Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-γ and TNF-α production

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A IMBALANCE of T helper cell type 1 (Th1) vs. type 2 (Th2) polarization in favor of Th1 cell subsets appears to be a key pathogenic mechanism in chronic inflammatory bowel disease (IBD). This concept is supported by studies of mucosal biopsies in patients with IBD, demonstrating an increased expression of proinflammatory cytokines, chemokines, and adhesion molecules (15, 22, 23, 35, 43, 44). The clinical relevance of suppression of Th1 responses in the treatment of IBD has been shown in animals and humans. In animal models, antibodies against interleukin (IL)-12, a Th1 cytokine, reduced the severity of the disease (39); in patients with Crohn’s disease, anti-tumor necrosis factor (TNF)-α treatment exhibited significant improvement (49, 53).

Consistent with the hypothesis of a Th1-mediated pathogenesis in Crohn’s disease, increased concentrations of IL-12, TNF-α, and interferon (IFN)-γ have been measured in the mucosa of patients with Crohn’s disease (31, 40, 43, 45). IL-18 is also a Th1 cytokine by its ability to induce IFN-γ (41). Pizarro and colleagues (44) could show that the mature form of IL-18 is indeed markedly overexpressed in intestinal lesions of patients with Crohn’s disease, but not ulcerative colitis. IL-12 acts synergistically with IL-18 in inducing IFN-γ synthesis by T lymphocytes and neurokinin cells (18, 27, 34). At least two different mechanisms account for the synergy between IL-12 and IL-18. IL-12 upregulates expression of both chains of the IL-18 receptor complex, thus rendering cells more responsive to IL-18 (1, 25, 58); additionally, IL-12 and IL-18 regulate the transcriptional activity of the IFN-γ promoter (4). Because endogenous IL-18 is a key inducer of IFN-γ, reducing IL-18 activity in Crohn’s disease is a rational strategy.

Dextran sulfate sodium (DSS)-induced colitis is characterized histologically by infiltration of inflammatory cells into the lamina propria, with lymphoid hyperplasia, focal crypt damage, and epithelial ulceration (9, 12, 42). These changes are thought to develop due to a toxic effect of DSS on the epithelium and by phagocytosis of lamina propria cells and production of TNF-α and IFN-γ (12, 13, 21, 42). Despite its common use, several issues regarding the mechanisms of DSS about the relevance to the human disease remain unresolved. DSS is regarded as a T cell-independent model because it is observed in T cell-deficient animals such as SCID mice (3, 13). In DSS-induced colitis
mice, TNF-α and IFN-γ are elevated at the site of inflammation and are diminished by the administration of either the adenosine kinase inhibitor G5P15 or the type 4 phosphodiesterase inhibitor rolipram (21, 50). To evaluate an agonist function of IL-18 in this model of experimental colitis, we first investigated the location and increase of IL-18 expression during DSS-induced colitis. Second, the effect of specific neutralization using anti-IL-18 antiserum during colitis was employed. The C57BL/6 strain, often used in models of Th1-mediated immune response, and the BALB/c strain were investigated. The study endpoints were the Th1-mediated immune response, and the BALB/c employed. The C57BL/6 strain, often used in models of induced colitis. Second, the effect of specific neutralization of either the adenosine kinase inhibitor GP515 or inflammation and are diminished by the administra-

**MATERIALS AND METHODS**

**Mice.** All experiments were approved by the regional animal study committees. Female, 8-wk-old BALB/c mice (Harlan Winkelmann, Borchern, Germany) or female, 8-wk-old C57BL/6 mice (The Jackson Laboratory, Bar Habor, ME) weighing 20 to 22 g were used. The animals were housed at controlled temperatures with light-dark cycles, fed standard mice chow pellets, had access to tap water from bottles, and were acclimatized before being studied. At the end of an experimental period, mice were killed by cervical dislocation under isoflurane anesthesia (Forene, Abbott, Wiesbaden, Germany). Clinical assessments and histological scoring of colitis were performed in a blinded fashion.

