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IL-18-Binding Protein Expression by Endothelial Cells and Macrophages Is Up-Regulated During Active Crohn’s Disease

Anne Corbaz,* Tessa ten Hove,† Suzanne Herren,* Pierre Graber,* Boris Schwartsburd,‡ Ilana Belzer,‡ Jillian Harrison,* Thomas Plitz,* Marie H. Kosco-Vilbois,* Soo-Hyun Kim,§ Charles A. Dinarello,§ Daniela Novick,¶ Sander van Deventer,† and Yolande Chvatchko* 2*

The pathogenesis of Crohn’s disease (CD) remains under intense investigation. Increasing evidence suggests a role for mature IL-18 in the induction of proinflammatory cytokines and Th1 polarization in CD lesions. The aim of this study was to investigate the contribution of the IL-18-neutralizing (a and c) and non-neutralizing (b and d) isoforms of IL-18-binding protein (IL-18BP) during active CD. Intestinal endothelial cells and macrophages were the major source of IL-18BP within the submucosa, and this IL-18BP production was also found to be relevant to other types of endothelial cells (HUVEC) and macrophages (peripheral monocytes). IL-18BP messenger transcript and protein were significantly increased in surgically resected specimens from active CD compared with control patients, correlating with an up-regulation of IL-18. Analysis of the expression of the four IL-18BP isoforms as well as being free or bound to IL-18 was reported and revealed that unbound IL-18BP isoforms a and c and inactive isoform d were present in specimens from active CD and control patients while isoform b was not detected. IL-18/IL-18BP complex was also detected. Interestingly, although most was complexed, free mature IL-18 could still be detected in active CD specimens even in the presence of the IL-18BP isoform a/c. These results demonstrate that the appropriate neutralizing isoforms are present in the intestinal tissue of patients with active CD and highlights the complexity of IL-18/IL-18BP biology. The Journal of Immunology, 2002, 168: 3608–3616.

C

rohn’s disease (CD) is a chronic inflammatory process involving the gastrointestinal tract. Although the clinical features of CD have been well characterized, its pathogenesis remains unclear. Histological observations and immunological studies demonstrate that T cell activation and cell-mediated immunity are key features of CD (1–3). Substantial evidence suggests that the normal balance between inflammatory and regulatory cytokines is perturbed in the gut in inflammatory bowel disease. This imbalance may induce uncontrolled T cell activation within the mucosa, perpetuating a prolonged cellular and humoral immune response, leading to subsequent tissue injury (4–6). Several studies suggest that the immune response within the affected mucosa leans toward a Th1 phenotype (7–9).

Certain cytokines, such as IL-18, originally described as the IFN-α-inducing factor (10), may play an important role in the dysregulation of Th1-mediated diseases. IL-18, mainly produced by APCs, can synergize with IL-12 to increase IFN-γ secretion, enhancing NK cell cytotoxicity and stimulating Th1 responses (11, 12). IL-18 also exerts proinflammatory effects by inducing TNF-α, IL-1β, chemokines such as IL-8, macrophage-inflammatory protein-1α, and monocyte chemoattractant protein-1 (13), NO (14), and adhesion molecules (15). Recent studies have reported an increase in IL-18 expression by intestinal epithelial cells and macrophages in the lesions of CD (16–19) and in the sera of patients with CD (19). The IL-18-induced cytokines IFN-γ, TNF-α, IL-1β, and IL-8 were also shown to be up-regulated in these lesions (18). These observations strongly imply that IL-18 plays a role in the pathogenesis of CD.

IL-18-binding protein (IL-18BP) has been previously described as a secreted protein that binds and neutralizes IL-18 (20). As such, it regulates IL-18-induced IFN-γ production and consequentially influences the Th1 and inflammatory responses. With a single Ig domain, IL-18BP resembles the extracellular segment of a cytokine receptor. However, IL-18BP is a novel protein distinct from the IL-1 and IL-18 receptor family members. The human IL-18BP gene encodes at least four distinct isoforms (IL-18BPa–d), which are derived by alternative splicing. The isoforms differ primarily in their carboxyl termini and biological activity. IL-18BP isoforms a and c neutralize the biological activity of IL-18, whereas b and d do not (21). IL-18BPa is constitutively expressed in human spleen and, to a lesser extent, in colon, small intestine, and prostate (20). It is presently unknown whether the other isoforms are expressed and secreted in vivo. In addition, little is known about the regulation of IL-18BP expression. IL-18BP mRNA has been shown to be induced by IFN-γ in several cell lines, such as keratinocytes, colon carcinoma/epithelial cells, and renal mesangial cells (22, 23). However, IL-18BP expression, cellular localization, and regulation during disease process remain to be defined. Therefore, in this study we evaluated the expression of the different isoforms of

