

Genetically Engineered and Synthetic Allergen Derivatives: Candidates for Vaccination against Type I Allergy

Rudolf Valenta^{1,*}, Susanne Vrtala¹,
Margit Focke-Tejkl¹,
Agnes Bugajska-Schretter², Tanja Ball¹,
Anna Twardosz¹, Susanne Spitzauer²,
Hans Grönlund³ and Dietrich Kraft¹

¹ Dept. of General and Experimental Pathology,

² Dept. of Medical and Chemical Lab. Diagn.,
Vienna General Hospital, University of Vienna,
Waehringer Guertel 18–20, A-1090 Vienna, Austria

³ Pharmacia and Upjohn Diagnostics AB,
S-75182 Uppsala, Sweden

* Corresponding author

Type I allergy, a hypersensitivity disease affecting almost 20% of the population worldwide, is based on the IgE recognition of otherwise harmless antigens (i.e., allergens). Allergen-induced crosslink of effector-cell-bound IgE antibodies leads to the release of biological mediators and thus to immediate disease symptoms (allergic rhinitis, conjunctivitis and asthma). Specific immunotherapy, the only causative treatment of Type I allergy, is based on the administration of increasing doses of allergens to allergic patients in order to yield allergen-specific non-responsiveness. Major disadvantages are 1. that current forms of allergen immunotherapy are performed with allergens difficult to standardize which cannot be matched to the patients reactivity profile and 2. that the administration of active allergen preparations can cause anaphylactic side effects. Through the application of molecular biological techniques many relevant environmental allergens have been produced as active recombinant proteins which allow component-resolved allergy diagnosis and thus represent the basis for patient-tailored forms of immunotherapy. Here we review molecular strategies which have been recently applied to generate genetically engineered and synthetic hypoallergenic allergen derivatives for patient-tailored and safe vaccination against Type I allergy.

Key words: Genetic engineering / Immunotherapy / Recombinant allergen / Synthetic peptide / Type I allergy / Vaccination.

Introduction

Type I allergy represents a genetically determined hypersensitivity disease which affects about 20% of the world population (Kay, 1997). The pathophysiological hallmark of Type I allergy is the production of immunoglobulin E (IgE) antibodies against otherwise harmless antigens (allergens) (Ishizaka *et al.*, 1966; Johansson and Bennich, 1967). IgE represents the least abundant class of immunoglobulins present in serum and other body fluids (tears, nasal, bronchial secretions, etc.). The immediate symptoms of Type I allergy (allergic rhinitis, conjunctivitis, asthma, dermatitis, anaphylactic shock) are due to allergen-induced crosslink of FcεR1-bound IgE antibodies which leads to the release of biological mediators (e.g., histamine, leukotrienes) from effector cells (mast cells, basophils) (Flavetch and Kinet, 1991; Beaven and Metzger, 1993). The chronic disease manifestations (late phase reactions in asthma and atopic dermatitis) result from the activation of allergen-specific T cells, a process which reportedly is increased when allergens are taken up by antigen presenting cells (macrophages, monocytes, dendritic cells) via IgE (Bieber, 1996; Stengl and Maurer, 1997).

Diagnosis of Type I allergy is performed via detection of allergen-specific IgE antibodies in serum (Wide *et al.*, 1967; Enksson and Ahlstedt, 1977). The clinical relevance of the presence of allergen-specific IgE in serum can be confirmed by provocation testing, which measures the immediate and late phase reactions elicited by exposing the patient to allergens (e.g., skin: skin prick-, intradermal testing; airways: nasal-, bronchial provocation; eyes: conjunctival provocation; gastrointestinal tract: food challenge) (Fuchs, 1979). The only causative form of allergy treatment, i.e., allergen-specific immunotherapy, represents the practice of administering increasing allergen doses to the patient in order to induce specific unresponsiveness and has been first described in 1911 (Noon 1911; Bousquet *et al.*, 1998; Durham and Till, 1998). While several studies have shown that allergen-specific immunotherapy is clinically effective, the underlying immunological mechanisms are not fully understood (Durham and Till, 1998).

What Can We Learn from Current Forms of Allergen Immunotherapy?

