

Humoral and Cellular Cross-Reactivity between Amb a 1, the Major Ragweed Pollen Allergen, and Its Mugwort Homolog Art v 6

This information is current as of January 19, 2012

Beatrice Jahn-Schmid, Michael Hauser, Nicole Wopfner, Peter Briza, Uwe E. Berger, Riccardo Asero, Christof Ebner, Fatima Ferreira and Barbara Bohle

J Immunol 2012;188:1559-1567; Prepublished online 28 December 2011;

doi:10.4049/jimmunol.1102445

<http://www.jimmunol.org/content/188/3/1559>

-
- | | |
|----------------------|--|
| References | This article cites 28 articles , 2 of which can be accessed free at:
http://www.jimmunol.org/content/188/3/1559.full.html#ref-list-1 |
| Subscriptions | Information about subscribing to <i>The Journal of Immunology</i> is online at
http://www.jimmunol.org/subscriptions |
| Permissions | Submit copyright permission requests at
http://www.aai.org/ji/copyright.html |
| Email Alerts | Receive free email-alerts when new articles cite this article. Sign up at
http://www.jimmunol.org/etoc/subscriptions.shtml/ |



Humoral and Cellular Cross-Reactivity between Amb a 1, the Major Ragweed Pollen Allergen, and Its Mugwort Homolog Art v 6

Beatrice Jahn-Schmid,* Michael Hauser,[†] Nicole Wopfner,[†] Peter Briza,[‡] Uwe E. Berger,[§] Riccardo Asero,[¶] Christof Ebner,^{||} Fatima Ferreira,[†] and Barbara Bohle*[#]

Ragweed and mugwort are closely related weeds that represent the major cause of pollen allergy in late summer. Concomitant sensitization and clinical cross-reactivity frequently occur in subjects who are coexposed to both pollen species, and have implications for diagnosis and specific immunotherapy. Molecules involved in this cross-reactivity might be Amb a 1, the major ragweed pollen allergen, and Art v 6, a highly homologous allergen from mugwort. Therefore, we investigated the IgE and T cell response to Art v 6 of 60 weed pollen-allergic patients and assessed its immunological cross-reactivity with Amb a 1. Results of ELISA inhibition experiments suggested that both allergens are largely cross-reactive, but Amb a 1 possesses more IgE epitopes than Art v 6. In patients with IgE to both allergens, Amb a 1-induced T cell lines and clones responded weakly to Art v 6. Moreover, Art v 6-induced T cell lines responded stronger to Amb a 1. T cell epitope mapping of Art v 6 revealed that it contains only a few cross-reactive epitopes, which is opposed to the multiple T cell-activating regions present in Amb a 1. In summary, Amb a 1 can elicit more diverse allergen-specific IgE and T cell responses than Art v 6 and dominates the cross-reactivity with its homolog. Nevertheless, Art v 6 can act as a primary sensitizing allergen in areas with high mugwort pollen exposure, and consequently may facilitate sensitization to Amb a 1 by epitope cross-recognition of T and B cells. *The Journal of Immunology*, 2012, 188: 1559–1567.

Ragweed and mugwort pollen are the most frequent triggers of weed pollen allergy. Common ragweed (*Ambrosia artemisiifolia*) represents the most prominent seasonal allergen source in North America and has also been introduced into parts of Europe and Asia. The prevalence of sensitization to ragweed pollen has been increasing (1, 2). In Austria, it increased from 8.5% in 1997 to 17.5% of pollen allergies in 2007 (3), and around Milan (Italy), ragweed today is the second most frequent cause of respiratory allergy next to grass pollen (1). The major allergen in ragweed pollen, Amb a 1, is a highly allergenic molecule that is recognized by >90% of ragweed-sensitized individuals (4, 5) and accounts for >90% of the allergenic activity in ragweed pollen (6). It is a 38-kDa nonglycosylated protein that belongs to the family of pectate-lyase proteins.

Mugwort (*Artemisia vulgaris*) pollen represents the main cause of seasonal IgE-mediated allergy in late summer in many areas in Europe and Asia (7–9). Ragweed and mugwort are botanically closely related as both belong to the subfamily *Asteroideae* of the *Asteraceae* plant family. In areas with coexposure to both pollen species, concomitant sensitization and clinical reactivity to either weed pollen has been observed (10). The pollen seasons of mugwort and ragweed overlap, and it is nearly impossible to diagnose the primary sensitizing allergen source by using ragweed and mugwort pollen extracts for in vitro or in vivo testing. However, for specific immunotherapy, it is of relevance whether these patients are actually sensitized to both weeds (cosensitized) or whether they are sensitized to one weed and cross-react to the other weed (cross-sensitized) (11). Panallergens present in ragweed and mugwort pollen only to a minor extent account for the cross-reactivity of IgE Abs (10, 12–14). Amb a 1 is another conceivable candidate molecule for causing cross-reactivity, because mugwort pollen contains a homologous allergen, Art v 6, with 65% amino acid sequence identity and 85% amino acid similarity (12, 13, 15). Previous attempts to investigate IgE cross-reactivity of Amb a 1 and Art v 6 have been hampered by the lack of properly folded, IgE-reactive recombinant or natural molecules (5, 16, 17). Therefore, we intended to define the IgE cross-reactivity between Amb a 1 and Art v 6 by using the purified natural weed pollen allergens. Because CD4⁺ allergen-specific T lymphocytes are important for initiation and perpetuation of IgE-mediated allergic immune responses, as well as for specific immunotherapy (18), we also investigated the T cell cross-reactivity between both allergens. Sixty ragweed- and/or mugwort pollen-allergic individuals from Vienna and Milan, areas with differential weed pollen exposure, were studied. IgE binding to and cross-reactivity between Amb a 1 and Art v 6 were investigated by means of ELISA and inhibition experiments. T cell epitopes of Art v 6 and cellular cross-reactivity between both allergens were assessed by using allergen-specific T cell lines (TCL) and T cell clones (TCC).

*Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, 1090 Vienna, Austria;

[†]Christian Doppler Laboratory for Allergy Diagnosis and Therapy, University of Salzburg, 5020 Salzburg, Austria; [‡]Department of Molecular Biology, University of Salzburg, 5020 Salzburg, Austria; [§]Department of Otorhinolaryngology, Medical University of Vienna, 1090 Vienna, Austria; [¶]Ambulatorio di Allergologia, Clinica San Carlo, 20037 Paderno Dugnano (MI), Italy; ^{||}Allergieambulatorium Reumannplatz, 1100 Vienna, Austria; and [#]Christian Doppler Laboratory for Immunomodulation, 1090 Vienna, Austria

Received for publication August 24, 2011. Accepted for publication November 18, 2011.

This work was supported by the Austrian Science Foundation (FWF Grants P20011-B13, T411-B12, SFB-F1807), the Austrian National Bank (Grant 13860), and Bio-may AG, Vienna, Austria.

Address correspondence and reprint requests to Dr. Beatrice Jahn-Schmid, Department of Pathophysiology and Allergy Research, Medical University of Vienna, Währinger Gürtel 18-20, AKH-3Q, A-1090 Vienna, Austria. E-mail address: beatrice.jahn-schmid@meduniwien.ac.at

Abbreviations used in this article: nAmb a 1, natural Amb a 1; nArt v 6, natural Art v 6; rAmb a 1, recombinant Amb a 1; rArt v 6, recombinant Art v 6; SI, stimulation index; TCC, T cell clones; TCL, T cell lines.

Copyright © 2012 by The American Association of Immunologists, Inc. 0022-1767/12/\$16.00

Table I. Characterization of weed pollen-allergic patients and IgE reactivity to ragweed and mugwort allergens

Patient No. ^a	Age (y)	Sex	Additional Sensitizations	Total IgE (kU/l)	ImmunoCAP (kIU/l)		ELISA (OD)		Amb a 1/Art v 6 Ratio
					Ragweed	Mugwort	nAmb a 1	nArt v 6	
1	38	f	g, hdm, dog	1355	12.1	<0.35	0.515	<0.202 ^b	— ^c
2	52	f	g, bi, cat	338	10.2	2.15	0.489	<0.202	—
3	45	f	bi	161	14.1	<0.35	0.959	<0.202	—
4	43	f	None	36.8	5.81	<0.35	0.386	<0.202	—
5	21	f	hdm	149	15.6	4.93	0.874	<0.202	—
6	37	f	g, bi, cat, mold	240	28.6	2.35	0.323	<0.202	—
7	39	f	g	713	18.7	<0.35	0.607	<0.202	—
8	40	f	g, bi, celery	87.7	16.5	0.81	0.283	<0.202	—
9	45	f	bi	41.4	15.1	<0.35	0.309	<0.202	—
10	17	m	None	103	20.4	<0.35	0.728	<0.202	—
11	47	f	None	346	10.4	<0.35	0.164	<0.202	—
12	16	m	None	128	6.16	<0.35	0.690	<0.202	—
13	44	m	g, cypress	304	80.4	1.62	1.272	<0.202	—
14	35	f	g, cypress	92.6	43.4	<0.35	1.100	<0.202	—
15	55	m	g, bi	16.4	2.36	<0.35	0.307	<0.202	—
16	71	f	None	44.9	10.5	<0.35	0.191	<0.202	—
17	59	f	None	83.5	16.4	<0.35	0.827	<0.202	—
18	46	f	g, hdm, par, cypress	648	>100	22.4	0.629	<0.202	—
19	27	f	g, hdm, cat	52.4	6.38	ND	0.603	0.301	2.00
20	43	f	None	557	30.3	13.5	0.846	0.419	2.02
21	23	f	bi, hdm	519	74.7	0.67	1.659	0.272	6.11
22	37	f	Bee, wasp	252	40.5	ND	1.316	0.530	2.48
23	35	f	g	78.2	22.6	<0.35	2.372	0.344	6.90
24	43	f	g, hdm, bi	672	17.1	30.0	0.653	0.567	1.15
25	25	m	g, hdm, bi	315	68.2	21.7	1.298	1.369	0.95
26	39	m	g, bi	294	13.9	2.5	0.632	0.879	0.72
27	40	f	Cat	53.4	5.93	1.73	0.435	0.530	0.82
28	45	f	g, bi	354	14.0	1.98	0.304	0.298	1.02
29	39	f	g, bi	779	61.7	21.1	1.611	3.112	0.52
30	24	f	g	25.8	2.06	2.16	0.241	0.416	0.58
31	30	f	g	110	11.0	7.3	0.678	0.692	0.98
32	65	f	g, bi	194	2.36	2.57	0.175	0.225	0.78
33	37	m	bi, hdm	157	7.48	0.37	0.336	0.226	1.49
34	24	m	g, bi, hdm	>2000	18.8	11.7	0.243	0.312	0.78
35	30	f	None	25.8	2.06	2.16	0.537	0.618	0.87
36	44	f	g, bi, latex	1028	ND	85.9	1.629	3.045	0.54
37	45	f	g, bi	108	ND	6.2	0.681	0.408	1.67
38	30	f	None	32.3	1.45	1.11	0.147	0.250	0.59
39	35	m	b, bi, peanut	197	24.8	6.52	0.618	0.527	1.17
40	49	m	g, hdm	111	13.7	2.07	0.775	0.529	1.47
41	44	f	g, bi, hdm	440	27.9	1.87	0.474	0.271	1.75
42	39	f	None	19.3	12.2	<0.35	0.313	0.259	1.21
43	75	f	Olive	65.1	27.4	4.22	0.409	0.271	1.51
44	44	f	hdm	589	>100	12.42	3.654	3.431	1.07
45	25	f	g, hdm	243	69.3	81.1	0.564	3.590	0.16
46	52	f	None	548	32.7	25.0	0.886	2.273	0.39
47	75	f	None	531	11.0	4.5	0.621	1.563	0.40
48	40	f	bi, spices	140	21.8	15.3	0.334	1.258	0.27
49	23	f	g	221	26.4	22.4	0.579	1.995	0.29
50	14	f	None	55.7	<0.35	11.4	0.144	1.420	0.10
51	35	f	hdm, g, bi, dog	191	59.4	81.3	0.270	2.661	0.10
52	20	m	g, bi, hdm	630	10.9	24.7	0.553	1.813	0.30
53	27	f	g, bi	337	24.4	18.5	0.242	1.086	0.22
54	26	f	g, dog	72.8	ND	9.0	0.261	0.569	0.46
55	20	m	g, hdm	>2000	ND	22.7	0.165	1.319	0.13
56	27	m	g, bi	330	ND	66.9	0.197	2.819	0.07
57	15	m	g	231	1.9	1.0	<0.130 ^b	0.213	—
58	45	m	g, bi	91.4	2.5	2.8	<0.130 ^b	0.246	—
59	44	f	g, ash	133	8.3	49.2	<0.130	0.800	—
60	14	f	g, hdm, horse, milk	>2000	ND	RAST 3	<0.130	0.465	—

