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The Immunoglobulin E-Allergen Interaction: A Target for Therapy of Type I Allergic Diseases

Key Words

Type I allergy · Immunoglobulin E · Recombinant allergens · Blocking antibody · Immunotherapy

Abstract

The interaction of immunoglobulin E and otherwise harmless antigens (allergens) leads in sensitized individuals through effector cell activation to the immediate induction of a cascade of inflammatory reactions, the hallmark of type I allergy. Recently, the molecular and structural characterization of allergens, specific IgE antibodies and their epitopes has made rapid progress. Here we discuss active and passive strategies for therapy of type I allergy, which are based on interfering with the IgE-allergen interaction.

Allergic patients tend to mount immunoglobulin E responses against innocuous antigens (allergens) that originate from pollen, mites, animal dander and moulds [1]. While healthy individuals display comparable immunoglobulin G and T cell responses against these *per se* harmless moieties without signs of disease [2–4], allergic patients with specific IgE antibodies show severe clinical symptoms (allergic rhinitis, conjunctivitis, dermatitis, asthma and even lethal anaphylactic shock) upon allergen contact. The immediate reaction mainly takes place at epidermal and mucosal surfaces (nose, eyes, lung), where allergic effector cells (mast cells, basophils, eosinophils), preloaded with Fcε-receptor-bound specific IgE antibodies, are prepared for allergen contact. Dimerization of effector cell-bound IgE antibodies, as it occurs when two allergen epitopes crosslink their paratopes, is the critical event that leads to the explosive release of biological mediators (e.g., histamine, leukotrienes [5]. Without such cellular amplifi-

cation mechanisms, IgE-allergen interaction would likely remain unnoticed because IgE itself represents the least abundant class of human immunoglobulins present in serum and other body fluids.

The molecular characterization of allergens by recombinant DNA technology has recently provided well-defined allergens and surprisingly has revealed that allergens from various origin display similar epitopes, thus suggesting that a limited number of cross-reactive model allergens may be defined for allergy diagnosis and therapy [6–8]. Current experimental approaches to specific immunotherapy are mainly focused to the T-cell-mediated control of IgE production either by shifting the increased TH2 activity in allergic patients to a TH1 response or by inducing T cell tolerance or anergy [9–11]. From the current viewpoint, we propose that preventing the interaction of allergens with cell-bound IgE may represent another target for specific therapeutic intervention.

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Mapping the IgE Epitopes of Allergens: How Do IgE Antibodies Recognize Allergens?

The cross-linking of IgE antibodies with specificity for at least two epitopes is required for the activation of allergic effector cells [5]. Any allergenic moiety needs therefore at least two epitopes which are available to IgE antibodies in order to induce mediator release. As B cell epitopes in general, IgE epitopes represent areas that are exposed on the antigen surface [12]. Basically two types of IgE epitopes have been defined. Epitopes which consist of a stretch of amino acids in a row are termed 'continuous epitopes' while those epitopes that are composed of at least two different areas of the molecule brought together by folding are termed 'discontinuous epitopes'. The determination of continuous epitopes may be achieved by mapping with synthetic overlapping peptides which are synthesized according to the allergen amino acid sequence [13]. While several IgE epitopes could be determined using peptide mapping [14, 15], many relevant IgE epitopes could not be identified because peptides frequently fail to assemble conformations suitable for binding [16, 17]. Screening of epitope libraries constructed from randomly DNase I-digested allergen cDNA fragments turned out to yield more information than did peptide mapping [16, 18, 19] (fig. 1a). Using this technology, epitopes linked to β -galactosidase may be better presented and available to IgE antibodies. IgE epitopes of several relevant allergens (e.g. phospholipase A2 [20], the major birch pollen allergen, Bet v 1 [21], the calcium-binding birch pollen allergens, Bet v 3 [22] and Bet v 4 [23, 24]) belong to the discontinuous type. Disruption of the three-dimensional structure of Bet v 1 and the major cow dander allergen, Bos d 2, as obtained by expressing two halves of the allergen, yielded protein fragments which presented random coil structure and had lost IgE-binding capacity and therefore anaphylactic activity [21, 25]. The determination of discontinuous IgE epitopes may be achieved by mimotope strategies including combinatorial peptide libraries [26] or more traditionally, by using antibody competition experiments (fig. 1b). From a panel of mouse monoclonal antibodies that potently inhibited binding of Bet v 1 to IgE, several mAbs identified a continuous epitope within an exposed area of Bet v 1 that may indeed be part of a discontinuous IgE epitope [27]. Ultimately, the detailed analysis of discontinuous IgE epitopes will require the resolution of the three-dimensional allergen structures by X-ray crystallography and/or nuclear magnetic resonance (NMR) spectroscopy as shown for the birch pollen allergens, Bet v 1 [28] and Bet v 2 [29], the grass pollen allergens, Phl p 2 [30] and Phl p 5 [31] and the major mite allergen, Der p 2 [32].