**Reagents.** RPMI 1640 medium was purchased from Biochrom KG (Berlin, Germany), and FCS was from Gibco Life Technologies (Karlsruhe, Germany). Murine recombinant IL-12 was a kind gift of Genetics Institute (Andover, MA). Murine recombinant IL-18 was a kind gift of Peprotech (Rocky Hill, NJ). The anti-IL-18 neutralizing antiserum was raised in rabbits against murine recombinant IL-18 (Peprotech) as previously reported (17, 18).

**Induction of colitis and experimental design.** Mice were fed various concentrations of DSS (molecular mass 30 to 40 kDa; ICN, Eschwege, Germany) dissolved in sterile, distilled water ad libitum throughout the experiment (days 1 to 10). The DSS solutions were changed on days 3, 6, and 9. Either normal rabbit serum (NRS) or rabbit antimurine-IL-18 antiserum was injected intraperitoneally. Control mice had access to water (without DSS) and were injected with either NRS or anti-IL-18 antiserum.

**Determination of clinical score, colon length, and histological scoring.** Body weight, the presence of occult or gross blood per rectum, and stool consistency were determined daily. The clinical score was assessed by trained individuals blinded to the treatment groups (21). The baseline clinical score was determined on day 1. Briefly, no weight loss was registered as 0, weight loss of 1 to 5% from baseline was assigned 1 point, 5 to 10% was assigned 2 points, 10 to 20% was assigned 3 points, and more than 20% was assigned 4 points. For stool consistency, 0 points were assigned for well-formed pellets, 2 points for pasty and semiformed stools that did not adhere to the anus, and 4 points for liquid stools that did adhere to the anus. For bleeding, 0 was assigned for no blood using hemoccult (Beckman Coulter, Palo Alto, CA), 2 points for positive hemoccult, and 4 points for gross bleeding. These scores were added and divided by three, resulting in a total clinical score ranging from 0 (healthy) to 4 (maximal activity of colitis). Postmortem the entire colon was removed from the caecum to the anus, and the colon length was measured as marker of inflammation. A 1-cm segment of the transverse colon was fixed in 10% buffered formalin for histological analysis. Paraffin sections were stained with hematoxylin/eosin. Histological scoring was performed in a blinded fashion by a pathologist as follows: presence of occasional inflammatory cells in the lamina propria was assigned a value of 0; increased numbers of inflammatory cells in the lamina propria as 1; confluence of inflammatory cells, extending into the submucosa, as 2; and transmural extension of the infiltrate as 3. For tissue damage, no mucosal damage was scored as 0; discrete lymphoepithelial lesions were scored as 1; surface mucosal erosion or focal ulceration was scored as 2; and extensive mucosal damage and extension into deeper structures of the bowel wall were scored as 3. The combined histological score ranged from 0 (no changes) to 6 (extensive cell infiltration and tissue damage).

**Colon organ culture.** Another segment of the colon was removed, cut open longitudinally, and washed in 0.01 M PBS containing penicillin (100 U/ml) and streptomycin (100 μg/ml). Tissue was homogenized in PBS using a tissue teearer (Biospec Products, Bartlesville, OK). The homogenized colon tissue was centrifuged at 10,000 g at 4°C for 15 min. Cytokine concentrations were determined in the supernatant. Protein concentrations of the homogenate were quantified by a Bradford assay as described previously (8).

**Colonic tissue cytokines.** Segments of colon (−4 cm in length) were cut open longitudinally and washed in 0.01 M PBS containing penicillin and streptomycin. The colon was then further cut into strips to ~1 cm² and placed in 24 flat-bottom well culture plates containing fresh RPMI 1640 supplemented with penicillin and streptomycin. Strips were incubated at 37°C in 1 ml of fresh supplemented RPMI 1640 medium for 24 h. Culture supernatants were harvested and assayed for cytokines.

