

V β 18.1⁺ and V α 2.3⁺ T-cell subsets are associated with house dust mite allergy in human subjects

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Background: The recognition of allergenic peptides by T cells through their T-cell receptor (TCR) represents a crucial step in the initiation of an allergen-specific immune response. In parallel to the superantigen-driven restricted expansion of V β subsets in autoimmune and infectious diseases, reports in animals and human subjects have shown a similar capacity of classical antigens.

Objective: The study was performed to analyze the V α /V β expression in house dust mite (HDM) allergy.

Methods: The TCR repertoire of 15 subjects with HDM allergy, 22 atopic subjects without HDM allergy, and 19 nonatopic individuals, members of 2 extended and 4 nuclear families, was determined. By using flow cytometry, the expression of 22 V β and 3 V α elements was analyzed in vivo and after in vitro allergen stimulation.

Results: In comparison with nonatopic and atopic individuals without HDM allergy, freshly isolated PBMCs of individuals with HDM allergy showed a significantly higher frequency of V β 18⁺ and V α 2.3⁺ T cells. Although members of all 3 groups had a similar lymphocyte proliferation response after in vitro stimulation with Der p 1 or Der p 1 peptide₁₀₁₋₁₃₁, a significant expansion of V β 18⁺ and V α 2.3⁺ T cells in vitro occurred only in individuals with HDM allergy. Moreover, the degree of expansion correlated with the levels of allergen-specific IgE antibodies. No expansion of V β 18⁺ and V α 2.3⁺ was observed after mitogen stimulation with PHA, indicating allergen specificity of the response.

Conclusion: Our results strongly suggest restricted TCR V α /V β gene use in HDM allergy and might be a step toward TCR-based immunotherapy. (J Allergy Clin Immunol 2002;109:517-23.)

Key words: T-cell receptors, allergy, T lymphocytes, flow cytometry

The specificity in the initiation and maintenance of an allergic sensitization is mediated by T cells and their receptor for antigen. Most often, the T-cell receptor (TCR) is a heterodimer composed of an α - and a β -chain, the variable regions of which are involved in antigen recognition.¹ The classification of T cells in V β or V α subsets is based on the similarity of their variable regions; chains of the same subset show more than 75% similarity in amino acid sequence.² The required diversity of these chains is based on random germline rearrangement of V-, D-, J-, and C-region genes and insertion and deletion of random nucleotides of the V-D-J junctional regions.³ Although this mechanism results in an enormous number of different TCRs, many immunologic diseases have been shown to be associated with selective TCR combinations.⁴⁻⁹ Recent studies on experimental encephalomyelitis and autoimmune carditis have demonstrated the feasibility of TCR-based immunotherapy,^{10,11} thus emphasizing the importance of investigating such relationships.

In the mouse model it was shown that specific V β T-cell subsets mediate the immediate hypersensitivity response to certain allergens, and even more importantly, with certain of these V β subsets, the IgE phenotype could be transferred from sensitized into nonsensitized animals of the same strain.^{5,6} Within the last several years, high- and low-responder mouse strains were identified with regard to their ability to mount an allergen-specific IgE antibody response. It was shown recently that a low IgE-responder strain was converted into an IgE-responder strain by introducing a V β gene segment into the TCR repertoire, which demonstrates the important role of T cells in the regulation of allergic responses.¹²

In human subjects we previously demonstrated that certain V β subsets were significantly increased in birch pollen (V β 16.1 and V β 20.1) and cat allergy (V β 17.1). The expansion of these V β T cells was dependent on allergen exposure.¹³ Investigating the T-cell repertoire in the lungs of

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Abbreviations usedHDM: House dust mite
TCR: T-cell receptor

atopic asthmatic subjects before and after segmental allergen challenge with ragweed, it was observed by others that in addition to a polyclonal influx of T cells, an oligoclonal activation of T cells within the lungs occurred.¹⁴ In the present study we will show that, similar to our findings in birch pollen and cat allergy, certain V β and V α subsets are associated with house dust mite (HDM) allergy and that the expansions in vivo could be simulated in vitro.