^aPatients 1–12, 19–22, 24–41, and 45–60 are from Vienna (Austria); patients 13–18, 23, and 42–44 are from Milan (Italy).^bCutoff was defined as mean OD + 5 SD of values obtained with sera from five nonallergic individuals. OD ratios of Amb a 1/Art v 6 within 0.5 and 2.0 were interpreted as “comparable” IgE reactivity (patients 23–44); OD ratios ≥ 2 or ≤ 0.5 were interpreted as “preferential” recognition of Amb a 1 (patients 19–22) or Art v 6 (patients 45–56), respectively.^cNot cross-reactive.bi, birch; g, grass; hdm, house dust mite; par, *Parietaria*; RAST, radioallergosorbent test.

Materials and Methods

Patients

Fifty subjects from Austria (Vienna) and 10 from Italy (Milan), regions with different pollen exposure, were included. Means of total pollen counts per year recorded in Vienna and Milan during the years 2006–2010 were 1630/m³ and 6189/m³ for ragweed and 585/m³ and 274/m³ for mugwort, respectively. All patients had recurrent rhinitis/conjunctivitis during late summer and positive skin prick tests to either ragweed or mugwort pollen or to both weeds. Sera from all patients also showed IgE reactivity in immunoblots to ragweed and/or mugwort pollen extract (data not shown). Specific IgE serum levels to ragweed and mugwort pollen (ImmunoCAP; Phadia, Uppsala, Sweden) are listed in Table I. All patients showed a positive T cell response to Amb a 1, Art v 6, or both. None of the patients had undergone specific immunotherapy. The study was approved by the local ethics committees, and all patients gave their informed consent.

Allergens

Extracts of ragweed or mugwort pollen (Allergon AB, Ängelholm, Sweden) were prepared by incubation of pollen in PBS for 2 h at room temperature followed by centrifugation at 4°C for 15 min at 15000 × *g*. Natural Amb a 1 (nAmb a 1) and natural Art v 6 (nArt v 6) were purified from pollen extract by ultrafiltration, followed by ion exchange and size exclusion chromatography. Recombinant Amb a 1 (rAmb a 1; Amb a 1.3, GenBank accession no. M62961, <http://www.ncbi.nlm.nih.gov/nucore/M62961>), Amb a 1.3 clone R2 with 3 aa substitutions L23Y, F364L, H367R (17) was obtained from Biomay (Vienna, Austria). Recombinant Art v 6 (rArt v 6) was expressed in *Escherichia coli* and purified as previously described (19).

Allergen-specific IgE binding

Binding of serum IgE to ragweed and mugwort pollen extracts was tested in immunoblots as described previously (20). Allergen-specific ELISAs were performed as described previously (21) using nAmb a 1 and nArt v 6, respectively. In brief, plates were coated overnight at 4°C with 50 µl per well of allergen (2 µg/ml). After blocking with TBS, pH 7.4, 0.05% Tween 20, and 0.5% BSA, patients' sera diluted 1:4 were incubated overnight at 4°C. Bound IgE was detected with alkaline phosphatase-conjugated monoclonal anti-human IgE (BD Biosciences, Franklin Lakes, NJ) diluted 1:3000; 4-nitrophenyl phosphate (Sigma-Aldrich, St. Louis, MO) was used as substrate, and OD was measured at 405/492 nm. As threshold for IgE positivity, the mean OD + 5 SD obtained with sera from five nonallergic individuals was defined. OD ratios of Amb a 1/Art v 6 within 0.5 and 2.0 were interpreted as "comparable" IgE reactivity, OD ratios ≥ 2 or ≤ 0.5 were interpreted as "preferential" recognition of Amb a 1 or Art v 6, respectively. For ELISA inhibition experiments, sera were diluted 1:5 or 1:10 and preincubated overnight at 4°C with nAmb a 1 or nArt v 6 at indicated protein concentrations before they were added to the plates coated with 2 µg/ml of either allergen.

Allergen-specific T cell cultures

Because the amounts of the natural purified allergens available were limited, rAmb a 1 and rArt v 6 were used for T cell stimulation. We have demonstrated previously that natural and recombinant proteins possess comparable T cell stimulation capacity (17). PBMCs (2×10^5) were stimulated in triplicates with concentrations from 0.3 to 20 µg/ml rAmb a 1 or rArt v 6. Allergen-specific TCL were established as described previously (20). In brief, 1.5×10^6 PBMCs were initially stimulated either with ragweed or mugwort pollen extract (10 µg/ml), Amb a 1 (2.5 µg/ml), or Art v 6 (2.5 µg/ml). TCC were established from TCL by limiting dilution as described previously (20), and most of them have been described elsewhere (22). The proliferative responses of TCL or TCC to Amb a 1 (2.5 µg/ml) or Art v 6 (2.5 µg/ml) or allergen-derived synthetic peptides (5 µg/ml) were measured after 48 h. Allergen concentrations used for *in vitro* stimulations had initially been optimized in titration experiments (data not shown). Unspecific T cell stimulation by the allergens was ruled out by nonreactivity of TCC of unrelated specificity. Results of proliferation assays are shown either as stimulation indices (SIs; ratio between cpm obtained in cultures stimulated with allergen and cpm obtained in control cultures without allergen) or as δ -cpm (dpm = mean cpm in stimulated cultures – mean cpm obtained with medium control cultures).

