

Immunotherapy of Allergic Disease

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I. Introduction

The term allergy was introduced by Clemens von Pirquet in 1906 to describe overwhelming pathological reactions of the body due to intercurrent contact with antigens (von Pirquet, 1906). More than 50 years later Coombs and Gell (1975) proposed a first detailed classification of allergic reactions in four types based on defined underlying pathomechanisms. We know today that the four principal types of allergic reactions (Types I–IV) described by Coombs and Gell do not occur in a mutually exclusive manner and there is now much more detailed information available about the molecular and cellular players in allergic reactions. However, the classification of Coombs and Gell still summarizes essential features of allergic reactions leading to tissue damage and hence is extremely useful for describing the mechanisms of human hypersensitivity disease.

When we discuss the immunotherapy of allergic diseases in this chapter we will restrict ourselves to the most common form of allergic disease in humans, which is actually characterized by Type I reactions as described by Coombs and Gell. The hallmark of Type I allergy is the formation of a unique class of antibodies, that is, immunoglobulin E (IgE) antibodies against harmless antigens (i.e., allergens). IgE antibodies were not characterized until 1966 because of their extremely low concentration in serum and other body fluids (Ishizaka *et al.*, 1966; Johansson and Bennich, 1967). However, upon contact with incremental doses of the corresponding allergens, they can cause severe inflammatory reactions through the activation of various cells of the immune system, especially mast cells and basophils (Kawakami and Galli, 2002; Marone *et al.*, 2002; Turner and Kinet, 1999). In 1921, long before the characterization of IgE antibodies, Prausnitz and Küstner (1921) demonstrated that the allergic reaction depends on three factors: the disease-eliciting allergen; a transferable serum factor, later identified as IgE antibodies, that distinguishes allergic patients from healthy persons; and a tissue factor (i.e., mast cells) that can be found in all individuals.

Type I allergy affects more than 25% of the population and shows a continuously increasing prevalence (Wüthrich *et al.*, 1995). The manifestations of Type I

allergy can be very diverse, ranging from mild to severe forms (e.g., allergic rhinoconjunctivitis, food allergy, dermatitis, asthma, fatal anaphylactic shock), and may occur locally and/or systemically. All of the diverse phenotypes of Type I allergy are, however, associated with the production of allergen-specific IgE antibodies and hence can be precisely diagnosed and distinguished from other forms of allergic diseases by the demonstration of allergen-specific IgE antibodies in serum or other body fluids and by the elicitation of immediate tissue reactions by provocation testing with allergens (Johansson *et al.*, 2001).

In this chapter we will discuss recent advances in the field of immunotherapy of IgE-mediated allergies because IgE-mediated allergies belong to the most common forms of allergic diseases with relatively well-defined immunological mechanisms. This chapter will focus on data obtained in allergic patients but, on certain occasions, will also refer to results from animal models with a close connection to human disease. First we will review the current knowledge regarding the immunological mechanisms operative in IgE-mediated allergy with special reference to disease manifestations and the time course of disease development. In addition to allergen-specific immunotherapy, several other forms of immunotherapy have been recently developed for the treatment of allergy. We will therefore summarize the general forms of immunotherapy without antigen specificity according to their molecular and cellular targets. Special emphasis will be given to results that add to our current knowledge about the mechanisms of human allergic disease. Next we will summarize the development of allergen-specific immunotherapy and provide an overview of immunological mechanisms that have been described in clinical studies performed in allergic patients. These observations suggest that different, but not mutually exclusive, immunological mechanisms may be operative in allergen-specific immunotherapy. Based on these mechanisms, several concepts have been developed to improve allergen-specific immunotherapy. These concepts are based on modifications of the mode of administration, of the adjuvant, and of the antigen. Due to recent rapid developments in the field of allergen characterization, we will finally focus on possible improvements of allergen-specific immunotherapy through the use of modified antigens that can be obtained by recombinant DNA technology and synthetic peptide chemistry. In the end, a possible scenario for the development of prophylactic vaccination concepts based on the use of recombinant and synthetic allergy vaccines will be created.

II. Time Course and Pathophysiology of Type I Allergy

A. THE CONCEPT OF ALLERGIC SENSITIZATION

Sensitization to allergens is the initial key event leading to the development of allergic disease. Table I summarizes some of the crucial factors involved in the sensitization process. These factors can be classified as environmental

TABLE I
FACTORS IN ALLERGIC SENSITIZATION^a

Environment	
Eliciting factors	Promoting/inhibiting factors
Allergens • Immunogenicity • Dose • Route/site	Adjuvant effect Hygiene Endotoxin Bacterial infection Parasites Vaccination
Atopic predisposition	• Allergen specific • Tendency for IgE production
Age	
Host	

^aSeveral environmental factors can promote or inhibit the development of an allergic immune response. Properties of allergens may influence their disease-eliciting potency, and numerous host factors may determine the susceptibility of a subject to develop allergy.

factors, which are required for the induction of sensitization (e.g., allergens), and those that act as promoting or inhibiting factors. The susceptibility of the host may be dictated by allergen-specific factors (e.g., HLA restriction of allergen-specific immune responses), by factors influencing the individual's tendency to mount IgE responses, and by age. Analysis of immune responses in allergic children, as well as in experimental animal models of allergy, suggests that allergic sensitization is induced by early, postnatal allergen contact (Niederberger *et al.*, 2002; Schiessl *et al.*, 2003; Wahn *et al.*, 1997). Some evidence also points to the possibility that prenatal maternal allergen contact may lead to the onset of allergy in offspring, but this is controversial (Edelbauer *et al.*, 2003; Herz *et al.*, 2001; Lange *et al.*, 2002; Melkild *et al.*, 2002; Platts-Mills *et al.*, 2001, 2003; Victor *et al.*, 2003). The susceptibility of the host to develop allergy, termed atopic predisposition, is controlled by a variety of genetic factors, of which some seem to be allergen related (i.e., allergen-specific HLA restrictions), whereas others affect the host's general ability to mount IgE responses upon antigen contact (e.g., cytokine expression) (Ansari *et al.*, 1989; Daniels *et al.*, 1996; Kim *et al.*, 2002; Lee *et al.*, 2000; Lonjou *et al.*, 2000; Marsh *et al.*, 1982a, 1982b, 1994; Sandford *et al.*, 1993; Texier *et al.*, 2002).

In addition to the previously-mentioned host factors, several environmental factors may influence whether an allergic immune response develops. However, controversial evidence exists on how factors affecting the innate immune system (e.g., bacillus Calmette-Guérin [BCG] vaccination, contact

with endotoxins, bacterial infection, parasitic infestation, hygiene) influence the onset of allergic immune responses (Wills-Karp *et al.*, 2001). For example, the hygiene hypothesis would predict that poor hygienic conditions and endotoxin contact in early life would prevent the development of allergic immune responses, but recent data suggest that in countries with poor hygienic conditions (e.g., Central Africa), allergies are a common problem (Braun-Fahrlander *et al.*, 2002; Westritschnig *et al.*, 2003). Likewise, greatly varying and sometimes opposite effects of BCG vaccination or parasite infestation on the development of allergy have been reported (Alm *et al.*, 1997; Herz *et al.*, 1998, 2000, 2001; Shirakawa *et al.*, 1997; Sibanda 2003; Van den Biggelaar *et al.*, 2000). In this context it will be interesting to obtain, in addition to the epidemiological data, more results from defined experimental animal models that precisely distinguish factors promoting allergy from those preventing it.

Considerable progress has been made regarding the characterization of those antigens (i.e., allergens) that induce and maintain allergic diseases in humans (Valenta and Kraft, 2002). It has been shown that most allergens fall into the class of proteins or glycoproteins with molecular masses between 5 and 80 kDa (Valenta and Kraft, 2001). IgE antibodies react primarily with proteins, whereas carbohydrate epitopes represent rather rare targets for IgE antibodies of uncertain biological significance (van Ree, 2002). Using recombinant DNA technology, cDNAs coding for the most common allergens have recently been isolated and allowed to reveal their identities, as well as their structures. Moreover, recombinant allergens mimicking their natural counterparts have allowed us to study in detail allergen-specific immune responses and to understand the structural basis for allergic cross-reactivity. The three-dimensional structures of many important allergens have been determined, and epitope mapping studies provide evidence that those allergens causing respiratory allergies contain mainly conformational IgE epitopes (reviewed in Valenta and Kraft, 2001). It has not been possible as yet to determine structural motifs or biological properties that predispose the allergenic nature of an allergen. Allergens may be composed of all α -helical, mixed α -helical and β -sheet structure, or all β -sheet structure and include storage proteins, cytoskeletal proteins, enzymes, calcium-binding proteins, or defense proteins. Results obtained from the molecular and epitope analysis of respiratory allergens indicate that allergic sensitization occurs mainly against intact, soluble, folded, and immunogenic proteins that are released from respirable particles in very low concentrations. Allergens that are released from their primary allergen source may also bind to nonallergenic environmental particles with a possible adjuvant effect. In this context it was reported that allergens coupled to diesel exhaust particles can induce strong IgE responses in animals (Fujieda *et al.*, 1998).

IgE recognition of many respiratory allergens not only requires the presence of conformational epitopes (Laffer *et al.*, 1996; Vrtala *et al.*, 1997), but was

even found to depend critically on a certain conformational state of the allergen (Akdis *et al.*, 1998), emphasizing the importance of conformational IgE epitopes. For example, it has been shown that certain calcium-binding allergens are primarily recognized in their calcium-bound, but not in their calcium-free, apoform, suggesting that the initial sensitization has occurred against a certain allergen conformation (Seiberler *et al.*, 1994; reviewed in Valenta *et al.*, 1998; Verdino *et al.*, 2002). The preferential recognition of certain conformational allergen epitopes by IgE antibodies hence allows us to reconstruct some of the molecular events during primary sensitization in patients. On the other hand, it seems possible to construct allergen derivatives with reduced IgE binding capacity and low allergenic activity for immunotherapy by rational disruption of the allergen's structure, a subject that will be discussed in the context of allergen-specific immunotherapy.

Other important allergen-related factors for sensitization are the dose, route, and mode of allergen contact (Constant *et al.*, 2000; Kolbe *et al.*, 1991; McCusker *et al.*, 2002). In principle, there are at least three possibilities for allergic sensitization of patients, depending on the organs involved. They include respiratory, gastrointestinal, and skin-mediated sensitization. Since the induction of robust IgE responses in experimental animal models requires the injection of adjuvant-bound allergen, primary sensitization against low doses of soluble allergen as it occurs in patients is difficult to study in animals. The question of whether respiratory, gastrointestinal, or skin-mediated sensitization represents the predominant event in allergic sensitization can therefore be reconstructed only indirectly.

It is known that allergy to typical food allergens precedes respiratory allergy in children, but almost no experimental data from allergic patients are available that demonstrate that dietary intake of food allergens can induce and increase allergen-specific IgE responses (Kulig *et al.*, 1999; Reininger *et al.*, 2003). Interestingly, many allergic children grow out of food allergies and develop respiratory allergy later, raising the possibility that early intake of large amounts of food allergens may induce tolerance. Evidence for the possibility of skin-mediated sensitization is scarce (Beck and Leung, 2000). Moreover, it has been demonstrated that allergic immune responses in the skin are frequently directed against respiratory or food allergens and that skin symptoms either occur after dietary allergen intake or after primary respiratory sensitization (Reekers *et al.*, 1999; van Reijssen *et al.*, 1998; Werfel *et al.*, 1999). The most important and frequent manifestations of IgE-mediated allergies affect the respiratory tract, and a large body of experimental evidence highlights the importance of the respiratory tract (especially the upper respiratory tract—the nose) in allergic sensitization, maintenance, and boosting of the allergic immune response (Durham *et al.*, 1997; Henderson *et al.*, 1975; McCusker *et al.*, 2002; Naclerio *et al.*, 1997; Simons,

1999; Smurthwaite *et al.*, 2001; Ying *et al.*, 2001). We will therefore discuss allergic sensitization using respiratory allergens as examples. Figure 1 gives an overview of the allergen-specific immune response during sensitization and memory. Furthermore, it describes mechanisms of allergic inflammation in sensitized allergic patients.

