Lamina Propria Mononuclear Cells Express and Respond to Interleukin-2 Differently in Crohn’s Disease and Ulcerative Colitis

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Lymphokine-activated killer (LAK) cell activity and messenger RNA (mRNA) expression for interleukin-2 (IL-2) were analyzed using lamina propria mononuclear cells (LPMC) in inflammatory bowel disease patients. Compared with control LPMC, Crohn’s disease (CD) LPMC exhibited significantly higher levels of LAK cell activity, whereas ulcerative colitis (UC) cells showed significantly lower levels of cytolytic activity with a difference in the frequency of CD3+, CD56+ and CD3+, CD56- LAK precursor cells. After incubation with IL-2, the proportion of CD3+, CD56+ lymphocytes continued to be higher in CD cultures and substantially lower in UC cultures. Freshly isolated CD LPMC exhibited significantly higher levels of IL-2 mRNA than controls. However, no significant difference was observed between UC and control cells. The level of IL-2 expression or responsiveness to IL-2 may be responsible for different mucosal immune reactivity between CD and UC patients.

Key words: lymphokine-activated killer cell activity, inflammatory bowel disease

Introduction

Substantial evidence indicates that underlying immunological mechanisms are important in the pathogenesis of inflammatory bowel disease (IBD). Thus, significant attention has focused on the investigation of local lymphocyte infiltrates in the intestinal mucosa (1, 2). There is increasing interest in the cytotoxic activity of lamina propria mononuclear cells (LPMC) in IBD under the assumption that the mucosal tissue damage can be mediated by the cytolytic function of the local immune cells. Most types of cytotoxic cells and their function have been studied using the cells isolated from the IBD-involved mucosa, including natural killer cell activity, antibody-dependent cellular cytotoxicity, antigen specific T cell cytotoxicity, anti-CD3-induced cytotoxicity and lymphokine-activated killer (LAK) cell activity (3). Among these cytotoxic cells, LAK cell activity has been investigated intensively because of its broad activity against the target cells including those of intestinal origin (4–7). The consistent and reliable nature of cytotoxic mechanisms can be used to reveal abnormalities of intestinal immune reactivity in IBD (7).

In our previous studies, we found that intestinal LAK cell activity differs among Crohn’s disease (CD), ulcerative colitis (UC) and controls: CD LPMC exhibit greater levels of LAK cell activity whereas UC LPMC display lower levels of activity than control cells (7). Using the cells isolated from the UC-affected and control mucosa, we also demonstrated that there is a positive correlation between the level of LAK cell activity and the proportion of CD56+ lymphocytes in the intestinal mucosa and that the precursor cells responsible for intestinal LAK cell activity may reside in CD56+ lymphocytes (8). Considering that CD56+ lymphocytes co-express IL-2 receptors (IL-2R) to a higher degree and proliferate well in response to IL-2 (9, 10), the local expansion of LAK precursor cells may be regulated by the level of IL-2 produced in the micro-environment of the intestinal mucosa. In fact, recent studies have demonstrated that mucosal IL-2 mRNA expression and IL-2 secreting cells are increased in CD but not in UC (11, 12). Therefore, we investigated the number of CD56+ lymphocytes, the LAK cell activity, the frequency of LAK precursor cells, and the expression of mRNA encoding IL-2 using LPMC isolated from the colonoscopic biopsy or surgical specimens to further elucidate...
the mechanisms involved in the different mucosal immune response of CD and UC patients.

