

Modulation of Allergen-Specific Antibody Responses by T-Cell-Based Peptide Vaccine(s)

Principles and Potential

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Introduction

Clinically asthma is considered a complex disorder with many variants, and several possible mechanisms exist apart from the events associated with chronic inflammation of the bronchial mucosa. On the basis of the inciting agents, asthmatics may be classified primarily into two categories: (1) those having atopic asthma (also referred to as extrinsic asthma) and (2) those having nonatopic asthma (referred to as intrinsic asthma). Extrinsic asthma is closely associated with external environmental factors that include, but are not limited to, diverse aeroallergens, and are primarily responsible for IgE-mediated atopy; and presence of allergen-specific IgE antibodies plays a critical role in the exacerbation of the asthmatic response (1). On the other hand, the antigen(s) that drives T-cells in the nonatopic form of asthma is unknown. However, a number of viral antigens, particularly of adenoviruses and rhinoviruses, appear to be involved, which may also elicit IgE antibodies, although the role of IgE in pathogenesis of the ongoing chronic disease is less certain.

Accumulating evidence suggests that asthma is immunologically driven and clearly involves diverse inflammatory cells, such as T-cells, mast cells, macrophages, epithelial cells, fibroblasts, neutrophils, and eosinophils, which contribute to the pathophysiology of

asthma (2–5). T-lymphocytes contribute to asthma by (1) directly initiating epithelial damage and bronchial hyperresponsiveness, and (2) orchestrating the ongoing chronic asthma by influencing the other effector cells by release of an armamentarium of interleukins and/or other mediators (6). Furthermore, the bronchial histopathology in patients with asthma has revealed that the CD4⁺ T-cells are unique among these cells, since they are capable of recognizing and responding to the processed antigen directly, rather than through cell-surface IgE antibodies as is the case with most of the other cells. The inter-relationships among these cells in allergen- and virus-induced asthma is schematically shown in Fig. 1.

Thus, irrespective of the antigen involved, T-cells play a pivotal role in the development and sustained progression of both atopic and nonatopic asthma, and therefore, T-cell-based therapies have become plausible. I shall review in this article the molecular basis of the T-cell recognition, the immunologic basis for the role of T-cells in an atopic/asthmatic immune response, the potential therapeutic approaches based on T-cell interactions, including those of the synthetic peptide vaccines, and their possible advantages in relation to the treatments in vogue. In some forms of nonatopic asthma, where IgE-mediated mechanisms are not obvious, the influences of local neuronal and hormonal networks may be operative. Clearly, these are outside the scope of this article.

Basis of T-Cell Peptide Vaccine(s)

Modulation of T-cell-dependent specific antibody responses is considered paramount in devising appropriate therapeutic strategies for several immunologic diseases, including allergies and asthma. During the last decade, advances in recombinant DNA and cell-manipulation technologies have led to the elucidation of some of the mechanisms, such as clonal deletion, clonal anergy, and suppressor T-cells, that underlie the induction of specific immune tolerance. These studies have primarily focused on the antigen-recognition apparatus of T-cells, i.e., the T-cell receptor (TCR).

T-cells are stimulated by foreign antigens on target cells only when presented as “processed” peptides in conjunction with the major histocompatibility complex (MHC) class I or class II molecules expressed on the antigen-presenting cells (7,8). The majority of the atopic antigens (which are water-soluble) are presented by MHC class II molecules, whereas the viral antigens are presented by class I molecules. As shown in Fig. 2, the antigen recognition occurs via the TCR that is embedded in the membrane of the T-cell and that interacts with the binary complex comprising the antigenic peptide and the MHC molecule.

