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Mucosal Cytokine Profiles Across Different Therapies in Ulcerative Colitis During Endoscopic Remission

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Research Article

Keywords: Colitis, Ulcerative, Cytokines, Ustekinumab, Interleukin-17, Biopsy

Posted Date: November 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11602219/v1

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Additional Declarations: Competing interest reported. Kazuhiko Uchiyama received collaborative research funds and lecture fees from Janssen Pharmaceutical K.K. Tomohisa Takagi received collaborative research funds from PreMedica Inc., and lecture fees from Mochida Pharma Co. Ltd., Janssen Pharmaceutical K.K., EA Pharma Co. Ltd., Takeda Pharmaceutical Co. Ltd, AbbVie GK, Bristol-Myers Squibb, and Pfizer Japan Inc. Yuji Naito received scholarship funds from EA Pharma Co. Ltd., a collaborative research fund from Taiyo Kagaku Co., Ltd., and lecture fees from Mylan EPD Co., Takeda Pharma Co., Ltd., Mochida Pharma Co. Ltd., EA Pharma Co. Ltd., Otsuka Pharma Co. Ltd., and Miyarisan Pharma Co. Ltd. The other authors declare no conflicts of interest.

Abstract

Background

Although advanced therapies for ulcerative colitis target distinct immune pathways, it remains unclear whether these agents differentially modulate mucosal cytokine expression in patients with deep mucosal healing.

Aims

To investigate mucosal cytokine profiles across colonic segments and treatments in patients with ulcerative colitis in endoscopic remission.

Methods

This observational study included 113 patients with ulcerative colitis in clinical and endoscopic remission (Mayo endoscopic subscore of 0). Biopsy specimens from the rectum, sigmoid colon, and cecum were analyzed by reverse transcription quantitative polymerase chain reaction for mRNA expression of 21 cytokines. Expression levels were compared across six treatment groups: 5-aminosalicylic acid, azathioprine, anti-tumor necrosis factor-α antibodies, vedolizumab, ustekinumab, and tofacitinib. Global cytokine patterns were evaluated using heatmaps and principal component analysis.

Results

Of the 21 cytokines analyzed, only IL-8 and IL-17A showed treatment-related differences. In rectal biopsy specimens, IL-8 expression was lower in the anti-tumor necrosis factor-α group than in the vedolizumab and tofacitinib groups, whereas IL-17A expression was lower in the ustekinumab group than in all other treatment groups. In the sigmoid colon, IL-17A expression was also lower in the ustekinumab group, whereas no significant treatment-related differences were observed in the cecum.

Conclusions

Among patients with ulcerative colitis in deep endoscopic remission, mucosal cytokine expression remains heterogeneous and is largely comparable across different therapeutic agents. IL-17A expression was selectively reduced in patients treated with ustekinumab, consistent with IL-23/Th17 pathway inhibition. Endoscopic healing may not necessarily reflect immunological uniformity in the colonic mucosa.

Trial registration:

UMIN000017990, registered June 20, 2015.

Introduction

Ulcerative colitis (UC), a chronic inflammatory bowel disease, is characterized by relapsing and remitting inflammation of the colonic mucosa[1]. Although advances in therapeutic strategies have improved clinical outcomes, UC remains clinically and biologically heterogeneous, with substantial variability in disease course, treatment response, and long-term prognosis[2]. Using pharmacological agents such as aminosalicylates, corticosteroids, immunomodulators, and biologics, current treatment strategies aim to induce and maintain remission. In recent years, therapeutic targets have evolved beyond symptom control toward deeper endpoints, including endoscopic and histological remission[3, 4]. Nevertheless, a proportion of patients experience relapse despite achieving clinical or endoscopic remission. Therefore, residual immunological activity may persist within the intestinal mucosa during the quiescent phase of the disease.

Evidence increasingly indicates that UC has considerable molecular and immunological heterogeneity. Differences in mucosal immune activation, cytokine signaling pathways, and cellular immune responses may contribute to variability in disease behavior and therapeutic outcomes observed among patients[5, 6]. The underlying mucosal immune environment may differ substantially even among individuals meeting conventional clinical or endoscopic criteria for remission. Such differences may reflect persistent subclinical immune activation or distinct patterns of immune regulation not captured by routine clinical assessments. Understanding this immunological diversity is increasingly recognized as an important step toward improving disease stratification and enabling more personalized therapeutic approaches.

Cytokines are key in regulating intestinal immune responses and maintaining mucosal homeostasis[7–13]. In UC, dysregulated cytokine networks contribute to epithelial barrier dysfunction, immune-cell recruitment and activation, and amplification of inflammatory responses. Previous studies have demonstrated that mucosal cytokine expression patterns correlate with disease activity and may reflect the immunological processes occurring within the intestinal mucosa[9]. Notably, mucosal cytokine expression has been reported to predict therapeutic efficacy in patients treated with anti-tumor necrosis factor (TNF) α antibodies[14–16]. Furthermore, several studies have suggested that cytokine expression profiles in the intestinal mucosa may predict subsequent clinical relapse, highlighting the potential prognostic value of local immune signatures in UC[17–19]. Nonetheless, although cytokine alterations during active inflammation have been extensively investigated, the cytokine milieu of the colonic mucosa during remission remains incompletely characterized. In addition, pharmacological agents used to treat UC exert therapeutic effects through distinct mechanisms of immune modulation. Anti-TNF antibodies inhibit TNF-mediated inflammatory signaling, whereas other therapeutic agents modulate immune responses through alternative pathways, including the regulation of lymphocyte trafficking or

intracellular cytokine signaling networks. These mechanistic differences suggest that the mucosal immune environment during remission may differ by therapeutic regimen. However, to our knowledge, no study has systematically evaluated whether different pharmacological treatments in patients with quiescent UC are associated with distinct cytokine expression profiles in the colonic mucosa.

