Acta Med. Nagasaki 39 : 40–44

Eosinophil Activation in Ulcerative Colitis

Shinichiro KANZAKI, Kazuya MAKIYAMA, Minoru ITSUNO, Yoshiyuki YAMASAKI, and Hidetoshi ODA

Second Department of Internal Medicine

To evaluate the activity level of eosinophils in patients with ulcerative colitis (UC), we analyzed peripheral blood eosinophil counts, serum levels of eosinophil cationic protein (ECP) and the percentage of hypodense eosinophils among all eosinophils in peripheral blood. Patients with active UC had significantly higher serum ECP levels and eosinophil counts, compared with patients in remission and healthy controls. Serum ECP levels correlated positively with eosinophil counts. In patients who could be followed from the active stage of UC to remission, ECP decreased significantly over time and eventually reached approximately normal levels, while eosinophil counts showed no significant change. The mean percentage of hypodense eosinophils (specific gravity < 1.082 g/ml) in peripheral blood was 5 % or less in patients in remission and healthy controls, but it was 31.7 % in patients with active UC.

These results suggest that eosinophils are activated in patients with UC, and that they are involved in injury to the colorectal mucosa by inducing the release of cytotoxic proteins such as ECP.

Key words : ulcerative colitis, eosinophil cationic protein, hypodense eosinophil

Introduction

The colorectal mucosa of patients with ulcerative colitis (UC) shows infiltration by various inflammatory cells, such as lymphocytes and plasma cells. Infiltration by neutrophils and eosinophils is especially marked in the mucosa affected by active UC[1),2)]. However, little is known about the roles of eosinophils in UC.

Regarding the roles of eosinophils in other diseases, eosinophils had been previously thought to control allergic inflammation by degrading histamine, leukotrienes and platelet activating factor (PAF) which are released following allergic responses and by englobing immune complexes in tissue. In the 1980's, however, Gleich et al.[3),4)] reported that marked eosinophil infiltration of the airway mucosa was noted in patients who died of asthma, that large amounts of major basic protein (MBP), a basic protein contained in some granules of eosinophils, had deposited on the airway mucosa of these patients, and that the MBP was found in vitro to be highly cytotoxic. This report and other reports published thereafter revealed that infiltrating eosinophils serve as effector cells that induce inflammation.

We speculate that eosinophils are proinflammatory cells in UC. The present study was undertaken to evaluate the activity level of peripheral blood eosinophils in patients with UC. As an indirect index of eosinophil activity, we measured serum levels of eosinophil cationic protein (ECP), which is a granular protein found in eosinophils[5)] and known to be highly cytotoxic[6)]. As a direct index of eosinophil activity, the percentage of hypodense eosinophils was also measured

Subjects and Methods

1. Subjects

The subjects were 106 patients with UC who had been treated as out-or inpatients at the Second Department of Internal Medicine, Nagasaki University Hospital or at its related facilities. Eighteen healthy volunteers served as controls. Cases complicated by allergic disease such as bronchial asthma or atopic dermatitis were excluded from this study. Patients who had been using steroids and/or immunosuppressors at the start of this study were also excluded, to avoid the influence of these drugs on the study. Of the 106 UC patients, 10 could be followed from the active stage of the disease to remission. A total of 116 samples (36 samples collected in the active stage and 80 samples during remission) were examined.

For 10 patients with UC (4 in the active stage and 6 in remission) and 4 healthy controls who consented to collection of 50 ml blood, the percentage of hypodense eosinophils among all eosinophils in peripheral blood was determined.

2. Methods

1) *Measurement of serum ECP*

Serum was separated from blood samples 30 mins after the samples were collected. The serum was then frozen at −80 ℃ until the ECP level was measured with an ECP RIA kit (Pharmacia, Uppsala, Sweden), according to Venge et al. 's method[7)].

2) *Percentage of hypodense eosinophils*

The distribution of the specific gravity of peripheral blood eosinophils was obtained in the following steps, using Yoshikuni's method of discontinuous Percoll density gradient centrifugation[8].

