1 Adverse Reactions to Food Antigens: Basic Science

1 The Mucosal Immune System

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Key Concepts

- The gastrointestinal tract is the largest lymphoid organ in the body. The mucosal immune system is unique in its ability to suppress responses against commensal flora and dietary antigens.
- The mucosal immune system is characterized by unique cell populations (intraepithelial lymphocytes, lamina propria lymphocytes) and antigen-presenting cells (epithelial cells, tolerized macrophages, and dendritic cells) that contribute to the overall nonresponsive state.
- Numerous chemical (extremes of pH, proteases, bile acids) and physical (tight junctions, epithelial membranes, mucus, trefoil factors) barriers reduce antigen access to the underlying mucosal immune system (nonimmune exclusion).
- Secretory IgA serves as a protective barrier against infection by preventing attachment of bacteria and viruses to the underlying epithelium (immune exclusion).
- Oral tolerance is the active nonresponse to antigen administered via the oral route. Factors affecting the induction of oral tolerance to antigens include the age and genetics of the host; the nature, form, and dose of the antigen; and the state of the mucosal barrier.

Introduction

An allergic response is thought to be an aberrant, misguided, systemic immune response to an otherwise harmless antigen. An allergic response to a food antigen then can be thought of as an aberrant mucosal immune response. The magnitude of this reaction is multiplied several fold when one looks at this response in the context of normal mucosal immune responses, that is, responses that are suppressed or downregulated. The current view of mucosal immunity is that it is the antithesis of a typical systemic immune response. In the relatively antigen pristine environment of the systemic immune system, foreign proteins, carbohydrates, or even lipids are viewed as potential pathogens. A coordinated reaction seeks to decipher, localize, and subsequently rid the host of the foreign invader. The micro- and macroenvironment of the gastrointestinal (GI) tract is quite different, with continuous exposure to commensal bacteria in the mouth, stomach, and colon and dietary substances (proteins, carbohydrates, and lipids) that, if injected subcutaneously, would surely elicit a systemic response. The complex mucosal barrier consists of the mucosa, epithelial cells, tight junctions, and the lamina propria (LP) containing Peyer's patches (PP), lymphocytes, antigen-presenting macrophages, dendritic cells (DCs), and T cells with receptors for major histocompatibility complex (MHC) class I- and II-mediated antigen presentation. Pathways have been established in the mucosa to allow such nonharmful antigens/organisms to be tolerated [1, 2]. In fact, it is thought that the failure to tolerate commensals and food antigens is at the heart of a variety of intestinal disorders (e.g., celiac disease and gluten [3, 4], inflammatory bowel disease, and normal commensals [5–7]). Those cells exist next to a lumen characterized by extremes of pH replete with digestive enzymes. Failure to maintain this barrier may result in food allergies. For example, studies in murine models demonstrated that coadministration of antacids results in breakdown of oral tolerance implying that acidity plays a role in the prevention of allergies and promotion of tolerance [8, 9]. Thus, it makes sense that some defect in mucosal immunity would predispose a person to food allergy. This chapter will lay the groundwork for the understanding of mucosal immunity. The subsequent chapters will focus on the specific pathology seen

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when the normal immunoregulatory pathways involved in this system are altered.

Mucosal immunity is associated with suppression: the phenomena of controlled inflammation and oral tolerance

As stated in the introduction, the hallmark of mucosal immunity is suppression. Two linked phenomena symbolize this state: controlled/physiologic inflammation and oral tolerance. The mechanisms governing these phenomena are not completely understood, as the dissection of factors governing mucosal immunoregulation is still evolving. It has become quite evident that the systems involved are complex and that the rules governing systemic immunity frequently do not apply in the mucosa. Unique compartmentalization, cell types, and routes of antigen trafficking all come together to produce the immunosuppressed state.

Controlled/physiologic inflammation

The anatomy of the mucosal immune system underscores its unique aspects (Figure 1.1). There is a single layer of columnar epithelium that separates a lumen replete with dietary, bacterial, and viral antigens from the lymphocyte-rich environment of the underlying loose connective tissue stroma, called the lamina propria. Histochemical staining of this region reveals an abundance of plasma cells, T cells, B cells, macrophages, and DCs [2, 10–12]. The difference between the LP and a peripheral lymph node is that there is no clear-cut organization in the LP and cells in the LP are virtually all activated memory cells. While the cells remain activated, they do not cause destruction of the tissue or severe inflammation. The cells appear to reach a stage of activation but never make it beyond that stage.

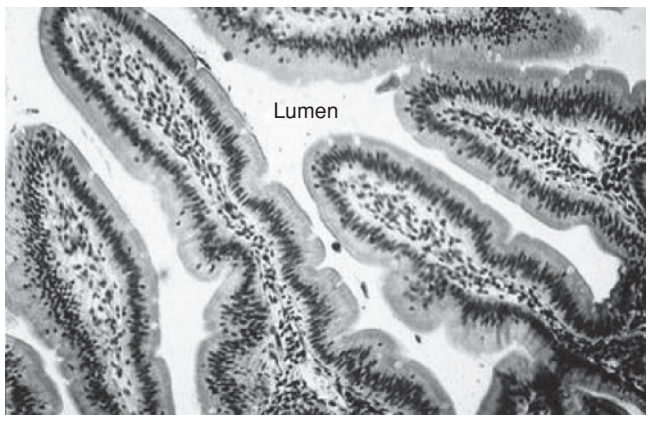

Figure 1.1 Hematoxylin and eosin stain of a section of normal small intestine (20×). Depicted is the villi lined with normal absorptive epithelium. The loose connective tissue stroma (lamina propria) is filled with lymphocytes, macrophages, and dendritic cells. This appearance has been termed controlled or physiologic inflammation.

