A Spontaneous CD8 T Cell-Dependent Autoimmune Disease to an Antigen Expressed Under the Human Keratin 14 Promoter

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A Spontaneous CD8 T Cell-Dependent Autoimmune Disease to an Antigen Expressed Under the Human Keratin 14 Promoter

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Using a previously described human keratin 14 (K14) promoter, we created mice expressing a peptide Ag (OVAp) in epithelial cells of the skin, tongue, esophagus, and thymus. Double transgenic mice that also express a TCR specific for this Ag (OT-I) showed evidence for Ag-driven receptor editing in the thymus. Surprisingly, such mice exhibited a severe autoimmune disease. In this work we describe the features of this disease and demonstrate that it is dependent on CD8 T cells. Consistent with the Ag expression pattern dictated by the human K14 promoter, an inflammatory infiltrate was observed in skin and esophagus and around bile ducts of the liver. We also observed a high level of TNF-α in the serum. Given that Ag expression in the thymus induced development of T cells with dual TCR reactivity, and that dual-reactive cells have been suggested to have autoimmune potential, we tested whether they were a causal factor in the disease observed here. We found that OT-I/K14-OVAp animals on a recombinase-activating gene-deficient background still suffered from disease. In addition, OT-I animals expressing OVA broadly in all tissues under a different promoter did not experience disease, despite having a similar number of dual-specific T cells. Thus, in this model it would appear that dual-reactive T cells do not underlie autoimmune pathology. Finally, we extended these observations to a second transgenic system involving 2C TCR-transgenic animals expressing the SIY peptide Ag with the hK14 promoter. We discuss the potential relationship between autoimmunity and self-Ags that are expressed in stratified epithelium. The Journal of Immunology, 2002, 169: 2141–2147.
mice expressed the OT-I TCR, specific for a peptide epitope from OVA in the context of the K\(^{b}\) MHC molecule. When the OVA peptide Ag was expressed in the thymus under the human keratin 14 (K14\(^{+}\)) promoter, only a modest reduction in thymic CD4\(^{+}\)CD8\(^{+}\) double positive cells was observed. TCR\(^{+}\) gene rearrangement was highly elevated in these precursors, and the predominant T cell population that matured expressed both the transgenic receptor and an endogenous TCR\(^{+}\) chain. Surprisingly, OT-I/K14-OVAp animals exhibited a lethal disease between 2 and 6 wk of age. In this report we describe the features of this disease and provide evidence that it is a CD8-mediated autoimmune disease with significant manifestation in the skin. Because of the large dual-reactive T cell population in these mice, and because such cells have previously been reported to have autoimmune potential, we tested the hypothesis that dual-reactive T cells play a dominant role in spontaneous disease induction in this model.

Materials and Methods

Mice

C57BL/6 (B6) and RAG1\(^{null}\)B6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). OT-I mice express a transgenic receptor specific for the OVA\(_{257-264}\) peptide (OVAp) in the context of the H-2K\(^{b}\) (13). C2 mice (14) express an allogeneic receptor that also has reactivity to a synthetic peptide (SIYRYYGL), in the context of H-2K\(^{b}\) (15). K14-OVAp and K14-SIYp transgenic constructs were generated using a multipurpose PCR procedure as previously described (16). Act-mOVA mice were obtained from B. Esh and M. Jenkins (University of Minnesota, Minneapolis, MN). These mice express a transmembrane form of OVA under control of the \(\beta\)-actin promoter and CMV immediate-early enhancer. OVA could be detected on the surface of all white blood cells by flow cytometry with an OVA-specific Ab (Sima-Aldrich, St. Louis, MO) and in all tissues by immunohistochemistry (data not shown). All four major thymic APCs (cortical and medullary epithelial cells, dendritic cells, and macrophages) from Act-mOVA mice were able to stimulate OT-I T cells (data not shown). All mice were treated in accordance with federal guidelines approved by the University of Minnesota Institutional Animal Care and Use Committee.

Flow cytometric analysis of lymphoid organs

Thymus, lymph nodes, and spleen were harvested from the indicated mice between 2 and 8 wk of age and stained with Abs to CD4 (L3T4, RMA-5), CD8 (53-6.7), CD69 (H1.2F3), Thy 1.1 (H551), and TCR\(^{\beta}\) (H57-597), all obtained from BD PharMingen (San Diego, CA). V\(_{\beta}\)5 (MR9.1)- and V\(_{\beta}\)2 (BZ0.1)-specific Abs were used to detect the OT-I transgene, although it should be noted that these Abs also recognize endogenous V\(_{\beta}\)5 and V\(_{\beta}\)2 TCR chains. A clonotype-specific Ab (1B2) was used to detect the 2C receptor along with an Ab to V\(_{\beta}\)8 (F23.1). Data were collected using a FACS Calibur (BD Biosciences, San Jose, CA) and analyzed with FlowJo software (TreeStar, San Carlos, CA).

