

Early sensitisation to food antigens – when and how?

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Introduction

Generally, the normal immunological response to orally introduced food antigens involves induction of tolerance, leading to unresponsiveness upon further exposure to the antigen. Thus, allergic sensitisation to a food antigen may be regarded as a failure of immunological tolerance. The mechanisms underlying allergic sensitisation to food allergens would then be supposed to be intimately related to those of tolerance induction. Development of tolerance can be readily demonstrated following antigen feeding in animal experiments. In humans the existence of oral tolerance was first demonstrated in 1994, when it was shown that feeding with keyhole limpet haemocyanin induced T cell but not B cell tolerance (1).

Mechanisms of tolerance and sensitisation

According to current concepts immunological tolerance may be induced via three different mechanisms: (i) clonal deletion of T cells, (ii) T cell anergy and (iii) active suppression by regulatory T cells that produce immunosuppressive cytokines. The latter of these mechanisms appears to be the most important factor in oral tolerance. Additionally, as is known from early animal experiments, IgG antibodies have a suppressive effect on IgE antibody formation (2) and therefore pre-existing, e.g. transplacentally acquired, antibodies may suppress IgE

antibody formation, leading to partial tolerance. Recently presented results suggest that such suppression may explain the relationship between development of pollen allergy and season at birth (3).

The different components of the immune response are differently sensitive to tolerance induction. Several studies have indicated that delayed type hypersensitivity (DTH) is unusually susceptible to induction of oral tolerance (4). Even if Th1 immunity may be more sensitive than is Th2 immunity in general, it seems clear, however, that the formation of IgE, as opposed to IgA antibodies is very easily suppressed by tolerogenic antigens. Regarding induction of oral tolerance to food antigens it appears that the rank of order of sensitivity of the various components of the immune system is similar to that suggested for antigens introduced into the airways, i.e. IgE>DTH>IgG>IgA (5).

The immune response is highly dependent on antigen concentration. Generally, very low or very high antigen doses may induce unresponsiveness. Therefore, the relationship between the immune response and antigen concentration follows a bell-shaped curve. In this regard it is important to note that the dependency on dose of sensitising antigen may be very different for IgG and IgE antibody formation. In experiments in mice it has been shown that parenteral sensitisation with a low dose of ovalbumin induces preferentially IL-4, IL-5 and IgE antibodies, whereas a high dose predominantly

induces IL-12, IFN- γ and IgG2a antibodies (6). These results, which were obtained in a murine model of atopic asthma, indicate clearly that Th1 and Th2 immune responses may be oppositely dependent on the dose of sensitising antigen, a finding that may have important implications regarding sensitisation to food antigens in humans.

Recent studies have indicated that the development of oral tolerance is highly dependent on the intestinal microflora. It is well known that it is difficult to induce tolerance in germ-free mice and that administration of lipopolysaccharide (LPS) prior to feeding antigen to these mice will restore their ability to develop immunological tolerance (7). LPS is a constituent of the walls of Gram-negative bacteria, e.g. *Escherichia coli*, which is a potent inducer of the immunosuppressive cytokine IL-10 (8). Therefore, a lack of adequate stimulation of the immune system by intestinal bacteria containing LPS may favour the development of allergic sensitisation, rather than tolerance. In this context it should also be noted that the Gram-positive bacteria have other characteristics than Gram-negative bacteria regarding cytokine induction, most notably a special ability to induce the formation of IL-12 (8). IL-12 is a key cytokine of Th1 immunity and stimulation of IL-12 production by intestinal bacteria such as lactobacilli or Gram-positive cocci may therefore preferentially stimulate Th1 immunity. According to the Th1/Th2 immunity concept this may counteract allergic sensitisation, which is Th2-dependent (9).

Possible causes of improper sensitisation to food allergens

As shown in numerous studies, sensitisation to food antigens, as evidenced by development of specific IgE antibodies, occurs regularly during infancy. In non-atopic children this is a transient phenomenon (10) and a key question in food allergy is thus why IgE antibodies persist and give rise to symptoms in atopic individuals. Possible explanations for this include the following.

Atopy is associated with an inherent imbalance in the Th1/Th2 system facilitating the production of i.a. IL-4 and IL-13, which in turn stimulate IgE responses. This is supported by the results of a plethora of studies (9). Since there already appears to be a deficiency of IFN- γ production at birth in babies who later develop atopy (11, 12) the physiological IgE response to food antigens that occurs in early infancy will not be adequately downregulated in these babies.

There might be a relative lack of the formation of immunosuppressive cytokines, in particular TGF- β and IL-10, associated with polymorphisms in the genes of these cytokines. This hypothesis has not been sufficiently well studied, although certain polymorphisms of both the IL-10 and the TGF- β genes have been found to be associated with elevated serum IgE (13).

Abnormalities of intestinal bacterial colonisation might lead to failing induction of immunological tolerance and insufficient stimulation of Th1 immunity. This possibility is currently being studied extensively. Although cross-sectional studies of the intestinal microflora in various groups of patients are unlikely to yield conclusive results, ongoing prospective studies will hopefully throw more light on the association between food allergies and the intestinal microflora.

