

T Cell Epitopes of House Dust Mite Major Allergen *Der p II*¹

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ABSTRACT. Recent work has indicated the significance of IL-4- and IL-5-secreting allergen-specific human Th2 lymphocytes in the control of immune responses to allergens in atopic individuals. The precise allergenic epitopes that activate these allergen-specific Th2 cells are, however, hardly known. We analyzed the epitope-specificity of T lymphocytes specific for *Der p II*, one of the major allergens of house dust mite *Dermatophagoides pteronyssinus*. Using a panel of overlapping synthetic peptides that span the entire *Der p II* molecule, we could demonstrate that polyclonal *Der p II*-specific T cell lines prepared from the peripheral blood of five atopic patients can react with at least 10 different epitopes of the molecule. Each donor showed a different pattern of reactivity with the synthetic peptides, suggesting that *Der p II* contains multiple T cell epitopes that may differ from individual to individual. We studied the specificity of the T cell response to *Der p II* in more detail in one atopic patient using a short term polyclonal T cell line that strongly reacted to one single peptide (116–129) of the allergen. From this patient we established a panel of 11 *Der p II*-specific TLC. Ten TLC were of the CD3⁺ CD4⁺ phenotype and showed a high IL-4/IFN- γ production ratio, whereas another TLC expressed CD3 and CD8 and failed to secrete substantial IL-4 and IFN- γ . The use of at least four different TCR V β gene segments was shown within this panel TLC. All TLC tested recognized the allergen in an HLA-DR1-restricted manner. Although this patient reacted to only one peptide on the polyclonal level, two T cell epitopes were identified on the clonal level by using synthetic peptides and autologous APC to stimulate the TLC. Combining data of CD4/CD8 expression, TCR V β usage, and epitope specificity, at least six different types of *Der p II*-specific TLC could be identified within this patient. Binding of IgE to all synthetic peptides of *Der p II* is low and of low affinity, which may be of particular importance with respect to possible desensitization protocols using such peptides. *Journal of Immunology*, 1993, 151: 2326.

The role of T lymphocytes in the regulation of IgE production in atopic disease is becoming increasingly clear. It is well documented that IgE synthesis by B lymphocytes is induced by IL-4 and inhibited by IFN- γ , both CD4⁺ T cell-derived lymphokines (1–3).

Allergen-specific Th2 cells, producing large amounts of IL-4 and IL-5, but little IFN- γ can be isolated from the peripheral blood of atopic patients (4–7). Allergen-specific Th2 cells have also been cloned from late phase inflammatory reactions in lesional skin of atopic dermatitis patients (8, 9). IL-4 and IL-5, but hardly any IFN- γ and IL-2, encoding mRNA can be detected in the skin of atopic patients after intradermal challenge with pollen allergens (10), suggesting that these allergen-specific Th2 cells are indeed activated in atopic disease.

Consequently, functional inactivation of these allergen-specific Th2 cells may be an important strategy for managing atopic disease. Currently used desensitization protocols with unfractionated allergens, however, frequently lead to IgE-mediated complications. These complications

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may not occur, if synthetic peptides are used for desensitization. At present, not much is known about the precise epitopes that are recognized by allergen-specific Th2 cells (11, 12). Therefore analysis of the T cell epitopes of allergens is required to investigate whether synthetic peptides that contain such T cell epitopes may eventually be used to design more efficient desensitization protocols.

T cell epitopes have only recently been described for the allergens *Der p* I (13), *Lol p* I (14), and *Amb a* I (15). However, because allergen extracts are complex mixtures of several allergenic proteins, detailed analysis of T cell epitopes of additional allergens will be important for establishing the feasibility of peptides for use in desensitization therapy.

Housedust mites are a major source of environmental inhalation allergens. The proteins of housedust mite Dp³ that are recognized by serum IgE molecules of atopic patients are quite diverse, and several major (*Der p* I, *Der p* II, and *Der p* III) as well as other allergens have been identified (16–23). At least two of the major Dp allergens, namely *Der p* I (24–27) and *Der p* II (24, 27), as well as the *Dermatophagoides farinae* analogue *Der f* II (28), have been shown to be recognized by T lymphocytes. In more detailed studies it was demonstrated that a majority of Dp-allergic patients show proliferative T cell responses toward *Der p* I and *Der p* II indicating that these major allergens are also recognized by T lymphocytes (26, 27).

We have focused on the analysis of T cell epitopes of *Der p* II, a major allergen of Dp that is recognized by the serum IgE of 70 to 90% of housedust mite allergic individuals (20, 29, 30). *Der p* II has a molecular mass of 15 kDa, and is heat stable (18, 20, 21). The gene encoding *Der p* II has recently been cloned and sequenced (22). It has no homology to any sequenced protein except for the *Dermatophagoides farinae* equivalent, *Der f* II (31, 32). The knowledge of the sequence of *Der p* II has enabled us to prepare synthetic peptides of *Der p* II.

To analyze the T cell epitopes of the allergen, we have tested the reactivity of *Der p* II-specific T cell lines of five atopic patients with a panel of overlapping synthetic peptides stretching the entire length of the allergen. For a more detailed analysis we generated a panel of *Der p* II-specific CD4⁺ TLC from the peripheral blood of one of these atopic patients. T cell epitope specificity of the TLC was assessed, lymphokine production was measured and MHC class II restrictions were determined. The diversity of these TLC was further studied by analyzing the TCR V β gene usage. Finally, the *Der p* II specificity of the serum IgE of the patient was studied using the same synthetic peptides.