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Abbreviations used in this paper: CD, Crohn’s disease; IL-18BP, IL-18-binding protein; rh, recombinant human; MFI, mean fluorescence intensity; CS, corticosteroid; h, human.

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IL-18BP and determined the balance between IL-18 and IL-18BP in active CD.

Materials and Methods

Specimens

Surgically resected specimens were obtained from patients with active CD undergoing a partial intestinal resection and were immediately snap-frozen. Sixteen CD patients (4 men and 12 women) with a mean age of 42.4 years (range, 27–78 years) and a mean disease duration of 9.5 years (range, 2–21 years) were included. The primary site of involvement was colonic in five patients, ileal in nine patients, and ileocolonic in two patients. The surgically resected specimens contained all layers of the intestinal wall. The diagnosis for the activity of the disease was made by histopathological examination of the surgically resected specimens based on the following criteria: presence of ulcerations, increased numbers of inflammatory cells, and transmural inflammation. At the time of surgery, six patients were on corticosteroids (CS), three on CS plus mesalazine, three on CS plus azathioprine, one on CS plus mesalazine and azathioprine, one on paracetamol, one on CS plus paracetamol, and the remaining one was off treatment. For all patients, indication for surgery was a chronic active course poorly responsive to medical treatment. Control surgically resected specimens (six males and nine females) were obtained from patients undergoing a partial intestinal resection for bowel cancer and were snap-frozen. Control specimens were from macroscopically and microscopically unaffected areas. The mean age of this group was 55.5 years (range: 24–81 years). The surgically resected specimens were used for immunohistochemical studies, real-time PCR, and Western blot analysis. The Medical Ethical Committee of the Academic Medical Center (Amsterdam, The Netherlands) approved the study.

rhIL-18 and rhIL-18BP isoforms

Recombinant human (rh)IL-18 was prepared in E. coli as mutated pro-rhIL-18 fused to GST, engineered to contain a caspase-8 cleavage site. Following purification, mature human IL-18 was released by caspase-8 cleavage. rhIL-18BP-6His isoforms a–d were transiently expressed in HEK 293 cells as previously described (21). Conditioned media were collected after 72 h and stored at −80°C. rhIL-18BPa-6His was purified to homogeneity from a stable Chinese hamster ovary cell line (21).

Abs to hIL-18BP

Anti-human (h)IL-18BP mAbs 582.10 and 657.27 were used for Western blot analysis, ELISA, and FACS analysis (24). Anti-hIL-18BP mouse polyclonal was generated by injecting BALB/c mice s.c. with rhIL-18BPa-6His and used for Western blot analysis.

Anti-hIL-18BP mAb 95-H20 was generated by injecting BALB/c mice s.c. into the four limbs as well as intranuchally, with 50 μg per injection of rhIL-18BPa-6His in PBS with adjuvant (monophosphoryl lipid A plus trehalose dicorynomycolate Emulsion; RIBI adjuvant system (Corixa, Hamilton, MT)) on days 0, 7, and 28. Four days after the third immunization, the lymph nodes were removed and digested with 2.4 μg/ml collagenase IV (Worthington Biochemical, Lakewood, NJ) and 0.1% DNase (Sigma-Aldrich, St. Louis, MO). Isolated cells were then fused with Sp2/0 myeloma cells using polyethylene glycol 1000 (Fluka, Buchs, Switzerland). Hybridomas were resuspended in DMEM-F12, 10% FCS (Life Technologies, Grand Island, NY) and plated in 96-well plates at a concentration of 5 × 10^4 cells/ml. Hybridomas were assayed using non-neutralizing Abs recognizing free and bound IL-18BP. After rehydration and blocking (as above), sections were incubated overnight with anti-hIL-18BP biotinylated mAb 95-H20, followed by biotinylated goat anti-mouse Ab (5 μg/ml; Jackson ImmunoResearch Laboratories) in PBS containing 0.5% BSA for 1 h and then avidin/biotinylated HRP complex (Vectorstain Elite ABC kit; Vector Laboratories, Burlingame, CA) for 30 min. Slides were developed using 3-ami-no-1-ethylcarbazole (Sigma-Aldrich) according to the manufacturer’s protocol, counterstained with hematoxylin (Sigma-Aldrich), and mounted.

