Novel Fundamental Approaches to Intervening in IgE-Mediated Allergic Diseases

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Recent progress in our understanding of the immunologic basis of allergic diseases has suggested that enhanced production of the cytokines IL-4 and IL-13 by allergen-specific T cells is responsible for increased IgE synthesis and the development of allergic disease in certain individuals. Based on these observations, it is clear that approaches to inhibition of allergen-specific IgE synthesis should be aimed at blocking the activation or preventing the synthesis of IL-4 and IL-13. In the present review, two approaches toward this goal are discussed.

First, an IL-4 mutant protein is described that acts as a powerful antagonist of both IL-4 and IL-13 activity, including induction of IgE synthesis by these cytokines in vitro.

Second, it is demonstrated that T-cell clones can be rendered non-responsive following incubation with allergen-derived peptides representing minimal T-cell activation-inducing epitopes. These non-responsive T cells fail to produce IL-4 and IL-13 and to proliferate following subsequent activation with the relevant allergen and antigen-presenting cells. In addition, these non-responsive T cells fail to provide B-cell help for IgE synthesis. It is tempting to speculate that induction of non-responsiveness in allergen-specific T-cell clones by allergen-derived immunogenic peptides may provide the basis for successful desensitization of allergic patients.

Allergen-specific IgE antibodies play an important role in mediating allergic reactions. They bind to high-affinity IgE receptors (FcεRI) expressed on mast cells and basophils. Binding of allergens to this receptor-bound IgE results in receptor activation that triggers the mastcells/basophils to release soluble mediators such as histamines, leukotrienes, prostaglandins, and proteases, which cause allergic reactions in various target organs, including the eyes, respiratory tract, intestines, and skin.

Early studies have shown that Ig production, including IgE synthesis, is T-helper-cell dependent [1], but only recently has it become clear that cytokines play an important role in Ig production [2]. They determine Ig production quantitatively through their action on B cells and differentiation inducing effects. In addition, cytokines direct the quality of the Ig isotype produced, because they determine Ig isotype switching. For example, the T-cell-derived interleukins (IL)-4 and IL-13 induce human B cells to produce IgG4 and IgE [3], whereas TGF-β induces IgA switching [4]. In addition to cytokines, contact-mediated co-stimulatory signals delivered by activated CD4+ T-helper cells are required for induction of Ig production both in vitro and in vivo [5].

In the present communication, cellular and molecular aspects of induction and regulation of human IgE synthesis and the role of allergen-specific T cells and cytokines produced by these cells will be discussed. In addition, novel approaches to intervene in early stages of the regulatory processes, which lead to induction of IgE synthesis, and their potential clinical utility will be described.

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Abbreviations: EAE, autoimmune encephalitis; PBMC, peripheral blood mononuclear cells.

IL-4 AND IL-13 INDUCE IGG4 AND IGE SYNTHESIS

B cells undergo Ig isotype switching and differentiation into Ig secreting cells in response to antigens that specifically bind to surface IgM receptors and co-stimulatory signals delivered by CD4+ T-helper cells. Antigen-specific T-B interactions require binding of the T-cell receptor to peptide class II major histocompatibility antigens (MHC) complexes on the B cells, which results in T-cell activation and cytokine production. Once T-helper cells are activated, they can stimulate B cells to proliferate and to differentiate in an antigen-dependent fashion. Cytokines play an essential role in these processes. IL-4, which is produced by T cells and mast-cells, induces IgE synthesis in cultures of mononuclear cells of peripheral or cord blood, tonsils, and spleens [3]. Single naive surface IgM+, IgD+ B cells can be induced to produce IgG4 and IgE, in the presence of activated CD4+ T-helper cells and IL-4, indicating that IgG4 and IgE production reflects Ig isotype switching [6]. In addition, these data suggested that during clonal expansion switching of a proportion of the B cells may proceed in two successive steps: from IgM to IgG4 and from IgG4 to IgE. The fact that Ig production in these cultures is restricted to IgM, IgG4, and IgE furthermore indicates that isotype switching is not a stochastic process but is directed by the way the B cells are activated.

