

The immunologic basis for intestinal food allergy

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There has been considerable recent broadening of basic concepts of intestinal food allergy, in particular the importance of non-IgE-mediated mechanisms. The traditional emphasis on IgE-mediated allergy now appears inappropriate in light of current studies of the basic mechanisms of oral tolerance to dietary antigen and of increasing recognition of the requirement for early infectious challenge in the prevention of allergic sensitization. This major change in emphasis has been forced both by basic scientific studies and by recognition of novel patterns of food allergic disease within the pediatric population, in which rapid increase in food-allergic sensitization has been noted in the last decade and previously rare phenomena such as multiple food allergies and sensitization of exclusively breast-fed infants to antigens eaten by the mother have become commonplace. It is thus emerging that the possession of exaggerated IgE responses may not be the direct cause of food allergic sensitization but may ensure that such sensitization is clinically obvious. Those without such immediate responses have a complex of symptoms, including diet-responsive eczema and a marked disturbance of intestinal motility. The clear demographic links with socioeconomic privilege and relative protection from gastrointestinal infections concord with recent murine data suggesting an obligatory input from innate immune responses to the gut flora in the establishment of oral tolerance. *Curr Opin Gastroenterol* 2000, 16:552–557 © 2000 Lippincott Williams & Wilkins, Inc.

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Abbreviations

ECP	eosinophil cationic protein
Ig	immunoglobulin
MHC	major histocompatibility
NK	natural killer complex
TGF	transforming growth factor

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Early life exposures

There has been substantial increase in the incidence of allergies of all kinds within the pediatric population. For food allergies in particular there has been both real increase and important changes in the pattern of presentation. Previously rare allergies, such as to peanuts and sesame, have become commonplace as early-life dietary exposures have changed [1,2]. Genetic predisposition is clearly important, and a family history of atopic disease is clearly a potent risk factor. Several candidate genes have indeed been identified by positional cloning, most concerned with generation of IgE responses [3]. However, the results of a large population-based study from Iceland showed that an IgA concentration in the lower quartile of the normal range (*ie*, distinct from true IgA deficiency) is more strongly predictive of allergic disease than elevated IgE level [4]. A developmental delay in maturation of IgA (transient IgA deficiency of infancy) had previously been suggested as an important cause of allergic sensitization [5]. Importantly, early-life IgA concentrations—even in cord blood—are substantially higher in infants born in the developing world, where dietary and other allergies are rare, than in the developed world [6]. There is also increasing evidence that cytokines within breast milk may regulate the infant's mechanisms of tolerance, and they appear to differ in atopic mothers [7]. However, there have been no comparisons between developing and developed-world mothers.

Improvement in social conditions in an individual country may cause very rapid increase in childhood allergies, as was seen in the decade after German reunification and in Singapore [8,9]. Epidemiologic assessment of Italian military recruits suggests that exposure to foodborne and orofecal pathogens in childhood protects against sensitization to allergens [10•]. A rural upbringing, particularly with exposure to animals, protects against the development of allergies in both developed-world and developing-world children [11,12]. This suggests a dominant role for early environmental exposures in the determination of immune tolerance, rather than genetic predisposition. There is huge current interest in the links between innate immune responses to pathogens and the enteric flora and specific immune response to dietary and systemic antigens [13], with recognition of an obligatory role for the gut flora and probably a facilitatory role for bacterial pathogens in the establishment of immune tolerance [14,15].

Lack of appropriate early infectious exposure has been postulated for some years as a cause of the overall increase in allergies [16]. These changes may be occurring from very early in life, and there is evidence that even the initial intestinal colonization of the developed-world neonate has altered dramatically compared with infants born in the developing world, with reduced colonization by previously dominant species such as *Bifidobacteria* and evidence of frequent discordance between maternal and infant gut flora [17,18]. Infants born by caesarean section may show prolonged abnormality in composition of the intestinal flora and distinct alterations in systemic immune function [19,20]. Comparison of the gut flora in Estonian and Swedish children with allergies supported the concept that this evolutionarily recent change may be important, as allergic children from either country showed reduced lactobacilli and anaerobes but higher numbers of coliforms and *Staphylococcus aureus* [21]. Study of the 1970 United Kingdom national birth cohort suggests that alterations in infant handling, even on the first day of life, with increased exposures to nonfamilial microorganisms, may be associated with later allergic disease [22]. Thus consideration of the epidemiology of allergies and recognition of the central role of innate immune responses in tolerance appear to be forcing a change in the basic understanding of food allergy—away from focus on IgE and immediate hypersensitivity, towards a broader concept based on the initial acquisition and subsequent loss of oral tolerance, in which the mode of response (immediate or delayed) becomes a secondary consideration and in which nondietary exposures may be a critical determinant.

