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Oral tolerance and its relation to food hypersensitivities

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The gastrointestinal tract is the largest immunologic organ in the body. It is constantly bombarded by a myriad of dietary proteins. Despite the extent of protein exposure, very few patients have food allergies because of development of oral tolerance to these antigens. Once proteins contact the intestinal surface, they are sampled by different cells and, depending on their characteristics, result in different responses. Antigens might be taken up by Microfold cells overlying Peyer's patches, dendritic cells, or epithelial cells. Different cells of the immune system participate in oral tolerance induction, with regulatory T cells being the most important. Several factors can influence tolerance induction. Some are antigen related, and others are inherent to the host. Disturbances at different steps in the path to oral tolerance have been described in food hypersensitivity. In this review we provide an overview of oral tolerance and cite data related to food hypersensitivity wherever evidence is available. (J Allergy Clin Immunol 2005;115:3-12.)

Key words: *Oral tolerance, food allergy, food hypersensitivity, mucosal immunity, antigen uptake, intestine*

Abbreviations used
APC: Antigen-presenting cell
DC: Dendritic cell
M cells: Microfold cells
PP: Peyer's patch

The gastrointestinal tract is the largest immunologic organ in the body. It is lined by a single layer of epithelium. Underneath this epithelial layer are abundant numbers of lymphocytes interspersed in a loose connective tissue stroma. The surface epithelium is directly exposed to the external environment, the lumen, which is bombarded daily by a myriad of bacteria and dietary proteins. Despite the large extent of dietary antigenic exposure, only a small percentage of individuals have food allergy. This is due to development of oral tolerance to dietary proteins. Oral tolerance, as characterized by Chase[1] in 1946, refers to a state of active inhibition of immune responses to an antigen by means of prior exposure to that antigen through the oral route (Fig 1).

The journey for a dietary protein antigen involves multiple steps before T cells can respond to it and cause either tolerance or food hypersensitivity. After undergoing modification in the lumen, the antigen is in contact with specific antigen-presenting cells (APCs) with distinct activation requirements, which then help to activate regulatory T cells, resulting in the net suppression of an immune response. It is postulated that a breakdown in oral tolerance mechanisms or a failure of induction of oral tolerance results in food hypersensitivity. In this article we provide an overview of protein processing and uptake in the gut, the different mechanisms of oral tolerance to that protein, and finally the factors that influence oral tolerance. Throughout the review, we will provide data related to food hypersensitivity wherever evidence is available.

ANTIGEN PROCESSING AND UPTAKE IN THE GUT

Proteins are essential for nutritional homeostasis. A normal healthy adult requires approximately 0.75 g/kg/d of protein to maintain positive nitrogen balance and to provide essential amino acids.[2] It is estimated that the average North American daily diet provides 70 to 100 g of protein. These proteins are assimilated in an efficient manner after the action of gastric, pancreatic, and small intestinal brush border proteases, resulting in a reduction of the majority of dietary proteins to a mixture of free

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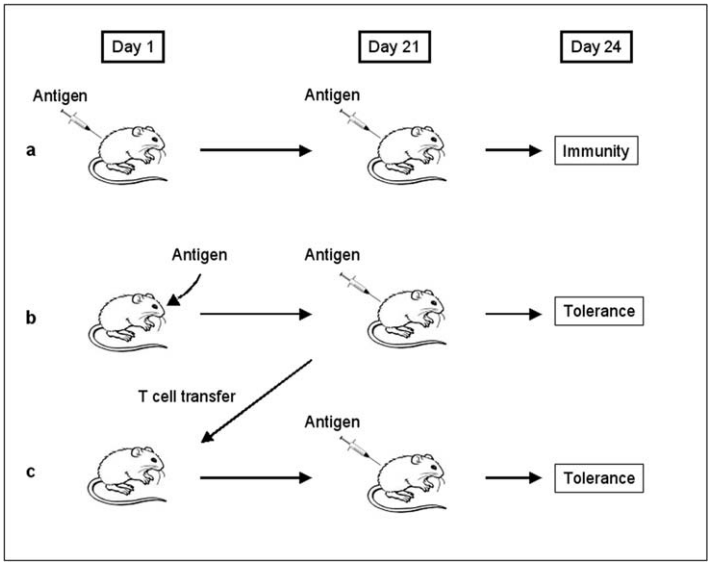

FIG 1. Induction of oral tolerance. **a**, When mice are immunized subcutaneously and then boosted subcutaneously with an antigen, strong *in vitro* cell-mediated and antibody responses to the antigen occur. **b**, When mice are first fed the antigen orally and then immunized subcutaneously, *in vitro* immune responses to the antigen are greatly reduced. **c**, When T cells from mice that were fed antigen are transferred to naive mice, subcutaneous immunization of these naive mice results in reduced *in vitro* immune responses as well. This shows that oral feeding of an antigen can induce a T cell–mediated active inhibitory immune response.

amino acids, dipeptides, and tripeptides, which are absorbed by intestinal epithelial cells.[3] Products of proteolysis, as well as intact proteins that escape digestion, can also be sampled by distinct immune cells, resulting in a state of immunologic tolerance through different mechanisms.