The Three-Dimensional Structure of Allergens: Defining Antigenic Surfaces

Due to the application of recombinant DNA technology large amounts of homogenous recombinant allergens have become available, which allowed the determination of their three-dimensional structures. NMR and X-ray crystallography of the major birch pollen allergen, Bet v 1, showed a seven-stranded antiparallel β -sheet that wraps around a C-terminal alpha helix [28]. The three-dimensional structures of birch pollen profilin and *Arabidopsis* profilin and a partial structure of the major timothy grass pollen allergens, Phl p 2 and Phl p 5, as well as of the mite allergen Der p 2 have been reported [29–33]. Profilins represent ubiquitous actin-binding proteins in eukaryotes that constitute a family of cross-reactive panallergens [34, 35]. Birch profilin is composed of three alpha helices, seven β -strands and ten turns. It displays the same seven-stranded incomplete, antiparallel up-and-down β -barrel structure observed in *Amoeba* and mammalian profilins, thus providing a solid basis for the extensive cross-reactivity of patients' IgE with different profilins. The experimentally determined IgE epitopes cluster in three major areas that are surface-exposed and overlap with the actin binding site [36] and the poly-L-proline binding region [37] (fig. 2). This finding strongly suggests that the unliganded profilin is responsible for the induction and probably maintenance of IgE antibody responses in profilin-allergic patients. Despite the relatively low molecular weight of profilin (i.e., 14 kD) sufficient space for binding of at least three different species of antibodies is available, thus permitting cross-linking of effector cell-bound IgE by monomeric profilin (fig. 3). The major timothy grass pollen allergen, Phl p 2, is composed of a compact eight-stranded β -barrel devoid of alpha helical elements [30].

Comparison of the three-dimensional allergen structures currently available could, however, not reveal common structural motifs shared by non-cross-reactive allergens. Nevertheless, these models have improved the definition of surface areas which are likely to interact with antibodies. Cocrystallization of allergens and specific IgE Fabs obtained from combinatorial libraries of patients' lymphocytes [38] will yield a detailed structural analysis of the allergen-IgE interaction. Ultimately these structural data may permit the design of allergen-derived peptide mimetics able to prevent the interaction of allergens with their specific IgE. Such peptide mimetics may prove useful for active as well as passive therapy.

