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Inhibition of IFN-γ-Inducible Protein-10 Abrogates Colitis in IL-10$^{-/-}$ Mice[1]

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A deficiency in understanding the steps responsible for colitis is the lack of comprehension for the role chemokines play in mucosal inflammation. IFN-γ-inducible protein-10 (IP-10) and CXCR3 are highly expressed at sites of colitis. Our findings show that IP-10 significantly contributes to the development of Th1 and inflammatory responses. Specifically, IP-10 inhibition in IL-10$^{-/-}$ mice attenuates the associated increases in serum and/or local amyloid A, IL-2, IL-6, TNF-α, IFN-γ, IL-1α, and IL-1β with colitis as compared with IL-10$^{-/-}$ mice that develop colitis similar to human Crohn's disease. Correspondingly, the rate or intensity of inflammation in IL-10$^{-/-}$ mice treated with anti-IP-10 Abs showed improved scoring of inflammation, compared with control IL-10$^{-/-}$ mice. This study provides important and novel information regarding IP-10 as a target for the treatment of colitis. ***The Journal of Immunology,*** **2003, 171: 1401–1406.**

The causes of inflammatory bowel diseases (IBD)[3] remain unknown, but the two major forms (Crohn's disease (CD) and ulcerative colitis) are defined by their clinical, pathologic, radiological, and endoscopic characteristics (1). Fortunately, mouse models of colitis that resemble human IBD provide important tools to develop new therapies for this devastating disease (2–4). Useful and noninvasive mouse models for IBD involve the spontaneous development of colitis in TCR and MHC knockout mice (4). Additionally, transfer of CD45BR^{high} T cells to SCID mice results in the development of IBD (5). Recently, it has been shown that IL-23, which is comprised of IL-12p40 and a different p19 subunit, is produced predominantly by APCs, and activates inflammatory T cells in a fashion that is similar to IL-12 (6, 7). In this regard, it has been suggested that CD is mediated by Th1-associated cytokines (e.g., IL-23, IL-12, and IFN-γ) that are overproduced by lamina propria macrophages and T cells (6, 8). IL-10$^{-/-}$ mice spontaneously develop colitis at ~3 mo of age. This murine disease is similar to that seen in human CD with small intestine and colon disease localization, chronic mucosal inflammation, alterations in mucosal architecture, bowel-wall thickening, and a chronic progressive disease course (2). However, this murine model differs from human CD, because colitis in IL-10$^{-/-}$ mice does not yield focal granulomatous, or transmural, inflammation.

IFN-γ-inducible protein-10 (IP-10) is a CXC chemokine that is secreted by endothelium, epithelium, fibroblasts, keratinocytes, and monocytes, and is chemotactic for monocytes as well as activated T cells that express CXCR3 (9, 10); CXCR3 is also expressed by epithelial, endothelial, and lymphoid cells (10). IP-10 ligation leads to preferential chemotaxis of activated Th1 lymphocytes that express CXCR3 (11). IP-10-CXCR3 interactions, with Th1-dependent immunity, have been observed in several inflammatory diseases, including multiple sclerosis and rheumatoid arthritis (12, 13). Most importantly, IP-10 and its ligand, CXCR3, are highly expressed by IL-10$^{-/-}$ mice (C57BL/6 background) that develop colitis (14), which corresponds to elevated levels of IP-10 expression during clinical colitis (15).

Similar to the natural antagonist of IL-1 (16), Ab therapy has some precedence in the prevention of CD. Administration of anti-TNF-α Ab or soluble TNF-α receptor has been shown to inhibit colitis (17). Ab blockade of ICAM-1 and VCAM-1 also ameliorates inflammation in an adoptive transfer mouse model of CD (18). Additionally, anti-IFN-γ Ab treatment significantly attenuates colitis developed by IL-10$^{-/-}$ mice (19). For the first time, we demonstrate that Ab therapy directed toward IP-10 is successful at impeding IBD development.

Materials and Methods

Animals

Female IL-10$^{-/-}$ mice, on C57BL/6 background and aged 8–12 wk, were purchased from The Jackson Laboratory (Bar Harbor, ME). Four- to five-month-old female New Zealand rabbits (Myrtle's Rabbitory, Thompson Station, TN) were used to generate IP-10 antisera. Experimental groups consisted of five mice, and experiments were repeated three times. The guidelines proposed by the committee for the Care of Laboratory Animal Resources Commission of Life Sciences-National Research Council were followed to minimize animal pain and distress.

