

Novel Approaches in Immunotherapy

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Introduction

Immunotherapy for allergic diseases, and in particular for allergic asthma, is more than ever a stimulating field of research. Consideration of the large number of publications on the subject is reasonably convincing that novel strategies for the regulation of the allergic immune response will emerge in the forthcoming years.

This renewed interest occurs at a time when the conventional form of desensitization—namely, a treatment consisting of regular injections of allergens at increasing doses and over prolonged periods of time—is fading away because of side effects and disputable efficacy in allergic bronchial asthma. It follows a number of recent decisive advances made in understanding the mechanisms by which IgE antibodies are produced after exposure to allergens. A summary of this current knowledge will be given below that will help define different levels at which an immune intervention can be envisioned.

A number of considerations should, however, be taken into account beforehand. The aim of immunotherapy is to improve the patient's clinical condition, and not to modulate the allergic immune response for itself. This means that, although everyone's interest is centered on methods by which the production of IgE antibodies could be downregulated or by which the IgE-dependent release of mediators could be controlled—and indeed this chapter will deal with such methods—we still do not know whether other components of the allergic response are not at least as important as IgE. A simple illustration of this is the very fragmentary understanding we currently have of the role of antiallergen IgG antibodies in the allergic reaction.

Improvement of the patient's symptoms implies that one should compare efficacy with that of other therapies. The current pharmacological treatment of asthma based on the use of inhaled corticosteroids combined with β -2-agonists is remarkably effective and includes a significant reduction of nonspecific bronchial reactivity, which is the hallmark of bronchial inflammation. However, the regular use of such drugs is burdened with side effects, not to mention that its efficacy is far from perfect and symptoms do recur within weeks after drug withdrawal. New drugs are unlikely to alter this fact: we treat the consequences of an allergic immune response in the bronchi rather than the initial event—the immune response itself—which is the very target for immunotherapy. A successful method of immunotherapy should therefore demonstrate a significant impact on the inflammatory process occurring in the bronchi, but it should also carry a long-term advantage over drug treatment, i.e., maintaining its effect as long as possible after discontinuation.

Brief Overview of the Mechanism of IgE Production

When an allergen encounters a genetically predisposed organism, be it through the respiratory or digestive mucosa or through the skin, it is first taken up by specialized cells called antigen-presenting cells (APC) (*see Fig. 1*). The function of such cells is to degrade the allergen into peptides that are then driven to the cell surface by major histocompatibility complex (MHC) class II determinants (1). The presentation of such peptides within the groove formed by the α chains of class II determinants is a prerequisite for T-cell recognition and activation. A number of cells can function as APC, depending on the site of the immune response. In the bronchial tree, macrophages, B-lymphocytes, and dendritic cells are "classical" APC (2), but recently, eosinophils, or even possibly mast cells, have been added to the list of these cells. In the skin, Langerhans' cells are the more important APC. We will see below that the type of APC that is processing the allergen can directly influence the final outcome of an immune response.

The APC migrates from the site where it has encountered the allergen to the regional lymph nodes, where specific T- and B-cells are activated (3). Specific T-cells recognize the complex made by MHC determinants and peptides derived from the nominal allergen by engaging their antigenic $\alpha\beta$ receptor, as well as other determinants that are not specific for the allergen, such as CD4/CD8 and CD2. This recognition brings about activation of T-cells, which is followed by a number of events, one of which is the production of

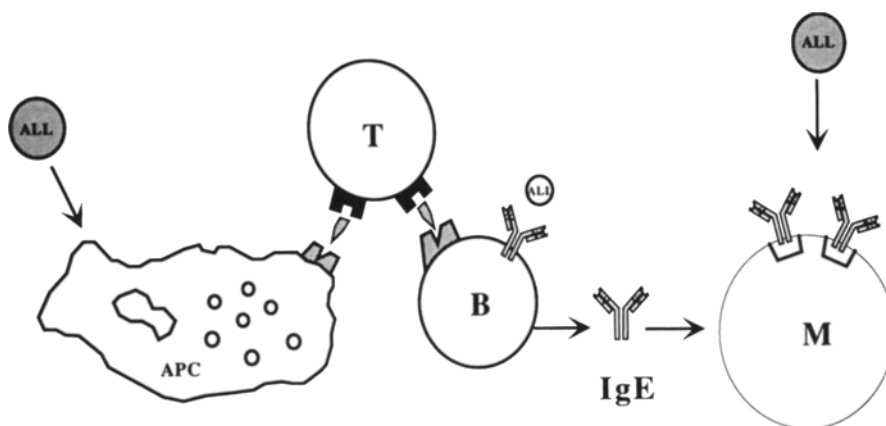


Fig. 1. Schematic view of the mechanism of IgE production (see text for explanation).

cytokines that will activate and trigger the differentiation of B-lymphocytes into IgE-secreting plasmocytes.

