Enhanced Oral Tolerance in Transgenic Mice with Hepatocyte Secretion of IL-10

Rifaat Safadi, Carlos E. Alvarez, Masayuki Ohta, Jens Brimnes, Thomas Kraus, Wajahat Mehal, Jonathan Bromberg, Lloyd Mayer and Scott L. Friedman

J Immunol 2005; 175:3577-3583; doi: 10.4049/jimmunol.175.6.3577
http://www.jimmunol.org/content/175/6/3577

References
This article cites 50 articles, 20 of which you can access for free at: http://www.jimmunol.org/content/175/6/3577.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at: http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at: 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
Enhanced Oral Tolerance in Transgenic Mice with Hepatocyte Secretion of IL-10

Rifaat Safadi,* Carlos E. Alvarez,* Masayuki Ohta,* Jens Brimnes,† Thomas Kraus,† Wajahat Mehal,§ Jonathan Bromberg,‡ Lloyd Mayer,† and Scott L. Friedman2*†

Several cytokines derived from Th3 and Tr1 cells, including IL-10, are believed to regulate oral tolerance, but direct evidence is lacking. We have explored the potential role of IL-10 by generating transgenic (TG) mice with sustained hepatocyte-specific expression of rat IL-10. TG mice expressed rat IL-10 downstream of a transthyretin promoter, which led to serum levels that were increased 10- to 100-fold compared with normal animals. Animals were orally administered 1 mg of whole OVA for 5 consecutive days, with control animals receiving PBS. There were six animal groups: Either OVA or PBS were fed orally to rat IL-10 TG mice, non-TG wild-type mice without IL-10 administration, and non-TG wild-type mice administered rat IL-10 systemically. On day 8, all mice were immunized with two injections of OVA, and then analyzed on day 18. T cell proliferation responses were reduced by 65.8 ± 14.3% after feeding of OVA in rIL-10 TG animals, compared with 39.4 ± 15.6% in the non-TG mice (p = 0.02). Anti-OVA titers were expressed as fold increase over naive non-TG mice. After feeding, titers decreased by ~33% (from 3- to 2-fold) in TG animals and, to a lesser extent, in non-TG animals. IFN-γ secretion by cultured popliteal lymphocytes decreased in TG animals by 83% after feeding and by 69% in non-TG animals. IL-4 secretion increased 4-fold in TG-fed mice, but did not significantly change in non-TG OVA-fed animals. In contrast to hepatic TG expression of rIL-10, systemic administration of rIL-10 had only a modest effect on tolerance. IL-10, when transgenically expressed in the liver enhances mucosal tolerance to an oral Ag. The Journal of Immunology, 2005, 175: 3577–3583.
CD80/86 expression on their surface (22, 23). In contrast, in B lymphocytes, IL-10 stimulates Ig secretion (24, 25). IL-10 also exerts potent anti-inflammatory effects. It down-regulates the synthesis of proinflammatory cytokines and chemokines by monocytes and Kupffer cells stimulated by endotoxin, including IL-1, TNF-α, IL-6, IL-8, and IL-12, and up-regulates the synthesis of the IL-1R antagonist (20, 26, 27). Neutrophil chemotaxis and chemokine expression are also down-regulated (28).

The liver has long been implicated in immunoregulatory functions. It is the largest reticulo-endothelial organ in the body, and several subpopulations of its cells are involved in Ag presentation and/or processing (29). For example, portocaval shunts or block-ade of Kupffer cell function may attenuate induction of oral toler-ance in animal models (30). Also, Ab titers to intestinal flora are elevated in humans with chronic liver diseases who undergo portocaval shunts (30, 31). Finally, portal vein administration of donor cells can promote allospecific hyporesponsiveness (31). Thus, the liver may be necessary for peripheral immune tolerance induction through first pass clearance of Ags.

Despite the growing familiarity with the activities of IL-10, its role in mediating oral tolerance remains unclear. To address this issue, and to explore the potential role of hepatic IL-10 in the oral tolerance response, we generated transgenic (TG) mice with hepatocyte-specific expression of IL-10 by expressing an active form of rat IL-10 driven by the transthyretin (TTR) promoter (32). Our results suggest that IL-10 expressed in liver in this model can significantly augment oral tolerance to a foreign Ag. This enhance-ment of the IL-1R antagonist (20, 26, 27). Neutrophil chemo-
taxis and chemokine expression are also down-regulated (28).

