

Development of Immunological Tolerance to Food Antigens

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KEY CONCEPTS

- Oral tolerance to ingested antigens is an active immunological process, mediated through two primary effector mechanisms, low doses favoring the induction of regulatory T-cells, and high doses promoting antigen driven tolerance.
- Early T-cell responses are subject to a broad range of regulatory mechanisms which are dictated by the concentration, frequency, and route of allergen exposure as well as age and developmental status of the individual at the time of exposure.
- Genetic risk of allergy is associated with delayed postnatal maturation of immune regulation.
- Interaction with microbes, especially the normal gut microbiota, is the principal environmental signal for postnatal maturation of T-cell function. Oral tolerance, for example, does not develop in germ-free animals and they also have other signs of impaired immune regulation.
- Immunological interaction between the mother and her offspring, during gestation and through breast milk, may play a significant role in tolerance induction.

Introduction

The prevalence of allergic diseases has increased progressively since the 1960s, in particular in First World countries, and the rises appear to be continuing in many countries. The most prominent increases have occurred in allergy to inhaled antigens. However, there is evidence to suggest that at least some forms of food allergy are also increasing. The most notable example is peanut allergy, which was previously rare in many countries. The increase may be due to altered dietary habits in previously unexposed populations coupled with changes in processing procedures resulting in increased allergenicity of peanut antigens, but the issue remains unresolved.

This chapter focuses primarily on the underlying immunological mechanisms governing host responses to ingested antigens. The focus will initially be on what has been learned from basic studies in animal models, what has been deduced from extrapolation of these systems to immunocompetent human adults, and then what is known of the immunology underlying sensitization to dietary versus inhalant allergens during childhood.

Immunological tolerance to dietary antigens in experiment animals: the phenomenon of oral tolerance

The first formal description of experimental oral tolerance (OT) dates back to the work of H.G. Wells in 1911 [1]. His studies in the guinea pig involving repeated feeding of egg white protein (ovalbumin; OVA) are prototypical of several generations of subsequent laboratory research viz. repeated antigen feeding of immunologically naive animals elicited initial “hypersensitivity” responses which in some animals resulted in fatal anaphylaxis, but in the majority the symptomatology was transient and was followed by permanent unresponsiveness to the antigen. We now recognize the initial hypersensitivity manifestations as a hallmark of the “Th2 default” response of the mucosal immune system to a soluble protein antigen [2], and the ensuing state of (antigen specific) unresponsiveness as indicative of the subsequent onset of immunological tolerance (i.e. OT).

It is recognized that most aspects of the adaptive immune response may be experimentally downregulated by antigen feeding, in many cases via a single dose. The range of susceptible immunological phenomena include cellular immunity measured as delayed-type hypersensitivity (DTH [3–5]), contact sensitivity [6], cytotoxic CD8+ T-cell responses [7,8], production of cytokines [9], and antibody

secretion [10]. There is some evidence that local secretory IgA responses to the eliciting antigen may be preferentially “spared” in this tolerance induction process via generation of IgA T-helper cells in gut associated lymphoid tissues during tolerogenesis [11]. This may be part of a generalized mucosal protection process, since IgA antibodies would prevent antigen penetration through the gut wall and thus limit the scope for allergic sensitization.

Many host and environmental factors have been identified as partial determinants of susceptibility to OT induction. Prominent among host factors are hormonal balance [12], genetic background [13], and in particular postnatal age. A transient temporal window defining increasing susceptibility to sensitization to dietary antigens is operative in the mouse during the early postnatal period [14–16]. This seems to be due to poorly developed immune regulation, both at the levels of T-regulatory and antigen-presenting cells (APC) [17,18]. Host immune competence is a key feature underlying efficient OT, as demonstrated by the fact that administration of cytotoxic immunosuppressants seriously compromises the process [4].

Additional exogenous factors which interfere with OT induction include inflammatory adjuvants [19], low dose irradiation [3], and changes in host microbial flora [20,21].

The type and dose of antigen are also important factors in the induction of OT. While tolerance can be readily induced to all thymus-dependent soluble protein antigens, replicating and particulate antigens tend to induce active immunity. The inflammatory response elicited by replicating antigens bypasses OT mechanisms, and this can be mimicked with inert soluble protein antigen by coupling to adjuvants and/or microbial toxins [22,23]. The tolerogenic dose range for some antigens can also in some situations be within the immunogenic range for others, as shown in recent studies contrasting cow's milk whey proteins and OVA [18]. It is also noteworthy that while OT can be induced over a wide range of dosages and using varying feeding frequency regimes, continuous exposure leads to the most profound tolerance [24,25]. Furthermore, very low doses below the tolerogenic range can in some circumstances prime animals for subsequent immune responses [26].

Antigen presentation and processing in oral tolerance induction

Several pathways are operative in sampling and processing of ingested antigen, and it is possible that the particular pathway which is dominant within an individual immune response may ultimately determine the nature of ensuing immunity. There are three principal pathways for sampling of antigens from the luminal surface of the gut: between lining epithelial cells, through the epithelial cells themselves, or via M-cells with subsequent delivery into Peyer's patches (PP). In each situation, distinct populations of

potential APC will be encountered, and it is not clear what the contribution of each is in the OT process.

The full range of known professional APC have been identified throughout the gut wall and associated lymphoid tissues, comprising dendritic cells (DC), macrophages, and B-cells [27]. Many sites contain multiple APC populations. For example, at least three populations of DC with APC activity have been defined in PP [28–30], one of which has been proposed to selectively prime T-cells for IL-10 production [28].