Materials and Methods

Donors

Donors L (HLA DR 1, 3; DRw52; DQw1,w3), D (HLA DRw13,w14; DRw52; DQw1), P (HLA DR 1, 4; DRw52; DQw1,w3), A (HLA-DR1, 12; DRw52; DQw1,w3), and S (HLA-DR 12,15; DRW52; DQW1,W3) are atopic patients who are clinically allergic to Dp. Radioallergosorbent tests indicated high titers of total Dp- and *Der p* II-specific IgE in the serum of all five patients.

Antibodies, reagents, and cytokines

mAb used for typing CD3 (OKT3)-, CD4 (OKT4)-, and CD8 (OKT8)-expressing cells were obtained from Ortho (Raritan, NJ). Subclasses of HLA class II restriction determinants were characterized by blocking Ag-specific T cell proliferation with the following mAb: anti-HLA-DR (B.8.11.2, a gift of Dr. F. Koning, Department of Immunohematology, University of Leiden, The Netherlands) (33), anti-HLA-DQ (SPV L3–8, a gift of Dr. H. Spits, DNAX, Palo Alto, CA) (34), and anti-HLA-DP (B21/7, Becton Dickinson, Mountain View, CA).

Culture medium and cell culturing

T cells were cultured in Iscove's modified Dulbecco's medium (GIBCO, Paisley, UK), supplemented with 10% pooled, c-inactivated normal human serum (Central Laboratory Blood Transfusion Service, Amsterdam, The Netherlands), gentamycin (80 mg/l) (Duchefa, Haarlem, The Netherlands), and where mentioned 10 U/ml rIL-2 (Cetus, Emeryville, CA). EBV-transformed B cell lines were maintained in Iscove's modified Dulbecco's medium supplemented with 10% FCS (HyClone, Logan, UT) and 80 μ g/liter gentamycin. All cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂ using tissue culture plates (Costar, Cambridge, MA).

Dp protein extract and purified *Der p* II

Protein extracts of Dp were prepared from lyophilized mite cultures (kindly provided by HAL, Haarlem, The Netherlands). After dialysis with PBS, the HAL Dp extract used in this study contained 3.75×10^6 U/ml *Der p* I and 38.3×10^6 U/ml *Der p* II, as compared with the International Reference Preparation for Dp extracts (NIBSC 82/518) (24). *Der p* II was affinity purified using an anti-*Der p* II mAb as described in detail elsewhere (20). The final preparation contained 8.5×10^6 U/ml (42.5 μ g/ml) *Der p* II and <160 U/ml (0.02 μ g/ml) *Der p* I. r*Der p* II (22) was prepared by G. Hakkaart, Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands.

Preparation of synthetic *Der p* II peptides

The preparation of the synthetic peptides used in this study is described in detail elsewhere (35). In short, the T-bag

³ Abbreviations used in this paper: Dp, *Dermatophagoides pteronyssinus*; TFA, trifluoroacetic acid; PCR, polymerase chain reaction; TLC, T lymphocyte clone.

method of Houghten (36–38) was adapted for 9-fluorenylmethoxycarbonyl-chemistry and peptides were synthesized on *p*-benzyloxybenzyl alcohol-resins (39, 40) prelinked with the first N-9-fluorenylmethoxycarbonyl-protected amino acids. All peptides, except peptide 1–15 (the N-terminus of *Der p* II), were acetylated at the N-terminus and all peptides, except peptides 1–15 and 116–129, were synthesized with an additional lysine residue at the C-terminus to facilitate coupling to Sepharose. The C-terminal peptide 116–129 was synthesized with an additional lysine residue at the N-terminus instead of at the C-terminus. Peptides were cleaved from the resin within 4 h with TFA containing 5% thianisole. The purity was checked by reversed-phase HPLC (Delta Pak C-18, gradient from 0.1% TFA in water to 0.1% TFA in acetonitrile, UV detection at 214 and 280 nm).

Cloning procedure of *Der p* II-specific T cells

Der p II-specific T cells were cloned from short-term *Der p* II-reactive T cell lines using a protocol as described previously (41). PBMC were isolated by density centrifugation on Lymphoprep (Nycomed, Torshov, Norway) and cultured for 11 days in the presence of affinity purified *Der p* II (5 µg/ml), the last 5 days in the presence of 10 U/ml rIL-2. On day 11, cloning was performed in 96-well flat-bottom culture plates, by limiting dilution at 0.3 cells/well and non-specific stimulation with 1 µg/ml PHA (Difco, Detroit, MI) in the presence of a feeder mixture of 3000 rad-irradiated PBMC (10^5 /well) from two different unrelated donors, 3000 rad-irradiated cells (10^4 /well) of an EBV-transformed B cell line (JY) and rIL-2 (10 U/ml). After about 2 wk, expanding clones were transferred to 24-well plates in culture medium supplemented with rIL-2 (10 U/ml). After another 5 to 10 days, most clones had produced enough cells to test for Dp-specificity in a proliferation assay. *Der p* II-reactive TLC were further expanded by restimulation with PHA (2.5×10^5 cells/well) in 24-well plates, and maintained in the same feeder mixture as described above. Fresh rIL-2 was added at 3-day intervals. Fresh feeder cells and PHA were added every 14 days. Clones MBBAA60, MBEEA40, and MBEEA42 have been described and characterized previously (24).

Cell typing

Cell typing was performed by labelling suspended cells with surface Ag-specific mouse mAb and detecting positive cells by fluorescence microscopy, or by flow cytometry (FACScan, Becton Dickinson) using FITC-conjugated F(ab')₂ fragments of a rabbit anti-mouse antiserum (Zymed, San Francisco, CA).

