Roles of $T_{H1}$ and $T_{H2}$ cytokines in a murine model of allergic dermatitis

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Skin lesions of atopic dermatitis (AD) are characterized by hypertrophy of the dermis and epidermis, infiltration by T cells and eosinophils, and expression of the cytokines IL-4, IL-5, and IFN-γ. The role of these cytokines in the pathogenesis of AD is not known. We took advantage of a recently described murine model of AD elicited by epicutaneous sensitization with ovalbumin (OVA) (1) and of the availability of mice with targeted deletions of the IL-4, IL-5, and IFN-γ cytokine genes to assess the role of these cytokines in this model.

OVA-sensitized skin from IL-5−/− mice had no detectable eosinophils and exhibited decreased epidermal and dermal thickening. Sensitized skin from IL-4−/− mice displayed normal thickening of the skin layers but had a drastic reduction in eosinophils and a significant increase in infiltrating T cells. These findings were associated with a reduction in eotaxin mRNA and an increase in mRNA for the T-cell chemokines macrophage inflammatory protein-2 (MIP-2), MIP-1β, and RANTES. Sensitized skin from IFN-γ−/− mice was characterized by reduced dermal thickening.

These results suggest that both the $T_{H2}$ cytokines IL-4 and IL-5 and the $T_{H1}$ cytokine IFN-γ play important roles in the inflammation and hypertrophy of the skin in AD.

Introduction
Atopic dermatitis (AD) is a chronically relapsing inflammatory disease of the skin. T cells and eosinophils are thought to play a major role in the pathogenesis of the disease (1). Affected skin lesions in AD reveal a mononuclear cell infiltrate, predominantly in the dermis, consisting of macrophages and activated memory CD4+ T cells bearing HLA-DR+ and CD45RO+ (2). The presence of eosinophils derived major basic protein and eosinophil cationic protein in the dermis, along with eosinophil degeneration, are indicative of eosinophil involvement in AD (3, 4).

In acute lesions of AD, there is a significant increase in the number of cells expressing IL-4, IL-5, and IL-13 mRNA and protein, suggesting preferential accumulation of $T_{H2}$ cells. Additionally, PBMCs show increased IL-4 and IL-5 expression in CD4+ and CD8+ cells in patients with AD (5, 6). Recent studies have revealed that expression of the $T_{H1}$ cytokine IFN-γ is predominant in the chronic eczematous AD skin lesions (7, 8). Furthermore, biopsies of skin lesions elicited by patch testing of AD patients with dust-mite antigens reveal that a majority of T cells in the lesions express IFN-γ mRNA and protein, alone or in combination with IL-4 (7, 9–11). These results indicate that both $T_{H1}$ and $T_{H2}$ cytokines may contribute to the lesions in AD (12).

The $T_{H1}$ cytokines (IL-2 and IFN-γ) play an important role in cell-mediated immunity and chronic inflammation. IL-2 is important for T-cell growth and activation. IFN-γ induces expression of MHC class I and II molecules, activates monocytes and macrophages, and favors the development of $T_{H1}$ cells (13). The $T_{H2}$ cytokines have a critical role in the initiation of the allergic response. IL-4 is important for IgE isotype switching, development of $T_{H2}$ cells, and induction of adhesion molecules on endothelial cells that recruit eosinophils (1, 14). IL-5 is important for eosinophil development and differentiation (15). Additional evidence for the role of $T_{H2}$ cytokines in allergic diseases comes from the study of mice with targeted gene deletions. For example, murine models of asthma show decreased tissue and bronchoalveolar lavage eosinophilia in both IL-4−/− and IL-5−/− mice, as well as decreased hyperresponsiveness to methacholine following allergen exposure (16–20).