Heparinized peripheral blood was left standing for 20 min after one-fourth its volume of 6% dextran-containing saline was added to it. The leukocyte-suspended fraction of this solution was layered onto a Ficoll-Conray, followed by 30-min centrifugation at 500× g. The plasma cell, lymphocyte and monocyte layers were discarded. The polymorphonuclear leukocytes (PMN) fraction was combined with 0.87% NH_4Cl solution for hemolysis. The mixture was then centrifuged, and the resulting pellets were washed in FCS-containing PIPES buffer.

A Percoll was diluted with PIPES buffer to yield four solutions with different specific gravities (1.100, 1.090, 1.085 and 1.080 g/ml). Each of these solutions (3 ml) were layered sequentially, using a peristaltic pump. PMN were then suspended in 2 ml of an FCS-containing solution (1.070 g/ml). This suspension was layered onto the discontinuous Percoll gradient. After 20-min centrifugation at 400×g, 1 ml of each fraction was collected, using a peristaltic pump.

The specific gravity of each fraction was determined by applying the refractive index of each fraction to the reference gravity-refraction index curve for Percoll solutions. Furthermore, the percentage of eosinophils was calculated based on the granulocyte counts of each fraction and the responses to May- Giemsa staining.

An eosinophil specific gravity distribution curve was obtained from the data concerning the percentage of each fraction's eosinophil count among all eosinophils. Eosinophils with a specific gravity below 1.082 g/ml were regarded as hypodense eosinophils, and their percentage among all eosinophils in peripheral blood was calculated.

3) *Statistical analysis*

The values of each parameter were expressed as mean ± SD. The significance of differences in averages was tested, using Student's t-test. $p < 0.05$ was regarded as significant.

Results

1. *Serum ECP level and eosinophil counts*

The serum ECP level for patients with active UC (27.5 ± 23.1 μg/L) was significantly higher than that for patients in remission (8.4±7.3 μg/L) and healthy controls (6.2±3.1 μg/L) ($p < 0.01$) (Figure 1).

The peripheral blood eosinophil counts for patients with active UC (362±268/mm³) was also significantly higher than that for patients in remission (136±113/mm³) ($p <$

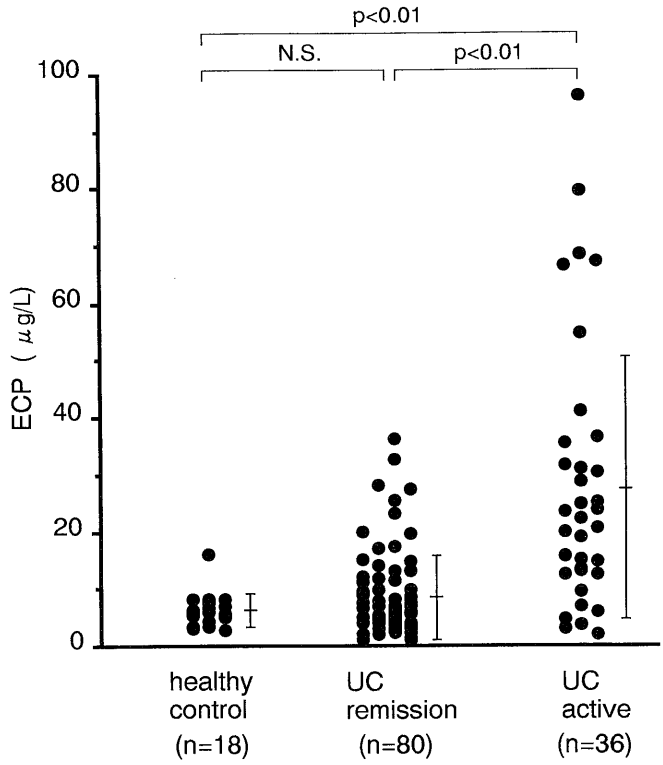

Figure 1. Serum ECP levels in patients with active UC, in remission and healthy controls.