**Confocal microscopy.** The transversing portion of the large intestine from DSS-exposed and -unexposed mice was excised, rinsed in PBS, and frozen on isopentane cooled with liquid nitrogen. Frozen sections (5 μm) were cut on a Leica CM 1850 cryostat (Leica, Deerfield, IL). The slides were fixed for 10 min in 4% paraformaldehyde, air-dried, and incubated for 20 min in PBS supplemented with 10% normal goat serum. Sections were incubated in a 1:50 dilution of affinity purified rabbit-antimurine IL-18 antibody (R&D Systems, Minneapolis, MN) or 1 μg/ml nonimmune rabbit IgG as negative control. The antibodies were diluted in PBS containing 1% bovine serum albumin. After an overnight incubation at 4°C, the sections were washed three times with 0.5% bovine serum albumin in PBS. The sections were then incubated with a secondary goat anti-rabbit antibody conjugated to Alexa488 (Molecular Probes, Eugene, OR) for 60 min at room temperature in the dark. Nuclei were stained blue using 1 μg/100 ml bisbenzimide (Sigma, St. Louis, MO). After further cut into strips to 1 cm² and placed in 24 flat-bottom well culture plates containing fresh RPMI 1640 supplemented with penicillin and streptomycin. Strips were incubated at 37°C in 1 ml of fresh supplemented RPMI 1640 medium for 24 h. Culture supernatants were harvested and assayed for cytokines.

**LPMC preparation and cultivation.** C57BL/6 mice were killed by isoflurane inhalation, and the colon was trimmed of fat, mesenteric tissue, and Peyer’s patches. LPMCs were isolated as described previously with minor changes (10, 57). Briefly, the colon was opened longitudinally and cut into 5-mm crosswise pieces. The tissue was incubated in calcium- and magnesium-free Hanks’ balanced salt solution (CMF-HBSS) containing 1 mM EDTA for 30 min under vigorous
vortexing at room temperature; this step was repeated once. After two washing steps with CMF-HBSS, the tissue was incubated for 60 min at 37°C in CMF-HBSS supplemented with 5% FCS and 100 U/ml of collagenases type II and VIII and 300 U/ml of hyaluronidase (Sigma). Cells were separated from tissue debris by filtration through a 100-μm cell strainer (BD Pharmingen, San Diego, CA) followed by purification through a discontinuous 20/40/70% Percoll gradient (Sigma) for 20 min at 800 g. Cells were then incubated at a concentration of 1 × 10^6/ml for 24 h under various experimental conditions.

Cytokine measurements. Murine IL-18 and TNF-α levels were measured using the electrochemiluminescence (ECL) method as described previously (11, 18, 19). The range of detection is 20 pg/ml to 10 ng/ml for both TNF-α and IL-18. IFN-γ was measured using a specific ELISA (Endogen, Woburn, MA).

Statistical analysis. The data are expressed as means ± SE. Statistical significance of differences between treatment and control groups was determined by factorial ANOVA and a Bonferroni-Dunn procedure as a post hoc test. Statistical analyses were performed using Stat-View 4.51 software (Abacus Concepts, Calabasas, CA).

RESULTS

DSS dose dependently induces colitis and IL-18 release. BALB/c mice were exposed to increasing concentrations of DSS for a 10-day period, resulting in a dose-dependent increase in colitis severity reaching a clear maximum at the 5% DSS concentration (Fig. 1A). To evaluate the role of IL-18 in this model, on day 10 colon segments of the different experimental groups were removed, incubated for 20 h, and IL-18 concentrations were determined in the supernatants (Fig. 1B). The spontaneous IL-18 synthesis was lowest in the organ culture of non-DSS-fed mice (0.4 ± 0.1 ng/mg protein), but it increased with the severity of colitis and DSS concentration to a maximum in the 4 and 5% DSS groups (2.5 ± 0.4 and 2.5 ± 0.5 ng/mg protein, respectively, n = 5, P < 0.01).