Construction of an epitope expression library

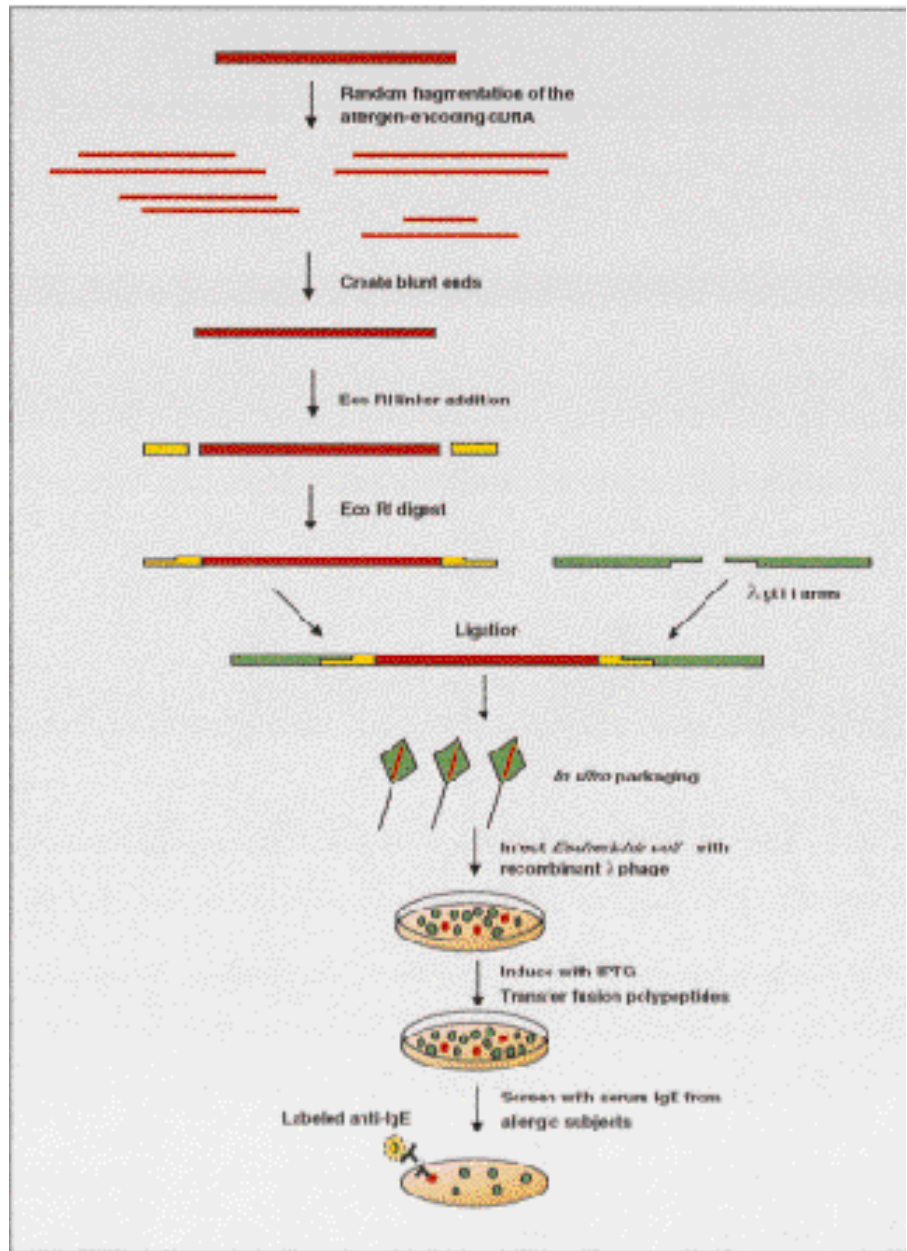


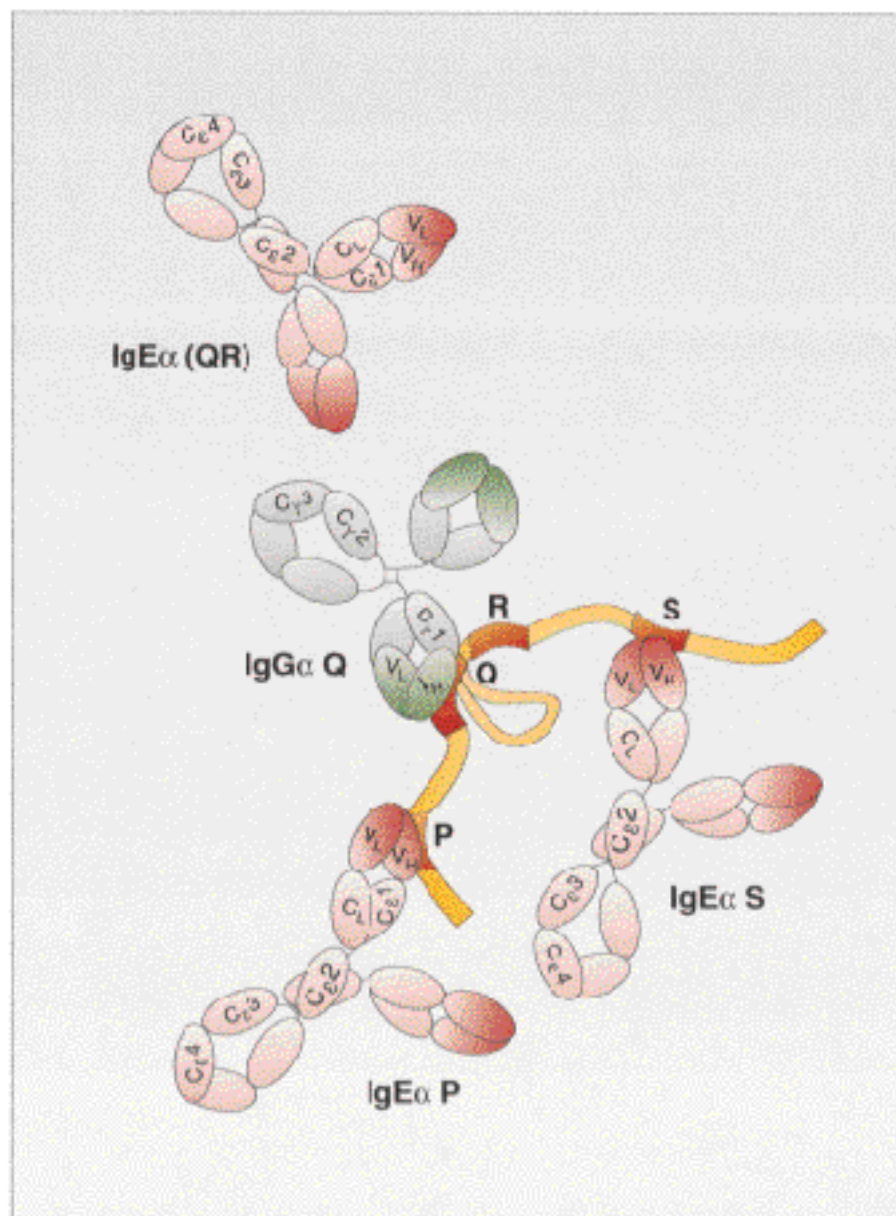
Fig. 1. Mapping of allergen IgE epitopes by gene fragmentation (**a**) and antibody competition (**b**). **a** By random DNase I digestion of the allergen-encoding cDNA, short DNA fragments are generated that are ligated into phage λ gt11. The resulting epitope expression cDNA library is then screened with IgE antibodies to obtain phage clones that express continuous IgE epitopes.