Respiratory allergens (e.g., allergens from pollen, dust mites, animals, and molds) are released from airborne particles (e.g., pollen) either after contact with mucosal surfaces by simple aqueous elution or via the release of small allergen-bearing and hence respirable particles (Grote, 1999; Grote *et al.*, 2000; Suphioglu *et al.*, 1992; Taylor *et al.*, 2002). According to measurements of allergen concentrations in the environment, only tiny amounts of antigen are sufficient for the initiation of allergic sensitization (Baur *et al.*, 1998).

Highly efficient mechanisms of allergen presentation must therefore be operative during initial allergic sensitization. Experimental data from patients are rare because sensitization occurs very early in childhood and hence was not studied in detail in humans, and data from animal studies are difficult to transfer to the human situation. In principle, at least two molecular and cellular scenarios are possible. Studies in mouse models suggest that antigen-presenting cells (APC; e.g., dendritic cells) take up allergens and prime allergen-specific T cells, which may then activate B cells by cognate T cell–B cell interactions (Constant *et al.*, 2000; Lambrecht, 2001; Masten and Lipscomb, 1999). However, since allergen-specific T and B cells recognize entirely different epitopes of the allergen, the APC involved in the initial sensitization process must present simultaneously T cell as well as B cell epitopes to act as a communication platform for B cells and T cells with specificity for the same allergen. In an alternative scenario in which allergen-specific B cells pick up the allergen via specific immunoglobulin, direct presentation to specific T cells would be possible and allow immediate interaction between the specific B cell and the specific T cell for the development of IgE and T cell responses (Constant *et al.*, 2000). In atopic individuals, CD4⁺ Th2 cells produce preferentially cytokines such as interleukin (IL)-4 and IL-13 that promote the immunoglobulin class switch of B cells to IgE (Paul, 1987; Romagnani, 1997; Vercelli and Geha, 1992). As a consequence of sensitization, allergic patients produce allergen-specific IgE antibodies, whereas low levels of allergen-specific IgG antibodies can be found in allergic as well as in nonallergic individuals (Valenta and Ball, 1997). The presence of low levels of allergen-specific IgE antibodies and skin sensitivity without manifestation of clinical disease may precede allergic disease (Bodtger *et al.*, 2003). The evolution of the allergen-specific antibody responses has recently been determined in children becoming allergic and provided evidence for a nonsequential, direct class switch mechanism to IgE (Niederberger *et al.*, 2002). This observation and the fact that the hypervariable regions of the few allergen-specific

IgE antibodies sequenced today showed relatively few somatic mutations suggest that IgE antibodies represent perhaps a rather early and primitive class of antibodies that may precede the development of allergen-specific IgG responses (Edwards *et al.*, 2002; Flicker *et al.*, 2002; Steinberger *et al.*, 1996; S. Flicker and R. Valenta, unpublished data). The latter assumption is indeed supported by data obtained in mouse models and by the monitoring of the development of humoral immune responses in allergic children (Niederberger *et al.*, 2002; Vrtala *et al.*, 1998).

B. PATHOPHYSIOLOGY OF THE ESTABLISHED ALLERGIC MEMORY IMMUNE RESPONSE

The previously described process of allergic sensitization leads to the establishment of an allergen-specific memory response on the humoral and cellular level (Fig. 1). Cells producing allergen-specific IgE antibodies can be detected in the peripheral blood of allergic patients (Dolecek *et al.*, 1995; Steinberger *et al.*, 1995, 1996). Interestingly, the allergen-specific IgE antibody production of these cells seems to be rather insensitive to cytokine stimulation (e.g., IL-4, IL-13) and cannot be abrogated by cytokine antagonists inhibiting a class switch to IgE (Dolecek *et al.*, 1995; Steinberger *et al.*, 1995). IgE-producing cells are also found in the mucosa of the respiratory tract (e.g., nasal and bronchial mucosa), and it has been demonstrated that seasonal allergen contact and nasal allergen exposure lead to strong increases of systemic allergen-specific IgE antibody levels, suggesting that allergen contact is a strong, if not the most important, stimulus for specific IgE production (Durham *et al.*, 1997; Henderson *et al.*, 1975; Naclerio *et al.*, 1997; Simons, 1999; Smurthwaite *et al.*, 2001; Ying *et al.*, 2001). In addition to IgE-producing cells, allergen-specific T cells also seem to form a pool of long-lived memory T cells that respond to repeated allergen contact (Mojtabavi *et al.*, 2002). These T cells can be detected specifically in the skin and peripheral blood of allergic patients via their T cell receptor sequences (Bohle *et al.*, 1998; van Reijssen *et al.*, 1997). Although the classical Th1/Th2 paradigm would predict a clear-cut distinction between allergic patients and nonallergic subjects based on their T helper cell and cytokine profile (Mosmann and Sad, 1996; Parronchi *et al.*, 1991; Romagnani, 1997; Wierenga *et al.*, 1991), several investigations demonstrate that allergen-specific T cells from allergic individuals also mount considerable interferon (IFN)- γ responses (Byron *et al.*, 1994; Hales *et al.*, 2000; Looney *et al.*, 1994; Oldfield *et al.*, 2001; van Neerven *et al.*, 1994a). Likewise, allergen-specific Th2 cells have been found in nonallergic patients, suggesting a less clear-cut distinction of the allergen-specific T cell responses in atopic versus nonatopic persons as originally anticipated (Ebner *et al.*, 1995; van Neerven *et al.*, 1994b).

In accordance with the classification of Coombs and Gell, the immediate reaction represents the hallmark of Type I allergy (Fig. 1). Allergen-induced

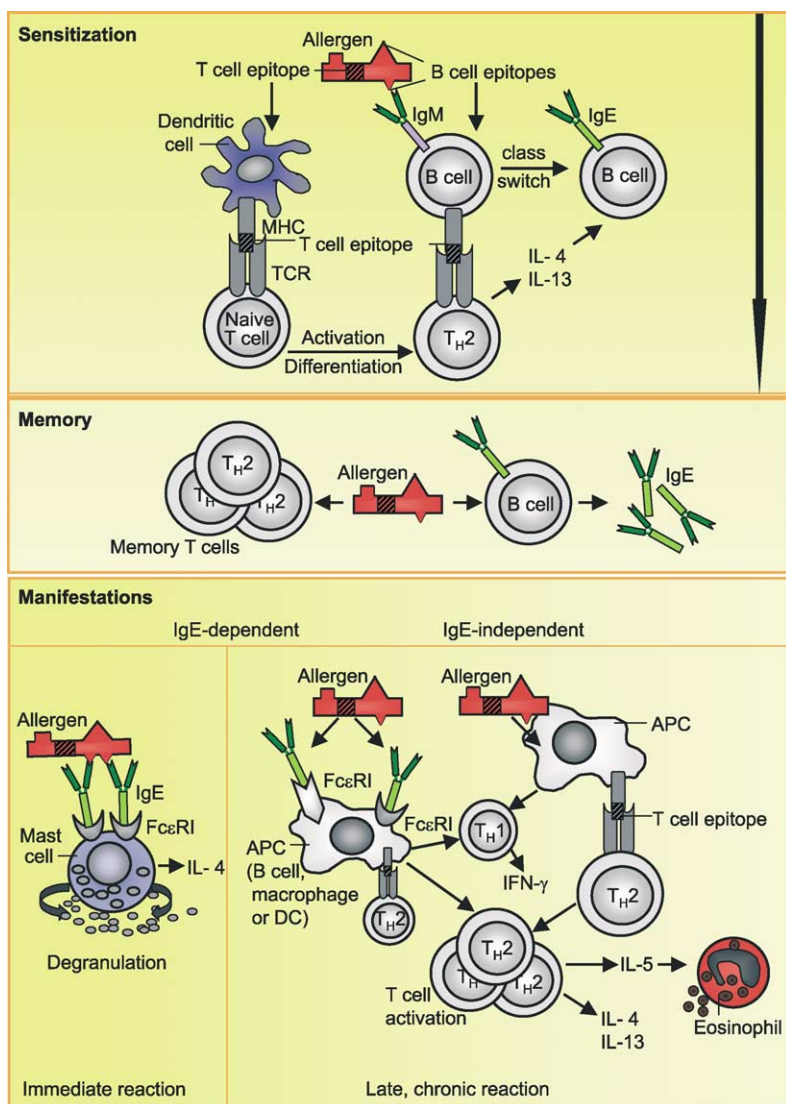


FIG 1 The allergic immune response (sensitization and memory) and mechanisms of allergic inflammation. The allergic immune response (top and middle panels). During initial sensitization, contact with minute amounts of intact, soluble allergen on mucosal surfaces, particularly of the respiratory tract, leads to allergen uptake by antigen-presenting cells (e.g., dendritic cells) and/or immunoglobulin-mediated recognition of allergens by B cells. In allergic individuals, T cells differentiate preferentially toward the T_H2 phenotype, which may be promoted by microenvironmental factors of the mucosa, the genetic background of the individual, the site of allergen contact,

cross-linking of IgE antibodies bound via high-affinity receptors to mast cells and basophils leads to the rapid release of inflammatory mediators (e.g., histamine, leukotrienes), but mast cells and basophils are also potent sources of Th2 cytokines (e.g., IL-4) (Bradding *et al.*, 1993; Brunner *et al.*, 1993; Burd *et al.*, 1989). Most of the immediate symptoms occurring a few minutes after allergen contact in sensitized patients (e.g., rhinitis, conjunctivitis, asthma, edema, urticaria) can be attributed to the rapid release of inflammatory mediators from mast cells and basophils. It is, however, becoming increasingly clear that Type IV-like T cell-mediated reactions can also occur in allergic individuals (Haselden *et al.*, 1999; Oldfield *et al.*, 2001).