METHODS**Study population**

A total of 56 individuals, including 4 monozygotic twin pairs, from 2 extended and 4 nuclear families participated in this study. As previously described, the families were recruited because of the high prevalence of atopy.¹³ Ages ranged from 3 to 78 years. A total of 37 individuals were atopic. Fifteen of the 37 individuals were sensitized against HDM. Their *Dermatophagoides pteronyssinus*-specific IgE levels ranged from 0.78 to 94.3 kU_A/L (median, 7.7 kU_A/L). All *D pteronyssinus*-sensitized individuals had allergic rhinitis, bronchial asthma, or both. Twenty-two individuals were allergic to one or more aeroallergens, but not to *D pteronyssinus*, and served as atopic control subjects. Their total IgE levels ranged from 2 to 1593 kU_A/L (median, 81.2 kU_A/L), and *D pteronyssinus*-specific IgE was not detectable (<0.35 kU_A/L). The other 19 individuals were nonatopic, with a negative history of allergic symptoms. Their total IgE levels ranged from 3.3 to 152 kU_A/L (median, 21.3 kU_A/L). Specific IgE antibodies against common aeroallergens were not detectable (<0.35 kU_A/L). Informed consent was obtained, and the study was approved by the local ethics committee.

Determination of total and specific serum IgE levels

Blood samples from all 56 individuals were clotted at room temperature and centrifuged. Concentrations of total and allergen-specific IgE antibodies against *D pteronyssinus*, mugwort, birch, *Cladosporium herbarum*, timothy grass, and dog and cat dander in the serum were determined by using a fluorescent enzyme immunoassay with the Pharmacia CAP system (Pharmacia, Uppsala, Sweden).

Isolation of PBMCs

PBMCs were purified from heparinized blood by means of density gradient centrifugation on Lymphoprep (Gibco, Paisley, Scotland) for 20 minutes at 600g at room temperature. Cells from the interface were washed 3 times with culture medium (10 minutes at 450g at room temperature).

Cell culture

Isolated PBMCs (2×10^6 /mL) were cultured in 24-well plates with or without antigen in AIM V medium supplemented with 2 mmol/L glutamine (Biochrom, Berlin, Germany), 100 U/mL penicillin (Biochrom), 100 μ g/mL streptomycin (Biochrom), and 0.2 μ g/mL amphotericin B (Life Technologies, Gaithersburg, Md) in the presence of IL-2 (50 U/mL) and IL-4 (200 U/mL). Der p 1 (10 μ g/mL; ALK, Copenhagen, Denmark) and recombinant Der p 1 peptide₁₀₁₋₁₃₁ (amino acid residues 101-131 of Der p 1, 10 μ g/mL)

were used as specific allergens, and PHA (5 μ g/mL) was used as a mitogen control. Cells were harvested after 7 days of culture (37°C in 5% CO₂) to determine V α - and V β -specific phenotypes.

Proliferation assay

Isolated PBMCs (2×10^5) were cultured in triplicate in 96-well plates (200 μ L) in the presence or absence of antigen in AIM V medium supplemented as described above. Der p 1 (1, 3, and 10 μ g/mL) and Der p 1 peptide₁₀₁₋₁₃₁ were used to determine antigen-specific proliferation. PHA (1 and 5 μ g/mL; Sigma, St Louis, MO) was used as a positive control. After 4 days of culture (37°C in 5% CO₂), Alamar Blue^{15,16} (Bio Source, Camarillo, Calif) was added (40 μ L/well). OD was measured by means of fluorescence counting, and proliferation was defined in percentages by the ratio of [OD_{allergen}-OD_{medium}]/[OD_{PHA}-OD_{medium}].

Expression of TCR V α and V β gene segments

The expression of the TCR-V α and TCR-V β repertoire on peripheral T cells was assessed by means of flow cytometry with a panel of FITC-labeled mAbs (TCR-V α 24.1, TCR-V β 2.1, TCR-V β 3.1, TCR-V β 5.1, TCR-V β 5.2, TCR-V β 5.3, TCR-V β 6.1, TCR-V β 8.1/8.2, TCR-V β 11.1, TCR-V β 12.2, TCR-V β 13.1, TCR-V β 13.6, TCR-V β 14.1, TCR-V β 16.1, TCR-V β 17.1, TCR-V β 18.1, TCR-V β 20.1, TCR-V β 21.3, and TCR-V β 22.1 [Immunotech, Hamburg, Germany]; V α 2.3, V α 12.1, V β 6.7, V β 12.1, and V β 13.1/13.3 [DPC Biemann, Bad Nauheim, Germany]; and V β 9.1 [PharMingen, San Diego, Calif]). For in vivo studies, freshly isolated PBMCs, and for in vitro experiments, cultured lymphocytes harvested at day 7 were incubated with anti-V α /V β antibodies for 30 minutes at 4°C in the dark. After washing 3 times with PBS (10 minutes at 250g at room temperature), cells were analyzed on a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany) with a gate for lymphocytes. Distributions of TCR-V α /V β elements were calculated and expressed as a percentage of CD3⁺ cells (as determined by staining with FITC-conjugated anti-CD3 mAb, Becton Dickinson) to determine the frequency of TCR-V α /V β -expressing T cells.

