

The T lymphocyte in food-allergy disorders

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Purpose of review

While much attention is focused upon the role of IgE antibodies in food-allergy disorders, the T cell remains central to all forms, both IgE and non-IgE-mediated, of food-hypersensitivity responses. This review considers the central role of the T cell in this group of disorders and provides a comprehensive overview of recent studies that elucidate our understanding of the mechanisms involved in the pathogenesis of food allergy in regard to the role of the T cell.

Recent findings

Recent studies have defined a dynamic process involving T cell homing receptors (e.g. cutaneous lymphocyte antigen) and activation markers in food-hypersensitivity disorders. Modulation of the T-cell responses occurs through the recognition of dominant allergenic epitopes, the elaboration of regulatory cytokines (e.g. transforming growth factor- β , IL-4, IL-5, tumor necrosis factor- α) and the influence of immunomodulatory microbial and environmental agents. The resulting disorders reflect T-cell dysregulation.

Summary

Significant recent advances in our understanding of the role of the T cell in food hypersensitivity have been made and will probably contribute to improved diagnostic and treatment methods in the near future.

Keywords

T cell, food allergy, food hypersensitivity

Curr Opin Allergy Clin Immunol 3:199–203. © 2003 Lippincott Williams & Wilkins.

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Current Opinion in Allergy and Clinical Immunology 2003, 3:199–203

Abbreviations

IFN- γ interferon- γ
TGF transforming growth factor
TNF tumor necrosis factor

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1528-4050

Introduction

Pioneering work clarifying the mechanisms of oral tolerance in food allergy have firmly established a key role for lymphocytes. The discovery of IgE antibodies in the 1960s confirmed initial hypotheses for a facilitating role of T cells in the production of IgE antibodies through specific cytokine production (IL-4 and IL-13), thus linking 'humoral' allergy with 'cellular' allergy. Current clinical classifications of food-allergy diseases are based mainly on the presence or the absence of food-specific IgE antibodies [1]. IgE-mediated food allergy is by far the most studied type of disease; there is good general knowledge of the mechanisms, reliable diagnostic tools, but no proactive treatment. In non-IgE-mediated food allergy, several lines of evidence implicate lymphocytes in the pathogenesis of the various disorders.

In recent years, three main areas of research in food allergy have emerged. First, mechanisms of oral tolerance in relation to food allergy were extensively studied and have contributed to a better understanding of the disease. Second, research aimed at improving the accuracy of diagnostic tools, and, most recently, at finding predictive tests for the natural history of food allergy, has been partly successful. Third, emphasis has been placed upon finding a proactive treatment for food allergy by exploring several ways in which lymphocytes are implicated [2*].

However, in all of these areas of research, the major drawback has been the difficulty, in human studies, of accessing and studying lymphocytes from gut compartments. Thus, much speculation has had to be made on the basis of the study of circulating lymphocytes, without definite proof of organ-linked mechanisms of sensitization or of relevance to clinical symptoms.

Mechanisms of food allergy

Food allergy, at least in its IgE-mediated form, is definitively linked to atopy, i.e. the predisposition to develop an allergic-type response to common environmental allergens. Thus, the presence of increased numbers of food allergen-specific Th2-type cells has been recognized, recently also in conjunction with interferon- γ (IFN- γ)-secreting lymphocytes. To investigate this observation further, Osterlund and Suomalainen [3] examined 27 breast-fed infants among whom 12 had challenge-proven cow's milk allergy. Interestingly, the symptoms had occurred during exclusive breast-feeding in all but one infant. Peripheral blood mono-

nuclear cells obtained from these infants were activated with phorbol myristate acetate (PMA) and ionomycin; this was followed by fluorocytometry for lymphocyte markers and intracellular cytokines. While there were no differences in the frequency of CD4-positive T cells between the infants with cow's milk allergy and the controls, the frequency of CD8-positive T cells in infants with cow's milk allergy was significantly lower. Children with cow's milk allergy also had significantly fewer CD4-positive T cells expressing IFN- γ . The authors suggest that the capacity of CD4-positive T cells to express IFN- γ is defective in cases of cow's milk allergy, and that the low number of CD8-positive T cells may contribute to the disease. The study did not verify either antigen specificity or an effect caused by an atopic disposition, but these interesting findings may have pinpointed a characteristic of T cells, early in life, that predisposes infants to cow's milk allergy.

