Gastroenterologia Japonica *Vol. 26, No. 1*
Copyright © 1991 by The Japanese Society of Gastroenterology *Printed in Japan*

Significance of interleukin-6 in patients with inflammatory bowel disease

Keiichi MITSUYAMA, Michio SATA, and Kyuichi TANIKAWA
Second Department of Medicine, Kurume University School of Medicine, Kurume 830, Japan

Summary: The significance of interleukin-6 (IL-6) in patients with inflammatory bowel disease (IBD) was studied by measuring the IL-6 level in serum and colonic tissue by means of an enzyme-linked immunosorbent assay (ELISA), and by examining its localization using an immunohistochemical method. The serum IL-6 level reflected the degree of disease activity, and the extent of affected area, and was also correlated with the serum C-reactive protein (CRP) level. In the colonic mucosa of active IBD, the tissue IL-6 level was markedly elevated, and immunoreactive products of anti-IL-6 antibody were present in infiltrative mononuclear cells in the lamina propria. This indicates that IL-6 production in these cells is enhanced at the site of affected intestine. These results, together with its biological activity and the type of cell producing it, suggest that IL-6 is an available marker to assess disease conditions of IBD and that it might be also involved in the pathophysiology of IBD. *Gastroenterol Jpn 1991;26:20–28*

Key words: *C-reactive protein; Crohn's disease; ELISA; immunohistochemistry; interleukin-6; ulcerative colitis*

Introduction

The recent progress in gene engineering led to separation of a large number of cytokines by cloning, allowing clarification of their structures and functions. These soluble factors were shown to play important roles in the biological defense mechanism. Interleukin-6 (IL-6) is produced by a variety of cells, including monocytes/macrophages, T cells, and B cells[1-3], and acts to induce acute phase protein synthesis in hepatocytes[4-6], differentiation of B cells[7,8], growth and differentiation of T cells[9-13], and maturation of megakaryocytes[14,15]. Therefore, this molecule has been regarded as one of the cytokines essential for inflammatory reactions and immune responses[1,2]. Recently, clinical studies on IL-6 were also conducted, revealing their important role in various inflammatory conditions and autoimmune diseases[16-22]. Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of unkonwn etiology associated with immunological abnormalities[23,24]. The type of cell producing IL-6, as well as its biological activity, suggests that IL-6 might be involved in the pathophysiology of IBD. In the present study, to assess its significance in IBD, the IL-6 level in serum and colonic tissue was measured using enzyme-linked immunosorbent assay (ELISA). Furthermore, immunohistochemical study was also performed.

Subjects and Methods

Subjects

Patients with ulcerative colitis (UC) and Crohn's disease (CD) were included in this study. In all patients, the diagnoses were established on the basis of clinical, radiologic, endoscopic, and histologic findings at the Second Department of medicine, Kurume University Hospital and affiliated hospitals.

Patients with UC lacking clinical and laboratory abnormalities were defined as having inactive disease. Patients with active UC were divided into

Received May 16, 1990. Accepted August 6, 1990.
Address for correspondence: Keiichi Mitsuyama, M.D., The Second Department of Medicine, Kurume University School of Medicine, 67 Asahimachi, Kurume 830, Japan.

Table 1 Clinical details of subjects

	ulcerative colitis		Crohn's disease		Normal controls
No. of cases	47		29		61
Age [yr] (range)	35.8 (13-68)		26.9 (13-63)		29.0 (21-46)
Sex [M:F]	20:27		14:15		29:32
Activity	Active	: 34	Active	: 20	—
	Inactive	: 13	Inactive	: 9	—
Extent of lesion	Proctitis	: 10	Ileitis	: 6	—
	Left-sided colitis	: 16	Colitis	: 5	—
	Entire colitis	: 21	Ileocolitis	: 18	—

3 degrees of severity according to the criteria by Truelove and Witts[25]; mild, moderate, and severe disease. In patients with CD, disease activity indexes were calculated according to the score of the International Organization for the Study of Inflammatory Bowel Disease (IOIBD)[26]; a total score of IOIBD $\leqq 1$ was defined as corresponding to inactive disease, and a score $\geqq 2$ was active disease.

