IL-10 modulates intestinal damage and epithelial cell apoptosis in T cell-mediated enteropathy

Pengfei Zhou, Cathy Streutker, Rajka Borojevic, Yufa Wang, and Ken Croitoru. IL-10 modulates intestinal damage and epithelial cell apoptosis in T cell-mediated enteropathy. Am J Physiol Gastrointest Liver Physiol 287: G599–G604, 2004; 10.1152/ajpgi.00063.2004.—In vivo T cell activation by anti-CD3 monoclonal antibody (mAb) results in intestinal damage characterized by loss of villi and epithelial cell apoptosis. The role of the increased interleukin (IL)-10 released during this process is not clear. We assessed the effects of IL-10 on T cell-induced mucosal damage in vivo using IL-10-deficient C57BL/6 [IL-10 knockout (KO)] mice. IL-10 KO and wild-type C57BL/6 mice were injected with anti-CD3 mAb and observed for diarrhea. Changes in serum cytokine levels were measured by ELISA. Histological changes and epithelial cell apoptosis were analyzed on hematoxylin- and eosin-stained tissue sections. Fas expression on intestinal epithelial cells was assessed by flow cytometry analysis of freshly isolated intestinal epithelial cells. Anti-CD3-treated IL-10 KO mice developed more severe diarrhea, a greater loss of intestinal villi, and an increase in the numbers of apoptotic cells in the crypt epithelium. This difference in IL-10 KO mice was associated with an increase in serum tumor necrosis factor-α and interferon-γ levels and with an increase in Fas expression on fresh, isolated, small intestinal epithelial cells. In addition, the enhanced intestinal tissue damage induced by anti-CD3 in IL-10 KO mice was significantly diminished by treatment with recombinant murine IL-10. Therefore, the lack of IL-10 allowed for an increased T cell-induced intestinal tissue damage, and this was associated with an increase in T cell cytokine release and an increase in epithelial cell Fas expression.

T cell receptor; interferon-γ; tumor necrosis factor-α; anti-CD3; Fas

INFLAMMATORY DISEASES of the bowel, such as celiac diseases, graft-vs.-host disease (GvHD), and the idiopathic inflammatory bowel diseases, are associated with excessive T cell activation (7, 24, 36). Systemic administration of a monoclonal antibody (mAb) against the e-chain of the CD3 molecule (145–2C11) induces polyclonal T cell activation that results in a severe diarrheal syndrome with intestinal damage characterized by increased intestinal epithelial permeability, crypt cell apoptosis, and villus flattening (31, 32, 34). The mechanism of anti-CD3-induced intestinal mucosal damage involves a combination of pathways, including tumor necrosis factor (TNF)-α, interferon (IFN)-γ, perforin, and Fas-FasL (10, 29, 31). The redundancy of cytotoxic T cell mechanisms involved in this enteropathy indicates that inhibiting any one pathway is unlikely to prevent all aspects of immunopathology associated with T cell-induced enteropathies.

The self-limiting feature of anti-CD3-induced enteropathy suggests that regulatory forces serve to minimize and turn off the tissue-damaging effects of T cell activation. The anti-inflammatory and immunosuppressive cytokine interleukin (IL)-10 is important in maintaining intestinal homeostasis, as evidenced by the observation that IL-10-deficient mice [IL-10 knockout (KO)] spontaneously develop colitis (19). In patients with Crohn’s disease, a decreased IL-10 concentration in the ileum predicted a higher risk of disease recurrence (30). Systemic IL-10 treatment or IL-10 gene transfer has been used in several animal models of inflammatory bowel disease, such as IL-10 KO mice (5, 22), severe combined immunodeficiency-transfer colitis (2), trinitrobenzenesulfonic acid, or dinitrobenzenesulfonic acid-induced colitis (4, 21), with some amelioration of colitic inflammation. Because most models of intestinal inflammation primarily involve the large intestine, the role of IL-10 in maintaining small intestine integrity during inflammatory conditions is less clear.

In the anti-CD3-induced enteropathy model, T cell activation stimulates both proinflammatory cytokine and anti-inflammatory cytokine release, including IL-10 (8, 16, 38). Given the potent anti-inflammatory and immunosuppressive effects of IL-10, we reasoned that IL-10 might serve to restore and maintain immunological balance and epithelial barrier integrity during local inflammatory events. This study shows that IL-10 significantly altered the severity of T cell-mediated intestinal damage in mice, and this was associated with changes in proinflammatory cytokine secretion and small intestinal epithelial cell (IEC) expression of Fas.