T cell epitope mapping

One hundred twenty-one overlapping, synthetic, 12-mer peptides (Biotrend/Mimotopes, Köln, Germany) spanning the complete amino acid sequence of Art v 6 or Amb a 1 (22) were used at a concentration of 5 µg/ml to

stimulate proliferation of TCL or TCC as described previously (20). Responses with SIs ≥ 3 were considered positive.

Statistics

Data from related samples were compared by using Wilcoxon signed rank test (SPSS 10.0; SPSS, Chicago, IL).

Results

IgE cross-reactivity between Amb a 1 and Art v 6

IgE reactivity of 60 weed-allergic patients to purified, nAmb a 1 or nArt v 6 was analyzed by ELISA to assess their reactivity to both weed allergens. The results are listed in Table I and are summarized in Fig. 1A. Eighteen patients showed exclusive IgE binding to Amb a 1 (patients 1–18) and four were sensitized only to Art v 6 (patients 57–60). Of the 38 patients with IgE against both allergens, 21 showed comparable IgE reactivity (patients 24–44), whereas preferential recognition of Amb a 1 or Art v 6 was found in 5 (patients 19–23) and 12 (patients 45–56) subjects, respectively.

Cross-inhibition experiments were performed with selected sera containing IgE to both allergens. Inhibition of IgE binding to Amb a 1 by preincubation with Art v 6 ranged from 20 to 60%, whereas inhibition of IgE binding to Art v 6 by preincubation with Amb a 1 ranged from 50 to 100% (Fig. 2). Self-inhibition of IgE binding by each allergen reached 100%. In control experiments, sera with exclusive IgE binding to either Amb a 1 or Art v 6 could not be blocked by preincubation with the homologous allergen (data not shown). Together, these data indicate that Amb a 1-specific IgE Abs largely cross-react with Art v 6 and possess a wider range of IgE epitopes. Austrian patients showed higher cross-reactivity than those from Italy (Table I). Of note, apart from cross-reactivity, Amb a 1-independent, Art v 6-specific IgE reactivity was identified.

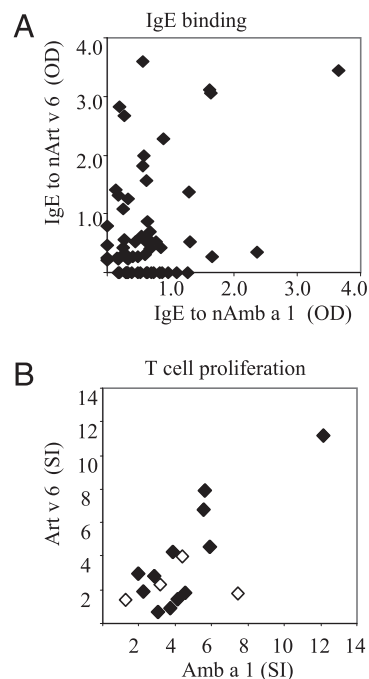


FIGURE 1. IgE binding and T cell proliferation to Amb a 1 and Art v 6. **A**, IgE binding to nAmb a 1 and nArt v 6 of sera from 60 different weed pollen-allergic patients was analyzed by ELISA. **B**, PBMCs from 12 weed pollen-allergic (filled diamonds) and 4 nonallergic subjects (open diamonds) were stimulated for 6 d. [³H]thymidine uptake was measured after another 16 h. SIs are shown.

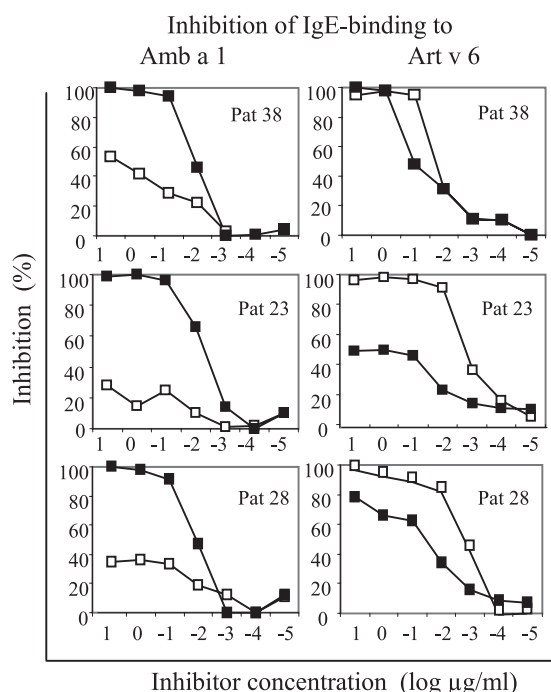


FIGURE 2. IgE cross-reactivity between Amb a 1 and Art v 6. Inhibition of IgE binding to nAmb a 1 or nArt v 6 was tested by preincubation of sera from cosensitized individuals with nAmb a 1 (filled squares) or nArt v 6 (open squares). Three representative sera with comparable IgE binding to both allergens are shown.

T cell proliferation in response to Amb a 1 and Art v 6

Proliferative responses to Amb a 1 and Art v 6 were tested in PBMCs of 12 individuals sensitized to both weed pollen allergens and 4 nonallergic, healthy individuals. At optimum concentrations, similar proliferation ranges were observed in allergic and nonallergic individuals in response to Amb a 1 (median SI: 4.1 [range: 2.0–12.2] and 4.0 [range: 1.5–7.6], respectively), as well as to Art v 6 (median SI: 2.9 [range: 1.0–11.2] and 2.2 [range: 1.5–4.2], respectively; Fig. 1B). The trend of stronger responses to Amb a 1 compared with Art v 6 was not statistically significant.