Materials and Methods

Study groups

Colonic mucosal tissues were obtained from colonoscopic biopsies (n=12) or surgical specimens (n=5) from 17 patients with CD. The group included 10 men and seven women, ranging from 18 to 46 years of age (mean age, 29.4 years). Three patients had disease limited to the colon and 14 had ileocolonic disease. The disease had been present from one month to 13 years. At the time of the study, two patients were receiving prednisolone alone (10-15 mg/day), three were on sulfasalazine alone (3.0 g/day), four were being treated with prednisolone (10-15 mg/day) plus sulfasalazine (3.0 g/day), and eight were receiving no specific drug therapy. Colonic mucosal specimens were also obtained from colonoscopic biopsies (n=14) or surgical specimens (n=2) from 16 patients with UC. Eight were men and eight were women, ranging from 20 to 60 years of age (mean age, 36.1 years). Ten patients had total colitis, five left-sided colitis, and one proctitis. The disease had been present from one month to 12 years. One was receiving prednisolone alone (60 mg/day), three were on sulfasalazine alone (3.0 g/day), eight were being treated with prednisolone (5-30 mg/day) plus sulfasalazine (1.5-4.0 g/day), and four were on no specific drug therapy. Control colonic mucosal specimens were obtained from surgical specimens from 18 patients with colonic adenocarcinoma, two with diverticular disease, and one with multiple colonic adenomas. The group was composed of eight men and 13 women, ranging from 22 to 70 years of age (mean age, 55.1 years).

All samples were obtained with informed consent in accordance with the Helsinki Declaration and the diagnoses were confirmed by clinical, radiological, endoscopic and histological criteria.

Isolation of lamina propria mononuclear cells

In CD and UC patients, colonoscopic biopsies or surgical specimens were obtained from the inflamed mucosa of the colon. Mucosal involvement by active inflammation was confirmed histologically in all specimens used in this study. Control colonic mucosa was taken from histologically normal sites with the tumor or diverticular disease. All specimens were weighed before isolation procedures of LPMC. The method used to isolate LPMC from colonoscopic biopsies or surgical specimens was an enzymatic method using collagenase and DNAase followed by a Ficoll-Hypaque gradient as described in detail elsewhere (7, 8, 13). After purification by a Ficoll-Hypaque gradient, the average mononuclear cell yield was 1.76±0.11×10⁶, 1.81±0.14×10⁶ and 0.63±0.07×10⁶ cells per 100 mg of the tissue in CD, UC and control patients, respectively. Cell viability was determined by 0.1% trypan blue dye exclusion and it was consistently >95% in all of the patient groups.

Flow cytometric analysis

The following murine monoclonal antibodies were used either in fluorescein or phycoerythrine conjugates for two-color flow cytometric analysis of cell surface markers: T3 (anti-CD3, Coulter Immunology, Hialeah, Florida), B1 (anti-CD20, Coulter), T4 (anti-CD4, Coulter), T8 (anti-CD8, Coulter), 2H4 (anti-CD45RA, Coulter), Mol1 (anti-CD11b, Coulter) and NKH-1 (anti-CD56, Coulter). Aliquots of cells were incubated with saturating concentrations of various monoclonal antibodies (single-color analysis) or combined antibodies (two-color analysis) for 30 minutes at 4°C and washed three times with phosphate-buffered saline containing 2% bovine serum albumin and 0.1% sodium azide. Flow cytometric analysis was performed using 1×10⁶ cells on a fluorescence-activated cell sorter (FACS) equipped with krypton and argon lasers (EPICS-C, Coulter). The background level of immunofluorescence was determined by incubating cells with fluorescein or phycoerythrine-conjugated isotype-matched mouse immunoglobulins (Coulter).

Induction of lymphokine-activated killer cell activity and cytotoxic assay

To induce LAK effector cells, freshly isolated LPMC were incubated at 1×10⁶ cells/ml in RPMI 1640 medium (Gibco, Grand Island, NY) with 5% heat-inactivated human AB serum, HEPES buffer and antibiotics (culture medium) in the presence of 100 U/ml recombinant IL-2 (rIL-2, kindly provided by Takeda Chemical Ind., Osaka) for 96 hours at 37°C in a 5% CO₂ incubator.