To further determine the IL-18-producing cells responsible for the dose-dependent increase, confocal laser microscopy was used to examine the transverse colon of mice receiving 3.5% DSS at day 10 of the experimental period. Sections of non-DSS-fed animals were comparable to controls stained with nonimmune rabbit IgG (data not shown). As shown in Fig. 2A, green staining reveals a marked increase of IL-18 in colon sections of DSS-fed animals compared with non-DSS-fed controls (Fig. 2B). In addition, IL-18 is clearly expressed in the epithelial cells.

Reduction of the clinical score by anti-IL-18 in BALB/c mice. BALB/c mice were injected intraperitoneally with either anti-IL-18 antiserum or NRS and started on a 10-day experimental course of 3.5% DSS in their drinking water. Additional injections of anti-IL-18 were given on days 4 and 8. Mice fed DSS developed signs of colitis indicated by a clinical score >0.5 starting from day 4 (Fig. 3A). Anti-IL-18 (400 μl) treatment markedly reduced the progression of colitis starting at day 6, as expressed by a significantly lower clinical score (0.7 ± 0.2) compared with the DSS-fed NRS control group (1.8 ± 0.3; n = 8, P < 0.01). This significant difference continued until the end of experiment on day 10 (1.7 ± 0.4 in the anti-IL-18-treated DSS group vs. 3.4 ± 0.2 in the NRS-treated DSS-exposed group, P < 0.01). In mice treated with 200 μl of anti-IL-18 antiserum on days 1 and 5, a nonsignificant improvement of colitis (2.9 ± 0.4; n = 5) was observed on days 9 and 10. Control mice without DSS treatment but receiving either NRS or 400 μl anti-IL-18 antiserum showed no signs of colitis.

The effect of anti-IL-18 (400 μl) treatment on the individual parameters of the clinical score, i.e., weight loss, stool consistency, and bleeding, is shown in Fig. 3, B-D. Figure 3B depicts a significantly reduced score for stool consistency starting at day 7 (0.8 ± 0.3 in the anti-IL-18-treated mice vs. 2.3 ± 0.2 in the NRS-treated mice; n = 8, P < 0.001) and persisting until the end of the experiment on day 10 (2.0 ± 0.5 in the anti-IL-18-treated mice vs. 3.5 ± 0.3 in the NRS-treated mice; n = 8, P < 0.001). Neutralization of IL-18 in DSS-exposed mice also reduced the bleeding score starting at day 7 (1.0 ± 0.4 in the anti-IL-18-treated group vs. 2.5 ± 0.3 in the NRS-treated group; n = 8, P < 0.001; Fig. 3C) and continuing until day 10 (2.0 ±
Neutralization of IL-18 decreases the clinical score in C57BL/6 mice. We evaluated the role of IL-18 in DSS-induced colitis in C57BL/6 mice, a strain often used in models of the Th1-directed immune response. As shown in Fig. 4, colitis severity in C57BL/6 mice fed 3% DSS was more pronounced on day 5 than in BALB/c mice (Fig. 3). Therefore, DSS administration was stopped on day 5 and followed by a 5-day observation period. Starting at day 4, the anti-IL-18 antiserum-treated DSS-fed mice showed a significantly lower clinical score (0.1 ± 0.1) than the NRS-treated DSS-fed group (1.6 ± 0.3; n = 5, P < 0.001; Fig. 4A). This difference continued until day 10 (1.1 ± 0.4 in the anti-IL-18-treated DSS-fed group compared with 2.3 ± 0.2 in the NRS-treated DSS-fed group; P < 0.01). Control mice without DSS treatment but receiving either NRS or 400 μL anti-IL-18 antiserum showed no signs of colitis.