Recently, we described a murine model of AD elicited by repeated epicutaneous sensitization with ovalbumin (OVA). This model operates in both BALB/c and C57BL/6 mouse strains (21). The skin lesions exhibit significant dermal and epidermal thickening, a cellular infiltrate consisting of T cells and eosinophils, and elevated levels of IL-4, IL-5, and IFN-γ mRNA. We have taken advantage of our model and the availability of mice with a targeted deletion of cytokine genes to explore the role of IL-4, IL-5, and IFN-γ in the pathogenesis of AD. The results obtained suggest that all three cytokines contribute to the lesions in AD. IL-4 and IL-5 are important for eosinophil infiltration, and IL-5 and IFN-γ are important for skin hypertrophy. In addition, IL-4 may play an anti-inflammatory role in AD by virtue of its ability to modulate the expression of T-cell chemokines and its subsequent recruitment of T cells in the skin lesion.
Table 1

Epidermal and dermal thickness

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>n</th>
<th>Saline (μm)</th>
<th>OVA (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>WT</td>
<td>10</td>
<td>20.7 ± 1.7</td>
<td>28.7 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>31.6 ± 1.2</td>
<td>39.8 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>IL-4–/–</td>
<td></td>
<td>21.4 ± 1.3</td>
<td>30.2 ± 1.7</td>
</tr>
<tr>
<td>BALB/c</td>
<td>WT</td>
<td>12</td>
<td>18.8 ± 0.8</td>
<td>29.6 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>IL-4–/–</td>
<td>12</td>
<td>24.6 ± 2.1</td>
<td>31.8 ± 0.2</td>
</tr>
</tbody>
</table>

Skin sections were stained with H&E at ×200. Results represent mean ± SEM calculated from 20 HPFs. *P < 0.05 saline-sensitized vs. OVA-sensitized. **P < 0.05 vs. similarly sensitized WT controls.

Table 2

Quantification of the skin cellular infiltrate in H&E sections

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>n</th>
<th>Sensitization</th>
<th>Total cells</th>
<th>Eosinophils</th>
<th>Mast cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>WT</td>
<td>10</td>
<td>Saline</td>
<td>22.3 ± 2.0</td>
<td>1 ± 0.4</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>OVA</td>
<td>34.2 ± 3.5</td>
<td>5.1 ± 0.8</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>IL-5–/–</td>
<td>6</td>
<td>Saline</td>
<td>19.5 ± 1.9</td>
<td>3.0 ± 0.03</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>OVA</td>
<td>28.5 ± 3.6</td>
<td>0.3 ± 0.03</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>BALB/c</td>
<td>WT</td>
<td>12</td>
<td>Saline</td>
<td>19.3 ± 3.8</td>
<td>2.1 ± 0.6</td>
<td>1.9 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>OVA</td>
<td>38.8 ± 6.4</td>
<td>6.3 ± 1.7</td>
<td>5.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>IL-4–/–</td>
<td>11</td>
<td>Saline</td>
<td>19.1 ± 6.5</td>
<td>0.87 ± 2.0</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>OVA</td>
<td>49.3 ± 5.5</td>
<td>1.1 ± 0.4</td>
<td>3.3 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>IFN-γ–/–</td>
<td>8</td>
<td>Saline</td>
<td>21.2 ± 4.0</td>
<td>1.5 ± 2.0</td>
<td>2.8 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>OVA</td>
<td>33.5 ± 7.1</td>
<td>5.5 ± 1.1</td>
<td>4.5 ± 0.6</td>
</tr>
</tbody>
</table>

Mean values are cells per HPF from 20 HPFs. Biopsies were taken 24 h after the patch from the third sensitization was removed. *P < 0.05 saline-sensitized vs. OVA-sensitized. **P < 0.05 vs. similarly sensitized WT controls.
RNA, followed by digestion with RNase A and T1. The samples were then treated with protease K-SDS mixture, extracted with phenol-chloroform (Roche Molecular Biochemicals, Indianapolis, Indiana, USA), and precipitated in the presence of ammonium acetate. The samples were loaded on an acrylamide-urea sequencing gel next to undigested label probes and run at 50 W with 0.5x Tris-borate/EDTA electrophoresis buffer (TBE). The gel was dried on filter paper under vacuum, exposed to XAR film (Eastman Kodak Co., Rochester, New York, USA) with intensifying screens, and developed at ~70°C. For quantification, gels were scanned with a QuantiScan densitometer, and analysis of integration peaks was done with ImageQuant software. Each band was normalized to L32 and GAPDH and expressed as a percentage of GAPDH + L32. We analyzed duplicate skin biopsies from 10 mice in each group.

