The Journal of Immunology

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J Immunol 1999; 163:143-147; ;
http://www.jimmunol.org/content/163/1/143

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Bioactive IL-18 Expression Is Up-Regulated in Crohn's Disease[1]

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An imbalance of immunoregulatory factors is believed to contribute to uncontrolled mucosal Th1 cell activation in Crohn's disease (CD). IL-18, a macrophage-like cell-derived cytokine, is involved in Th1 clone development, and IFN-γ production. Therefore, IL-18 expression was investigated in CD. Whole mucosal intestinal tissue and lamina propria mononuclear cells (LPMC) of 12 CD and 9 ulcerative colitis (UC) patients and 15 non-inflammatory bowel disease (IBD) controls were tested for IL-18 by semiquantitative RT-PCR and Western blot analysis. Transcripts for IL-18 were found in all samples tested. However, increased IL-18 mRNA accumulation was detected in both mucosal and LPMC samples from CD in comparison to UC and controls. In CD, transcripts for IL-18 were more abundant in the mucosal samples taken from involved areas. An 18-kDa band consistent with mature IL-18 was predominantly found in CD mucosal samples. In mucosal samples from non-IBD controls, IL-18 was present as a 24-kDa polypeptide. Consistently, active IL-1β-converting enzyme (ICE) subunit (p20) was expressed in samples from either CD or UC, whereas, in colonic mucosa from non-IBD controls, ICE was synthesized as precursor (p45) only. To confirm that IL-18 produced in CD tissue was functionally active, CD LPMC were treated with a specific IL-18 antisense oligonucleotide. In these cultures, IL-18 down-regulation was accompanied by a decrease in IFN-γ expression. In aggregate, our data indicate that IL-18 up-regulation is a feature of CD and suggest that IL-18 may contribute to the local immunoinflammatory response in CD. ***The Journal of Immunology*****, 1999, 163: 143–147.**

Crohn's disease (CD)[4] is a chronic inflammatory process involving the gastrointestinal tract. Both histological and immunological observations indicate that cell-mediated immunity and T cell activation are key features of CD (1–5). Substantial evidence also supports the concept that an imbalance of immunoregulatory factors may lead to uncontrolled T cell activation within the mucosal compartment (6). Studies from humans and experimental models suggest that, in CD, the local immune response tends to be predominantly Th1 in type and that locally released cytokines contribute to promote and expand Th1 immune responses (7–11).

IFN-γ-inducing factor is a recently described cytokine, designed IL-18 (12). IL-18, originally purified from the livers of mice treated with the bacterium *Propionibacterium acnes* and subsequently challenged with LPS, is produced by macrophage-like cells (12, 13). The IL-18 gene encodes a precursor protein that is processed and cleaved to bioactive IL-18, a 18.3-kDa polypeptide, by IL-1β-converting enzyme (ICE) (14).

IL-18 has a variety of biologic effects consistent with its role in promoting Th1 cell clone development. These functions include the stimulation of both T cell proliferation and IL-2R α-chain expression, the enhancement of the lytic activity of NK cells, and the promotion of IFN-γ synthesis (13, 15). Therefore, IL-18 exerts functions similar to those reported with IL-12 (16), a macrophage-derived cytokine actively expressed and released in CD tissue (17, 18). In addition, IL-18 is capable of promoting inflammatory cascade by enhancing TNF-α, IL-8, and IL-1 release in human PBMC cultures (19).

The present study was therefore designed to explore whether IL-18 is involved in the immunoinflammatory response in CD. Specific aims were: 1) to demonstrate IL-18 at both the mRNA and protein levels, in freshly obtained mucosal tissue and lamina propria mononuclear cell (LPMC) samples from CD patients; 2) to investigate whether bioactive IL-18 expression is related to the presence of active ICE; and 3) to explore whether IL-18 produced in CD is biologically active. We report here data showing that: 1) transcripts for IL-18 are constitutively expressed in human intestine; 2) IL-18 mRNA accumulation is more pronounced in mucosal samples from CD patients; 3) bioactive IL-18 is expressed in either CD or ulcerative colitis (UC) but not in non-inflammatory bowel disease (IBD) control mucosal samples; 4) in CD and UC, the expression of bioactive IL-18 is related to the presence of active ICE subunit p20; and 5) in CD LPMC cultures, antisense-induced IL-18 down-regulation is accompanied by a decrease in IFN-γ production.

