

APPLICATION OF MOLECULAR BIOLOGY FOR DIAGNOSIS AND TREATMENT OF ALLERGIC DISEASES

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Allergic diseases comprise a complex array of disorders, including allergic rhinitis and asthma, and are characterized by persistently elevated IgE responses and increased airway inflammation. Only genetically predisposed individuals develop allergies as a result of exposure to diverse aeroallergens.^{44, 93} Initial sensitization involves recognition of allergens by the T cells and synthesis of IgE antibodies to specific allergens such as pollens, molds, or mites. This constitutes one of the hallmarks of human allergic diseases.^{6, 72} Antigen-presenting cells (APCs), such as dendritic cells or B cells, ingest and break down the incoming allergen to its constituent peptidic fragments and then present these fragments to the T cells. The latter recognize the peptide via its receptor (TCR), which interacts with the peptide-major histocompatibility (MHC) II complex (binary complex) on the APC. The formation of this trimolecular interaction is essential and provides the foundation for a specific immune response (Fig. 1).¹⁵ Further, interaction of the B7-family of molecules on APC and CD28 on T cells generates a second antigen-non specific signal, which is required for the full activation of the T cells and leads to the production of effector cytokines.³⁵ Cytokines,

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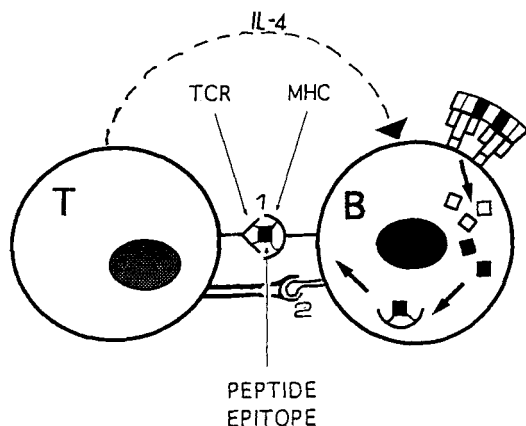


Figure 1. Requirement for two signals for the induction of an immune response. The first signal (1) involves TCR ligation with MHC-peptide complex and the second signal (2) involves interaction between CD28 with B7. TCR ligation in the absence of a second signal may lead to anergization of the T cell in question. T-cell activation leads to IL-4 production, which in turn induces B cells to produce IgE.

such as IL-4 and IL-13, are known to be involved in the class switch of the antibodies and production of IgE antibodies.⁷²

The IgE antibodies bind to the Fc receptors on the mast cells and basophils. Subsequent exposure of these cells to multivalent allergens results in bridging of the cell-bound IgE antibodies and leads to release of the inflammatory mediators, including histamines.⁶ In allergic asthma, airway hyperresponsiveness may contribute to the exacerbation of asthma as a result of exposure to aeroallergens. Although the IgE antibodies are produced by most individuals, it is the sustained production of allergen-specific IgE antibodies above a certain threshold level in conjunction with other inflammatory factors that are responsible for the clinical symptoms.

Currently, pharmacotherapy comprises a major form of therapy against chronic allergies and asthma. This form of therapy leads to the suppression of secretion of cytokines from T cells and release of mediators from mast cells and basophils. Such therapy, however, is palliative, directed toward the final effector phase of the disease. Thus, it does not address the immunologic origin of the disease and has a number of adverse side effects. Additionally, allergen immunotherapy, which comprises repeated injections of the appropriate allergen(s) over prolonged periods, has been practiced since its introduction in early 1900 as the one and only immunologically specific therapy against allergic diseases such as rhinitis and asthma.⁶ Although allergen immunotherapy is known to be effective,⁸ as it is currently practiced has a number of disadvantages. A major limitation of presently practiced diagnosis and allergen immunotherapy is the utilization of crude allergen extracts. Such

Table 1. DISADVANTAGES OF EXTRACTS USED IN CONVENTIONAL DIAGNOSIS AND IMMUNOTHERAPY

I. AL Source
Seasonal dependence
Geographic variation
Method of collection
Lack of purity (admixtures)
II. Quality of Extract
Extraction procedures vary with the allergens
Storage conditions
Lack of standardization
III. Allergen Content
Multiplicity of components
Heterogeneity of components
Minute quantity of major allergens
IV. General
Batch-to-batch variation
Lack of reagents for standardization
Time-consuming and labor intensive

allergen preparations exhibit batch to batch variations (often up to a hundred-fold) in both quality and allergen content (Table 1). A few standardized allergens are available, but these are not devoid of the limitations of crude extracts. In addition, the other drawbacks that compromise the efficacy and use of allergen immunotherapy are mode of administration (both frequency and route); duration of the treatment, which may last as long as 1 to 3 years; the dosage of allergens administered; and the possibility of systemic reaction.^{13, 67}

To circumvent these limitations, several laboratories recently have used rDNA procedures for the molecular characterization of major aero-allergens.^{47, 49, 50} Indeed, the last 5 years have seen spectacular advances in the field of allergen characterization. The advent of the recombinant allergen, in particular, has played a significant role in furthering our knowledge of the structure-function relationships of allergens and our understanding of the allergen-induced IgE-mediated allergies. In this report, we briefly review the recent advances in the field of molecular biology and immunology of allergens and how this knowledge has led to several potential new approaches to the diagnosis and therapy of allergic diseases.