The cytotoxic assay was performed by a ⁵¹Cr release assay using HT-29 cells derived from human colonic carcinoma (kindly provided by Dr. Toshifumi Hibi, Keio University, Tokyo) and a human B lymphoblastoid line, Daudi cells (kindly provided by Japanese Cancer Resources Bank, Tokyo) as previously described (7). Briefly, the target cells were labeled with 100 μCi ⁵¹Cr sodium chromate for 60 minutes. Then, the target cells were washed three times and added (5×10⁵ cells) to the wells of the round-bottom microtiter plates containing various numbers of effector cells (total volume, 200 μl). Microtiter plates were incubated for 4 hours at 37°C in a 5% CO₂ incubator. At the end of the incubation period, the plates were spun and the radioactivity of the supernatant (100 μl) from each well was measured in a γ-counter. Cytotoxicity was calculated as the percentage of specific ⁵¹Cr release using the following formula: Specific release (%)=(Experimental cpm – Spontaneous cpm)/(Maximal cpm – Spontaneous cpm)×100. The results were expressed in lytic unit (LU) per 10⁷ effector cells required to induce 20% specific ⁵¹Cr release from 5×10⁵ target cells (14).

In some experiments with the surgical specimens, a combination of a modified panning technique and complement-mediated lysis was utilized to deplete the cells bearing CD3 or CD56 antigens from LPMC prior to incubation with rIL-2 (8). The contamination of CD3+ or CD56+ cells in the depleted fractions was less than 3.0% and 0.5%, respectively, as demonstrated by FACS analysis.
Limiting-dilution analysis for lymphokine-activated killer precursor cells

A limiting-dilution assay was performed according to the method of Vie et al (15) to determine the frequency of LAK precursor cells among LPMC. Increasing numbers of freshly isolated LPMC (1 x 10^3, 5 x 10^3, 1 x 10^4, 5 x 10^4 cells/200 µl) were put in the wells of the round-bottom microtiter plates with 100 U/ml rIL-2, with 24–36 wells seeded for each cell concentration. The plates were incubated for 8 days at 37°C in a 5% CO2 incubator. At the end of the incubation period, ^51Cr-labeled HT-29 cells (5 x 10^5) were added to each well and cytotoxic tests were performed as described in the preceding paragraph. Individual wells were classified as positive or negative in reference to the threshold calculated as 3 SD above the mean cpm of control wells. Frequencies were determined using the ß-minimization method of Taswell (16).

Analysis of messenger RNA by polymerase chain reaction and dot blot hybridization

To obtain mRNA from freshly isolated LPMC, polyadenylated RNA was directly extracted from cell pellets (2 x 10^5 cells) using the oligo(dT)-cellulose (Quick Prep Micro mRNA Purification Kit, Pharmacia, Uppsala, Sweden). Extracted mRNA was reverse-transcribed for 1 hour at 42°C with 0.5 µl of Rous-associated virus 2 reverse-transcriptase (Takara Biomedicals, Ohtsu) and 100 pmol of oligo (dT) (Pharmacia) in a 24 µl reaction mixture. Twelve microliters of complementary DNA (cDNA) from each sample were amplified separately with specific 5' and 3' primers for IL-2 and ß-actin (200 pmol) in a 100 µl polymerase chain reaction (PCR) mixture containing 0.5 µl of Taq DNA polymerase (Promega Corporation, Madison, WI) with the cycles of 1 minute at 94°C, 1 minute at 60°C and 2 minutes at 72°C in a thermal cycler (Perkin Elmer Cetus, Norwalk, CT). The following oligonucleotide 5' and 3' primers were synthesized using a DNA synthesizer (Applied Biosystems, Foster City, CA): IL-2, ATGTACAGGATGCTCCTTAATGTCACGCACGATTTC. The amplified product was electrophoresed in a 2% agarose gel with ethidium bromide and visualized by UV transillumination.

To quantitate the expression of mRNA for IL-2 and ß-actin, dot blot hybridization was performed using the PCR product. Three microliters of amplified cDNA were spotted directly onto a nylon membrane (Amersham International plc, Buckinghamshire, England), denatured with 0.5 mol NaOH, neutralized, and cross-linked by UV light for 2 minutes. After an overnight prehybridization, the membrane was hybridized for 24 hours at 42°C with 32P-labeled oligonucleotides internal to each amplified product. Radioactive blots were quantitated using an image scanner (Fujix BAS 2000, Fuji Photo Film Co., Tokyo).