Data of the study indicate that IL-18 up-regulation is a feature of CD and suggest that IL-18 contributes to the local IFN-γ synthesis.

Materials and Methods

Patients and samples

Mucosal samples were taken from freshly obtained intestinal resection specimens of eight patients with CD. The primary site of involvement was

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Received for publication December 31, 1998. Accepted for publication April 12, 1999.

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[1] This work is supported by Grant CNR 96.03133.CT04 from the Italian National Research Council.

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[4] Abbreviations used in this paper: CD, Crohn's disease; ICE, IL-1β-converting enzyme; LPMC, lamina propria mononuclear cells; UC, ulcerative colitis; IBD, inflammatory bowel disease; CS, corticosteroids; SEB, staphylococcal enterotoxin B.

0022-1767/99/$02.00

ileal in four patients and ileocolonic in four patients. The disease was active in all patients, as defined by a Crohn's Disease Activity Index (CDAI) of >150 (20). At the time of surgery, three patients were on corticosteroids (CS), two were on CS plus mesalazine, and three were on mesalazine plus antibiotics. In all patients, indication for surgery was a chronic active course poorly responsive to medical treatment. From three patients with ileocolonic involvement, mucosal samples were taken from involved (gross lesions) and spared, ileal and colonic, areas. Additional mucosal samples were taken during endoscopy from colon of four CD patients. In these patients, the primary site of involvement was ileal in one, ileocolonic in two, and colonic in one. At the time of endoscopy, no patient had active disease (CDAI >150). One of four patients was on CS, two were on mesalazine, and the remaining one was off treatment.

Mucosal samples were also taken from involved areas of nine UC patients (five undergoing colectomy and four endoscopy). All UC patients had active disease at the time of study, defined by clinical criteria (21) supplemented by endoscopic and histopathological data (22, 23). In all patients who underwent colectomy, disease extent was substantial. Indication for surgery was a chronic active course poorly responsive to CS treatment. In all patients, preoperative endoscopy showed moderate to severe changes. In no case was dysplasia or extraintestinal manifestation the indication for surgery. In UC patients whose biopsy specimens were available, disease activity was moderate in one and mild in three. Disease extent was substantial in one, left-sided in one, and distal in two patients. One patient was on CS, whereas the remaining three were on mesalazine.

Normal controls included mucosal samples taken from four patients with irritable bowel syndrome undergoing endoscopy for recurrent abdominal pain and macroscopically and microscopically unaffected areas of eight colon cancer specimens. As additional disease control group, mucosal specimens were obtained from three patients with diverticular disease. Tissues from normal controls and patients with diverticular disease were categorized as non-IBD controls.

Autologous PBMC were obtained from three CD patients, three UC patients, and five non-IBD control patients. PBMC from 10 healthy subjects were also available. The study was approved by the Department Ethical Committee.

LPMC and PBMC isolation and cultures

LPMC were isolated by the DTT-EDTA-Collagenase sequence, as previously described in detail (4). The isolated cells were counted and checked for viability using 0.1% trypan blue (viability ranged from 90 to 94%). PBMC were isolated by density gradient centrifugation (Lymphoprep; Nycomed Pharma, Oslo, Norway) from 10 ml heparinized blood samples.

