Correlations between Interleukin-8, and Myeloperoxidase or Luminol-Dependent Chemiluminescence in Inflamed Mucosa of Ulcerative Colitis

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Interleukin-8 (IL-8) is a peptide which induces not only chemotaxis of neutrophils but also the release of reactive oxygen metabolites from the neutrophils. There are few reports which clarify the relationships between IL-8 and mucosal infiltration of neutrophils or reactive oxygen metabolites produced by neutrophils in the colonic mucosa of ulcerative colitis (UC). Biopsy specimens of colonic mucosa obtained from 26 patients with active UC and 21 patients with inactive UC were studied in order to clarify the relationships among the inflammation factors in UC. Levels of IL-8 and myeloperoxidase in organ culture media of the biopsy specimens from active UC (measured by ELISA and EIA) were significantly higher than those from inactive UC and controls. Reactive oxygen metabolites of biopsy specimens in active UC (measured by luminol-dependent chemiluminescence) were also markedly increased compared to those in inactive UC and controls. The levels of IL-8 were closely correlated to luminol-dependent chemiluminescence or myeloperoxidase levels. However, the levels of IL-8 and myeloperoxidase did not correlate with the grades of activity on colonoendoscopic findings. These findings suggest that IL-8 may play a role in the pathophysiology of UC but it does not define the endoscopic activity grades of UC.

Key words: inflammatory bowel disease, interleukin (IL)-8, free radical, neutrophil

Introduction

The main role of polymorphonuclear leukocytes (neutrophils) in inflammatory and immune responses had long been thought to be only phagocytosis and killing of bacteria and they were thought to be terminally differentiated cells. However, recent studies have shown that neutrophils, when activated, release tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), the interleukin-1 receptor antagonist (IL-1ra), interleukin-8 (IL-8), transforming growth factor β (TGFβ) and reactive oxygen metabolites (1). Although the etiology of ulcerative colitis (UC) has not yet been established, numerous immunological substances, such as IL-1β, IL-6, IL-8 and TNFα, and vasoactive and chemotactic substances, such as platelet activating factor (PAF) and leukotriene B4, have been found to be markedly increased in the inflamed mucosa of active UC (2–4). IL-8, PAF and leukotriene B4 may induce infiltration of neutrophils into the inflamed mucosa.

On the other hand, neutrophil-derived reactive oxygen metabolites have been highlighted in various inflammatory diseases (5, 6) and in carcinogen induction (7). Myeloperoxidase is involved in the regulation of the respiratory burst of human activated neutrophils and these oxidants are thought to be toxic to tissues (8). Infiltration of numerous neutrophils in the colonic mucosa is not specific but characteristic of activity of UC, suggesting acute on chronic inflammation. The histopathological features of the colonic mucosa in UC may somewhat resemble those of chronic active gastritis induced by Helicobacter pylori (9). To understand the endoscopic features and pathophysiology in acute on chronic inflammation, it seems very important to clarify the relationship among production of IL-8, mucosal infiltration of neutrophils and reactive oxygen metabolites produced by neutrophils.

For editorial comment, see also p 229.
Materials and Methods

Patients with UC

Forty-seven patients with UC (22 males and 25 females; median age, 36.6 years, range 18–75) were entered in this study. The diagnosis of UC was based on clinical, radiological, endoscopic and histological findings. Disease activity was assessed by clinical symptoms such as rectal bleeding, fever, tachycardia and so on, and by laboratory findings (anemia, sedimentation rate, C reactive protein and so on) and endoscopic mucosal appearance (10). 26 patients were active and 21 inactive. Seventeen patients had entire colitis, 24 left-sided colitis and 6 proctitis. The disease duration from the first diagnosis ranged from 1 day to 9 years and 2 months (median, 2 years and 4 months). At the time of study, 3 of 26 active patients were on corticosteroids only, 11 patients on sulphasalazine only, 6 patients on corticosteroids and sulphasalazine, and 6 patients without therapy. On the other hand, 1 of 21 inactive patients was on corticosteroids only, 15 patients on sulphasalazine only, and 5 patients on sulphasalazine and corticosteroids.

Mucosal biopsy specimens were obtained from the distal sigmoid colon of patients with UC and 15 control subjects undergoing colonoscopy for a surveillance of colonic polyp or cancer. Informed consent was obtained from each patient of UC and controls.