Quantification of Der p 1 in mattress dust by ELISA

Mattress dust was collected by means of vacuum cleaning of the mattresses from 38 of the 56 individuals. ELISA was performed with a commercially available kit (ALK, Copenhagen, Denmark), following the protocol of the manufacturer to determine the amount of Der p 1 in the collected dust.

Statistical analysis

Nonparametric analysis (Wilcoxon test) was performed to assess differences in TCR distribution between stimulated and nonstimulated T cells by using SPSS 10.0 software. Results are presented as means $\pm$ SE. In addition, nonparametric Spearman rank correlation was used to test for a correlation between V α /V β expression and specific IgE levels.

RESULTS

The V α and V β distribution of 15 patients with HDM allergy was compared with that of 22 atopic subjects without HDM allergy and 19 nonatopic individuals to describe differences in the TCR repertoire between individuals with and without HDM allergy. All individuals were members of 2 extended and 4 nuclear families. In these families we previously described the TCR distribution in the peripheral blood of atopic and nonatopic individuals, as well as in monozygotic twin pairs.¹³

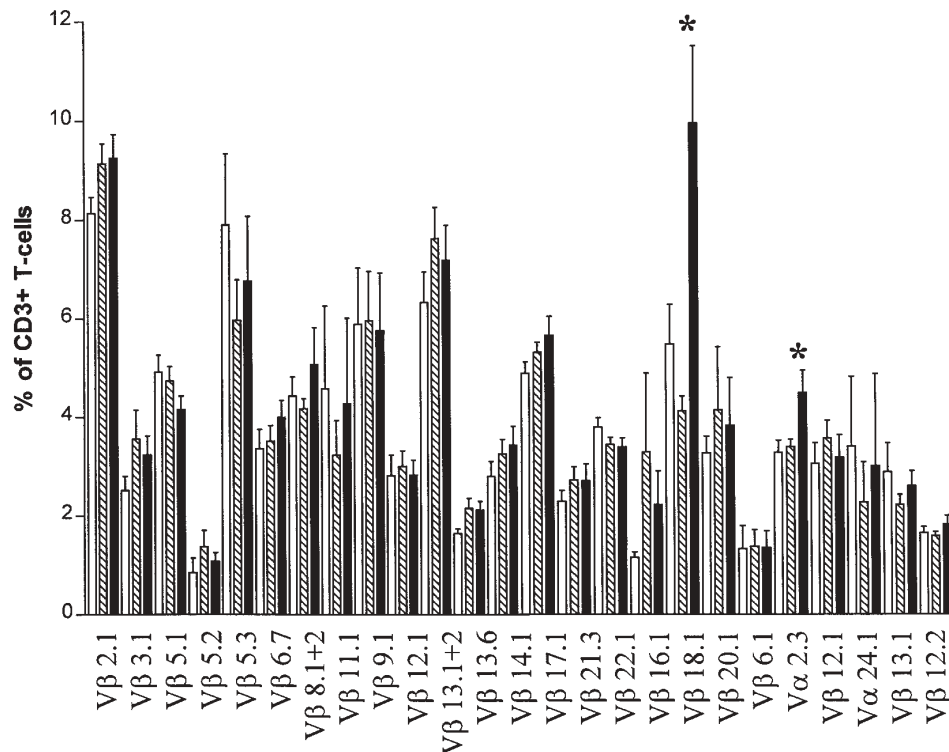


FIG 1. In vivo expression of the TCR-V α and TCR-V β repertoire on peripheral T cells in patients with HDM allergy (filled bars), atopic subjects without HDM allergy (hatched bars), and nonatopic individuals (open bars). The expression of the TCR-V α and TCR-V β repertoire on the freshly isolated cells was assessed by means of flow cytometry with mAbs and expressed as a percentage of CD3⁺ cells. Shown are means and SEs. * $P < .05$ in comparison with the control groups. Data for the nonatopic individuals are from Beyer et al.¹³ Copyright 1999, The American Association of Immunologists.

