

A major allergen gene-fusion protein for potential usage in allergen-specific immunotherapy

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Background: Specific immunotherapy is a common treatment of allergic diseases and could potentially be applied to other immunologic disorders. Despite its use in clinical practice, more defined and safer allergy vaccine preparations are required. Differences between epitopes of IgE that recognize the 3-dimensional structure of allergens and T cells that recognize linear amino acid sequences provide a suitable tool for novel vaccine development for specific immunotherapy.

Objective: The aim of the study was to delete B-cell epitopes and prevent IgE crosslinking, but to preserve T-cell epitopes by fusion of 2 major allergens of bee venom because of a change in the conformation.

Methods: By genetic engineering, we produced a fusion protein composed of the 2 major bee venom allergens: phospholipase A₂ (Api m 1) and hyaluronidase (Api m 2).

Results: The Api m [1/2] fusion protein induced T-cell proliferation and both T_H1-type and T_H2-type cytokine responses. In contrast, IgE reactivity was abolished, and profoundly reduced basophil degranulation and type 1 skin test reactivity was observed. Pretreatment of mice with Api m [1/2] fusion protein significantly suppressed the development of specific IgE as well as other antibody isotypes after immunization with the native allergen.

Conclusion: The novel fusion protein of 2 major allergens bypasses IgE binding and mast cell/basophil IgE FcεRI crosslinking and protects from IgE development. (J Allergy Clin Immunol 2005;115:323-9.)

Key words: Specific immunotherapy, allergy, bee venom, T cells, IgE

Abbreviations used

BV: Bee venom
PCA: Passive cutaneous anaphylaxis
SIT: Specific immunotherapy
SPT: Skin prick test

The symptoms of immediate and late-type allergic reactions can be ameliorated by pharmacotherapy, but specific immunotherapy (SIT) remains the only causative approach.¹ SIT is based on the administration of increasing amounts of disease-eliciting allergens in the form of allergen-containing extracts. The disadvantage with allergen extracts is that they consist of insufficiently characterized allergen mixtures that are not designed for a patient's individual allergen profile and contain nonallergenic or even toxic proteins, which can induce severe side effects on therapeutic administration.^{2,3} With the introduction of recombinant DNA technology, it became possible to produce recombinant allergens for diagnosis of type 1 allergy and to reduce the allergenic activity of allergens by recombinant technology or peptide chemistry.³

Despite its use since the beginning of the century, the underlying immunological mechanisms of SIT are still a matter of debate.⁴⁻⁷ It has been demonstrated recently that the induction of allergen-specific peripheral T-cell tolerance represents an essential step for successful therapy.⁴⁻⁶ Accordingly, it appears that a basic requirement for an allergen vaccine in achieving successful SIT without risk of anaphylaxis is to express T-cell epitopes, which induce T-cell tolerance and lack antibody-binding sites that mediate IgE-mediated allergic responses.² Conformation dependence of B-cell epitopes and linearity of T-cell epitopes may induce a different regulation of allergen-specific T-cell cytokine toward a nonallergic phenotype.⁸ Native allergens use IgE-facilitated antigen presentation by dendritic cells and B cells, which activates T cells to produce T_H2-type cytokines and B cells to produce further IgE in a secondary response.⁹⁻¹¹ In contrast, B-cell epitope-deleted allergens, which do not

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bind IgE, do not initiate effector cell degranulation. They use phagocytic or pinocytic antigen uptake mechanisms in dendritic cells, macrophages, and B cells. T cells subsequently may be induced to generate a balanced T_H0/T_H1 -type cytokine pattern in lower quantities as well as T-cell tolerance, which involves T-regulatory cells.^{2,12} Moreover, targeting T cells by modified allergens will enable the administration of higher doses of allergens, which is required to induce T-cell tolerance without the risk of anaphylaxis.¹³

The response to bee venom (BV) allergens provides a specially suited model to study the cellular and molecular mechanisms of specific immune response regulation in human beings.^{4,5,8} The BV phospholipase A_2 (Api m 1) represents the major allergen in both normal immunity and bee sting allergy. Hyaluronidase (Api m 2) represents the second major allergen in BV, with more than 70% reactivity of individuals allergic to BV.¹⁴ We generated a fusion protein consisting of the 2 major allergens of BV, Api m 1 and Api m 2. Destroyed conformational B-cell epitopes but intact T-cell epitopes of the 2 allergens characterize this protein. By providing decreased allergenicity with preserved T-cell tolerance inducing capacity, the Api m [1/2] fusion protein represents a novel vaccine prototype for allergen-specific immunotherapy.