Serum samples were obtained from 47 patients with UC (20 males and 27 females, mean age 35.8 years, range 13-68 years), and 29 patients with CD (14 males and 15 females, mean age 26.9 years, range 13-63 years) for serological study (**Table 1**). Among the 47 UC patients, the disease was active in 34 (consisting of 16 mild, 14 moderate, and 4 severe diseases), and inactive in 13. Ten patients had proctitis, 16 patients had left-sided colitis, and 21 patients had entire colitis. Thirty-six patients had received salazosulfapyridine and/or corticosteroids, and 11 patients were untreated. Among the 29 CD patients, the disease was active in 20 and inactive in 9. Six patients had ileitis, 5 patients had colitis, and 18 patients had ileocolitis. Twenty-two patients had received salazosulfapyridine and/or corticosteroids, and 7 patients were untreated. Follow-up blood sampling was conducted in 11 patients with active UC and 8 patients with active CD. Serum samples obtained from 61 healthy volunteers (29 males and 32 females, mean age 29.0 years, range 21-46 years) served as controls.

Endoscopic biopsy specimens of the colon were obtained from 10 patients with UC (4 active and 6 inactive) and 4 patients with CD (3 active and 1 inactive). All these patients were subjected to the above serological study. IL-6 levels in the mucosal tissue were measured in all specimens. Some of them underwent immunohistochemical staining of IL-6. In cases with active disease, the biopsy specimen was obtained from the site where the most intense inflammation was macroscopically observed. When there were ulcerative lesions, the mucosal tissue near the lesion was obtained. In cases with inactive disease, the specimen was obtained from the site where inflammation had been evident during the active phase. Specimens obtained from 9 patients having no endoscopic abnormality in the colon served as controls. This biopsy was performed after informed consent was obtained. It was confirmed before use that all these control tissues were histologically normal.

Methods

1. Measurement of serum IL-6 levels

Blood samples were obtained from patients with IBD and from healthy control subjects by standard venipuncture. The sera were immediately separated by centrifugation, frozen, and stored at −20°C until analyzed for IL-6[27]. ELISA (SRL Inc., Tokyo) was employed to determine IL-6 level. Mouse anti-human IL-6 monoclonal antibody (αBSF2-166, provided by Dr. T. Kishimoto, Osaka University, Osaka) was coated on a 96-well microplate (Nunc, Roskilde, Denmark) and treated for blocking. Test sample or IL-6 standard (Ajinomoto Co., Inc., Tokyo) was added to each well. The plate was incubated for

18 hours at 4°C, and then the well was rinsed. Goat anti-IL-6 polyclonal antibody[28] (Fujirevio Inc., Tokyo) was added to the well, the plate was incubated for 3 hours at room temperature, and then the well was rinsed again. Horseradish peroxidase-labelled anti-goat IgG (Tago Inc., Burlingame, U.S.A.) was added to the well. After incubation for 2 hours at room temperature, the well was rinsed. After addition of a substrate solution, the plate was incubated for 30 minutes at room temperature, and optical density was measured at 490 nm. IL-6 levels were determined according to the standard curve prepared concurrently. Intra-assay and inter-assay replicates gave results with a coefficient of variation of less than 3% and 6%, respectively. Furthermore, the specificity of the ELISA result was confirmed by using recombinant human IL-1α, IL-1β, IL-3 IL-4, tumor necrosis factor α, granulocyte/macrophage-colony stimulating factor (CSF), macrophage-CSF, and granulocyte-CSF. Quantitation of serum C-reactive protein (CRP) levels was performed by laser nephelometry (NA latex CRP kit, Hoechst Japan, Tokyo).

2. Measurement of IL-6 contents in the colonic mucosa

Tissue samples obtained by biopsy were suspended in 0.7 ml of 0.01 M phosphate buffered saline and homogenized in a glass homogenizer. The homogenate was centrifuged at 4°C at 10,000x g for 30 minutes and filter sterilized before assay[29]. IL-6 was measured with the filtrate by ELISA as described above for serum samples. Total protein in the filtrate was also measured by the pyrogallol-red method (Micro TP test, Wako Pure Chemical Industries, Ltd., Osaka). The content of IL-6 (pg/mg protein) was calculated.

3. Immunohistochemistry of IL-6

The biopsy specimen was embedded in OCT compound (Miles Inc., Elkhart, U.S.A.) immediately after the removal, and frozen quickly with dry ice ethanol. Thin sections, 6 μm thick, were cut on a cryostat, and immunohistochemical staining of IL-6 was performed by the immunoperoxidase method utilizing the Biotin-Streptavidin Amplified System (Biogenex Laboratories, San Ramon, U.S.A.). Goat anti-human IL-6 polyclonal antibody[28] (provided by Drs. T. Kishimoto and T. Matsuda, Osaka University, Osaka) was used as the first antibody. After incubation with this antibody, the tissue was treated with 3,3'-diaminobenzidine 4HCl (DAB) in 0.1 M Tris-HCl buffered solution, and then the nuclei were stained with hematoxylin. The preparations were observed under light microscopy. To block endogenous peroxidase activity, the tissue was immersed in 5 mM periodic acid solution for 10 minutes and then 3 mM sodium borohydride solution for 30 minutes and was treated with DAB solution containing 10 mM sodium azide. Sections incubated with preimmune goat serum in place of the first antibody served as controls.