Tissues

We used at least four biopsy specimens obtained at endoscopic examinations. Tissues were washed in CMRL 1066 (Gibco, Rockville, MD) solutions containing penicillin G (100 U/ml) and streptomycin sulfate (100 μg/ml) three times, placed on plastic culture dishes (Falcon, Lincoln Park, NJ) containing culture medium consisting of 1.0 ml CMRL 1066 solutions containing 10% fetal bovine serum (FBS; Nipro, Osaka), penicillin G (100 U/ml) and streptomycin sulfate (100 μg/ml). The culture dishes were placed in an incubator at 37°C for 24 hours gassed with a mixture of 5% CO₂ and 95% air (11, 12). After that, cultured tissues were centrifuged at 10,000 g for 10 minutes, and the supernatant was stored at –80°C until further analysis.

There was no obvious histological evidence of tissue damage due to process of organ culture in our study like other workers’ study (13). To measure IL-8 peptide levels in the mucosa, supernatants of mucosal organ culture were used. As IL-8 is produced from various cells such as macrophages, neutrophils, vascular endothelial cells and intestinal epithelial cells, levels of IL-8 released in the medium of organ culture were measured in this study. Levels of myeloperoxidase released in the medium of organ culture of mucosal biopsy specimens were determined as one of the indices for inflammation. Myeloperoxidase is an enzyme found predominantly in the azurophilic granules of polymorphonuclear leukocytes (neutrophils) and has been used as a quantitative index of mucosal inflammation (14). Myeloperoxidase activity is a useful but limited index for the assessment of mucosal inflammation of neutrophils because it is inhibited by 5-amino salicylic acid, one of the metabolites of sulphasalazine, and corticosteroids which are used in many patients with UC (15).

Measurement of IL-8 and myeloperoxidase

IL-8 peptide was determined by a solid phase double ligand enzyme-linked immunosorbent assay (ELISA) kit (Amersham, Buckinghamshire, England) and myeloperoxidase was measured by an enzyme immunoassay (EIA) kit (BIOXYTECH, Marne, France). And the absorbance of reaction was determined by using a microplate reader (Titertek; Flow Laboratories, Scotland). Both mucosal levels of IL-8 and myeloperoxidase measured in the culture supernatants were converted to be expressed as per 1 mg dry weight of specimen. After the end of experiment, the samples were blot-dried and weighed. In addition, the minimum detectable levels of kit used this time was 4.7 pg/ml by IL-8 ELISA kit, and was 1.5 ng/ml by myeloperoxidase EIA kit.

Measurement of luminol-dependent chemiluminescence (L-CL)

Another biopsy specimens obtained serially at endoscopic examinations were washed immediately in 0.01M phosphate-buffered saline (pH 7.4). The tissue was then put into scintillation test tubes containing 0.1 ml of CMRL 1,066 solutions. After an addition of 0.02 mg luminol (Sigma, St. Louis, MO) and 0.1 μg phorbol myristate acetate (PMA; Sigma, St. Louis, MO) to the test tubes, L-CL values were determined on a CL analyzer (Lumat LB9501; Berthold, Germany). L-CL values were expressed as counts/s/mg dry weight of specimen after subtraction of background as described previously. Grades of mucosal activity of colonooendoscopic findings in UC were assessed according to Marts’ classification of endoscopy (16). Briefly, grade 1 is normal, grade 2 mild granularity of the mucosa, with mild contact bleeding, grade 3 marked granularity and edema of the mucosa, contact bleeding and spontaneous bleeding and grade 4 severe ulceration of mucosa with hemorrhage. Endoscopic findings of higher than grade 2 were defined to be active.

Statistical Analysis

Data are expressed as the mean ± SD. Differences among groups were assessed using the Kruskal-Wallis rank test and differences between groups by the Scheffe’s F test. p values of <0.05 for the differences between groups were considered significant. Correlation between levels of myeloperoxidase and levels of IL-8 or luminol-dependent chemiluminescence values was assessed using Spearman’s rank correlation test.