Analysis of data

Statistical analysis was performed using the paired t, Kruskal-Wallis, Wilcoxon’s rank-sum tests. All data were expressed as mean±SEM.

Results

Cell surface markers

The cell surface markers of freshly isolated LPMC were determined using flow cytometric analysis with a variety of monoclonal antibodies in nine CD, eight UC and nine control patients. The percentage of lymphocytes bearing pan T (CD3) and B (CD20) markers did not differ significantly among all patient groups (CD3: 66.1±2.8%, 64.7±2.2% and 67.1±3.3%, CD20: 16.8±2.8%, 20.0±2.4% and 15.9±2.9%, in CD, UC and controls, respectively). When T cell subpopulations were analyzed in dual immunofluorescence, the composition of CD4+, CD45RA+ (suppressor-inducer), CD4+, CD45RA- (helper-inducer), CD8+, CD11b+ (suppressor) and CD8+, CD11b+ (cytotoxic) cells was comparable among CD, UC and control LPMC (CD4+, CD45RA+: 5.2±1.2%, 5.3±0.9% and 4.7±0.9%, CD4+, CD45RA+: 40.2±4.8%, 40.6±4.7% and 46.2±5.8%, CD8+, CD11b+: 3.4±0.7%, 4.2±0.7% and 4.0±0.5%, CD8+, CD11b+: 19.8±1.9%, 19.6±1.5% and 18.4±1.9%, in CD, UC and controls, respectively). The proportion of CD56+ lymphocytes was determined using freshly isolated LPMC because our previous preliminary study has shown that CD56+ lymphocytes were almost exclusively responsible for LAK cell activity in the colonic mucosa (8, 9). Two-color flow cytometric analysis demonstrated that lamina propria CD56+ cells were either CD3+ (CD3+, CD56+ cells) or CD3- (CD3-, CD56+ cells) and the proportion of these subsets was different among CD, UC and control LPMC. As shown in Table 1, CD LPMC contained more (p<0.05), whereas UC LPMC had fewer (p<0.05) CD3+ CD56+ lymphocytes than control LPMC. However, the percentage of CD3-, CD56+ lymphocytes was comparable in CD LPMC and lower (p<0.05) in UC LPMC compared with control cells. After a 96-hour incubation with 100 U/ml rIL-2, an increase of CD3+, CD56+ lymphocytes was observed in CD and control LPMC but not in UC LPMC, and their proportion continued to be higher in CD cultures and substantially lower in UC cultures.

Lymphokine-activated killer cell activity and frequency of lymphokine-activated killer precursor cells

In the bulk cultures stimulated with 100 U/ml rIL-2 for 96 hours, CD LPMC exhibited significantly (p<0.01) higher levels of LAK cell activity against colonic carcinoma-derived HT-29 cells, while UC LPMC showed significantly (p<0.05) lower levels of cytotoxic activity than control LPMC. Almost identical results were obtained when human B lymphoblastoid Daudi cells were used as a target (data not shown). When the frequency of LAK precursor cells was determined by the limiting-dilution analysis, LPMC derived from CD specimens contained more (p<0.05) LAK precursor cells and those from UC specimens had fewer (p<0.05) cells compared with controls (Table 2). To determine the phenotypes responsible for LAK cell activity, the cells bearing CD3 or CD56 antigens were depleted before 96 hour culture with rIL-2. As shown in Table 3, depletion of CD56+ lymphocytes prior to incubation with rIL-
### Table 1. Percentages of CD3+, CD56+ and CD3–, CD56+ Lymphocytes among Freshly Isolated and Cultured Lamina Propria Mononuclear Cells

<table>
<thead>
<tr>
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<th>Fresh cells*</th>
<th>Cultured cells†</th>
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<tr>
<td></td>
<td>CD3+, CD56+</td>
<td>CD3+, CD56+</td>
</tr>
<tr>
<td>CD</td>
<td>8.9±1.4‡</td>
<td>3.9±0.6</td>
</tr>
<tr>
<td>UC</td>
<td>2.3±0.3‡</td>
<td>1.2±0.3‡</td>
</tr>
<tr>
<td>Control</td>
<td>5.6±0.6</td>
<td>3.7±0.6</td>
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*The surface marker of freshly isolated LPMC was analyzed by two-color flow cytometry in nine CD, eight UC and nine control patients. †After incubation with 100 U/ml rIL-2 for 96 hours, the surface marker of the cultured cells was analyzed by two-color flow cytometry in eight CD, seven UC and seven control patients. ‡p<0.05 compared with control.

