

# Oral tolerance: lessons on treatment of food allergy

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Oral tolerance is the active non-response by the immune system to an antigen administered through the oral route. It is postulated that food hypersensitivity results from a breakdown in oral tolerance induction, and the importance of oral tolerance in food hypersensitivity can be traced back to classic experiments from 1911 in which guinea pigs were protected from anaphylaxis by prior feeding of antigen. Host and antigenic factors play a role in determining the pathways and mechanisms to which a fed antigen can gain tolerance. Recent studies have demonstrated the potential of using oral tolerance to treat food allergies, and additional studies are necessary to further our understanding of mechanisms of oral tolerance

induction. *Eur J Gastroenterol Hepatol* 17:1299–1303 © 2005 Lippincott Williams & Wilkins.

*European Journal of Gastroenterology & Hepatology* 2005, 17:1299–1303

**Keywords:** oral tolerance, food allergy, food hypersensitivity, mucosal immunity

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Received 24 May 2005 Accepted 31 August 2005

## Introduction

Oral tolerance is the active non-response by the immune system to an antigen administered through the oral route. This phenomenon was first reported in 1829 by Dakin, who described Native Americans eating poison ivy leaves to avoid the contact hypersensitivity reaction to urushiol [1]. Tolerance, which can also be elicited by antigen administration via the anterior chamber of the eye [2], portal vein injection [3], or nasal [4] or airway mucosa [5], is in direct contrast to active cellular and humoral immune responses generated by antigens administered by cutaneous injury or subcutaneous or intramuscular injection [6]. Oral tolerance has been demonstrated in several animal models, including guinea pig [7], mice [8,9], rats [10], pigs [11] and dogs [12]. In humans, oral tolerance has been shown experimentally using keyhole limpet hemocyanin as a neo-antigen [13], and defective oral tolerance induction is seen in patients with inflammatory bowel disease [14]. While it is postulated that food allergy results from a breakdown in oral tolerance induction, defective oral tolerance has yet to be demonstrated experimentally in food allergic patients.

The centrality of oral tolerance to food hypersensitivity can be traced back to classic experiments by Wells in which guinea pigs were protected from anaphylaxis by prior feeding of hen's egg [7]. Insights into the mechanisms of oral tolerance have been gained using animal models and in-vitro systems; much of this study has been driven by the potential of utilizing oral tolerance as a treatment for autoimmune and chronic inflammatory conditions and, to a lesser extent, food hypersensitivity. In this review, we will provide a framework for understanding oral tolerance by outlining the major antigenic and host factors responsible for the induction of oral

tolerance. Data related to food hypersensitivity will be provided when available, and recent advances in oral tolerance research that impact food allergy will be discussed.

## Antigenic versus host factors

A review of specific antigenic and host factors is necessary for a discussion of the pathways to which a fed antigen can gain tolerance and the cellular and molecular constituents involved in oral tolerance (Table 1). A more comprehensive overview of the mucosal immune system can be found in recent articles detailing special anatomic features of the gastrointestinal tract relating to oral tolerance [15] and animal and human studies of oral tolerance [16].

## Antigenic factors: form and dose

### Form of antigen

After ingestion, food proteins are broken down by acids and proteases in the stomach and small intestine. While most proteins are degraded from immunologically recognizable forms into non-immunogenic free amino acids or short peptides, approximately 2% of proteins absorbed reach the intestinal lymph and portal circulation intact [17]. In animal models, digestion of proteins is an important part of oral tolerance induction. Direct administration of undigested bovine serum albumin (BSA) into the mouse ileum led to an active immune response, whereas administration of a peptic digest of BSA was tolerogenic [18]. Food-allergic mice fed antacid were found to have increased caviar-specific IgE and T-cell reactivity to caviar [19]. An experiment in which soluble ovalbumin was protected from digestion in a non-soluble encapsulated form illustrated that soluble antigen is more tolerogenic than particulate antigen [20].