METHODS

Study population

Blood and serum samples from 12 individuals allergic to BV were selected before BV SIT was started. For diagnosis of BV allergy, clinical allergic reaction grade II to IV, type 1 skin reactivity, and serum IgE levels (Unicap-system; Pharmacia, Uppsala, Sweden) were analyzed. Five individuals not allergic were studied as controls. Skin prick tests (SPTs) were performed in 13 patients allergic to BV and 5 healthy controls. The study was approved by the Ethical Committee of the University of Zürich. All patients and controls gave written informed consent.

Expression and purification of recombinant fusion protein Api m [1/2]

The fusion of the genes from Api m 1 and Api m 2 was performed by conventional PCR methods. The resulting fusion protein with N-terminal His₆ tag was expressed in *Escherichia coli* BL21 (Stratagene, East Kew, Australia) and purified by immobilized metal ion affinity chromatography following the manufacturer's protocol (Qiagen, Clifton Hill, Australia; for supplementary material, see the Journal's Online Repository at www.mosby.com/jaci).

ELISA and ELISA inhibition

Allergen-specific serum IgE was detected by a standard solid phase ELISA.¹⁵ For ELISA inhibition proteins (equimolar concentration, 25 μ mol/L) and BSA as a control were serially diluted 1:4 in blocking buffer and incubated with 1:50 diluted patients' pooled serum for 2 hours at room temperature. The preincubated sera were transferred to the coated plates and incubated for 2 hours at room temperature. Residual IgE-binding capacity was determined as described. Percentage of inhibition was calculated from the absorbency of serial dilutions containing BSA in fluid phase set as 0% inhibition.

Generation and characterization of mAbs against Api m 2

Mouse IgG mAbs against Api m 2 generated and tested for their binding capacity to Api m 2 revealed that 2 types of mAbs were obtained against Api m 2. The binding of 1 group was strongly inhibited by serum of individuals allergic to BV. These mouse mAbs presumably recognize same epitopes as human antibodies (clones a-d). The second group of mouse mAb binding was not or was very weakly inhibited by serum of individuals allergic to BV, suggesting that these mouse mAbs recognize linear and nonconformational epitopes (clones e-h). These mAbs were further characterized for their binding to 3 non-IgE-binding linear fragments of Api m 2. Again, mAbs a to d did not bind to these linear nonconformational peptides, but mAbs e to h showed strong IgE reactivity. A human mAb against Api m 1 (BVA2) was used to characterize further the Api m [1/2] fusion protein.¹⁶

Immunoblotting

For Western blot analysis, equimolar amounts (1.5 μ mol/L) of the 3 recombinant proteins, Api m 1, Api m 2, and Api m [1/2], were electrophoresed by 4% to 12% Bis-Tris polyacrylamide NuPAGE gels (Invitrogen, Basel, Switzerland) under denaturing, reducing conditions and transferred to nitrocellulose membrane (Amersham, Little Chalfont, United Kingdom).¹⁷ The binding of IgE in pooled serum from 4 individuals allergic to BV was detected with rabbit antihuman IgE (Dako Inc, Burlingame, Calif) and developed by goat antirabbit biotin conjugated IgG antibody (Dako Inc) and streptavidin-horseradish peroxidase (Sigma Chemical Co, St Louis, Mo).¹⁷ Bands were visualized with enhanced chemiluminescent detection reagents and Hyperfilm ECL (Amersham, Otelfingen, Switzerland). Horseradish peroxidase-labeled sheep antimouse antibody was used as a second antibody for the analysis of mouse mAb binding (Amersham). A human mAb against Api m 1 (BVA2) was used to characterize further the Api m [1/2] fusion protein in dot blot assays.¹⁶ Peroxidase-conjugated rabbit antihuman IgG antibody (Dako Inc) was used as a second antibody.

