Proinflammatory properties of IL-4 in the intestinal microenvironment

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Van Kampen, C., J. Gauldie, and S. M. Collins. Proinflammatory properties of IL-4 in the intestinal microenvironment. Am J Physiol Gastrointest Liver Physiol 288: G111–G117, 2005; doi: 10.1152/ajpgi.00014.2004.—IL-4 is involved in type 2 T helper cell (Th2)-type immune responses and, in some cases, can promote Th1 responses. However, the proinflammatory potential of IL-4 alone is unclear. In this study, we examined the ability of IL-4 to induce colitis after its overexpression in the colon using an adenoaviral vector (Ad5) and compared results with those obtained after overexpression of IL-12, a cytokine implicated in several models of colitis. Overexpression of IL-4 or IL-12 caused a fatal colitis within 24 h in 60% of animals and was dose and strain dependent. IL-12-induced colitis was accompanied by the local expression of IFN-γ and TNF-α but not IL-4 mRNA and protein. Conversely, IL-4-induced colitis was accompanied by the local expression of IL-4 and TNF-α but not IFN-γ mRNA and protein. The Ad5-IL4-induced colitis did not persist beyond 3 days and was present in recombinase activation gene-2 (RAG-2)-/− mice but not in STAT6−/− mice. Acute lethal colitis induced by Ad5IL12 was T cell mediated and IFN-γ receptor (IFN-γR) dependent. Furthermore, TNF-α was found to be important in the pathogenesis of Ad5IL-4 and Ad5IL-12-induced colitis. Results of this study indicate that IL-4 alone can act as a proinflammatory cytokine in the gut of normal mice, inducing a rapid onset and short-lived colonic injury while maintaining a Th2-type cytokine profile that functions via a local T cell-independent mechanism involving TNF-α.

colitis; adenovirus; cytokine; gene transfer

CYTOKINE REGULATION OF HOST responses has been implicated as a primary mechanism of pathogenesis in Crohn’s disease and ulcerative colitis. The importance of the cytokine balance in the gastrointestinal tract has been established due to the development of mice deficient in regulatory cytokines and from studies of the cytokine profiles of patients with inflammatory bowel disease (IBD). These studies have identified T lymphocytes as being of principal importance in the development and chronicity of IBD. Initially, murine cytokine profiles categorized T helper cells into Th1 and Th2 subsets (20). Th1 cells produce IL-2, IFN-γ, and lymphotoxin and regulate cell-mediated immune responses, such as delayed-type hypersensitivity reactions, whereas Th2 cells produce IL-4 and IL-5 and are important regulators of antibody-mediated immune responses. Subsequently, it has been shown that the Th1/Th2 paradigm described in mice is much more complicated and is not necessarily identical in other species.

IL-4 is a pleiotropic cytokine with regulatory effects on B cell growth, T cell growth and function, immunoglobulin switching, hematopoietic cells, and tumor cells (21). There is evidence to support both anti- and counterinflammatory roles for IL-4 in experimental models of intestinal inflammation. IL-4 downregulates Th1-associated injury in T cell receptor α-chain-deficient mice (15), but ameliorates colitis induced by hapten (14) or dextran sulfate sodium (23). Dohi et al. (7, 8) found that hapten-induced colitis shifts from Th1 to a Th2 response during the resolution phase, again suggesting the counterinflammatory properties of Th2 cytokines. The number of studies that support IL-4 as a proinflammatory mediator of injury in the intestine are quite limited. In contrast to extensive literature on Th1-based models of colitis, Th2-driven models of colitis are few and include colitis induced by oxazalone (2) and that seen in T cell receptor (TCR)-α−/− mice (18). IL-4 has also been shown to enhance inflammation in a Th1 cell-mediated transfer model of colitis (11).

Studies examining the effects of IL-4 in the intestine have used several established models of experimental colitis and have shown that the effects of IL-4 are dependent on the particular model of colitis. Thus the aims of this study were to determine the potential for IL-4 to induce intestinal inflammation in normal naive mice and to examine underlying mechanisms. We also determined the extent to which IL-4-induced colitis resembles that induced by a classic proinflammatory cytokine such as IL-12. This was determined by the use of a replication-defective adenovirus vector (Ad5) to overexpress either IL-4 or IL-12 directly in the colonic mucosa. We showed that the overexpression of IL-4 in the colonic mucosal environment of healthy mice induces a potentially fatal colitis but that this does not occur when Ad5IL-4 is administered systemically. In contrast to colitis induced by IL-12, the damage induced by IL-4 is less severe, of shorter duration, and is T cell independent. These results provide support for the proinflammatory properties of a Th2 cytokine in the intestinal compartment.