This phenomenon has been called controlled/physiologic inflammation. The entry and activation of the cells into the LP is antigen driven. Germ-free mice have few cells in their LP. However, within hours to days following colonization with normal intestinal flora (no pathogens), there is a massive influx of cells [13–16]. Despite the persistence of an antigen drive (luminal bacteria), the cells fail to develop into aggressive, inflammation-producing lymphocytes and macrophages. Interestingly, many groups have noted that cells activated in the systemic immune system tend to migrate to the gut. It has been postulated that this occurs due to the likelihood of reexposure to a specific antigen at a mucosal rather than a systemic site. Activated T cells and B cells express the mucosal integrin $\alpha_4\beta_7$ which recognizes its ligand, MadCAM [13–20], on high endothelial venules (HEV) in the LP. They exit the venules into the stroma and remain activated in the tissue. Bacteria or their products play a role in this persistent state of activation. Conventional ovalbumin-T-cell receptor (OVA-TCR) transgenic mice have activated T cells in the LP even in the absence of antigen (OVA) while OVA-TCR transgenic mice crossed on to a RAG-2-deficient background fail to have activated T cells in the LP [21]. In the former case, the endogenous TCR can rearrange or associate with the transgenic TCR generating receptors that recognize luminal bacteria. This tells us that the drive to recognize bacteria is quite strong. In the latter case, the only TCR expressed is that which recognizes OVA and even in the presence of bacteria no activation occurs. If OVA is administered orally to such mice, activated T cells do appear in the LP. So antigen drive is clearly the important mediator. The failure to produce pathology despite the activated state of the lymphocytes is the consequence of suppressor mechanisms in play. Whether this involves regulatory cells, cytokines, or other, as yet undefined, processes is currently being pursued. It may reflect a combination of events. It is well known that LP lymphocytes (LPLs) respond poorly when activated via the TCR [22, 23]. They fail to proliferate although they still produce cytokines. This phenomenon may also contribute to controlled inflammation (i.e., cell populations cannot expand, but the cells can be activated). In the OVA-TCR transgenic mouse mentioned above, OVA feeding results in the influx of cells. However, no inflammation is seen even when the antigen is expressed on the overlying epithelium [24]. Conventional cytolytic T cells (class I restricted) are not easily identified in the mucosa and macrophages respond poorly to bacterial products such as lipopolysaccharide (LPS) because they downregulate a critical component of the LPS receptor, CD14, which associates with Toll-like receptor-4 (TLR-4) and MD2 [25]. Studies examining cellular mechanisms regulating mononuclear cell recruitment to inflamed and noninflamed intestinal mucosa demonstrate that intestinal macrophages express chemokine receptors but

do not migrate to the ligands. In contrast, autologous blood monocytes expressing the same receptors do migrate to the ligands and chemokines derived from LP extracellular matrix [26]. These findings imply that monocytes are necessary in maintaining the macrophage population in noninflamed mucosa and are the source of macrophages in inflamed mucosa. All of these observations support the existence of control mechanisms that tightly regulate mucosal immune responses.

Clearly, there are situations where the inflammatory reaction is intense, such as infectious diseases or ischemia. However, even in the setting of an invasive pathogen such as *Shigella* or *Salmonella*, the inflammatory response is limited and restoration of the mucosal barrier following eradication of the pathogen is quickly followed by a return to the controlled state. Suppressor mechanisms are thought to be a key component of this process as well.

Oral tolerance

Perhaps the best-recognized phenomenon associated with mucosal immunity and equated with suppression is oral tolerance (Figure 1.2) [27–32]. Oral tolerance can be defined as the active, antigen-specific nonresponse to antigens administered orally, characterized by the secretion of interleukin (IL)-10 and transforming growth factor beta (TGF-β) by T lymphocytes. Many factors play a role in tolerance induction and there may be multiple forms of tolerance elicited by different factors. The concept of oral tolerance arose from the recognition that we do not frequently generate immune responses to foods we eat, despite the fact that they can be quite foreign to the host. Disruption in oral tolerance results in food allergies and food intolerances such as celiac disease. Part of the explanation for this observation is trivial, relating to the properties of digestion. These processes take large macromolecules and, through aggressive proteolysis and carbohydrate and lipid degradation, render potentially immunogenic substances nonimmunogenic. In the case of proteins, digestive enzymes break down large polypeptides into nonimmunogenic di- and tri-peptides, too small to bind to MHC molecules. However, several groups have reported that upwards of 2% of dietary proteins enter the draining enteric vasculature intact [33]. Two percent is not a trivial amount, given the fact that Americans eat 40–120 g of protein per day in the form of beef, chicken, or fish.

The key question then is: How do we regulate the response to antigens that have bypassed complete digestion? The answer is oral tolerance. Its mechanisms are complex (Box 1.1) and depend on age, genetics, nature of

Box 1.1 Factors affecting the induction of oral tolerance.