Antigen processing in the lumen

Ingested dietary proteins are subject to degradation and destruction of their conformational epitopes by gastric acidity and luminal digestive enzymes, resulting, in many cases, in the destruction of immunogenic epitopes (immunologic ignorance) to the protein. In animal models a disturbance in these factors has been shown to lead to food hypersensitivity rather than tolerance or ignorance.

To address gastric acidity, Untersmayr et al[4] examined the effect of antacids on food allergy induction in a murine model of gastrointestinal hypersensitivity to caviar proteins. Groups of mice fed caviar extract in combination with different types of antacids had significant levels of caviar-specific IgE antibodies and demonstrated positive immediate-type skin reactivity to the protein subsequent to immunization with the extract. Furthermore, T-cell reactivity to caviar was shown to be increased in stimulated spleen cell cultures. These responses were not seen in the group fed the extract without antacids, pointing toward a potential role of acidity in the prevention of allergies and possibly promoting tolerance.

The importance of digestive enzymes was demonstrated in an elegant experiment by Michael,[5] who observed that a peptic digest of BSA was tolerogenic when administered orally or directly injected into the ileum of mice. In contrast, untreated BSA was tolerogenic when administered orally but immunogenic after direct ileal administration. To address both acidity and digestive enzyme effects, Barone et al[6] were able to demonstrate interruption of already established tolerance to egg protein, ovalbumin, by protecting it from digestion through encapsulation in water-soluble, low-pH-resistant acrylic microspheres. This technique likely protects the protein from both acid and enzymatic digestion but might also alter its site of entry into the host. Hogan et al[7] were able to successfully use this technique to create a murine model of ovalbumin-induced eosinophilic gastrointestinal allergy.

Other luminal factors affecting proteins in the lumen include gastrointestinal peristalsis and the protective mucus layer that lines the intestinal epithelium and prevents some proteins from contacting the epithelium.

Those dietary proteins that escape luminal digestion and processing subsequently contact the epithelium, beneath which is both an organized and disorganized mucosal immune system, to generate a wide range of immunologic responses. Peyer's patches (PPs) sit underneath Microfold cells (M cells), dendritic cells (DCs), antigen-presenting macrophages, T cells bearing receptors for MHC class I– and II–mediated antigen presentation, and other cytokine-producing cells. Protein antigens can be taken up by different cell types, depending on their specific properties (Fig 2). The site of entry for these proteins might also dictate the nature of the immune response to these antigens.

Sites of antigen sampling in the gut

PPs. PPs are organized lymphoid structures distributed in the small intestine and rectum. They consist of a germinal center comprised of B lymphocytes surrounded

