# Interleukin-18 and its Binding Protein in Patients with Inflammatory Bowel Disease during Remission and Exacerbation

Timna Naftali MD[1], Daniella Novick PhD[2], Gila Gabay MSc[1], Menahem Rubinstein PhD[2] and Ben Novis MD[1]

[1]Institute of Gastroenterology and Liver Diseases, Meir Hospital, Kfar Saba, Israel
Affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel
[2]Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

**Key words:** interleukin-18, interleukin-18 binding protein, Crohn's disease, ulcerative colitis

## Abstract

**Background:** Crohn's disease and ulcerative colitis are inflammatory bowel diseases with an unknown etiology. Interleukin-18 is a pro-inflammatory cytokine that is up-regulated in Crohn's disease. IL-18 binding protein neutralizes IL-18. The relationship of IL-18 and IL-18BP and disease activity in these diseases is not fully understood.

**Objectives:** To investigate the correlation of IL-18 and IL-18BP with disease activity and other disease parameters in inflammatory bowel disease.

**Methods:** IL-18 and IL-18BP isoform $\alpha$ were measured in 129 patients and 10 healthy individuals. Patients' mean age was 40.5 (range 15–70 years) and 43 were women; 58 Crohn's and 28 colitis patients were in remission and 52 and 14, respectively, were in exacerbation. Twenty-three (19 and 4 respectively) were studied in both remission and exacerbation.

**Results:** The mean level of free IL-18 was significantly different between healthy individuals and Crohn patients, and between Crohn patients during exacerbation and remission (167 ± 32 vs. 471 ± 88 and 325 ± 24 pg/ml, respectively, $P < 0.05$). Mean level of IL-18BP was significantly different between healthy individuals and Crohn patients, and between Crohn patients during exacerbation and remission (2.1 ± 1.1, 7.5 ± 4 and 5.23 ± 2.8 ng/ml, respectively, $P < 0.01$). In the colitis patients, mean free IL-18 level and IL-18BP were significantly different between healthy individuals and patients, but not between disease remission and exacerbation (167 ± 32, 492 ± 247 and 451± 69 pg/ml for IL-18, and 2.1 ± 1.1, 7.69 ± 4 and 6.8 ± 7 ng/ml for IL-18BP, respectively, $P = 0.05$).

**Conclusions:** IL-18 and IL-18BP levels are higher in patients with inflammatory bowel disease compared to healthy individuals. In Crohn's disease, but not in ulcerative colitis, IL-18 (but not free IL-18) and IL-18BP levels are significantly higher during exacerbation compared to remission. This observation highlights the importance of IL-18 in the pathogenesis of inflammatory bowel diseases, especially in Crohn's disease.

*IMAJ 2007;9:504–508*

Interleukin-18 is a cytokine that initiates and promotes host defense and inflammation. It is characterized by both structural and functional homology to the IL-1β family of cytokines [1,2]. IL-18 appears to be a pivotal mediator of T helper type 1 cell responses [3], as was manifested by the induction of T helper 1 cytokines such as interferon gamma [4] synergistically with IL-12 [5]. IL-18 was also shown to facilitate the development of Th2 response [6] in the absence of IL-12.

IL-18 exerts its functions by binding to the IL-18 receptor complex. This complex consists of two chains, the ligand binding chain, IL-18Rα and the signaling chain IL-1Rβ. IL-18Rα is a member of the IL-1 receptor family and was previously called IL-1 receptor-related protein. As is the case for the IL-1R complex, both chains are required for IL-18 signaling [7].

IL-18 is unique among other cytokines in that it has a specific binding protein, named IL-18 binding protein, which was purified from urine by ligand affinity chromatography [8,9]. IL-18BP is expressed in the spleen, colon and small intestine, and is found in plasma. It belongs to the immunoglobulin superfamily and has limited homology to the IL-1 type II receptor. Its gene was localized on the human chromosome 11q13. There is no exon coding for a trans-membrane component. Administration of IL-18BP abolishes the induction of IFNγ, IL-8 and activation of nuclear factor kappa B [10]. Thus, IL-18BP acts as an inhibitor in the early Th-1 response. It is of note that several viruses produce proteins homologous to IL-18BP [9], suggesting that viral products may interfere with cytotoxic T cell response. The balance between IL-18 and IL-18BP is complex and poorly understood.

