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Treatment of T Cell-Dependent Experimental Colitis in SCID Mice by Local Administration of an Adenovirus Expressing IL-18 Antisense mRNA

Stefan Wirtz,* Christoph Becker,* Richard Blumberg,† Peter R. Galle,* and Markus F. Neurath2*

Recent studies have shown that IL-18, a pleiotropic cytokine that augments IFN-γ production, is produced by intestinal epithelial cells and lamina propria cells from patients with Crohn’s disease. In this study, we show that IL-18 is strongly expressed by intestinal epithelial cells in a murine model of Crohn’s disease induced by transfer of CD62L⁺CD4⁺ T cells into SCID mice. To specifically down-regulate IL-18 expression in this model, we constructed an E1/E3-deleted adenovirus expressing IL-18 antisense mRNA, denoted Ad-asIL-18, and demonstrated the capacity of such a vector to down-regulate IL-18 expression in colon-derived DLD-1 cells and RAW264.7 macrophages. Local administration of the Ad-asIL-18 vector to SCID mice with established colitis led to transduction of epithelial cells and caused a significant suppression of colitis activity, as assessed by a newly developed endoscopic analysis system for colitis. Furthermore, treatment with Ad-asIL-18 induced a significant suppression of histologic colitis activity and caused suppression of mucosal IFN-γ production, whereas IFN-γ production by spleen T cells was unaffected. Taken together, these data indicate an important role for IL-18 in the effector phase of a T cell-dependent murine model of colitis and suggest that strategies targeting IL-18 expression may be used for the treatment of patients with Crohn’s disease. The Journal of Immunology, 2002, 168: 411–420.

Interleukin-18 is a multifunctional cytokine with structural similarities to the IL-1 cytokine family (1). It was originally identified as IFN-γ-inducing factor in the liver of mice infected with Propionibacterium acnes (2). IL-18 is produced as an inactive precursor polypeptide, which is intracellularly processed and activated by the cysteine protease caspase-1 (IL-1β-converting enzyme) (3, 4). IL-18 mediates its function through binding to a specific receptor complex consisting of two chains: a ligand-binding chain termed the IL-18Rα chain (IL-1R related protein); and a signal-transducing chain termed the IL-18Rβ chain (5, 6). Whereas the IL-18Rα chain has a weak affinity for the ligand, the complete IL-18R complex has a high binding affinity for IL-18 (7). The functions of IL-18 on cells of the immune system are controlled by IL-18-binding proteins (8, 9).

IL-18 has a variety of important immunomodulatory effects on many different cells of the immune system through binding to low and high affinity receptors on the cell surface (1, 5, 10–12). In particular, IL-18 triggers NK cell activity; induces production of IL-2, IFN-γ, and GM-CSF by T lymphocytes, and up-regulates the expression of Fas ligand by NK cells (3, 7). Furthermore, IL-18 is a coinducer of IL-12 driven Th1 development and synergizes with IL-12 for IFN-γ production from Th1 cells (7, 13, 14). Hereby, IL-18 activates the transcription factors NF-κB and AP-1 that synergize with the IL-12-inducible transcription factor STAT-4 in activating the IFN-γ promoter in T cells (13, 15). In addition, IL-18 augments inflammatory immune responses by up-regulating the production of the proinflammatory TNF-γ, IL-1, and IL-6 cytokines (3). It is therefore believed that IL-18 expression could be a major contributing factor for the pathophysiology of Th1-related chronic inflammatory diseases and organ-specific autoimmunity.

IL-18 production has been detected in many cell types including activated macrophages, keratinocytes, osteoblasts, and airway epithelial cells (1, 3). In addition, pro and mature forms of IL-18 are also present in the colonic mucosa (16, 17). Interestingly, highly elevated IL-18 levels have been detected in lamina propria mononuclear cells (LPMC)3 and colon epithelial cells of subjects suffering from Crohn’s disease (16, 17), one of the major forms of inflammatory bowel diseases in humans (18–20). Furthermore, it has been suggested that IL-18 is a potent regulatory factor for both proliferation and Th1 cytokine production by lamina propria T lymphocytes in this disease (16, 17, 21, 22). However, little is known about the function of IL-18 in animal models of Crohn’s disease such as the CD45Rb(high) transfer model. In this model, SCID mice are reconstituted with CD45Rb(high) or CD45Rb(low) CD4⁺ T cells leading to a chronic transmural colitis in ~80% of the recipient mice 6–12 wk after the T cell transfer (23–25). This colitis is mediated by Th1 T lymphocytes that produce large amounts of IFN-γ (23, 26).