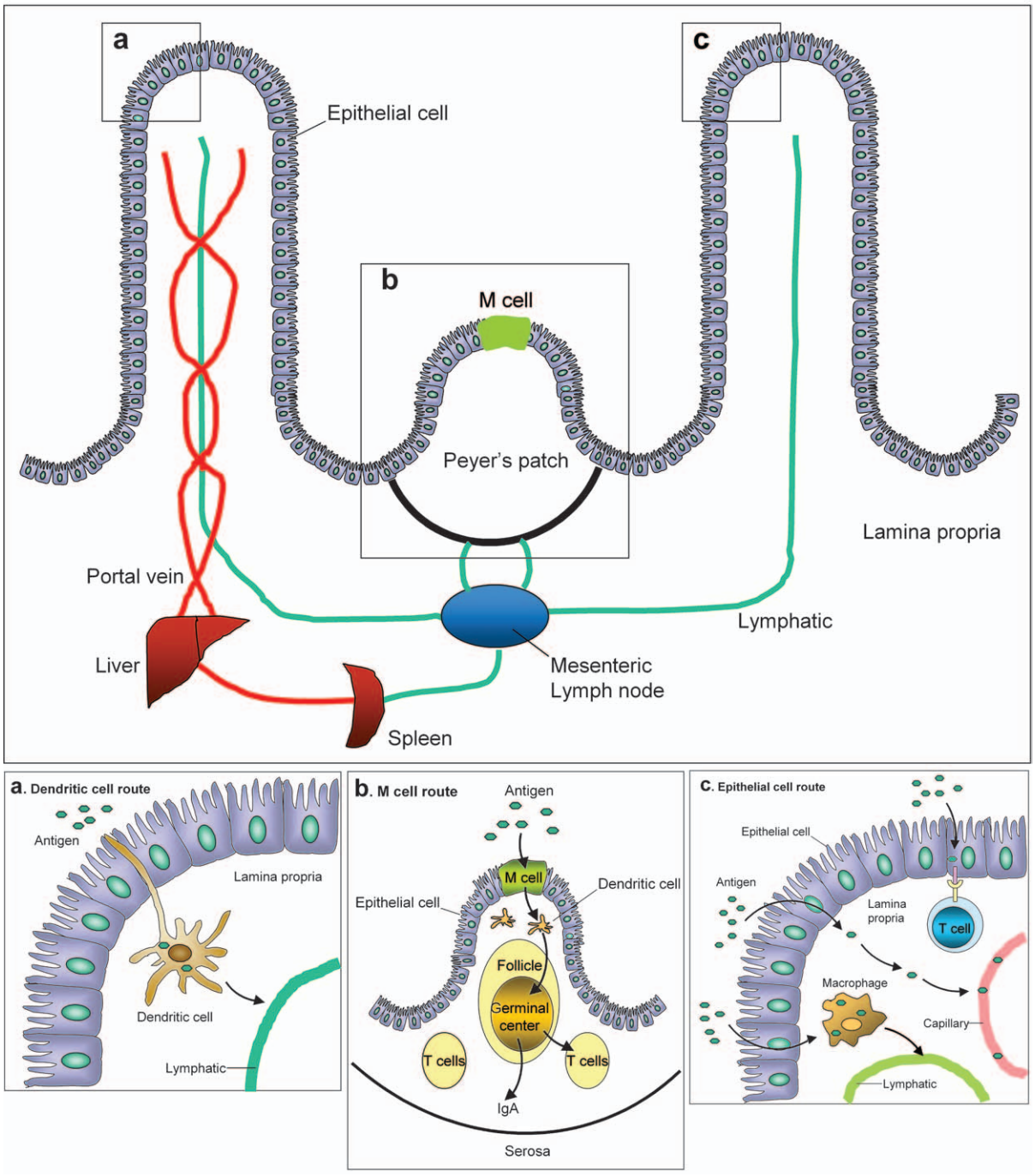

FIG 2. Sites of antigen uptake in the gut. **a**, Antigen can be sampled by DCs that extend processes into the lumen. **b**, Particulate antigens are taken up by M cells overlying PPs and then delivered to DCs in the subepithelial dome region and then to underlying B-cell follicles, where IgA commitment occurs. **c**, Soluble antigens might cross the epithelium through transcellular or paracellular routes and then might encounter T cells or macrophages in the lamina propria or might reach the circulation.

by smaller numbers of T lymphocytes. These B lymphocytes are committed to the production of IgA. On the appropriate signal, PP B cells migrate to the mesenteric lymph node, where they mature into plasma cell precursors, which then migrate to the intestinal lamina propria, where they differentiate and secrete dimeric IgA.[8]

PPs are overlaid by specialized epithelial cells, referred to as M cells. M cells are inefficient at uptake of soluble protein antigens. They are specialized to take up particulate antigens or those antigens for which these cells express receptors (eg, poliovirus).[9] The antigen is in turn delivered into the subepithelial dome region of the PP, an

area rich in DCs, that in turn ingests the antigen and delivers it to the underlying B-cell follicles of the PPs.[10] IgA switching then occurs in these cells, mediated by TGF-β–secreting T cells, and this is thought to contribute to oral tolerance as well.[11] Santos et al[12] were able to recover antigen-specific TGF-β–secreting regulatory cells from PPs after oral administration of the antigen to mice. TGF-β is capable of suppressing cell-mediated immune responses while serving as a switch factor for IgA.[13] TGF-β receptor signaling was shown to be critical for mucosal IgA responses.[14]

With regard to food allergy, Frossard et al[15] compared antigen-specific IgA-secreting cells in PPs from mice actively sensitized to β-lactoglobulin with resultant anaphylaxis versus mice actively tolerized to the same protein by using different feeding protocols. Tolerant mice were found to have a higher number of β-lactoglobulin–specific IgA-secreting cells in PPs, as well higher fecal β-lactoglobulin-specific IgA titers, than anaphylactic mice. An increase of β-lactoglobulin-induced IL-10 and TGF-β production by T cells from PPs was observed in tolerant mice.

How essential PPs are in oral tolerance induction is debatable, however, because tolerance to soluble antigen could be induced in mice lacking PPs.[16] In addition, PP-deficient mice were shown to have the same frequency of APCs, including DCs and macrophages, in various lymphoid organs after oral administration of a soluble antigen as mice with intact PPs.[17]

DCs. DCs are potent APCs. They are present in different compartments of the gut, including intestinal lamina propria, PPs, and mesenteric lymph nodes.[18-20] DCs can also intercalate between intestinal epithelial cells while preserving the integrity of the epithelial barrier through expression of tight junction proteins. They can send dendrites into the lumen and sample antigen directly from the lumen.[21] Antigen-carrying DCs might then traffic through the lymphatics to the mesenteric lymph nodes,[20,22] although this has not been directly shown.

