CHAPTER 1

Overview of Mucosal Immunity and Development of Oral Tolerance

Corinne Keet and Robert Wood

KEY CONCEPTS

- The GI mucosa is the major immunologic site of contact between the body and the external world.
- The manner in which immune cells encounter antigen determines the subsequent immunologic response.
- Oral tolerance is a complicated process, probably proceeding by several overlapping mechanisms.
- Many factors, including developmental stage, microbial exposures, diet and genetics, influence the balance between allergy and tolerance.

Introduction

The mucosa is the principal site for the immune system's interaction with the outside environment. Unlike the skin, which is characterized by many layers of stratified epithelium, the intestinal mucosa is lined with a single layer of columnar epithelium. Almost two tons of food travel past this thin barrier each year. More than one trillion bacteria representing about 500 distinct species live in contact with it. The vast majority of these bacteria are nonpathogenic commensals, but pathogens lurk in this diverse antigenic stew, and even the commensal bacteria have the potential to cause harm if not kept in check. The mucosal immune system performs the essential job of policing this boundary and distinguishing friend from foe.

Not only must the mucosal immune system determine the local response to an antigen, but, as the primary site of antigenic contact for the body, it also plays a central role in directing the systemic response to antigens. Oral tolerance – the modulation of the immune response to orally administered antigens – is a fundamental task of the mucosal immune system. In general, as befits the ratio of benign to pathogenic antigens it encounters, the default response of the mucosal immune system is tolerance. The tendency to tolerize to fed antigen can even be used to overcome already developed systemic sensitization, something known and exploited long before the specific cells comprising the immune system were identified. Yet, despite the general bias toward tolerance, the mucosal immune system is capable of producing protective responses to pathogens. This response is controlled by recognition of inherent characteristics of the antigen, or contextual cues such as tissue damage. In general, the immune system is remarkably skilled at responding properly to the antigens it encounters. Failures, albeit uncommon, can be very serious. Food allergy is a prime example of the failure of oral tolerance.

How the mucosal immune system determines when to sound the alarm and when to remain silent is the focus of this chapter. In it, we examine

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the normal response to food proteins, how that response can go awry, and the factors that tip the balance.

Structure and function

The primary role of the GI tract is to absorb food and liquid and eliminate waste. To achieve this goal, the surface of the tract is both enormous (100 m^2) and extremely thin. The lumen of the intestinal tract provides a hospitable environment for bacteria that help break down foods into absorbable nutrients. However, the thinness of the barrier between external and internal creates a grave danger. It is not just nutrients, but toxins, pathogenic bacteria, viruses and parasites that are kept out by a single cell layer only. Breaks in this thin barrier create a risk of systemic infection. The complex task of protecting this border involves both non-specific and highly targeted techniques.

Chemical defenses

Protection begins with chemical and physical measures that keep some of the potentially harmful antigens (both food and microbial) from contact with the mucosal immune system and thus from generating an inflammatory response. Although the intestinal lumen is one of the most microbiologically dense environments in the world, bacteria and large antigens are actually maintained at some distance from the epithelial cells that line the GI tract. This is accomplished by a rich glycocalyx mucin layer (the mucus), which is produced by specialized intestinal epithelial cells. Antimicrobial peptides are caught in the mucous layer in a concentration gradient that provides a zone of relative sterility immediately proximal to the epithelial layer. In mouse models, deficiency of either the mucins or the antimicrobial peptides results in chronic inflammation. In humans, mutations causing abnormal production of the antimicrobial peptides are associated with the autoimmune syndrome Crohn's disease.[1,2] Whether dysfunction in the mucous layer or antimicrobial peptides play a role in the development of food allergy is an area yet to be explored.

What is known is that the enzymatic degradation of food proteins is a first line of protection against allergic sensitization, and that defects in digestion of food antigens contribute to allergy. Many food proteins never get a chance to cause the systemic immune responses characteristic of allergy because they are labile and are denatured by the acidic contents of the stomach. Allergens tend to be proteins that are resistant to this degradation, and thus capable of reaching immune cells to cause sensitization and reaction. For example, β-lactoglobulin and Ara h2, some of the relevant allergens for milk and peanut allergy, respectively, are not denatured by the conditions of the GI tract. Other potential allergens, such as the birch homologs found in many fruits, are easily broken down: although they can induce oral symptoms in cross-reactive individuals, they do not typically initiate sensitization by themselves. Several studies have lent evidence to the importance of the normal enzymatic processes in preventing allergy by showing that antacids impair oral tolerance in both animals and humans. Further, in mice, encapsulation of potentially allergenic foods facilitated allergy by allowing intact allergen to be present in the small intestine.[3]

The fact that most proteins are broken down by acid and enzymes may help explain why most foods tend not to be allergens, but it does not explain why allergy to stable proteins remains relatively rare. Peanut, for example, contains several proteins that are not degraded, yet only about 1% of the US population is allergic to it, despite near universal exposure. Clearly, other factors come into play after the digestive processes of the stomach.

Trafficking of antigen across the epithelium

Proteins that are not degraded by enzymatic processes can come into contact with the immune system in a number of ways. Transport across the epithelium is both active and passive, occurring both in the spaces between the cells and across them (Fig. 1.1).