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1 Abbreviations used in this paper: LPMC, lamina propria mononuclear cells; Ad5, adenovirus serotype 5; GFP, green-fluorescent protein; CD62L, CD62 ligand; MEICS, murine endoscopic index of colitis severity; Erk2, extracellular signal-related kinase 2.

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Of the various types of viral and nonviral vector systems, recombinant adenoviruses of serotype 5 (Ad5) have shown promising results in transducing a broad spectrum of both dividing and nondividing eukaryotic cells in many different tissues (27, 28). We and others have recently shown that replication-defective Ad5 vectors are able to efficiently transduce epithelial and subepithelial areas of the intestines of mice with or without experimentally induced colitis (29–32). In the current study, we investigate the utility of an IL-18-based gene therapy as a potential treatment of experimental inflammatory bowel disease. Thus far, there are several studies showing an effective down-regulation of endogenous gene expression by adenovirus-mediated expression of corresponding antisense RNA constructs (33–35). We have created, therefore, a mucosal gene transfer model using adenoviral vectors producing IL-18 antisense RNA to achieve selective down-regulation of IL-18 mRNA and protein levels in the colonic mucosa in a mouse model of experimental colitis. We show that rectal administration of recombinant adenoviruses with an expression cassette for IL-18 antisense RNA was able to reduce IL-18 expression and suppress experimental colitis in vivo.

Materials and Methods

Construction and purification of recombinant adenoviruses

The E1E3-deleted recombinant adenoviruses Ad-asaIL-18 and Ad-saIL-18 were constructed with the AdEasy system (kindly provided by B. Vogelstein), as shown in Fig. 1. Briefly, total RNA obtained from LPS-stimulated RAW cells was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Roche, Basel, Switzerland). Next, the full length IL-18 cDNA was amplified by PCR using IL-18-specific oligonucleotide primers (5′-CTCCCATCCAATTGATG-3′ and 5′-CCAGGAACATTGCT GCCAT-3′) and subcloned into the eukaryotic expression vector pCR3.1 (Invitrogen, Heidelberg, Germany). An EcoRI/HindIII fragment was treated with Klenow enzyme to create blunt ends and cloned in either sense or antisense orientation into the EcoRV site of pAdTrackCMV yielding the pAdTrackCMVsaIL-18 or pAdTrackCMVsIL-18 plasmids, respectively. These plasmids were cotransfected together with pAdEasy-1 into electrocompetent BJ5183 bacterial cells for homologous recombination. For generation of viral particles, the resulting plasmids were transfected into 293 cells using the Effectene reagent (Qiagen, Hilden, Germany). The resulting adenoviral vectors, denoted Ad-saIL-18 and Ad-asaIL-18, were amplified by sequential culture on 293 cells and purified by CsCl density gradient centrifugation. Banded viral particles were dialyzed several times against Tris-HCl (pH 8), 10% glycerol and stored in aliquots at −80°C. The centrifugation of viral particles were determined by fluorescence-forming units or plaque assay on 293 cells essentially as described (30). Both vectors expressing IL-18 sense (Ad-saIL-18) and antisense (Ad-asaIL-18) RNA contained as an additional feature an expression cassette for green-fluorescent protein (GFP) as a reporter gene.