The production of IgE antibodies is triggered by the presence of allergen, which is recognized by B-cell-surface immunoglobulins, but requires a double signal from T- to B-cells (4). The first signal is obtained by direct interaction between the two cells. Two types of interactions can take place, cognate or noncognate (5). Cognate interaction is achieved when a T-cell carrying an $\alpha\beta$ receptor for an allergen-derived T-cell epitope encounters this epitope on a peptide carried by a B-cell within MHC class II determinants. The B-cell itself is indeed an efficient APC (6). Noncognate interaction means a direct contact between activated T-cells and nonspecific B-cells by other surface molecules than MHC determinants; B-cells activated in such a way will produce IgE of unrelated specificity, if the second signal is also provided.

The second signal is supplied by cytokines produced by activated T-cells. Interleukin 4 (IL-4) is the main cytokine that triggers the C_ϵ gene transcription and, therefore, the isotypic switch toward the production of IgE antibodies (7). The main source of IL-4 in this case is a subset of CD4⁺ T-cells, called Th2. This second signal is antagonized by IFN- γ , a cytokine produced by Th1 cells.

The two signals provided by T-cells are sufficient to drive B-cell proliferation and differentiation into IgE-producing plasmocytes. However, the direct cognate interaction between T- and B-cells can be bypassed by B-cell activators, such as LPS (8), B-cell infection with EBV (9), or clustering of the CD40 B-cell surface antigen (10). Moreover, the ongoing production of IgE is independent of IL-4 (11).

The fact that the two signals are not necessarily required in a number of situations has an important impact on the design of immunotherapy, as will be explained below.

The functional division between the Th1 and Th2 subsets is, however, less clear-cut in humans than in rodents. Th0, namely T-cells producing IL-4 and IFN- γ together with IL-2, predominate in the peripheral blood T-cell population, in contrast with the situation in mice (12). An interesting question is therefore how Th0 cells differentiate into either the Th1 or Th2 subset. Much remains to be learned here, yet several factors are known to affect this differentiation. Since these factors have direct repercussions on the design of immunotherapy, a brief description is given here. First, the environment into which T-cells differentiate is of primary importance. Thus, Th0 will preferentially differentiate into Th1 in the presence of IFN- γ , whereas Th2 will be formed in the presence of IL-4 (13). Interestingly, it may well be that the main source of IL-4 in this case is the mastocyte, which, on activation, produces large amounts of this cytokine (14). Second, the type of cells presenting the antigen may condition how Th0 differentiates. Dendritic cells drive T-cells toward Th2, whereas macrophages preferentially induce Th1 cells (15). Third, the amount of allergen presented to T-cells also alters the profile of cytokines produced by T-cells. Thus, T-cell clones specific for phospholipase A2 of bee venom produce mainly IL-4 when exposed to low or intermediate doses of allergen; increasing the amount of allergen switches the same T-cell clones mostly to the production of IFN- γ (16). Lastly, both the MHC class II determinants and the peptide bound to it influence the selection of either Th1 or Th2 cells (17).

B-cells committed to the production of IgE can still be modulated by different factors. Soluble CD23, for instance, can act in synergy with IL-4 to increase the production of IgE antibodies (18), and IL-6 increases proliferation and late-phase differentiation of B-cells (19). In addition, B-cells, which carry surface IgE, are amenable to downregulation by interaction with surface molecules, as will be seen below.

IgE antibodies, which are produced mainly in lymph nodes draining the region where the initial contact with allergen occurred, circulate briefly in the serum (t one-half of 2.5 d) and locate in tissues in which they bind to specific receptors. These receptors include a high-affinity receptor (sFc ϵ RI) that is expressed at the surface of basophils, mastocytes, and Langerhans' cells (20), a low-affinity receptor (sFc ϵ RII), which is present on B-lymphocytes, eosinophils, Langerhans' cells, macrophages, and platelets (21), and an

S-type lectin receptor (Mac-2) present on eosinophils and neutrophils (22). The binding of IgE to type I and II receptors results in a number of physiological events, and this level can also be considered for possible immunotherapy.