Gut-derived DC are currently the focus of intense interest, as potential candidates for generation of the rate-limiting tolerogenic signal(s) in the OT process. In other organ systems, DC are recognized as the ultimate regulators of the immune response [17,31]. These cells are the most potent APC for activation of T-cells in primary immune responses, and they are increasingly being implicated in regulation of tolerance to self-antigens and to exogenous antigens. Administration of the growth factor Flt3L to mice markedly expands the numbers of DC in the intestine and associated lymphoid tissues, and at the same time increases susceptibility to OT induction [32].

An additional antigen presentation pathway which may also contribute to OT development involves the direct absorption into the circulation of breakdown products of ingested antigens (i.e. low molecular weight peptides) which are potentially tolerogenic [33,34].

Cellular mechanisms governing the induction and maintenance of oral tolerance

There are two primary effector mechanisms of OT. The first mechanism involves the induction of regulatory T-cells that mediate active suppression and the second is caused by clonal anergy. Low doses of antigen favor the generation of regulatory T-cell driven tolerance, whereas high doses of antigen promote antigen driven tolerance [35].

The initial evidence that OT is an active process came from studies involving adoptive transfer of OT to naive recipients via CD8+ splenocytes from tolerant donors [36]. However, this maneuver failed in many laboratories until careful studies revealed that several apparently distinct mechanisms are operative at the two extremes of the antigen exposure dose-response curve [35]. At one end of the spectrum, high-dose oral antigen exposure leads to functional elimination of antigen-specific T-cells via either deletion or anergy induction.

An alternative to deletion is T-cell anergy induction, in which antigen-response cells are functionally paralyzed. This also occurs in the high-dose range, most likely via aberrant antigen presentation by MHC class II-bearing APC which lack key costimulator molecules, such as CD80/CD86 [37]. These anergized cells do not apoptose and instead persist at the periphery. They presumably maintain their surface antigen receptors, but lose the capacity to clonally expand and secrete

the full repertoire of cytokines following encounter with antigen. In particular, in some model systems cytokine secretion by anergized T-cells appears limited to IL-10. Consistent with the potential importance of this mechanism, evidence exists in some models of OT for the presence of tolerized T-cells whose ability to respond to antigen *in vivo* can be restored by exogenous IL-2 [38]. Further supporting evidence has been provided in transgenic mice [39].

In contrast, exposure of animals to low dose oral antigen is proposed to induce a form of “low zone tolerance” involving active antigen-specific suppression of immunity which can in some systems be adoptively transferred by CD8⁺ T-cells, and by CD4⁺ T-cells in others [40]. However, it should be noted that OT induction proceeds normally in CD8-knockout mice [7], suggesting that the role of these cells may be restricted to maintenance, rather than induction of OT. There is additional evidence to suggest that a subset of TcR γ/δ T-cells may also participate in the OT process [41].

Currently, much of the OT literature is focused on CD4⁺ T-regulatory cells and the cytokines they secrete. Two principal subtypes have been identified (i.e. Th3 cells which secrete TGF β with or without IL-10 [42,43], and Tr1 cells which secrete IL-10 [42]). IL-4 has also been implicated in the OT inducing activity of Th3 cells; however, its role may be restricted to that of a non-essential growth factor [44] given that IL-4-knockout mice can still generate OT [45].

TGF β has many roles in the control of epithelial growth and differentiation and in local control of secretory IgA production in the gut [46]. This may explain the selective preservation of secretory IgA antibody production during systemic OT induction [47]. Additionally, TGF β has a number of potent immunosuppressive effects [46], including those targeted at APC functions. IL-10 is recognized as a powerful anti-inflammatory and immunomodulatory agent which plays a critical role in local homeostasis in the gastrointestinal mucosa, particularly via its damping effects on Th1 activity [27,48].

One interpretation of these findings is that many of the mechanisms defined experimentally may represent redundancies. However, it is more likely that each constitutes one component of a multi-layered integrated regulatory process, each of which is operative as dictated by prevailing conditions of antigen dosage and exposure frequency [18]. An intriguing additional possibility is that some of the T-regulatory populations may in fact be partially anergized T-cells, which despite functional downregulation have conserved their ability to secrete certain cytokines, such as IL-10 and TGF β .

Oral tolerance in humans: How well do mouse models mimic the human situation?

As noted above, the baseline “default” response of laboratory mice appears biased toward a Th2-like cytokine profile,

admixed with TGF β production [2]. This Th2 default is also consistent with the pioneering studies of Wells in guinea pigs [1], wherein the first manifestations of immunological reactivity in a subset of his test animals during feeding was what we now recognize as anaphylaxis. However, this Th2 bias is clearly a transient state in most situations, given the fact that the hallmark of successfully induced OT is tolerance in the IgE antibody class.

Selective priming of T-cells in PP for production of IL-4 and TGF β is a hallmark of this bias toward baseline Th2 in murine immune responses to oral antigen [49,50]. These cells migrate to peripheral sites and function as T-regulators to dampen Th1 immunity [51]. Such studies have not been performed in man. However, studies indicate that unlike the murine situation, the immunological milieu in human gut associated lymphoid structures such as PP is strongly Th1 biased [52], at least in adults. Thus, freshly isolated T-cells from the human gut lamina propria produce high levels of IFN γ relative to IL-4, IL-5, and IL-10 [53,54], and high levels of IL-12 production are observed in human PP [52,55]. This difference between mouse and humans may be a direct reflection of the markedly differing levels of microbial stimulation in humans versus specified pathogen-free mice housed under controlled conditions and fed exclusively sterilized food [52]. Under extreme conditions (i.e. in germ-free mice) OT does not even develop [20].