The high-volume route for fluid is via the paracellular spaces, and the overall permeability of the mucosa is regulated by tight junctions that seal the space between epithelial cells. The leakiness of these junctions is subject to a variety of factors, including cytokines, medications and nutritional status. Permeability varies along the GI tract, and even within a short area, as the pores of the villi allow passage of larger solutes than those of the

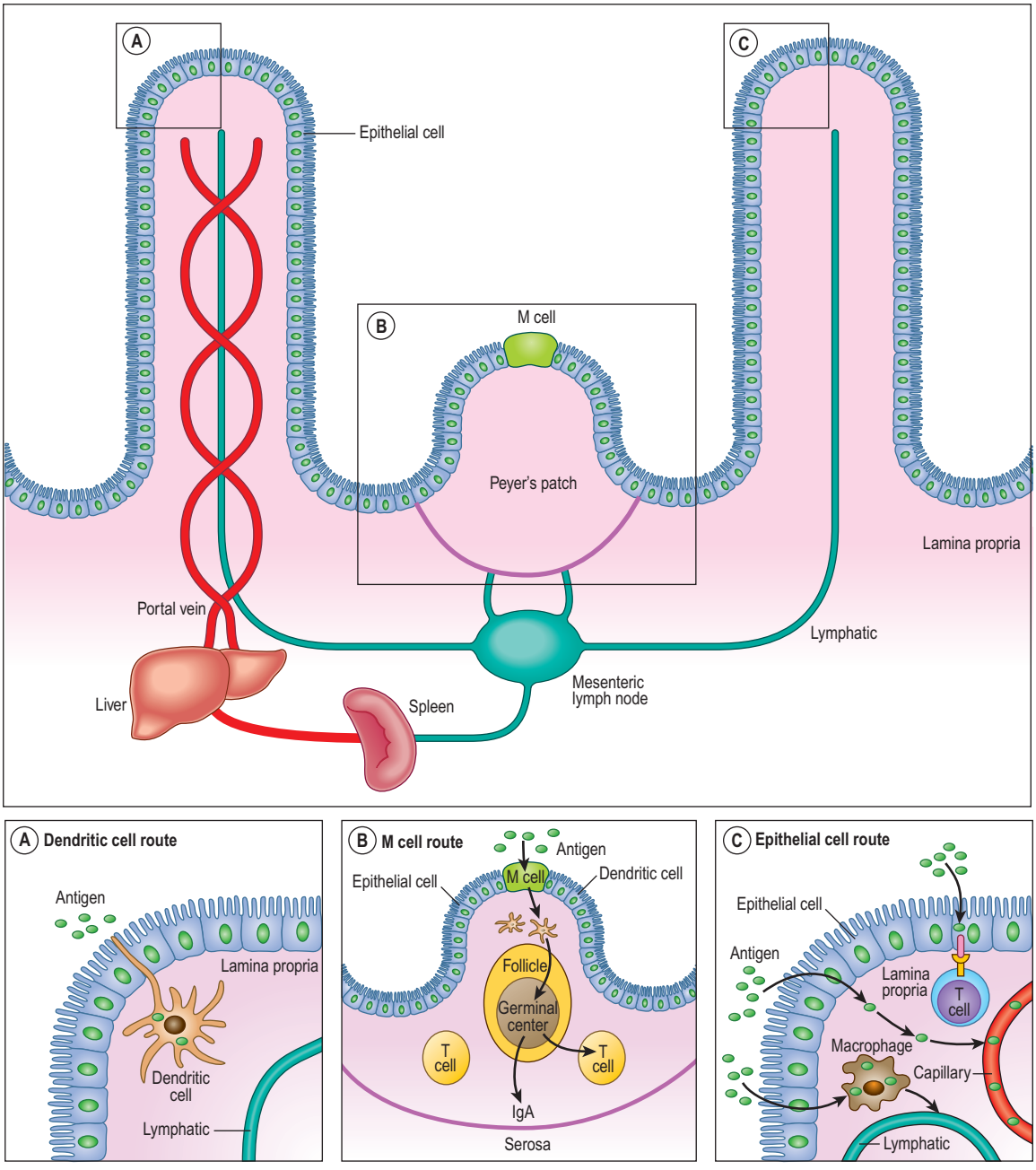

Figure 1.1 Antigen sampling in the gut. (A) Dendritic cells sample antigen directly by extending processes into the lumen. (B) Antigen taken up by M cells travels to the underlying Peyer's patches. (C) Antigen can cross the epithelium for transport to antigen-presenting cells, T cells, or into the lymphatic circulation. Reproduced with permission from: Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. J Allergy Clin Immunol 2005; 115: 3–12.

crypt.[2,4] Cytokines associated with both autoimmune and allergic disease disrupt barrier function and increase permeability.[5] Children with food allergy have been shown to have increased intestinal permeability, both at a time when they are regularly consuming the relevant allergen and after a long period of avoidance.[6,7] Other evidence for the importance of barrier function in allergy is the high rate of new sensitization in people taking the anti-rejection medicine tacrolimus, which causes mucosal barrier dysfunction. Although tacrolimus has other effects on the immune system, the high rate of new food allergies after solid organ transplantation is thought to be due its effects on mucosal integrity.[5]

In addition to the paracellular route, several alternative transport systems actively carry proteins, electrolytes, fatty acids and sugars across cells. Specialized modified epithelial cells called M (or microfold) cells act as non-professional antigen-presenting cells. These cells stud the follicle-associated epithelium overlying specialized collections of immune cells called Peyer's patches. They express receptors that recognize microbial patterns and aid in the endocytosis and transfer of antigen to the basal surface of the epithelium. This is especially important for bacteria, but may also be relevant for food allergens.[4]

Other non-specialized columnar epithelial cells form vesicle-like structures that allow transport of dietary proteins across cells. The formation of these vesicle-like structures seems to be dependent on MHC class II binding, but transocytosis can also occur via binding of antigen to IgA, IgE, and IgG. Transport via IgE may be especially important in the acute allergic response and in the amplification of allergy.[4] In contrast, secretory IgA, which accounts for the majority of the immunoglobulin produced by the body, complexes with antigen and facilitates transport across the epithelium to antigen-presenting cells, with a tolerogenic outcome.