Anti-IP-10 Ab preparation and treatment

Murine IP-10 was purchased from PeproTech (Rocky Hill, NJ). IP-10 plus CFA or IFA (Sigma-Aldrich, St. Louis, MO) were used to generate anti-IP-10 Ab titers ~1:10^6. Normal or anti-IP-10 sera were purified using an IgG isotype-specific protein A column (Pierce Biotechnology, Rockford, IL). The specificity of these IgG Abs was determined by direct ELISA, and no cross-reactivity was detected when tested against chemokines (monokine induced by IFN-γ, IFN-inducible T cell α chemoattractant, RANTES, macrophage-inflammatory protein-1α, macrophage-inflammatory protein-1β, IL-8, monocyte chemoattractant protein-1 (MCP-1), MCP-2, MCP-3, Lptn, B cell-attracting chemokine-1, and eotaxin), cytokines (IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α), or CCR5, CXCR4, or CCR3 transfectants. Anti-IP-10 polyclonal Ab titers were adjusted to 1:4 × 10^5 (i.e., 50× dilution) in PBS (IP-10 Ab solution). The body weight of mice was monitored twice per week. We did not notice a major weight change until the onset of severe and symptomatic colitis. Therefore, serum amyloid A (SAA) and serum

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[3] Abbreviations used in this paper: IBD, inflammatory bowel disease; CD, Crohn's disease; IP-10, IFN-γ-inducible protein-10; MCP, monocyte chemoattractant protein; SAA, serum amyloid A.

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IL-6 levels were monitored every other week; SAA and IL-6 levels exceeding 200 μg/ml and 500 pg/ml, respectively, corresponded with the onset of asymptomatic colitis. At this stage, mice received 200 μl by i.p. injection of the IP-10 or preimmune Ab solution every 3 days.

Sample and tissue collection

Fecal samples were dissolved in PBS containing 0.1% sodium azide (e.g., 1 ml per 100 mg of fecal pellet), vortexed, and centrifuged, and supernatants were collected for analysis (20). The blood samples were collected by tail vein bleeding, and serum was obtained following centrifugation. Serum and mucosal secretions were collected every 2 wk after the start of IP-10 Ab treatment. Mice were sacrificed by CO_2 inhalation, and intestinal tissues were preserved using Streck fixative (Streck Laboratories, LaVista, NE).

Histology

Fixed tissues were sectioned at 6 μm, and stained with H&E for microscopic examination. Fourteen sections from proximal, middle, and distal regions of the colon from each mice were examined by two different pathologists. Sections from the liver and stomach were also examined by same pathologist for quality control. The investigator was blinded throughout the entire experiment, and the results presented, as mean score, were obtained from both pathologists. A score (0–4) was given, based on the following criteria: grade 0, no change from normal tissue; grade 1, 1 or a few multifocal mononuclear cell infiltrates in the lamina propria, minimal hyperplasia, and no mucus depletion; grade 2, intestinal lesions involved with several multifocal, mild, inflammatory cell infiltrates in the lamina propria composed of mononuclear cells with no inflammation in the submucosa; grade 3, lesions involved moderate inflammation and epithelial hyperplasia; grade 4, inflammation involved most of the intestinal sections. Each segment of the colon was given a score based on the criteria described above. The summation of these scores provided a total colonic disease score that could range from 0 to 12, with grade 4 lesions in proximal, middle, and distal colon segments.

Ab, cytokine, and SAA quantification by ELISA and Luminex analysis

Total IgG subclass Ab levels in sera as well as IgA and IgG Abs in fecal extracts were measured by ELISA (21). Ab-specific ELISA were capable of detecting >25 pg/ml. The serum levels of IL-2, IL-6, TNF-α, and IFN-γ were determined by ELISA capable of detecting >15 pg/ml for each assay, following manufacturer's instructions (E-Biosciences, San Diego, CA). Serum IL-1α, IL-1β, IL-2, IL-12, IFN-γ, and TNF-α were also determined by multicytokine detection system (Bio-Rad, Hercules, CA) capable of detecting >5 pg/ml for each analyte, following manufacturer's instructions, measured using a Luminex System (Austin, TX) and calculated using Bioplex software (Bio-Rad). SAA levels were determined by ELISA (Biosource International; Camarillo, CA) capable of detecting 5 ng/ml of SAA.