4. Statistical analysis

The chi-square test was used for comparison of serum IL-6 levels, and Student's *t*-test for serum CRP levels and IL-6 levels in the colonic mucosa. All observed values were expressed as mean$\pm$ standard error (mean$\pm$SE). P values of <0.05 were considered significant.

Results

1. IL-6 levels in the serum

Serum samples containing IL-6 at the level of 4.0 pg/ml or higher were considered IL-6 positive, and those containing less than 4.0 pg/ml, negative; the level of 4.0 pg/ml being the detection limit of the ELISA. IL-6 was positive in only 9.8% (6/61) of the healthy controls, and IL-6 levels among positive cases ranged from 5.5 to 14.9 pg/ml. In contrast, the positive rates in patients with active UC and CD were 41.2% (14/34) and 90.0% (18/20), respectively. Both rates were significantly higher than that of the healthy controls ($P<0.001$ in both groups). The levels among positive cases ranged from 4.7 to 218.3 pg/ml in patients with active UC and from 4.2 to 84.1 pg/ml in patients with active CD. The high serum level was found more frequently in patients with active disease than in healthy controls. The positive rates in patients with inactive UC and CD were

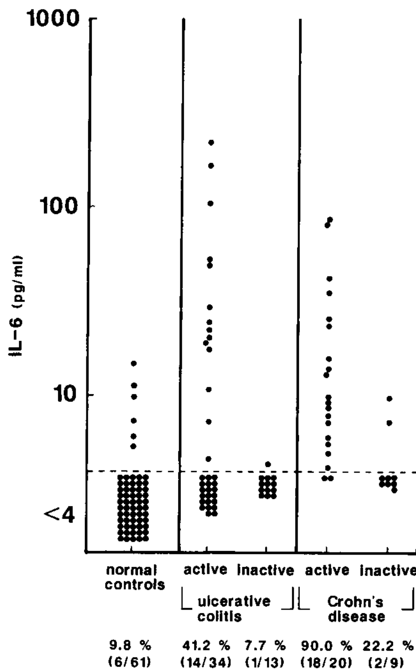

Fig. 1 Serum IL-6 levels in patients with ulcerative colitis and Crohn's disease. IL-6 levels were determined by ELISA. Each point represents the value in a different individual. The horizontal dotted line represents the detection limit of the assay (4.0 pg/ml).

7.7% (1/13) and 22.2% (2/9), respectively. The level was 4.4 pg/ml in the patient with inactive UC and ranged from 7.2 to 9.7 pg/ml in patients with inactive CD. The low serum level was found more frequently in patients with inactive disease than in those with active disease (**Fig. 1**).

Serum IL-6 levels were determined during both the active phase and inactive phase after treatment in 11 and 8 patients with UC and CD, respectively. Comparison of individual levels before and after treatment revealed that the serum IL-6 level during the inactive phase was lower than that during the active phase in all patients except two UC patients whose serum samples contained no detectable IL-6 during the active phase (UC: P<0.01, CD: P<0.05) (**Fig. 2**).

In classifying the severity among patients with active UC, serum IL-6 was positive in 1 (6.3%) of 16 patients with mild disease, 9 (64.3%) of 14 patients with moderate disease, and in all (100%) 4 patients with severe disease. The positive rates

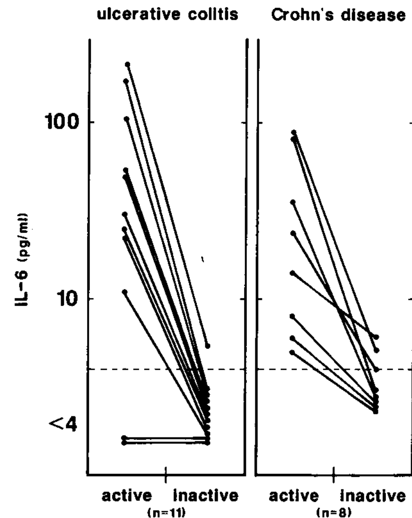

Fig. 2 Individual serum IL-6 levels during the active and inactive phase in 11 patients with ulcerative colitis (left panel) and 8 patients with Crohn's disease (right panel).

