

Sensitization and tolerance

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The mechanisms responsible for sensitization, in particular within the gastrointestinal tract, are IgE-mediated as well as of a non-IgE-mediated, immunological origin. The phenomenon that is the opposite of sensitization is the maintenance of tolerance and is exemplified by the phenomenon 'oral tolerance'. The cytokines transforming growth factor beta and interferon gamma have been shown to be key immunoregulatory cytokines in oral tolerance. A new experimental model of eosinophilic allergic gastroenteritis has been developed with the use of encapsulated dietary antigen. The model was used in mice with genetic deletions of the eosinophil chemokine eotaxin or of the cytokine IL-5, demonstrating the importance of eotaxin. In clinical allergy research, a major question has been to explain the global increase in asthma and allergy. The 'hygiene hypothesis' states that a lack of maturation of the infant immune system from a T helper 2 to a T helper 1 type of immune response may be caused by less microbial stimulation in Western societies. Several lines of data support this hypothesis. However, apart from the genetic constitution of the individual, breastfeeding in infancy may be the most important single determinant for the development of clinical tolerance. *Curr Opin Allergy Clin Immunol* 1:237–241. © 2001 Lippincott Williams & Wilkins.

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Abbreviations

CT-B cholera toxin B subunit
iNOS inducible nitric oxide synthase
RSV respiratory syncytial virus
TGF- β transforming growth factor beta
Th T helper

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Introduction

The subject of sensitization versus tolerance may be divided into two. First, an immunological area that has a focus on the mechanisms of inflammation. This area includes oral tolerance in which the encounter with antigen/allergen occurs at the mucosal surfaces. Oral tolerance allows the immune response to be down-regulated towards innocuous dietary components and at the same time enables the individual to mount a vigorous immune response to potentially dangerous microorganisms [1•]. Second, the subject includes a clinical area dominated by studies of the development of allergy in humans and with the use of both immunological and epidemiological methods. The main question in allergy research in the new millennium is how to understand and explain the recent epidemic of asthma and allergy, particularly in the Western world. A current view is the so-called 'hygiene hypothesis', which suggests that a reduction of the infectious load during the first months and years of life influences the immune system towards a less mature state, promoting allergy [2•]. The aim of the present review is to present and combine recently published information from both the more basic and the clinical aspects of the subject.

News in oral tolerance

Oral tolerance as defined above has long been recognized as a basic principle in normal mucosal immunity. However, species differences are recognized and for example rabbits and chickens seemingly do not react with oral tolerance [3]. The mechanisms of oral tolerance may be either active immune suppression, clonal deletion or anergy. Classically, suppression occurs at low-dose administration of an antigen, whereas anergy or clonal deletion occur at high doses of antigen (Table 1). The paradigm of T helper (Th) type 1- and 2-like responses has been useful but not all-encompassing for the further characterization of mucosal immunity. In oral

Table 1. Mechanisms of immune tolerance and their relationship to oral tolerance

Mechanism of tolerance	Oral dose administered
Deletion	High dose
Anergy	
Receptor downregulation	Low dose
Cellular regulation:	
Th1-type cells (IFN- γ production)	
Th2-type cells (IL-4, IL-5)	
Th3-type cells (TGF- β)	
Tr1 cells (IL-10)	

TGF- β , Transforming growth factor beta; Th, T helper; Tr, T regulatory.

tolerance the locally involved T cells show both characteristics of Th1 producing IFN- γ and of Th2 producing IL-4 and IL-5, but cells also appear that primarily produce the suppressive cytokine transforming growth factor beta (TGF- β), called Th3, and the picture is not quite clear [4•]. The production of IFN- γ in intestinal lymphoid tissue has previously been reported. Interestingly, Lee *et al.* [5•] used a model with the transfer of T cells specific for ovalbumin into recipient mice. The feeding of ovalbumin and the formation of oral tolerance occurred concurrently with the upregulation of IFN- γ production in the Peyer's patches of the gut, whereas the blocking of IFN- γ with antibody prevented the formation of tolerance in a cause-and-effect relationship. The local IFN- γ production blocked the entry of further T cells into the site of antigen delivery. Another new model for oral tolerance has been provided by the injection of genes within an adenovirus vector directly into the Peyer's patches [6]. In this model, the injection of genes for the antigen B7 or of IL-12 reversed oral tolerance accompanied by the production of IFN- γ . Receptor downregulation is another mechanism that is of importance for the induction of oral tolerance [7•]. Such studies illustrate the importance of the interaction between the systemic immune system and the local gastrointestinal immune system.