A final method of antigen transport involves direct sampling of the luminal contents by extensions of antigen-presenting cells. Dendritic cells found in the lamina propria form their own tight junctions with intestinal epithelial cells and can project directly into the intestinal lumen. These projections increase when invasive bacteria are present, and sampling via this route seems to be especially important for the transport of commensal and invasive bacteria.[4]

Initial contact with the mucosal immune system

Once the antigen has been captured by dendritic cells, either by direct sampling or after processing through epithelial cells, the fate of the immune response depends on the interaction between dendritic cells and naive CD4+ T cells. Of the professional antigen cells associated with the gut, dendritic cells are the most important. They are found throughout the mucosal-associated lymph tissue and comprise a large class of phenotypically and functionally diverse cells. Subspecialization of these cells is thought to depend on their derivation (some develop from lymphoid precursors and some from myeloid precursors), their maturity, and environmental cues. This interaction can occur in specialized aggregations of antigen-presenting cells, T cells and B cells, such as Peyer's patches, in the loose aggregations of lymphocytes in the lamina propria, or, most importantly for food antigens, in the draining mesenteric lymph nodes.

Although there is communication between the mucosal and systemic immune systems, contact that is essential for both protective immune responses and oral tolerance, there is significant compartmentalization of responses at the mucosal level. The mesenteric lymph nodes act as a 'firewall', keeping the systemic immune system ignorant of much of the local immune response. In animals whose mesenteric lymph nodes have been removed, massive splenomegaly and lymphadenopathy develop in response to typical exposure to commensal organisms. In fact, much of the interaction with commensal organisms never even reaches the level of the mesenteric lymph nodes. IgA+ B cells, which collectively produce the majority of the immunoglobulin in the body, are activated at the level of the Peyer's patches and lamina propria and act locally. Induction of this IgA response can proceed normally in mice deficient in mesenteric lymph nodes. Although the response to commensals happens largely at the level of the Peyer's patches and lamina propria, for food antigens it seems that the mesenteric lymph nodes are key for the active response that constitutes oral tolerance. Mice without Peyer's patches develop oral tolerance normally, but those without mesenteric lymph nodes cannot. For food antigens, it seems that the typical path is for dendritic cells in the lamina propria to traffic to the mesenteric lymph nodes for presentation to CD4+ cells.[7,8]

Different experimental models have shown somewhat different kinetics of traffic to mesenteric lymph nodes after oral antigen. However, within days after exposure, dendritic cells carry orally fed antigen to the mesenteric lymph nodes and cause T-cell proliferation. T cells stimulated in this way then travel back to the mucosa and to the systemic lymph nodes.[9]

Once captured and processed, antigen presented by dendritic cells can cause several distinct immune responses. It is this interaction that determines whether allergy or oral tolerance develops.

What is oral tolerance?

Before we can begin to discuss what factors influence the development of oral tolerance, we must discuss what is meant by oral tolerance. There is disagreement at a fundamental level about how oral tolerance to foods develops. Not only are the specific mechanisms of oral tolerance imperfectly understood, but also the overall paradigm. Here we explore different theories about the development of oral tolerance.

Immune deviation

Starting in the 1980s, with work from Coffman and Mosmann, researchers began to describe distinct subsets of CD4+ T cells that were characterized by distinctive cytokine milieus and resulting disease or protective states.[10] A central paradigm in immunology for the past two decades has been this division of effector CD4+ T cells into Th1 and Th2 cells, both responsible for different mechanisms of clearing infection and both causing different pathological states when overactive. The cytokines that Th1 cells secrete (such as IFN-γ) activate macrophages and facilitate clearance of intracellular pathogens. In contrast, Th2 cells produce cytokines that promote class switching and affinity maturation of B cells, and signal mast cells and eosinophils to activate and proliferate. Th2 responses are important for clearance of extracellular parasites.

Allergy is dominated by the Th2 response and is characterized by IgE production, eosinophilia, mast cell activation, and, in some cases, tissue fibrosis. For many years it has been posited that the central defect in allergy is an imbalance between Th1 and Th2 responses. This model, although an oversimplification, has proved helpful in identifying factors that promote allergy. In the original model naive T-helper cells were stimulated by dendritic cells to develop either as Th1 or Th2 cells. Cytokines necessary and sufficient for Th1 polarization include IL-12 and INF-γ, but the mechanisms of Th2 differentiation have remained elusive. Two cytokines, IL-4 and IL-13, play a role, but are not essential for the development of high numbers of Th2 cells in the mouse model. Until recently, a leading hypothesis was that Th2 differentiation is the default response that occurs in the absence of Th1-directing signals. The theory of Th2 as a default has appeal because it harmonizes nicely with the so called 'hygiene hypothesis', in which inadequate infectious stimuli create the conditions for allergy. If Th2 deviation were the default, allergic responses would naturally develop in the absence of Th1 driving infectious stimuli. Recent work, however, suggests that Th2 differentiation requires other signals, including OX40L from dendritic cells, but that the signals essential for Th1 differentiation are stronger and predominate if present.[11]

Despite the compelling qualities of this theory, it is now clear that the reality is much more complicated. Although allergy is characterized by a Th2 response, an increasing body of evidence calls into question whether it is simply the balance between Th1 and Th2 responses that lies at the crux of the problem of allergy. Epidemiologic studies do not consistently show a reciprocal relationship between incidence of Th1 imbalance (i.e. autoimmunity) and Th2 imbalance.[12] Adoptive transfer of Th1 cells in mice cannot control Th2-induced lung inflammation.[13] A recent study showed that allergic subjects had low-level Th1-type cytokine responses to allergenic stimulation that matched the non-allergenic responses but were simply overwhelmed by the massive Th2 cytokine response.[14] Most importantly, other types of CD4 cells important in the control of both allergy and autoimmunity have been identified.