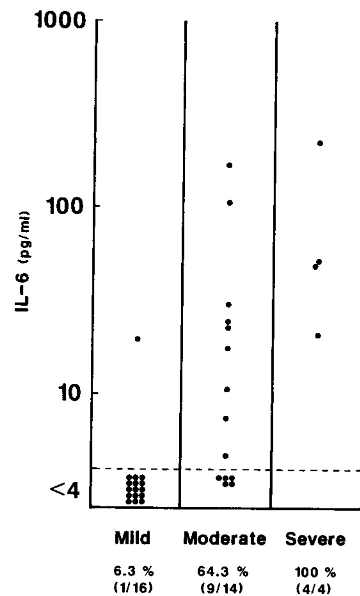

Fig. 3 Serum IL-6 levels in patients with active ulcerative colitis according to the severity of disease evaluated by the criteria described by Truelove and Witts.

of moderate and severe disease were significantly higher than the rate of mild disease (P<0.01 in both severities of disease). The serum level among positive cases also tended to rise as the severity of the disease increased (**Fig. 3**).

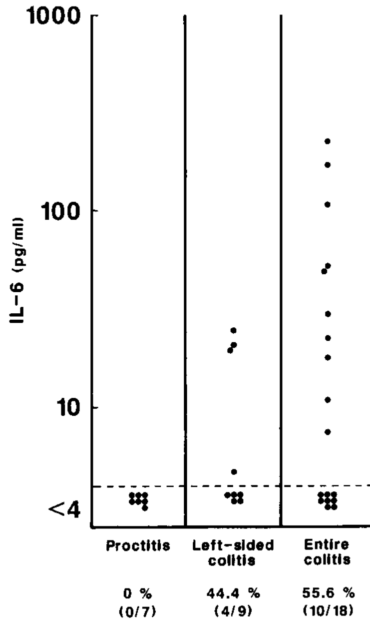

Fig. 4 Serum IL-6 levels in patients with active ulcerative colitis according to the extent of the affected area.

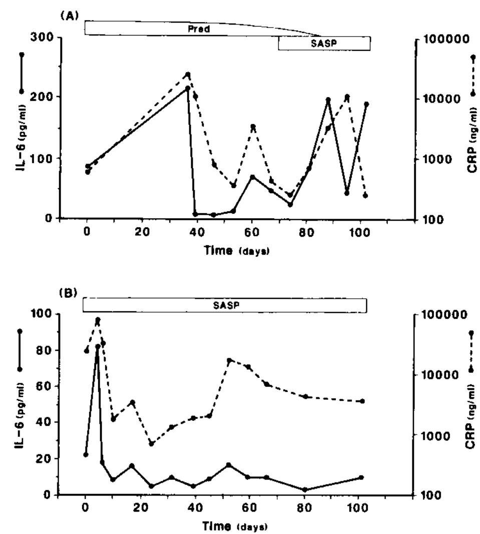

Fig. 5 Serial changes in serum IL-6 and CRP levels in a 30-year-old female patient with ulcerative colitis (A) and a 23-year-old female patient with Crohn's disease (B). Pred: prednisolone, SASP: salazosulfapyridine.

In classifying the extent of affected area among patients with active UC, serum IL-6 was positive in none (0%) of 7 patients with proctitis, 4 (44.4%) of 9 patients with left-sided colitis, and 10 (55.6%) of the 18 patients with entire colitis. The positive rate of entire colitis was significantly higher than that of proctitis ($P<0.05$). The serum level also tended to rise as the affected area expanded (**Fig. 4**).

2. Association between serum IL-6 and CRP levels

Serum CRP levels in serum IL-6 positive patients were compared with those in negative patients in **Table 2**. Among patients with UC, the serum CRP level was 27,748.7±9,308.7 ng/ml for the IL-6 positive group (n=15) and 2,955.2±1,126.5 ng/ml for the IL-6 negative group (n=32). Among patients with CD, CRP levels for positive (n=20) and negative (n=9) groups were 29,843.0±5,347.8 and 3,142.4±951.3 ng/ml, respectively. In both groups of patients with UC and CD, IL-6 positive patients had significantly higher serum CRP levels than IL-6 negative patients ($P<0.0005$ and $P<0.005$, respectively).