Results

Levels of IL-8 in the mucosa

Levels of IL-8 in the mucosa were significantly higher in patients with active UC (n=23; 36.0 ± 14.6 pg/mg) than in patients with inactive UC (n=21; 5.3 ± 6.2 pg/mg, p<0.01) and
in control subjects (n=15; 3.2 ± 3.8 pg/mg, p<0.01) (Fig. 1). Levels of IL-8 in the mucosa from patients with active UC (n=23) had no significant differences among the three groups of grade 2 (n=3), grade 3 (n=13), and grade 4 (n=7) when they were compared for Matts’ classification of endoscopy (Fig. 2).

**Levels of myeloperoxidase in the mucosa**

Levels of myeloperoxidase in the mucosa were significantly higher in patients with active UC (n=23; 54.6 ± 27.6 ng/mg) than in patients with inactive UC (n=21; 12.2 ± 13.4 ng/mg, p<0.01) and in control subjects (n=15; 0.13 ± 0.52 ng/mg, p<0.01) (Fig. 3). Levels of myeloperoxidase in the mucosa from patients with active UC (n=23) had no significant differences among three groups of grade 2 (n=3), grade 3 (n=13), and grade 4 (n=7) when they were compared for Matts’ classification of endoscopy (Fig. 4).

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**Figure 1.** Levels of IL-8 in the mucosa from patients with ulcerative colitis and control subjects. Bars represent the mean ± SD.

**Figure 2.** Levels of IL-8 in the mucosa from patients with active ulcerative colitis (n=23) by Matts’ classification of endoscopy. Bars represent the mean ± SD.

**Figure 3.** Levels of myeloperoxidase in the mucosa from patients with ulcerative colitis and control subjects. Bars represent the mean ± SD.

**Figure 4.** Levels of myeloperoxidase in the mucosa from patients with active ulcerative colitis (n=23) by Matts’ classification of endoscopy. Bars represent the mean ± SD.
L-CL values in the mucosa

L-CL values in the mucosa were significantly higher in patients with active UC (n=13; 3,601.1 ± 1,372.2 counts/s/mg) than in patients with inactive UC (n=11; 1,052.6 ± 782.9 counts/s/mg, p<0.01) and in control subjects (n=11; 166.2 ± 111.2 counts/s/mg, p<0.01) (Fig. 5). L-CL values in the mucosa from patients with active UC (n=13) had no significant differences among the three groups of grade 2 (n=2), grade 3 (n=6), and grade 4 (n=5) when they were compared for Mats’ classification of endoscopy (data not shown).

In addition, levels of IL-8, levels of myeloperoxidase and L-CL values in the mucosa from patients with active UC (n=26) had no significant differences between the non-medication group (n=6) and medication group (n=20) (data not shown).

Correlation among levels of IL-8 and myeloperoxidase and L-CL values

Levels of myeloperoxidase were positively correlated with levels of IL-8 in patients with UC (n=44; Spearman’s ρ=0.97, p<0.0001) (Fig. 6).

L-CL values were positively correlated with levels of myeloperoxidase in patients with UC (n=15; Spearman’s ρ=0.89, p=0.0009) (Fig. 7).

Discussion

UC is a chronic disease of unknown etiology affecting the mucosa of the large intestine. This disease is thought to usually begin in the rectum and extends to involve all or part of the remaining colon. The histopathological features of UC are characterized by two major features (17). One is architectural distortion of colonic crypt with frequent depletion of goblet cell mucin and the other is diffuse inflammation with mucosal infiltration of plasma cells, lymphocytes, histiocytes, eosinophils and neutrophils. In the active stage of UC, aggregates of neutrophils near and invading the crypt epithelium form crypt abscesses and massive infiltration of neutrophils in the colonic lamina propria is observed.
Neutrophils are the first cells that migrate into tissues in response to invading pathogens. Therefore, their main role in inflammatory and immune responses has been thought to be the phagocytosis and killing of bacteria through reactive oxygen metabolites and the release of lytic enzymes stored in granules (18).

Reactive oxygen metabolites (ROMs) are involved in inflammatory diseases and are reported to injure the intestinal epithelial cells in vitro (19). The hydroxyl radical is more injurious for cellular injury of the intestinal epithelium than superoxide and hydrogen peroxide. Neutrophils have been shown to produce hypochlorous acid which is a myeloperoxidase-derived product. Many studies on UC and Crohn’s disease have shown that excessive ROMs are generated by peripheral blood leukocytes and inflamed colonic mucosa (20-22). These ROMs were produced by monocytes and neutrophils. Simmonds et al have proposed that neutrophil-derived oxidants (superoxide, hydrogen peroxide, hydroxyl radical and hypochloride) are generated in the colorectal mucosa of active inflammatory bowel disease and that luminol-dependent chemiluminescence is correlated with microscopic inflammation (23).

