

Characterization of the human T cell response to antigen 5 from *Vespula vulgaris* (Ves v 5)

B. Bohle*, B. Zwölfer*, G. F. Fischer†, U. Seppälä‡, T. Kinaciyan§, C. Bolwig‡, M. D. Spangfort‡ and C. Ebner*

Departments of *Pathophysiology and †Blood Group Serology, Medical University of Vienna, Vienna, Austria, ‡ALK-Abelló Research, Hørsholm, Denmark and §Division of Immunology, Allergy and Infectious Diseases, Department of Dermatology, Medical University of Vienna, Vienna, Austria

Summary

Background The T cell reactivity to the major allergen of bee venom, phospholipase A2, has been thoroughly characterized. In contrast, only little is known about the human cellular response to major allergens from wasp venom.

Objective To characterize the human T cell response to antigen 5 from *Vespula vulgaris*, Ves v 5.

Methods Recombinant Ves v 5 was used to establish allergen-specific T cell lines (TCL) and T cell clones (TCC) from the peripheral blood of vespid-allergic and non-allergic individuals. Ves v 5-specific TCL were mapped for T cell epitopes using overlapping synthetic peptides representing the complete amino acid sequence of Ves v 5. Ves v 5-specific TCC were analysed for antigen-induced secretion of IL-4, IFN- γ and IL-10.

Results Seventeen distinct T cell epitopes were recognized by allergic individuals among which Ves v 5_{181–192} was identified as a dominant T cell epitope. Partially different epitopes were observed in TCL from non-allergic subjects and the dominant epitope Ves v 5_{181–192} was not prevalent in these cultures. Ves v 5-specific TCC isolated from allergic individuals did not show the typical T helper type 2 (Th2)-like cytokine profile in response to specific stimulation, i.e. high amounts of IL-4 and low IFN- γ . TCC from non-allergic individuals showed a Th1-like cytokine pattern.

Conclusions Our findings provide evidence that the allergic T cell response to Ves v 5 is not Th2-dominated and that different immunogenic sites on this major wasp venom allergen are recognized by allergic and non-allergic individuals.

Keywords human, insect venom allergy, T cell epitopes, Th1/Th2

Submitted 29 June 2004; revised 22 November 2004; accepted 16 December 2004

Introduction

It is well documented that stings from insects belonging to the order Hymenoptera, e.g. honey bees (*Apoidea*) and vespids (*Vespidae*), can induce allergic reactions and occasionally fatal anaphylaxis [1, 2]. The prevalence of sensitization against these insects was estimated between 9.3% and 28.5% in the adult population [3, 4]. Vespid venom contains three major allergens: phospholipase A1, hyaluronidase and antigen 5 (Ag 5). Whereas phospholipase A1 and hyaluronidase represent two allergens with enzymatic activities, the biological function of Ag 5 is still not fully understood [5]. The complete amino acid (aa) sequences of Ag 5 from different vespid species have been determined [6]. Ag 5 from *Vespula vulgaris* (Ves v 5) is a protein of 23 kDa and consists of 204 aa. RVes v 5 was expressed in bacteria and – in order to obtain the native conformation of the natural protein – in

Pichia pastoris [7]. Recently, the structure of Ves v 5 was solved by X-ray crystallography enabling the analysis of B cell epitopes that may participate in allergic cross-reactivity [8].

The cloning of the complementary DNA (cDNA) of allergens and their subsequent expression in recombinant form allow to elucidate the cellular response to these proteins. Several relevant respiratory allergens have been characterized with respect to their T cell epitopes and the phenotype of responding T cells in the recent past [9–16]. Regarding Hymenoptera venom allergens, however, only a limited number of allergens have been investigated so far. The T cell reactivity to the major allergen of bee venom, phospholipase A2 (PLA2), has been characterized in allergic and non-allergic individuals [17]. Several T cell epitopes of PLA2 distributed throughout the entire molecule have been identified [18, 19]. Based on these findings, therapeutic strategies have been developed which involve the use of peptides that contain important T cell epitopes but are not able to induce IgE-mediated adverse side-effects [20–22]. In clinical studies overlapping peptides deduced from the PLA2 molecule have been shown to be clinically effective as vaccines and to provide increased safety of specific venom immunotherapy [23, 24]. The cellular response to Ag 5 from *Dolichovespula maculata* (American hornet), Dol m 5, has been analysed in mice [25, 26].

Correspondence: Barbara Bohle, Medical University of Vienna, Center of Physiology and Pathophysiology, Department of Pathophysiology, Währinger Gürtel 18–20, A-1090 Vienna, Austria.

E-mail: barbara.bohle@meduniwien.ac.at

The intention of the present study was to characterize the human T cell response to Ag 5 from *V. vulgaris*, Ves v 5. Peripheral blood mononuclear cells (PBMC) from human leucocyte antigen (HLA)-typed allergic and non-allergic individuals were incubated with rVes v 5 and T cell lines (TCL) were established from those cultures. To delineate relevant T cell epitopes, the TCL were stimulated with overlapping synthetic peptides representing the complete aa sequence of Ves v 5. Ves v 5-specific T cell clones (TCC) were established and served as tools to characterize the phenotype of responding T lymphocytes to the major *V. vulgaris* venom allergen.