Cross-reactivity of ragweed-generated T cell cultures

To study T cell cross-reactivity, we generated Amb a 1-specific TCL from patients sensitized to both allergens using ragweed extract as initial stimulus ($n = 32$; Fig. 3A). Restimulation of these cultures with Art v 6 resulted in a much weaker proliferation as compared with Amb a 1 with medians of 1085 versus 41,896 dpm ($p < 0.001$). For control, Amb a 1-specific TCL were established from eight exclusively Amb a 1-sensitized, Art v 6-negative individuals. These TCL did not cross-react with Art v 6 (Fig. 3E).

To analyze which Amb a 1 epitopes were involved in the cross-reactivity with Art v 6, we mapped ragweed-induced TCL responsive for Amb a 1 for epitope recognition and then tested them in parallel with Amb a 1- and corresponding Art v 6-derived peptides (Table II). In the 27 T cell-activating regions found in Amb a 1, proliferative responses were induced by Art v 6 peptides matching Amb a 1_{343–357} in 96%, Amb a 1_{301–315} in 88%, and Amb a 1_{46–60} in 50% of the respective cultures. Fifteen additional Art v 6-derived peptides induced proliferative responses in 8–38% of Amb a 1-reactive TCL, and nine epitope regions were not cross-reactive at all (Table II). The Art v 6 peptides corresponding to the three major epitopes of Amb a 1 (Amb a 1_{178–189}, Amb a 1_{199–216}, and Amb a 1_{343–357}) (22) were cross-recognized by 9, 33, and 96% of the TCL, respectively.

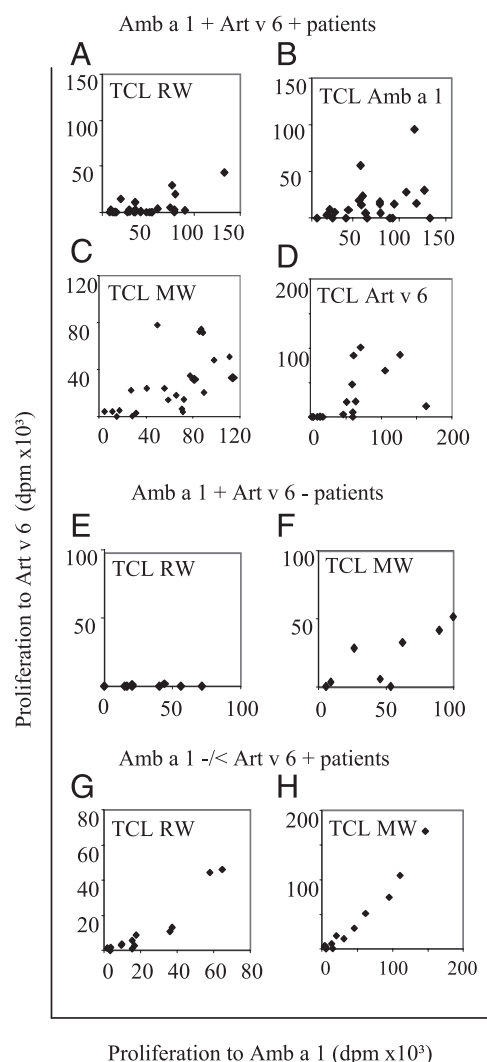


FIGURE 3. T cell cross-reactivity between Amb a 1 and Art v 6. TCL were stimulated with Amb a 1 or Art v 6. After 48 h, proliferation was determined by [³H]thymidine uptake. A, B, TCL induced with ragweed pollen extract (RW; $n = 32$); Amb a 1 ($n = 25$); C, D, TCL induced with mugwort pollen extract (MW; $n = 22$); Art v 6 ($n = 18$); E, F, TCL from exclusively ragweed-sensitized individuals initiated either with RW or MW (both $n = 8$); G, H, TCL from predominantly mugwort-sensitized individuals generated with RW ($n = 16$) or MW ($n = 13$).

In addition, 35 Amb a 1-specific TCC derived from 7 patients and recognizing 12 different T cell epitopes of Amb a 1 were tested for their reactivity with both allergens. Most of these TCC showed either no or weaker proliferative responses to Art v 6 as compared with Amb a 1 (Table III). Cross-reactivity with Art v 6 at the clonal level was mainly found for the regions Amb a 1_{292–306} and Amb a 1_{343–357}, and sporadically for Amb a 1_{178–189}, Amb a 1_{136–150}, and Amb a 1_{202–216}. Of note, Amb a 1_{292–306} has a prevalence of only 16% in ragweed-allergic patients (22). Together, these data show that the poor stimulation of Amb a 1-expanded T cells by Art v 6 is due to both the restricted number of cross-recognized T cell epitopes and better recognition of Amb a 1 sequences in cross-activating epitopes.

T cell epitope mapping of Art v 6

Because T cell epitopes of Art v 6 have not been described yet, epitope mapping with TCL that had been established with mugwort pollen extract was performed using a peptide panel spanning the complete Art v 6 amino acid sequence. Of 26 patients studied, 6 were

Table II. Cross-reactivity of Amb a 1- and Art v 6-reactive TCL with corresponding peptides derived from the homologous allergen

