A Role for IL-10-Mediated HLA-DR7-Restricted T Cell-Dependent Events in Development of the Modified Th2 Response to Cat Allergen


*J Immunol* 2004; 172:2763-2772; doi: 10.4049/jimmunol.172.5.2763

http://www.jimmunol.org/content/172/5/2763

---

**References**  This article cites 52 articles, 12 of which you can access for free at: [http://www.jimmunol.org/content/172/5/2763.full#ref-list-1](http://www.jimmunol.org/content/172/5/2763.full#ref-list-1)

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at: [http://jimmunol.org/subscription](http://jimmunol.org/subscription)

**Permissions**  Submit copyright permission requests at: [http://www.aai.org/About/Publications/JI/copyright.html](http://www.aai.org/About/Publications/JI/copyright.html)

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: [http://jimmunol.org/alerts](http://jimmunol.org/alerts)
A Role for IL-10-Mediated HLA-DR7-Restricted T Cell-Dependent Events in Development of the Modified Th2 Response to Cat Allergen


Although high dose exposure to inhaled cat allergen (Fel d 1) can cause a form of tolerance (modified Th2 response), the T cell mechanism for this phenomenon has not been studied. T cell responses to Fel d 1 were characterized in both allergic (IgEpos) and modified Th2 (IgEmnegIgGpos) responders as well as serum Ab-negative controls (IgEnegIgGneg). Fel d 1 stimulated high levels of IL-10 in PBMC cultures from all individuals, with evidence of Th2 and Th1 cytokine skewing in allergic and control subjects, respectively. Using overlapping peptides, epitopes at the N terminus of Fel d 1 chain 2 were shown to stimulate strong T cell proliferation and to preferentially induce IL-10 (peptide 2:1 (P2:1)) or IFN-γ regardless of the allergic status of the donor. Injection of cat extract during conventional immunotherapy stimulated expansion of IL-10- and IFN-γ-producing chain 2 epitope-specific T cells along with increased Fel d 1-specific serum IgG and IgG4 Ab. Six of 12 modified responders expressed the major HLA-DRB1 allele, *0701, and both P2:1 and P2:2 were predicted ligands for this allele. Cultures from DR7-positive modified responders produced the highest levels of IL-10 to P2:1 in addition to other major and minor epitopes within chains 1 and 2. In the presence of anti-IL-10 mAb, both T cell proliferation and IFN-γ production were enhanced in a Fel d 1- and epitope-specific manner. We conclude that IL-10-producing T cells specific for chain 2 epitopes are relevant to tolerance induction, and that DR7-restricted recognition of these epitopes favors a modified Th2 response. The Journal of Immunology, 2004, 172: 2763–2772.

*Asthma and Allergic Diseases Center, Department of Internal Medicine, and †Department of Rheumatology, University of Virginia, Charlottesville, VA 22908; and ‡Department of Genomics and Information Sciences, Hoffmann-LaRoche, Nutley, NJ 07110

Received for publication June 16, 2003. Accepted for publication December 15, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by National Institutes of Health Grants AI50989 and AI20565, and the 2002 University of Virginia Beirne Carter Center of Immunology Research Award (to J.A.W.).

2 Address correspondence and reprint requests to Dr. Judith A. Woodfolk, Asthma and Allergic Diseases Center, University Health System, P.O. Box 801355, Charlottesville, VA 22908-1355. E-mail address: jaw4tn@virginia.edu

© 2004 by The American Association of Immunologists, Inc.

Materials and Methods

Classification of allergic, modified Th2, and nonallergic subjects

Study participants were recruited from patients attending the University of Virginia Allergic Diseases Clinic or by advertisement. Seventy-four patients were skin tested with commercial cat extract (Greer, Lenoir, NC) and with affinity-purified natural Fel d 1 obtained from cat hair (23, 24). Natural Fel d 1 was filtered through a 0.2-μm pore size, sterile, nitrocellulose disposable filter (Micron Separations, Westborough, MA) and diluted for prick testing to 20 μg/ml in 0.05% human serum albumin in phenol-saline solution. Skin test sites were examined 10 min after injection of Ag and compared with a diluent control. Criteria for classifying patients in the allergic experimental group (n = 14) were a positive immediate skin prick test to cat extract and the presence of serum IgE Ab to cat (>0.7 IU/ml). Subjects with a modified Th2 response (n = 12) had a...
negative skin test to cat and Fel d 1, no measurable IgE Ab to cat, and high
titer anti-Fel d 1 IgG Ab (>500 U/ml). Nonallergic subjects (controls, n =
11) were identified on the basis of negative skin test reactivity and no
measurable serum IgG (<0.125 U/ml) or IgE (<0.35 IU/ml) Ab to cat. These
studies were approved by the University of Virginia human investigation
committee.