The classical experiment by Cooke and colleagues showing that transfusion of blood from a ragweed allergic pa-

tient who had successfully received immunotherapy abolished symptoms in another ragweed allergic recipient, provided the first evidence that blocking antibodies might be involved in protection against Type I allergy (Cooke *et al.*, 1935). These blocking antibodies were then classified as allergen-specific IgG antibodies which belonged mainly to the IgG₁ and IgG₂ subclasses (Loveless, 1940, 1943; Lichtenstein *et al.*, 1968; Adkinson *et al.*, 1979; Sobotta *et al.*, 1976; Gleich *et al.*, 1981; Aalberse *et al.*, 1983). Other investigators reported a reduction of mediator concentrations (Oreticos *et al.*, 1985), decreased numbers of mast cells and eosinophils in target tissues of atopy (Rak *et al.*, 1988; Otsuka *et al.*, 1991; Furin *et al.*, 1991) and the generation of suppressor cells during immunotherapy (Rocklin *et al.*, 1990). The analysis of allergen-specific CD4-positive T cells in the course of immunotherapy indicates a reduction of these cells together with a decrease in their IL-4 production (Schnitz *et al.*, 1993; Jutel *et al.*, 1995; Durham *et al.*, 1996; Ebner *et al.*, 1997; O'Brien *et al.*, 1997). Recent studies using purified recombinant allergens and B cell epitopes demonstrate that specific immunotherapy may induce a *de novo* immune response to the administered allergens, which can be directed to IgE epitopes and will then compete with the existing IgE response (Ball *et al.*, 1999a). In this context it is of note that several other studies support the idea that successful immunotherapy requires the administration of high allergen doses to induce a preferential Th1 immune response which is accompanied by the production of 'blocking' IgG antibodies (Kolbe *et al.*, 1991; Schnitz *et al.*, 1995). There is also good evidence that non-native allergens which are not recognized by IgE antibodies are processed in a way that preferentially Th1 type cytokines are secreted which gives rise to allergen-specific IgG production (Yang *et al.*, 1993; Axidis *et al.*, 1998). In summary it appears that allergen-specific immunotherapy has vaccination character. Depending on their structure and dose as well as dose of administration, allergens can elicit different immune responses which may either trigger or antagonize IgE production.

Disadvantages of Current Forms of Immunotherapy

The major disadvantage of allergen-specific immunotherapy seems to be related to the use of natural allergen extracts which consist of difficult to standardize mixtures of allergenic and non-allergenic moieties. Therapy with allergen extracts thus cannot be performed according to the patient's reactivity profile (Valenta *et al.*, 1993). It is possible that certain allergens are not present in the administered extract or that patients can become sensitized to new components in the course of treatment (van Ree *et al.*, 1997; Ball *et al.*, 1999a). Another disadvantage of extract-based therapy results from the fact that the administration of biologically active allergen preparations can induce anaphylactic side effects. The latter problem can be re-

duced by adsorbing the allergen extract to an adjuvant which prevents systemic release of allergens after injection and by chemical denaturation of the extract yielding denatured allergens with reduced IgE binding capacity and lower anaphylactic activity (e. g., allergoids) (Marsh *et al.*, 1981; Norman *et al.*, 1982; Meriney *et al.*, 1986; Bousquet *et al.*, 1990; Lockey and Bukantz, 1998).

Recombinant Allergen-Based Component-Resolved Allergy Diagnosis: Selection of Crossreactive Marker Allergens for Immunotherapy