Histology

Tissues from OT-I/K14-OVAp or OT-I control animals were arranged in aluminum foil cups, snap-frozen in liquid nitrogen, and stored at -80°C. Cryosections were cut 5 mm thick, mounted onto glass slides, fixed for 5 min in acetone, and stained with H&E.

Serum cytokine measurement

Serum was tested in duplicate with TNF-\(\alpha\) or IL-1\(\beta\) ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s protocol. A standard curve was generated with known amounts of cytokine provided in the kit, and the values in the serum were extrapolated from this.

Ab treatment

Mice were injected i.p. with depleting Abs to CD8a (2.43), CD8\(\beta\) (H35-17), or rat Ig beginning at day 1 after birth and continuing every other day until 6 wk of age. An initial dose of 50 \(\mu\)g was increased to 100 \(\mu\)g after 2 wk of age and to 150 \(\mu\)g after 4 wk. A neutralizing Ab to TNF-\(\alpha\) (TN3-19.12; a gift of K. Sheehan, Washington University, St. Louis, MO) was injected i.p. every fourth day from birth to wk 1 (50 \(\mu\)g), from wk 1 to wk 3 (100 \(\mu\)g), and from wk 3 to wk 6 (150 \(\mu\)g). All mice were weighed and monitored for signs of disease every other day. Mice remaining at the end of wk 6 were sacrificed. Serum was taken for analysis as described above. Lymph node and spleen were analyzed for T cell depletion by flow cytometry. Depletion >95% was observed in all animals.

Results

We studied TCR-transgenic mice that expressed the antigenic peptide ligand in the thymus, skin, and esophagus (9). The transgenic TCR was the OT-I TCR that recognizes a peptide from chicken OVA (OVAp) in the context of the MHC class I molecule, K\(^{b}\). The antigenic peptide, OVAp, was also expressed as a transgene under the control of a human keratinocyte-specific promoter, K14. This promoter directs expression in epithelial cells of the thymus as well as the skin, esophagus, and tongue (17). We previously reported that in double transgenic (OT-I/K14-OVAp) mice the presence of the antigenic peptide in the thymus did not delete the OT-I transgenic T cells. On the contrary, extensive endogenous TCR\(^{+}\) gene rearrangement occurred (9). This receptor editing was required for efficient development of mature T cells. Thus, a significant number of CD4 and CD8 T cells were present in periphery of OT-I/K14-OVAp mice (Table I). These cells expressed the transgenic TCR\(^{\beta}\) chain (V\(_{\beta}\)5) at a high level and the transgenic \(\alpha\)-chain (V\(_{\alpha}\)2) at a reduced level (9), implying the usage of an endogenous TCR\(^{+}\) chains. Interestingly, the level of V\(_{\alpha}\)2 on CD8 T cells from the spleen or lymph nodes of OT/K14-OVAp mice was consistently higher than the level of V\(_{\alpha}\)2 on mature CD8 thymocytes in the same animals (Fig. 1A). In addition, the cell surface phenotype of CD8 T cells from OT-I/K14-OVAp animals differed from control OT-I animals, being CD44\(^{low}\), CD62L\(^{low}\), and CD69\(^{hi}\) (9). Together these features suggest that peripheral CD8 T cells in OT-I/K14-OVAp mice were activated.

Double transgenic mice develop an acute disease

OT-I/K14-OVAp double transgenic animals experienced a lethal disease. The outward signs included hair loss, skin lesions, weight loss, and a hunched appearance (Fig. 2). Disease was not observed in OT-I or K14-OVAp single transgenic littermates (Fig. 3A and data not shown). Onset occurred at an average age of 2–4 wk and was incompletely penetrant, with 20% of animals showing no signs of disease (Fig. 3A). Animals with outward signs of the disease showed, on average, higher levels of TNF-\(\alpha\) in the serum (Fig. 4). In addition, the level of TNF-\(\alpha\) in the serum was elevated in a few of the double transgenic mice that were not acutely ill. However, there were no major differences in cellular composition or T cell surface phenotype of lymph node and spleen cells from acutely sick double transgenic and age-matched littermates that showed no signs of disease (data not shown).