**Immunosassays for IgG, IgG4, and IgE Abs to cat**

Serum IgG and IgG4 Ab to Fel d 1 were measured using an Ag binding
radioimmunoassay with methods described previously (1, 25).

Quantification of anti-Fel d 1 IgG and IgG4 Ab was conducted using separate
control curves established with serum obtained from cat-allergic patients
assigned to contain 2000 U of IgG or 1500 U of IgG4 Ab/ml. IgE Ab to cat was
measured by CAP assay (Pharimacia Biotech, Uppsala, Sweden).

**Measurement of Fel d 1 levels in floor dust samples**

Floor dust samples were collected from the living rooms of the homes of
study patients using standard techniques, and samples were assayed for Fel
d 1 content (micrograms per gram of dust) by ELISA (23).

**Preparation of Ag and peptides for T cell studies**

A set of 20 peptides was synthesized by standard F-moc chemistry (Uni-
versity of Virginia Biomolecular Research Facility) using a Symphony
automated peptide synthesizer (50 μm scale; Rainin, Woburn, MA), and
peptides were purified to >90% reverse phase HPLC. Peptides spanning both
polypeptide chains of the heterodimeric Fel d 1 molecule were de-
signed with a 10-aa overlap (26). Peptides spanning chain 1 were design-
nated P1:1 through P1:9, and those spanning chain 2 were designated P2:1
through P2:11. Peptides contained 17 aa, with the exception of P1:9 (14-
mer) and P2:11 (19-mer), which spanned the C termini of chains 1 and 2.
Stock solutions of peptides (1 mM) were solubilized in sterile water or 50%
DMSO. All peptides were determined to be free of endotoxin by quanti-
tative chromogenic Limulus amebocyte lysate assay (BioWhittaker, Walk-
tersville, MD),Fel d 1, tetanus toxoid (TT),7 and PHA were sterile-filtered
before use.

**PBMC cultures**

PBMC were isolated by density gradient centrifugation over Ficoll-Paque
(Pharimacia Biotech) from 90 cc of blood and cultured in complete medium
(C-RPMI) containing RPMI 1640 (Life Technologies, Gaithersburg, MD)
with 1-glutamine, 10% autologous human serum (heat-inactivated), 100
U/ml penicillin, and 100 μg/ml streptomycin.

**Proliferation assays.** PBMC were cultured in the presence of 10 μg/ml
Fel d 1 (2 × 106 cells in quadruplicate wells) or 7.5 μM Fel d 1-derived peptide
(100 cells in 12 replicate wells) for 6 days at 37°C in 96-well plates. TT (10 μg/ml)
and PHA (20 μg/ml) in quadruplicate wells were used as positive controls, and 60
unstimulated wells (12 wells on each assay plate) were negative controls. Cells were pulsed with 1μCi of
[3H]thymidine/well during the final 8 h of culture before harvesting and
counting (Topcount NXT; Packard Instrument, Meriden, CT). Results were
expressed as stimulation indexes for Fel d 1 and TT. Data for peptides were
log-transformed to derive a standardized index value as described previ-
ously (27).

**Cytokine assays.** Cells were cultured in 24-well plates (4 × 105 cells/well
in 1-ml volume) in the presence of Fel d 1 (10 μg/ml) or peptide (7.5 μM).
Positive control wells included PHA and TT, whereas unstimulated and medium-only wells served as negative controls. Culture supernatants were
harvested on day 6 and assayed by ELISA for IL-4, IL-5, IFN-γ, and IL-13,
and IFN-γ content using matched mAb pairs (Pierce-Endogen, Woburn, MA).
Values were derived from standard curves to a sensitivity of 2 pg/ml for
IL-4, IL-5, IFN-γ, and IL-13, and 4 pg/ml for IL-10.

**HLA class II typing**

Genomic DNA was isolated from 2 ml of whole blood, and HLA typing
was conducted in the Molecular Pathology Laboratory at University of
Virginia as described previously (27). Sequences were evaluated using
HLA SequiTyper software (Pharimacia Biotech) and compared with the
European Bioinformatic Institute HLA database (European Bioinformatic
Institute, Cambridge, U.K.) for allele assignments.

---

7 Abbreviations used in this paper: TT, tetanus toxoid; IT, immunotherapy.
conducted using S-Plus version 2000 (Insightful, Seattle, WA) and SPSS 10.1 (SPSS, Chicago, IL) software packages.

