

Original article

Characterization of the T-cell epitopes of a major peanut allergen, Ara h 2

Background: The development of safe and effective immunotherapy for peanut allergy has been complicated by the high anaphylactic potential of native peanut extracts. We sought to map the T-cell epitopes of the major peanut allergen, Ara h 2 in order to develop T-cell targeted vaccines.

Methods: A panel of eight peanut-specific CD4⁺ T-cell lines (TCL) was derived from eight peanut-allergic subjects and proliferative and cytokine responses to stimulation with a set of overlapping 20-mer peptides representing the entire sequence of Ara h 2 determined. Proliferation was assessed in 72 h assays via tritiated thymidine incorporation, while interleukin (IL)-5 and interferon (IFN)- γ production were assessed via sandwich enzyme-linked immunosorbent assay (ELISA) of cell culture supernatants.

Results: Eight of the 17 Ara h 2 peptides were recognized by one or more subjects, with the two peptides showing highest reactivity [Ara h 2 (19–38) and Ara h 2 (73–92)] being recognized by three subjects each. Adjoining peptides Ara h 2 (28–47) and Ara h 2 (100–119) induced proliferative responses in two subjects. Each of these peptides was associated with a Th2-type cytokine response.

Conclusion: Two highly immunogenic T-cell reactive regions of Ara h 2 have been identified, Ara h 2 (19–47) and Ara h 2 (73–119), providing scope for the development of safe forms of immunotherapy for peanut allergy.

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Key words: allergens; Ara h 2; peanut; T-cell epitopes.

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Accepted for publication 10 April 2004

Peanut allergy is associated with a significant risk of mortality, while the need for vigilant avoidance of exposure produces considerable psychological morbidity in both sufferers and carers (1, 2). As a consequence, there is an urgent need for a treatment for this disorder. Despite the existence of efficacious specific immunotherapy for a variety of clinically important allergens for almost a century, to date, efforts at specific immunotherapy for peanut allergy have met with limited success (3). Two studies have provided proof of concept that desensitization is possible for peanut allergy (4, 5). Each has shown that tolerance of peanut can be induced using a rush immunotherapy protocol, but that tolerance is lost in approximately half of the subjects during maintenance dosing. Additionally, injections with unmodified crude peanut extract (CPE) were associated with frequent episodes of anaphylaxis in the majority of subjects during both the induction and maintenance phases.

It has long been established that the morbidity associated with allergen immunotherapy is due to the cross-linking of immunoglobulin E (IgE) on mast cells and basophils, and that this action is not required for such therapy to be efficacious (6). Because B cells and antibodies recognize conformational epitopes dependent on molecular tertiary structure, in contrast to CD4⁺ T cells which recognize short linear peptides from the

primary structure, approaches that alter or bypass the need for allergenic tertiary structures within vaccines have provided a route to the development of novel immunotherapy strategies (7). Of these strategies, T-cell epitope-based peptide immunotherapy is the method for which the best evidence of efficacy exists, being documented for both bee venom allergy (8) and cat dander allergy (9).

Crucial to the development of T-cell targeted strategies is the identification and retention of critical T-cell epitopes in hypoallergenic immunotherapy preparations (10). To date, no primary data have been published identifying the T-cell epitopes of peanut allergens. We present here an analysis of the T-cell epitopes of the major peanut allergen, Ara h 2. Peanut-specific oligoclonal T-cell lines (TCL) are stimulated with a set of overlapping Ara h 2 peptides representing the entire sequence of Ara h 2, and proliferation and cytokine production are measured.

Methods

Subjects

Eight peanut-allergic subjects were recruited from the Alfred Hospital Asthma and Allergy Clinic, Melbourne, Australia. Donors were selected on the basis of a history of anaphylaxis following

peanut ingestion, and either a positive serum peanut-specific IgE immunoassay (Pharmacia CAP score ≥ 1 ; Pharmacia Diagnostics, Uppsala, Sweden), or a positive skin prick test (wheal diameter > 3 mm, and $> 50\%$ of histamine 10 mg/ml). Four nonpeanut-allergic controls were also recruited, three of whom were also nonatopic (as defined by the lack of symptoms of allergic disease and negative skin prick testing to common environmental aeroallergens). The study was approved by the Alfred Hospital Ethics Committee, and informed written consent was obtained from all donors.