An important final common pathway for the inflammatory immune responses of the gut is the production of nitric oxide in the gastrointestinal epithelial cells. Intraepithelial lymphocytes induce the production of nitric oxide in intestinal epithelial cells by cell-to-cell contact [8]. Mice without inducible nitric oxide synthase (iNOS) had augmented oral tolerance and a high systemic and local production of TGF- β compared with wild-type mice [9•], indicating that iNOS inhibitors may strengthen oral tolerance. As mentioned by the authors this avenue may be exploited therapeutically.

The form of the antigen is of importance. It was thus shown that oral tolerance could be abrogated by feeding ovalbumin as the antigen, when it was encapsulated within a specific pH-resistant polymer preparation [10•]. This procedure protects the antigen from partial degradation in the acidic milieu of the stomach and promotes the immune response in the small intestine. Conversely, the potential human therapeutic use of oral tolerance in autoimmune or allergic disorders will demand the amelioration of an existing disease process. It has proved difficult to achieve oral tolerance in primed experimental animals regardless of the repetitive administration of antigen, either because of inherent resistance by antigen-experienced T cells or because of the inhibition of tolerogenic signals to antigen-specific T cells [11]. Of immediate therapeutic interest is the coupling of antigen to the cholera toxin B

subunit (CT-B), which is the non-toxic receptor-binding part of the cholera toxin and which has previously been shown to strengthen oral tolerance. Experimental allograft rejection in mice was diminished by the oral administration of allogeneic thymic cells coupled to CT-B [12•], and this process was accompanied by a general reduction in the number of T cells producing IL-2, IFN- γ and IL-4. In the Peyer's patches the production was increased both of the cytokine IL-4 and the suppressive cytokine TGF- β . The same research group investigated IgE antibody responses to ovalbumin in mice [13•]. They showed that the development of systemic IgE antibody responses to ovalbumin could be suppressed by the previous oral administration of CT-B coupled to the antigen. Furthermore, the oral administration of CT-B coupled to ovalbumin suppressed already established IgE antibody responses. In conclusion, it was demonstrated that CT-B had a profound effect on the development of oral tolerance. All in all, these recent data indicate that the manipulation of cytokine production and the subsequent inflammatory cascade or alteration of the antigen form may be of potential human therapeutic use.

Disease models and chemokines

A number of experimental disease models for mucosal inflammation have been developed in the past decade. The Th1–Th2 paradigm has continued to be a useful framework. The mechanisms for oral tolerance as described in the preceding section are also relevant for disease models. When colitis was induced by trinitrobenzene sulfonic acid in a number of different cytokine knock-out mice, IFN- γ $-/-$ mice and IL-12 $-/-$ mice developed mild mucosal colitis, whereas IL-4 $-/-$ mice had a penetrating colitis with a high mortality rate [14•]. These data are dependent on the pathogenetic differences between the two main inflammatory bowel diseases, ulcerative colitis and Crohn's disease. In mice experimentally infected with *Leishmania major* infection a Th1 response is typically obtained. However, after the intranasal administration of parasites an influx of Th2 type cytokines (IL-4 and IL-5) as well as eosinophilic leukocytes [15] was observed in the airways, indicating organ specificity as a determinant for the general T cell differentiation. A recent report and review [16•] demonstrated that a plasmid with TGF- β could locate both in the lungs and the gut and exert suppressive actions at these sites. The data indicate that TGF- β and IL-10 are key elements of the down-regulation of mucosal inflammation, but local differences could be important.

In the human, a novel chemokine called MEC (mucosae-associated epithelial chemokine) has been identified in mucosal tissues [17•], in particular in bronchial and mammary glands and the colon. The same

researchers described another chemokine ligand called epithelial thymus-expressed chemokine, which bound to CCR10 and CCR3 and was expressed by epithelial cells in the jejunum and ileum, but not in other tissues [18•]. As an important principle, the differential expression of these receptors thus seems to determine the migration of leukocytes to mucosal surfaces. Furthermore, the lack of the chemokine receptor CCR5 protected mice from experimental colitis [19], indicating that CCR5 is critical for a Th1 type of inflammation in the colon.

