Epitope Peptides and Immunotherapy

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Abstract: Allergic diseases affect atopic individuals, who synthesize specific Immunoglobulins E (IgE) to environmental allergens, usually proteins or glycoproteins. These allergens include grass and tree pollens, indoor allergens such as house dust mites and animal dander, and various foods.

Because allergen-specific IgE antibodies are the main effector molecules in the immune response to allergens, many studies have focused on the identification of IgE-binding epitopes (called B cell epitopes), specific and minimum regions of allergen molecules that binds to IgE. Our initial studies have provided evidence that only four to five amino acid residues are enough to comprise an epitope, since pentapeptide QQQPP in wheat glutenin is minimally required for IgE binding. Afterwards, various kinds of B cell epitope structures have been clarified. Such information contributes greatly not only to the elucidation of the etiology of allergy, but also to the development of strategies for the treatment and prevention of allergy.

Allergen-specific T cells also play an important role in allergy and are obvious targets for intervention in the disease. Currently, the principle approach is to modify B cell epitopes to prevent IgE binding while preserving T cell epitopes to retain the capacity for immunotherapy. There is mounting evidence that the administration of peptide(s) containing immunodominant T cell epitopes from an allergen can induce T cell nonresponsiveness (immunotherapy). There have been clinical studies of peptide immunotherapy performed, the most promising being for bee venom sensitivity. Clinical trials of immunotherapy for cat allergen peptide have also received attention. An alternative strategy for the generation of an effective but hypoallergenic preparation for immunotherapy is to modify T cell epitope peptides by, for example, single amino acid substitution.

In this article, I will present an overview of epitopes related to allergic disease, particularly stress on allergen specific immunotherapy. In addition, our ongoing study of immunotherapy by 'eating' T cell epitope peptides will be described. Eating T cell epitope peptides as food provides a more practical way of inducing tolerance and a challenge to prevent allergy in daily life, as opposed to therapy by ingesting peptides as medicine.

Keywords: Allergy, allergen, B cell epitope, IgE binding epitope, T cell epitope, immunotherapy.

1. IgE-MEDIATED ALLERGIC DISEASE

More than 25% of the population in industrialized countries suffers from IgE-mediated (type I) allergic symptoms [1]. The disease arises when mechanisms controlling responses to innocuous environmental antigens (allergens) break down. Allergens are proteins characterized by their ability to induce a pathogenic IgE response in susceptible individuals, giving rise to asthma, rhinitis, and atopic dermatitis. Although the reasons that certain individuals suffer particular hypersensitivities are unclear, there is evidence that both genetic and environmental factors influence susceptibility [2]. Analyses of genes contributing to allergic disorders have shown that susceptibility arises from complex multigenic interactions [3]. The dramatic recent increase in the prevalence of allergic sensitization provides evidence for the additional roles of environmental factors in the pathogenesis of immune hypersensitivity.

It has been explained that allergic disease is induced by the following mechanisms [1].

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Exogeneous antigens are processed by endosomal proteases to peptides in antigen presenting cells (APC), and are presented to T cells by appropriate major histocompatability complex (MHC) class II molecules present on the cell surface (Fig. **1-A**). $CD4^+$ helper T cells are activated upon recognition of these peptide-MHC complexes by T cell receptor (TCR) molecules and exert effector functions through various biological activities of secreted cytokines (Fig. **1-B**). Allergen-specific $CD4^+$ T cells that produce Th2-type cytokines play a major role in the elicitation and progression of allergic diseases. The most important Th2 cytokine is interleukin-4 (IL-4), which drives IgE class switching by allergen-stimulated B cells (Fig. **1-B**).

IgE binds with high affinity to IgE receptors (FcεRI) located on the surface of mast cells in tissues and basophils in the blood (Fig. **1-C**). Mast cells play a central role in inflammatory allergic reactions. The multivalent binding of antigen to receptor-bound IgE and the subsequent aggregation of FcεRI provide the trigger for the activation of mast cells (Fig. **1-D**). FcεRI aggregation results in the release of inflammatory mediators from secretory granules, which contain preformed mediators such as histamine (Fig. **1-E**).

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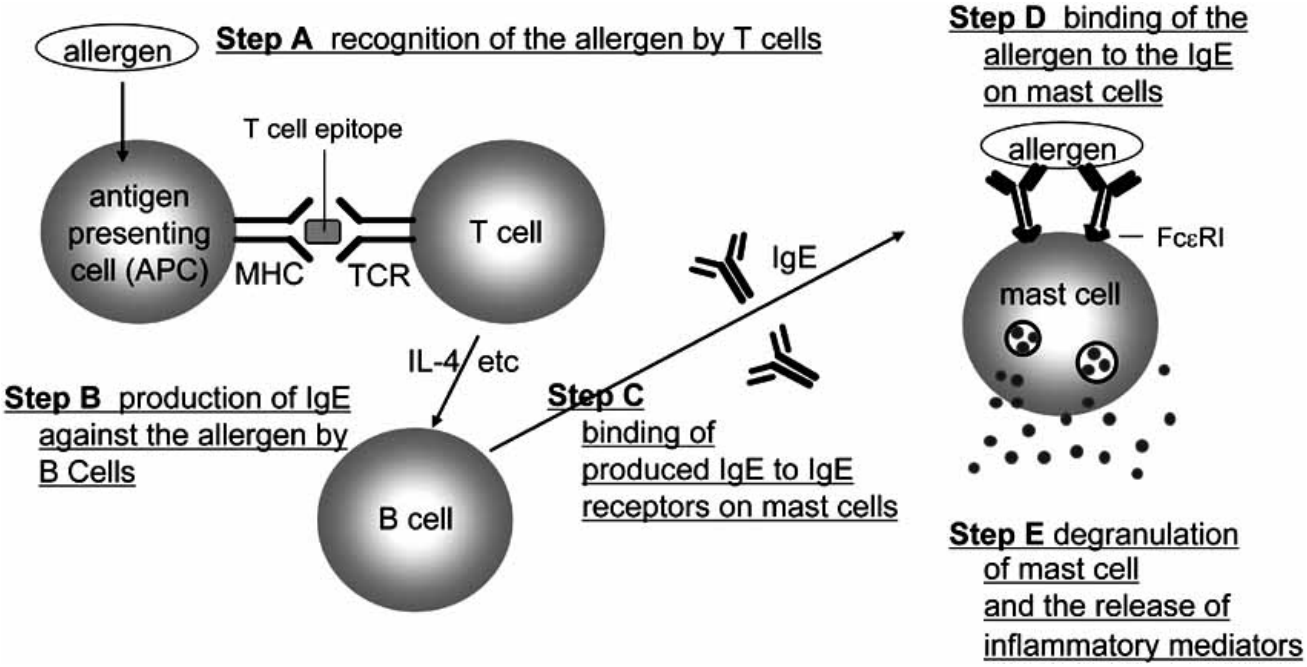