- Age of host (reduced tolerance in the neonate)
- Genetics of the host
- Nature of the antigen (protein → carbohydrate → lipid)
- Form of the antigen (soluble → particulate)
- Dose of the antigen (low dose → regulatory T cells; high dose → clonal deletion or anergy)
- State of the barrier (decreased barrier → decreased tolerance)

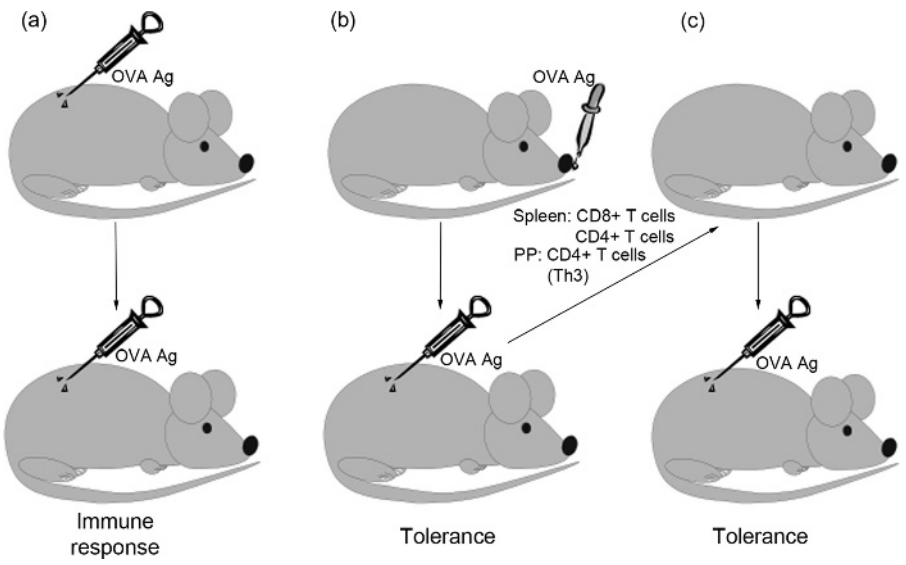

Figure 1.2 Comparison of immune responses elicited by changing the route of administration of the soluble protein antigen ovalbumin. (a) The outcome of systemic immunization. Mice generate both T-cell and antibody responses. (b) If mice are fed OVA initially, systemic immunization fails to generate a T- or B-cell response. (c) When T cells are transferred from mice initially fed OVA antigen to naïve mice, systemic immunization fails to generate a T- or B-cell response. Tolerance is an active process since it can be transferred by either PP CD4+ T cells (Strober, Weiner) or splenic CD8+ T cells (Waksman). These latter findings suggest that there are multiple mechanisms involved in tolerance induction. Adapted from Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. *J Allergy Clin Immunol* 2005; 115:3–12; quiz 13.

the antigen, form of the antigen, dose of the antigen, and the state of the mucosal barrier.

Several groups have noted that oral tolerance is difficult to achieve in neonates [34]. This may relate to the rather permeable barrier that exists in the newborn and/or the immaturity of the mucosal immune system. The limited diet in the newborn may serve to protect the infant from generating a vigorous response to food antigens. However, several epidemiological studies have suggested that delayed introduction may contribute to food allergies [35, 36], though these studies were retrospective and difficult to control. Thus, recent guidelines for introduction of allergenic solid foods were revised to reflect that insufficient evidence exists to support delayed weaning as a strategy to prevent allergies [37]. In contrast, early introduction may also not be the solution to prevent food allergies as there may exist a time for immune regulation to mature. Interestingly, in humans, despite the relatively early introduction of cow's milk (in comparison to other foods) it remains one of the most common food allergens in children [38]. A study by Strobel demonstrated enhancement of immunologic priming in neonatal mice fed antigen in the first week of life, whereas tolerance developed after waiting 10 days to introduce antigen [39].

The next factor involved in tolerance induction is the genetics of the host. Berin et al. examined allergic sensitization in TLR4+ and TLR4− mice on two genetic backgrounds, C3H and Balb/c, and found Th2 skewing in TLR4-deficient C3H mice compared with TLR4-sufficient C3H mice. This pattern of Th2 skewing was not observed in TLR4-deficient mice on a Balb/c background [40]. Lamont et al. [41] published a report detailing tolerance induction in various mouse strains using the same protocol. Balb/c mice tolerize easily while others failed to tolerize at all. Furthermore, some of the failures to tolerize were antigen specific; upon oral feeding, a mouse could be rendered tolerant to one antigen but not another. This finding suggested that the nature and form of the antigen also play a significant role in tolerance induction.

Protein antigens are the most tolerogenic while carbohydrates and lipids are much less effective in inducing tolerance [42]. The form of the antigen is critical; for example, a protein given in soluble form (e.g., OVA) is quite tolerogenic whereas, once aggregated, it loses its potential to induce tolerance. The mechanisms underlying these observations have not been completely defined but appear to reflect the nature of the antigen-presenting cell (APC) and the way in which the antigen trafficks to the underlying mucosal lymphoid tissue. Insolubility or aggregation may also render a luminal antigen incapable of being sampled [2]. In this setting, nonimmune exclusion of the antigen would lead to ignorance from lack of exposure of the mucosa-associated lymphoid tissue (MALT) to the antigen in question. One study examining the characteristics of milk allergens involved in sensitization and elicitation of allergic response demonstrated that pasteurization led to aggregation of whey proteins but not casein and that the formation of aggregates changed the path of antigen uptake, away from absorptive enterocytes to PP. Subsequently, pasteurized β-lactoglobulin leads to enhanced IgE as well as Th2 cytokine responses in the initial sensitization step, and in contrast only soluble milk proteins triggered anaphylaxis in mice, since transepithelial uptake across the small intestinal epithelium was not impaired [43].

