Enriched CD45RA<sup>-</sup>CD62L<sup>+</sup> central memory T and decreased CD3<sup+</sup>CD56<sup+</sup> natural killer T lymphocyte subsets in the rectum of ulcerative colitis patients

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Abstract

Objectives: To investigate the distinctive features of lymphocytes promoting inflammation in ulcerative colitis.

Methods: We performed flow cytometric analysis of peripheral blood mononuclear cells (PBMCs) and colorectal mucosa lymphocytes in ulcerative colitis patients (n = 13) and control patients (n = 5).

Results: CD62L<sup>+</sup>/CD3<sup+</sup>CD4<sup+</sup> (35.7 ± 14.0% vs. 19.9 ± 6.4%) and CD62L<sup+</sup>/CD3<sup+</sup>CD4<sup+</sup>/CD8<sup+</sup> cells (17.1 ± 17.4% vs. 2.4 ± 3.9%) were higher in the rectum of ulcerative colitis patients than in control patients. Subpopulation analysis revealed that CD45RA<sup>+</sup>CD62L<sup+</sup>/CD3<sup+</sup>CD4<sup+</sup>, that is, central memory T cell fraction in CD4<sup+</sup> T cells, was significantly increased in the rectum of ulcerative colitis, compared to that in control patients (23.3 ± 10.5% vs. 8.2 ± 4.0%). Comparison of rectum and colon samples in ulcerative colitis patients indicated that CD56<sup+</sup>/CD3<sup+</sup> was decreased in the rectum compared to that in the colon (11.3 ± 12.5% vs. 21.3 ± 16.5%). The ratio of CD56<sup+</sup>/CD3<sup+</sup> was also decreased in the rectum of active ulcerative colitis patients compared to that in ulcerative colitis patients at the endoscopic remission stages (2.8 ± 1.7% vs. 18.5 ± 13.3%).

Conclusion: We demonstrated that CD62L<sup+</sup> T lymphocytes, particularly the CD45RA<sup>+</sup>CD62L<sup+</sup> T cell subset that represents central memory T cells, were increased in the rectum of patients with ulcerative colitis. In addition, the CD56<sup+</sup>/CD3<sup+</sup> subset (natural killer T cells) was decreased in the rectum compared to that of less inflamed colonic mucosa. These results suggest that the enrichment of central memory T lymphocytes and the reduction of natural killer T cells in the gut mucosa are involved in the pathogenesis of ulcerative colitis.

Keywords

central memory T cell, flow cytometry, natural killer T cells, peripheral blood mononuclear cell, ulcerative colitis

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Background

Ulcerative colitis is an inflammatory bowel disease that causes prolonged inflammation primarily in the colorectum, which leads to mucosal erythema, friability, erosions, and/or ulcer formation. Although the exact pathophysiology of this disease has not been elucidated yet, immune-mediated tissue destruction secondary to a combination of barrier dysfunction, genetic risk, and dysbiosis is essential for the development and deterioration of inflammation in the gut. Since T lymphocytes play a central role in cell-mediated immunity, they are thought to be involved in the pathogenesis of ulcerative colitis.

Flow cytometry enables the typing of immune cells based on the presence of specific cell surface markers, expression of cytokines, and phosphorylation of key proteins. Therefore, flow cytometry can analyze lymphocytes in patients with ulcerative colitis. In previous studies, peripheral blood mononuclear cells (PBMCs) and colorectum-resident lymphocytes were primarily used as sample cell sources for flow cytometric analysis. The composition of T lymphocytes in intestinal tissues differs from that in the blood due to intestinal microbiota responses and homing receptor/ligand interactions; however, there is a lack of data on the in-depth surface marker profiles of PBMCs and colorectum-resident T lymphocytes in patients with ulcerative colitis. This study aimed to perform a comprehensive flow cytometric characterization of T lymphocytes in ulcerative colitis and reveal the distinctive features of lymphocytes that promote inflammation in this disease.

Materials and methods

Overview

To characterize the lymphocyte surface markers of PBMCs and the colorectum and reveal the distinctive features of lymphocytes that promote inflammation in ulcerative colitis, comparative analyses were performed between (i) rectum-resident lymphocytes in patients with ulcerative colitis and those in control patients, (ii) PBMCs in patients with ulcerative colitis and those in control patients, (iii) lymphocytes from the inflamed mucosa (rectum) and those from less-inflamed mucosa (colon) in patients with ulcerative colitis, and (iv) rectum-resident lymphocytes in the active stage and those in the remission stage in patients with ulcerative colitis.

