

Letter to the Editor

The T-cell response to Amb a 1 is characterized by 3 dominant epitopes and multiple MHC restriction elements

To the Editor:

Common ragweed (*Ambrosia artemisiifolia*) represents the most prominent seasonal allergen in North America and Mexico. The plant has also been introduced into parts of Europe and Asia, and the prevalence of sensitization to ragweed pollen has been increasing steadily.¹ Ragweed pollen contains several allergens, of which Amb a 1, a 38-kd protein belonging to the pectate-lyase protein family, is the most important. More than 90% of ragweed-sensitized subjects react to Amb a 1 in skin prick tests, and at least 90% of the allergenic activity in ragweed pollen can be attributed to this protein.² The dominance of 1 major allergen makes ragweed pollen allergy especially suitable for new molecule-based immunotherapeutic strategies. Pollinosis to ragweed can be cured by specific immunotherapy (SIT) using vaccines that contain solely the major allergen or hypoallergenic variants thereof. In fact, conjugates containing purified natural Amb a 1 showing reduced IgE binding have been used successfully in clinical trials,³ indicating that the single major ragweed allergen can replace the pollen extract in SIT.

CD4⁺ allergen-specific T lymphocytes play a major role in the initiation and perpetuation of allergic immune responses. Although Amb a 1 represents a highly relevant seasonal allergen, information on the T-cell response to Amb a 1 is still scarce. Proliferative T-cell responses of 12 patients with ragweed allergy to crude *Escherichia coli* lysates of recombinant isoforms of Amb a 1 were reported in the early 1990s.⁴ A non-peer-reviewed publication (patent WO 96/13589) has described 4 relatively large (34–59 amino acids) T-cell activating regions of Amb a 1.⁵ However, a precise definition of T-cell epitopes and a functional characterization of Amb a 1-specific T cells are still lacking. We investigated the T-cell response to Amb a 1 in detail. Allergen-specific T-cell lines (TCLs) and T-cell clones (TCCs) from 48 subjects with ragweed pollen allergy were established and used for epitope mapping, determination of cytokine responses, and HLA restriction. The methods used are included in the Methods section of this article's Online Repository at www.jacionline.org.

Four isoallergens of Amb a 1 with 70% to 80% amino acid sequence identity have been identified.^{4,6,7} We have previously shown that the recombinant isoform Amb a 1.3 and natural Amb a 1 induce equivalent proliferation and cytokine responses in Amb a 1-reactive TCLs.⁷ Therefore rAmb a 1.3 was regarded as a suitable isoform to study T-cell responses to Amb a 1. We used 121 overlapping peptides spanning the entire amino acid sequence of Amb a 1.3 to identify the T-cell epitopes recognized by TCLs established with ragweed pollen extract from 9 Italian and 39 Austrian subjects with ragweed pollen allergy (Fig 1). Because Italian (I 1–9) and Austrian (V 1–39) patients showed similar epitope recognition profiles, data from both patient groups were combined for analysis. The number of peptides recognized in a single subject ranged from 2 to 39 (median, 10). In total, 26 T cell-activating regions comprising 12 to 18 amino acids with a prevalence of greater than 10% were identified. Four regions were recognized by 31% to 40%, epitope Amb a 1_{343–357} was recognized by 45%, epitope Amb a 1_{178–189} was recognized by 51%,

and epitope Amb a 1_{199–216} was recognized by 57% of the patients. Together, the latter 3 dominant epitopes covered 65% of the patients. The fact that 32 TCLs established with rAmb a 1.3 recognized the same epitope regions as TCLs induced with ragweed pollen extract (data not shown) confirmed that rAmb a 1.3 is a representative isoform to study the T-cell response to the major ragweed pollen allergen.

In addition, 41 Amb a 1-specific TCCs expressing TCR $\alpha\beta$ were isolated from 8 different ragweed-sensitized patients (see Table E1 in this article's Online Repository at www.jacionline.org). All but 2 TCCs were CD4⁺. On allergen stimulation, 20 TCCs produced predominantly IL-4 (T_H2-like; 49%; Table I), 15 TCCs produced similar levels of IL-4 and IFN- γ (T_H0-like; 37%), and only 4 CD4⁺ TCCs and both CD8⁺ TCCs produced predominantly IFN- γ (T_H1/T_C1-like; 15%). High levels of IL-10 (>500 pg/mL) were secreted by 38% of TCCs. The production of IL-10 correlated with neither IL-4 nor IFN- γ production and was not confined to special epitopes of Amb a 1. The epitopes recognized by Amb a 1-specific TCCs within single subjects were concordant with those determined for the respective TCLs. Twelve TCCs from 5 different donors recognized amino acids 343 to 357, 6 TCCs (4 donors) recognized amino acids 199 to 213, 3 TCCs recognized amino acids 292 to 306 and 369 to 378 (2 donors each), and 3 TCCs recognized amino acids 136 to 150 (same donor). For 2 TCCs, no epitope could be identified in spite of strong proliferation to rAmb a 1.3 and natural Amb a 1.