Lastly, prior sensitization to an antigen through extraintestinal routes affects the development of a hypersensitivity response. For example, sensitization to peanut protein has been demonstrated by application of topical agents containing peanut oil to inflamed skin in children [44]. Similar results were obtained by Hsieh's group in epicutaneous sensitized mice to the egg protein ovalbumin [45].

The dose of antigen administered during a significant period early in life is also critical to the form of oral tolerance generated. In addition, frequent or continuous exposure to relatively low doses typically results in potent oral tolerance induction. In murine models, high-dose exposure to antigen early in life can produce lymphocyte anergy while low doses of antigen appears to activate regulatory/suppressor T cells [38, 46, 47] of both CD4 and CD8 lineages. Th3 cells were the initial regulatory/suppressor cells described in oral tolerance [47–49]. These cells appear to be activated in the PP and secrete TGF-β. This cytokine plays a dual role in mucosal immunity; it is a potent suppressor of T- and B-cell responses while promoting the production of IgA (it is the IgA switch factor) [34, 50–52]. An investigation of the adaptive immune response to cholera toxin B subunit and macrophage-activating lipopeptide-2 in mouse models lacking the TGF-βR in B cells (TGFβRII-B) demonstrated undetectable levels of antigen-specific IgA-secreting cells, serum IgA, and secretory IgA (SIgA) [53]. These results demonstrate the critical role of TGF-βR in antigen-driven stimulation of SIgA responses *in vivo*. The production of TGF-β by Th3 cells elicited by low-dose antigen administration helps explain an associated phenomenon of oral tolerance, bystander suppression. As mentioned earlier, oral tolerance is antigen specific, but if a second antigen is coadministered systemically with the tolerogen, suppression of T- and B-cell responses to that antigen will occur as well. The participation of other regulatory T cells in oral tolerance is less well defined. Tr1 cells produce IL-10 and appear to be involved in the suppression of graft-versus-host disease (GVHD) and colitis in mouse models, but their activation during oral antigen administration has not been as clear-cut [54–56]. Frossard et al. demonstrated increased antigen-induced IL-10-producing cells in PP from tolerant mice after β-lactoglobulin feeding but not in anaphylactic mice suggesting that reduced IL-10 production in PP may support food

allergies [57]. There is some evidence for the activation of CD4+CD25+ regulatory T cells during oral tolerance induction protocols but the nature of their role in the process is still under investigation [58–61]. Experiments in transgenic mice expressing TCRs for OVA demonstrated increased numbers of CD4+CD25+ T cells expressing cytotoxic T-lymphocyte antigen 4 (CTLA-4) and cytokines TGF-β and IL-10 following OVA feeding. Adoptive transfer of CD4+CD25+ cells from the fed mice suppressed *in vivo* delayed-type hypersensitivity responses in recipient mice [62]. Furthermore, tolerance studies done in mice depleted of CD25+ T cells along with TGF-β neutralization failed in the induction of oral tolerance by high and low doses of oral OVA suggesting that CD4+CD25+ T cells and TGF-β together are involved in the induction of oral tolerance partly through the regulation of expansion of antigen-specific CD4+ T cells [63]. Markers such as glucocorticoid-induced TNF receptor and transcription factor FoxP3, whose genetic deficiency results in an autoimmune and inflammatory syndrome, have been shown to be expressed CD4+CD25+ Tregs [64, 65]. Lastly, early studies suggested that antigen-specific CD8+ T cells were involved in tolerance induction since transfer of splenic CD8+ T cells following feeding of protein antigens could transfer the tolerant state to naïve mice [66–69]. Like the various forms of tolerance described, it is likely that the distinct regulatory T cells defined might work alone depending on the nature of the tolerogen or in concert to orchestrate the suppression associated with oral tolerance and more globally to mucosal immunity.

As mentioned, higher doses of antigen lead to a different response, either the induction of anergy or clonal deletion. Anergy can occur through T-cell receptor ligation in the absence of costimulatory signals provided by IL-2 or by interactions between receptors on T cells (CD28) and counterreceptors on APCs (CD80 and CD86) [70]. Clonal deletion occurring via FAS-mediated apoptosis [71] may be a common mechanism given the enormous antigen load in the GI tract.

The last factor affecting tolerance induction is the state of the barrier. Several states of barrier dysfunction are associated with aggressive inflammation and a lack of tolerance. In murine models the permeability of the barrier is influenced by exposures to microbial pathogens such as viruses, alcohol, and nonsteroidal anti-inflammatory drugs, which can result in changes in gene expression and phosphorylation of tight junction proteins such as occludins, claudins, and JAM-ZO1, which have been associated with changes in intestinal mast cells and allergic sensitization [72, 73]. Increased permeability throughout the intestine has been shown in animal models of anaphylaxis by the disruption of tight junctions, where antigens are able to pass through paracellular spaces [74–76]. More recently, mutations in the gene encoding filaggrin have been linked to the barrier dysfunction in patients with atopic dermatitis, which has been associated with increased prevalence of food allergy. Similarly, barrier defects associated with decreased filaggrin expression have been demonstrated in patients with eosinophilic esophagitis [77]. It is speculated that barrier disruption leads to altered pathways of antigen uptake and failure of conventional mucosal sampling and regulatory pathways. For example, treatment of mice with interferon gamma (IFN-γ) can disrupt the inter-epithelial tight junctions allowing for paracellular access by fed antigens. These mice fail to develop tolerance to OVA feeding [78, 79]. However, as IFN-γ influences many different cell types, mucosal barrier disruption may be only one of several defects induced by such treatment.