Gene expression analysis by RT-PCR

Mouse mRNA sequences of IP-10, CXCR3, IFN-γ, TNF-α, IL-12, 18S rRNA were obtained from the National Institutes of Health-National Center for Biotechnology Information (NCBI) gene bank database accession number IP-10 M33266 (95); CXCR3 AF045146 (96); IFN-γ K00083 (98); IL-12p40 M86671 (102); and TNF-α NM 013693 (97) used for RT-PCR primer design, which generated amplicons of 95, 96, 98, 102, 97, and 87 bp in size, respectively. Primers were designed using the primer 3 software program from Whitehead Institute/MIT (Cambridge, MA). Thermodynamic analysis of the primers was conducted using computer programs: Primer Premier (Integrated DNA Technologies, Coralville, IA) and MIT Primer III (Whitehead Institute). The resulting primer sets were compared against the entire mouse genome using NCBI homepage to confirm specificity and insured to flank mRNA splicing regions. To analyze mRNA expression levels by RT-PCR, mRNA was isolated from colon and mesenteric lymph nodes using TriReagent (Molecular Research Center, Cincinnati, OH), according to manufacturer's protocols. Potential genomic DNA contamination was removed from these samples by treatment with 10 U/μl of RNase-free DNase (Invitrogen, San Diego, CA) for 15 min at 37°C. RNA was then precipitated and resuspended in RNA Secure (Ambion, Austin, TX). cDNA was generated by reverse transcribing ~2 μg of total RNA using Taqman reverse-transcription reagents (Applied Biosystems, Foster City, CA). Subsequently, cDNAs were amplified with specific cDNA primers to using SYBR Green PCR master mix reagents (Applied Biosystems). The level of copies (>10) of mRNA relative to 18S rRNA copies of these targets was evaluated by RT-PCR analysis using the Bio-Rad Icycler and software.

Statistics

The data are expressed as the mean ± SEM and compared using a two-tailed Student's t test or an unpaired Mann-Whitney U test. The results were analyzed using the Statview II statistical program (Abacus Concepts, Berkeley, CA) for Macintosh computers and were considered statistically significant if p values <0.05.

Results

IP-10 and CXCR3 expression during murine colitis

To investigate IP-10 and CXCR3 increases during colitis, mRNA expression of CXCR3 and IP-10 was measured by semiquantitative RT-PCR analysis. A significant increase in CXCR3 and IP-10 was noticed in mesenteric lymph nodes of the IL-10$^{-/-}$ with colitis mice when compared with normal wild-type mice (Table I). We also noticed a significant increase in CXCR3 and IP-10 expression in inflamed colons of IL-10$^{-/-}$ mice, as compared with controls (Table I).

IP-10 Ab therapy prevents associated weight loss and elevated SAA/IL-6 in colitis

IL-10$^{-/-}$ mice spontaneously developed colitis, with features associated with weight loss (19). Intraperitoneal injections of anti-IP-10 Ab were given on every third day at the onset of asymptomatic colitis, which was associated with SAA levels >200 μg/ml (Fig. 1). Specifically, SAA levels 1 wk before the initiation of antisera treatment were ~100 μg/ml and quickly rose to >250 μg/ml 1 wk later. The results clearly show that IP-10 blockage inhibited both the weight loss and elevated SAA levels that are associated with IBD. Control groups showed signs of colitis with continuous body weight decline to ~82.5% of initial body mass (Fig. 2). The weight of IP-10 Ab-treated mice remained within 95% of their initial body mass. SAA and IL-6 serum concentrations in IP-10 antisera-treated IL-10$^{-/-}$ mice showed a significant ($p < 0.01$) decline, as compared with control mice (Fig. 3). SAA