DCs have a pivotal role in directing the balance between tolerance and active immunity in the intestine. This role depends on the cytokine microenvironment and the expression of costimulatory molecules by these cells. Viney et al[23] were able to expand DCs *in vivo* by using the hemopoietic factor FMS-like tyrosine kinase 3 ligand. DCs presented soluble fed antigen and induced greater tolerance. These cells minimally express CD80/CD86, indicating an immature state.[23] On exposure to inflammatory stimuli, however, DCs were shown to upregulate CD80 and CD86 and cause a potent stimulatory response. This was demonstrated in FMS-like tyrosine kinase 3 ligand–treated mice by means of oral feeding with cholera toxin (which elicits intestinal production of the proinflammatory cytokines IL-1 and IL-6 in the intestine) or by means of direct administration of IL-1, resulting in strong immunologic responses.[24]

The role of DCs in food allergy has still not been fully investigated. In a mouse model of type I milk hypersensitivity, Chambers et al[25] were able to induce antigen-specific IgE responses in naive mice on adoptive transfer of splenic and PP-derived DCs from the allergic mice, even without challenging the naive mice with antigen. The same group also examined the level of apoptosis in splenic DCs and PP-derived DCs from allergic and nonallergic mice after interaction with antigen-specific T cells. DCs from mice with milk allergy showed a reduced degree of apoptosis compared with DCs from control animals without allergy when cultured in the presence of both the antigen and antigen-specific T cells.[26] The role of the DCs intercalating between epithelial cells has not yet been studied in relation to oral tolerance and food hypersensitivity.

Intestinal epithelial cells. Normally, when in contact with soluble dietary antigens that escape proteolysis in the lumen, intestinal epithelial cells take them up through fluid-phase endocytosis by means of the microvillous membrane. Antigens are then transported in small vesicles and larger phagosomes and digested when lysosomes combine to form phagolysosomes. Intact molecules that remain after digestion are deposited in the extracellular space by means of exocytosis.[27] As a result, approximately 2% of intact proteins reach the intestinal lymph and portal circulation under physiologic conditions.[28]

Intestinal epithelial cells might also act as nonprofessional APCs. They constitutively express MHC class II molecules on their basolateral membranes[29,30] and are capable of presenting antigen to primed T cells. In contrast to professional APCs, intestinal epithelial cells normally selectively activate $CD8^+$ suppressor T cells, playing a role in local suppression of immune responses.[31,32] This process appears to be regulated by the nonclassical class I molecule CD1d and the CD8 ligand gp180, an intestinal epithelial membrane glycoprotein.[33,34] The role of intestinal epithelial cells in the regulation of mucosal immunity was supported in studies of inflammatory bowel disease, a disease of the intestine characterized by chronic inflammation. In coculture experiments *in vitro*, intestinal epithelial cells from patients with inflammatory bowel disease stimulate potent helper $CD4^+$ cells rather than suppressive $CD8^+$ cells.[35] Furthermore, patients with inflammatory bowel disease fail to have oral tolerance to soluble fed protein antigens.[36] This has not been studied in patients with food hypersensitivity.

Intestinal epithelial cells were also shown to have the ability to assemble and release exosome-like structures that carry MHC class II with bound antigenic peptides sampled from the lumen. These structures were recovered by Karlsson et al[37] in the serum of animals shortly after feeding an antigen and were capable of inducing antigen-specific oral tolerance. Bruce and Fergusson[38] had also measured tolerogenic peptides in serum after feeding with a protein antigen. Serum collected from mice after feeding with an antigen, when injected intraperitoneally into recipient mice, induced antigen-specific suppression of systemic delayed-type hypersensitivity.

In relation to allergic phenomena, IL-4 was shown to induce expression of FcεRII/CD23 on the surface of intestinal epithelial cells in a rat model, allowing rapid internalization and transcytosis of IgE-allergen com-

plexes.[39] Whether this induction occurs in human subjects and its potential relevance to food allergy remain to be determined.

Paracellular spaces. Once in contact with the epithelial layer, an antigen might not pass between the cells because of tight junctions joining adjacent enterocytes. These junctions are so impermeable that they prevent even small peptides and amino acids from passing through. In anaphylaxis and in gastrointestinal allergy animal models, intestinal permeability was shown to be increased.[40,41] This permeability increase is due not only to increased transcellular uptake but also to disruption of tight junctions and opening of paracellular spaces.[42] This mechanism was also shown in a T_H2 murine model of intestinal parasitic infestation.[43] In human subjects intestinal permeability was shown to increase on milk provocation in children with cow's milk–sensitive enteropathy by using a lactulose-mannitol test.[44] Whether the increase in permeability to sugars is reflective of an increased permeability to proteins remains to be proved. Furthermore, whether the increase in intestinal permeability in turn affects further food hypersensitization remains to be studied.

