

Intravenous injection of major and cryptic peptide epitopes of ribotoxin, Asp f 1 inhibits T cell response induced by crude *Aspergillus fumigatus* antigens in mice[☆]

Elena Svirshchevskaya^{a,*}, Ella Frolova^a, Ludmila Alekseeva^b, Olga Kotzareva^a,
Viswanath P. Kurup^c

^aDepartment of Immunology, Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, RAS, Miklukho–Maklaya str, 16/10,
117871 Moscow, Russia

^bLaboratory of Peptide Chemistry, Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, RAS, Miklukho–Maklaya str, 16/10,
117871 Moscow, Russia

^cDepartment of Medicine, The Medical College of Wisconsin, Milwaukee, V.A. Medical Center, Milwaukee, WI 53295-1000, USA

Received 22 July 1999; accepted 4 October 1999

Abstract

Aspergillus fumigatus, a ubiquitous fungus, is implicated in the pathogenesis of a number of clinically different allergic diseases in man. Peptide-based immunotherapy may offer an alternative in patient care and management. The purpose of this study was to evaluate the role of T cell epitopes of *A. fumigatus* ribotoxin, Asp f 1 in inducing tolerance in mice exposed to *A. fumigatus* antigen. The epitope analysis in BALB/c mice using synthetic peptides of Asp f 1 demonstrated both cryptic and dominant epitopes detected from 42 through 54 and 155 through 167 aa, accordingly. Intravenous injection of these peptides markedly inhibited the response induced by the exposure to crude *A. fumigatus* extract in mice as evidenced by the in vitro interleukin-2 (IL-2) production and proliferation of T-lymphocytes. Cytokine transcription studies indicate that, when stimulated with the peptides in immunogenic conditions, the major peptide (aa 155–167) specific T cell clone produced only IFN- γ , but not IL-4. The ability of both dominant and cryptic peptide epitopes of a single molecule to induce tolerance against the immune response to a multi-molecular allergen complex has significant implication for peptide-based immunotherapy. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Allergy; *Aspergillus fumigatus*; Peptide-induced tolerance; Immunotherapy

1. Introduction

Aspergillus fumigatus, a ubiquitous fungus, is implicated in the pathogenesis of a number of clinically different diseases in man ranging from saprophytic colonization, hypersensitivity diseases to systemic and local invasive infections. The hypersensitivity diseases caused by *A. fumigatus* include asthma, allergic bronchopulmonary aspergillosis (ABPA) and hypersensitivity pneumonitis [5,18,32]. Among the allergic diseases caused by *A. fumigatus*, ABPA

is the most difficult to treat. This may be partly because of the role of underlying and associated condition such as asthma and cystic fibrosis, and due to the complex immunopathogenesis of ABPA. The demonstration of *A. fumigatus* specific IgE and IgG antibodies has been used as a significant parameter in the diagnosis of ABPA. Although crude *A. fumigatus* antigens have been used to demonstrate the specific antibodies in the sera of patients, in recent years recombinant allergens from *A. fumigatus* have shown potential usefulness in the antibody assays due to their specificity and sensitivity [3,9]. The IUIS lists 18 allergens from *A. fumigatus*. Patients with active ABPA exhibit IgG and IgE reactions with up to 12 major *A. fumigatus* allergens, including Asp f1, f2, f3, f4, f6, and f12 [1,2,4,19,28]. Patients in remission react weakly to fewer than five major *A. fumigatus* allergens [33]. It has also been reported that IgE against secreted *A. fumigatus* proteins Asp f 1 and f 3 were

[☆] Supported by grant 97-04-48722 from Russian Foundation for Basic Research and by the National Institute of Health (AI42349) and by the Veterans Affairs Medical Research.

* Corresponding author. Tel.: +7-095-330-4011; fax: +7-095-335-7103.

E-mail address: esvir@ibch.siocb.ras.ru (E. Svirshchevskaya)

detected during the early phase of the disease [19,24]. If the initial sensitization starts with these secreted proteins, it is reasonable to assume that the immunotherapy directed against such sensitization can arrest the progression of the disease thereby reducing or eliminating the lung damage in both ABPA and ABPA in cystic fibrosis. Immunotherapy using peptides from these early appearing secreted molecules, such as *Asp f 1* and *Asp f 3*, could ameliorate the immune responses and arrest the progress of allergy resulting from exposure and sensitization to other *A. fumigatus* molecules and, thus, abrogate ABPA development.

Early studies using human and mouse T cell clones showed that interaction of the T cell receptor with antigen in the absence of co-stimulation did not result in activation of the cells, but rather led to a state of nonresponsiveness or anergy during which the cells no longer proliferated or produced IL-2 following stimulation with antigen [25]. Several in vitro and in vivo studies indicate that application of high doses of dominant T cell epitope can induce a state of antigen-specific nonresponsiveness (anergy) [16,37]. For efficient T cell activation two signals have to be activated. These include a T cell receptor signaling and a second signal via ligation of CD28 or CTLA-4 on T cells with CD80 or CD86 on antigen presenting cells [14,34]. Anergy is induced when only one signal is operational. For example it was demonstrated that administration of one immunodominant peptide from cat allergen *Fel d 1* [6] and house dust mite allergen *Der p 1* [21] in naive and primed mice inhibited T cell reactivity as well as specific IgG antibody production. Treatment with T cell peptides could therefore make CD4⁺ Th2 cells tolerant and at the same time make specific immunotherapy safer since it does not bear the potential risk of cross-linking IgE antibodies on mast cells [11]. In recent years, T cell epitopes of several major allergens from grass pollens, tree pollens, bee venom, and house dust mites have been identified [10,11,22,38,39], and tolerance was induced by immunizing with the dominant peptides from these allergens [6,27,36]. The results of these studies suggest the probable usefulness of T cell peptide immunotherapy in ABPA and other fungal allergy also. In the present study, we have evaluated dominant and cryptic T cell epitopes of *A. fumigatus* ribotoxin *Asp f 1* in a murine model. Our results from in vitro and in vivo studies indicate that the immune response to multi-component allergen can be modulated by i.v. injection of synthetic peptides representing dominant and cryptic T cell epitopes from a single major molecule suggesting the feasibility of such intervention in fungal allergy.