Do these phenomena relate to food allergy? There is no clear answer yet, though both allergen-specific and non-specific techniques to induce tolerance are being studied in clinical trials in food-allergic patients [80–83]. While these studies are interventional and may not provide insight into the mechanisms involved in the naturally occurring mucosal tolerance, they are valuable in determining successful treatment approaches to food-allergic patients.

The nature of antibody responses in the gut-associated lymphoid tissue

IgE is largely the antibody responsible for food allergy. In genetically predisposed individuals an environment favoring IgE production in response to an allergen is established. The generation of T-cell responses promoting a B-cell class switch to IgE has been described (i.e., Th2 lymphocytes secreting IL-4). The next question, therefore, is whether such an environment exists in the gut-associated lymphoid tissue (GALT) and what types of antibody responses predominate in this system.

Antibodies provide the first line of protection at the mucosal surface with IgA being the most abundant antibody isotype in mucosal secretions. In fact, given the surface area of the GI tract (the size of one tennis court), the cell density, and the overwhelming number of plasma cells within the GALT, IgA produced by the mucosal immune system far exceeds the quantity of any other antibody in the body. IgA is divided into two subclasses, IgA1 and IgA2, with IgA2 as the predominant form at mucosal surfaces. The production of a unique antibody isotype SIgA was the first difference noted between systemic and mucosal immunity. SIgA is a dimeric form of IgA produced in the LP and transported into the lumen by a specialized pathway through the intestinal epithelium (Figures 1.1–1.3) [84]. SIgA is unique in that it is anti-inflammatory in nature. It does not bind classical complement components but rather binds to luminal antigens, preventing their attachment to the epithelium or promoting agglutination and subsequent

removal of the antigen in the mucus layer overlying the epithelium. These latter two events reflect "immune exclusion," as opposed to the nonspecific mechanisms of exclusion alluded to earlier (the epithelium, the mucus barrier, proteolytic digestion, etc.). SIgA has one additional unique aspect—its ability to bind to an epithelial cell-derived glycoprotein called secretory component (SC), the receptor for polymeric Ig (pIgR) [85–88]. SC serves two functions: it promotes the transcytosis of SIgA from the LP through the epithelium into the lumen, and, once in the lumen, it protects the antibody against proteolytic degradation. This role is critically important, because the enzymes used for protein digestion are equally effective at degrading antibody molecules. For example, pepsin and papain in the stomach digest IgG into $F(ab')_2$ and Fab fragments. Further protection against trypsin and chymotrypsin in the lumen allows SIgA to exist in a rather hostile environment.

IgM is another antibody capable of binding SC (pIgR). Like IgA, IgM uses J chain produced by plasma cells to form polymers—in the case of IgM, a pentamer. SC binds to the Fc portions of the antibody formed by the polymerization. The ability of IgM to bind SC may be important in patients with IgA deficiency. Although not directly proven, secretory IgM (SIgM) may compensate for the absence of IgA in the lumen.

What about other Ig isotypes? The focus for years in mucosal immunity was SIgA. It was estimated that upwards of 95% of antibody produced at mucosal surfaces was IgA. Initial reports ignored the fact that IgG was present not only in the LP, but also in secretions [89, 90]. These latter observations were attributed to leakage across the barrier from plasma IgG. However, recent attention has focused on the potential role of the neonatal Fc receptor, FcR_n, which might serve as a bidirectional transporter of IgG [91, 92]. FcR_n is an MHC class I-like molecule that functions to protect IgG and albumin from catabolism, mediates transport of IgG across epithelial cells, and is involved in antigen presentation by professional APCs. FcR_n is expressed early on, possibly as a mechanism to transport IgG from mother to fetus and neonate for passive immunity [93–95]. Its expression was thought to be downregulated after weaning, but studies suggest that it may still be expressed in adult lung, kidney, and possibly gut epithelium. Recent studies have explored the possibility of utilizing these unique properties of FcR_n in developing antibody-based therapeutics for autoimmune diseases [96–98].

We are left then with IgE. Given the modest amounts present in the serum, it has been even more difficult to detect IgE in mucosal tissues or secretions. Mucosal mast cells are well described in the gut tissue. The IgE Fc receptor, FcεRI, is present and mast cell degranulation is reported (although not necessarily IgE related). FcεRI is not expressed by the intestinal epithelium, so it is unlikely