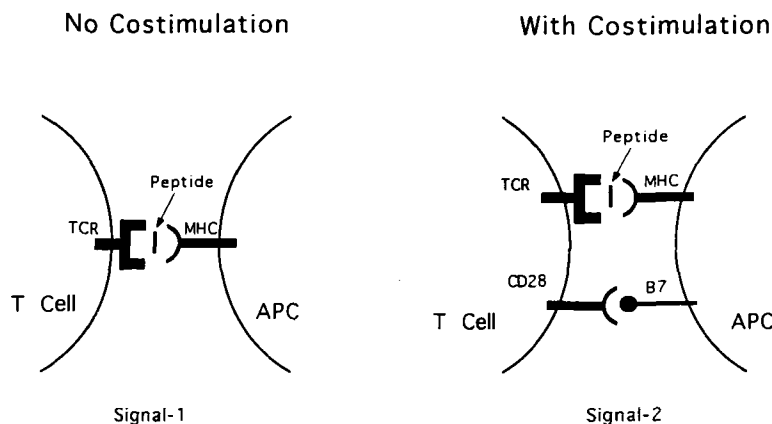


Fig. 2. Requirement for two signals for the induction of an immune response. The first signal involves TCR ligation with MHC-peptide complex and the second signal involves interaction between CD28 (or CTLA4, not shown) with B7. TCR ligation in absence of second signal may lead to anergization of the T-cell in question.

The TCRs of helper (Th), cytotoxic T (Tc), and suppressor T (Ts) cells consist of cell-surface heterodimers of $\alpha\beta$ and $\gamma\delta$ chains (9). Each of these chains is composed of regions encoded by separate gene segments: variable (v), junctional (j), and constant (c) elements; in addition, the β chain contains a diversity (D) segment (8). These gene elements are organized and rearranged in a fashion similar to those of the immunoglobulins, and three clusters of complementarily determining regions (CDR1, CDR2, and CDR3) can also be recognized on the TCR. Modeling studies have suggested that the CDR1 and CDR2 regions mainly interact with the MHC, whereas the CDR3 region interacts with the peptide when it is associated with the MHC molecule (8). Some evidence in favor of this model has been documented (10–12).

As stated previously and illustrated in Fig. 2, in addition to the above trimolecular interaction, activation of T-cells requires an additional costimulatory signal arising from the interaction between CD28 or CTLA4 on T-cells, and B7 molecule on the professional antigen-presenting cell. In atopy and asthma, activation of CD4⁺ Th cells, which in turn stimulate antigen-specific B-cells via the formation of trimolecular interaction as alluded to above, and initiates the cascade of regulatory elements for IgE synthesis by B-cells, and the differentiation and expansion of effector cells, including mast cells and eosinophils. The disruption of the trimolecular interaction constitutes the very first step in the initiation of an immune response in atopy and asthmatics; as shown in Fig. 3, by direct

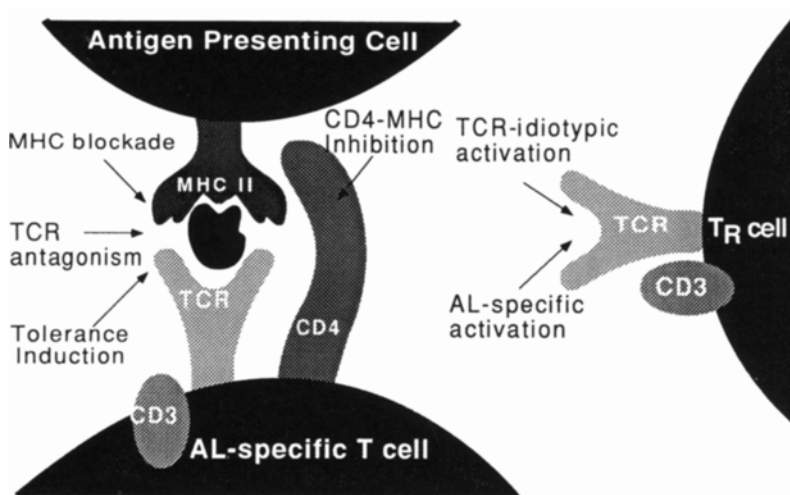


Fig. 3. Specific immunomodulation via interference with the trimolecular interaction. Antigen-specific tolerance may be induced by (1) blocking the TCR ligation with structural analogs of the antigenic peptide *per se*, or (2) by inhibition of interaction of coreceptor (CD4) with MHC, or (3) by idiotypic regulation using the peptides of antigen-specific TCR.

manipulation of the T-cell responses through their specific antigen receptors, or by modifying allergenic epitope(s) of the antigen-combining site of the appropriate MHC molecules, it may therefore be possible to prevent the activation of the disease-related CD4⁺ T-cells (13). Thus, modulation of specific immune response by manipulation of CD4⁺ T-cells of appropriate specificity and function has generated considerable interest.

