

Characterization of Cat Dander-Specific T Lymphocytes from Atopic Patients¹

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Fel d I, the major cat dander allergen, is recognized by serum IgE of more than 80% of all cat-allergic patients. Because IgE synthesis by B lymphocytes is under the control of T lymphocytes, we studied the specificity and lymphokine production profiles of cat dander-specific T lymphocytes. Polyclonal cat dander-specific T cell lines were found to react with purified *Fel d I*, but not with cat albumin, the only other characterized cat allergen. Similarly, within a panel of CD4⁺ T lymphocyte clones (TLC) that was generated from these cat dander-specific T cell lines, 5 of 16 TLC were found to react with *Fel d I*, and 0 of 16 with cat albumin. The remaining 11 TLC were shown to recognize at least two different proteins. In general, the TLC had a high IL-4/IFN- γ production ratio, and could recognize the cat dander extract in an HLA-DR, HLA-DQ, or HLA-DP restricted manner. In addition, five distinct T cell epitopes of *Fel d I* were identified by using a panel of overlapping synthetic peptides of both chains of *Fel d I*. The data presented here indicate that, even though multiple proteins in cat dander extract are recognized by T lymphocytes of allergic patients, *Fel d I*, the major IgE binding allergen, is also important in T cell activation. The fact that the cat-specific TLC are Th2-like indicates that these cells may play an important role in the pathophysiology of allergic responses to cat allergens. However, the diversity of HLA-class II restriction of cat dander- and *Fel d I*-specific TLC and the presence of multiple T cell epitopes in the allergen may complicate future immunotherapies. *Journal of Immunology*, 1994, 152: 4203.

CD4⁺ T lymphocytes have been shown to play an important role in the pathophysiology of allergic disease (1–3). These CD4⁺ T lymphocytes can be subdivided in T helper 1 (Th1), T helper 2 (Th2) cells, and intermediate T helper 0 (Th0) cells, as based on lymphokine production (1, 4–8). Allergen-specific CD4⁺ cells of the Th2 type are probably responsible for atopic allergy in humans (1, 2, 5, 9, 10). Because the synthesis of IgE by B lymphocytes is induced by IL-4 and inhibited by IFN- γ (11–13), these Th2 cells are able to induce IgE synthesis and play a strong promoting role in the differentiation of eosinophils, two characteristic features of atopic disease (5, 14).

T cell responses have only been studied against a few allergens, such as proteins of house dust mites (1, 15–18), and some pollen (2, 19–22). Information about the number and diversity of T cell epitopes of allergens is sparse to this date (3, 18–20, 23, 24). 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 of desensitization therapy.

The domestic cat, *Felis domesticus*, is an important source of indoor allergens. IgE binding studies with serum from atopic patients has shown that several proteins in cat dander extracts are recognized by IgE, of which *Fel d I* appears to be the major allergen for most patients (25–28). *Fel d I* is a complex heterodimer with a molecular mass of approximately 38 kDa. It consists of two chains that together have a molecular mass of approximately 18 kDa (29). The genes encoding both chains recently have been cloned and sequenced (30). T cell reactivity of polyclonal T cell lines with both recombinant chains of *Fel d I* recently has been described (31). In addition to *Fel d I*, only one other allergenic cat protein allergen has been

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characterized so far, namely cat albumin, to which serum IgE of only some cat-allergic patients binds (25). In the present study, we have analyzed T cell responses to allergens present in a protein extract prepared from cat dander to establish the relative contribution of the major allergen *Fel d I* for T cell activation and to get an insight into the number and location of T cell epitopes of *Fel d I*.

To this aim we have studied the patterns of reactivity of short-term cat dander-specific polyclonal T cell lines of eight atopic patients with cat dander extract, purified *Fel d I* and cat albumin, and a panel of overlapping synthetic peptides of *Fel d I*. In addition, we have analyzed the (epitope) specificity, HLA restriction, and lymphokine production patterns of a panel of cat dander-specific CD4⁺ T lymphocyte clones (TLC)³ generated from these T cell lines, to evaluate their role in atopic disease.