Ag-specific T cell proliferation assays

Ten to 14 days after the last stimulation, T cells were washed three times in HBSS + 2% FCS to remove all rIL-2. Subsequently, Ag-specific proliferation of the TLC was assayed by culturing the cells (2×10^4 /well) in 96-well flat-bottom culture plates in the presence of specific Ag, using 3000 rad-irradiated autologous PBMC (10^5 /well) or EBV-transformed B cells (10^4 /well) as APC. Cells were cultured for 40 h, the last 16 h in the presence of 0.3 µCi (=11.1 kBq)/well of [³H]TdR (Amersham International, Amersham, UK). Proliferation was expressed as the mean cpm of duplicate cultures. *Der p* II-specific proliferation of T cells in PBMC ($1-2 \times 10^5$ /well) was assayed after 6 days of culture in the presence of affinity-purified *Der p* II (5 µg/ml), also terminated by a 16-h [³H]TdR pulse. Proliferation was expressed as the mean cpm of triplicate cultures.

Production of TLC supernatants for cytokine measurements

Ten to 14 days after the last stimulation T cells were washed three times in HBSS + 2% FCS and stimulated in 96-well tissue culture plates at 4×10^4 cells/well. T cell supernatants were obtained by incubating the TLC for 24 h in the presence of a mitogenic pair of mAb directed against CD2 (CLB-T11.1/1 and CLB-T11.2/1; 1/1000 dilution of ascites) in combination with a mAb directed against CD28 (CLB-28/1; 1/1000 dilution of ascites). This method has previously been shown to yield optimal lymphokine production (6).

IL-4 and IFN-γ assays

Estimation of the IL-4 and IFN-γ contents of TLC supernatants was performed with specific solid-phase sandwich ELISA as described in detail elsewhere (42, 43)

PCR analysis of TCR Vβ gene segment usage

Total RNA was isolated from 10^7 T cells by the RNazol B method (Campro Scientific, Elst, The Netherlands). A 10-µl mixture containing 10 µg RNA and 500 ng oligo-dT was heated at 55°C for 10 min and cooled to room temperature. The first-strand DNA was synthesized in a 20 µl volume using 200 U MMLV-derived reverse transcriptase (Superscript; GIBCO-BRL, Eggenstein, FRG) and applying the reaction conditions as recommended by the manufacturer. The mixture was incubated at 42°C for 1.5 h and then heated at 68°C for 5 min to inactivate the reverse transcriptase. Of this first strand mix 0.4 µl was made up to a final volume of 100 µl containing 0.2 mM of dATP, dCTP, dGTP, and dTTP each, 50 mM KCl, 10 mM Tris-HCl, pH 9.0, at 25°C, 1.5 mM MgCl₂, 0.01% gelatin, 0.1% Triton X-100, 200 ng of both the Cβ and the Vβ gene family-specific primers (44, 45) and 1 U Taq DNA poly-