In this context it has been reported that patients suffering from severe and chronic forms of allergy (e.g., atopic dermatitis, chronic asthma) exhibit more pronounced T cell responses to allergens compared with patients suffering only from allergic rhinoconjunctivitis (Rawle *et al.*, 1984). The occurrence of T cell-dependent late responses is thus perceived as a sign of severe and chronic allergic disease, whereas in most allergic patients, classic immediate, Type I-like reactions dominate. It is well established that cross-linking of FcεRI-bound IgE antibodies on mast cells and basophils by allergens induces the rapid release of inflammatory mediators and Th2 cytokines (Turner and Kinet, 1999). However, recently additional effects of monomeric IgE on mast cells have been reported. In this context it has been demonstrated that IgE promotes the survival of mast cells and also of FcεRI-bearing antigen-presenting cells by preventing apoptosis (Asai *et al.*, 2001; Kalesnikoff *et al.*, 2001; Katoh *et al.*, 2000) and it was also demonstrated that high IgE concentrations up-regulate the expression of FcεRI (Kubo *et al.*, 2001; MacGlashan *et al.*, 2001; Saini *et al.*, 2000; Yamaguchi *et al.*, 1999). A close linkage between IgE and T cells has been established by demonstrating that various cells express (e.g., B cells, monocytes) the low-affinity receptor for IgE, FcεRII, or the high-affinity

allergen dose and conformation, as well as by other factors. The T_H2 cells secrete factors (e.g., IL-4, IL-13) that favor the immunoglobulin switch of specific B cells to immunoglobulin E. The process of allergic sensitization leads to the establishment of an allergen-specific memory T cell pool, as well as of an IgE memory B cell pool, both of which can be strongly activated by contact with allergens. Mechanisms of allergic inflammation (bottom panel). The manifestations of allergic disease occur primarily as immediate and, under certain circumstances, as late/chronic symptoms. Immediate reactions are caused by the cross-linking of effector-cell (i.e., mast cell, basophil)-bound IgE by allergens, leading to the release of biologically active mediators (e.g., histamine, leukotrienes) and proinflammatory cytokines (e.g., IL-4). Late and chronic reactions are caused by the presentation of allergens to T cells, which then become activated, proliferate, and release cytokines. Allergen presentation to T cells can occur in a highly efficient manner by IgE-dependent mechanisms using FcεRI or FcεRII on APCs. In addition, allergens can be taken up by APCs in an IgE-independent manner and then be presented to T cells. Activated T_H2 cells release IL-4, IL-13, and IL-5 and thus cause tissue eosinophilia, but also T_H1-cells secreting IFN-γ may be activated by allergens, especially during late phase reactions. APC, antigen-presenting cell; DC, dendritic cell; TCR, T cell receptor.

IgE receptor, FcεRI (e.g., monocytes, dendritic cells, eosinophils, thrombocytes, epithelial cells) (Campbell *et al.*, 1998; Hasegawa *et al.*, 1999; Gounni *et al.*, 1994, 2001; Kita *et al.*, 1999; Smith *et al.*, 2000) and can use receptor-bound IgE for highly efficient presentation of allergens to T cells (Kraft *et al.*, 1998; Maurer *et al.*, 1996; Mudde *et al.*, 1990; van der Heijden *et al.*, 1993). In addition, it has been found that histamine, a mast cell and basophil-derived mediator, can regulate T cell responses (Jutel *et al.*, 2001) and that T cell-derived cytokines can prime mast cells and basophils for enhanced degranulation (Bischoff *et al.*, 1990a,b). All these findings demonstrate that IgE-mediated effects do not exclusively comprise the classic Type I reaction described by Coombs and Gell (i.e., allergen-induced mast cell degranulation) but also have effects on T cell activation and thus may be of importance for the late responses and chronic manifestations of allergic disease.

The chronic manifestations of allergic disease (e.g., chronic allergic asthma, atopic dermatitis) are dominated by T cells and are characterized by the influx of eosinophils (Larché *et al.*, 2003; Leung, 2000). Interestingly chronic allergen exposure of the skin in patients with atopic dermatitis induces not only the influx of CD4⁺ Th2 cells, but also of Th1 cells secreting IFN-γ (Thepen *et al.*, 1996). The latter finding is interesting in the context of recent findings, suggesting that IFN-γ may induce keratinocyte apoptosis, a hallmark of eczematous diseases (Trautmann *et al.*, 2000). The activation of allergen-specific T cells is strongly enhanced when allergens are presented via IgE antibodies bound to APCs via FcεRI or FcεRII, but it has also been demonstrated that IgE-independent activation of allergen-specific T cells can occur during late-phase reactions. The injection of T cell epitope-containing peptides of the major cat allergen Fel d 1, which lacked IgE binding sites, was shown to induce late-phase reactions in cat-allergic patients in an MHCII-restricted manner (Haselden *et al.*, 1999; Oldfield *et al.*, 2001). These data demonstrate that non-IgE-dependent, T cell-mediated mechanisms can also play a role in late-phase allergic inflammation. T cell-mediated late reactions predominate in certain severe and chronic manifestations of allergic disease (e.g., atopic dermatitis) and are frequently associated with sensitization to a great variety of different allergens (i.e., polysensitization) (Leung, 2000). It should, however, be kept in mind that the frequent manifestations of atopy (e.g., allergic rhinoconjunctivitis, acute allergic asthma, urticaria, oral allergy syndrome) are caused preferentially by immediate reactions, whereas late-phase allergic reactions occur in fewer allergic patients. On the other hand, there is considerable evidence that untreated acute manifestations of allergy, especially under chronic allergen exposure, can progress into chronic forms and that manifestations in the upper respiratory tract may lead to symptoms in the deeper airways (Fuhlbrigge and Adams, 2003; Simons, 1999).

III. Possible Targets for Immunotherapy of Allergic Disease

The major subject of this chapter is allergen-specific immunotherapy, an active vaccination approach based on the therapeutic administration of the disease-eliciting allergens. Before we discuss allergen-specific immunotherapy, we would like to briefly discuss other therapies for the treatment of IgE-mediated allergies that are based on immunological strategies and hence may be referred to as immunotherapies. [Table II](#) summarizes these therapy forms according to the targeted structures and mechanisms. The aim of allergen-specific passive forms of immunotherapy is the inhibition of the interaction between allergens and IgE antibodies, which may be achieved at least in two ways. First, it is possible to produce allergen-specific antibodies or antibody fragments that may capture allergens before they can form complexes with IgEs. Such therapeutic allergen-specific antibodies may be administered locally into the target organs of allergy (e.g., nasal or bronchial mucosa, conjunctiva) to form a first line of defense against intruding allergens (reviewed in [Valenta *et al.*, 1997](#)). Human antibodies with therapeutic potential have been isolated by classic tissue culture and combinatorial cloning technology using B cells from allergic patients as a source ([Flicker *et al.*, 2002](#); [Lebecque *et al.*, 1997](#); [Sun *et al.*, 1995](#); [Visco *et al.*, 1996](#)). These antibodies were shown to inhibit the allergen-IgE interaction and to prevent allergen-induced basophil degranulation.

Second, it has been shown that allergen-derived IgE-reactive haptens obtained by proteolytic digestion of allergen extracts, by recombinant DNA technology, by chemical approaches, or by synthetic peptide chemistry (e.g., mimotopes) bind to IgE, but due to monovalent IgE binding, fail to cross-link and activate effector cells ([Attallah and Schon, 1969](#); [Ball *et al.*, 1994](#); [de Weck and Schneider, 1972](#); [Jensen-Jarolim *et al.*, 1998](#)). When effector cell-bound IgE is saturated with IgE-reactive haptenic structures, complete allergens should fail to induce the release of biologically active mediators and thus inflammation will be suppressed. The studies mentioned document that allergen-specific passive therapy concepts are effective in reducing effector cell activation (e.g., basophil degranulation), but several technical problems need to be overcome before these strategies may enter clinical applications. For example, it will be necessary to review the complexity of the natural allergen and epitope repertoire in order to obtain a representative panel of blocking antibodies or hapten-like structures, which may be achieved by the application of combinatorial cloning and peptide library technology. In addition, it is likely that passive therapy strategies will be preferentially applied in the target organs of atopy and it will hence be necessary to develop technologies for preventing the rapid wash-out of therapeutic components and to avoid

TABLE II
MOLECULAR AND CELLULAR TARGETS FOR IMMUNOTHERAPY OF ALLERGY

Target structure	Mechanism	References ^a
Allergens	Active immunotherapy	Bousquet <i>et al.</i> , 1998
	Passive immunotherapy	
	Blocking antibodies	Flicker <i>et al.</i> , 2002; Lebecque <i>et al.</i> , 1997; Sun <i>et al.</i> , 1995; Valenta <i>et al.</i> , 1997; Visco <i>et al.</i> , 1996
	Haptens	Attalah and Sehon, 1969; Ball <i>et al.</i> , 1994; de Weck and Schneider, 1972; Hedin and Richter, 1982
IgE	Mimotopes	Jensen-Jarolim <i>et al.</i> , 1998
	Therapeutic administration of α IgE	Heusser and Jardieu, 1997; MacGlashan <i>et al.</i> , 1997; Milgrom <i>et al.</i> , 1999
	Induction of α IgE antibodies by immunization	Haba and Nisonoff, 1987, 1990; Hellman <i>et al.</i> , 1994; Rudolf <i>et al.</i> , 1998; Vemersson <i>et al.</i> , 2002; Zuercher <i>et al.</i> , 2000
	Removal of IgE	Dau, 1988; Laffer <i>et al.</i> , 2001; Lebedin <i>et al.</i> , 1991
Receptors	Blockade of IgE/Fc ϵ RI interaction	Hamburger, 1975; Helm <i>et al.</i> , 1988, 1997; Kelly <i>et al.</i> , 1998; McDonnell <i>et al.</i> , 1996; Naito <i>et al.</i> , 1996; Vangelista <i>et al.</i> , 1999
	α Fc ϵ RI	Nechansky <i>et al.</i> , 2001
	α CD23	Dasic <i>et al.</i> , 1999; Sherr <i>et al.</i> , 1989
	Co-cross-linking of Fc ϵ RI and Fc γ RII	Daeron <i>et al.</i> , 1995; Zhu <i>et al.</i> , 2002
Cytokines	IL-4 antagonists	Borish <i>et al.</i> , 2001; Grunewald <i>et al.</i> , 1998
	α IL-5, IL-12	Bryan <i>et al.</i> , 2000; Flood-Page <i>et al.</i> , 2003; Leckie <i>et al.</i> , 2000
	Immune response modifier	Brugnolo <i>et al.</i> , 2003
IgE ⁺ B cells	Inhibition of IgE production	Yanagihara <i>et al.</i> , 1994
T cell	Anti-CD4 antibody	Kon <i>et al.</i> , 1998
	Immunosuppressors	Alexander <i>et al.</i> , 1992; Fleischer, 1999; Kapp <i>et al.</i> , 2002; Lock and Kay, 1996
Mast cell/basophil	Inhibition of mediator release	Sperr <i>et al.</i> , 1997; Triggiani <i>et al.</i> , 1989; Majlesi <i>et al.</i> , 2003; Zuberbier <i>et al.</i> , 2001
APC	Immunosuppressors	Panhans-Gross <i>et al.</i> , 2001

(continues)

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TABLE II (continued)

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the development of unwanted immune responses against the therapeutic competitors.

Another possible target for therapy of allergy is the IgE molecule. It has been debated whether IgE may have beneficial roles as protective antibodies that defend against parasites and perhaps cancer (El Ridi *et al.*, 1998; Gounni *et al.*, 1994; Reali *et al.*, 2001), but no disease related to a defect in IgE antibodies has been described in humans or in mouse strains that are deficient in IgE responses (Oettgen *et al.*, 1994). Therefore different therapeutic strategies for the removal of IgE antibodies have been developed. One approach evaluated in clinical studies in a large number of patients is the systemic administration of anti-IgE antibodies to allergic patients with the aim to capture and complex IgE antibodies so that they cannot bind to effector cells (Heusser and Jardieu, 1997; MacGlashan *et al.*, 1997; Milgrom *et al.*, 1999). For this purpose, anti-IgE antibodies preventing the binding of IgE to the high-affinity receptor have been produced and humanized for therapeutic application. Successful clinical outcomes have been reported, but it is not clear

whether the approach of injecting anti-IgE antibodies is suitable to treat patients containing high levels of IgE antibodies (Milgrom *et al.*, 1999). Furthermore, results from long-term applications that would allow long-term side effects to be evaluated are not yet available. One interesting finding made in these studies was that the removal of IgE antibodies led to a strong reduction of the expression of the high-affinity IgE receptor on mast cells and basophils, suggesting that IgE up-regulates its own receptors (MacGlashan *et al.*, 2001).