The application of molecular biological techniques in the field of allergen characterization has allowed to isolate the cDNAs coding for many of the relevant environmental allergens and allowed the production of recombinant allergens (Valenta and Kratt, 1995). Using recombinant allergens it has become possible to determine the individual patient's reactivity profile either by *in vitro* diagnostic methods (i.e., detection of allergen-specific IgE antibodies in serum) (Valenta *et al.*, 1991; Valenta *et al.*, 1992) or by *in vivo* testing (i.e., skin testing) (Menz *et al.*, 1996; Pauli *et al.*, 1996). Recombinant allergen-based component-resolved diagnosis would allow to identify precisely the disease-eliciting molecules whereas previous extract-based diagnosis at best allowed to define the allergen-containing source (Valenta *et al.*, 1998a, 1999). The possibility to identify the components against which a patient is sensitized would have little consequences for immunotherapy if there was a great variety of immunologically diverse allergens and if patients would change their reactivity profile in the natural course of their disease. However, the analyses of the DNA and deduced sequences of allergens has revealed unexpected structural similarities of many relevant allergens (Valenta *et al.*, 1996). As a consequence of the structural similarities and crossreactivities it turned out that a few recombinant allergens can resemble the relevant IgE epitopes of even complex allergen extracts (Niederberger *et al.*, 1998a, b). Immunological competition experiments showed that a few pollen allergens contained most of the IgE epitopes present in natural pollen extracts of a variety of plant species. Moreover, the monitoring of IgE antibody reactivity profiles in allergic patients sera that were collected in the course of the natural disease indicated that while there is variation in the allergen-specific antibody levels depending on natural allergen exposure, the profile of recognized components remains stable (Ball *et al.*, 1999b).

Based on the notions above it appears possible to develop novel component-based vaccination strategies against Type I allergy which are tailored to the patient's sensitization profile (Valenta *et al.*, 1998a). Using recombinant allergen-based component-resolved diagnosis (CRD) the disease-eliciting allergens can be selected and used for component-resolved immunotherapy (CRIT) (Valenta *et al.*, 1999). Diagnosis should be performed with

recombinant molecules which closely mimic the allergenic activity of their natural counterparts in order to detect most of the relevant sensitivities. However, in order to improve immunotherapy the allergenic activity of the allergens has to be reduced which will allow to increase the dose of administered allergens with a low risk of anaphylactic side effects. Based on the detailed knowledge of allergen sequences and structures several strategies are currently applied to convert allergens into hypoallergenic derivatives.

Requirements for the Conversion of Allergens into Hypoallergenic Allergen Derivatives

The crosslinking of effector cell (mast cell, basophil)-bound IgE antibodies by allergens represents the key event leading to the release of biological mediators and thus to the immediate symptoms in Type I allergy (Figure 1). In order to behave as an allergen, an antigen must therefore contain several binding sites for IgE antibodies (IgE epitopes) (Figure 1: F, Q, R). Most of the relevant environmental allergens represent low molecular weight proteins or glycoproteins. The molecular and structural analysis of allergens indicates that the IgE epitopes are almost exclusively of protein nature. IgE epitopes are distinct identities rather than repeats of the same binding site and are therefore recognized by IgE antibodies with different specificities (Figure 1: P, Q, R). IgE epitopes can be represented by a stretch of amino acids in a row (i.e., continuous epitope) (Figure 2: D) or may be composed by non-adjacent elements of the allergen which are brought together by fold-

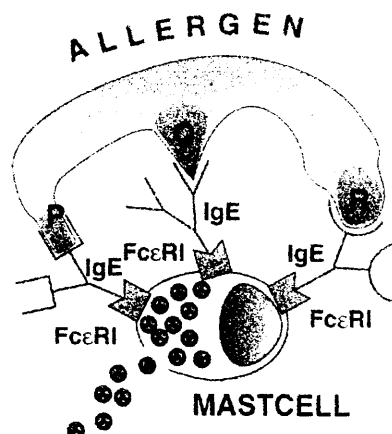


Fig. 1 Allergen-Induced Crosslinking of Effector-Cell-Bound IgE Causes Mediator Release. Allergens contain several IgE epitopes (F, Q, R) which by crosslinking of FcεRI-bound IgE antibodies lead to release of mediators.