HLA class II restriction of 6 TCCs and 5 TCLs derived from 9 different patients specific for 10 T-cell epitopes was investigated (Table I). Of note, 50% of the tested epitopes were presented by more than 1 HLA class II molecule. For instance, Amb a 1_{343–357} was recognized in the context of 1 to 3 different HLA class II molecules (DR, DP, and DQ), depending on the patient, the T-cell receptor, or both.

It is not exactly clear why ragweed pollen possesses such a high allergenic potential within the Asteraceae plant family, which comprises about 1,600 genera and more than 20,000 species. Our immunologic investigations suggest that the major ragweed allergen might have a high intrinsic allergenicity. First, Amb a 1 contains multiple epitopes, including 3 dominant epitopes recognized by more than 45% of subjects with ragweed allergy. Second, the presentation of several Amb a 1 epitopes, including the frequently recognized regions Amb a 1_{202–213} and Amb a 1_{343–354}, was found to be inhibited by antibodies to HLA-DR, HLA-DQ, and HLA-DP molecules (Table I), suggesting highly promiscuous binding of these peptides. Interestingly, the response of individual TCCs to these peptides was inhibited by antibodies specific for 2 or even all 3 types of HLA class II molecules (see Table E1), indicating that TCR recognition of these epitopes is also exceptionally degenerate. Promiscuity of peptides and TCRs commonly resides within different HLA-DR alleles and has only rarely been found across HLA class II loci. Because the HLA-DP and HLA-DQ gene loci encode fewer polymorphisms in their alleles, these molecules are more widely distributed in the population than HLA-DR. Therefore T-cell epitopes presented by HLA-DP or HLA-DQ molecules, as observed in Amb a 1, might sensitize a large part of the population. In contrast, Amb a 5, which contains only a single T-cell epitope exclusively presented by HLA-DRB1*1501/02,⁸ is only a minor