MATERIALS AND METHODS

Animals. Specific pathogen-free, 8- to 12-wk-old male Balb/c (Jackson Laboratories, Bar Harbor, ME), C57BL/6 (Taconic, Germantown, NY), and NIH Swiss (NCI, Frederick, MD) mice were housed in Level B clean rooms. Various knockout mouse strains including CD4, IFN-γ receptor (IFN-γR), TNF-α receptor (TNF-αR), and recombinase activation gene-2 (RAG-2)-deficient animals were obtained from Jackson Laboratories. Breeding pairs of STAT6−/− mice were generously provided by Dr. K. I. Matthaei of the John Curtin School of Medical Research (Canberra, ACT Australia). Mice lacking the IFN-γ gene were generously provided by Dr. M. Inman, McMaster University (Hamilton, ON, Canada). C57BL/6 mice, also from Taconic were used as controls for the CD4, TNF-αR, and STAT6−/− mice, and Balb/c mice from Jackson Laboratories were used as controls for all other knockout strains.

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Mast cell-deficient mice (WWv+/−) and littermate controls (WWv++/+) were purchased from Jackson Laboratories. All mice were kept in sterilized filter-topped cages and had ad libitum access to autoclaved food and water during the study. Each experimental group of animals, controls, and test subjects, including all wild-type and knockout strains, contained 6–10 animals. The protocols used in this study were in accordance with guidelines drafted by McMaster Animal Care Committee and Canadian Council on the Use of Laboratory Animals.

Reagents. Human Ad5 lacking the E1 replication region of its genome were transfected with murine IL-12 cDNA (Ad5IL-12) or murine IL-4 cDNA (Ad5IL-4) according to established procedures (3, 25). Cytokine ELISA kits for detection of TNF-α, IFN-γ, and IL-4 were purchased from R&D Systems (Minneapolis, MN), and for IL-12, they were purchased from Biosource International (Medicorp, Montreal, PQ, Canada). All reagents for RT-PCR procedures were purchased from GIBCO-BRL and Sigma (St. Louis, MO). Primers for cytokine mRNA analysis were synthesized by MOBIX (McMaster University).

Study protocol. Mice were anesthetized with enflurane (Abbott Laboratories, St. Laurent, PQ, Canada). Three hours before administering the Ad5 vectors, 150 μl of 50% ethanol in PBS were introduced into the colon at ~4 cm proximal to the rectum, using a polyethelene 90 catheter, attached to a 20-gauge needle and 1-ml syringe. Ad5 vectors were suspended in PBS at concentrations of 10⁷-10⁹ plaque-forming units (pfu)/mouse and administered intrarectally in 100-μl volumes. Some mice received only the vector intraperitoneally at 10⁹ pfu/mouse. Control mice were given 50% ethanol only. There were 6–10 mice in each experimental group, and each experiment was repeated at least twice.

On days 1, 3, 7, and 14, after Ad5 administration, full-thickness samples were removed from an area ~6 cm proximal to the anus in control mice or from the upper margin of the macroscopically damaged area in the inflamed distal colons. Samples for histology were fixed in 10% formalin, and samples for cytokine mRNA and protein measurements were snap frozen in liquid nitrogen and stored at −70°C until needed. Assessment of macroscopic and microscopic damage was based on a modification of a previously published scoring system (Table 1). All tabular data, graphs, and micrographs were generated from results using the 108 pfu of Ad5IL-12 and Ad5IL-4.

Measurement of colon cytokine concentrations. Tissue samples were homogenized in a solution of 100 μM PMSF plus 10 mg/ml aprotinin using a Brinkman Polytron (Switzerland). Suspensions were centrifuged at 12,000 rpm for 10 min to remove large debris, and supernatants were transferred into clean Eppendorf tubes and stored at −20°C until needed.

Concentrations of IFN-γ, IL-4, IL-12, and TNF-α were measured by enzyme-linked immunosorbent assay technique using commercially available kits purchased from R&D Systems or Biosource International.