Table I.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reverse Primer</th>
<th>Forward Primer</th>
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<td>β-actin (X00351)</td>
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<td>5′-GCTCACCAGTTGATGATAAGCC-3′</td>
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RNA, cDNA, and protein preparation

RNA and protein were extracted from control or active CD surgically resected specimens using TRIzol (Life Technologies) according to the manufacturer’s instructions, which allowed total RNA and protein recovery from the same tissue sample. RNA integrity was assessed by electrophoresis on 1% agarose gels. cDNA was synthesized from 1 μg of total RNA using a reverse transcription system (Promega, Madison, WI) according to the manufacturer’s protocol. After RNA recovery, proteins were extracted by sequential precipitation. Total protein was quantified using the bicin- choninic acid protein assay (Pierce, Rockford, IL). Protein extraction from TRIzol was validated by comparison of proteins directly lysed in Laemmli sample buffer and proteins extracted from TRIzol. Both methods gave identical protein profiles by SDS-PAGE and Coomassie blue staining. Western blotting of both protein preparations also gave identical specificity and intensity.

Real-time PCR

SYBR green real-time PCR primers for human IL-18BP, IL-18, and GAPDH (housekeeping control) were designed using Primer Express software from PE Applied Biosystems (Foster City, CA) (Table I). The specificity and optimal primer concentrations were tested. The IL-18BP primers did not distinguish among the various isoforms. Potential genomic DNA contamination was excluded by performing PCR with specific intron-GAPDH primers. The absence of nonspecific amplification was confirmed by analyzing the PCR products by agarose gel electrophoresis. Real-time PCR was performed with 5 μl/well of reverse transcription products (0.5 ng total RNA), 25 μl/well SYBR Green PCR master mix (PE Applied
Biosystems) with 0.5 U/well of AmpErase (PE Applied Biosystems) uracil-N-glycosylase and 300 nM primers. PCR was performed at 50°C for 2 min and 95°C for 10 min, and then run for 40 cycles at 95°C for 15 s and 60°C for 1 min on the ABI PRISM 7700 Detection System (PE Applied Biosystems). The reverse-transcribed cDNA samples were thus amplified and their cycle threshold values were determined. All cycle threshold values were normalized to the housekeeping gene GAPDH.

Semiquantitative PCR

Semiquantitative PCR primers for human IL-18BP and β-actin (housekeeping control) were designed (Table I). PCR were performed in a total volume of 50 μl in the presence of 1.25 U of AmpliTaq DNA Polymerase (Perkin-Elmer, MA), 2.5 mM dNTPs (Amersham, Arlington Heights, IL), and 50 pmol of forward and reverse PCR primers. Reactions were incubated in a PTC-200 Peltier Effect Thermal Cycler (MJ Research, Cambridge, MA) under the following conditions: denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 1 min at 72°C. Absence of contaminating genomic DNA was confirmed by performing PCR on the nontranscribed RNA. PCR products were analyzed by agarose gel electrophoresis and the band size was verified by comparison with a 1-kb ladder (Life Technologies).

ELISA

Maxisorp microtiter 96-well ELISA plates (Labsystems, Chicago, IL) were coated with 100 μl/well anti-hIL-18BP mAb 582.10 (2 μg/ml) overnight at room temperature. Soluble hIL-18BP was then detected using biotinylated anti-hIL-18BP mAb 657.27 (0.5 μg/ml) followed by incubation with peroxidase-conjugated extravidin (1/10,000; Sigma-Aldrich). The sensitivity of the ELISA was 100 pg/ml. Conditioned media from HEK 293 cells transfected with the different hIL-18BP isoforms illustrated that the ELISA recognized only isoforms a and c. IL-18/IL-18BP complexes were then formed using rhIL-18 with conditioned media containing either isoform a or c for 30 min at room temperature before electro- phoresis and Western blotting.