Tolerance protocol

Eight-week-old mice were used in this study, both IL-10 TG animals (fourth generation on a mixed C57BL/6 and C3H background) and their non-TG littermates as a control. Tolerance induction was performed by repeated oral low-dose administration of OVA (Sigma-Aldrich). The Ag administration schedule was 1 mg of whole OVA by gastric intubation for 5 consecutive days (days 1–5). To compare the effect of systemic vs hepatic IL-10 on oral tolerance, we separately treated non-TG animals with i.p. rIL-10 injections (65 ng/day for 14 days) that achieved serum concentra-
tions similar to those seen in IL-10-TG mice. There were six animal groups: Either OVA or PBS were fed orally to rat IL-10 TG mice, non-TG wild-type mice without IL-10 administration, and non-TG wild-type mice administered rat IL-10 systemically. Each animal subgroup contained 8 male mice in the first experiment (total of 32 mice) and 5 in the second independent experimental set. Only male mice were used, similar to prior studies (33).

On day 8, all mice were immunized with two footpad injections of a total of 200 μg of OVA emulsified in IFA (Sigma-Aldrich) at a 1:1 ratio. On day 18, mice were sacrificed, blood was collected, and serum was separated. Spleens and the popliteal lymph nodes (PLN) were aseptically removed and teased into single-cell suspensions. Each mouse was analyzed as a separate data point.

Serum anti-OVA Ab measurements

Anti-OVA serum Ab titers were measured by ELISA. Diluted serum samples were incubated for 1 h on ELISA plates (Nalg Nunc International) previously coated with 5 μg/ml of OVA in 0.1 M carbonate buffer (pH 9.5). Plates were washed and incubated for 1 h with 100 μl/well HRP-conju-
gated goat anti-mouse IgG (Roche Molecular Biochemicals) diluted to 0.2 μg/ml. A/B substrate solution (BD Pharmingen) was added and colorimetric-
ance was performed on an ELISA reader (Bio-Tek Instruments) at a wavelength of 650 nm. As controls, nonimmunized (normal mouse sera) and OVA-immunized (i.p.) mouse sera were used at concentrations identi-
tical to the samples.

In vitro analysis of T cell anti-OVA responses

The spleen and PLN from each mouse were analyzed separately. Spleno-
cytes and cells from the PLN were teased into single-cell suspensions and washed twice with PBS. Cells were then cultured at 2 × 10^6 cells/ml in RPMI 1640 supplemented with 10% FCS (Atlantic Biologicals), 2-ME (5 × 10^-3 M) and 1% penicillin-streptomycin-glutamine (Invitrogen Life Technologies). Cultured cells were analyzed for Ag-specific cytokine se-
cretion in T cell proliferation.

Cytokine measurements

Cells (2 × 10^5/ml) were collected in the presence or absence of OVA (25 μg/ml), and supernatants were collected at intervals of 24 h, 3 days, and 5 days. Culture supernatants were analyzed for the presence of murine IL-10, IL-4, and IFN-γ by OptEIA ELISA kits (BD Pharmingen) according to the manufacturer’s protocol. A standard curve was generated using recombinant cytokines and concentrations of samples were determined by a poly-
nomial curve fit analysis. A similar kit and measurements were used for determination of rIL-10 serum levels. Cells were also exposed separately to Con A (Sigma-Aldrich) stimulation in vitro (1 μg/ml) as a positive control. T cell proliferation

Splenocytes and PLN cells were cultured in the above medium in the presence or absence of OVA (25 μg/ml), and supernatants were collected at intervals of 24 h, 3 days, and 5 days. Culture supernatants were analyzed for the presence of murine IL-10, IL-4, and IFN-γ by OptEIA ELISA kits (BD Pharmingen) according to the manufacturer’s protocol. A standard curve was generated using recombi-
nant cytokines and concentrations of samples were determined by a poly-
nomial curve fit analysis. A similar kit and measurements were used for determination of rIL-10 serum levels. Cells were also exposed separately to Con A (Sigma-Aldrich) stimulation in vitro (1 μg/ml) as a positive control.