While these observations suggest significant differences between human immune responses to oral antigens and those seen in mice under experimental conditions, it is clear from several lines of evidence that the fundamental process of OT nevertheless occurs in man. Notably, feeding of keyhole limpet hemocyanin to immunologically naive volunteers selectively downregulated subsequent cellular immune responses to the antigen [56,57]. Furthermore, prospective studies have shown transient IgE antibody production to foods to be common during the first 2 years of life and then downregulated [58]. Also deliberate parenteral immunization of volunteers with the common dietary antigen bovine serum albumin elicited little or no antibody production [59]. Additionally, clinical trials aimed at amelioration of autoimmune disease by autoantigen feeding have provided varying levels of clinical effects [60], and have also provided evidence of the induction of Th3 responses in blood lymphocytes which are comparable to those reported in murine model [43].

Comparative studies on T-cell responses of PP-derived T-cells (PPT) versus peripheral blood T-cells (PBT) from normal subjects indicated consistent lymphoproliferation in PPT in response to the dietary antigen β -lactoglobulin (BLG), in contrast to low/non-responsiveness in PBT [55]. Moreover, the peripheral blood mononuclear cell responses were dominated by Th1 cytokines [55], mirroring the overall Th1-biased milieu of gut associated lymphoid tissues [49]. This apparent OT at the periphery with the concomitant presence of antigen-specific IFN γ -secreting T-cells in lymphoid compartments

draining the intestinal mucosa, mimics precisely the situation reported for mice fed repeated doses of OVA [9], and may be indicative of the contribution of locally activated regulatory T-cells in the maintenance of systemic tolerance. It is clear that more fundamental studies are needed on human immune responses to dietary antigens, and in particular on the potential interactions between these and parallel responses to microbial stimuli provided by the local commensal flora.

Food allergy in humans: the clinical reality

Dietary antigen-induced enteropathies are believed to be central to the pathogenesis of a broad range of chronic inflammatory diseases in humans, including IgE-mediated food allergy, celiac disease, inflammatory bowel disease, and other enteropathies. The discussion below will focus on allergic-like manifestations of aberrant immunity to dietary antigens, in particular issues relating to the initiation of dietary allergies in early life. From a clinical point of view it is important to appreciate that similar clinical symptoms may be induced by immune reactions, various biochemical intolerances and toxic reactions. Thus, various food intolerances caused by non-immunologically mediated mechanisms are often erroneously called “food allergy”. For example, the majority of adults in the world are lactose intolerant. Drinking milk may induce symptoms that may be interpreted as evidence of food allergy.

Allergy to food antigens in an infant is often the first manifestation of atopy and may be the forerunner of subsequently developing IgE-mediated allergy to inhalant allergens. IgE-mediated food allergy and atopic dermatitis in infancy may thus be the first steps in the “atopic march” [61–63]. In most instances, clinical tolerance to the food develops within the first 3 years of life; however, the atopic march continues with manifestations of allergic asthma and subsequently allergic rhinoconjunctivitis. The reasons for this switch from the gastrointestinal to the respiratory tract are unknown.

Clinical tolerance does not develop equally to all food allergens. For unknown reasons, IgE-mediated allergy to cow’s milk and egg are uncommon after the age of 4 years, while allergy to soy protein tends to last for a longer time period, peanut allergy usually for many years, and celiac disease is regarded as a lifetime condition which will relapse at any age if the individual is exposed to gluten for some time.

The resolution of clinically manifest food allergy is accompanied by the development of tolerance to food antigens. This process has mostly been studied for allergens causing IgE-mediated reactions, while less is known regarding, for example, the kinetics of immune responses to antigens involved in the pathogenesis of celiac disease and other enteropathies. Prospective studies, in which immune responses to food antigens have been studied through the first several years of life, show that transient IgE antibody responses to, for example, egg and cow’s milk are common

in healthy non-atopic infants [62]. In contrast, the IgE antibody responses are of higher magnitude and more prolonged in infants who develop food allergy and/or who will manifest respiratory allergy later during childhood. Indeed, high levels of IgE antibodies to egg or milk in an apparently healthy infant predict the appearance of respiratory allergy some years later [61–63].

Cellular mechanisms underlying control of T-cell immunity to dietary antigens in humans: lessons from studies on responses to aeroallergens

As noted above, studies on antibody production indicate that immune responses against environmental allergens are initiated very early in life in most individuals. These observations have prompted detailed investigations in many laboratories on the nature of underlying T-cell immunity during this life phase. The salient findings are reviewed below.

Firstly, it is clear that T-cells responsive to both dietary and inhalant allergens, as measured by lymphoproliferation and cytokine secretion, are present in cord blood from virtually all subjects [64–67]. Additionally, T-cell cloning and subsequent genotyping studies indicate that the responsive cells are of fetal origin and exhibit a Th2-polarized and/or Th0 cytokine profile [68]. It has been suggested [65,67] that these T-cells may have been primed by processed antigen crossing the placenta, perhaps bound to maternal IgG. Evidence showing the presence of detectable levels of allergen in complex with IgG antibodies in cord blood supports this suggestion [69]. However, it is also feasible that these T-cell responses may be directed against cross-reacting antigens or anti-idiotypic antibodies.