**Results**

**Tolerance to cats is associated with high dose exposure to cat allergen**

The relationship among exposure to Fel d 1, distinct allergic phenotypes, and Ab responses to cat allergen was examined (Table I). The significantly higher level of Fel d 1 measured in the homes of modified Th2 compared with allergic responders (mean, 711 vs 17 μg/g floor dust; \( p = 0.024 \)) was consistent with the high prevalence of cat ownership in the modified group (>90%) compared with other experimental groups (<40%). In individuals who had an allergen-specific IgG Ab response, concentrations of Fel d 1 in floor dust were strongly correlated with Ab titers (\( r = 0.77; \ p < 0.0001 \)); however, the absence of a humoral response was not restricted to individuals with low exposure to Fel d 1 (Table I).

**Table I. Characteristics of patients with distinct immune responses to cat**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (year), Sex, Race ( ^a )</th>
<th>Fel d 1 (μg/g) and (No. of Cats)</th>
<th>Anti-Fel d 1 Ab ( ^b ) (U/ml)</th>
<th>IgG</th>
<th>IgG4</th>
<th>IgE to Cat ( ^c ) (U/ml)</th>
<th>DRB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic</td>
<td>6( ^d )</td>
<td>22 FW</td>
<td>4.3 (0)</td>
<td>153</td>
<td>&lt;140</td>
<td>5.12</td>
<td>09011501</td>
</tr>
<tr>
<td></td>
<td>7( ^e )</td>
<td>26 FW</td>
<td>9.5 (0)</td>
<td>4,878</td>
<td>208</td>
<td>17</td>
<td>03010701</td>
</tr>
<tr>
<td></td>
<td>9( ^f )</td>
<td>29 FW</td>
<td>0.3 (0)</td>
<td>421</td>
<td>&lt;140</td>
<td>2.59</td>
<td>03010404</td>
</tr>
<tr>
<td></td>
<td>9( ^g )</td>
<td>28 FA</td>
<td>0.4 (0)</td>
<td>233</td>
<td>&lt;140</td>
<td>1.35</td>
<td>03010405</td>
</tr>
<tr>
<td></td>
<td>11( ^h )</td>
<td>28 MW</td>
<td>0.4 (0)</td>
<td>1,410</td>
<td>&lt;140</td>
<td>14.7</td>
<td>03011501</td>
</tr>
<tr>
<td></td>
<td>12( ^i )</td>
<td>23 FB</td>
<td>0.7 (0)</td>
<td>338</td>
<td>&lt;140</td>
<td>0.94</td>
<td>04001401</td>
</tr>
<tr>
<td></td>
<td>13( ^j )</td>
<td>24 MW</td>
<td>1,272 (1)</td>
<td>9,159</td>
<td>5,733</td>
<td>17.1</td>
<td>03010407</td>
</tr>
<tr>
<td></td>
<td>14( ^k )</td>
<td>31 FW</td>
<td>0.4 (0)</td>
<td>384</td>
<td>&lt;140</td>
<td>0.77</td>
<td>11041302</td>
</tr>
<tr>
<td></td>
<td>15( ^l )</td>
<td>24 MA</td>
<td>44 (0)</td>
<td>849</td>
<td>&lt;140</td>
<td>3.86</td>
<td>08001308</td>
</tr>
<tr>
<td></td>
<td>45( ^m )</td>
<td>32 FW</td>
<td>1,752 (4)</td>
<td>43,700</td>
<td>34,145</td>
<td>1.99</td>
<td>01031104</td>
</tr>
<tr>
<td></td>
<td>46( ^n )</td>
<td>53 FB</td>
<td>1,007 (2)</td>
<td>11,443</td>
<td>5,230</td>
<td>31.1</td>
<td>01031104</td>
</tr>
<tr>
<td></td>
<td>47( ^o )</td>
<td>26 FW</td>
<td>401 (3)</td>
<td>1,987</td>
<td>1,242</td>
<td>6.46</td>
<td>03010401</td>
</tr>
<tr>
<td></td>
<td>49( ^p )</td>
<td>36 FW</td>
<td>3.4 (0)</td>
<td>1,609</td>
<td>&lt;140</td>
<td>4.15</td>
<td>04011201</td>
</tr>
<tr>
<td></td>
<td>53( ^q )</td>
<td>50 FW</td>
<td>3.5 (0)</td>
<td>2,432</td>
<td>1,406</td>
<td>2.89</td>
<td>04041101</td>
</tr>
<tr>
<td>Mean</td>
<td>31</td>
<td>17( ^b )</td>
<td>1,536( ^b )</td>
<td>496( ^b )</td>
<td>4.40( ^b )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified Th2</td>
<td>1</td>
<td>48 FW</td>
<td>145 (2)</td>
<td>8,962</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>03011501</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>42 FW</td>
<td>4,700 (2)</td>
<td>2,374</td>
<td>1,064</td>
<td>&lt;0.35</td>
<td>04040701</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22 MW</td>
<td>166 (0)</td>
<td>2,916</td>
<td>1,273</td>
<td>&lt;0.35</td>
<td>07011001</td>
</tr>
<tr>
<td></td>