T-cell cultures

PBMCs were isolated by Ficoll (Biochrom, Berlin, Germany) density gradient centrifugation of peripheral venous blood.⁴ Antigen-specific proliferation and cytokine production were determined with equimolar amounts of Api m 1, Api m 2, and Api m [1/2] fusion protein, as described.⁴ The solid phase sandwich ELISAs for IFN- γ , IL-4, IL-5, IL-10, and IL-13 were performed in duplicate.⁴ Four T-cell clones specific to Api m 1 were derived from a hyperimmunized healthy individual as described.¹⁸ BuT 6.1 F10 recognizes an epitope spanning amino acids 81 to 92, and BuT 6.6 F4 recognizes an epitope spanning amino acids 113 to 124. In addition, BuT 5.1 F12 and BuT 7.3B4 T-cell clones, which are not specific to Api m 1, were used as controls. Autologous EBV-transformed B cells served as autologous antigen-presenting cells.¹⁸ A total of 50,000 T cells, 25,000 irradiated (3000 rad) autologous PBMCs, and 25,000 irradiated EBV-B cells were cultured in 200 μ L RPMI 1640 medium in 96-well round-bottom microtiter plates in quadruplicate. Cultures were incubated for 48 hours at 37°C in 5% CO₂ in the presence of equimolar amounts of Api m 1, Api m 2, and Api m [1/2], and proliferation was measured.¹⁸

Immunization of mice

All experiments complied with the Home Office 1986 Animals Scientific Act and were approved by the King's College animal committee for review of ethics and welfare or by the Kantonales Veterinäramt Zürich (#136/2000). Female C57/J mice (4-6 weeks old; n = 6) were immunized subcutaneously with 100 μ g/100 μ L Api

m [1/2] in saline, and the control group ($n = 6$) saline alone, 14, 10, and 6 days before intraperitoneal immunization with 100 $\mu\text{g}/100 \mu\text{L}$ Api m 1/Al(OH)₃. Sera were collected at days 0, 7, and 14 and assayed for Api m 1-specific IgE, IgG1, and IgG2a antibodies. Api m 1-specific IgE antibodies were assayed by passive cutaneous anaphylaxis (PCA) by using Wistar rats (Harlan Olac, Bicester, United Kingdom) and represented the highest dilution of serum that produced a positive mast cell-dependent PCA reaction. Api m 1-specific IgG1 and IgG2a were monitored by ELISA.¹⁹ For the analysis of epitope similarity between Api m [1/2] and Api m 1, mice (2 groups of 5 mice each) were immunized intraperitoneally 3 times every 2 weeks with 0.1 μg Al(OH)₃-adsorbed Api m 1 and Api m [1/2/3]. Serum Api m 1-specific IgE and IgG2a were detected by ELISA at the sixth week.

Basophil mediator release

Leukotriene release from basophils was analyzed by cellular antigen stimulation test–ELISA (Bühlmann Laboratories AG, Allschwil, Switzerland). Briefly, granulocytes were isolated from 3 different patients allergic to BV and incubated with equimolar concentrations of recombinant Api m 1, Api m 2, and Api m [1/2]. BV (dilutions as high as 1 $\mu\text{g}/\text{mL}$; Aquagen/Pharmalgen; ALK, Hørsholm, Denmark) and an antibody directed against the IgE high-affinity receptor (FcεRI) provided by the manufacturer were used as positive controls. Leukotrienes were determined in the cell supernatant by ELISA.²⁰ Similarly, released histamine was measured by ELISA (Bühlmann Laboratories AG). Results are expressed as mean values of triplicate determinations and represent the percentage of total histamine.