Genetic predisposition to food allergy

Although a history of other atopic diseases is common in food-allergic individuals, major genetic studies have so far only been carried out in classic atopy and thus may not be a true representation of susceptibility for food allergy itself. Studies of susceptibility to asthma identified regions on chromosomes 2q, 5q, 6q, 12q, and 13q that are consistently linked with atopic disorders, with candidate genes including the IL-1 cluster, the IL-4/IL-9/IL-13 cluster, the major histocompatibility complex (MHC), and the interferon- γ gene [23,24]. Intriguingly there appear to be several susceptibility regions shared with chronic inflammatory bowel disease, in which tolerance is lost to the enteric flora rather than dietary antigen [23], and individual regions shared with several other autoimmune conditions.

The results of epidemiologic studies point to a more central role for T-cell responses and MHC type in food allergy than previously recognized, even when the response appears to be a classic type I response. The results of a large community-based study of infants in

Australia and Southeast Asia identified cow's milk, egg, and peanut hypersensitivity as the most common food allergies in Australian infants but showed a wide variety of allergies in different regions of Southeast Asia [25•]. Although peanut hypersensitivity is also common in Indonesia, there is a very low incidence of peanut allergy in Malaysia, Japan, and the Philippines despite broadly similar exposures. Several other food allergies show clear difference with racial origin. However, little is known of MHC susceptibility to individual food allergies.

Mechanisms of IgE-mediated allergy

Although it is now recognized that non-IgE-mediated food allergy may be an important cause of chronic symptomatology, IgE-mediated mechanisms account for the majority of immediate hypersensitive reactions to foods. Transient IgE responses to foods can be seen in many healthy children, and these may thus not be clinically relevant [26]. However, there is little doubt that exaggerated IgE responses may be important in severe food allergies and anaphylaxis. There have been substantial advances in understanding the genetic basis and molecular regulation of IgE responses [27]. Isotype shift within the B-cell population towards IgE is directly affected by products of Th1 and Th2 T cells. The cytokine secretion pattern of Th1 cells (particularly IFN- γ and IL-2) directly affects B-cell isotype shift to limit IgE production, as do Th1-associated cytokines such as IL-12 and IL-18 [28]. By contrast, products of activated Th2 cells, particularly IL-4 and IL-13, directly promote IgE synthesis [29,30]. This striking Th1 effect may be important in explaining the protection against allergy afforded by childhood within the developing world, although Th2 responses are required for defense against helminths [31]. In addition to T cells, eosinophils, mast cells, and basophils may produce IL-4 and IL-5, providing the potential for autoamplification of mucosal Th2 responses [32]. Natural killer (NK) cells, a central component of the antiviral response, have been shown to produce IL-13 in murine studies. If confirmed in man, this may potentially contribute to the triggering of allergy in infancy by pathogens such as respiratory syncytial virus [33]. IL-4 production and Th2 cell differentiation may be promoted by surface molecules on antigen-presenting cells, such as CD86 (formerly B7.2) or CD30 ligand, and soluble C-C chemokines such as RANTES released by macrophages [13].

Class-switching to IgE in response to IL-4 and IL-13, whose receptors share a common α chain (IL-4R α), is mediated by a signal cascade involving Stat-6 (signal transducer and activator of transcription-6), and mice deficient in either IL-4R α or Stat-6 cannot mount IgE responses to antigen challenge [34,35]. This appears to be clinically relevant in humans, as gain-of-function

mutations in IL-4R α are associated with an increased atopic response [36,37]. In addition to cytokine-mediated signaling, direct cell-cell contacts are also required for induction of IgE synthesis. In particular, signalling through cell-surface CD40 is required for IgE class shifting, and deficiency of CD40 or its ligand CD40-L is the molecular basis of hyper-IgM immunodeficiency [38].