In principle, on the basis of our current knowledge of T-cell activation, the following approaches can be envisaged for the T-cell-based immunomodulation of atopic and/or asthmatic disorders:

1. Blocking of interactions involving TCR, CD4⁺, or MHC molecule, and of costimulation, adhesion, or migration of T-cells responsible for the disease;
2. Manipulation of antigen/allergen presentation in the context of the presenting cells;
3. Interference of differentiation and/or recruitment of T-cells to the site of inflammation; and
4. Induction of regulatory T-cell circuits that may lead to down-regulation of specific antibody production by altered profile of cytokine secretion or by anti-idiotypic regulation.

Atopic Asthma

Allergens are pragmatically defined as a class of antigens that on inhalation, ingestion, or contact are capable, at extremely low doses, of inducing the synthesis of IgE antibodies by precommitted lymphocytes in genetically predisposed individuals. In allergic patients with asthma, environmental allergens present in a number of airborne particles, such as house dust, molds, animal dander, pollen, and certain foods, are the major cause of airway hyperresponsiveness, i.e., airway constrictive response to a variety of chemical, physical, and pharmacologic stimuli (14). Although there is a positive relationship between the degree of airway hyperresponsiveness and the severity of patients' asthma, there is considerable overlap (15).

Currently used pharmacological therapy for atopic asthmatics consists of medications that include anti-inflammatory agents and bronchodilators that are used to reverse and prevent airflow obstruction. Although the bronchodilators reverse and/or inhibit bronchoconstriction and related symptoms of acute asthma, they do not reverse bronchial inflammation or hyperresponsiveness. After a long course of the disease, inflammation becomes the primary cause of symptoms and bronchial hyperresponsiveness, suggesting that the immunologic treatments should be replaced by anti-inflammatory drugs. The main objective of the immunologic treatment has been to reduce the allergic triggers that exacerbate the symptoms, and in the long run, to decrease bronchial inflammation and bronchial hyperresponsiveness when it is not severe.

Several studies aimed at evaluating the use of "time-honored" allergen-specific immunotherapy for asthma have led to the conclusion that such therapy is only effective under optimal conditions, including an appropriate selection of patients and the use of high-quality extract administered with an adequate schedule (16). Despite the clinical improvement from this therapy (16), the inconvenience to the patient owing to the lengthy course of the therapy and its high cost, and the finite risk of severe asthmatic or anaphylactic reactions that may occasionally result in death limit the utility of this treatment. The most important factor responsible for the discontinuation of this form of immunotherapy in some countries, in particular, the UK (17), has been due to the lack of methods for (1) the exact definition, (2) consistent reproducibility of the composition of the relatively crude commercially available mixtures of allergenic extracts, and (3) the difficulty of isolating in pure form and sufficient amounts the compounds responsible for the allergic condition to be treated.

Parallel with the increase in effectiveness of immunotherapy with highly purified allergens, rapid progress has been made within the last few years in relation to the molecular characterization of allergens by the application of recombinant DNA procedures. Thus, cDNAs of several allergens from an array of aeroallergenic sources have been cloned, and the corresponding recombinant allergens (RAs) have been produced or are in the process of being synthesized. A list of RAs published at the end of 1992 is presented in Table 1 (18–59). Overlapping cDNAs and/or synthetic peptides derived from the sequence of allergens have aided in the definition of the B- and T-cell epitopes, which is expected to lead to the development of new therapies for the RA-specific downregulation or even abrogation of IgE antibodies (60,61). Moreover, the determination of the primary structures of these allergens has facilitated comprehensive analysis of crossreactivities at the B- and T-cell levels (13,62,63). Motif analysis of pollen allergens led to the identification of two putative motifs on these allergens (64). Lastly, for some allergens, molecular cloning and sequencing have aided in the determination of the biological activity of some of these proteins.

The relevance of these advances in the field of allergens and advances in molecular allergology in general have suggested several potentially effective therapeutic approaches. For instance, it is envisaged that a major improvement in allergen-specific immunotherapy may be possible by the chemical modification of RA in the form of allergoids (65) or pegylated allergens (66), that render these molecules essentially nonallergenic. The therapeutic effect of these modified allergens is also mediated by T-cells and is dealt with elsewhere in this compendium (67). The approaches based on T-cell vaccination, which are mostly at the experimental stage at present, are reviewed below.