Results

Eosinophils are virtually absent in OVA-sensitized skin sites of IL-5–/– mice. We have shown previously that epicutaneous sensitization with OVA induced significant thickening of dermis, epidermis, and a cellular infiltrate in the dermis (Table 1) that contained T cells, eosinophils, and mast cells (Tables 2 and 3). Importantly, equivalent skin lesions were elicited in C57BL/6 mice, a strain with a propensity for T(1) responses, as in BALB/c mice, a strain with a propensity for T(2) responses (Ref. 21; Figure 1, Tables 1 and 2).

Table 1 shows that in contrast to WT mice of the same C57BL/6 genetic background, IL-5–/– mice had no significant increase in the thickness of the epidermal or dermal layers following OVA sensitization. However, the number per HPF of infiltrating cells—including CD45+, CD3+, CD4+, and CD8+ cells—in sensitized skin of IL-5–/– mice was equivalent to that in similarly sensitized WT controls (Figure 1, Tables 2 and 3). IL-5–/– mice had virtually no eosinophils in either saline-sensitized skin or in OVA-sensitized skin (Table 2). There were no significant differences in the expression of chemokines in OVA-sensitized skin sites between IL-5–/– mice and WT controls (data not shown).

OVA-sensitized skin sites of IL-4–/– mice have increased inflammatory cells but decreased eosinophils. The thickness of epidermis and dermis was equivalent in the OVA-sensitized skin sites of IL-4–/– mice and WT mice (Table 1). Saline-sensitized skin sites from IL-4–/– mice had a higher total number of cells per HPF in the dermis compared with WT controls but had significantly reduced numbers of eosinophils (Table 2). More importantly, OVA-sensitized skin from IL-4–/– mice had significantly more infiltrating cells but significantly less eosinophils in the dermis than OVA-sensitized skin from WT controls (Figure 1, Tables 2 and 3). IL-4–/– mice had virtually no eosinophils in either saline-sensitized skin or in OVA-sensitized skin (Table 2). There were no significant differences in the expression of chemokines in OVA-sensitized skin sites between IL-4–/– mice and WT controls (data not shown).

Detailed immunohistochemical analysis revealed that saline-sensitized skin sites of IL-4–/– mice had increased numbers of CD45+, CD4+, and CD8+ cells compared with saline-sensitized skin sites of IL-5–/– mice.

Figure 1

Histological features of OVA-sensitized and saline-sensitized skin sites. Skin sections were stained with H&E. Magnification for large panels: ×200; small panels: ×800. Arrowheads point to eosinophils. The epidermal layer of IL-5–/– mice does not contain as many cell layers as its WT control, and there is less thickening of the dermal layer. IL-4–/– mice have an increased cellular infiltrate and decreased eosinophils compared with WT mice. The IFN-γ–/– mice have decreased thickness of dermal layer.