To investigate whether LPMC IL-18 expression was modulated by bacterial products, LPMC were isolated from mucosal samples of surgical specimens obtained from either CD and UC patients or non-IBD controls. LPMC or autologous PBMC were resuspended in complete medium (RPMI 1640 supplemented with 10% FCS, 1% L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (all available from Sigma, St. Louis, MO)) at a concentration of 2×10^6 cells/ml and cultured in 24-well culture plates (Falcon Plastic, Becton Dickinson, Lincoln Park, NJ) with and without the initial addition of LPS (*Escherichia coli*) (1 μg/ml) (Sigma) or staphylococcal enterotoxin B (SEB; 1 μg/ml) (Sigma) for 4, 8, 12, 24, 48, and 72 h. Caco-2 cells were resuspended in complete medium and cultured in 6-well culture plates until they reached confluence (3 wk). Before assessment of IL-18 expression, Caco-2 were stimulated by replacement with fresh complete medium in the presence or absence of LPS (1 μg/ml) or SEB (1 μg/ml). Duplicate cell cultures were run for 4, 8, 12, 24, 48, and 72 h. At the end of the culture period, cells were collected and used for IL-18 analysis at both the mRNA and protein levels.

Tissue homogenate preparation

Biopsy or surgical mucosal samples taken from all patients enrolled for this study were used for both RNA and protein analysis on freshly obtained whole tissue. Mucosal samples were separately placed in sterile tubes containing 1–2 ml cold guanidine thiocyanate buffer (for RNA extraction) or 0.5 ml lysis buffer (for protein extraction). The latter contained 0.0625 mol/L Tris (pH 6.8), 2% SDS, 3% 2-ME, 10% glycerol, 100 mmol/L sodium fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mmol/L phenylmethylsulfonyl fluoride (all available from Sigma). Tissue samples were homogenized using a tissue homogenizer (Ystral GmbH, D-7801; PBI International, Dottingen, Germany).

RNA and cDNA preparation

Total RNA was extracted from freshly obtained mucosal samples and (both unstimulated and stimulated) LPMC by using phenol/chloroform procedure, according to Chomczynsky and Sacchi (24). The sample obtained was quantitated by absorbance at 260 nm. RNA integrity was assessed by electrophoresis on a 1.5% agarose gel. cDNA was synthesized from 0.5 to 1 μg of total RNA using 0.2 U of murine leukemia virus reverse transcriptase (Promega, Madison, WI), 2.5 μM random hexamers (Boehringer-Mannheim, Mannheim, Germany), 1 mM dNTP (Boehringer-Mannheim), 2 U RNase inhibitor (Promega) in a total volume of 20 μL. The reaction was performed at 37°C for 60 min.

RT-PCR

Before examining transcripts for IL-18, sample cDNA content was normalized on β-actin signal. For this purpose, varying amounts of cDNA were incubated in a PCR reaction for 19, 20, 21, 22, and 23 cycles with β-actin-specific primers. IL-18 primers were assayed on all samples by incubating an equivalent amount of cDNA for 35 cycles. PCR reactions were performed in a total volume of 50 μl in presence of 1 U of *Taq* DNA Polymerase (Boehringer-Mannheim), 200 μmol dNTPs (Boehringer-Mannheim), and 25 pmol/L 5′ and 3′ primers. Reactions were incubated in a Robocycler thermal cycler (Stratagene, La Jolla, CA) (denaturation 1 min at 94°C, annealing for 1 min at 46°C for IL-18 and 57°C for β-actin, and extension for 1 min at 72°C). PCR primers (Genosys, Cambridge, U.K.) were as follows: IL-18, 5′-GAATCTAAATTATCAGTCATAAG-3′; 3′-GATAGATCTATAATGTTCACTG-5′; β-Actin, 5′-CGAGGCCCAGAG CAAGAGA-3′; 3′-CGTGACATTAAGGAGAAGCTGTG-5′. To exclude the amplification of genomic DNA contaminating the samples, experiments were also performed using RNA as substrate for PCR assay. A total of 10 μl of PCR product was combined with 1 μl of loading buffer and electrophoresed on a 1.5% agarose gel (in Tris ethylenediaminetetracetic acid buffer). A 123-bp ladder was used to assess sample size. Specificity of PCR products were confirmed by specific restriction enzymes.

Southern blot analysis

To assess IL-18 mRNA semiquantitatively, RT-PCR was performed by using the primers mentioned above. An equivalent amount of cDNA samples was incubated for 19 or 23 cycles with β-actin or IL-18-specific primers, respectively. RT-PCR reactions were performed in a total volume of 50 μl, as indicated above. RT-PCR products were run, transferred to a nylon membrane, fixed with UV light, and hybridized with α-^{32}P-labeled DNA fragments encoding the full-length PCR product of IL-18 and β-actin. RT-PCR products were used as probes only after each product was cloned and its sequence verified.