We therefore hypothesized that, during remission in UC, mucosal cytokine expression profiles differ according to the ongoing pharmacological therapy. This study aimed to investigate whether distinct cytokine expression patterns in the colonic mucosa of patients with quiescent UC are associated with different pharmacological treatments. We sought, by characterizing mucosal cytokine expression across different treatment regimens, to clarify the immunological heterogeneity of UC during remission and to explore whether treatment-associated immune signatures can be identified within the colonic mucosa.

Materials and Methods

Ethics

The study protocol was approved by the Ethics Committee of Kyoto Prefectural University of Medicine (approval No. ERB-C-464-8). All patients provided written informed consent before undergoing the study procedures. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN000017990) on June 20, 2015.

Patients

Patients with UC who were in clinical and endoscopic remission and had maintained remission for at least one year both before and after the index endoscopy were included. Endoscopic remission was defined as a Mayo endoscopic subscore (MES) of 0. Patients were enrolled from April 1, 2016, to February 28, 2024, and followed at the gastroenterology outpatient clinic of the study institution. A total of 313 patients with UC in clinical remission provided informed consent for this study. At the time of biopsy, patients were excluded if they met any of the following criteria: (1) a MES of 1–3 at sampling; (2) an MES of 0 with clinical relapse within 12 months after sampling; (3) an MES of 0 with less than 12 months of follow-up after the index endoscopy; or (4) treatment with azathioprine in combination with biologics or small-molecule agents. After applying these criteria, 113 patients were included in the final analysis and classified into six groups. The 5-aminosalicylic acid (5-ASA) group included patients receiving oral 5-ASA only. The azathioprine (AZA) group comprised patients receiving AZA, with or without concomitant 5-ASA, who were not receiving advanced therapy. The anti-TNFα antibody, vedolizumab (VDZ), ustekinumab (UST), and tofacitinib (TOF) groups comprised patients treated with anti-TNFα antibody, VDZ, UST, and TOF, respectively, with or without concomitant 5-ASA.

Assessment of disease activity

Clinical activity was assessed using the partial Mayo score (pMayo score) [20], and endoscopic activity was assessed using the MES. All enrolled patients were in clinical remission, defined as a pMayo score of 2 or below, and in endoscopic remission (MES of 0).

Sample collection

For each patient, two biopsies were obtained from each of the cecum, sigmoid colon, and rectum to assess histologic activity and cytokine mRNA expression. Within 15 s of collection, the biopsy tissue was placed in an RNase inhibitor-containing reagent and stored at − 80°C.

Histological assessment

An expert pathologist used the Geboes Score (GS) to assess histological inflammation in the biopsy specimens [21]. Biopsies were collected from the same sites assessed using MES, and the GS was used to grade histological inflammation from 0 to 3.

Definition of relapse

Relapse of UC was defined as worsening clinical symptoms and/or endoscopic findings requiring modification of medical therapy. Clinical activity was assessed using the partial Mayo score, whereas endoscopic activity was assessed using the MES. Relapse was considered to have occurred when treatment was intensified because of worsening clinical symptoms and/or endoscopic activity, including initiation of a new medication or escalation or addition of existing therapy.

mRNA analysis

Reverse transcription quantitative polymerase chain reaction (RT-PCR) was used to determine the mRNA expression levels of human colonic mucosal cytokines in biopsy samples from patients with UC. Total RNA was isolated from human biopsy samples using the acid guanidinium phenol-chloroform method with an ISOGEN kit (Nippon Gene, Tokyo, Japan). RNA concentration was determined from the absorbance ratio at 260 and 280 nm (A260/280), and the isolated RNA was stored at − 80°C until RT-PCR. Extracted RNA (1 μg) was reverse-transcribed into cDNA at 42°C for 40 min using 100 U/mL reverse transcriptase (Takara Bio Inc., Shiga, Japan) and 0.1 μM oligo (dT)-adapter primer (Takara Bio Inc.) in a 50 μL reaction mixture. Human cytokine RT-PCR was performed using the 7300 RT-PCR system and SYBR Green I DNA-binding dye to detect PCR products. The reaction mixture (RT-PCR kit, Code RRO43A; Takara Bio Inc.) contained 12.5 μL of Premix Ex Taq, 2.5 μL of SYBR Green I, custom-synthesized primers, ROX reference dye, and cDNA (equivalent to 20 ng total RNA), in a final reaction volume of 25 μL. PCR began with an initial denaturation step of 15 s at 95°C, followed by 40 amplification cycles of 3 s at 95°C and 31 s at 60°C. Melting-curve analysis followed, with the temperature increasing from 60°C to 95°C. A total of 21 cytokine mRNAs reported to be related to the

pathogenesis of UC (TNFα, interferonγ, interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17A, IL-17F, IL-18, IL-21, IL-22, IL-23, IL-27, and IL-33) were quantified in biopsy specimens; primer sequences are shown in Supplementary Table 1. GAPDH served as the internal control. Analysis was performed according to a previously reported method[22]. Based on our previous observation that mucosal cytokine expression was generally higher in the rectum than in the sigmoid colon and cecum [19], rectal biopsy specimens were used for the primary cytokine expression analyses; sigmoid colon and cecal specimens were used for supplementary analyses.

Statistical analysis

Continuous variables are presented as the mean ± standard deviation for normally distributed data or as the median and interquartile range (IQR) for non-normally distributed data. The Mann–Whitney U test was used to compare mucosal cytokine expression between two independent groups, including comparisons according to histological activity. The Kruskal–Wallis test was used to compare mucosal cytokine expression among the six treatment groups and among the three colonic segments (cecum, sigmoid colon, and rectum) within each treatment group. When significant differences were identified, post hoc pairwise comparisons were performed using Dunn's test with Bonferroni correction.

Relapse-free survival was estimated using the Kaplan–Meier method. Heatmaps were generated using JMP Pro version 14.0.0 (SAS Institute Inc., Cary, NC, USA). Principal component analysis (PCA) was performed using ClustVis to visualize variation in mucosal cytokine expression profiles among treatment groups. Data were mean-centered and scaled to unit variance before PCA using the default settings of ClustVis[23]. All statistical analyses, except PCA, were performed using JMP Pro version 14.0.0. A two-sided P value < 0.05 was considered statistically significant.