APPLICATION OF rDNA TECHNOLOGIES TO CHARACTERIZATION OF ALLERGENS

A diverse arsenal of rDNA and allied chemical synthesis technologies have been utilized for the molecular-immunologic characterization of several allergens. These are shown schematically in Figure 2. With the advances in the field of molecular biology in general, new techniques are used for the isolation of clones of allergens, characterization of

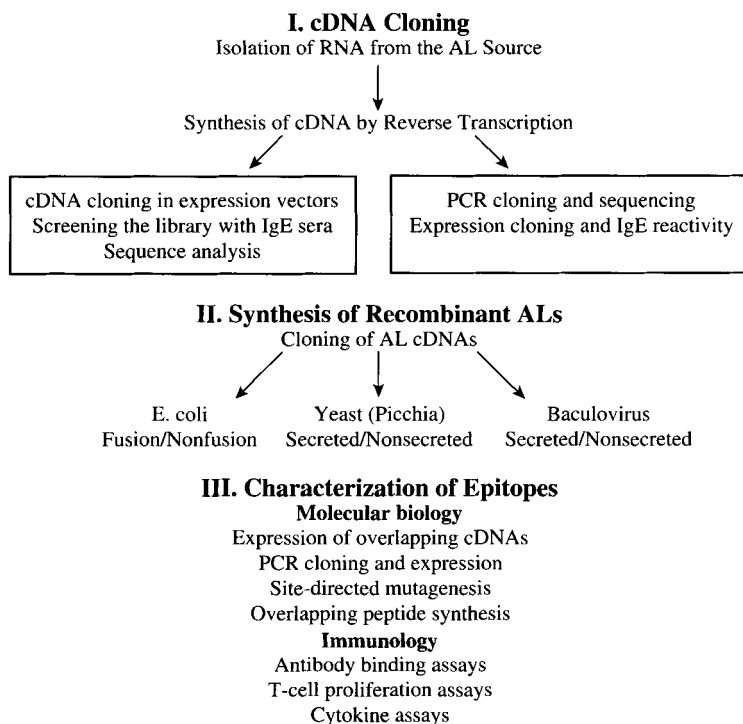


Figure 2. Steps involved in molecular cloning and characterization of allergens.

isoallergens, and identification of epitopes.⁵⁴ Recent progress in different areas is summarized in the following sections.

Molecular Cloning and Sequencing of Allergens

Molecular cloning of allergens has led to the determination of the primary structure of numerous allergens. To date, a majority of the allergens have been cloned in the phage expression vector λ gt 11. More recently, cloning and sequencing based on polymerase chain reaction (PCR) has been reported.³⁸ A list of allergens that have been molecularly cloned and sequenced using this strategy has been published recently.^{47, 49, 50} Thus, about 70 of the major allergens have been cloned from diverse sources such as mites, animals, molds, pollens (grasses, weeds and trees), insects, and foods. This number has tripled since 1992, and five to ten new allergenic proteins are being added to this list every year. In addition to the cloned allergens, new allergens continue to be reported; presumably the cloning of these allergens is currently in progress.

Synthesis of Recombinant Allergens

A major application of molecular biology technologies for allergen characterization has been the expression cloning of allergens which enable the production of the recombinant proteins in virtually unlimited amounts for use in basic as well as clinical studies. To date, a majority of these allergens have been cloned in plasmid vectors for high-level expression in *Escherichia coli*, which allow synthesis of the allergenic protein either (1) as a fusion protein, that is, linked to β -galactosidase^{64, 92} or glutathione S-transferase,⁶² or (2) as a recombinant allergen by itself, using vectors such as the pKK233 series of expression plasmids.¹¹ Recently, both yeast¹⁷ and baculovirus⁷⁹ expression systems have been used for the synthesis of certain recombinant allergens.

One of the concerns with the synthesis of these proteins is whether the recombinant allergen in question is functionally comparable to its native counterpart from the corresponding source. Recent studies in several laboratories have shown that the baculovirus expression system is more efficient not only in terms of the amount of recombinant allergen produced but also with respect to the IgE binding of these recombinant allergens. The IgE binding presumably is caused by glycosylation of the recombinant proteins, which allows appropriate folding similar to that of the native protein. A schematic diagram of the baculovirus expression of antigens is shown in Figure 3. Given that several allergens are glycoprotein, this system may be the optimal choice for production of recombinant allergens for therapeutic purposes.

Delineation of B- and T-Cell Epitopes

Knowledge of the B- and T-cell epitopes is mandatory for the formulation of any therapeutic modality(ies) based on epitopes. Therefore, determination of the complete repertoire of epitopes of diverse environmental aeroallergens remains one of the most arduous and formidable tasks. The availability of structural information has prompted studies on delineation of B-cell* and T-cell† epitopes of a number of major allergens from mites, cat, and pollens of trees, ragweed and grasses, and so on. These studies used both human and model animal systems. Rapid characterization of epitopes has been possible through the techniques that allowed production of overlapping peptides, including expression cloning of overlapping cDNAs and the synthesis of the corresponding peptides in *E. coli*, and site-directed mutagenesis in conjunction with PCR, which allowed synthesis of the allergens and peptides with desired amino acid substitutions. Additionally, overlapping peptides can be synthesized chemically by automated methods or

*References 1, 2, 19, 27, 65, 71, 77, 82, 97, 98.