In the bronchi, mastocytes and basophils are located in the submucosa. When a sensitized individual is exposed to an allergen, the latter binds to specific IgE antibodies located at the surface of those cells. The minimum requirement for basophils or mastocytes to be activated is that at least two IgE molecules are bridged by a multivalent allergen. Activation involves two phases. The immediate phase is characterized by the release of preformed mediators, such as histamine and enzymes, together with heparin. The second, delayed-type phase is dependent on the *de novo* production of mediators that derive from the oxidative metabolism of arachidonic acid; these newly formed mediators include prostaglandins, leukotrienes, and PAF. Mediators of both phases produce the characteristic symptoms of the allergic reaction, vasodilatation, edema, cell infiltration, smooth muscle contraction, hyperproduction of mucus, and so on, which lead to asthma symptoms. In addition, it has recently become clear that on activation mastocytes produce a large array of cytokines, such as IL-1, IL-3, IL-4, and IL-5, that participate in the cell recruitment at the activation site (23).

This rapid overview of the mechanisms by which exposure to an allergen leads to the development of allergic symptoms allows an easy identification of steps that might be amenable to modulation by immunotherapy. The aim of this chapter is to survey novel approaches to immunotherapy that might become available for immunotherapy of asthma in the near future. A few of these approaches have in fact already entered into a phase of clinical evaluation.

For the sake of clarity, I will divide the chapter into four main headings, each corresponding to a level at which immune intervention may be feasible. However, one should keep in mind that nearly all the possibilities that will be envisaged here could have several points of interference in the cascade of events described above. We will first review the presentation of allergen, namely, how physicochemical alterations of allergen can influence the final outcome—IgE production—and why the type of APC handling the allergen is of importance. We will next describe how T-cell activation could be modulated, between antigen recognition at the MHC class II level of an APC to differentiation and interaction with specific B-cells and how IgE-committed B-cells could be downregulated. Last, we will envisage possibilities for immunotherapy at the level of the target cell itself, i.e., the basophil or mastocyte.

Allergen Processing and Presentation

For more than two decades, the physicochemical properties of allergens have been altered with, broadly speaking, two aims: either to produce tolerogens or increase the allergen immunogenicity while reducing allergenicity, namely, its capacity to boost the production of IgE antibodies. One example of tolerogen is the derivative made by covalent coupling of allergens with nonspecific isologous gammaglobulins. Thus, in a mouse model, coupling a hapten or a protein, such as ovalbumin, with gammaglobulins produces a conjugate that renders the animal tolerant to further immunization (24). Transfer of cells from these animals showed that tolerance was dependent on a T-cell population (25). However, variable results were obtained using different animal models; in rats, for instance, similar conjugates induced anaphylactic reactions and no reduction in IgE production (26).

Another example of structural alteration of allergen is given by its polymerization with glutaraldehyde, a procedure described a long time ago, for it reduced the production of IgE antibodies compared to native allergen while maintaining its capacity to mount an IgG immune response. This procedure has recently received much attention following the observation that high-molecular-weight polymers of allergens preferentially triggered the production of IFN- γ , which inhibits IgE synthesis, over that of IL-4 (27). Suppression of IgE production resulting from the use of such derivatives is long-living and resistant to even repeated attempts to immunize with native antigen, as well as being specific for both the antigen used and for the IgE isotype (28). Although much remains to be learned about the genetic factors that influence this outcome (29) and about the precise molecular mechanism of this effect, it illustrates how recent knowledge of the mechanisms of IgE production may be used in the design of novel allergen derivatives. Glutaraldehyde derivatives are in fact already used in clinical practice. Substitution of allergens with monomethoxy-polyethylene glycol (mPEG) yields derivatives that have strong inhibitory effects on both IgE and IgG production in rodents (30), an effect dependent on the induction of CD8⁺ T-suppressor cells (31).

In clinical trials with mPEG derivatives, higher doses could be administered without significant side effects, when compared to native allergen. However, the clinical usefulness of such derivatives has not proven to be superior to those used in conventional desensitization, in either grass pollen or hymenoptera venom hypersensitivity (32). Moreover, in contrast to results obtained in rodents, mPEG

derivatives did not reduce the production of specific IgE antibodies in humans, and instead increased the level of specific IgG, much like conventional desensitization. The use of mPEG derivatives made from highly purified allergen molecules, such as those produced by recombinant DNA technology, instead of allergen extracts of poorly defined composition, might, however, clarify the reasons for such discrepancies.