Methods

Allergic and non-allergic individuals

Fifteen vespid-allergic and 15 non-allergic individuals were included in the study. IgE-mediated allergy to *V. vulgaris* venom was documented by typical case histories, positive skin test titration (skin prick test or intracutaneous test) and positive CAP/RAST (median: 25.2 kU/mL; Pharmacia, Uppsala, Sweden). In general, the patients were stung two to three times and blood samples were taken within a time period of 3–6 months after they had developed severe systemic reactions. Non-allergic individuals were stung by vespids at least twice and did not experience abnormal local or systemic reactions. In skin tests all non-allergic individuals were negative. The serum of each allergic and non-allergic individual was tested for Ves v 5-specific IgE in immunoblot experiments. HLA-A, B, C, DRB1, DRB3/4/5 and DQB1 typing was performed at low resolution by reverse-blot SSO technique (Reli, Dynal, Uppsala, Sweden) and ligation-based typing. In addition, DRB1 alleles were typed by nucleotide sequencing as previously described [27].

Allergens

V. vulgaris venom (12019726YJ) was kindly provided by Vespa Labs (Spring Mills, PA, USA). The gene coding for Ves v 5 was obtained from Dr T. P. King [28]. The gene was subcloned into pGapZαA expression vector into *Xho*I and *Xba*I restriction site(s). The vector was transformed into *P. pastoris* X33 cells by electroporation [28]. Expression of rVes v 5 was performed in yeast peptone dextrose medium with 100 µg/mL Zeocin at 30 °C. The *Pichia pastoris*-expressed rVes v 5 was concentrated from the culture supernatants by stepwise ammoniumsulfate precipitation. The precipitated rVes v 5 was dissolved and dialysed against 20 mM Tris-Cl, pH 7.5. The sample was then subjected to a 6 mL Resource Q (Amersham Biosciences, Uppsala, Sweden) anion exchange column. The column was first equilibrated with 20 mM Tris-Cl, pH 7.5 buffer. The elution was performed with a linear 20 min gradient from 0% to 100% in a 20 mM Tris-Cl, 0.5 M NaCl pH 7.5. The flow-through fractions containing rVes v 5 were pooled and further subjected into the 5/5HR (Amersham Biosciences) SE-53 cation exchange column. The column was equilibrated with 20 mM Tris-Cl, pH 7.5 and the elution was performed as described above. The eluted fractions containing rVes v 5 were pooled and put on the 16 × 60 Superdex 75 (Amersham Biosciences) column equilibrated by 10 mM (NH₄)₂HCO₃

buffer. RVes v 5-containing fractions were pooled and subsequently lyophilized and stored at –20 °C.

Immunoblots and inhibition assays

For immunoblotting experiments *V. vulgaris* venom (6 µg/lane) or rVes v 5 (1 µg/lane) were separated by 12% SDS-PAGE and blotted onto a nitrocellulose membrane. The membrane was incubated with patients' sera overnight at 4 °C. After incubation with a ¹²⁵I-labelled anti-human IgE antibody (IBL, Hamburg, Germany) bound IgE was visualized by autoradiography. For inhibition experiments sera were incubated overnight at room temperature with rVes v 5 (5 µg/mL) or bovine serum albumin (BSA) (5 µg/mL). After pre-incubation, sera were subjected to immunoblotting experiments as described above.

Proliferation assays

PBMC were obtained from peripheral blood by Ficoll-Hypaque density gradient centrifugation (Pharmacia Diagnostics, Uppsala, Sweden). PBMC (2×10^5) were cultured in triplicate in 96-well plates (Nuncclone, Nunc, Denmark) in 200 µL of serum-free Ultra Culture Medium (Bio Whittaker, Walkersville, MD, USA) supplemented with 2 mM/L-glutamine and 2×10^{-5} M 2-ME in the presence of allergen for 6 days. RVes v 5 was titrated in the concentration range of 3 µg to 50 µg/mL. During the last 16 h of culture [³H]-thymidine (0.5 µCi/well) was added and the incorporated radioactivity was measured by scintillation counting in counts per minute (cpm). Δcpm are cpm in cultures stimulated with rVes v 5 minus cpm in cultures with medium alone. Background cpm ranged from 981 to 37912 cpm in vespid-allergic and from 1114 to 31487 cpm in non-allergic individuals.

T cell epitope mapping

For epitope mapping studies a panel of 65 overlapping dodeca peptides (5 µg/mL; Mimotopes/Biotrend, Köln, Germany) representing the entire aa sequence of Ves v 5 was used. Since in PBMC no significant proliferative responses to peptides were observed, allergen-specific T lymphocytes were enriched in oligoclonal TCL. For this purpose, PBMC (1.5×10^6 /mL) were stimulated with rVes v 5 (10 µg/mL) or *V. vulgaris* venom (20 µg/mL) in 24-well flat-bottomed culture plates (3524 Costar, Cambridge, MA, USA) under conditions as described above. After 5 days, 10 U/mL of human rIL-2 (Boehringer, Mannheim, Germany) were added. At day 7, T cell blasts were enriched by density gradient centrifugation and expanded in 96-well-round-bottomed plates (Nuncclone) by adding 2×10^5 irradiated PBMC and rIL-2 (4 U/well) at weekly intervals. TCL were stimulated with rVes v 5 (5 µg/mL), *V. vulgaris* venom (25 µg/mL) or single peptides in the presence of 1×10^5 irradiated (60 Gy) autologous PBMC. After 48 h, [³H]-thymidine was added and cell proliferation was evaluated as described above. The stimulation index (SI) was calculated as ratio between cpm obtained in T cell cultures plus PBMC plus allergen and cpm obtained in cultures plus PBMC without allergen. The proliferative response to selected peptides in TCL from allergic compared with non-allergic subjects was statistically analysed by the Mann–Whitney U-test.