MATERIALS AND METHODS

Mice. Four- to six-week-old wild-type C57BL/6 (BL/6) and C57BL/6-IL-10(−/−) (IL-10 KO) mice were purchased from Jackson Laboratory (Bar Harbor, ME). All mice were housed under specific pathogen-free conditions. This study was approved by the McMaster University Animal Care Committee and conforms to the guidelines of the Canadian Council on Animal Care.

Reagents and in vivo treatments. The hybridoma for anti-mouse CD3-ε (mAb; 145–2C11) was provided by Dr. J. Bluestone (University of Chicago). Hamster IgG (HlgG) control mAb was purchased from Rockland (Gilbertsville, PA). Recombinant murine IL-10 (rmIL-10) was kindly provided by Dr. S. Narula (Shering-Plough Research Institute, Kenilworth, NJ). Groups of mice were treated with a single intraperitoneal injection of 25–50 µg anti-CD3 or control HIgG mAb diluted in 200 µl PBS, pH 7.4. Some of the IL-10 KO mice were given rmIL-10 (10 µg/mouse, in 100 µl PBS) 1 h before and 16 h after anti-CD3 treatment. Mice were monitored for change in body temperature and diarrhea. Body temperature was recorded before and 3, 6, 12, and 24 h after anti-CD3 administration, by means of a digital microthermometer.

Histology evaluation. Mice were killed at 24 or 48 h after anti-CD3 treatment. Hematoxylin- and eosin (H&E)-stained tissue sections of...
The body temperature and mortality in IL-10 KO mice and wild-type mice after anti-CD3 treatment are shown in Table 1.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Rectal Temperature, °C</th>
<th>Mortality at 24 h, %</th>
<th>n/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10 KO</td>
<td>29.8 ± 0.6*</td>
<td>36.5 ± 0.9</td>
<td>64* 7/11</td>
</tr>
<tr>
<td>Wild type</td>
<td>34.4 ± 0.2</td>
<td>35.9 ± 0.4</td>
<td>0       0/9</td>
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</tbody>
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Values represent means ± SE; n, no. of mice. KO, knockout. *P < 0.05 vs. wild-type mice.

Evaluation of Fas expression on the surface of IECs by flow cytometry. Freshly isolated IEC were suspended in PBS/0.1% BSA at 1 × 10⁶ cells/ml. The cell suspension (100 μl) was added to Eppendorf tubes, washed one more time in PBS/0.1% BSA, and stained with phycoerythrin (PE)-conjugated anti-Fas mAb (BD Biosciences, Mississauga, ON). Subsequently, cells were fixed in 0.5 ml 1.0% paraformaldehyde and analyzed by flow cytometry showing that <3% of isolated cells expressed CD3.

RESULTS

T cell activation-induced enteropathy was more severe in mice deficient in IL-10. IL-10 KO and wild-type mice were injected with a single intraperitoneal dose of anti-CD3 mAb (50 μg). Mice were observed for clinical signs of diarrhea, rectal prolapse, piloerection, and changes in overall mobility. No lethality was observed in wild-type mice injected with...
anti-CD3; whereas IL-10 KO mice treated with anti-CD3 developed severe hypothermia, and 64% of mice had to be killed in the first 24 h after anti-CD3 treatment (Table 1). To avoid the high rate of lethality in the IL-10-deficient mice, wild-type and IL-10 KO mice were injected intraperitoneally with 25 μg anti-CD3 in subsequent experiments.

Anti-CD3 mAb treatment of wild-type BL/6 mice caused a decrease in villus length and an increase in the number of apoptotic bodies in the crypt region over 24 and 48 h, as previously described (Fig. 1, A and B). In IL-10 KO mice, anti-CD3 treatment resulted in a more significant shortening of villi, at both 24 and 48 h after treatment (Fig. 2B). Therefore, in the absence of IL-10, T cell activation resulted in more severe mucosal damage. No histopathological damage was noted in either wild-type or IL-10 KO mice treated with HlgG.

IL-10 deficiency alters anti-CD3-induced cytokine release. To evaluate the mechanisms by which endogenous IL-10 can modulate T cell-induced enteropathy, we measured serum TNF-α and IFN-γ levels 2 and 24 h after anti-CD3 injection. Consistent with a previous study (11), anti-CD3 injection induced an increase in serum levels of TNF-α and IFN-γ in wild-type mice, peaking at 2 h and decreasing by 24 h. In IL-10 KO mice, there was a threefold increase of serum levels of TNF-α and a sixfold increase of IFN-γ at 2 h. These remained elevated 24 h after anti-CD3 treatment compared with that of wild-type mice (Fig. 3, A and B). Therefore, in the absence of IL-10, anti-CD3 activation of T cells in vivo induced a significantly higher and protracted increase of proinflammatory cytokine secretion.