†References 5, 14, 16, 20–22, 24, 29, 36, 61, 70, 76, 83, 91, 94.

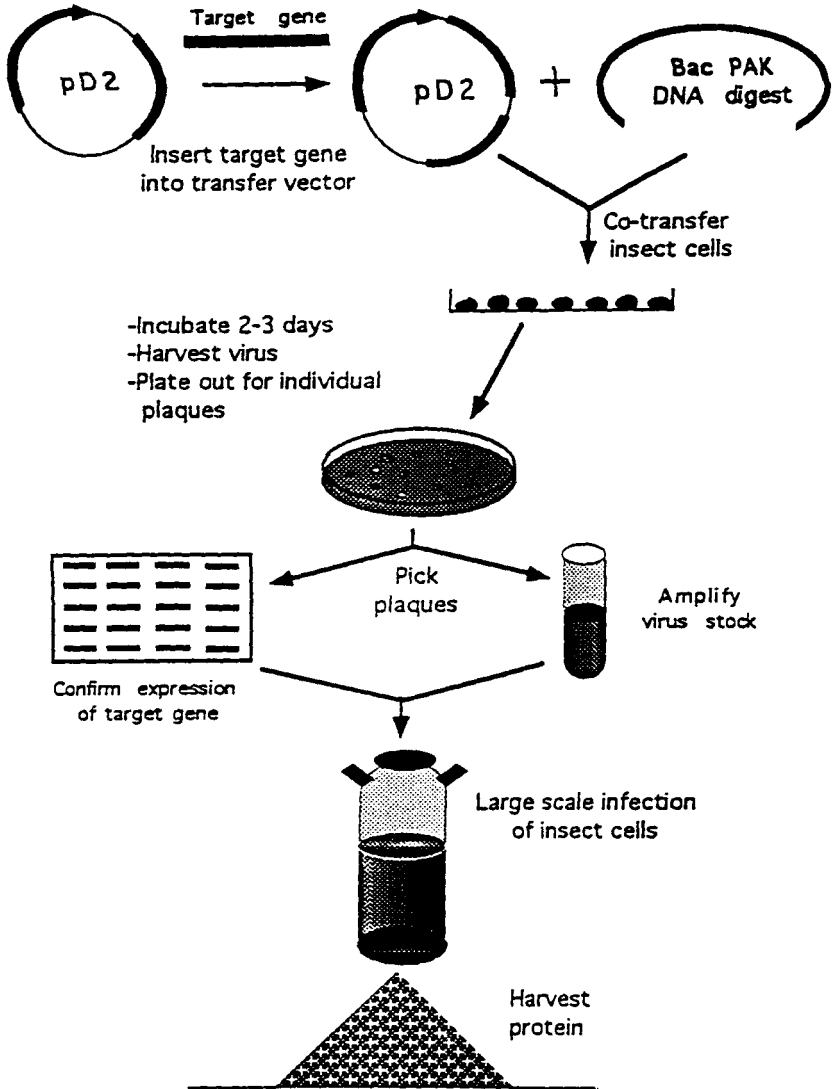


Figure 3. High-level production of recombinant allergens using baculovirus expression system.

by manual methods such as through the use of polypropylene pins. These methods have aided in the identification of the B- and T-cell epitopes of a number of allergens (Fig. 2). Also, availability of allergens and peptides facilitated the analysis of B-cell epitopes including the

human IgE binding epitopes. The T-cell epitopes of many allergens also were examined by T-cell proliferation assay using peripheral blood mononuclear cells (PBMC) or T-cell lines and clones. Studies to date suggest that (1) the major allergens have a number of epitopes, (2) the B- and T-cell epitopes often appear to overlap, and (3) the epitopes recognized by individuals differ.

Furthermore, knowledge of the primary structures of allergens as well as of B- and T-cell epitopes has led to the elucidation of cross-reactivities among different allergens. Cross-reactivities have been studied both at the B- and T-cell level not only among allergens belonging to the same aeroallergen source, but also among different aeroallergens.^{46, 47, 50, 75, 96} In principle, the same methods that have been discussed previously for the identification of B- and T-cell reactivities have been used for the delineation of cross-reactivities among allergens and epitopes. Conservation of the immunodominant residues among various allergens and their epitopes appears to be a principal reason for the extensive cross-reactivities observed among allergens.

APPLICATION OF MOLECULAR BIOLOGY TO THE DIAGNOSIS AND THERAPY OF ALLERGIC DISEASE

As stated previously, all of the presently available therapies have the common disadvantage of using ill-defined allergenic extracts, although some purified allergens were shown to be effective. Because the recombinant allergens have only been synthesized relatively recently, however, they have yet to be used for clinical studies. Obviously, these products have to undergo rigorous testing by the regulatory agencies prior to their approval for such studies.