The two inflammatory bowel diseases, Crohn's disease and ulcerative colitis, are characterized by a chronic inflammatory process involving the gastrointestinal tract. The pathogenesis of these diseases remains unclear, but evidence suggests that the normal balance between inflammatory and regulatory cytokines is disturbed. This has been exploited in recent treatment modalities, such as anti-tumor necrosis factor alpha antibody that manipulates the immune system and returns the balance of the inflammatory process to normal. Cytokines that are up-regulated

IL = interleukin
IL-18BP = IL-18 binding protein
IL-18R = IL-18 receptor
IFNγ = interferon gamma

during the inflammatory process are likely candidates for future therapeutic interventions.

In several studies, IL-18 was found to be up-regulated in Crohn's disease. Monteleone et al. [11] used polymerase chain reaction and Western blot to examine whole mucosa intestinal tissue and lamina propria mononuclear cells for IL-18 in 12 patients with Crohn's disease, 9 with ulcerative colitis, and 15 healthy individuals. Transcripts for IL-18 were found in all samples. However, increased transcripts were present in the samples from Crohn patients as compared to the samples from colitis patients and controls. The levels of IL-18 were higher in samples from sites of more active disease. Only patients in an active phase of disease were examined in that study. In another study, Furuya and co-workers [12] measured IL-18 levels in the serum of five patients with Crohn's disease and found that the level was 1.7 times higher than in the control group. They did not measure IL-18BP. They did not find a high level of IL-18 in colitis patients. In another study Ten Hove and team [13] found that in experimental models of colitis, neutralization of IL-18 reduced the severity of the disease. In another study, Ludwiczek et al. [14] showed that IL-18 but not IL-18BP is higher in the serum of patients with Crohn's but not with colitis. The purpose of our study was to measure IL-18 and IL-18BP in the sera of Crohn and colitis patients, in both active and non-active stages of the disease, and to correlate these levels with disease activity index and other disease parameters.

## Patients and Methods

Our study population included 129 patients with inflammatory bowel diseases, 91 with Crohn's and 38 with colitis. Patients' average age was 38 (range 10–72) and 44.8 years (range 14–84) in those with Crohn's and colitis respectively. Male to female ratio was 52/39 in Crohn patients and 24/14 in colitis patients. The disease parameters studied are described in Table 1. They included the type of disease (fistulizing, inflammatory, or fibrosing), extra-intestinal manifestations, duration of disease, family history of disease, and the involved part of the intestine. Crohn's Disease Activity index or Ulcerative Colitis Activity Index at the time of the study were calculated using the BEST score [15] or ulcerative colitis clinical activity score [16]. Briefly, the BEST score calculates Crohn's disease activity by taking into account the patients' well-being, number of bowel movements, extra-intestinal disease manifestations, anemia and weight loss. The UCAI takes into account the number of bowel movements, the proportion of bloody stool, nocturnal diarrhea incontinence, and patient well-being. Remission was defined as CDAI below 150 and UCAI below 6. In total, 110 blood samples were obtained from 91 Crohn patients to measure IL-18 and IL-18BP: 58 during remission and 52 during an exacerbation. Nineteen blood samples were obtained from the same patient twice, once during remission and once during an exacerbation.

Forty-two blood samples were obtained from 38 colitis patients. In only four patients were blood samples available during both exacerbation and remission. (Because of the small number they were not analyzed separately.) Results of patients with active disease and patients in remission were compared. Ten healthy volunteers picked from the hospital staff served as a control group. Male to female ratio was 4/6 and average age was 45. The study was approved by the medical ethics committee of the medical center.