### Table 2. Lymphokine-activated Killer Cell Activity and Frequency of Lymphokine-activated Killer Precursor Cells in Lamina Propria Mononuclear Cells

<table>
<thead>
<tr>
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<th>LAK cell activity* (LU/10⁷ cells)</th>
<th>Frequency† (/10⁶ cells)</th>
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<tbody>
<tr>
<td>CD</td>
<td>203±27‡</td>
<td>85±24§</td>
</tr>
<tr>
<td>UC</td>
<td>22±5†</td>
<td>9±3§</td>
</tr>
<tr>
<td>Control</td>
<td>54±8</td>
<td>27±5</td>
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</table>

*After incubation with 100 U/ml rIL-2 for 96 hours, LAK cell activity was measured by a ⁵¹Cr release assay using HT-29 target cells in 10 CD, 10 UC and 17 control patients. †The frequency of LAK precursor cells was determined by the limiting-dilution analysis (100 U/ml rIL-2, 8 days) in eight CD, eight UC and nine control patients. ‡p<0.01 compared with control. §p<0.05 compared with control.

### Table 3. Lymphokine-activated Killer Cell Activity Exhibited by Unseparated Lamina Propria Mononuclear Cells and CD3– or CD56– depleted Lamina Propria Mononuclear Cells

<table>
<thead>
<tr>
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<th>LAK cell activity* (LU/10⁷ cells)</th>
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<tbody>
<tr>
<td>Unseparated LPMC</td>
<td>CD3– LPMC‡</td>
</tr>
<tr>
<td>CD</td>
<td>240</td>
</tr>
<tr>
<td>CD</td>
<td>175</td>
</tr>
<tr>
<td>CD</td>
<td>292</td>
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<td>UC</td>
<td>21</td>
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<td>UC</td>
<td>16</td>
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<td>Control</td>
<td>56</td>
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<td>Control</td>
<td>69</td>
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<td>Control</td>
<td>45</td>
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<td>Control</td>
<td>34</td>
</tr>
<tr>
<td>Control</td>
<td>52</td>
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</table>

*After incubation with 100 U/ml rIL-2 for 96 hours, LAK cell activity was measured by a ⁵¹Cr release assay using HT-29 target cells. ‡ § A combination of a panning technique and complement-mediated lysis was utilized to deplete the cells bearing CD3 or CD56 antigens from LPMC prior to incubation with rIL-2.

### Expression of messenger RNA for interleukin-2 in lamina propria mononuclear cells

In the preliminary experiments of reverse-transcriptase PCR (RT-PCR) analysis using freshly isolated LPMC, IL-2 cDNA was detected as a band on the ethidium bromide-stained gel from 26 or 28 PCR cycles and saturated in the range over 34 or 36 cycles (Fig. 1, left panel). In contrast, β-actin cDNA was detected from 24 or 26 PCR cycles and reached a saturation over 34 PCR cycles (Fig. 1, right panel). Therefore, we chose 30 PCR cycles to compare the levels of IL-2 mRNA expressed in LPMC and amplified all samples at once to avoid the variability between the assays. The original levels of IL-2 mRNA in individual LPMC were quantitated by the radioactivity ratio of IL-2 to β-actin cDNA in each dot blot (Fig. 2).

When normalized by the level of β-actin mRNA, CD LPMC (n=10) exhibited significantly (p<0.01) higher IL-2 mRNA levels (0.227±0.034) compared with control LPMC (n=10, 0.069±0.028). In contrast, there was no significant difference of IL-2 mRNA levels between UC LPMC (n=8, 0.086±0.021) and control cells.