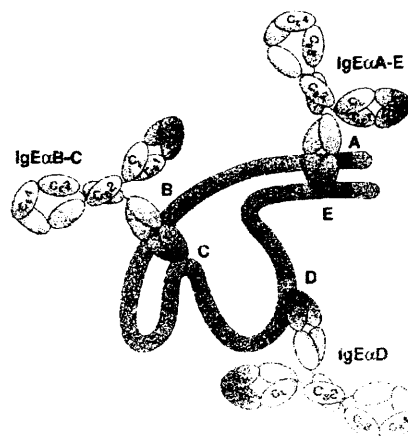


Fig. 2 The major Allergen Contains Continuous and Discontinuous Epitopes (A-E, B-C) and Continuous IgE Epitopes. Discontinuous epitopes result from joining of distant continuous allergen segments which are brought together by folding.

ing of the protein (i.e., discontinuous epitope) (Figure 2: A-E, B-C). The fact that the proper assembly of discontinuous epitopes depends on a certain conformation of the molecule has led to the synonymous designation of such epitopes as conformational epitopes although recognition of a continuous epitope may depend as well on its conformation. In order to reduce the allergenic activity of an allergen it has to be changed in such a way that it cannot crosslink effector cell-bound IgE antibodies (Singh *et al.*, 1999). To reach this goal at least two strategies can be applied: first, it may be considered to influence exclusively the activity of a allergen-specific T cells by administration of peptides containing T cell epitopes only (Rothbard, 1992; Roland and O'Hair, 1998). T cell epitopes represent small peptides which result from the proteolytic digestion of intact allergens by antigen presenting cells. T cell epitopes are in most cases distinct from IgE epitopes and therefore cannot crosslink effector cell-bound IgE. They can be produced as synthetic peptides. Second, hypoallergenic allergen derivatives or fragments may be produced by recombinant DNA technology or peptide synthesis. The latter molecules bear T cell epitopes and can induce IgG antibodies that compete with IgE recognition of the native allergen (Valenta *et al.*, 1998b).

T cell Epitope-Containing Peptides

The idea of using T cell epitope-containing peptides for immunotherapy of Type I allergy is based on the fact that allergen-specific CD4⁺ T cells can secrete either cytokines (e.g., Th2 cells: IL-4, IL-13) which favor class switch to IgE

or produce cytokines (e.g., Th1 cells: γ -IFN) which favor IgG antibody production. Depending on the dose and mode of administration it was found that T cell epitope peptides can induce proliferation or anergy of T cells *in vitro* (Roiland and O'Héhir, 1998). Peptides containing T cell epitopes of the major cat allergen Fel d 1 and of bee venom allergen phospholipase A2 were therefore administered to allergic patients. While there was some evidence that phospholipase A2-derived peptides induced T cell anergy in bee venom allergic patients (Müller et al., 1998), administration of cat allergen-derived peptides failed to induce significant immunological or clinical effects (Simons et al., 1996; Norman et al., 1996, 1997). It rather appeared that in case of certain patients, T cell peptides induced T cell activation as indicated by the early appearance of T cell-dependent reactions. Several explanations for the low efficacy of T cell peptide-based therapy may be considered: first, it may be difficult to administer the optimal dose to achieve T cell tolerance instead of activation. Second, small T cell epitope peptides will have a short half-life in the body. Third, there is considerable evidence that IgE production in atopic individuals represents a memory immune response which does not require *de novo* class switching and thus cannot be controlled by T cell-derived cytokines (Romagnani et al., 1990; Steinberger et al., 1995; Dolecek et al., 1995). Therapy forms which are based exclusively on the administration of T cell epitopes may therefore modulate the activity of allergen-specific T cells but may have little influence on the production of allergen-specific IgE antibodies by already switched memory B cells.

Recombinant Allergen Fragments Representing IgE-Binding Haptens

Several studies performed more than 20 years ago demonstrated that proteolytic digestion of allergens yielded small peptide fragments which in part retained their IgE binding capacity but failed to elicit immediate type reactions (Malay et al., 1975; Chakrabarty et al., 1980). The reason for this behaviour is that allergen fragments which contain the IgE binding site for only one species of IgE (i.e., IgE-binding haptens) are unable to crosslink effector cell-bound IgE antibodies. Low molecular weight allergen fragments obtained by proteolytic digestion were successfully used for immunotherapy long ago (Malley et al., 1969; Litwin et al., 1988) and the hapten principle was also applied for the treatment of penicillin and dextran allergy (DeWeck and Schneider, 1972; Richter et al., 1981; Hedin and Richter, 1982). There it was demonstrated that monovalent penicillin-derived haptens inhibited allergic reactions to penicillin.