Regulatory T cells

The existence of T cells with suppressive capacity was first recognized in the 1980s. Initially, centrally derived T-regulatory cells were identified. These cells are important in regulating autoimmunity and are generated in the thymus, in a process of T-cell selection that has been compared to Goldilocks' sampling of the bears' oatmeal. T cells with too strong an attraction to self antigens are deleted, as

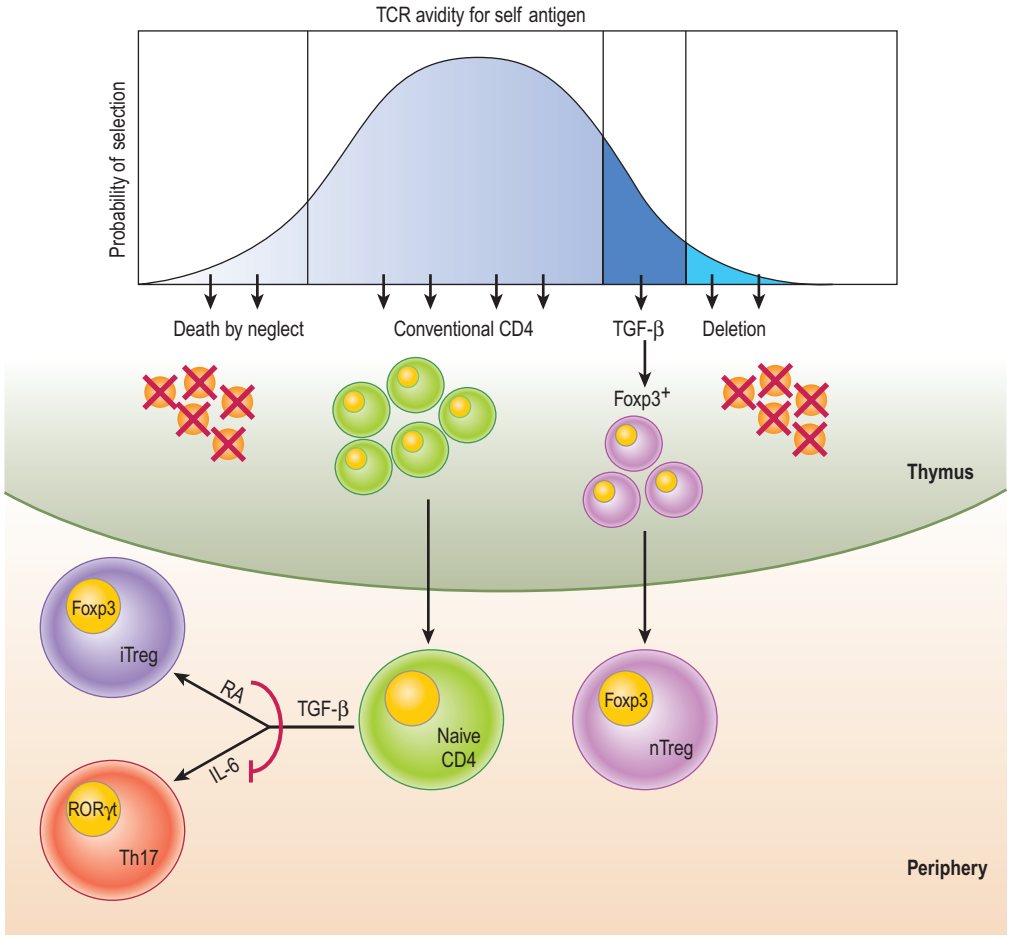

Figure 1.2 The development of regulatory T cells. In the thymus, avidity of the T-cell receptor for self antigen determines the fate of the T cell. In the periphery, naive Foxp3– CD4+ T cells can develop into FoxP3+ T-regulatory cells or Th17 cells, depending on the cytokine milieu. Reproduced with permission from: Mucida D, Park Y, Cheroutre H. From the diet to the nucleus: vitamin A and TGF-beta join efforts at the mucosal interface of the intestine. Semin Immunol 2009; 21: 14–21.

are those that do not bind well at all, and thus will not be effective antigen presenters. The majority of the remaining cells bind 'just right' at a moderate level and are destined to become effector T cells, but a subset that binds to self antigens more strongly persists and becomes suppressive T cells (Fig. 1.2).[15] A transcription factor, FOXP3, is essential for the suppressive nature of these cells and has served to identify them. The importance of these cells in autoimmune disease has been amply demonstrated, both in animal models – autoimmune disease can be induced by depletion of these cells – and in natural human diseases. Children with IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome have mutations in the FOXP3 gene leading to absent or abnormal levels of regulatory T cells. These children have early and severe autoimmune gastrointestinal and endocrine disease. Bone-marrow transplant that replaces the T-regulatory cells successfully reverses the disease.

Children with IPEX also have food allergy and eczema, demonstrating a failure of tolerance to antigens that are not present in the thymus. More recently, the importance of peripherally generated T-regulatory cells has become clear. As with the centrally generated T-regulatory cells, FoxP3 marks these cells (called iTregs), although other related

subsets of suppressor T cells generated in the periphery do not express Fox P3. T-regulatory cells are preferentially induced in the mesenteric lymph nodes, where the cytokine TGF-β is a key mediator of T-cell differentiation. In the past decade, it has been determined that T-regulatory cells and a newly described T-cell subset, Th17 cells, develop reciprocally under the influence of TGF-β. A cytokine, IL-6, drives differentiation to Th17 cells, whereas a metabolite of vitamin A, retinoic acid, was recently discovered to inhibit Th17 differentiation and promote T-regulatory development in the presence of TGF-β.[16] Vitamin A, which is not produced by the human body, is converted to its active form, retinoic acid, by epithelial cells and dendritic cells. The fact that generation of suppressor cells is dependent on an orally derived factor that is converted to an active form by the intestinal epithelium may help explain how the gut is maintained as a tolerogenic site.[17]