The individual parameters of the clinical score of the C57BL/6 mice are presented in Fig. 4, B-D. Neutralization of IL-18 in DSS-exposed mice significantly reduced the bleeding score starting from day 4 (0.4 ± 0.4 in the anti-IL-18-treated group vs. 3.2 ± 0.4 in the NRS-treated group; n = 5, P < 0.001; Fig. 4C) until day 8 (1.2 ± 0.4 in the anti-IL-18-treated group vs. 2.8 ± 0.4 in the NRS-treated group; P < 0.01). Figure 4D depicts reduced weight loss starting from day 4 (0.0 ± 0.0 in the anti-IL-18-treated mice vs. 0.8 ± 0.3 in the NRS-treated mice; n = 5, P < 0.05) and persisting until day 7 (2.2 ± 0.3 in the anti-IL-18-treated mice vs. 3.2 ± 0.3 in the NRS-treated mice; n = 5, P < 0.05). A reduced score for stool consistency in anti-IL-18-treated DSS-fed mice was also observed, but this trend was not statistically significant (Fig. 4B).

Colon length. The degree of reduction in colon length correlated with the severity of the clinical score at day 10 in both strains of mice (Fig. 5). Comparing the colon length in both strains of the healthy control groups, it was remarkable that the colon length in BALB/c mice was significantly longer than in C57BL/6 mice (14.4 ± 0.2 vs. 10.7 ± 0.1 cm, respectively, n = 8, P < 0.001). The 10-day course of DSS in BALB/c mice resulted in a 35.5 ± 1.4% reduction of colon length (Fig. 5A). Administration of either 200 or 400 μL anti-IL-18 antiserum dose dependently reduced colon shortening (30.6 ± 3.5 and 22.9 ± 2.8%, respectively). In the C57BL/6 mice, the 10-day experimental period resulted in a 23.4 ± 2.8% shortening in the untreated DSS-fed group (Fig. 5B). However, anti-IL-18 treatment in the DSS-exposed mice almost completely prevented colon shortening in this group (4.7 ± 1.9%).

Histological score. Histology of the transverse colon in DSS-treated BALB/c mice revealed multiple erosive lesions and inflammatory cell infiltrations mainly composed of macrophages with few lymphocytes and occasional eosinophils and neutrophils. After 10 days of continuous DSS administration, neutralization of IL-18 resulted in a lower histological score in BALB/c mice, from 4.8 ± 0.2 in the NRS-treated to 4.0 ± 0.4 in the 400-μL anti-IL-18-treated mice (n = 8, P < 0.01). In the non-DSS groups, histological signs of inflammation were not detected (0.9 ± 0.2; n = 11), with no differences between anti-IL-18- and NRS-treated mice.

In vivo IFN-γ content in the colon. The concentration of IFN-γ in the colon at the end of the experiment (day 10) was determined in BALB/c and C57BL/6 mice. As shown in Fig. 6A, anti-IL-18 treatment led to a 43.0 ± 16.7% reduction in the IFN-γ concentration in the colon of DSS-fed BALB/c mice. In C57BL/6 mice, the anti-IL-18 treatment resulted in a 72.2 ± 6.1% reduction of the IFN-γ concentration. In both strains, the lowest concentrations were detected in the non-DSS-exposed control groups.

Spontaneous ex vivo synthesis of TNF-α, IFN-γ, and IL-18 in colon organ cultures. Colon segments of the different experimental groups were incubated for 20 h, and cytokine concentrations were determined in the supernatant. As shown in Fig. 7A, spontaneous TNF-α synthesis was clearly higher in the C57BL/6 strain; however, in both strains, anti-IL-18 treatment resulted in a significantly lower TNF-α production (70.0 ± 8.1% in BALB/c mice and 55.9 ± 11.8% in C57BL/6 mice).
compared with NRS-treated DSS-fed mice. As shown in Fig. 7B, the synthesis of IL-18, also significantly higher in the C57BL/6 strain, showed a marked suppression in the anti-IL-18-treated groups (47.8 ± 4.3% in BALB/c and 41.1 ± 5.7% in C57BL/6 mice) compared with NRS-treated DSS-fed mice. In contrast, the amount of spontaneous DSS-induced IFN-γ production is comparable among the supernatants of BALB/c and C57BL/6 mice. In both strains, the anti-IL-18 treatment led to a significant decrease in IFN-γ production (44.3 ± 14.8% reduction in BALB/c and 98.1 ± 1.5% reduction in C57BL/6 mice). The non-DSS-fed controls treated with either NRS or anti-IL-18 antiserum showed significantly lower concentrations than the untreated DSS-fed groups in both strains.