Antigens

Crude peanut extract was prepared as described previously (11) and dialyzed against phosphate-buffered saline (PBS) at 4°C, using a 3.5 kDa cut-off dialysis membrane (Pierce, Rockford, IL, USA). For cell culture, the supernatant was filter sterilized through a 0.2 μ m filter.

Peanut cDNA was synthesized from peanut mRNA using the Timesaver cDNA synthesis kit (Amersham Biosciences, Uppsala, Sweden). The cDNA encoding Ara h 2 was then amplified by polymerase chain reaction (PCR) following our standardized methods (12) using primers based on the published sequence of Ara h 2 [(13); GenBank accession number L77197] as outlined below:

Forward: 5'-GCGGAATTCCTCACCATACTAGTAGCC-3'

Reverse: 5'-CGCCTGCAGTTAGTATCTGTCTCTGCC-3'

Sequence comparisons showed 100% identity with the published sequence. The Ara h 2 cDNA was subcloned into the pPROEX-HTa vector (Invitrogen, Carlsbad, CA, USA) as previously described (12) and the plasmid construct was transformed into *Escherichia coli* strain ER1793 (New England Biolabs, Beverly, MA, USA) using the heat shock procedure. The rAra h 2 expression was performed as outlined previously (12) and affinity purification using Ni-NTA agarose (Qiagen, Doncaster, Australia) was performed as described by the manufacturer but using modified denaturing wash (50 mM NaH_2PO_4 , 300 mM NaCl, 50 mM imidazole and 8 M urea, pH 8.0) and elution (50 mM NaH_2PO_4 , 300 mM NaCl, 500 mM imidazole and 8 M urea, pH 8.0) buffers.

Ara h 2 peptides (20-mers, 11 amino acid overlap) were synthesized by Mimotopes (Victoria, Australia) according to the Ara h 2 sequence published by Stanley et al. (13).

Electrophoresis and Western immunoblotting

Crude peanut extract proteins were resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on 14% gels under reducing conditions, as described in de Leon et al. using the Xcell II Mini-Cell apparatus (Invitrogen) (11). Proteins were then stained with Coomassie brilliant blue (Sigma, St Louis, MO, USA).

For Western immunoblotting, peanut proteins separated by 14% SDS-PAGE were transferred onto nitrocellulose membranes as outlined previously (11) using an Xcell II blotting apparatus (Invitrogen). Membranes were blocked in 10% milk powder-PBS for 1 h. After washing in PBS, membranes were incubated overnight with sera diluted (1 : 5) with 1% milk powder-PBS-0.05% Tween. Following washing, rabbit antihuman IgE horseradish peroxidase (HRPO) conjugate (Dako, Carpinteria, CA, USA) diluted (1 : 500) in 1% milk powder-PBS-0.05% Tween was applied and incubated 1–2 h at room temperature. Following washing, membranes were incubated for 1 min with chemiluminescence substrate (Du Pont, Wilmington, DE, USA) then analysed using Labworks image acquisition software (UVP Laboratory Products, Cambridge, UK).