Recently, we cloned, expressed, and characterized another T-cell-derived cytokine, IL-13 [7], which was found to induce IgG4 and IgE production by human B cells in an IL-4-independent manner [8]. Interestingly, mouse IL-13 failed to induce IgE synthesis by mouse B cells in culture systems where IL-4 was effective. This is compatible with the observation that mice in which the IL-4 gene is disrupted by homologous recombination fail to produce IgE following parasite infections [9]. Thus IL-13 is only effective in inducing human IgE synthesis. Whether this implies that IL-13 plays a unique role in inducing IgE-mediated allergic diseases remains to be determined and depends on the relative contributions of IL-13 and IL-4 to this process.
IL-4/IL-13-INDUCED IgE SYNTHESIS REQUIRES CO-STIMULATORY SIGNALS PROVIDED BY T-B CELL CONTACTS

In addition to cytokines, contact-mediated co-stimulatory signals delivered by activated CD4+ T-helper cells are required for induction of Ig production in vivo [5,10,11]. These T-B cell contacts are mediated through interactions between the CD40 ligand (CD40L) (which is transiently expressed on activated T cells) and CD40 (constitutively expressed on B cells). Productive CD40L-CD40 interactions are also required for Ig isotype switching in vivo. Patients with the hyper IgM syndrome, who have normal, or enhanced serum IgM levels, but none, or low levels, of the other Ig isotypes have mutations in their CD40L genes that lead to defective CD40L expression and non-productive CD40L-CD40 interactions, which accounts for the defective Ig isotype switching in these patients. Patients with hyper IgM syndrome have normal B cells that can easily be induced to Ig isotype switching and Ig isotype production in vitro by crosslinking of CD40 by anti-CD40 monoclonal antibodies, or transfectants expressing "normal" CD40L in the presence of the relevant cytokines [12-15].

IgE SYNTHESIS IN VIVO

Murine studies have indicated that IL-4 is responsible for IgE production in vivo, because parasite-infected mice treated with neutralizing anti-IL-4 antibodies failed to produce IgE [16]. However, no circulating IL-4 or IL-13 could be detected in the serum of patients with very high serum IgE levels, but convincing indirect evidence has been obtained, indicating that enhanced IL-4 production is also associated with elevated serum IgE levels in humans [17]. For example, peripheral blood mononuclear cells (PBMC) of some patients with high serum IgE levels spontaneously produce IL-4 in vitro, a phenomenon that is never observed with PBMC of non-atopic individuals. In addition, PBMC of such patients produce levels of IL-4 following activation that are significantly higher than those produced by PBMC of normal control donors [16]. The failure to detect measurable levels of circulating IL-4 and IL-13 in atopic patients is probably related to the fact that these cytokines are biologically active at femtomolar concentrations, which are below the sensitivity of the detection assays and that they are predominantly produced at the site of allergic reactions. Indeed, T cells producing IL-4 and IL-5 have been identified in situ in skin biopsies taken from late-phase cutaneous reactions in atopic asthma patients [18] or in bronchial lavage fluids of atopic patients, following allergen challenge [19].

Recent information has shown that allergen-specific T cells are responsible for the enhanced levels of IL-4 production in allergic patients. Allergen-specific CD4+ T cells established from allergic patients generally belong to the T-helper cell 2 (Th2) subset, producing relatively high levels of IL-4, IL-5, and IL-13 and no, or low levels of, IFN-γ after allergen-specific activation in vitro. Because of their IL-4 and IL-13 contents, supernatants of these T-cell clones induce B cells to synthesize IgG4 and IgE under the appropriate culture conditions in vitro.*

MODULATION OF IN VITRO AND IN VIVO IgE SYNTHESIS BY CYTOKINES

IL-4 and IL-13 are thus far the only cytokines that induce IgE synthesis. However, many other cytokines can modulate IL-4/IL-13-induced IgE synthesis both in vitro and in vivo. IFN-γ and IFN-α, IL-8, IL-10, IL-12, and transforming growth factor (TGF)-β all inhibit IL-4-induced IgE synthesis in vitro, whereas IL-5, IL-6, and tumor necrosis factor (TNF)-α have enhancing effects. The mechanism by which these cytokines modulate IgE synthesis are still poorly understood and therefore will not be further discussed here. Interferon (IFN)-γ and IFN-α are also effective in reducing serum IgE levels in vivo. Administration of IFN-γ or IFN-α to patients with the hyper IgE syndrome resulted in a rapid, but transient, reduction in serum IgE levels, whereas the serum levels of the other Ig isotypes in these patients remained unaffected [20,21]. In the single patient treated with IFN-α, complete but transient remission of the skin lesions associated with this syndrome was observed, despite her serum IgE levels still being elevated as compared to those of normal controls [21]. Such case reports, however, indicate that the use of IFN-γ and/or IFN-α for the treatment of severe atopic diseases, like atopic dermatitis, deserves further exploration.