Table I. *mRNA expression[a] during murine colitis*

Mucosal Tissue	Colitis State/Ab Treatment	IP-10	CXCR3	IL-12p40	IFN-γ	TNF-α
Colon	Before onset	BD[b]	BD	BD	BD	BD
	Anti-IP-10	1.8×10^2	3.4×10^1	3.5×10^4	BD	1.9×10^1
	Severe colitis/sham	4.7×10^4	9.3×10^4	7.9×10^5	BD	8.8×10^4
Mesenteric lymph nodes	Before onset	8.0×10^5	8.9×10^4	BD	6.0×10^3	BD
	Anti-IP-10	1.8×10^7	1.8×10^4	3.0×10^6	1.6×10^{11}	4.6×10^3
	Severe colitis/sham	1.0×10^9	1.1×10^4	1.6×10^{11}	8.2×10^{11}	7.0×10^5

[a] Before or after the onset of severe colitis in IL-10$^{-/-}$ mice, on C57BL/6 background, mucosal cells from the colon or mesenteric lymph nodes were isolated for RT semiquantitative-PCR analysis. The number of copies are expressed relative to copies of 18S rRNA.

[b] The RT-PCR assay was capable of detecting >50 copies of mRNA. Hence, message <50 are shown as BD.

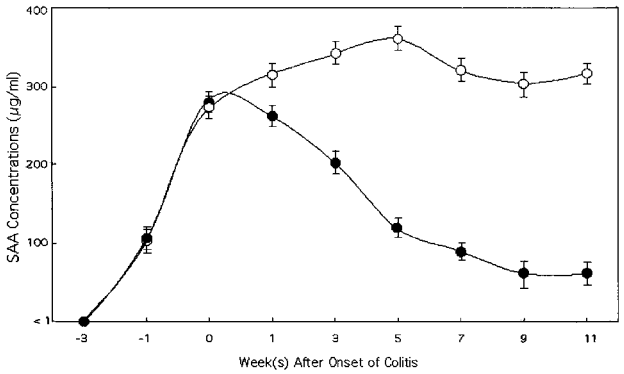

FIGURE 1. SAA levels and the development of colitis in IL-10$^{-/-}$ mice. SAA concentrations >200 μg/ml were associated with the onset of asymptomatic colitis at wk 0. Mice received 200 μl of preimmune (○) or anti-mouse IP-10 (●) Ab solutions every 3 days. Sera were collected every 2 wk, and the data presented are the mean SAA concentrations ± SEM.

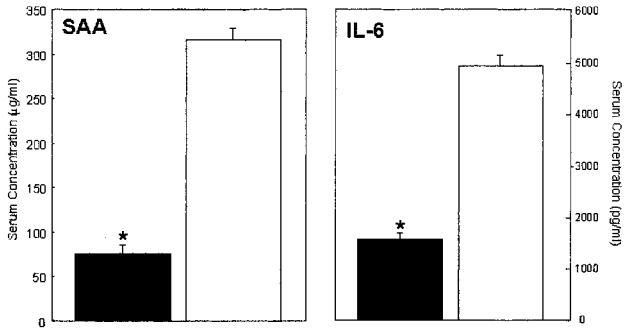

FIGURE 3. Association of serum IL-6 and SAA levels with murine CD. IL-10$^{-/-}$ mice received 200 μl of preimmune (□) or anti-mouse IP-10 (■) Ab solutions every 3 days. The levels of SAA and serum IL-6, at wk 11, were determined by ELISA. The data presented are the mean SAA or IL-6 concentrations ± SEM.

and IL-6 also correlated with colitis severity (Fig. 3). The results show that SAA and IL-6 levels as well as weight loss associated with colitis in IL-10$^{-/-}$ mice were abrogated by IP-10 blockade. Moreover, the results also suggest a utility of using SAA levels as an indicator for the switch from acute (i.e., asymptomatic) to chronic colitis in this murine model of CD.