Serial changes in serum IL-6 and CRP levels along the clinical course in a 30-year-old female patient with UC involving the entire colon are shown in **Figure 5A**. The patterns of a 23-year-old female patient with CD involving both small and large bowel are shown in **Figure 5B**. In both cases,

Table 2 CRP levels in IL-6 positive and negative sera collected from patients with ulcerative colitis and Crohn's disease

	disease	IL-6 positive	IL-6 negative	P
CRP (ng/ml)	ulcerative colitis (n=47)	27748.7±9308.7 (n=15)	2955.2±1126.5 (n=32)	<0.0005
	Crohn's disease (n=29)	29843.0±5347.8 (n=20)	3142.4±951.3 (n=9)	<0.005

The values are expressed as mean±SE.

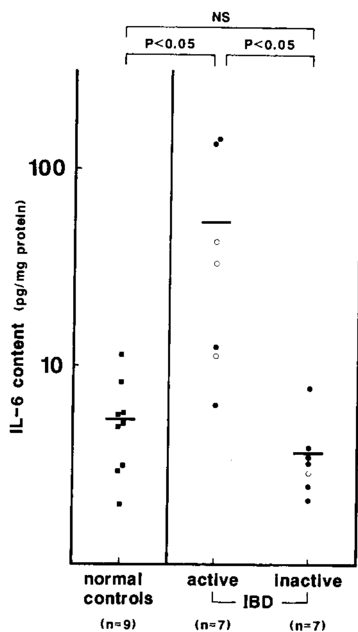

Fig. 6 IL-6 content in the colonic mucosa obtained from normal controls (■) and patients with inflammatory bowel disease (● ulcerative colitis, ○ Crohn's disease). The horizontal bars represent mean values. IBD: inflammatory bowel disease, NS: not significant.

changes in IL-6 levels were correlated with those in CRP levels.

3. IL-6 contents in the colonic mucosa

The tissue IL-6 content was 5.5±1.0 pg/mg protein (n=9) in the healthy control subjects, 54.1±21.9 pg/mg protein (n=7) in patients with active IBD, and 3.7±0.7 pg/mg protein (n=7) in patients with inactive IBD. The content was significantly higher in active IBD than in inactive IBD or controls ($P<0.05$ in both groups) (**Fig. 6**).

4. Immunohistochemistry of IL-6

In order to examine the localization of IL-6 in the colonic mucosa of IBD, an immunohistochemical study was performed. As shown in **Table 3**, immunoreactive products of goat anti-human IL-6 polyclonal antibody were observed in many infiltrative mononuclear cells in the lamina propria in all four cases of active IBD (**Fig. 7**). However, all tissues of inactive IBD (n=3) and normal controls (n=3) were negative for anti-IL-6 antibody.

Table 3 Expression of IL-6 in the colonic mucosa obtained from patients with inflammatory bowel disease

Tissue		No. of positive cases / no. of cases examined	
active IBD	ulcerative colitis	2/2	4/4
	Crohn's disease	2/2	
inactive IBD	ulcerative colitis	0/2	0/3
	Crohn's disease	0/1	
normal controls			0/3

IBD: inflammatory bowel disease.

Discussion

IL-6 was originally identified by Kishimoto and Hirano[30] as T cell-derived B cell stimulatory factor 2 (BSF-2) that induced terminal differentiation of activated B cells into antibody producing cells. Subsequent studies have revealed that IL-6 is produced by a variety of cells and has a wide range of biological activities[1,2]. The present study was conducted to determine its significance in patients with IBD.

Serum IL-6 levels were elevated in patients with active IBD. The level was associated with the severity of disease, especially in patients with UC, indicating that the serum IL-6 level may reflect the degree of disease activity. The elevation with expansion of disease as observed in patients with UC suggests that the serum IL-6 level may reflect the extent of affected area as well. Recent *in vitro*[4,5] and *in vivo*[6] studies with the recombinant molecule have shown that IL-6 is the most important factor which regulates induction of acute phase protein synthesis in hepatocytes. Clinical studies on rheumatoid arthritis[19] and Kawasaki disease[21] also revealed a close correlation between IL-6 and acute phase proteins in the blood. In this study on IBD, the serum CRP level was significantly higher in IL-6 seropositive patients than seronegative patients. In individual patients, serum IL-6 and CRP levels altered following courses of similar pattern, suggesting a relationship between them. Nishimoto et al.[31] analyzed serum levels of IL-6 and CRP following surgical operation. Detectable IL-6 levels were already observed within 3 hours of surgery, reached maximum in 24 hours, and leveled off within 48 hours.