Saturation of Effector Cell-Bound IgE with Allergen-Derived Haptens: Strategies to Prevent Effector Cell Activation

'IgE-binding haptens' which comprise the binding site for only one IgE antibody will bind to effector cells without cross-linking and induction of mediator release (fig. 4). Nonanaphylactic IgE-binding haptens may be considered

for passive and active therapy of allergy. Passive treatment may be performed by exposing effector cells to a cocktail of IgE binding haptens comprising most IgE epitopes prior to allergen exposure. It would be expected that a hapten cocktail locally applied to the mucosa of the nose, eyes and lung will saturate effector cell-bound IgE and subsequently prevent cell activation by complete allergens (fig. 4; upper part). Alternatively nonanaphylactic IgE binding haptens

Fig. 1b. Allergens harbor continuous IgE epitopes (e.g. P, S) and discontinuous IgE epitopes [e.g., (QR)] assembled by two continuous segments Q and R]. Continuous IgE epitopes P and S may be identified by screening an IgE epitope library as shown in **a**. The discontinuous IgE epitope comprising (QR) can be defined with blocking IgG (IgG α Q) antibodies that recognize only a continuous segment (e.g., Q) of it. IgG α Q will block IgE α (QR) binding and therefore represents an antibody potentially useful for therapeutic purposes. IPTG = Isopropyl- β -thiogalactopyrenoside.



may be used for active immunization with reduced side effects in order to induce blocking IgG antibodies against the IgE epitopes (fig. 4; lower part). The use of haptens in therapy is not a new idea as it has been utilized more than 20 years ago to treat drug-induced allergy [39, 40]. The application of the hapten concept for protein allergens requires that relevant cross-reactive allergens which harbor most of the relevant IgE epitopes can be identified. For tree and grass pollen allergy it has indeed been shown that a panel of a few recombinant allergens account for most of the tree and grass pollen-specific IgE [41, 42]. As illustrated in figure 1a, IgE epitopes can then be efficiently mapped by IgE

immunoscreening of epitope expression cDNA libraries built from randomly fragmented allergen genes. A major IgE binding hapten of the major timothy grass pollen allergen, Phl p 1, was recently obtained by this technique and it was further shown that incubation of effector cells with allergen fragments generated by proteolytic digestion [43] prevented mediator release by the complete allergen. Although these results are encouraging, tremendous work will be required regarding the molecular and structural characterization of allergens and their IgE epitopes to provide the IgE binding haptens needed for therapeutic purposes.