<td>4( ^r )</td>
<td>59 FW</td>
<td>71 (1)</td>
<td>786</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>03010304</td>
</tr>
<tr>
<td></td>
<td>5( ^s )</td>
<td>34 FW</td>
<td>271 (1)</td>
<td>5,603</td>
<td>1,998</td>
<td>&lt;0.35</td>
<td>07011302</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>24 MW</td>
<td>2,380 (3)</td>
<td>5,418</td>
<td>2,060</td>
<td>&lt;0.35</td>
<td>07011501</td>
</tr>
<tr>
<td></td>
<td>31( ^d )</td>
<td>28 FW</td>
<td>3,004 (3)</td>
<td>5,883</td>
<td>2,349</td>
<td>&lt;0.35</td>
<td>07011501</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>26 MB</td>
<td>68 (2)</td>
<td>2,025</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>04010901</td>
</tr>
<tr>
<td></td>
<td>33( ^u )</td>
<td>26 MW</td>
<td>563 (3)</td>
<td>1,669</td>
<td>964</td>
<td>&lt;0.35</td>
<td>01010401</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>50 FW</td>
<td>3,700 (7)</td>
<td>10,239</td>
<td>10,876</td>
<td>&lt;0.35</td>
<td>01021401</td>
</tr>
<tr>
<td></td>
<td>44( ^v )</td>
<td>59 FW</td>
<td>2,605 (14)</td>
<td>50,500</td>
<td>43,472</td>
<td>&lt;0.35</td>
<td>04010701</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>37 FW</td>
<td>2,908 (3)</td>
<td>9,935</td>
<td>3,569</td>
<td>&lt;0.35</td>
<td>01011101</td>
</tr>
<tr>
<td>Mean</td>
<td>38</td>
<td>711( ^b )</td>
<td>4,804( ^b )</td>
<td>1,404( ^b )</td>
<td>&lt;0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td>24 FW</td>
<td>1,272 (1)</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>03011501</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>21 FW</td>
<td>2.3 (0)</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>13021501</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>26 FW</td>
<td>4,656 (3)</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>03011501</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>32 MW</td>
<td>5.6 (0)</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>13021501</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>24 MW</td>
<td>24.5 (0)</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>07011101</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>22 FW</td>
<td>1.1 (0)</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>03011501</td>
</tr>
<tr>
<td></td>
<td>35( ^x )</td>
<td>28 FB</td>
<td>2.4 (0)</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>03010901</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>25 MW</td>
<td>1,197 (1)</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>03011001</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>19 MA</td>
<td>132 (1)</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>01020403</td>
</tr>
<tr>
<td></td>
<td>42( ^y )</td>
<td>25 MW</td>
<td>5.3 (0)</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>07011301</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>34 FW</td>
<td>738 (4)</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td>01010701</td>
</tr>
<tr>
<td>Mean</td>
<td>26</td>
<td>48( ^a )</td>
<td>&lt;60</td>
<td>&lt;140</td>
<td>&lt;0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunotherapy</td>
<td>42( ^z )</td>
<td>31 MW</td>
<td>ND (0)</td>
<td>3,395</td>
<td>211</td>
<td>0.43</td>
<td>01020401</td>
</tr>
<tr>
<td></td>
<td>60( ^a )</td>
<td>69 MW</td>
<td>ND (0)</td>
<td>1,229</td>
<td>&lt;140</td>
<td>0.35</td>
<td>11011501</td>
</tr>
<tr>
<td></td>
<td>62( ^a )</td>
<td>37 FW</td>
<td>ND (1)</td>
<td>14,893</td>
<td>6,083</td>
<td>1.01</td>
<td>03010404</td>
</tr>
<tr>
<td></td>
<td>63( ^a )</td>
<td>25 MW</td>
<td>ND (0)</td>
<td>2,706</td>
<td>&lt;140</td>
<td>0.35</td>
<td>04080701</td>
</tr>
</tbody>
</table>