Cell isolation, staining, and flow cytometric analysis

The spleen and Peyer’s patches were teased, and lymphocytes were washed and counted before staining for FACS analysis. Intrahepatic lymphocytes (ILH) were isolated by perfusion of the liver with digestion buffer (3 ml of medium (in 1 min) containing collagenase (2 mg/10 ml) and DNase I (0.2 mg/10 ml) at 37°C). After perfusion, the liver was homogenized with an addi-
tional 10 ml of digestion buffer, completed to 40 ml by RPMI 1640 plus 5%
Detection of rIL-10 mRNA in the liver by semiquantitative RT-PCR was apparent in both TG lines (Fig. 2). The same size product was also seen in the spleen and intestine, whereas less expression was observed in lungs of line 1 TG animals.

The composition of lymphocyte subsets from spleens, IHL, lymph nodes, and Peyer’s patches among naïve rIL-10 TG vs non-TG animals is illustrated in Table I. Importantly, both intrahepatic CD4 and TCR α/β cells were significantly reduced in rIL-10 TG mice compared with non-TG mice. Although CD4 subsets from peripheral lymph nodes demonstrated the same pattern, total CD4 cells were significantly increased in the TG Peyer’s patch lymphocytes. Significant decreases were seen in total CD8 lymphocytes obtained from TG spleens and livers. No significant alterations were seen in NK cells obtained from all organs in both TG and non-TG mice. Unlike IL-4-secreting CD4 lymphocytes, significant changes were recorded in IFN-γ CD4 splenocytes. They significantly decreased in the TG splenocytes, reflecting attenuation of the Th1 profile. Results were similar in both TG lines 1 and 2 (data not shown). Although line 1 appeared to have more promiscuous rIL-10 mRNA expression than line 2 (Fig. 2), the distribution of lymphocyte subsets from the Peyer’s patches was similar in both TG lines.

Because the rIL-10 TG mice contain the LPS-responsive C3H/HeN genetic background, they are an appropriate model to investigate the response to oral tolerance. In contrast to these LPS-responsive C3H/HeN mice, lack of oral tolerance was recently reported in the LPS nonresponsive C3H/HeJ strain (35).

Oral tolerance to OVA in rIL-10 TG and wild-type mice

Gastric feeding of OVA significantly reduced PLN T cell proliferative responses to OVA in vitro (Fig. 3). After feeding and systemic immunization, in vitro proliferation of T cells stimulated with 10 μg of OVA per milliliter was reduced 39.4 ± 15.6% in non-TG groups (p = 0.044 between fed and nonfed states), compared with 65.8 ± 14.3% in line 1 TG animals and 72 ± 8% in line 2 TG mice (p < 0.0001 between fed and nonfed states; and p = 0.02 between reduction in non-TG and TG rodents. No significant differences were found between both TG lines). To compare the effect of systemic vs hepatic TG rIL-10 on oral tolerance, non-TG animals were treated with daily i.p. rIL-10 injections. Mean serum rIL-10 by ELISA in rIL-10-treated mice was 128 ± 53 and 176 ± 58 pg/ml within OVA-fed and nonfed groups, respectively. After feeding and systemic immunization, in vitro proliferation of PLN T cells stimulated by 10 μg of OVA per milliliter was reduced 47.5 ± 17.8% in non-TG groups with IL-10 administration (p = 0.02). Subsequently,
reduction of T cell proliferation after OVA feeding was significantly enhanced in IL-10 TG mice (65.8 ± 14.3%) compared with 47.5 ± 17.8% (p = 0.05) and 39.4 ± 15.6% (p = 0.02) in non-TG groups treated with or without systemic IL-10 administration, respectively (Fig. 3). Thus, the effect of hepatic IL-10 on enhanced tolerance was not necessarily due to increased cytokine (IL-10) levels in the blood.

The same pattern was seen in cells stimulated with 100 μg of OVA per milliliter in vitro (data not shown). No significant differences were observed in proliferation of splenocytes. After nonspecific in vitro stimulation by Con A, T cell proliferative responses from PLN and splenocytes were identical in all non-TG and TG-fed or nonfed mice (data not shown). Results were similar in two independent experiments (eight in each subgroup). Animals were sacrificed on day 18, because earlier or later time points failed to show significant changes (data not shown).