Materials and Methods

Donors

All eight atopic donors (numbered 1 to 8) are clinically allergic to *F. domesticus*, suffer from allergic asthma, and did not receive immunotherapy. Patient 8 suffers from severe atopic dermatitis and has a history of atopic asthma. Radioallergosorbent tests indicated high titers of cat dander and *Fel d I*-specific IgE in the serum of all patients.

Abs

mAb used for detection of CD3 (OKT3), CD4 (OKT4), and CD8 (OKT8) expression were obtained from Ortho Pharmaceutical Corp. (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, ascites used at a dilution of 1:200, a kind gift of Dr. F. Koning, Department of Immunohematology, University of Leiden, The Netherlands) (32), anti-HLA-DQ (SPV L3-8, ascites used at a dilution of 1:200, a kind gift of Dr. H. Spits, the Netherlands Cancer Institute, Amsterdam, The Netherlands) (33) and anti-HLA-DP (B7/21, purified Ab used at 10 µg/ml, a kind gift of Dr. J. Higgins, St. Mary's Hospital Medical School, London, UK).

Culture medium and cell culturing

T cells were cultured in Iscove's modified DMEM (GIBCO, Paisley, UK), supplemented with 10% pooled, complement-inactivated normal human serum (Central Laboratory Blood Transfusion Service, Amsterdam, The Netherlands.), gentamicin (80 mg/l) and where mentioned rIL-2 (10 U/ml, Cetus, Emeryville, CA). All cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂.

Allergen extracts and purification of *Fel d I*

Protein extracts of cat dander (a 10% w/v extract) and house dust mite Dermatophagoides pteronyssinus were a kind gift from Haarlem Allergen Laboratory (HAL, Haarlem, The Netherlands). After dialysis against PBS, the cat dander-extract used in this study contained 160 U/ml *Fel d I* and 60 µg/ml cat albumin. *Fel d I* was affinity purified using anti-*Fel d I* mAb Fd1a as described in detail elsewhere (34). Affinity purified cat albumin was a kind gift of Dr. S. Stapel, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands.

Size fractionation of cat dander extract

The cat dander extract was size fractionated using a 100-ml Sephadex G-75 column (Pharmacia, Uppsala, Sweden) in PBS (pH 7.4).

Synthetic peptides of *Fel d I*

The preparation of the 12 to 16 amino acid long synthetic peptides of *Fel d I* (with an overlap of 7 amino acids) used in this study is described in detail elsewhere (35, 36). In short, the T bag method of Houghten was adapted for 9-fluorenyl-methocarbonyl (Fmoc)-chemistry and peptides were synthesized on *p*-benzyloxybenzyl alcohol resins prelinked with the first N-Fmoc-protected amino acids. All peptides were acetylated at the N-terminus and synthesized with an additional lysine residue at the C-terminus, to facilitate coupling to Sepharose. Peptides were cleaved from the resin within 4 h with trifluoroacetic acid (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).

Preparation of cat dander-specific T cell lines and clones

Short-term cat dander-reactive T cell lines were prepared using a protocol as described previously (1, 18). In short, PBMC were isolated by density centrifugation on Lymphoprep (Nycomed, Torshov, Norway) and cultured for 11 days in the presence of cat dander extract at a concentration corresponding to 5 µg/ml *Fel d I*, the last 5 days in the presence of 10 U/ml rIL-2 (Cetus). On day 11, cloning was performed in 96-well flat bottom culture plates, by limiting dilution at 0.3 cells/well and nonspecific stimulation with 1 µg/ml PHA (Difco, Detroit, MI) in the presence of 3000 rad-irradiated PBMC (10⁵/well) from two different unrelated donors, 3000 rad-irradiated cells (10⁵/well) of an EBV-transformed B cell line (JY) and rIL-2 (10 U/ml). After approximately 2 wk, expanding TLC were transferred to 24-well plates in culture medium supplemented with rIL-2 (10 U/ml). After another 5 to 10 days, TLC cultures contained enough cells to test for cat dander-specificity in a proliferation assay. Cat dander-reactive TLC were further expanded by restimulation with PHA (2.5 × 10⁵ 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.