Another possible way to prevent IgE binding to the high-affinity receptor and to remove IgE from the circulation by the formation of IgE immune complexes is the induction of anti-IgE antibodies by active immunization (Haba and Nisonoff, 1987, 1990; Hellman, 1994; Rudolf *et al.*, 1998; Vernersson *et al.*, 2002; Zuercher *et al.*, 2000). For this purpose, complete IgE antibodies, recombinant fragments of the IgE constant region implicated in the Fc ϵ RI binding and peptides mimicking the receptor-binding domains of IgE, have been used for immunization in animals (Table II). It could be shown that immunized animals developed anti-IgE antibodies that inhibited the IgE–Fc ϵ RI interaction and that these immunized animals exhibited reduced immediate-type skin reactions (Hellman, 1994).

Furthermore, it has been shown that the therapeutic removal of IgE by selective plasmapheresis can reduce allergic symptoms (Dau, 1988; Lebedin *et al.*, 1991), and antibodies have been developed that are suitable for the depletion of IgE antibodies, as well as of IgE-bearing effector cells from the blood of allergic patients (Laffer *et al.*, 2001).

The effect of IgE on immune cells is mediated by two different receptors (i.e., the high-affinity receptor, Fc ϵ RI and the low-affinity IgE receptor, Fc ϵ RII [CD23]) (Bonney *et al.*, 1997; Heyman, 2000; Kinet, 1999; Novak *et al.*, 2003). Fc ϵ RI is the key structure mediating immediate-type inflammation via the IgE-dependent degranulation of mast cells and basophils and, more recently, has been found to be important for IgE-mediated activation of eosinophils and IgE-mediated allergen presentation (Gounni *et al.*, 1994; Kraft *et al.*, 1998; Maurer *et al.*, 1996). Fc ϵ RII seems to have regulatory functions on IgE synthesis and is involved in allergen presentation to T cells (Mudde *et al.*, 1990; van der Heijden *et al.*, 1993). Therapeutic approaches to prevent the interaction of IgE and Fc ϵ RI include IgE-derived peptides or recombinant fragments, as well as the Fc ϵ RI α -chain or portions thereof (Hamburger, 1975; Helm *et al.*, 1988, 1997; Kelly *et al.*, 1998; McDonnell *et al.*, 1996; Naito *et al.*, 1996; Vangelista *et al.*, 1999). Furthermore, antibodies against Fc ϵ RI have been shown to inhibit IgE-mediated basophil activation (Nechansky *et al.*, 2001). Antibodies against Fc ϵ RII have been shown to inhibit IgE-allergen presentation and IgE synthesis (Dasic *et al.*, 1999; Sherr *et al.*, 1989).

The interesting observation that co-cross-linking of Fc ϵ RI and Fc γ RII causes a reduction of effector cell activation has led to the construction of a chimeric molecule consisting of the receptor-binding portions of human IgG and IgE that inhibited mast cell and basophil function (Daeron *et al.*, 1995; Zhu *et al.*, 2002). Therapeutic approaches aiming to antagonize the effects of Th2 cytokines have mainly focused on IL-4, the key cytokine involved in the class-switch to IgE production, and IL-5, a potent activator of eosinophils. In this context it has been shown that the administration of IL-4 antagonists (e.g., an IL-4 mutant protein) during primary sensitization prevented the development of IgE responses in mice, and continuing clinical trials investigate the potential of IL-4 antagonists for the treatment of allergic patients (Borish *et al.*, 2001; Grunewald *et al.*, 1998). In recent clinical studies, recombinant IL-12 and anti-IL-5 monoclonal antibodies, both known to suppress eosinophilic inflammation, have been used for the treatment of patients with allergic asthma (Bryan *et al.*, 2000; Flood-Page *et al.*, 2003; Leckie *et al.*, 2000). Although a strong reduction of tissue eosinophilia was observed in treated patients, no effects on airway hyperresponsiveness and late asthmatic responses were found, thus questioning the pathological role of eosinophils in asthma.

A synthetic immune response modifier capable of shifting T cells from Th2 to Th1 cytokine production has recently been evaluated *in vitro* and may be a therapeutic substance for the treatment of allergies (Brugnolo *et al.*, 2003). The inhibition of IgE production in B cells using recombinant portions of Fc ϵ RI indicates that it may be possible to target IgE-producing cells for therapeutic intervention (Yanagihara *et al.*, 1994).

Support for a critical role of T cells in late allergic reactions comes from a number of studies in which T cell-specific reagents (e.g., anti-CD4 antibodies) or immunosuppressive drugs with a strong focus on T cell reactivity (e.g., cyclosporine, tacrolimus, pimecrolimus) were shown to be effective in the treatment of chronic forms of atopy (e.g., chronic asthma, atopic dermatitis) (Alexander *et al.*, 1992; Fleischer, 1999; Kapp *et al.*, 2002; Kon *et al.*, 1998; Lock and Kay, 1996).

It should, however, be mentioned that several *in vitro* findings indicate that cyclosporine, as well as tacrolimus, is also effective in reducing allergen-induced immediate inflammation due to degranulation of mast cells and basophils and may affect antigen-presenting cells (Panhans-Gross *et al.*, 2001; Sperr *et al.*, 1997; Triggiani *et al.*, 1989; Zuberbier *et al.*, 2001). Furthermore, previously unknown suppressive effects of already known drugs on allergen-specific mast cell and basophil activation have been reported (Majlesi *et al.*, 2003).

The risk of side effects limits the systemic application of immunosuppressive drugs for the treatment of allergy, but the macrolactams tacrolimus and pimecrolimus seem to be very effective and safe for the local treatment of atopic dermatitis.

IV. Allergen-Specific Immunotherapy

Allergen-specific immunotherapy is the administration of increasing doses of the disease-eliciting allergens to allergic patients with the aim to induce a state of allergen-specific nonresponsiveness. An overview regarding immunological and clinical studies related to allergen-specific immunotherapy, together with recommendations for the use of this treatment, has been prepared by an international expert consortium (Bousquet *et al.*, 1998). More than 90 years ago, when allergen-specific immunotherapy was applied for the first time to treat grass pollen-allergic patients, nothing was known about the immunological mechanisms operative in Type I allergy (Noon, 1911). At that time pollen-induced hay fever was erroneously interpreted as an intoxication caused by a grass pollen toxin. Nevertheless, patients having received injections containing grass pollen extract showed improvement, and this protection was found to last for at least 1 year after treatment was discontinued. After the first successful trials, the treatment was also applied for desensitization to other allergen sources. In 1935 Cooke and co-workers provided the first evidence for mechanisms operative in allergen-specific immunotherapy by demonstrating that symptoms of allergy could be suppressed in untreated patients by the transfer of blood from successfully treated patients (Cooke *et al.*, 1935). As a protective factor, “blocking antibodies” were identified by Mary Loveless, who showed that the injection of serum from successfully treated patients into the skin of untreated patients suppressed allergen-induced immediate skin reactions (Loveless, 1940). A major improvement of safety of allergen-specific immunotherapy was achieved by the use of adjuvant-bound allergen extracts, which caused fewer systemic allergenic side effects than the injection of aqueous allergen extracts (Sledge, 1938). Analyzing the development of allergen-specific immunotherapy, it becomes evident that this treatment has been practiced in patients with considerable success for a long time, although the molecular and cellular mechanisms involved in the pathogenesis of Type I allergy were not known. For example, IgE antibodies were discovered in the late 1960s, when immunotherapy had already been routinely used for more than 50 years (Ishizaka *et al.*, 1966; Johansson and Bennich, 1967). Likewise, many immune cells and cytokines involved in the regulation of IgE synthesis were characterized in the 1980s (reviewed in Romagnani, 1997; Paul, 1987), and the nature of the most common disease-eliciting allergens was revealed by molecular cloning techniques only in the past 15 years (reviewed in Valenta and Kraft, 2002). It is therefore not surprising that the operative immunological mechanisms behind allergen-specific immunotherapy are not fully understood even today, although it is a frequently practiced allergy treatment and perhaps one of the few causative treatment forms (Bousquet *et al.*, 1998).

V. Possible Mechanisms Underlying Allergen-Specific Immunotherapy

Currently, the most common form of immunotherapy is injection immunotherapy, which is based on the repeated subcutaneous injection of gradually increasing amounts of adjuvant-bound allergen extracts (Bousquet *et al.*, 1998). In Table III we summarize possible mechanisms and effects observed during the treatment of allergic patients according to the targeted structure, cell, or process. Viewing the various immunological and clinical effects allows us to propose at least three mutually nonexclusive models for the immunological mechanisms underlying allergen-specific immunotherapy, which will be briefly described and discussed.

A. MODEL 1: ALLERGEN-SPECIFIC IMMUNOTHERAPY SHIFTS THE TH2-DOMINATED ALLERGEN-SPECIFIC IMMUNE RESPONSE TOWARD A TH1 RESPONSE

The identification of Th2 cytokines as the classic factors initiating IgE antibody production and thus Type I allergic immune responses, together with the finding that in allergic patients allergen-specific T cell clones, but not T cell clones specific for bacterial antigens exhibit a preferential Th2 cytokine profile, has led to the Th2/Th1 paradigm (Romagnani, 1997). This paradigm is extremely useful in explaining major features of allergic diseases as being due to a predominance of Th2 cytokines (e.g., IL-4, IL-13, IL-5) that are responsible for the predisposition of atopic individuals to mount IgE antibody responses against allergens. According to this paradigm, it has been suggested that it would be desirable to shift the allergen-specific immune response in allergic patients from the dominating Th2 response toward a preferential Th1 response. Support for the assumption that allergen-specific immunotherapy may indeed be capable of inducing such a shift came from observations that among allergen-specific T cell clones isolated from the peripheral blood of allergic patients after immunotherapy, the number of Th1 clones increased and there was also an increase in Th1 cytokine production (Ebner *et al.*, 1997; Jutel *et al.*, 1995; McHugh *et al.*, 1995; Secrist *et al.*, 1993). The reduction of eosinophils and IL-5 production after immunotherapy has been interpreted as another sign of the proposed shift toward Th1 immunity (Rak *et al.*, 2001; Wilson *et al.*, 2001). The Th2 to Th1 shift model, however, does not explain the increase of IgE antibodies during the initial phase of immunotherapy and the heavy production of allergen-specific IgG₄ and IgG₁ antibodies while the Th1-indicative antibody subclasses (IgG₂, IgG₃) fail to increase after treatment. Furthermore, the enthusiasm about shifting allergen-specific immune responses toward a Th1 profile has been reduced by the following considerations. First, it turned out that allergen-specific T cell responses in allergic versus nonallergic persons are not as strictly polarized as was originally

anticipated, and there is even evidence that allergen-specific Th1 responses may be responsible for pathogenetic effects in patients suffering from chronic forms of atopy (Byron *et al.*, 1994; Hales *et al.*, 2000; Looney *et al.*, 1994; Oldfield *et al.*, 2001; van Neerven *et al.*, 1994a, 1994b). Second, the finding that overwhelming Th1 responses are implicated in a number of autoimmune diseases (Del Prete, 1998; Ghoreschi *et al.*, 2003; Kaufman *et al.*, 1993; Mirakian *et al.*, 2001) has raised the question of whether it will be desirable to shift Th2 responses toward a strong Th1 response in allergic patients.