**Cytokine production**

Cells from the PLN were cultured in vitro for 1, 3, and 5 days in the presence or absence of 25 μg of OVA per milliliter. After OVA feeding, both non-TG and rIL-10 TG mice exhibited significantly reduced IFN-γ secretion at day 5 in the presence (Fig. 4A) but not in the absence (data not shown) of OVA stimulation in vitro. Serum IFN-γ secretion was decreased from 7536 ± 3325 to 2142 ± 1402 pg/ml in TG mice (p = 0.02), and from 9731 ± 3315 to 3819 ± 3843 pg/ml (p = 0.04) in non-TG animals. However, the reduction in IFN-γ at day 3 (Fig. 4B) was significant only in the TG animals (from 3569 ± 1642 to 1521 ± 276 pg/ml, p = 0.045) and not non-TG mice.

After feeding, in vitro stimulation of PLN with OVA in the TG mice induced a 4-fold increase in IL-4 secretion on day 1 (p = 0.05), but showed no change in the non-TG-fed animals (Fig. 4C). Murine IL-10 secretion after OVA feeding was higher (8.1-fold, p = 0.026) in non-TG but not in TG mice (1.3-fold). This may reflect feedback inhibition of IL-10 by high levels of TG rIL-10 (Fig. 4D).

All animal groups displayed similar cytokine responses after nonspecific stimulation by Con A (data not shown). Results of cytokines were similar in two independent experiments of 32 mice (eight in each subgroup). Cytokine measurements from all other time points fail to show significant differences between animal groups (data not shown).

Serum IgG anti-OVA titers were reduced after OVA feeding in both non-TG and TG groups (Fig. 5). At a 1/1000 dilution, the anti-OVA titer in non-TG animals decreased from 1 ± 0.27 to 0.54 ± 0.24 OD after OVA feeding (p = 0.02). Anti-OVA Ab titers followed the same pattern but were lower in both TG groups, as titers decreased from 0.85 ± 0.32 to 0.48 ± 0.16 OD after OVA feeding (p = 0.05). Results were similar in two independent experiments in a total of 52 mice (13 in each subgroup). Results were not significant at other dilutions including 1/5,000, 1/25,000 (Fig. 5), 1/2,000, or 1/125,000 (data not shown). Animals were sacrificed on day 18, because earlier or later time points failed to show significant changes (data not shown).

All tolerization findings in TG line 1 were similar to those found in line 2, including T cell proliferation (Fig. 3), cytokines, and anti-OVA Abs (data not shown).

**Discussion**

Accumulating evidence has identified a role for IL-10 in mediating oral tolerance. Initial reports suggested that the production of IL-10 was enhanced in low-dose oral tolerance (12, 13, 36), and IL-10-producing clones have been isolated from animals tolerized by feeding myelin basic protein (12, 13, 36). Moreover, mucosal administration of IL-10 orally enhances tolerance after mucosal autoantigen exposure in an experimental model of autoimmune encephalomyelitis (37). IL-10-dependent, OVA-specific TCR TG