\( ^a \) F, female; M, male; W, white; B, black; A, Asian.

\( ^b \) Measured by Ag binding radioimmunoprecipitation assay.

\( ^c \) Measured by Pharmacia CAP assay.

\( ^d \) Patients with rhinitis.

\( ^e \) Patients with asthma.

\( ^f \) Patients with livers.

\( ^g \) Patient received immunotherapy.

\( ^h \) Geometric mean values.
Fel d 1 induces high levels of IL-10 in allergic and nonallergic donors

Fel d 1-specific T cell responses were compared between allergic and nonallergic responders. T cell proliferation in the modified group was comparable to that in the allergic group, with lowest levels in the control group (Fig. 1A). Surprisingly, Fel d 1 induced high levels of IL-10 in PBMC cultures established from all patient groups, with slightly lower levels in modified responders (p < 0.023; Fig. 1, B and C). The level of IFN-γ was significantly increased in controls compared with allergic subjects (p < 0.025). In addition, there was a trend toward decreased IL-5 production in modified (mean, 4.6 ± 2.2 pg/ml) and control (mean, 2.2 ± 0.2 pg/ml) subjects compared with allergics (mean, 16 ± 7 pg/ml), with a significant difference between allergics and controls (p = 0.025; Fig. 1, B and C). No IL-4 was measurable in any of these primary cultures stimulated with Fel d 1. The cytokine profiles observed in this study provide evidence of Th1 and Th2 skewing in nonallergic and allergic subjects, respectively, but do not fit classical Th1/Th2 patterns. Furthermore, cytokine profiles in Fel d 1-stimulated cultures were in marked contrast to the IFN-γ-dominated profile observed in cultures stimulated with TT (Fig. 1B).

Novel chain 2 major epitopes of Fel d 1 selectively induce IL-10 and IFN-γ

To resolve the significance of cytokine profiles induced in cultures stimulated with cat allergen, peptides spanning chains 1 and 2 of the Fel d 1 molecule were used to stimulate T cell responses in vitro. Major epitopes of Fel d 1 (defined as those with a mean standardized index ≥ 1.5) localized to chain 1 (P1:2 and P1:6) as described previously (32, 33); however, a new immunodominant region was also identified, which mapped to the N terminus of chain 2 (P2:1, P2:2, P2:3, P2:4; Fig. 2A). By cross-validated linear discriminant analysis, P1:2 was the best single peptide marker for distinguishing allergic and control groups (68% of patients classified correctly) (Student’s t test, p = 0.038) compared with P1:1 for allergic and modified groups (69% of patients classified correctly) (Student’s t test, p = 0.007). Induction of IL-5 also localized to P1:1 and P1:2 (Fig. 2B), with a trend toward significantly increased levels in allergics compared with controls (p = 0.098 and p = 0.072 for P1:1 and P1:2, respectively). Furthermore, mean production of IL-5 to all 20 peptides was significantly increased in the allergic group compared with modified and control groups (p < 0.04), but not in the modified group compared with controls.

FIGURE 1. Fel d 1 induces IL-10-dominated responses in allergic and modified responders. Freshly isolated PBMC were stimulated for 6 days with 10 μg/ml Fel d 1 or TT. A, Proliferation was measured by [3H]thymidine incorporation (cpm × 1000) and expressed as the mean stimulation index ± SEM. B, Culture supernatants were assayed for cytokines, and values expressed as the mean ± SEM. C, Comparison of the distribution of Fel d 1-induced cytokines between patient groups. Dotted lines represent the sensitivity of the ELISA. Allergics, n = 14; modified Th2, n = 12; controls, n = 11. *, p < 0.025.
In contrast, the novel chain 2 major epitopes, P2:1 through P2:4, stimulated strong T cell reactivity in all patient groups, with overlapping peptides P2:1 and P2:2 inducing the highest levels of IL-10 and IFN-γ, respectively (Fig. 2B). Mean levels of IL-10 in P2:1-stimulated cultures were highest in the modified group (326 ± 152 pg/ml vs 238 ± 113 pg/ml for allergics and 187 ± 88 pg/ml for controls), whereas P2:2 induced comparable levels of IFN-γ in all patient groups (allergic, 494 ± 226 pg/ml; modified, 562 ± 215 pg/ml; controls, 563 ± 227 pg/ml; Fig. 2B). Flow cytometric analysis of lymphocytes from subjects with distinct immune responses identified IL-10-producing CD4+ T lymphocytes in cultures stimulated with pooled chain 2 peptides (P2:1 through P2:4; Fig. 3).

Subcutaneous injection of whole allergen is associated with activation of T cells that target chain 2 epitopes

Injection of proteins from diverse sources, including cat, during conventional allergen immunotherapy has been reported to induce T cell hyporesponsiveness mediated by IL-10 and/or immune deviation from Th2 to Th1 responses (5–7, 10, 34, 35). In addition, elevated allergen-specific IgG4 Ab and decreased IgE Ab frequently accompany successful IT (8, 36–38). We theorized that, by virtue of their ability to induce IL-10 and IFN-γ, chain 2 epitopes of Fel d 1 would fulfill an important role in tolerance induction during IT. T cell cultures were studied at monthly intervals for 4 mo in five cat-allergic patients (no. 52, 53, 60, 62, and 63) starting IT. Immunotherapy was associated with increases in production of IgG and IgG4 Ab specific for Fel d 1 in all patients.