Allergen Standardization

A major drawback of conventional diagnosis and immunotherapy is the lack of standardized allergens. To date, only a few allergen mixtures are available in standardized form. Although efforts are underway to obtain standardized extracts, including grass pollen extracts, the process is labor-intensive and time-consuming. For some other allergens, monoclonal antibody-based standardization is not as yet possible because of the paucity of such antibodies. Availability of recombinant allergens is considered a major breakthrough, as the use of the recombinant allergens per se for the purpose of diagnosis and immunotherapy should obviate the requirement for conventional standardization: Effective dosages have to be determined based on the protein concentration itself.⁵¹ Furthermore, where an allergen extract is poorly characterized and contains many constituents, one or more recombinant allergens can

facilitate the standardization of the extract by the production of the monoclonal antibodies to the cloned allergens, and by allowing the comparison of the stability of the allergens in the extracts with the reference recombinant proteins.

Diagnosis of Allergic Disease by Use of Recombinant Allergens

Accurate diagnosis of allergy by commonly used skin prick or intradermal testing in conjunction with *in vitro* tests such as radioallergen sorbent test (RAST) is dependent upon the quality of the extracts prepared from the natural allergens, which in turn are affected by several often uncontrollable factors (Table 1). As stated previously, only a limited number of standardized allergen extracts are currently available, and even the standardized extracts are not devoid of the disadvantages associated with the natural extracts (Table 1). Despite these limitations, diagnosis of allergies to many aeroallergens with the use of the currently available extracts is efficient (>80%).²⁸ Nonetheless, diagnosis of allergies to foods and a few indoor (e.g., molds) and outdoor (e.g., pollens of cypress) allergens is far from being satisfactory.^{7, 9}

The major limitations of the natural allergen extracts are (1) presence of certain major allergens in very low concentrations, (2) poor extractability of some allergens, and (3) rapid denaturation of the allergens during or after their extraction due to the presence of proteases.^{7, 9, 28, 33} Clearly, use of recombinant allergens for diagnosis can eliminate these disadvantages inherent with the crude allergen extracts.^{63, 81} Furthermore, recombinant allergens allow diagnosis of a patient's sensitivity to specific allergens and, therefore, may aid in the tailored allergen immunotherapy. Prior to their use in diagnosis, however, recombinant allergens have to be subject to the following: First, the recombinant allergens should be tested thoroughly for their IgE binding properties using a large panel of sera (at least 50 samples). Second, adequacy of the required components for diagnosis has to be addressed. Often, more than one recombinant allergen may be required to diagnose allergy to major aeroallergens. For instance, a combination of Group I and Group 5 recombinant allergens is required to diagnose grass pollen allergy.^{63, 81} Last but not least, a detailed characterization of the biochemical and immunochemical properties of the recombinant allergens, as summarized in Table 2, is necessary prior to their use in diagnosis.

Considering that the presently available diagnostic technologies are efficient and often satisfactory, general use of recombinant allergens for diagnosis may not be favored because of their cost. It is, however, possible that, for certain allergens for which the conventional diagnosis is not satisfactory, recombinant allergens may be useful. Furthermore, it is likely that new molecular technologies may be available in the future that will enable cost-effective and highly sensitive diagnosis of allergies using recombinant allergens.

Table 2. CHARACTERIZATION OF RECOMBINANT ALLERGENS PRIOR TO THEIR USE IN DIAGNOSIS AND THERAPY

I. Biochemical	
	Purity (recommended >95%)
	Lack of toxicity in animal models
	Stability under appropriate storage conditions
	Lack of biologic activity when possible
II. Immunochemical	
	IgE-binding properties satisfying at least two of three following):
	ELISA (human) and PCA (rodents) assays
	ELISA and/or PCA inhibition using native allergens as the competitor
	Histamine release
	T-cell stimulation (satisfying at least two of three following):
	PBMC proliferation with a stimulation index of 3 or more
	Proliferation of T-cell lines
	Cytokine assays (IL-2, IL-4, IFN- γ)
III. In vivo tests (only after approval of regulatory agencies)	
	Skin-test reactivity with recombinant allergens
	Challenge tests

Allergen-Specific Immunotherapy

A large body of evidence in both human and animal systems suggests a preponderance of type 2 helper T-cell (Th2) responses in allergic disorders.^{55, 70} Th2 cells produce cytokines such as IL-4, IL-5, IL-10 that support elevated IgE synthesis, as opposed to the "protective" Th1 type of cells, which produce IL-2 and IFN- γ . The ratio of the major IgE-switch factor IL-4 and its regulator IFN- γ determines the level of IgE synthesis; these two cytokines also mediate the differentiation of Th2 and Th1 subsets, respectively.^{26, 37} Although research endeavors continue in several fronts directed at suppressing allergic inflammation, immunomodulation of T-cell responses has become a major target. In the context of modulating allergen-specific immune responses, the strategies of immune intervention of allergic diseases may be categorized pragmatically as those aimed at altering the functional specialization of allergen-specific T cells and those directed at vaccinations or immunotherapy with appropriate allergens, each of which has its constraints and advantages (Fig. 4). It is to be noted that despite this classification, these intervention strategies may work sequentially, that is, vaccination and immunotherapy with allergens may lead to functional specialization and therapeutic efficacy.