2. Materials and methods

2.1. Animals

Ten to 12-week-old inbred BALB/c (H-2^d) mice bred at the Institute of Bioorganic Chemistry Animal Department,

Table 1
Amino acid sequences of synthetic peptides of ribotoxin *Asp f 1*

Peptide no.	Ribotoxin sequence	Amino acid sequence
Peptide 1	42–54 (13-mer)	NKWEDKRLLYSQA
Peptide 2	80–95 (16-mer)	NGYDGNGLIKGRTP
Peptide 3	114–126 (13-mer)	GKDDHYLLEFPTF
Peptide 4	155–167 (13-mer)	KVFCGIVAHQQRGN

Moscow, were used in this study. The protocol for experimental animals used was approved by the ethics committee of the Institute of Bio-organic Chemistry, RAS, Moscow, Russia.

2.2. Antigen and peptides

A. fumigatus antigen extract, cited further as crude extract, was prepared as previously described [29]. A mixture of culture filtrate and mycelial extracts of *A. fumigatus* was diluted to 5 mg/ml and stored frozen at –20°C. The peptides were synthesized using standard Fmoc chemistry [7,13,20] and purified by reverse-phase high-pressure liquid chromatography (HPLC). The identities of the synthesized peptides were verified by electrospray mass-spectrometry. The peptides from *Asp f 1* allergen were selected using a computer program developed for the prediction of H-2I^d matched motif [12,15,23]. The peptides used in this study, namely, aa 42–54, aa 80–95, aa 114–126, and aa 155–167 are listed in Table 1.

2.3. Antigen and peptide treatment

Mice (3 to 7 per group) were immunized by subcutaneous (s.c.) injection of 100 µg of crude *A. fumigatus* or 50 µg peptides in incomplete Freund's Adjuvant (IFA) in the hind footpads of each animal. Mice were sacrificed 10 days after injection with antigen and the blood was collected by cardiac puncture. The spleen and popliteal lymph nodes were obtained and single cells isolated. Animals injected similarly with PBS in IFA served as controls. Tolerance in mice was induced by a single i.v. injection of 50 µg of peptides aa 42 to 54 or aa 155 to 167 5 days before s.c. immunization with the same dose of peptides in IFA or by three repeated injections of 50 µg mixture of these peptides on Days 10, 5, and 2 before the immunization with *A. fumigatus* crude extract in IFA. Animals injected similarly with PBS and immunized with either peptides or *A. fumigatus* crude extract in IFA served as positive controls, and mice injected with PBS and immunized with PBS in IFA served as negative controls. The spleen and popliteal lymph nodes from these animals were obtained as described below.

2.4. Spleen and lymph node cell cultures

Single cells from the popliteal lymph nodes (LN) were obtained by pressing cells through a stainless steel wire

mesh. The cells were washed and cultured at 4×10^5 cells in 0.2 ml of RPMI10 medium (Gibco-BRL), at 37°C in 5% CO₂ incubator, in a 96 well flat-bottomed tissue culture plate (Linbro, UK). *A. fumigatus* crude antigen and peptides were added at various concentrations and the LN cells were incubated for 24 h to analyze for IL-2 production or for 72 h to estimate proliferation. Proliferation was studied by [³H]TdR incorporation during the last 12 h of the culture. Adherent and B cells from spleens enriched by panning procedure (rabbit anti-murine IgG serum, Institute of Bioorganic Chemistry, Moscow) and treated with Mitomycin C (40 µg/ml) (Sigma, St. Louis, MO, USA) were used as antigen presenting cells (APC) for in vitro stimulation of lymph node cells from mice immunized with the peptides.

2.5. Cytokine mRNA transcription in splenocytes

The total RNA was extracted from immune splenocytes stimulated in vitro for 1, 4, and 8 days with *Aspergillus* antigen and peptides. Two ml of splenocytes (5×10^6) was incubated in a 24-well plate (Linbro, UK) with either 50 µg/ml of *A. fumigatus* extract or 5 µg/ml of peptides. The spleen cells from the cultures were washed and the RNA was isolated by guanidine isothiocyanate phenol chloroform single step method [8]. The cDNA was prepared from the mRNA with Maloney's murine leukemic virus (MMLV) reverse transcriptase (Bion, Moscow, Russia). The specific genes were amplified by PCR using sense and anti-sense primers of IL-4 and IFN-γ as described before [17]. β-actin primers were similarly used for housekeeping control. The PCR products were run on a 2% agarosa gel and the amplified bands were visualized after staining with ethidium bromide. The size of the amplified fragments was determined by comparison with 1-Kb DNA ladder and the β-actin band.