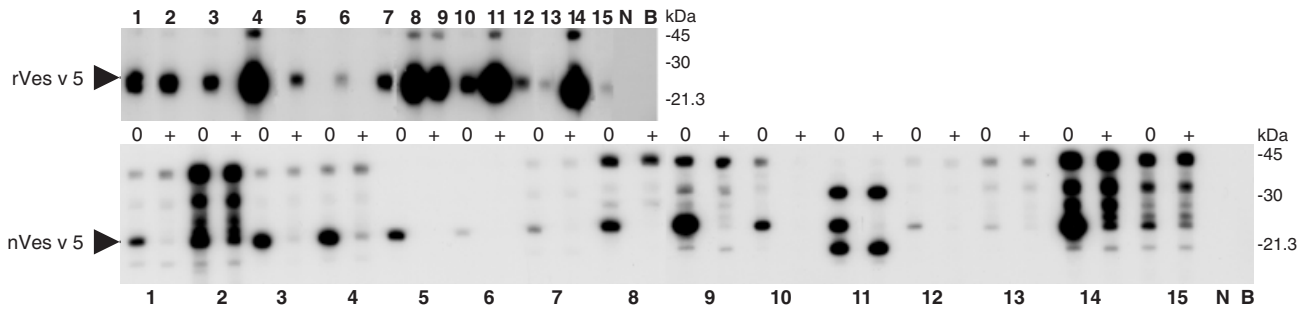


Fig. 1. IgE immunoblot. The IgE reactivity of vespid-allergic individuals to rVes v 5 (upper level) and *Vespula vulgaris* venom (lower level) is shown. Pre-incubation of each serum with rVes v 5 (lower level, lane +) reduced IgE binding to natural Ves v 5 (lower level, lane 0) in *V. vulgaris* venom. N, serum pool of non-allergic individuals; B, buffer control.

Ves v 5-specific T cell clones

After density gradient centrifugation of TCL at day 7, the enriched T cell blasts were seeded by limiting dilution technique in 96-well round bottom plates (Nunc) in the presence of 2×10^5 irradiated PBMC, 0.5% v/v PHA (Gibco, Grand Island, NY, USA) and rIL-2 (4 U/well). Growing monoclonal microcultures were expanded at weekly intervals with irradiated PBMC and rIL-2. To determine allergen-specificity, TCC were stimulated with rVes v 5 (5 µg/mL) and *V. vulgaris* venom (25 µg/mL) in the presence of 1×10^5 irradiated (60 Gy) PBMC. After 48 h, [^{3} H]-thymidine was added and cell proliferation was evaluated as described above. The phenotype of TCC was analysed by flow cytometry as previously described [15]. Cytokine levels in supernatants collected after 48 h of allergen-specific stimulation were measured by ELISA using matched Ab pairs (Endogen, Woburn, MA, USA) according to instructions of the manufacturer (sensitivity limits: IL-4: 9 pg/mL, IFN- γ : 9.5 pg/mL, IL-10: 4.7 pg/mL). Cultures containing TCC and PBMC alone served as negative controls. TCC were distinguished into Th-subsets according to their cytokine pattern in response to allergen-specific stimulation as previously described [15]: TCC synthesizing >47.5 pg/mL IL-4/ <9.5 pg/mL IFN- γ and IL-4 (pg/mL) $>5 \times$ IFN- γ (pg/mL) were attributed to the Th2-like subset. TCC producing >45 pg/mL IFN- γ / <9 pg/mL IL-4 and IFN- γ (pg/mL) $>5 \times$ IL-4 (pg/mL) to the Th1-like subset. TCC synthesizing similar levels of IL-4 and IFN- γ were classified as Th0.

Results

Immunoglobulin E immunoblots

IgE binding patterns of 15 patients with wasp sting allergy are depicted in Fig. 1. All patients reacted with rVes v 5 (Fig. 1, upper level) and the 23 kDa allergen in *V. vulgaris* venom, i.e. natural Ves v 5 (Fig. 1, lane 0, lower level). Pre-incubation of the sera with rVes v 5 markedly reduced IgE binding to natural Ves v 5 in all patients (lane +), lower level) confirming the correct three-dimensional structure of the recombinant protein. None of the 15 non-allergic individuals showed IgE binding to recombinant or natural Ves v 5 nor to any protein contained in *V. vulgaris* venom (individual data not shown).