Protein extraction and Western blot analysis

Total proteins were extracted from both freshly obtained mucosal samples and LPMC by using the lysis buffer mentioned above. After cell lysis, the supernatant was collected, run at $4000 \times g$ for 40 min (4°C) and stored at −80°C until assay.

For the detection of IL-18 or ICE, 40 μg of total protein lysate were separated on a 15% SDS-polyacrylamide gel and electrophoretically transferred onto Immobilon-P membrane (Amersham International, Little Chalfont Buckinghamshire, U.K.) for 12 h at 4°C. IL-18 or ICE proteins were detected after incubation with an anti-IL-18 (1:600 final dilution) or anti-ICE (p20) Ab (1:200 final dilution) (Santa Cruz Biotechnology, Santa Cruz, CA) and subsequent incubation with HRP peroxidase-conjugated goat anti-mouse IgG mAb (Santa Cruz Biotechnology) diluted 1:3500 in 10 mmol/L Tris (pH 7.5), 100 mmol/L NaCl, and 0.1% Tween 20 containing 0.1% BSA. Ab reaction was detected with a chemiluminescence detection kit (Amersham International). To confirm the equal loading and transfer of proteins, ponceau S staining was performed.

Effect of IL-18 antisense oligonucleotide on CD LPMC IFN-γ expression

To determine whether IL-18 produced in CD mucosa was functionally active, we tested the effect of an IL-18 antisense oligonucleotide on IFN-γ secretion by CD LPMC. LPMC isolated from three CD patients, as indicated above, were resuspended in complete medium and cultured for 4 days. Parallel LPMC cultures were added of a specific IL-18 antisense phosphorothioate oligonucleotide (final concentration 2 μg/ml). This oligonucleotide targeted the translation initiation site of human IL-18. Since we showed in preliminary experiments that the IL-18 antisense oligonucleotide was degraded at 30–36 h after initiation of culture, it was administered at 0, 36, and 72 h to obtain the desired activity over the culture period. LPMC viability upon antisense oligonucleotide treatment was consistently >84%. The sequences of phosphorothioate oligonucleotides were as follows: IL-18 antisense, 5′-TCAGCAGCCATCTTTATTCC-3′; IL-18 nonsense, 5′-GGAATAAAGATGGCTGCTGA-3′.

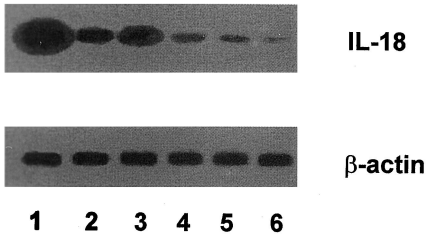

FIGURE 1. Southern blot analysis of transcripts for IL-18 and β-actin in mucosal tissue homogenates (*lanes 1, 3,* and *5*) and lamina propria mononuclear cells (LPMC) (*lanes 2, 4,* and *6*) from CD (*lanes 1* and *2*) or UC (*lanes 3* and *4*) patients and non-IBD controls (*lanes 5* and *6*). Total RNA, extracted as indicated in *Materials and Methods*, was used for cDNA preparation. A total of 1 or 3 μl of cDNA was amplified by using specific primers for β-actin (19 cycles) and IL-18 (23 cycles), respectively. RT-PCR products were then run on gel, blotted, and hybridized with probes specific for IL-18 or β-actin. One representative experiment of eight independent experiments is shown.