To comprehensively analyze the profiles of T lymphocytes in patients with ulcerative colitis using flow cytometry, the expressions of helper T-lymphocyte marker CD4, cytotoxic T-lymphocyte marker CD8, regulatory T (Treg) lymphocyte markers CD25 and CD127, natural killer cell marker CD56 (also known as neural cell adhesion molecule), mature T-lymphocyte marker CD7, central inhibitory receptor programmed death receptor-1 (PD1, also known as CD279), activated T-lymphocyte marker CD30 (also known as tumor necrosis factor receptor superfamily member 8), human leukocyte antigen DR isotype (HLADR), and type-2 helper T-lymphocyte marker C-C chemokine receptor type 4 (CCR4, also known as CD194) were assessed. The expressions of CD45RA and CD62L were assessed to categorize lymphocytes into subpopulations such as effector memory T cells, central memory T cells, and naive T cells.

Ulcerative colitis patients

Flow cytometry was prospectively performed between April and November 2020 at Okayama University Hospital (Okayama, Japan) on endoscopic biopsy specimens obtained from 14 patients who had been diagnosed with ulcerative colitis. The inclusion criteria for ulcerative colitis patients were: (i) individuals who had never undergone surgical resection of the colorectum, and (ii) those who had not been taking biologic drugs, including infliximab, adalimumab, golimumab, vedolizumab, and ustekinumab, or the Janus kinase inhibitor, tofacitinib, for at least 6 months prior to study enrollment. Disease activity was determined according to the Mayo endoscopic subscore (MES). Briefly, normal or inactive ulcerative colitis indicated MES 0; mild disease showing erythema, decreased vascular pattern, and/or mild friability indicated MES 1; moderate disease with marked erythema, absent vascular pattern, friability, and/or erosions indicated MES 2; and severe disease with spontaneous bleeding and/or ulceration indicated MES 3. As no patients presented an MES of 3, all patients in this study were classified as having MES 0, 1, or 2, as described below.

Follow-up endoscopy and biopsy were performed as part of standard care for screening neoplasia, mostly on a yearly basis. In one of the ulcerative colitis patients, flow cytometry analysis was solely performed on two samples from the sigmoid colon mucosa, as specimens were not taken from the rectum. Therefore, this patient was excluded from the study. For each of the remaining 13 ulcerative colitis patients, a single specimen was obtained using disposable biopsy forceps from the rectal mucosa during endoscopy, and another specimen was obtained from the colon, where inflammation was absent or mild. Lymphocytes were isolated from each biopsied specimen using the one-step lymphocyte isolation procedure, which was reported in our earlier work. In addition, PBMCs were isolated from 5 mL of peripheral venous blood by density centrifugation using Lymphoprep (Alere Technologies AS, Oslo, Norway).

Patients with colon polyp

Flow cytometry analysis was also performed in five patients who underwent endoscopic mucosal resection
of ulcerative colitis patients, we compared the population of patients with colon polyps (control) and that

**Analysis**

First, to assess the differences between the lymphocyte population of patients with colon polyps (control) and that of ulcerative colitis patients, we compared the flow cytometry results for PBMC samples and rectal samples between the two groups. Second, to reveal the characteristics of the rectum-resident lymphocytes in ulcerative colitis patients, we contrasted the flow cytometric results from rectum samples with those of colon samples or PBMCs. Third, to evaluate the relationship between inflammation severity and lymphocyte composition in ulcerative colitis, we compared the flow cytometry results of PBMC, rectum, and colon samples between MES 0 patients and MES 1 and 2 patients. Fourth, to evaluate the relationship between the clinical courses of patients within 6 months after colonoscopy and the lymphocyte composition in ulcerative colitis, we compared the flow cytometry results of rectal samples between patients with and without relapse. The relapse group was defined as patients in whom clinical symptoms, such as increased bloody stool, diarrhea, abdominal pain, uncontrolled fever, and increased levels of inflammatory markers, worsened within 6 months of colonoscopy or those who required induction of biologic drugs or tofacitinib. The non-relapse group was defined as patients without worsening of clinical symptoms within 6 months following colonoscopy.

Statistical analyses were performed using JMP 14.0.0 software (SAS Institute Inc., Cary, NC, USA). Significant differences between samples were determined using paired or unpaired t-tests, where indicated. Differences were considered significant if the p value was less than 0.05.