2.6. Western blot analysis

A. fumigatus extract (50 µg per lane) was subjected to electrophoresis on a 4 to 30% gradient sodium dodecylsulfate-polyacrylamide gel (SDS-PAGE). The gels were stained with Coomassie brilliant blue for demonstrating the proteins and ascertaining their sizes. Proteins were transferred after electrophoresis onto a nitrocellulose membrane (Millipore Corp., Bedford, MA, USA) with a semidry electrotransfer system (Bio-Rad Laboratories, Richmond, CA, USA) according to manufacturer's instructions. The membranes were washed and the free protein binding sites on the membranes were blocked with TBS with 3% bovine serum albumin (BSA) (TBS-BSA) for 1 h. After washing, the membranes were incubated overnight at 4°C with 1:100 dilution of serum in TBS-BSA from mice immunized with *A. fumigatus* for IgG and IgG1 antibodies detection. After overnight incubation, membranes were washed with TBS containing 0.1% Triton X-100 (TBST) (Sigma), and incubated for 1 h with goat anti-murine IgG1- or IgG-peroxidase

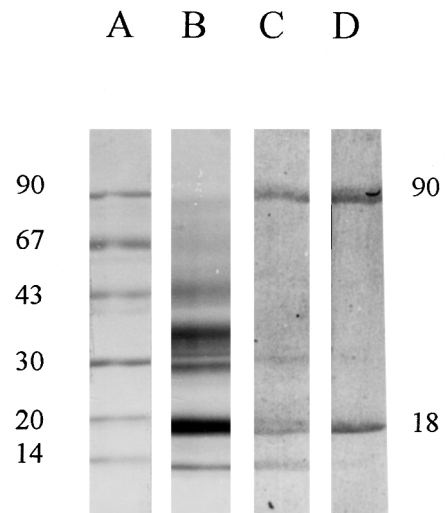


Fig. 1. SDS-PAGE and Western blot analysis of crude *A. fumigatus* extract. Shown are low molecular weight markers, kDa (lane A), SDS-PAGE profile of crude antigen (lane B), IgG reactivity (lane C), and IgG₁ reactivity (lane D) of *A. fumigatus* proteins with serum from mice immunized s.c. with 100 µg/mouse of *A. fumigatus* extract 2 weeks earlier. IgG₁-binding band with MW of 18 kDa represents *Asp f1*.

conjugate (Sigma). Membranes were washed once with TBST and twice with TBS. The peroxidase activity of the bands was detected by using 4-chloro-1-naphthol as a chromogen. The reactive bands were compared.

3. Results

3.1. Antibody response in mice immunized with *A. fumigatus* antigen

The analysis of early immune response in mice demonstrated that ribotoxin *Asp f1* is recognized by IgG1 antibodies in sera from mice immunized with crude *Aspergillus* extract. Western blots showed IgG1 binding to only two out of more than 10 *Aspergillus* antigen bands resolved whereas IgG antibodies of other subclasses recognized about five proteins (Fig. 1). IgG1 subclass of antibodies is known to be associated with IgE production, and mediates allergy even in the absence of IgE, as demonstrated in mice with a null mutation of the Cε locus [35]. There was a high molecular weight band corresponding to an unidentified protein (>90 kDa) reacting strongly with IgG1 of sera from immunized mice. The low molecular weight protein reacting with IgG1 antibodies, *Asp f1* or *Asp f3* (molecular sizes 18 and 18.5 kDa respectively) was well discernible. No IgE binding bands were observed with the sera from mice sacrificed 10 days after immunization.

3.2. T cell response to synthetic peptides of *Asp f1*

Mice immunized s.c. with *A. fumigatus* antigens were sacrificed 10 days after the injection of the antigen. The

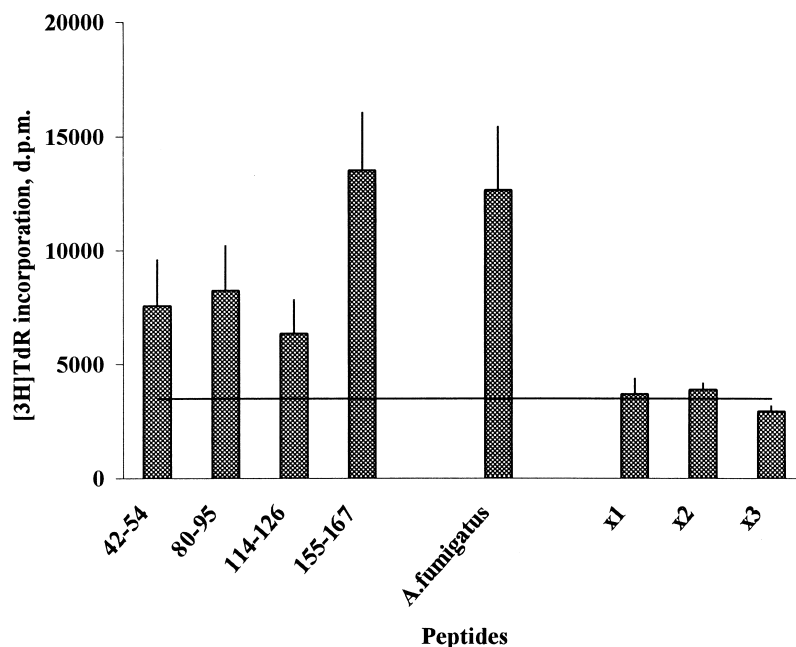


Fig. 2. Identification of T cell epitopes on *Asp f 1*. BALB/c mice (7 per group) were immunized s.c. in hind footpads with 100 μ g of crude *A. fumigatus* in IFA. On Day 10 after immunization splenocytes from individual mice were cultured with either 50 μ g/ml of crude extract or 5 μ g/ml of peptides for 72 h. The peptide 155 through 167 was able to induce proliferation in six out of seven mice and the peptide 42 through 54 induced proliferation in three out of seven mice. Each data point represents the mean $\pm$ SD responses of mice in each group. The data are shown in comparison to three nonrelevant peptides $\times 1$, $\times 2$, $\times 3$.