Results

Patients' baseline demographic characteristics

Of the 113 patients included in this study, 45 (39.8%) received 5-ASA, 13 (11.5%) AZA, 12 (10.6%) an anti-TNFα antibody, 18 (15.9%) vedolizumab, 20 (17.7%) ustekinumab, and 5 (4.4%) tofacitinib (Fig. 1). Table 1 summarizes the enrolled patients' characteristics. The groups did not differ in clinical characteristics, such as sex, age, disease duration, or disease extent.

Table 1
Baseline characteristics of the study population.

Group		5-ASA	AZA	anti-TNFα	VDZ	UST	TOF
Total number		45	13	12	18	20	5
Sex (female / male)		25 / 20	5 / 8	6 / 6	6 / 12	8 / 12	0 / 5
Age (years)		56.1 ± 13.6	57.6 ± 15.2	45.8 ± 16.3	44.2 ± 19.4	46.9 ± 14.3	62.6 ± 12.9
Disease duration (months)		147 ± 115	150 ± 90	137 ± 148	94.5 ± 92.8	136 ± 102	53 ± 37
Disease location (%)							
	Extensive	30 (66.7)	11 (84.6)	11 (91.7)	14 (77.8)	17 (85.0)	4 (80.0)
	Left-sided	9 (20.0)	2 (15.4)	1 (8.3)	4 (22.2)	3 (15.0)	1 (20.0)
	Proctitis	6 (13.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Current medication (%)							
5-Aminosalicylates		45 (100)	10 (76.9)	9 (75.0)	9 (50.0)	12 (60.0)	5 (100)
Azathioprine		0 (0)	13 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Advanced therapy	IFX	0 (0.0)	0 (0.0)	6 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
	ADA	0 (0.0)	0 (0.0)	5 (41.7)	0 (0.0)	0 (0.0)	0 (0.0)
	GLM	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)
	VDZ	0 (0.0)	0 (0.0)	0 (0.0)	18 (100)	0 (0.0)	0 (0.0)
	UST	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (100)	0 (0.0)
	TOF	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (100)

Histological activity and cytokine expression

Supplementary Table 2 presents the expression of various cytokines in the rectal mucosa in relation to histological activity. This study included patients in endoscopic remission (MES 0); histological activity was present in only 10 patients. No cytokine showed increased expression in relation to histological activity.

Site-specific cytokine expression by therapeutic agent

Cytokine expression at different colonic sites for each therapeutic agent is shown in the Supplementary tables 3–8. In the 5-ASA group, expression of IL-1β, IL-8, and IL-33 was higher in the rectum, but expression of the other cytokines did not differ significantly by site (Supplementary Table 3). In the AZA group, IL-8 expression was higher in the rectum, but expression of the other cytokines did not differ

significantly by site (Supplementary Table 4). The other groups showed no differences in cytokine expression across colonic sites (Supplementary Tables 5–8).

Mucosal cytokine profiles in rectal biopsies: an overview by treatment

Our previous finding that mucosal cytokine expression was generally higher in the rectum than in the sigmoid colon and cecum informed our decision to focus the main analyses on rectal biopsy specimens[19]. Figure 2 summarizes the expression profiles of 21 cytokine-related genes in rectal biopsy specimens across the six treatment groups (5-ASA, AZA, anti-TNF, VDZ, UST, and TOF). Within each treatment group, heatmap analysis showed heterogeneous cytokine expression patterns among individual patients (Fig. 2a). All patients had achieved endoscopic remission (MES 0), yet substantial interindividual variability in mucosal cytokine expression remained. Nonetheless, overall cytokine expression patterns were broadly comparable across treatment groups, and no distinct cytokine signature clearly distinguished one group from another. As in the heatmap analysis, PCA showed substantial overlap among the six treatment groups, with no clear clustering or separation by treatment class (Fig. 2b). Thus, rectal mucosal cytokine expression was heterogeneous despite endoscopic remission, and treatment class did not generate a clearly segregated cytokine expression profile in unsupervised analyses. Nevertheless, treatment-related differences became apparent upon separate examination of individual cytokines. Similar patterns were observed in biopsy specimens from the sigmoid colon and cecum, where cytokine expression also showed substantial interindividual heterogeneity without clear clustering or separation according to treatment class (Supplementary Fig. 1).

Treatment-related differences in mucosal cytokine expression across colonic segments

We next compared the expression of individual cytokine genes across the six treatment groups. Among the 21 cytokine-related genes analyzed in rectal biopsy specimens, IL-8 and IL-17A differed significantly among treatment groups. IL-8 expression was significantly lower in the anti-TNF group than in the VDZ and TOF groups (Fig. 3a). Conversely, IL-17A expression was significantly lower in the UST group than in all other treatment groups, including the 5-ASA, AZA, anti-TNF, VDZ, and TOF groups (Fig. 3b). Figure 4 shows expression levels of the remaining 19 cytokine-related genes in rectal biopsy specimens; none differed significantly among treatment groups. We further examined the expression of all 21 cytokine-related genes in sigmoid colon and cecal biopsy specimens (Supplementary Figs. 2 and 3, respectively). In the sigmoid colon, IL-17A expression was significantly lower in the UST group than in the 5-ASA, VDZ, and TOF groups, whereas no significant differences were observed for the other 20 cytokine-related genes (Supplementary Fig. 2n). In cecal biopsy specimens, no significant differences were observed among the six treatment groups for any of the 21 cytokine-related genes (Supplementary Fig. 3). Thus, treatment-related differences in mucosal cytokine expression were most evident in the rectum, particularly for IL-8 and IL-17A, whereas IL-17A also showed treatment-related differences in the sigmoid colon.