Amino Acid No.	Peptide Sequences of Amb a 1 and Art v 6	Amino Acid Identity (%)	Cross-Reactive TCL (%)	No. of TCL	No. of Patients
46–60	EAYNIIDKCWRGKAD AAHNIIDKCWRCKAD	80	50	6	4
82–93	GKWGDVYTVTSN GLGGEIYVVTDC	42	0	6	6
103–117	EGTLRFAAAQNRPLW PGTLRCGVTQDKPLW	53	0	11	9
112–126	QNRPLWIIIFKNDMVI QDKPLWIIIFKNDMVI	80	9	11	9
121–132	KNDMVINLNQEL KKDMVIKLNKHEL	67	10	10	8
130–144	QELVVNSDKTIDGRG HELVINKDKTIDGRG	80	0	12	10
136–150	SKTIDGRGVKVEII KDKTIDGRGANVEIT	73	75 ^a 27	4 ^a 11	3 ^a 10
148–165	EIINGGLTLMNVKNIIH EITCGGLTIHNVCNVIIH	67	0	13	11
160–177	KNIIHNINIHDKVLP CNVIIHNINIHDIKVTEG	67	33 ^a 33	3 ^a 9	3 ^a 8
178–189	GMIKSNDGPPIL GIIKATDAKPGH	42	78 ^a 9	9 ^a 22	7 ^a 18
199–216	NVAGSSQIWDHCSLSKS CVAGSSKIWDHCTLSHG	72	81 ^a 33	16 ^a 27	12 ^a 20
220–231	LVDVTLGSTHVT LIDVTLGSTAVT	83	0	5	3
226–237	GSTHVTISNCKF GSTAVTISNCKF	92	17	12	9
253–261	VQDKG.MLATVAFNMF VDDKKMHVTVAFNRF	67	0 ^a 17	3 ^a 6	3 ^a 5
262–276	VAFNMFNDVDQRMF VAFNRFACDQRMF	67	13	8	8
271–285	VDQRMPCRFRGFQV CDQRMPCRFRGFQV	93	38	8	8
280–291	FGFFQVVNNNDYRWG FGFFQVVNNNDYTSWG	80	67 ^a 25	3 ^a 4	3 ^a 4
286–300	VNNNDYRWGTYAIGG VNNNDYTSWGTYAIGG	80	0	8	8
292–306	RWGTYAIGGSSAPTI SWGTYAIGGSANPTI	80	100 ^a 20	4 ^a 10	3 ^a 7
301–315	SSAPTILCQGNRFLA SANPTILSQGNRFHA	73	87 ^a 88	15 ^a 8	11 ^a 8
310–327	GNRFLAPDDQIKKNVLAR GNRFHAPNDPNKKNVLVR	72	0	7	7
322–339	KNVLARTGTGAESMAWN KNVLVRADAPHTESMKWN	56	13	8	6
343–357	DKDLENGAIFVTSG EKDLENGAIFVASG	87	94 ^a 96	35 ^a 27	26 ^a 20
364–378	PVQSAGMIPAEPGEA PEQKSHLIPAEPGSA	60	8	13	9
349–360	NGAIFVTSGSDPVL NGAIFVASGCDPHLT	80	22	9	8
355–369	TSGSDPVLTPVQSAG ASGCDPHLTPEQKSH	53	0	9	7
376–393	GEAAIKLTSSAGVLSRCP GSAVLQLTSCAGTLKCVP	56	0	11	9

TCL were initiated with ragweed pollen extract unless noted otherwise. Cross-reactivity of Amb a 1- or Art v 6-reactive TCL with the corresponding peptides was tested in proliferation assays.

^aTCL were initiated with mugwort pollen extract.

primarily sensitized to Amb a 1, 6 to Art v 6, and 14 to both allergens at comparable IgE levels (Fig. 4). Epitope recognition was similar in these three groups, except for the fact that epitope region Art v 6_{301–315} was more frequently recognized in subjects with preponderant sensitization to Art v 6. In contrast with Amb a 1, which contains >20 T cell epitope regions (22), only 5 T cell-activating regions with a prevalence rate of >11% were identified in Art v 6, namely, Art v 6_{178–189} (15%), Art v 6_{199–216} (41%), Art v 6_{253–267} (15%), Art v 6_{301–315} (33%), and Art v 6_{343–357} (74%; Fig. 5). The latter can be considered as the dominant T cell epitope in Art v 6.

Cross-reactivity of mugwort-generated T cell cultures

To analyze cross-recognition of Art v 6-reactive T cells by Amb a 1, 22 mugwort-induced TCL from patients sensitized to both allergens were generated. These TCL showed stronger proliferation with Amb a 1 than with Art v 6 (65,768 versus 20,717 dpm; $p < 0.001$; Fig. 3C). In five of eight mugwort-generated TCL from subjects with IgE reactivity to Amb a 1 but not to Art v 6, we detected Art v 6-reactive T cells (Fig. 3F). As mentioned earlier, the ragweed-induced TCL of the same patients did not react with

Table III. Reactivity of Amb a 1-specific TCC to Art v 6

TCC	Amb a 1 Epitope Amino Acid Sequence	Proliferation (dpm $\times 10^3$)	
		Amb a 1	Art v 6
Pat47 12	Negative	2.6	41.3
Pat5 154	KNDMVINLNQEL aa 121–132	83.0	0.9
Pat5 19		42.9	2.0
Pat19 50	SDKTIDGRGVKVEII aa 136–150	97.2	34.7
Pat19 51		39.6	1.1
Pat19 81		25.1	1.1
Pat30 66	GMIKSNGGPPII aa 168–179	196.4	46.7
Pat19 45		204.0	0.5
Pat5 12	GSSQIWIHDHSLSKS aa 202–216	106.6	0
Pat5 375		34.7	0.2
Pat35 20		35.3	0
Pat6 135		146.0	0
Pat6 266		33.8	19.8
Pat7 13		54.2	0
Pat19 64	LVDVTLGSTHVT aa 220–231	143.6	0
Pat7 151	VAFNMFTDNVDQ aa 262–273	28.5	0
Pat6 233	RWGTYAIGGSSAPTI aa 292–306	42.2	31.2
Pat19 31		296.0	274.0
Pat19 101		405.7	35.8
Pat19 17	GNRFLAPDDQIKKNVLAR aa 310–327	151.5	0
Pat19 63		119.4	2.2
Pat5 130		57.8	2.6
Pat5 148		9.9	2.3
Pat5 159	DKDLLENGAIFVTSG aa 343–357	57.4	0.2
Pat5 184		84.9	12.1
Pat5 249		60.7	10.6
Pat30 40		13.8	7.5
Pat6 55		114.3	18.0
Pat6 27		7.3	0.2
Pat5 35	TSGSDPVLTPVQSAG aa 355–369	17.9	0
Pat47 29	PVQSAGMIPAEP aa 367–378	202.6	0
Pat19 68		65.0	1.3
Pat19 111		188.9	1.2
Pat5 227	GEAAIKLTSSAGVLSCRIP aa 376–393	93.8	0.6
Pat5 62		105.7	0.7

T cell reactivity to rAmb a 1 and rArt v 6 was determined by parallel stimulation of TCC derived from seven different Amb a 1- (patients 5–7) or Amb a 1- and Art v 6-sensitized patients (patients 19, 30, 35, and 47) and specific for the indicated epitope regions of Amb a 1. Background proliferation TCC plus APC alone ranged from 1310 to 4654 cpm.