Table I. T lymphocyte cellularity in various strains of mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>CD8 (%)</th>
<th>CD4 (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>6.7 ± 1.1</td>
<td>8.1 ± 2.8</td>
<td>14.8 ± 3.9</td>
</tr>
<tr>
<td>OT-I</td>
<td>14.6 ± 4.9</td>
<td>19.5 ± 7.7</td>
<td>34.1 ± 12.6</td>
</tr>
<tr>
<td>OT-I/K14-OVAp</td>
<td>12.8 ± 3.8</td>
<td>14.9 ± 1.9</td>
<td>27.7 ± 5.7</td>
</tr>
<tr>
<td>OT-I/Act-mOVA</td>
<td>4.1 ± 4.7</td>
<td>4.3 ± 5.7</td>
<td>8.4 ± 10.4</td>
</tr>
<tr>
<td>OT-I/IRAG(^{null})</td>
<td>5.3 ± 5.7</td>
<td>7.1 ± 8.9</td>
<td>12.4 ± 14.6</td>
</tr>
<tr>
<td>OT-I/K14-OVAp RAG(^{null})</td>
<td>3.0 ± 3.7</td>
<td>4.1 ± 5.2</td>
<td>7.1 ± 8.9</td>
</tr>
</tbody>
</table>

The average number of CD4 or CD8 T cells (×10\(^3\)) from spleens of various transgenic mice is indicated, along with the SD. The data are from 6–18 adult animals per group for B6 or OT-I strains. The data are from six 2-wk-old C2 or 2C/K14-SIY mice.

Abbreviations used in this paper: K14, human keratin 14; RAG, recombine-activating gene; LC, Langerhans cells.
Histology of tissue sections revealed an extensive infiltrate of mononuclear cells directly beneath the epidermis and also around the hair follicles (Fig. 5). This infiltrate consisted of CD4 T cells, CD8 T cells, and macrophages, but not B cells (data not shown). Hyperkeratosis and sloughing were observed in some areas of the skin. In the esophagus (Fig. 5) and tongue (data not shown), inflammation was again localized to the epithelial layer. Double transgenic animals showed occasional incidence of diarrhea and intestinal necrosis. However, this was not a consistent feature of this disease and in most animals the epithelium of the stomach, small intestine, and colon appeared intact, with no significant mononuclear cell infiltrate. Transgenes driven by the hK14 promoter were shown to be expressed in a manner similar to endogenous K14, specifically in basal cells of stratified epithelium in skin, esophagus, and tongue (17–21). Therefore, the pattern of infiltration reproducibly observed in skin and esophagus is consistent with the predicted Ag expression pattern in these animals. We also observed an inflammatory infiltrate in the liver (Fig. 5). The infiltrate was not observed in the parenchyma of the liver or around hepatic venules, but surrounded the bile duct. Although transgene expression was not detected in total liver RNA by PCR (9), it was detected in RNA from bile duct (data not shown), perhaps explaining the unique pattern of infiltration in this organ. Nonetheless, bile duct was not obliterated and function was not impaired, at least as

![FIGURE 1. T cell phenotype in lymph nodes of various transgenic strains. A, Lymph node cells from various adult transgenic mice were stained for CD4, CD8, Va2, and CD69. Histograms show the level of Va2 or CD69 on the CD8-positive cells. For comparison, the level of Va2 on CD8 single positives in the thymus of the double transgenic is also included. B, Lymph nodes from OT-1 RAGnull and the OT-1/K14-OVAp RAGnull mice were stained as above. These cells were also stained for Thy 1 due to a high number of cells in the OT-1/K14-OVAp RAGnull lymph nodes that were not T cells. C, Spleens from 2C or 2C/K14-SIY mice at 2 wk of age were harvested and stained as in A.](http://www.jimmunol.org/)

![FIGURE 2. Disease in OT-1/K14-OVAp mice. Photograph was taken at 4 wk of age. The OT-1/K14-OVAp mouse is on the left next to an OT-1 control littermate.](http://www.jimmunol.org/)
reflected by the normal levels of bilirubin and γ-glutamyl transpeptidase present in the serum (data not shown). In skin, esophagus, and liver, the composition of the infiltrate was similar, being comprised of both CD4 and CD8 T cells and macrophages but not B cells (data not shown). Other organs that were analyzed and appeared normal included heart, lung, pancreas, and kidney (data not shown).