Application of molecular biology has aided in characterization of a plethora of cytokines that are involved in pathogenesis of allergies. This has led to a number of therapeutic approaches, including an engineered mutant IL-4 protein that may block IL-4 responses. The cytokine-based approach(es), however, may be inadequate, as (1) therapeutic effects of cytokines are not specific and not always predictable and (2) most cytokines, including IL-12, which is one of the most promising of all, may have unwanted side effects.⁴⁸ Specifically in the context of allergy,

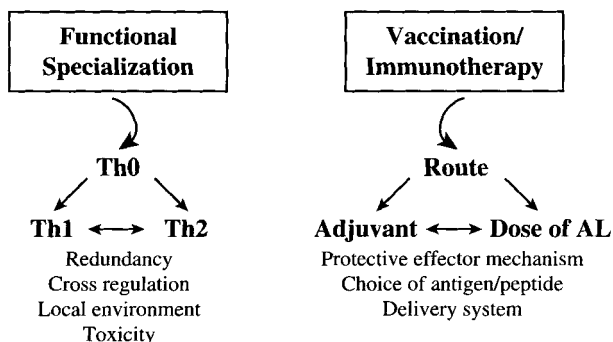


Figure 4. An outline of strategies for immune intervention of allergic diseases. Functional specialization involves modulating T-cell differentiation profiles from pathogenic “Th2-like” responses to protective “Th1-like” responses. Vaccination/immunotherapy involves development of a strategy with optimized conditions comprising an appropriate dose of allergen peptide, route, and adjuvant, if any. Functional specialization may serve as an approach to vaccination and vice versa. Factors that influence the optimization of each specific strategy are listed.

cytokine therapies are impractical because (1) IL-4 is not only released by T cells but also by mast cells and basophil-like cells and CD8+ or CD4- CD8- T cells^{55, 73}; (2) it functions locally; and (3) its activity is affected by the presence in the local milieu of soluble receptors and other cytokines such as IL-10, IL-12 and IL-13, which are also involved in Th1-Th2 regulation.^{26, 37, 55, 72} Nonetheless, although cytokines by themselves may not be adequate adjuvants, in conjunction with allergens, they may serve as effective therapeutic agents.¹

On the basis of our current knowledge of allergy and asthma, the development of allergen-specific therapies and vaccines remains an important choice. Recent studies have revealed that (1) development of sensitization to allergens occurs within the first 2 years of life,⁵ (2) a significant proportion of individuals (about 20%–30%) remain sensitized to a few major offending aeroallergens,²³ and (3) specific immunotherapy with one aeroallergen prevented general sensitization to other allergens.¹⁸ These findings support the notion of specific immunotherapy and prophylaxis as logical approaches to the prevention and/or management of allergies and asthma. Three potential approaches that use recombinant allergen(s) as a therapeutic modality are described in the following sections.

Allergen Immunotherapy Using Recombinant Allergen(s)

The advent of recombinant allergens makes it possible to identify the unique protein(s) to which the individual is sensitized; hence, it may be visualized that future allergen immunotherapies will be based on the use of selective allergens. The other advantages of recombinant allergens

instead of crude extracts for allergen immunotherapy include the use of tailored extracts based on the major allergens to which the patient is sensitized, administration of allergens in adequate amounts, better and more effective dosage control, and the possibility to modify IgE binding structures thereby increasing the safety of the immunotherapy. It is to be noted, however, that, in case of humans, because of the purity of recombinant allergen preparations, their anaphylactogenic index may be extremely high even at very low doses. As a result, extreme care may be required when these preparations are administered by allergy specialists. Moreover, enteric coated⁸⁹ or liposome-packed^{4, 57} recombinant allergens also may be useful for immunotherapy.

Further, allergoids produced by formaldehyde polymerization⁴² of recombinant allergens, the "r-allergoids," may be useful for immunotherapy. The problem with presently available polymerized extracts⁴² is that they are based on crude extracts. Because several allergens are present in varying concentrations in the extracts, it is difficult to ascertain whether all molecules have been polymerized to the same degree. Often batch-to-batch variation is excessive, and analysis of each individual allergen is impossible. It is envisaged that a major improvement in allergen-specific immunotherapy may be achieved by the use of chemically modified recombinant allergens, which should render these regimens essentially nonallergenic, while retaining the antigenicity. Also, conjugation with N-formyl-methionyl-leucyl-phenylalanine may be useful.⁹⁰ Therefore, it is reasonable to predict that, in contrast to conventional immunotherapy, the use of relatively fewer injections of these modified recombinant allergens would lead to safer and more efficacious therapies.

Novel Approaches to Immunotherapy

Immunotherapy with Recombinant Allergens and Cytokines as Adjuvants. Previously, with the use of a recombinant antigen-allergen chimeric protein, we demonstrated that it was possible to modulate, in an animal model, antigen and isotype-specific immune responses.⁹⁵ Recent demonstrations that (1) Th2 cells play a critical role in pathogenicity of allergic disorders and (2) IFN- γ and IL-4, respectively, promote polarization to Th1 and Th2 cell responses have led to the possibility of developing methods for shifting allergen-specific responses from Th2 type to Th1 type for suppression of allergic responses.^{25, 49} Conceivably, allergen-induced changes in cytokine profiles could be a potential strategy in the treatment of allergic diseases. One approach has emerged from our recent in vitro studies performed in collaboration with S. Romagnani's laboratory, using human peripheral blood lymphocytes. It was shown that the culture of T-cell lines from atopic individuals with a recombinant allergen of *Poa p* IX family of allergens and IFN α led to the development of "protective," that is, Th1 cells instead of the "pathogenic" Th2 cells.²⁵ Thus, it is visualized that administration of recombinant allergens in conjunction with IFNs may aid in diverting

allergen-specific immune responses from default Th2 to Th1 type. Indeed, recent use of IFN α for treatment of Th2-dominant diseases, such as allergies and atopic dermatitis, has supported our *in vitro* results.