**Ethics approval**

The patients were prospectively registered and analyzed in this study. Written informed consent was obtained from all participants. This study adhered to the principles of the Declaration of Helsinki and was approved by the ethics committee of Okayama University Hospital (approval number: 2003-011 and 2009-014). The study protocol was registered in the UMIN Clinical Trials Registry (UMIN000039868).

**Results**

**Patients’ characteristics**

The clinical characteristics of the enrolled patients are shown in Table 1. In the ulcerative colitis group, there were eight men and five women. The average age was 52.6 years (range, 21–72 years). Eight patients were non-smokers, three patients were ex-smokers, and one patient was a current smoker. Data on smoking status was not available for the remaining patient. The average duration of the disease was 16.1 years (range, 2–40 years). Seven patients were in remission (MES 0), two patients had an MES of 1, and four patients had an MES of 2. None of the patients presented a severe form of the disease, showing spontaneous bleeding and/or ulceration (MES 3). Ulcerative colitis patients were treated with mesalazine (n = 7), salazosulfapyridine (n = 6), mesalazine suppositories (n = 2), azathioprine (n = 4), and/or mercaptopurine (n = 1). Although one patient had been taking celecoxib and another patient used hydrocortisone ointment for hemorrhoids, no patient had been treated with oral or injectable corticosteroids for at least 6 months prior to study enrollment.
In the control group, which consisted of five patients with colon polyps, there were three men and two women; average age was 64.5 years (range, 37–86 years). All patients, except one who was a current smoker, were non-smokers. One patient had been taking diclofenac sodium, whereas the other patients had not been taking anti-inflammatory agents.

Statistical differences were not observed in terms of sex and age between the ulcerative colitis and control groups. Further, significant differences were not observed in terms of patient characteristics between the inactive (MES 0) and active (MES 1 and 2) ulcerative colitis subgroups.

**Lymphocyte composition in control and ulcerative colitis patients**

The flow cytometry results for all samples are shown in Figure 1. Comparison between control and ulcerative colitis groups revealed that ratios of CD62L⁺/CD3⁺ CD4⁺ (35.7 ± 14.0% vs. 19.9 ± 6.4%) and CD62L⁺/CD3⁺ CD4⁻ cells (17.1 ± 17.4% vs. 2.4 ± 3.9%) were higher in the rectum of patients in the ulcerative colitis group than those of the control group. In PBMCs from ulcerative colitis patients, HLADR⁺/CD3⁺ ratio was decreased (5.1 ± 6.1% vs. 19.6 ± 7.3%), while CD45RA⁺/CD3⁺ CD4⁻ ratio was

**Table 1. Characteristics of the enrolled patients.**

| No | Age | Sex  | Diagnosis     | Smoking status | Age at UC diagnosis | Rectum MES | Colon MES | Intestine samples that were used for flow cytometry | Anti-inflammatory medication |
|----|-----|------|---------------|----------------|---------------------|------------|-----------|--------------------------------------------------|----------------------------|
| 1  | 45  | M    | Ulcerative colitis | Ex-smoker       | 29                  | 0          | 0         | Rectum and ascending colon                      | Azathioprine, salazosulfapyridine |
| 2  | 48  | F    | Ulcerative colitis | Unknown         | 37                  | 0          | 0         | Rectum and transverse colon                     | Salazosulfapyridine          |
| 3  | 49  | F    | Ulcerative colitis | Non-smoker      | 31                  | 0          | 0         | Rectum and sigmoid colon                        | Mesalazine                  |
| 4  | 50  | M    | Ulcerative colitis | Non-smoker      | 40                  | 0          | 0         | Rectum and ascending colon                      | Azathioprine, mesalazine, salazosulfapyridine, celecoxib |
| 5  | 56  | M    | Ulcerative colitis | Smoker          | 54                  | 0          | 0         | Rectum and ascending colon                      | Mercaptopurine               |
| 6  | 62  | M    | Ulcerative colitis | Non-smoker      | 40                  | 0          | 0         | Rectum and ascending colon                      | Mesalazine                  |
| 7  | 64  | F    | Ulcerative colitis | Non-smoker      | 24                  | 0          | 0         | Rectum and transverse colon                     | Mesalazine, hydrocortisone ointment |
| 8  | 53  | F    | Ulcerative colitis | Non-smoker      | 36                  | 1          | 0         | Rectum and ascending colon                      | Salazosulfapyridine, mesalazine suppositories |
| 9  | 55  | M    | Ulcerative colitis | Ex-smoker       | 48                  | 1          | 0         | Rectum and ascending colon                      | Mesalazine suppositories     |
| 10 | 21  | M    | Ulcerative colitis | Non-smoker      | 18                  | 2          | 0         | Rectum and ascending colon                      | Azathioprine, mesalazine     |
| 11 | 42  | M    | Ulcerative colitis | Non-smoker      | 30                  | 2          | 1         | Rectum and ascending colon                      | Mesalazine                  |
| 12 | 61  | M    | Ulcerative colitis | Ex-smoker       | 51                  | 2          | 0         | Rectum and transverse colon                     | Azathioprine, mesalazine, salazosulfapyridine |
| 13 | 72  | F    | Ulcerative colitis | Non-smoker      | 32                  | 2          | 1         | Rectum and ascending colon                      | Salazosulfapyridine         |
| 14 | 37  | F    | Colon polyp      | Non-smoker      | NA                  | NA         | NA        | Rectum                                          | Diclofenac sodium            |
| 15 | 55  | M    | Colon polyp      | Non-smoker      | NA                  | NA         | NA        | Rectum                                          | None                        |
| 16 | 69  | M    | Colon polyp      | Smoker          | NA                  | NA         | NA        | Rectum                                          | None                        |
| 17 | 73  | F    | Colon polyp      | Non-smoker      | NA                  | NA         | NA        | Rectum                                          | None                        |
| 18 | 86  | M    | Colon polyp      | Non-smoker      | NA                  | NA         | NA        | Rectum                                          | None                        |