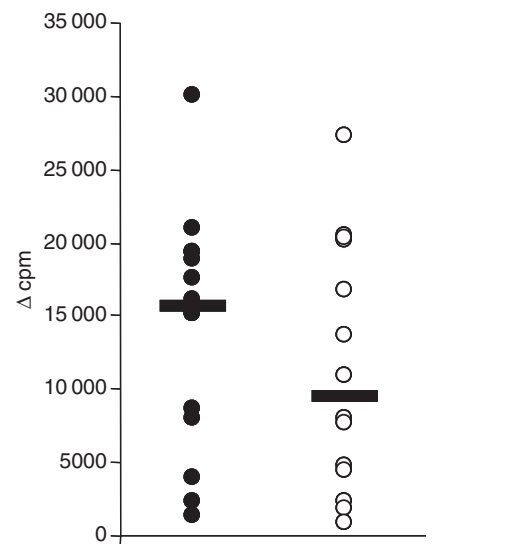


Fig. 2. Lymphoproliferative response to rVes v 5. Peripheral blood mononuclear cells from 15 vespid-allergic (filled circles) and 15 non-allergic (open circles) donors were stimulated with 25 µg/mL of rVes v 5. The horizontal line indicates the median. Δ cpm = counts per minute (cpm) in cultures stimulated with rVes v 5 minus cpm in cultures with medium alone.

The serum pool of all non-allergic individuals is shown in lane N (Fig. 1).

Proliferation assays

In initial experiments, PBMC of vespid-allergic and non-allergic individuals were stimulated with titrated concentrations (3–50 µg/mL) of rVes v 5 which revealed 25 µg/mL as optimum concentration (data not shown). Proliferative responses of PBMC from 15 vespid-allergic and 15 non-allergic individuals in response to 25 µg/mL rVes v 5 are shown in Fig. 2. Overall, rVes v 5-induced lymphoproliferation in cells from vespid-allergic individuals was stronger although no statistically significant difference between the two groups was observed.

T cell epitopes of Ves v 5

To delineate relevant T cell epitopes, TCL generated with rVes v 5 were mapped with a panel of 65 synthetic peptides covering the complete aa sequence of Ves v 5. Results are depicted in Fig. 3. By considering a SI >4 as positive, 17