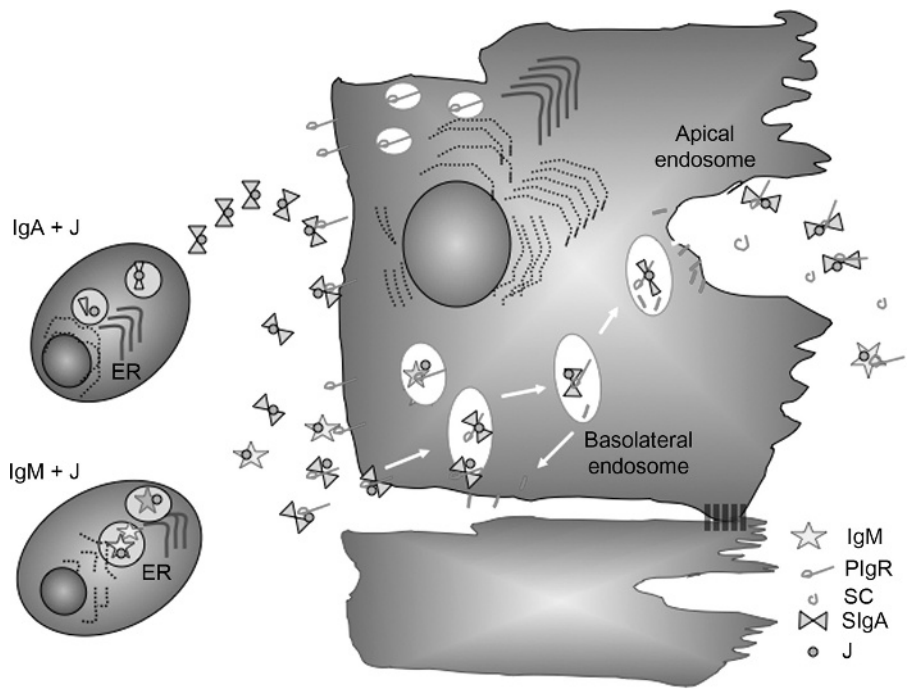

Figure 1.3 Depiction of the transport of secretory IgA (SIgA) and SIgM. Plasma cells produce monomeric IgA or IgM that polymerizes after binding to J chain. Polymeric immunoglobulins are secreted into the lamina propria and taken up by the polymeric Ig receptor (PIgR) or secretory component (SC) produced by intestinal epithelial cells and expressed on the basolateral surface. Bound SIgA or SIgM are internalized and transcytosed in vesicles across the epithelium and releases with SC into the intestinal lumen. SC protects the SIgA from degradation once in the lumen.

that this molecule would serve a transport function. CD23 (FcεRII), however, has been described on gut epithelial cells, and one model has suggested that it may play a role in facilitated antigen uptake and consequent mast cell degranulation [99–101]. In this setting, degranulation is associated with fluid and electrolyte loss into the luminal side of the epithelium, an event clearly associated with an allergic reaction in the lung and gut.

Thus, the initial concept that IgA was the be-all and end-all in the gut may be shortsighted and roles for other isotypes in health and disease require further study.

The anatomy of the gut-associated lymphoid tissue: antigen trafficking patterns

The final piece of the puzzle is probably the most critical for regulating mucosal immune responses: the cells involved in antigen uptake and presentation (Figure 1.4). As alluded to earlier, antigens in the GI tract are treated very differently than in the systemic immune system. There are additional hurdles to jump. Enzymes, detergents (bile salts), and extremes of pH can alter the nature of the antigen before it comes in contact with the GALT. If the antigen survives this onslaught, it has to deal with a thick mucous barrier, a dense epithelial membrane, and intercellular tight junctions. Mucin produced by goblet cells and trefoil factors produced by epithelial cells provide a viscous barrier to antigen passage. However, despite these obstacles antigens manage to find their way across the epithelium and immune responses are elicited.

Probably the best-defined pathway of antigen trafficking is in the GI tract through the specialized epithelium overlying the organized lymphoid tissue of the GALT, the Peyer's patches (PPs). PPs consist of germinal centers comprising switched IgA B cells. The specialized epithelial surface overlying the PPs and lymphoid follicles is called follicle-associated epithelium (FAE). Within the FAE reside specialized M (microfold) cells derived from enterocytes under the influence of Notch signaling pathways. The M cell, in contrast to the adjacent absorptive epithelium, has few microvilli, a limited mucin overlayer, a thin elongated cytoplasm, and a shape that forms a pocket around subepithelial lymphocytes, macrophages, and DCs. The initial description of the M cell documented not only its unique structure, but also its ability to take up large particulate antigens from the lumen into the subepithelial space [102–105]. M cells contain few lysosomes, so little or no processing of antigen can occur [106]. M cells protrude into the lumen, pushed up by the underlying PP. This provides a larger area for contact with luminal contents. The surface of the M cell is special in that it expresses a number of lectin-like molecules, which help promote binding to specific pathogens [107, 108]. For example, poliovirus binds to the M cell surface via a series of glycoconjugate