popliteal lymph nodes were obtained and tested for specific antigen induced lymphocyte stimulation (Fig. 2). Six of seven animals studied showed marked stimulation with peptide aa 155 through 167, whereas only three out of seven mice showed stimulation with peptide aa 42 through 54. The remaining two peptides aa 80 through 95 and aa 114 through 126 failed to show stimulation more than detected with no antigen control or irrelevant peptide control cultures. The stimulation measured as [^{3} H]thymidine uptake with peptide aa 155 through 167 showed values comparable to those obtained with *A. fumigatus* antigens stimulated T cell cultures.

To identify cryptic T cell epitopes of *Asp f 1*, mice were immunized with the peptides individually and responses to different peptides and *A. fumigatus* antigen were evaluated. As the lymph node cells are reported to be poor antigen presenting cells splenic adherent cells and B-cells were used as antigen presenting cells. The results indicate that peptides aa 155 through 167 and aa 42 through 54 are the only peptides capable of stimulating the lymph node cells (Fig. 3), but not the other two peptides.

3.3. Tolerance induction by cryptic and dominant peptides

To examine how the i.v. injection of dominant or cryptic epitope containing peptides affected the response to the same peptides, mice were injected i.v. with either aa 42 through 54 or aa 155 through 167 and after 5 days they were immunized with the same peptide in IFA (Fig. 4). The

lymph node cells were studied 10 days after the immunization with the peptides for IL-2 production. Animals injected with the dominant peptide (aa 155–167) showed marked decrease in IL-2 production ($P < 0.05$) whereas when injected with the cryptic peptide (aa 42–54) only a tendency was observed ($P < 0.1$). Animals injected with irrelevant peptides or PBS alone demonstrated no effect on IL-2 production ($P = 0.35$, data not shown). This result demonstrated a marked inhibition of the response induced by peptides in mice tolerized with a single i.v. injection of the same peptides. When mice are immunized with the peptides the response to only a single determinant is induced, whereas immunization with the whole extracts results in multi-determinant immune responses. It is likely that the tolerization signal sufficient to silence a single peptide induced immune response will not be effective at silencing the immune response to the crude *A. fumigatus* extract. It was found that only marginal effect was induced by a single peptide injection when mice were subsequently immunized with crude extract (data not shown).

To induce tolerance to crude *A. fumigatus* extract, mice were injected with a mixture of peptides aa 155 through 167 and aa 42 through 54 on 10, 5, and 2 days before immunization with *A. fumigatus* extract. Popliteal LN cells collected seven days after immunization were cultured in vitro with either a mixture of peptides aa 155 through 167 and aa 42–54 (Fig. 5A) or with crude *A. fumigatus* extract (Fig. 5B). Proliferative T cell responses from the group injected with the peptides aa 155 through 167 and aa 42 through 54

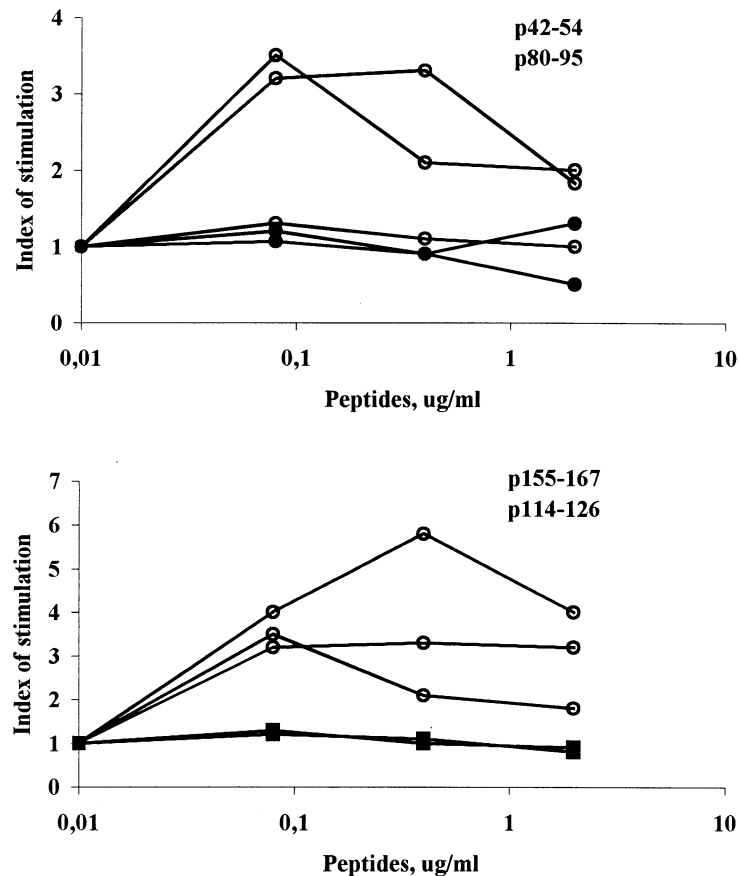


Fig. 3. Identification of cryptic epitope on *Asp f 1*. Groups (three per group) BALB/c mice were immunized with 50 μ g of peptides in IFA and 10 days later lymph node cells were cultured for 72 h with spleen adherent cells and the peptide used for immunization. Individual data are presented. The peptide p 42 through 54 (upper window, ○) was able to induce proliferation in two out of three mice immunized whereas the dominant peptide p155 through 167 (lower window, ○) was able to induce proliferation in all mice studied. The data are given in comparison with peptides p 80 through 95 ● and p 114 through 126 ■ which did not induce proliferation. The standard deviation was within 15%.

were markedly down-regulated, whereas T cells from control mice were able to proliferate when stimulated in vitro with either *A. fumigatus* extract or the peptides aa 155 through 167 and aa 42 through 54 (Fig. 5).