While the proteolysis of allergen extracts is difficult to control and will yield a difficult to standardize mixture of fragments and enzymes, molecular biology has opened new avenues for the production of IgE-binding haptens. By gene fragmentation it has become possible to dissect

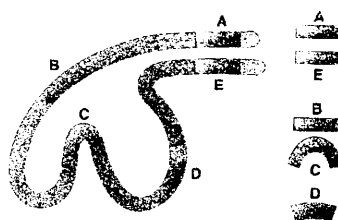


Fig. 3 IgE-Binding Haptens Containing the Binding Sites for One Species of IgE.
IgE-binding haptens can be represented by continuous (D) or discontinuous (A–E, B–C) epitopes.

the IgE binding sites of major allergens and to produce defined allergen fragments. IgE-binding haptens were isolated for the major timothy grass pollen allergen Phl p 1 and for birch profilin, a highly crossreactive plant allergen (Ball et al., 1994; Fedorov et al., 1997). Figure 3 illustrates the identification of IgE binding haptens. While continuous IgE-binding haptens (Figure 3: D) can be easily defined by fragmentation of the allergen-encoding cDNA and subsequent expression of the DNA fragments, discontinuous IgE-binding haptens (Figure 3: A–E, B–C) need to be rebuilt by more sophisticated technologies (e.g., mimotope strategies) (Leitner et al., 1998; Jensen-Jarolim et al., 1998).

At a first glance it may appear difficult to exactly rebuild the IgE binding sites of allergens but one has to consider that IgE-binding haptens may be useful for active immunization with reduced risk of anaphylactic side effects to focus IgG antibodies against IgE epitopes. Moreover IgE-binding haptens could be used for passive therapy to saturate effector cell-bound IgE prior to allergen contact and thus to block allergen-induced mediator release (Ball et al., 1994).

Hypoallergenic Allergen Isoforms, Mutants and Oligomers

The observation that allergens can naturally occur as isoforms which differ in only a few amino acids (e.g., major pollen allergens from hazel, Cor a 1, (Breiteneder et al., 1993) and birch, Bet v 1 (Swoboda et al., 1995)) or in conformations with low IgE binding capacity (e.g., apo-forms of EF-hand calcium-binding allergens; Seiberler et al., 1994; Twardosz et al., 1997; Engel et al., 1997; Suphioglu et al., 1997; Smith et al., 1996; Bugajska-Schretter et al., 1998; Hayek et al., 1998) has stimulated ideas that by means of genetic engineering hypoallergenic allergen versions can be produced. Oligomerization of the major birch pollen allergen, Bet v 1, by genetic engineering yielded a recombinant trimer with greatly reduced allergenic activity (Vrtala et al., 1999; Pauli et al., 1999 and submitted; van Hage-Hamsten et al., 1999).

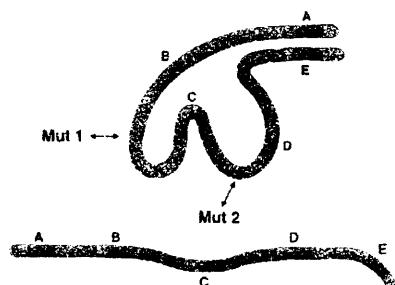


Fig. 4 Changing the Fold and Native-Like Structure of an Allergen by Point Mutations.

Changes of a few amino acids (arrows) can severely compromise the folding of an allergen and thus disrupt discontinuous (conformational) IgE epitopes (A-B, B-C).

Basically two types of point mutations capable of reducing the IgE binding capacity of allergens may be conducted by site-directed mutagenesis:

1. Point mutations outside the IgE binding sites may lead to conformational changes in the allergen structure and thus disrupt discontinuous IgE epitopes (Figure 4). In this context it was shown that point mutations in the calcium-binding sites of EF-hand allergens yielded stable apo-forms with reduced IgE binding capacity (Engel *et al.*, 1997; Okada *et al.*, 1998). Mutations of cysteine residues in major mite allergens likewise decreased the IgE binding capacities and *in vivo* allergenic activity of these molecules (Smith and Chapman, 1996; Takai *et al.*, 1997; Olsson *et al.*, 1998).