Generation of short-term peanut-specific TCL

The peripheral blood mononuclear cell (PBMC) proliferative responses to stimulation with CPE demonstrated a dose-response for all peanut-allergic subjects and nonpeanut-allergic subjects. For each subject, the lowest dose that produced maximal stimulation at 7 days was used to drive peanut-specific TCL for use in peptide assays; this dose ranged between 50 and 200 μ g/ml. The TCL generation was performed using our standard methodology (14). Briefly, PBMC were cultured in 24-well plates (Greiner Biotechnik, Frickenhausen, Germany) at 2.5×10^6 cells per well (2 ml volume) in complete medium with CPE at an individually optimized concentration for 7 days at 37°C in a 5% CO_2 humidified incubator. At day 7 and day 14, cells were washed and resuspended at $1\text{--}1.5 \times 10^6$ cells/ml and added together with 1×10^6 /ml washed irradiated (3000 rads; Gammacell 1000 Elite, Nordion International, Inc., Kanata, Canada) autologous PBMC and antigen into fresh 24-well plates. At day 2 following restimulation, 25 U/ml of recombinant human interleukin-2 (rIL-2) was added and at day 4, 1 ml of culture medium was removed and replaced with fresh medium and 25 U/ml rIL-2. In all experiments, T cells were rested for 6–7 days after the last addition of antigen and antigen-presenting cell (APC).

TCL proliferation assays

Oligoclonal T cells (5.0×10^4 /well) were cultured in triplicate in 96-well U bottom plates (Linbro ICN Biomedicals, Aurora, OH, USA) with 10 μ g/ml Ara h 2 peptides, along with CPE in concentrations between 25 and 100 μ g/ml, and rAra h 2 in concentrations between 1 and 10 μ g/ml, in the presence of autologous irradiated (3000 rads) PBMC (5.0×10^4 /well). Cell culture medium alone and rIL-2 (50 U/ml) were used as negative and positive controls respectively. Cultures were incubated for 3 days. In the last 16 h, wells were pulsed with ^3H -thymidine (1 μ Ci/well), then harvested onto glass fibre filters with a 96-well automatic cell harvester. The ^3H -thymidine incorporation was measured by liquid scintillation spectroscopy and proliferation determined according to the mean counts per minute (c.p.m.) for triplicate cultures. Responses were considered positive if the stimulation index (SI, c.p.m. of antigen-stimulated T cells divided by c.p.m. of unstimulated T cells) was ≥ 2.5 .

Cytokine detection in culture supernatants

The IL-5 and interferon (IFN)- γ levels in 48 h culture supernatants were measured by sandwich enzyme-linked immunosorbent assay (ELISA). White 96-well ELISA plates (Corning, Acton, MA, USA) were coated with capture monoclonal antibody (mAb) (IL-5 and IFN- γ , 2 μ g/ml; 30 μ l/well) diluted in 0.1 M bicarbonate buffer overnight at 4°C. After washing in PBS/0.05% Tween (wash buffer), wells were blocked with 100 μ l/well of 1% bovine serum albumin (BSA)/PBS (blocking buffer). Following washing, 30 μ l/well of serial dilutions of recombinant human IL-5 or IFN- γ (5000–0.15 pg/ml) in blocking buffer plus 0.05% Tween, or culture supernatants were incubated overnight at 4°C. After washing, plates were incubated with biotinylated detection mAb (IL-5, 1 μ g/ml; IFN- γ , 0.5 μ g/ml; 50 μ l/well) for 1 h then incubated with streptavidin-peroxidase diluted 1 in 2000 in blocking buffer (50 μ l/well) for 45 min. Following washing, 100 μ l/well of freshly prepared chemiluminescent substrate (Perkin-Elmer, Boston, MA, USA) was added and plates read in a Lumicount microplate glow luminometer (Packard Instrument Company, Meriden, CT, USA). The lower limit of detection for IL-5 and IFN- γ ELISAs were 2 pg/ml and 4 pg/ml, respectively.

Results

Subject characterization

Clinical characteristics of the eight peanut-allergic subjects are detailed in Table 1. The mean age of subjects was 38 years (range: 28–55). Each subject described typical features of anaphylaxis on exposure to peanut, beginning within minutes of that exposure. There was no correlation between the level of serum specific IgE and the severity of reactions subjects experienced with exposure to peanut.