---

**Table I. The composition of lymphocyte subsets from spleens, Peyer’s patches, and lymph nodes**

<table>
<thead>
<tr>
<th></th>
<th>αβ TCR (%) (total)</th>
<th>CD4 (%) (total)</th>
<th>CD8 (%) (total)</th>
<th>γδ TCR (%) (total)</th>
<th>NK (%) (total)</th>
<th>IL-4+CD4 (%)</th>
<th>IFNγ+CD4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-TG</td>
<td>39.3 ± 1.2</td>
<td>59.6 ± 1.5</td>
<td>16.6 ± 3.2</td>
<td>0.9 ± 0.3</td>
<td>1.31 ± 0.6</td>
<td>1.7 ± 0.2</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>TG</td>
<td>2 ± 1.3</td>
<td>8.2 ± 4</td>
<td>2.3 ± 1.1</td>
<td>1.2 ± 0.6</td>
<td>1.8 ± 0.9</td>
<td>2.3 ± 1.2</td>
<td>1 ± 0.5</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.001</td>
<td>0.4</td>
<td>&lt;0.001</td>
<td>0.4</td>
<td>0.07</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-TG</td>
<td>54.7 ± 2.7</td>
<td>45.2 ± 2.9</td>
<td>3.8 ± 2.4</td>
<td>1 ± 0.7</td>
<td>0.2 ± 0.1</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>8.7 ± 0.4</td>
<td>5.6 ± 1.7</td>
<td>0.3 ± 0.4</td>
<td>0.4 ± 0.4</td>
<td>0.4 ± 0.5</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.001</td>
<td>&lt;0.0001</td>
<td>0.14</td>
<td>0.29</td>
<td>0.09</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Peyer’s patch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-TG</td>
<td>74 ± 20</td>
<td>31 ± 6</td>
<td>36 ± 6</td>
<td>2 ± 1</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>TG</td>
<td>1.6 ± 1.4</td>
<td>0.8 ± 0.6</td>
<td>0.8 ± 0.7</td>
<td>0.4 ± 0.4</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>p value</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-TG</td>
<td>68.7 ± 50</td>
<td>11.8 ± 6.8</td>
<td>33 ± 19</td>
<td>2 ± 1</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>TG</td>
<td>1.5 ± 1.3</td>
<td>0.5 ± 0.51</td>
<td>0.7 ± 0.6</td>
<td>0.4 ± 0.4</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>p value</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>αβ TCR (%) (total)</td>
<td>32.5 ± 7.8</td>
<td>56.5 ± 12</td>
<td>13.3 ± 6.7</td>
<td>1.9 ± 0</td>
<td>1.4 ± 0.3</td>
<td>2.1 ± 1</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>p value</td>
<td>0.10</td>
<td>0.32</td>
<td>0.8 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>1.3 ± 0.4</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>library</td>
<td>0.2</td>
<td>0.06</td>
<td>0.04</td>
<td>0.4</td>
<td>0.07</td>
<td>0.1</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Total millions of cells. Bold text indicates statistical significance.
TG, Rat IL-10 transgenic mice. IFN-γ.
NA, Not available.
T lymphocytes can also mediate nonspecific bystander suppression of experimental colitis when adoptively transferred in vivo followed by oral administration of OVA (10). Similarly, there is now solid evidence for the existence of CD4\(^+\) cells (Th3, Tr1) that produce IL-4, IL-10, and TGF-β. Analogous populations of IL-10-dependent regulatory cells (Tr1) that produce TGF-β are capable of suppressing a murine model of inflammatory bowel disease (38), further highlighting a role of IL-10 in mucosal immune regulation. Finally, in low-dose oral tolerance to Ag in a uveitis model, both IL-4 and IL-10 are required for induction of protective oral tolerance that is dependent upon regulatory cytokines (34).

In contrast, some studies have refuted a role for IL-10 in oral tolerance. For example, marked suppression of IL-10 occurs in mice fed OVA (14), and anti-IL-10 treatment did not block either the induction or the maintenance of orally induced tolerance to OVA (39). Studies of oral tolerance in IL-10 KO animals have shown that IL-10 is required for oral tolerance induction (3). Although IL-10 is not required for experimental autoimmune uveitis induction in IL-10 KO animals, both IL-10 and IL-4 are required for the induction of protective oral tolerance (34).

One interpretation of these conflicting results is that the effects of IL-10 might either depend on the site of production and/or whether elevated levels are intermittent (for example, after exogenous administration) or sustained. In the present study we have assessed the impact of IL-10 on oral tolerance to OVA when secreted from TG hepatocytes in a sustained manner. We find a significant reduction of T cell proliferation in TG animals tolerized to OVA, compared with non-TG mice. This reduction was less clear when IL-10 was administered systemically instead (Fig. 3) (\(p = 0.20\), NS).