3.4. Cytokine mRNA transcription

To determine the type of T-helper cells recognizing 155 through 167 and 42 through 54 peptides the total RNA was extracted from splenocytes of mice immunized 10 days earlier with a crude *A. fumigatus* extract and stimulated in vitro for 1, 4, and 8 days with *Aspergillus* antigen or peptides. In this instance no tolerance was induced in mice prior the immunization, and hence normal T cell sensitization occurred. When stimulated in vitro with the extract or relevant peptides these T cells were able to produce lymphokines and proliferate. After 24 h of incubation no difference was found between cultures with or without antigens as cells activated in vivo continued to produce high levels of IL-2 and lower levels of IL-4 and IFN- γ mRNA transcriptions. On Day 4 the cells stimulated in vitro with *A. fumigatus* still produced IL-2 and IFN- γ but the amount of IL-4

greatly increased. No clear difference was seen at this time between cultures stimulated with the peptides. The mRNA for IL-4 and IFN- γ transcription in LN cells stimulated with dominant and cryptic peptides for 8 days of in vitro culture studied by PCR are shown in Fig. 6. The dominant peptide promoted the transcription of IFN- γ mRNA, but not IL-4 mRNA, whereas the lymph node cells stimulated with the cryptic epitope aa 42 through 54 demonstrated IL-4 mRNA, but not IFN- γ mRNA. The control cultures stimulated with the irrelevant peptide showed no mRNA transcription of cytokines and low level of β -actin. A high level of β -actin was observed in the experimental cultures demonstrating that only cells undergoing activation cycles were able to survive in these conditions.

4. Discussion

A. fumigatus is implicated in the pathogenesis of the hypersensitivity diseases including asthma, ABPA, and hypersensitivity pneumonitis [5,18,32]. These disorders are allergic in nature and are caused by exposure to *A. fumiga-*

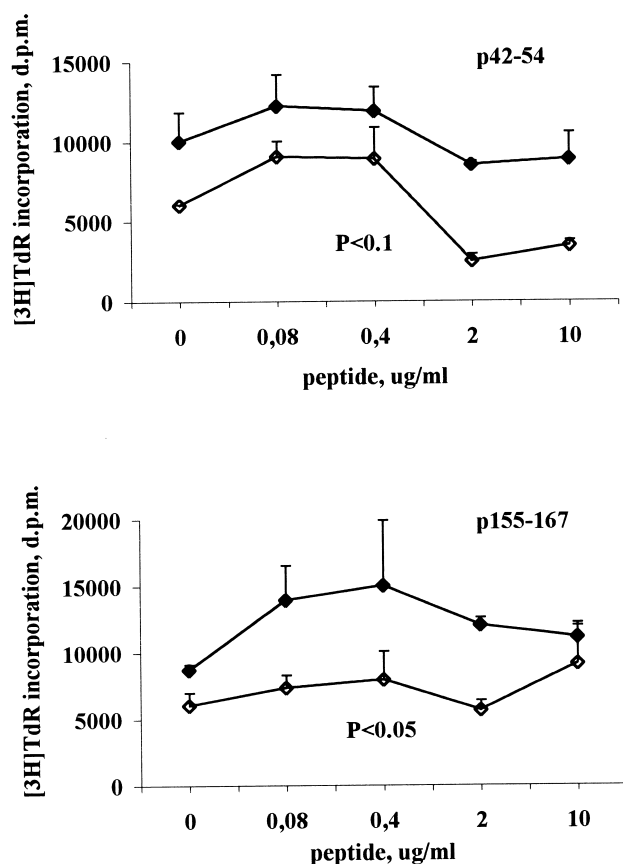


Fig. 4. Intravenous peptide injection induces tolerance to the peptide. BALB/c mice (three per group) were injected either with PBS (closed symbols) or peptides (open symbols) 5 days before immunization with the corresponding peptide. Ten days later draining lymph node cells from immunized mice were pooled and cultured in vitro with the immunizing peptide. The 24 h supernatants were assayed for IL-2 content. Each data point represents the mean $\pm$ SD response of mice in each group. The effect of i.v. injection of p 155 through 167 peptide was significant ($P < 0.05$) whereas the suppressive effect of p 42 through 54 seemed to be mainly unspecific ($P < 0.1$).

tus or related species leading to a CD4⁺ T-helper 2 (Th2) cell response. This results in the production of IL-4, IL-5, and IL-10 and the suppression of INF- γ leading to specific IgE and IgG1 expression and the inflammatory response that follows. The serum IgE response is significantly elevated in allergic asthma and ABPA, whereas both *Af*-specific serum IgE and IgG are common features of ABPA. Hypersensitivity pneumonitis demonstrates *Af*-specific serum IgG but not IgE. Moreover, the *Af*-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in mice with a null mutation of the C ϵ locus [35]. It has also been reported that IgE against secreted *A. fumigatus* proteins, *Asp f1* and *f3*, were detected during the early phase of the disease [19,24]. If the initial sensitization starts with these secreted proteins, it is reasonable to assume that the immunotherapy directed against such sensitization can arrest the progression of the disease thereby reducing or