Cell typing

Cell typing was performed by labeling 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 fourteen days after the last stimulation, TLC or polyclonal T cell lines were washed three times in HBSS + 2% FCS to remove rIL-2. Subsequently, Ag-specific proliferation of the TLC (or lines) was assayed by culturing TLC (2 × 10⁴/well) in 96-well flat-bottom culture plates in the presence of cat dander extract or purified *Fel d I*, using 3000 rad-irradiated autologous PBMC (10⁵/well) as a source of 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 counts per minute of triplicate cultures. Cat dander-specific proliferation of T cells in PBMC (1 to 2 × 10⁵/well) was assayed after 6 days of culture in the presence or absence of cat dander extract (at a *Fel d I* concentration of 5 µg/ml), or affinity-purified *Fel d I* (5 µg/ml). These cultures were also terminated by a 16-h [³H]TdR-pulse. Proliferation was expressed as the mean counts per minute of triplicate cultures.

Production of TLC supernatants for cytokine measurements

Ten to fourteen days after the last stimulation, T cells were washed three times in HBSS 2% FCS and stimulated in Costar 96-well plates at 4 × 10⁴

³ Abbreviations used in this paper: TLC, T lymphocyte clone; TFA, trifluoroacetic acid.

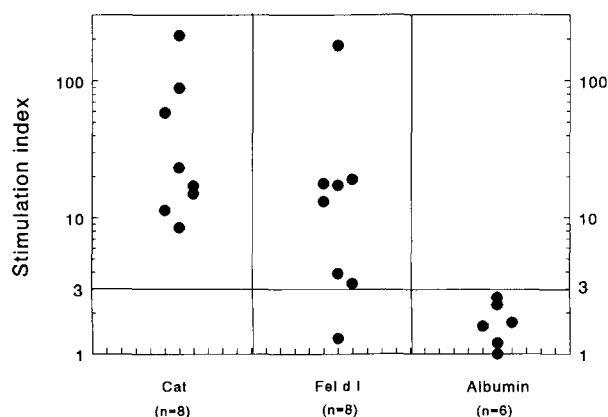


FIGURE 1. Cat dander extract-specific polyclonal T cell lines (2 to 4×10^4 /well) were tested for reactivity with total cat dander extract, *Fel d I*, and cat albumin in the presence of autologous PBMC (10^5 /well) as APC. Values shown are stimulation indexes as determined by [^{3} H]TdR incorporation. Background [^{3} H]TdR incorporation ranged from 0.1 to maximal 2.3×10^3 cpm.

cells/well. T cell supernatants were obtained by incubating the TLC for 24 h with Ag and APC for Ag-specific supernatants, or in the presence a combination of mAb directed against CD2 (CLB-T11.1/1 and CLB-T11.2/1 were used as a pair; $1:1000$ dilution of ascites) and CD28 (CLB-28/1; $1:1000$ dilution of ascites) in the presence of PMA (1 ng/ml), both methods normally yielding similar results (9).

IL-4 and IFN- γ assays

Estimation of IL-4 and IFN- γ contents of 24 h TLC supernatants was performed with specific solid-phase sandwich ELISAs as described in detail elsewhere (37, 38).