In the author's laboratory, another approach for immunotherapy that makes use of immune complexes constructed by allergen and specific antibodies was chosen. As will be described below, such complexes can interfere at different levels within the sequence leading from allergen exposure to the production of IgE antibodies. One early level is the capacity to alter the way the antigen is processed. It has indeed been shown *in vitro* that the presence of a specific antibody together with an antigen alters the antigen processing so as to activate T-cell clones different from those activated with the antigen alone (33).

The route by which allergen is administered is of importance. Thus, antigen given orally results in tolerance rather than immunization, a phenomenon that has been known for long (34) and has resuscitated several attempts to desensitize allergic patients (35). Acid denaturation of allergen in the stomach can be circumvented by the use of encapsulated allergen. Few convincing data have, however, emerged from such studies. One obstacle to such treatment is the very large dose of allergens that has to be given (up to 100-fold—the amount currently used in conventional desensitization) making this approach a very costly one. It also remains to be demonstrated whether the incidence of side effects is lower than in conventional desensitization.

More promising perhaps are the experiments made in rats to which antigen is given by inhalation. Rats repeatedly exposed to antigen like ovalbumin by such a route develop a short-lasting production of specific IgE, followed by a prolonged state of IgE unresponsiveness (36). This tolerance is owing to the induction of specific CD8⁺ T-lymphocytes (37), whose main production is in the lymph nodes draining the upper respiratory tract (38). Interestingly, newborn rats lack this tolerance-inducing mechanism, a finding that can be of relevance when one considers the high degree of susceptibility to allergen sensitization observed in human neonates (39). Whether administration of allergen by inhalation will become an efficient treatment for the ongoing IgE immune response, which is the hallmark of hypersensitive patients, is still speculative. However, attempts to downregulate the production of IgE antibodies by

administration of allergen by the intranasal or sublingual route are already under way.

T-Cells

T-cells recognize a processed allergen in the context of MHC class II determinants, which is followed by cell activation. One can therefore envision different approaches to interfere with this recognition in a highly specific manner (Fig. 2). The antigen receptor of T-cells can be lured by being presented with a peptide or denatured allergen that binds to it without activating the cells; specific T-cells could therefore be rendered unresponsive to further stimulation by native allergen. However, the TCR itself might be functionally inactivated, using reagents with affinity for certain V β chains. In addition to these possible methods, if one keeps in mind how important the microenvironment under which T-cells are activated is for their differentiation into either the Th1 or Th2 subset, it is quite conceivable that specific means to alter this environment will be at hand in the very near future.

Human T-cell clones specific for *Der p* I, one of the main allergens of the house dust mite *Dermatophagoides pteronyssinus*, can be anergized by a single exposure to supraoptimal amounts of peptides derived from the allergen (40). This anergy, which is obtained in the absence of APC, is long-lasting and is accompanied by decreased expression of the TCR. T-cells are functionally inactivated, but keep their reactivity to IL-2. Interestingly, the pattern of cytokines they produce changes, for instance, from a predominantly IL-4 secretion profile to an IFN- γ one (41). Inhibition of specific T-cell activation can therefore be obtained directly at the level of the TCR, using allergen-derived peptides. However, even molecules as small as *Der p* I, with a mol wt of 25,000, contain many T-cell epitopes. A complete control of the immune response toward such molecules would therefore require several peptides representative of T-cell epitopes, which would in practice limit this approach to small molecules, such as haptens (the benzyl-penicilloyl group may be one example of this). Alternatively, one may envisage constructing molecules comprising all T-cell epitopes, but lacking B-cell epitopes (42). It could also be possible that some of the T-cell epitopes dominate the T-cell immune response; in such a case, a single or a small number of peptides could well be sufficient to control the entire antiallergen immune response. This is a matter for further research, but at least we know that in vitro specific T-cells can be anergized for a long period of time.

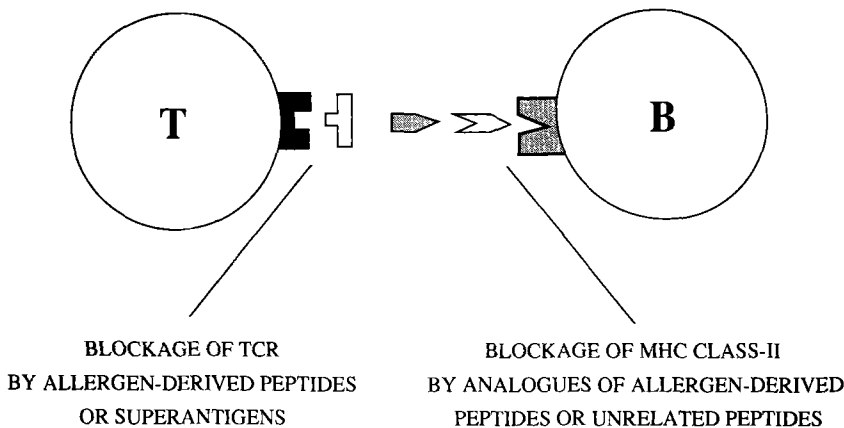


Fig. 2. Possible methods by which the MHC-restricted T-cell recognition of allergen-derived peptides could be inhibited (*see text* for explanation).