Cell culture and in vitro infection experiments

293 cells, DLD-1 cells, and HT-29 cells were obtained from American Type Culture Collection (Manassas, VA). RAW264.7 cells were a gift of X. Ma (Cornell University, Ithaca, NY) (36). The cells were cultured in DMEM supplemented with 10% FCS (PAA, Colbe, Germany), 2 mM glucose, 100 U/ml penicillin and 100 µg/ml streptomycin (Biochrome, Berlin, Germany) at 37°C in the presence of 5% CO2. In vitro experimental studies with DLD-1 cells were performed as follows. Confluent DLD-1 cells in T60 flasks were split 1:3 the day before infection. For infection, cells were incubated at 37°C for 1 h with different PFU of Ad-saIL-18 or Ad-asaIL-18 in 2 ml culture medium. Subsequently, the viral particles were removed, and 10 ml of culture medium were added. After 48 h, supernatants and cells were harvested for subsequent ELISA or Western blot analysis. RAW264.7 cells were stimulated with LPS (Sigma, St. Louis, MO) as previously described and infected with indicated amounts of Ad-asaIL-18 cells, as specified in Results. In some experiments, the percent of infected cells was monitored by FACS analysis (FACScan; BD Biosciences, Mountain View, CA) of GFP-positive cells.

Western blots

For IL-18 Western blot analysis, intestinal specimens were snap frozen in liquid nitrogen and homogenized in MPER reagent (Pierce, Heidelberg, Germany) supplemented with 0.5 mM PMSF, 1 mM DTT, 10 µM leupeptin, and 1 µg/ml aprotinin and centrifuged at 10,000 rpm. Proteins in the supernatants were separated on Tris-Tricine SDS-PAGE gels (50 µg) and transferred to nitrocellulose membranes (Schleicher & Schuell). Nonspecific binding sites were blocked with PBS, 5% milk powder, 0.1% Tween 20, followed by sequential incubation in 0.75 µg/ml rat anti-mouse IL-18 (R&D, Heidelberg, Germany) and 1:2000 HPO-labeled anti-rat IgG (Santa Cruz Biotechnology, Santa Cruz, CA). Detection of IL-18-specific complexes was performed with the ECL system (Amersham, Arlington Heights, IL) and Biomax MR films (Kodak, Rochester, NY).

ELISA

IL-18 in cell culture supernatants was detected and quantified using a specific sandwich ELISA (OptiEIA System; BD Biosciences) and 96-well plates (MaxiSorb; Nunc, Naperville, IL).

In vivo gene transfer studies

CB.17 SCID and BALB/c mice (6–8 wk old) were obtained from Charles River (Sulzfeld, Germany) and bred under specific pathogen-free conditions. Chronic colitis in SCID mice was induced by i.p. injection of 1 × 106 CD4+ CD62 ligand (CD62L)+ T cells isolated from spleens of BALB/c mice into SCID mice. In brief, CD4+ T cells were purified from spleen mononuclear cells of healthy BALB/c mice with the use of FITC-conjugated mAbs, anti-FITC immunomagnetic beads, and MACS (Milteny, Bergisch Gladbach, Germany) followed by enzymatic removal of the beads (25). The resulting CD4+ T cells (purity, >95%) were further separated by immunomagnetic beads into CD62L+ and CD62L− T cells. The former cells (purity, >95%) showed high expression of CD45RB by FACS analysis, CD62L− CD45RB+ with CD4+ T cells (1 × 106) were finally transferred into CB-17 SCID mice. SCID mice were maintained in isolated ventilated cages in the S2 animal facility of the I Medical Clinic.

After onset of colitis, mice were anesthetized with avertin. The colon was flushed several times with PBS, and 1 × 1010 PFU Ad-asIL-18 or AdCMVβGal in a volume of 100 µl were injected intrarectally into the lumen of the colon through a small 3.5 French catheter. This procedure was repeated 2 days and 4 days later. Six days after the first injection, mice were sacrificed, and spleens and colons were removed for subsequent analysis.

Analysis of transduction by fluorescence studies and immunohistochemical analysis

For the analysis of cell transduction, 7-µm colon cryosections were analyzed by fluorescence microscopy (Olympus fluorescence microscope; Olympus, Melville, NY). For immunohistochemical analysis, cryosections were fixed in ice cold acetone for 10 min followed by sequential incubation with methanol, 3% H2O2, avidin/biotin (Vector Laboratories, Burlingame, CA), and protein blocking reagent (DAKO, Wiesbaden, Germany) to eliminate unspecific background staining. Slides were then incubated overnight with biotinylated monoclonal (38) (1/200 dilution) or unlabeled rabbit polyclonal (Biotrend, Cologne, Germany; 1/100 dilution) IL-18-specific Abs. Subsequently, the latter samples were incubated for 1 h at room temperature with biotinylated secondary Abs (Sigma). All samples were finally treated with streptavidin and stained with diaminobenzidine chromogen according to the manufacturer’s instructions (DAKO). Before examination, the nuclei were counterstained with hematoxylin.