Peripherally generated T-regulatory cells have a multitude of effects on other immune cells. Through the action of secreted cytokines, such as IL-10 and TGF-β, they act on B cells, reducing IgE production and inducing the blocking antibody IgG4; on Th1 and Th2 cells, suppressing their inflammatory activities; and on dendritic cells, inducing them to produce IL-10 and further stimulate the development of regulatory T cells. In addition, they have direct interaction with mast cells through cell surface ligands (Fig. 1.3). In sum, they control both Th1- and Th2-mediated inflammatory responses.[18]

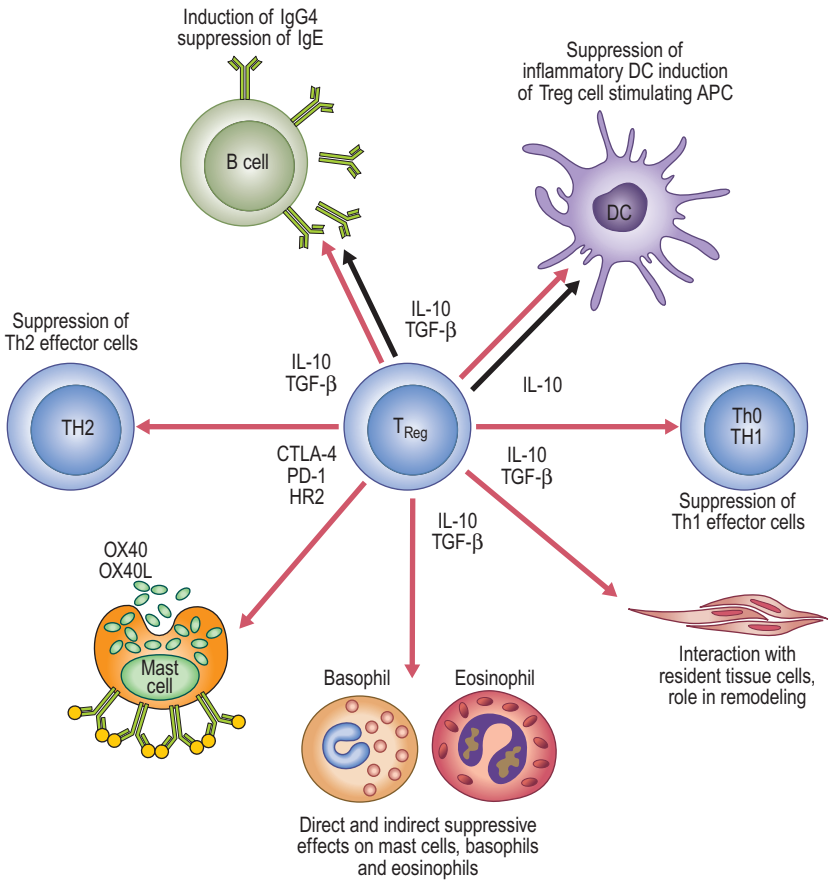

Figure 1.3 T-regulatory cells have direct and indirect effects on many different types of effector cells. Suppressive cytokines include interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). Another mechanism of suppression is by cell–cell contact via OX40-OX40ligand (red arrows: suppression; black arrows: induction). Reproduced with permission from: Akdis M. Immune tolerance in allergy. Curr Opin Immunol 2009; 21: 700–7.

Antigen-specific peripherally induced T cells are essential for oral tolerance. Oral tolerance proceeds normally in mice lacking centrally derived T-regulatory cells, but fails in mice unable to induce regulatory cells peripherally.[16] In humans, T-regulatory cell function has been implicated in both IgE- and non-IgE mediated food allergy. Children with active non-IgE mediated milk allergy had lower T-regulatory cells than controls in one study, whereas another, also of non-IgE mediated milk allergy, showed that T-regulatory function was associated with outgrowing the disease. In IgE-mediated milk allergy, increased numbers of T-regulatory cells were found in children with a milder phenotype who were better able to tolerate cooked milk than those with a more severe phenotype who reacted to cooked milk.[6]

T-regulatory cells seem also to be important for the effectiveness of allergen-specific immunotherapy. Oral and sublingual immunotherapies (reviewed in Chapter 17) have emerged as a very promising treatment for food allergy. Although the precise mechanisms by which they work are not yet known, an increase in FOXP3+ T-regulatory cells was found in the initial stages of peanut immunotherapy, with a return to baseline by 2 years on therapy.[6]

Th17 cells, which develop reciprocally with T-regulatory cells, promote inflammatory responses at the gut and seem to be especially important for protection against infection.[19] Deficiency of Th17 cells, as in Job's syndrome (also known as hyper-IgE syndrome), is characterized by abnormal responses to infectious stimuli, as well as very high levels of IgE. However, despite these high levels, specific sensitization is less common and the causes of high IgE in this syndrome are not clear.[20] Th17 cells do seem to be important in certain types of asthma that are less atopic, but whether they have a role in either prevention or promotion of food allergy has not been determined.

Other methods of tolerance

Other mechanisms of oral tolerance overlap with those discussed above. For control of self-reactivity, besides deviation and responsiveness to suppression, T cells have other mechanisms that allow them to be switched off or killed. In general, activation of the cell in the absence of co-stimulatory signals results in anergy. Anergy refers to a T-cell state where proliferation to antigen on rechallenge is impaired, but can be reversed with sufficient quantities of the T-cell growth cytokine IL-2. Blockage of co-stimulatory receptors can induce anergy, as can other methods of TCR cross-linking without co-stimulation, such as stimulation with soluble peptides. Deletion is a related process, and can follow anergy.

Several studies have shown that anergy and deletion can be important in oral tolerance to food antigens. In a key paper, Chen and colleagues[21] found that high doses of a model antigen caused initial activation of T cells followed by apoptosis of antigen-specific T cells. Low doses led to increases in what we now know to be regulatory T cells. Similarly, Gregerson et al.,[22] in a model of autoimmune uveoretinitis, found that low doses of fed antigen caused suppressive mechanisms to kick in, and that transfer of lymphocytes from treated animals transferred suppression to untreated animals. At higher doses, anergy was the predominant mechanism, and this could not be transferred to a naive animal.