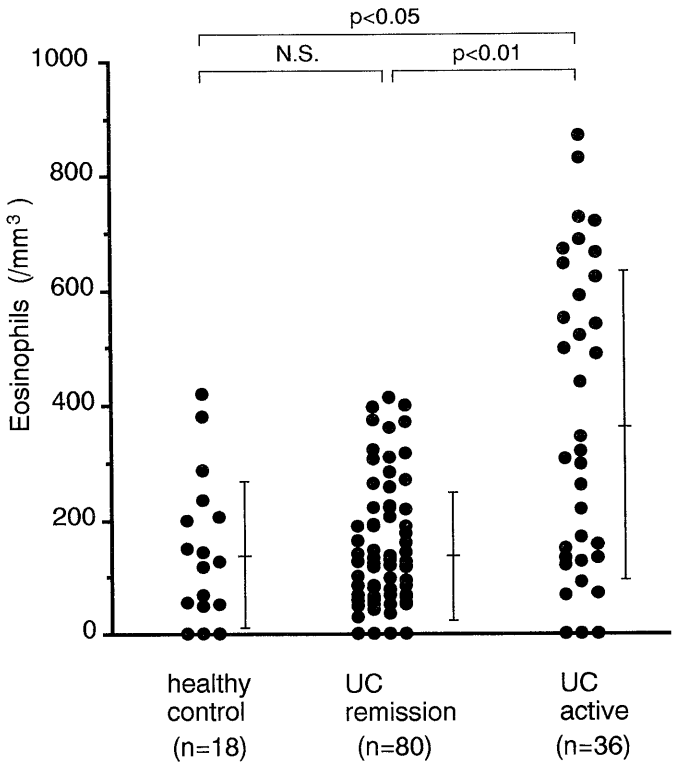

Figure 2. Peripheral blood eosinophil counts in patients with active UC, in remission and healthy controls.

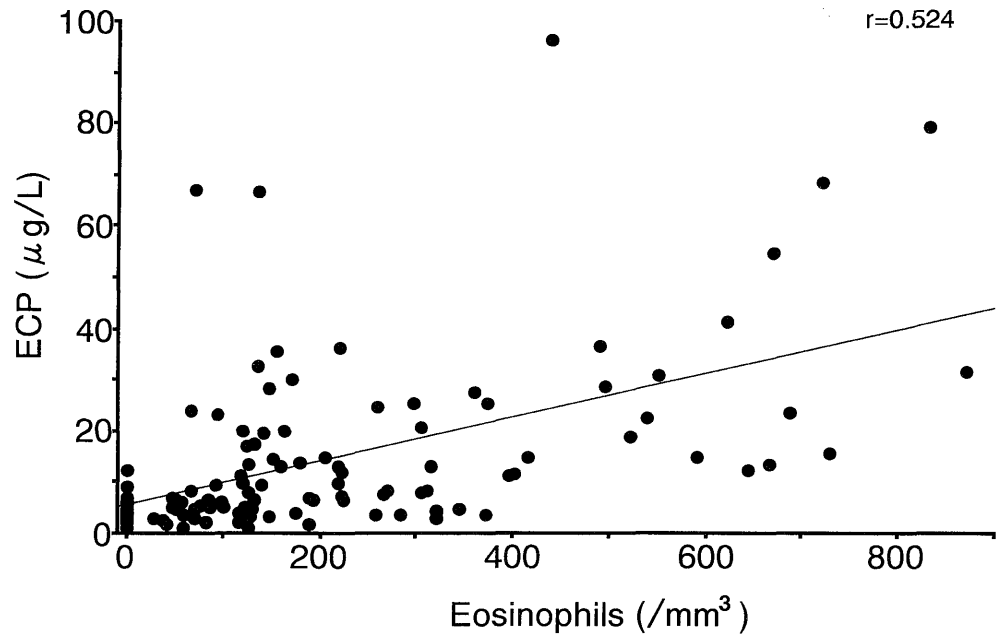

Figure 3. Correlation between serum ECP levels and peripheral blood eosinophil counts in UC patients (n = 116).