### Experimental data and clinical parameters

There were no significant differences or associations of cell surface markers, LAK cell activity, LAK precursor cells and expression of IL-2 mRNA with sex and age of the patients, length of disease, anatomical segment of the large bowel and type of drug therapy.
Figure 1. RT-PCR analysis of IL-2 (left panel) and β-actin (right panel) mRNA levels in freshly isolated LPMC. After RNA was extracted from LPMC and reverse-transcribed, the resultant cDNA was amplified for 22–36 cycles. The amplified product was electrophoresed in a 2% agarose gel.

![IL-2 and β-actin PCR cycles](image)

Frequency of lymphokine-activated killer precursor cells and clinical course

To assess whether LAK cell activity is associated with disease activity (17), we repeated the limiting-dilution analysis to monitor the frequency of LAK precursor cells three to six months after introduction of the therapy with total parenteral nutrition or elemental diet in three cases of active CD patients without any specific drug therapy during the observation period. A substantial decrease was observed in the number of LAK precursor cells after nutritional therapy (case 1: 220 vs 22 per 10⁶ cells, case 2: 92 vs 42 per 10⁶ cells, case 3: 67 vs 17 per 10⁶ cells, before and after therapy, respectively) in parallel with the decrease in disease activity evaluated by the IOIBD score (case 1: 5 vs 0, case 2: 4 vs 1, case 3: 4 vs 0, before and after therapy, respectively).

Discussion

The results of the present study further support the observation that mucosal immune reactivity is different between the two forms of IBD (7). When compared with control cells, CD-derived LPMC exhibited a greater level of LAK cell activity...
against colon carcinoma HT-29 and Daudi target cells, whereas UC-derived LPMC displayed lower cytotoxic activity. Furthermore, the parallel experiments using the limiting-dilution analysis demonstrated that the number of LAK precursor cells was correlated with the cytotoxic activity in the bulk culture, suggesting that the differences in LAK cell activity among CD, UC and control LPMC can be attributed to the alteration in the number of LAK precursor cells. This study also showed that IL-2 mRNA expression was increased in CD LPMC, but not in UC LPMC, which may be responsible for the increased number of IL-2-responsive LAK precursor cells in the intestinal mucosa affected with CD (9).

The mechanisms of LAK cell development and lytic activity in the intestine have not been clearly defined. Earlier studies using peripheral blood have shown that the majority of LAK cell activity is derived from both CD3+, CD56+ T lymphocytes and CD3+, CD56+ NK cells which comprise the cells capable of mediating non-MHC restricted cytotoxicity (18). Previously, using depletion and enrichment experiments with control LPMC, we found that the precursor cells responsible for intestinal LAK cell activity are also restricted to these two distinct subpopulations (8, 10). Consistent with this observation, the depletion experiment in the present study demonstrated that lamina propria CD56+ lymphocytes are also the main precursors for LAK cell activity in the IBD-affected mucosa. However, since the number of CD56+ lymphocytes was much higher than that of LAK precursor cells obtained in the limiting-dilution analysis, LAK precursor cells may be present as a small subpopulation among lamina propria CD56+ lymphocytes. In this study CD-derived LPMC contained more CD56+ lymphocytes, especially CD3+, CD56+ lymphocytes, and UC-derived LPMC had fewer CD3+, CD56+ and CD3+, CD56+ lymphocytes compared with control cells, when freshly isolated LPMC were analyzed in the flow cytometry. Van Tol et al also demonstrated that non-MHC restricted cytotoxicity mediated by CD56+ lymphocytes was increased in CD LPMC compared with UC LPMC (19). Taken together, the numerical alteration in LAK precursors among CD56+ lymphocytes may be responsible for the observed difference of intestinal LAK cell activity between CD and UC.

