Studies on Lymphocyte Subpopulations in Human Colonic Biopsy Specimens by Colonoscopy

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Lymphocytes, isolated by the enzymatic technique, from colonic mucosal specimens obtained from eight patients with ulcerative colitis (UC), two with ischemic colitis, two with antibiotic-associated colitis, and 15 controls by colonoscopic biopsy were used to examine T and B cells. In control biopsy specimens, the mononuclear cell yield averaged $3.6 \pm 0.3$ (SEM) $\times 10^6$/g with a viability of $93 \pm 1\%$, while T and B cells expressed as a percentage of total lymphocyte counts were $65 \pm 1\%$ and $15 \pm 1\%$, respectively. T and B cells in the ascending, transverse, and sigmoid colon and rectum did not present any differences. In involved tissue at the active stage of UC, the mononuclear cell yield averaged $11.4 \pm 2.1 \times 10^6$/g, and T and B cells constituted $49 \pm 3\%$ and $43 \pm 4\%$, respectively. After treatment for two months with salazosulfapyridine, mononuclear cell yields in five cases of UC were significantly reduced to an average of $3.9 \pm 0.8 \times 10^6$/g as compared to the pretreatment level ($P < 0.01$). The percentage of T cells was significantly increased from $48 \pm 2\%$ to $62 \pm 2\%$ ($P < 0.01$), and that of B cells was significantly reduced from $39 \pm 2\%$ to $27 \pm 3\%$ ($P < 0.01$). These results revealed a significant difference in the subpopulations of mucosal lymphocytes in the involved sites in the active stage of UC as compared to normal controls, suggesting that immunological abnormalities may be implicated in the etiology of this disease and that disease remission on salazosulfapyridine treatment is associated with a correction of the colonic lymphocyte abnormalities.

The etiology of ulcerative colitis (UC) and Crohn's disease, here termed inflammatory bowel disease (IBD), is yet unknown. Recently, however, the involvement of some type of autoimmunity has been considered as the triggering mechanism. Hereafter, various immunological studies have been carried out using peripheral blood lymphocytes of patients with IBD (1–3), but it is doubtful whether peripheral blood lymphocytes properly reflect inflammatory responses in the involved sites of IBD. Recent papers have reported isolation of human colonic mucosal lymphocytes and identification of their subpopulations using surgical specimens (4–13). However, it is difficult to obtain an adequate amount of material for biopsy specimens of the human colon. Furthermore, as an effective isolation method of human colonic mucosal lymphocytes obtained through colonoscopic biopsies has not yet been established, there are few accurate reports on normal and diseased human colonic mucosal lymphocytes.

This report presents our preliminary studies on a method to isolate lymphocytes from the colonic mucosa.
mucosa, which is a modification of the enzymatic technique developed by Bull and Bookman (4, 5) and Crofton et al (6). It uses human colonic mucosal biopsy specimens obtained with a colonoscope and involves examination of subpopulations of human colonic mucosal lymphocytes in active and inactive UC.

**MATERIALS AND METHODS**

The colonic mucosal specimens were obtained from eight patients with UC (three males and five females, mean age 44 ± 7 years, range 24–77 years) in the active stage, two patients with ischemic colitis in the healing stage (two males, 50 and 56 years of age), and two patients with antibiotic (ampicillin) -associated colitis (two males, 62 and 47 years of age). The diagnosis of UC was based on the clinical picture and laboratory findings, including barium contrast radiographic studies of the small and large bowel, colonoscopy, and histological features of the biopsy. The extent of disease was determined by radiological and colonoscopic findings. Seven patients had left-sided colitis and one had total colitis.

Active and inactive UC was defined by clinical features including fever, abdominal pain, diarrhea, rectal bleeding, elevated erythrocyte sedimentation rate, and C-reactive protein, colonoscopic findings, and histological features of the biopsy when available. The UC patients were initially untreated. The diagnosis of ischemic colitis or antibiotic-associated colitis was based on the clinical history and radiological, colonoscopic, and histological features. Control specimens were obtained from five cases with surgically excised colonic mucosa (all colonic cancer, three males and two females, mean age 55 ± 3 years, range 52–67 years) and 15 biopsy cases (all colonic polyps, nine males and six females, mean age 52 ± 2 years, range 47–77 years). Each specimen was histopathologically normal colonic mucosa taken 20 cm from the involved site to a depth extending to the lamina propria.