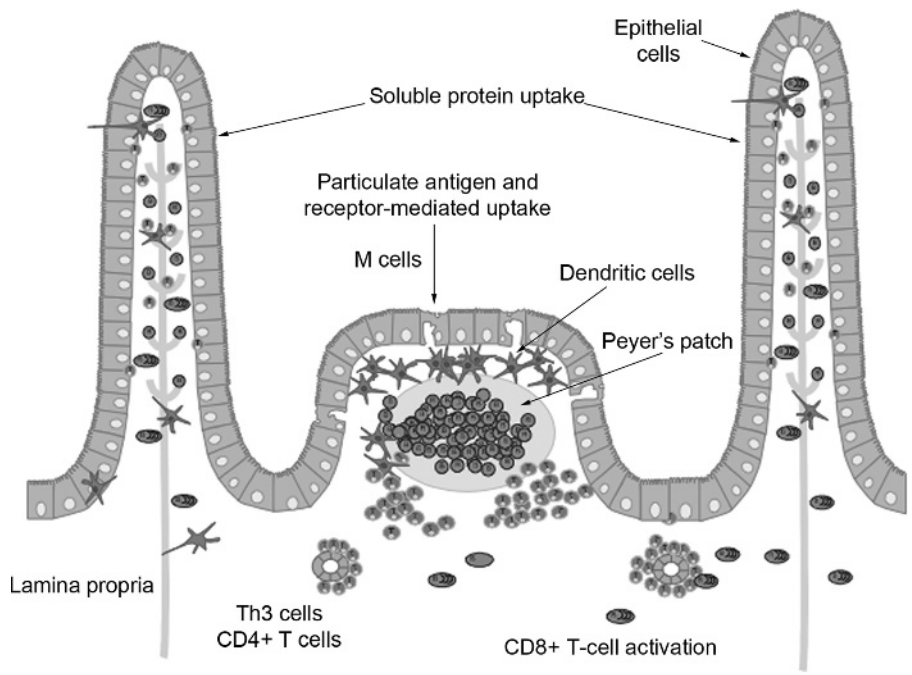

Figure 1.4 Sites of antigen uptake in the gut. Antigen taken up by M cells travel to the underlying Peyer's patch where Th3 (TGF-β-secreting) T cells are activated and isotype switching to IgA occurs (B cells). This pathway favors particulate or aggregated antigen. Antigen taken up by intestinal epithelial cells may activate CD8+ T cells that suppress local (and possibly systemic tolerance) responses. This pathway favors soluble antigen.

interactions [109]. Interestingly, antigens that bind to the M cell and get transported to the underlying PP generally elicit a positive (SIgA) response. Successful oral vaccines bind to the M cell and not to the epithelium. Thus, this part of the GALT appears to be critical for the positive aspects of mucosal immunity.

The M cell is a conduit to the PP. Antigens transcytosed across the M cell and into the subepithelial pocket are taken up by macrophages/DCs and carried into the PP. Once in the patch, TGF-β-secreting T cells promote B-cell isotype switching to IgA [52]. These cells leave the patch and migrate to the mesenteric lymph node, and eventually to other mucosal sites, where they undergo terminal maturation to dimeric IgA-producing plasma cells. In relation to food allergy and tolerance mechanisms, Frossard et al. compared antigen-specific IgA-secreting cells in PP from mice sensitized to β-lactoglobulin resulting in anaphylaxis versus tolerant mice. Tolerant mice were found to have higher numbers of β-lactoglobulin-specific IgA-secreting cells in PPs, in addition to higher fecal β-lactoglobulin-specific IgA titers compared to anaphylactic mice. The increase in antigen-specific SIgA is induced by IL-10 and TGF-β production by T cells from PPs [110].

Several groups have suggested that M cells are involved in tolerance induction as well. The same TGF-β-producing cells activated in the PP that promote IgA switching also suppress IgG and IgM production and T-cell proliferation. These are the Th3 cells described by Weiner's group initially [46]. Other observations, however, must also be considered. First, M cells are more limited in their distribution, so that antigen sampling by these cells may be modest in the context of the whole gut. Second, M cells are rather inefficient at taking up soluble proteins. As stated earlier, soluble proteins are the best tolerogens. These two factors together suggest that sites other than PPs are important for tolerance induction.

Studies have attempted to clearly define the role of M cells and the PP in tolerance induction [111–113]. Work initially performed by Kerneis et al. documented the requirement of PP for M-cell development [114]. The induction of M-cell differentiation was dependent upon direct contact between the epithelium and PP lymphocytes (B cells). In the absence of PP, there are no M cells. In B-cell-deficient animals (where there are no PP), M cells have not been identified [115]. Several groups looked at tolerance induction in manipulated animals to assess the need for M cells in this process. In most cases, there appeared to be a direct correlation between the presence of PP and tolerance; however, each manipulation (LTβ−/−, LTβR−/−, treatment with LTβ-Fc fusion protein *in utero*) [116–118] is associated with abnormalities in systemic immunity as well (e.g., no spleen, altered mesenteric LNs), so interpretation of these data is clouded. Furthermore, compared to mice with intact PPs, PP-deficient mice were found to have the same frequencies of APCs in secondary lymphoid organs after oral administration of soluble antigen [113]. More recent data demonstrate that tolerance can occur in the absence of M cells and PPs. Kraus et al. created a mouse model of surgically isolated small bowel loops (fully vascularized with intact lymphatic drainage) that either contained or were deficient in M cells and PPs. They were able to generate comparable tolerance to OVA peptides in the presence or absence of PPs. These data strongly support the concept that cells other than M cells are involved in tolerance induction [111–113].