The number of IL-2-responsive LAK precursor cells may be regulated by the level of IL-2 produced by mucosal helper T cells. With regard to this hypothesis, the RT-PCR analysis of the present study demonstrated that the expression of IL-2 mRNA was enhanced in freshly isolated LPMC from the inflamed mucosa with CD than in control LPMC but there was no significant difference in IL-2 mRNA levels between the cells from the diseased mucosa with UC and control cells. Recently, Mullin et al showed that IL-2 mRNA was increased in the whole biopsy tissues from the inflamed mucosa with CD but not in the tissues from the mucosa with UC (11, 20). Using the reverse hemolytic plaque assay of isolated LPMC, Breese et al demonstrated that IL-2 secreting cells were readily detectable in the mucosa involved with CD whereas they were mostly absent or present at only low levels in the mucosa with UC (12). Together, these findings strongly suggest that functional heterogeneity of mucosal helper T cells may contribute to the difference of LAK cell activity between these two forms of IBD. Since IL-2 is a pivotal cytokine for the proliferation of LAK precursor cells possessing IL-2R (7, 9), enhanced production of endogenous IL-2 may be involved in the increased number of CD56+ LAK precursor cells in the mucosa with CD (7). This concept is further strengthened by the observation in this study that there was a prominent increase of CD56+ cells, especially CD3+, CD56+ cells in CD LPMC after in vitro culture with exogenous rIL-2. On the other hand, the number of LAK precursor cells was decreased in UC LPMC compared with control cells in spite of comparable levels of IL-2 mRNA expression. The reason for this observation is not clear, but it may be due to compartmentalization or altered homing patterns of LAK precursor cells as described in other intestinal lymphoid cells (21, 22) or limited responsiveness of LAK precursor cells to IL-2. The last hypothesis is most likely because in this study UC LPMC exhibited a restricted expansion of CD3+, CD56+ cells even in the presence of an optimal concentration of rIL-2 (7).

The differential expression of IL-2 between CD and UC LPMC may be explained by a selective activation or proportional shift of mucosa helper T cell subsets. In the murine models, helper T cells are divided into T helper cells type-1 (Th1), marked by the production of IL-2 and IFN-γ, and T helper cells type-2 (Th2), marked by the production of IL-4, IL-5, IL-6 and IL-10 (23). In the murine parasitic infection, a differential activation of Th1 or Th2 cells is associated with a resistance or susceptibility to the parasitic agents (24, 25). The involvement of T helper subsets was also suggested by Yamamura et al in the study of human leprosy lesions in which the selected expression of Th1 or Th2 cytokines is critical to the formation of the characteristic disease, tuberculoid or lepromatous lesions, respectively (26). Since the proportion of cells positive for the conventional cell surface marker for helper T cells (CD4+, 2H4- cells) was similar between CD and UC LPMC (8, 27), the future development of cell surface markers which distinguish Th1 and Th2-like cells will clarify whether abnormalities of mucosal helper T cell subsets are associated with the pathogenesis of IBD (20).

Increasing evidence suggests the involvement of T cells in the pathogenesis of CD. These include elevated IL-2 and soluble IL-2R levels in the serum of the patients with CD (28, 29), the benefit of cyclosporine for the treatment of CD (30), the remission of CD associated with the onset of the human immunodeficiency virus infection which affects CD4+ T helper cells (31), and symptomatic exacerbation in patients with CD after infusion of high-dose IL-2 for the treatment of associated malignancy (32). However, in regard to mucosal T cells, little is known about the role in the immune effector function in the inflamed mucosa with CD. The data of the present study demonstrate that a subset of mucosal T cells with CD56 antigens could mediate substantial levels of LAK cell activity as a predominant phenotype in the patients with CD. Considering its broad action against mucosal target cells including epithelial and stromal cells (7, 33), these results suggest that mucosal T cells may be involved in the tissue damage and resultant inflammatory responses in the patients with CD.
Mucosal Immune Reactivity in IBD

In summary, this study suggests that LPMC express and respond to IL-2 differently in CD and UC. A detailed investigation of mucosal helper T cell subsets can provide important clues for the clarification of the pathogenesis of IBD.

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