Patients with HDM allergy, all of them sensitized against *D pteronyssinus*, showed, in vivo, a significantly higher frequency of V β 18.1- and V α 2.3-expressing T cells compared with that in the atopic subjects without HDM allergy and the nonatopic individuals (Fig 1). Interestingly, no significant differences in HDM exposure were found among the 3 study groups ranging from 0 to 270 ng of Der p 1/g mattress dust in patients with HDM allergy (median, 40 ng of Der p 1/g mattress dust; measured in 8 of 15 individuals), 0 to 46,000 ng of Der p 1/g mattress dust in atopic subjects without HDM allergy (median, 10 ng of Der p 1/g mattress dust; measured in 15 of 22 individuals), and 0 to 130,000 ng of Der p 1/g mattress dust in nonatopic individuals (median, 80 ng of Der p 1/g mattress dust; measured in 15 of 19 individuals; data not shown).

PBMCs from all individuals were incubated with Der p 1, Der p 1 peptide₁₀₁₋₁₃₁, and PHA as a control to test whether the same V α and V β subsets proliferate after allergen stimulation. Fig 2 shows the overall lymphocyte proliferation under respective conditions; no significant differences could be detected among the 3 groups. However, in contrast to nonatopic and atopic individuals with-

out sensitization against Der p 1, subjects with HDM allergy showed a significant expansion of V β 18.1- and V α 2.3-expressing T cells after stimulation of T cells with Der p 1 or Der p 1 peptide₁₀₁₋₁₃₁ (Fig 3). No significant shift in the distribution of other V α /V β ⁺ T-cell subsets were noted after allergen stimulation (data not shown). Furthermore, no expansion of V β 18.1 and V α 2.3 was observed under PHA stimulation, suggesting allergen specificity of the TCR expansion (Fig 3).

In addition, a significant correlation between *D pteronyssinus*-specific IgE and Der p 1- and Der p 1 peptide₁₀₁₋₁₃₁-specific V β 18.1⁺ T-cell expansion in vitro was observed ($P < .0005$ for Der p 1 [Fig 4] and $P < .0005$ for Der p 1 peptide₁₀₁₋₁₃₁ [data not shown]). Similar results were found for V α 2.3 ($P < .005$ and $P < .05$, respectively; data not shown).

We previously reported that healthy, nonatopic twins have a striking concordance in their TCR repertoires.¹³ Two of 4 monozygotic twins included in the present study were discordant for the sensitization to HDM. Interestingly, V β 18.1 and V α 2.3 expression in monozygotic twins discordant for HDM sensitization differed markedly. Table I shows the expression of V β 18.1 and V α 2.3 on T cells. In

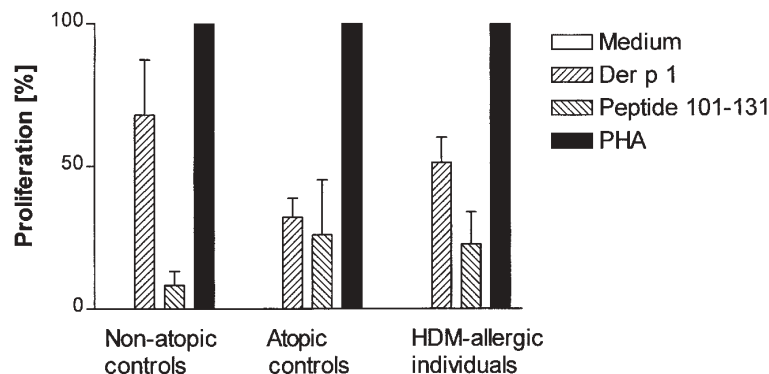


FIG 2. Proliferation of PBMCs from patients with HDM allergy and nonatopic individuals after stimulation with Der p 1, Der p 1 peptide₁₀₁₋₁₃₁, PHA, or medium alone, as determined with Alamar Blue staining. OD was measured, and proliferation was defined in percentages by the ratio of $[\text{OD}_{\text{allergen}} - \text{OD}_{\text{medium}}] / [\text{OD}_{\text{PHA}} - \text{OD}_{\text{medium}}]$. Per definition, the values for *Medium* and *PHA* are set to 0% and 100%, respectively. Shown are means and SEs.