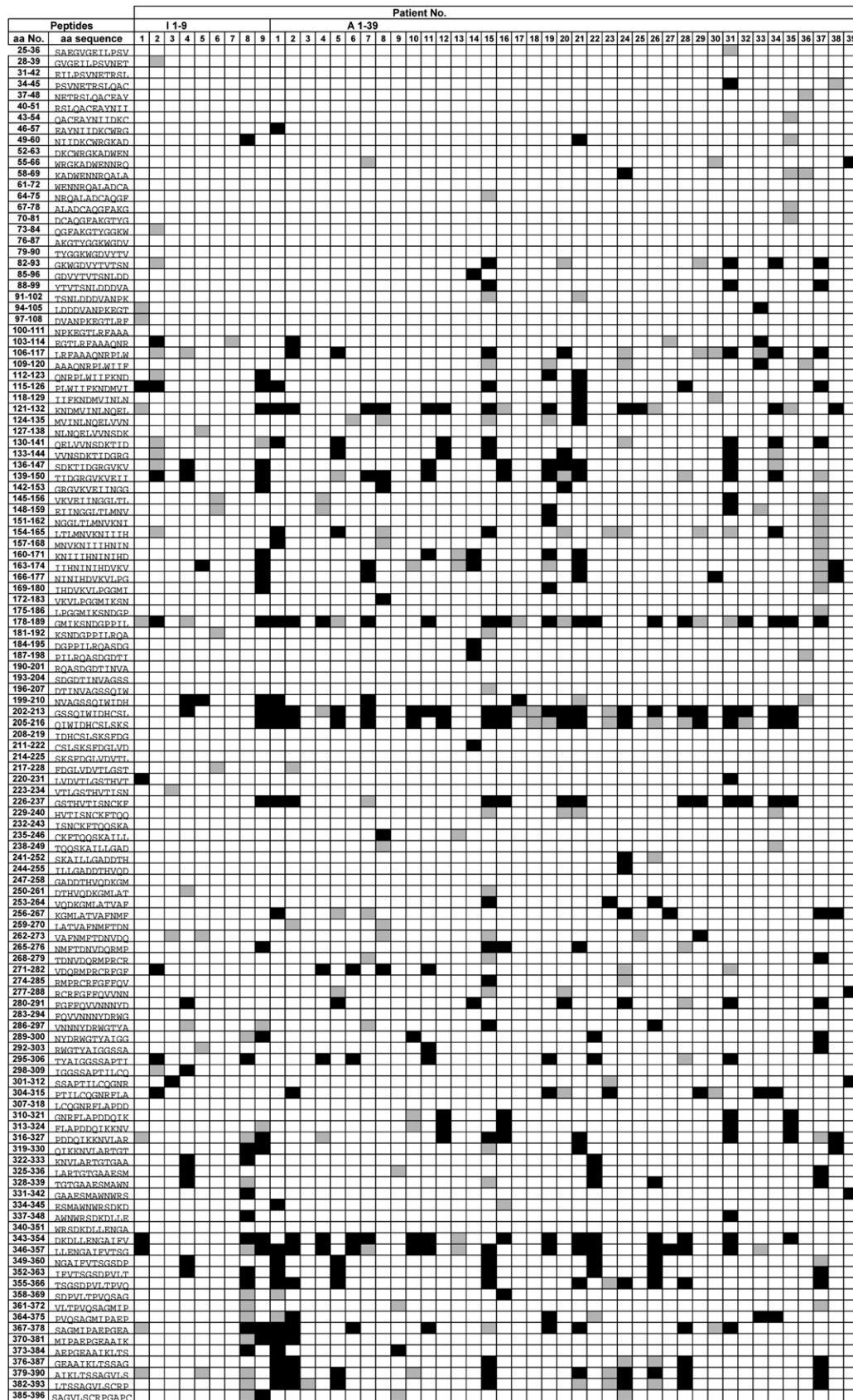


FIG 1. Mapping of T-cell epitopes in Amb a 1. Amb a 1-specific TCLs derived from 48 patients with ragweed pollen allergy (I 1-9 from Milan, Italy and V 1-39 from Vienna, Austria) were stimulated with 121 overlapping synthetic 12-mer peptides covering the complete sequence of Amb a 1.3 (excluding the leader peptide). Peptides inducing proliferation of a stimulation index of 3 or greater are indicated in *gray*, and those with a stimulation index of 5 or greater are indicated in *black*.

TABLE I. HLA class II restriction of Amb a 1-specific T cells

Amb a 1 specificity	T cells	Proliferation (dpm $\times 10^3$) anti-HLA antibody				Restriction
		None	DR	DP	DQ	
Amino acids 106-117	TCL patient 1	8.3	3.9	8.9	nt	DR
Amino acids 130-144	TCL patient 1	18.9	0.4	14.3	25.0	DR
	TCL patient 2	5.4	0.0	0.3	16.5	DR, DP
Amino acids 178-189	TCL patient 3	23.9	11.7	22.6	26.5	DR
Amino acids 202-213	TCL patient 3	15.3	3.9	14.1	10.8	DR
	TCC patient 4	55.5	57.5	17.1	0.6	DP, DQ
Amino acids 262-273	TCC patient 5	28.2	14.2	6.7	0.8	DR, DP, DQ
Amino acids 295-306	TCC patient 6	53.5	47.8	34.2	1.7	DP, DQ
	TCL patient 2	20.4	19.1	21.9	8.4	DQ
Amino acids 325-339	TCL patient 1	18.8	0.2	22.5	15.9	DR
Amino acids 343-357	TCL patient 3	26.0	22.4	26.3	12.5	DQ
	TCL patient 2	6.6	4.1	8.7	8.2	DR
	TCC patient 4	24.8	14.9	3.6	7.4	DR, DP, DQ
	TCC patient 7	19.7	17.3	5.8	10.0	DP, DQ
	TCL patient 8	60.2	10.9	11.6	14.5	DR, DP, DQ
	TCL patient 9	20.7	3.5	1.4	2.7	DR, DP, DQ
Amino acids 349-363	TCL patient 1	19.0	13.2	20.4	18.0	DR
Amino acids 367-378	TCL patient 3	13.4	8.1	7.1	13.4	DR, DP
	TCC patient 7	16.1	15.4	4.6	4.3	DP, DQ

Six TCCs and 13 TCLs from 9 different patients and specific for diverse Amb a 1 epitopes were stimulated in duplicates with peptides presented by autologous antigen-presenting cells in the presence or absence of inhibitory antibodies against HLA-DR, HLA-DP, or HLA-DQ. Inhibition of net proliferation of 30% or greater was considered evidence for restriction and is shown in boldface. For a definition of dpm, see the Methods section in this article's Online Repository.

nt, Not tested.

allergen in ragweed pollen recognized by 12% of patients.⁹ Thus the diversity of T-cell epitopes together with their broad MHC class II restriction profile might account for the high allergenicity of Amb a 1.