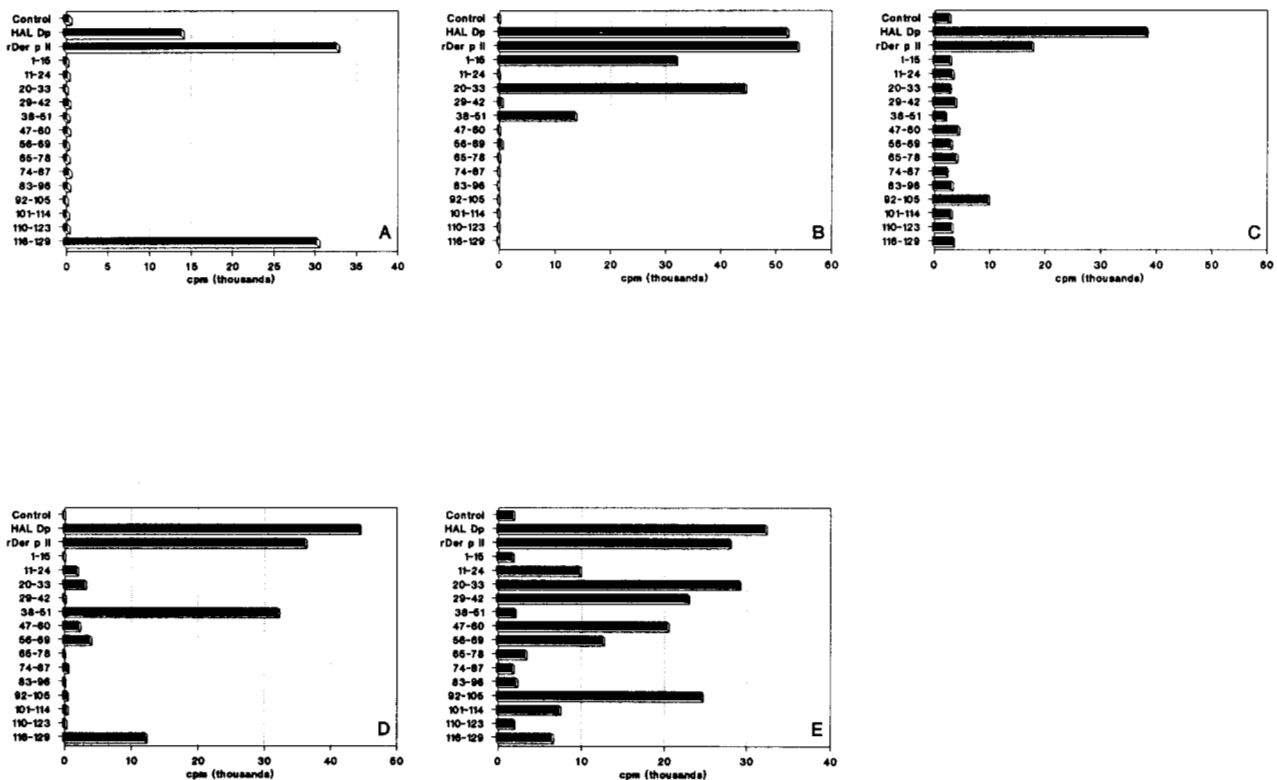


FIGURE 1. Proliferative responses of short-term *Der p II*-reactive T cell lines from atopic donors L (A), D (B), P (C), A (D), and S (E) to a total Dp protein extract (HAL), *rDer p II* and to synthetic peptides of *Der p II*. The T cells (2×10^4 /well) were cultured in the presence of autologous irradiated PBMC (10^5 /well) as a source of APC. Proliferation was determined by [^{3} H]Tdr incorporation. Values shown are the mean cpm of triplicate cultures (SD < 10%).

merase I (Promega, Madison, WI). The mixture was overlaid with 100 μ l paraffin oil (Merck, Darmstadt, FRG) and subjected to 25 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. In all experiments controls without template were included. Under these conditions no bands could be detected on ethidium bromide-stained agarose gels or after Southern blot hybridization.

Ten μ l of the PCR products were fractionated by electrophoresis on a 2% agarose gel and DNA was transferred to nitrocellulose filters (Schleicher & Schull, Basel, Switzerland). Baked filters were hybridized overnight at 65°C with a probe specific for the human C β region. This probe was the *Bg*/II fragment of clone JUR- β 2. Probes were labeled with ^{32} P-dCTP by random primer extension. Sp. act. was 5 to 10×10^8 cpm/ μ g. The hybridization buffer (10–15 ml) consisted of 7% SDS, 1% BSA, 2 mM EDTA, 0.5 M NaHPO₄, pH 7.0, and denaturated salmon sperm DNA (100 μ g/ml). Stringency wash was in $0.3 \times$ SSC/0.1% SDS at 65°C for 30 min. Autoradiography was performed for 1 to 7 days at -70°C using Kodak XAR-5 films.

IgE binding to synthetic peptides

After lyophilization, the synthetic peptides of *Der p II* were coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) without further purification. The coupling was performed in 0.1 M bicarbonate, pH 8.5 (10 ml), to

which the peptides, dissolved in N,N-dimethylformamide were added. Each peptide (4 mg) was coupled to 500 mg CNBr-activated Sepharose-4B. After 4 h, remaining binding sites on the Sepharose were blocked with 0.5 M glycine, pH 8.5. The Sepharose was suspended in PBS 0.3% BSA 0.1% Tween 20 (250 ml). In the RIA-test this suspension (250 μ l) was incubated with 50 μ l of antibody sample under shaking conditions for 16 h at room temperature. After washing the Sepharose five times with PBS, anti-IgE reagent (1 ng), radiolabeled with 400 Bq ^{125} I, was added in PBS 0.3% BSA, 0.1% Tween 20, 4.5% normal bovine serum, and 0.5% normal sheep serum (500 μ l). After 16 h of incubation, the Sepharose was washed four times with PBS and the γ -radiation was counted. Results are given as percent of added radioactivity bound by the solid-phase Ag.

Results

Der p II epitopes recognized by polyclonal *Der p II*-reactive T cell lines

Short-term *Der p II*-specific T cell lines were prepared from the peripheral blood of five atopic donors. These lines were tested for reactivity with total Dp protein extract, purified *Der p II*, and a panel of overlapping synthetic peptides of *Der p II*. As shown in Figure 1, 10 different T cell epitopes were identified in these five donors (peptides 1–15, 11–24,

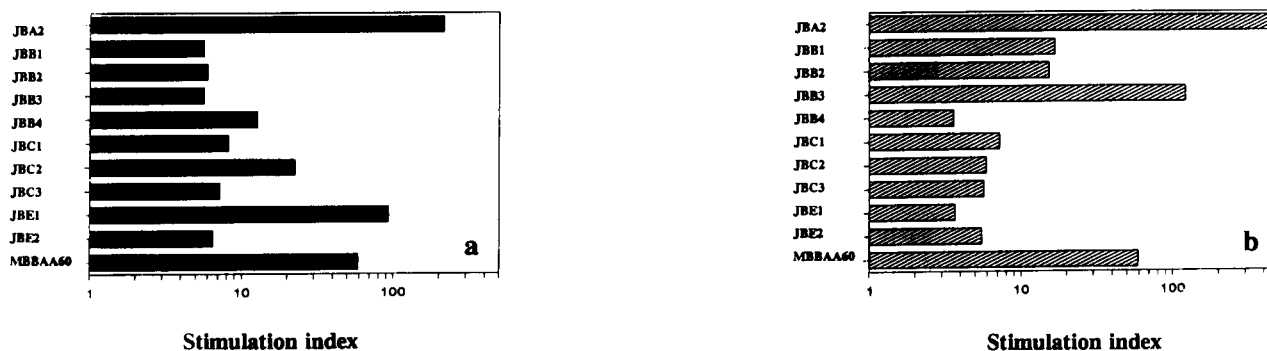


FIGURE 2. Proliferative responses of *Der p* II-specific TLC to a total Dp protein extract (A) and to *Der p* II (B). TLC were washed three times and cultured (2×10^4 /well) for 40 h with irradiated autologous PBMC (10^5 /well) or EBV-transformed B cells (10^4 /well) as a source of APC in the presence or absence of Ag. Values shown are stimulation indexes, as determined by [^{3} H]TdR incorporation. Presented data are obtained from several comparable experiments.