The abrogation of oral tolerance by acid-resistant antigen-containing biospheres, as mentioned above [10•], has been employed for the development of an experimental model for eosinophilic gastrointestinal allergy [20•]. When this model was used in mice with a deletion of the gene for the eosinophil chemokine eotaxin, the researchers showed that eosinophils did not accumulate in gut tissue. The data thus indicate that eotaxin was essential for the recruitment of eosinophils to the small intestine in experimental gastrointestinal food allergy. In mice with the genetic absence of IL-5, eosinophils still accumulated in the small intestine but eosinophil levels declined in the systemic circulation [20•]. At the tissue level, after allergen exposure eosinophils accumulate in the intestinal Peyer's patches in the close vicinity of lymphocytes. Eotaxin was essential for this process when mice were exposed to allergen in the presence of IL-5 [21•]. Similar mechanisms may apply to the lung [22•].

Risk factors for clinical sensitization

Current clinical research in the area of sensitization emphasizes the further study of factors in atopic inflammation such as the cytokines, chemokines and their receptors, mentioned in the previous sections. Early events after or even before birth seem to be particularly important. It is generally recognized that a recent epidemic of asthma and allergy has occurred, in particular in the Western world. As an example, a cross-sectional study of the development of asthma and allergy was performed in Estonia, one of the former Eastern Bloc countries in Europe, in 10-year-old children. The prevalence of asthma and allergy was examined in 1996–1997 and compared with 1992–1993 [23]. The study showed a constant prevalence of asthma of 2.5–3.2%. However, in a longitudinal study of 0–2-year-old infants and children in the same population the manifestations and sensitization to foods and inhalants were equally common in Estonian as compared with Scandinavian children [24], with a prevalence of definite atopy of 17% at 2 years of age. This Estonian cohort will be interesting to follow in the future as the sensitization may be transient as a developmental phenomenon, or may persist corresponding to the frequency of sensitization of older Scandinavian children.

Several environmental risk factors have been suggested to be responsible for the sensitization of a significant proportion of the infant and childhood population in the Western world. The combination of atopic dermatitis and food allergy at 12 months of age seems to be a strong indicator for the development of asthma-like disease at 24 months of age [25] and further at 7 years of age [26]. An independent risk factor could be infection with respiratory syncytial virus (RSV) in infants who were subsequently followed-up to the age of 7 years [27]. The question of whether the RSV infection was indeed an independent risk factor has been thoroughly debated previously, but the study did not clarify whether the RSV-positive infants were initially hospitalized because of previously silent hypersensitivity and were thus positively selected for infection intensity.

Genes and sensitization

Specifically in relation to food allergy, a recent twin study of peanut allergy [28•] obtained a heritability estimate of 81.6% (95% confidence limits 42–100%). This result clearly indicates a strong genetic influence on food allergy, as exemplified by allergy to peanuts. The genes responsible for sensitization and the development of allergy have been intensively studied and are not the subject of this review. One polymorphism of particular interest will be mentioned. A mutation in the promoter region of the gene for the leukocyte antigen CD14, which has been mapped to chromosome 5q31–33, has been associated with protection against increased s-IgE levels as well as asthma [29]. CD14 is a glycoprotein present in tissues in two forms: a membrane-bound form (mCD14) present on monocytes and a soluble form (sCD14) present in serum, and functions as a receptor for bacterial lipopolysaccharide. The CD14 molecule may have important functions in the maturation of the immune response, see below, and particular mutations may constitute a partial protection against the development of atopic disease.