Results

Specific reactivity of short-term, polyclonal cat dander-specific T cell lines

Short-term, polyclonal cat dander-specific T cell lines were prepared by culturing PBMC for 6 days in the presence of cat dander extract. Cell lines of eight patients were expanded by stimulation with PHA in the presence of rIL-2 (10 U/ml) and irradiated feeder cells. After one cycle of stimulation, the lines were tested for reactivity with cat dander extract, purified *Fel d I*, or cat albumin. Figure 1 shows that 7 to 8 cat dander-specific T cell lines reacted to *Fel d I*, of which two lines were only borderline reactive, whereas none of the lines reacted with cat albumin.

Characterization of cat-specific TLC

To study the specificity of the cat dander-specific T lymphocytes in more detail, T cells were cloned from six of the short-term cat dander-reactive T cell lines. The specificity of the generated TLC was screened in an Ag-specific proliferation assay using autologous irradiated PBMC as a source of APC in the presence or absence of cat dander extract. Sixteen cat-specific TLC were obtained, all of

Table I. Ag reactivity of cat dander-specific TLC^a

TLC	Donor	Cat	<i>Fel d I</i>	Albumin	Dp
PBB1	1	37.0 ^b	1.2	0.5	1.4
PBB2	1	410.8	456.9	1.8	1.7
PBB3	1	115.1	167.9	0.6	1.0
PBB4	1	148.7	181.0	0.9	1.1
PBC1	2	216.2	0.9	2.7	0.5
PBD1	3	14.6	2.9	0.8	1.9
PBE1	4	70.2	0.5	0.7	1.0
PBF2	5	59.3	0.8	1.0	0.5
PBF3	5	302.3	2.9	3.1	1.7
PBF4	5	125.1	99.7	0.5	1.1
PBF7	5	209.0	52.4	1.1	1.0
PBF8	5	103.5	0.6	0.5	0.5
PBG2	6	21.5	0.6	0.6	0.2
PBG3	6	36.1	0.5	0.8	2.9
PBG4	6	41.8	2.5	1.8	1.4
PBG5	6	62.2	2.1	0.8	1.8

^a Proliferative responses of cat dander-specific TLC (2 to 4×10^4 /well) were determined to cat dander extract, purified *Fel d I*, purified albumin, and house dust mite (DP) extract in the presence of autologous PBMC (10^5 /well) as APC. Values shown are stimulation indexes as determined by [^{3} H]TdR incorporation. Background [^{3} H]TdR incorporation was less than 1×10^3 cpm in all cases.

^b Stimulation indexes >3 have been underlined.

which were of the CD3⁺ CD4⁺ CD8[−] phenotype. These cat-specific TLC were further tested for reactivity with cat dander extract, purified *Fel d I*, cat albumin, and, as a control, a house dust mite *D. pteronyssinus* extract. As expected, all TLC tested reacted with total cat dander extract, but not with house dust mite extract (Table I). Notably, 5 of the 16 TLC also reacted with purified *Fel d I*, whereas none of the TLC reacted with cat albumin. These five TLC were derived from two separate atopic donors. To further characterize the specificity of the cat-specific TLC that did not react with *Fel d I*, four TLC were tested with fractionated cat-dander extract. The TLC all had different reactivity patterns with the fractions, indicating that they reacted to at least three different proteins other than *Fel d I* (Figure 2). TLC PBD1 and PBE1 have a partial overlap, whereas TLC PBB1 and PBB4 have an almost complete overlap with the gel filtration fractions. Therefore we can conclude that at least two, but possibly three or four, different proteins are recognized by cat dander-specific T lymphocytes.