Flow cytometry analysis of IL-18BP expression

Intracellular IL-18BP was detected in PBMC by flow cytometry using a FACSCalibur (BD Biosciences, Mountain View, CA). Brefeldin A (5 μg/ml; Sigma-Aldrich) was added 4 h before assay. FITC-labeled anti-CD64 mAb (BD Pharmingen) was used as a marker to identify monocytes/macrophages. The proportion of CD64+ cells in PBMC preparations was 12%. Intracellular IL-18BP was detected using biotinylated mouse anti-IL-18BP mAb 582.10 followed by streptavidin-PE conjugate. All isotype-matched controls were negative. Data are presented as the difference (Δ) between the mean fluorescence intensity (MFI) of the positive label (i.e., anti-IL-18BP) and the MFI of the negative control (i.e., mouse IgG1).

Statistical analysis

For IL-18BP and IL-18 mRNA and protein levels, results are expressed as mean ± SEM. Results were analyzed using the Wilcoxon rank test by the statistical program S-Plus 2000 Professional (Release 3; Insightful, Seattle, WA). Differences were considered significant when p < 0.05.

Results

Immunohistochemical expression and localization of IL-18BP in intestinal tissue

To assess the expression and localization of IL-18BP, immunohistochemistry was performed on cryosections of surgically resected specimens from patients with active CD or from control patients. Evaluating the control sections, vessels and a few scattered cells expressed IL-18BP (Fig. 1, A and B). IL-18BP was also observed associated with the vessels of active CD patients (Fig. 2, A and B). Interestingly, an increased number of IL-18BP-positive cells were scattered in the submucosa (Fig. 2, A–C) and overlying lymphoid aggregates (Fig. 2C). Thus, the endothelial cells within the vessels and the cells infiltrating the submucosa appeared to be the major source for IL-18BP during active CD.

To further characterize the positive infiltrating cells, immunofluorescent microscopy was used. The IL-18BP-positive cells in active CD cryosections were formally identified as macrophages using an anti-CD68 mAb. Endothelial cell labeling was confirmed using an anti-CD31 mAb. CD68-positive cells (Fig. 3A), CD31-positive cells (Fig. 3D), and IL-18BP-positive cells (Fig. 3, B and E) were detected in the submucosa of active CD. Superimposing the two fluorescent images confirmed that the macrophages and endothelial cells were indeed positive for IL-18BP (Fig. 3, C and F, respectively). Other cell specific markers, e.g. anti-epithelial Ag clone Ber-Ep4 (epithelial cells), anti-CD94 (NK cells), anti-CD3 (T cells), anti-CD19, and anti-CD20 (B cells) were analyzed but did not colocalize with the IL-18BP staining (data not shown). Thus, macrophages and endothelial cells appear to be the major source of IL-18BP within intestinal tissues obtained from active CD patients.

Balance between IL-18BP and IL-18 expression in active CD intestinal tissue

Analysis of IL-18BP and IL-18 expression was performed by real-time PCR on the surgically resected specimens obtained from patients with active CD and from control patients (Fig. 4). IL-18BP mRNA was detectable in control patients (mean, 1.74; SD, 0.1) and the levels were significantly increased in active CD patients (mean, 8.92; SD, 2.4, p < 0.001). Similarly, IL-18 mRNA was present in control patients (mean, 2.83; SD, 0.2), again with increased levels in active CD patients (mean, 4.40; SD, 0.5, p < 0.05).
To quantify the levels of IL-18BP (free isoform a/c) and total IL-18 (free and bound to IL-18BP), protein extracts from control and active CD surgically resected specimens were analyzed by ELISA. IL-18BP levels were 2-fold higher in specimens from active CD patients (49.5 ± 6 ng/ml for CD vs 20.9 ± 3.2 ng/ml for control; \( p < 0.001 \)) (Fig. 5A). Total IL-18 in the specimens from active CD patients was also significantly increased compared with control patients (1576 ± 246.3 vs 410.4 ± 75.1 pg/ml, respectively; \( p < 0.001 \)) (Fig. 5B). Analysis of IL-18BP and IL-18 levels of individual specimens demonstrated a significant positive correlation between the two proteins (\( r = 0.87; \ p < 0.0001 \)), revealing that the increase in IL-18BP parallels the IL-18 up-regulation known to be associated with active CD (16–19). Unbound IL-18BP isoform a/c was in excess compared with total IL-18 (49.5 ng/ml vs 1570 pg/ml, respectively) in active CD specimens.