B. MODEL 2: ALLERGEN-SPECIFIC IMMUNOTHERAPY INDUCES ALLERGEN-SPECIFIC T CELL TOLERANCE

The idea that induction of T cell tolerance may be a relevant mechanism in allergen-specific immunotherapy is supported by several observations. For example, one early study reported that allergen-specific suppressor cells are induced by immunotherapy (Rocklin *et al.*, 1980). Based on the idea that a down-regulation of allergen-specific T cell activity may be beneficial, allergen-specific T cell epitope-containing peptides may be used for the induction of tolerance/anergy by specific immunotherapy. Studies in mice have shown that it is possible indeed to induce T cell tolerance/anergy with T cell epitope-containing peptides (Briner *et al.*, 1993; Hoyne *et al.*, 1993), but clinical trials conducted in patients with allergen-derived peptides provided controversial results (Müller *et al.*, 1998; Oldfield *et al.*, 2001; Pene *et al.*, 1998; Simons *et al.*, 1996). It has been proposed that induction of T cell anergy may also play a role in the currently used allergen-specific immunotherapy (i.e., injection immunotherapy) because reduced proliferation of allergen-specific T cells was demonstrated after immunotherapy (Akdis and Blaser, 1999; Ebner *et al.*, 1997). In this context IL-10 has been suggested as a crucial factor that may be produced in an autocrine manner by allergen-specific T cells and may down-regulate T cell responses (Akdis *et al.*, 1996).

However, several other immunological changes observed during allergen-specific immunotherapy are difficult to explain with the tolerance model. First, it is difficult to explain the strong therapy-induced increase of allergen-specific IgG₁ and IgG₄ antibody levels by an induction of allergen-specific tolerance. Furthermore, it is difficult to explain how T cell tolerance/anergy may be linked to the immunotherapy-associated reduction of immediate reactions. Allergen-specific effects on immediate reactions cannot be easily understood by general cytokine effects (e.g., IL-10) because they are caused by strictly allergen- and IgE-dependent degranulation of mast cells and basophils. Moreover, there is considerable evidence that the down-regulation of allergen- and IgE-dependent effector cell degranulation is mediated mainly by therapy-induced allergen-specific blocking antibodies (Ball *et al.*, 1999a, 1999b; Clinton *et al.*, 1989; Mothes *et al.*, 2003).

TABLE III
POSSIBLE MECHANISMS OF ALLERGEN-SPECIFIC IMMUNOTHERAPY

Targeted structure/process	Mechanism	References
Mast cell and basophil degranulation	Mast cell and basophil degranulation	Ball <i>et al.</i> , 1999a, 1999b; Clinton <i>et al.</i> , 1989; Loveless, 1940; Mothes <i>et al.</i> , 2003
	Reduction of histamine releasability	Shim <i>et al.</i> , 2003
	Reduction by T cell-derived IL-10 and IFN- γ	Pierkes <i>et al.</i> , 1999
Mast cell number	Reduction by unknown mechanism	Durham <i>et al.</i> , 1999
IgE-mediated allergen presentation to T cells, T cell activation, and cytokine release	Blocking IgG competing with IgE	van Neerven <i>et al.</i> , 1999
B cells	Down-regulation of CD23	Hakansson <i>et al.</i> , 1998; Roever <i>et al.</i> , 2002
T cell proliferation	Generation of suppressor cells; T cell tolerance/anergy	Akdis and Blaser, 1999; Akdis <i>et al.</i> , 1996; Baskar <i>et al.</i> , 1997; Briner <i>et al.</i> , 1993; Ebner <i>et al.</i> , 1997; Hoyne <i>et al.</i> , 1993; Rocklin <i>et al.</i> , 1980; Müller <i>et al.</i> , 1998; Oldfield <i>et al.</i> , 2001, 2002
Th1/Th2 cell ratio	Shift from Th2 to Th1 pattern	Ebner <i>et al.</i> , 1997; Hakansson <i>et al.</i> , 1998; Jutel <i>et al.</i> , 1995; Kammerer <i>et al.</i> , 1997; McHugh <i>et al.</i> , 1995; Pene <i>et al.</i> , 1998; Secrist <i>et al.</i> , 1993

Th2 cytokine production	Preferential apoptosis of Th2 cells Reduction of T cell-derived IL-4 and IL-13	Guerra <i>et al.</i>, 2001 Ebner <i>et al.</i>, 1997 ; Gabrielsson <i>et al.</i>, 2001 Jutel <i>et al.</i>, 1995 ; McHugh <i>et al.</i>, 1995 ; Pene <i>et al.</i>, 1998 ; Secrist <i>et al.</i>, 1993
Th1 cytokine production	Preferential apoptosis of Th2 cells Induction of Th1 cells	Guerra <i>et al.</i>, 2001 Ebner <i>et al.</i>, 1997 ; Jutel <i>et al.</i>, 1995 ; Kammerer <i>et al.</i>, 1997 ; McHugh <i>et al.</i>, 1995
Eosinophil number/activation	Eosinophil reduction and IL-5 reduction by unknown mechanism	Rak <i>et al.</i>, 2001 ; Wilson <i>et al.</i>, 2001
IgE production by memory cells; allergen-induced boost of IgE production	Possible suppression by blocking antibodies	Mothes <i>et al.</i>, 2003
Prevention of bronchial hyperresponsiveness	Unknown mechanism	Rak <i>et al.</i>, 2001
Prevention of disease progression	Unknown mechanism	Moller <i>et al.</i>, 2002

C. MODEL 3: ALLERGEN-SPECIFIC IMMUNOTHERAPY HAS VACCINATION CHARACTER AND INDUCES AN IMMUNE RESPONSE THAT COUNTERACTS THE ALLERGIC IMMUNE RESPONSE

The third model suggests that allergen-specific immunotherapy has a vaccination character. In this model the injection of adjuvant-bound allergens induces a new type of allergen-specific immunity, which, in the case of the most frequently used adjuvant (i.e., aluminium hydroxide) has features of a Th2-like immunity. This immune response is dominated by the induction of strong allergen-specific IgG₁ and IgG₄ responses and an initial boost of allergen-specific IgE responses (Ball *et al.*, 1999a, 1999b; Mothes *et al.*, 2003). The therapy-induced humoral immune response may be directed against new epitopes as well as against IgE epitopes, depending on the allergen preparation used. If immunotherapy is tolerated and can be continued by injection of increasing allergen doses, the initial IgE response is overwhelmed by strong IgG responses (Ball *et al.*, 1999a, 1999b). Furthermore, if allergen-specific IgG antibodies recognize IgE epitopes, they can compete with allergen-specific IgE antibodies and accordingly inhibit allergen-induced mast cell, as well as basophil activation (Ball *et al.*, 1999a, 1999b; Clinton *et al.*, 1989; Mothes *et al.*, 2003). The latter effect explains the suppression of immediate, IgE-dependent allergic reactions. In addition to a simple blocking of IgE allergen recognition, it is also possible that allergen-specific IgG leads to a co-cross-linking of FcεRI and FcγRII receptors, which may down-regulate effector cell activation (Daeron *et al.*, 1995).

Using recombinant allergen fragments, it has been demonstrated that allergen-specific immunotherapy induces antibody responses against epitopes that were not recognized before treatment, suggesting that the treatment does have a vaccination character (Ball *et al.*, 1999b). The newly induced IgG responses not only may have beneficial effects regarding the suppression of mast cell degranulation, but also may inhibit IgE-mediated allergen presentation to T cells and thus suppress T cell activation and the release of proinflammatory cytokines (van Neerven *et al.*, 1999). Furthermore, it has been shown that patients developing allergen-specific IgG antibodies after immunotherapy exhibit a reduced boost of allergen-specific IgE antibodies due to allergen exposure compared with placebo-treated patients lacking allergen-specific IgG (Mothes *et al.*, 2003). The latter result suggests that immunotherapy-induced allergen-specific IgG antibodies may also suppress the boost of allergen-specific IgE production in memory cells caused by allergen exposure.

The vaccination model thus would explain the reduction of immediate, late-phase responses and possibly the long-term effects observed during injection immunotherapy. According to the vaccination model, immunotherapy would induce a new allergen-specific immune response that competes with the

established allergic immune response and is characterized by the formation of “blocking antibodies” as already suggested by [Loveless \(1940\)](#) and [Cooke *et al.* \(1935\)](#). These blocking antibodies exhibit their protective role simply by antagonizing the effects of IgE. Due to the fact that aluminium hydroxide almost exclusively is permitted for use as adjuvant for allergen-specific immunotherapy, the therapy-induced immune response is characterized by Th2 features (i.e., initial boost of IgE responses, induction of Th2-indicative IgG₄ and IgG₁ responses).

However, the protective role of therapy-induced antibodies has been questioned because it has been observed that clinical success of immunotherapy does not always correlate with the induction of IgG antibodies ([Birkner *et al.*, 1990](#); [Djurup and Malling, 1987](#)). In this context it must, however, be stated that these immunotherapy studies and the corresponding clinical, as well as immunological, analyses were performed with crude allergen extracts. Using such allergen extracts, it is not possible to determine whether therapy-induced IgG antibodies react with allergens and inhibit IgE recognition of allergens. It will hence be necessary to reinvestigate the influence of blocking antibodies on clinical outcome parameters with defined allergens and epitopes.

VI. Modifications of Traditional, Allergen Extract-Based Immunotherapy

Several variations of traditional allergen extract-based injection immunotherapy have been introduced and evaluated ([Table IV](#)). The aim of these variations has been to improve the disadvantages of immunotherapy (e.g., risk of anaphylactic side effects). We have summarized these variations according to the target structure (i.e., the administered antigen, the mode of administration, and the adjuvant used to formulate the vaccine) ([Table IV](#)). Major disadvantages of allergen-specific immunotherapy have been the risk of inducing anaphylactic side effects in sensitized patients, the inconvenience of frequent injection treatments, and difficulties in obtaining defined allergen preparations. Successful attempts to reduce the allergenic activity of the vaccines were based on the coupling of allergen extracts to adjuvants that were thought to prevent the spreading and systemic release of the administered allergens ([Sledge, 1938](#)). Compared to the injection of aqueous allergen extracts, the rate of systemic anaphylactic side effects observed in the course of immunotherapy could indeed be drastically reduced by using aluminium hydroxide-adsorbed allergen extracts (reviewed in [Bousquet *et al.*, 1998](#)). Although aluminium hydroxide is known to induce antigen-specific Th2 responses it is still today the most frequently, if not the only adjuvant permitted for vaccination treatment and allergen-specific immunotherapy. Other adjuvants such as liposomes or monophosphoryl lipid A (MPL) are rarely used ([Drachenberg *et al.*, 2001](#); [Santeliz *et al.*, 2002](#)).