Western immunoblotting for serum IgE reactivity to Ara h 2

Seven of the eight peanut-allergic subjects demonstrated specific IgE reactivity for a double band of approximately 15 kDa corresponding to Ara h 2 (Fig. 1), as described by Burks et al. (15). Interestingly, the same seven subjects also recognized a band of approximately 11 kDa, likely to represent either Ara h 3, Ara h 5 or Ara h 6 (16, 17). Subject 8, despite an excellent history of peanut-induced anaphylaxis, demonstrated IgE reactivity to CPE on SPT, but not by Pharmacia CAP or Western immunoblotting (Fig. 1). Two of the four nonpeanut-allergic controls also showed binding to the lower of the two Ara h 2 bands and three showed reactivity to the 11 kDa protein, but these reactions were weak and used as the cut-off for positive IgE-binding to CPE in the peanut-allergic subjects.

Mapping of Ara h 2 T-cell epitopes

Oligoclonal CPE-specific TCL were generated from PBMC of the eight peanut-allergic donors. The TCL

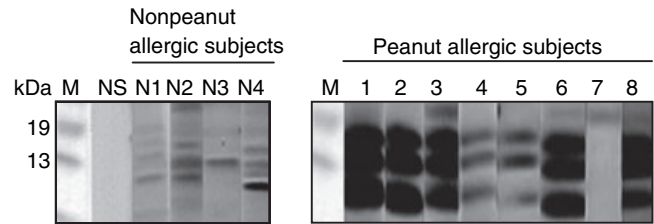


Figure 1. Western blot for serum immunoglobulin E (IgE) reactivity to Ara h 2 of peanut-allergic and nonpeanut-allergic subjects. M, molecular mass; NS, no serum. Following resolution of crude peanut extract (CPE) on a 14% polyacrylamide gel, proteins were transferred to nitrocellulose and probed with sera from the eight peanut-allergic subjects and four nonpeanut-allergic controls. IgE was detected using HRP-conjugated mouse antihuman IgE and enhanced chemiluminescence.

could not be generated from any of the nonpeanut-allergic donors, cells becoming nonviable after two stimulations or demonstrating a nondiscriminatory ‘high background’ response to all antigens assayed (data not shown). Individual responses of peanut-allergic donor TCL to peptide, Ara h 2 and CPE are summarized in Table 2 and Fig. 2. Of the 17 peptides tested, eight (47%) induced a proliferative response. Proliferative responses to rAra h 2 showed best correlation with responses to Ara h 2 (19–38) and Ara h 2 (28–47), in that where a subject had a response to these peptides, 60% also had a proliferative response to Ara h 2.

Ara h 2 (19–38) and Ara h 2 (73–92) were associated with the greatest frequency of response, each producing proliferative responses in three of the eight

Table 1. Clinical features of peanut-allergic subjects

Subject	Sex	Age (years)	Allergic disease	Peanut-induced symptoms	Known nut allergens	Other food allergens	Age at first reaction (years)	Time since last reaction (months)	Serum specific IgE		
									Level (kU _A /l)	Score	Atopic status
1	M	28	Asthma	GIT upset, laryngeal oedema, urticaria	Peanut, hazelnut, egg, milk		5	1	100	6	HDM
2	F	36	Asthma, eczema	GIT upset, asthma, hypotension, facial angioedema	Peanut, hazelnut		4	2	3.09	2	GP, HDM, C
3	F	49	Nil	GIT upset, asthma, laryngeal oedema, loss of consciousness, urticaria	Peanut, cashew nut	Peas	2	18	17.6	4	GP
4	F	33	Nil	GIT upset, urticaria	Peanut, hazelnut, pine nuts		0.5	3	13.3	3	GP, B, HDM, C
5	M	55	Asthma, eczema	Asthma, laryngeal oedema, facial angioedema	Peanut, walnut	Banana	1.5	120	2.01	2	GP, HDM, A
6	M	35	Asthma, rhinitis, eczema	Asthma, urticaria, facial angioedema	Peanut, hazelnut		8	12	2.82	2	NA
7	F	37	Rhinitis, eczema	GIT upset, asthma, laryngeal oedema, hypotension	Peanut, hazelnut, pine nuts		10	36	Peanut SPT: 14 mm		GP, HDM
8	F	30	Asthma	Asthma, urticaria, laryngeal oedema, facial angioedema	Peanut, Brazil nut, almond		3	228	0.93	2	NA

GIT, gastrointestinal tract; GP, grass pollen; B, birch; HDM, house dust mite; C, cat; A, alternaria; SPT, skin prick test.