Therapeutic Approaches

Modulation of De Novo T-Cell Cytokine Patterns

CD4⁺ T-cells of both mice (68) and humans (69) have been subdivided into Th1 and Th2 types on the basis of their cytokine secretion patterns; thus, Th1 cells produce IL-2, IFN γ , and LT, whereas Th2 cells produce IL-4, IL-5, IL-6, and IL-10; of these, IL-4 and IFN γ have reciprocal effects on IgE synthesis. Th2 cells are of particular interest with regard to the pathogenesis of atopic asthma because of the role of IL-4 in IgE synthesis (70) and that of the IL-5 in eosinophil maturation, survival, and activation (2–5). In several studies with T-cells both in vitro and in vivo, atopic asthmatics showed elevated expression of IL-4 and IL-5 mRNA as compared to nonatopic controls, reflecting a Th2-like pattern (5,6).

Table 1
List of Recombinant Allergens

Allergen source	Allergens, systematic and original names	Mol wt, kDa	Refs
Weed pollens			
<i>Ambrosia artemisiifolia</i> (short ragweed)	<i>Amb a</i> I; antigen E	38	18,19
	<i>Amb a</i> II; antigen K	38	20
	<i>Amb a</i> III; Ra3	11	21
	<i>Amb a</i> V; Ra5	5	22
<i>Ambrosia trifida</i> (giant ragweed)	<i>Amb t</i> V; Ra5G	4.4	23,24
Grass pollens			
<i>Dactylis glomerata</i> (orchard)	<i>Dac g</i> II;	11	
<i>Lolium perenne</i> (rye)	<i>Lol p</i> I; group I	27	25,26
	<i>Lol p</i> II; group II	11	27
	<i>Lol p</i> III; group III	11	28
	<i>Lol p</i> IX; <i>Lol p</i> Ib	31	29
<i>Poa pratensis</i> (Kentucky blue)	<i>Poa p</i> IX;	28–33	30
Tree pollens			
<i>Alnus glutinosa</i> (alder)	<i>Aln g</i> I;	17	31
<i>Betula verrucosa</i> (birch)	<i>Bet v</i> I;	17	32
	<i>Bet v</i> XI; profilin	15	33
<i>Carpinus betulus</i> (hornbeam)	<i>Car b</i> I;	17	34
Animals			
<i>Felis domesticus</i> (cat)	<i>Fel d</i> I; cat-1	38	35
<i>Mus musculus</i> (mouse)	<i>Mus m</i> I; MVP	19	36,37
<i>Rattus norvegicus</i> (rat)	<i>Rat n</i> I	17	38,39
Fungus			
<i>Aspergillus fumigatus</i>	<i>Asp f</i> I;	18	40
<i>Candida albicans</i>	<i>Can a</i> I;	40	41
Arthropods			
<i>Apis mellifera</i> (honey bee)	<i>Api m</i> I; phospholipase A ₂	16	42
	<i>Api m</i> IV; melittin	3	43
<i>Chironomus thummi</i> (midges)	<i>Chi t</i> I; hemoglobin	16	44
<i>Dermatophagoides pteronyssinus</i> (mites)	<i>Der p</i> I; antigen P ₁	25	45
	<i>Der p</i> II;	17	46
	<i>Der p</i> V;	14	47
	<i>Der p</i> VII;		

(continued)

Table 1 (continued)

Allergen source	Allergens, systematic and original names	Mol wt, kDa	Refs
<i>Dermatophagoides farinae</i> (mites)	<i>Der f</i> I;	25	48
	<i>Der f</i> II;	14	49
	<i>Der f</i> III;	28/30	50
<i>Dolichovespula maculata</i> (white face hornet)	<i>Dol m</i> I; phospholipase A ₁	35	51
	<i>Dol m</i> V; antigen 5	23	52,53
<i>Dolichovespula arenaria</i> (yellow hornet)	<i>Dol a</i> V; antigen 5	23	54
<i>Polistes exclamans</i> (wasp)	<i>Pol e</i> V; antigen 5	23	54
<i>Vespula maculifrons</i> (yellowjacket)	<i>Ves m</i> I; phospholipase A ₁	33.5	55
	<i>Ves m</i> V; antigen 5		
<i>Vespula vulgaris</i> (yellowjacket)	<i>Ves v</i> V; antigen 5	23	54
Others			
<i>Gadus callarias</i> (cod)	<i>Gad c</i> I; allergen M	12	56
<i>Gallus domesticus</i> (chicken)	<i>Gal d</i> I; ovomucoid	28	57,58
	<i>Gal d</i> II; ovalbumin	44	57,58
	<i>Gal d</i> III; conalbumin (Ag22)	78	57,58
<i>Sinapis alba</i> (yellow mustard)	<i>Sin a</i> I;	14	59