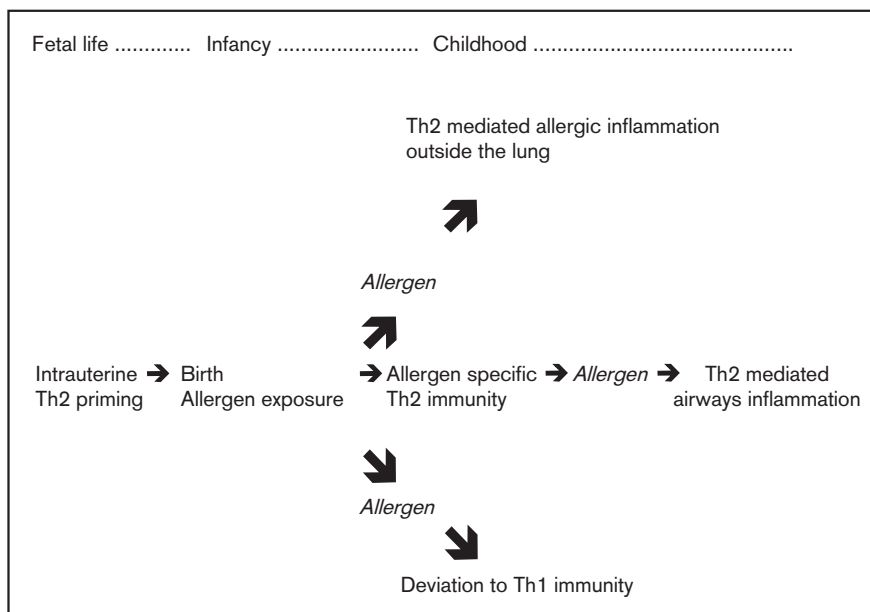
Breastfeeding

Human breast milk contains hormones, growth factors and other bioactive molecules with the capacity to modify the infant's immune responses. Previous investigations have been directed at the elimination of antigens in the breast milk. Reports now indicate that exposure to cow's milk proteins via the breast milk may in fact decrease antibody levels in the infant, such as antibodies to bovine insulin [30•]. This finding represents an example of the early induction of tolerance. Along the same lines, low levels of IgA in breast milk were obtained from mothers whose infants later developed cow's milk allergy [31]. Also, factors of the innate immune system are present in the mother's milk. Lower numbers of TNF- α producing leukocytes in the mother's milk given to cow's milk allergic infants were

Figure 1. Model for the development of allergic inflammation in relation to exposure and age

Th, T helper.

Modified from Holt and Sly [34], with permission.



observed compared with the milk given to healthy infants [32•]. As mentioned above a mutation of the pattern recognition receptor CD14, which binds to bacterial lipopolysaccharide was implicated as a protection against allergic disease [29]. Soluble CD14 was observed at high concentrations in breast milk, and may play a significant part in the innate immune protection of the breast milk [33••]. Taken together, these data, even as yet fragmentary, support the evidence for the protective role of the mother's milk in relation to allergy.

Support for the hygiene hypothesis

In a broader context, the Western lifestyle has been suspected of promoting asthma and allergy, giving rise to the 'hygiene hypothesis' [34]. The fetus has an immune response inherently skewed towards the Th2 phenotype, which after birth matures into a Th1 phenotype, provided it is exposed to bacterial, viral as well as parasitic infestations [35•]. A combination of genetic risk and of exposure to allergens, including foods instead of microorganisms, may lead to allergic inflammation dominated by a Th2 phenotype (Fig. 1, modified from Ref. 34). In support of the hypothesis, the immune responses to diphtheria–tetanus–acellular pertussis (DTaP) vaccine in infants were initially dominated by IL-4 as a Th2 cytokine response [36]. Later, at 6 years of age, atopic children responded to vaccination with a Th1 type response with higher IFN- γ levels than non-atopic children [37•]. Interestingly, early bacillus Calmette–Guérin vaccination appeared in African children to modify the later development of atopy, as 57 out of 271 (21%) of the vaccinated children had atopy

compared with 21 out of 53 (40%) of the non-vaccinated children [38•]. Live vaccines may thus generate a general stimulation of the immune system, which then influences the development of atopy – a finding that gives impetus for further research.

Conclusion

The mechanisms responsible for oral tolerance are increasingly well understood. Combined with the use of suitable animal models, this concept has led to tools of potential therapeutic significance. The year 2000 brought forward support for the 'hygiene hypothesis' for understanding the increase in prevalence of allergic diseases. Breastfeeding is probably the single most important factor that modifies the degree of sensitization. Also, genetic factors are of profound significance.

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