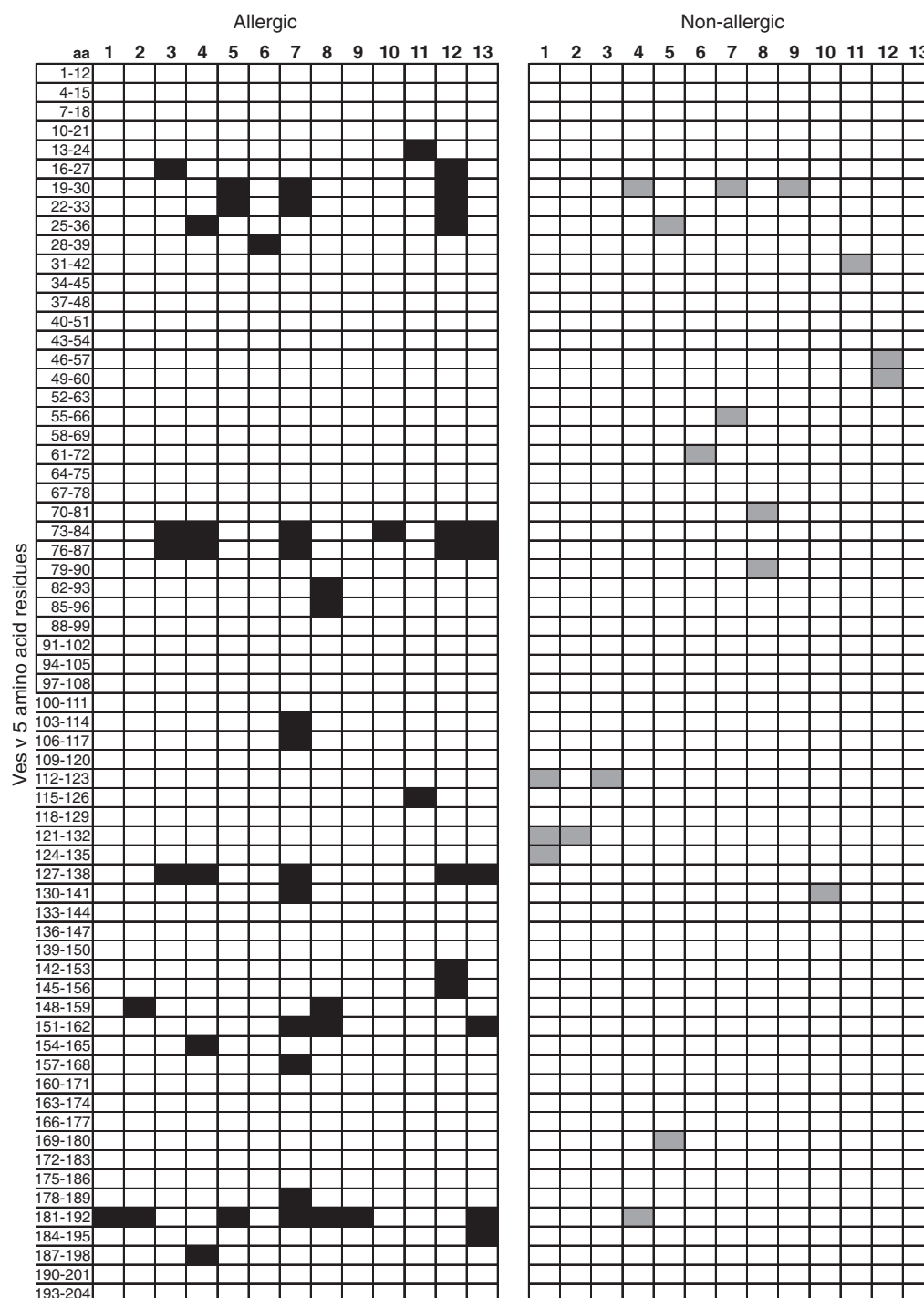


Fig. 3. T cell epitopes of Ves v 5. T cell lines from 13 allergic and 13 non-allergic individuals were established with rVes v 5 and proliferation was tested with overlapping peptides spanning the Ves v 5 sequence. Peptides inducing a SI > 4 in allergic patients and a SI > 2 in non-allergic donors are shown as boxes.

T cell epitopes were identified in TCL derived from 13 vespid-allergic patients. The peptide Ves v 5₁₈₁₋₁₉₂ was recognized by seven individuals, Ves v 5₇₃₋₈₇ by six, Ves v 5₁₂₇₋₁₃₈ by five, Ves v 5₁₄₈₋₁₆₂ by four, Ves v 5₁₉₋₃₃ by three, Ves v 5₁₆₋₂₇ and Ves v 5₂₅₋₃₆ by two individuals each. The peptides Ves v 5₁₋₁₂, Ves v 5₁₃₋₂₄, Ves v 5₂₈₋₃₉, Ves v 5₈₂₋₉₆, Ves v 5₁₀₃₋₁₁₇, Ves v 5₁₁₅₋₁₂₆, Ves v 5₁₄₂₋₁₅₆, Ves v 5₁₅₄₋₁₆₅, Ves v 5₁₅₇₋₁₆₈ and Ves v 5₁₈₇₋₁₉₈ were recognized by one allergic patient each.

By considering a SI > 2 as positive, 15 T cell epitopes were identified in TCL derived from 13 different non-allergic individuals (Fig. 3). The peptide Ves v 5₁₉₋₃₀ was recognized by three individuals, Ves v 5₁₁₂₋₁₂₃, Ves v 5₁₂₁₋₁₃₂, Ves v 5₁₄₈₋₁₅₉, and Ves v 5₁₆₉₋₁₈₀ by two and the peptides Ves v 5₂₅₋₃₆, Ves v 5₃₁₋₄₂, Ves v 5₄₆₋₆₀, Ves v 5₅₅₋₆₆, Ves v 5₆₁₋₇₂, Ves v 5₇₀₋₈₁, Ves v 5₇₉₋₉₀, Ves v 5₁₃₀₋₁₄₁, Ves v 5₁₃₉₋₁₅₀ and Ves v 5₁₈₁₋₁₉₂ by one individual each. One donor although able to recognize the

full-length Ves v 5 molecule failed to respond to any of the peptides tested.

In parallel to rVes v 5-initiated TCL, TCL were generated with *V. vulgaris* venom of each individual. Epitope mapping of these cultures revealed comparable epitope recognition patterns as observed in TCL generated with rVes v 5 (data not shown). The SI induced by the most frequently recognized peptides in TCL of allergic patients but not recognized by non-allergic individuals were statistically compared. A significant difference between allergic and non-allergic individuals was found: $P=0.039$ for Ves v $S_{181-192}$; $P<0.001$ for Ves v S_{73-87} and $P<0.001$ for Ves v $S_{127-141}$.

Distribution of human leucocyte antigen alleles

Twelve of the vespid-allergic patients and 13 of the non-allergic individuals were typed for HLA class II alleles (Table 1). All seven individuals of the patients' group who recognized peptide Ves v $S_{181-192}$ possessed an HLA-DRB3*0202 allele

while the remaining six patients did not possess this allele. In the group of non-allergic individuals four persons possessed an HLA-DRB3*0202 allele. No other peptide-defined subgroup of patients showed an association with any HLA class I or class II alleles.

Ves v 5-specific T cell clones

In total, 10 Ves v 5-specific TCC were isolated from six different vespid-allergic patients and six Ves v 5-specific TCC from two non-allergic donors (Table 2). All TCC responded with a SI >10 to rVes v 5 and proliferated equally after stimulation with *V. vulgaris* venom (data not shown). The TCC expressed the TCR $\alpha\beta$ and the majority were CD4⁺CD8⁻. Three TCC were CD4⁺CD8⁺. In response to stimulation with rVes v 5, two of 10 TCC isolated from allergic donors were classified as Th2 like, two of 10 as Th1 like and six of 10 as Th0. Three Th0-like TCC synthesized IL-10. Two of six TCC isolated from non-allergic individuals