MES: Mayo endoscopic subscore, NA: not available.
Figure 1. Flow cytometry analysis. Lymphocyte composition was analyzed in samples from control and ulcerative colitis patients. Regulatory T (Treg) cells were defined as CD3⁺CD4⁺CD25⁺CD127low/− cells. *, p < 0.05 (paired t-test); **, p < 0.01 (paired t-test); †, p < 0.05 (unpaired t-test); ††, p < 0.01 (unpaired t-test); PBMC, peripheral blood mononuclear cells; UC, ulcerative colitis.

Figure 2. Images depicting differences in lymphocyte composition between patients with colon polyps (control) and ulcerative colitis patients. In the rectum of ulcerative colitis patients, CD62L⁺/CD3⁺CD4⁺ and CD62L⁺/CD3⁺CD4⁺ were increased, compared to those of control patients. HLADR⁺/CD3⁺ was decreased and CD45RA⁺/CD3⁺CD4⁺ was increased in peripheral blood mononuclear cells (PBMCs) of ulcerative colitis patients, compared to those of control patient PBMCs.
increased (55.1 ± 15.1% vs. 43.9 ± 5.0%) compared to PBMCs from control patients (Figure 2).

Comparison between the rectum and colon samples in ulcerative colitis patients revealed that CD56+/CD3+ ratio was decreased (11.3 ± 12.5% vs. 21.3 ± 16.5%), while CD4+/CD3+ (71.2 ± 11.0% vs. 59.7 ± 19.4%) and PD1+/CD3+ (78.1 ± 6.6% vs. 69.1 ± 10.8%) ratios were increased in the rectum compared to those in the colon of ulcerative colitis patients.

Characteristics of the rectum-resident lymphocytes in ulcerative colitis

Comparison between the rectum and colon samples in ulcerative colitis patients revealed that CD56+/CD3+ ratio was decreased (11.3 ± 12.5% vs. 21.3 ± 16.5%), while CD4+/CD3+ (71.2 ± 11.0% vs. 59.7 ± 19.4%) and PD1+/CD3+ (78.1 ± 6.6% vs. 69.1 ± 10.8%) ratios were increased in the rectum compared to those in the colon mucosa (Figure 3). Furthermore, lymphocyte composition from the rectum and from PBMC samples in ulcerative colitis patients differ in various aspects: the ratios of CCR4+/CD3+, CD45RA+/CD3+CD4+, CD62L+/CD3+CD4+, CD45RA−/CD3+CD4−, and CD62L+/CD3−CD4− were significantly lower, whereas the ratios of CD8+/CD3+, PD1+/CD3+, CD30+/CD3+, and HLADR+/CD3+ were significantly higher in the rectum than in PBMCs.