Failing maturation of the immune system because of abnormal production of certain factors, such as prostaglandins and reactive oxygen species, such as hydrogen peroxide, might influence allergic sensitisation. Abnormal production of prostaglandins may be caused by high omega-6/omega-3 fatty acid ratios, and increased levels of hydrogen peroxide by low content of antioxidants in the food. The increase in the prevalence of allergy in the western world has occurred in parallel with changed food habits regarding omega-3 and omega-6 fatty acids (14) as well as antioxidants (15). In our laboratory we have found that both prostaglandin E2 and hydrogen peroxide may increase the Th2/Th1 (IL-4/IFN- γ) ratio more than 100-fold in *in vitro* cultures (Strannegård Ö and Strannegård I-L, unpublished results).

When does sensitisation to food antigens occur?

IgE antibodies, which do not pass the placenta barrier, may occur in cord blood in minute amounts. However, the ability to produce IgE antibodies is already developed at a fetal age of 11 weeks (16). Furthermore, fetal T cell priming can occur at gestational age of 22 weeks (17). Sensitisation *in utero* to food allergens, ingested by the mother should therefore be possible. In fact, several studies have suggested that sensitisation to various allergens may indeed take place *in utero* (17). Since the intrauterine milieu is skewed towards Th2 immunity (18) the fetus might be assumed to be prone to develop IgE antibody responses. The human fetus, however, seems to develop similar patterns of cytokine production to those observed in adults after antigenic stimulation (19) and cord blood cells from atopic babies appear to be deficient in producing Th1 as well as Th2 cytokines (20). Furthermore newborns have been

shown to be perfectly able to mount a Th1 response to BCG vaccination that is of a similar magnitude to that observed after vaccination later in life (21). Thus, according to these results Th2 polarisation does not seem to be a prominent feature of fetal immunity. Other studies, however, have indicated that prenatal and neonatal sensitisation to a variety of antigens involves a predominant Th2 response (22, 23).

Allergic sensitisation to food allergens is common in non-atopic as well as in atopic individuals during infancy (10). In non-atopic babies the peak of concentration of IgE antibodies to food allergens is usually observed during the first year of life, whereafter the antibody titres decline, in most cases to undetectable levels. By contrast, in infants who later develop atopic disease the IgE antibodies persist, frequently at high titres (10). The occurrence of skin prick positivity or high titres of IgE antibodies to, in particular, egg white in infancy has been found to be a good predictive marker for later development of atopy (10, 24).

Since infants seem to be particularly prone to develop allergic sensitisation to food antigens the existence of a window of sensitisation has been postulated. Studies on the effect of introducing cow's milk at different times during infancy suggest that tolerance develops less easily after early than after late introduction of the milk (25). Thus, it seems that oral tolerance is poorly developed in infants during early life, similar to that observed in rodents (26). The window of sensitisation may therefore be more dependent on a relative lack of oral tolerance than on the state of the Th1/Th2 immunity balance. In this regard it should, however, be noted that tolerance and Th1/Th2 immunity are interrelated, since the Th1 cytokine IFN- γ may be necessary for induction of tolerance (27).

The window of sensitisation may be quite narrow regarding food allergens, usually not extending beyond infancy. By contrast, sensitisation to aeroallergens may certainly occur much later. The possible allergy-stimulating effect of antibiotics, thought to act by abolishing the tolerogenic effect of commensal bacteria, seems to be most pronounced before the age of 2 years, although it may have some effect also after this age (28). These findings agree with the notion that sensitisation to food antigens is most likely to occur during the first months of life, i.e. at the time when the mechanisms of tolerance are not well developed.

Although generally allergic sensitisation to food allergens occurs in infancy, well before the development to airborne allergens, it may also occur considerably later, even in adults. In these

cases the sensitisation may be associated with a breaking of tolerance, caused for instance by intercurrent infections (29) or with the introduction of new food antigens during periods of inadequately functioning tolerance-inducing mechanisms, according to the concept of "allergic breakthrough" (30).

Does sensitisation to food antigens occur solely via the gut?

As is well known from animal experiments, allergic sensitisation, i.e. formation of specific IgE antibodies, is much more easily achieved when an antigen is introduced parenterally rather than orally. It may therefore be questioned whether, for instance, cutaneous exposure to foodstuffs in small children rather than ingestion of the food may be responsible for the sensitisation. In experiments in mice it has been demonstrated clearly that primary sensitisation to aeroallergens as well as food allergens may take part through the skin and it seems likely that similar mechanisms may occur in children (31).

In this context it should be mentioned that feeding with antigen may suppress the allergy-sensitising effect of cutaneously applied antigen (32). Although this phenomenon is well described regarding contact allergy, it may also be assumed to occur in IgE-mediated food allergy. Therefore it seems possible that at least a part of sensitisation to food allergens may take place via the skin e.g. around the mouth of infants, and that this sensitisation is normally counteracted by tolerance-inducing mechanisms of the gut.

Conclusion

Sensitisation to food antigens can be considered a failure of normal induction of oral tolerance. Oral tolerance is mainly dependent on activation of antigen-specific regulatory T cells that produce the immunosuppressive cytokines TGF- β and IL-10. Allergic sensitisation seems to occur preferentially following stimulation with low rather than high amounts of antigen and is highly sensitive to tolerance induction. Sensitisation to food antigens may occur *in utero* but is particularly pronounced during infancy when mechanisms of tolerance are poorly developed. Although allergic sensitisation can be assumed to be induced mainly via orally introduced food it seems possible that sensitisation to foodstuffs may also occur percutaneously.

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