HLA class II restriction of cat-specific TLC

To determine the HLA class II restriction of the TLC, proliferation experiments were performed using anti-HLA-DP, anti-HLA-DQ and anti-HLA-DR mAb. As shown in Table II, Ag-specific proliferation of TLC PBB1 and PBB4 was inhibited by the addition of anti-HLA-DQ mAb, Ag-specific proliferation of TLC PBB2 was inhibited by the addition of anti-HLA-DP mAb, and all other TLC tested ($7/10$) were inhibited by anti-HLA-DR mAb. These results indicate that cat-specific TLC can be either HLA-DR-, HLA-DQ-, or HLA-DP-restricted. Additional

% of maximal proliferation

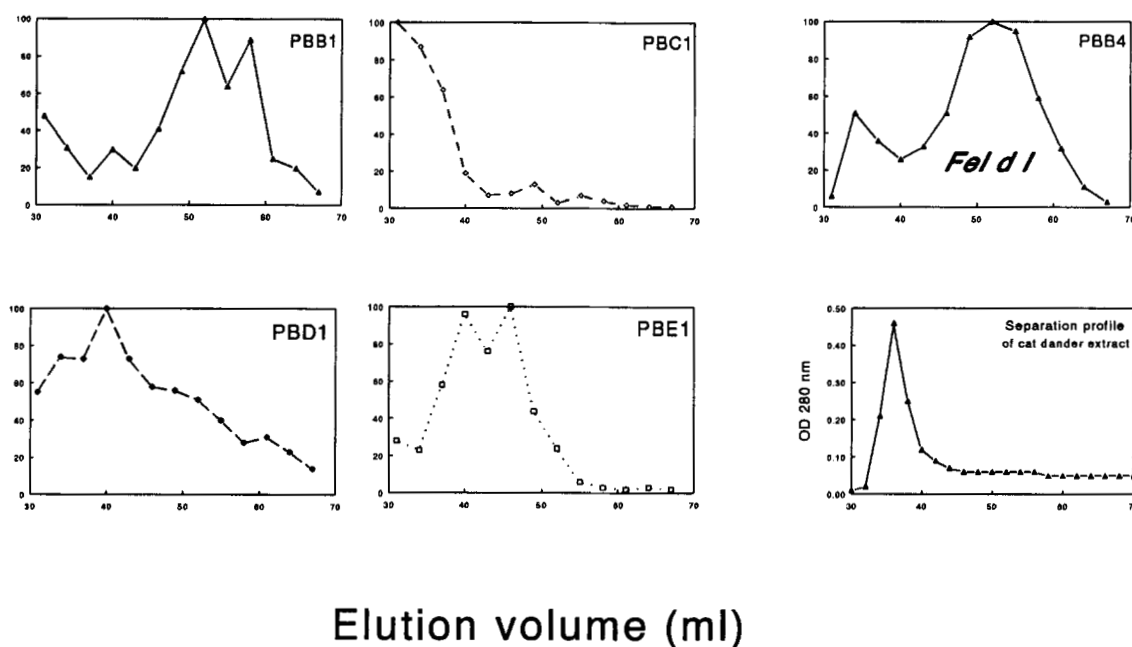


FIGURE 2. One ml of cat dander extract containing a total of 160 U of *Fel d 1* was separated by gel filtration on a Sephadex G-75 column (see *Materials and Methods*). Cat-specific TLC (2 to 4×10^4 /well) were tested with different cat-dander extract fractions (elution volume 30 to 70 ml of the Sephadex G-75 column) in the presence of autologous PBMC (10^5 /well). Maximal proliferative response was set at 100%. The illustration shows that several peaks can be discerned which are recognized by the four TLC that do not react with *Fel d 1* (see Table I). As a control, the Sephadex G-75 elution profile (OD 280) of the cat dander extract is shown.