CELLS INVOLVED IN ORAL TOLERANCE INDUCTION

Oral tolerance can be induced in mice after administration of either a single high dose of antigen or repeated lower doses.[45] These 2 forms of tolerance, termed high-dose and low-dose tolerance, respectively, are mediated by 2 different mechanisms (Fig 3). A high dose of an oral antigen can induce lymphocyte anergy[46] or deletion.[47]

High-dose tolerance is mediated by lymphocyte anergy, which can occur through T-cell receptor ligation in the absence of costimulatory signals provided either by soluble cytokines, such as IL-2, or by interactions between costimulatory receptors on T cells (CD28) and counter-receptors on APCs (CD80 and CD86).[48] High-dose induced deletion occurs by means of FAS-mediated apoptosis,[47] which can be blocked by the proinflammatory cytokine IL-12.[49]

Low-dose tolerance is mediated by regulatory T cells. In addition to the suppressor $CD8^+$ cells described above, different types of regulatory $CD4^+$ cells have also been shown to be important for oral tolerance induction to an antigen.[50-52] These cells can be divided into 3 subgroups: T_H3 cells, T_R1 cells, and $CD4^+CD25^+$ cells (Table I).

Oral tolerance can also be mediated by liver-associated lymphocytes carrying the NK1.1 marker ($NK1.1^+$ T cells). After passage through intestinal epithelial cells, antigens might be absorbed into capillaries that drain into the portal vein and, in the liver, might lead to tolerance to these antigens, a phenomenon referred to as portal tolerance. Evidence for this mechanism was provided by performing a portacaval shunt in rats, a procedure that bypasses processing of antigen in the liver. This procedure led to abrogation of tolerance to a soluble protein antigen.[53] $NK1.1^+$ T cells were shown to be the mediators of

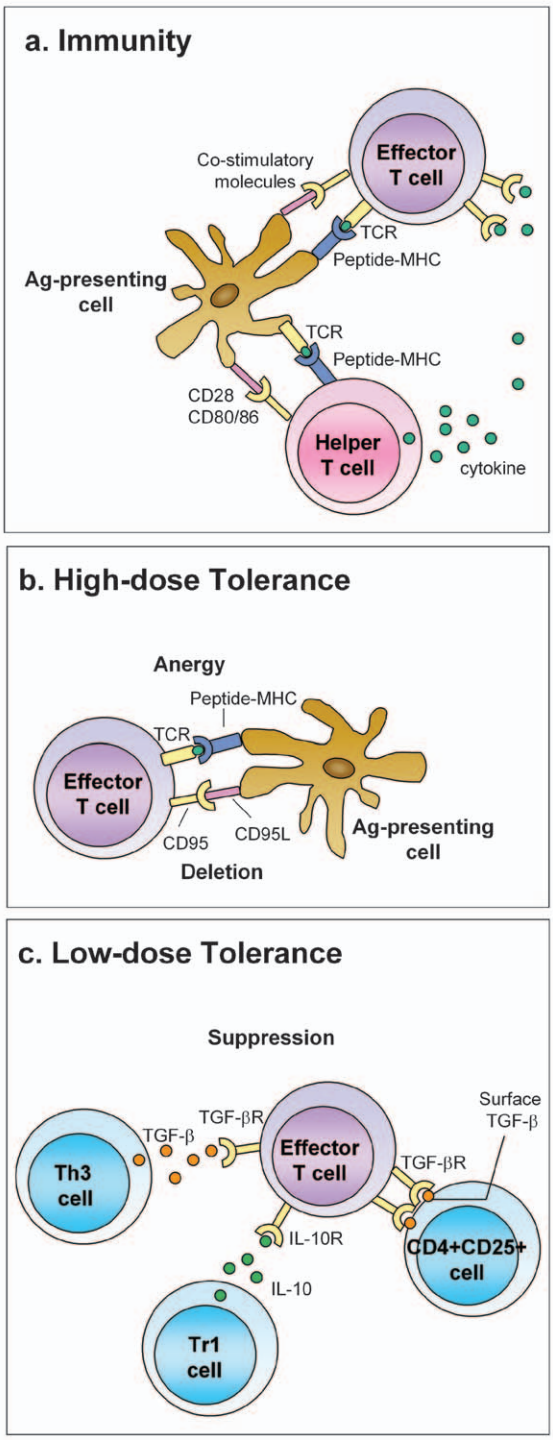

FIG 3. Mechanisms of oral tolerance. **a**, Generation of an immune response requires ligation of the T-cell receptor with peptide-MHC complexes in the presence of appropriate costimulatory molecules (CD80 and CD86) and cytokines. **b**, With high doses of oral antigen, T-cell receptor cross-linking can occur in the absence of costimulation or in the presence of inhibitory ligands (CD95 and CD95 ligand), leading to anergy or deletion, respectively. **c**, Low doses of oral antigen lead to the activation of regulatory T cells, which suppress immune responses through soluble or cell surface–associated suppressive cytokines (IL-10 and TGF-β). *L*, Ligand; *R*, receptor.