Table 1. HLA alleles of vespid-allergic and non-allergic individuals

Vespid-allergic individuals				Non-allergic individuals		
1	DRB1*0301,*1104	DRB3*0101,*0202	DQB1*02,*03	DRB1*0404,*15	DRB4*0101/03; DRB5*0101	DQB1*03,*06
2	DRB1*1101	DRB3*0202	DQB1*03	DRB1*0101		DQB1*05
3	n.d.			DRB1*0301,*1401	DRB3*0101; *0202	DQB1*02,*05
4	DRB1*0102,*1301	DRB3*0101	DQB1*05,*06	DRB1*0401,*0701	DRB4*0101/03	DQB1*02,*03
5	DRB1*0801,*1104	DRB3*0202	DQB1*03,*04	DRB1*0301,*0701	DRB3*0101; DRB4*0101/03	DQB1*02
6	DRB1*0701,*1303	DRB3*0101; DRB4*0101/03	DQB1*02,*03	DRB1*1101,*1301	DRB3*0202	DQB1*03,*06
7	DRB1*1101,*1401	DRB3*0202	DQB1*03,*05	DRB1*0101,*1302	DRB3*0301	DQB1*05,*06
8	DRB1*0404,*1101	DRB3*0202; DRB4*0101/03	DQB1*03	DRB1*1301,*1501	DRB3*0202; DRB5*0101	DQB1*06
9	DRB1*0101,*1103	DRB3*0202	DQB1*03,*05	DRB1*0408,*1301	DRB3*0202; DRB4*0101/03	DQB1*03,*06
10	DRB1*1501,*0101	DRB5*0101	DQB1*05,*06	DRB1*0301,*0401	DRB3*0101; DRB4*0101/03	DQB1*02,*03
11	DRB1*0404,*1501	DRB4*0101/03; DRB5*0101	DQB1*03,*06	DRB1*0301,*0402	DRB3*0101; DRB4*0101/03	DQB1*02,*03
12	DRB1*0101,*1501	DRB5*0101	DQB1*05,*06	DRB1*0701,*1501	DRB4*0101/03; DRB5*0101	DQB1*02,*06
13	DRB1*1201/06,*1401	DRB3*0202	DQB1*03,*05	DRB1*0701,*1501	DRB4*0101/03; DRB5*0101	DQB1*02,*06

HLA, human leucocyte antigen.

Table 2. Characterization of Ves v 5-specific TCC

Clone	Phenotype	IL-4 (pg/mL)	IFN- γ (pg/mL)	IL-10 (pg/mL)	Th subset
Allergic individuals					
B 71	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	21.5	<9.5	71.1	Th0*
F 38	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	<9	<9.5	<4.7	Th0
K 109	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	<9	<9.5	241	Th0
M 216	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	475	39.5	45.3	Th2
O 104	CD4 ⁺ CD8 ⁺ TCR $\alpha\beta$ ⁺	<9	28.3	125	Th0
O 106	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	<9	105	22.7	Th1
T 26	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	<9	<9.5	<4.7	Th0
T 45	CD4 ⁺ CD8 ⁺ TCR $\alpha\beta$ ⁺	<9	143.7	<4.7	Th1
T 57	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	<9	30	<4.7	Th0
T 149	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	794	<9.5	<4.7	Th2
Non-allergic individuals					
A 4	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	<9	242	13.3	Th1
A 6	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	40.2	>500	303	Th1
A 9	CD4 ⁺ CD8 ⁺ TCR $\alpha\beta$ ⁺	<9	>500	35.7	Th1
A 43	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	18.5	<9.5	9.7	Th0
K 6	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	<9	35.7	58.2	Th0
K 28	CD4 ⁺ CD8 ⁻ TCR $\alpha\beta$ ⁺	<9	417	56.3	Th1

*TCC synthesizing >47.5 pg IL-4/<9.5 pg IFN- γ and pg IL-4>5 pg IFN- γ were defined as Th2, TCC synthesizing >45 pg IFN- γ /<9 pg IL-4 and pg IFN- γ >5 pg IL-4 as Th1 and all other constellations as Th0. TCC, T cell clones; Th1, T helper type 1; Th2, T helper type 2.

were attributed to the Th0 and four of six to the Th1 subset. One Th1-like TCC produced IL-10.

Discussion

The T cell response to Ag 5 from *V. vulgaris* was characterized and compared between vespid-allergic and non-allergic individuals. In total, 28 distinct T cell epitopes of Ves v 5 were identified (Fig. 3). Most allergic patients recognized multiple epitopes whereas non-allergic individuals reacted in general with one or two distinct peptides. In addition, the peptide-induced proliferation was more pronounced in TCL from allergic than non-allergic individuals. These differences are also reflected in the weaker proliferative responses to rVes v 5 of PBMC from non-allergic individuals (Fig. 2). Of interest, several peptides were recognized by allergic but not by non-allergic subjects (Fig. 3). The T cell response to the relevant epitopes Ves v 5_{181–192}, Ves v 5_{73–87} and Ves v 5_{127–141} differed significantly from non-allergic subjects. On the other hand, individual non-allergic individuals harboured T cells specific for single peptides located in the region of aa 31–72 and aa 169–180 whereas allergic patients did not. Thus, in contrast to other previously analysed allergens, the T cell recognition sites of Ves v 5 differ between vespid-allergic and non-allergic individuals [19, 29, 30].