Fig. (1). Mechanisms of allergic reactions.

2. ALLERGENS

As described above, allergens are proteins that bind to IgE. In general, they have been isolated from extracts of symptom producing pollens, house dust mites, or foods by a series of chromatographies. The cDNA nucleotide sequences of almost all allergens have been determined.

The number of characterized allergens is increasing rapidly. The Allergen Nomenclature Sub-Committee of the **I**nternational **U**nion of **I**mmunological **S**ocieties (IUIS) maintains a list of 'certified' protein allergens [4]. By June 2005, about 500 allergens had been classified (http://www. allergen.org/List.htm).

Allergen nomenclature was established by the allergen nomenclature sub-committee of IUIS and is now well recognized [4]. Allergens are designated according to the accepted taxonomic name of their source as follows: the first three letters of the genus, *space*, the first letter of the species, *space*, and an Arabic number. Numbers are assigned to allergens in the order of their identification, and the same number is generally used to designate homologous allergens of related species. As an example, Lol p 1 refers to the first pollen allergen identified from *Lolium perenne*, rye grass.

The following are some common pollen allergens with their nomenclature as shown in the above example. Birch pollen allergy is one of the main causes of allergy from spring to early summer in northern and central Europe. The major birch pollen allergen protein (Bet v 1) is recognized by specific IgE antibodies in more than 95% of individuals with birch pollen allergy [5]. Pollen from Japanese cedar (*Cryptomeria japonica*) is a seasonal aeroallergen in Japan. More than 10% of the population suffers from pollinosis caused by exposure to this pollen [6]. Two major allergens, Cry j 1 and Cry j 2, have been isolated from this pollen [6,7]. IgE specific to Cry j 1 is detected in up to 95% of patients suffering from Japanese cedar pollinosis, while that for Cry j 2 is found in about 70% of patients [7,8] A Western immunoblot of a crude extract of Japanese cedar pollen showed that more than 50% of the IgE from individual patients binds to 40- to 50-kDa polypeptides, corresponding to Cry j 1 and Cry j 2 [9]. Therefore, both Cry j 1 and Cry j 2 are thought to be important in the pathogenesis of Japanese cedar pollinosis [8]. Rye grass pollen is also an important aeroallergen in cool temperate climates during the grass flowering season; Lol p 1 and Lol p 5 are the major allergens in rye grass pollen [10].

Among indoor allergens, house dust mites of two species of *Dermatophagoides* (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) associated with allergic diseases, including bronchial asthma, rhinitis, and atopic dermatitis, are the most important causative factors [11,12]. Group 1 and group 2 allergens are major allergens derived from house dust mites on the basis of frequency of patients sensitized, amount of specific IgE, and content in mite extract [13]. Group 1 allergens (Der p 1 and Der f 1) are the most clinically relevant allergens because they exist in abundance in mite fecal pellets, and account for more than 50% of IgE antibodies against total mite extract. Der p 1 and Der f 1, which show 82% sequence homology to one another, belong to the papain-like cysteine protease family [12]. Contact with animal hair or dander allergens frequently leads to perennial IgE-mediated allergy. It has been reported that cat and dog allergies are often associated [14], and a cross-reactive animal allergen has been identified as their albumins [15]. Immunotherapy for mite and cat allergies has been attempted extensively, as will be discussed later (section 4).

It is estimated that up to 8% of children under the age of 3 years and 2% of adults are affected by food allergies [16].

Chicken egg is one of the most common causes of food allergic reactions in children; in one study, approximately two-thirds of children diagnosed with food allergies were reactive to egg [17]. As major egg allergens, ovomucoid (Gal d 1), ovalbumin (Gal d 2), and others, have been identified. Cow's milk, nuts (including peanut and walnut), wheat flour, and crustaceans (shrimp, crab) are also common causes of allergy. Of note, a recent study indicated that the incidence of peanut allergy in children under 5 years of age has doubled in the past 5 years [18]. Major allergens in these foods have mostly been clarified, that is, α_{S1}-casein and β-lactoglobulin in cow's milk, Ara h 1 (63.5kDa) and Ara h 2 (17kDa) in peanut, gluten and α-amylase inhibitor in wheat flour, and tropomyosin in shrimp [19].