TABLE I. Regulatory T cells in the gut

$CD4^+$ cells
T_H3 cells → suppression mainly through secreted TGF-β
T_R1 cells → suppression mainly through secreted IL-10
$CD4^+CD25^+$ cells → suppression possibly through surface-bound TGF-β
$CD8^+$ cells
Natural killer T cells

tolerance by using an experimental model of colitis. Depletion of these cells exacerbated colonic inflammation in these animals.[54]

T_H3 cells

T_H3 cells were first described by Chen et al,[55] who administered low doses of myelin basic protein orally to animals and derived myelin basic protein–specific $CD4^+$ T-cell clones from the mesenteric lymph nodes of these animals. Adoptive transfer of these cells suppressed experimental allergic encephalomyelitis.[55] T_H3 cells produce TGF-β with various amounts of IL-4 and IL-10. TGF-β might play an important role in oral tolerance. As stated above, it also serves as a switch factor for IgA.[13] Furthermore, TGF-β was shown to reduce delayed-type hypersensitivity reactions in a murine model when this cytokine was administered systemically to mice at the time of antigen challenge.[56] Children with food allergy were found to have fewer TGF-β1–expressing duodenal lymphocytes in both epithelial and lamina propria compartments when assayed by means of flow cytometry.[57] Beyer et al[58] earlier demonstrated the lack of expression of TGF-β and IL-10 by milk-specific duodenal mucosal lymphocytes from children with milk-induced allergic gastroenteropathies when cultured *in vitro* in the presence of milk.

The regulatory cytokine TGF-β is not only produced by T_H3 cells but was also found to be expressed by epithelial cells of the duodenum and was shown by means of immunohistochemical staining to be decreased in children with different forms of food hypersensitivity, namely multiple food allergies[57] and food protein–induced enterocolitis,[59] although the latter group of patients studied had intestinal villous atrophy, which could have exaggerated the observed decrease.

T_R1 cells

T_R1 cells, first described by Groux et al,[60] secrete IL-10, which also drives generation of these cells. IL-10 was shown to suppress antigen-specific immune responses and prevent colitis in a mouse model. Chronic enterocolitis develops in IL-10–deficient mice.[61] IL-10 can be produced by gut lamina propria lymphocytes in response to orally administered ovalbumin in ovalbumin-transgenic mice.[62] IL-10 was also shown to be produced by cells in the PPs in an antigen-specific murine model of oral tolerance to β-lactoglobulin, a whey protein.[63] Further evidence of its role in oral tolerance was provided by Frossard et al,[64] who demonstrated the presence of IL-10 in PP cells from tolerant mice after β-lactoglobulin feeding but not in mice with β-lactoglobulin–induced anaphylaxis.

$CD4^+CD25^+$ cells

$CD4^+CD25^+$ regulatory T cells arise from the thymus as early as day 3 of life.[65] They are characterized by a naive phenotype, low proliferative capacity, and IL-2 production. $CD4^+CD25^+$ cells have been implicated in oral tolerance by using experiments in transgenic mice that express a T-cell receptor for an ovalbumin peptide. These mice had an increased number of $CD4^+CD25^+$ cells when fed ovalbumin, which expressed high levels of cytotoxic T lymphocyte antigen 4 and the regulatory cytokines TGF-β and IL-10. Adoptive transfer of $CD4^+CD25^+$ cells from fed mice suppressed *in vivo* delayed-type hypersensitivity responses in mice.[66] Further studies have shown that the immunosuppression by $CD4^+CD25^+$ cells is mediated by cell surface-bound TGF-β.[67] The importance of TGF-β in the suppressor function of $CD4^+CD25^+$ cells has been challenged, however.[68]

$CD4^+CD25^+$ cells also express the transcription factor forkhead box P3 (FOXP3).[69,70] FOXP3 is thought to ensure blockade of T_H1 and T_H2 induction.[71] Mutations of FOXP3 result in a fatal disorder in human subjects characterized by immune dysregulation, polyendocrinopathy, and enteropathy.[72]

The role of $CD4^+CD25^+$ cells in food hypersensitivity has not been thoroughly investigated yet. Karlsson et al[73] studied T-cell responses in allergic children who, after a milk-free period, had a cow's milk oral challenge. Children who outgrew their milk allergy had higher frequencies of circulating $CD4^+CD25^+$ cells and decreased *in vitro* proliferative responses to bovine β-lactoglobulin in PBMCs compared with children who maintained a clinically active allergy. Furthermore, depletion of $CD25^+$ cells from PBMCs of tolerant children led to an increase in *in vitro* proliferation against β-lactoglobulin, suggesting that mucosal induction of tolerance against dietary antigens is associated with the development of $CD4^+CD25^+$ cells.[73] Note, however, that the group studied consisted of patients with non–IgE-mediated milk allergy. No intestinal biopsies were performed to confirm that the gastrointestinal symptoms were indeed allergic in etiology in all patients.