Total IgG and IgA in fecal and serum IgG subclass Ab profile in murine colitis

We also measured total fecal IgG and IgA to determine correlative changes in intestinal Abs during CD. IgA Ab levels in fecal extracts, present in both groups, did not change. A significant decline in fecal IgG Abs was observed in IL-10$^{-/-}$ mice that received the IP-10 Ab solution (Fig. 4). These results clearly indicate that blockade of IP-10 attenuated the excretion of IgG Abs from the periphery to the lumen of the intestinal mucosa during murine CD. The previously described imbalance of Th1≫Th2 cytokine levels during CD suggests a possibility that there may also be an associated Th1-biased humoral response. To test this hypothesis, we measured the level of total IgG1, IgG2a, IgG2b, IgG3, and IgM Abs in the sera of control mice and those treated with anti-IP-10 Abs. Control and IP-10 Ab-treated mice had similar levels of IgM, IgG1, IgG2b, and IgG3 Abs. However, total serum IgG2a levels were significantly higher in mice with active colitis, as compared

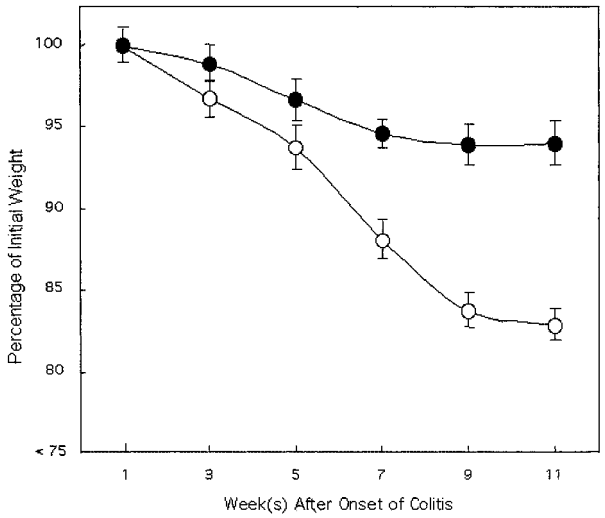

FIGURE 2. Change in body weight of IL-10$^{-/-}$ mice. The wasting disease associated with murine CD was observed by monitoring the change in initial body mass at wk 0. IL-10$^{-/-}$ mice received 200 μl of preimmune (○) or anti-mouse IP-10 (●) Ab solutions every 3 days. Body masses were recorded every 2 wk, and the change from initial body mass was expressed as a percentage: weight at wk 0 minus weight at wk 1, 3, 5, 7, 9, or 11 divided by the weight at wk 0.

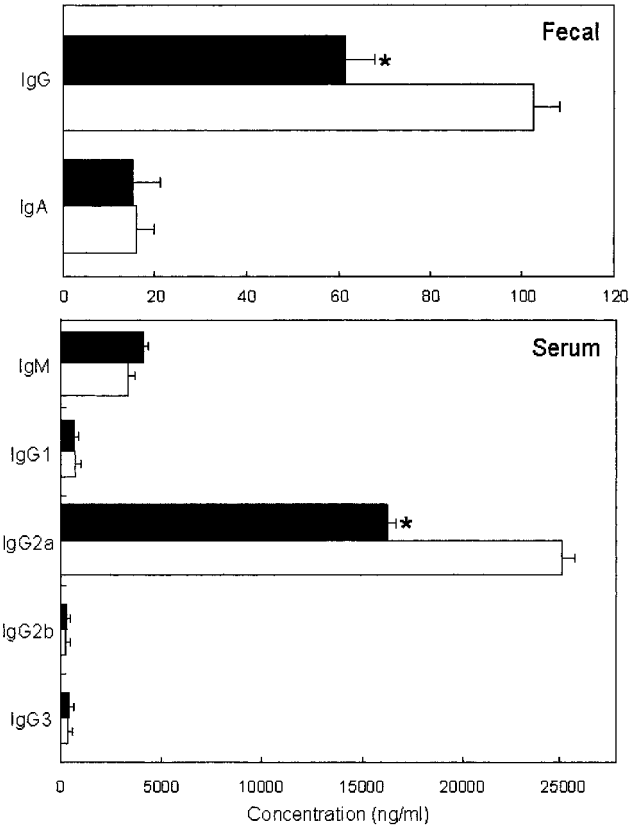

FIGURE 4. Total fecal and serum Ab levels in IL-10$^{-/-}$ mice. Groups of five IL-10$^{-/-}$ mice received 200 μl of either preimmune (□) or anti-mouse IP-10 (■) Ab solutions every 3 days. The data presented are the mean concentration of total Ig Abs (nanograms per milliliter) ± SEM. Total IgA and IgG Abs in fecal extracts or IgM, IgG1, IgG2a, IgG2b, and IgG3 Abs in serum were collected at wk 11, and levels were determined by ELISA. Asterisk(s) indicates statistically significant differences, i.e., $p < 0.05$ (*), between the two groups.

with anti-IP-10 Ab-treated mice (Fig. 4). These results clearly indicate that blockade of IP-10 attenuated total IgG2a levels and the excretion of IgG Abs during CD.