Anergy, apoptosis and suppressive mechanisms are not mutually exclusive and have been shown to work simultaneously.[23,24] In all likelihood, the normal response to food proteins involves a combination of immune deviation, regulatory factors and anergy/deletion of reactive clones. It makes sense that something as important as oral tolerance would have highly redundant mechanisms.

Factors that influence the development of oral tolerance versus allergy

Factors both intrinsic to the individual and related to environmental exposures influence the development of allergy. Those that have been identified so far include age, microbial exposures, genetics, nutritional factors, and dose and route of antigen.

Developmental stage

The neonatal GI tract differs from the adult tract in significant ways, including the robustness of physical and chemical barriers, the composition of the microbial flora, and the maturity of the gut-associated immune system. Overall, these differences predispose the infant to the development of allergy, although the precise developmental window

of risk and the optimal strategy to prevent allergy in infants are among the most contentious areas in the field of allergy.

Part of the difficulty of resolving these controversies lies in the inadequacy of the animal models. Both human and rodent neonates have increased intestinal permeability compared to their adult counterparts. However, in humans, the transition from the highly permeable fetal gut to a more mature gut barrier occurs in the first few days of life, compared to more than a month in rats.[25]

One well-studied area is the difference in gastric pH and pancreatic enzyme output between infants and adults. With their immature barriers to regurgitation of caustic gastric contents, infants secrete much less acid into the stomach and have decreased pancreatic enzyme output, and do not reach adult levels of pH for the first few years of life.[25] As discussed above, acidic and enzymatic digestion is a first-line defense preventing some potentially sensitizing proteins from reaching relevant immune cells. Combined with somewhat increased intestinal permeability, this increases the chances of intact allergen crossing the epithelial border.

Once across the epithelial border, the immune system that the antigen encounters is very different in neonates than in adults. Both cellular and humoral branches of the immune system are immature. Total numbers of dendritic cells are lower, as is their ability to respond to co-stimulatory factors that typically elicit a Th1-type response. Further, CD4+ T cells are themselves highly skewed in a Th2 direction in the neonate, and have poor production of IL-12, a cytokine involved in Th1 responses. The inability to mount Th1 responses but ability to mount Th2 responses leads to an environment where potential autoimmunity or reactivity to maternal antigens is dampened, responses to microbial insults are deficient, and allergic responses are relatively favored.[26]

The fetal and neonatal immune system is also characterized by varying levels of T-regulatory cell function. At the time of birth, T-regulatory cells are found less frequently in cord blood than in adult blood, and those found have less efficient suppressive function after stimulation.[28] However, there is some evidence that, at least in mice, neonatal T cells have a propensity to develop into T-regulatory cells.[27] Given the uniquely stressful experience of birth, one could question whether what is found in cord blood is a valid reflection of the intrinsic qualities of the neonate. Regardless, the T-regulatory cell compartment is one area where neonatal and adult responses vary considerably, with important implications for the development of allergy.

The humoral immune system is also immature in the infant. Immaturity of the humoral immune system is at least partially compensated for by unique features of breast milk. Breast milk contains large amounts of secretory IgA and some IgG. Maternally supplied IgA substitutes for the infant's relative lack, complexing with dietary proteins and promoting non-inflammatory responses.[25] IgG found in breast milk plays a similar role, with added nuances. Neonates express a receptor for IgG in their intestinal epithelium (the FcRn receptor). This allows for active transport of IgG from breast milk into the neonatal circulation. In addition to absorbing maternal antibody to be used in fighting infections, the FcRn receptor can also transport intact antigen complexed with IgG directly from the lumen to lamina propria dendritic cells, contributing to oral tolerance. In mice, antigen complexed to IgG in breast milk has been shown to induce antigen-specific T-regulatory cells in a manner independent of the other ingredients in breast milk. Interestingly, this was enhanced in mothers who were sensitized to the allergen.[29]

Other components of breast milk are important in oral tolerance. Pro-forms of the tolerogenic cytokine TGF-β are abundant in breast milk. They are thought to be physiologically active after exposure to the acidic gastric environment, and epidemiologic work in humans suggests that higher levels are associated with protection from atopic disease.[30,31]

Despite these pro-tolerogenic features, the presence of allergen in breast milk does not always lead to oral tolerance. Allergens are found both free and complexed to antibody in breast milk, and infants can become sensitized to proteins encountered in breast milk and react to them. Complicating the picture further, maternally ingested or inhaled allergens have also been found in the placenta, although whether this allergen is transferred to the fetal circulation remains unclear. Studies in mice have shown variation in the results of prenatal exposure by the dose of antigen. Mice whose mothers had low doses of prenatal exposure to a model allergen developed tolerance to that allergen. With higher doses there was transient inhibition of IgE production upon challenge, but after the immediate neonatal period the mice had increased susceptibility to the development of allergy to that allergen.[32]

Whether sensitization or oral tolerance to these antigens occurs probably depends on a complex interaction between the non-allergen components of breast milk, infant factors, and the dose and timing of the allergen.

Route of exposure

Some have suggested that the primary route of sensitization leading to food allergy is via the skin. In this model, oral exposure is almost always tolerogenic. Allergy happens when the skin encounters potentially allergenic foods prior to oral contact. Eczema, which creates breaks in the skin and an inflammatory backdrop, predisposes to allergic sensitization. Evidence supporting this model includes the fact that mice can be sensitized via low-dose skin exposure, some epidemiologic evidence tying peanut oil-containing lotions to peanut allergy, and the differences in immune responses induced by antigen-presenting cells in the skin and in the gut. However, this theory has not been conclusively proven.[33]

Microbial influences

The most compelling theory for the wide variation in incidence in allergic disease remains the so called 'hygiene hypothesis'. In general terms, this theory posits that the decreased burden of infection, especially childhood infections, characteristic of the western lifestyle does not adequately stimulate the developing immune system into a non-allergic phenotype. The beauty – and the limitation – of this theory is that it is sufficiently broad to encompass a wide range of theoretical mechanisms by which infection might prevent allergy, including Th1 skewing and induction of T-regulatory cells, and that it does not specify what infections are actually essential.