Several studies have suggested a specific lymphocyte response that varies in relation to the age of the patient. Ng *et al.* [4] recruited two groups of egg-allergic children: one comprised infants less than 18 months of age, and the other comprised children over 3 years of age. Peripheral blood mononuclear cells were obtained and stimulated with phytohemagglutinin (PHA) or with major egg antigens (ovalbumin and ovomucoid) or house-dust mites. Cytokines were measured in the culture supernatants after 48 h. In egg-allergic infants, the lymphocyte response following stimulation with allergen was associated with a strong production of Th2-type cytokines, particularly in the group of infants under 3 years of age. More specifically, IL-5 levels were closely correlated with the clinical status of the patients. Interestingly, IFN- γ was detected in cells from egg-allergic infants, suggesting a mixed Th1/Th2 response rather than an exclusive Th2 bias. The group of older patients included children who had lost their egg allergy, and, in these patients, lymphocyte activation by egg antigens also resulted in IL-10 and IFN- γ production. Summarizing the results, the authors suggest that food allergies result from a dynamic process influenced through regulation by specific T-cell cytokines. Cells from egg-allergic patients were producing IFN- γ , and this cytokine was also found in supernatants from cells obtained from egg-tolerant subjects; in the latter case together with IL-10, however. The authors speculate, therefore, that IL-10 has an important regulatory function in this process.

Food protein-induced enterocolitis syndrome is characterized by a delayed onset (1–2 h following ingestion of a causal food) of severe vomiting, diarrhea and sometimes lethargy and hypotension in the absence of positive food-specific IgE antibody tests. A group from Korea [5] recently raised the hypothesis that the disease may be

related to the activity of transforming growth factor (TGF)- β . They recruited 28 patients clinically diagnosed with enterocolitis syndrome. Biopsy specimens were obtained during upper gastro-duodenoscopy. Immunohistochemical studies were done for TGF- β receptor 1 as well as the TGF- β receptors and tumor necrosis factor (TNF)- α . Epithelial expression of TGF- β receptor 1 was significantly lower in patients with villous atrophy (observed in 16/28 patients) than in those with normal mucosa or in controlled subjects. An inverse relationship was observed with TNF- α , as its expression was significantly higher in patients with villous atrophy. TGF- β receptor 1 expression was generally not detected in the duodenal mucosa. In their conclusion, these workers also suggest that the release of TNF- α and the decreased activity of TGF- β receptor 1 might be implicated in the pathogenesis of food-induced enterocolitis syndrome. However, one might also speculate that TGF- β receptor 1 is associated non-specifically with villous atrophy and could also be present in other diseases, so further investigation is needed.

Although gastrointestinal food allergies are most often diagnosed in children, adults may present similar clinical pictures, which are often related to non-IgE-mediated mechanisms. In a study by Lin *et al.* [6], duodenal biopsies were obtained from 14 adults with food-related gastrointestinal symptoms but with negative tests for food-specific IgE antibodies (skin-prick tests/radioallergosorbent test). Immunostaining of the cells revealed that there was an increased number of IgE-positive duodenal cells, eosinophils and activated T cells, despite the negative allergy tests. This work suggests that negative investigations for food-specific IgE does not exclude the possibility of an IgE-antibody-associated inflammatory response. However, food-antigen specificity of the IgE antibodies was not demonstrated, which does not exclude a non-specific, allergic inflammation.

Most recently, our understanding of T-cell-related mechanisms of food allergy has evolved from the concept of pure Th2-type disease to one of an antigen-specific, mixed Th1- and Th2-type cytokine response. The important role of regulatory T cells (e.g. those secreting IL-10) has also emerged. One might speculate, from the results in the studies reviewed above, that the cytokine production pattern expressed by lymphocytes could profoundly affect the type of immune response and contribute to the emergence of symptoms of food allergy, but also influence the favorable outcome of food allergy seen in most patients – that of eventual tolerance.