**Table 1 Factors involved in the development of oral tolerance**

|                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Antigenic factors</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Form                     | <p>Digestive enzymes and acids break down antigen, leaving ~2% immunologically intact</p> <p>Soluble antigens are taken up by intestinal epithelial cells and are more tolerogenic</p> <p>Particulate antigens tend to be taken up by M cells overlying Peyer's patches</p> <p>Food allergy patients with IgE against linear peptide sequences are less likely to develop tolerance</p> <p>Food allergy patients with IgE directed towards conformational sequences often 'outgrow' their allergy or can tolerate cooked forms of food</p> |
| Dose                     | <p>High doses of fed antigen result in lymphocyte anergy or deletion</p> <p>Low doses result in activation of regulatory T cell populations</p> <p>CD4<sup>+</sup> T cell populations</p> <p>Th3 secrete TGF-<math>\beta</math></p> <p>Tr1 secrete IL-10</p> <p>CD4<sup>+</sup>CD25<sup>+</sup> have surface-bound IL-10</p> <p>CD8<sup>+</sup> T cells</p> <p>NK1.1<sup>+</sup> T cells</p>                                                                                                                                               |
| <b>Host factors</b>      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Age                      | <p>In animal models, neonates have increased intestinal permeability and positive immune responses to fed antigen</p> <p>Oral desensitization to food was successful in 15 of 21 children age 6 to 10, unclear if advanced age has an effect on oral tolerance induction</p>                                                                                                                                                                                                                                                               |
| Genetics                 | <p>Murine models of oral tolerance and food hypersensitivity vary by genetic strain</p> <p>C3H/HeJ mice lack TLR4, exhibit anaphylaxis to egg and peanut protein</p>                                                                                                                                                                                                                                                                                                                                                                       |
| Flora                    | <p>Mice raised in germ-free conditions are unable to generate oral tolerance in some studies</p> <p>Probiotics have been found to have a beneficial effect on eczema in infants</p>                                                                                                                                                                                                                                                                                                                                                        |

Protection from acid digestion may also affect the tolerogenicity of encapsulated antigens [21].

The site of absorption differs between soluble and particulate antigens. Antigens can be absorbed in the intestines by intestinal epithelial cells (IECs), microfold cells (M cells), dendritic cells or paracellular spaces. IECs form the majority of the single epithelial layer lining the intestine and take up soluble antigens by fluid phase endocytosis [22]. IECs can also serve as non-professional antigen presenting cells and selectively activate CD8<sup>+</sup> T cells, resulting in local immune suppression [23].

M cells are specialized epithelial cells overlying organized lymphoid structures distributed in the small intestine and rectum called Peyer's patches. M cells are adept at taking up particulate antigens or antigens for which surface receptors are expressed [24]. TGF- $\beta$  appears to play an important role in oral tolerance induction in the Peyer's patches, in addition to serving as a switch factor for the secretory immunoglobulin, IgA [25]. In a food allergy model, mice tolerized to  $\beta$ -lactoglobulin were

found to have higher numbers of antigen-specific IgA secreting cells in the Peyer's patches, increased TGF- $\beta$  and IL-10 production by Peyer's patch T cells, and higher fecal IgA levels compared to  $\beta$ -lactoglobulin sensitized mice [26]. The role of Peyer's patches in oral tolerance induction remains controversial since tolerance to soluble antigen has been demonstrated in mice lacking Peyer's patches [27].

Dendritic cells can be found in Peyer's patches, intestinal lamina propria and mesenteric lymph nodes [28–30], and play a pivotal role in balancing tolerance and immunity in the gastrointestinal tract. Dendritic cells can intercalate between IECs, sampling antigen directly from the lumen while preserving the integrity of the epithelial barrier [31]. Dendritic cells can serve as potent antigen presenting cells, but their role depends on the local cytokine milieu and co-stimulatory molecule expression [32,33]. In mouse models of milk hypersensitivity, adoptive transfer of splenic and Peyer's patch-derived dendritic cells from milk-allergic mice results in antigen-specific IgE responses in naïve mice [34].