FACTORS INVOLVED IN ORAL TOLERANCE INDUCTION

Several factors affect the induction of oral tolerance to a dietary antigen. Some are antigen related, namely the dose and nature of the antigen. Other factors are inherent to the host, including age, genetics, and intestinal flora (Table II).

Dose of the antigen

As detailed above, oral tolerance can be divided into 2 forms, high dose and low dose, each mediated by a distinct mechanism.

Much of the focus in food allergy in human subjects is on disturbances in low-dose tolerance mechanisms. Zivny et al,[74] however, described a state of tolerance to dietary proteins in human subjects through the mechanism of anergy. When human volunteers were fed antigens, such as ovalbumin and soybean protein, their peripheral blood humoral and T-cell proliferative responses were low, which is consistent with immune tolerance. Furthermore, PBMCs stimulated with antigens failed to express IL-2, which is consistent with T-cell anergy. However, when recombinant human IL-2 was added, T-cell proliferation to the antigen significantly increased. This experiment points to anergy as a possible mechanism of tolerance to antigens in the face of massive antigen challenge in the intestine. Whether disturbances in this tolerance mechanism are present in individuals with food allergy remains to be seen.

Form of the antigen

A soluble antigen is more tolerogenic than a particulate one. When mice were fed the soluble protein ovalbumin, their antigen-specific splenic T cells were unresponsive and secreted little, if any, IL-4 and IFN-γ, but they showed antigen dose-dependent T-cell proliferation and secretion of high levels of IL-4 when fed the same antigen in an encapsulated form.[75] This could, however, be due to the protection from acid and enzymatic degradation of the protein through encapsulation, as detailed above.

Another noticeable phenomenon related to antigenic nature is that of pollen-related food allergy. Individuals with pollen allergy frequently present allergic symptoms after ingestion of several kinds of plant-derived foods. This phenomenon is thought to be secondary to cross-reactions of human IgE antibodies directed against pollen allergens with homologous allergens in plant foods.[76]

Another factor influencing the development of food hypersensitivity responses is prior sensitization to that antigen through extraintestinal routes. This was observed in children with peanut allergy, whereby sensitization to peanut protein was thought to have occurred through the application of skin preparations containing peanut oil to inflamed skin.[77] Hsieh et al[78] demonstrated this phenomenon of food allergy induction by means of allergy exposure through the skin in a mouse model. Epicutaneous sensitization of mice to the egg protein ovalbumin induced a high level of ovalbumin-specific IgE. Subsequent oral challenge with ovalbumin resulted in symptoms of anaphylaxis with increased levels of plasma histamine, as well as histologic changes in the intestines.

Genetics of the host

Genes play an important role in oral tolerance and food hypersensitivity. This has been demonstrated in murine models of oral tolerance induction to the egg protein ovalbumin. Although tolerance was achieved in a variety of mouse strains, the magnitude of tolerance achieved was highly variable among the different strains.[79] These differences in immune responses were not secondary to differences in intestinal processing of the antigens because feeding ovalbumin to the 2 MHC-congenic mice BALB/c and BALB/b generated tolerogenic peptides in the serum of both strains, although BALB/b mice had much less tolerance of delayed-type hypersensitivity and humoral immunity than the BALB/c strain. The differences in immune responses are therefore likely to reflect a genetically determined defective recognition of tolerogens in the BALB/b strain.[80]

TABLE II. Factors involved in oral tolerance

Dose of the antigen
High-dose results in lymphocyte anergy/deletion
Low-dose results in activation of regulatory T cells
Form of the antigen
Soluble antigen is more tolerogenic than particulate
Genetics of the host
Normal flora of the host
Age of the host
Neonates have stronger immunologic reactions

The genetic influence of the development of food hypersensitivity was supported in a murine model of protein-induced anaphylaxis,[81] in which strain-dependent susceptibility to food allergy was observed. C3H/HeJ but not BALB/c mice exhibited anaphylactic reactions and antigen-specific IgE production on sensitization and intragastric challenge with cow's milk or peanut. Furthermore, splenocytes from the sensitized C3H/HeJ mice exhibited increased IL-4 and IL-10 secretion when cultured *in vitro* in the presence of the antigen, whereas splenocytes from BALB/c mice secreted significant amounts of IFN-γ. This strain-dependent difference in susceptibility to food allergy associated with differential T_H2 versus T_H1 reactions might, however, be influenced by the fact that C3H/HeJ mice lack functional toll-like receptor 4, a receptor for LPS. Toll-like receptor signals provided by intestinal commensal flora were shown to inhibit the development of allergic responses to food antigens in mice.[82]