Table 2. Oligoclonal T-cell proliferative responses of peanut-allergic subjects to Ara h 2 peptides

Subjects	Ara h 2 peptides																	rAra h 2	CPE
	1–20	10–29	19–38	28–47	37–56	46–65	55–74	64–83	73–92	82–101	91–110	100–119	109–128	118–137	127–146	136–155	138–157		
1	0.9	1.0	26.8	1.3	0.7	0.9	1.9	1.0	1.1	1.1	1.3	1.3	1.0	0.9	0.9	1.0	2.0	20.9	89.9
2	2.4	0.8	5.2	0.8	0.7	0.6	0.8	0.7	0.4	0.6	0.6	0.7	1.2	0.8	0.9	0.8	0.7	2.3	4.8
3	1.5	1.1	3.1	1.3	1.7	1.6	1.4	0.8	1.5	0.8	1.5	1.1	1.0	1.5	2.2	1.0	1.2	5.8	52.0
4	1.1	1.5	1.5	2.8	0.9	2.3	1.2	2.1	3.3	2.4	5.3	2.9	1.4	1.6	1.2	2.6	2.0	5.7	34.2
5	0.9	1.1	2.3	2.6	1.7	1.7	2.2	1.9	1.8	2.3	1.7	1.7	1.4	1.4	1.8	1.6	0.9	0.8	4.4
6	2.3	1.1	1.6	1.8	1.1	1.1	1.6	1.9	6.7	1.5	1.1	2.5	1.0	1.3	1.2	1.1	1.1	1.4	13.9
7	1.3	1.8	1.9	1.3	1.7	2.1	2.7	1.5	2.7	1.9	2.2	2.1	1.4	1.7	1.6	1.3	1.5	1.2	3.2
8	1.1	1.4	1.2	1.4	1.2	1.1	1.1	1.2	1.3	2.9	2.4	1.2	1.6	1.7	1.5	1.4	1.4	1.2	15.7

Proliferative responses for Ara h 2 peptide-responsive T-cell lines (TCL) are shown as stimulation indices and values ≥ 2.5 are shaded grey. For the entire cohort, background responses of T cells cultured with irradiated antigen-presenting cells (APC) in the absence of antigen were 6269 ± 2579 c.p.m. (mean $\pm$ SEM). The maximal crude peanut extract (CPE) and rAra h 2 response is shown for each donor.

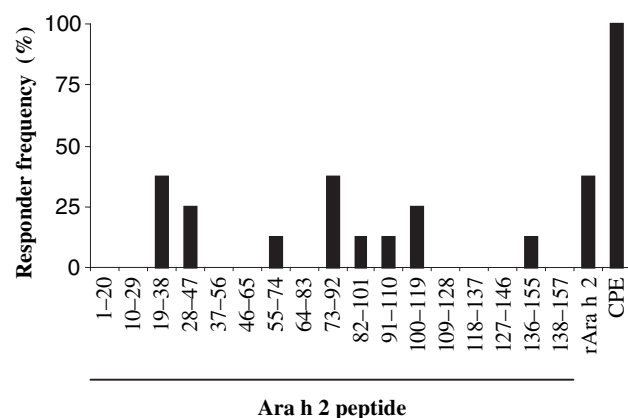


Figure 2. Percentage responder frequency to Ara h 2 peptides. Responder frequency of T-cell lines (TCL) from a panel of eight peanut-allergic donors to individual Ara h 2 peptides. A stimulation index of ≥ 2.5 was considered positive and the number of donor TCL responding to each peptide is shown as a percentage.

peanut-allergic subjects although not the same three subjects. Other peptides inducing proliferative responses in two subjects each were located at Ara h 2 (28–47) and Ara h 2 (100–119), while Ara h 2 (55–74), Ara h 2 (82–101), Ara h 2 (91–110), and Ara h 2 (136–155) induced proliferation in one subject each. A range of magnitudes of response to each peptide was seen but when response magnitude was ranked for each subject, Ara h 2 (19–38) responses ranked highest, with three subjects demonstrating their greatest response to this peptide.