Lastly, antigen can pass through paracellular spaces that are normally sealed by tight junctions. This may be important in food hypersensitivity since intestinal permeability was shown to be increased in animal models of anaphylaxis and gastrointestinal allergy [35,36]; this increased permeability seems to be due to disruption of tight junctions and the opening of paracellular spaces [37]. Increased intestinal permeability has also been seen in children with cow's milk-sensitive enteropathy [38].

The epitope on the antigen that is recognized is important in patients who 'outgrow' food allergy and become tolerant to the food that previously caused allergic reactions. In egg and milk allergy, patients with IgE antibodies directed against linear or sequential epitopes have persistent allergy whereas those with IgE to conformational epitopes tend to develop tolerance [39,40]. Digestion may play a role in the type of epitope presented to the immune system. This finding also has implications for patients who are able to tolerate cooked forms of food (such as egg or certain fruits), where breakdown of the antigenic structure allows for tolerance to be achieved. In pollen-related food allergy (or oral allergy syndrome), patients with allergic rhinitis are sensitized to tree, grass or weed pollens and have IgE cross-reactivity with homologous food allergens [41].

#### Dose of antigen

Different mechanisms of tolerance have been demonstrated in animal models depending on the dose of antigen used. Oral tolerance can be induced in mice after either a single high dose or repeated low doses of antigen [42]. High-dose oral tolerance can be mediated by one of

two mechanisms: Fas-mediated apoptosis [43] or induction of anergy due to T cell receptor ligation in the absence of co-stimulatory signals, such as IL-2 or CD28 interaction with CD80 or CD86 [44]. Low-dose tolerance is mediated by regulatory T cells. Three subgroups of CD4<sup>+</sup> regulatory T cells have been well characterized: Th3 cells [45], Tr1 cells [46] and CD4<sup>+</sup>CD25<sup>+</sup> T cells [47] (Table 1). Suppressor CD8<sup>+</sup> T cells have been shown to play a central role in oral tolerance induction [48], while the role of NK1.1<sup>+</sup> T cells in tolerance induction has been more recently elucidated [49].

Anergy as a mechanism of oral tolerance to food was described by Zivny *et al.* [50]. In that study, human subjects were fed food antigens such as ovalbumin and soy protein with resulting low antigen-specific IL-2 production and T cell proliferation, consistent with T cell anergy. In studies of oral desensitization protocols for patients with milk allergy and other food hypersensitivities, it has been postulated that tight control of antigen dose is a vital part of the success of these treatment regimens [51,52] (see below for discussion).

### **Host factors: age, genetics and normal flora**

#### **Age of the host**

Age of the host appears to be important in intestinal permeability and oral tolerance induction. Ovalbumin fed to newborn mice elicited immune responses, whereas adult mice, fed similarly, developed tolerance [53]. Also, in a rat model of multiple sclerosis (MS), early feeding of myelin basic protein led to worsening of MS-type symptoms, whereas feeding animals in adulthood (after 6 weeks) had a protective role [54]. It has been speculated that this response is related to increased intestinal permeability, which is supported by studies in which indomethacin-treated mice had increased intestinal permeability and a reversal in tolerance to ovalbumin [55]. However, other studies have demonstrated that rapid antigen entry into the bloodstream leads to enhanced oral tolerance [56]. It is not clear how much intestinal permeability is altered in infancy. One human study showed that mature intestinal permeability is achieved within 4 days of birth [57].