Although we have studied IFN α ,²⁵ it clearly has been shown recently that IL-12 also has the potential to convert allergen-specific Th2 responses to Th1 responses.¹ Because IL-12 generates a strong Th1 response, it has been suggested as an adjuvant in designing vaccines against diseases with the preponderance of Th2 responses. Using a recombinant allergen (ovalbumin) and IL-12 (P40) fusion protein, Umetsu and associates reported (AAAAI, International Conference, 1996) down-regulation of OVA-specific IgE responses *in vivo*. IL-12, however, has a number of side effects. In addition, its effectiveness requires IFN- γ production by the cell in question and its capacity to convert established Th2 cells to Th1 cells has been contested.⁴⁸ Nonetheless, IL-12 as an adjuvant for the treatment of allergy appears to show great promise and requires further investigation.

The potential approaches involving such therapy with allergens and cytokines as adjuvants is shown schematically in Figure 5. It is envisaged that recombinant allergen(s), either singly or in cocktails, can be used as immunotherapeutic modalities against certain forms of specific allergies. For this purpose, recombinant allergen(s) *per se* or in combination with appropriate Th1-inducing adjuvants can be used. For tailored immunotherapy, individuals allergic to certain antigens would be treated with therapy comprising injection of the same allergens. In principle, the peripheral blood mononuclear cells of these patients may be cultured *in vitro* with the corresponding antigen and IFN α and may be injected back to the same individual to boost Th1-like responses to these antigens followed by a booster of allergen(s). Alternatively, immunotherapy may

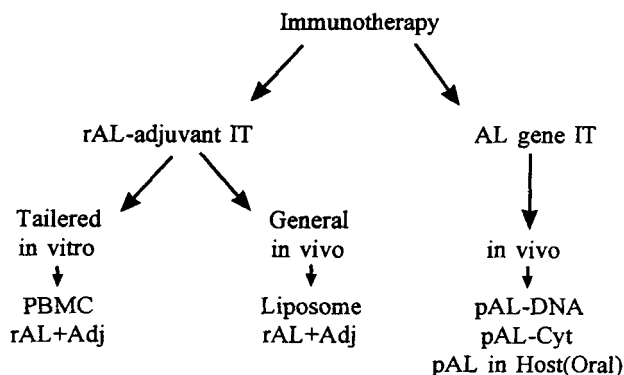


Figure 5. Novel approaches to allergen immunotherapy using recombinant allergens (rAL) and allergen genes (see text for details). PBMC-peripheral blood mononuclear cells; Adj = adjuvant; pAL = plasmid carrying allergen DNA; pAL-cyt = AL linked in-frame to a cytokine gene capable of inducing AL-specific Th1-like response.

be carried out with liposomes containing mixtures of recombinant allergen(s) and a selected adjuvant which could induce a persistent Th1-like response to specific allergens.

Allergen Gene Immunotherapy. Availability of cDNAs encoding allergens has prompted investigations to address the possibility of using the allergen genes and cDNAs for suppression of allergen-specific IgE responses. The development of live vaccines through the use of allergen genes expressed in appropriate hosts was suggested previously.⁴⁷ Two recent developments are noteworthy in this context. Raz and associates⁶⁸ reported that immunization with an allergen-cDNA cloned in a plasmid vehicle resulted in the development of allergen-specific IgG2a and Th1-like response and no detectable IgE response in mice. This was in marked contrast to immunization with the antigen in alum, which induced IgE Ab response.⁶⁸ Further, the Th1 response induced by gene immunization dominated over an ongoing protein-induced Th2 response in an antigen-specific manner. This suggested that immunization of DNA encoding for the allergen may provide a novel type of immunotherapy for allergic diseases. In another study, immunization (by oral route) of *Salmonella typhimurium* expressing allergen-cDNA also led to the development of IgG2a responses instead of default IgE Ab responses.⁸⁶ IgG2a responses specific for *Bet v* 1, however, could be detected in only 10% of the immunized mice. Although these are early studies, such approaches hold promises in terms of developing new approaches to immunotherapy.

It is noteworthy that with the advances in the field of cytokines and allergens, the scope of gene immunotherapy may be extended to covaccination with plasmids that enable expression of certain allergen(s) and cytokine(s) in vivo. Despite recent advances and the fact that these experimental approaches appear promising, further studies are necessary prior to pilot clinical studies.