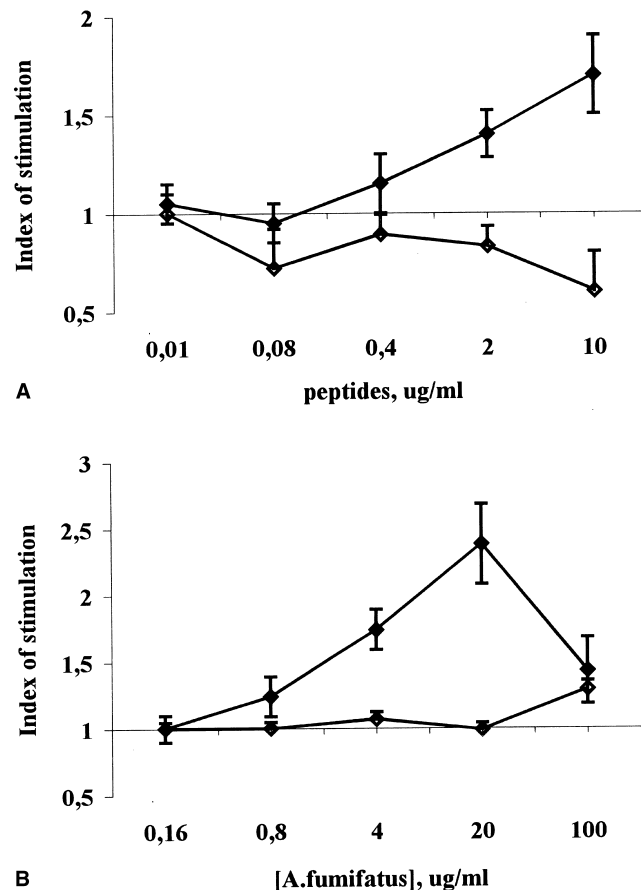


Fig. 5. Multiple i.v. injections of peptides containing both dominant and cryptic epitopes induce tolerance to crude *A. fumigatus* proteins. BALB/c mice (three per group) were injected either with PBS (closed symbols) or a mixture of peptides 42 through 54 and 155 through 167, 50 μ g of each per mouse (open symbols) on Days 10, 5, and 2 before immunization with 50 μ g of crude *A. fumigatus* in IFA. Seven days later draining lymph node cells were cultured in vitro with a mixture of peptides 42 through 54 and 155 through 167 (A) or *A. fumigatus* extract (B) for 72 h and the proliferation was tested. Each data point represents the mean $\pm$ SD response of mice in each group.

eliminating the lung damage in both ABPA and ABPA in cystic fibrosis.

To determine what *A. fumigatus* proteins are recognized by allergy associated antibody subclasses IgE and IgG1 in murine model during early immune response, we have immunized BALB/c mice with crude extract, and IgG, IgG1, and IgE content was studied two weeks later. Three major IgG1 bands with MW of 18, 18.5, and 90 kDa were found whereas other IgG subclass antibodies bound proteins with MW of 14, 18, 18.5, 30, 37, and 90 kDa (Fig. 1). The band of 18 kDa corresponds to ribotoxin *Asp f1*, demonstrating that *Asp f1* is a major protein taking part in mice sensitization, as it is also observed in men [19,24]. Other proteins could be immunogenic in the extract since blotting technique primarily detects linear epitopes on denatured proteins, but molecules whose linear epitopes are recognized earlier during immune response are more immunogenic.

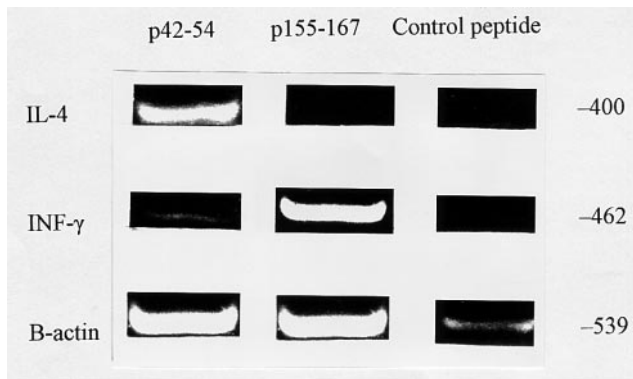


Fig. 6. Peptide-induced mRNA production. BALB/c mice were immunized s.c. in hind footpads with 100 μ g of crude *Asp f* in IFA and 8 days later splenocytes were cultured with either 50 μ g of crude *A. fumigatus* extract or 5 μ g/ml peptides. After 8 days of cultivation mRNA was extracted and analyzed using RT-PCR. The peptide p 155 through 167 was able to support IFN- γ mRNA transcription whereas peptide 42 through 54 induced IL-4 mRNA. The data from a single representative experiment are shown. This experiment has been performed on three occasions and all experiments have given equivalent results.

Later, after repeated immunizations, epitopes on other proteins, both conformational and linear, could be recognized.