In addition to a specific cytokine pattern, the expression of cell-surface receptors might also contribute to the pathogenesis of food allergy. Schade *et al.* [7•] examined the expression of CD25, CD26 and CD30 on allergen-

specific T cells from children with or without food allergy. They found that food-specific T cells from patients with food allergy expressed much higher levels of CD25 (the IL-2 receptor α) together with a higher expression of CD30 (a marker for the activation state of T cells). After the development of tolerance to cow's milk, the expression of CD25 and CD30 was significantly decreased, whereas the previously low expression of CD26 (associated with a Th1 response) increased after tolerance acquisition. These results suggest a clear relationship between cell-surface markers and the activation state of lymphocytes, as well as with the clinical activity of the disease.

Another way in which characteristics of T cells influence the expression of food allergy is their tropism to specific organs. Beyer *et al.* [8^{*}] examined expression of the skin homing receptor cutaneous lymphocyte antigen on CD4-positive T cells obtained from patients with milk-induced urticaria or atopic dermatitis. They found that both patient populations had increased expression of cutaneous lymphocyte antigen and antigen-specific CD4-positive T cells, suggesting that this homing receptor may be of importance in the pathogenesis of not only atopic dermatitis [9] but also urticaria. They also confirmed the previous findings showing the absence of cutaneous lymphocyte antigen on the lymphocytes obtained from patients with gastrointestinal food allergy.

Diagnostic and preventive tools

The determination of food antigen-specific IgE antibodies is of great help in the diagnosis of food-related diseases. So far, concentrations of food-specific IgE antibodies have been shown to be helpful for discriminating between sensitization and clinical allergy [10], and were found to be partially predictive of the natural history of the disease in a specific patient. There is increasing emphasis on epitope mapping of major food allergens as a means of improving diagnostic and therapeutic strategies. Three recent studies have highlighted a distinction between patients with IgE binding to linear, as opposed to conformational, epitopes. Vila *et al.* [11] found higher levels of IgE antibodies to specific linear epitopes from casein in children who have persistent cow's milk allergy compared with those who were to develop tolerance. Similarly, Jarvinen *et al.* [12] discovered that the presence of IgE antibodies to certain linear allergenic epitopes of α -lactalbumin and β -lactoglobulin might serve as a marker of persistent cow's milk allergy, and the same group showed that five IgE-binding epitopes of caseins were not recognized by any of the patients with transitional cow's milk allergy but were recognized by the majority of patients with persistent cow's milk allergy [13]. In future, these data might facilitate the provision of a better screening tool for predicting the outcome of food allergy and for

identifying patients at risk for persistent allergy as potential candidates for proactive treatment.

In addition, these data help to clarify the immunogenic capacities of food antigens. Further studies might exploit these results to design epitope-specific immunotherapy or to engineer foods with decreased antigenic potency.

While much work has concentrated upon IgE-mediated food allergy, only limited advances have been made recently in the understanding and diagnosis of non-IgE-mediated food-allergy syndromes. T-cell epitope mapping, like IgE epitope mapping, might be an important goal for further studies. Recent studies that have considered the role of the T cell in diagnostic purposes include a study by Benlounes *et al.* [14], who found increased secretion of TNF- α in antigen-specific peripheral blood mononuclear cells from children with gastrointestinal manifestations of cow's milk allergy. Similarly, antigen-specific T cells could be postulated to be an important source of diagnostic information for evaluating children with food-induced enterocolitis syndrome [15].

Perspective for treatments of food allergy

Early studies in humans demonstrated the potential risk of severe side-effects of conventional immunotherapy for food allergy [16,17]. The recent development of animal models for food allergy has greatly enhanced the progress of research for potential treatments. Animal models have facilitated studies on several modalities in the treatment of food allergy (specific immunotherapy with modified antigens/epitopes; regulatory cytokines, e.g. IL-10 or IL-12; immunomodulatory micro-organisms; immunomodulatory pharmaceutical agents; anti-IgE antibodies; DNA vaccination; Chinese herbal medicine). They have also been used to study the clinical outcome of specific treatments, and to research potential mechanisms involved in secondary tolerance acquisition.