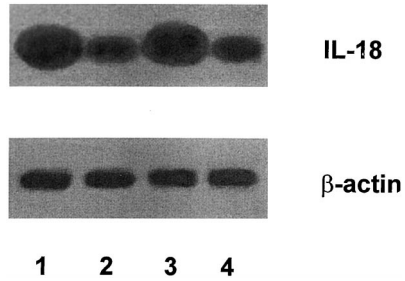

FIGURE 2. Southern blot analysis of transcripts for IL-18 and β-actin in mucosal tissue homogenates from patients with CD. Mucosal samples were taken from both involved (*lanes 1* and *3*) and spared (*lanes 2* and *4*) ileal (*lanes 1*and *2*) or colonic (*lanes 3* and *4*) areas. Total RNA, extracted as indicated in *Materials and Methods*, was used for cDNA preparation. cDNA samples were amplified by using specific primers for IL-18 (23 cycles) or β-actin (19 cycles). RT-PCR products were then run on gel, blotted, and hybridized with probes specific for IL-18 or β-actin. One representative experiment of three independent experiments is shown.

At the end of the culture period, cells were collected and used for RNA extraction. Transcripts for IFN-γ in both unstimulated and stimulated cells were analyzed by semiquantitative RT-PCR. An equivalent amount of cDNA samples was incubated for 19 or 24 cycles with β-actin or IFN-γ-specific primers, respectively. PCR primers for IFN-γ were as follows: 5′-AATGCAGGTCATTCAGATG-3′; 3′-AACTGACTTGAATGTCCAA-5′.

The PCR products were detected by Southern blot hybridization. The cDNA probes were generated by RT-PCR using the primers mentioned above. Cell-free supernatants were collected, concentrated 10 times by using commercially available concentrators (Amicon, Beverly, MA), and tested for IL-18, IFN-γ, and IL-6 content by Western blot analysis. Western blot experiments were performed as indicated previously, using specific primary Abs (all from Santa Cruz Biotechnology) at a final dilution of 1:600, 1:300, and 1:250 for IL-18, IFN-γ, and IL-6, respectively.

Results

IL-18 mRNA expression is enhanced in CD tissue

β-Actin was found in all samples tested. Transcripts for IL-18 were detected in tissue homogenates from both involved and uninvolved intestinal mucosal areas of CD patients. Similarly, transcripts for IL-18 were found in all tissue homogenates from either UC patients or non-IBD controls. IL-18 mRNA was also present in freshly isolated LPMC from either CD or UC patients and non-IBD controls. In addition, a spontaneous IL-18 mRNA expression was observed in all freshly isolated PBMC from either disease groups or controls, as well as in Caco-2 cell lines. In contrast, no IL-18 mRNA was found in EBV-transformed B cell lines (data not shown).

When IL-18 mRNA expression was analyzed by a semiquantitative RT-PCR, an increased accumulation was seen in whole mucosal tissue and LPMC from CD in comparison to UC and non-IBD controls (Fig. 1). The amount of transcripts for IL-18 detected in UC was greater than that found in non-IBD controls (Fig. 1). In both CD and UC, transcripts for IL-18 appeared to be more pronounced in whole mucosal tissue than LPMC samples (Fig. 1). In CD, IL-18 expression was more abundant in mucosal samples taken from involved areas, whereas no difference was found in the accumulation of IL-18 mRNA between ileal and colonic mucosal samples (Fig. 2).

To examine whether IL-18 mRNA was enhanced by bacterial stimuli, LPMC isolated from either CD and UC patients or non-IBD controls were stimulated with LPS or SEB for 4, 6, 12, and 24 h. At the end of the culture period, RNA was extracted and used for IL-18 analysis. Neither LPS nor SEB proved to efficiently increase the accumulation of IL-18 mRNA in the LPMC tested (data not shown). In addition, no significant IL-18 mRNA change was observed in Caco-2 cell lines after LPS or SEB exposure (data not shown). These data are consistent with the observation that the expression of IL-18 mRNA in circulating mononuclear cells is not enhanced by mitogens, including LPS (13).