Fig. 2. Model depicting the IgE and ligand binding sites of birch pollen profilin. Profilin backbone is in white, with the experimentally identified epitopes in green. This orientation shows the actin binding surface which is composed of the C-terminal helix and the three bottom strands of the β -sheet. Also shown is a model of the poly-*L*-proline binding site (poly-*L*-proline in yellow). As actin (43 kD) and proline-rich peptides overlap with the IgE binding sites, it is likely that the native (unliganded) profilin is responsible for the elicitation of the allergic response.

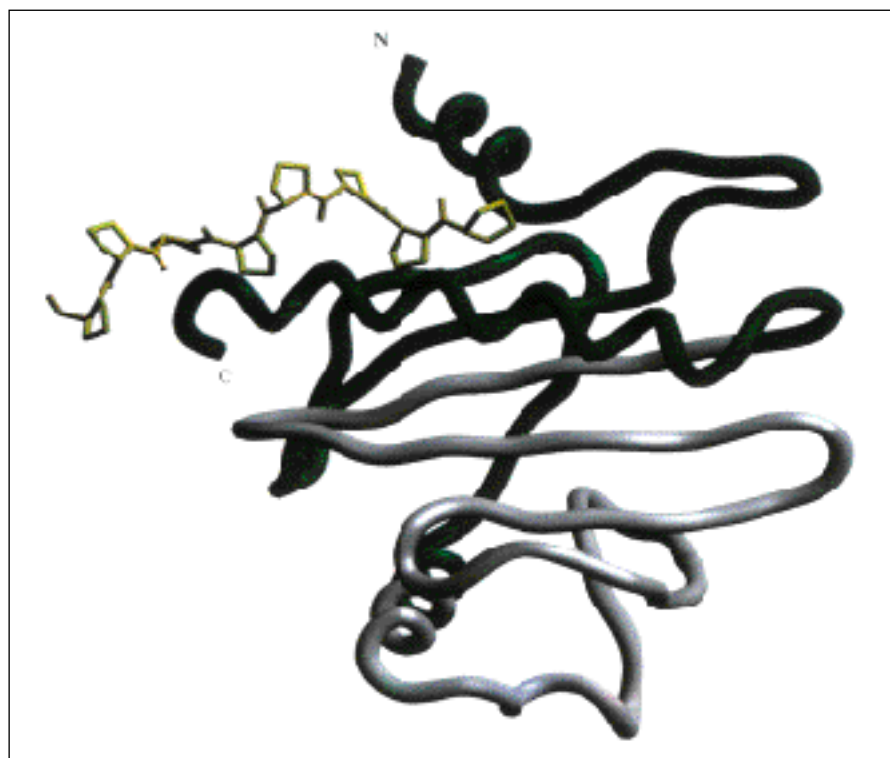
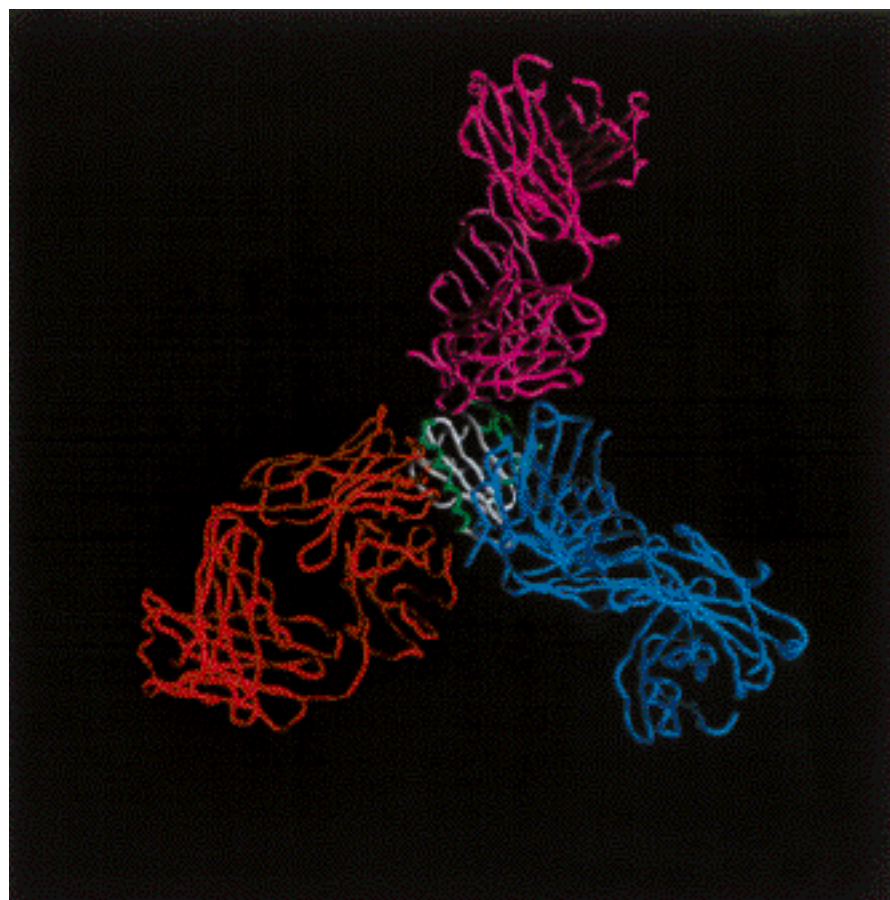