The analysis of the murine T cell response to Dol m 5, the Ag 5 of *D. maculata* (American hornet), has defined a major T cell epitope located within aa 176–195 of Dol m 5 which shows strong cellular cross-reactivity with Ves v 5 [26]. This peptide is highly conserved among Ag 5 molecules from different vespid species [31]. In accordance, an epitope located within aa 181–192 of Ves v 5 (HKHYLVLCNYGPS) was recognized by 54% of the allergic patients included in this study. In all but one of the patients, Ves v 5_{181–192} induced the highest proliferation among all peptides. Hence, with respect to vespid-allergic humans Ves v 5_{181–192} can also be regarded as immunodominant. This epitope was present in only one of 13 TCL from non-allergic donors (Fig. 3). All seven allergic patients recognizing Ves v 5_{181–192} possessed the HLA-DRB3*0202 allele (Table 1), a phenotype with a frequency of 45% in the Austrian population. None of the other more frequently recognized Ves v 5-peptides, e.g. Ves v 5_{73–87}, could be associated with a particular HLA phenotype. However, no statistical significance for an association of Ves v 5_{181–192} with HLA-DRB3*0202 was found and a higher number of individuals needs to be studied to confirm whether this HLA phenotype is truly linked with the recognition of the immunodominant epitope Ves v 5_{181–19} in vespid-allergic patients.

To evaluate the specific cytokine response to Ves v 5 we employed allergen-specific TCC because cytokine levels in PBMC were constantly below the sensitivity limit. We have used this approach before to analyse the cellular immune response to respiratory allergens and the majority of these TCC showed a Th2 phenotype, i.e. synthesized high amounts of IL-4 but no IFN- γ [15, 16, 29, 32]. However – applying comparable culture conditions – most Ves v 5-specific TCC from allergic individuals did not produce noticeable amounts of IL-4 (Table 2) although all patients had a CAP-RAST > 3 for *V. vulgaris* venom and Ves v 5-specific IgE in their sera

(Fig. 1). Moreover, Ves v 5-specific TCC from three different individuals were CD4⁺CD8⁺ which we have not observed for TCC specific for inhalant allergens [15, 16, 29, 32]. Thus, our data provide evidence that the T cell response to Hymenoptera allergens differs from the typical Th2-dominated response to inhalant allergens. Ves v 5-specific TCC isolated from non-allergic individuals predominantly expressed the Th1-phenotype. With respect to the secretion of IL-10, which is associated with the suppression of allergen-specific T cell responses in healthy individuals, no differences between Ves v 5-specific TCC from allergic and non-allergic individuals were observed [33].

Based on the identification of major T cell determinants of bee venom allergens novel immunotherapeutic agents were designed and successfully applied to desensitize bee venom-allergic patients [21–24]. The characterization of the T cell response to the major allergen in *V. vulgaris* venom, Ves v 5, represents a first step for similar approaches to the therapy of vespid-allergic patients with increased safety and efficacy.

Acknowledgements

We thank T. P. King, Rockefeller University, and Miles Guralnick, Vespa Labs, for support and valuable discussions. This work was supported by the grant from the European commission within the 5th Framework Program (EU project: Therapeutic Recombinant Allergens from Structural Allergology) and by Grant SFB F018-07 from the Fonds zur Förderung der Wissenschaftlichen Forschung, Vienna, Austria.

References

- 1 Lichtenstein LM, Valentine MD, Sobotka AK. Insect allergy: the state of the art. *J Allergy Clin Immunol* 1979; 64:5–12.
- 2 Fernandez J, Rodes F, Marti J, Blanca M. Wasp sting anaphylaxis as a cause of death: a case report. *Allergol Immunopathol (Madr)* 1992; 20:40–1.
- 3 Golden DB, Marsh DG, Freidhoff LR et al. Natural history of Hymenoptera venom sensitivity in adults. *J Allergy Clin Immunol* 1997; 100:760–6.
- 4 Antonicelli L, Bilo MB, Bonifazi F. Epidemiology of Hymenoptera allergy. *Curr Opin Allergy Clin Immunol* 2002; 2:341–6.
- 5 King TP, Lu G, Gonzalez M, Qian N, Soldatova L. Yellow jacket venom allergens, hyaluronidase and phospholipase: sequence similarity and antigenic cross-reactivity with their hornet and wasp homologs and possible implications for clinical allergy. *J Allergy Clin Immunol* 1996; 98:588–600.
- 6 Lu G, Villalba M, Coscia MR, Hoffman DR, King TP. Sequence analysis and antigenic cross-reactivity of a venom allergen, antigen 5, from hornets, wasps, and yellow jackets. *J Immunol* 1993; 150:2823–30.
- 7 Monsalve RI, Lu G, King TP. Expressions of recombinant venom allergen, antigen 5 of yellowjacket (*Vespa vulgaris*) and paper wasp (*Polistes annularis*), in bacteria or yeast. *Protein Expr Purif* 1999; 16:410–6.
- 8 Henriksen A, King TP, Mirza O et al. Major venom allergen of yellow jackets, Ves v 5: structural characterization of a pathogenesis-related protein superfamily. *Proteins* 2001; 45:438–48.
- 9 O'Brien RM, Thomas WR, Wootton AM. T cell responses to the purified major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1992; 89:1021–31.