Measurement of cytokine mRNA expression by RT-PCR. Total cellular RNA was isolated from each tissue sample using a previously described (4) guanidium isothiocyanate method. The concentration of RNA was determined by measuring absorbance at 260 nm, and purity was assessed by the ratio of absorbency of 260/280 nm. During the RT-PCR, the mRNA was reverse transcribed to yield cDNA and was then amplified by PCR using gene-specific primers. The housekeeping gene GAPDH was used as an internal control and detected by using commercially available kits purchased from R&D Systems or Biosource International.

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>Macroscopic</td>
<td>Appearance</td>
</tr>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Hyperemia, no ulcers</td>
</tr>
<tr>
<td>2</td>
<td>Ulceration w/o hyperemia or wall thickening</td>
</tr>
<tr>
<td>3</td>
<td>Ulceration with inflammation</td>
</tr>
<tr>
<td>4</td>
<td>2 or more sites of inflammation and ulceration</td>
</tr>
<tr>
<td>5</td>
<td>Sites of damage more than 1 cm</td>
</tr>
<tr>
<td>6–10</td>
<td>Additional point for each centimeter of damage beyond 2 cm</td>
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<table>
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<tr>
<th>Microscopic*</th>
<th>Parameter</th>
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<tr>
<td>0, 1, 2</td>
<td>Loss of architecture (none-severe)</td>
</tr>
<tr>
<td>0, 1</td>
<td>Edema</td>
</tr>
<tr>
<td>0, 1</td>
<td>Goblet cell depletion</td>
</tr>
<tr>
<td>0, 1, 2</td>
<td>Cellular Infiltrate (none-severe)</td>
</tr>
</tbody>
</table>

Criteria for scoring was modified from Wallace and Keenan (24a); *Total after addition of values for each parameter.
with buffer and distilled water. Messages for IFN-γ, IL-4, TNF-α, and GAPDH were amplified by using the following parameters: denaturation at 94°C for 30 s, followed by annealing at 55°C for 30 s and extension at 72°C for 60 s. The number of cycles varied for each set of primers with 42 cycles for IFN-γ, 48 cycles for IL-4, 40 cycles for TNF-α, and 27 cycles for GAPDH. PCR products were loaded onto a 2.5% agarose gel and visualized under ultraviolet light after ethidium bromide staining.

**Statistical analysis.** Data were analyzed by using either Student’s unpaired t-test or Mann-Whitney U-test with 95% confidence intervals being considered significant. All data were normally distributed and are expressed as means ± SD.

**RESULTS**

**Effect of Ad5 plus DL-70 control virus on colonic tissue.** Administration of the Ad5 plus DL-70 control virus (Ad5DL70) at doses of $5 \times 10^8$ and $10^9$ pfu/mouse was nonlethal and caused mild hyperemia at the site of instillation within 24 h. Histological damage scores were lower than in animals treated with the Ad5 cytokine constructs (Fig. 1). Tissue from animals receiving the DL-70 control virus showed no mucosal damage and no infiltrate or edema at any time point (Fig. 2E).

**Overexpression of IL-4 in distal colon is lethal and causes severe damage.** Intrarectal administration of Ad5IL-4 resulted in severe damage to the colonic tissue with high macroscopic and histological damage scores (Fig. 1). At a dose of $10^9$ pfu/mouse, Ad5IL-4 caused 50–60% mortality in NIH Swiss and Balb/c mice within 24 h. Lethality was dose dependent with 10–20% mortality at $10^8$ pfu and none at lower doses. Strain differences were evident; Balb/c and NIH Swiss required 10-fold less Ad5 to induce the same degree of damage as seen in C57BL/6 mice.

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Fig. 2. Representative hematoxylin and eosin micrographs of tissue sections from colons of mice that received $10^9$ pfu/mouse of either Ad5IL-12 for 24 (A) or 72 h (B), Ad5IL-4 for 24 (C) or 72 h (D), or Ad5DL70 for 24 h (E).
Gross examination of the colon within 3 days after gene transfer revealed severe ulceration and hemorrhage in NIH Swiss and Balb/c mice that received Ad5IL-4 (Fig. 2, C and D). Damage was significantly less by 3 days and reached baseline by day 7. Severity of damage was less at the lower doses but with the same time course as the high doses (Fig. 3).