TABLE IV
MODIFICATIONS OF TRADITIONAL ALLERGEN-SPECIFIC IMMUNOTHERAPY

Target	Type of variation	Mechanism/advantage	References
Antigen	Chemically modified allergen extracts (haptens, PEG, allergoids)	Reduction of allergenic activity; induction of tolerance; induction of Th1 responses	Attallah and Sehon, 1969; HayGlass and Stefura, 1991; Lee and Sehon, 1977; Litwin <i>et al.</i> , 1988, 1991; Malley <i>et al.</i> , 1976; Marsh <i>et al.</i> , 1970; Norman <i>et al.</i> , 1982
	Recombinant allergens and modified recombinant allergens	Allergen specificity; induction of blocking antibodies; increase of safety; immunomodulation; induction of T cell tolerance	Valenta, 2002; Valenta and Kraft, 2002
	T cell peptides	Allergen specificity; increase of safety; induction of T cell tolerance	Briner <i>et al.</i> , 1993; Hoyne <i>et al.</i> , 1993; Müller <i>et al.</i> , 1998; Oldfield <i>et al.</i> , 2001, 2002; Simons <i>et al.</i> , 1996
	B cell peptides	Allergen specificity; increase of safety; induction of blocking antibodies	Ball <i>et al.</i> , 1999a; Focke <i>et al.</i> , 2001
	Mimotopes	Allergen specificity; induction of blocking antibodies	Jensen-Jarolim <i>et al.</i> , 1998
	DN Vaccines	DNA vaccines Allergen specificity; induction of Th1 responses	Hartl <i>et al.</i> , 1999; Hochreiter <i>et al.</i> , 2003; Hsu <i>et al.</i> , 1996; Raz <i>et al.</i> , 1996; Slater <i>et al.</i> , 1998

Mode of administration	Oral/sublingual administration	Safety; induction of T cell anergy; easy to perform; convenient for patients	Andre et al., 2000 ; Bjorksten et al., 1986 ; Clavel et al., 1998 ; Fanta et al., 1999 ; Quirino et al., 1996 ; Taudorf et al., 1989 ; Urbanek et al., 1990
	Nasal administration	Safety; convenient for patients	Andri et al., 1996 ; Passalacqua et al., 1997 ; Welsh et al., 1983
Adjuvant	Al(OH) ₃	Reduction of anaphylactic side effects	Sledge, 1938
	CpG, MPL, liposomes	Induction of Th1 responses; reduction of allergenic activity (CpG)	Alvarez et al., 2002 ; Drachenberg et al., 2001 ; Horner et al., 2002 ; Marshall et al., 2001 ; Mothes et al., 2003 ; Santeliz et al., 2002 ; Tighe et al., 2000
	Chitosan-nanoparticles	Induction of T cell tolerance	Roy et al., 1999
	Carbohydrate-based particles	Reduced tissue damage; ease of production	Grönlund et al., 2002
	Live vaccines	Induction of Th1 responses	Vrtala et al., 1995
	Surface layers	Induction of Th1 responses	Jahn-Schmid et al., 1996

Modifications of allergen extracts were already performed more than 30 years ago in order to reduce anaphylactic side effects (Attallah and Sehon, 1969; Lee and Sehon, 1977; Litwin *et al.*, 1988; Malley *et al.*, 1976; Marsh *et al.*, 1970). These modifications were achieved by chemical modifications of the allergen extracts and their proteolytic digestion, by coupling of allergen extracts to polyethyleneglycol (PEG), or by chemical denaturation of allergen extracts using various aldehydes. Proteolytic digestion of allergen extracts and coupling to PEG have indeed yielded material with reduced allergenic activity. Allergoids produced by chemical denaturation had a strongly reduced allergenic activity and even induced strong Th1 immune responses (HayGlass and Stefura, 1991). Due to the difficulties in manufacturing chemically modified allergen extracts of consistent quality, today only allergoids are frequently used for routine treatment.

Another major problem of allergen-specific immunotherapy is that allergen extracts are mixtures of allergenic and nonallergenic components that cannot be tailored according to the individual patient's sensitization profile (reviewed in Bousquet *et al.*, 1998). Since great variations regarding allergen content have been found for various allergen extracts, antibody-based assays have been developed that at least have allowed us to detect and quantify some of the common allergens in the therapeutic extracts (van Ree, 1997). However, the composition of allergen extracts of natural allergen sources depends on the representation of the individual allergen molecules in the sources and cannot be changed during manufacturing. Furthermore, the extraction process and many other factors (e.g., proteolysis) may influence the presence of allergenic and nonallergenic components in natural allergen extracts. Due to the progress made in the field of allergen characterization, the cDNAs coding for the most common environmental allergens have been isolated, and it has become possible to produce recombinant allergens that closely mimic the properties of the corresponding natural allergens (reviewed in Valenta and Kraft, 2002; Valenta *et al.*, 1999a). Moreover, it has been demonstrated that the complete allergen/epitope repertoire of natural allergen sources can be replaced by recombinant allergens. The finding that recombinant allergens can resemble the epitope repertoire of natural allergen sources represents a prerequisite for the development of new diagnostic and therapeutic strategies for Type I allergy (reviewed in Valenta, 2002). The advantages of using recombinant allergens for diagnosis and therapy will, however, be discussed separately.

Based on the DNA and deduced amino acid sequences obtained for the most common allergens by molecular cloning, highly specialized and experimental forms of therapy strategies have been developed. They include, for example, the use of allergen-derived T cell epitope-containing peptides for immunotherapy. Studies performed in mouse models have indicated that it is possible to induce T cell tolerance against the major allergens from mites and

cats by using only a few major T cell epitope-containing peptides (Briner *et al.*, 1993; Hoyne *et al.*, 1993). However, T cell peptide-based therapy evaluated in cat and bee venom allergic patients yielded controversial results (Müller *et al.*, 1998; Oldfield *et al.*, 2001; Pene *et al.*, 1998; Simons *et al.*, 1996). Another concept suggests using recombinant fragments or synthetic peptides derived from B cell epitopes of allergens or mimotopes (i.e., peptides mimicking B cell epitopes) for the induction of blocking antibodies (Ball *et al.*, 1999a; Focke *et al.*, 2001; Jensen-Jarolim *et al.*, 1998).

Based on the finding that injection of allergen-encoding DNA can induce Th1-prone immune responses in animals, it was also suggested that DNA vaccination be applied for the treatment of Type I allergy (Hartl *et al.*, 1999; Hsu *et al.*, 1996; Raz *et al.*, 1996). A major problem limiting the clinical application of DNA vaccination to allergic patients was the finding that it leads to a rather uncontrolled spreading of allergen-encoding DNA in various organs, bearing the risk that active allergen may be synthesized at these sites (Slater *et al.*, 1998). The latter problem may, however, be overcome if DNA coding for hypoallergenic allergen versions is used for vaccination (Hochreiter *et al.*, 2003).

More than 10 years ago oral or nasal administration of allergens was studied as a possible alternative to injection immunotherapy (Bjorksten *et al.*, 1986; Taudorf *et al.*, 1989; Urbanek *et al.*, 1990; Welsh *et al.*, 1983). Recently, a renaissance of clinical studies investigating again whether oral, sublingual, or nasal application of allergens may represent an alternative to current forms of injection immunotherapy has been observed (Andri *et al.*, 1996; Andre *et al.*, 2000; Clavel *et al.*, 1998; Fanta *et al.*, 1999; Passalacqua *et al.*, 1997; Quirino *et al.*, 1996). These forms of administration appear safe and convenient, and evidence for clinical efficacy has been provided in certain studies. However, it appears that these forms of immunotherapy seem to have little detectable immunological effects. While it has been speculated that they may induce tolerance preferentially, the immunological mechanisms of these alternative forms of treatment are currently completely elusive.

The discovery that certain bacterial DNA sequences, that is, immunostimulatory oligodeoxynucleotides (ISS-ODN, CpG oligonucleotides) promote strong Th1 immune responses has led to several *in vitro* and animal studies exploring the usefulness of immunostimulatory DNA sequences as general immunomodulators and as adjuvants for allergen-specific immunotherapy (Horner *et al.*, 2002; Marshall *et al.*, 2001; Tighe *et al.*, 2000). Recently, a clinical study with a CpG-coupled version of the major ragweed allergen, Amb 1, has been initiated in patients.

Likewise, chitosannanoparticles, bacterial surface layers, and carbohydrate-based particles have been suggested as possible adjuvants for allergy vaccination (Grönlund *et al.*, 2002; Jahn-Schmid *et al.*, 1996; Roy *et al.*,

1999). In addition, attempts have been made to express allergens in bacterial strains that can be used as live vaccines (Vrtala *et al.*, 1995).

VII. Possible Improvement of Immunotherapy by Using Recombinant Allergens

A. THE DEVELOPMENT OF RECOMBINANT ALLERGENS

New possibilities for the improvement of diagnosis and allergen-specific immunotherapy evolved from the molecular and structural characterization of allergens (reviewed in Valenta, 2002; Valenta and Kraft, 2002). Our knowledge about the molecular nature of the disease-eliciting allergens has dramatically increased through the application of molecular cloning techniques to the field of allergen characterization. By the end of the 1980s, several research groups started to isolate cDNAs coding for allergens, and soon thereafter recombinant allergens mimicking the natural allergens became available. The characterization of allergens by molecular cloning was initially thought to reveal the molecular nature of paradigmatic allergen molecules and to allow studies of the allergen-specific immune responses using well-defined allergens that are frequently recognized by allergic patients. Unexpectedly, immunological studies (i.e., IgE reactivity, T cell proliferations) showed that only a few recombinant allergens were required to cover the majority of disease-eliciting epitopes that are present in natural allergen extracts (reviewed in Valenta *et al.*, 1999a, 1999b).

More than 10 years ago, two studies demonstrated that allergy to birch pollen and grass pollen could be diagnosed with a few recombinant allergens and suggested replacing diagnostic allergy tests based on crude allergens with recombinant allergen tests (Valenta *et al.*, 1991, 1992). Due to the work of several research groups, the allergen repertoires of the most common allergen sources have now been reconstructed with recombinant allergens and new types of multiallergen tests based on microarrayed recombinant allergens have been developed (Harwanegg *et al.*, 2003; Hiller *et al.*, 2002). There is a fundamental difference between previously used diagnostic tests based on crude allergen extracts and the new test systems using recombinant allergen molecules. Allergen extract-based tests only determine if a patient is sensitized against a particular allergen source, but provide no information about the immune reactivity to the individual allergenic molecules in the allergen source. The analysis of the reactivity profiles of allergic patients with recombinant allergens, termed component-resolved diagnosis (CRD), has revealed that allergic patients are characterized by individual reactivity to the allergens in a given allergen source (Valenta *et al.*, 1999a, 1999b). This IgE reactivity profile evolves in the first years of life and seems to remain unchanged in the further natural course of the disease. Studies analyzing the sensitization profiles in different populations with recombinant allergens have demonstrated that

the IgE reactivity profiles of patients also vary depending on the locally predominating allergens and thus are an imprint of the local allergen exposure. For example, it was found that individuals living in the northern parts of Europe, where birch predominates, are more frequently and exclusively sensitized against Bet v 1, the major birch pollen allergen, suggesting that these patients were genuinely sensitized to birch pollen, whereas patients from the more southern parts of Europe more frequently reacted with birch pollen due to sensitization to cross-reactive allergens from sources other than birch (Moverare *et al.*, 2002a). Similarly, it was found that allergic individuals from Central Africa were preferentially sensitized against marker allergens that reflect the predominance of certain grass species in their local environment (Westritschnig *et al.*, 2003). The differences of reactivity profiles in populations may have considerable implications for selecting the suitable composition of allergy vaccines.