The nature of the allergen or pathogen may itself direct towards either a Th1 or Th2 response. Bacterial pathogens such as tuberculosis contain sequences of DNA with repeated cytosine and guanine residues (CpG repeats) that induce production of IL-12 by antigen-presenting cells and thus skew responses towards Th1 [39]. Intriguingly, dietary nucleic acids may have a similar effect in skewing towards Th1 responses and supplementation of nucleic acids in the diet of BALB/c mice reduced both circulating and ovalbumin-specific IgE and IgG1, as well as decreasing IL-4 and increasing IFN- γ secretion *in vitro* [40•]. By contrast, allergens are clearly likely to skew responses towards Th2; suggested mechanisms include enzymatic activity (a characteristic of many allergens), as the *Der p 1* allergen from house dust mites causes loss of the low-affinity IgE receptor CD23, which negatively regulates IgE production, from the B-cell surface [41]. In addition, the strength of signal transduction from the T-cell receptor may determine ultimate Th1 or Th2 polarity of precursor Th0 cells, and peptides with either an extremely strong or extremely weak affinity for MHC molecules, or present at very low concentrations, would tend to favor Th2 responses [42].

There is important evidence of compartmentalized IgE responses within both the intestine and lung, with transportation of mucosally-produced IgE into the gut lumen or airway by a IL-4-dependent mechanism currently unknown but distinct from the polyimmunoglobulin transporter that mediates IgA secretion [43,44]. This compartmentalized response may partly explain the negative skin prick tests or RASTs in patients with a clear history of rapid onset responses to dietary antigens. In addition, there is murine evidence of an IgE-independent pathway through which anaphylaxis can be triggered by IgG1 bound to Fc γ RIII [45].

Mechanisms of oral tolerance

Food allergy requires by its nature a breakdown of oral tolerance, in which systemic immunologic tolerance to an antigen is induced by taking it orally. The molecular mechanisms of oral tolerance to dietary antigens have become increasingly understood. The dose of ingested antigen appears to be critical in determining the means by which tolerance is maintained [46,47,48]. Tolerance for high doses of antigen occurs through anergy of potentially responsive T cells. This may reflect antigen presentation by gut epithelium in the absence of costim-

ulatory ligands, or active suppression of the lymphocytes by suppressor cell populations or regulatory cytokines [49]. This form of tolerance may be abrogated by breakdown of the epithelial barrier and presentation of dietary antigen by activated antigen-presenting cells in a proinflammatory context; it is probably not coincidental that infant sensitization to cow's milk formula often occurs in the aftermath of gastroenteritis. It has also been demonstrated that feeding extremely high doses of dietary antigen to mice induces apoptosis of antigen-specific lymphocytes within Peyer patches, but this has not yet been demonstrated in humans [50]. By contrast, tolerance to low-dose antigen is an activation-dependent process and appears to occur within Peyer patches, in which antigen-specific regulatory lymphocytes producing transforming growth factor- β (TGF- β) are generated (Th3 cells). Both Th3 cells and other regulatory lymphocytes producing IL-10 (Tr1 cells) play a critical role in suppressing immune reactivity within the intestine by a process termed "bystander tolerance," in which they home to the intestinal mucosa and release TGF- β or IL-10 upon encountering antigen, thus suppressing potential reactivity of all surrounding lymphocytes [51,52]. If this process breaks down, immunologic tolerance may be lost and allergy or gut inflammation the consequence. Importantly, both gut colonization and local inflammation appear to be required to establish oral tolerance. Mice maintained germ-free may not establish normal enteric tolerance for antigen [14]. In a remarkable study using transgenic mice, possessing only T cells reactive to a class II MHC-restricted peptide in hen egg lysozyme, Newberry and colleagues [53••] demonstrated an obligatory role for physiologic inflammation in the establishment of oral tolerance. These mice were fully tolerant to dietary hen egg lysozyme in normal conditions. However, when production of prostaglandin E2 (PGE2) was prevented by cyclooxygenase-2 (COX-2) antagonists, tolerance was abrogated and enteropathy developed in response to antigen ingestion. Thus PGE2 appears to be a pivotal molecule in prevention of immune reactivity to dietary antigens. Production of PGE2 occurs in lamina propria macrophages in response to the enteric flora, and it functions as a potent inducer of IL-10 production in lymphocytes [53]. In turn, IL-10 is now recognized to play an obligatory role in the generation of mucosal suppressor cells, and the CD45RB^{low} T-cell population, potent regulatory cells in wild-type mice, cannot prevent inflammation in IL-10-deficient mice [54••]. As this population produces TGF- β , and may be a murine equivalent of human Th3 cells, this raises intriguing questions about the links between these two molecules in the maintenance of normal enteric tolerance. Somewhat contrasting results, in which blockade of IL-10 did not prevent TGF- β -mediated oral tolerance in experimental colitis, were provided in an impor-