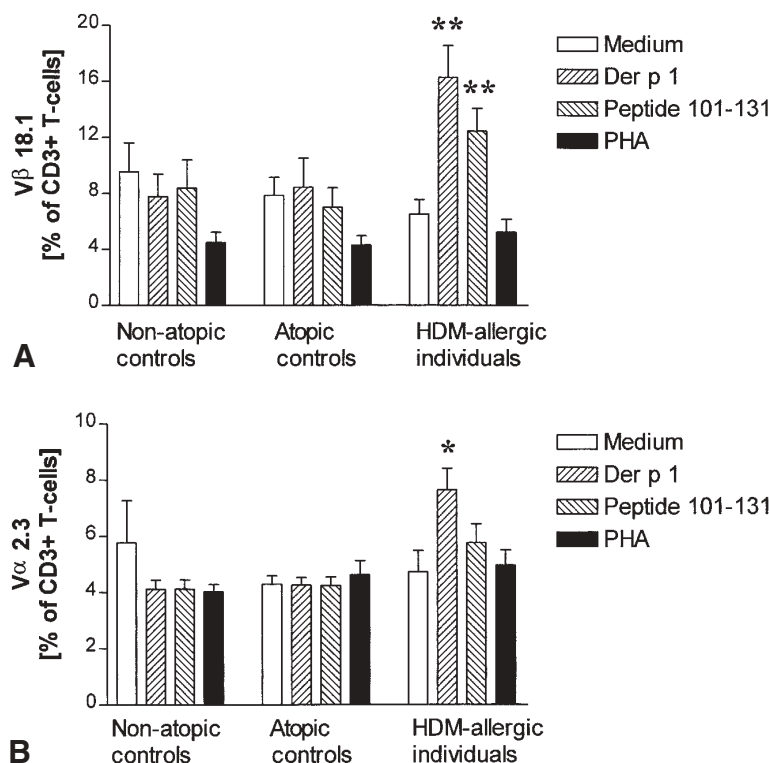


FIG 3. Expression of Vβ18.1⁺ (A) and Vα2.3⁺ (B) T cells in nonatopic subjects, atopic subjects without HDM allergy, and patients with HDM allergy after in vitro stimulation with Der p 1, Der p 1 peptide₁₀₁₋₁₃₁, PHA, or medium alone. Shown are means and SEs. ** $P < .005$ and * $P < .05$ in comparison with medium alone.

vivo, the sensitized twin in both twin pairs had a frequency of Vβ18.1 that was almost twice as high as that of his non-sensitized brother. When expression of Vα2.3 was compared, the *D pteronyssinus*-sensitized brother in twin pair 1 also had a 2-fold higher frequency than his nonsensitized

twin, whereas twin pair 2 had similar Vα2.3 expression. Correspondingly, the sensitized twins 1A and 2A showed a marked expansion of Vβ18.1⁺ T cells after stimulation of PBMCs with Der p 1 in vitro, whereas for Vα2.3, an increase in frequency was only seen in twin pair 1 (Table I).