Latex allergy is also an important occupational disease in health-care workers. Latex allergens are primarily residual allergenic proteins that originate from the rubber tree, *Hevea brasiliensis*. Hev b 5 is a major latex allergen and a proline-rich acidic protein, 151 amino acids in length with a predicted molecular mass of about 16 kDa (although it migrates in SDS-PAGE gels at about 36kDa), and a reported prevalence of specific serum IgE of 52% to 92% among health-care workers allergic to latex [20]. It is well known that latex allergy is commonly associated with immunologic evidence of an IgE-mediated sensitivity to certain fruits, particularly avocado, bananas, or kiwis (latex-fruit syndrome) [21]. Similar to the cross-reactivity between latex and fruits, there is cross-reactivity between pollen and fruits or vegetables; birch pollinosis is strongly associated with food allergy to certain fruits of the Rosaceae family (such as apples, cherries, and apricots), tree nuts (such as hazelnuts), and vegetables of the Apiaceae family (such as celery and carrots) [5]. The major birch pollen allergen Bet v 1 plays a substantial role in the initiation of these food allergies (birch-fruit syndrome).

There are some allergen databases that seem to be very useful [22,23]. For example, the Allergome web site (http://www.allergome.org/) has been designed to supply information about allergens. Molecules identified as causing an IgE-mediated allergic disease (anaphylaxis, asthma, atopic dermatitis, conjunctivitis, rhinitis, urticaria) have been selected from international scientific journals and from web-based resources. It also contains data on allergenic sources, whether the specific allergens have been identified or not. Allergens and their sources are included in the Allergome database independently of the sources (animals or plants) and their tissues (dander, fruit, pollen, seed, spores, venoms, whole bodies etc.), or the routes of exposure (contact, ingestion, inhalation, injection, and so on). The Allergome is based on literature published since 1960's. The current database includes 5800 selected papers (November 2004) and is continuously updated.

The **s**tructural **d**atabase of **a**llergenic **p**roteins, SDAP-Food, http://fermi.utmb.edu/SDAP/, has been developed to aid in predicting the IgE-binding potential of novel food proteins and cross-reactivities among known allergens [24]. The site is designed to facilitate the first steps of a decision tree approach to determining the allergenicity of a given protein based on their sequence and structural similarity to known allergens and their IgE binding sites. Immunological tests can then be used to confirm the predictions.

3. IgE-BINDING EPITOPES (B CELL EPITOPES)

The allergenic regions of protein recognized by the binding sites of IgE molecules are called IgE-binding epitopes (or as B cell epitopes hereafter). These epitopes can be classified into two categories: i) conformational epitopes, in which the involved residues are distantly separated in the sequence and brought into physical proximity by protein folding, and ii) linear/sequential epitopes, comprised of a single continuous stretch of amino acids within a protein sequence that can react with anti-protein antibodies. It has been thought that both conformational and sequential epitopes are responsible for allergic reactions.

Our initial studies [25] provided evidence that, since the pentapeptide QQQPP in wheat glutenin can bind to patient IgE, only four or five amino acid residues are sufficient to comprise a linear epitope. Afterwards, various kinds of IgE-binding epitope structures have been clarified; for example, QQPFP and PQQPF motifs in wheat gliadin [26], EYAV in bovine serum albumin (BSA), a major beef allergen, as described later [27,28], RGEE (aa 36-39) in the English walnut allergen Jug r 1 [29], KWVNGREI (aa 124-131) in the cedar pollen allergen Cry j 2 [30], and IPGEFGLPGP (aa 495-494) in bovine type I collagen [31]. This information contributes greatly not only to the elucidation of the etiology of allergy, but also to the development of strategies for the treatment and prevention of allergy.

To find linear epitopes on allergens, epitope mapping with synthetic peptides has been demonstrated to be a suitable tool. On the basis of the amino acid sequence of an allergen, about 50 overlapping peptides (Fig. **2**) are usually synthesized by a solid-phase method. These are then evaluated for their IgE-binding abilities by a method based on enzyme-linked immunosorbent assay (ELISA) using patient sera. For example, 34 overlapping 20-mer peptides (offset by 10 amino acids) covering the entire length of an allergen would be used to map the epitope of an allergen comprising 350 amino acid residues (aa 1-20, aa 11-30, aa 21-40, to be continued to 331-350). Since fewer than ten amino acid residues are generally enough to form an epitope, the overlapping peptide method can find linear/ sequential epitope(s) on the allergen comprehensively.

However, epitope mapping requires a large number of IgE-binding studies. Thus, several studies have evaluated allergenic fragments derived by limited proteolysis of allergens, especially allergens with high molecular weights. Taking BSA as an example, the experimental procedures for the determination of its B cell epitope by the limited proteolysis method [32] and another method devised by our group [27] will be described.

Beretta *et al.* performed limited tryptic proteolysis on BSA, which comprises about 580 amino acid residues, and detected eleven polypeptides liberated by enzymatic hydrolysis [32]. Four bands (Nos. 3, 4, 5, and 6) among the eleven were recognized by all four patients tested, and two bands (Nos. 11 and 12) were recognized by two patients each. Amino acid sequencing of these bands (Nos. 4, 5, 6,