Cytokine production of IL-10$^{-/-}$ mice with active colitis

To address the question of whether the cytokine environment of the host was responsible for the elevated IgG2a Abs observed in this murine model of CD, we next examined the expression of important Th1 cytokines (i.e., IL-12 and IFN-γ) in sera. Control groups showed moderately higher levels of serum IL-12 p40, compared with IP-10 Ab-treated mice (Fig. 5). In contrast, anti-IP-10 Ab therapy dramatically decreased IFN-γ levels in IL-10$^{-/-}$ mice. Overproduction of IL-2, TNF-α, IL-1α, and IL-1β during IBD has been well documented (22). Indeed, the administration of neutralizing anti-TNF-α Abs in human patients with active CD leads to improvement of symptoms. Serum IL-2, TNF-α, IL-1α, and IL-1β were significantly decreased by IP-10 blockade (Fig. 5). Similarly, local production of IFN-γ, IL-6, IL-12 p40, and TNF-α was significantly elevated in IL-10$^{-/-}$ mice with colitis, compared with wild-type or anti-IP-10 Ab-treated mice (Table I). Taken together, the inflammatory state of a host with active colitis was significantly reduced by anti-IP-10 Ab treatment.

Characteristics of enterocolitis

The colitis developed after anti-IP-10 Ab and preimmune Ab treatment was also studied by histology. The mean histological scores of IL-10$^{-/-}$ mice treated with anti-IP-10 Abs were significantly lower than control mice (Table II, Fig. 6). Pathologic changes included small multifocal infiltrates in the lamina propria of the ascending and transverse colon. These infiltrates consisted of lymphocytes and occasional small numbers of neutrophils. Epithelial cells were not hypertrophied in the IP-10-inhibited group. Multinucleated, enlarged epithelial, and elongated glandular cells were also present in control mice. However, colitis progression was more aggressive in control groups, as noted by multifocal lesions in all regions of the large intestine, especially in colon. These results show a marked improvement in colitis associated with IP-10 blockade.

Table II. *Histological evaluation of IL-10$^{-/-}$ mice after IP-10 Ab treatment*[a]

Treatment/Group	Number of Mice	Colitis Score (0–12)	SAA (μg/ml)
Anti-IP-10 Ab solution	15	2.13 ± 0.20	74.6 ± 11
Preimmune Ab solution	15	6.89 ± 0.71	357.6 ± 15
Untreated wild-type C57BL/6	2	0	10.2 ± 0.4

[a] Colitis was monitored by evaluating SAA levels and histopathological changes in the colon of mice that received 200 μl of preimmune or anti-IP-10 Ab solutions every 3 days. Following sacrifice at wk 11, the intestines of mice were fixed, sectioned at 6 μm, and stained. Sections were examined at a magnification view of ×40–100 and scored for severity of colitis.

Discussion

Previous studies demonstrated that Th1 responses associated with CD could be prevented by early treatment with neutralizing anti-IL-12 or anti-IFN-γ Abs as well as IL-10 (22, 23). IP-10 is part of a family of non-ELR (glutamic acid-leucine-arginine) chemokines that bind CXCR3, which is associated with Th1 immune responses (24). The association of IP-10-CXCR3 and Th1-dependent immunity has been observed in several models of diseases, including multiple sclerosis and rheumatoid arthritis (12, 25). We hypothesized that blocking IP-10 interactions would prevent the development of colitis in IL-10$^{-/-}$ mice by negatively modulating Th1 and inflammatory cytokines produced by T lymphocytes and monocytes. We have shown that anti-IP-10 Ab treatment abrogates colitis, weight loss, SAA, and serum IL-1α, IL-1β, IL-12, IL-6, TNF-α, and IFN-γ increases observed in murine colitis.