20–33, 29–42, 38–51, 47–60, 56–69, 92–105, 101–114, and 116–129). All donors reacted in a different pattern. The results suggest that the epitopes recognized by human T cells are quite diverse.

Characterization of *Der p* II-specific TLC

To study the phenotype and specificity of the T cells present in the *Der p* II-specific cell lines in more detail, T cells were cloned from a *Der p* II-specific T cell line of atopic donor L. This patient was selected because the *Der p* II-reactive T cell line strongly reacted with only one peptide (116–129), suggesting a restricted *Der p* II-specific T cell repertoire. All clones reacting with the Dp extract also reacted with affinity-purified *Der p* II (Fig. 2). A total of 11 *Der p* II-specific TLC were generated in four cloning procedures. The phenotype of the TLC was determined by flow-cytometric analysis using mAb reacting with CD3, CD4, and CD8. Ten clones expressed both CD3 and CD4, although clone JBB1 expressed CD3 and CD8. Cytokine production by the TLC was assayed after polyclonal stimulation with a mitogenic pair of two anti-CD2 mAb directed against different epitopes of CD2 in combination with a mAb directed against CD28 (6). After 24 h, the supernatant was collected and the IL-4 and IFN- γ contents were measured. After polyclonal stimulation, high amounts of IL-4 were found in the supernatants of all clones except JBB1, whereas no or low amounts of IFN- γ were found (Fig. 3). As a reference, the previously described Dp-specific Th1 clones MBEAA40 and MBEAA42 from an HLA class II identical nonatopic individual and the *Der p* II-specific Th2 clone MBBAA60 from atopic donor L were used as a control, stressing that the present *Der p* II-specific TLC are of the Th2 type (4).

PCR analysis of TCR V β gene segment usage

To further characterize the *Der p* II-specific TLC, TCR V β gene usage was determined in five of the TLC. This analysis revealed that clones JBB2 and JBB4 expressed V β 6.1–3,

clone JBA2 expressed V β 14, and clones JBB1 and JBB3 expressed V β 15. To investigate whether the usage of these particular V β gene segments reflects a restricted repertoire of *Der p* II-specific T cells, the remaining six TLC were tested using only the primers for the V β 6.1–3, V β 14, and V β 15 gene families. Of these six TLC, clone JBE2 expressed V β 6.1–3 and clone JBC1 expressed V β 15. The remaining four clones (MBBAA60, JBC2, JBC3, and JBE1) did not yield PCR products using the V β 6.1–3-, V β 14-, or V β 15-specific primers and apparently these TLC use other TCR V β gene segments, indicating that the diversity of the *Der p* II-specific TLC may be even larger.

HLA-DR restriction of *Der p* II-specific TLC

Proliferation inhibition experiments using anti-HLA-DP, anti-HLA-DQ, and anti-HLA-DR mAb were performed to determine the HLA class II restriction of the TLC. Addition of anti-HLA-DR mAb inhibited Ag-specific proliferation of all TLC, whereas anti-HLA-DP and anti-HLA-DQ had no effect (Table I), indicating that these TLC all recognize *Der p* II in association with HLA-DR. Notably, this is also the case for TLC JBB1 that expresses CD8, but not CD4. Experiments in which PBMC of two donors with different HLA-DR haplotypes were used as APC showed that all seven TLC tested recognize *Der p* II in association with the HLA-DR1 molecule. Clones JBB3 and JBC1, however, seem to be able to recognize *Der p* II not only in association with autologous APC, but also, to a lesser extent, with APC that express HLA-DR3 and HLA-DR4 (Table II).

Determination of *Der p* II epitopes recognized by *Der p* II-specific TLC

The epitope specificity of the *Der p* II-specific TLC was determined by testing the reactivity of the TLC with overlapping synthetic peptides in a proliferation assay. In accordance with the results obtained with the polyclonal *Der p* II-specific cell line (Fig. 1A), 7 of 11 TLC tested reacted