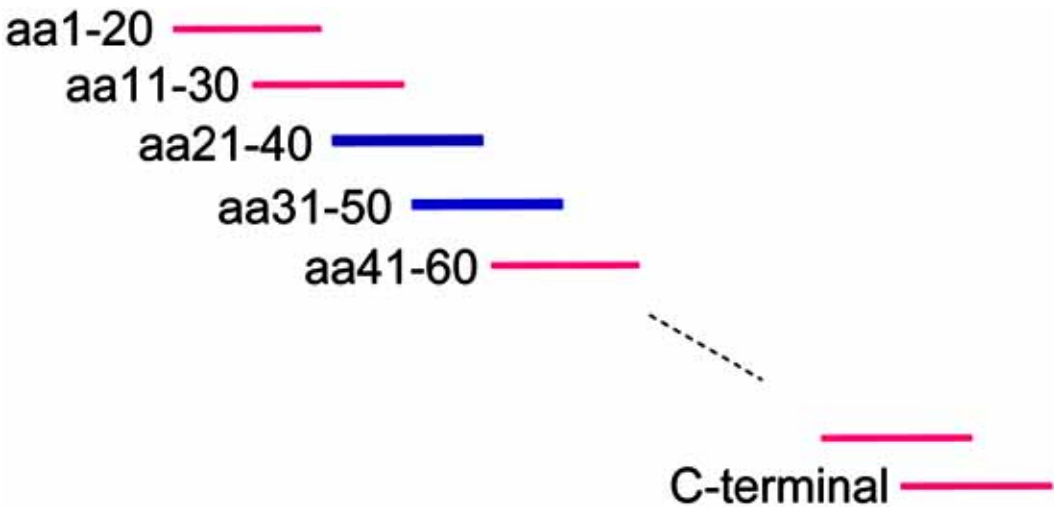

Fig. (2). Epitope mapping with overlapping peptides.
For analyses of IgE-binding and T cell epitopes, overlapping peptides are frequently synthesized and evaluated. In this figure, overlapping 20-mer peptides (offset by 10 amino acids) that cover the entire length of the allergen are shown as an example. If peptides aa21-40 and aa31-50 are positive, it is highly probable that the one of the epitope structures exists in the aa31-40 region.

and 11) revealed them to be aa 264-607, 310-607, 402-607, and 524-598, respectively. After further investigations, the authors concluded that aa 524-542 was the most critical epitopic sequence.

In the meantime, we conducted a break-through study in which we identified the precise regions of the B cell epitopes of BSA [27]. Prior to our experiments, we hypothesized that BSA-specific IgE antibodies would react primarily with sequential epitopes in which the amino acid sequences differ greatly between the bovine protein and human serum albumin (HSA). To clarify this hypothesis, sixteen peptides (Nos. 1-16) corresponding to such regions were synthesized as candidate epitopes (Table **1**). Among them, at least two regions, aa336-345 (No. 7) and aa451-459 (No. 15), were found to be major B cell epitopes (Fig. **3**). In further analyses by inhibition ELISA, EYAV (aa338-341) and LILNR (aa453-457) were found to bind to patient IgE antibodies and were to be the cores of the B cell epitopes.

Among eight IgE-reactive peptides, three peptides were found to contain EXXV motifs (HPEYAVSVLL (No. 7), PVESKVT (No. 12), and VMENFVAF (No. 15)). The corresponding sequences in HSA are HPDYSVVLLL, PVSDRVT, and VMDDFAAF, respectively. After comparing two epitopic sequences (Nos. 7 and 15) in BSA with their corresponding sequences in HSA, it appears likely that E residues (E338 and E547) are important for recognition by IgE-antibodies since the corresponding residues in HSA are D in both peptide Nos. 7 and 15. Therefore, we synthesized two analog peptides, E338D (HPDYAVSVLL) and E547D (VMDNFVAF), with amino acid substitutions from E to D (see Table **1**), and characterized their IgE-binding abilities [28]. As a result, the replacement of the glutamic acid in the EXXV sequence with aspartic acid led to a remarkable reduction in IgE-binding ability. Thus, ^{338}E and ^{547}E in BSA were thought to be important for recognition by patient IgE antibodies. In other words, the difference between D (human type) and E (bovine type) at positions 338 and 547 seems to be a major cause for the allergenicity of BSA. According to the three-dimensional structure of HSA, these two D residues are located on the surface of the molecule (Fig. **4**). Although the three-dimensional structure of BSA is not available, the corresponding E residues at positions 338 and 547 in BSA are assumed to be located similarly to the D residues in HSA, since the tertiary structures of BSA and HSA are very similar. Therefore, it is possible that IgE-antibodies in allergic patients easily recognize E residues at positions 338 and 547 on the surface of BSA, with the subsequent allergic reaction taking place [28].

In addition, recombinant proteins of an allergen and its fragment have been prepared to examine the epitopic area in the secondary and tertiary conformations. These would allow the analysis of discontinuous conformational epitopes [33], in contrast to the epitope mapping or other methods that have limited value for the identification of such epitopes.

4. T CELL EPITOPES AND IMMUNOTHERAPY

To analyze T cell epitopes, the epitope mapping method is also frequently used. For T cell analyses, peripheral blood mononuclear cells (PBMC), T cell lines (TCL) and T cell clones (TCC) from patients are required, instead of using IgE antibodies as for B cell analyses. T-cell epitope mapping studies involving binding or functional assays of multiple overlapping peptides have led to large numbers of T cell epitopes having been reported, and all allergens studied to date contain multiple T cell epitopes dispersed throughout the molecule. Since T-cell epitope mapping of an allergen requires significant experimental effort, computational methods for the prediction of T-cell epitopes have recently been reported [34].

A detailed characterization of T cell epitopes is indispensable for immunotherapy, since allergen-specific T cells are obvious targets for intervention in the disease as described below.