**FIGURE 2.** Chain 2 epitopes of Fel d 1 selectively induce IL-10 and IFN-γ. PBMC were stimulated for 6 days in the presence of Fel d 1 peptides spanning chains 1 and 2. A, Proliferation assays incorporated 12 replicate wells for each peptide (n = 20), and the mean standardized index ± SEM was used for between-group comparisons. The dotted line represents an arbitrary reference line corresponding to a mean standardized index of 1.5. *, p = 0.007 for P1:1 (allergics vs modified responders) and p = 0.038 for P1:2 (allergics vs controls). B, Cytokine values represent the mean ± SEM. The arrows in three panels denote peptides that induce IL-5 (P1:2), IL-10 (P2:1), or IFN-γ (P2:2), respectively. U, unstimulated wells.

**FIGURE 3.** IL-10+CD4+ T lymphocytes that recognize chain 2 major epitopes are a component of the Fel d 1-specific T cell repertoire in patients with diverse serological responses. Detection of live IL-10-secreting T cells was conducted after in vitro culture of PBMC with pooled chain 2 peptides (16 h). MACS was used to separate cells labeled with PE-conjugated anti-IL-10 mAb using anti-PE microbeads, and cells were counterstained with FITC-CD4 mAb before analysis. Live lymphocytes were gated according to light scatter properties and propidium iodide exclusion. Representative data from experiments on six patients is shown.
Maximal increases in production of peptide-induced IL-10 and IFN-γ localized to the N-terminal region of Fel d 1 spanned by peptides 2:1, 2:2, 2:3, and 2:4 (Fig. 4B). Strong T cell reactivity to chain 2 epitopes persisted, whereas levels of IL-5 and IL-13 generally remained unchanged throughout the course of therapy (data not shown). The results show that enhancement of in vitro T cell responses to these chain 2 epitopes is a consistent feature of the response to cat allergen injected in vivo.

**IL-10 production in modified Th2 responders is associated with expression of DR7**

The ability for the IL-10- and IFN-γ-inducing chain 2 epitopes, P2:1 and P2:2, to induce strong T cell responses in individuals with diverse HLA-DR types (Table I) was consistent with the presence of multiple DR ligands within these peptides (Fig. 5A). HLA analysis revealed an increased frequency of HLA-DR7 in the modified group (6 of 12 patients) compared with allergics (one of 14; \( p = 0.002 \); Table I). Of the four DR7 peptide binding motifs identified within Fel d 1, two localized to the major epitopes P2:1 and P2:2 (Fig. 5A), whereas the remaining two mapped to weakly stimulatory peptides (P1:5 and P2:9), suggesting that these epitopes were subdominant or not relevant in vivo. IL-10 production to P2:1 was markedly increased in DR7-positive modified responders compared with their DR7-negative counterparts (mean, 560 ± 278 vs 93 ± 49 pg/ml) and compared with allergic (238 ± 88 pg/ml) and control (187 ± 88 pg/ml) groups (Fig. 5B). Furthermore, P2:1 induced increased IL-10 production in DR7-positive subjects with an allergic or modified response (711 ± 280 pg/ml; \( n = 7 \)) compared with DR7-negative allergic or modified responders (119 ± 32 pg/ml; \( n = 19 \); \( p < 0.01 \)). Mean levels of IL-10 production in response to all 20 peptides were enhanced in DR7-positive vs DR7-negative modified subjects (\( p = 0.029 \)). Differences in the pattern of peptide-induced IL-10 induction were also observed for DR7+ modified subjects compared with each of the other patient groups (\( p < 0.001 \); Fig. 5B).

**IL-10 down-regulates Fel d 1- and epitope-specific Th responses independent of allergic phenotype**

The association between IL-10 production and the modified Th2 response coupled with activation of chain 2 epitope-specific T cells during IT support a role for IL-10 in tolerance to cat allergen. The effects of anti-IL-10 mAb on Fel d 1-specific T cell responses were examined in cultures from two allergic, two modified, and two nonallergic high IL-10 producers (>200 pg/ml IL-10 in Fel d 1-stimulated cultures). Anti-IL-10 mAb caused a significant increase in T cell proliferation in response to Fel d 1 and pooled chain 2 epitopes in all cultures (Fig. 6A). Marked increases in IFN-γ production were also observed for all cultures in the presence of anti-IL-10 mAb, whereas increases in IL-5 and IL-13 were restricted to allergic and modified responders (Fig. 6B). There was generally no effect of anti-IL-10 mAb on cultures stimulated with TT (Fig. 6A and data not shown). Findings support the view that IL-10 down-regulates Fel d 1-specific T cell responses associated with distinct allergic phenotypes.