To study if we can induce tolerance to a crude allergen using dominant epitopes of a single, major, early recognized, protein from multi-component allergen we identified dominant epitopes of *Asp f*1. The epitope analysis using synthetic peptides of *Asp f*1 demonstrated both cryptic and dominant epitopes detected from 42 to 54 aa and 155 through 167 aa, accordingly. The i.v. administration of synthetic peptides from *A. fumigatus* ribotoxin *Asp f*1 representing one dominant and one cryptic epitopes inhibited the T cell responses to s.c. immunization with the whole multi-component crude antigen of *A. fumigatus*. The lymph node T cells of mice tolerized and then immunized with crude *A. fumigatus*, when stimulated in vitro with the peptides or *A. fumigatus* crude extract showed depressed IL-2 release and decreased proliferation of the cells. This inhibition was directed to the multi-component extract of *A. fumigatus* consisting of several other major proteins in addition to the targeted *Asp f*1 molecule. The decrease in antigen-dependent IL-2 release during the induction of tolerance can be explained by in vivo “silencing” of the dominant and cryptic peptide epitope binding T cells. The encounter of these T cells with the MHC II/peptide complex without costimulatory molecules such as CD80 or CD86 present on activated APC leads to tolerance [14,34]. This type of antigen presentation is thought to be non-immunogenic and leads to induction of anergy in peptide-specific T cells. These anergized T cells remain immunologically unresponsive even when the antigens are presented to these in the usual manner. It is also important to note that the suppression can be induced by “silencing” an immune response to only one major molecule from multi-molecule allergen complex.

Our results indicate that peptides representing both dom-

inant and cryptic epitopes are able to induce tolerance. This is in agreement with the findings reported in [22], where feeding the fusion peptide GEX p131 through 187 present in the allergen of the house dust mite Der p I containing the cryptic epitopes p 144 through 169 and p 120 through 143 significantly inhibits the immune response to the whole antigen. The degree of inhibition induced by ‘cryptic’ peptide was not always as marked as that for the peptides containing dominant epitopes, but presumably could be increased by extending the feeding regimen or increasing the dose.

Allergic immune response is mediated by type 2 helper cells (Th2), which produce IL-4 and lead to B-cell switch to IgE producing plasma cells. This mechanism has been shown in both human diseases and in animal models [26, 30]. Of particular interest is the finding that the dominant peptide epitope of *Asp f*1 aa 155 through 167 is recognized with a Th1 like T cell response, producing IFN- γ , but not IL-4. This type of Th1 and Th2 polarized responses have been reported with specific epitopes studied using T cells from ABPA patients and in murine model of ABPA [30,31]. There may be other possible dominant epitopes on *Asp f*1 capable of inducing a Th2 response as high levels of IgE is demonstrable in the sera of ABPA patients for which a strong Th2 switch is essential. In conclusion, peptide aa 155 through 167 specific T cell clones have a regulatory role and may be of potential use in redirecting immune response toward a Th1 type rendering from allergy.

References

- [1] Arruda KL, Mann BJ, Chapman MD. Selective expression of a major allergen and cytotoxin, *Asp F*1, in *Aspergillus fumigatus*. Implications for the immunopathogenesis of *Aspergillus*-related diseases. *J Immunol* 1992;149:2385–90.
- [2] Arruda KL, Platts-Mills TAE, Fox JW, Chapman MD. *Aspergillus fumigatus* allergen I, a major IgE-binding protein, is a member of the mitogillin family of cytotoxins. *J Exp Med* 1990;172:1529–32.
- [3] Banerjee B, Kurup VP. Molecular biology of *Aspergillus* allergens. In: Kurup VP, Apter AJ, editors. Allergic Bronchopulmonary Aspergillosis, Vol. 18. *Immunol Allergy Clin N Am* 1998:601–18.
- [4] Banerjee B, Kurup VP, Greenberger PA, Hoffman DR, Deepu SN, Fink JN. Purification of a major allergen, *Asp f*2 binding to IgE in allergic bronchopulmonary aspergillosis, from culture filtrate of *Aspergillus fumigatus*. *J Allergy Clin Immunol* 1997;99:821–7.
- [5] Bateman ED. A new look at the natural history of aspergillus hypersensitivity in asthmatics. *Resp Med* 1994;88:325–7.
- [6] Briner TJ, Kuo MC, Keating KM, Rogers BL, Greenstein JL. Peripheral T cell tolerance induced in naive and primed mice by s.c. injection of peptides from the major cat allergen, *Fel d*1. *Proc Natl Acad Sci USA* 1993;90:7608–12.
- [7] Chang CD, Meisenhofer J. Solid-phase peptide synthesis using mild base cleavage of N9-fluorenyl methyloxycarbonylamino acids exemplified by a synthesis of dihydrosomatostatin. *Int J Pept. Res* 1978; 137:179–83.
- [8] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem* 1987;162:156.