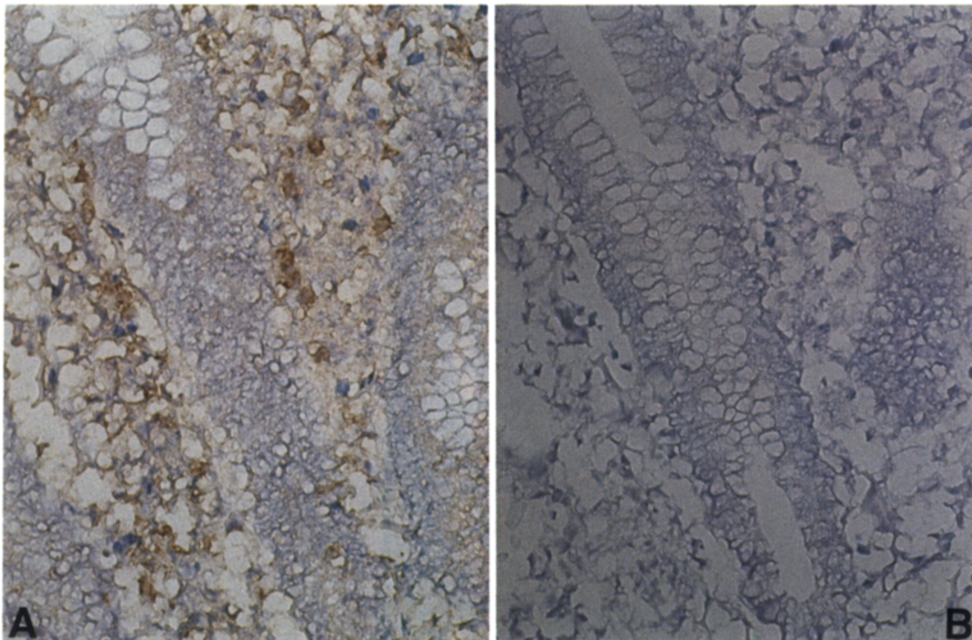

Fig. 7
Immunohistochemical localization of IL-6 in the colonic mucosa of active Crohn's disease (×100). (A) Immunoreactive products of goat anti-human IL-6 antibody are observed in the lamina propria. (B) The control section incubated with preimmune goat serum. Specific staining is not observed.

On the other hand, CRP could not be detected within the first 6 hours, rose to the highest in 48 hours, and then declined gradually. Nijsten et al.[32] reported an elevation of the serum IL-6 level preceding an elevation of the CRP level in patients with severe burns. These findings may suggest that IL-6 acts to induce acute phase protein synthesis such as CRP in humans. Consequently, the measurement of serum IL-6 may be available for rapid and precise evaluation of inflammatory status, and it may be used to assess disease conditions in IBD as well.

It has been already reported that high levels of IL-6 are detected in synovial fluid of patients with rheumatoid arthritis[18,19] and in cerebrospinal fluid of patients with acute infection of the central nervous system[33]. These results indicate that production of IL-6 is enhanced topically at the site of inflammation. In the present investigation, homogenized tissues of the intestine obtained from patients with active IBD contained IL-6 at significantly higher levels than those from patients with inactive disease or from healthy controls, suggesting increased local IL-6 production in the affected intestine. In addition, the presence of IL-6 in many infiltrative mononuclear cells in the lamina propria, as demonstrated by immunohistochemical methods, indicated that these cells were mainly responsible for the production. IL-6 observed in the intestinal tissue may be involved in an increased number of immunoglobulin-producing cells[34] and in autoantibody production[23,24], as observed in the intestine of IBD, because IL-6 is known to regulate terminal differentiation of B cells[7,8]. Additionally, IL-6 also has been known to have effects on T cells, leading to their growth and differentiation[9-13]. Therefore, IL-6 may be involved in a wide range of immunological abnormalities observed locally in the intestine of IBD[23,24].