The extensive studies on the pathophysiology of asthma, particularly the analysis of T-cells and their cytokine secretion patterns in airways, lungs, and bronchial lavage, have led to the conclusion that atopic asthma is a "Th2-type disease." Moreover, in both mice and humans, it has been demonstrated that IFN γ and IL-4, respectively, promote the differentiation of Th1 and Th2 subsets, both in vitro (71) and in vivo (72). In humans, treatment of patients with IFN α has been reported to ameliorate atopic diseases, although the effectiveness of such therapy depended on the degree of IgE sensitization. All these taken together and the fact that asthma is localized to the airways and lungs, have suggested the idea of designing therapeutic regimens for the purpose of modulating T-cell cytokine patterns *de novo*. It is to be pointed out that in line with the above idea, a promising novel approach to specific therapy directed at T-cells has emerged from our recent in vitro studies in collaboration with S. Romagnani's laboratory, utilizing human T-cell clones (72).

It was thus shown that the incubation of the peripheral blood mononuclear cells of atopic individuals with a recombinant grass allergen (73) and IFN led to the selective development of Th1/Th0 type of cells instead of Th2 type of cells. Remarkably, most of these

cell clones (22 of 24) possessed specificity to a single peptide of the allergen. Furthermore, analysis of the cytokine profile of the T-cell clones developed with the above peptide as stimulant revealed that all four clones induced were of Th0/Th1 type, even if they were derived from PBMC that had been incubated initially with peptide and IL-4. More recent studies have demonstrated that IL-12 (which is relatively less toxic) is a more potent inducer of Th1 cells. These studies taken together suggest that specific allergenic peptides may play a definite role in the differentiation of Th cells and suggested that the treatment with recombinant allergen(s) and IFN α or IL-12 may comprise a feasible specific immunotherapy approach for the atopic asthma.

Vaccination with T-Cell Epitopes of Allergens

The concept of vaccination with T-cell epitopes of allergens is based on advances in two areas of immuno-allergology. First, as stated previously, rapid progress in cloning and sequencing of a diverse array of allergens made it possible to define the allergen-derived peptides recognized by allergen-specific T-cell clones; these peptides are referred to as minimal T-cell epitopes (MTCEP). Second, as described earlier, induction of immune response requires two signals: signal 1, involves the trimolecular interaction between T-cell and the antigen-presenting cell, and signal 2, also referred to as costimulating signal, arises from the interaction between CD28 and B7 molecules of the T-cell and professional antigen-presenting cell, which is nonspecific in nature. Stimulation of T-cells, by MHC-peptide-TCR interaction in the absence of costimulatory signal delivered by professional APC, results in T-cell unresponsiveness or anergy by subsequent challenges by antigen and APC (74).

The rationale for vaccination with MTCEP stems from the studies of antigen recognition by B- and T-cells that suggest that contrary to B-cell epitopes, T-cell epitopes are sequential in nature. Thus, vaccination with MTCEP, which do not bind to IgE antibodies on the effector cells, has been considered an effective immunotherapeutic approach. Since 1980, a number of pilot clinical studies have addressed this issue of immunotherapy with antigenic peptides (75,76), although these studies did not investigate the precise immunologic and clinical parameters affecting the symptomatic relief. Moreover, these peptides were not completely devoid of systemic side effects (75,76). Thus, *in vivo* data utilizing MTCEP of allergens as therapeutic regimens are presently lacking. It is noteworthy, however, that treatment of mice with an immunodominant T-cell determinant of another protein, i.e., myelin basic protein, which induced experimental autoimmune encephalomyelitis (EAE) (77).

Thus, treatment resulted in induction of T-cell tolerance to native antigens *in vivo*, and virtually blocked the progression and decreased the severity of EAE, after the disease had begun.