Secondly, it is evident that these early T-cell responses are subject to a variety of regulatory mechanisms post-natally, which are driven by direct exposure of the infant immune system to incoming environmental allergen. Given the experience from animal models, it is likely that a broad range of regulatory mechanisms will be involved, which are dictated by the concentration, frequency and route(s) of allergen exposure, the age and hence developmental status of the individual at the time of exposure, and potentially by allergen structure (e.g. susceptibility to proteolytic degradation). The relevant immunoregulatory mechanisms involved are likely to span the full range from classical low zone tolerance (essentially Th1/Th2 cross-regulation) through to high-zone tolerance phenomena (anergy and/or deletion via apoptosis), and will inevitably include important, but as yet uncharacterized contributions from recently described subsets of T-regulatory cells which appear to participate in a wide range of immunological control mechanisms.

Cross-sectional and prospective studies indicate that in atopic children, consolidation of Th2-polarized immunity against inhalant allergens is initiated in early infancy

[64,70,71] and may be completed by the end of the pre-school years in children who develop clinically manifest allergy [72], or even earlier [64]. In contrast, in infants who develop allergic manifestations, low level Th1 responses are established [64]. Prospective studies from Estonia, with a low, and Sweden with a high prevalence of allergy, indicate that the regulatory mechanisms are established more rapidly and that also other mechanisms may be operative [64,73,74]. This is suggested by the observation that during the first 2 years of life, the incidence of positive skin prick tests was similar to that in recent studies from Western Europe, while at 5 years the prevalence was only 3%. At the same time the prevalence of circulating IgE antibodies to milk or egg increased to 36% and 47% to inhalant allergens. The discrepancy between positive skin prick tests and circulating IgE antibodies is interesting in a country with a low prevalence of atopic allergy, as well as Th1-dependent type I diabetes and a lifestyle similar to that prevailing in Scandinavia some 30–40 years ago. The findings may also suggest that clinical tolerance to a food does not exclude the presence of IgE antibodies and possibly other indicators of Th2 immunity. It is possible that a traditional lifestyle is associated with an early induction of a general regulation of T-cell immunity. This notion is supported by the close correlation globally between the prevalence of wheezing and type I diabetes [75].

In contrast to what appears to be positive selection for different forms of active T-cell immunity against inhalant allergens during infancy, the majority of subjects manifest active downregulation of T-cell responses to dietary allergens such as egg, as demonstrated by diminishing lymphoproliferative responses and by a progressive reduction in the number of egg-specific T-cell epitopes recognized *in vitro* [70,71]. This finding suggests the operation of control mechanisms akin to high-zone tolerance (anergy/deletion) in the mouse, although additional regulatory pathways may operate in parallel.

Microbial stimulation in the gastrointestinal tract as a potential modulator of human oral tolerance

Genetic risk for allergy is associated with delayed postnatal maturation of T-helper cell function [76]. Both the cloning frequency of CD4⁺ T-cells and the capacity of cloned CD4⁺ T-cells to secrete IFN γ and IL-4 were reduced in infants with positive atopic family history (AFH⁺) and the reduction was most marked in IFN γ , indicating an overall Th2 bias in T-cell function in this group [76]. Several groups have since reported similar findings in cord blood, both in countries with a high- and low-allergy prevalence [64,77–80]. It is recognized that T-cell function in fetal life is constitutively Th2 based, as part of a set of control mechanisms to limit potential damage to the placenta via toxic Th1 cytokines [81,82], and the more pronounced Th2 bias in AFH⁺ children may

reflect inappropriate persistence of one or more of these control mechanisms after birth [83].

It is also recognized that interaction with microbes, especially the normal microbial flora of the gastrointestinal tract, is the principal environmental signal for postnatal maturation of T-cell function (in particular the Th1 component) [84,85]. Recognition of these signals is mediated by a series of Toll-like receptors expressed on cells of the innate immune system, and other receptors such as CD14, and it is noteworthy that a polymorphism in the CD14 gene has been associated with high IgE levels [86].

International studies have drawn attention to the wide variations in allergy prevalence between different countries [87–89], and a variety of evidence suggests that these changes have been particularly pronounced over the last 30–40 years [90,91]. Variations in patterns of microbial colonization of the gastrointestinal tract, linked with lifestyle and/or geographic factors, may be important determinants of the heterogeneity in allergy prevalence throughout the world [84]. Ongoing cohort studies are focusing in detail on this complex question. These suggestions are supported by observations that germ-free mice do not develop tolerance in the absence of a gut flora [20,21] and by the demonstration of differences in the composition of the gut flora between infants living in countries with a high and a low prevalence of allergy [74,92] and between healthy and allergic infants [93–96]. Although all the studies confirm such differences, no particular protective or potentially harmful bacterial species can be identified so far. In the three prospective studies [94,96,97] and one cross-sectional study [93], the presence of bifidobacteria was associated with less allergy, while the presence of *Clostridium difficile* has been linked to allergy. The administration of lactobacilli to mothers or their infants was recently reported to be associated with less atopic dermatitis during the first 2 years of life [96] but there was no reduction in respiratory allergies at 4 years of age [98].

Maternal influences

There is a close immunological interaction between the mother and her offspring, not only during pregnancy as already discussed, but also as long as the baby is breast-fed. Human milk contains numerous immunological components, including IgA and other antibodies, various chemokines [99] as well as cytokines, mainly with anti-inflammatory and IgA stimulatory properties, such as TGF- β , IL-8, and IL-10 [100]. It is well established that human milk often contains food antigens that may induce IgE antibody formation. Less is known regarding the immunological consequences of introducing foreign antigens while the infant is still breast-feeding. As indicated by studies of immunity to infectious agents, it is possible that this represents a mechanism by which immune responses are modulated [101]. In the early 1990s, there was a pronounced increase in the

incidence of celiac disease among Swedish infants [102]. Prior to the increase in celiac disease, gluten was gradually introduced while the baby was still breast-fed. Then, gluten was avoided for the first 6 months. When the national recommendations were changed back to gradual introduction of gluten while the babies were still partly breast-fed, the incidence of celiac disease dropped rapidly.