Disease activity and lymphocyte composition in ulcerative colitis

A comparative analysis between the flow cytometry results of MES 1 and 2 ulcerative colitis patients (n = 6) and those of MES 0 patients (n = 7) showed that CD8+/CD3+ (28.7 ± 10.7% vs. 52.7% ± 18.2%) and CD56+/CD3+ (2.8 ± 1.7% vs. 18.5 ± 13.3%) ratios were decreased in the rectum of MES 1 and 2 patients, compared to those of MES 0 patients (Figure 4). With respect to the colon mucosal lymphocytes, we found a lower ratio of CD30+/CD3+ (3.1 ± 2.0% vs. 7.9 ± 3.3%), and a higher ratio of CD7+/CD3+ (91.8 ± 4.0% vs. 80.4 ± 12.2%) in MES 1 and 2 patients than in MES 0 patients. In addition, CCR4+/CD3+ ratio was increased in PBMCs from MES 1 and 2 patients compared to that from MES 0 ulcerative colitis patients (28.7 ± 7.9% vs. 15.5 ± 5.6%) (Figure 5).

Central memory T cells among the rectum-resident lymphocytes in ulcerative colitis

As described above, we discovered that ulcerative colitis patients had increased ratios of CD62L+/CD3+CD4+ and CD62L+/CD3−CD4− cells in the rectal mucosal lymphocytes compared to those in patients from the control group. To explore the changes in lymphocyte composition in depth, we further separated CD3+CD4+ and CD3−CD4− cells into four subpopulations based on their CD45RA and CD62L expression: CD45RA−CD62L− cells were considered as effector memory T cells, CD45RA−CD62L+ cells as central memory T cells, CD45RA+CD62L+ cells as naïve T cells, and CD45RA−CD62L− cells (Figure 6). As shown in Figure 7, the CD45RA−CD62L−/CD3+CD4+ fraction was increased in the rectum of ulcerative colitis patients, compared to that of control patients (23.3 ± 10.5% vs. 8.2 ± 4.0%). Although the ratio of CD45RA−CD62L+/CD3−CD4− was also higher in the rectum of ulcerative colitis patients than in control patients (8.1 ± 6.7% vs. 1.5 ± 2.9%), this difference was not statistically significant (p = 0.051).

Comparison between MES 1 and 2 ulcerative colitis patients and MES 0 ulcerative colitis patients revealed that the CD45RA−CD62L−/CD3+CD4+ fraction was higher in the MES 1 and 2 group than in MES 0 group (12.6 ± 4.8% vs. 4.3 ± 5.8%) (Figure 8). Despite the CD45RA−CD62L+/CD3−CD4− fraction being higher in MES 1 and 2 than in MES 0 (29.2 ± 7.6% vs. 18.2 ± 10.4%), this difference was not statistically significant (p = 0.052).

Central memory T cells and natural killer T cells as potential predictors of a relapsing clinical course

Within 6 months after the colonoscopy, one patient (Case 8) developed bloody stool, one patient (Case 11) developed worsening diarrhea, and another patient (Case 10) required administration of vedolizumab to control intestinal inflammation. Thus, the relapse group comprised these three patients, while the non-relapse group comprised the other 10 patients.

Based on the results obtained, we investigated the ratio of central memory T cells (CD45RA−CD62L+ cells) and natural killer T (NKT) cells (CD3+CD56− cells) as a potential predictor of relapsing clinical course in patients with
ulcerative colitis (Figure 9). The CD45RA⁺/CD62L⁺/CD3⁺CD4⁺ fraction was higher in the relapse group than in the non-relapse group (31.1 ± 3.8% vs. 21.0 ± 10.9%, p = 0.03). Furthermore, the CD45RA⁺/CD62L⁺/CD3⁺CD4⁺ fraction was higher (15.1 ± 4.4% vs. 6.0 ± 5.9%, p = 0.04), while the CD3⁺CD56⁺ fraction was lower in the relapse group than in the non-relapse group (2.8 ± 1.5% vs. 13.8 ± 13.2%, p = 0.03). A cutoff value of CD45RA⁺CD62L⁺/
CD3+CD4 fraction ≥10% versus <10% provided a sensitivity of 100% and specificity of 80%. In addition, CD45RA−CD62L+/CD3+CD4+ fraction ≥25% had a sensitivity of 100% and specificity of 60% for differentiating patients with relapse from those without relapse. CD3+CD56+ fraction <5% had a sensitivity of 100% and specificity of 60% for differentiating patients with relapse.