Some evidence in support of potential of MTCEP therapy has been reported recently. Thus, utilizing a major dust mite allergen, *Der p* I, which has been implicated in atopic asthma, human T-cells were tolerized *in vitro* following activation with MTCEP (78,79). Subsequent exposure of these tolerized cells to corresponding antigens in the presence of APC did not result in proliferation (78,79). Moreover, these anergic T-cell clones failed to produce IL-2; decreased production of this cytokine and other cytokines by the T-cells has been proposed as the mechanism for clonal unresponsiveness. Most recent efforts in some laboratories have focused on immunotherapy using defined T-cell epitopes of Cat allergen *Fel d* I as a model. A phase I trial is currently under way with two *Fel d* I epitopes in 16 patients with atopic and/or asthmatic symptoms.

It is worth pointing out that immunotherapy with T-cell peptides of allergens appears to be a promising approach for the modulation of allergen-specific IgE responses. However, two major limitations need to be overcome. The first is the multiplicity of epitopes on certain atopic antigens, particularly the pollen allergens (13,60–64). It has been suggested that the total T-cell responses to antigens implicated in atopic asthma may be limited to a few epitopes. This latter process, referred to as immunodominance, involves selection of determinants by their MHC-binding affinity; thus, immunodominant peptides have the highest affinity (80,81). However, whether the utilization for immunotherapy of peptides representing immunodominant epitopes would obviate the need for “individual” T-cell-inducible epitopes is questionable, in view of the recent demonstration that T-cells can escape tolerance induction because of minor epitopes that only became available after *in vivo* processing of the whole antigen (80). In addition, the observation that Th2 T-cell clones recognizing many different allergen-derived peptides can be readily isolated from atopic patients is not compatible with a determinant selection model (69–73).

Secondly, multiple T-cell epitopes, which may be restricted by various class II MHC molecules (70), implies that for each patient, all individual T-cell epitopes are to be determined with precise HLA typing, although the ability to deliver multiple T-cell epitopes in the form of a few non-IgE binding peptides may only partially overcome the problem of HLA differences (67). Considering that atopic asthmatics may be sensitized to diverse antigens and the fact that an increasing number of allergens are being found in different aero-allergenic extracts, it is premature to judge the feasibility

of T-cell peptide vaccination for therapeutic purposes. Clearly, more research is required on this issue.

Vaccination with TCR Peptides

As alluded to earlier, in atopic asthma, the role of IgE-inducing antigens that are recognizing TCRs of the CD4⁺ T-cells is critical in initiation of the response. The nonatopic asthma, on the other hand, is less defined since it is possible that IgE-mediated reactions, which are not detectable systemically, may occur in such patients. Thus, it has been postulated that the nonatopic asthma may constitute a chronic cell-mediated disease that occurs independently of IgE- or other antibody-mediated immune mechanism. Irrespective of the involvement of humoral or cell-mediated immune mechanisms, the CD4⁺ T-cells and their TCRs play a very important role in development and sustained inflammatory responses. Moreover, recently, CD8⁺ T-cells have also been implicated in regulating immune responses (77,80).

Modulation of immune responses utilizing the TCR is of considerable importance in this regard. A plethora of evidence in the last decade has pointed out the role of TCR in regulating the T-cell development and in acquired immune tolerance (82). It has been clearly demonstrated in many antigenic systems and diseases that T-cells that play a role in recognition of the antigen utilize a restricted repertoire of TCR V β genes (83). As stated previously, remarkable progress has been made recently in the field of autoimmune diseases, such as EAE, in which restricted usage of TCR V β genes has been demonstrated (83). Thus, pretreatment of rats with a V β 8.2 peptide 39-49 was shown to induce crossreactive and EAE-protective T-cells and antibodies. More recently, it was shown that CD8⁺ T-cells regulated the protective response induced by the treatment with TCR peptides (80). A schematic diagram with the postulated mechanisms underlying this regulation has been shown in Fig. 4.