**Discussion**

We have identified major T cell epitopes localizing to chain 2 of the cat allergen, Fel d 1, which selectively induce IL-10 and IFN-γ.
Lymphocytes that target this site were activated after systemic administration of whole allergen as part of a conventional IT regimen, suggesting that chain 2 epitopes fulfill a tolerogenic role. These results provide strong support for the argument that the response to allergens is influenced by both the dose received and intrinsic properties of the allergen molecule. The ability of an immunodominant region of an Ag to induce both IL-10 and IFN-γ has been described previously for the hepatitis C virus core protein (39). In that study the authors speculated that suppression of IFN-γ production by IL-10 could contribute to the persistence of infection, because blocking IL-10 had been shown to restore IFN-γ production to hepatitis C virus Ags (40). Similarly, we have shown that blocking IL-10 enhances IFN-γ production in response to Fel d 1 and chain 2 epitopes. The potent capacity for chain 2 peptides to induce IFN-γ in the absence of IL-10 (Fig. 6) suggests that responses to the N-terminal region of chain 2 are regulated by IL-10. To our knowledge, the current study is the first report of an immunoregulatory region within an allergen molecule that could play a central role in determining allergic phenotype.

The similar patterns of T cell epitope recognition observed for all patient groups implicates involvement of similar Ag processing and presentation pathways in distinct immune responses to Fel d 1. Given that the primary mode of exposure to cat allergen in patients studied is via the respiratory route, we would anticipate that similar T cell and non-T cell populations participate in the generation and recognition of Fel d 1-derived antigenic determinants. In contrast, we recently showed that T cell responses to chain 2 epitopes are altered in patients with atopic dermatitis (41). In that study we speculated that delivery of cat allergen through the skin and involvement of distinct APC types (e.g., Langerhans cells or inflammatory dendritic epidermal cells) from those in the respiratory tract could contribute to these differences. Lung dendritic cells have a propensity to secrete IL-10, which may favor the induction of IL-10-producing T regulatory cells (14). Indeed, we have confirmed that chain 2 epitope-specific, IL-10-producing CD4+ T cells are a component of the T cell repertoire associated with both allergic and nonallergic responses. The ability for IL-10 to exert a regulatory effect in allergic subjects may seem paradoxical, especially in light of our study of highly allergic atopic dermatitis patients (mean IgE Ab, 20.1 IU/ml), in whom blocking IL-10 had little or no effect (41). However, given that allergic subjects in the present study had relatively low titer IgE Ab to cat (mean, 4.4 IU/ml), IL-10 may fulfill a central role in controlling the magnitude or quality of the allergic response.

Definition of an Ab response that is not associated with IgE Ab or allergic symptoms has provided a new avenue for investigating immune responses associated with high dose allergen exposure. Based on the presence of the Th2-dependent Ab isotype IgG4, it seems likely that the modified response reflects a variation of the allergic response. Thus, in those subjects with an allergic predisposition and the appropriate genetic type (i.e., DR7-positive), high dose exposure to cat allergen could favor production of IL-10 by chain 2 epitope-specific T cells within the lung beyond a critical threshold, resulting in tolerance. In keeping with this allergic-modified Th2 balance model, enhanced IL-10 secretion to chain 2 epitopes along with increased IgG4 Ab in patients receiving IT demonstrates plasticity of the allergic response consistent with a shift toward a modified phenotype. Furthermore, when analyzing IL-10 production to P2:1 among allergic and modified responders collectively, significant differences were observed for DR7-positive and DR7-negative subjects. These data support the view that DR7 expression favors IL-10 production to specific epitopes of Fel d 1 in both allergic and modified responders. Our findings do not support a role for IL-10-mediated T cell hyporesponsiveness in tolerance to cat allergen induced either by inhalation or systemically. To the contrary, strong persistent CD4+ T cell responses that target chain 2 epitopes appear to be important.