- [9] Cramer R. Recombinant *Aspergillus fumigatus* allergens: from the nucleotide sequences to clinical application. *Int Arch Allergy Immunol* 1998;115:99–114.
- [10] Dhillon M, Roberts MC, Nunn T, Kuo M. Mapping human T cell epitopes on phospholipase A2: the major bee-venom allergen. *J Allergy Clin Immunol* 1992;90:42–51.
- [11] Ebner C, Szepefalusi Z, Ferreira F, et al. Identification of multiple T-cell epitopes on Bet v 1, the major birch pollen allergen using specific T-cell clones and overlapped peptides. *J Immunol* 1993;150:1047–54.
- [12] Feller DC, de la Cruz VF. Identifying antigenic T-cell sites. *Nature* 1991;349:720–1.
- [13] Fields GB, Noble RL. Solid phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids. *Int J Pept Protein Res* 1990;35:161–214.
- [14] Freeman GJ, Freeman G, Boussiotis VA, Ng JW, Restivo VA Jr, Lombard A, Gray GS, Nadler LM. B7 a B cell restricted antigen that identifies preactivated B cells. *J Immunol* 1987;139:3260–7.
- [15] Gammon G, Geysen M, Apple RJ, et al. T cell determinant structure: core and determinant envelopes in three mouse major histocompatibility complex haplotypes. *J Exp Med* 1991;173:609–17.
- [16] Gaur A, Wiers B, Lin A, Rothbard J, Fathman CG. Amelioration of autoimmune encephalomyelitis by myelin basic protein synthetic peptide-induced anergy. *Science* 1992;258:1491–4.
- [17] Granucci F, Girolomoni G, Lutz MB, et al. Modulation of cytokine expression in mouse dendritic cell clones. *Eur J Immunol* 1994;24(10):2522–6.
- [18] Hearn VM. Antigenicity of *Aspergillus Fumigatus*. *J Med Vet Mycol* 1992;30:11–25.
- [19] Hemmann S, Nikolaizik W, Schoni M, Blaser K, Cramer R. Differential IgE recognition of recombinant *Aspergillus fumigatus* allergens by cystic fibrosis patients with allergic bronchopulmonary aspergillosis or *Aspergillus* allergy. *Eur J Immunol* 1998;28:1155–60.
- [20] Houghton RA. General method for the rapid solid-phase synthesis of large numbering of peptides: specificity of antigen antibody interaction at the level of individual amino acids. *Proc Natl Acad Sci USA* 1985;82:5131–35.
- [21] Hoyne GF, Callow MG, Kuo MC, Thomas WR. Inhibition of T-cell responses by feeding peptides containing major and cryptic epitopes: studies with the *Der p I* allergen. *Immunology* 1994;83:190–5.
- [22] Hoyne GF, O'Hehir R, Wraith DC, Thomas WR, Lamb JR. Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. *J Exp Med* 1993;178:1783–8.
- [23] Hunt DF, Michel H, Dickinson TA, et al. Peptides presented to the immune system by the murine class II major histocompatibility complex molecule I-A^d. *Science* 1992;256:1817–20.
- [24] Hutcheson PS, Rejent AJ, Slavin RG. Variability in parameters of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *J Allergy Clin Immunol* 1991;88:390–4.
- [25] Jenkins MK, Schwartz RH. Antigen presentation by chemically-modified splenocytes induced antigen-specific T cell unresponsiveness in vitro and in vivo. *J Exp Med* 1987;167:302.
- [26] Knutsen AP, Mueller KR, Levine AD, Chouhan B, Hutchenson PS, Slavin RG. Asp f 1 CD4+ TH2-like T-cell lines in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1994;94:215–21.
- [27] Ku G, Kronenberg M, Peacock DJ, et al. Prevention of experimental autoimmune arthritis with a peptide fragment of type II collagen. *Eur J Immunol* 1993;23:591–9.
- [28] Kumar A, Reddy LV, Sochanik A, Kurup VP. Isolation and characterization of a recombinant heat shock protein of *Aspergillus fumigatus*. *J Allergy Clin Immunol* 1993;91:1024–30.
- [29] Kurup VP, Semour BW, Choi H, Coffman RL. Particulate *Aspergillus fumigatus* antigens elicit a Th2 response in BALB/c mice. *J Allergy Clin Immunol* 1994;93:1013–20.
- [30] Kurup VP, Banerjee B, Murali PS, Greenberger PA, Krishnan M, Hari V, Fink JN. Immunodominant peptide epitopes of allergen, *Asp f 1* from the fungus *Aspergillus fumigatus*. *Peptides* 1998;19:1469–77.
- [31] Kurup VP, Hari V, Guo J, Murali PS, Resnick A, Krishnan M, Fink JN. *Aspergillus fumigatus* peptides differentially express Th1 and Th2 cytokines. *Peptides* 1996;17:183–90.
- [32] Kurup VP, Kumar A. Immunodiagnosis of aspergillosis. *Clin Microbiol Rev* 1991;4:439–56.
- [33] Leser C, Kauffman HF, Virchow C, Menz G. Specific serum immunopatterns in clinical phases of allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1992;90:589–99.
- [34] Linsley PS, Clark EA, Ledbetter JA, T cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. *Proc Natl Acad Sci USA* 1990;87:5031–5.
- [35] Mehlhop PD, van de Rijn M, Goldberg AB, et al. Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc Natl Acad Sci USA* 1997;94:1344–9.
- [36] Metzler B, Wraith DC. Inhibition of experimental autoimmune encephalomyelitis by inhalation but not oral administration of the encephalitogenic peptide: influence of MHC binding affinity. *Int Immunol* 1993;5:4184–94.
- [37] O'Hehir RE, Garman RD, Greenstein JL, Lamb JL. The specificity and regulation of T cell responsiveness to allergens. *Ann Rev Immunol* 1991;9:67–95.
- [38] Schenk S, Breiteneder H, Susani M, et al. T cell epitopes of Ph1 p 1, major allergen of timothy grass (*Phleum pratense*). Evidence for crossreacting and non-crossreacting T cell epitopes within grass group I allergens. *J Allergy Clin Immunol* 1995;98:986–98.
- [39] Spiegelberg HL, Beck L, Stevenson D, Ishioka GY. Recognition of T cell epitopes and lymphokine secretion by rye-grass allergen Lolium perenne-specific human T cell clones. *J Immunol* 1994;152:4203–10.