![FIGURE 3. Ag-specific T cell proliferation. PLN cells isolated 10 days after footpad immunization with OVA/adjuvant were cultured in the presence or absence of OVA (10 \(\mu\)g/ml) for 72 h, followed by a 16-h pulse with 1 \(\mu\)Ci of \(^{3}H\)thymidine. T cell proliferation was reduced after feeding from 45,018 ± 18,452 to 25,836 ± 16,304 cpm in wild-type mice (\(p = 0.027\)), from 51,542 ± 24,302 to 17,563 ± 6,017 cpm in all TG animals (\(p = 0.001\)) and from 51,342 ± 10,722 to 38,553 ± 39,854 cpm in systemic IL-10-treated groups (\(p = 0.05\)). T cell proliferative responses were significantly reduced after feeding by 65.8 ± 14.3% in line 1 rIL-10 TG animals and 72 ± 8% in line 2 (\(p < 0.0001\) between fed and nonfed states), no significant differences were seen between both TG lines) compared with a less significant (\(p = 0.02\)) reduction (39.4 ± 15.6%, \(p = 0.044\) between fed and nonfed states) in the non-TG mice. To compare the effect of systemic vs the hepatic IL-10 on oral tolerance, non-TG animals were treated with daily i.p. rIL-10 injections instead of using the rIL-10 TG mice. After feeding, in vitro proliferation of T cells stimulated by 10 \(\mu\)g of OVA per milliliter was reduced 47.5 ± 17.8% in non-TG groups (\(p = 0.02\) between fed and nonfed states), which was less than IL-10 TG (\(p = 0.05\)) and similar to the reduction seen in the non-TG that were not treated with IL-10. Results were generated in two independent experiments (eight mice in each subgroup).]

![FIGURE 4. Cytokine production in low-dose OVA oral tolerance in rIL-10 TG mice. Cells from the PLNs of unfed or OVA-fed and from rIL-10 TG and wild-type OVA-immunized mice were cultured with 10 \(\mu\)g of OVA for 24 –120 h (A) or without (data not shown), and cytokine secretion was measured by ELISA. Although results indicate that fed mice had a decrease of IFN-γ at 120 and 72 h (A and B) and increased secretion of IL-4 at 24 h (C), rIL-10 TG mice showed enhanced changes compared with non-TG animals. Mouse IL-10 secretion at 72 h (D) was increased in fed mice compared with unfed mice, but was less prominent in the rIL-10, compared with non-TG immunized mice, possibly due to an inhibitory effect of TG rIL-10 on mouse IL-10 secretion. Results were similar in two independent experiments of 32 mice (eight each subgroup).]
Moreover, these TG mice displayed more marked suppression of proinflammatory responses by switching to an anti-inflammatory cytokine profile, manifested by a more significant decrease in IFN-γ secretion and a greater enhancement of IL-4 secretion.

IL-10 may enhance oral tolerance in our model by a number of mechanisms, but the hepatic secretion of the cytokine appears the most important because systemic administration of IL-10 did not fully replicate the findings in TG IL-10 mice. There may also be indirect actions from the systemic effects of high circulating levels of rIL-10 and/or after Ag processing and exposure to especially high concentrations of IL-10 in the liver.

Mucosal tolerance is thought to reflect the impact of the local mucosa-associated lymphoid tissue environment that influences Ag presentation where regulatory cells are being generated. Alternatively, mucosal tolerance might be mediated by modulating MHC class-II expression on APCs or effects upon costimulatory molecules (CD80/86). Mucosal tolerance is also regulated by the cytokine microenvironment as well as the immunogenic properties of the specialized cells that line the intestinal surface, the M cell and the absorptive epithelial cell (40, 41). In humans, these absorptive epithelial cells express MHC class I, class II, and nonclassical class I molecules (42), as well as a unique costimulatory molecule that induces the proliferation of CD8⁺ T cells in culture (43). Treatment of dendritic cells with IL-10 decreases their capacity to stimulate CD4⁺ T cells in a MLR. IL-10 also stimulates immature DC to become tolerogenic APCs (44) and preferentially primes naïve T cells to generate Th2-type cells in vitro and in vivo (45). More recent studies suggest that production of IL-10 by lamina propria APCs might participate in local immunoregulation in the gut (46, 47).

Our model allows us to address the liver’s role in oral tolerance and to examine the impact of high local concentrations of IL-10. The liver is increasingly recognized as an immunoregulatory organ that participates in oral tolerance (29). Induction of peripheral immune tolerance appears to require first-pass clearance of specific subpopulations of cells or peptides via the portal circulation from the intestine through the liver (30, 48). As noted above, diversion of portal blood from the liver by portacaval shunt, or blockade of Kupffer cell function abrogates oral tolerance in animal models (30). In support of this conclusion, elevated titers of Abs to intestinal flora have been reported in humans with chronic liver diseases who have undergone portocaval shunts (30, 31). Additionally, by administering properly modified alloantigen into the hepatic environment by portal vein inoculation, prolonged T cell anergy can be induced, which allows for the indefinite acceptance of donor-specific heterotopic cardiac allografts (31).