Very recent studies with probiotics suggest that the maternal influences may be more pronounced in tolerance induction than previously appreciated. There are now at least three studies trying to prevent food allergy and infantile eczema with lactobacilli [96,103,104]. In the study with a negative outcome [104], the bacteria were given only to the babies, while in the two studies with some protective effect [96,98,103] they were also given to the mothers during the last month of gestation.

Concluding remarks

The development of immunological tolerance to food antigens is a complex process and depends on an intense interaction between the host and the environment, including microbial stimulation. It is intriguing that microbial stimulation, in particular via the gastrointestinal tract, has also been implicated as an etiological factor in respiratory allergic diseases. This suggests that microbial stimuli exert effects beyond the mucosal tissue microenvironments adjacent to sites of exposure, and presumably can influence systemic precursor compartments such as bone marrow and thymus. The underlying mechanism(s) are likely to include stimulation of functional maturation of cells within the innate and adaptive immune systems during the early postnatal period, a process which may ultimately determine the overall efficiency of immune/tolerance induction during early life, with major flow-on effects into adulthood. A full understanding of the underlying mechanisms may open new venues for the prevention by modification of the gut microflora, not only of food allergy, but also conceivably of respiratory allergies.

References

- 1 Wells HG, Osborne TB. The biological reactions of the vegetable proteins. I. Anaphylaxis. *J Infect Dis* 1911;8:66–124.
- 2 Weiner HL, Friedman A, Miller A, *et al.* Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Ann Rev Immunol* 1994;12:809–37.
- 3 Bruce MG, Strobel S, Hanson DG, Ferguson A. Irradiated mice lose the capacity to “process” fed antigen for systemic tolerance of delayed-type hypersensitivity. *Clin Exp Immunol* 1987;70:611–18.
- 4 Mowat AM, Strobel S, Drummond HE, Ferguson A. Immunological responses to fed protein antigens in mice. I. Reversal of oral tolerance to ovalbumin by cyclophosphamide. *Immunology* 1982;45:105–13.
- 5 Peng HJ, Turner MW, Strobel S. The kinetics of oral hyposensitization to a protein antigen are determined by immune status and the timing, dose and frequency of antigen administration. *Immunology* 1989;67:425–30.
- 6 Asherson GL, Zembala M, Perera MA, *et al.* Production of immunity and unresponsiveness in the mouse by feeding contact sensitizing agents and the role of suppressor cells in the peyer’s patches, mesenteric lymph nodes and other lymphoid tissues. *Cell Immunol* 1977;33:145–55.
- 7 Garside P, Steel M, Liew FY, Mowat AM. CD4+ but not CD8+ T cells are required for the induction of oral tolerance. *Int Immunol* 1995;7:501–4.
- 8 Ke Y, Kapp JA. Oral antigen inhibits priming of CD8+ CTL, CD4+ T cells, and antibody responses while activating CD8+ suppressor T cells. *J Immunol* 1996;156:916–21.
- 9 Hoyne GF, Callow MG, Kuhlman J, Thomas WR. T-cell lymphokine response to orally administered antigens during priming and unresponsiveness. *Immunology* 1993;78:534–40.
- 10 Thompson HS, Staines NA. Could specific oral tolerance be a therapy for autoimmune disease? *Immunol Today* 1990;11:396–9.
- 11 Richman LK, Graeff AS, Yarchoan R, Strober W. Simultaneous induction of antigen-specific IgA helper T cells and IgG suppressor T cells in the murine Peyer’s patch after protein feeding. *J Immunol* 1981;126:2079–83.
- 12 Mowat AM. Depletion of suppressor T cells by 2′-deoxyguanosine abrogates tolerance in mice fed ovalbumin and permits the induction of intestinal delayed-type hypersensitivity. *Immunology* 1986;58:179–84.
- 13 Holt PG, Britten D, Sedgwick JD. Suppression of IgE responses by antigen inhalation: studies on the role of genetic and environmental factors. *Immunology* 1987;60:97–102.
- 14 Strobel S, Ferguson A. Immune responses to fed protein antigens in mice. 3. Systemic tolerance or priming is related to age at which antigen is first encountered. *Pediatr Res* 1984;18:588–94.
- 15 Hanson DG. Ontogeny of orally induced tolerance to soluble proteins in mice. I. Priming and tolerance in newborns. *J Immunol* 1981;127:1518–24.
- 16 Miller A, Lider O, Abramsky O, Weiner HL. Orally administered myelin basic protein in neonates primes for immune responses and enhances experimental autoimmune encephalomyelitis in adult animals. *Eur J Immunol* 1994;24:1026–32.
- 17 Niess J, Reinecker H. Dendritic cells: the commanders-in-chief of mucosal immune defenses. *Curr Opin Gastroenterol* 2006;22:354–60.
- 18 Strobel S, Mowat AM. Immune responses to dietary antigens: oral tolerance. *Immunol Today* 1998;19:173–81.
- 19 Hornquist E, Grdic D, Mak T, Lycke N. CD8-deficient mice exhibit augmented mucosal immune responses and intact adjuvant effects to cholera toxin. *Immunology* 1996;87:220–9.
- 20 Moreau MC, Coste M, Gaboriau V, Dubuquoy C. Oral tolerance to ovalbumin in mice: effect of some parameters on the induction and persistence of the suppression of systemic IgE and IgG antibody responses. *Adv Exp Med Biol* 1995;371B:1229–34.
- 21 Sudo N, Sawamura S-A, Tanaka K, *et al.* The requirement of intestinal bacterial flora for the development of an IgE production