- 10 O'Hehir RE, Verhoef A, Panagiotopoulou E et al. Analysis of human T cell responses to the group II allergen of *Dermatophagoides* species: localization of major antigenic sites. *J Allergy Clin Immunol* 1993; 92:105–13.
- 11 Ebner C, Szepefalusi Z, Ferreira F et al. Identification of multiple T cell epitopes on Bet v I, the major birch pollen allergen, using specific T cell clones and overlapping peptides. *J Immunol* 1993; 150:1047–54.
- 12 Counsell CM, Bond JF, Ohman JL Jr, Greenstein JL, Garman RD. Definition of the human T-cell epitopes of Fel d 1, the major allergen of the domestic cat. *J Allergy Clin Immunol* 1996; 98:884–94.
- 13 Schenk S, Breiteneder H, Susani M et al. T cell epitopes of Phl p 1, major pollen allergen of timothy grass (*Phleum pratense*). Cross-reactivity with group I allergens of different grasses. *Adv Exp Med Biol* 1996; 409:141–6.
- 14 Muller WD, Karamfilov T, Kahlert H et al. Mapping of T-cell epitopes of Phl p 5: evidence for crossreacting and non-cross-reacting T-cell epitopes within Phl p 5 isoallergens. *Clin Exp Allergy* 1998; 28:1538–48.
- 15 Bohle B, Wagner B, Vollmann U et al. Characterization of T cell responses to Hev b 3, an allergen associated with latex allergy in spina bifida patients. *J Immunol* 2000; 164:4393–8.
- 16 Jahn-Schmid B, Kelemen P, Himly M et al. The T cell response to Art v 1, the major mugwort pollen allergen, is dominated by one epitope. *J Immunol* 2002; 169:6005–11.
- 17 Carballido JM, Carballido-Perrig N, Terres G, Heusser CH, Blaser K. Bee venom phospholipase A2-specific T cell clones from human allergic and non-allergic individuals: cytokine patterns change in response to the antigen concentration. *Eur J Immunol* 1992; 22:1357–63.
- 18 Dhillon M, Roberts C, Nunn T, Kuo M. Mapping human T cell epitopes on phospholipase A2: the major bee-venom allergen. *J Allergy Clin Immunol* 1992; 90:42–51.
- 19 Carballido JM, Carballido-Perrig N, Kagi MK et al. T cell epitope specificity in human allergic and nonallergic subjects to bee venom phospholipase A2. *J Immunol* 1993; 150:3582–91.
- 20 Kammerer R, Kettner A, Chvatchko Y et al. Delineation of PLA2 epitopes using short or long overlapping synthetic peptides: interest for specific immunotherapy. *Clin Exp Allergy* 1997; 27:1016–26.
- 21 Texier C, Pouvelle S, Busson M et al. HLA-DR restricted peptide candidates for bee venom immunotherapy. *J Immunol* 2000; 164:3177–84.
- 22 Texier C, Pouvelle-Moratille S, Buhot C et al. Emerging principles for the design of promiscuous HLA-DR-restricted peptides: an example from the major bee venom allergen. *Eur J Immunol* 2002; 32:3699–707.
- 23 Muller U, Akdis CA, Fricker M et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998; 101:747–54.
- 24 Fellrath JM, Kettner A, Dufour N et al. Allergen-specific T-cell tolerance induction with allergen-derived long synthetic peptides: results of a phase I trial. *J Allergy Clin Immunol* 2003; 111:854–61.
- 25 King TP, Kochoumian L, Lu G. Murine T and B cell responses to natural and recombinant hornet venom allergen Dol m 5.02 and its recombinant fragments. *J Immunol* 1995; 154:577–84.
- 26 King TP, Lu G. Hornet venom allergen antigen 5, Dol m 5: its T-cell epitopes in mice and its antigenic cross-reactivity with a mammalian testis protein. *J Allergy Clin Immunol* 1997; 99: 630–9.
- 27 Fischer GF, Fae I, Petrsek M, Haas H, Mayr WR. An HLA-DR11 variant (HLA-DRB1*1115) segregating in a family of Turkish origin. *Tissue Antigens* 1995; 45:143–4.
- 28 King TP, Jim SY, Monsalve RI, Kagey-Sobotka A, Lichtenstein LM, Spangfort MD. Recombinant allergens with reduced allergenicity but retaining immunogenicity of the natural allergens: hybrids of yellow jacket and paper wasp venom allergen antigen 5s. *J Immunol* 2001; 166:6057–65.
- 29 Ebner C, Schenk S, Najafian N et al. Nonallergic individuals recognize the same T cell epitopes of Bet v 1, the major birch pollen allergen, as atopic patients. *J Immunol* 1995; 154:1932–40.
- 30 Mark PG, Segal DB, Dallaire ML, Garman RD. Human T and B cell immune responses to Fel d 1 in cat-allergic and non-cat-allergic subjects. *Clin Exp Allergy* 1996; 26:1316–28.
- 31 Hoffman DR. Allergens in Hymenoptera venom. XXV: the amino acid sequences of antigen 5 molecules and the structural basis of antigenic cross-reactivity. *J Allergy Clin Immunol* 1993; 92:707–16.
- 32 Schenk S, Breiteneder H, Susani M et al. T-cell epitopes of Phl p 1, major pollen allergen of timothy grass (*Phleum pratense*): evidence for crossreacting and non-crossreacting T-cell epitopes within grass group I allergens. *J Allergy Clin Immunol* 1995; 96:986–96.
- 33 Jutel M, Akdis M, Budak F et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003; 33:1205–14.