SPTs

The SPTs were performed in duplicate in the volar forearm with commercial BV (Aquagen/Pharmalgen) and equimolar amounts of Api m 1 and Api m [1/2]. Sodium chloride 0.9% and histamine hydrochloride were used as negative and positive controls.

Statistical analysis

Data are presented as means $\pm$ SDs. Paired Student *t* tests and Mann-Whitney *U* tests were used for statistical analysis.

RESULTS

Characterization of the recombinant Api m [1/2] fusion protein

We generated a recombinant fusion protein composed of the whole sequence of the 2 *Apis mellifera* (honeybee venom) major allergens, phospholipase A₂ and hyaluronidase. The fusion protein has a molecular weight of 58 kD, which is the sum of the 2 fusion partners (Fig 1, A). To characterize the Api m [1/2] fusion protein, we used human and mouse mAbs generated against Api m 1 and Api m 2. We found that mAbs, which recognize same epitopes as human antibodies on Api m 2, did not bind to the Api m [1/2] fusion protein (Fig 1, B; clones a-d). In contrast, the mAbs, which recognize epitopes different from human antibodies (nonconformational), bound to the Api m [1/2] (clones e-h). In addition, human anti-Api m 1 mAb, which recognizes Api m 1 in its 3-dimensional structure, did not bind to Api m 2, Api m [1/2] fusion protein, and BSA (Fig 1, C). To investigate whether there were remaining cross-reactive antibody epitopes between Api m 1 and Api

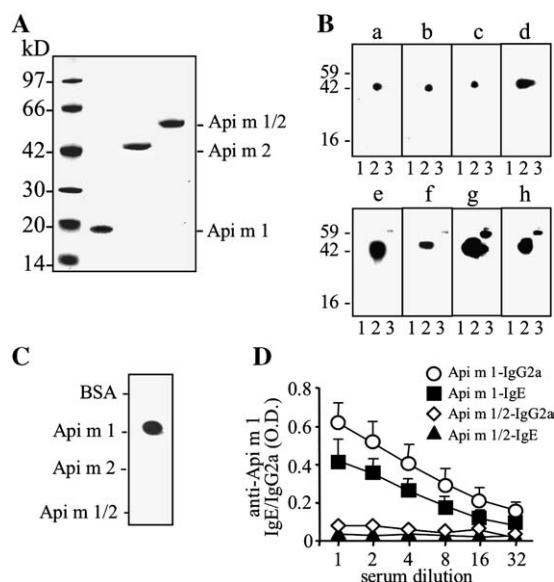


FIG 1. Characterization of Api m [1/2] fusion protein. **A**, Coomassie staining of 0.5 $\mu\text{mol}/\text{L}$ recombinant Api m 1, Api m 2, and Api m [1/2] fusion protein. **B**, Binding of 8 different mouse anti-Api m 2 IgG mAbs to Api m 1 (lane 1), Api m 2 (lane 2), and Api m [1/2] (lane 3). **C**, Dot blot analysis of human anti-Api m 1 IgG4 mAb binding to Api m 1, Api m 2, and Api m [1/2]. **D**, Api m [1/2] does not induce IgE and IgG2a against native Api m 1. Api m 1-specific IgE and IgG2a were determined by ELISA.

m [1/2], mice were immunized with Al(OH)₃-adsorbed Api m 1 and Api m [1/2]. Api m [1/2] did not induce any IgE and IgG2a against the native Api m 1 *in vivo* (Fig 1, D). In summary, the data indicate that Api m [1/2] fusion protein did not preserve the Api m 1-specific and Api m 2-specific B-cell epitopes and assumed a structure different from that of the fusion partners.