Histological examination of hematoxylin and eosin sections from Ad5IL-4 mice showed structural damage within 24 h (Fig. 2C) but cellular infiltrate and damage were almost completely absent by 72 h (Fig. 2D).

Effect of IL-4 overexpression in knockout mice. Animals lacking either the STAT6 gene, which regulates the Th2 pathway of cytokine production, or the TNF-α receptor did not develop colitis after administration of Ad5IL-4 (Table 2). Mast cell-deficient mice (WWv−/−) and the wild-type strain (WWv+/+), however, showed comparable damage to the colon after overexpression of IL-4. Overexpression of IL-4 in the colon of RAG−2−/− animals caused the same amount of damage as in the wild-type strain (Fig. 4, A and C).

Ad5IL-4 administration into naive mice induces a Th2 cytokine profile. Significantly higher ratios of IL-4/GAPDH mRNA, which peaked at 24 h, were present in Ad5IL-4 animals (1.21 ± 0.2) compared with control animals (0.01 ± 0.002). IL-4 protein concentrations also peaked at 24 h (166 ± 41 pg/ml vs. 22.4 ± 1.8 pg/ml). No IFN-γ mRNA or protein was detected in samples of colon from any time points; however, TNF-α mRNA expression (0.88 ± 0.1) and protein production (195 ± 58 pg/ml) was detected at 24 h and was absent by 3 days. There was no IFN-γ production in STAT6 and RAG-2 knockout mice given Ad5IL-4.

Ad5IL-4 overexpression in IFN-γ knockout mice induced comparable amounts of IL-4 protein and mRNA expression as wild-type mice at 24 h; however, the amount of IL-4 protein and mRNA expression in IFN-γ−/− knockout mice was significantly less (Table 3). Minimal IL-4 mRNA and protein production was found in STAT6 knockout mice at days 1 and 3 (Table 3).

Overexpression of IL-12 causes severe damage and acute lethality. At a dose of 10⁹ pfu/mouse, intrarectal administration of Ad5IL-12 caused 50–60% mortality in NIH Swiss and Balb/c mice within 24 h. Lethality was dose dependent (Fig. 3) and strain dependent, as with Ad5IL-4. High mortality occurred in mice that received intraperitoneal administration of Ad5IL-12 at 10⁸ pfu; however, there was no mortality at 2.5–5.0 × 10⁸ pfu/mouse and minimal damage to the colon (data not shown).

Macroscopic damage was severe within 3 days after IL-12 gene transfer with extensive ulceration and hemorrhage in all strains of mice. Damage scores were significantly less after 3 days and reached baseline by day 7 (Fig. 1). Lower doses of the Ad5IL-12 construct caused less damage (Fig. 2), but with a similar time course to the higher doses (data not shown).

Histological examination of hematoxylin and eosin-stained sections from Ad5IL-12 mice showed complete destruction of the mucosal architecture within 24 h accompanied by inflammatory and mononuclear infiltrate (Fig. 2). Mucosal damage lessened by 3 days with some evidence of repair (Fig. 2B); however, disruption of the mucosal layer persisted until 14 days, after which the colon looked structurally normal.

Effect of IL-12 overexpression in knockout mice. There was minimal damage and no lethality in mice deficient in the IFN-γR gene in response to overexpression of IL-12 in the Table 2. Macroscopic and microscopic damage scores of colons from wild-type and knockout (KO) mouse strains given adenovirus 5 plus interleukin (Ad5IL)-12 or Ad5IL-4 (10⁸ plaque-forming units (pfu/mouse) for 24 h