DCs play an important role in the tolerance and immunity of the gut. They function as APCs, directly sampling antigen from the lumen through transepithelial projections; help in maintaining gut integrity through expression of tight junction proteins; and orchestrate immune responses. DCs continuously migrate within lymphoid tissues even in the absence of inflammation and present self-antigens, likely from dying apoptotic cells, to maintain self-tolerance [119]. DCs process internalized antigens slower than macrophages, allowing adequate accumulation, processing, and eventually presentation of antigens [120]. They have been found within the LP and their presence is dependent on the chemokine receptor CX3CR1 to form transepithelial dendrites, which allows for direct sampling of antigen in the lumen [121, 122]. Studies are ongoing to determine the chemokines responsible for migration of DCs to the LP. However, what has been found is that epithelial cell-expressed CCL25, the ligand for CCR9 and CCR10, may be a DC chemokine in the small bowel, and CCL28, ligand for CCR3 and CCR10, may be a DC chemokine in the colon [123–125]. DCs in the LP were found to take up the majority of orally administered protein suggesting they may be tolerogenic [126]. Mowat, Viney, and colleagues expanded DCs in the LP by treating mice with Flt-3 ligand. The increase in gut DCs directly correlated with enhanced tolerance [127]. The continuous sampling and migration by DCs is thought to be responsible for T-cell tolerance to food antigens [128]. Several studies have examined the pathways by which DCs may be tolerogenic, including their maturation status at the time of antigen presentation to T cells; downregulation of costimulatory molecules CD80 and CD86; production of suppressive cytokines IL-10, TGF-β, and IFN-α; and interaction with costimulatory molecules CD200 [122, 129, 130]. Man et al. examined DC–T-cell cross-talk in relation to IgE-mediated allergic reactions to food, specifically investigating T-cell-mediated apoptosis of myeloid DCs from spleen and PPs of mice with a cow's milk allergy. DCs from mice with milk allergy exhibited reduced apoptosis compared to DCs from control nonallergic donors. This suggests that dysregulation of DCs, both systemic and gut derived, influences the development of food allergy and is necessary for controlling immune responses [131].

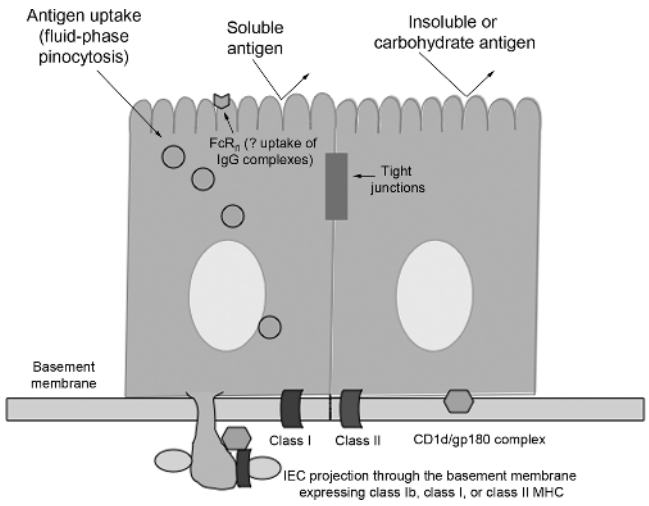

Figure 1.5 Antigen uptake by intestinal epithelial cells. Soluble proteins are taken up by fluid-phase endocytosis and pursue a transcellular pathway (endolysosomal pathway). Particulate and carbohydrate Ags are either not taken up or taken up with slower kinetics. Paracellular transport is blocked by the presence of tight junctions. In the case of antigen presentation by the intestinal epithelial cell, a complex of a nonclassical class I molecule (CD1d) and a CD8 ligand, gp180, is recognized by a subpopulation of T cells in the lamina propria (possibly intraepithelial space as well). The interaction of IEC with the LPL occurs by foot processes extruded by the IEC into the lamina propria through fenestrations in the basement membrane. Antigens can also be selectively taken up by a series of Fc receptors expressed by IEC (neonatal FcγR for IgG or CD23 for IgE). The consequences of such uptake may affect responses to food antigens (food allergy).

The other cell type potentially involved in antigen sampling is the absorptive epithelium (intestinal epithelial cells, IECs) based on its location between the lumen and a wide array of mucosal lymphocytes. The exact role of IECs in the adaptive and innate mucosal immune responses is still being investigated though it is likely the epithelium maintains homeostasis by modulating lymphocyte activation and controlling local inflammation through more than one mechanism and secreted products. This cell not only takes up soluble proteins but also expresses MHC class I, II, as well as nonclassical class I molecules to serve as restriction elements for local T-cell populations (Figure 1.5). Indeed, a number of groups have documented the capacity of IECs to serve as APCs, to both CD4+ and CD8+ T cells, recognizing and responding to bacterial and viral motifs by expression of the nucleotide-binding oligomerization domain and TLRs, and in turn producing cytokines and chemokines, which influence immune responses [132–140]. Furthermore, studies have shown that intestinal epithelial cells can influence T-regulatory cell expansion in the intestine [141]. In man, *in vitro* studies have suggested that normal IECs used as APCs selectively activate CD8+ suppressor T cells [137]. Activation of such cells could be involved in controlled inflammation and possibly oral tolerance. The studies by Kraus et al. described earlier (loop model) strongly support a role of IECs in tolerance induction. However, a role for IECs in the regulation of mucosal immunity is best demonstrated in studies of inflammatory bowel disease [142,143]. In *in vitro* coculture experiments with IECs from patients with inflammatory bowel disease, stimulated CD4+ T cells, rather than suppressive CD8+ cells, were activated by normal enterocytes [142]. Furthermore, Kraus et al. demonstrated that oral antigen administration does not result in tolerance in patients with inflammatory bowel but rather results in active immunity [144].

Once again, how does this fit into the process of food allergy? Do allergens traffic differently in predisposed individuals? Is there a Th2-dominant environment in the GALT of food-allergic patients? The real key is how the initial IgE is produced and what pathways are involved in its dominance. The answers to these questions will provide major insights into the pathogenesis and treatment of food allergy.

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