Cytokine responses to peptides associated with a proliferative response

To identify the phenotype of T cells associated with a peptide proliferative response, supernatants were collected from TCL cultures 48 h after stimulation with these peptides or with CPE and assayed for the presence of

Table 3. IL-5 and IFN- γ production by CPE-specific TCL stimulated with Ara h 2 peptides

Subject	Peptide	Proliferation-inducing peptides		Nonproliferation-inducing peptides		CPE	
		IL-5	IFN- γ	IL-5	IFN- γ	IL-5	IFN- γ
1	19–38	2053	92	9	5	2004	157
2	19–38	3	UD	2	UD	4	UD
3	19–38	506	32	211	34	921	86
4	28–47	6	UD	3	UD	36	64
	73–92	UD	UD				
	91–110	2	UD				
	100–119	UD	UD				
	136–155	UD	UD				
5	28–47	UD	UD	UD	384	97	937
	118–137	UD	UD				
6	73–92	2	UD	UD	UD	6	UD
	100–119	UD	UD				
7	55–74	6	UD	3	36	3	91
	73–92	2	28				
8	82–101	UD	UD	UD	UD	UD	20

Supernatants were harvested at 48 h from T-cell cultures and tested by enzyme-linked immunosorbent assay (ELISA) for the presence of interleukin (IL)-5 and interferon (IFN)- γ . Cytokine levels (pg/ml) induced by immunoreactive peptides, mean responses to the two unreactive peptides, and crude peanut extract (CPE) are demonstrated. UD, undetectable.

IL-5 and IFN- γ . All TCL demonstrated detectable cytokine levels upon stimulation with CPE and six of the eight TCL produced cytokines on stimulation with proliferation-inducing peptides (Table 3). The magnitude of cytokine responses associated with individual peptides varied greatly between subjects, from the lower limits of detection for both cytokines, up to 2053 pg/ml for IL-5, and 937 pg/ml for IFN- γ . The greatest individual IL-5 response to a peptide was to Ara h 2 (19–38) with subject 1 producing 2053 pg/ml of IL-5 and subject 3 producing 506 pg/ml of IL-5 towards this peptide. Similar high IL-5 responses were also produced to CPE by these subjects.

The IL-5/IFN γ ratios were skewed towards greater IL-5 production for all peptide responses in the six subjects for whom cytokines could be detected, except for one peptide, Ara h 2 (73–92), which induced greater IFN- γ production in one subject. The greatest ratio occurred for Ara h 2 (19–38), being approximately 20 for subject 1, and seven for subject 3.

Discussion

Ara h 2 is a major peanut allergen, recognized by serum-specific IgE of at least 80% of peanut-allergic subjects (18, 19). In order to develop a T-cell targeted vaccine for use in peanut-allergic patients it is crucial to identify dominant T-cell epitopes of Ara h 2. In this study, oligoclonal CPE-specific TCL were stimulated with CPE, rAra h 2 and a set of 20-mer peptides representing the entire length of Ara h 2. A distinct pattern of proliferative responses to Ara h 2 peptides was demonstrated amongst the TCL, with certain cluster of peptides being more frequently associated with proliferative responses and producing a predominantly IL-5 cytokine response, suggesting that those peptides contain epitopes relevant to the T-cell immune response of peanut-allergic subjects.