Association between mucosal cytokine profiles and subsequent clinical relapse

We next examined how mucosal cytokine profiles were associated with subsequent clinical relapse. A subset of patients relapsed during long-term follow-up (> 12 months) after biopsy sampling, as shown by the relapse-free survival curves in Fig. 5a. Figure 5b shows the cytokine expression heatmap annotated with relapse status; red triangles below the heatmap indicate patients who subsequently relapsed. Notably, patients who relapsed did not necessarily show higher cytokine expression at the time of biopsy sampling. These findings suggest that mucosal cytokine profiles at the time of endoscopic remission were not sufficient to predict subsequent relapse in all cases. One possible explanation is that mucosal cytokine expression patterns may change dynamically between biopsy sampling and clinical relapse.

Table 2 summarizes relapse outcomes by treatment group: relapse occurred in 0–33.3% of patients, with no clear trend favoring a particular therapy. The median interval to relapse varied by treatment group, ranging from several months to several years. Collectively, these observations suggest that endoscopic remission does not necessarily represent stable immunological quiescence.

Table 2
Clinical relapse outcomes according to maintenance therapy.

Patients with maintained remission, n (%)	**5-ASA**	**AZA**	**anti-TNFα**	**VDZ**	**UST**	**TOF**
	41 (91.1)	**11 (84.6)**	**8 (66.7)**	**17 (94.4)**	**17 (85.0)**	**5 (100)**
Patients with relapse, n (%)	4 (8.9)	2 (15.4)	4 (33.3)	1 (5.6)	3 (15.0)	0 (0)
Follow-up duration in patients with maintained remission (months) Median (IQR)	51 (31–81)	57 (32–81)	70 (22.5–86.5)	26 (16–43.5)	29 (15.5–40)	57 (36.5–67.5)
Follow-up duration among patients who experienced relapse (months) Median (IQR)	42.5 (34.5–59)	38.5 (31–46)	32 (18.5–54.5)	51	19 (16–33)	NA

Discussion

This study demonstrates that mucosal cytokine profiles remain heterogeneous among patients with UC who have achieved deep endoscopic remission (MES 0), despite treatment with different therapeutic agents. Although IL-17A and IL-8 exhibited treatment-related differences, most cytokines were comparable across therapies. Therefore, endoscopic remission may not necessarily represent complete immunological quiescence at the mucosal level. Specifically, IL-17A expression was consistently

reduced in the UST group compared with the other therapies, whereas IL-8 demonstrated variable treatment-related differences without a consistent therapy-specific pattern. When cytokine expression was compared across different colonic segments, only limited regional variation was observed, and apart from IL-8 and IL-17A, no consistent site-specific differences in cytokine expression were detected across the colon. To our knowledge, few studies have directly compared mucosal cytokine expression profiles across different therapeutic agents in patients with UC with deep remission. Because currently available therapies for refractory UC target distinct inflammatory pathways, it could be hypothesized that mucosal cytokine expression profiles would vary substantially among treatment groups. Nonetheless, our findings suggest that once deep mucosal healing is achieved, the overall mucosal cytokine environment becomes broadly similar across treatment classes, even though certain cytokines may still be selectively influenced by specific therapies.

The reduction in IL-17A observed in the UST group is biologically plausible in the context of Th17 signaling. IL-1β and IL-6 generally promote the differentiation of Th17 cells from $CD4^+$ naïve T cells, whereas TGF-β and IL-23 contribute to their expansion, maintenance, and survival [24, 25]. The balance between IL-23 and TGF-β also influences Th17 cell pathogenicity [26, 27]. Activated Th17 cells produce cytokines of the IL-17 family, including IL-17A and IL-17F [28]. These cytokines are elevated in the intestinal mucosa of patients with UC and have been implicated in mucosal inflammation and tissue injury [9, 29]. However, accumulating evidence indicates that Th17 responses may also exert protective functions in the intestinal mucosa, such as promoting epithelial barrier integrity and antimicrobial peptide production [30–32].

Activated Th17 cells differentiate into subpopulations expressing IL-17A, IL-17A/F, or IL-17F. Several factors influence this differentiation, including the cytokine environment, strength of antigen-induced signaling through the T-cell receptor, and the duration of stimulation [33, 34]. IL-17A is typically produced rapidly following T-cell activation, whereas IL-17F expression increases more gradually during later phases of immune activation [35]. Unlike IL-17F expression, IL-17A expression is not sustained during prolonged T-cell activation. This difference suggests that IL-17A may play a more prominent role during the early phase of inflammation, whereas IL-17F may be more relevant during chronic inflammatory responses. Consistent with this concept, previous studies have reported that targeting IL-17A alone may not provide sustained disease control [36–40].

Here, mucosal IL-17A expression was significantly lower in patients treated with UST than in those treated with other therapies, whereas IL-17F expression was comparable among treatment groups. Although the mechanisms underlying this difference remain unclear, the study population's relatively quiescent inflammatory state may partially explain the finding: all patients achieved complete endoscopic mucosal healing, and only a limited proportion showed histological activity. Because UST targets the p40 subunit shared by IL-12 and IL-23, inhibition of IL-23 signaling may suppress pathogenic Th17 responses and consequently reduce IL-17A production. These findings are consistent with the mechanism of action of UST and suggest that IL-23-Th17 signaling may remain partially active even in patients with endoscopic remission.

Importantly, our findings also indicate that mucosal immune activity may persist even in patients with deep endoscopic remission. Among patients with MES 0, heterogeneity in cytokine expression was observed, suggesting that endoscopic healing alone may not fully reflect the immunological state of the intestinal mucosa. To our knowledge, only limited data are available on mucosal cytokine profiles in patients with UC with deep endoscopic remission. Direct comparisons across different therapeutic agents have rarely been reported. These findings highlight the complexity of mucosal immune regulation in UC and suggest that immunological heterogeneity may persist even when macroscopic inflammation has resolved.