To obtain additional information on the expression of the four IL-18BP isoforms as well as being free or bound to IL-18, proteins extracts from CD and control surgically resected specimens were subjected to Western blot analysis. The anti-IL-18BP, mAb 657.27, was first shown to recognize recombinant isoforms a and c with a molecular mass of 42 and 40 kDa, respectively (Fig. 6A), as well as the complexes IL-18/IL-18Pa and IL-18/IL-18Pc with an approximate molecular mass of 58 kDa (Fig. 6B). Bands of 42, 40, and 58 kDa, comigrating with the free and complexed recombinant proteins, were also observed in the specimens, demonstrating that free IL-18BP isoform a and c as well as the IL-18/
IL-18BP complex were present in intestinal tissue (Fig. 6C). Using an anti-IL-18 polyclonal Ab further confirmed the presence of the IL-18/IL-18BP complex in the active CD and control specimens (Fig. 6D), suggesting that a proportion of IL-18 is bound to IL-18BP. Free mature IL-18 and pro-IL-18 were also present (Fig. 6D).

The presence of inactive (b and d) vs active (a and c) isoforms in active CD and control specimens was further evaluated by Western blot analysis using an anti-IL-18BP mouse polyclonal Ab that recognizes all four recombinant isoforms as shown in Fig. 6E. Bands of 42, 40, and 35 kDa (isoforms a, c, and d, respectively) were observed in the active CD and control specimens, whereas isoform b was not detected (Fig. 6F). These results further confirm the presence of the IL-18/IL-18BP complex (Fig. 6F). An additional band of ~19 kDa revealed by the anti-IL-18BP mouse polyclonal Ab was analyzed by mass spectrometry and found to be the actin binding protein (data not shown).

**IL-18BP production by endothelial cells and macrophages**

To further investigate whether the IL-18BP production by intestinal macrophages and endothelial cells was relevant to other types of macrophages and endothelial cells, experiments were performed with HUVEC and peripheral blood monocytes/macrophages. Interestingly, IL-18BP mRNA was found to be constitutively expressed in HUVEC, which then increased following 24 h of stimulation with inflammatory cytokines (IL-1β, TNF-α, IFN-γ) (Fig. 7A). Similar to our in situ observations, a significant increase of IL-18BP production was observed (30-fold) (Fig. 7B). As observed with the endothelial cells, IL-18BP was constitutively expressed in PBMC. Inflammatory cytokines (IL-1β, TNF-α, IFN-γ) combined with LPS also induced a significant increase in IL-18BP secretion (p < 0.05) (Fig. 8A). A similar induction was observed after 48 h of stimulation with LPS, IFN-γ, and TNF-α alone, demonstrating their individual potential to induce IL-18BP production (Fig. 8A). IL-1β and IL-18 alone had no significant effect (Fig. 8A).

The demonstration that peripheral blood monocytes/macrophages produced IL-18BP was confirmed by IL-18BP intracellular FACS analysis on PBMC (Fig. 8B). IL-18BP was observed primarily in CD64+ monocytes/macrophages. Thus, CD64-positive cells appear to be the major source of IL-18BP in PBMC. The ability of these cells to produce IL-18BP was further confirmed

**FIGURE 3.** Double immunofluorescence of active CD intestinal sections. CD68, a macrophages marker (A), or CD31, an endothelial cell marker (D, green), were used. IL-18BP-positive cells (B and E, red) were revealed by biotinylated anti-hIL-18BP mAb 95-H20 followed by streptavidin-Texas Red. Double-immunolabeled images (C and F) reveal the colocalization (yellow) of IL-18BP-positive macrophages (C) and IL-18BP-positive endothelial cells (F). Magnification is ×700.