In vivo endoscopic analysis of the colon

A novel method was created to perform endoscopy in mice using a minioscopescope (length, 65 mm; diameter, 0.89 mm) with an Intralux vision light source (Volpi, Schlieren, Zurich, Switzerland). This technique was established in >150 mice with and without colitis. In brief, mice were anesthetized with avertine, and the colon was flushed with PBS. The colon was assessed by macroscopic analysis. It was found that SCID mice reconstituted with CD62L+ CD4+ T cells showed endoscopic signs of colitis earlier than weight loss. Prominent endoscopic signs of inflammation in SCID mice were masking of the normal vascular pattern, the presence of mucosal granularity, and the appearance of ulcers. Based on our data in colitic SCID mice, a murine endoscopic index of colitis severity (MEICS) was created, as specified in Table I. This index allowed monitoring of colitis activity in individual SCID mice over several months, because the endoscopic procedure could be performed without problems up to eight times in the same SCID mouse. To determine colitis activity in SCID mice after treatment with the IL-18 antisense RNA-expressing adenovirus, mice were monitored by endoscopy at the indicated time points.
Isolation of spleen mononuclear cells

At the indicated time points, mononuclear cells from the spleen of Ad-asIL-18- or control-treated mice were isolated as described previously (25). To measure cytokine production, $1 \times 10^6$ splenic cells per ml were activated with 10 μg/ml purified hamster anti-mouse CD3ε (clone 145-2C11) and 1 μg/ml soluble hamster anti-mouse CD28 (clone 37.51) and cultured in complete medium (RPMI 1640 supplemented with 3 mM l-glutamine, 10 mM HEPES buffer, 100 U/ml penicillin-streptomycin, 0.05 mM 2-ME, 10% heat-inactivated FCS) at 37°C in a humidified atmosphere containing 5% CO₂. After 48 h, culture supernatants were removed and assayed for cytokine concentration. Cytokine concentrations were determined by specific ELISA using commercially available recombinant cytokines and Abs (BD PharMingen, San Diego, CA).

**FIGURE 1.** Construction of recombinant adenoviruses. A, The entire coding region of murine IL-18 was inserted in either sense or antisense orientation into the Ad5 genome via homologous recombination in bacterial cells using a shuttle vector (pAdTrackCMV). Large scale amplification of Ad-sIL-18 and Ad-as-IL-18 was performed in 293 cells. Successful transduction was monitored in vitro and in vivo using enhanced GFP expression (B). LITR, left inverted terminal repeat; RITR, right inverted terminal repeat.
Isolation of LMPC

LPMC were isolated from resected colon specimens by a previously described technique (39). Briefly, after removal of Peyer’s patches, the colon was opened longitudinally, washed several times in PBS to remove feces and debris, and cut into small pieces. Tissues were incubated at 37°C in PBS supplemented with 0.145 mg/ml DTT and 0.37 mg/ml EDTA for 15 min. The tissue was afterwards digested in RPMI 1640 containing 0.15 mg/ml type II collagenase (Worthington, Munich, Germany) and 0.1 mg/ml DNase (Roche Molecular Biochemicals, Mannheim, Germany) for 75–90 min at 37°C on a shaking platform. LPMCs were finally isolated from the interface of a 40%/100% Percoll gradient (Biochrom). The cells were stimulated for 48 h with Abs to CD3 and CD28 (BD PharMingen), as previously described (39) followed by the collection of culture supernatant and analysis of the IFN-γ content by specific ELISA (BD PharMingen).

Histologic monitoring of colitis activity

Mouse colon was taken at indicated time points for histologic analysis. Cryosections were made from frozen samples and stained with H&E. Histologic scoring of colitis activity was made, as previously described (25). Data were compared by the Wilcoxon test using the program Statworks for Macintosh (Apple, Cupertino, CA).