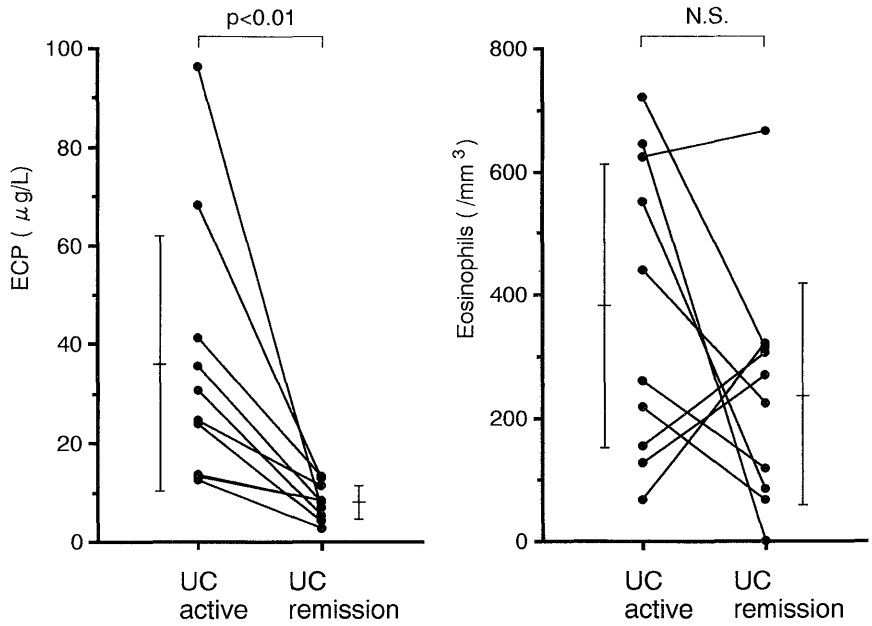

Figure 4. Individual changes of ECP and peripheral blood eosinophil counts from active stage to remission in UC patients (n = 10).

0.01) and healthy controls (138 ± 129/mm³) ($p < 0.05$) (Figure 2.).

In patients with UC, serum ECP levels correlated positively with peripheral blood eosinophil counts (r = 0.524) (Figure. 3).

2. Individual changes of ECP and eosionphil counts from active stage to remission

In the 10 patients who could be followed to remission, the serum ECP level, which was 36.0 ± 25.7 μg/L in the active stage, decreased significantly to an approximately normal level (8.1±3.4 μg/L) during remission ($p < 0.01$).