Peptide-Based Therapies

Advances in our knowledge of the cellular and molecular factors that determine IgE-mediated allergies, in conjunction with the definition of increasing number of epitopes of allergens, provide a sound basis for the development of new approaches for therapy of allergies using peptide epitopes. Because T cells play a dominant role in IgE synthesis, a number of strategies have been proposed that are directed at anergizing these cells or at interfering with the formation of trimolecular complexes involving the interaction of the MHC-peptide duplex with the appropriate Th cell receptor.^{49, 87} Whether treatment with peptides would be a feasible alternative to existing immunotherapy remains to be elucidated. A brief overview and a critical appraisal of the potential for treatment of allergic disorders with peptides is provided in the following section.

Rationale for Design of Peptide Therapeutics. During the process of antigen presentation, Th cells are activated and secrete cytokines when costimulatory signals are provided by the APCs. A state of T-cell

nonresponsiveness, however, is achieved upon subsequent stimulation by antigens and APCs if costimulating signals are absent or supra-immunogenic doses of antigenic peptides are provided. Recently, it has been demonstrated that T-cell nonresponsiveness can be achieved in vitro with allergen-specific human T-cell clones, and that administration of peptides corresponding to T-cell epitopes leads to T-cell tolerance to the complete antigen in mice.^{12, 49, 87} These studies have led to the current view that the immunodominant T-cell epitopes of an allergen also may function as strong inducers of T-cell nonresponsiveness in humans.

Based on our knowledge of the three players involved in trimolecular interaction (i.e., MHC–allergen peptide–TCR, see Fig. 1), a number of peptide based immune-intervention strategies can be derived:

1. *Direct intervention at the level of peptide/MHC interaction:* MHC antagonist peptides/analogues are used to simply block antigenic peptides from binding MHC and stimulating T cells. Although HLA association has been observed for some allergens, these are limited in number and, for major allergens, HLA-association studies have led to discordant results.^{10, 32} Responses can be blocked easily in vitro,^{29, 59–61} although in vivo responses may be difficult to block.
2. *Intervention at the level of TCR:* T-cell antagonist peptides are used to bind MHC, engage the TCR, and prevent stimulatory complexes from binding to the TCR. Also, T-cell anergized peptides are sought that will bind to MHC, engage TCR, and induce nonresponsiveness in the T cell.
3. *Intervention by immunization with peptides derived from Igs and TcRs:* Intervention with peptides from either Ig receptors or TCRs can be achieved by establishing an anti-idiotypic regulatory network.

Treatment with Allergen-Peptides and Peptoids. A strategy for modulating immune responses involved treatment with the peptidic fragments of the desired allergens produced by proteinase digestion, as was shown with the ragweed allergen, *Amb a 1*.^{40, 43, 45} Although the precise mechanism underlying this approach is not known, the treatment led to reduction of clinical symptoms and was considered to be successful. The study involved only a few individuals, however, and was incomplete. TCR–peptide ligation plays a critical role in the differentiation of T cells into Th1 or Th2 type; thus, it may be possible to manipulate this interaction in favor of the desired (protective) response. A number of studies have addressed the use of defined minimal T-cell peptides for treatment of allergies.

The allergen peptides may enable induction of peripheral T-cell tolerance as has been achieved recently in experimental animal studies by injection with T-cell peptides of the major cat allergen, *Fel d 1*,¹² and the house dust mite allergen, *Der p 1*.³¹ In the murine system, activation of allergen-specific T cells and IgE Ab synthesis were inhibited by in vivo administration of peptides by intranasal, oral, and subcutaneous

routes. Pretreatment of mice with dominant *Fel d I* or *Der p I* allergen prevented the development of an immune response to the corresponding native proteins. Further, primary response to native *Der p I* or *Fel d I* could be down-regulated substantially by peptide treatment. Interestingly, tolerance to dominant peptides was achieved by treatment with minor (cryptic) epitopes of *Der p I*.³¹

The encouraging results from the murine studies have prompted clinical studies using peptides. So far, studies with bee venom phospholipase A₂-derived peptides have shown good clinical efficacy in combination with no adverse reactions.⁵⁶ This study involved the treatment of five patients given 400 µg each of three PLA₂ peptides, 12 to 20 amino acids in length, followed by a challenge with complete native PLA₂ after 7 days. The treatment was considered to be effective in three of five patients as judged by lymphocyte proliferation, but no decrease in IgE was seen in these patients. The largest clinical study with allergen peptides is being conducted using two peptides from the cat allergen, *Fel d I* (a total of four injections of 750 µg each at 2-week intervals).⁵⁸ Preliminary results from about 2000 patients enrolled so far suggest that the treated group exhibited about 50% efficacy in comparison with the placebo group, which showed about 30% efficacy. Some late bronchial reaction was seen in a small number of patients. Studies with three ragweed peptides encompassing the entire ragweed extract currently are underway, and preliminary results look somewhat better than did those from the *Fel d I* study.