Results

Construction of a recombinant adenovirus expressing IL-18 antisense RNA

On the basis of previous studies showing a major role of IL-18 in Crohn’s disease (3, 16, 17), we thought to down-regulate the expression levels of IL-18 in a murine model of Crohn’s disease (3, 16, 17), we thought to down-regulate the expression levels of IL-18 in a murine model of Crohn’s disease and to assess the potential relevance of IL-18 in chronic experimental colitis. To specifically regulate the levels of IL-18 gene expression in the colonic mucosa in vivo, we constructed replication-deficient Ad5 vectors expressing IL-18 antisense and sense RNA under the control of a CMV promoter (Fig. 1). In these experiments, we inserted the full-length IL-18 cDNA in either sense or antisense orientation into the EcoRV site of pAdTrackCMV construct (see Materials and Methods) yielding the pAdTrackCMVasIL-18 or pAdTrackCMVasIL-18 plasmids. These plasmids were cotransfected with pAdEasy-1 into BJ5183 cells for homologous recombination. For generation of viral particles, the resulting plasmids were transfected into 293 cells. Both adenoviral vectors expressing IL-18 sense (Ad-sIL-18) and antisense (Ad-asIL-18) RNA, respectively, contained as additional feature an expression cassette for GFP as reporter gene.

Down-regulation of IL-18 expression in colon-derived cell lines and RAW264.7 macrophages with a recombinant adenovirus expressing IL-18 antisense RNA

For the analysis of modulation of IL-18 by recombinant adenoviruses, we selected the colon-derived DLD-1 adenocarcinoma cell line for in vitro studies. After infection of DLD-1 cells with Ad-asIL-18 or Ad-sIL-18 more than 60% of DLD-1 cells were GFP positive, as assessed by fluorescence microscopy, indicating that these cells express sufficient receptors for binding and entry of adenoviruses (Fig. 2A). Indeed, the transduction efficiency of both adenoviral vectors in DLD-1 cells was up to 100% depending on...
the viral titer, as assessed by FACS (data not shown). In addition, Western blot analysis revealed a strong expression of IL-18 precursor protein in Ad-sIL-18-transduced DLD-1 cells 48 h after incubation with viral particles (Fig. 2A). Interestingly, the coinfection of DLD-1 cells with Ad-sIL-18 and various amounts of the antisense IL-18 mRNA-expressing construct, Ad-asIL-18, resulted in a significant decrease in the amount of IL-18 protein in cell lysates, suggesting that Ad-asIL-18 application causes down-regulation of cellular IL-18 expression. To explore IL-18 secretion patterns in cotransduced cells, we collected culture supernatants and performed ELISA for IL-18 protein. Whereas no detectable IL-18 protein secretion was found in noninfected control cells, DLD-1 cells infected with Ad-sIL-18 released IL-18 into the supernatant (Fig. 3). Coexpression of increasing amounts of adenovirus-derived antisense RNA led to a significant (p < 0.05) down-regulation of IL-18 levels in a dose-dependent manner. Similar treatment of human HT-29 colon adenocarcinoma cells produced similar results (data not shown).

To test whether Ad-as-IL-18 treatment would cause down-regulation of endogenous intracellular IL-18 production, we infected LPS-stimulated RAW264.7 macrophages with Ad-asIL-18 and analyzed IL-18 expression by Western blot analysis. As shown in Fig. 2C, Ad-asIL-18 treatment resulted in a down-regulation of intracellular IL-18 expression. Interestingly, higher virus titers were required to observe similar effects to DLD-1 cells most likely due to the strikingly lower transduction efficiency of this cell line under our experimental conditions (Fig. 2, D and E). However, 1011 PFU of Ad-asIL-18 led to a marked down-regulation of IL-18 protein expression, whereas extracellular signal-related kinase 2 (Erk2) expression was unaffected.

Taken together, these data suggest that the constructed Ad-asIL-18 adenovirus is capable to suppress IL-18 expression in vitro cell culture systems.