**FIGURE 4.** IL-18BP and IL-18 mRNA expression in active CD and control intestinal tissue. Quantification by real-time PCR of IL-18BP (A) and IL-18 (B) mRNA transcripts normalized to the housekeeping gene GAPDH in surgically resected specimens from active CD patients (n = 16) and from control patients (n = 15). Results are reported as the mean ± SEM. Asterisks indicate significant differences according to the Wilcoxon test between experimental groups (*, p < 0.05; ***, p < 0.001).
using culture supernatants of purified monocytes/macrophages from total PBMC with or without stimulation. A 4-fold increase in IL-18BP levels was observed in 48-h supernatants of TNF-α- or IFN-γ-stimulated monocytes/macrophages (Fig. 8C). A similar induction of IL-18BP was observed using differentiated THP-1 cells (data not shown).

**FIGURE 6.** Western blot analysis of IL-18BP and IL-18 protein expression in active CD and control intestinal tissue. A, The specificity of anti-hIL-18BP mAb 657.27 was demonstrated using conditioned media from transiently transfected HEK 293 cells containing rhIL-18BP-6His isoform a–d. Anti-hIL-18BP mAb 657.27 detected only rhIL-18BP-6His isoforms a (42 kDa) and c (40 kDa). B, rhIL-18BP-6His isoform a or c was complexed with rhIL-18 before electrophoresis and Western blotting using anti-hIL-18BP mAb 657.27, which detected IL-18/IL-18BP a/c (~58 kDa). C, IL-18BP expression in protein extracts from surgically resected specimens of active CD and control patients using the anti-hIL-18BP mAb 657.27. Bands of 42, 40, and 58 kDa (isoform a, isoform c, and IL-18/IL-18BP a/c complex, respectively) comigrating with free and bound rIL-18BP were detected. D, IL-18 expression in protein extracts from surgically resected specimens of active CD and control patients using an anti-hIL-18 Ab. These protein extracts were from the same patients as in C. Bands of 18, 24, and 58 kDa corresponding to mature IL-18, pro-IL-18, and complex IL-18/IL-18BP a/c, respectively, were detected. E, The specificity of anti-hIL-18BP mouse polyclonal Ab was demonstrated using conditioned media from transiently transfected HEK 293 cells containing rhIL-18BP-6His isoform a–d. Anti-hIL-18BP mouse polyclonal Ab detected all four isoforms at 42, 16, 40, and 35 kDa, respectively. F, Protein extracts from active CD and control specimens previously analyzed in C and D were further tested for IL-18BP a–d expression using the anti-hIL-18BP mouse polyclonal Ab. Bands of 42, 40, and 58 kDa comigrating with the rIL-18BP isoforms a, c, and d were detected. A 58-kDa band corresponding to the complex IL-18/IL-18BP a/c was also visualized. Cross-reaction of the polyclonal Ab with a protein of ~19 kDa not related to IL-18BP was also observed. Each blot is representative of three independent experiments.
Discussion
In this study, we investigated the expression of the natural inhibitor of IL-18, IL-18BP, in CD using intestinal surgically resected specimens. Several studies have reported increased IL-18 production in chronic CD lesions, suggesting its role in the pathogenesis of the disease (16–19). However, these studies did not take into account the existence of IL-18BP, which exhibits an exceptionally high affinity for IL-18 (21). Hence, it was of interest to evaluate the expression of IL-18BP, IL-18-neutralizing (a and c) and non-neutralizing (b and d) isoforms, as well as the balance between IL-18 and these IL-18BP isoforms in intestinal tissue obtained from control and active CD patients.

Intestinal endothelial cells and macrophages were found to be the major source of IL-18BP in the submucosa, and an increased number of IL-18BP-expressing macrophages were present within CD specimens. Our results document for the first time a significant increase in IL-18BP transcripts in specimens from active CD patients compared with control patients. Unbound IL-18BP isoform...
a/c protein levels were quantified and found to be significantly higher in active CD specimens compared with control tissue and, interestingly, higher than the amount of total IL-18 (49.5 ± 6 ng/ml vs 1570 ± 246 pg/ml, respectively). Thus, IL-18BP up-regulation correlated with the increase of IL-18 previously shown to be associated with CD. IL-18BP and IL-18 expression were also analyzed in surgically resected specimens from patients with ulcerative colitis, another form of inflammatory bowel disease in which polarized Th2 responses are believed to play a dominant role. These experiments revealed that IL-18BP and IL-18 transcript and protein levels in ulcerative colitis specimens were comparable to the ones in control specimens (data not shown).