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Ad5IL-12</th>
<th>Ad5IL-12</th>
<th>Ad5IL-4</th>
<th>Ad5IL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balb/c⁶⁺</td>
<td>6.7 ± 0.58</td>
<td>5.3 ± 1.12</td>
<td>4.5 ± 1.3</td>
<td>5.75 ± 0.96</td>
</tr>
<tr>
<td>C57BL/6⁶</td>
<td>5.3 ± 2.1</td>
<td>3.3 ± 2.2</td>
<td>2 ± 1.5</td>
<td>4.3 ± 1.5</td>
</tr>
<tr>
<td>CD4 KO</td>
<td>0</td>
<td>1.25 ± 0.13</td>
<td>5.1 ± 2.2</td>
<td>4.5 ± 1.6</td>
</tr>
<tr>
<td>RAG2 KO</td>
<td>2.1 ± 0.73</td>
<td>2.04 ± 1.1</td>
<td>4.85 ± 1.8</td>
<td>4.75 ± 2.5</td>
</tr>
<tr>
<td>IFN-γ KO</td>
<td>6.1 ± 1.3</td>
<td>5.14 ± 1.2</td>
<td>4.62 ± 2.2</td>
<td>4.19 ± 1.95</td>
</tr>
<tr>
<td>IFN-γR KO</td>
<td>1.7 ± 0.54</td>
<td>2.4 ± 0.8</td>
<td>4.1 ± 1.5</td>
<td>4.05 ± 2.3</td>
</tr>
<tr>
<td>TNF-αR KO</td>
<td>0</td>
<td>1.5 ± 0.52</td>
<td>0.75 ± 0.95</td>
<td>1.0 ± 0.81</td>
</tr>
<tr>
<td>STAT6 KO</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>1.8 ± 0.95</td>
</tr>
</tbody>
</table>

Bold-face values indicate significant differences compared with controls. 
⁶⁺ Wild-type (control) strain for IFN-γ and IFN-γR knockout (KO) mice; 
⁶⁻ Wild-type (control) strain for CD4 KO, STAT6 KO, TNF-αR KO; ND, not done. RAG, recombinase activation gene; IFN-γR, INF-γ receptor; TNF-αR, TNF-α receptor.
colon (Table 2). Histological damage was completely absent by 3 days. There was, however, considerable damage in the colons of IFN-γ-deficient mice given Ad5IL-12 (Fig. 5B) and that was comparable to the Balb/c wild-type controls (Fig. 5B).

Mice lacking CD4+ T cells, the TNF-α receptor, or the RAG-2−/− developed significantly less macroscopic and microscopic damage at all time points (Table 2). Histological damage in RAG-2−/− mice was minimal compared with the destruction caused by Ad5IL-12 in wild-type controls (Fig. 4).

Cytokine mRNA expression and protein production in colonic tissue after administration of Ad5IL-12. IFN-γ mRNA and protein (0.96 ± 0.2 IFN-γ/G3, and 205 ± 63 pg/ml of IFN-γ) was detected in animals that overexpressed IL-12 but not IL-4 in the colon. Peak expression occurred at 24 h. There was no measurable IL-4 mRNA or protein in the colons of NIH Swiss, Balb/c, or C57BL/6 mice that received the Ad5IL-12 virus, or the control virus. TNF-α mRNA (1.1 ± 0.04 TNF-α/G3) and protein (654 ± 101 pg/ml) expression occurred within 24 h of IL-12 overexpression and persisted at elevated levels until day 14.

Expression of IFN-γ mRNA and protein was present at very low levels in the IFN-γ knockout (0.005 IFN-γ/G3 ratio, and 0 pg/ml), IFN-γR knockout (0.005 IFN-γ/G3 and 0 pg/ml), and in the CD4 or RAG-2 knockout (0.015 or 0.18 IFN-γ/G3 ratio, and 1.5 or 2.1 pg/ml) mice after Ad5IL-12 administration. High amounts of TNF-α mRNA and protein were present in the IFN-γ knockout mice (1.31 TNF-α/G3 and 526 pg/ml) and were comparable to amounts in wild-type mice (1.1 TNF-α/G3 and 654 pg/ml). TNF-α mRNA and protein were found at much lower concentrations in the IFN-γR knockout and the CD4 and RAG-2 knockout mice (e.g., 0.23 TNF-α/G3 and 25 pg/ml).

**DISCUSSION**

This study demonstrates that the isolated expression of IL-4 in mouse colon induces a colitis that is transient, T cell- and mast cell-independent, and characterized by the infiltration of mononuclear and granulocytic cells. In contrast, IL-12 induced more severe clinical damage and a colitis that was T cell dependent. The proinflammatory effect of IL-4 overexpression contrasts with the anti-inflammatory effect of overexpressing this cytokine in hapten-induced colitis (14). The microenvironment in which Ad5IL-4 was introduced and the response measured, in the present study, differs from that used in the previous work in rats, possibly accounting for some of the