#### **Genetics of the host**

Studies in humans and in animal models have demonstrated the effects of varied genetic backgrounds on oral tolerance and food hypersensitivity. In mice, it is known that oral tolerance induction varies by strain [58]. In murine models of food hypersensitivity, C3H/HeJ mice, which lack functional toll-like receptor 4 (TLR4), are much easier to sensitize to cow's milk or peanut than BALB/c and other strains of mice [59]. TLR4 signals provided by intestinal flora were shown to inhibit development of allergic responses to food antigens in mice [60]. In humans, no differences were observed

when comparing HLA-A, HLA-B, and HLA-C haplotypes between patients with severe cow's milk allergy and control subjects [61]. In sibling pairs discordant for peanut allergy, no association with HLA class II alleles was found [62]. However, a family study of peanut allergic patients comparing HLA class II loci using PCR-based techniques revealed a higher frequency of three class II genotypes [63]. Also, in a study of monozygotic and dizygotic twins, heritability of peanut allergy was calculated to be 81.6%, a number similar to asthma (87%) and atopic dermatitis (74%) [64].

#### **Normal flora in the host**

Initial studies in animal models showed that mice raised in germ-free conditions were sensitized when fed sheep erythrocytes, a particulate antigen, whereas oral tolerance was induced in mice with normal intestinal flora [65]. However, this finding was not confirmed when using soluble ovalbumin, raising the possibility that the form of antigen played a role [66]. The role of intestinal flora in oral tolerance was re-emphasized when it was found that TLR4 deficient mice with normal flora and germ-free TLR4 wild-type mice are both more susceptible to food allergy sensitization when compared to wild-type mice with normal flora [60].

In humans, the role of intestinal flora was first raised in children with cow's milk allergy and atopic dermatitis [67]. Administration of *Lactobacillus rhamnosus* GG with protein hydrolysate formula led to skin improvement and improved intestinal barrier function compared to formula alone. Subsequent studies have shown a positive clinical effect of probiotics on eczema [68] but not asthma [69] and the efficacy of perinatal administration of probiotics to atopic mothers and infants up to 6 months of age [70]. An extensive review of probiotics was recently published [71] and a discussion of probiotics and food allergy is included in this issue.

### **Oral tolerance in relation to food allergy treatment**

Methods of achieving tolerance in patients with food allergy have for the most part proven unsuccessful, and the standard of care for most food allergic patients is strict avoidance of offending foods. Subcutaneous allergen immunotherapy has been attempted for peanut allergy. Four of six subjects showed improved tolerance to peanut; however, there was an unacceptably high rate of serious adverse events, and further investigation was halted [72].

Two recent studies have examined the use of oral desensitization protocols for treating IgE-mediated food allergy. Meglio *et al.* described a 6-month protocol to treat IgE mediated cow's milk allergy and, in their study, 15 of 21 children were able to reach the full dose of milk

(200 ml), and an additional three children were able to tolerate 40–80 ml [51]. Subjects with higher allergen-specific IgE levels were more likely to fail desensitization, and while prick skin test size to allergen decreased, allergen-specific IgE levels did not change significantly from the beginning to the end of the study. In comparing their protocol to previously published protocols, the authors speculated that dose of antigen played a key role (specifically, starting with extremely low doses led to a suppressive response). Patriarca *et al.* were similarly successful in desensitizing to cow's milk in a less well-characterized patient population; oral desensitizations to egg, fish and several fruits were also reported to be successful [52]. Attempted desensitization to peanut failed. Adults were included in this trial, and their overall success rate was similar to children although the rates of success were not necessarily matched when subdivided by food. The role of intestinal flora and administration of probiotics in these protocols has not been examined. These studies demonstrating successful induction of oral tolerance in food allergic patients are promising, and further studies are warranted to confirm these results and help gain mechanistic insights into how oral tolerance induction is occurring.

## Conclusion

Development of oral tolerance to food proteins is of central importance to prevent or treat food allergies. As demonstrated in animal models and limited human studies, host factors and antigenic factors play important roles in complex and interrelated ways in oral tolerance induction (Table 1). Additional studies are necessary to further our understanding of mechanisms of oral tolerance induction and develop treatments for food hypersensitivities.

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