In the eight cases of active UC, comparison was made at two different sites for involved tissue and noninvolved tissue which was at a distance of at least 20 cm orally and normal, both colonoscopically and histopathologically. In five of the eight cases of UC, examination of colonic mucosal lymphocytes was carried out twice, in the active stage before treatment and during remission after salazopyridine treatment consisting of 3–4 g daily for two months.

The surgically excised colonic mucosa was removed as promptly as possible and thoroughly washed in sterilized physiological saline solution. After separation by a scalpel, the tissue was sectioned and 100 mg was used for study. The amount of colonic mucosa biopsy material used was five or six pieces of tissue (mean weight of 35 mg) taken from one site under direct vision, followed immediately by thorough washing in sterilized physiological saline solution.

The isolation of human colonic mucosal lymphocytes was made by a modification of previously described methods (4–6). Briefly, the tissue was placed gently in Hanks' balanced salt solution (HBSS, Nissui Seiyaku Co., Ltd., Tokyo, Japan) with the pH adjusted to 7.0–7.2, and incubated for 15 min at 22° C in HBSS containing 1 mM dithiothreitol (DTT, Nakarai Chemicals, Ltd., Kyoto, Japan), after which it was washed again in HBSS. Each aliquot of 35 mg or 100 mg of tissue was placed in 10 ml of HBSS containing the appropriate concentrations of collagenase (0.5 mg/ml) (Sigma Chemical Co., St. Louis, Missouri) and hyaluronidase (1 mg/ml) (Sigma) in a 20-ml sterile glass universal container, which was left standing for 20 hr at 4° C. The tissue was then stirred gently for 30 min at 37° C. The lymphocyte suspension was filtered through a platinum sieve (150 mesh), centrifuged, and washed twice in phosphate-buffered saline (PBS). The lymphocytes were separated by Ficoll–Hypaque density gradient (Japan Immunoresearch Laboratories Co. Ltd., Tokyo, Japan) and resuspended in PBS. The lymphocytes of the peripheral blood were also separated by Ficoll–Hypaque density gradient. Viability was assessed by complete exclusion of 0.3% trypan blue (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Lymphocytes were counted using a Burker-Türk hemacytometer (Erma Optical Works Ltd., Tokyo, Japan).

The percentages of T and B cells were determined on a cell suspension of lymphocytes and macrophages. T and B cells were analyzed by the microtest plate method (14) (Japan Immunoresearch). One microliter of lymphocyte suspension in PBS, 1 × 10⁶ cells/ml, was added to the wells of plates using poly-L-lysine for T cells, B cells, and macrophages. The plate was allowed to stand for 15 min at room temperature. Four microliters of undiluted fetal calf serum (FCS) was added to the wells, after which they were allowed to stand for 30 min at room temperature. Then, for the demonstration of neuraminidase-treated sheep erythrocyte (E) -binding lymphocytes (T cells), 4 μl of E suspension in PBS was added to the wells, and for erythrocyte-antibody complement (EAC) -binding lymphocytes (B cells), the same volume of EAC suspension in PBS was added to the wells, followed by centrifugation at 100 g for 5 minutes. To remove E and EAC solution which had not reacted, the plate was inverted and allowed to stand in this position for 30 min and subsequently washed in PBS for 30 min.

Rosettes were counted using 0.01% brilliant cresyl blue–0.25% glutaraldehyde stain, and the percentage of rosette-forming lymphocytes was determined by counting 200 cells. Identification of T cells was carried out using E rosettes and B cells with EAC rosettes. The number of T, B, and null cells was expressed as the percentage of total lymphocyte counts. Null cells were estimated by the difference between 100% and the sum of T and B cells. Macrophages were defined by use of peroxidase stain and the percentage of macrophages was determined by counting 200 cells. The percentage of macrophages was calculated as follows:

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\text{macrophages} \times 100
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\text{lymphocytes + macrophages}
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Identification of T and B cells in the peripheral blood in all cases was carried out using E and EAC rosettes. All