Reduced IgE binding of Api m [1/2] fusion protein

We further determined whether Api m [1/2] exhibits reduced IgE binding in comparison with native allergens. Western blot analysis with pooled serum from 4 highly sensitized patients allergic to BV showed only very weak IgE binding to the fusion protein compared with equimolar amounts of the recombinant allergens Api m 1 and Api m 2 (Fig 2, A). In all tested individuals, the serum specific IgE showed significantly less binding to the fusion protein compared with Api m 1 or Api m 2 in ELISA ($P = .002$; Fig 2, B). Sera of 9 of 12 patients showed higher IgE reactivity to Api m 1, 2 showed higher IgE reactivity to Api m 2, and 1 showed equal IgE reactivity to both Api m 1 and Api m 2. ELISA inhibition experiments confirmed the reduced ability of the purified Api m [1/2] fusion protein to inhibit IgE binding to the native allergens in patients with allergy. The binding of patients' serum IgE to Api m 1 (Fig 2, C) and Api m 2 (Fig 2, D) coated on solid phase was completely inhibited by increasing amounts of the native proteins, but not the Api m [1/2] fusion protein.

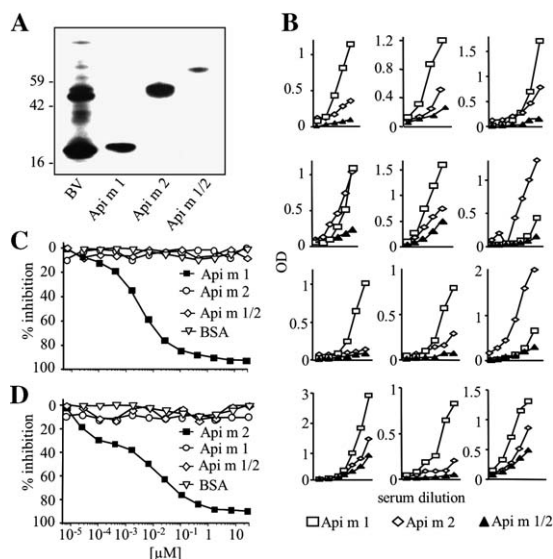


FIG 2. Low IgE binding to Api m [1/2] fusion protein. **A**, Serum IgE binding to BV, Api m 1, Api m 2, and the Api m [1/2] fusion protein. **B**, Serum IgE binding (12 different patients allergic to BV) to equimolar amounts of Api m 1, Api m 2, and Api m [1/2] coated on the solid phase by ELISA. There was no inhibition of serum IgE binding to Api m 1 (**C**) and Api m 2 (**D**) coated on a solid phase by Api m [1/2]. BSA was used as control.

Reduced allergenic activity of Api m [1/2] fusion protein

In the next step, we analyzed whether the fusion protein still induces type 1 hypersensitivity reaction *in vitro* by basophil mediator release assays and *in vivo* by SPTs. The *in vitro* allergenic activity was studied by exposing basophils from 3 patients sensitized to BV to equimolar concentrations of Api m [1/2] fusion protein, Api m 1, Api m 2, and the whole BV. In all cases, the fusion protein showed a significantly reduced release of de novo synthesized (leukotriene) mediators (**Fig 3, A**) and histamine (**Fig 3, B**) compared with Api m 1, Api m 2, and whole BV ($P < .05$). The hypoallergenic nature of Api m 1 [1/2] was demonstrated in type 1 hypersensitivity SPTs, with a strongly reduced skin test reactivity compared with BV and Api m 1 (**Table I**).

Api m [1/2] fusion protein retains the ability to stimulate T cells

To examine the T-cell-stimulating capacity of the Api m [1/2] fusion protein, specific proliferation and cytokine responses of PBMCs from patients allergic to BV were compared with those of healthy controls. In experiments using equimolar antigen doses, the Api m [1/2] fusion protein stimulated PBMC proliferation and IFN- γ , IL-10, IL-4, IL-5, and IL-13 release as strong as the single fusion partners (**Fig 4**). In addition, Api m [1/2] fusion protein induced significantly higher proliferation compared with the control protein Api m 2 in a dose-dependent manner in Api m 1-specific T-cell clones (**Fig 5**).