Table 1. Amino Acid Sequences of the Synthesized Peptides and Comparison with the Corresponding Sequences in HSA (Ref. [27], Modified)

Peptide	Sequence synthesized (amino acid position in BSA)	Corresponding sequences in HSA
No. 1	ESHAGCEKS (57-65)	ESAENCDKS
No. 2	DDSPDLPKLKPDPNTLC (107-123)	DDNPNLPRLVRPEVDVMC
No. 3	CDEFKADEKKFWGKY (123-137)	CTAFHDNEETFLKKY
No. 4	LLYANKYNGVFQEC (153-166)	LLFFAKRYKAAFTEC
No. 5	PKIETMREKVLTSS (178-191)	PKLDELRDEGKASS
No. 6	EKDAIPEDLPPLTADFAEDK (292-311)	ENDEMPADLPSLAADFVESK
No. 7	HPEYAVSVLL (336-345)	HPDYSVVLLL
No. 8	PHACYTSVFDKLKHLVDEP (364-382)	PHECYAKVFDEFKPLMEEP
No. 9	NCDQFEKLG (389-400)	NCELFEQLG
No. 10	VGTRCCTKPESERM (431-444)	VGSKCCKHPEAKRM
No. 11	LSLILNRLC (451-459)	LSVVLNQLC
No. 12	PVESKVT (466-472)	PVSDRVT
No. 13	PKAFDEKLFT (497-506)	PKEFNAETFT
No. 14	TLPDTEKQI (513-521)	TLSEKERQI
No. 15	VMENFVAF (545-552)	VMDDFAAF
No. 16	LVVSTQTAL (573-581)	LVAASQAAL
	EYAV (338-341)	
	LILNR (453-457)	
E338D	HPDYAVSVLL (336-345)	
E547D	VMDNFVAF (545-552)	

Underlined residues represent amino acid differences between BSA and HSA.

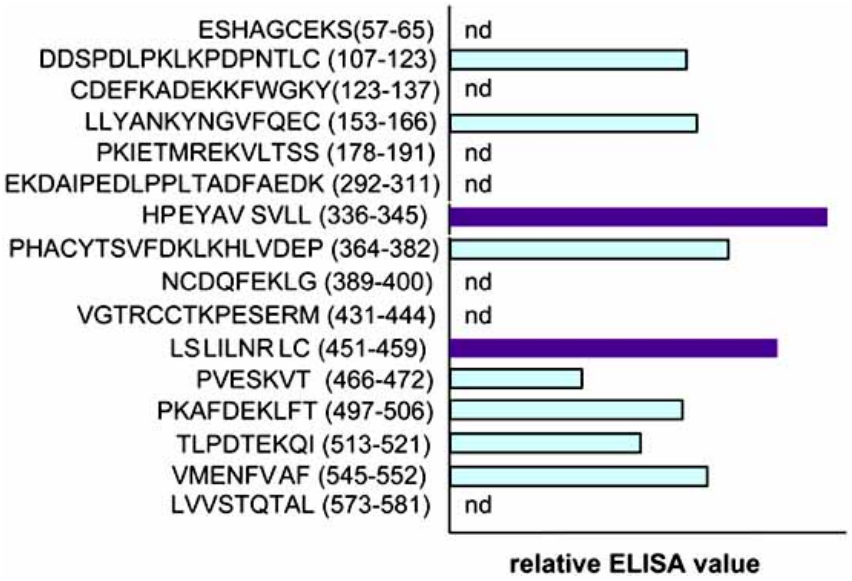

Fig. (3). IgE-binding abilities of BSA peptides.
The synthesized peptides were covalently immobilized on immunoplates and applied to ELISA using patient sera. Several peptides were found to be positive; peptide No. 7 (aa336-345) gave the highest ELISA value, and peptide No. 11 (aa451-459) gave the second highest value among them. (Tanabe *et al.* (2002) ref. [27])

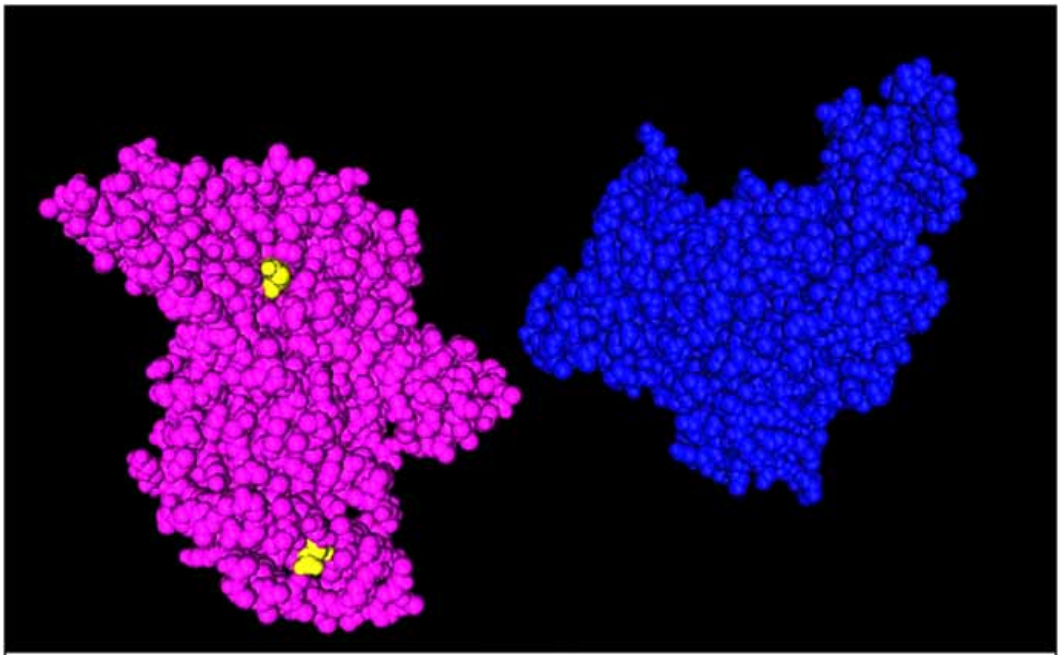

Fig. (4). Molecular structure of HSA.
The molecular structure of HSA is shown in gray, and the two aspartic acid residues (upper, D338; lower, D547) are highlighted in white. The drawing was generated with Cn3D ver. 4.1 software (www.ncbi.nlm.nih.gov). (Tanabe *et al.* (2004) ref. [28]).