**Discussion**

Comprehensive flow cytometry analysis revealed that the composition of T lymphocytes is different in various aspects between PBMCs, lymphocytes from the less-inflamed mucosa (colon), and lymphocytes from inflamed mucosa (rectum) in patients with ulcerative colitis (Figure 1). The present results reinforce that sample cell sources for flow cytometric analysis should be selected cautiously in research studies on ulcerative colitis. We consider that isolation of lymphocytes from the rectal mucosa and its use for flow cytometric analysis is the most desirable to discover essential features involved in the pathogenesis of ulcerative colitis.

In this study, we performed flow cytometric analysis of colorectal lymphocytes and PBMCs, primarily to reveal
distinctive features of the rectal lymphocyte composition in ulcerative colitis patients, by comparing the rectum samples of ulcerative colitis patients to those of control patients. The results showed that CD62L+/CD3+CD4+ and CD62L+/CD3+CD4− cells were increased and further subpopulation analysis revealed that the CD45RA−CD62L+/CD3+CD4+ fraction was increased in the rectum of ulcerative colitis patients compared to those of control patients. Additionally, we found a trend toward an increased ratio of CD45RA−CD62L+/CD3+CD4− in ulcerative colitis patients compared to control patients. Furthermore, CD45RA−CD62L+/CD3+CD4− and CD45RA−CD62L+/CD3−CD4+ fractions tended toward being increased in more inflamed rectal mucosa (MES 1 and 2) than in the rectal mucosa in remission (MES 0). Based on these results, we speculate that central memory T lymphocytes (CD45RA−CD62L−/CD3+ cells) are involved in the pathogenesis of ulcerative colitis.

Central memory T cells express the adhesion molecule CD62L, also known as L-selectin or leukocyte adhesion molecule 1,12 and mainly reside in the T cell areas of the secondary lymphoid organs. In response to antigenic

**Figure 8.** Subpopulation analysis based on CD45RA and CD62L expression comparing the rectum-resident lymphocytes between ulcerative colitis patients with Mayo endoscopic subscore (MES) of 0 and those of MES 1 or 2. CD45RA−CD62L− cells represent effector memory T cells, CD45RA−CD62L+ cells represent central memory T cells, and CD45RA−CD62L− cells represent naive T cells. †, p < 0.01 (unpaired t-test).

**Figure 9.** Flow cytometry analysis of rectum samples from ulcerative colitis patients with relapse within 6 months after colonoscopy and those without relapse. *, p < 0.05 (unpaired t-test).
stimulation, these cells produce IL-2, rapidly proliferate, and efficiently differentiate into effector cells after proliferation. Long-term maintenance of the memory T-cell response is the hallmark of immune protection, allowing the activation of a rapid response against re-exposure to the pathogen. Horjus Talabur Horje et al. investigated T lymphocyte subsets in patients with inflammatory bowel disease and defined the CD45RA+CD27+ population as naïve T cells and CD45RA−CD27+ as central memory T cells. Patients with inflammatory bowel disease had a higher frequency of central memory cells than control patients (42% in ulcerative colitis and 37% in Crohn’s disease vs. 25% in the control group). In addition, naïve T cell subsets were larger in ulcerative colitis (28%) and Crohn’s disease (31%) than in controls (19%). Furthermore, patients with endoscopic active inflammatory bowel disease had increased percentages of central memory T cells compared to those in healthy controls and patients with endoscopic inactive inflammatory bowel disease. Moreover, high endothelial venules, which express specific adhesion molecules named addressins that attach to CD62L and anchor lymphocytes, were increased in the inflamed colon of patients with ulcerative colitis compared to those in healthy controls. An increased number of colonic high endothelial venules was associated with enhanced infiltration of naïve T cells and central memory T cells in the inflamed gut, compared to the low density of colonic high endothelial venules in control patients. These results support our speculation of central memory T cells possibly contributing to the development and worsening of ulcerative colitis.

PBMCs of ulcerative colitis showed decreased HLA DR+CD3−, while CD45RA+/CD3+CD4+ cells were increased, compared to PBMCs of control patients. As HLA DR is a marker of T lymphocyte activation, the number of cytotoxic T lymphocytes expressing HLA DR is known to increase in autoimmune diseases. CD4+CD45RA+ cells were defined as suppressor-inducers of T lymphocytes. Suppressor-inducer T cells induce cytotoxic T cells to become suppressor cells, that is, Treg cells, which in turn reduce inflammation. Therefore, the results of our study imply that inflammatory reactions in circulating T-cell populations may be suppressed in ulcerative colitis patients. As most of the patients had been taking 5-aminosalicylic acid and/or immunomodulatory drugs, such as azathioprine and mercaptopurine, these drugs might have affected the circulating T-cell compositions.