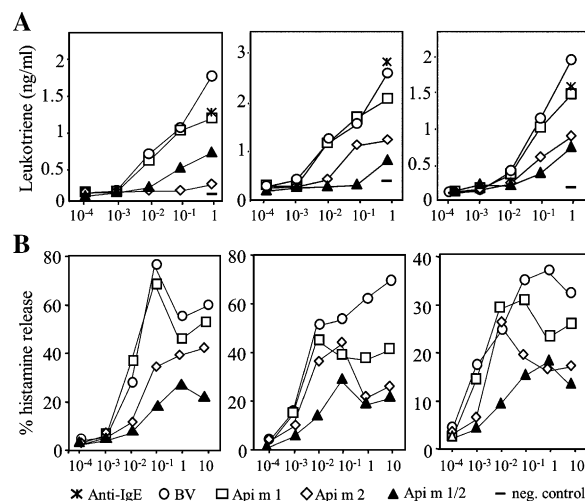


FIG 3. Reduced allergenic activity of the Api m [1/2] fusion protein. Induction of leukotriene release (**A**) and histamine release (**B**) from basophils of 3 patients allergic to BV stimulated with equimolar amounts of Api m 1, Api m 2, Api m [1/2] fusion protein, and BV.

Api m [1/2] vaccination suppresses both IgE and IgG antibodies against native allergen

We further investigated whether Api m [1/2] may serve as an allergy preventive vaccine. Mice were subcutaneously pretreated with Api m [1/2] in saline or saline alone as control before intraperitoneal immunization with Api m 1/Al(OH)₃. Control mice showed significantly enhanced Api m 1-specific antibodies; in contrast, Api m [1/2] pretreatment resulted in the inhibition of Api m 1-specific IgE, IgG1, and IgG2a (**Fig 6**). These data demonstrate that Api m [1/2] vaccination induces an efficient suppression of antibody response, including IgE directed to native allergen, without showing a switch between isotypes.

DISCUSSION

The current study demonstrates the first genetically engineered candidate allergen SIT vaccine, which is composed of 2 different major allergens. The rationale for the construction of the Api m [1/2] fusion protein is based on the exquisite conformational dependence of B-cell epitopes^{2,8,13,21} and on the linearity of T-cell epitopes²² in allergenic molecules. High-level expression of genetically engineered proteins in *E coli* mostly results in the accumulation of the gene product as insoluble and inactive protein aggregates called *inclusion bodies*. The recovery of bioactive proteins from inclusion bodies is a complex process that requires solubilization of the aggregates in strong denaturing agents (eg, guanidinium chloride or urea) and the subsequent development of sophisticated *in vitro* refolding protocols.²³ Under folding conditions, unfolded protein molecules rapidly equilibrate between different conformations before complete refolding.²³ In cysteine-containing proteins, folding is

TABLE I. SPTs*

	Age (y)	CAP (kU/L)	BV (mm)	Api m 1 (mm)	Api m [1/2] (mm)	histamine (mm)	NaCl (mm)
Patients (n = 13), mean ± SD	38.1 ± 16.3	8.3 ± 7.4	5.6 ± 1.4	3.4 ± 1.3	1.3 ± 0.9†	4.2 ± 0.9	0 ± 0
Healthy (n = 5), mean ± SD	40.6 ± 15.5	<0.35	All positive 0 ± 0	12 Positive, 1 negative 0 ± 0	1 Positive, 12 negative 0 ± 0	All positive 3.4 ± 2.3	All negative 0 ± 0

*SPTs for Api m 1, BV, and Api m [1/2] were performed in 13 patients allergic to BV and 5 healthy individuals who were not allergic. Mean diameter of wheal and SD are shown (†P in comparison with Api m 1 and BV, <.001).