We identified more than 20 distinct epitopes within Amb a 1, including 3 epitopes with a prevalence of greater than 45% and 7 epitopes recognized by more than 25% of patients (Fig 1). The knowledge of T-cell epitopes is an essential prerequisite to developing allergen-derived vaccines with low/no IgE-binding activity and retained T-cell reactivity to modulate the allergen-specific T_H2 response. Amb a 1 consists of 2 subchains, the N-terminal Amb a 1 β chain (amino acids 26-180) and the C-terminal Amb a 1 α chain (amino acids 181-396).⁷ The majority of T-cell epitopes found (17/26), including 2 dominant epitopes (amino acids 199-216 and 343-357, being recognized by 45% and 57% of patients, respectively), are located in the C-terminal part of Amb a 1. Because the Amb a 1 α subchain exhibits a much lower IgE-binding capacity than the Amb a 1 β subchain, the present data strongly support the use of the Amb a 1 α subchain as a vaccine for SIT of ragweed pollen allergy.⁷ However, the potential risk of T cell-mediated side effects should be considered.

In summary, this study provides a detailed characterization of the T-cell response to Amb a 1 that allows the design of T cell-based vaccines for SIT of ragweed pollen allergy. Moreover, the diversity of T-cell epitopes together with broad HLA class II restriction profiles might account for the high allergenicity of Amb a 1.

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METHODS

Patients

In total, 48 allergic subjects (9 from Milan, Italy, and 39 from Vienna, Austria) with recurrent rhinitis/conjunctivitis during late summer and a positive skin prick test response and serum IgE level against ragweed pollen (ImmunoCAP class ≥ 2 ; Phadia, Uppsala, Sweden) were included in this study. None of the patients had undergone SIT. The study was approved by local ethics committees, and all patients provided informed consent.

Allergens

Natural Amb a 1 was kindly provided by Professor T. P. King (Rockefeller University, New York City, NY).^{E1} Recombinant Amb a 1 (Amb a 1.3; GenBank accession no. C53240 with 2 amino acid substitutions^{E2}) was obtained from Biomay (Vienna, Austria). Ragweed pollen was extracted with PBS, as previously described.^{E3}

Allergen-specific T-cell cultures

Allergen-specific TCLs were established as previously described.^{E3} Briefly, 1.5×10^6 PBMCs were stimulated either with ragweed pollen extract (10 $\mu\text{g/mL}$) or rAmb a 1 (2.5 $\mu\text{g/mL}$). TCCs were established from TCLs by means of limiting dilution. The proliferative responses of TCLs or TCCs to rAmb a 1 (2.5 $\mu\text{g/mL}$) were measured after 48 hours. Results of proliferation assays are shown either as stimulation indices (ratio between cpm obtained in cultures stimulated with allergen and cpm obtained in control cultures without allergen) or as dpm ($\text{dpm} = \text{mean cpm in stimulated cultures} - \text{mean cpm obtained with medium control cultures}$). Allergen-specific cytokine production was assessed in supernatants harvested after 48 hours by means of ELISA, according to instructions from the manufacturer (Endogen, Woburn, Mass; sensitivity limits: IL-4, 4 pg/mL; IFN- γ , 4 pg/mL; IL-5, 5 pg/mL; and

IL-10, 5 pg/mL). Cultures containing TCCs and antigen-presenting cells alone served as negative controls. TCCs with an IFN- γ /IL-4 ratio of greater than 5 were classified as T_H1 , those with an IFN- γ /IL-4 ratio of 0.2 to 5 were classified as T_H0 , and those with an IFN- γ /IL-4 ratio of less than 0.2 were classified as T_H2 . The phenotype of the TCCs was analyzed by means of staining with antibodies to CD3, CD4, CD8, TCR $\alpha\beta$, and TCR $\gamma\delta$ (all from BD Biosciences, San Jose, Calif) and flow cytometry with a FACScan (BD Biosciences) and Cellquest software.^{E3}