tant study in which mice were treated by intranasal administration of a plasmid encoding active TGF- β 1 [55••]. In this study it was evident that TGF- β 1 induced mucosal IL-10 production and attenuated Th1 responses by downregulating IL-12 signalling. This whole area of basic research, involving both intestinal inflammation and allergy because of shared tolerance mechanisms, may eventually provide a link with the findings of increased food allergic sensitization in privileged children. It is probable that a lack of appropriate early immune priming through infectious exposure may specifically hinder the development of normal gut tolerance. However, the induction and regulation of Th3 cells and Tr1 cells remain unclear, and studies of the links between innate and adaptive immune mechanisms in human infants are awaited. It will be intriguing to see whether the therapeutic effects of probiotics in some children with food allergies are mediated through such tolerance mechanisms [56,57].

Multiple food intolerance, food-allergic dysmotility, and the eosinophil response

In addition to clear increase in IgE-mediated and non-IgE-mediated food allergies, a remarkable alteration in disease presentation has been noted in several countries, where increasing numbers of infants are now sensitizing to multiple antigens despite exclusive breastfeeding, often within the first weeks of life. [3,25•,58–61]. This was rare a generation ago and is still almost unknown in the developing world. These children represent a major clinical challenge, and extensive dietary exclusions are often required. There is a clear association with eczema, food-allergic colitis, or enteropathy, and these infants demonstrate a prominent disruption of intestinal motility. This pattern of disease suggests a primary failure to establish basic oral tolerance mechanisms, rather than the loss of previously acquired tolerance of classic food allergy. It is notable that many are intolerant of hydrolyzed whey or casein formulas, also previously rare, and require an amino acid formula [3,25•,58–63].

There is now increasing evidence that both gastroesophageal reflux and constipation may be features of the food-allergic dysmotility syndrome, which may present as infant colic [64] and then continue well beyond infancy to cause chronic symptoms that may require prolonged dietary exclusions [3,25•,63,65–67]. There are so far few studies of food-allergic dysmotility in adults, but this area may be directly relevant to irritable bowel syndrome in atopic patients.

The histologic characteristic of food-allergic dysmotility is local eosinophilic infiltration. Elevated levels of eosinophil cationic protein (ECP), along with TNF- α , were detected in the stools of infants with diet-responsive eczema [68].

By contrast, fecal ECP does not increase after oral antigen challenge in children or adults with immediate hypersensitive responses, although TNF- α and other mast cell activation products such as tryptase may be detected [69,70]. Thus a demonstration of eosinophil activation in the immediate response to dietary antigen in humans is lacking, although the time course of these collections may miss late recruitment of eosinophils upon chronic administration. Clearly eosinophil accumulation within the mucosa is a frequent finding in food allergies [71], although there are no standardized diagnostic criteria. The results of an important murine study suggest a critical role for the eosinophil chemokine eotaxin in the dietary antigen-specific recruitment of eosinophils within the intestine [72••]. Challenge of mice sensitized by peritoneal injection of ovalbumin mice with oral administration of ovalbumin-coated beads resulted in an allergen-specific Th2 response, with increased IL-4 and IL-5 production and generation of allergen-specific IgE and IgG1, together with mucosal eosinophil recruitment. In eotaxin-deficient mice, mucosal eosinophilia did not occur, although increased circulating numbers were seen. By contrast, IL-5 deficient mice showed a partial recruitment of mucosal eosinophils but reduced circulating numbers. Both IL-5 and eotaxin-deficient mice showed similar antigen-specific IgE and IgG1 responses to the wild-type mice. In a separate study, monoclonal anti-IL-5 antibody inhibited eosinophil recruitment after ovalbumin challenge [73], whereas unattenuated challenge caused upregulation of IL-5 mRNA within the lamina propria over a time course of several hours. These studies confirm a role for both IL-5 and eotaxin in eosinophil recruitment in late-phase food allergic responses in rodents. In humans, there is also evidence of increased IL-5 mRNA in the gut mucosa of food allergic patients but not in atopic or nonallergic controls [74•]. This IL-5 dependent pathway of eosinophil recruitment thus appears to be a specific characteristic of the delayed hypersensitive response to dietary antigen, and it will be important to see whether this phenomenon holds in those patients with non-IgE-mediated food allergies.

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