AN IL-4 ANTAGONIST INHIBITS BOTH IL-4 AND IL-13-INDUCED IgE SYNTHESIS

Although the relative contribution of IL-4 and IL-13 to IgE production in vivo remains to be determined, it is clear that effective inhibition of IgE synthesis in vivo requires prevention or inhibition of both IL-4 and IL-13 production by allergen-specific T cells, or blocking of the activity of these cytokines by using specific IL-4 and IL-13 antagonists. Recently an IL-4 mutant protein has been described that binds with high affinity to the IL-4 receptor (IL-4R), without receptor activation [22,23]. Interestingly, this IL-4 mutant protein, in which the tyrosine residue at position 124 is replaced by an aspartic acid (IL-4.Y124D), effectively inhibits both IL-4- and IL-13-induced IgE synthesis in vitro [24]. A 10-50-fold molar excess of IL-4.Y124D inhibited IgE synthesis by optimal IL-4- and IL-13 concentrations by >95%, indicating that IL-4. Y124D acts as a powerful antagonist for both IL-4 and IL-13 activity. Although IL-13 competes with IL-4 for binding to functional IL-4R, and vice versa, it does not bind to the cloned 130-kD IL-4 receptor expressed on heterologous cells. In addition, the affinity of IL-4 for its functional "wild type" receptor is higher than that for the cloned IL-4R. These, and other data, have led to the conclusion that the IL-4 and IL-13R are complex receptors that share a common component, which confers high-affinity binding for IL-4, and which is required for signal transduction [23]. The observation that the IL-4 antagonist inhibited both IL-4- and IL-13-induced IgE production in vitro supports this notion. Therefore, antagonistic IL-4 mutant proteins may provide the most efficient way to reduce IgE synthesis in allergic patients and may have therapeutic potential for the treatment of allergies. However, more information is required regarding the stability and the effects of these molecules in vivo.

INDUCTION OF NON-RESPONSIVENESS IN ALLERGEN-SPECIFIC T CELLS IN VITRO

The most specific approach to prevent allergen-specific IgE synthesis in vivo should be aimed at rendering allergen-specific T cells non-responsive to allergen challenge. Such non-responsive T cells will not proliferate and will not produce IL-4 and IL-13 in response to subsequent activation by allergen and antigen-presenting cells (APC), which will result in prevention of allergen-specific IgE synthesis in vivo.

The minimal requirement for an allergen-specific immune response is effective binding of T-cell receptors on allergen-specific T cells to allergen-derived peptide-class II MHC complexes on APC. Whether these T-cell—receptor peptide/MHC complex interactions result in T-cell activation depends on co-stimulatory signals provided by "professional" APC. TCR-peptide/MHC complex interactions in the absence of co-stimulatory signals delivered by professional APC renders these cells non-responsive to subsequent challenges by antigens and APC [25,26].

Human T cells can also be tolerized in vitro following activation with supra immunogenic doses of antigenic peptides in the absence of professional APC. Subsequent exposure of these tolerantized T cells to antigens presented by APC does not result in proliferation [27,28]. Because anergic T-cell clones fail to produce IL-2, de-

CREASED production of this cytokine by the T cells has been proposed as the mechanism of clonal non-responsiveness [25,26].

Similar observations were made with allergen-specific human T-cell clones. Incubation of T-cell clones specific for the major house dust mite allergen Der pI with super immunogenic doses of Der pI-derived peptides (representing the minimal T-cell activation inducing epitopes) in vitro rendered these clones non-responsive to subsequent stimulation by whole Der pI and APC. The non-responsive state was associated with the strong downregulation of T-cell receptors, suggesting that in this situation reduced TCR expression may account for the non-responsiveness of these cells.* The observation that stimulation of these T-cell clones via their TCR failed to induce significant intercellular Ca++ fluxes is compatible with this notion. In contrast to their non-activated counterparts, the "tolerized" Der pI-specific T cells failed to produce cytokines, including IL-4 and IL-13, and to induce IgE synthesis when co-cultured with purified B cells, indicating that these cells also lost their B-cell helper activity resulting in IgE switching and IgE production.

INDUCTION OF TOLERANCE IN VIVO

Immunotherapy with immunogenic peptides corresponding to immunodominant T-cell determinants administered to mice can induce T-cell tolerance to subsequent challenge with native antigens in vivo. Such peptides have recently been successfully used to treat autoimmune disease in mice. For example, tolerance-inducing regimens for the treatment of myeloid basic protein induced autoimmune encephalitis (EAE), blocked the progression and decreased the severity of EAE, after the disease had begun. The peptide-induced tolerance resulted from the induction of anergy in proliferative, antigen-specific T cells [32]. Comparable data have recently been obtained with allergen-derived peptides. Administration of two peptides, which contain some, but not all, T-cell epitopes on one of the chains of the cat allergen Fel dl also decrease the T-cell responses to this entire chain of Fel dl in mice [32].