The presence of IL-18BP in CD lesions would suggest neutralization of IL-18 activity, unless patients with active CD preferentially undergo differential splicing to produce more of the inactive isoforms (b and d) than the active ones (a and c). These patients would then have a reduced ability to regulate the activity of IL-18 during the course of the disease. In fact, our results demonstrated for the first time that, although two of seven patients appeared to have higher levels of IL-18BP isoform d, free isoform a was always present at comparable or elevated levels to that of b, c, or d. In the future, a quantitative comparison will be needed, because reagents are currently unavailable to obtain an exact quantification of the inactive isoforms. Furthermore, Western blot analysis demonstrated that, while IL-18 was complexed with IL-18BP, free mature IL-18 was also observed. These observations suggest that, despite the presence of IL-18BP, some IL-18 activity may be available for perpetuating the pathogenesis of CD.

The presence of free mature IL-18 in active CD specimens which also express an excess of free IL-18BP isoform a was astonishing and may indicate another function of IL-18BP in addition to its ability to bind IL-18. Like most cytokine receptors, the IL-18 receptor is composed of a ligand binding chain (IL-18Rα), originally described as IL-1R-related protein, and an accessory chain (IL-18Rβ), named accessory protein-like. Recently, IL-1H4, an IL-1-related protein, has been shown to bind IL-18R but not the IL-1R. IL-1H4 has a high degree of homology to IL-18 (25, 26). It is therefore possible that IL-1H4 could bind IL-18BP. Although to date there is no reported biological effect of IL-1H4, binding of IL-1H4 to IL-18BP may account for the reduced capability of IL-18 to bind IL-18. Another possible hypothesis to explain the presence of free IL-18 found by Western analysis together with an excess of active free IL-18BP isoform a would be that, in the tissue, IL-18 was not accessible to endogenous IL-18BP.

Interestingly, using the murine model of TNBS-induced colitis, we found that administration of rIL-18BPa significantly ameliorated the disease and reduced parameters of colonic inflammation (27). Similar findings using the murine model of dextran sulfate sodium-induced colitis and neutralizing IL-18 Abs were also reported (28, 29). Although the IL-18BP excess found in CD lesions is not understood, the results obtained with experimental animal models suggest that neutralizing IL-18 may be beneficial and that administration of rIL-18BP may be considered as a possible therapy.

Our results thus demonstrate that IL-18BP transcript and protein are up-regulated in active CD intestinal specimens by endothelial cells and macrophages. As observed in our in vitro studies, this increase of IL-18BP production was also found in other types of endothelial cells (HUVEC) and monocytes/macrophages (purified from total PBMC) after stimulation with proinflammatory cytokines (TNF-α, IFN-γ). Because these proinflammatory cytokines are reported to be increased in the intestinal tissue during CD (18), one could hypothesize that they significantly contribute to the up-regulation of IL-18BP observed in active CD specimens. Interestingly, gene induction of IL-18BP by IFN-γ has been recently observed using cultures of colonic biopsy specimens (23). However, direct isolation of intestinal endothelial cells and macrophages would be further needed to verify this hypothesis and to study the IL-18BP regulation in the intestinal tissue. Moreover, the production of IL-18BP by macrophages is a surprising finding, because activated macrophages are the main source of IL-18 (30). One needs also to further investigate whether macrophages found in inflamed intestinal tissue produce both IL-18 and IL-18BP as well as how this expression is regulated.

In summary, our results show that endothelial cells and macrophages were the major source of IL-18BP within the intestinal submucosa. In addition, levels of IL-18BP transcript and protein are significantly up-regulated in active CD specimens compared with control specimens, with isoform a, c, and d being detected. Despite the presence of the appropriate neutralizing isoforms, free IL-18 is still observed in specimens from active CD and highlights the complexity of the regulation of IL-18 activity. Further studies are needed to elucidate the role of IL-18BP in intestinal tissue during CD.

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