T-cell epitope mapping and HLA class II restriction

Overlapping, synthetic 12-mer peptides (Biotrend/Mimotopes, Köln, Germany) spanning the complete amino acid sequence of Amb a 1.3 ($n = 121$) were used at a concentration of 5 $\mu\text{g/mL}$ to stimulate proliferation of TCLs or TCCs in single experiments, each as described previously.^{E3} Responses with a stimulation index of 3 or greater were considered positive. To determine HLA restriction, T cells were stimulated in duplicates with 2.5 $\mu\text{g/mL}$ Amb a 1 peptide in the presence of 2.5×10^4 autologous antigen-presenting cells and 10 $\mu\text{g/mL}$ blocking antibodies against HLA-DR (L243), HLA-DP (B7/21), or HLA-DQ (SK10; all from BD Biosciences).

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TABLE E1. Characterization of Amb a 1–specific TCCs

Patients' TCCs	Peptide recognition (amino acids)	Cytokine production (pg/mL)			T _H subset	Phenotype (CD4/8, TCRαβ)
		IFN-γ	IL-4	IL-10		
Patient A						
A 154	121-132	65	25	>670	T _H 0	4/αβ
A 19	121-132	717	233	>670	T _H 0	4/αβ
A 186	160-171	<4	<4	23	T _H 0	4/nt
A 12	202-213	10	126	294	T _H 2	4/αβ
A 375	202-213	<4	<4	89	T _H 0	4/αβ
A 101	343-354	16	347	260	T _H 2	4/αβ
A 184	343-354	<4	>440	308	T _H 2	4/αβ
A 159	343-354	90	104	104	T _H 0	4/αβ
A 130	343-354	<4	82	149	T _H 2	4/αβ
A 221	343-354	<4	157	370	T _H 2	4/αβ
A 148	343-354	48	<4	51	T _C 1	8/αβ
A 249	343-354	389	<4	61	T _H 1	4/αβ
A 203	379-393	26	<4	85	T _H 1	4/αβ
Patient B						
B 66	178-189	<4	12	610	T _H 0	4/αβ
B 40	346-357	<4	<4	39	T _H 0	4/αβ
Patient C						
C 12	Negative	<4	<4	66	T _H 0	4/αβ
C 18	346-357	<4	124	34	T _H 2	nt/nt
Patient D						
D 178	199-210	<4	>440	179	T _H 2	4/αβ
D 20	202-213	382	<4	207	T _C 1	8/αβ
Patient E						
E 50	136-150	<4	225	>1,500	T _H 2	4/αβ
E 51	139-150	<4	<4	135	T _H 0	4/αβ
E 81	136-150	<4	87	>1,500	T _H 2	4/αβ
E 45	178-189	<4	>440	>1,500	T _H 2	4/αβ
E 64	220-231	<4	>440	>670	T _H 2	4/αβ
E 31	295-306	146	>440	>1,700	T _H 0	4/αβ
E 101	295-306	<4	>440	>1,700	T _H 2	4/αβ
E 17	310-327	1,035	61	>1,700	T _H 1	4/αβ
E 63	310-327	206	>440	>1,700	T _H 0	4/αβ
E 111	369-378	<4	301	>1,700	T _H 2	4/αβ
E 68	369-378	<4	40	220	T _H 2	4/αβ
Patient F						
F 13	202-216	<4	119	11	T _H 2	4/αβ
F 151	262-273	46	231	5	T _H 0	4/αβ
Patient G						
G 25	Negative	116	313	204	T _H 0	4/αβ
G 44	343-354	18	300	938	T _H 2	4/αβ
G 35	355-369	1,908	430	776	T _H 0	4/αβ
G 62	367-378	86	92	6	T _H 0	4/αβ
Patient H						
H 125	154-165	271	<4	27	T _H 1	4/αβ
H 135	202-213	13	>440	>670	T _H 2	4/αβ
H 233	292-306	<4	>440	>670	T _H 2	4/αβ
H 55	343-354	<4	21	58	T _H 2	nt/nt
H 27	343-354	<4	54	340	T _H 2	nt/nt

Forty-one Amb a 1–reactive TCCs were derived from PBMCs of 8 patients with ragweed pollen allergy and analyzed for epitope recognition and allergen-induced cytokine production, and surface marker expression was assessed by means of flow cytometry. TCCs with an IFN-γ/IL-4 ratio of greater than 5 were classified as T_H1 (T_C1), those with an IFN-γ/IL-4 ratio of 0.2 to 5 were classified as T_H0, and those with an IFN-γ/IL-4 ratio of less than 0.2 were classified as T_H2.

nt, Not tested.