It is early to predict the utility of peptides as immunotherapeutic regimens against allergies. Peptide therapeutics generally is considered advantageous because of its defined chemical nature, simplicity of preparation, prolonged shelf-life under normal storage conditions, and low risk of adverse effects particularly in allergies, for the IgE binding epitopes often are conformational in nature and the T-cell peptides especially may be void of IgE binding capability. Because human responses to allergens are more complex than those seen in mice, however, a number of theoretical constraints can be predicted on the basis of present knowledge, and a number of limitations are already apparent. These include the following: (1) Some of the major allergens, particularly pollen allergens, contain a number of T-cell epitopes; (2) certain major allergens appear to have a number of isoforms that contain cross-reacting and non-cross-reacting epitopes that increase the repertoire of allergenic epitopes^{41, 78}; (3) allergic individuals differ with respect to their recognition of these epitopes; (4) B- and T-cell epitopes may be present on the same peptide, thus increasing the risk of adverse side effects; and (5) excess dosages of some peptides may induce autoimmune reactions. Thus, in individuals allergic to certain complex aeroallergens, treatment with the peptide(s) may not be effective.

It is noteworthy that peptides used for treatment may be modified with respect to their structure on the basis of the knowledge of the core residues involved in interactions with TCR. In principle, certain structural analogs of those peptides that do not cause cross-linking (i.e., those

that are devoid of B-cell binding activity and that engage the TCR but do not stimulate or, if stimulated, do not lead to IL-4 production), may be found. Furthermore, the "peptoids," which contain peptides with appropriate chemical modification, may lead to induction of antigen-specific tolerance. Obviously, all approaches targeted via trimolecular interaction are MHC-restricted and will only be effective if there exists considerable immunodominance for the antigen in question.

Treatment with TCR Peptides of Allergen-Specific T Cells. As stated previously, TCR plays a pivotal role in antigen recognition. A number of studies in autoimmunity have revealed that the TCR gene usage is restricted, which led to the formulation of TCR peptide-based antigen-specific therapy.⁸⁵ Studies in murine models suggested that the TCR V β 8 gene is involved in the regulation of IgE responses. In addition, analysis of TCRs in a complex *Leishmania* also revealed the expansion of a TCR-restricted population of T cells.⁶⁹ In humans, TCR V gene analysis has been performed thus far only for the dust mite and grass allergen-specific T-cell clones.^{53, 88} The results of mite studies indicate a limited usage of TCR V β genes (V β 3 or V β 6), which recognize the main mite allergens, *Der p* I. Similarly, a limited usage of V α genes was demonstrated in T-cell clones specific for grass allergens.⁵³ Recently, analysis of TCR V β 8 expression in monozygotic twins showed that the expression of this TCR gene was elevated significantly in the twin with asthma and not in the twin without asthma. Further longitudinal clonal studies of allergen-specific T-cell repertoires in allergic individuals suggested in vivo clonal dominance with respect to TCR gene usage.^{22, 88} These studies have led to formulation of a TCR peptide immunotherapy approach.

A method to down-regulate specific immune response using TCR peptides was proposed for aberrant immune responses, such as autoimmune⁸⁰ and allergic responses. The advantage of these peptides is that they are not related or may not share the structure of the allergen; therefore, they may not induce any adverse reactions. These findings reinforce further examination of the idea of TCR peptide vaccines as an experimental therapeutic approach for allergies. However, TCR peptide-based therapy may not be applicable to all aeroallergens because of lack of immunodominance of T-cell response.

Development of Preventive Therapies Against Allergies

As stated previously, the concept of developing preventive vaccines against allergies was suggested⁴⁷ and recently has been reinforced by other investigators.³⁰ Studies in murine models demonstrated that vaccination with a recombinant allergen prior to sensitization resulted in a state of immune deviation, that is, a shift in development of Th1 and IgG2a responses instead of the default Th2 and IgE responses.⁵² A recent study of asthma prevention through early immunotherapy showed that after 1 year of treatment with pollen immunotherapy, fewer children in the immunotherapy group developed asthma as opposed to the children

in the open control group.³⁴ Also a study by Bousquet et al⁸ showed that early immunotherapy in children with a single aeroallergen resulted in no new sensitization to other allergens. In view of the recent finding that the immune systems of neonates and adult mice are similar in terms of their ability to develop immune deviation and the advances in the genetics of atopy and asthma, which may enable the prediction of susceptibility to allergies and asthma, the preventive approach to immunotherapy using recombinant allergens holds great promise and needs to be investigated further.

SUMMARY

Despite the progress in our understanding of the molecular immunologic properties of allergens, the application of this knowledge in clinical arenas is in its infancy. Synthesis of certain major recombinant allergens by fermentation and allied technologies as reported recently, however, is anticipated to expedite the use of these allergens for therapeutic studies. Furthermore, the knowledge of epitopes of allergens has advanced to a point whereby some of these epitopes are in clinical trial. At present, one can be optimistic that the application of these recent advances will lead toward either revival of the existing immunotherapy or development of certain new immunotherapeutic procedures based on the rational immunologic principles. Taking into account the complexity of allergic conditions, it is naive to expect that one form of allergen-specific immunotherapy would be effective in the treatment of allergies. Obviously, for certain allergies (e.g., ragweed or grass) protocols would involve a battery of allergens to target the polyclonal populations of T cells, thereby increasing the likelihood of successful treatment. Also, it is more likely that different forms of this type of immunotherapy used in concert or with other therapeutic agents may prove to be generally efficacious, particularly in the case of chronic allergic conditions. As we continue to learn in greater detail the mechanisms involved in the establishment of allergic conditions, we may be able to devise additional and more effective protocols for treatment.

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