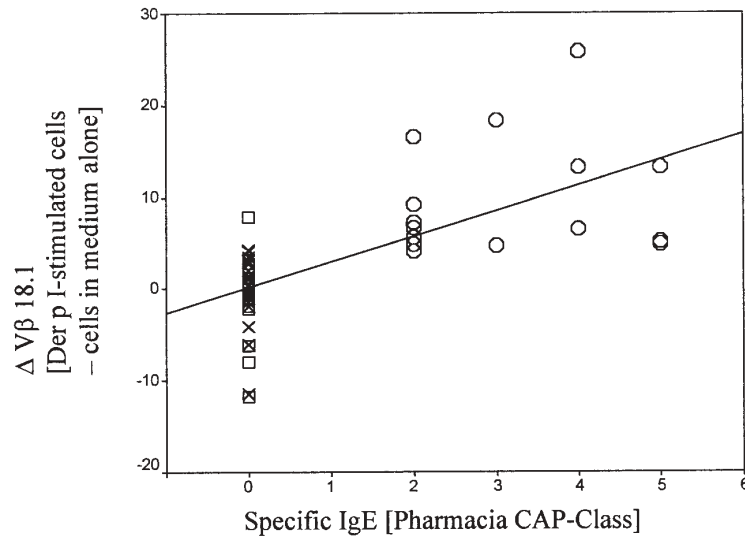


FIG 4. Correlation of Vβ18.1 expression on allergen-specific T cells and *D pteronyssinus*-specific IgE levels. Open circles represent patients with HDM allergy, open squares represent nonatopic subjects, and crosses represent atopic subjects without HDM allergy.

TABLE I. Frequency of Vβ18.1+ and Vα2.3+ T cells in vivo and after stimulation with Der p 1 in vitro in 2 pairs of monozygotic twins

Twin	Patients with HDM allergy	HDM-specific IgE (KU _A /L)	In vivo		In vivo			
					Vβ18.1 (%)		Vα2.3 (%)	
			Vβ18.1 (%)	Vα2.3 (%)	Medium	Der p 1	Medium	Der p 1
1A	Yes	0.92	18.0	7.5	13.3	18.9	6.5	9.4
1B	No	<0.35	9.7	3.2	10.1	9.7	4.4	4.8
2A	Yes	85.2	7.1	3.3	3.2	8.0	3.6	3.7
2B	No	<0.35	4.2	3.5	4.8	6.8	6.2	4.3

Both twin pairs were discordant for HDM allergy.

DISCUSSION

Experimental and epidemiologic data indicate preferential expansion of specific Vβ/Vα⁺ T cells in various immunologic diseases. Knowledge of such associations is important because it offers, in principle, the possibility of TCR-based immunotherapy.

The TCR repertoire is shaped during early maturation of the immune system and has been described as stable over time.^{17,18} However, several studies have described a restricted expansion of certain Vβ subsets in autoimmune, infectious, and atopic diseases.^{5,6,13,19-24} In human subjects a restricted Vβ repertoire was found in ragweed (Vβ5.2), birch pollen (Vβ16.1 and Vβ20.1), cat (Vβ17.1), insect (Vβ8), and peanut (Vβ2 and Vβ11) allergy, as well as in nickel-mediated contact dermatitis.^{7,13,25-28} Furthermore, we have previously demonstrated that the TCR repertoire can be transiently shaped by antigen exposure in predisposed individuals.¹³ In the present study we were able to show a restricted TCR repertoire in HDM allergy. In addition, the in vivo expansion could be simulated in

vitro. As in the present study, the in vivo accumulation of TCR-Vβ-expressing T cells in a mouse model of ragweed-specific airway inflammation correlated with in vitro expansion of the same T-cell subsets.⁶

Thus far, few investigators have focused on the TCR distribution in HDM allergy and reported partially contradicting results. In an animal model of HDM-sensitized mice, molecular analysis of HDM-specific T-cell clones suggested that the TCR use is restricted to members of the Vα8 and Vβ6 subfamilies.²⁹ Similar results regarding Vα8, but not Vβ6, were found in human subjects. Studying the TCR use of HDM-specific CD4⁺ T-cell clones isolated from one atopic individual, Wedderburn et al³⁰ observed a dominant expression of genes encoding TCR-Vα8 and TCR-Vβ3. However, all 10 analyzed clones were *Dermatophagoides farinae* specific, and only 4 of 10 clones showed cross-reactivity with *D pteronyssinus*. Jarman et al³¹ were able to reduce the polyclonal response to HDM allergen in the majority of their examined individuals by using a Vβ3-CDR2 peptide and con-