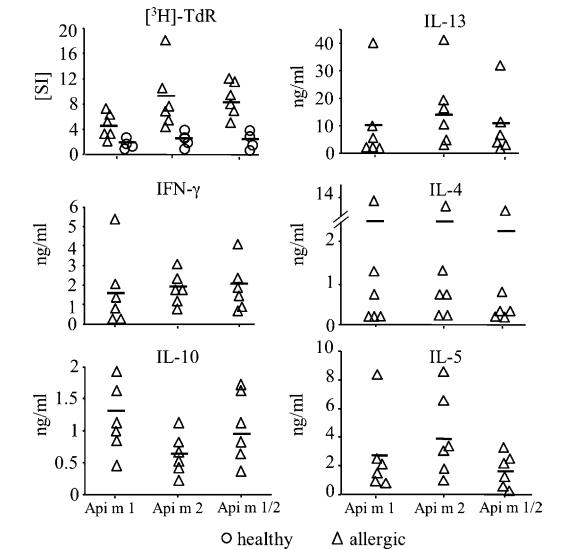


FIG 4. Induction of T-cell proliferation and cytokine production by Api m [1/2]. PBMCs were stimulated with equimolar amounts of Api m 1, Api m 2, and the Api m [1/2] fusion protein. Thymidine incorporation (³[H] TdR) was measured after 5 days. Background proliferation of unstimulated cultures was between 1570 and 3355 cpm. IL-4, IFN-γ, IL-13, IL-10, and IL-5 were determined in parallel culture supernatants by ELISA. Cytokine production of healthy PBMCs was less than 10% and was omitted from the figure. Horizontal bars represent mean values. SI, Stimulation index.

complicated by the formation of intramolecular and inter-molecular disulphide bond formation, whereas any formed disulphide bond can fix the conformation and limit the freedom of further folding processes. With an increasing number of cysteines, the probability of a correct or nativlike folding rapidly decreases because of the increasing probability of incorrect disulphide bond formation. In fact, it has been shown that no more than 5% of a mutant of bovine pancreatic ribonuclease A containing 6 cysteines refolds into wild-type-like ribonuclease A.²⁴ On the basis of these considerations, we must assume that the proportion of incorrectly folded Api m [1/2] fusion protein, which contains 14 cysteine residues, must approach 100%. Because the proportion of nativlike folded protein drastically decreases with an increasing number of cysteines, we can predict that any fusion containing more than 4 cysteines will display a reduced

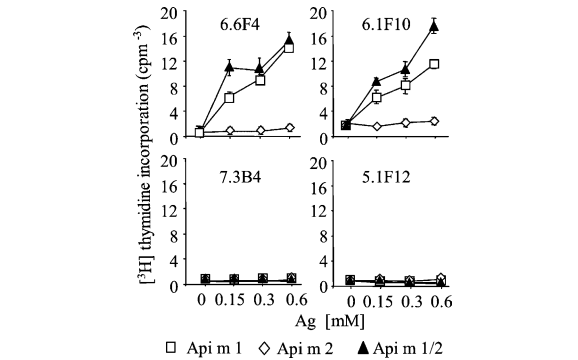


FIG 5. Api m [1/2] retains T-cell epitopes of Api m 1. Api m 1-specific T-cell clones (BuT 6.1 F10 and BuT 6.6 F4) and Api m 1-nonspecific T-cell clones (BuT 5.1 F12 and BuT 7.3 B4) were stimulated with equimolar amounts of Api m 1, Api m 2, and Api m [1/2] fusion protein for 48 hours. ³[H] thymidine incorporation was measured 12 hours after addition. Ag, Antigen.

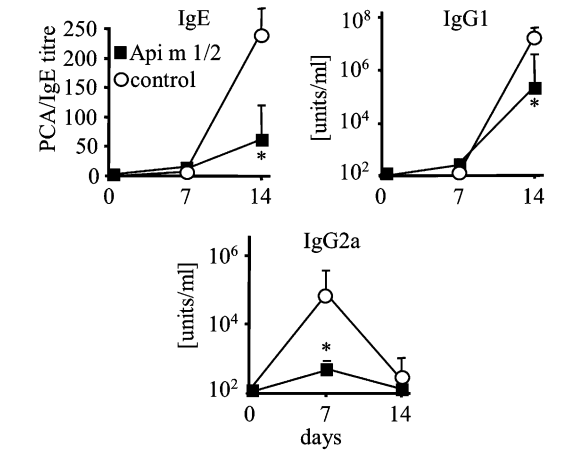


FIG 6. Api m [1/2] suppresses antibody development against native allergen. Mice (n = 6) were pretreated subcutaneously with Api m [1/2] in saline or saline alone as control 14, 10, and 6 days before intraperitoneal immunization with Api m 1/AL(OH)₃. Serum Api m 1-specific IgE, IgG1, and IgG2a were determined. *P < .001.