![Fig. 4. Representative hematoxylin and eosin micrographs of tissue sections from colons of wild-type (A and B) or recombinase activation gene-2 (RAG-2−/−) knockout (C and D) mice.](image)

Table 3. **IL-4 mRNA expression and protein production in KO mice 24 h after administration of Ad5IL-4**

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>IL-4 Protein Expression, pg/ml</th>
<th>IL-4 mRNA Expression (IL-4/G3 ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>145 ± 29</td>
<td>0.82 ± 0.18</td>
</tr>
<tr>
<td>IFN-γ KO</td>
<td>158 ± 21</td>
<td>0.92 ± 0.12</td>
</tr>
<tr>
<td>IFN-γR KO</td>
<td>23 ± 4.1</td>
<td>0.31 ± 0.09</td>
</tr>
<tr>
<td>STAT6 KO</td>
<td>27 ± 1.5</td>
<td>0.27 ± 0.08</td>
</tr>
<tr>
<td>Control</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD. Wild-type mice were treated with adenovirus 5 (Ad5) plus IL-4; control mice were treated with Ad5 plus DL-70 control virus. ND, not detectable.
by DSS, which is generally accepted to be a T cell-independent mechanism. This is overexpression was evident in CD4 and RAG-2-deficient mice, indicating a T cell-independent mechanism. This is associated with helminth infection (13). Colitis induced by IL-4 deficient mice (18), and in intestinal inflammation associated with IL-4 overexpression was evident in CD4 and RAG-2-deficient mice, indicating a T cell-independent mechanism. This is supported by a previous study (23) in which colitis induced by DSS, which is generally accepted to be a T cell-independent model, was ameliorated in IL-4-deficient mice. The ability of IL-4 to exacerbate a Th-1-driven model of colitis suggests that IL-4 may also exert proinflammatory effects in a context of T cell-mediated injury (11).

The results of this study implicate TNF-α as an important mediator of colitis induced by IL-4 and by IL-12. This cytokine is, of course, well known as a mediator of intestinal inflammation. The higher and more sustained levels of this cytokine are associated with the more severe colitis seen after overexpression of IL-12. TNF-α has also been implicated in intestinal inflammation that accompanies helminth infection, in which IL-4 plays a critical role (1). However, in contrast to the models of parasitic infection in which mast cells are prominent, the proinflammatory effect of IL-4 seen here is mast cell independent. The identity of the cells involved in this response remain to be identified. Weaver and colleagues (16), have shown that Th1 and Th2 cells can mediate intestinal inflammation, but each in a qualitatively distinct manner. An examination and quantification of specific cell types that infiltrate the site would be a useful comparison between IL-12- and IL-4-induced mechanisms of action. Because colitis was seen in RAG-2 as well as mast cell-deficient mice, a role for innate immune cells seems likely and should be examined further.

As expected, overexpression of IL-12 induced a severe colitis which, in contrast to that induced by IL-4, was T cell dependent. The effect of IL-12 was dose dependent, and similar to findings from other studies (6, 10, 19). Davidson et al. (6) reported that IL-12 of a particular dose played a major role in sustaining the chronic phase of colitis in the IL-10-deficient spontaneous model of colitis. Increasing the dose of IL-12 used in vitro to stimulate fetal gut explants led to mucosal degradation and an increase in stromelysin-1 concentrations (19). In the present study, IFN-γ did not appear critical for IL-12-induced injury. Colitis was attenuated in IFN-γ R-deficient mice but not in IFN-γ-deficient mice. Perhaps IFN-γR is stimulated by some other molecule that contributes to inflammation. This is a possible focus of future work in this area. Whereas IFN-γ was absent in both knockouts, it was the marked reduction in TNF-α in the IFN-γR-deficient mouse that accompanied the attenuation of colitis. These results suggest that TNF, rather than IFN-γ, is critical for the proinflammatory effects of IL-12 under these conditions. Our finding of an IFN-independent action of IL-12 is consistent with conclusions drawn by others in different experimental contexts (5, 22).

In conclusion, this study provides direct evidence of the potential for a counterinflammatory Th2 cytokine to cause acute and lethal damage in a healthy mouse colon, through IFN-γ- and T cell independent mechanisms. These results establish the basis for more in-depth examination of the effects of Th2 cytokine overexpression, and its implications for the design of drugs and therapies that steer the immune response toward a Th2 response.

**GRANTS**

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REFERENCES