Interestingly, the relapse analysis in this study provides additional insight into the relationship between mucosal immune activity and disease recurrence. Several previous studies have reported associations between mucosal cytokine expression and subsequent relapse in UC. Nonetheless, our study design differed from those used in previous studies in several important respects. First, we restricted our analysis to patients with deep endoscopic remission (MES 0), a population with minimal residual macroscopic inflammation. Second, we excluded patients who relapsed within 12 months after biopsy sampling, ensuring the cohort represented a relatively stable remission phase at the time of sampling. Third, the relapse events analyzed in this study occurred more than 12 months after biopsy sampling, reflecting longer-term disease outcomes. Under these conditions, patients who subsequently relapsed did not necessarily show elevated mucosal cytokine expression at the time of biopsy sampling. These findings suggest that mucosal cytokine expression measured at a single time point during mucosal healing may still provide meaningful information about mucosal immune status, but may be less predictive of long-term relapse occurring more than one year later.

The study had several limitations. First, it was conducted at a single center. However, this allowed the biopsy collection procedure to be standardized, thereby minimizing potential variability in sample collection. Second, the number of patients was not balanced across treatment groups, with a particularly small number of patients in the TOF group. Because this was a real-world observational study, patient recruitment reflected actual treatment patterns in clinical practice, resulting in unequal sample sizes across treatment groups. In addition, given the exploratory nature of the study, no formal sample size calculation was performed a priori. Therefore, the relatively small and unequal sample sizes may have limited the statistical power to detect treatment-related differences in mucosal cytokine expression, particularly in the smaller treatment groups. Third, the number of biopsies was limited to minimize the invasiveness of sampling; consequently, cytokine protein expression could not be examined. Finally, because selective IL-23p19 inhibitors have only recently been introduced into clinical practice and few patients met the eligibility criteria, these agents could not be included in the present analysis.

In conclusion, this study demonstrates that mucosal cytokine expression remains heterogeneous among patients with UC who have achieved deep endoscopic remission. Overall mucosal cytokine profiles were broadly comparable despite different therapeutic agents, although IL-17A expression was specifically reduced in patients treated with ustekinumab. These findings suggest that endoscopic healing does not necessarily indicate complete immunological remission at the mucosal level. Further studies are

warranted to clarify both how mucosal immune heterogeneity during endoscopic remission influences long-term disease outcomes and whether immunological markers may help refine therapeutic targets and monitoring strategies in UC.

Declarations

Competing Interests

Kazuhiko Uchiyama received collaborative research funds and lecture fees from Janssen Pharmaceutical K.K. Tomohisa Takagi received collaborative research funds from PreMedica Inc., and lecture fees from Mochida Pharma Co. Ltd., Janssen Pharmaceutical K.K., EA Pharma Co. Ltd., Takeda Pharmaceutical Co. Ltd, AbbVie GK, Bristol-Myers Squibb, and Pfizer Japan Inc. Yuji Naito received scholarship funds from EA Pharma Co. Ltd., a collaborative research fund from Taiyo Kagaku Co., Ltd., and lecture fees from Mylan EPD Co., Takeda Pharma Co., Ltd., Mochida Pharma Co. Ltd., EA Pharma Co. Ltd., Otsuka Pharma Co. Ltd., and Miyarisan Pharma Co. Ltd. The other authors declare no conflicts of interest.

Ethics approval

The study was approved by the Ethics Committee of Kyoto Prefectural University of Medicine (approval No. ERB-C-464-8). The study was conducted in accordance with the Declaration of Helsinki.

Consent to participate

Written informed consent was obtained from all individual participants included in the study.

Funding

This work was supported by the MAFF-commissioned “Project for the realization of foods and dietary habits to extend healthy life expectancy” (grant number JPJ009842 to Y.N.); and Grants-in-Aid for Scientific Research (KAKENHI) (C) from the Japan Society for the Promotion of Science (grant numbers 24K09440 and 24K11092 to K.U. and T.T., respectively).

Author Contribution

K.U. conceived and designed the study, collected and analyzed the data, interpreted the results, and drafted the manuscript. T.T., R.O., M.H., H.H., J.Y., E.M., K.A. contributed to the study design, data interpretation, and critical revision of the manuscript. K.M., M.Kaj., R.K., H.K., N.I., Y.H., M.T., K.I., O.D., and K.K. contributed to the experimental analyses and interpretation of the results. M.Kis. performed

histopathological assessments and contributed to the interpretation of the findings. Y.N. supervised the study and critically revised the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgement

We thank all members of the Department of Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, and the Graduate School of Medical Science, Kyoto Prefectural University of Medicine, for helping with this study.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