Superantigens belong to a special class of antigens that can stimulate powerful polyclonal proliferation by direct binding to the V β chain of particular TCR (43). In some cases, however, the outcome of the binding is functional inactivation or deletion of the T-cells carrying the corresponding V β chain (44). Thus, enterotoxins from *S. aureus* can activate and induce anergy of human T-cell clones carrying specific V β chains, such as the V β 3 chain. This functional inactivation is accompanied by downregulation of TCR and increased expression of IL-2 receptors (45). The clinical use of such toxins would require the elimination of side effects, such as pyrogenicity, which should be at hand through molecular engineering of the molecule. It would also be necessary to demonstrate that a restricted number of β chains is recruited for a given allergen immune response, and that these β chains are not crucial for other relevant immune response, as for example toward infectious agents. Restriction in TCR-V β gene product usage has been demonstrated in autoimmune diseases, such as multiple sclerosis (46), and extrapolation to the allergen immune response may be justified to some extent. Indeed, V β 3 or V β 6 chains are frequently used in the immune response toward house dust mite allergens, and T-cell clones carrying these β chains have been tolerized by treatment with *S. aureus* enterotoxins (47). Moreover, in a mouse system, the IgE immune response to ovalbumin administered by inhalation was found to be associated with the preferential recruitment of T-cells carrying V β 8 chains (48). Up to now, however, this promising approach is still too fragmentary, and very little is known about the

capacity of T-cells to use a different TCR to respond to the initial allergenic molecule.

The growing understanding of how the microenvironment can influence T-cell differentiation in either Th1 or Th2 may well have direct practical consequences for immunotherapy. For instance, the medication taken by patients might be of relevance: vitamin D3 or corticosteroids have been shown to trigger Th2 differentiation preferentially over Th1 (49). The way by which allergen is administered is another example: antigen given by other routes than transmucosal favors Th1 over Th2 (50), and since a source of IL-4 is required to switch from Th0 to Th2, one may surmise that mastocyte-rich tissues should be avoided.

B-Cells

The B-cell is understandably a privileged target for immune modulation of the allergic reaction. Again, both allergen-specific and nonspecific approaches can be outlined (Fig. 2).

At the B-cell level, an efficient presentation of a processed peptide has to satisfy two criteria: the peptide should bind firmly to the groove formed by the α helical walls of MHC class II determinants and should contain the sequence that is recognizable by specific T-cell TCR. T-cell epitopes on a number of allergens have been defined (51,52); the engineering of analogs of T-cell-activating peptides might result in efficient inhibition of T-cell activation by competitive displacement from MHC determinants, provided these analogs keep their ability to bind to MHC determinants, but lose their capacity to activate specific T-cells (53). A very limited amino-acid substitution may be all that is required to construct such peptides (54). However, as described above for the interaction of T-cells with peptides, this approach is conceivable if all T-cell epitopes, or at least dominant epitopes if they exist, are defined on each allergen molecule, a condition that, in practice, could be met only for very simple allergenic molecules.

An alternative at the B-cell level would be the functional inactivation of MHC class II determinants. If allergen-derived peptides are presented by a restricted number of MHC determinants, and provided that these determinants are not essential for the presentation of unrelated, relevant antigens, one can envisage suppressing the expression or the function of these class II determinants at the B-cell surface. Peptides unrelated to allergens, such as those obtained from the carboxyl terminus of the HA-1 molecule of influenza hemagglutinin, are capable of selectively binding to DRB1 and

DRB3 gene products, MHC determinants that are preferentially used in the activation of T-cells specific for house dust mite (HDM) allergens, such as *Der p* II (55). Indeed, the binding of this non-allergen-derived peptide results in efficient inhibition of HDM-specific T-cell activation, at both the polyclonal and clonal cell levels. The question is therefore to determine whether there is a preferential association between MHC class II determinants and the capacity to activate allergen-specific T-cells and, hence, the capacity to mount an IgE antibody response. This type of association has been repeatedly reported, first in the IgE immune response toward short ragweed pollen allergen Ra5 found to be linked to HLA-Dw2 (56), and more recently, in the immune response to grass pollen allergens (57) and to house dust mites of the *Dermatophagoides* species (58). Significant associations are encountered when the IgE response to highly purified allergen molecules is analyzed, and might even be stronger when screening it at the level of single epitopes. Again, however, the practical issue here is blurred by the fact that the repertoire of MHC class II determinants used to mount an immune response toward allergen mixtures, such as house dust mite, is likely to be much wider than just a few determinants.