**Table 1.** Disease parameters

| | |
|---|---|
| **Crohn's disease** | |
| Disease duration (yrs) | 9.47 (0.1–35) |
| Disease in family | 15/91 |
| Extra-intestinal manifestations | 21/91 |
| Involved organ | |
| Terminal ileum | 52 |
| Colon | 23 |
| Small intestine | 7 |
| Terminal ileum + colon | 8 |
| Terminal ileum + esophagus | 1 |
| Treatment* | |
| Before treatment | 22 |
| 5 ASA | 37 |
| Steroids | 26 |
| Immunssuppressive | 20 |
| Anti-TNF | 111 |
| **Ulcerative colitis** | |
| Disease duration (yrs) | 15 (1–43) |
| Disease in family | 1/38 |
| Extra-intestinal manifestations | 3/38 |
| Involved organ | |
| Pan colitis | 9 |
| Left colon | 23 |
| Proctitis | 6 |
| Treatment* | |
| Before treatment | 19 |
| 5 ASA | 13 |
| Steroids | 2 |
| Immunosuppressive | 6 |

* Note that 22 patients received more than one drug
5 ASA = 5 aminosalicilic acid, anti-TNF = anti-tumor necrosis factor

### Laboratory tests

- IL-18 *enzyme-linked immunosorbent assay*: IL-18 was measured in patient's serum by ELISA (MBL, Naka-Ku Nagoya, Japan). The assay uses two monoclonal antibodies against two different epitopes of human IL-18. Serum samples, blank and standards, were incubated in duplicates in 96-well microplates coated with anti-human IL-18 monoclonal antibodies. After washing, peroxidase conjugated anti-human IL-18 monoclonal antibodies were added and incubated. Then after another

UCAI = Ulcerative Colitis Activity Index
CDAI = Crohn's Disease Activity index

ELISA = enzyme-linked immunosorbent assay

washing a peroxidase substrate mixed with chromogen was added. The enzyme reaction was terminated by adding a sulfuric acid solution. Optical density of the developed color was measured at 450 nm in an ELISA microplate reader. The concentration of IL-18 in pg/ml was calibrated from a dose response curve, based on the five reference standards. The minimum detection limit of the kit is 12.5 pg/ml.

- A *double antibody* ELISA *for* IL-18BP [17] *to measure isoforms a+c*: The ELISA consisted of two anti-rIL-18BP-His6 antibodies: Mab 582.10 served as the capture antibody and rabbit polyclonal antibody was used for detection. A highly purified recombinant human rIL-18BPa, quantitated by amino acid analysis, was used as a standard and a linear standard curve was obtained between 0.062 and 2 ng/ml.

- *Calculation of free* IL-18: Antibodies used for the IL-18 ELISA detect both free IL-18 and IL-18 bound to IL-18BP and are not able to distinguish between the free and the bound form of IL-18. Therefore, the levels of free IL-18 were calculated. Free IL-18 was calculated according to the law of mass action as recently described [1,18]. In brief, the calculation was based on a molecular weight of 18 kD and 20 kD of IL-18 and IL-18BP, respectively. A 1:1 stoichiometry in the complex is known from earlier cross-linking studies [9]. The dissociation constant (K*d*) is 0·4 nM.

#### Statistical analysis

Data were analyzed using SPSS; the Mann-Whitney test was used to compare results between the different groups. Paired sample *t*-test was used to compare results of the same patients in exacerbation and remission.

## Results

Levels of IL-18 and IL-18BP in Crohn's patients are presented in Table 2. The levels of IL-18 and IL-18BP were significantly different between the control group and the patients, and between patients during disease remission and exacerbation. The level of free IL-18 was statistically different between patients and controls, but the difference between patients in exacerbation and remission did not reach statistical significance.

Table 3 presents the results in 19 of 91 Crohn's patients in whom blood samples were available during both exacerbation and remission. A paired sample *t*-test showed a significant difference between IL-18 and IL-18BP levels during exacerbation and remission. When free IL-18 was calculated a significant difference was not found between exacerbations and remissions.

No significant correlation was found between the levels of IL-18 or IL-18BP and the patients' age, gender, duration of disease, phenotype of disease (inflammatory, fistulizing or fibrosing), family history of inflammatory bowel disease, region of the intestine involved, or extra-intestinal manifestations of disease.