Disease was dependent on CD8αβ T cells

The activated phenotype of the CD8 T cells and the fact that there was a mononuclear cell infiltrate found in areas of Ag expression suggested that the disease in the OT-I/K14-OVAp mice might be due to the autoimmune CD8 T cells. To test this, CD8 T cells were depleted with administration of Abs to CD8α or CD8β from birth to 6 wk of age. As a control, littermates were injected with rat Ig, and the mice were monitored for signs of disease. All mice injected with CD8 Abs survived, whereas only 3 of the 10 mice injected with the control Ab survived (Table II). The percentage of survival in mice injected with the control Ab was similar to untreated OT-I/K14-OVAp mice. Thus, CD8 T cells are required for disease induction.

It had previously been shown that T cells expressing CD8α homodimers could mature extrathymically in TCR-transgenic mice (22, 23). FACS analysis of the spleen and lymph nodes from OT-I/K14-OVAp mice indicated that there was only a very small increase in CD8α cells, but that these cells also had an activated phenotype (data not shown). To test whether it was these cells or conventional CD8αβ heterodimer expressing T cells of thymic origin that induced disease, we treated mice with a CD8α Ab. Similar to the CD8α depletions, all of the mice injected with the CD8α Ab survived, indicating that the CD8α T cells alone did not cause disease. Mice depleted with either CD8α or CD8β Ab did not display elevated levels of TNF in the serum (data not shown). Because of the correlation between elevated levels of TNF-α and disease, we also tested whether TNF-α was required for disease. Six OT-I/K14-OVAp mice were injected with a neutralizing Ab to TNF-α (24), beginning at day 1 after birth and continuing every 4 days until 6 wk of age. Surprisingly, five of the six mice suffered from acute disease and died by 6 wk of age (Table II). This indicated that, while TNF-α levels were high in...
Table II. Disease was dependent on CD8αβ T cells

<table>
<thead>
<tr>
<th>CD8α</th>
<th>Number of Mice That Died Before 6 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0/6</td>
</tr>
<tr>
<td>CD8β</td>
<td>0/5</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5/6</td>
</tr>
<tr>
<td>Rat Ig</td>
<td>7/10</td>
</tr>
</tbody>
</table>

* Neonatal animals were given 50 μg of Ab i.p. every other day (for CD8 depletion) or every fourth day (for TNF neutralization) until 6 wk of age.

Discussion

We report in this work the observation of spontaneous autoimmune disease in OT-I TCR-transgenic animals when the Ag is expressed under control of the human K14 promoter. This is a lethal disease, although the precise cause of death was not determined. An inflammatory infiltrate was observed surrounding the bile ducts of the liver, presumably related to transgene expression in the bile duct. Nonetheless, liver and bile duct function were apparently normal. The high levels of TNF-α observed in the serum of these mice might account for the lethality, because aspects of this disease are similar to those in mice expressing TNF-α as a transgene (20). Nonetheless, serum levels of TNF-α varied, and a TNF-α neutralization treatment did not prevent lethal disease. IL-1α and IL-1β were also elevated in double transgenics (Fig. 4), and in the absence of TNF-α these cytokines might contribute to disease. Thus, disease initiation does not require TNF-α, and the end stages of the disease process could involve multiple factors including TNF-α. CD8 T cells were required for the disease, because treatment with Ab to either CD8α or CD8β prevented lethality and no disease characteristics were observed in treated animals.

T cells with dual TCR reactivity were previously shown to have autoimmune potential (10, 11). Because the OT-I/K14-OVAp animals showed a high number of dual-reactive CD8 T cells, we considered whether this was a factor in disease induction. OT-I/K14-OVAp animals on a RAGnull background still experienced disease, although the average age of onset was delayed by ~4 wk. This experiment formally proved that dual-reactive T cells are not required for disease. However, the delayed onset of disease in RAGnull animals might suggest that dual-reactive T cells can accelerate or exacerbate disease. However, such a conclusion would be premature, because RAGnull animals also have a B cell deficiency, severe lymphopenia, and a greatly reduced total number of...
CD8 T cells. Such factors could also contribute to the age of disease onset. The study of TCRα\textsuperscript{null} double transgenics and development of an adoptive transfer system to mimic disease are being used to approach this issue further.