The mucosal and systemic humoral immunity of IL-10$^{-/-}$ mice, with active colitis, varied from controls. There is no known active transport mechanism for IgG secretion by epithelial cells into the

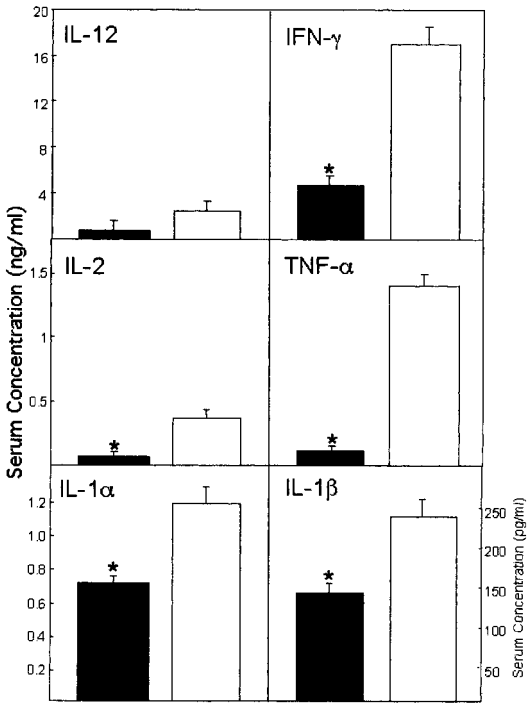

FIGURE 5. Serum IL-12, IFN-γ, IL-2, TNF-α, IL-1α, and IL-1β levels in IL-10$^{-/-}$ mice with IBD. IL-10$^{-/-}$ mice received 200 μl of either preimmune (□) or anti-mouse IP-10 (■) Ab solutions every 3 days. Serum cytokine levels, at wk 11, were determined by ELISA. The data presented are the mean cytokine concentrations ± SEM (ng/ml).

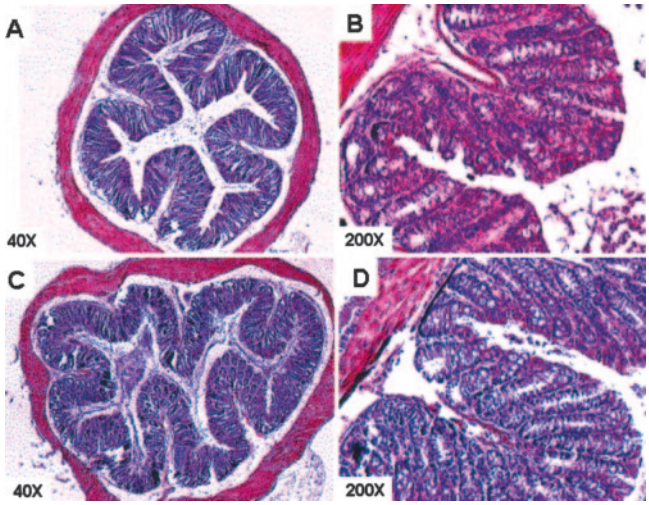

FIGURE 6. Histological characteristics of colitis presented by IL-10$^{-/-}$ mice. Changes in mice that received 200 μl of either preimmune (*C* or *D*) or anti-mouse IP-10 (*A* or *B*) Ab solutions every 3 days. Following sacrifice at wk 11, the intestines were fixed, sectioned at 6 μm, and stained. Sections were examined microscopically at a magnification view of ×40 (*A* and *C*) or ×200 (*B* and *D*). Qualitative analysis of these differences is shown in Table II.

lumen; perhaps in correlation with the anemia observed in CD, mice with active colitis displayed excretion of IgG Ab in fecal extracts, as compared with controls. Correspondingly, serum IgG2a Ab levels in IL-$10^{-/-}$ mice that received anti-IP-10 Abs were dramatically reduced, as compared with controls. The relatively high level of serum IFN-γ was closely associated with the similar increases observed in IgG2a Ab concentrations in mice with active colitis. IFN-γ production would support the Th1 help for IgG2a Ab responses (26).