Table II. HLA-restriction of cat dander-specific TLC^a

TLC	HLA Type Donor			Proliferation (cpm $\times 10^{-3}$)					Restriction ^b Specificity
	DR	DRw	DQ	Control	Cat	Cat + α -DP	Cat + α -DQ	Cat + α -DR	
PBB1	11,w9;	w52,w53;	w3	0.1	16.3	16.4	0.1 ^c	16.9	HLA-DQw3
PBB2	11,w9;	w52,w53;	w3	0.1	12.2	0.2	3.7	4.4	HLA-DP ^d
PBB4	11,w9;	w52,w53;	w3	0.3	12.7	10.6	0.2	6.8	HLA-DQw3
PBC1	4;	w53;	w3	0.2	33.1	23.9	21.2	0.1	HLA-DR4
PBD1	4,11;	w52,w53;	w3	0.8	3.8	3.9	3.3	0.8	HLA-DR11
PBE1	1,4;	w53;	w1,w3	0.2	7.0	6.5	4.3	0.1	HLA-DR1
PBF2	3,4;	w52,w53;	w2,w3	0.1	9.5	6.4	3.0	0.1	HLA-DR3
PBF3	3,4;	w52,w53;	w2,w3	0.4	13.2	11.3	6.8	0.2	HLA-DR3
PBF7	3,4;	w52,w53;	w2,w3	0.1	24.3	26.5	18.5	0.0	HLA-DR(3 or 4) ^e
PBF8	3,4;	w52,w53;	w2,w3	0.2	5.4	5.3	2.4	0.1	HLA-DR3

^a Cat dander-specific TLC (2 to 4×10^4 /well) were stimulated with cat dander extract in the presence of autologous PBMC (10^5 /well) as APC. Proliferative responses are shown as the mean cpm of triplicate cultures as determined by [³H]TdR incorporation.

^b All TLC shown were tested with a panel of at least six differently HLA-typed PBMC to determine the precise HLA-DR, HLA-DP, or HLA-DQ-restriction specificity (individual data not shown).

^c Underlined numbers represent inhibition by anti-HLA mAb of $>75\%$.

^d PBMC were not typed for HLA-DP.

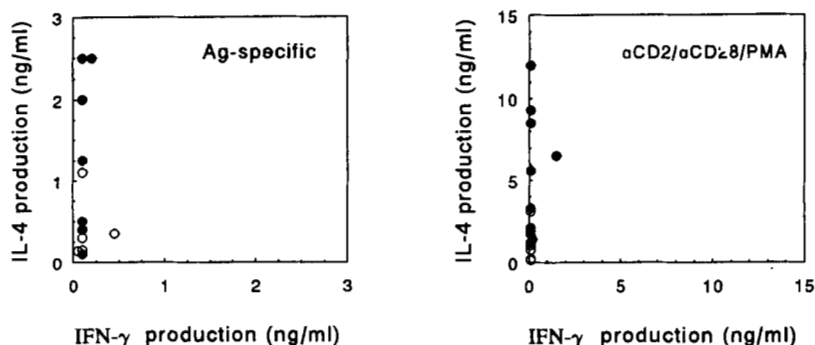
^e TLC PBF7 only reacts in the presence of autologous PBMC.

experiments using a panel of serologically HLA-typed PBMC were performed to identify the HLA-restriction of the TLC more precisely (Table II, *right column*, individual data not shown). These results show that several HLA-DR molecules are able to present cat allergens to T lymphocytes, indicating that the HLA type may not be a decisive factor for T cell responses to cat allergens.

Lymphokine production profiles of cat-specific TLC

IL-4 and IFN- γ production was measured after Ag-specific stimulation. Upon stimulation, most TLC are capable of producing large amounts of IL-4 but not IFN- γ , classifying these TLC as Th2-like cells (Fig. 3). Similar secretion profiles were obtained after stimulation with a

FIGURE 3. Cat dander-specific (●) and *Fel d* I-specific (○) TLC were stimulated for lymphokine production as described in the *Materials and Methods* section. IL-4 and IFN- γ production was measured in 24-h supernatants of Ag-specific ($n = 12$) or α -CD2/ α -CD28/PMA ($n = 16$) stimulated TLC.



Fel d I chain 1.

EICPAVKRDV DLFLTGTPE YVEQVAQYKA LPVVLENA
1..... 11..... 21..... 31.....

7 1
RI LKNCVDAKMT EE DKENALSL LDKIYTSPIC
.. 41..... 51 61.....70.