![Figure 2](http://ajpgi.physiology.org/)

**Fig. 2.** Mucosa tissue damage induced by T cell activation is enhanced in the absence of IL-10. A: T cell activation induces villus blunting in the small intestine. Intestinal villus and crypt lengths were measured in hematoxylin- and eosin (H&E)-stained tissues from mice 24 and 48 h after anti-CD3 treatment. Intestinal villus-to-crypt ratios were calculated by measuring at least 10 villus/crypt units under ×200 magnification. B: T cell activation induces apoptosis in crypt enterocytes in the small intestine. Apoptosis was determined as the presence of pyknotic nuclei, condensed chromatin, and nuclear fragmentation on H&E-stained small intestinal tissue sections. The apoptotic index was calculated as no. of apoptotic enterocytes/5 villus/crypt units. Results are presented as means ± SE and are from 3 independent experiments with 3–5 mice in each group. *P < 0.05, IL-10 KO vs. wild-type mice. #P ≤ 0.05, anti-CD3 vs. hamster IgG (HlgG) treatment.

![Figure 3](http://ajpgi.physiology.org/)

**Fig. 3.** Increases in serum tumor necrosis factor (TNF)-α and interferon (IFN)-γ levels were greater after anti-CD3 injection of IL-10 KO mice compared with wild-type mice. Serum levels of TNF-α (A) and IFN-γ (B) were measured at 2 and 24 h after anti-CD3 injection. The results represent mean values ± SE from 3 independent experiments with 3–5 mice in each group. *P < 0.01.
T cell activation-induced enhancement of Fas expression on IEC was increased in the absence of IL-10. We previously reported that Fas-FasL interactions were required for T cell activation-induced IEC apoptosis. The absence of Fas-FasL significantly diminished anti-CD3-induced apoptosis and mucosal tissue damage (31). To evaluate whether IL-10 influenced the Fas/FasL pathway of apoptosis, we examined the effect of IL-10 on Fas expression on IEC isolated after anti-CD3 treatment. Small IEC isolated from mice 12 h after anti-CD3 injection were stained with PE-conjugated anti-Fas mAb and analyzed by flow cytometry. The level of Fas expression was evaluated by measuring the mean fluorescence intensity (MFI) of staining with PE-anti-Fas mAb. As shown in Fig. 4, A and B, Fas was constitutively expressed on both wild-type and IL-10 KO mice, albeit at a low level of fluorescence intensity in control mice (MFI 23.5 ± 6.9 and 22.3 ± 2.6, respectively, P > 0.05). After anti-CD3 treatment (12 h), there was a significant increase in the MFI of Fas expression on both IL-10 KO and wild-type mice-derived IEC (Fig. 4, A and B); however, the increase in Fas expression was more pronounced on IEC from IL-10 KO mice compared with wild-type mice (MFI 56.5 ± 2.9 and 35.8 ± 2.4, respectively, P < 0.05; Fig. 4C). IL-10 deficiency alone had no effect on the constitutive expression of Fas on epithelial cells (data not shown).

Exogenous IL-10 significantly diminished the enhanced intestinal tissue damage resulting from anti-CD3 treatment of IL-10 KO mice. To further determine if the enhanced intestinal damage that occurred in the IL-10 KO mice after anti-CD3 injection was the result of the lack of IL-10, rmIL-10 (10 μg/mouse) was injected intraperitoneally into 5- to 6-wk-old IL-10 KO mice 1 h before and 16 h after anti-CD3 (25 μg/mouse) treatment. This dose of IL-10 treatment was previously shown to prevent the development of enterocolitis in IL-10 KO mice (5). In our study, two doses of IL-10 treatment significantly diminished the enhanced intestinal tissue damage in IL-10 KO mice after anti-CD3 treatment. The villus-to-crypt ratio was significantly increased when compared with anti-CD3-treated IL-10 KO mice not receiving rmIL-10 (2.3 ± 0.2 vs. 1.6 ± 0.1, P ≤ 0.01; Fig. 5A), whereas the number of apoptotic cells per 5 villus/crypt units was reduced (19.0 ± 2.3 vs. 41.9 ± 5.6, P ≤ 0.05; Fig. 5B). Both the villus-to-crypt ratio and number of apoptotic cells in IL-10 KO mice treated with rmIL-10 and anti-CD3 were comparable to that of anti-CD3-treated BL/6 mice. These results showed that exogenous IL-10 treatment diminished the enhanced T cell activation-mediated intestinal tissue damage observed in the IL-10 KO mice.