In vivo down-regulation of IL-18 levels in the colon mucosa

Because overexpression of IL-18 antisense RNA significantly inhibited IL-18 protein production in vitro, we next assessed the effectiveness of the Ad-asIL-18 construct to down-regulate IL-18 expression in experimental colitis in vivo. In these studies, we used an adoptive transfer model of chronic colitis in which a subfraction of CD4+ T cells expressing CD62L from BALB/c mice was transferred into CB17 SCID mice (23–25, 40). About 80% of the reconstituted SCID mice displayed clinical signs of colitis with diarrhea and weight loss 6–12 wk after cell transfer. This was associated with an increase in the expression of mature IL-18 in the inflamed colonic mucosa compared with the colon of unreconstituted SCID mice, as shown by Western blot analysis (Fig. 4A). Subsequent immunohistochemical analysis using two independent Abs to detect IL-18 showed that the highest IL-18 expression in colitic SCID mice was seen in intestinal epithelial cells and to a lesser extent in LPMC (Fig. 4B). Because local administration of recombinant adenoviruses with normal fiber structure in colitic mice has been shown to result in high transduction efficiency in intestinal epithelial cells and to a lesser extent in LPMCs (30), these data thus provided a rationale for local administration of Ad-asIL-18 adenoviruses to suppress intestinal IL-18 expression in colitic SCID mice. Accordingly, reconstituted SCID mice with established colitis that showed diarrhea and at least 10% weight loss were treated by intrarectal administration of Ad-asIL-18 or control adenovirus. Accordingly, 1 × 1010 PFU Ad-asIL-18 or AdCMV/Gal were injected intrarectally into the lumen of the colon, and this procedure was repeated 2 and 4 days later. Six days after the first injection, mice were sacrificed, and spleens and colons were removed for subsequent analysis. Treatment with AdasIL-18 led to transduction of intestinal cells in the inflamed colon, as shown by fluorescence analysis of colon cryosections (Fig. 4C). To determine whether expression of IL-18 antisense RNA affected the endogenous IL-18 production in the colon mucosa in vivo, we performed Western blot analysis with colon lysates. As shown in Fig. 4D, the levels of endogenous IL-18 protein were reduced on treatment with the Ad-asIL-18 adenovirus compared with treatment with the AdLacZ control virus. These data indicated that local treatment with the Ad-asIL-18 adenovirus suppressed local IL-18 production in the mucosa of colitic mice in vivo.

Suppression of chronic intestinal inflammation in reconstituted SCID mice by IL-18 antisense mRNA-expressing adenoviruses

To monitor colitis activity in reconstituted SCID over prolonged periods of time, we developed a novel method to assess colitis activity using a minendoscope. This method allows detection of colitis activity that correlates well with histologic analysis of colitis. Based on our endoscopic experience in >150 mice, we created the MEICS score (Table I) to assess colitis in Ad-asIL-18- and control-treated SCID mice. This score considers the major endoscopic features of colitis in reconstituted SCID mice such as ulcer formation, granularity of the mucosa, and abrogation of the normal vascular pattern and correlated well with blinded histopathologic scoring data (correlation coefficient, 0.9). As shown in Fig. 5, treatment with Ad-asIL-18 improved the endoscopic appearance of the colon compared to control-treated SCID mice. This finding was further supported by blinded endoscopic scoring of colitis activity, because there was a time-dependent significant reduction in the MEICS score after treatment with Ad-asIL-18 compared with the control vector (Fig. 6).

To further document the changes in the colon on treatment with Ad-asIL-18, we finally performed histopathologic analysis of colitis activity. As shown in Fig. 7, there was a suppression of colitis activity on treatment with Ad-asIL-18 adenoviruses compared with control-treated animals. This finding was further supported by a significant reduction of histologic colitis activity, as assessed by quantification of pathologic changes in three independent experiments (Fig. 8).
Suppression of IFN-γ production by lamina propria but not spleen mononuclear cells on Ad-asIL-18 treatment

Because IL-18 has been shown to be a key regulator of IFN-γ production by T cells (3), we focused in a final series of experiments on the production of IFN-γ by LPMC from Ad-asIL-18-treated SCID mice. Accordingly, we isolated LPMC from Ad-asIL-18- and control virus-treated mice and stimulated them in the presence of Abs to CD3 and CD28 for 48 h. As shown in Fig. 9, there was a profound down-regulation of IFN-γ production by LPMC in Ad-asIL-18-treated mice compared with control-treated SCID mice.
animals. In contrast, however, IFN-\(\gamma\) production by spleen mononuclear cells was not affected by Ad-asIL-18 treatment. Taken together, these data suggested that Ad-asIL-18 treatment modulates mucosal IFN-\(\gamma\) production, whereas systemic IFN-\(\gamma\) production was not affected.