Epidemiologic evidence supporting the hypothesis includes the fact that allergy is more common in developed than in developing countries, in city than in farming communities, in children who do not attend daycare, and in older siblings than in younger siblings, especially younger siblings in large families. A thorough analysis of farming communities in Europe identified unpasteurized milk and the presence of multiple species of farm animals living under the same roof as key protective factors of the rural life. In other populations, markers for parasitic infections, such as Schistosoma, are associated with reduced rates of allergy. In addition, differences in the microbial content of drinking water have been linked to the disparate rates of atopic disease found in genetically similar populations of people living on different sides of the Finnish/Russian border. Similar epidemiologic studies also associate infection with protection from autoimmune disease.[34]

Evidence tying actual differences in gut flora to allergy has been mixed, with some finding that allergic children have different colonization patterns, and others failing to replicate the result. Birth by Caesarean section, which does not expose the infant to the normal maternal vaginal and fecal flora, has been associated with alterations in the infant's fecal flora. In one study,[35] Caesarean delivery was associated with an increased risk of wheezing, although this was not replicated in another study. Methodological problems with how gut flora were analyzed may be a part of the confusion, as the relevant bacteria may be hard to culture.

In rodent models, intestinal colonization is essential for normal development of the immune system and for the ability to induce oral tolerance. Recent work has identified certain bacterial components as being essential for the development of the normal gut immune system.[36] Specific mechanisms for prevention of allergy by infection are still being worked out. In humans, the mechanisms have been most carefully explored in prospective studies of children growing up on European farms. In these studies, several mechanisms of protection from allergy were identified, including upregulation of Toll-like receptors (TLRS), increased T-regulatory cell function and alterations in prenatal serum cytokine levels.[37–39] Prenatal farm exposure has been identified as particularly protective for the development of allergy. Whether the prenatal exposure is mediated by colonization of the infant, epigenetic changes passed from mother to child, or by so far unidentified features of the intrauterine environment, is unknown.

Nutritional factors

Nutritional factors are one way in which the prenatal environment or early life could modify the risk for allergic disease. Because diet has changed so rapidly in developed countries over the last half century, nutritional factors are candidates to explain the rapid increase in allergic disease and the geographic variation in disease. The Mediterranean diet in general during pregnancy has been associated with

protection from respiratory allergy and wheeze in children.[40] It has been suggested that an important difference between more 'westernized' diets and the Mediterranean diet is the presence of different isoforms of vitamin E found in cooking oils. D-α-tocopherol, found in olive oil and sunflower oil, has anti-inflammatory effects by reducing cell adhesion molecules on epithelial cells. D-γ-tocopherol, the predominant isoform of vitamin E found in vegetable oils in westernized diets, has opposite effects on epithelial cells.[41] The effects of these isoforms on food allergy have not been adequately explored.

Another dietary factor that may have a role in protection from allergy is polyunsaturated fatty acids (such as those found in fish oil). In a randomized placebo-controlled study, supplementation with omega-3 polyunsaturated fatty acids during pregnancy and breastfeeding was associated with lower sensitization to food proteins and eczema.[42] Epidemiologic studies have found similar results, although not uniformly.[43]

Besides fatty acids, vitamin D is also found in fish oil. Vitamin D levels vary significantly within westernized populations. Vitamin D is found in the diet, both naturally in foods such as fatty fish and in fortified dairy products, and is also produced by the skin with exposure to sun. Populations living at very northern or southern latitudes, as is the case in most developed countries, are at risk for deficiency. Vitamin D is a steroid hormone with pleotropic effects. Its many effects on the immune system can vary by dose. To innate cells, it promotes the production of antimicrobial peptides, while also downregulating some TLRs. The effects on Th1 cells include downregulation of IFN-γ at the gene level. Effects on Th2 cells depend on the dose, with very high or low levels associated with increased Th2 deviation. Overall, T-regulatory cells are upregulated. Epidemiologic studies of the relationship between vitamin D supplementation and allergy or wheeze have found mixed results, and have typically been very susceptible to recall bias. Several recent population studies have linked latitude and season of birth with acute food allergy episodes, implicating lack of sun exposure in the pathogenesis of food allergy. Studies that prospectively assess the relationship between vitamin D and development of allergy are under way.[44,45]

Vitamin A, which has a clear role in the development of oral tolerance, is found in sufficient amounts in almost all western diets. Blood levels are tightly controlled, and so although vitamin A may be necessary for the development of oral tolerance, differences in intake may not be an important risk factor for food allergy. Whether variations in intake relate to the development of oral tolerance has not been explored.

The role of folic acid in allergy and asthma is another area of intense study, although its specific role in oral tolerance has not been determined. The interest in folic acid is driven by its potential role in the modification of DNA expression through epigenetics, and by the fact that folic acid intake has changed markedly in the past two decades. Epigenetics refers to heritable changes in gene expression that are not due to changes in the underlying DNA sequence. The major mechanism of epigenetic change is through changes in methylation of DNA. Folic acid, which is a methyl donor, was added to all grain products in the US in 1998 by FDA mandate. In 2008, Hollingsworth et al.[46] showed in a mouse model that maternal supplementation with folate led to suppression of a gene known to be important for the balance between Th1 and Th2 skewing, among other effects. In contrast, in a cross-sectional epidemiologic study, Matsui and Matsui[47] found an inverse relationship between folic acid levels and total IgE, atopy and wheeze. The role of folic acid in allergy and airway disease remains highly controversial.