Our results showed that luminol-dependent chemiluminescence in active UC mucosa was higher than that in inactive UC mucosa and controls, but it did not correlate with activity grades of colonoendoscopic findings. The addition of sodium azide, a myeloperoxidase inhibitor, to the media of inflamed mucosal scrapings significantly decreased their chemiluminescence, suggesting that most of the chemiluminescence is derived from the myeloperoxidase system (23, 24).

Myeloperoxidase activity is derived primarily from neutrophils and partly from monocytes well correlated with acute intestinal inflammation (24).

Therefore, myeloperoxidase activity is used as one of the indicators of neutrophil infiltration. As myeloperoxidase is a stable substance and an enzymatic protein which is found in polymorphonuclear leukocytes, we measured the myeloperoxidase levels in the media of organ cultures of mucosal biopsy specimens by using an EIA method in place of myeloperoxidase activity, because myeloperoxidase activity is partly inhibited by 5-aminosalicylic acid and corticosteroids as mentioned in Methods. Measurement of the extracellular myeloperoxidase concentration can be used to monitor polymorphonuclear leukocyte activation, because myeloperoxidase is released into the extracellular medium as a consequence of polymorphonuclear leukocyte activation (8, 25).

Luminol-dependent chemiluminescence was generated near and on the plasma membrane of neutrophils and spread out into the extracellular space of tissues (26). Therefore, it may be reasonable to measure the levels of myeloperoxidase released from neutrophils in the medium for an assessment of mucosal infiltration by neutrophils. The mechanism of how neutrophils infiltrate the colonic mucosa of patients with UC is important to the understanding of the pathogenesis of UC. IL-8 is one of the chemotactic substances against leukocytes including complement C5a, leukotriene B4, PAF and formyl-met-leu-phe (fMLP).

IL-8 is produced by many cells such as monocytes, macrophages, neutrophils, lymphocytes, fibroblasts, vascular endothelial cells, hepatocytes and tumor cell lines (27). In UC, IL-8 mRNA was found mainly in macrophages, and also in neutrophils and colonic epithelial cells using in situ hybridization with IL-8 antisense RNA probes (28). Increased production of IL-8 peptide and expression of IL-8 mRNA is observed in the inflamed mucosa of patients with UC (29, 30).

The present results also supported an increased production of IL-8 in the colonic mucosa of UC. Izzo et al reported that the increase in IL-8 levels measured by radioimmunoassay in UC patients is correlated with increased activity of myeloperoxidase in all regions of the colonic mucosa (31). The present results also supported that the increase in IL-8 levels measured by ELISA in UC mucosa well correlate with the increased levels of myeloperoxidase and also the increased chemiluminescence, but these IL-8 levels did not correlate with activity grades of colonoendoscopic findings. Since IL-8 is not only a chemokinetic substance but also a neutrophil-activating substance to release free radicals from neutrophils, our results suggest that most of the infiltrating neutrophils in the colonic mucosa of UC might be activated. However, there has been a report that in the colonic mucosa of patients with Crohn’s disease, the increased levels of IL-8 were not correlated with the levels of myeloperoxidase (32). However, it seems that levels of IL-8 in mucosal tissue homogenates from patients with Crohn’s disease were much lower than those from patients with UC (28).

The report by Raab et al showed that increased values of TNFα were correlated with those of IL-8 in the perfusion fluid from the sigmoid and rectum of patients with UC (33). An increased combination of the proinflammatory cytokines such as IL-1β, IL-6, IL-8 and TNFα may be very important for defining the mucosal inflammation of UC (34). However, the present results suggest that there may be factors defining mucosal endoscopic activity grades of UC other than neutrophils, because there are several papers discussing that MHC-restricted T cell cytotoxicity or antibody-dependent cytotoxicity via anticolon antibody (ADCC) may play a role in mucosal damage in some patients of UC (35, 36). Further study to clarify other factors defining the grade of endoscopic activity is needed.

References