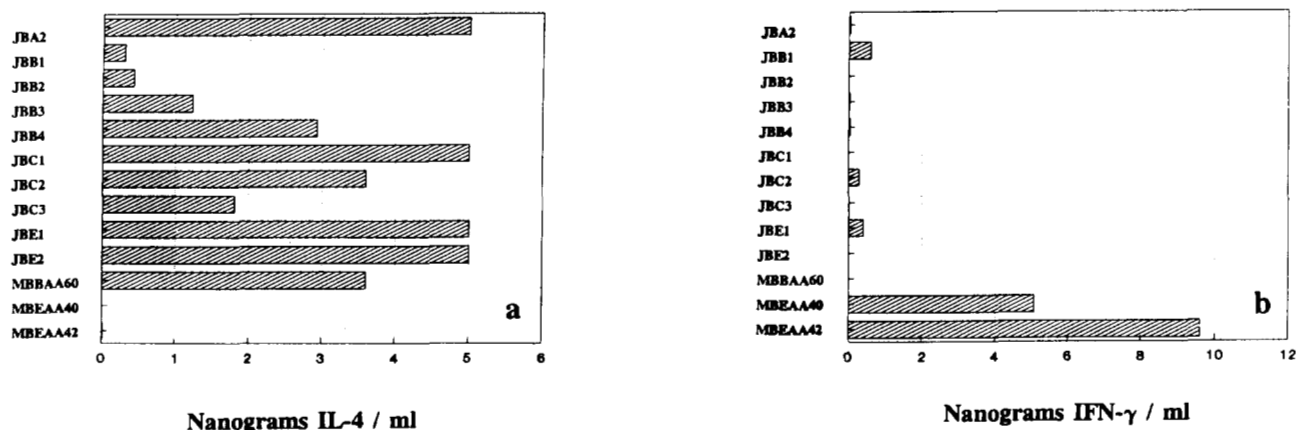


FIGURE 3. IL-4 and IFN- γ production by *Der p II*-specific TLC. Supernatants were prepared by stimulating T cells simultaneously with 2 mAb directed against CD2 and 1 mAb directed at CD28 at 4×10^4 cells/well in a 96-well plate. After 24 h, the supernatants were collected and IL-4 and IFN- γ production measured by ELISA. Clones MBBA42 and MBBA40 are control TLC of a HLA-DR-matched nonatopic donor that are *Dp* specific and of the Th1 type, and TLC MBBA60 has previously been described to be of the Th2 type (6). Production is shown as the production of IL-4 and IFN- γ per 4×10^4 cells.

Table I
Inhibition of *Dp*-specific proliferation by anti HLA mAb^a

TLC	Proliferation (cpm $\times 10^{-3}$)				% Inhibition Anti-HLA-DR ^b
	No mAb	Anti-HLA-DP	Anti-HLA-DQ	Anti-HLA-DR	
JBA2	35.2	34.5	31.1	0.4	98.8
JBB1	10.8	10.5	11.9	0	100
JBB2	22.3	23.0	22.4	1.7	92.4
JBB3	66.5	65.8	63.8	0.9	98.7
JBB4	20.5	15.5	15.0	0	100
JBC1	11.8	13.3	7.7	0	100
JBC2	2.0	1.7	1.0	0	100
JBC3	8.5	6.7	4.3	0	100
JBE1	5.6	4.6	3.5	0	100
JBE2	14.1	12.7	10.6	0.9	99.9
MBBA60	23.7	16.5	11.2	0	100

^a *Der p II*-specific TLC ($2-4 \times 10^4$ /well) were stimulated with *Dp* Ag and autologous APC in the presence or absence of anti-HLA class II blocking mAb. Background values are subtracted.

^b % inhibition by Anti HLA-DR mAb is expressed as:

$$\% \text{ inhibition} = \frac{\text{cpm} (-\text{antibody}) - \text{cpm} (+\text{antibody})}{\text{cpm} (-\text{antibody})} \times 100\%$$

strongly with peptide 116–129, showing some residual reaction with peptide 110–123 (Fig. 4). Interestingly, two other TLC (JBB3 and JBC1) reacted with peptide 29–42, being the same TLC that can also recognize *Der p II* with HLA-DR3 HLA-DR4 expressing APC. This epitope was not detected in the polyclonal *Der p II*-specific cell line, and may therefore represent a minor T cell epitope for this patient. The remaining two TLC did not react with any of the synthetic peptides. At least six different types of *Der p II*-specific TLC can be identified as based on their phenotype, TCR V β usage, HLA class II restriction, and epitope specificity (Table III). Four different clone types (types 1, 2, 3, and 6) recognize peptide 116–129, clone type 4 rec-

Table II
HLA restriction of *Der p II*-specific T cell clones^a

TLC	HLA Haplotype of APC					
	DR 1,3 ^b DRw52		DR1,2		DR3,4 DRw52,w53	
	Proliferation (cpm × 10 ⁻³)					
	No Ag	Dp	No Ag	Dp	No Ag	Dp
JBA2	1.5	29.1	0.2	12.8	0.2	0.8
JBB3	1.0	41.8	0.2	27.0	0.2	6.6
JBB4	1.7	21.2	0.4	2.8	0.4	0.4
JBC1	0.4	5.0	0.1	9.2	0.1	3.2
JBE1	0.1	5.6	0.1	9.2	0.0	0.1
JBE2	0.1	14.2	0.1	13.6	0.1	0.2
MBBAA60	0.3	18.2	1.7	47.8	0.3	0.2

^a Proliferative responses of *Der p II*-specific TLC to *Dp* Ag were tested after presentation by APC (irradiated PBMC, 10^5 /well) from three donors with different HLA class II haplotypes. Values given are cpm $\times 10^{-3}$ as determined by [³H]TdR incorporation.

^b Autologous PBMC.

ognizes peptide 29–42, and clone type 5 does not recognize any of the synthetic peptides.

Reactivity of serum IgE of patient L with synthetic peptides of *Der p II*

The use of peptides that contain T cell epitopes of allergens may be useful for the development of desensitization protocols only when circulating IgE does not bind to such peptides, thus preventing adverse reactions to therapy. Therefore, reactivity of serum IgE of patient L with synthetic peptides was determined. As is shown in Figure 5, approximately 47% of the serum IgE of patient L bound to affinity purified *Der p II*. Binding of the serum IgE to the synthetic peptides was less efficient. Only to peptide 74–87 significant amounts of the IgE bound. Binding both with