- system fully susceptible to oral tolerance induction. *J Immunol* 1997;159:1739–45.
- 22 Maloy KJ, Donachie AM, Mowat AM. Induction of Th1 and Th2 CD4+ T cell responses by oral or parenteral immunization with ISCOMS. *Eur J Immunol* 1995;25:2835–41.
 - 23 Pierre P, Denis O, Bazin H, *et al.* Modulation of oral tolerance to ovalbumin by cholera toxin and its B subunit. *Eur J Immunol* 1992;22:3179–82.
 - 24 Melamed D, Fishman-Lovell J, Uni Z, *et al.* Peripheral tolerance of Th2 lymphocytes induced by continuous feeding of ovalbumin. *Int Immunol* 1996;8:717–24.
 - 25 Melamed D, Friedman A. *In vivo* tolerization of Th1 lymphocytes following a single feeding with ovalbumin: anergy in the absence of suppression. *Eur J Immunol* 1994;24:1974–81.
 - 26 Mowat AM. The regulation of immune responses to dietary protein antigens. *Immunol Today* 1987;8:93–8.
 - 27 Faria AM, Weiner HL. Oral tolerance. *Immunol Rev* 2005;206:232–59.
 - 28 Iwasaki A, Kelsall BL. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J Exp Med* 1999;190:229–39.
 - 29 Iwasaki A, Kelsall BL. Mucosal immunity and inflammation. I. Mucosal dendritic cells: their specialized role in initiating T cell responses. *Am J Physiol* 1999;276:G1074–8.
 - 30 Strobel S, Mowat AM. Oral tolerance and allergic responses to food proteins. *Curr Opin Allergy Clin Immunol* 2006;6:207–13.
 - 31 Akbari O, Umetsu DT. Role of regulatory dendritic cells in allergy and asthma. *Curr Opin Allergy Clin Immunol* 2004;4:533–8.
 - 32 Viney JL, Mowat AM, O'Malley JM, Williamson E, Fanger NA. Expanding dendritic cells *in vivo* enhances the induction of oral tolerance. *J Immunol* 1998;160:5815–25.
 - 33 Bruce MG, Ferguson A. The influence of intestinal processing on the immunogenicity and molecular size of absorbed, circulating ovalbumin in mice. *Immunology* 1986;59:295–300.
 - 34 Webb KE. Amino acid and peptide absorption from the gastrointestinal tract. *Fed Proc* 1986;45:2268–71.
 - 35 Friedman A, Weiner HL. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *PNAS* 1994;91:6688–92.
 - 36 Mowat AM. The role of antigen recognition and suppressor cells in mice with oral tolerance to ovalbumin. *Immunology* 1985;56:253–60.
 - 37 Mueller DL, Jenkins MK, Schwartz RH. Clonal expansion versus functional clonal inactivation: a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu Rev Immunol* 1989;7:745–80.
 - 38 Whitacre CC, Gienapp IE, Orosz CG, Bitar DM. Oral tolerance in experimental autoimmune encephalomyelitis. *J Immunol* 1991;147:2155–63.
 - 39 Van Houten N, Blake SF. Direct measurement of anergy of antigen-specific T cells following oral tolerance induction. *J Immunol* 1996;157:1337–41.
 - 40 Chen Y, Inobe J, Weiner HL. Induction of oral tolerance to myelin basic protein in CD8-depleted mice: both CD4+ and CD8+ cells mediate active suppression. *J Immunol* 1995;155:910–6.
 - 41 Ke Y, Pearce K, Lake JP, *et al.* Gamma delta T lymphocytes regulate the induction and maintenance of oral tolerance. *J Immunol* 1997;158:3610–18.
 - 42 Fowler E, Weiner HL. Oral tolerance: elucidation of mechanisms and application to treatment of autoimmune diseases. *Biopolymers* 1997;43:323–35.
 - 43 Fukaura H, Kent SC, Pietruszewicz MJ, *et al.* Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-beta1-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients. *J Clin Invest* 1996;98:70–7.
 - 44 Inobe J, Slavin AJ, Komagata Y, *et al.* IL-4 is a differentiation factor for transforming growth factor-beta secreting Th3 cells and oral administration of IL-4 enhances oral tolerance in experimental allergic encephalomyelitis. *Eur J Immunol* 1998;28:2780–90.
 - 45 Garside P, Steel M, Worthey EA, *et al.* T helper 2 cells are subject to high dose oral tolerance and are not essential for its induction. *J Immunol* 1995;154:5649–55.
 - 46 Faria AM, Weiner HL. Oral tolerance and TGF-beta-producing cells. *Inflamm Allergy Drug Targets* 2006;5:179–90.
 - 47 Challacombe SJ, Tomasi TJ. Systemic tolerance and secretory immunity after oral immunization. *J Exp Med* 1980;152:1459–72.
 - 48 Battaglia M, Gianfrani C, Gregori S, Roncarolo M. IL-10-producing T regulatory type 1 cells and oral tolerance. *Ann NY Acad Sci* 2004;1029:142–53.
 - 49 Gonnella PA, Chen Y, Inobe J, *et al.* *In situ* immune response in gut-associated lymphoid tissue (GALT) following oral antigen in TCR-transgenic mice. *J Immunol* 1998;160:4708–18.
 - 50 Santos LM, al-Sabbagh A, Londono A, Weiner HL. Oral tolerance to MBP induces regulatory TGFβ secreting T cells in Peyer's patches of SJL mice. *Cell Immunol* 1994;157:439–47.
 - 51 Khoury SJ, Hancock WW, Weiner HL. Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor β, interleukin-4, and prostaglandin E expression in the brain. *J Exp Med* 1992;176:1355–64.
 - 52 MacDonald TT, Monteleone G. IL-12 and Th1 immune responses in human Peyer's patches. *Trends Immunol* 2001;22:224–47.
 - 53 Carol M, Lambrechts A, Van Gossum A, *et al.* Spontaneous secretion of interferon gamma and interleukin 4 by human intraepithelial and lamina propria gut lymphocytes. *Gut* 1998;42:643–9.
 - 54 Hauer AC, Breese EJ, Walker-Smith JA, MacDonald TT. The frequency of cells secreting interferon-gamma and interleukin-4, -5, and -10 in the blood and duodenal mucosa of children with cow's milk hypersensitivity. *Pediatr Res* 1997;42:629–38.
 - 55 Nagata S, McKenzie C, Pender SLE, *et al.* Human Peyer's patch T cells are sensitized to dietary antigen and display a Th cell type 1 cytokine profile. *J Immunol* 2000;165:5315–21.
 - 56 Husby S, Mestecky J, Moldoveanu Z, Elson CO. Oral tolerance in humans: T cell but not B cell tolerance to a soluble protein