Since atopic and/or asthmatic responses involve T-cells initially, it is possible that a regulatory T-cell network may be operational. The availability of pure allergens or RAs in sufficient amounts has promoted the investigation of allergen-specific T-cell clones and examination of the repertoire of TCR V genes (13). TCR V gene analysis of T-cell clones specific to dust mite allergens, which constitute a predominant indoor allergen responsible for exacerbation of asthmatic responses, indicated that a limited number of V β genes, i.e., V β 3- and V β 6-encoded TCR proteins, recognized the main mite allergen, *Der p* I (84). Moreover, it was demonstrated that staphylococcal enterotoxins (referred to as superantigens), when devoid of toxicity, may be employed to inactivate T-cells expressing the above

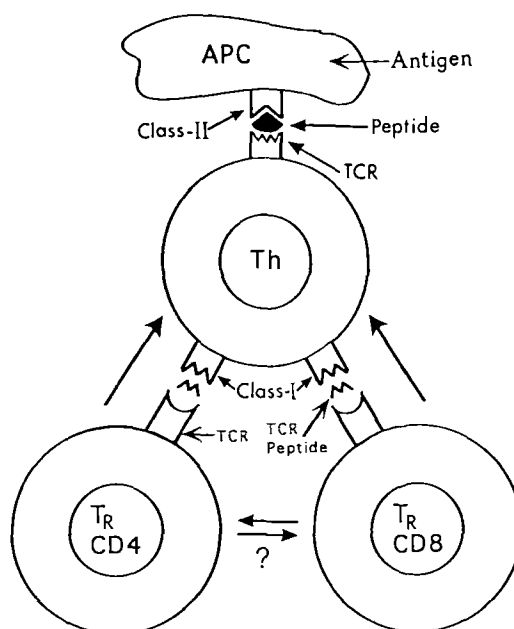


Fig. 4. Proposed mechanism of TCR peptide-induced tolerance of specific antibody production. The antigen- c or allergen-specific Th cell expresses (1) TCR, which interacts with MHC class II-peptide complex on the APC's surface, and (2) the TCR peptide, which interacts with MHC class I of CD4⁺ Th cell. In the latter case, the Th cell activity is modulated by the idiotypic regulatory T-cells (TR) which may be of either CD4⁺ or CD8⁺ phenotype.

antigen-specific TCRs. More recently, analysis of grass-allergen-specific T-cell clones also pointed out the possibility of restricted usage of TCR V α genes. However, in atopy and/or asthma, the studies of TCR gene usage are very limited at present.

It is noteworthy that an approach similar to the one suggested for autoimmune disorders for downregulating antigen-specific antibody responses has emerged from our recent studies in collaboration with A. Sehon's laboratory of TCRs of CD8⁺ T-cell clones induced by tolerogenic conjugates of ovalbumin (OVA), used as a model antigen and allergen, and monomethoxypolyethylene glycol (85,86). In this study, analysis of TCRs of a number of OVA-specific CD8⁺ T-cell clones indicated a restricted repertoire of TCR V α -J α genes used by these clones. Whereas pretreatment with TCR- α -CDR3 peptide suppressed >90% OVA-specific antibody production (both IgG1 and IgE), treatment with TCR- β -CDR3 peptide induced a suppression of about 60%. However, maximal tolerance was induced when mice were treated with a mixture of CDR3 peptides of TCR α and β chains.

Remarkably, a single course of treatment of mice with TCR-CDR3 peptide(s) led to persistent suppression of anti-OVA antibody responses (i.e., up to 216 d) in spite of the fact that the mice received six subsequent injections of OVA at about 4–6 wk intervals (87). Moreover, the injection of this TCR-CDR3 peptide(s) also abrogated the established, i.e., ongoing IgG1 and IgE antibody responses. Taken together, the above studies have pointed out that in adult animals with established diseases as characterized by humoral and cellular responses, antigen-specific tolerance can be induced by treatment with TCR peptide vaccination. It is to be pointed out, however, that the TCR peptides used in the above study were designed on the basis of restricted TCR V-J genes of the antigen-specific CD8⁺ T-cells, which were isolated from tolerized spleen cells of mice. Although regulatory CD8⁺ T-cells have also been reported in humans, the generality of the overall approach of TCR-peptide-induced specific tolerance in humans remains to be elucidated.

Virus-Induced Asthma

Epidemiological evidence gathered to date confirms a close temporal association between exacerbations of asthma and intercurrent viral infections; the predominant associated viruses are human rhinovirus and respiratory syncytial virus (RSV) (88). A number of mechanisms have been proposed whereby viral infections may lead to exacerbation of asthma, which include, but are not limited to, certain IgE-mediated mechanisms, such as increased total IgE, virus-specific IgE antibodies, and the subsequent allergic sensitization and mediator release. However, such sensitization is local and is not reflected in a systemic IgE-induced symptomology, leading to a lack of prick-test.