Table 3

Quantification of the skin cellular infiltrate in immunohistochemical sections

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Sensitization</th>
<th>CD45+</th>
<th>CD3+</th>
<th>CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>WT</td>
<td>Saline</td>
<td>23.8</td>
<td>10.5</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OVA</td>
<td>44.3</td>
<td>70.2</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>IL-5–/–</td>
<td>Saline</td>
<td>21.7</td>
<td>12.9</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OVA</td>
<td>40.9</td>
<td>22.8</td>
<td>22.1</td>
</tr>
<tr>
<td>BALB/c</td>
<td>WT</td>
<td>Saline</td>
<td>21.2</td>
<td>14.1</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OVA</td>
<td>45.3</td>
<td>25.6</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>IL-4–/–</td>
<td>Saline</td>
<td>32.7</td>
<td>15.8</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OVA</td>
<td>75.6</td>
<td>40.2</td>
<td>39.1</td>
</tr>
<tr>
<td>IFN-γ–/–</td>
<td>Saline</td>
<td>28.5</td>
<td>12.8</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>OVA</td>
<td>44.1</td>
<td>21.0</td>
<td>14.2</td>
</tr>
</tbody>
</table>

Mean values are cells per HPF from 20 HPFs. Biopsies were taken 24 h after the patch from the third sensitization was removed. *P < 0.05 saline-sensitized vs. OVA-sensitized. **P < 0.05 vs. similarly sensitized WT controls.
WT controls, with only the increase in CD4+ cells reaching a significant level (Table 3). Following OVA sensitization, there was a significant increase \((P < 0.05)\) in CD45+, CD3+, CD4+, and CD8+ cells in both IL-4−/− mice and WT controls. More importantly, OVA-sensitized skin sites in IL-4−/− mice had significantly increased numbers of cells bearing CD45, CD3, CD4, and CD8, compared with OVA-sensitized skin sites in WT controls (Figure 2 and Table 3). These results suggest that T-cell infiltration into OVA-sensitized skin is increased in the absence of IL-4.

Chemokines are 8- to 10-kDa proteins that are key components of the leukocyte recruitment process. Chemokines and their receptors have specific tissue and cellular distribution leading to a selective inflammatory process in response to different stimuli (26). For example, eotaxin, MCP-3, and MCP-4 are chemotactic factors for eosinophils and T\(_{H}2\) cells, while MIP-1\(\beta\), MIP-2, RANTES, and IP-10 are chemotactic factors for T\(_{H}1\) cells (27–31). To explore the cause of the increased numbers of infiltrating T cells and the decreased numbers of eosinophils in OVA-sensitized skin of IL-4−/− mice, we examined chemokine expression in sensitized skin sites. For this purpose, we performed an RNase protection assay that detects mRNA for nine chemokines: LTN, RANTES, eotaxin, MIP-1\(\alpha\), MIP-2, MIP-1\(\beta\), IP-10, MCP-1, and TCA-3. Figure 3a depicts a representative experiment. Each band represents a radiolabeled, protected probe specific for each chemokine.

For this purpose, we performed an RNase protection assay that detects mRNA for nine chemokines: LTN, RANTES, eotaxin, MIP-1\(\alpha\), MIP-2, MIP-1\(\beta\), IP-10, MCP-1, and TCA-3. Figure 3a depicts a representative experiment. Each band represents a radiolabeled, protected probe specific for each chemokine. In the experiment shown, there was general increased expression of chemokine genes in OVA-sensitized skin compared with saline-sensitized skin in both WT and IL-4−/− mice. To quantify the results, we analyzed duplicate skin biopsies from 10 mice in each group. The bands were scanned using a densitometer, and the intensity of each chemokine signal was expressed as a percentage of the combined signals for the two housekeeping genes, L32 and GAPDH. There was a significant increase in the expression of MIP-1\(\alpha\) in OVA-sensitized sites in both WT and IL-4−/− mice, whereas MIP-2, MIP-1\(\beta\), IP-10, and RANTES increased significantly only in IL-4−/− mice (Figure 3b). More importantly, MIP-2, MIP-1\(\beta\), and RANTES expression was significantly increased in IL-4−/− mice compared with WT controls following OVA sensitization. Increased expression of MIP-1\(\beta\), MIP-2, and RANTES chemokines may underlie the increased accumulation of T cells in the skin of OVA-sensitized IL-4−/− mice.