Other methods for immunotherapy at the B-cell level are essentially nonspecific, with the possible exception of anti-idiotypic antibodies (Fig. 3). The synthesis of IgE antibodies could be interrupted at two levels at least. Two steps have been described in the transcription of the C ϵ gene. The first ϵ -germline transcript is a $\pm$ 1.9-kb mRNA that can be detected very early after B-cell activation by IL-4 (59). This transcript is not functional since it contains several stop codons, but is a necessary step for the induction of a second 2.2-kb ϵ -mRNA requiring IL-4 and a second T-cell signal, which is then translated into IgE molecules. This two-step gene transcription may provide a method by which IgE synthesis can be blocked; specific inhibitors of the first gene product could be designed in the very near future. The second level is the translation from mRNA to protein. Indeed, antisense DNA can effectively block the synthesis of sFc ϵ R II and proliferation of B-cells (60); the highly specific and nonimmunogenic nature of such DNA may well become an advantage for clinical applications.

Molecules expressed at the surface of B-cells are possible targets for downregulating the IgE immune response in an isotypic-specific manner. The CD23 cell-surface antigen, a marker for early B-cell activation as well as a low-affinity receptor for IgE, belongs to the lectin type-II receptor family and exhibits a number of properties that have been recently reviewed (61). Some controversy still

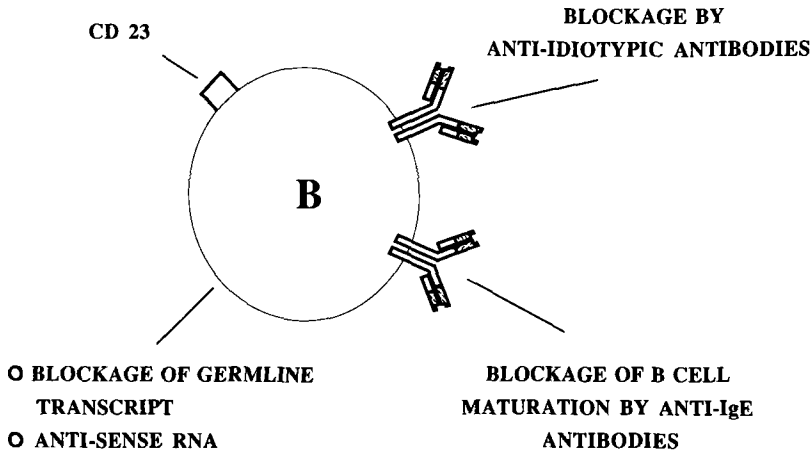


Fig. 3. Possible interventions at the IgE-committed B-cell level (see text for explanation).

exists about the influence of CD23 on IgE production because of the significant differences that have been encountered between humans and rodents. In humans, the CD23 molecule is cleaved by an autolysis, and the released fragments, mostly a 22-kDa fragment, have been shown to act in synergy with IL-4 in the induction of IgE synthesis in humans (62). On the other hand, addition of anti-CD23 antibodies to B-cells results in the blockage of both the maturation and differentiation into IgE-secreting plasmocytes (63). More recently, the demonstration that CD21, another cell-surface molecule known as the Epstein-Barr virus and C3d receptor, is the natural ligand for CD23 (64) has opened yet another possibility: interference with the binding of CD21 to CD23, as for instance with a specific antibody toward the first, may block IgE synthesis. One of the difficulties of targeting CD23 for therapy is its expression on cells of other lineages than B-cells, which may practically limit the usefulness of such a method.