B. THE IMPACT OF RECOMBINANT ALLERGEN-BASED DIAGNOSTIC TESTING ON TRADITIONAL ALLERGEN-SPECIFIC IMMUNOTHERAPY

Adequate diagnosis of IgE-mediated allergy is a prerequisite for the inclusion of patients for allergen-specific immunotherapy. However, as mentioned earlier, the currently used allergen extract-based diagnostic tests cannot identify the disease-eliciting allergen molecules and therefore do not allow a precise selection of patients for immunotherapy. It has therefore been suggested that recombinant allergen-based tests be used for an improved selection of patients for immunotherapy (Kazemi-Shirazi *et al.*, 2002). For example, it has been shown that certain allergen molecules occur abundantly and rather exclusively in a given allergen source and therefore can be used as diagnostic markers for a genuine sensitization toward this allergen source. On the other hand, sensitization to highly cross-reactive allergens that occur in many different unrelated allergen sources may give rise to positive test reactions to all these sources when diagnostic tests are performed with allergen extracts. On the basis of these findings, it has been suggested that species-specific marker allergens and highly cross-reactive allergens be used to differentiate patients with a genuine sensitization to a given allergen source from cross-sensitized patients who appear polysensitized to many allergen sources and hence may benefit less from allergen-specific immunotherapy (Bousquet *et al.*, 1991; Kazemi-Shirazi *et al.*, 2002).

To accommodate the wealth of individual allergen molecules, chip-based allergy tests that utilize microarrayed recombinant allergens have recently been developed (Harwanegg *et al.*, 2003; Hiller *et al.*, 2002). They allow rapid screening of the sensitization profile toward numerous allergen molecules and epitopes with minute amounts of serum in single determinations. Using recombinant allergen-based diagnostic tests, it has thus become

possible to obtain additional diagnostic information for the selection of patients for traditional immunotherapy and also to monitor the development of immune responses toward individual allergen molecules in the course of immunotherapy (Ball *et al.*, 1999a, 1999b; Mothes *et al.*, 2003).

Analyses of patients undergoing allergen extract-based immunotherapy with purified recombinant allergens and epitopes have provided evidence for several potential weaknesses of traditional immunotherapy that are related to the use of allergen extracts instead of defined allergen molecules. For example, it was found that the injection of allergen extracts can induce *de novo* IgE sensitizations against allergens that were not recognized before the therapy (Ball *et al.*, 1999b; Moverare *et al.*, 2002b). Although the clinical relevance of these new IgE sensitizations has not yet been demonstrated in all of the reported studies, these findings strongly suggest that treatment with defined allergen molecules selected according to the patient's sensitization profile should be preferred to treatment with allergen extracts. Another weakness of therapeutic allergen extracts is that these extracts may lack important allergens or that certain allergens are not present as immunogenic molecules and hence fail to induce protective immune responses (Mothes *et al.*, 2003). Since it is technically impossible to influence the composition of natural allergen extracts, the replacement of these extracts by recombinant allergens seems to be a logical next step toward the improvement of allergen-specific immunotherapy.

C. RECONSTRUCTING THE NATURAL ALLERGEN REPERTOIRE BY RECOMBINANT ALLERGENS: A PREREQUISITE FOR NEW THERAPEUTIC CONCEPTS

The potential replacement of natural allergen extracts by recombinant allergens depends on whether it is possible to replace the majority of disease-eliciting epitopes of a given allergen source by recombinant molecules. For this purpose, the immunological equivalence of natural and recombinant allergens has to be demonstrated, and, as a next step, a panel of recombinant allergens resembling the relevant IgE epitopes and T cell epitopes of the natural allergen source has to be defined. For the most common allergen sources (e.g., pollens, mites, animal dander), recombinant allergens containing most of the IgE and T cell epitopes present in the natural allergen sources have now been identified (reviewed in Valenta *et al.*, 1999a, 1999b). In principle, it is therefore possible to formulate recombinant allergen-based vaccines that can be tailored according to the patient's sensitization profiles. However, recombinant allergens that equal the natural allergens will exhibit allergenic activity and hence may induce anaphylactic side effects. It has therefore been suggested that the safety and immunological features of recombinant allergen-based vaccines be improved by the use of genetically engineered recombinant allergen derivatives (reviewed in Valenta *et al.*, 1999a, 1999b; Singh *et al.*,

1999; Valenta, 2002). A major aim of allergen engineering work has been reduction of the allergenic activity of recombinant allergen derivatives, but additional unexpected results have been obtained in the course of the experiments. They include changes of the immunological properties of the engineered allergen derivatives (e.g., alterations of immunogenicity, cytokine profiles) and the possibility of fusing unrelated allergens/epitopes to obtain combination vaccines for the treatment of complex sensitization profiles.

VIII. Genetic Engineering of Modified Allergens for Immunotherapy

A. REDUCTION OF THE ALLERGENIC ACTIVITY BY ALLERGEN ENGINEERING

For most of the common respiratory allergens, IgE recognition depends on the presence of conformational epitopes on intact and structurally folded allergens (reviewed in Valenta and Kraft, 2001). Using allergen-encoding cDNAs as templates, it has now become possible to engineer defined recombinant allergen derivatives that can be produced in a standardized manner. Table V displays a summary and brief characterization of hypoallergenic allergen derivatives obtained by genetic engineering for several important allergen sources.

A reduction of IgE reactivity and allergenic activity of allergens can be obtained by splitting them into defined recombinant fragments or by the deletion of IgE-reactive portions. The reduced allergenic activity of recombinant fragments of the major timothy grass pollen allergen, Phl p 1, was demonstrated by basophil histamine release studies 9 years ago (Ball *et al.*, 1994). A similar reduction of IgE reactivity was also noted for several allergens from pollen, mites, and molds when truncated proteins were produced that lacked IgE-reactive portions (Hayek *et al.*, 1998; Tamborini *et al.*, 1997; Tang *et al.*, 2000; Twardosz *et al.*, 1997; Vrtala *et al.*, 1997, 1999; Zeiler *et al.*, 1997). Advantages of the fragmentation approach are that a profound reduction of allergenic activity can be obtained for most allergens by this technology and that different fragments from one molecule can be combined so that no primary sequence information, and hence T cell epitope, is lost. Furthermore, it is possible to reassemble fragments derived from a given molecule in a different order in the form of a new mosaic protein that preserves the repertoire of T cell epitopes of the wild-type allergen (Mothes and Valenta, unpublished data).

The second type of modifications is based on the observation that natural isoforms of allergens exist in allergen sources that exhibit a strongly reduced IgE reactivity (Breiteneder *et al.*, 1993; Ferreira *et al.*, 1996). Such isoforms with reduced IgE reactivity were found to differ from the highly IgE-reactive versions in only a few amino acids. The reduction of IgE reactivity of such

TABLE V
MODIFIED RECOMBINANT ALLERGENS FOR IMMUNOTHERAPY

Allergen source	Allergen	Type of modifier	IgE reactivity	T cell reactivity	Basophil activation	Cytokine profile	Animal model	References
Pollen								
Trees								
Birch	Bet v 1	Oligomerization	+	+	—	Th1	+	Vrtala <i>et al.</i>, 2001
		Fragmentation	—	+	—	Th1	+	Vrtala <i>et al.</i>, 1997, 2000
		Mutation	—	+	n.d. ^a	n.d.	n.d.	Ferreira <i>et al.</i>, 1998
		Isoform	—	+	n.d.	n.d.	n.d.	Ferreira <i>et al.</i>, 1996
	Bet v 4	Deletion	—	n.d.	n.d.	n.d.	n.d.	Twardosz <i>et al.</i>, 1997
		Mutation	—	n.d.	n.d.	n.d.	n.d.	Engel <i>et al.</i>, 1997
Hazel	Cor a 1	Isoform	—	n.d.	n.d.	n.d.	n.d.	Breiteneder <i>et al.</i>, 1993
Alder	Aln g 4	Fragmentation	—	n.d.	n.d.	n.d.	n.d.	Hayek <i>et al.</i>, 1998
Grasses								
Timothy grass	Phl p 1	Fragmentation	+/-	n.d.	n.d.	n.d.	n.d.	Ball <i>et al.</i>, 1999a
	Phl p 2	Mosaic	—	n.d.	—	n.d.	+	Mothes <i>et al.</i> (unpublished data)
	Phl p 5b	Deletion	—	+	—	n.d.	n.d.	Schramm <i>et al.</i>, 1999
	Phl p 6	Deletion	—	n.d.	—	n.d.	n.d.	Vrtala <i>et al.</i>, 1999
	Phl p 1+Phl p 5	Hybrid molecule	+	+	+	n.d.	+	Linhart <i>et al.</i>, 2002
	Phl p 2+Phl p 6	Hybrid molecule	+	+	+	n.d.	+	Linhart <i>et al.</i>, 2002
	Phl p 1+Phl p 2+Phl p 5+Phl p 6	Hybrid molecule	+	+	+	n.d.	+	Linhart <i>et al.</i>, submitted

	Ryegrass	Lol p 1	Deletion	—	n.d.	—	n.d.	n.d.	Tamborini et al., 1997
		Lol p 5	Mutation	—	+	—	n.d.	n.d.	Swoboda et al., 2002
	Weeds								
	Pellitory	Par j 1	Mutation	—	+	n.d.	n.d.	n.d.	Bonura et al., 2001
	Oil seed rape	Bra r 1	Mutation	—	n.d.	n.d.	n.d.	+	Okada et al., 1998
	Mites								
	House dust mites	Der p 2	Mutation	—	n.d.	n.d.	n.d.	n.d.	Smith and Chapman, 1996
		Der f 2	Mutation	—	+	—	Th1	+	Korematsu et al., 2000 ; Noguchi et al., 1996 ; Takai et al., 1997
			Deletion	—	n.d.	n.d.	n.d.	n.d.	Takai et al., 1999
	Storage mites	Lep d 2	Mutation	—	+	n.d.	n.d.	n.d.	Olsson et al., 1998
	Mould								
	<i>Aspergillus fumigatus</i>	Asp f 2	Deletion	—	n.d.	n.d.	n.d.	n.d.	Tang et al., 2000
	Animals								
	Cow	Bos d 2	Fragmentation	—	+	n.d.	n.d.	n.d.	Zeiler et al., 1997
			Mutation	—	+	n.d.	n.d.	n.d.	Kauppinen et al., 1999
	Venoms								
	Yellow jacket/paper wasp	Ves v 5+Pol a 5	Hybrid molecule	—	+	—	n.d.	+	King et al., 2001
	Food								
	Peanut	Ara h 1	Mutation	—	+	n.d.	n.d.	+	Bannon et al., 2001
		Ara h 2	Mutation	—	+	n.d.	n.d.	+	Bannon et al., 2001
		Ara h 3	Mutation	—	+	n.d.	n.d.	+	Bannon et al., 2001 ; Rabjohn et al., 2002

^an.d., not done.

isoforms may be explained either by effects on the general fold of the molecule or by their direct involvement in IgE recognition.

Based on rational considerations mutations can be introduced by site-directed mutagenesis that may profoundly alter the IgE reactivity of the mutated protein. For example, it has been shown that the mutation of cysteine residues in the group 2 mite allergens leads to a disruption of disulfide bonds and thus causes reduced IgE reactivity (Olsson *et al.*, 1998; Smith and Chapman, 1996; Takai *et al.*, 1997). Likewise, it was shown that mutation of the acidic amino acids in the calcium-binding domains of the birch pollen allergen, Bet v 4, reduced its IgE-binding capacity (Engel *et al.*, 1997). A rather unexpected finding was that oligomerization (i.e., formation of recombinant dimers, trimers) of the major birch pollen allergen, Bet v 1, led to a strong reduction of the allergenic activity of this molecule, but preserved its immunogenic activity and even part of its IgE reactivity (Vrtala *et al.*, 2001).