Fig. 3. Model of IgE-allergen complex showing the binding of multiple IgE antibodies to distinct epitopes on birch pollen profilin. The distribution of epitopes on the profilin surface allows for the simultaneous binding of multiple IgE antibodies. The profilin backbone is in white with the experimentally identified epitopic regions in green. Fab fragments (red, blue, magenta) are modeled so as to contact the three epitopic regions with minimal steric interactions between antibodies.



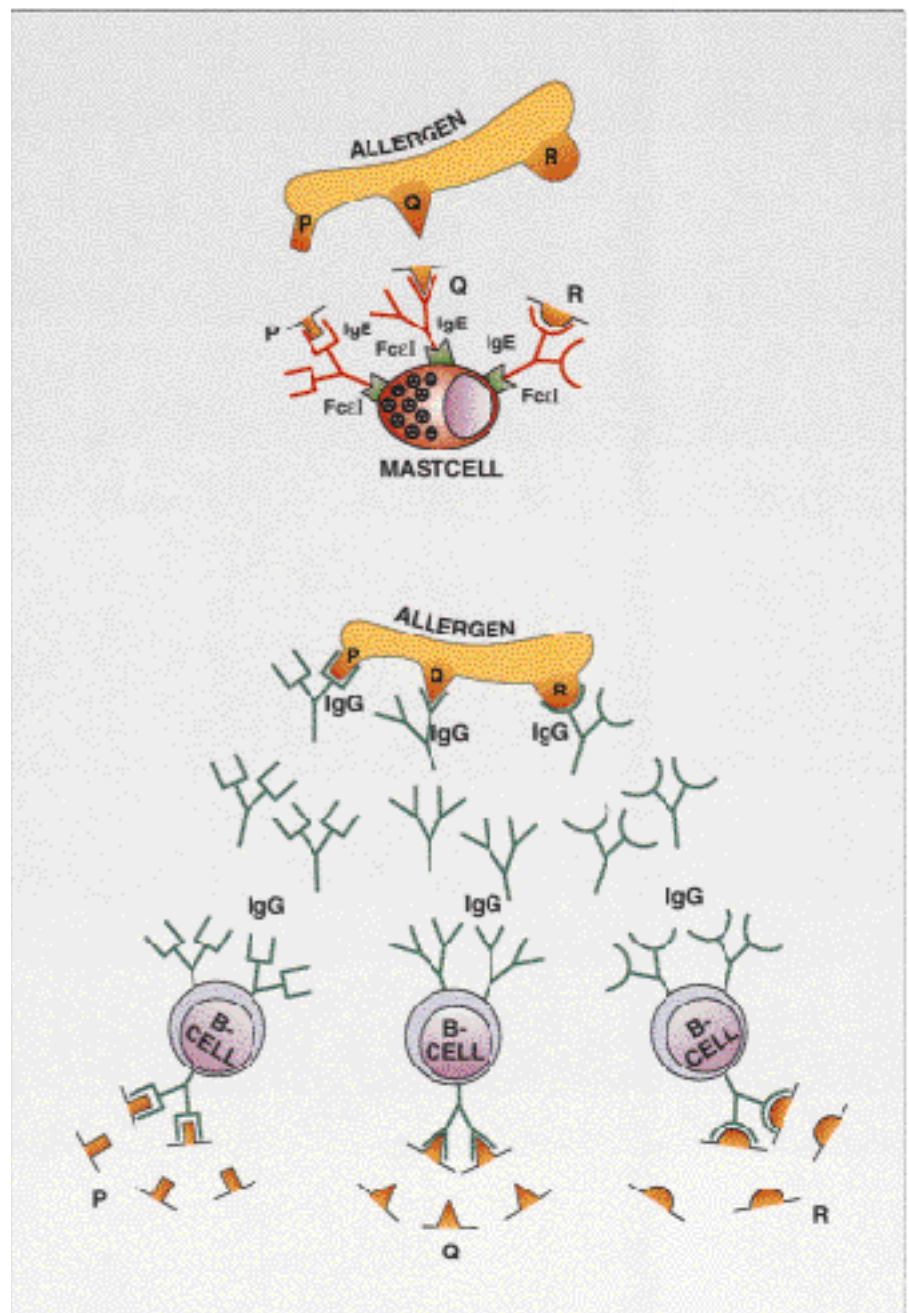


Fig. 4. Use of allergen-derived haptens to saturate effector cell-bound IgE prior to allergen exposure and to induce blocking antibodies. Allergen-derived haptens (P, Q, R) can be used to saturate mast cells without induction of mediator release. Subsequent allergen exposure will fail to cross-link surface-bound IgE antibodies. High-dose active immunization with the allergen-derived haptens or portions of IgE epitopes which by themselves cannot bind IgE antibodies may induce high titers of blocking IgG antibodies which subsequently prevent the interaction between allergens and specific IgE.

Therapy of Allergy with Allergen-Specific Antibodies and Recombinant Antibody Fragments: Building a Defense Line in Exposed Tissues

The physiological role of allergen-specific IgG antibodies is not yet completely understood and the question of whether allergen-specific IgG may have beneficial effects represents a controversial issue. However, there are at least

two mechanisms of how allergen-specific IgG may contribute to the amelioration of allergic disease. In this respect, it has been reported that cross-linking of Fcγ receptors via bound IgG can inactivate effector cells and prevent release of biological mediators [44]. The most direct mechanism for a beneficial role of IgG is plain competition of IgG with IgE antibodies regarding epitope recognition. Already years ago, the presence and efficacy of blocking anti-

bodies has been clearly demonstrated by passive transfer of serum into the skin of allergic individuals resulting in reduced skin reactions [45, 46]. Recently, the combination of CD40 activation and Epstein-Barr virus transformation has dramatically improved our ability to clone human B cells secreting allergen-specific, high-affinity IgG antibodies. A cell line secreting a human monoclonal IgG anti-Bet v 1 antibody, designated BAB1, was established from peripheral blood lymphocytes of a hyposensitized birch pollen allergic patient [47]. The human antibody and several anti-Bet v 1 monoclonal antibodies generated by immunization of mice [27] were shown to inhibit IgE binding to Bet v 1 as well as Bet v 1-induced histamine release.