DISCUSSION

In this study, we showed that mice deficient in IL-10 developed a more severe intestinal enteropathy in response to direct in vivo anti-CD3-induced T cell activation. We observed that IL-10 KO mice were more susceptible to anti-CD3 (50 μg)-induced lethality. Treating IL-10 KO mice with 25 μg anti-CD3 also led to a significantly more severe mucosal damage compared with wild-type mice. This mucosal damage was characterized by an increase in loss of villi and an increase in the number of apoptotic bodies in the intestinal crypts over that seen in wild-type mice. In addition, treatment of IL-10 KO mice with rmIL-10 significantly reduced the enhanced intestinal tissue damage induced by anti-CD3-mediated T cell activation.

To further understand the potential mechanisms by which the absence of IL-10 contributes to the enhanced immune-mediated mucosal damage, we measured the cytokine responses and the changes in Fas expression on IEC. Shortly after anti-CD3 injection, IL-10 KO mice produced significantly
greater levels of the proinflammatory cytokines TNF-α and IFN-γ. These results are in agreement with previous studies showing that IL-10 can influence T cell responses to in vivo T cell receptor-mediated activation (12). The increase in both TNF-α and IFN-γ could account for the increase in mucosal tissue damage in that previous studies have shown that TNF-α and IFN-γ act synergistically to induce apoptosis in IEC (14). Systemic administration of TNF-α alone induced an enteropathy characterized by epithelial cell apoptosis and villous shortening, and IFN-γ is involved in the apoptotic response in the intestine during GvHD (13, 15, 39). Neutralization or deletion of TNF-α or IFN-γ was shown to decrease IEC apoptosis (27, 39). Therefore, one explanation for the increased mucosal tissue damage is that the absence of IL-10 regulation of T cell activation results in the dramatically enhanced secretion of cytokines that directly damage IEC. On the other hand, we had previously shown (31) that the absence of either IFN-γ alone or both TNF-α receptors was not sufficient to prevent anti-CD3-induced damage associated with this enteropathy. Even though increases in proinflammatory cytokines such as TNF-α and IFN-γ in IL-10-deficient mice may contribute to the increased epithelial cell apoptosis after anti-CD3 treatment, we cannot exclude the possibility that IL-10 influences other pathways involved in T cell activation-induced tissue damage.

We have previously shown (31) that the Fas/FasL pathway was required for T cell activation-induced IEC apoptosis and T cell-induced enteropathy. Mice lacking either Fas (lpr mice) or FasL (gld mice) have a significantly reduced enteropathy after anti-CD3 injection (31). Proinflammatory cytokines such as TNF-α can act synergistically with Fas ligation in causing target cell damage (28, 37). In agreement with others, our data showed that Fas is expressed constitutively on small IEC (20). In vitro studies (1, 33) have shown that Fas-expressing cells can be resistant to Fas-mediated cell death under normal conditions but become susceptible in certain inflammatory conditions. Our data demonstrated that in vivo anti-CD3-induced T cell activation upregulated Fas expression on IEC, and this increase was more significant in the IL-10 KO mice compared with wild-type mice. The mechanism by which IL-10 regulates Fas expression on IEC is not clear. It is possible that this is because of indirect effects on proinflammatory cytokine release, although it remains possible that IL-10 has a direct role on epithelial cells as well. Indeed, it has been shown that IEC express IL-10 receptors (6).

The ability of IL-10 to regulate intestinal inflammation, predominantly through the suppression of the activation and effector function of T cells, monocytes, and macrophages, is well described (3, 19, 26). The ability of IL-10 to modulate the immune-mediated epithelial cell damage that characterizes enteropathies has not been well defined. Given that IEC express IL-10 receptors, IL-10 could have direct effects on epithelial function, e.g., IL-10 influences epithelial cell barrier function (23, 25), modulating major histocompatibility antigen class II expression and changing epithelial cell growth and viability (6, 17). Therefore, under inflammatory conditions such as those initiated by activation of T cells, both Fas expression and the susceptibility of IEC to Fas-mediated killing are markedly enhanced in IL-10-deficient mice. The resulting increase in mucosal damage in IL-10 KO mice suggests that IL-10 plays an important role in the modulation of epithelial cell injury incited by immune cell activation.

The findings described in this study demonstrate the modulatory function of IL-10 on T cell activation-induced enteropathy and the accompanying epithelial cell death. These findings are relevant to inflammatory disorders of the intestine in which there is extensive cytokine release. This study provides direct evidence that IL-10 can modulate IEC death in vivo and further suggests that IL-10 may contribute to therapeutic strategies for the treatment of a wide range of inflammatory bowel diseases in spite of disappointing results from clinical studies using recombinant human IL-10 in the treatment of Crohn’s disease (9, 35).

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