cluded that TCR-V β 3 gene use may represent a major component of the human HDM repertoire. In contrast to these studies, Yssel et al³² examined Der p 1-specific T-cell clones from 2 atopic patients allergic to *D pteronyssinus* and observed that these clones did not share any of the examined TCR V α and V β gene products. The present study demonstrates a restricted use of V β 18.1 and V α 2.3 in *D pteronyssinus*-sensitized individuals. *D pteronyssinus*-derivatized allergens were chosen for our investigation because 60% to 85% of all patients with allergic bronchial asthma are sensitized against *D pteronyssinus*. More than 50% of IgE against HDM has been shown to be specific for Der p 1, and Der p 1-specific IgE can be found in about 70% of allergic individuals.^{34,35} Different T-cell recognition sites exist at different locations within Der p 1. Yssel et al³² mapped 3 distinct regions at residues 45-67, 94-104, and 117-143. On the other hand, Higgins et al³⁶ mapped a cluster of 3 overlapping T-cell epitopes, amino acids 107-119, 110-119, and 110-131. The finding that the Der p 1 peptide₁₀₁₋₁₃₁ used in our study provoked a similar expansion of the same TCR subsets as the whole Der p 1 allergen would support the proposal that amino acids 101-131 contain the major allergenic sites of Der p 1.

Our finding that PBMCs from those individuals in our study population who did not have detectable IgE against *D pteronyssinus* had a similar proliferative response in vitro as the *D pteronyssinus* sensitized group may seem somewhat surprising. However, our data are in line with several investigations that found T cells from both atopic and nonatopic individuals to be reactive with HDM allergens.³⁷⁻⁴⁰ In particular, Upham et al⁴⁰ showed that the use of serum-free medium, as used in our investigation, enhances T-cell proliferation and lowers the threshold level for allergens to trigger T-cell activation. In their study the unmasking effect of serum-free medium compared with serum-supplemented medium resulted in a similar T-cell proliferative response of individuals with specific IgE compared with subjects without specific IgE; especially for ubiquitous inhalant allergens, activated T cells were found in close to 100% of individuals of similar age as in our study. The proliferative response in our nonatopic and atopic control group, however, did not show TCR restriction and therefore leads to the assumption of a broad polyclonal response without a relative shift in TCR distribution.

Using linkage analysis, Moffatt et al⁴¹ have suggested that genes of the TCR- α δ , but not TCR- β , complex are linked to specific IgE responses to HDM, Der p 1, and Der p 2. This would support our finding of V α 2.3, but not V β 18.1, expansion in Der p 1 sensitization. However, Moffatt et al studied British and Australian subjects, whereas we present data from individuals with bilateral German ethnicity. In addition, the same group showed, more recently, a strong allelic association between V α 8.1 polymorphism and reactivity to Der p 2, but not Der p 1, allergens.⁴² Of note, studies in the Japanese population found linkage between TCR- β genes and IgE production against HDM allergens.⁴³

The twin data from the present study support the finding that V α 2.3 and V β 18.1 are associated with HDM allergy. The pathogenesis of allergic diseases is multifactorial: A complex interaction between genetic variants and environmental factors determine disease expression. The genetic determination of TCR use in the monozygotic twins in our study should not differ. All twins were raised together; however, environmental differences must be considered that resulted in distinct TCR expression patterns in twins discordant for HDM sensitization. These findings and observations from our previous studies¹³ strongly suggest that environmental influences play an important part in shaping the TCR repertoire.

Through inclusion not only of nonatopic but also of atopic control subjects without HDM allergy, we could exclude that the observed V-gene use is a consequence of atopy per se but is really driven by *D pteronyssinus* exposure. In addition, the fact that there was no significant difference in *D pteronyssinus* exposure (amount of Der p 1 in the mattress dust) makes it unlikely that the observed V-gene use is the result of *D pteronyssinus* acting as a superantigen. There were also 8 nonatopic individuals who did not show any in vitro proliferative response to stimulation with Der p 1. In the case of involvement of a superantigen, one would expect proliferative response in all individuals, regardless of their state of sensitization.

In conclusion, we were able to identify expanded populations of T cells expressing certain TCR-V α /V β chains in *D pteronyssinus*-sensitized individuals in vivo. In vitro stimulation with Der p 1 resulted in expansion of the same T-cell subsets, showing the importance of antigen exposure in the development of individual TCR phenotypes and suggesting restricted TCR use in individuals with HDM allergy. Junctional region sequencing of TCR variable chain CDR3 regions would need to be performed in future studies to prove that the observed expansions are clonal antigen-specific response. TCR-based immunotherapy has made promising progress for diseases that are associated with distinct V α /V β subsets.^{10,11} Further investigations are now required to determine whether such therapy can be developed in allergic diseases.

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