2. It is equally possible that point mutations may change those amino acid residues which are responsible for the interaction with the paratope of the corresponding antibody and thus directly affect the IgE binding capacity (Figure 5). Single point mutations were shown to abolish the binding of monoclonal antibodies (Wiedemann *et al.*, 1996; Swoboda *et al.*, 1999) and, although not yet proven,

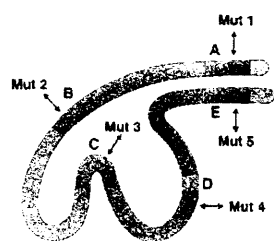


Fig. 5 Point Mutations which Alter the IgE-Binding Sites of the Folded Native-Like Allergen.

Exchange of those amino acids within the IgE epitope (arrows) which are required for the interaction with the paratope of the corresponding IgE antibody will lead to loss of binding.

may be responsible for the reduced IgE binding capacity of certain isoforms or mutants of the major birch pollen allergen, Bet v 1 and the major rye grass allergen, Lol p 5 (Ferreira *et al.*, 1996, 1998; Swoboda *et al.*, 1999). Deletions or mutations of several amino acids in a row (Figure 6) were found to be effective in reducing the IgE binding capacity of the major timothy grass pollen allergen Phi p 5 (Schramm *et al.*, 1999), the major rye grass pollen allergens Lol p 1 and Lol p 5 (Tamborini *et al.*, 1997; Swoboda *et al.*, 1999).

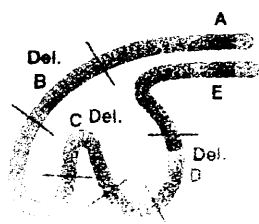


Fig. 6 Deletions which Reduce the IgE Binding Capacity of a Folded Native-Like Allergen.

Recombinant Allergen Fragments with Reduced or No IgE Binding Capacity

Two recombinant fragments representing amino acid 1–74 and 75–160 of the major birch pollen allergen were expressed in *Escherichia coli* and purified (Vitala *et al.*, 1997). The recombinant Bet v 1 fragments had almost completely lost their IgE binding capacity and allergenic activity due to a loss of their native-like fold as is indicated in Figure 7 (Pauli *et al.*, 1999; Van Hage-Hamsten *et al.*, 1999). Nevertheless both fragments contained the Bet v 1-specific T cell epitopes and induced IgG antibodies in mice and rabbits which recognized the native Bet v 1 molecule and inhibited binding of patient's IgE to rBet v 1 wild

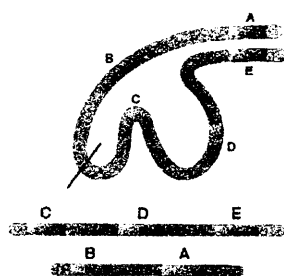


Fig. 7 Cutting or Fragmentation Changes the Allergens Fold and Destroys Discontinuous (Conformational) IgE Epitopes.

type. A similar behaviour was reported for two EF-hand pollen allergens from alder, *Aln g 4* (Hayek et al., 1998), and birch, *Bet v 4* (Twardosz et al., 1997) as well as for a bovine dander allergen (Zeiler et al., 1997). Two recombinant fragments containing the first and second EF-hand domain of *Aln g 4* exhibited a greatly reduced IgE binding capacity. Recombinant *Bet v 4*, the *Aln g 4*-homologous 2EF-hand birch pollen allergen, lacking the N-terminal first 16 amino acids had a more than 90% reduced IgE reactivity. We thus assume that fragmentation of proteins containing primarily discontinuous/conformational IgE epitopes will lead to a substantial reduction of the allergens IgE binding capacity. In a next step it needs to be investigated in specific animal models (Vrtala et al., 1998) whether such hypoallergenic allergen fragments can induce protective immune responses *in vivo*.