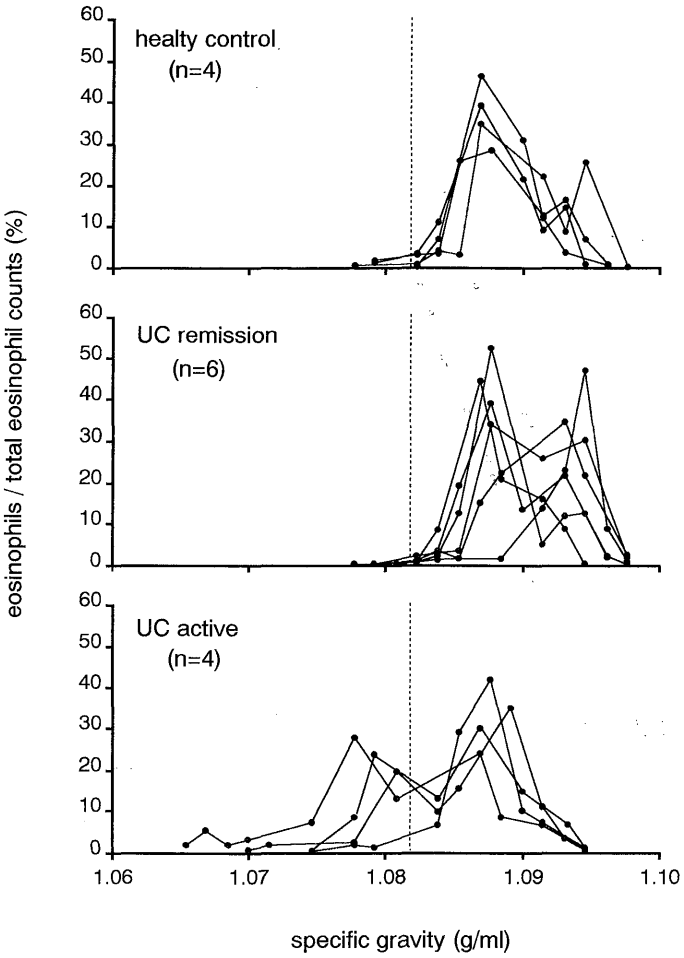

Figure 5. Eosinophil specific gravity distribution curve in patients with active UC (n = 4), in remission (n = 6) and healthy controls (n = 4). The percentage of hypodense eosinophils (specific gravity<1.082 g/ml) increases in the active stage of UC.

In terms of eosinophil counts, however, there was no significant difference between the active stage and remission of these 10 patients (Figure 4).

3. *Percentage of hypodense eosinophils*

The percentage of hypodense eosinophils in peripheral blood was significantly higher in patients with active UC (31.7±21.7 %) than in patients in remission (2.1±2.2 %) ($p < 0.01$) and healthy controls (1.8±1.1 %) ($p < 0.05$) as shown in Figure 5.

Discussion

Because marked infiltration by eosinophils is often noted in the colorectal mucosa of UC patients, we have been interested in the possible roles of eosinophils in UC, especially the possible cytotoxicity of eosinophils. When activated, eosinophils undergo degranulation and serve as effector cells by releasing ECP and MBP[9]. In the present study, we measured serum ECP levels as an index of eosinophil activation, and analyzed the relationship between serum ECP levels and UC activity levels. Serum ECP levels and peripheral blood eosinophil counts in patients with active UC were significantly higher than those for patients in remission and healthy controls. When changes in these parameters in individual subjects were analyzed, the serum ECP level correlated better with the activity level of UC than did the eosinophil count.

The distribution of the specific gravity of eosinophils is known to be heterogenous[10]. Hypodense eosinophils with a specific gravity below 1.082 g/ml are 'activated eosinophils'. Compared with normodense eosinophils with a specific gravity over 1.082 g/ml, hypodense eosinophils show enhanced functions as proinflammatory cells, e. g., increased expression of cell surface receptors[11], increased production of LTC_4 [12] and enhanced chemotaxis and cytotoxicity[13]. Patients with bronchial asthma or atopic dermatitis have been reported to show an increase in hypodense eosinophils[14].

In the present study, the percentage of hypodense eosinophils in peripheral blood was analyzed as a direct index of eosinophil activation in peripheral blood. This analysis revealed an increase of hypodense eosinophils during the active stage of UC. This supports the view that eosinophils are activated in UC patients.

Berstad et al.[15], who examined feces instead of peripheral blood, reported that fecal ECP levels were significantly elevated in patients with UC and Crohn's disease. As we previously reported, a significant increase of eosinophils was also noted in the colorectal mucosa of patients in the active stage of UC[16]. We also reported that, the secreted type ECP, which reacts with EG_2 antibody, was immunohistochemically positive in the lamina propria of the colorectal mucosa[17]. EG_2 antibody is a monoclonal antibody which Tai et al[18]. prepared by immunizing mice with the supernatant of the cultures of zymosan-C3b-stimulated eosinophils. This antibody does not react with the stored type ECP of normal eosinophils. Furthermore, by electron microscopy, we found that the eosinophils invading the colorectal mucosa of patients with active UC showed varying degrees of degranulation (unpublished data). Taking these findings together, we speculate that eosinophils are activated in patients with UC, and involved in injury to the colorectal mucosa through thier release of ECP.

Although the causes of UC remains unknown, it has been widely accepted that abnormal local immune responses in the mucosa are involved in the etiology of this disease. It is likely that the colorectal mucosa of humans with UC contains activated T cells, which produce ECF and IL-5, resulting in chemotaxis of eosinophils. It is also likely that GM-CSF, IL-3 and IL-5 activate eosinophils, resulting in degranulation of eosinophils and the release of ECP from eosinophils. Since we found increased LTB_4 in the colorectal mucosa of UC patients (unpublished data), we speculate that the LTB_4 and PAF, which are produced by mast cells, are also involved in the chemotaxis and activation of eosinophils.