Fel d I chain 2.

1 7 PBB2-4 5
VKMAETCPIF YDVFFAVANG N ELLLDLSLT KVNATEPERT
1..... 11..... 21 31.....

6 7
AMKKIQDCYV ENGLISRVL D GLVMTTISSS KDCMGEAVQN
41..... 51..... 61..... 71.....

TVEDLKLNTLGR
81.....92.

FIGURE 4. T cell epitopes of *Fel d* I recognized by polyclonal cat dander-specific T cell lines of atopic patients (1, 5, 6 and 7) or *Fel d* I-specific TLC from patient 1 (PBB2 to 4). Lines and TLC (2 to 4×10^4 /well) were tested for proliferation with cat dander extract, *Fel d* I and all synthetic peptides of *Fel d* I in the presence of autologous PBMC (10^5 /well) as APC. Data shown represent proliferation values with stimulation indexes >3 as determined by [^{3} H]TdR incorporation.

mitogenic pair of anti-CD2 mAb in combination with anti-CD28 mAb in the presence of PMA (data not shown). Interestingly, the IL-4 production of cat-specific TLC seemed to be even higher than that of *Fel d* I-specific TLC.

T cell epitopes of *Fel d* I

T cell epitope-specificity of cat-specific T cell lines reactive with *Fel d* I (Fig. 1) and *Fel d* I-specific TLC (see also Table 1) was determined by testing their reactivity with a panel of overlapping synthetic peptides stretching both chains of *Fel d* I. Figure 4 shows that the reactivity of polyclonal T cell lines of four atopic donors and three TLC of donor 1 revealed at least five distinct T cell epitopes (chain 1, amino acid 39–52 and 53–66; chain 2 amino acids 9–21, 22–35 and 57–70), indicating the prevalence of T cell epitopes throughout both chains of *Fel d* I. Peptide 9–21 of chain 2 is recognized by three TLC and by two polyclonal T cell lines and, thus, may appear to be immunodominant. However, all these three *Fel d* I-specific TLC are derived from donor 1, whose cell line also recognized the same peptide. Interestingly, even though the TLC recognized the same peptide, the HLA-restriction of TLC PBB2 and PBB4 were not the same (see Table II). The two additional *Fel d* I-specific TLC did not react with any of the synthetic peptides.

Discussion

In the present study, we demonstrate that in atopic patients cat dander proteins are recognized by CD4 $^+$ T lymphocytes with a Th2-like lymphokine secretion profile. This suggests that cat dander-specific CD4 $^+$ T lymphocytes play an important role in the development of cat allergy. A substantial proportion of these T lymphocytes were specific for *Fel d* I, the major allergen of which at least five distinct T cell epitopes were identified, but several additional proteins are recognized as well. HLA restriction was diverse, both for cat dander and for *Fel d* I-specific TLC. This diversity of the T cell response may change our view on the usefulness of peptides of one allergen only for immunotherapy.

IL-4 is a potent IgE-inducing factor, and human Th2 cells have been shown to be able to induce IgE production in human B lymphocytes (1, 10, 39). In support of the in vitro evidence for the role of Th2-like cells in the pathophysiology of atopic disease, allergen-specific Th2-like T lymphocytes have been cloned with high frequency from

peripheral blood and affected atopic dermatitis skin (1, 2, 10, 40) in skin tissue and nasal mucosa after allergen challenge (41, 42), and from bronchoalveolar lavage fluid from asthmatic patients (43). For this reason, lymphokine production of the cat-specific TLC was studied. This analysis revealed that they conform to the Th2 subset, producing large amounts of IL-4, but no or nondetectable amounts of IFN- γ , suggesting that the cat-specific TLC may also be able to induce IgE production. Notably, the TLC that did not react with *Fel d* I appeared to produce even more IL-4 than the *Fel d* I-specific TLC. To show the role of these cat-specific T cells in IgE production, we currently are identifying the proteins that are recognized by the TLC to investigate whether IgE that is directed against the same proteins is present.