The work of Welliver et al. (89) demonstrated that RSV infections induced the production of virus-specific IgE bound to exfoliated respiratory epithelial cells, and that the magnitude of this response correlated with subsequent wheezing, increased histamine release, and degree of arterial hypoxia. Genetic predisposition to atopy has been proposed as a basis for this virus-induced IgE synthesis, and recent population studies support this notion (90). Studies by Frick (91) have suggested an IgE immunoregulatory role for viral respiratory infections in children who are genetically prone to allergy and to increased production of IgE. All these studies together indicate that viral-specific IgE production may be a factor linking atopy to asthma via the release of mediators and cytokines leading to the amplification of inflammation in the already diseased airways of asthmatics. In this connection, it is to be noted that recent analy-

sis of allergen structures has revealed that there exist some meaningful homologies between certain atopic aeroallergens and viral genes implicated in asthma. Crossreactivities between these antigens at the B- and T-cell level may contribute also to the link between atopy and asthma.

Therapeutic Approaches

In principle, the approaches outlined above, which were directed toward the downregulation of allergen-specific antibody response, may also be applicable to the virally induced IgE production. However, a number of limitations exist:

1. The viral antigens responsible for induction of IgE antibodies have as yet not been identified;
2. In the absence of the knowledge of the antigen, the T-cell peptide immunotherapy cannot be utilized; and
3. The mature T-cells, and their cytokines and receptor gene expression, have not been aptly characterized to permit any rational approach to emerge even for experimental research.

In addition, since the viral-induced asthma devoid of IgE synthesis may involve pathways different than that of the environmental allergens, other approaches may also be feasible. Recombinant virus vaccines that express a limited number of epitopes are currently being developed to prevent disease by changing the relative balance between viral spread and immune response (92). Although vaccination usually induces protective antibodies, in the case of respiratory syncytial virus, induction of antibodies by vaccination may enhance subsequent infection and disease (93), and therefore, these approaches may not lead to amelioration of the disease. However, a CD8⁺ MHC class I restricted CTL response to a virus may be important in limiting the spread of virus in the infected host as has been demonstrated for HIV (94).

Palmer et al. (95) have proposed an experimental approach for making a potent peptide vaccine based on the above principle. Thus, self-peptides were eluted from H-2K^d molecules, and an epitopic motif on these peptides was determined by analyzing the amino acid sequence of the peptide (95). Tyr-2 and Ile-9 were found to be critical for binding of the self-peptides to K^d molecules. These investigators then searched for a similar amino acid motif in listeriolysin of *Listeria monocytogenes* and identified 11 peptide fragments that carried a tyrosine at position 2, and isoleucine, valine, leucine, or alanine at position 9. These 11 peptides were synthesized and analyzed for antigenicity. It was demonstrated that 1 of the 11 synthetic peptides could be a determinant of CTL clone specific for

L. monocytogenes. It was, therefore, suggested that this approach might be useful for preparing peptide vaccines to generate a CTL response to *L. monocytogenes*. By analogies with these studies, when the viral antigens responsible for the induction and/or exacerbation of asthma are identified, a similar approach may be utilized for designing of peptide vaccine(s) appropriate for asthma.

Summary and Concluding Remarks

Extensive studies of immunopathology of asthma have revealed the predominant role of T-cells in driving the inflammatory responses associated with bronchial asthma and the role of allergen-induced IgE responses in exacerbating the asthmatic responses. With recent advances in molecular immunoallergology, in general, and the role of T-cells in asthma in particular, it has become apparent that therapy involving T-cell-based vaccines may provide a rational immunologic approach(es) for the treatment of specific allergen induced asthma. Three main approaches appear to have potential:

1. Immunotherapy with allergen in combination with appropriate cytokines;
2. Vaccinations with T-cell epitopic peptides; and
3. Vaccinations with T-cell receptor peptides.

It is to be noted that these therapeutic approaches are presently at the experimental stage. Given that asthma is a very complex disorder and each of these approaches has its own advantages and disadvantages, it is difficult at present to predict which of these would succeed. It is clear, however, that each of these immunologic approaches has sufficient potential to be useful either by itself or in combination with other therapies and/or in conjunction with pharmacotherapy, depending on the prognosis of asthma in individuals on a case by case basis.

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