It is well established that IL-23 and IL-12 drive Th1 differentiation and subsequent IFN-γ production (6, 7, 27). IL-12 has been implicated to play a central role in the induction and progression of colitis in rodent models of IBD, including trinitrobenzenesulfonic acid ethanol-induced colitis (28) and IL-$2^{-/-}$ mouse models (29). Moreover, anti-IL-12 mAb treatment significantly reduces IFN-γ production by colonic lamina propria cells associated with IBD. In a recent study in IL-$10^{-/-}$ mice, IL-12 was shown to play a role in the progression of IBD, independent of IFN-γ (30). In the present study, we report that both serum IL-12 and IFN-γ levels significantly decline as a result of anti-IP-10 Ab treatment. It has also been reported that Th1 responses may be maintained by other cytokines, such as IL-23 (6, 7) and IL-18 (31). Hence, it is also likely that these cytokines may also contribute to the chronic IBD phase in IL-$10^{-/-}$ mice.

TNF-α is produced by macrophages and has multiple biological activities involved in CD (32). In CD, TNF-α levels are elevated in tissue and secretary fluids; correspondingly, there are increased numbers of TNF-α-producing lamina propria cells (33–35). TNF-α also leads to and exacerbates the development of colitis in certain murine models of IBD (22). In the present study, serum TNF-α levels were reduced in IL-$10^{-/-}$ mice that received anti-IP-10 Abs, as compared with controls. The sustained acute-phase responses associated with both human and murine CD are well documented (19, 36). SAA is elevated and alters inflammatory conditions (37). Our present study clearly shows that anti-IP-10 Ab treatment decreases SAA levels in mice with colitis. This regression correlates with other studies; SAA regression is an indication of clinical improvement of CD (38). IL-1α and IL-1β are potent mediators that activate many immune and inflammatory cells; monocytes, macrophages, neutrophils, and endothelial cells produce these inflammatory cytokines. IL-1α and IL-1β expression is increased in patients with IBD, by activated macrophages (39). In the present study, we have demonstrated that both IL-1α and IL-1β expression was decreased by IP-10 blockade.

mRNA analysis shows that both IP-10 and CXCR3 are up-regulated in the inflamed colon of IL-$10^{-/-}$ mice, while IP-10 is predominantly expressed in the mesenteric lymph nodes. The expression of CXCR3 has clearly been associated with Th1 cells (12). We find a reduction in both IP-10 and CXCR3 expression in the mesenteric lymph nodes of mice that received anti-IP-10 Ab therapy, as compared with mock Ab-treated mice. Our findings of IP-10 and CXCR3 expression differ slightly from earlier reports (40). This difference is most likely due to the background (C57BL/6 vs 129.EvSv) of the mice used in these studies, phase of colitis, and severity of disease studied. Possibly, IL-$10^{-/-}$ mice display large amounts of IP-10 in the colon at the initial phase of colitis, followed by the recruitment of the $CXCR3^{+}$ leukocytes that migrate from the mesenteric lymph nodes for propagation of colitis. To this end, we show local IP-10, CXCR3, IL-12p40 (i.e., IL-12 and IL-23), IFN-γ, and TNF-α mRNA expression during colitis, which has been well supported by other murine colitis studies (14, 15, 19, 22, 30, 40–42).

IP-10 was initially characterized as a chemoattractant for T lymphocytes (9, 43, 44). CXCR3-expressing T cells have been shown to produce predominantly classical Th1 cytokines, which supports the concept that IP-10 selectively mobilizes Th1 lymphocytes (11). IP-10 is strongly induced by IFN-γ in a range of cell types, including: monocytes, keratinocytes, and endothelial cells (45). Neutrophils also produce IP-10 (46). It has been reported that IP-10 triggers maximal lymphocyte adhesion to immobilized integrin ligands (47). Possibly, neutrophil-, monocyte-, or macrophage-derived IP-10 is important for diapedesis and recruitment of immunocompetent T lymphocytes to sites of inflammation. Hence, the described anti-IP-10 Ab therapy may block the recruitment and maintenance of inflammatory lymphocytes, which potentiates colitis. Although additional studies will be required to ascertain the precise role and source of IP-10 in colitis, our studies both highlight the importance of IP-10-CXCR3 interactions in CD and present a new target for immunotherapy for the treatment of colitis.

Acknowledgments

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