4.1. Mechanisms of T Cell Epitope-Targeted Immunotherapy [1,2,35,36]

Allergen-specific immunotherapy (SIT) involves the administration of native allergen extracts to modify or abolish allergic symptoms. The process is specific, in that the treatment is targeted at those allergens recognized by the patient. Usually, patients receive a course of injections, starting with a very low dose of allergen, and building up gradually through a series of weekly injections until a plateau or maintenance dose is achieved. Maintenance injections are then given at 4-6 weekly intervals for 3 to 5 years [35].

Several mechanisms have been proposed to explain the beneficial effects of immunotherapy, including the induction of IgG (blocking) antibodies, reduction in specific IgE over the long term, reduced recruitment of effector cells, altered T cell cytokine balance (shift from Th2 to Th1), T cell anergy, and the induction of regulatory T cells [35]. Although the mechanisms have not been fully clarified, there is general agreement that allergen-specific T-cell responses are affected by SIT. Both in the skin and in the nose, successful SIT is accompanied by a reduction in allergen-specific T-cell and eosinophil recruitment in response to allergen challenge. In parallel, there is a shift in the balance of expression of T helper 1 (Th1) cytokines (such as interferon γ) and Th2 cytokines (such as IL- 4 and 13). Since cytokines elaborated by the Th2 subset of T lymphocytes govern the production of IgE antibodies, altered cytokine balance from Th2 to Th1 may contribute to the treatment of allergic symptoms. Venom SIT induces regulatory T cells that produce IL-10, which is regarded as an immunosuppressive or regulatory cytokine [37]. Similar findings have also been reported following SIT with inhalant allergens [38].

However, adverse reactions occur occasionally during SIT with native allergens as a result of the binding of injected allergens to IgE bound to mast cells and basophils (Fig. **1-D**). In this regard, since short linear peptide sequences generally lack the ability to crosslink IgE molecules on mast cells and basophils, a particular advantage of synthetic T cell epitopes (section 4.2) in allergic disease is the avoidance of IgE-mediated adverse reactions. Other new forms of immunotherapy have been devised using modified forms of allergens designed to elicit a Th1-pattern cytokine response (section 4.3).

4.2. Examples of Immunotherapy Using T Cell Reactive Peptides

4.2.1. Bee Venom Hypersensitivity

SIT for hypersensitivity to bee venom is of proven efficacy [39,40]. In particular, immunotherapy with peptides containing major T cell epitopes of the major bee venom allergen phospholipase A2 (PLA2, Api m 1) provides a safe and effective strategy without allergic side effects. Five patients with IgE-mediated systemic allergic reactions to bee stings were treated with a mixture of three T cell PLA2 epitope peptides [39]. Increasing doses of the peptide mixture were administered subcutaneously over a period of 2 months. The patients were then challenged with PLA2, and 1 week later, with a bee sting, and the cellular and humoral immune responses were measured in vitro. There were no allergic side effects caused by the peptide immunotherapy, and all patients tolerated the challenge with PLA2 without showing systemic allergic symptoms. After peptide immunotherapy, specific proliferative responses to PLA2 and the peptides in PBMCs were successfully decreased in the treated patients, and the production of Th1 and Th2 cytokines was inhibited [39].

However, two patients developed mild systemic allergic reactions after the bee sting challenge, maybe because the restricted set of T cell epitope peptides was not sufficient due to genotype variety among patients. This may be overcome by using overlapping peptides (aa 1-60, 47-99, and 90-134) covering entire 134 amino acid sequence of PLA2 [40]. The results of a double-blind, placebo-controlled study have demonstrated that overlapping peptide-based SIT is safe and able to induce Th1-type immune deviation, allergen-specific IL-10 production, and T-cell hyporesponsiveness. Thus, it was concluded that long overlapping peptides, which offer the advantage of covering all possible T-cell epitopes for any genotype, should be considered for a novel and safe approach to SIT [40]. However, because of the length of these peptides, there may be a risk that one will possess at least two B cell epitopes, thus raising the possibility of IgE-crosslinking.

4.2.2. Cat Allergy

Several groups have attempted to treat cat-allergic patients by subcutaneous injection of two peptides (IPC1 and IPC2) that span a large proportion of chain 1 of Fel d 1, a major cat allergen [41-43]. In these studies, IPC1 and IPC2 were effective at high doses, but the administration of these peptides was associated with some allergic symptoms. Because of the length of this peptide (27 amino acid residues), the immediate reactions induced by IPC1 and IPC2 may have been the result of the presence of at least two B cell epitopes and IgE-crosslinking [44]. Alternatively, Oldfield *et al.* chose to use twelve overlapping Fel d 1-derived peptides (16-17 residues) spanning most of chain 1 and chain 2, and performed a double-blind, placebo-controlled study [44]. These smaller peptides inhibited both the early- and late-phase skin reactions to whole cat allergen and improved tolerance to cats. This was associated with a decrease in IL-4, IL-13, and IFN-γ production, and an increase in IL-10 from PBMC cultures. Very recently, the same group reported that treatment with these kinds of peptides results in a recruitment of IFN-γ^+ Th1 cells to the sites of allergen-induced late-phase skin reactions in allergic subjects [45].