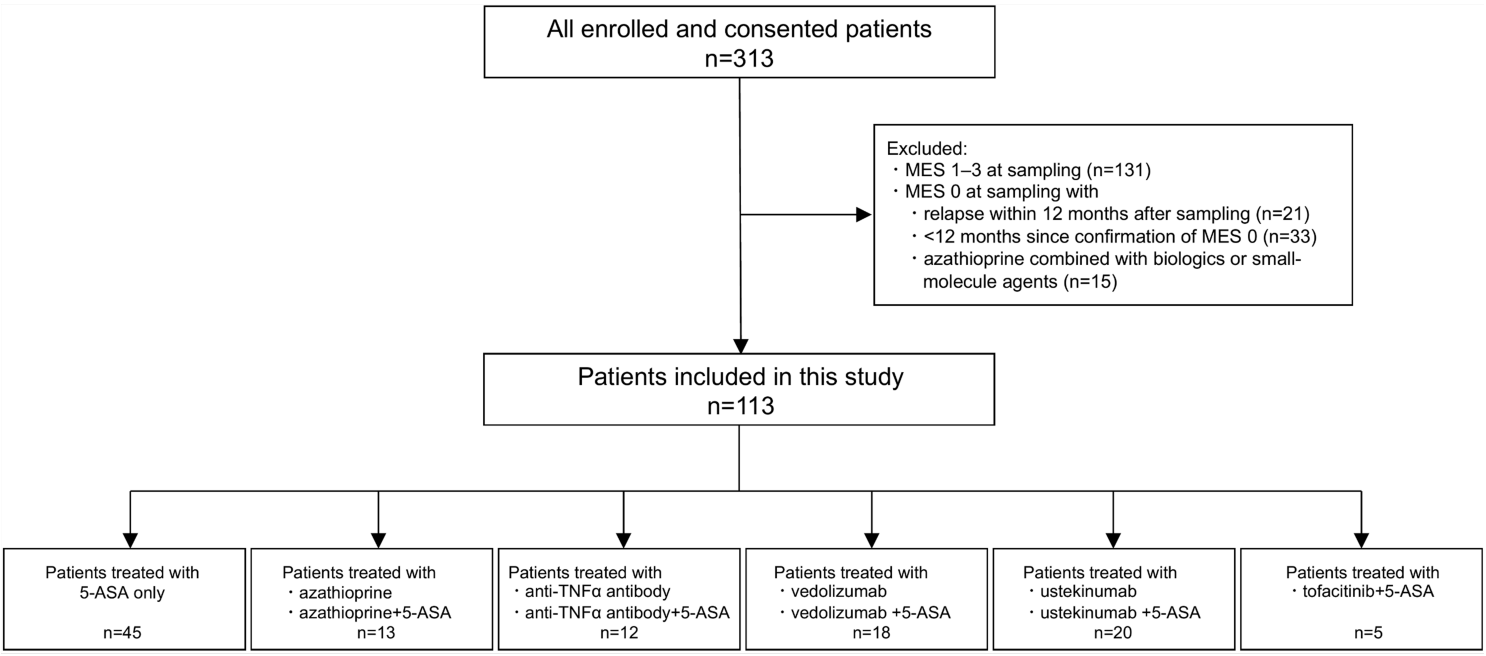


Figure 1

Study flow diagram of patient selection and treatment groups.

Of 313 patients with ulcerative colitis who enrolled and provided consent, 113 met the study criteria and were included in the analysis. Patients were excluded for an MES of 1–3 at the time of biopsy sampling (n=131), relapse within 12 months after sampling despite MES 0 (n=21), less than 12 months since confirmation of MES 0 (n=33), or azathioprine in combination with biologics or small-molecule agents (n=15). The final cohort included patients receiving maintenance therapy with 5-aminosalicylic acid (5-ASA) alone (n=45), azathioprine with or without 5-ASA (n=13), anti-tumor necrosis factor-α antibodies with or without 5-ASA (n=12), ustekinumab with or without 5-ASA (n=20), vedolizumab with or without 5-ASA (n=18), or tofacitinib with 5-ASA (n=5).

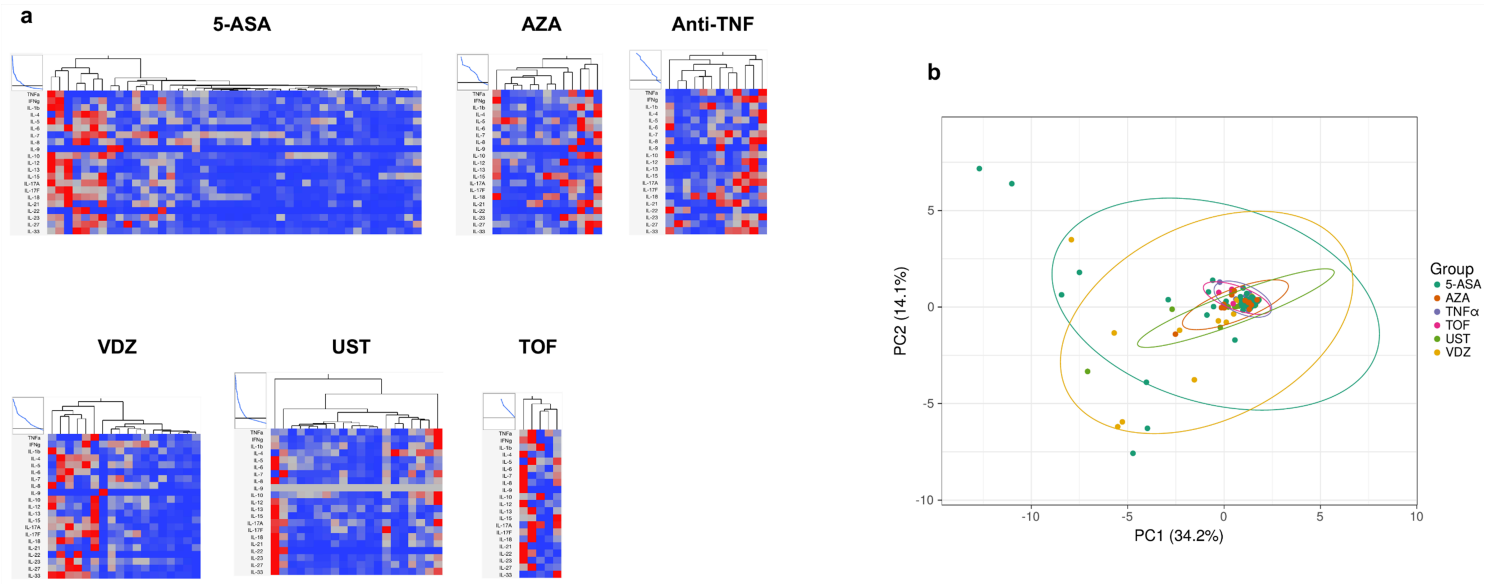


Figure 2

Mucosal cytokine expression profiles in rectal biopsy specimens according to treatment.

(a) Heatmap showing the expression profiles of 21 ulcerative colitis–related cytokine genes in rectal biopsy specimens from patients treated with 5-aminosalicylic acid, azathioprine, anti–tumor necrosis factor-α antibodies, vedolizumab, ustekinumab, or tofacitinib. Each column represents an individual patient, and each row represents a cytokine-related gene. (b) Principal component analysis of mucosal cytokine expression profiles across the six treatment groups.