Figure 3b shows that following OVA sensitization, eotaxin expression increased significantly (3.5-fold) only in WT mice. Furthermore, eotaxin expression was significantly decreased in IL-4−/− mice compared with WT controls following OVA sensitization. The decreased eotaxin expression in IL-4−/− mice may contribute to reduced eosinophil infiltration.

Given the role of IL-5 in eosinophil differentiation and survival, we investigated whether reduced serum IL-5 levels and diminished blood eosinophilia are additional causes of decreased eosinophilic infiltration in the skin of IL-4−/− mice. There were no significant differences in either absolute blood eosinophil count or in serum IL-5 level between IL-4−/− and WT mice sensitized with saline or between IL-4−/− and WT mice sensitized with OVA (WT-saline 1.23 ± 0.53 \(\times\) 10⁴ eosinophils/ml; WT-OVA 1.90 ± 0.75 \(\times\) 10⁴ eosinophils/ml; IL-4−/−-saline 0.85 ± 0.33 \(\times\) 10⁴ eosinophils/ml; IL-4−/−-OVA 2.15 ± 0.65 \(\times\) 10⁴ eosinophils/ml). In both IL-4−/− and WT mice, serum IL-5 levels increased following OVA sensitization; however, the increase was not statistically significant (WT-saline 216 ± 42 pg/ml; WT-OVA 300 ± 90 pg/ml; IL-4−/−-saline 191 ± 53 pg/ml; IL-4−/−-OVA 401 ± 173 pg/ml). These results suggest that the decrease in skin infiltration with eosinophils is not due to inefficient IL-5-driven differentiation of eosinophils.

Vascular cell adhesion molecule-1 (VCAM-1) is the counter-ligand to very late activation-4 antigen (VLA-4), which is expressed on eosinophils. VCAM-1 is essential...
for efficient adhesion of eosinophils to endothelium and their subsequent migration into tissue. We showed previously that VCAM-1 expression is increased in OVA-sensitized skin sites. IL-4 has been shown to upregulate the expression of VCAM-1 on endothelial cells (32). We investigated the possibility that lack of upregulation of VCAM-1 in sensitized skin of IL-4−/− mice underlies the decrease in tissue eosinophilia. Immunohistochemical analysis using an mAb to VCAM-1 showed that VCAM-1 expression was similar in IL-4−/− and WT mice sensitized with saline and in IL-4−/− and WT mice sensitized with OVA (data not shown). Possibly this is because other cytokines, such as TNF-α, also upregulate VCAM-1 expression (32).

OVA-sensitized skin sites of IFN-γ−/− mice have decreased thickening of the dermal layer. Although the dermis in IFN-γ−/− mice thickened after OVA sensitization, the increase in thickness was not significant — in contrast to WT controls (Table 1). The number of cells per HPF and the distribution of infiltrating cells (e.g., CD45, CD3, CD4, CD8), eosinophils, and mast cells were equivalent in IFN-γ−/− mice and WT controls (Figure 1, Tables 2 and 3). There were no significant differences in the expression of chemokines in OVA-sensitized skin sites between IFN-γ−/− mice and WT controls (data not shown).

Cytokine expression in skin of cytokine deletion mice. We have shown previously, using a semiquantitative RT-PCR, that epicutaneous sensitization with OVA increased local expression of mRNA for IL-4, IL-5, and IFN-γ (21). We used the same strategy to examine cytokine mRNA expression in sensitized skin sites of cytokine-deficient mice. OVA-sensitized skin sites from IL-4−/− mice demonstrated skewing to Th1 cytokines, with a 15-fold increase in IL-2 expression and a 6.8-fold increase in IFN-γ expression compared with WT control (Figure 4). In contrast, OVA-sensitized skin sites from IFN-γ−/− mice demonstrated skewing to IL-4 expression, with a significant twofold increase in IL-4 expression compared with WT control. OVA-sensitized skin from IL-5−/− mice had a significant twofold decrease in IL-4 expression and a significant threefold increase in IFN-γ expression.