In conclusion, the colorectal mucosa of UC patients shows infiltration by various inflammatory cells, and that injury to the colorectal mucosa due to activation of eosinophils and the release of ECP from activated eosinophils is closely associated with UC.

ACKNOWLEDGMENTS

We wish to thank Prof. K. Hara for his marvelous advice, and Mr. M. Harada for his technical assistance.

We are also grateful to Prof. Y. Tsuji of the Department of Pediatrics for his kind support.

References

1) Truelove SC, WCD Richards. Biopsy studies in ulcerative colitis. Brit Med J. 1: 1315-1319 (1956)
2) Lumb G. Pathology of ulcerative colitis. Gastroenterology. 40: 290-298 (1961)
3) Filley WV, Holley KE, Kephat GM, Gleich GJ. Identification by immunofluorescence of eosinophil granule major basic protein lung tissues of patient with bronchial asthma. Lancet. 2: 11-15 (1982)
4) Frigas E, Loegering DA, Gleich GJ. Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. Lab Invest. 76: 595-603 (1980)
5) Olsson I, Venge P, Spitznagel JK, Lehrer RJ. Arginine rich cationic proteins of human eosinophil granules: comparison of the constituents of eosinophilic and neutrophilic leukocytes. Lab Invest. 39: 493-500 (1977)
6) Motojima S, Frigas E, Loegering DA, Gleich GJ. Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium in vitro. Am Rev Respir Dis. 139: 801-805 (1989)
7) Venge P, Roxin IE, Olsson I. Radioimmunoassay of human eosinophil cationic proteins. Br J Haematol. 37: 331-335 (1977)
8) Yoshikuni H, Hayashi K, Tsuji Y. Isolation of human peripheral blood eosinophils. Clin Immunol. 22: 22-27 (1990) (Japanese)
9) Kay AB. Eosinophils as effector cells in immunity and hypersensitivity disorders. Clin Exp Immunol. 62: 1-12 (1985)
10) Fukuda T, Gleich GJ. Heterogeneity of human eosinophils. J Allergy Clin Immunol. 83: 369-373 (1989)
11) Capron M. Eosinophils: Receptors and mediators in hypersensitivity. Clin Exp Allergy. 19: 3-8 (1989)
12) Kajita T, Yui Y, Mita H, Taniguti N, Saito H, Mishima T, Shida T. Release of leukotrien C4 from human eosinophils and its relation to the cell density. Int Arch Allergy Appl Immunol. 78: 406-410 (1985)
13) Prin L, Capron M, Tonnel AB, Bletry O, Capron A. Heterogeneity of human peripheralblood eosinophils. I. Variability in cell density and cytotoxic ability in relation to the level and the origin of hypereosinophilia. Int Arch Allergy Appl Immunol 72: 336-346 (1983)
14) Fukuda T, Donnette SL, Reed CE, Ackerman SJ, Peters MS, Gleich GJ. Increased numbers of hypodense eosinophils in the blood of patients with bronchial asthema. Am Rev Respir Dis. 132: 981-985 (1985)
15) Berstad A, Borkje B, Riedel B, Elsayed S. Increased fecal eosinophil cationic protein in inflammatory bowel disease. Hepato-Gastroenterol. 40: 276-278 (1993)
16) Senju M. Reaginic hypersensitivity in ulcerative colitis. Jpn J Gastroenterol. 85: 2168-2177 (1988) (Japanese)
17) Yamasaki K, Makiyama K, Iwanaga S. Eosinophil cationic protein in ulcerative colitis. Jpn J Gastroenterol. 85: 1723 (1988) (Japanese)
18) Tai PC, Spry CJF,Peterson C, Venge P, Olsson I. Monoclonal antibodies distinguish between storage and secreted forms of eosinophil cationic protein. Nature. 309: 265-275 (1984)