Stimulation of LPMCs with IL-18. LPMCs were isolated from healthy C57BL/6 mice as described in Materials and Methods.
RIALS AND METHODS. Cells were incubated for 24 h in the presence or absence of IL-12 (2 ng/ml), IL-18 (10 ng/ml), or a combination of both (Fig. 8). Stimulation by IL-12 alone did not result in an increase of IFN-γ production (0.3 ± 0.1 ng/ml in controls compared with 0.2 ± 0.1 ng/ml in the presence of IL-12; n = 4). Also, IL-18 alone did not induce a significant increase in IFN-γ production compared with control levels (0.4 ± 0.2 ng/ml; n = 4). However, the combination of IL-12 and IL-18 led to a significant elevation of IFN-γ production (1.5 ± 0.8 ng/ml; n = 4, P < 0.05).

DISCUSSION

In the present study, we demonstrated the importance of IL-18 as a mediator during DSS-induced colitis in both BALB/c and C57BL/6 mice. The data are derived from in vitro and in vivo determinations. At the site of inflammation, IL-18 was dose dependently induced by DSS, and IL-18 expression could be clearly localized to the colon epithelium by confocal laser microscopy. Most importantly, neutralizing anti-IL-18 antiserum resulted in a dose-dependent protection from DSS-induced colitis in BALB/c and C57BL/6 mice as shown by a mitigation in weight loss, improved stool consistency, and reduced rectal bleeding, the three parameters that comprise the clinical score. In agreement with the reduction in clinical and histological scores, anti-IL-18 treatment also prevented colon shortening, colon length representing the parameter with the least intraindividual variation in this model (42). Neutralization of IL-18 led to a significant inhibition of the IFN-γ content at the site of inflammation as determined in the homogenate of colonic tissue. Moreover, there was an impressive inhibition of TNF-α, IL-18, and IFN-γ secretion into the supernatant of colon organ culture. Stimulation of LPMC in vitro with either IL-12 or IL-18 alone did not induce an increase in IFN-γ; however, the combination of IL-12 and IL-18 synergistically increased IFN-γ synthesis.

The model DSS-induced colitis has a number of advantages, including its simplicity and high degree of uniformity of the lesions (16). With short-term administration, DSS causes a self-limited colitis; with continuous exposure, colitis with chronic features develops (9). DSS-induced colitis is viewed as a T cell-independent model because it is observed in T cell-deficient animals such as SCID mice (3, 13). These changes are thought to develop due to a toxic effect of DSS on the colonic epithelium followed by phagocytosis by lamina propria cells resulting in regional inflammation within

Fig. 5. Effect of anti-IL-18 on colon shortening in DSS-induced colitis. A: either 200 µl (days 1 and 5) or 400 µl (days 1, 4, and 8) of anti-IL-18 antiserum were injected intraperitoneally in BALB/c mice. B: four-hundred microliters (days 1, 4, and 8) of anti-IL-18 were injected in C57BL/6 mice. Colons were obtained at day 10 from the groups for which the clinical scores are shown in Figs. 3A and 4A. In BALB/c mice, 400 µl, n = 8, **P < 0.001; 200 µl, n = 5, *P = 0.003. In C57BL/6 mice, n = 5, *P < 0.001. The mean length ± SE of the colons in each group is depicted.

Fig. 6. Content of colonic interferon (IFN)-γ in DSS-induced colitis. Four-hundred microliters (days 1, 4, and 8) of anti-IL-18 antiserum were administered to BALB/c (A) and C57BL/6 mice (B). Colon segments were sliced longitudinally, homogenized in 400 µl PBS, and centrifuged as described in MATERIALS AND METHODS. IFN-γ was quantified by ELISA. Values are means ± SE, n = 5 mice/group. *P < 0.05 vs. DSS + NRS.
the colon. Increased production of Th1 and Th2 cytokines, as well as adhesion molecules and chemokines, likely mediates the inflammation (6, 12). Sulfasalazine and anti-TNF-α antibodies, both in clinical use for patients with Crohn’s disease, have been shown to reduce DSS-induced colitis (2, 28, 36). In addition, administration of the anti-intercellular adhesion molecule-1 (ICAM-1) antibodies or the anti-inflammatory cytokine IL-10 is effective in this model and is now in clinical trials (52, 54).