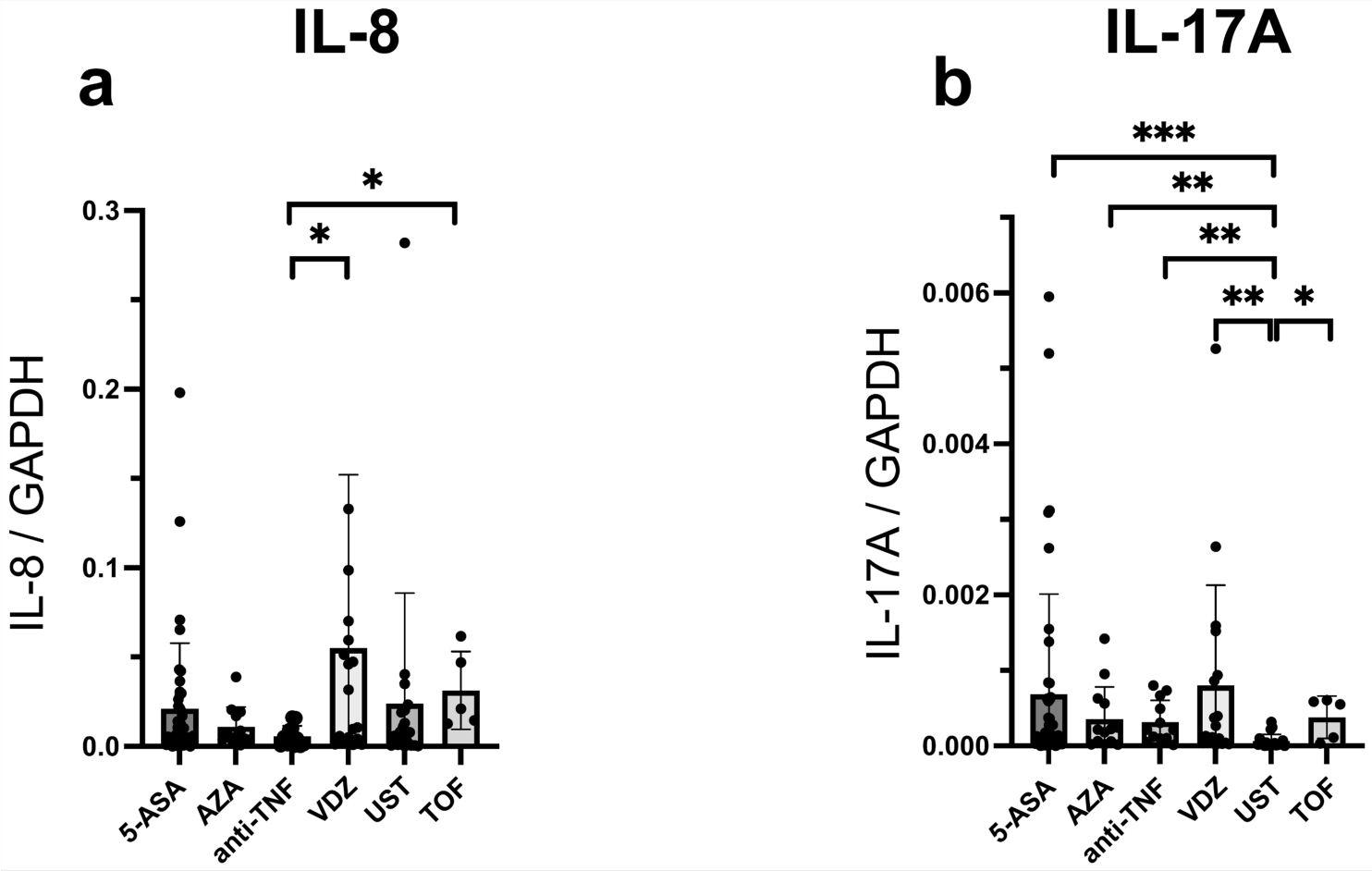


Figure 3

Mucosal IL-8 and IL-17A expression in rectal biopsy specimens according to treatment.

Mucosal mRNA expression levels of IL-8 (a) and IL-17A (b) in rectal biopsy specimens were compared among patients treated with 5-aminosalicylic acid, azathioprine, anti–tumor necrosis factor-α antibodies, vedolizumab, ustekinumab, or tofacitinib. Differences among treatment groups were analyzed using the Kruskal–Wallis test, followed by Dunn's post hoc test with Bonferroni correction when appropriate. *P < 0.05, **P < 0.001, ***P < 0.0001