**Discussion**

Recent studies have shown that IL-18 production is up-regulated in the lamina propria of patients with Crohn’s disease and indicated an important regulatory function of this cytokine for both proliferation and IFN-\(\gamma\) production of lamina propria T cells in this disease (16, 17, 21). The potential therapeutic relevance of blocking IL-18 in an animal model of Crohn’s disease has not been tested thus far, however. Here, we demonstrate that an adenovirus producing IL-18 antisense RNA is able to suppress IL-18 production by intestinal cell line and RAW264.7 macrophages. Furthermore, administration of this adenovirus to mice with established experimental colitis led to suppression of mucosal IFN-\(\gamma\) production and colitis activity, as assessed by endoscopic and histologic criteria. These data suggest the potential therapeutic utility of strategies to suppress IL-18 in intestinal inflammation.

IL-18 has been recently shown to contribute to Th1-mediated immune responses and chronic inflammation (3), and its production in the intestine is up-regulated in Crohn’s disease (16, 17), a major form of chronic intestinal inflammation in humans thought to be mediated by IL-12-driven Th1 effector cells (41, 42). Furthermore, it was found that serum levels of IL-18 were higher in patients with Crohn’s disease than in control patients, whereas no such increase was observed in patients with ulcerative colitis (21), a disease thought to be mediated by T cells producing high amounts of IL-5 rather than IFN-\(\gamma\) (42–44). In addition, increased amounts of bioactive IL-18 have been demonstrated to be produced in inflamed Crohn’s disease mucosa (16, 17). However, in addition to intestinal epithelial cells, recent data suggest that infiltrating macrophages and dendritic cells are a major source of IL-18 production in Crohn’s disease (21). Interestingly, IL-18 induces proliferation of LP T cells even in the absence of anti-CD3 stimulation, possibly due to interaction of IL-18 with the IL-1R related protein on LP T cells (6). Furthermore, IL-18 induces IFN-\(\gamma\) production by lamina propria T cells in Crohn’s disease and

![FIGURE 5.](image)

**FIGURE 5.** In vivo endoscopic analysis of the colon in healthy unreconstituted SCID mice top) and in reconstituted SCID mice (other panels). SCID mice were infected intrarectally with \(1 \times 10^{10}\) PFU Ad-asIL-18 or control virus (control Ad) or left untreated, as indicated. Note the normal vascular pattern of the mucosa in the upper and lower panels and an abrogation of vessels (a typical sign of colitis in SCID mice; see arrows) in untreated and control virus-treated (day 6 after administration of adenoviral particles) mice.

![FIGURE 6.](image)

**FIGURE 6.** Down-regulation of IL-18 levels in the colon of chimeric SCID mice with colitis causes a reduction in MEICS. SCID mice reconstituted with \(1 \times 10^6\) CD4+CD62L+ T cells from donor BALB/c mice were infected with \(1 \times 10^{10}\) PFU Ad-asIL-18, control adenovirus, or PBS via the rectum after the T cell transfer. Colitis activity was assessed before and 4 and 6 days after the treatment with Ad-asIL-18 or control adenovirus, as indicated. Data represent mean values ± SEM of the MEICS score in three mice per group. Treatment with Ad-asIL-18 caused a time-dependent, significant (\(\ast\), \(p < 0.05\); **\(\ast\ast\), \(p < 0.01\)) reduction in colitis severity in reconstituted SCID mice.
could thus be an important factor for the pathophysiology of this disease (16, 17). On the basis of these findings, we chose in the present study to target IL-18 production in an animal model of Crohn’s disease using an adenovirus-based strategy.