Finally, IgE molecules expressed at the surface of IgE-switched B-cells may represent yet another target for regulation. Murine monoclonal antibodies have been produced toward the ϵ -CH3 domain, which recognize surface IgE molecules on B-cells, but not soluble IgE or IgE located on basophils or mastocytes, and are therefore nonanaphylactogens. Instead, these antibodies have the capacity to inhibit the binding of IgE to Fc ϵ RI. Interestingly enough, such antibodies are able to inhibit both IL-4-dependent and IL-4 independent IgE production (65), the latter prevailing in the ongoing

IgE immune response of atopic individuals, as well as in situations in which the second signal produced by IL-4 is replaced by LPS or *S. aureus*. Thus, mice immunized with benzylpenicilloyl-KLH in alum produce 80% less IgE antibodies to BPO on subsequent immunization if they are treated with an anti-IgE antibody to the ϵ -CH3 domain just before the boost injection of BPO-KLH (66). A human-mouse chimeric antibody exhibiting the same properties has been prepared and is expected to be tested in clinical trials in the forthcoming months. A variant of this method has been described in which monoclonal anti-IgE antibodies to B-cell-surface IgE led to cell deletion (67).

Surface-bound IgE molecules express idiotypes, namely, determinants carried by antibodies at the level of their variable part, that could be used to downregulate the production of specific antibodies sharing the same idiotypic determinants. Anti-idiotypic antibodies have indeed the capacity to downregulate the *in vitro* IgE production in humans, either with xenogeneic anti-idiotypic antibodies (68) or with autologous antibodies (manuscript submitted). This approach has the potential advantage of reducing specific antibodies of both the IgE and IgG isotypes. Treating patients with allergen-antibody complexes results in an increased production of anti-idiotypic antibodies (*see Allergen-Antibody Complexes*).

Target Cells

The last level at which one can define targets for immunotherapy is the level of the basophil or mastocyte, the target cell itself. Several possibilities have recently emerged (Fig. 4).

Since activation of the target cell requires IgE antibodies to bind to high-affinity receptors located at its surface, preventing this binding will also prevent the release of mediators responsible for allergic symptoms. The cloning of the high-affinity receptor for IgE (sFc ϵ RI) has recently been achieved (69). The complete receptor is a tetramer constructed from one α chain, one β chain, and two γ chains. The α chain contains a long hydrophilic extracellular segment that confers the receptor with its specificity for IgE (70). The β chain does not seem to be essential for full physiological activity of the receptor in humans, since cotransfection of α and γ results in the expression of functional receptors. The γ chain, which is shared by receptors for IgG, transduces the signal inside the cell. The α chain is therefore a first candidate for interaction between IgE and the receptor; its soluble form has an affinity for IgE comparable to that of the whole, cell-bound receptor. With the precise identification of the regions of the α chain, on the one hand, and of the ϵ -CH3 domain, on the other

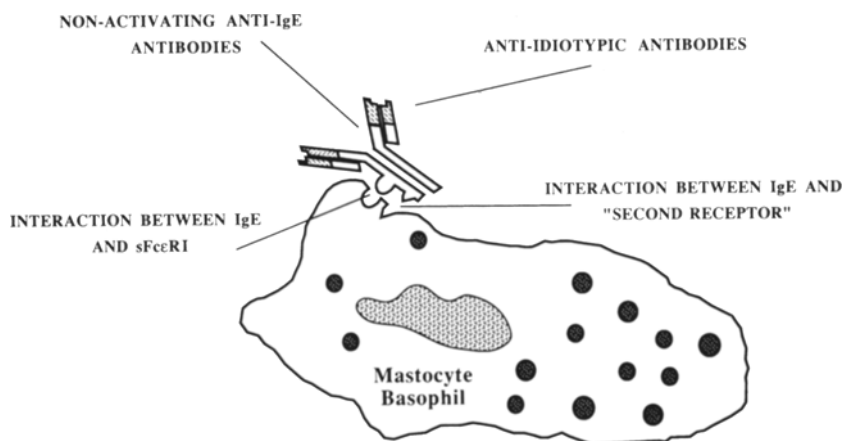


Fig. 4. Possible interventions at the target cell level (*see text for explanation*).

hand, that interact with each other, the design of drugs or peptides capable of preventing such an interaction will be feasible (71). As such, drugs or peptides would be highly specific for reactions involving the sFcεRI, with the additional advantage of the restricted cell distribution of this receptor, since it has been described only on mastocytes, basophils, and Langerhans' cells. Inhibitors of transduction may be developed together with our increased understanding of how surface-bound IgE triggers cell activation. Finally, the expression of the sFcεRI itself might be amenable to modulation.