As demonstrated for Bet v 1, blocking antibodies for therapy may be obtained by different approaches. Using routine tissue culture techniques cell lines may be established from immunized animals or directly from allergic patients. Allergen-specific monoclonal antibodies that compete with IgE antibodies can be selected and manipulated by molecular biological techniques (humanization, production of Fabs with reduced immunogenicity) [48].

Alternatively combinatorial library technology may be employed to generate allergen-specific recombinant Fabs directly from lymphocytes of allergic individuals. Using recombinant major timothy grass pollen allergens, Phl p 1, Phl p 2 and Phl p 5, for panning, phage expressing specific IgE Fabs were isolated from a combinatorial IgE library based on a grass pollen allergic patient [38]. Recombinant human IgE Fabs cross-reacted with homologous allergens from other grass species showed binding comparable to human IgE antibodies and could be used to block IgE binding to the allergens. The recombinant IgE Fabs lack most parts of their constant regions that bind to the Fc ϵ receptors, and therefore represent nonanaphylactic IgE fragments. In contrast to conventional monoclonal antibodies, which are used for standardization or allergen measurements, recombinant Fabs can be easily produced in bacterial expression systems. These bacterially expressed Fabs can readily be modified by standard molecular biological techniques and engineered according to the desired application. A lowering of their immunogenicity may be obtained by reducing their size and/or by eliminating particularly immunogenic portions. Furthermore, mutagenesis or CDR rearrangements may permit to improve the affinity of a recombinant Fab to its antigen, a process called 'in vitro maturation' [49]. Such a manipulation should increase the blocking activity of allergen-specific Fabs. Consequently we propose to use allergen-specific IgG antibodies as well as recombinant allergen-specific Fabs (1) to standardize allergen extracts for their content of relevant epitopes, (2) to measure allergen