Chinese herbal medicine has provided interesting treatment options for several allergic conditions, including food allergy. The group by Li *et al.* [18] examined the protective effect of a food-allergy herbal formula called FAHF-1. They administered the formula to mice previously sensitized with peanut antigen in the presence of cholera toxin. Mice treated with FAHF-1 had no signs of anaphylaxis to antigen challenge, while sham treated mice experienced severe anaphylaxis. These results correlated with objective measurement of rectal temperature (which decreases during anaphylaxis). Measurement of biological parameters showed a decreased peanut-specific IgE antibody concentration in FAHF-1-treated mice compared to controls, as well as a decrease in IL-4, IL-5 and IL-13 production by splenocytes after antigen and mitogen activation. Inter-

estingly, antigen-specific T-cell proliferation was maintained in the treated group. These promising results may need to be confirmed for other antigens, and further studies must be carried out to determine the active component(s) of FAHF-1, but, again, such studies show the interesting role of the T cell.

As described above, a dysregulation or imbalance of Th1- and Th2-type responses might contribute to the pathogenesis of food allergy. Lee *et al.* [19] examined IL-12 as a potential candidate in the treatment of peanut hypersensitivity. In a mouse model of food allergy, they orally administered liposome-encapsulated recombinant IL-12, either prior to, or following, sensitization of the mice with peanut antigen. They found that primary administration of IL-12 greatly diminished the production of peanut-specific serum IgE, while the same effect was seen to a lesser extent with secondary administration of IL-12. Primary administration of IL-12 also greatly reduced the clinical scores for anaphylaxis. These scores were also reduced to a lesser extent by secondary administration of IL-12. Regarding immunological parameters, peanut-specific IgG₁ was greatly reduced in both treatments, while IgG₂ levels were not significantly different. Splenocyte proliferation in response to the antigen was maintained in the IL-12-treated groups. However, significantly less peanut-specific fecal IgA was observed in both IL-12-treated groups. This work showed that, by administering a cytokine that shifts the immune response towards Th1, the investigators could either prevent or suppress the Th2-type response.

Another potential approach for the treatment of food allergy might involve the immunomodulatory effects of microorganisms that may prevent allergy. Several clinical studies have shown the potential effect of lactobacilli strains in this regard. Shida *et al.* [20••] administered heat-killed *Lactobacillus casei* intraperitoneally to ovalbumin T-cell receptor-transgenic mice prior to antigen sensitization [20••]. They found an increased production of IL-12 in the group of mice given lactobacilli. The rise in IL-12 correlated with a decrease in IL-4 and IL-5 and an increase in IFN- γ production by splenocytes. Upon egg challenge, the clinical anaphylaxis score also decreased in the mice treated with IL-12. These results also correlate well with those of Lee *et al.* [19] showing a similar effect of orally administered IL-12. However, the administration of an IL-12-promoting bacterial strain might be a much easier approach in further therapeutic trials.

The importance of T cells in food allergy is also demonstrated by the observation that infestation by helminths is protective against allergy. Bashir *et al.* [21••] examined the influence of administering *Heligmosomoides polygyrus* on allergy by using a mouse model similar to

the one described above. They found that the group of mice infected with *H. polygyrus* before sensitization had greatly diminished peanut-specific IgE levels and anaphylactic symptoms upon antigen challenge. Marked reduction of secretion of IL-13 by peanut specific T cells was also observed in the treated group. Furthermore, a role for IL-10 in clinical tolerance was observed. Taken together, these studies indicate a key role for modulation of T cell responses in the treatment of food allergy.

Conclusion

Our understanding of the involvement of T and B lymphocytes in food allergy has progressed significantly over the last few years. The concept that a pure Th2 response underlies the mechanism of food allergy has been revisited and supplanted by the theory that immune dysregulation is at work and in some way also involves Th1 and/or Th0 cells. In addition, a role for regulatory cytokines such as IL-10 is strongly suggested, but further work will be needed to verify the variables involved in tolerance acquisition. The recirculation of T cells is a dynamic process strongly influenced by the presence of surface receptors either indicating the activation state of the lymphocyte or a preference for specific organ homing, and this process clearly influences the clinical expression of food allergy. Our understanding of epitope-specific T-cell or B-cell responses has progressed greatly in recent years and may contribute in the near future to the creation of better diagnostic tests and treatment approaches. Finally, mouse models of food allergy are an invaluable tool and provide new insights into mechanisms and therapies for food allergy, at the center of which, it appears, is the role of T-cell responses.

Acknowledgement

The author wishes to thank Sabine Cuenod for excellent secretarial assistance.

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