Although our data cannot conclusively distinguish between effects of circulating vs local hepatic rat IL-10, serum rIL-10 levels were not elevated in all TG mice. Despite this, effects on oral tolerance were consistently observed in all animals. In fact, 20% of rIL-10 TG mice had normal IL-10 serum levels (Fig. 1), yet all had tolerance responses indistinguishable from animals with high serum IL-10 levels, including equivalent T cell proliferation, anti-OVA Abs and cytokine alterations (data not shown).

Based on these data, locally enhanced hepatic expression of rIL-10 is likely to play an important role in the enhanced oral tolerance in our TG mice. This conclusion is supported by evidence of altered ILH composition in TG animals. ILH normally include αβ TCR (TCR αβ⁺) cells, T cells with the TCR γδ⁺, classic NK cells (NK), NK cells with the TCR (NK-T), and dendritic cells (49). The hepatic CD4 to CD8 ratio is the opposite of the lymph node, with CD8⁺ cells predominating, but ~20% of TCR αβ⁺ cells are TCR αβ low and negative for CD4 and CD8 (TCR low-DN). The liver also contains small numbers of a lin-“c-kit” population, which may have pluripotent potential (48–50). In contrast, IL-10 hepatocyte TG mice displayed a significant decrease of intrahepatic CD4 and TCR αβ cells compared with non-TG animals (Table I). These changes would favor the persistence of oral tolerance by down-regulation of Th1 CD4⁺ cells or CD4⁺ T-reg as a result of prolonged exposure to high IL-10 levels. Similar localized anti-proliferative changes were also reported in an IL-10 gene transfer model in transplanted hearts, which showed an increase of Fas/FasL mediated apoptosis of CD4 and CD8 cells, accompanied by decreased apoptosis of myocytes and a decrease in rejection episodes (51).

The data presented in this paper are in line with a recent study (52), which reported that the liver plays an important role in inducing peripheral tolerance in a mucosal tolerance model, especially after feeding high-dose OVA. In this report, the livers from BALB/c mice fed with OVA at either a low or high dose were transplanted into syngeneic recipients. Nonfed recipients were controls. Orthotopic liver transplantation was followed by OVA immunization and delayed-type hypersensitivity challenge. Livers from all OVA-fed mice after 10 days transferred tolerance to OVA-naive mice. The in vitro proliferative response of the liver nonparenchymal cells to OVA revealed a decreased response in both dosage groups over the control group.

The pharmacokinetics of IL-10 secretion in rIL-10 TG mice might also favor development of oral tolerance. Specifically, elevated levels of IL-10 are sustained through constant production by TG hepatocytes, in contrast to intermittent administration of exogenous IL-10, as previously evaluated (37, 46). The specific impact of sustained elevations of IL-10 merits further evaluation. In summary, our data establish IL-10 as an important immunomodulatory protein that enhances oral tolerance toward OVA when expressed locally via hepatocyte secretion. In this model, IL-10 modifies cytokine secretion (with less proinflammatory responses in TG animals), reduces T cell proliferation, and decreases anti-OVA titers. Based on these data and related studies (39, 50), as well as the acceptable safety profile of IL-10 in human studies, in vivo augmentation of IL-10 might enhance the effectiveness of oral tolerance regimens currently being tested in a variety of chronic diseases. Additionally, our model of hepatic overexpression of TG rat IL-10 may prove useful for exploring the role of IL-10 in other hepatic responses, including immunologic injury, cholestasis, metabolic derangements, or hepatic fibrosis.
Acknowledgments
We acknowledge the Mount Sinai Mouse Genetics Shared Research Facility for the production of the founder TG animals.

Disclosures
The authors have no financial conflict of interest.

References

ton nonresponsiveness is maintained in the absence of functional Th1 or Th2 cells. J. Immunol. 163: 5143–5148.
23. Arese, L. S., F. Cardillo, D. A. De Albuquerque, N. M. Vaz, and J. Mengel. 1995. Anti-IL-10 treatment does not block either the induction or the mainte
tion of antigen trafficking to MHC class II-positive late endosomes of entero
32. Fleming, K. A. 1999. The anatomy of the normal liver and the hepatic lympho
cyte. In T Lymphocytes in the Liver; Immunobiology, Pathology and Host De