IL-18 is produced in CD and UC

To investigate the capability of human intestinal cells to translate IL-18 mRNA, Western blot analysis was performed by using total proteins extracted from freshly obtained mucosal tissue and LPMC samples. In all CD and UC patients and non-IBD controls, anti-IL-18 Ab detected a larger protein with a molecular size of 24 kDa (Fig. 3). Since we used a polyclonal Ab (Santa Cruz Biotechnology; *sc-6177*) reacting with an epitope corresponding to an amino acid sequence mapping at the carboxy terminus of the human IL-18 precursor, the 24-kDa band may represent IL-18 propeptide precursor (Fig. 3). In contrast, tissue homogenate and LPMC samples from all CD and UC patients, but not non-IBD controls, contained an 18-kDa protein that was stained by the anti-IL-18 Ab (Fig. 3). The 18-kDa polypeptide comigrated with recombinant human IL-18 upon SDS-PAGE (Fig. 3). The amount of IL-18 detected in CD was apparently greater than that found in mucosal samples of UC patients (Fig. 3). No 18-kDa protein was found in normal LPMC and Caco-2 cells after LPS or SEB exposure (Fig. 4). Similar results were observed when culture supernatants of both LPS- or SEB-stimulated LPMC were tested.

No 18-kDa protein was observed in unstimulated PBMC isolated from either CD or UC patients or non-IBD controls (Fig. 4). However, the IL-18 18-kDa polypeptide was induced in these cells by LPS or SEB stimulation (Fig. 4).

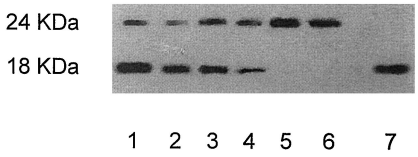

FIGURE 3. Western blot analysis of IL-18 protein expression in freshly obtained mucosal tissue (*lanes 1, 3,* and *5*) and LPMC (*lanes 2, 4,* and *6*) from CD (*lanes 1* and *2*) or UC (*lanes 3* and *4*) patients and non-IBD controls (*lanes 5* and *6*). Anti-IL-18 Ab detected a protein corresponding to the size of human recombinant IL-18 (*lane 7*) in mucosal and LPMC samples from both CD and UC patients. A 24-kDa protein was also detected in all samples tested. Sizes of protein standards are given in kDa. One representative experiment of six independent experiments is shown.

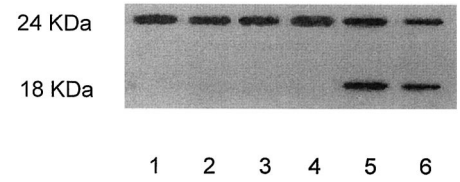

Figure 4. Western blot analysis of IL-18 protein expression in LPMC (*lanes 1, 2,* and *3*) and autologous PBMC (*lanes 4, 5,* and *6*) isolated from non-IBD controls. Cells were cultured with medium alone (*lanes 1* and *4*), LPS (*lanes 2* and *5*) or SEB (*lanes 3* and *6*) for 24 h. Anti-IL-18 Ab detected a protein of 18 kDa in PBMC exposed to LPS or SEB stimulation only. A 24-kDa protein was detected in all samples tested. Sizes of protein standards are given in kDa. One representative experiment of three independent experiments is shown.

IL-18 expression correlates with the presence of active ICE in CD and UC intestinal mucosa

Production of active IL-18 requires the presence of the intracellular cysteine proteinase ICE (25). This enzyme processes pro-IL-18 in mature IL-18 (14). ICE is, in turn, synthesized as a 45-kDa polypeptide precursor, which may be transformed in active subunits of 20 (p20) and 10 (p10) kDa (25). To examine whether the expression of the mature form of IL-18 was correlated with the presence of active ICE, total proteins extracted from both freshly obtained whole mucosal tissue and LPMC samples were tested for the ICE p20 subunit by Western blot analysis. In all CD and UC, but not normal, samples, a 20-kDa protein was stained by an anti-ICE p20 Ab (Fig. 5). No difference in the intensity band of p20 was observed between CD and UC samples. The anti-ICE p20 Ab also detected a larger protein of ~30 kDa, which may represent an intermediate form of ICE (Fig. 5). A 45-kDa polypeptide consistent with the precursor form of ICE was moreover detected in all samples tested from either disease groups or controls (Fig. 5).

