these components mature, formation of a physiologically immunosuppressed state evolves. This inhibits responses to food Ags and may also reverse an allergic response that developed during a more immature state of the MALT. It is the hope that a better understanding of this mechanism will open new approaches to therapy and prevention of food allergy.

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