contents in the environment and different food and (3) to evaluate the IgE-allergen interaction as a target for the therapy of type I allergy. Blocking Fabs might be applied at the mucosal surfaces of the eyes, nose and lung prior to anticipated allergen contact [48].

Focusing IgG Antibodies to IgE Epitopes by Vaccination with 'Hypoallergenic' Allergen Derivatives

The occurrence of allergen-specific antibodies without 'reaginic' activity was reported more than 50 years ago [45, 46]. It was demonstrated that allergen-specific IgG antibodies were able to inhibit anaphylactic reactions [45, 46, 50]. Although the induction of allergen-specific IgG4 antibodies in response to specific immunotherapy was documented [51], the presence and/or increase of allergen-specific IgG and IgG subclass antibody levels could not be correlated with successful immunotherapy [52]. The studies on the role of allergen-specific IgG antibody responses, previously hampered by the lack of well-defined allergen preparations, are now possible because of the availability of purified recombinant allergens and their derived epitopes. Studies performed with recombinant Bet v 1, the major birch pollen allergen [53, 54], with recombinant major timothy grass pollen allergens (Phl p 1, Phl p 2, Phl p 5) [55] and with recombinant Der p 2 [56, 57] showed that: (1) allergen-specific IgE and IgG subclass responses are not correlated, and (2) IgE and IgG antibodies of allergic patients may bind to different epitopes [57, 58].

As a logic consequence of these observations, specific immunotherapy approaches should focus on the induction of blocking IgG antibodies against IgE epitopes. Support for the idea that allergen-specific immunotherapy can induce blocking antibodies which prevent the allergen-induced effector cell activation comes from experiments demonstrating that immunization of mice with recombinant allergens yielded high titers of mouse IgG1 antibodies which bound to epitopes recognized by human IgE antibodies and blocked the allergen-induced histamine release from allergic patients' basophils [59]. Unfortunately, the induction of blocking antibodies requires a high dose of allergens, which may result in anaphylactic reactions. Nonanaphylactic allergen derivatives (mutants [60–63], fragments [21, 25], haptens [16]) generated by recombinant or peptide technology may help to overcome this problem. Immunization with high doses of these derivatives involves pathways of antigen presentation that favor a TH1 environment where high levels of IgG antibodies are induced [64].

Conclusions and Perspectives

The IgE-allergen interaction represents the crucial event that triggers immediate type reactions in allergic patients. Current therapeutic strategies for treating type I allergy include either a general pharmacological suppression of the cascade of inflammatory events resulting from an IgE-allergen encounter or specific immunotherapy. Specific immunotherapy necessitates that the disease-inducing antigens (allergens) are exactly defined and available. Recent progress in the molecular and structural characterization of allergens as well as their B cell epitopes together with advances regarding the molecular characterization of allergen-specific IgE antibodies allow to understand how IgE antibodies recognize allergens. It turned out that due to extensive cross-reactivities among allergens, a limited panel of B cell epitopes may be defined for diagnosis and possibly therapy of allergy. Based on this knowledge, we pro-

pose that interfering with the IgE-allergen interaction may be a promising therapeutic strategy of type I allergy. We suggest using competitors of the IgE-allergen interaction (e.g. IgE binding haptens, synthetic mimetics of IgE epitopes, blocking antibodies or antibody fragments, synthetic ligands for IgE epitopes) for passive therapy of type I allergy in the target organs (nose, eyes, lung). In order to induce high titers of protective blocking antibodies in allergic patients, active vaccination using allergen derivatives with reduced anaphylactic activity (e.g. IgE binding haptens, synthetic allergen fragments/epitopes or mutants) is proposed as a second therapeutic concept.

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Announcement

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