In the present report, we observed a dose-dependent correlation of the clinical score and spontaneous IL-18 secretion in colon culture supernatants. The IL-18 assay used in this study detects both the precursor and mature forms; however, IL-18 measured in the extracellular compartment of colon culture supernatants is mostly the mature form (18). Interestingly, although there was a worsening in the clinical score from 4 to 5% DSS, no further increase in the IL-18 release could be detected. This suggests that IL-18 may not be the sole mediator responsible for the pathological changes induced by DSS. In fact, it is likely that the combination of IL-18 plus IL-12 accounts for the induction of IFN-γ production. In vivo, the expression of IL-18 was clearly present in the epithelial cells using confocal laser microscopy. This observation is of significant importance for this model. In fact, Pizarro and colleagues (44) demonstrated that the mature form of IL-18 is markedly overexpressed in the intestinal lesions of patients with Crohn’s disease, but not with ulcerative colitis. In particular, the increased expression of IL-18 during Crohn’s disease localized to the epithelial cells as in DSS-induced colitis (44). Consequently, in terms of IL-18 expression, inflammation in the DSS-induced colitis mimics human Crohn’s disease.

Fig. 7. Spontaneous release of IFN-γ, tumor necrosis factor (TNF)-α, and IL-18 into the supernatants of colon cultures from DSS-fed mice. After a 24-h incubation period, TNF-α (A), IL-18 (B), and IFN-γ (C) concentrations in the supernatant of anti-IL-18- and NRS-treated mice were determined. Non-DSS controls were injected with either anti-IL-18 or NRS. TNF-α and IL-18 were detected by electrochemiluminescence, IFN-γ by ELISA. Bars represent means ± SE, n = 5. *P < 0.05 vs. DSS + NRS.

Fig. 8. Induction of IFN-γ synthesis in lamina propria mononuclear cells (LPMC). LPMC from healthy C57BL/6 mice were cultured for 24 h in the presence or absence of murine IL-12 (2 ng/ml), murine IL-18 (10 ng/ml), or a combination of both. IFN-γ was detected in both the cell lysate and supernatant by ELISA. Data represent means ± SE, n = 4. *P < 0.05 vs. IL-12 and IL-18 alone.
Two different mouse strains were purposefully chosen for these experiments: BALB/c and C57BL/6 mice. The BALB/c mouse strain is a high producer of IL-4, and the Th2 response is characteristic of the cytokines produced in these mice (7, 24). In BALB/c mice, IL-18 is a clear cofactor with IL-12 for the development of the Th1 response (51). In contrast, C57BL/6 mice are often used in models of autoimmune disease (33, 59). However, C57BL/6 mice do not exhibit a requirement for IL-18 for the development of the Th1 responses (48). Mahler and colleagues (32) previously demonstrated a difference in susceptibility to colitis associated with DSS depending on the mouse strain used. Consistent with this finding, we observed a marked difference between BALB/c and C57BL/6 mice. This difference was apparent at day 5, when the untreated BALB/c mice showed a clinical score of 0.3 ± 0.2 compared with 2.6 ± 0.1 in the C57BL/6 strain (Figs. 3 and 4). Nevertheless, in both strains, anti-IL-18 treatment protected against colitis, resulting in a lower clinical score and reduced colon shortening, as well as significant changes in the expression of the proinflammatory cytokines TNF-α, IFN-γ, and IL-18. In both strains, anti-IL-18 treatment led to suppression of the IFN-γ content in the colon to levels comparable to the background IFN-γ concentration detected in the non-DSS-fed control groups. Interestingly, in the colon culture supernatants, IFN-γ was the only cytokine that showed similar concentrations in both strains. However, the suppression by anti-IL-18 treatment was more pronounced in the C57BL/6 strain.