IL-18 produced in CD mucosal tissue is biologically active

To test whether IL-18 expressed in CD was functionally active, we designed a phosphorothioate oligonucleotide targeting the translation initiation site of human IL-18. Coincubation of CD LPMC with IL-18 antisense oligonucleotide resulted in a reduced expression of IL-18 at both the mRNA (data not shown) and protein levels (Fig. 6*b*).

No change in CD LPMC IL-18 release was seen after stimulation with nonsense control oligonucleotide (Fig. 6). As assessed by densitometry of both Southern and Western blots, a significant decrease in IFN-γ production by IL-18 antisense-stimulated CD LPMC was documented (Fig. 6). IFN-γ inhibition seemed to be dependent on IL-18 down-regulation, rather than the result of a general state of LPMC hyporesponsiveness induced by antisense oligonucleotide, because the expression level of a control cytokine, such as IL-6, was not affected in the same samples (data not shown).

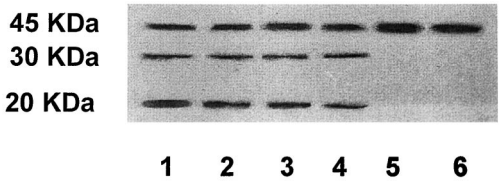

FIGURE 5. Western blot analysis of ICE protein expression in freshly obtained mucosal tissue (*lanes 1, 3,* and *5*) and LPMC (*lanes 2, 4,* and *6*) from CD (*lanes 1* and *2*) or UC (*lanes 3* and *4*) patients and non-IBD controls (*lanes 5* and *6*). Anti-ICE p20 Ab detected a protein of 20 kDa in both mucosal and LPMC samples from either CD or UC patients. A 30-kDa protein was also detected in samples from either CD or UC. A 45-kDa protein was found in all samples tested. Sizes of protein standards are given in kDa. One representative experiment of six independent experiment is shown.

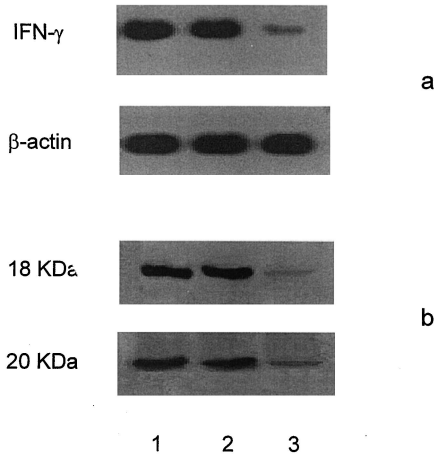

FIGURE 6. IL-18 and IFN-γ expression in CD LPMC cultured with medium alone (*lane 1*), IL-18 nonsense (*lane 2*), or antisense (*lane 3*) oligonucleotides for 4 days. *a,* The Southern blot analysis of RT-PCR products for IFN-γ and β-actin. Total RNA, extracted as indicated in *Materials and Methods*, was used for cDNA preparation. cDNA samples were amplified by using specific primers for IFN-γ (24 cycles) or β-actin (19 cycles). RT-PCR products were then run on gel, blotted, and hybridized with probes specific for IFN-γ or β-actin. One representative experiment of three independent experiments is shown. *b,* The Western blot analysis of IL-18 and IFN-γ protein in supernatants of CD LPMC. Sizes of protein standards are given in kDa. One representative experiment of three independent experiments is shown.

Discussion

The present study was designed to explore whether active IL-18 is expressed in CD. We have shown here that IL-18 is up-regulated in CD mucosal tissue, and we have provided evidence that IL-18 expression clearly differentiates both CD and UC from non-IBD controls.

A constitutive expression of IL-18 mRNA was found in all intestinal mucosal samples tested from either patients or controls. Transcripts for IL-18 were found in both ileal and colonic whole mucosa, as well as in intestinal epithelial cell lines and lamina propria mononuclear cells, consistent with studies showing that IL-18 is expressed in a wide range of tissues and cell types (13, 26).