Genetics

A family history of food allergy in particular, and atopy in general, is a major risk factor for the development of food allergy. Teasing apart the role of environment and genetics in failures of oral tolerance has been complicated by the lack of uniform definitions for food allergy, and by the probability that what we call food allergy actually comprises several distinct phenotypes. Further, as has been demonstrated best for asthma, it is likely that gene –environment interactions mandate precise determinations of environmental factors when trying to determine the role of genetics (and vice versa). For example, in studies of asthma, a genetic variant in the receptor for lipopolysaccharide (a bacterial product important in stimulating innate immune responses) is protective at high levels of endotoxin (such as might be found on a farm), but increases the risk of asthma when levels of endotoxin are low.[48] Exposure to both microbial products and allergens probably modifies whatever genetic risk factors there are for food allergy.

However, no matter how it is defined, and under what environmental conditions, it is clear that there is a large genetic component to food allergy. For example, a British study found that a child with a peanut-allergic sibling had a five times increased risk of peanut allergy than the general population. Depending on how food allergy is defined, and on the population studied, the heritability of specific food allergies has been estimated to be 15–80%.[48] Despite the clear heritability of food allergy, it is not yet clear which genes are most important for the normal development of oral tolerance. The genes that most obviously cause food allergy when mutated, such as FOXP3, in which food allergy is part of a larger syndrome, are probably only responsible for a fraction of the overall burden of disease.

Candidate genes that have been explored with varying levels of success include those for antigen presentation, cytokines, and intracellular signaling. Human leukocyte antigens, which determine the antigenic epitopes presented to the immune system, were early targets for study. Although initial studies showed an association with certain food allergies, repeat studies did not replicate those results. Two genes known to be involved in Th2 differentiation, SPINK5 (serine protease inhibitor Karzal type 5) and the gene for IL-13, have shown association with food allergy in preliminary studies. Studies of two other genes that would be logical to be involved, the gene for the receptor for lipopolysaccharide, discussed above, and the gene for IL-10 (which is important in T-regulatory cell development), have found inconsistent results. Larger studies are under way to try to further elucidate the genetic factors important in the normal development of food tolerance.[48]

In summary, the balance between oral tolerance and allergy is influenced by a complicated array of factors, including genetic susceptibility, microbial exposure, dietary factors, and the route, dose and timing of allergen exposure. Environmental influences begin in the womb, and perhaps before, and are modified by the mother's genetics and own allergic history. So far we have only scratched the surface of this field.

Opportunities for prevention

With the steep rise in allergy in general, and food allergy in particular, the need for interventions that might prevent allergy has become more imperative. However, implementing a successful preventive strategy is like threading a narrow needle: any intervention can have unintended consequences. So far, preventive strategies have focused most heavily on the timing of antigen exposure, with some attention to trying to alter the gut flora and to non-allergen related dietary factors.

The history of recommendations about the timing of allergen exposure serves as a cautionary tale about the dangers of making policy for populations without clear evidence. Although previous AAP recommendations suggested that pregnant and lactating women with a family history of allergy avoid peanuts and tree nuts, and possibly eggs, fish and milk, more recent reviews of the literature have concluded that there is no good evidence that maternal avoidance is beneficial. Indeed, small interventional studies have suggested that maternal avoidance is not risk-free, and that maternal egg and milk avoidance can be harmful nutritionally. The most recent advisory statement by the AAP retracts the previous recommendation, stating instead that there are not enough data to make any recommendation.[49]

The best time to introduce allergens directly to the infant is even more contentious. Previous recommendations were that at-risk children avoid cows' milk until their first birthday, egg until the second, and peanut, tree nuts and fish until the third. In the decade since those recommendations were made in the US and the UK, the incidence of food allergy has continued to grow rapidly, and prominent allergists are questioning whether more harm than good is being done by avoiding allergens early in life. Some tentative epidemiologic evidence supports the notion that early introduction could be helpful. Evidence includes the low rate of peanut allergy in Israel, where peanuts are eaten early, compared to the high rate in genetically similar populations in the UK, where peanuts typically are not eaten early. A large interventional study of early peanut introduction in children with eczema or egg allergy currently under way in the UK will hopefully shed light on this question. In the meantime, pediatricians, allergists and parents are left without clear guidance about when to start highly allergenic foods.

Probiotics for the prevention of allergy are another area where initial high promises have not been met. Given the data for the importance of gut microbiota in the development of the intestinal immune response, it would make sense that one

could alter the microbial contents with beneficial results. Prebiotics, which contain elements that stimulate specific bacterial growth, and probiotics, which contain the bacteria themselves, have been used in many small studies for the prevention and treatment of allergic disease. In sum, the studies suggest a small beneficial effect for the prevention of atopic dermatitis, but no benefit for the treatment of established disease or for the prevention of other atopic conditions. Larger, well-designed studies are required before probiotics can be confidently recommended.[50]

Other dietary factors are promising, although they have not yet been fully evaluated. As discussed above, the single randomized controlled study of fish oil found some protection from food allergy, but this needs to be replicated. It is not yet clear whether an increase or reduction in vitamin D and folic acid would be the best intervention for prevention of food allergy. Well-designed prospective epidemiologic studies are the first necessary step to sort this out.

Conclusions

Oral tolerance is a complex, active process that occurs in the gut-associated immune system. Although the precise mechanisms have not been completely elucidated, regulatory T cells seem to be essential for its development and maintenance. Other, overlapping mechanisms, including immune deviation, anergy and deletion, also play a role. Many factors affect the balance between allergy and oral tolerance. They include genetic variations, the dose, timing and route of antigen exposure, the microbial milieu, and probably other dietary factors. This field is still young, and much remains to be done to identify the mechanisms of allergic sensitization. Because of the complexity of the system, some things will not be known until interventional studies in humans are carried out.

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