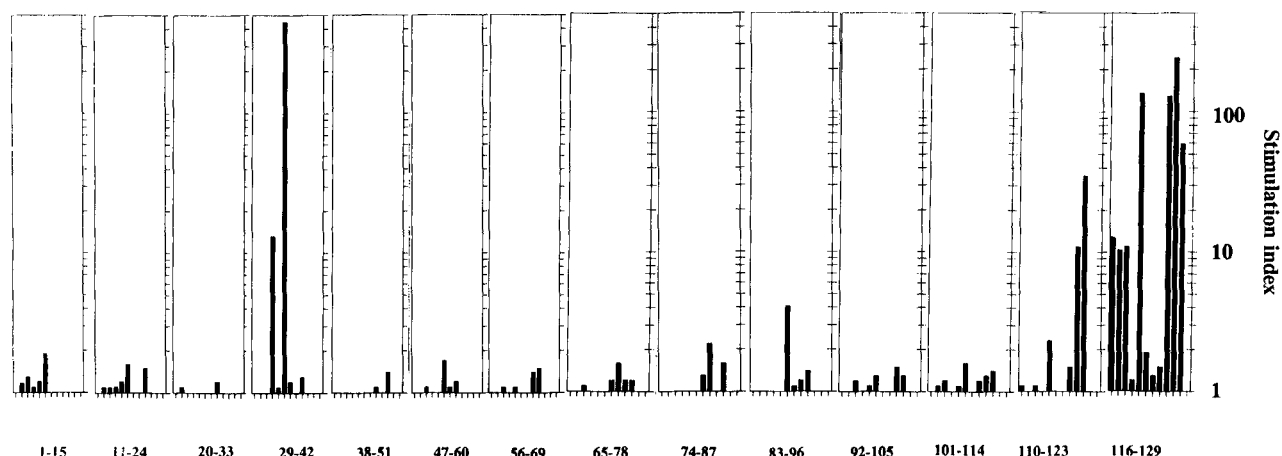


FIGURE 4. Determination of epitope specificity of all 11 *Der p II*-specific TLC of atopic donor L using a panel of overlapping synthetic peptides. All TLC were tested for their proliferative response in the presence of autologous APC to all 14 synthetic peptides of *Der p II*. Bars in each panel represent reactivity of all TLC to the indicated peptide. The order of all TLC is the same for each panel. Values given are stimulation indexes as determined by [^3H]TdR incorporation.

Table III
Characteristics of *Der p II*-specific TLC^a

TLC	CD	V β	HLA-DR Restriction	Peptide	TLC Type
JBA2	4	14	DR1	116–129	1
JBB1	8	15	NT ^b	116–129	2
JBB2	4	6.1–3	NT	116–129	3
JBB3	4	15	DR1(3,4)	29–42	4
JBB4	4	6.1–3	DR1	116–129	3
JBC1	4	15	DR1(3,4)	29–42	4
JBC2	4	Other ^c	NT	NO ^d	5
JBC3	4	Other	NT	NO	5
JBE1	4	Other	DR1	116–129	6
JBE2	4	6.1–3	DR1	116–129	3
MBBAA60	4	Other	DR1	116–129	6

^a Table III summarizes all results of phenotyping, TCR-V β analysis, MHC restriction, and peptide specificity of all clones described. At least six different clone types can be distinguished on the basis of these results.

^b NT, not tested.

^c No product could be observed after PCR with the V β 6, 14, and 15 primers.

^d NO, none of the peptides were recognized by these TLC.

peptide 29–42 and peptide 116–129, that contain the T cell epitopes, was less than 2%.

Discussion

Largely due to the inavailability of amino acid sequence data of allergens, not much is known about the precise epitopes recognized by allergen-specific T cells. The recent cloning of Dp major allergen *Der p II* (22) has enabled us to evaluate the epitope specificity of *Der p II*-specific T cell lines and clones. To this aim we have analyzed the epitope specificity of polyclonal, *Der p II*-reactive T cell lines from the peripheral blood of five atopic donors. We could demonstrate 10 distinct T cell epitopes, that are different from donor to donor. This indicates that multiple T cell epitopes exist that may be scattered throughout the entire *Der p II* molecule. The fact that multiple T cell epitopes may be present in allergenic proteins is also

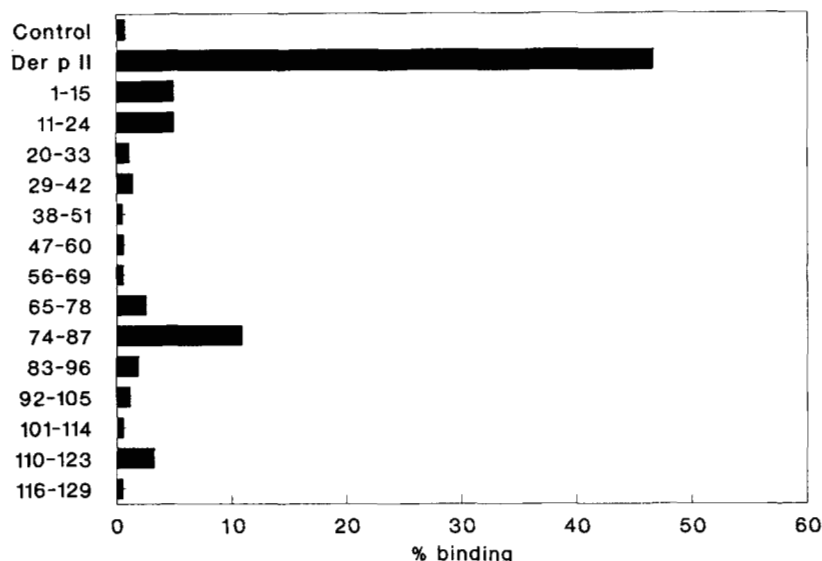
suggested by a study of IJssel et al. (13) for the Dp major allergen *Der p I*.