When transcripts for IL-18 were analyzed by a semiquantitative RT-PCR, an increased accumulation was found in both mucosal tissue and LPMC samples from CD and UC in comparison to non-IBD controls. In addition, the amount of IL-18 transcripts appeared to be greater in CD than in UC. However, further studies are required to verify whether the observed differences in IL-18 expression in patients with CD and UC may at least in part depend on the semiquantitative nature of RT-PCR and/or differences in disease expression. Taken together, these data seem to suggest, however, that IL-18 production is differently regulated during chronic intestinal inflammation, where local factors may be involved in enhancing IL-18 gene activation.

The accumulation of IL-18 mRNA in CD intestinal cells did not seem to be dependent on the sampling site since ileal and colonic mucosal samples expressed similar amounts of transcripts for IL-18. However, a more pronounced expression of IL-18 was seen in both whole mucosal tissue and LPMC samples taken from involved areas. As IL-18 is capable of promoting the synthesis of proinflammatory

mediators (19), it is conceivable that the up-regulation of IL-18 occurring in CD may contribute to the local inflammatory cascade.

In all CD and UC whole mucosal tissue and LPMC samples, IL-18 mRNA was efficiently translated, as indicated by the presence of an 18-kDa protein in Western blot experiments. In either CD or UC, IL-18 was more expressed in whole mucosal tissue than LPMC, indicating that different cell types are likely involved in IL-18 production. In contrast, no mature form of IL-18 was detected in freshly isolated autologous CD and UC PBMC, suggesting that IL-18 production is compartmentalized to the human intestine as shown for other cytokines (17). No IL-18 18-kDa polypeptide was found in control mucosal and LPMC samples, as well as in Caco-2 cells. In addition, neither LPS nor SEB stimulation proved to induce the mature form of IL-18 in both normal LPMC and Caco-2 cell cultures. However, these stimuli were efficient in promoting PBMC IL-18 synthesis. Taken together, these observations would seem to indicate that the production of the mature form of IL-18 is a down-regulated function in normal human intestine. However, it is not possible to exclude that, in normal gut mucosa, IL-18 is produced in amounts too low for detection by Western blot analysis. Morphological and immunohistochemical studies support the concept that, in chronic intestinal inflammatory diseases, the vast majority of mononuclear cells infiltrating inflamed mucosa and submucosa are recruited from circulation (27, 28). In this context, our data suggest that, in CD and UC, IL-18 might at least in part be produced by recently recruited monocytes exposed to bacterial products.

IL-18 is produced as a precursor molecule that is cleaved to active form by ICE, an enzyme also involved in the synthesis of mature IL-1β (25). In monocytes or monocytic cell lines, ICE exists as a 45-kDa zymogen, an intermediate form of 30 kDa, and active subunits of 20 (p20) and 10 kDa (p10) (25). In CD and UC mucosal samples, the expression of the mature form of IL-18 was invariably associated with the presence of ICE p20 subunit. All CD and UC samples also contained a 30-kDa subunit, which may be the result of an incomplete autocatalysis. In contrast, mucosal samples from non-IBD controls expressed inactive ICE form (p45) only, further supporting the notion that the synthesis of IL-18 requires the presence of active ICE subunits (14). In agreement with our data, a recent study reported that CD and UC, but not normal, LPMC are capable of activating ICE and releasing mature IL-1β (29).

IL-18 produced in CD mucosal tissue is functionally active. Down-regulation of CD LPMC IL-18 expression by specific antisense oligonucleotide resulted in a reduced release of IFN-γ but not IL-6.

Evidence has been accumulated to indicate that Th1 cytokines are predominantly produced in CD tissue and that locally released molecules contribute in promoting IFN-γ-producing cell development (7–11, 17, 18). IL-18 plays an important role in favoring IFN-γ synthesis and inducing Th1 cell proliferation (15). Therefore, it is conceivable that IL-18 may contribute to the local immune response in CD, promoting the expansion of Th1-primed intestinal lymphocytes (15, 30). In conclusion, our data indicate that IL-18 up-regulation is an immunological feature of CD and suggest that IL-18 may play a role in promoting the local immunoinflammatory response. However, further studies are required to understand whether manipulating IL-18 expression may have relevance to the treatment of CD.

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