Table V summarizes recombinant allergen derivatives with altered allergenic and/or immunological properties. A major advantage of recombinant allergen derivatives is that they can be engineered in a manner to preserve most of the allergens and T cell epitopes and to retain the immunogenicity of the wild-type allergen that is required for the induction of protective antibody responses. As indicated in Table V, T cell reactivity, reduced IgE reactivity and allergenic activity, cytokine profile, and immunogenic activity (animal models) need to be evaluated before the application of genetically modified allergens in patients should be considered. The molecular engineering of allergens has, however, not only provided us with derivatives with reduced allergenic activity but also demonstrated that changes in a particular allergen can profoundly alter its immunological properties.

B. GENETIC ENGINEERING OF ALLERGENS CAN ALTER THEIR IMMUNOLOGICAL PROPERTIES

To illustrate unexpected changes of immunological properties of genetically engineered allergens, we will briefly discuss a few concrete examples. A recently described recombinant trimer consisting of three covalently linked copies of the major birch pollen allergen, Bet v 1, breaks the rule that IgE reactivity must be completely abrogated if a strong reduction of allergenic activity needs to be achieved (Vrtala *et al.*, 2001). The described recombinant Bet v 1 trimer exhibited a structural fold similar to the allergenic Bet v 1 monomer and maintained its IgE reactivity. Despite these characteristics, the trimer had strongly reduced activity in inducing basophil degranulation and skin reactions in Bet v 1-allergic patients. The mechanism for the reduced allergenic activity remains unknown, but the study points to the possibility that either partial hiding of IgE epitopes or perhaps their reorientation has reduced

the allergenic activity of the engineered molecule. Another striking feature of the hypoallergenic trimeric form of Bet v 1 was its altered capacity to induce lymphoproliferative and cytokine responses. T cells from Bet v 1-allergic patients showed increased lymphoproliferative responses to the trimer, and their cytokine release profile was strongly diverted toward a Th1 profile (Vrtala *et al.*, 2001).

That the engineering of allergens may also have profound effects on their ability to induce cytokine responses has been also demonstrated by the finding that a recombinant Bet v 1 fragment and mutated versions of the major house dust mite allergen, Der f 2, more strongly induced the release of Th1 cytokines from patient's peripheral blood mononuclear cells (PBMC) than the allergenic wild-type allergens (Korematsu *et al.*, 2000; Vrtala *et al.*, 2000). Whether the altered immunological properties of the engineered allergen versions may be due to a different presentation of folded versus unfolded proteins depends on the type of APC, the MHC molecule repertoire, or is due to differences between presentation with or without antibodies remains to be answered. Although there are by now only a few examples demonstrating profound alterations of the immunological properties of engineered allergen variants, these findings lend support to the idea that molecular engineering of a given allergen can strongly alter its immunological characteristics without requiring the addition of adjuvants.

C. HYBRID ALLERGENS WITH INCREASED IMMUNOGENICITY FOR THE TREATMENT OF COMPLEX SENSITIZATION PROFILES

Another unexpected example of a profound alteration of the immunological features of genetically engineered allergens was discovered when hybrid allergens were constructed out of two or more immunologically unrelated allergens. The latter approach was chosen to investigate whether it is possible to engineer hybrid molecules that can cover the allergen/epitope repertoire of complex allergen sources (Linhart *et al.*, 2002, submitted). For this purpose, unrelated grass pollen allergens were fused by polymerase chain reaction (PCR) based on their corresponding cDNAs, and the resulting hybrid allergens were then characterized. The unexpected finding in this study was that the fusion of weakly immunogenic allergens yielded hybrid molecules with strongly enhanced immunogenicity (Linhart *et al.*, 2002). The grass pollen hybrid allergens induced stronger IgG antibody responses in animals and lymphoproliferative responses in PBMC cultures than equimolar mixtures of the individual allergens. This observation may have important implications for vaccine development in general, as it suggests that it is possible to increase the immunogenicity of weakly immunogenic antigens by using another unrelated antigen as a kind of scaffold. It will thus be possible to engineer improved

allergy vaccines, especially for complex allergen sources where certain allergens are present in low immunogenic form.

IX. Clinical Results with Modified Recombinant Allergens

The immunological features of genetically engineered recombinant allergen derivatives can be evaluated using different types of *in vitro* assays. These assays include the measurement of IgE and antibody reactivity, the induction of lymphoproliferative and cytokine responses, and especially the induction of basophil degranulation as a very sensitive marker for the allergenic activity of the molecule. Small allergen fragments and, in particular, allergen-derived peptides may either preferentially target allergen-specific T cells or induce antibody responses against new epitopes and thus favor only certain immunological mechanisms, as described in the three possible models explaining the effects of immunotherapy. A major advantage of genetically modified allergens containing most of the primary sequence information of the originating wild-type allergens is that they will comprise most of the T cell epitopes of the wild-type allergen, exhibit reduced anaphylactic activity (i.e., reduced basophil degranulation), and preserve the immunogenicity/tolerogenicity of the wild-type allergens in experimental animal models. These molecules may therefore be used simultaneously for different therapy strategies (e.g., modulation of T cells, induction of protective antibody responses) (Valenta, 2002).

However, before genetically engineered allergen derivatives are used in immunotherapy trials, a clinical evaluation regarding their *in vivo* allergenic activity must be performed by provocation testing. Table VI shows that many recombinant allergen derivatives have already been compared regarding their allergenic activity with the corresponding wild-type allergens in patients. The induction of immediate inflammatory reactions can be precisely determined and quantified by skin testing and by nasal and conjunctival provocation testing. Since skin testing allows us to perform simultaneous end point titrations for several antigens and dilutions, it has been the preferred method used for the evaluation of genetically modified allergens. A strong reduction of allergenic activity was found for genetically modified versions of the major birch pollen allergen, Bet v 1, in representative numbers of patients (Arquint *et al.*, 1999; Pauli *et al.*, 2000; van Hage-Hamsten *et al.*, 1999). Similar data were obtained for genetically modified mite, weed, and cow dander allergens (Bonura *et al.*, 2001; Kauppinen *et al.*, 1999; Kronqvist *et al.*, 2001; Kusunoki *et al.*, 2000; Ruoppi *et al.*, 2001). For other genetically modified allergens, only a few patients have been tested so far.

The results obtained by nasal and conjunctival provocation obtained for genetically engineered versions of the major birch pollen allergen, Bet v 1, were similar to those obtained by skin testing and hence suggest that skin

TABLE VI
CLINICAL EVALUATION OF MODIFIED RECOMBINANT ALLERGENS

Type of modification	Allergen	Evaluation ^a	Number of patients	References
Fragments	Bet v 1	SPT, IDT	23	van Hage-Hamsten <i>et al.</i> , 1999
		SPT, IDT	36	Pauli <i>et al.</i> , 2000
		SC	9	Nopp <i>et al.</i> , 2000
		NPT	10	van Hage-Hamsten <i>et al.</i> , 2002
Isoforms	Bos d 2	NPT	10	Ruoppi <i>et al.</i> , 2001
	Bet v 1	SPT, IDT, CPT	48	Arquint <i>et al.</i> , 1999
	Phl p 5	SPT	4	Gehlhar <i>et al.</i> , 1997
Mutants	Bet v 1	SPT	11	Ferreira <i>et al.</i> , 1998
	Lol p 5	SPT	2	Swoboda <i>et al.</i> , 2002
	Phl p 5	SPT	5	Schramm <i>et al.</i> , 1999
	Par j 1	SPT	10	Bonura <i>et al.</i> , 2001
	Der f 2	SPT	3	Takai <i>et al.</i> , 1997
	Der f 2	SPT	1	Takai <i>et al.</i> , 1999
	Der f 2	SPT	20	Kusunoki <i>et al.</i> , 2000
	Der p 2	SPT, IDT	4	Smith and Chapman, 1996
	Lep d 2	SPT	1	Olsson <i>et al.</i> , 1998
		SPT	17	Kronqvist <i>et al.</i> , 2001
Oligomers	Bos d 2	SPT	9	Kauppinen <i>et al.</i> , 1999
	Bet v 1	SPT, IDT	23	van Hage-Hamsten <i>et al.</i> , 1999
		SPT, IDT	36	Pauli <i>et al.</i> , 2000
		SC	9	Nopp <i>et al.</i> , 2000
		NPT	10	van Hage-Hamsten <i>et al.</i> , 2002

^aCPT, conjunctival provocation test; IDT, intradermal test; NPT, nasal provocation test; SC, skin chamber; SPT, skin prick test.

testing is an appropriate method for the evaluation of their allergenic activity (Arquint *et al.*, 1999; van Hage-Hamsten *et al.*, 2002). The fact that genetically modified Bet v 1 derivatives could be safely administered to the nasal mucosa suggests that these derivatives are also suitable for therapeutic nasal application (van Hage-Hamsten *et al.*, 2002).

Using an *in vivo* skin chamber model that allows us to investigate allergen-mediated late effects on cell migration, cell activation, cytokine, and mediator release, it could be demonstrated that genetically modified Bet v 1 derivatives not only exhibited reduced allergenic activity, but also caused reduced eosinophil activation and release of proinflammatory cytokines (Nopp *et al.*, 2000).

Most of the genetically engineered allergen derivatives described in Tables V and VI contain many of the T cell epitopes of the corresponding wild-type allergens, and several of them were found to induce protective antibody responses in experimental animal models. They should therefore be useful for conventional injection immunotherapy in an adjuvant-bound form or,

alternatively, may be used for T cell immunomodulation strategies (e.g., tolerance induction) by mucosal administration. As yet one double-blind, placebo-controlled multicenter immunotherapy trial has been carried out with recombinant Bet v 1 fragments and the Bet v 1 trimer (Vrtala *et al.*, 1997, 2001) in more than 100 patients so that data about clinical efficacy, safety, and immunological mechanisms will be available in the near future.

X. Formulating Prophylactic Allergy Vaccines

The fact that prophylactic vaccination against a great variety of infectious diseases is performed routinely and safely very early in childhood raises the question of whether specific prophylactic vaccinations against the most common allergies might become possible in the future. The advantage of early prophylactic treatment would be that the pathological allergic immune response has not yet started or has not led to severe clinical disease. It may thus be easier to prevent or counteract the development of the allergic response early in childhood. To achieve this goal, several prerequisites need to be fulfilled and a number of critical issues must be addressed. The requirement for defined allergens is nearly fulfilled due to molecular allergen characterization work. Today most of the common allergens covering most of the relevant allergen sources have been reconstructed by recombinant DNA technology. However, these recombinant allergens are almost equivalent to the natural allergens and, hence, if given for prophylactic vaccination, may also induce IgE responses. Therefore, either alternative routes of administration or adjuvants preventing the induction of allergen-specific immune responses need to be used if prophylactic vaccination with wild-type recombinant allergens is considered. The danger of inducing unwanted allergic sensitization may be overcome by the use of genetically modified allergen derivatives that differ substantially from the naturally occurring allergens. However, regardless of the type of allergen/allergen modification, route of administration, or adjuvant used, it must be demonstrated in animal and later in clinical studies that prophylactic vaccination does not induce allergic sensitization and that it is capable of preventing allergic sensitization. The genetic blueprints of the most common environmental allergens are now available to approach prophylactic vaccination strategies for allergic diseases. With such an approach, it may be possible to prevent the development of allergic diseases.

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