We also demonstrated that, CD56+/CD3+ in the rectum is lower than that in the colon in ulcerative colitis patients. In addition, MES 1 and 2 patients had a lower ratio of CD56+/CD3+ in the rectum, compared to MES 0 patients. This is relevant because CD3+CD56− cells are known as NKT cells, which share properties of both T cells and natural killer cells. NKT cells possess multiple functions, including enhancement of microbial and tumor immunity, suppression of autoimmune disease, and promotion of tolerance. For instance, NKT cell deficiencies have been reported in systemic lupus erythematosus mouse models. A decreased number of NKT cells has also been described in peripheral blood from patients with autoimmune diseases, such as rheumatoid arthritis, systemic sclerosis, type 1 diabetes mellitus, and systemic lupus erythematosus. In ulcerative colitis patients, a selective decrease in colonic CD56+ T lymphocytes in the inflamed mucosa has been reported. Taken together, our results reinforce that gut inflammation in ulcerative colitis patients is inversely correlated with the number of NKT cells.

A comparison between control (patients with colon polyp) and patients with ulcerative colitis with low or high disease activity revealed that the CD56+/CD3+ ratio was lower in the rectum of patients with MES 1 and 2 ulcerative colitis than in the rectum of control (p = 0.03); however, there was no difference between patients with MES 0 ulcerative colitis and control. Similarly, the CD45RA−CD62L+CD3+CD4+ ratio was higher in the rectum of patients with MES 1 and 2 ulcerative colitis than in the rectum of controls (p < 0.01); however, no difference was observed between patients with MES 0 ulcerative colitis and controls. Our results complement the findings of Smids et al., who reported that intestinal T lymphocyte subsets of inactive ulcerative colitis were partly comparable to those of healthy controls. In contrast, the CD45RA−CD62L+CD3+CD4+ ratio was higher in the rectum of patients with MES 1 and 2 (p < 0.01) and MES 0 (p < 0.05) than in the rectum of controls. These memory cells may exist in a functionally quiescent state in inactive disease states and rapidly expand the effector functions on antigen stimulation.

A comparison between the relapse and non-relapse groups revealed that the ratio of central memory CD4+ T cells and central memory CD4− T cells was higher and the ratio of NKT cells was lower in the rectum samples of patients who relapsed, as assessed from flow cytometry. Thus, these fractions may be potential biomarkers for predicting future relapse in patients with UC. However, as the relapse group consisted of MES 1 and 2 patients, underlying inflammation in this group might have affected the results. Future studies incorporating a larger number of patients and their clinical courses may reveal the key to flow cytometry results to predict relapse of this disease.

Our study had several limitations. First, all ulcerative colitis patients had been diagnosed prior to enrollment in this study and had been taking aminosalicylates and/or immunosuppressive drugs. Although ultimate ideal objects are treatment-naïve ulcerative colitis patients, such studies are difficult to conduct, considering the low incidence of this disease. Therefore, medications used in our study groups might have affected lymphocyte composition. The second limitation is the small number of participants.
However, we found several significant differences in lymphocyte composition despite the small sample size. Third, diet and other lifestyle factors can affect the gut microbiota, which may in turn alter the immune system of the host. However, except smoking, no other lifestyle factors were included in this study. Fourth, although flow cytometry analyzed T lymphocyte markers using a panel of 13 commercially available antibodies, other markers may be involved in the pathogenesis of this disease. The tissue-resident memory T cells that mediate the local immune protection against infections and cancer have attracted attention. Dendritic cells, which are responsible for the initiation of adaptive immune responses, have also been implicated in pathogenesis of ulcerative colitis based on genetics and their central role in controlling microbial interactions. Therefore, further research is needed to reveal the role of these lymphocytes in ulcerative colitis.

Conclusions

Our study showed that CD62L+ T lymphocytes, particularly the CD45RA−CD62L+ T cell subset, were increased in the rectum of patients with ulcerative colitis. In addition, CD56+/CD3+ was lower in the rectum than in the less-inflamed mucosa. These results suggest that the enrichment of central memory T lymphocytes and depletion of NKT cells in the gut mucosa are involved in the pathogenesis of ulcerative colitis. These findings offer potentially useful information for this patient population. Further investigations with a greater number of patients, including treatment-naïve individuals, will reveal the true nature of colorectal lymphocytes in ulcerative colitis.