- antigen. In: Mestecky J (ed.) *Advances in Mucosal Immunology*. New York: Plenum Press, 1995:1225–8.
- 57 Husby S, Mestecky J, Moldoveanu Z, *et al.* Oral tolerance in humans: T cell but not B cell tolerance after antigen feeding. *J Immunol* 1994;152:4663–70.
 - 58 Hattevig G, Kjellman B, Björkstén B. Appearance IgE antibodies to ingested and inhaled allergens during the first 12 years of life in atopic and non-atopic children. *Pediatr Allergy Immunol* 1993;4:182–6.
 - 59 Kornblat PE, Rothberg RM, Minden P, Farr RS. Immune response of human adults after oral and parenteral exposure to bovine serum albumin. *J Allergy* 1968;41:226–35.
 - 60 Faria AM, Weiner HL. Oral tolerance: therapeutic implications for autoimmune diseases. *Clin Dev Immunol* 2006;13:143–57.
 - 61 Kulig M, Klettke U, Wahn V, *et al.* Development of seasonal allergic rhinitis during the first 7 years if life. *J Allergy Clin Immunol* 2000;106:832–9.
 - 62 Sigurs N, Hattevig G, Kjellman B, *et al.* Appearance of atopic disease in relation to serum IgE antibodies in children followed up from birth for 4 to 15 years. *J Allergy Clin Immunol* 1994;94:757–63.
 - 63 Van Asperen PP, Kemp AS, Mukhi A. Atopy in infancy predicts the severity of bronchial hyperresponsiveness in later childhood. *J Allergy Clin Immunol* 1990;85:790–5.
 - 64 Böttcher MF, Jenmalm MC, Voor T, *et al.* Cytokine responses to allergens during the first 2 years of life in Estonian and Swedish children. *Clin Exp Allergy* 2006;36:619–28.
 - 65 Holt PG, O’Keeffe PO, Holt BJ, *et al.* T-cell “priming” against environmental allergens in human neonates: sequential deletion of food antigen specificities during infancy with concomitant expansion of responses to ubiquitous inhalant allergens. *Pediatr Allergy Immunol* 1995;6:85–90.
 - 66 Kondo N, Kobayashi Y, Shinoda S, *et al.* Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch Dis Child* 1992;67:1003–7.
 - 67 Piastra M, Stabile A, Fioravanti G, *et al.* Cord blood mononuclear cell responsiveness to beta-lactoglobulin: T-cell activity in “atopy-prone” and “non-atopy-prone” newborns. *Int Arch Allergy Immunol* 1994;104:358–65.
 - 68 Prescott SL, Macaubas C, Holt BJ, *et al.* Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T-cell responses towards the Th-2 cytokine profile. *J Immunol* 1998;160:4730–7.
 - 69 Casas R, Björkstén B. Detection of Fel d 1-IgG immune complexes in the cord blood and sera from allergic and non allergic mothers. *Pediatr Allergy Immunol* 2001;12:59–64.
 - 70 Prescott SL, Macaubas C, Smallacombe T, *et al.* Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999;353:196–200.
 - 71 Yabuhara A, Macaubas C, Prescott SL, *et al.* Th-2-polarised immunological memory to inhalant allergens in atopics is established during infancy and early childhood. *Clin Exp Allergy* 1997;27:1261–9.
 - 72 Macaubas C, Sly PD, Burton P, *et al.* Regulation of Th-cell responses to inhalant allergen during early childhood. *Clin Exp Allergy* 1999;29:1223–31.
 - 73 Julge K, Vasar M, Björkstén B. Development of allergy and IgE antibodies during the first five years of life in Estonian children. *Clin Exp Allergy* 2001;31:1854–61.
 - 74 Sepp E, Naaber RP, Voor T, *et al.* Development of intestinal microflora during the first month of life in Estonian and Swedish infants. *J Microbiol Ecol Health* 2000;12:22–6.
 - 75 Stene LC, Nafstad P. Relation between occurrence of type 1 diabetes and asthma. *Lancet* 2001;257:607–8.
 - 76 Holt PG, Clough JB, Holt BJ, *et al.* Genetic “risk” for atopy is associated with delayed postnatal maturation of T-cell competence. *Clin Exp Allergy* 1992;22:1093–9.
 - 77 Liao SY, Liao TN, Chiang BL, *et al.* Decreased production of IFN γ and increased production of IL-6 by cord blood mononuclear cells of newborns with a high risk of allergy. *Clin Exp Allergy* 1996;26:397–405.
 - 78 Martinez FD, Stern DA, Wright AL, *et al.* Association of interleukin-2 and interferon- γ production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy. *J Allergy Clin Immunol* 1995;96:652–60.
 - 79 Rinas U, Horneff G, Wahn V. Interferon- γ production by cord-blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE-levels. *Pediatr Allergy Immunol* 1993;4:60–4.
 - 80 Tang MLK, Kemp AS, Thorburn J, Hill DJ. Reduced interferon- γ secretion in neonates and subsequent atopy. *Lancet* 1994;344:983–6.
 - 81 Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: Is successful pregnancy a Th2 phenomenon? *Immunol Today* 1993;14:353–6.
 - 82 Zenclussen AC. Regulatory T cells in pregnancy. *Springer Semin Immunopathol* 2006;28:31–9.
 - 83 Holt PG, Macaubas C. Development of long term tolerance versus sensitisation to environmental allergens during the perinatal period. *Curr Opin Immunol* 1997;9:782–7.
 - 84 Björkstén B. Genetic and environmental risk factors for the development of food allergy. *Curr Opin Allergy Clin Immunol* 2005;5:249–53.
 - 85 Demengeot J, Zelenay S, Moraes-Fontes MF, *et al.* Regulatory T cells in microbial infection. *Springer Semin Immunopathol* 2006;28:41–50.
 - 86 Baldini M, Lohman IC, Halonen M, *et al.* A polymorphism in the 5’-flanking region of the CD14 gene is associated with circulating soluble CD14 levels with total serum IgE. *Am J Resp Cell Mol Biol* 1999;20:976–83.
 - 87 Asher MI, Montefort S, Björkstén B, *et al.* Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 2006;368:733–43.
 - 88 European Community Respiratory Health Survey (ECRHS). Variation in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the