Art v 6 (Fig. 3E), indicating that stimulation with mugwort allergen expanded a different set of T cells than ragweed, namely, cross-reactive rather than non-cross-reactive.

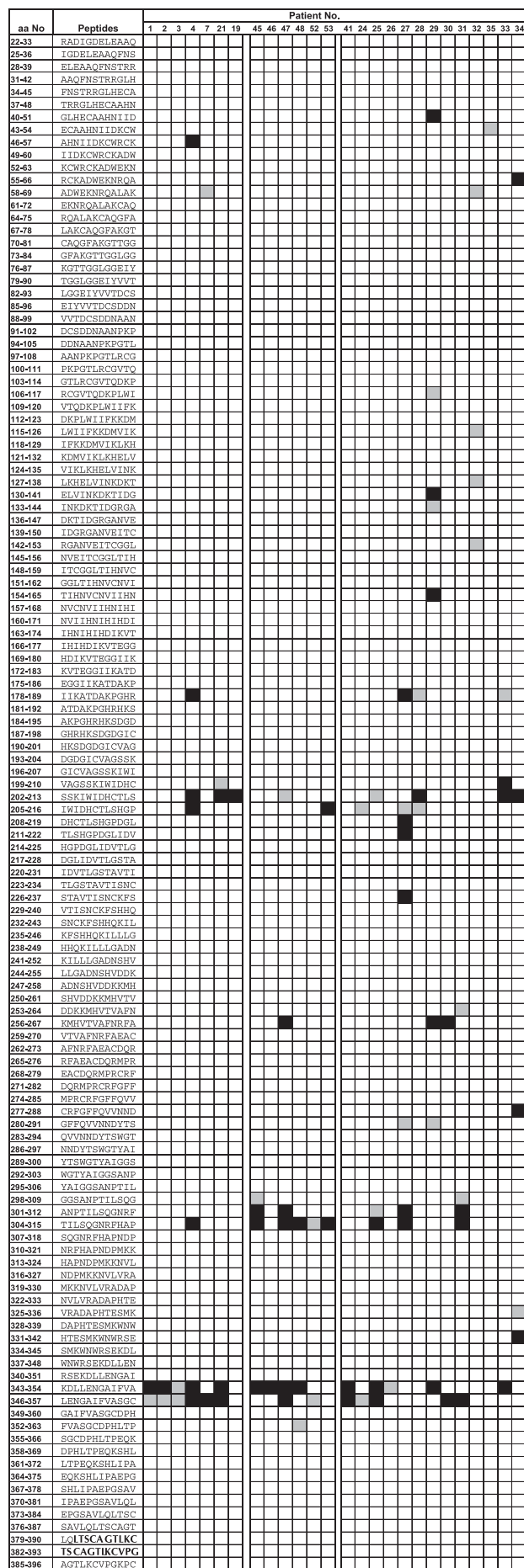
To analyze which Art v 6 epitopes were involved in the cross-reactivity with Amb a 1, we mapped Art v 6-reactive TCL with Art v 6 peptides and then cross-stimulated with the corresponding Amb a 1-derived peptides. Seven of nine epitope regions were highly cross-reactive: Art v 6_{343–357} in 94%, Art v 6_{301–315} in 87%, Art v 6_{292–306} in 100%, Art v 6_{280–291} in 67%, Art v 6_{199–216} in 81%, Art v 6_{178–189} in 78%, and Art v 6_{136–150} in 75% (Table II). Notably, the cross-reactivity for five epitope regions was markedly higher than in the TCL that had been induced with ragweed extract described earlier, indicating a selective expansion of Art v 6-reactive T cells in culture. The observation that TCL from 12 patients with preferential or exclusive mugwort sensitization that had been initiated with mugwort or ragweed pollen extract responded markedly stronger to Art v 6 (Fig. 3G, 3H) than the respective TCL from subjects with exclusive sensitization to Amb a 1 (Fig. 3E, 3F) or both allergens (Fig. 3A–D) suggests that an analogous expansion of Art v 6-reactive T cells also occurs in vivo in subjects with substantial exposure to mugwort pollen.

Discussion

In this study, the humoral and cellular cross-reactive potential of Amb a 1, the major ragweed allergen, and Art v 6, its homolog in mugwort pollen, was examined. In patients with IgE reactivity to

both weed pollen allergens, cross-inhibition experiments indicated that Amb a 1-specific IgE Abs largely cross-react with Art v 6 and also show a broader epitope range or, alternatively, possess higher affinities than IgE Abs to Art v 6. Thus, Amb a 1 generally is the stronger sensitizing molecule.

Previous attempts to evaluate IgE cross-reactivity between Amb a 1 and Art v 6 by immunoblot inhibition experiments performed with total pollen extracts and patients from the Milan area had suggested a limited cross-reactivity between Amb a 1 and Art v 6 (12). Accordingly, the 10 Italian ragweed-allergic patients also showed no or low cross-reactivity to Art v 6. In contrast, most mugwort-allergic subjects from Austria exhibited marked cross-reactivity (Table I). This finding indicated that the extent of cross-reactivity depends on the exposure to mugwort pollen, because the pollen counts for mugwort are higher in Austria than in Milan. This notion is further supported at the individual level by sensitization against the major allergen in mugwort pollen, Art v 1 (23), in patients with high levels of cross-reactivity. Art v 1-specific IgE was observed in 75% of the patients with preferential or exclusive recognition of Art v 6, opposed to 25% of those with preferential recognition of Amb a 1 (data not shown). Nevertheless, Art v 6-positive, Amb a 1-negative patients were found in Vienna (Table I), and inhibition experiments indicated the presence of additional, non-cross-reactive IgE epitopes in Art v 6 (Fig. 2). Thus, apart from pronounced cross-reactivity with Amb a 1, Art v 6 can act as primary sensitizing allergen.