IgE-binding capacity, making our approach broadly applicable. Recently, stable recombinant Bet v 1 dimers and trimers were generated.^{13,25} The trimer consists of 3 covalently linked copies of the major birch pollen allergen Bet v 1. It

exhibits reduced allergenic activity but contains similar secondary structures of native Bet v 1, composed of both specific B-cell and T-cell epitopes. The difference of Api m [1/2] fusion protein from the Bet v 1 oligomers is that IgE-binding B-cell epitopes of the fusion protein are almost totally disrupted, whereas the T-cell epitopes remain intact. The reduced IgE reactivity of the Api m [1/2] fusion protein shown in ELISA inhibition and Western blot analysis resulted in a reduction of its allergenic activity. In basophil leukotriene and histamine release experiments, the Api m [1/2] fusion protein had a >100-fold reduced biological activity. Patients allergic to BV showed significantly fewer type 1 skin reactions as proof of *in vivo* reduced IgE crosslinking by Api m [1/2]. Accordingly, it can be expected that the hypoallergenic nature of the modified allergen allows much higher allergen doses to be administered to patients with allergy. This might permit SIT with fewer injections, favoring allergen-specific tolerance in T_H2 cells and suppression of IgE.²⁶

T-cell reactivity patterns of the 2 native allergens and the fusion protein did not show any difference. The initiation and maintenance of allergic reactions are strongly influenced by allergen-specific T-cell responses that not only regulate IgE production but also affect the activation and differentiation of allergic effector cells. These immunogenic determinants are recognized by specific T cells of both allergic and nonallergic individuals and are able to generate a IgE-inducing T_H2 and IgG4-inducing T_H0 cytokine pattern.¹⁸ Moreover, the same immunogenic T-cell epitopes that activate T-cell responses can induce anergy in T_H0 and T_H2 clones.¹⁷ The use of Api m [1/2] fusion protein in a mouse allergy vaccine model demonstrated that it can suppress the generation of native allergen-induced specific IgE as well as IgG1 and IgG2a. The suppression of all 3 isotypes without showing a switch to T_H1-related IgG2a suggests induction of specific tolerance *in vivo*.

To overcome the principal problems of SIT, earlier studies were mainly based on chemical modification by formaldehyde-treated and glutaraldehyde-treated allergen extracts termed *allergoids*.²⁷ Allergoids are hypoallergenic, but IgE epitopes are not fully destroyed, and they still may possess the risk of anaphylaxis. In addition, it is not clear whether chemical modification destroys some of the T-cell epitopes of allergoids. Several therapeutic approaches of targeting T-cell epitopes by peptide immunotherapy without the presence of B-cell epitopes have already been performed for the treatment of allergy.^{28,29} The Api m [1/2] fusion protein offers advantages over T-cell peptide-based immunotherapy, because it is composed of the complete repertoire of T-cell epitopes. Complete allergens with the full T-cell repertoire are apparently necessary, because multiple T-cell epitopes are recognized individually by different patients as a result of the large diversity of MHC and T-cell receptors.³⁰

In conclusion, the use of Api m [1/2] fusion protein can lead to decreased anaphylactic side effects compared with the whole venom-based treatment. Safe administration

of the fusion protein may increase the efficacy of SIT directing the allergic immune response toward a tolerized and balanced healthy immune response. The vaccine development strategy presented in this study can be extended to hybrids of major allergens from different extracts for the treatment of other allergies.

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Correction

With regard to the December 2004 article entitled “HLA-DQB1*03 in allergic fungal sinusitis and other chronic hypertrophic rhinosinusitis disorders” (2004;114:1376-83): The second sentence of the fourth paragraph of the Discussion should have appeared as follows:

Furthermore, virtually all patients with AFS who underwent skin tests with *B spicifera* had positive skin test responses (Table II).