Expression of HLA-DR7 in modified Th2 responders was associated with increased IL-10 production, not only to P2:1, but also to Fel d 1 peptides in general. This observation is consistent with our view that DR7-restricted recognition of chain 2 epitopes in individuals exposed to high dose cat allergen favors IL-10 induction to the whole molecule by intramolecular epitope spreading (42). This phenomenon has been described in relation to cancer treatment and, more recently, induction of tolerance to cat allergen, where vaccination with specific peptides resulted in an immune response to peptides or Ags not included in the vaccine (13, 43, 44). For such an event to occur during natural exposure, a high dosage of allergen is likely to be critical. Exactly why DR7 expression would favor such a response is more difficult to answer. Although two DR7 peptide ligands reside within the regulatory region, major epitopes of chain 2 also stimulate strong T cell responses in allergic subjects, presumably by virtue of their ability to bind to multiple HLA-DR molecules. However, increased affinity and/or stability of chain 2 peptide binding to DR7 coupled with increased density of DR7-peptide complexes at the APC surface could alter T cell signaling events to favor production of regulatory T cells.
The increased expression of HLA-DR7 observed in the modified group compared with allergic subjects was striking. Although decreased expression of HLA-DR7 has been reported among atopic patients compared with nonallergic healthy controls (45), other studies report an association between DR7 and sensitization to certain allergens (46–50). Our interpretation of these findings is that development of the modified Th2 response to cats is an allergen-specific phenomenon that does not preclude sensitization to other allergens (51). The ability for IL-10 to selectively enhance IgG4 Ab while suppressing IgE Ab (6, 52) formed the basis for our initial hypothesis that the modified response to cats was mediated by IL-10. Indeed, we have shown that the production of Fel d 1-specific IgG and IgG4 Ab in patients receiving IT is accompanied by enhancement of epitope-specific IL-10. Nevertheless, it is clear that some individuals living with cats develop an IgG Ab response independent of DR7 expression. The lack of IgG4 Ab in some of these DR7-negative modified responders coupled with low levels of IL-10 suggest an alternate mechanism governing the immune response in these individuals.

A notable finding in our study was the discrepancy in IL-10 induced by whole allergen and peptides in the modified group compared with other groups. These data highlight the importance of analyzing allergen- and epitope-specific responses in parallel in individuals with defined HLA haplotypes (27, 53). Although Fel d 1 stimulated reduced IL-10 in PBMC from modified responders (Fig. 1, B and C), levels of IL-10 were nevertheless very high. Recent studies in our laboratory that have compared endotoxin-high and endotoxin-low preparations of Fel d 1 showed that endotoxin associated with Fel d 1 can contribute, at least in part, to IL-10 production by PBMC (our unpublished observations); however, induction of IL-10 appears to be an intrinsic property of the Fel d 1 molecule itself. This latter statement is supported by the observation that in cultures stimulated with endotoxin-free Fel d 1 peptides, IL-10 production localizes to a defined region of the molecule, and peptides spanning this region induce IL-10 secretion by CD4+ T cells. It remains to be determined which other allergens exhibit similar features.

Characterization of tolerogenic epitopes of Fel d 1 chain 2 has important implications for therapy. It is of note that previous epitope-mapping studies identified immunodominant epitopes of Fel d 1 in chain 1, but not chain 2, of the molecule (32, 33). It was largely as a result of those studies that a subsequent vaccine incorporated only chain 1 peptides (54). More recent studies examined the effects of injecting short peptides (~16 mer) spanning chain 1 or both chains of the Fel d 1 molecule (9, 13, 55). In those studies a subset of cat-allergic patients receiving peptides experienced late asthmatic reactions that were attributed to MHC-restricted T cell-dependent events; however, diminished late asthmatic reactions and peptide-specific T cell hyporesponsiveness resulted from a second injection of peptides. Notably, peptides spanning the N terminus of Fel d 1 chain 2 were not administered as part of the peptide preparations, although T cell hyporesponsiveness to all four noninjected peptides was observed (13). The strong reactivity to IL-5-inducing chain 1 epitopes in allergic responders with asthma suggests that chain 1 peptides may be relevant to peptide vaccine-related adverse events. Furthermore, the lack of evidence for T cell hyporesponsiveness related to either IT or the modified Th2 response in our study argues against diminished T cell proliferation as the primary goal of therapy. Instead, identification of regulatory epitopes may provide an opportunity for developing a more tailored approach to peptide-based treatments for allergy by preferentially inducing T cell responses that target defined regions of the allergen molecule.

**FIGURE 6.** Blocking IL-10 enhances Fel d 1- and chain 2 epitope-specific T cell proliferation and cytokine responses regardless of allergic phenotype. PBMC from six donors with distinct immune responses were stimulated with Fel d 1 or pooled chain 2 peptides (P2:1, P2:2, P2:3, and P2:4) with or without 10 μg/ml anti-IL-10 mAb. Proliferation (A) and cytokine (B) responses were measured on day 7. Proliferation values represent the mean cpm for 12 replicate wells ± SEM. *, p < 0.05. Data are representative of duplicate experiments.
Acknowledgments

We thank all participants who agreed to donate blood for this study. We also thank Karen Siegrist for determining HLA haplotypes of the donors, and Lucy Goddard; Dr. Anne McLaughlin; and Deborah Murphy for assistance with drawing blood. The statistical support provided by Dr. Jae K. Lee is also greatly appreciated.

References


Downloaded from http://www.jimmunol.org/ by guest on April 14, 2017