In the ulcerative colitis patients, the levels of IL-18 and IL-18BP and free IL-18 were significantly different between patients and controls, but no significant difference was detected between patients during disease remission or exacerbation [Table 4].

**Table 2.** Levels of IL-18 and IL-18 BP in Crohn's disease

| | Control | Crohn's disease in exacerbation | Crohn's disease in remission | *P* (control/ Crohn's disease) | *P* (exacerbation/ remission) |
|---|---|---|---|---|---|
| Number * | 10 | 52 | 58 | | |
| CDAI | | 264 ± 91 | 80.6 ± 48 | | < 0.01 |
| IL-18 (pg/ml) | 218 ± 102 | 819 ± 866 | 539 ± 300 | < 0.01 | < 0.01 |
| Free IL-18 | 167 ± 32 | 471 ± 88 | 325 ± 24 | < 0.05 | NS |
| IL-18BP (ng/ml) | 2.1 ± 1.1 | 7.5 ± 4 | 5.23 ± 2 .8 | < 0.01 | < 0.05 |

Values are presented as mean ± SD

* Blood samples were taken from 19 patients twice: during remission and during exacerbation, so the numbers of blood samples is higher than the number of patients

CDAI = Crohn's Disease Activity Index

**Table 3.** Levels of IL 18 and IL 18 BP in the same Crohn patients during exacerbation and remission

| | Exacerbation (n=19) | Remission (n=19) | *P* |
|---|---|---|---|
| CDAI | 255 ± 84 | 88 ± 41 | < 0.001 |
| IL-18 | 621 ± 362 | 446 ± 293 | < 0.05 |
| Free IL-18 | 377 ± 193 | 257 ± 145 | NS |
| IL-18 BP | 8.5 ± 4.5 | 5.7 ± 2.6 | < 0.01 |

Values are presented as mean ± SD

CDAI = Crohn's Disease Activity Index

**Table 4.** Levels of IL-18 and IL-18 BP in Ulcerative colitis

| | Control | Ulcerative colitis in exacerbation | Ulcerative colitis in remission | *P* (control/ colitis) | *P* (exacerbation/ remission) |
|---|---|---|---|---|---|
| Number * | 10 | 14 | 28 | | |
| UCAI | | 11.3 ± 3 | 1.85 ± 2 | | < 0.05 |
| IL-18 (pg/ml) | 218 ± 102 | 853 ± 1300 | 822 ± 500 | < 0.01 | NS |
| Free IL-18 | 167 ± 32 | 492 ± 247 | 451 ± 69 | < 0.01 | NS |
| IL-18BP (ng/ml) | 2.1 ± 1.1 | 7.69 ± 4 | 6.8 ± 7 | < 0.01 | NS |

Values are presented as ± SD

* Blood samples were taken from four patients twice: during remission and during exacerbation, so the numbers of blood samples is higher than the number of patients

UCAI = Ulcerative Colitis Activity Index.

## Discussion

IL-18 is a pro-inflammatory cytokine that is an important contributor to systemic and local inflammation. IL-18 plays an important role in the activation and regulation of T1 helper cells (Th1) [4] that are mandatory in the inflammatory reaction of Crohn's disease. IL-18 and IL-18BP have been found to be sig-

nificantly higher in both serum and tissue specimens of Crohn's disease patients [14,19,20], but the changes of IL-18 and IL-18BP during disease remission and exacerbation in Crohn's disease and in colitis were not fully explored. In Crohn patients, IL-18 and IL-18BP were elevated compared to healthy individuals, but their relation to disease activity was not clear. In patients with colitis, levels of free IL-18 did not seem to rise [14], despite the fact that in animal models suppression of IL-18 ameliorates colitis [10]. The purpose of our study was to examine the relationship between IL-18, IL-18BP and disease activity in both diseases.