Synthetic Peptides Derived from Surface-Exposed Areas or from IgE Epitopes of Allergens

For binding, IgE antibodies require the availability of certain critical amino acids on the surface of the allergen molecule. IgE epitopes can either be determined by experimental epitope mapping strategies or the three dimensional structure of a given allergen can give information what portions of the molecule contain surface exposed amino acid residues likely to be involved in the interaction with IgE antibodies. By epitope mapping and structural approaches it is possible to select portions of an allergen molecule which are involved in IgE binding but *per se* lack allergenic activity or even IgE binding capacity. Synthetic peptides containing allergen fragments bearing amino acids involved in IgE binding (portions of IgE epitopes) (Figure 8 parts A-E) can then be used for active immunization in order to focus IgG antibodies to the IgE binding sites of the allergen. This concept would be generally applicable to construct hypoallergenic peptide vaccines for any allergen where the IgE epitopes or the three dimensional structure is known (Balle et al., 1999b; Focke et al.,



Fig. 8 Synthetic Peptides or Recombinant Allergen Fragments Containing Portions of the IgE Epitopes.

Peptides without IgE binding capacity (parts of IgE epitopes) representing surface-exposed portions of an allergen may be used to focus blocking antibodies to IgE epitopes.

1999). The application of IgE-epitope derived peptides for vaccination may however be limited by the low capacity of synthetic peptides to induce antibody responses *in vivo*. The latter problem might be overcome by increasing the immunogenicity of the allergen-derived peptides through coupling to suitable carriers or multimerization.

The Future of Allergen-Specific Immunotherapy: Vaccination with Recombinant or Synthetic Hypoallergenic Allergen Derivatives?

We envisage that future allergen-specific immunotherapy may be based on established injection- and adjuvant-based vaccination protocols currently applied with allergen extracts. Future vaccination strategies will however differ from the established treatment in as much as they may use defined allergen molecules instead of allergen extracts. The appropriate components will be selected by component-resolved diagnosis for component-resolved immunotherapy (Valenta et al., 1999). Treatment should then be performed with hypoallergenic recombinant or synthetic allergen derivatives which were developed on the basis of a detailed molecular and structural characterization of the disease-eliciting molecules. Component-resolved immunotherapy will have no risk to sensitize pa-

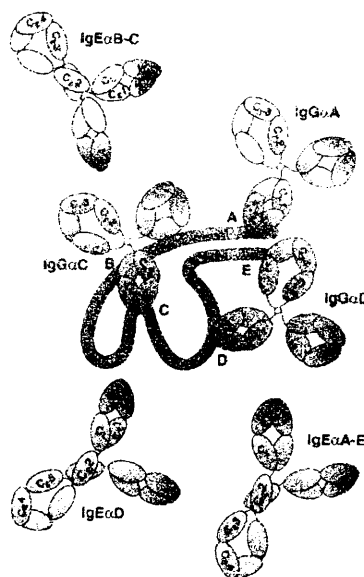


Fig. 9 Vaccination with Hypoallergenic Allergen Derivatives Induces Synthesis of Blocking IgG Antibodies.

Allergens hidden by IgG antibodies cannot be recognized by IgE.

tients against new components. The possibility of treatment with hypoallergenic molecules holds also promise that a reduced rate of anaphylactic side effects will be observed in the course of immunotherapy. From the administration of higher doses of hypoallergenic molecules we expect the induction of a preferential Th1-like immune response which will be accompanied by the production of blocking antibodies that compete with the ongoing IgE response. The induction of blocking antibodies will to a great extent require the presence of motifs/sequences on the hypoallergenic immunogen which mimic structures within the IgE epitopes of the wildtype allergen. Therapy-induced blocking antibodies will be able to compete with IgE for its binding sites on the natural allergen (Figura 9) and thus prevent allergen-induced release of biological mediators as well as of Th2-like cytokines from the effector cells (Haas *et al.*, 1999). Blocking antibodies present in local secretions and serum will also suppress allergen-dependent activation of IgE production which reportedly may take place in target organs of atopy (Durham *et al.*, 1999). If blocking IgG antibodies interfere with IgE-mediated presentation of allergens to T cells, it may be expected that T cell dependent late reactions are also ameliorated. As far as the mode of allergen administration is concerned, it appears likely that the use of hypoallergenic molecules will allow to develop new therapy protocols which are based on the less frequent injection of high doses as in classical vaccination treatment.

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