Frei et al.[35] stated that in mice with experimentally induced viral meningitis, the IL-6 level rose more rapidly in the cerebrospinal fluid than in the serum and that the level in the cerebrospinal fluid was far higher than that in the serum. This difference may suggest that the circulating level of IL-6 is raised by the transfer of IL-6 from the inflammatory site where it is produced. In the present study, IL-6 levels were elevated both in serum and colonic tissue in patients with active IBD, suggesting a linkage between IL-6 in the circulation and that at the inflammatory site. The

occurrence of transcription of the gene for IL-6 at high levels in the spleen, liver, kidney, and peripheral blood leucocytes of normal individuals has been reported recently[36]. In addition, enhanced IL-6 production has been observed in cultured peripheral blood mononuclear cells from patients with alcoholic liver cirrhosis[22]. Although these findings cannot be linked directly to changes in IL-6 production in the body, tissues, other than inflammatory sites, may also produce IL-6. Consequently, it remains to be clarified yet whether IL-6 in the circulation arises solely from the tissue involved in inflammation.

Hirano[37] reported that the mechanism of regulating the expression of the gene for IL-6 may be impaired by unknown causes, leading to persistent expression of the gene and to a subsequent chronic inflammatory process. In addition, as a result of interaction among cytokines including IL-6, the individual activities are either enhanced or suppressed to control biologic reactions in the body[2]. If this system become disordered due to some causes, it may induce another disorder, triggering off a series of events leading to persistence of inflammation. These possible mechanisms may also contribute to the chronicity of the inflammatory process in IBD.

It is therefore concluded that IL-6 is an available marker to assess disease conditions of IBD and that it also might be involved in the pathophysiology of IBD. If the mechanism of the IL-6 gene expression and the cytokine network are elucidated in future, the findings will provide new insights into the mechanisms of the pathogenesis of IBD.

The authors would like to thank Associate Prof. Atsushi Toyonaga and Dr. Ei Sasaki for their advice relating to this work. The authors also thank Prof. Tadamitsu Kishimoto and Dr. Tadashi Matsuda, Institute for Molecular and Cellular Biology, Osaka University, for their kind gift of anti-IL-6 antibody, and Mr. Naoki Takanashi and Mrs. Taeko Morimoto, SRL.Inc., for their invaluable cooperation.

References

1. Kishimoto T: The biology of interleukin 6. Blood 1989;74:1-10
2. Le JM, Vilcek J: Interleukin 6. A multifunctional cytokine regulating immune reactions and the acute phase protein response. Lab Invest 1989;61:588-602
3. Horii Y, Muraguchi A, Suematsu S, et al: Regulation of BSF-2/IL-6 production by human mononuclear cells. J Immunol 1988;141:1529-1535
4. Andus T, Geiger T, Hirano T, et al: Recombinant human B cell stimulatory factor 2(BSF-2/IFN- β_2) regulates β-fibrinogen and albumin mRNA levels in Fao-9 cells. FEBS Lett 1987;221:18-22
5. Castell JV, Gomez-Lechon Mj, David M, et al: Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulated the synthesis of acute phase proteins in human hepatocytes. FEBS Lett 1988;232:347-350
6. Geiger T, Andus T, Klapproth J, et al: Induction for rat acute-phase proteins by interleukin-6 in vivo. Eur J Immunol 1988;18:717-721
7. Muraguchi A, Hirano T, Tang B, et al: The essential role of B-cell stimulatory factor 2(BSF-2/IL-6) for the terminal differentiation of B cells. J Exp Med 1988;167:332-344
8. Takatsuki F, Okano A, Suzuki C, et al: Human recombinant interleukin 6/B cell stimulatory factor 2(IL-6/BSF-2) augments murine antigen-specific antibody responses in vitro and in vivo. J Immunol 1988;141:3072-3077
9. Lotz M, Jirik F, Kabouridis R, et al: BSF-2/IL-6 is costimulant for human thymocytes and T lymphocytes. J Exp Med 1988;167:1253-1258
10. Helle M, Brakenhoff JPJ, De Groot ER, et al: Interleukin 6 is involved in interleukin 1-induced activities. Eur J Immunol 1988;18:957-959
11. Uyttenhove C, Coulie PG, Van Snick J: T cell growth and differentiation induced by interleukin-HP1/IL-6, the murine hybridoma/plasmacytoma growth factor. J Exp Med 1988;167:1417-1427
12. Le J, Fredrickson G, Reis LFL, et al: Interleukin 2-dependent and interleukin 2-independent pathways of regulation of thymocyte function by interleukin 6. Proc Natl Acad Sci USA 1988;85:8643-8647
13. Ceuppens JL, Baroja ML, Lorre K, et al: Human T cell activation with phytohemagglutinin. The function of IL-6 as an accessory signal. J Immunol 1988;141:3868-3874
14. Ishibashi T, Kimura H, Uchida T, et al: Human inteleukin 6 is a direct promoter of maturation of megakaryocytes in vitro: Proc Natl Acad Sci USA 1989;86:5953-5957
15. Ishibashi T, Kimura H, Shikawa Y, et al: Interleukin-6 is a potent thrombopietic factor in vivo in mice. Blood 1989;74:1241-1244
16. Hirano T, Taga T, Yasukawa K, et al: Human B-cell differentiation factor defined by an anti-peptide antibody and its possible role in autoantibody production. Proc Natl Acad Sci USA 1987;84:228-231
17. Yoshizaki K, Matsuda T, Nishimoto N, et al: Pathogenic significance of interleukin-6(IL-6/BSF-2) in Castleman's disease. Blood 1989;74:1360-1367
18. Hirano T, Matsuda T, Turner M, et al: Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. Eur J Immunol 1988;18:1797-1801
19. Houssiau FA, Devogelaer JP, Damme JV, et al: Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides. Arth Rheum 1988;31:784-788
20. Horii Y, Muraguchi A, Iwano M, et al: Involvement of IL-6 in