In the conventional model of mastocyte/basophil activation by IgE antibodies, the minimum requirement for a mastocyte to degranulate is that at least two molecules of IgE are linked together by, for instance, a multivalent allergen. This is followed by activation of the cell, influx of calcium into the cell, and degranulation. In such a model, IgE acts only as a surrogate receptor. However, the presence of a second activation site on the mast cell surface has been suspected for some years. The binding of IgE to this so-called second receptor might be a necessary step to obtain full activation of the cell (72). Thus, an amphipathic decapeptide has been defined from the amino-acid sequence of the ε-CH4 domain that exhibits a remarkable primary structural similarity with substance P (73), a neuropeptide released from afferent neurons in response to antidromic stimuli, and that induces the release of histamine from mast cells. In an attempt to interfere with this second signal, antibodies have been generated toward the ε-CH4-derived decapeptide. These antibodies strongly inhibit the release of histamine from rat

mastocytes (74). Therefore, immunization toward this decapeptide might lead to inhibition of mast cell activation in human immediate hypersensitivity. Phase I clinical trials with such a peptide are expected in the forthcoming months.

Auto-anti-IgE antibodies are a normal constituent of the IgE immune response in humans (75). Their precise role is unknown, but they may be similar to rheumatoid factors described in IgG immune responses in which they help mounting secondary immune responses (76). Anti-IgE antibodies with specificity for different regions of IgE and various biological activities have been described. The properties of some of them are most interesting. Thus, antibody binding to IgE at the mast cell can inhibit the degranulation resulting from the addition of another, anaphylactogen anti-IgE antibody or potentially an allergen (77). Of interest is the observation that such an antibody has also been shown to displace IgE from CD23, its low-affinity sFcεRII receptor (78). Active production of such antibodies by immunization with the corresponding IgE-derived peptides or passive transfer may be envisaged to modulate the allergic reaction at the target cell level.

Antibodies toward the variable part of IgE, namely, anti-idiotypic antibodies, share some of the characteristics of anti-IgE antibodies, insofar as they can be potent anaphylactogens or, on the contrary, strong inhibitors of degranulation (79), the main difference being their selectivity for a given immune response. As explained below, the target cell level might be a further point of impact for anti-idiotypic antibodies.

Allergen–Antibody Complexes

Complexes constructed from allergens and specific antibodies have been used in the author's laboratory and clinical settings for the last few years in an attempt to design a method by which IgE production could be suppressed in a specific way. These studies had been initiated after it was demonstrated in animal models that anti-idiotypic antibodies suppressed the production of IgE (80), that complexes of antigen and antibody were potent boosters of anti-idiotypic antibody production (81), and that the presence of a specific antibody altered the processing of antigen (82). We reasoned that allergen–antibody complexes could interfere at different levels in the cascade of events from allergen exposure to IgE production: alteration of allergen processing, selection of specific B-cells as APC, induction of anti-idiotypic antibodies with the potential of blocking the differentiation of IgE-committed B-cells, and perhaps the target cell itself. We first demonstrated that anti-idiotypic antibodies were

in fact part of the normal response made by hypersensitive subjects toward allergens (83) and that specific IgG antibodies could be used as a substitute for IgE to make complexes (84).

Over recent years, we have treated more than 250 patients with either chronic allergic asthma or atopic dermatitis, and sensitivity to *D. pteronyssinus* or grass pollens. In both diseases and with those two allergens, a significant clinical improvement was observed within a few months after starting therapy, using an amount of specific antibodies of 10–100 μ g and an average of 2–25 μ g allergen (85, 86). With regard to allergic asthma, we have shown that bronchial reactivity to inhalation of not only allergen, but also of a nonspecific reagent, such as acetylcholine, was significantly reduced if not completely lost (87). The long-term followup of treated patients has shown that the benefit of such treatment was maintained long after discontinuation of injections.

The biological findings that have been regularly associated with clinical improvement include a significant reduction in the level of both specific IgE and IgG antibodies (85), with no concomitant reduction of total IgE during the first months and a significant and early increase in the production of anti-idiotypic antibodies toward allergen-specific antibodies (manuscript submitted).

Conclusions

This article summarizes the current approaches for immunotherapy, from concepts that have a reasonable chance to give birth to practical methods to those that have already proven their efficacy through clinical trials. At this time-point, it remains risky to predict which of these methods—or combination of methods—will ultimately prove to be of benefit for hypersensitive patients, but there is a high likelihood of having at hand alternatives to conventional desensitization within the next few years. There is no doubt that the intensive work presently devoted to allergen cloning and production of recombinant molecules, together with the availability of methodologies to produce human monoclonal antibodies, will efficiently hasten the pace at which this field of research grows.

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