PEPTIDE VACCINATION IN ALLERGIC PATIENTS

The potential use of peptide induced T-cell tolerance as a means to treat severe human allergies is a consequence of these studies. The question, however, is how to proceed. As mentioned above, allergen-specific T-cell clones can be rendered non-responsive to subsequent allergen challenges in vitro by incubation with super immunogenic doses of antigenic peptides in the absence of professional APC. In addition, traditional immunotherapy (IT) with standard-dose allergens seems to become more effective, when patients are desensitized with increasing (maximally tolerated) concentrations of allergens. Although it remains to be established, it is tempting to speculate that mechanisms resulting in peptide-induced allergen-specific T-cell non-responsiveness also may account for successful IT. Therefore, based on the present information, ideally one would like to administer high concentrations of allergen-derived peptides, containing T-cell activation inducing epitopes, to bypass the professional APC and to "hit" the allergen-specific T cells directly and render them anergic. Theoretically this is feasible, because peptides containing minimal T-cell-inducing epitopes do not react with specific IgE antibodies and do not induce histamine release from sensitized basophils, and thus are unable to induce anaphylactic reactions (H. Yssel et al, unpublished). Therefore, such peptides seem to be ideal tools for inducing allergen-specific T-cell tolerance. A major problem, however, is that most, if not all, allergens contain multiple T-cell activation inducing (T-cell epitopes), which are restricted by various class II MHC molecules [33–35]. This implies that for each patient all "individual" T-cell epitopes should be determined together with precise human leukocyte antigen typing. Such an approach is, at this stage, impractical and cannot be carried out on a large scale for general treatment of allergies. However, the principles of this type of IT could be worked out in individual, well-defined patients.

A more practical approach could be the use of overlapping peptides of approximately 30–50 amino acids, representing whole known (recombinant) allergens that can be produced at an industrial scale. Assuming that such peptides contain all the T-cell epitopes of the corresponding allergen, administration of high concentrations of these mixtures of allergen-derived peptides seems feasible without inducing anaphylaxis. The advantage of this approach is that it does not require mapping of minimal T-cell activation inducing epitopes. In addition, there is no necessity of class II MHC typing of the patients. One clinical trial, which is in part based on this approach, is presently in progress. In this trial, patients with cat allergies are treated with two Fel dl-derived peptides representing the immunodominant T-cell epitopes.

CONCLUDING REMARKS

Treatment of allergies is presently predominantly focused on late phases of the allergic response, namely, an inhibition of mediator release by mastcells or basophils or on blocking of binding of soluble mediators to their receptors. The present data indicate that enhanced IL-4 and IL-13 production by allergen-specific T cells is associated with enhanced allergen-specific serum IgE levels. Therefore, it is clear that novel approaches to block IgE synthesis in an early stage should be aimed at the inhibition, or neutralization, of IL-4 and IL-13 production in vivo by using IL-4 and IL-13 antagonists like IL-4 mutant protein or antibodies thereof, described here. However, much more information is required about the stability of such antagonistic proteins and their activities in vivo.

The mechanisms by which high IL-4– and IL-13–producing allergen-specific CD4+ T cells are selected in atopic patients remain to be elucidated. A better understanding of these selection mechanisms will enable us to define ways to intervene in these processes, and to reduce the frequencies of high IL-4– and IL-13–producing T cells. One possible way is to render these T cells non-responsive by administration of mixtures of allergen-derived peptides, representing all T-cell activation inducing epitopes on the corresponding allergen. As discussed here, such peptides induce T-cell unresponsiveness, and fail to provide B-cell help, resulting in IgE synthesis in vitro. They also induce a state of T-cell unresponsiveness in mice in vivo. Because these peptides do not react with IgE antibodies and thus are unable to induce anaphylactic reactions, they seem to be ideal tools for inducing specific T-cell tolerance in vivo.

The outcome of the clinical trial with the Fel dl–derived peptides will provide more information about the efficacy and safety of such therapies, although it has to be kept in mind that this trial is carried out with only two peptides containing "immunodominant" T-cell epitopes, whereas the Fel dl molecules contain many more T-cell epitopes, which may prevent efficient tolerance induction in all patients.

REFERENCES

cell clones can be induced to proliferate and switch to IgE and IgG4 synthesis by IL-4 and a signal provided by activated CD4+ T cell clones. *J Exp Med* 173:747–750, 1991


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**ANNOUNCEMENT**

The Ninth Combined Skin Pathology Course and The Fifth Annual Workshop in Dermatopathology sponsored by Scripps Clinic and Research Foundation, will be held at the Sheraton Grande Torrey Pines Hotel, La Jolla, California, September 17–21, 1994.

The program will be based on a rigorous, comprehensive, and practical review of dermatopathology. Microscopic sessions will be conducted, and the workshop approach will include demonstration and unknown slides. The course will offer 40 hours of Category 1 CME credit.

The Course Director will be James H. Graham, M.D., Head, Division of Dermatopathology.

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