4.2.3. Pollinosis

Several Japanese groups have tried peptide-based SIT for Japanese ceder pollinosis using polypeptides in which several T cell epitopes of Cry j 1 and Cry j 2 are linked together [46,47]. Sone *et al.* [46] designed a recombinant polypeptide (Cry-consensus) containing the major five T cell epitopes (aa 106-120 and aa 211-225 from Cry j 1 and aa 66-80, aa 182-200, and aa346-360 from Cry j 2). Separately, Hirahara *et al.* [47] also prepared a hybrid peptide comprising seven T cell epitopes (aa 212-224, aa 235-247, and aa 312-330 from Cry j 1 and aa 77-89, aa 96-107, aa 192-204, and aa356-367 from Cry j 2). Since these recombinant polypeptides were recognized by PBMCs from patients more strongly than individual peptides, they would be candidates for the management of pollinosis.

4.3. Analog Peptides or Recombinant Allergens for Immunotherapy

Various groups have investigated the use of analog peptides of T cell epitopes for not only allergic but also autoimmune diseases [2, 28, 48]. Such peptides share T cell epitopic characteristics with the native peptide sequence but, as a result of amino acid substitutions, deliver antagonist or partial agonist signals, modifying T cell activation and cytokine production [2]. For example, a single amino acid substitution of ^{339}Thr to Val on peptide aa 335-346 of Cry j 1 resulted in a significant increase in IFN-γ, with no remarkable change in IL-4 production [48]. Our preliminary results [28] also demonstrated that substitution of the glutamic acid in the EXXV sequence of BSA (section 3) to aspartic acid induced potent T cell proliferation (Fig. **5-A**). Moreover, in response to this substitution, the INF-γ:IL-4 ratio was altered by selectively enhancing INF-γ secretion in patient PBMCs (Fig. **5-B**).

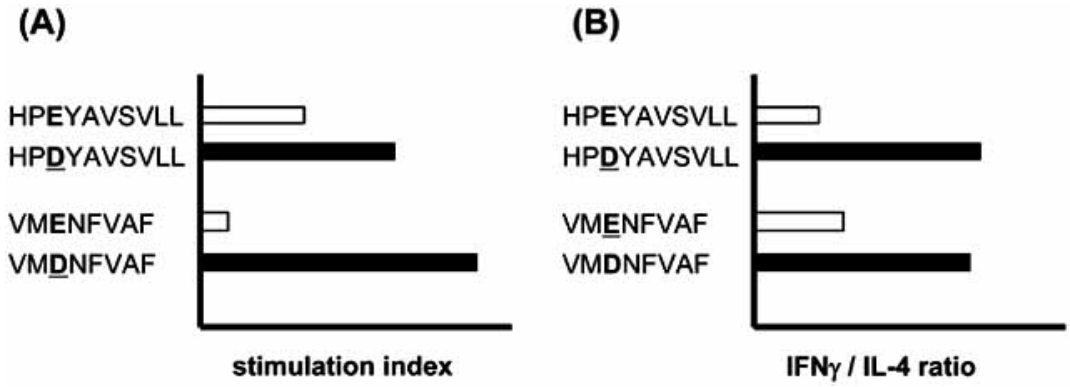

Fig. (5). (A) Lympho-proliferation assay of BSA peptides and their analogs.
PBMCs from patients were stimulated with BSA peptides or their analogs (50 μM) for 72 hrs, and 5-bromo-2'-deoxyuridine incorporation was measured. The substitutions of E to D induced potent T cell proliferation.
(B) Cytokine secretion by PBMCs following incubation with BSA peptides and their analogs.
PBMCs from the same patients were stimulated with BSA peptides or their analogs. Supernatants were harvested 24 hrs later for the determination of IFN-γ and IL-4 concentrations, and the IFN-γ/ IL-4 ratios induced by BSA peptides and their analogs were calculated.
While the production of INF-γ induced by the analogs was markedly higher than that elicited by the native peptides, the analogs induced almost the same level of IL-4 secretion as the native peptides (data not shown). Consequently, these divergences in the cytokine profiles between the native and analog peptides are clearly reflected by the higher ratio of INF-γ:IL-4 secretion induced by the two analog peptides. (Tanabe *et al.* (2004) ref. [28]).

Gene-engineering of allergens has been also tried for SIT. Takai *et al.* [49] prepared mutant Der f 2, the major house dust allergen. One of the mutants, C8/119S, in which the disulfide bond that links the N- and C-terminal sequences of Der f 2 is disrupted, showed markedly reduced capacity to induce skin reactivity and histamine release from basophils in mite-allergic patients. At the same time, it retained T cell epitopes essential for SIT. Korematsu *et al.* [50] demonstrated that the mutant induces exclusive Th1 cell differentiation in patient PBMCs. For the major birch pollen allergen, bet v 1, recombinant bet v 1 mutants were prepared with four or nine amino acid residue substitutions [51]. These mutations induced a distortion of the IgE-binding epitopes, but still retained their protective blocking-antibody responses. These groups concluded such engineered allergens are potentially useful for safer and more effective immunotherapy for allergies.

5. THE FUTURE – FROM THERAPY TO PREVENTION

Contrary to bee venom hypersensitivity, cat allergy and pollinosis (section 4.2), the efficacy of SIT for food allergies by 'eating' T cell epitope peptides remains controversial. A peptide mixture of the major peanut allergen, Ara h 2, is currently being evaluated in a mouse model of peanut allergy. Pretreatment of the peptide mixture has been shown to prevent anaphylactic reactions in peanut-sensitized mice [52]. However, further analyses are needed to clarify the efficacy.