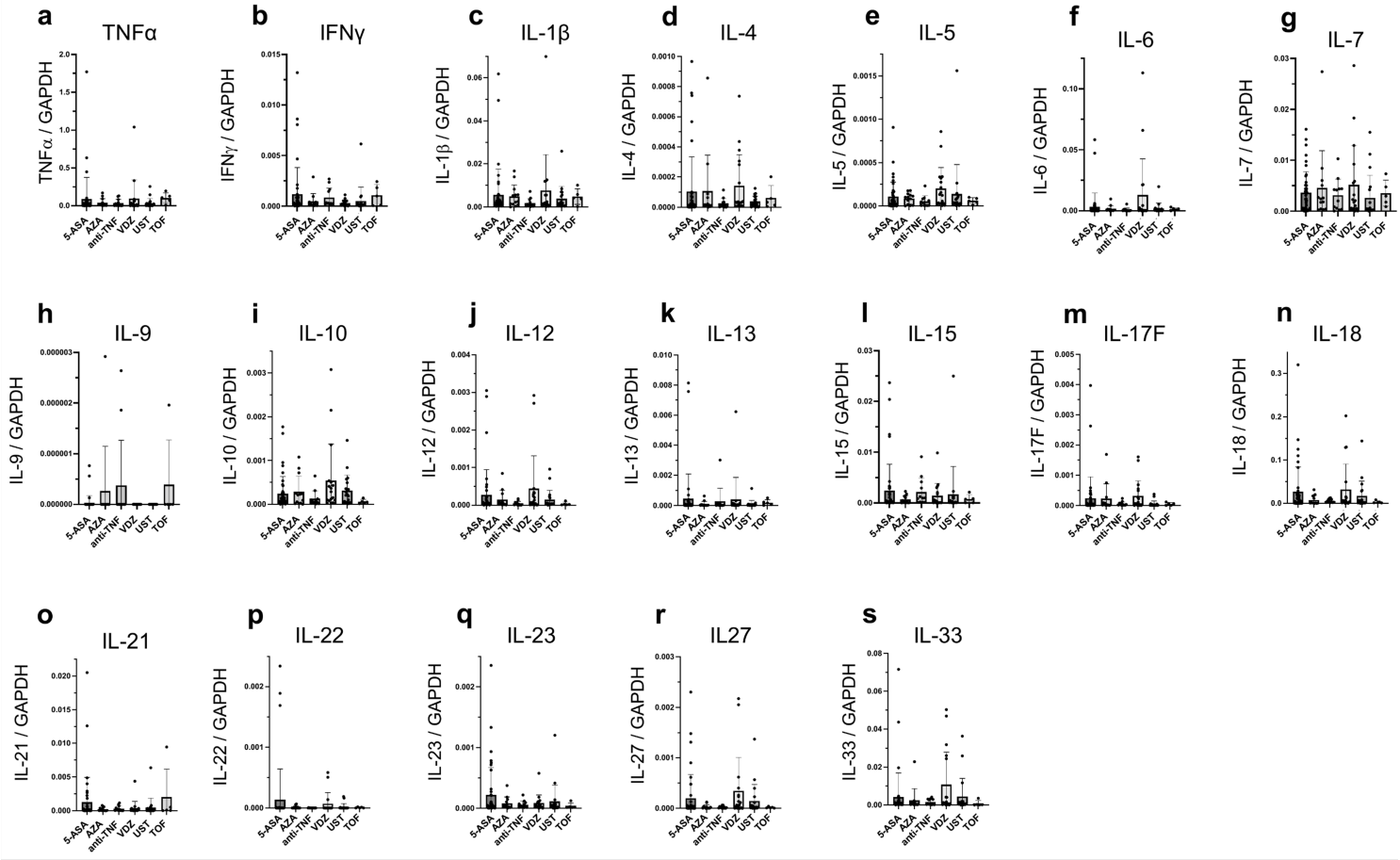


Figure 4

Mucosal expression of cytokine-related genes in rectal biopsy specimens according to treatment.

Mucosal mRNA expression levels of (a) TNFα, (b) IFNγ, (c) IL-1β, (d) IL-4, (e) IL-5, (f) IL-6, (g) IL-7, (h) IL-9, (i) IL-10, (j) IL-12, (k) IL-13, (l) IL-15, (m) IL-17F, (n) IL-18, (o) IL-21, (p) IL-22, (q) IL-23, (r) IL-27, and (s) IL-33 were evaluated in rectal biopsy specimens from patients treated with 5-aminosalicylic acid (5-ASA), azathioprine (AZA), anti-tumor necrosis factor-α antibodies (anti-TNF), vedolizumab (VDZ), ustekinumab (UST), or tofacitinib (TOF). Cytokine expression levels were normalized to GAPDH and compared among the six treatment groups using the Kruskal–Wallis test. Dunn's post hoc test with Bonferroni correction was then used when appropriate. We observed no significant differences in the expression of these cytokine-related genes among the treatment groups.

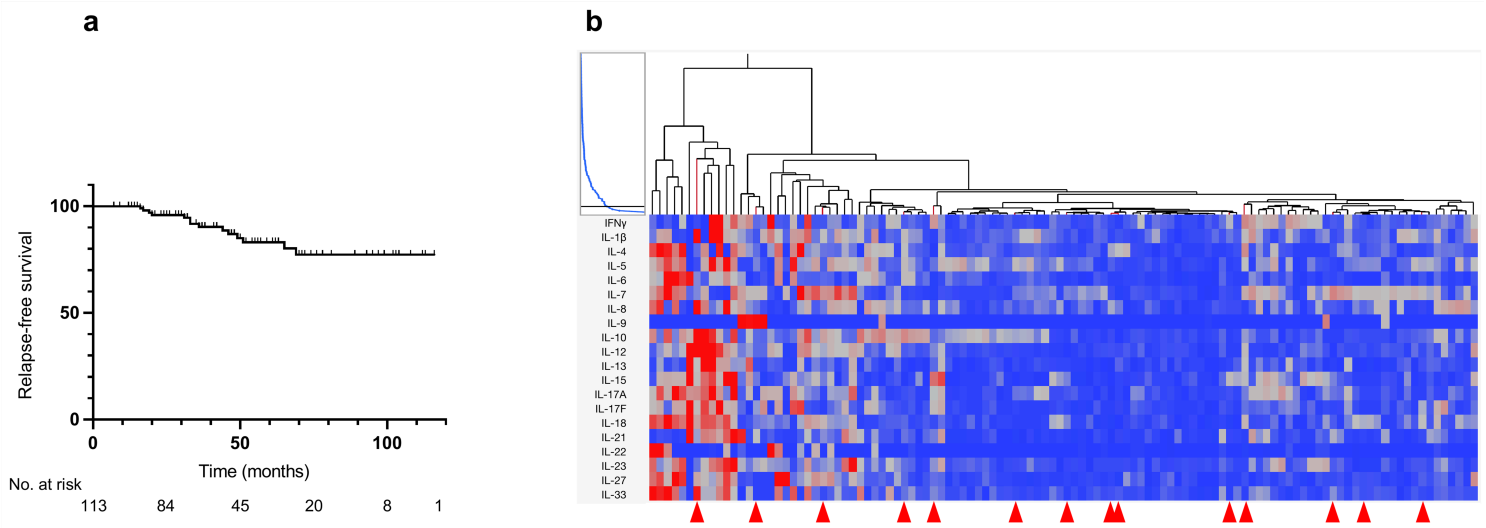


Figure 5

Association between mucosal cytokine expression and subsequent clinical relapse.

(a) The Kaplan–Meier method was used to analyze relapse-free survival after biopsy sampling. (b) The heatmap shows the expression profiles of 21 cytokine-related genes in rectal biopsy specimens and is annotated according to subsequent clinical relapse. Each column represents an individual patient, whereas each row represents a cytokine-related gene. Below the heatmap, triangles indicate which patients experienced clinical relapse during follow-up.

Supplementary Files

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