Skewing of antibody isotypes in mice with targeted deletion of cytokine genes following epicutaneous sensitization with OVA. We showed previously that epicutaneous sensitization with OVA induces a rise in total serum IgE, as well as in OVA-specific IgE, IgG1, IgG2a, and IgG2b antibodies. Cytokines are known to affect immunoglobulin isotype switching. IL-4 plays an important role in switching to IgE, and IFN-γ plays an important role in switching to IgG2a (33, 34). This is demonstrated by the failure of IL-4−/− mice to mount an IgE antibody response and of IFN-γ−/− mice to make an IgG2a response (35, 36). These principles held true in our epicutaneous sensitization model. IL-4−/− mice had drastically less total and OVA-specific IgE and significantly more total and OVA-specific IgG2a in serum than did WT controls (Figure 5). Conversely, total and OVA-specific IgE were increased in IFN-γ−/− mice, whereas total and OVA-specific IgG2a were significantly decreased (Figure 5). Total and OVA-specific IgE and IgG2a in IL-5−/− mice were equivalent to those in WT C57BL/6 controls (Figure 5).

IgE antibody is not essential for the induction of skin inflammation by epicutaneous sensitization with OVA. Serum IgE lev-
Table 5
Skin cellular infiltration in IgE−/− and controls

<table>
<thead>
<tr>
<th></th>
<th>WT (129Sv)</th>
<th>IgE−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline (n = 7)</td>
<td>OVA (n = 8)</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cells</td>
<td>22.9 ± 3.1</td>
<td>22.9 ± 3.1</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>3.6 ± 1.2</td>
<td>6.9 ± 1.8a</td>
</tr>
<tr>
<td>Mast cells</td>
<td>1.8 ± 1.3</td>
<td>4.5 ± 1.7a</td>
</tr>
<tr>
<td><strong>Histochemistry</strong></td>
<td></td>
<td></td>
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<tr>
<td>CD45+</td>
<td>32.5 ± 4.2</td>
<td>63.6 ± 5.1a</td>
</tr>
<tr>
<td>CD3+</td>
<td>8.3 ± 2.2</td>
<td>19.8 ± 4.2a</td>
</tr>
<tr>
<td>CD4+</td>
<td>14.2 ± 3.4</td>
<td>28.3 ± 4.8a</td>
</tr>
<tr>
<td>CD8+</td>
<td>0.5 ± 0.1</td>
<td>0.8 ± 0.4</td>
</tr>
</tbody>
</table>

Results represent mean values ± SEM. *P < 0.05 saline-sensitized vs. OVA-sensitized.

The results of the present study suggest that the Th2 cytokines IL-4 and IL-5 contribute to skin inflammation and that the Th1 cytokine IFN-γ contributes to dermal thickening in a murine model of AD.

Eosinophils were absent from the skin of IL-5−/− mice. These findings are consistent with the nonredundant role of IL-5 in the differentiation of eosinophils from bone marrow precursors (37–39). More importantly, absence of eosinophils in OVA-sensitized skin sites of IL-5−/− mice was associated with a lack of increase in the thickness of the epidermal and dermal skin layers, a common feature of the skin lesion in AD (2). These results suggest that eosinophils play an important role in the hypertrophy of the skin in AD. Eosinophil major basic protein and eosinophil cationic protein are known to have cytotoxic effects (38). This may lead to tissue hypertrophy during the repair process, resulting in dermal thickening.