In human subjects, studies examining potential associations of specific HLA antigens with allergies to different foods show variable results. No differences were observed when HLA-A, HLA-B, and HLA-C locus antigens were compared between patients with severe cow's milk allergy and control subjects comprised of healthy blood donors and cadaver kidney donors.[83] When individuals with peanut allergy and unrelated control subjects were typed for the HLA-class II DRB1, DQB1, and DPB1 loci by using PCR-based techniques, 3 class II genotypes, DRB1*08, DRB1*08/12tyr16, and DQB1*04, were found at higher frequency in those with peanut allergy than in control subjects.[84] These findings indicate that allergic reactions to peanut are at least in part under genetic control. Causality still needs to be demonstrated, however. Sibling comparisons would also be informative in these studies. They are difficult to perform, however, because individuals with peanut monoallergy versus

nonallergic sibling pairs would be needed, and peanut allergy rarely exists as an isolated manifestation of allergy.

Normal flora of the host

Early studies by Wannemuehler et al comparing germ-free and conventionally reared rodents have demonstrated that intestinal colonization by bacterial flora might affect the development of oral tolerance to sheep erythrocytes.[85] Daily oral administration of sheep erythrocytes induced a state of tolerance in conventional BALB/c mice but primed their germ-free counterparts for anamnestic responses when systemically challenged with sheep erythrocytes. This lack of oral tolerance was not found to be strain related. Note, however, that comparison between germ-free and conventional mice might not be very accurate because of inherent immunologic differences between them. PP cells in germ-free mice are less activated than in conventional mice in response to an antigen,[86] and germ-free mice lack IgA secretion in their intestines.[87]

Subsequent experiments by Moreau and Corthier[88] using ovalbumin as the antigen did not find intestinal microflora to be important when germ-free and conventional C3H/HeJ mice were compared. These conflicting results with those of Wannemuehler et al might raise the possibility that the difference relates to the different nature of the antigen used (ovalbumin is a soluble antigen, whereas sheep erythrocyte is a globular antigen).

Recently, however, intestinal flora were again demonstrated to be important in inhibiting allergic responses by using toll-like receptor 4–deficient mice and MHC-matched or congenic control animals.[82] In addition, Sudo et al[89] were able to demonstrate the importance of intestinal flora in oral tolerance induction by using reconstitution of bifidobacteria into germ-free mice. However, this was possible only when added during the neonatal period.

In human subjects the potential importance of commensal flora was raised in a clinical trial conducted in children with cow's milk allergy and atopic dermatitis.[90] Concomitant administration of *Lactobacillus* species and a protein hydrolysate formula to these children, as compared with the formula alone, improved eczema and possibly the intestinal barrier function, as measured on the basis of fecal α1-antitrypsin levels. No formal intestinal permeability testing was done in these children.

Age of the host

Age has been suggested to be important in both animal and human studies. Strobel and Ferguson[91] demonstrated that feeding a weight-related dose of ovalbumin within the first week of life results in priming of both humoral and cell-mediated immune responses, despite the profound tolerance found in adult animals when treated in the same way. In human subjects Eastham et al[92] demonstrated stronger immunologic reactions to dietary antigens in neonates during the first 3 months of life. Speculation relating to an increase in intestinal permeability being the underlying cause has been challenged because mature intestinal permeability is achieved within 4 days after feeds in newborns.[93] Differences in immunologic reactivity could be playing a role in these findings.

CONCLUSION

Oral tolerance to dietary proteins is crucial to prevent the development of food hypersensitivities. The mode of antigen uptake in the gut and different regulatory immune cells play a role in its maintenance, as demonstrated in multiple animal studies. In addition to intestinal epithelial cells acting as nonprofessional APCs, DCs, $CD8^+$ cells, a variety of regulatory $CD4^+$ cells, namely T_R1, T_H3, and $CD4^+CD25^+$ cells, play an important role in maintaining oral tolerance to low doses of antigen through suppression of immune responses. Other mechanisms are important in response to high antigen doses, including induction of lymphocyte anergy or deletion. A breach in any of these mechanisms has been demonstrated to result in loss of oral tolerance to an antigen in animal models. More mechanistic studies are needed to test whether these phenomena are similar in human subjects and to determine their relative importance in predisposing individuals to food hypersensitivities. Understanding processes leading to tolerance versus hypersensitivity to foods is crucial for the possible development of novel therapeutic strategies.

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