- European Community Respiratory Health Survey (ECRHS). *Eur Resp J* 1996;9:687–95.
- 89 The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variations in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis and atopic eczema. *Lancet* 1998;251:1225–32.
 - 90 Bråbäck L, Hedberg A. Perinatal risk factors for atopic disease in conscripts. *Clin Exp Allergy* 1998;28:936–42.
 - 91 Jögi R, Janson C, Björnsson E, *et al.* Atopy and allergic disorders among adults in Tartu, Estonia compared with Uppsala, Sweden. *Clin Exp Allergy* 1998;28:1072–80.
 - 92 Sepp E, Julge K, Vasar M, *et al.* Intestinal microflora of Estonian and Swedish infants. *Acta Paediatr* 1997;86:956–61.
 - 93 Björkstén B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year old children. *Clin Exp Allergy* 1999;29:342–6.
 - 94 Björkstén B, Sepp E, Julge K, *et al.* Allergy development and the intestinal microflora during the first two years of life. *J Allergy Clin Immunol* 2001;108:516–20.
 - 95 Böttcher M, Sandin A, Norin E, *et al.* Microflora associated characteristics in faeces from allergic and non-allergic children. *Clin Exp Allergy* 2000;30:590–6.
 - 96 Kalliomaki M, Kirjavainen P, Eerola E, *et al.* Distinct patterns of neonatal gut microflora in infants developing or not developing atopy. *J Allergy Clin Immunol* 2001;107:129–34.
 - 97 Sepp E, Julge K, Mikelsaar M, Björkstén B. Intestinal microbiota and immunoglobulin E responses in 5-year-old Estonian children. *Clin Exp Allergy* 2005;35:1141–6.
 - 98 Kalliomaki M, Salminen S, Poussa T, *et al.* Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 2003;361:1869–71.
 - 99 Böttcher FM, Jenmalm MC, Björkstén B, Garofalo RP. Chemoattractant factors in breast milk of atopic and nonatopic mothers. *Pediatr Res* 2000;47:592–7.
 - 100 Böttcher MF, Jenmalm MC, Garofalo RP, Björkstén B. Cytokines in breast milk from allergic and nonallergic mothers. *Pediatr Res* 2000;47:157–62.
 - 101 de Martino M, Resti M, Appendino C, Vierucci A. Different degree of antibody response to hepatitis B virus vaccine in breast- and formula-fed infants born to HBsAg-positive mothers. *J Pediatr Gastroenterol Nutr* 1987;6:208–11.
 - 102 Ivarsson A, Persson LA, Nystrom L, *et al.* Epidemic of coeliac disease in Swedish children. *Acta Paediatr* 2000;89:165–71.
 - 103 Abrahamsson T, Jakobsson T, Fagerås Böttcher M, *et al.* Probiotics in prevention of IgE-associated eczema: a double blind randomised placebo-controlled trial. *J Allergy Clin Immunol* 2007;119:1174–80.
 - 104 Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 2007;119:184–91.

PART 2

Adverse Reactions to Food Antigens: Clinical Science