A panel of 11 *Der p II*-specific TLC was prepared from the *Der p II*-reactive polyclonal T cell line from donor L (Fig. 1A) that reacted to one peptide only (116–129). In accordance with the results obtained with a polyclonal *Der p II*-specific T cell line, 7 of 11 *Der p II*-specific TLC reacted to peptide 116–129. However, two other TLC reacted to another peptide (29–42), with which no reactivity was observed on the polyclonal level. This suggests that additional T cell epitopes of *Der p II* may be recognized by the other two atopic patients that were not detected in polyclonal cell lines. The remaining two TLC did not react with any of the synthetic peptides, which may be explained by too little overlap of the synthetic peptides.

Table I shows that Ag-recognition of all TLC could be completely inhibited by the addition of an anti-HLA-DR mAb, indicating HLA-DR restriction of the TLC. This was confirmed by testing the TLC with three differentially typed PBMC as source of APC (Table II). However, TLC JBB3 and JBC1 that recognize peptide 29–42 appeared to be able to recognize the peptide in the presence of HLA-DR1⁺ PBMC, but also, to a lesser extent, on HLA-DR3,4⁺ PBMC. However, several additional Dp-specific as well as cat allergen-specific TLC generated in our laboratory show allele-specific HLA-restriction, indicating that degenerate HLA-restriction of allergen-specific TLC does not frequently occur.

All *Der p II*-reactive TLC, irrespective of their epitope specificity, have a lymphokine production profile that fits the profile of the human Th2 subset (4, 6, 7). The CD8⁺ TLC JBB1 is the only TLC that did not show a Th2 lymphokine production profile. These data indicate that no correlation exists between epitope specificity and lymphokine production by *Der p II*-specific TLC. This is confirmed by the fact that at least two of the epitopes described can also

FIGURE 5. Reactivity of serum IgE of patient L with overlapping synthetic peptides of *Der p* II. Peptides were coupled to Sepharose 4B, and binding of IgE was measured in an RIA. Results are given as percentage of added label bound by the solid-phase Ag.



be recognized by T lymphocytes of non atopic donors that have no detectable Dp-specific IgE (R. J. J. van Neerven, unpublished observations).

TCR V β gene usage by the *Der p* II-specific TLC was diverse. No strict correlations between TCR V β usage and epitope specificity or lymphokine production by the TLC could be found. This indicates that V β gene usage in atopic disease gives no evidence for a largely biased T cell repertoire, in contrast to V β gene usage of T cells in some murine and rat autoimmune models (46).

When all data of the TLC were combined, they were grouped in at least six distinct TLC types of which four different types recognize the epitope contained in peptide 116–129. These data indicate that, even though responses of some *Der p* II-specific T cell lines to peptides of *Der p* II may seem to be limited in their repertoire, in fact several distinct types of TLC are present.

With knowledge of T cell epitopes of allergens, some new strategies for immune intervention in allergic diseases may be explored by specifically interfering with the interaction between APC, allergen, and T lymphocyte. This can be established by either preventing the interaction to occur (e.g., by designing blocking peptides that bind to HLA molecules), or by means of making the T cells unresponsive to the allergens (e.g., by tolerizing the T lymphocyte). O'Hehir et al. (47–49) have described several distinct methods to induce clonal anergy in house dust-mite specific TLC in vitro as models for immune intervention therapies. This could be done either by preincubating these TLC with supraoptimal doses of a T cell epitope-containing peptide (47), or by incubating TLC with superantigens that bind to specific V β regions (47, 48). In addition, T cell nonresponsiveness has been induced in vitro by blocking the HLA molecule that presents the Ag with competing allele-specific HLA binding peptides (49).

Interestingly, some of the TLC we described are able to

recognize the synthetic peptide at low dosage (1 μ g/ml) in the absence of APC (data not shown). Experiments are currently performed to study if this leads to functional inactivation of these TLC, as was demonstrated earlier for supraoptimal doses of peptide (47).

A prerequisite for the use of synthetic peptides for such tentative immunotherapies is that the IgE of atopic patients should not bind to these peptides to prevent adverse reactions to therapy. A total of 70 to 90% of the sera of house dust mite allergic patients contain IgE that binds to group II (*Der p* II, *Der f* II) allergens (20, 29, 30). Van 't Hof et al. (35) have shown that only a low percentage of circulating *Der p* II-specific IgE binds to these synthetic peptides with the exception of peptide 65–78 that is recognized by the IgE in some sera. However, the binding of IgE to these peptides has a relatively low affinity. In addition, Chua et al. (50) reported that IgE binding to *Der p* II is largely dependent on the intact sequence of the molecule. Also, the IgE of the patient used in our study did not bind significantly to the peptides that contained T cell epitopes. This may imply that peptides containing a T cell epitope of *Der p* II may not be recognized by circulating allergen-specific IgE, thereby ensuring that no adverse reactions to peptide therapy will occur.

The prevalence of T cell epitopes in both Dp major allergens, *Der p* II and *Der p* I (13) form an obstacle for clinical trials, because mixtures of peptides of both major allergens will be necessary to cover all possible T cell epitopes. Inasmuch as it is known that HLA-haplotype may determine T cell epitope specificity (13, 51, 52) we are currently investigating if the epitopes we have identified both on the clonal as on the polyclonal level are dependent on HLA haplotype, and if HLA phenotype may predict T cell epitopes of *Der p* II.

Taken together, the use of peptides that contain T cell epitopes of allergens may be promising for the improve-

ment of effective desensitization protocols because they are hardly recognized by circulating IgE. However, detailed information of T cell epitopes of major allergens and HLA haplotype will be required to make such therapies operational.

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