In an animal model of Crohn’s disease induced by reconstitution of SCID mice with CD62L⁺CD4⁺ T cells (23, 25), we found in the present study that IL-18 is strongly up-regulated in the inflamed colon compared with unreconstituted SCID mice. By immunohistochemical analysis, we observed that most of the IL-18-expressing cells in colitis appeared to be intestinal epithelial cells and that some of them were lamina propria cells. Because local administration of adenoviruses with normal fiber structure to the inflamed colon mainly targets intestinal epithelial cells and to a lesser extent lamina propria cells (30), these data suggested that local targeting of IL-18 expression by adenoviruses is an attractive approach to target the expression of this cytokine in the inflamed colon. Indeed, local administration of Ad-asIL-18 adenoviruses in colitic SCID mice led to transduction of epithelial cells and some lamina propria cells (the cells that were shown to express IL-18 in this model) and was associated with a down-regulation of mucosal IL-18 expression and IFN-γ production by purified LPMC. These data indicate that the Ad-asIL-18 adenovirus efficiently transduces intestinal cells after local administration and causes down-regulation of IL-18 expression in the inflamed intestine.

Various cytokines such as IL-12, IL-18, and IL-23 have been shown either to induce Th1 T cell development or to augment the Th1 effector function (13, 45, 46). It has been shown that IL-12 stimulates IL-18 responsiveness of T cells through induction of IL-18R expression (1) and that IL-18 has synergistic effects to IL-12 on IFN-γ production in T cells (13, 47). This is at least in part thought to be mediated by synergistic activation of the IFN-γ promoter through STAT-4 and AP-1 sites (15). High affinity receptors for IL-18 are predominantly expressed on Th1 but not Th2 cells (6), and the functional importance of IL-18 for Th1 cells is underlined by the finding that IL-18Rα chain-deficient mice exhibit impaired Th1 development (12). These observations have important implications for the mucosal immune system, in that recent studies showed that administration of rIL-18 as well as IL-12 induces acute intestinal inflammation in mice (48). However, IL-18-transgenic mice did not show chronic intestinal inflammation (Finotto et al., manuscript in preparation), suggesting that other cytokines such as IL-12 and IL-12-dependent signaling events are important to initiate a chronic inflammatory Th1 response in the intestine. In support of this hypothesis, recent data showed that transgenic mice for the IL-12-responsive transcription factor STAT-4 can exhibit chronic intestinal inflammation (49). The data from the present study, however, clearly show that IL-18 is highly important for the effector phase of chronic colitis, as adenoviral-induced suppression of IL-18 expression led to significant improvement of established colitis in SCID mice reconstituted with CD4⁺CD62L⁺ T cells. This effect was demonstrated using quantitative histologic assessment of colitis activity as well as endoscopic scoring of colitis activity using a newly developed technique for miniendoscopy of the colon in mice. This latter technique might be particularly useful in monitoring colitis activity in SCID mice, because it allows daily monitoring of colitis activity in mice with chronic intestinal inflammation.

Despite recent advances in our understanding of mucosal immunology, effective manipulation of the gastrointestinal immune system as a novel therapeutic strategy remains a challenging task (18, 20, 50–52). In our study, we have used an adenoviral vector expressing antisense DNA to IL-18 to treat chronic intestinal inflammation in SCID mice. Such local administration of adenoviruses blocking the synthesis of IL-18 is a novel concept that has the theoretical advantage of suppressing the local immune function in the intestine rather than the entire immune system. One might speculate that this is also important with regard to the neutralization of IL-18, given that IL-18 has pleiotropic effects on various cell types of the immune system with important regulatory function (2, 3, 12). In support of this hypothesis, we have observed that IFN-γ cytokine production by spleen cells is virtually unaffected by local administration of IL-18 antisense RNA-producing adenoviruses, whereas mucosal IFN-γ production was profoundly suppressed. However, colitis activity was suppressed by adenoviral gene transfer of our IL-18 antisense RNA-producing construct even after colitis was already established, suggesting that IL-18 plays a key role in the effector phase of chronic intestinal inflammation in SCID mice. Taken together with the recent demonstration that IL-18 production is up-regulated in patients with Crohn’s disease, these data thus provide a rationale for the treatment of patients with Crohn’s disease using strategies to suppress the production and or function of IL-18.
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