The role of IL-4 in our murine model of AD appears to be complex. Our data indicate that IL-4 is important for eosinophil recruitment into the skin. This is consistent with the previously established role of IL-4 in tissue eosinophilia in the allergic model of airway inflammation mice (19, 20, 40). OVA-sensitized skin sites from IL-4−/− mice did not exhibit the increased expression of eotaxin mRNA that we observed in WT controls. This observation agrees with previous observations that IL-4 increases eotaxin gene expression in skin and in dermal fibroblasts (41, 42). Taken together with the observation that serum IL-5 levels, blood eosinophil counts, and VCAM-1 expression at sensitized skin sites are equivalent in IL-4−/− mice and WT controls, our results suggest that IL-4 induction of eotaxin expression plays an important role in the eosinophilic infiltration into the skin lesions in AD. Expression of RANTES, another chemokine with chemotactic activity on eosinophils, remained increased in IL-4−/− mice, suggesting that it does not play a major role in recruiting eosinophils to the skin in our model. We cannot, however, rule out a role for additional chemokines such as MCP-3 and MCP-4, neither of which we have studied. An additional factor in the drastic reduction of eosinophils in the inflamed skin lesions of IL-4−/− mice may be the decreased local expression of mRNA for IL-5, a cytokine that plays a critical role in eosinophil survival. Whether the decrease in IL-5 mRNA and eotaxin expression in the skin of IL-4−/− mice following OVA sensitization can be detected at the induction phase of sen-
Sensitization is a subject for future experiments.

In contrast to its role in promoting eosinophil infiltration, our results suggest that IL-4 modulates skin infiltration by T cells. OVA-sensitized skin sites in IL-4+/− mice had significantly higher numbers of infiltrating CD3+ cells than did WT controls. This was associated with a significantly increased expression of MIP-1β, MIP-2, and RANTES, chemokines that recruit Th1 cells (29–31, 43–45). These results suggest that IL-4 normally down-regulates the expression of T-cell chemokines and the subsequent infiltration and expansion of T cells into the skin lesion. The persistence of a T-cell infiltrate in the OVA-sensitized skin of IL-4−/− mice does not rule out a role for TH2 cells in our model of AD. Indeed, IL-5 expression was still increased in OVA-sensitized skin sites of IL-4−/− mice. Similarly, there was no change in the size of the T12 granuloma or in IL-5 expression in IL-4−/− mice sensitized with Schistosoma mansoni (31).

The thickness of the epidermal and dermal layers of OVA-sensitized skin sites of IL-4−/− mice did not differ from that of similar sites in WT controls. This may reflect the balance between the dual activities of IL-4 in AD. IL-4 may function as a proinflammatory cytokine by virtue of its ability to recruit eosinophils and to enhance collagen synthesis (46). IL-4 may also function

Figure 5
Serum immunoglobulin and OVA-specific antibody levels. (a) Total IgE and IgG2a were determined by ELISA (four mice per group). (b) OVA-specific IgE and IgG2a antigen-specific antibodies were determined by ELISA (four mice per group). The columns and error bar represent mean ± SE. *P < 0.05 compared with WT controls.

Figure 6
Histological features of OVA-sensitized and saline-sensitized skin sites of IgE−/− and WT 129Sv controls.
as an anti-inflammatory cytokine, by virtue of its ability to suppress the infiltration of T cells, including Th1 cells that secrete IFN-γ, a cytokine that appears to play a role in enhancing dermal thickening.

We found significantly less thickening of the dermal layer in IFN-γ−/− mice than in WT mice following sensitization with OVA. These findings suggest that IFN-γ plays a role in the skin hypertrophy of AD. This is consistent with the predominant expression of IFN-γ in the hypertrophic skin lesions of patients with chronic AD and may be related to the ability of IFN-γ to activate tissue macrophages (47), which play an important role in the skin hypertrophy of delayed hypersensitivity reactions.

OVA-sensitized IL-4−/− mice had drastically reduced levels of IgE (Figure 5). Residual IgE probably is due to the skin hypertrophy of delayed hypersensitivity reactions. Macrophages (47), which play an important role in the be related to the ability of IFN-γ for both the T H2 cytokines IL-4 and IL-5 and the T H1 cytokine IFN-γ plays an anti-inflammatory role in AD by virtue of its ability to downmodulate T-cell infiltration into the skin. These findings have important implications for designing therapeutic strategies aimed at modulating cytokines and chemokines in AD.

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