We demonstrated again that levels of both IL-18 and IL-18BP are higher in patients with inflammatory bowel disease than in healthy individuals. In Crohn's we found that both IL-18 and IL-18BP rise with disease relapse. Our findings that levels of IL-18 and IL-18 BP increase with disease activity further support a major role for this cytokine in the pathogenesis of Crohn's disease. This rise is similar to a parallel rise of both IL-18 and IL-18BP in patients with sepsis [17], rheumatoid arthritis [21] and chronic hepatitis [22]. The high levels of IL-18 in Crohn patients compared to healthy individuals even during disease remission indicates that remission in Crohn's is only relative and a certain degree of inflammation is constant.

IL-18 is not completely free in the serum and is bound by IL-18BP. The amount of free IL-18 that is left depends on the initial concentration of IL-18, the concentration of IL-18BP and their dissociation constant (K*d*) and can be calculated using the law of mass action [1,17]. That is why, despite the seemingly high concentrations of IL-18BP, IL-18 is not completely neutralized and levels above normal still remain free and biologically active. Though IL-18 level was always above normal in our patients, the rise of IL-18 during disease relapse was modest and not significant when free IL-18 was calculated. The lack of difference of free IL-18 levels between disease remission and exacerbation may be the result of suppression of this cytokine by IL-18BP. The possibility of increasing the inhibition by using IL-18BP needs to be explored.

In their study, Ludwiczek and colleagues [22] did not find a correlation between CDAI and IL-18 levels. We, however, found that as a group, Crohn patients in relapse have a significantly higher level of IL-18 but not free IL-18 compared to Crohn patients during remission. It seems that the range of IL-18 levels in different individuals is wide; some have higher levels in remission than others would during relapse, but when examining the same individual during both remission and relapse we found that relapse levels were higher.

IL-18 also stimulates Fas ligand-mediated cytotoxic activity of natural killer cells [23]. This activity is part of the Th2 response, mandatory in the inflammatory reaction causing ulcerative colitis. Thus IL-18 may be a key cytokine, playing an important role in both colitis and Crohn's disease [6].

Pizarro et al. [24] found that in tissue from patients with active colitis, IL-18 levels were only minimally elevated. Contrary to this, Ludwiczek et al. [14] found that colonic explant cultures from disease-involved areas from colitis patients showed elevated secretion of IL-18, but that blood levels of IL-18 did not differ between colitis patients and healthy individuals. Several studies in animal models of colitis have shown that neutralization of IL-18, by using either IL-18BP or by specific anti-IL-18 antibodies, led to a significant amelioration of colitis. [10,13,25].

When combined, all these data indicate that IL-18 does participate in the inflammatory process of ulcerative colitis. Our finding that total IL-18 and IL-18BP serum levels were significantly increased in colitis patients compared to healthy controls further supports this role, though no significant difference between disease remission and exacerbation was observed. Further studies are needed to precisely understand the significance of the constant rise of cytokines during both disease remission and exacerbation.

In conclusion, our findings indicate a role for IL-18 and IL-18BP not only in Crohn's disease but in ulcerative colitis too. In view of the rise of IL-18BP during disease exacerbation, it may be used as a surrogate marker of ongoing inflammatory activity. Whether these cytokines could be used for therapeutic intervention, as was shown in experimental mouse models, is a possibility that should be explored.

**Acknowledgments.** Menachem Rubinstein is the Edna and Maurice Weiss Professor of Cytokine Research. This study was supported by the Serono Group of companies.