Authors’ contributions

MI designed and performed the research, interpreted the data, and wrote the manuscript; T Takahashi and NW contributed to the flow cytometry analysis; KO, T Tanaka, TI, SH, and FO substantively revised the manuscript; and HO supervised the research. All authors have read and approved the final manuscript.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval

Written informed consent was obtained from all participants. This study adhered to the principles of the Declaration of Helsinki and was approved by the ethics committee of our institution (approval number: 2003-011 and 2009-014). The study protocol was registered in the UMIN Clinical Trials Registry (UMIN000039868).

Consent for publication

All authors consented to the publication of the manuscript.

References

1. Khan I, Ullah N, Zha L, et al. (2019) Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): cause or consequence? IBD treatment targeting the gut biome. *Pathogens* 8(3): 126.
2. Yu LC (2018) Microbiota dysbiosis and barrier dysfunction in inflammatory bowel disease and colorectal cancers: exploring a common ground hypothesis. *Journal of Biomedical Science* 25(1): 79.
3. Corridoni D, Antanaviciute A, Gupta T, et al. (2020) Single-cell atlas of colonic CD8+ T cells in ulcerative colitis. *Nature Medicine* 26(9): 1480–1490.
4. Crothers JW, Chu ND, Nguyen LTT, et al. (2021) Daily oral FMT for long-term maintenance therapy in ulcerative colitis: results of a single-center, prospective, randomized pilot study. *BMC Gastroenterology* 21(1): 281.
5. Horjus Talabur Horje CS, Middendorp S, van Koolwijk E, et al. (2014) Naive T cells in the gut of newly diagnosed, untreated adult patients with inflammatory bowel disease. *Inflammatory Bowel Disease* 20(11): 1902–1909.
6. Horjus Talabur Horje CS, Smids C, Meijer JW, et al. (2017) High endothelial venules associated with T cell subsets in the inflamed gut of newly diagnosed inflammatory bowel disease patients. *Clinical and Experimental Immunology* 188(1): 163–173.
7. Hegazy AN, West NR, Stubbington MJT, et al. (2017) Circulating and tissue-resident CD4+ T cells with reactivity to intestinal microbiota are abundant in healthy individuals and function is altered during inflammation. *Gastroenterology* 153(5): 1320–1337.
8. Schroeder KW, Tremaire WJ and Istrup DM (1987) Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *The New England Journal of Medicine* 317(26): 1625–1629.
9. Iwamuro M, Takahashi T, Watanabe N, et al. (2020) Technique for single-step lymphocyte isolation from an endoscopic biopsy specimen for the diagnosis of gastrointestinal lymphoma. *Methods (Orlando)* 7: 101095.
10. Iwamuro M, Matsueda K, Takahashi T, et al. (2020) An endoscopic biopsy specimen contains adequate lymphocytes for flow cytometric analysis of light chain expression in the gastrointestinal mucosa. *Annals of Clinical & Laboratory Science* 50(3): 348–353.

11. Matsueda K, Iwamuro M, Takahashi T, et al. (2019) Feasibility of flow cytometric analysis of restricted light chain in endoscopic biopsy specimens from patients with gastrointestinal tract B cell lymphoma: a pilot study. *BMC Research Notes* 12(1): 571.

12. Ivetic A, Hoskins Green HL and Hart SJ (2019) L-selectin: a major regulator of leukocyte adhesion, migration and signaling. *Frontiers in Immunology* 10: 1068.

13. Smids C, Talabur Horje HCS, Drylewicz J, et al. (2018) Intestinal T cell profiling in inflammatory bowel disease: linking T cell subsets to disease activity and disease course. *Journal of Crohns and Colitis* 12(4): 465–475.

14. Saraiva DP, Jacinto A, Borralho P, et al. (2018) HLA-DR in cytotoxic T lymphocytes predicts breast cancer patients’ response to neoadjuvant chemotherapy. *Frontiers in Immunology* 9: 2605.

15. Cho YN, Kee SJ, Lee SJ, et al. (2011) Numerical and functional deficiencies of natural killer T cells in systemic lupus erythematosus: their deficiency related to disease activity. *Rheumatology* 50(6): 1054–1063.

16. Shimamoto M, Ueno Y, Tanaka S, et al. (2007) Selective decrease in colonic CD56(+) T and CD161(+) T cells in the inflamed mucosa of patients with ulcerative colitis. *World Journal of Gastroenterology* 13(45): 5995–6002.