In this study, CPE driven TCL showed significant proliferation (SI $\geq$ 2.5) to eight of the 17 peptides. Examination of the pattern of response suggests that T-cell reactivity is clustered within two regions of the Ara h 2 molecule, located at Ara h 2 (19–47) and Ara h 2 (73–119). These regions produced stimulation in five and four of the eight subjects respectively; one subject demonstrated a SI $\geq$ 2.5 at both sites. Interestingly, only subjects with responses in the region Ara h 2 (19–47) had responses to rAra h 2. The T-cell reactivity to Ara h 2 was discussed previously in a review but no primary data was presented (18). The regions cited as showing T-cell responses were amino acid residues 19–28, 45–53, 96–114, and 131–139. The dominant T-cell reactive sites identified in the current study correspond to two of these regions, providing further support for their clinical importance. Since individual reactivity to the two dominant regions of Ara h 2 generally showed responses to only one of the peptides spanning that region, it seems likely that each region contains more than one T-cell epitope. Further, delineation of the precise T-cell epitopes and their core sequence requires stimulation with truncated peptide series.

Examination of the magnitude of proliferative responses to peptides also provides support for there being two dominant T-cell reactive regions within Ara h 2 located at Ara h 2 (19–47) and Ara h 2 (73–119). Of the three peptides that produced responses $>$ 5.0 SI, two were associated with these regions. Ara h 2 (19–38) produced the largest responses, with an SI of 26.8 in subject 1, as well as 5.2 in subject 3. No peptides outside

of the two identified regions produced a SI of this magnitude.

The clinical significance of T-cell peptide reactivity was further examined by assessing the cytokine response associated with each peptide. Peptides that contain T-cell epitopes relevant to the allergic response would be expected to cause secretion of cytokines associated with a T_H2 response, such as IL-5. Data with regard to cytokine secretion were available for six subjects, and revealed that the IL-5 : IFN- γ ratio was significantly skewed towards IL-5 production for peptides associated with proliferative responses. The largest responses again occurred for Ara h 2 (19–38), both on the basis of absolute concentration of IL-5 and the IL-5/IFN- γ ratio, with one subject recording an IL-5 concentration of 2053 ng/ml and an IL-5 : IFN- γ ratio of 20. Thus, these data provide further support for those Ara h 2 peptides producing proliferative responses being those associated with the allergic response to this allergen. Examination of cytokine production on a single cell basis via flow cytometry would be helpful in confirming the T_H2-type phenotype of these cell lines (20).

A caveat to the use of peptide immunotherapy for allergy is that peptides are unable to elicit significant IgE-induced effector cell reactivity, thus reducing the risk of systemic allergic reactions (6). Immunodominant regions for serum IgE-binding for Ara h 2 have been identified at Ara h 2 (27–36), Ara h 2 (57–66) and Ara h 2 (65–74) (13). The presence of an IgE immunodominant region within the T-cell reactive peptide Ara h 2 (19–38) mandates the need for further clarification of the precise T-cell epitope to assess the degree of overlap with the IgE immunodominant epitope, along with functional assays of effector cell activation by that region.

The data presented within this study provide a platform for the development of novel allergen immunotherapy. One such approach could be via the production of an hypoallergenic mutant whereby IgE conformational epitopes are destroyed as has been performed for Ara h 3 (21). Because Ara h 2 in its reduced form demonstrates significant alteration in secondary and tertiary structure, it is likely to prove suitable for similar strategies (22). Alternatively, with the identification of the precise T-cell epitope using truncated peptide sequences, peptide-based immunotherapy approaches could be generated, as has been performed successfully for bee venom and cat dander (8, 9). Although the major histocompatibility complex (MHC) haplotype of responding subjects has not been determined in this study, it is likely that our findings will be applicable to the general population. It has been shown previously that individual allergenic proteins retain their immunodominance despite presentation by MHC molecules of different haplotypes (14, 23).

In conclusion, this study has identified two highly immunogenic T-cell reactive regions of Ara h 2, namely

Ara h 2 (19–47) and Ara h 2 (73–119). Within these regions, the peptide Ara h 2 (19–38) induced the greatest T-cell proliferative responses, with corroboration by cytokine data that this region is clinically relevant. Our results provide the basis for the development of safe and effective T-cell targeted immunotherapy.

Acknowledgments

This research was funded by the National Health and Medical Research Council of Australia, The Alfred Hospital, the Cooperative Research Centre for Asthma, Sydney, Australia, and a Monash University Graduate Scholarship.

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