mesangial proliferative glomerulonephritis. J Immunol 1989;143:3949-3955

21. Ueno Y, Takano N, Kanegane H, et al: The acute phase nature of interleukin 6. Studies in Kawasaki disease and other febrile illnesses. Clin Exp Immunol 1989;76:337-342
22. Deviere J, Content J, Denys C, et al: High interleukin-6 serum levels and increased production by leucocytes in alcoholic liver cirrhosis. Correlation with IgA serum levels and lymphokines production. Clin Exp Immunol 1989;77:221-225
23. MacDermott RP, Stenson WF: Inflammatory bowel disease. In: Targan SR, Shanahan F, eds. Immunology and Immunopathology of the Liver and Gastrointestinal Tract, 1st Ed. Igaku-Shoin, New York · Tokyo. 1990;459-486
24. Brandtzaeg P, Halstensen TS, Kett K, et al: Immunobiology and immunopathology of human gut mucosa. Humoral immunity and intraepithelial lymphocytes. Gastroenterology 1989;97: 1562-1584
25. Truelove SC, Witts LJ: Cortisone in ulcerative colitis. Final report on a therapeutic trial. Br Med J 1955;2:1041-1048
26. Myren J, Bouchier IAD, Watkinson G, et al: The OMGE multinational inflammatory bowel disease survey 1976-1982. A further report on 2657 cases. Scand J Gastroenterol 1984;19 (suppl.95):1-27
27. Breen EC, Rezai AR, Nakajima K, et al: Infection with HIV is associated with elevated IL-6 levels and production. J Immunol 1990;144:480-484
28. Miki S. Iwano M, Miki Y, et al: Interleukin-6(IL-6) functions as an in vitro autocrine growth factor in renal cell carcinomas. FEBS Lett 1989;250:607-610
29. Rachmilewitz D, Simon PL, Schwartz L, et al: Inflammatory mediators of experimental colitis in rats. Gastroenterology 1989;97:326-337
30. Kishimoto T, Hirano T: Molecular regulation of B lymphocyte response. Ann Rev Immunol 1988;6:485-512
31. Nishimoto N, Yoshizaki K, Tagoh H, et al: Elevation of serum interleukin 6 prior to acute phase proteins on the inflammation by surgical operation. Clin Immunol Immunopathol 1989;50: 399-401
32. Nijisten MWN, De Groot ER, Ten Duis HJ, et al: Serum levels of interleukin-6 and acute phase responses. Lancet 1987;2:921
33. Houssiau FA, Bukasa K, Sindic CJM, et al: Elevated levels of the 26K human hybridoma growth factor (interleukin 6) in cerebrospinal fluid of patients with acute infection of the central nervous system. Clin Exp Immunol 1988;71:320-323
34. Brandtzaeg P, Baklien K, Fausa O, et al: Immunohistochemical characterization of local immunoglobulin formation in ulcerative colitis. Gastroenterology 1974;66:1123-1136
35. Frei K, Leist TP, Meager A, et al: Production of B cell stimulatory factor-2 and interferon γ in the central nervous system during viral meningitis and encephalitis. Evaluation in a murine model infection and in patients. J Exp Med 1988;168:449-453
36. Tovey MG, Content J, Gresser I, et al: Genes for IFN-β_2 (IL-6), tumor necrosis factor, and IL-1 are expressed at high levels in organs of normal individuals. J Immunol 1988;141:3106-3110
37. Hirano T: Metabolism and Disease 1988;25(suppl.):135-143 (in Japanese)