Allergen-specific CD4⁺ T lymphocytes previously have been shown to have diverse HLA-restrictions, including HLA-DR, HLA-DQ, HLA-DP, and the supertypic HLA-DRB3 specificities HLA-DRw52 and HLA-DRw53 (16, 18, 22–24, 44). This is consistent with the finding that, with the exception of responses to *Amb a* V, *Lol p* I, and *Lol p* II (45, 46), no clear-cut correlations between HLA phenotype and the presence of allergen-specific IgE have been described (47). The HLA restriction of the cat-specific TLC described in the present report showed strong diversity as well. Seven of the ten TLC tested were HLA-DR restricted, two were HLA-DQ restricted, and one was HLA-DP restricted. Notably, *Fel d* I itself could be recognized in the context of HLA-DR, HLA-DQ, or HLA-DP. Therefore, the lack of correlation between cat allergy and HLA phenotype may be explained not only by the fact that cat-specific TLC may recognize several proteins, but also by the fact that several different HLA molecules can present cat allergens to T lymphocytes.

Although multiple HLA molecules are involved in T cell recognition by cat-specific TLC, there may still be one immunodominant peptide that binds to all these HLA molecules. Peptide binding to HLA molecules is generally allele-specific (48–50), but degenerate binding of peptides to multiple HLA-DR molecules has been reported (49–51). The putative presence of such degenerate peptides in allergens, would be very useful for the development of immunotherapies, as only a limited number of peptides would be necessary. We show here that within the T cell population of one individual, T cells recognize multiple epitopes in the same allergen, arguing against the presence of immunodominant epitopes in *Fel d* I. This also seems to be the case for other allergens, such as *Der p* I (23), *Der p* II (18, 52) and *Bet v* I (20), suggesting that allergens do not generally contain immunodominant epitopes. It should be noted however, that peptide 9–21 of chain 2 can be presented to the T cells in the context of two different HLA-molecules, suggesting that degenerate binding of some peptides of allergens to HLA molecules may still take place.

Moreover, because atopic and nonatopic individuals are able to recognize the same epitopes of allergens (24, 52, and R. J. J. van Neerven, unpublished observations), lymphokine production profiles of allergen-specific TLC do not seem not to be dictated by the T cell epitope of the allergen that is recognized. There is increasing evidence suggesting that lymphokines, such as IL-4 (53–56), IL-10 (54), IL-12 (57), IFN- γ (53), TGF- β (58) and prostaglandin E2 (59) regulate lymphokine profiles of T cells and the selective outgrowth of Th1 or Th2 cells. The presence of T cell epitopes in both chains of *Fel d* I and the fact that several other proteins are recognized by Th2-like cells may complicate immunotherapies using synthetic peptides, as complex mixtures of peptides will be needed. Van Milligen et al. (36) previously have reported the binding of IgE to the same peptides as were used in this study.

Interestingly, 65% of cat-allergic patients reacted with at least one of the three identified IgE binding peptides (Chain 1 25–38 and 46–59; Chain 2 15–28) that show a partial overlap with the T cell epitopes described here, indicating that some of the peptides may still lead to adverse reactions in vivo. This is in contrast to findings with peptides of the dust mite allergen *Der p* II (60).

Future research is needed to evaluate whether identification of T cell epitopes of allergens, such as *Fel d* I, may eventually lead to improvement of immunotherapy that is directed at silencing the T cells that are responsible for support of IgE production. Care should be taken, however, in developing such novel therapies, because IgE specific for one allergen may decrease after targeted immunotherapy, but, as additional proteins in allergen extracts can be recognized by Th2 cells, there is a possibility that this will result in a shift of specificity of Th2 responses and serum IgE from one allergen to other allergens.

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