At the T cell level, Amb a 1 was the stronger stimulant for both ragweed- and mugwort-induced, allergen-reactive T cell cultures. The cross-reactivity between the two weed allergens was found to be restricted to a few T cell-activating regions despite their high sequence homology (Fig. 3, Tables II, III). This finding was unexpected, because, for example, the food allergens homologous to the major birch pollen allergen, Bet v 1, all possess multiple epitope regions similar to Bet v 1 (24, 25), and T cell cross-stimulation has been observed for epitopes with 60–80% amino acid similarity and 40–67% amino acid identity (26). For Amb a 1, we have previously found >20 distinct T cell epitope regions, including 3 dominant epitopes (Amb a 1_{178–189}, Amb a 1_{199–216}, and Amb a 1_{343–357}) recognized by >45%, and 7 epitopes recognized by >25% of patients (22). In contrast, cellular cross-reactivity with Art v 6 was restricted to a few Amb a 1 epitopes, excluding the two immunodominant regions Amb a 1_{178–189} and Amb a 1_{199–216}. The three most cross-reactive epitopes (Art v 6_{343–357}, Art v 6_{301–315}, and Art v 6_{64–60}) matched the corresponding amino acid sequences of Art v 6 with an identity of 73–87% (Fig. 5). Interestingly, for other highly similar epitope-containing regions, less pronounced T cell cross-reactivity was observed. For instance, Art v 6_{226–237} with 92% amino acid identity to the corresponding Amb a 1 peptide caused cross-reactivity in only 17% of Amb a 1_{226–237}-reactive TCL. However, because the Viennese patients were exposed to both ragweed and mugwort pollen, this dual exposure could contribute to the more extensive recognition of Amb a 1 epitopes.

The small number of T cell epitopes discovered in Art v 6 provided an explanation for the limited T cell cross-reactivity between Art v 6 and Amb a 1. Only five epitope regions including one major T cell-activating region, Art v 6_{343–357}, with a prevalence rate of 74% were identified in Art v 6 (Fig. 4). Although these regions corresponded to epitope regions of Amb a 1 to a great extent, the prevalence of their recognition was different for the two allergens (Fig. 5).

A possible reason for the less diverse T cell response to Art v 6 compared with its homolog Amb a 1 could be different binding capacities of the respective homologous peptides to MHC class II molecules for presentation. Therefore, we used the consensus method of the Immune Epitope database (<http://www.lii.org/pages/immune-epitope-database>) (27) to compare the presumed binding capacity to the most common HLA-DR, -DP, and -DQ molecules of 14 immunogenic Amb a 1 peptides with their counterparts derived from Art v 6. However, no correlation between T cell responses to Art v 6 and HLA binding of the respective peptides was observed (data not shown).

In contrast with the Amb a 1-dominated T cell response in concomitantly sensitized individuals, Art v 6-generated T cell cultures from solely or primarily mugwort-sensitized subjects, were activated strongly by both Amb a 1 and Art v 6 (Fig. 3G, 3H). This finding suggested that only T cells recognizing cross-reactive epitopes had been expanded *in vivo* by Art v 6. Once sensitization to Art v 6 is established, such "pre-priming" of cross-reactive T cells and B cells, supported by the overlap of the weed pollen seasons, might pave the way to sensitization against Amb a 1 (28).

FIGURE 4. T cell-activating regions in Art v 6. Art v 6-specific TCL from 26 mugwort pollen-allergic Austrian patients were stimulated with 12-mer peptides covering the complete sequence of Art v 6. Proliferation is indicated in gray (SI ≥ 3) or in black (SI ≥ 5). Patients were sensitized preferentially to Amb 1 (*left columns*), to Art v 6 (*middle columns*), or to both allergens (*right columns*).

23. Himly, M., B. Jahn-Schmid, A. Dedic, P. Kelemen, N. Wopfner, F. Altmann, R. van Ree, P. Briza, K. Richter, C. Ebner, and F. Ferreira. 2003. Art v 1, the major allergen of mugwort pollen, is a modular glycoprotein with a defensin-like and a hydroxyproline-rich domain. *FASEB J.* 17: 106–108.
24. Bohle, B., A. Radakovics, D. Lüttkopf, B. Jahn-Schmid, S. Vieths, and C. Ebner. 2005. Characterization of the T cell response to the major hazelnut allergen, Cor a 1.04: evidence for a relevant T cell epitope not cross-reactive with homologous pollen allergens. *Clin. Exp. Allergy* 35: 1392–1399.
25. Bohle, B., A. Radakovics, B. Jahn-Schmid, K. Hoffmann-Sommergruber, G. F. Fischer, and C. Ebner. 2003. Bet v 1, the major birch pollen allergen, initiates sensitization to Api g 1, the major allergen in celery: evidence at the T cell level. *Eur. J. Immunol.* 33: 3303–3310.
26. Jahn-Schmid, B., A. Radakovics, D. Lüttkopf, S. Scheurer, S. Vieths, C. Ebner, and B. Bohle. 2005. Bet v 1142-156 is the dominant T-cell epitope of the major birch pollen allergen and important for cross-reactivity with Bet v 1-related food allergens. *J. Allergy Clin. Immunol.* 116: 213–219.
27. Wang, P., J. Sidney, C. Dow, B. Mothé, A. Sette, and B. Peters. 2008. A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. *PLOS Comput. Biol.* 4: e1000048.
28. Aalberse, R. C. 1991. The IgE response and atopy. *Eur. Respir. J. Suppl.* 13: 78s–84s.