The lack of correlation between the number of dual-reactive T cells and disease occurrence in two additional strains we examined also suggested that dual reactivity was not a key factor in this model of autoimmune disease. OT-I/Act-mOVA animals had a slightly higher number of dual receptor-expressing CD4 and CD8 T cells in the spleen compared with OT-I/K14-OVAp animals (Fig. 1 and Table I). However, this strain showed no signs of disease. The fact that OT-I/K14 and OT-I/mOVA strains had a similar thymic tolerance phenotype but differed absolutely in disease occurrence suggests that the immune dysregulation in OT-I/K14 may be related to how or where the Ag is presented in the peripheral tissues of K14 mice. Consistent with this, we also observed disease in 2C animals expressing Ag under control of the human K14 promoter. Thymic tolerance (via clonal deletion) was very profound and these animals matured few CD8 T cells in thy-mus, similar to 2C animals expressing L\textsuperscript{d} (14). Thus again, the very profound and these animals matured few CD8 T cells in thy-mus, similar to 2C animals expressing L\textsuperscript{d} (14). Thus again, the very profound and these animals matured few CD8 T cells in thy-

In light of this possibility, it is interesting that class II molecules might be a cofactor for disease induction or escalation in this model. Recent studies suggest that the thymus also directs the selection of regulatory CD25\textsuperscript{+}CD4\textsuperscript{T} cells (27). A potential deficiency of thymus-derived regulatory CD4 T cells in the above models has not been addressed. In our model, we might consider the possibility that Ags expressed under the K14 promoter do not efficiently select regulatory CD25\textsuperscript{+}CD4\textsuperscript{T} T cells in the thymus. Such cells have recently been shown to suppress CD8 T cell responses (28). In light of this possibility, it is interesting that class II molecules expressed under the control of the K14 promoter did allow selection of CD25\textsuperscript{+} regulatory CD4 T cells (29). However, in our case, where the transgene encodes only the 8-aa class I binding peptide, this would not be sufficient to select class II-restricted, OVA-specific regulatory CD4 T cells. Nonetheless, it is unlikely that this would be the sole factor contributing to disease in this situation. If the absence of Ag-specific regulatory CD4 T cells resulted in disease, then disease should be a universal feature of all transgenic animals expressing class I binding peptide minigenes. Two other articles describing peptide transgenic mice did not report health problems, even when crossed to TCR transgenics specific for the Ag (30, 31).

Based on the data presented in this work, it is possible that use of the K14 promoter to express self-AgS is associated with the breakdown of peripheral tolerance. We note with interest that the localization of hK14-driven transgenes correlates rather tightly with that of the specialized subset of dendritic cells termed Langerhans cells (LC). These cells reside specifically in stratified epithelial tissue, including the epidermis, hair follicle, and esophagus (32). Within the epidermis, LC form a semicontinuous network directly underneath the basal cell layer of keratinocytes. LC become activated and migrate to the lymph node during skin irritation, injury, or infection. Of APCs, LC represent a likely candidate APC for the presentation of OVA to lymph node T cells in OT-I/K14-OVAp mice, although this remains to be tested directly. Thus, it is interesting to consider whether skin injury or infection might be a cofactor for disease induction or escalation in this murine autoimmune disease model. Perhaps in the context of other predisposing factors, peripheral tolerance more frequently fails to control the T cell response to self-Ags that are repeatedly or chronically presented by APC from the skin but not from other tissues. Skin injury has been shown to lead to pathologic lesions in individuals predisposed to the human autoimmune disease psoriasis—a phenomenon referred to as Koebner’s effect (33).

A predisposing factor in this model is clearly the high Ag-specific T cell clone frequency. We emphasize that both K14-OVAp and K14-SIYp single transgenic animals appear healthy and viable; disease was observed only after crossing K14 transgenic animals to a TCR-transgenic specific for the Ag. This is consistent with CTL tolerance to a viral Ag driven by the K14 promoter, observed in a non-TCR-transgenic system (34). Thus, an exaggerated frequency or continued onslaught of T cells may be required to shift the tolerance/immunity balance in this model, as in all TCR-transgenic models of spontaneous disease (10, 35–39). In humans, expansion of T cell clones occurs during the immune response (40, 41) and under conditions of lymphopenia (42, 43). However, it is not clear whether this is a predisposing factor in human autoimmune disease (44). Because of this, murine TCR-transgenic models may not represent ideal systems to study the basis of immune tolerance loss. Nonetheless, TCR transgene-based models of autoimmune disease can potentially be useful to define pathogenic processes and autoimmune spreading mechanisms, and to test treatment protocols. Future experiments will be aimed at determining the potential roles of skin injury in disease induction and escalation in this model.

\section*{Acknowledgments}
We thank Kelly McCarthy, Melinda Berthold, and John Hermanson for excellent technical assistance and members of the Hogquist and Jameson labs for their input.

\section*{References}