Several years ago, our group succeeded in producing hypoallergenic wheat flour by hydrolyzing IgE-binding epitope structures using food-usable enzymes [53]. Wheat proteins were hydrolyzed into small peptides, and so lacked of IgE-crosslinking activity. At the same time, the material flour was unfortunately changed to a batter state due to the hydrolysis of the gluten, and this characteristic limits its use as a material for food processing. To solve this problem, we gelatinized the starch that remained in the hypoallergenic batter. The viscosity of the batter was increased by gelatinization, and the resulting product was more easily handled. The partially and/or exhaustively gelatinized batters are suitable for making cupcakes, pizza, cookies, wafers, pasta-like noodles and puffed items [53], and are now supplied to patients. Next, we clarified the safety and usefulness of the hypoallergenic flour in 15 patients with atopic dermatitis and wheat allergy [54]. All patients had a history of severe urticaria when cereal-based products were ingested. Among them, 13 patients showed no adverse reaction after consuming the hypoallergenic flour. The results of provocation by the hypoallergenic cupcake produced a immediate positive reaction (severe urticaria) in only two patients. Therefore, the hypoallergenic flour is safe for most patients with wheat allergy [54].

In the meantime, we demonstrated that there are T-cell reactive peptides in the hypoallergenic flour. Actually, by taking the hypoallergenic flour over a long period (more than one year), some patients were hyposensitized and became able to eat normal wheat products (Fig. **6**). This suggests that the hypoallergenic flour can act as an anti-allergenic via SIT. A more sophisticated study is currently under way to clarify the precise mechanisms of the anti-allergic effect of our hypoallergenic flour.

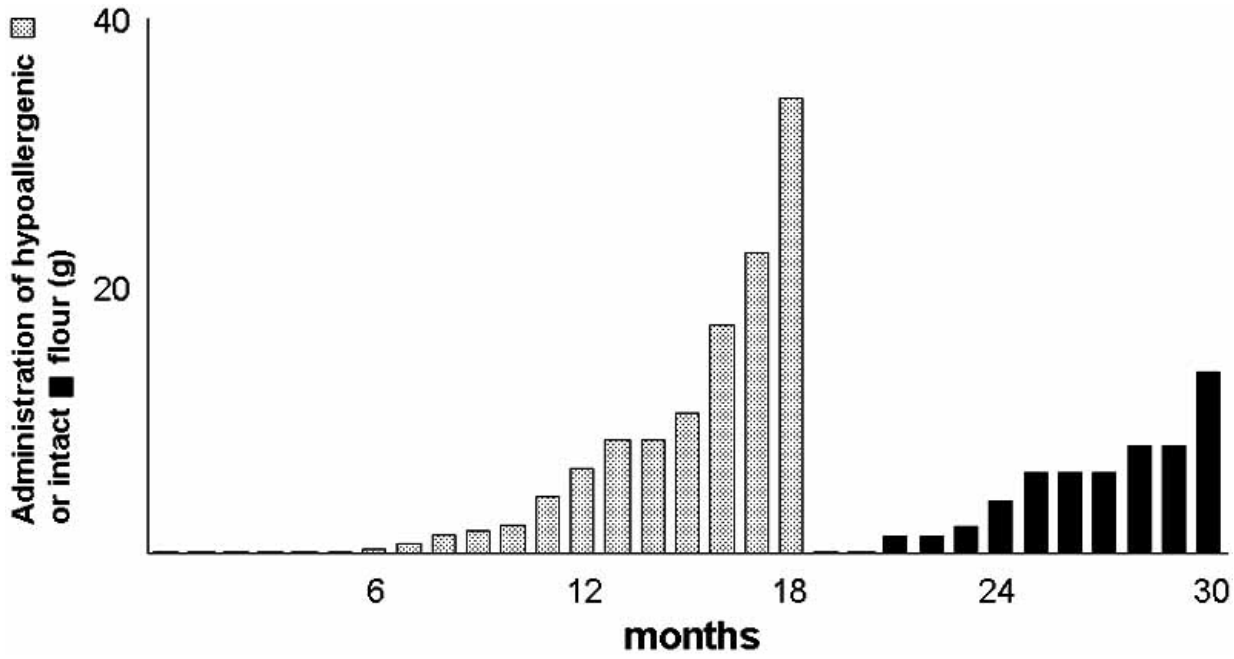

Fig. (6). An example of our clinical trial of immunotherapy for food allergy - The case of a ten-year-old boy suffering from severe wheat allergy.

The patient received a course of weekly administrations, starting with a very low amount of hypoallergenic flour, and building up gradually over 18 months. After the administration of the hypoallergenic flour, he then received a course of increasing amounts of intact flour in the same manner without any severe side effects (unpublished data). Peripheral blood was obtained every month for the measurement of the IgE value and so on.

In concluding, it is important to mention perspective. Although immuno'therapy' using peptides as medicines, or 'vaccines', is mainly emphasized in this article, the importance of 'preventing' allergies in daily life should be greatly stressed. In this regard, as described above, the treatment of allergies (including food allergies) by 'eating' T cell epitopes deserves much close attention. This method has an advantage over medicine, not only in convenience for patients, but also because of the practicality in the production of materials; this could change the direction of immunotherapy. Continuous research on the bioavailability of orally-administered T cell epitopes would strengthen this proposition. Food science and technology will make a pertinent contribution to the future.

ABBREVIATIONS USED

APC = Antigen presenting cells

BSA = Bovine serum albumin

ELISA = Enzyme-linked immunosorbent assay

FcεRI = High-affinity IgE receptor

HSA = Human serum albumin

IgE = Immunoglobulin E

IL = Interleukin

MHC = Major histocompatability complex

PBMC = Peripheral blood mononuclear cells

PLA_2 = Phospholipase A_2

SIT = Allergen-specific immunotherapy

TCC = T cell clone

TCL = T cell line

TCR = T cell receptor

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