IL-18 has several biological properties consistent with its role in enhancing Th1 cell development and inflammation (14). In particular, the administration of exogenous IL-18 plus IL-12 to mice induces high serum levels of IFN-γ (37). These levels were 1,000 times higher than those in mice treated with IL-18 alone and 200 times higher than those in mice treated with IL-12 alone (37). In our in vitro stimulation of LPMC with either IL-12, IL-18, or a combination of both, we could confirm this synergism for the immune regulatory cells of the intestine. Two mechanisms may account for the enhancing effects of IL-12 on IFN-γ production as previously examined in peripheral blood mononuclear cells (29, 58). First, IL-12 upregulates production of IL-18 itself (18, 29) and, second, IL-12 increases the responsiveness (IFN-γ synthesis) of T and B cells to IL-18 by upregulation of IL-18Rα and IL-18Rβ chains mRNA expression (25, 58). Interestingly, in view of our experimental colitis model, Nakamura and colleagues (37) have observed that administration of IL-12 plus IL-18 led to weight loss in mice that did not occur in an IFN-γ-deficient strain; this could be compared with subclinical colitis.

Our results strengthen the evidence for a role of IFN-γ in colitis. Remarkably, IL-18 production in the C57BL/6 strain was significantly higher than in the BALB/c strain. Anti-IL-18 treatment resulted in a reduced secretion of IL-18, which cannot be explained by a direct effect of the antiserum. This effect is likely to be mediated by the suppression of IFN-γ synthesis by anti-IL-18 treatment, because IFN-γ induces cleavage of pro-IL-18 to the mature form by inducing the synthesis of interleukin-1β-converting enzyme (18). The suppression of TNF-α synthesis in the colon culture supernatants can also be explained by two different pathways. First, IL-18 can directly induce TNF-α production as previously demonstrated in whole blood assays (46). Therefore, suppression of IL-18 could result in the inhibition of TNF-α expression. Second, TNF-α suppression can also be mediated by suppression of IFN-γ by the anti-IL-18 treatment. In fact, IFN-γ is known to increase TNF-α production in the presence of lipopolysaccharide (38, 47).

IFN-γ-independent mechanisms might also be involved in the effect described, as IL-18 induces other proinflammatory mediators that contribute to inflammation in IBD. First, the upregulation of ICAM-1 is an IFN-γ-independent direct effect of IL-18 (26). ICAM-1 deficiency as well as administration of either antibodies against ICAM-1 or ICAM-1 antisense oligonucleotides protect mice against DSS-induced colitis (5, 6, 20). Second, IL-18 induces CC and CXC chemokine expression independently of IFN-γ (46). Cells infiltrating the lamina propria of patients with Crohn’s disease or ulcerative colitis show increased expression of CC and CXC chemokines such as IFN-γ-inducible protein (IP-10), IL-8, monocyte-chemoattractant protein-1 (MCP-1), and MCP-3 (30, 55). Studies with colonic epithelial cell lines suggest that TNF-α and IFN-γ are responsible for IL-8, MCP-1, and regulated upon activation, normal T cell expressed and secreted (RANTES) induction, thereby facilitating the development of chronic inflammatory infiltrates (56). IFN-γ and TNF-α are both elevated in patients with IBD and in DSS-induced colitis at the site of inflammation (12, 50).

We conclude that IL-18 is a key inflammatory mediator in this model of experimental colitis, as IL-18 expression can be induced by DSS, whereas neutralization of IL-18 dose dependently suppressed induction of colitis in BALB/c and C57BL/6 mice. In addition, anti-IL-18 treatment resulted in reduction of the in vivo colonic IFN-γ content as well as the ex vivo synthesis of TNF-α, IL-18, and IFN-γ, the prime secondary mediators of IL-18. Consequently, suppression of IL-18 represents a rational strategy for anti-inflammatory therapy in IBD.

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