## References

1. Dinarello C. Interleukin 18. *Methods* 1999;19:121–32.
2. Bazan JF, Timans JC, Kastelein RA, A newly defined interleukin-1? *Nature* 1996;379(6566):591.
3. Okamura H, Tsutsi H, Komatsu M, et al. Cloning of a new cytokine that induces INF-γ production by T cells. *Nature* 1995;378:88–91.
4. Dinarello C, Novick D, Puren AJ, et al. Overview of interleukin 18: more than an interferon γ inducing factor. *J Leukoc Biol* 1998;63:658–64.
5. Munder M, Mallo M, Eichmann K, et al. Murine macrophages secrete interferon gamma upon combined stimulation with interleukin (IL)-12 and IL-18: a novel pathway of autocrine macrophage activation. *J Exp Med* 1998;187:2103–8.
6. Nakanishi KT, Yoshimoto H, Tsutsui H, et al. Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. *Cytokine Growth Factor Rev* 2001;12:53.
7. Born TL, Thomassen E, Bird TA, et al. Cloning of a novel receptor subunit, AcPL, required for interleukin-18 signaling. *J Biol Chem* 1998;273:29445–50.
8. Novick D, Dinarello CA, Rubinstein M. Soluble IL 18 receptor in human urine: characterization and purification. *Cytokine* 1997;9: A963.
9. Novick D, Kim SH, Fantuzzi G, et al. Interleukin 18 binding protein: a novel modulator of the Th1 cytokine response. *Immunity* 1999;10:127–36.
10. Siegmund B, Fantuzzi G, Reider F, et al. Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-gamma and TNF-alpha production. *Am J Physiol Regul Integr Comp Physiol* 2001;281:R1264–73.
11. Monteleone G, Trapasso F, Parrello T, et al. Bioactive IL 18 is up-regulated in Crohn's disease. *J Immunol* 1999;163:143–7.
12. Furuya D, Yagihashi A, Komatsu M, et al. Serum interleukin-18 concentrations in patients with inflammatory bowel disease. *J Immunother* 2002;25(Suppl 1):S65–7.

13. Ten Hove T, Corbaz H, Amitai S, et al. Blockade of endogenous IL-18 ameliorates TNBS induced colitis by decreasing local TNFα production. *Gastroenterology* 2001;120:A687.
14. Ludwiczek O, Kaser A, Novick D, et al. Elevated systemic levels of free IL-18 in patients with Crohn´s disease. E*ur Cytokine Netw* 2005;16:27–33.
15. Best WR, Becktel JM, Singelton JW, et al. Development of a Crohn's disease activity index. *Gastroenterology* 1976;70:439.
16. Lichtiger S, Present D, Kornbluth A, et al. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. N E*ngl J Med* 1994;330:1841–5.
17. Novick D, Schwartsburd B, Pinkus R, et al. A novel IL–18 BP ELISA shows elevated serum IL–18 BP in sepsis and extensive decrease of free IL-18. *Cytokine* 2001;14:334–42.
18. Kaser A, Novick D, Rubinstein M, et al. Interferon-a induces interleukin-18 binding protein in chronic hepatitis C patients. C*lin Exp Immunol* 2002;129:332–8.
19. Puren AJ, Fantuzzi G, Gu Y, et al. Interleukin 18 (INF γ inducing factor) induces IL-1 β and IL 8 via TNF α production from non CD 14+ human blood mononuclear cells. J C*lin Invest* 1998;101: 711–21.
20. Corbaz A, Hove T, Herren S, et al. IL 18 binding protein expression by endothelial cells and macrophages is up regulated during active Crohn's disease. J I*mmunol* 2002;168:3608–16.
21. Gracie JA, Forsey RJ, Chan WL, et al. A proinflammatory role for IL-18 in rheumatoid arthritis. J C*lin Invest* 1999;104:1393–401.
22. Ludwiezek O, Kaser A, Novick D, et al. Plasma levels of interleukin-18 binding protein are elevated in patients with chronic liver disease. J C*lin Immunol* 2002;22:331–7.
23. Tsutsui H, Nakanishi K, Matsui K, et al. INF-γ-inducing factor up regulates Fas ligand mediated cytotoxic activity of murine natural killer cell clones. J Immunol 1996;157:3967–73.
24. Pizarro T, Michie M, Bentz M, et al. IL-18, a novel immunoregulatory protein, is up regulated in Crohn's disease: expression and localization in intestinal mucosal cells. J I*mmunol* 1999;162:6829–35.
25. Sivakumar PV, Westrich GM, Kanaly S, et al. Interleukin 18 is a primary mediator of the inflammation associated with dextran sulphate sodium induced colitis; blocking interleukin 18 attenuates intestinal damage. G*ut* 2002;50::812–20.

**Correspondence:** Dr. T. Naftali, Gastroenterology Institute, Meir Hospital, Kfar Saba 44281, Israel.
Phone: (972-9) 747-2523
Fax: (972-9) 747-2725
email: naftali@post.tau.ac.il