MAIT cells are activated and accumulated in the inflamed mucosa of ulcerative colitis

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Complete List of Authors:	Haga, Keiichi; Juntendo University School of Medicine, Department of Gastroenterology Chiba, Asako; Juntendo University School of Medicine, Department of Immunology Shibuya, Tomoyoshi; Juntendo University School of Medicine, Department of Gastroenterology Osada, Taro; Juntendo University School of Medicine, Department of Gastroenterology Ishikawa, Dai; Juntendo University School of Medicine, Department of Gastroenterology Kodani, Tomohiro; Juntendo University School of Medicine, Department of Gastroenterology Nomura, Osamu; Juntendo University School of Medicine, Department of Gastroenterology Watanabe, Sumio; Juntendo University School of Medicine, Department of Gastroenterology Miyake, Sachiko; Juntendo University School of Medicine, Department of Immunology
Key Words:	MAIT cells, ulcerative colitis, activation marker, inflammatory bowel diseases

Title page

Title

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Short title

MAIT cell activation reflects mucosal inflammation

Authors

Keiichi Haga[1,2], Asako Chiba[1], Tomoyoshi Shibuya[2], Taro Osada[2], Dai Ishikawa[2], Tomohiro Kodani[2], Osamu Nomura[2], Sumio Watanabe[2], and Sachiko Miyake[1].

Institution

[1]Department of Immunology, Juntendo University School of Medicine, Tokyo, Japan

[2]Department of Gastroenterology, Juntendo University School of Medicine, Tokyo, Japan

Correspondence

Sachiko Miyake, M.D, Ph.D, and Asako Chiba M.D, Ph.D.

Department of Immunology, Juntendo University School of Medicine

2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

Tel: 81-33-813-1044

Fax: 81-33-813-0421

E-mail: s-miyake@juntendo.ac.jp, a-chiba@juntendo.ac.jp

Disclosure

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Abstract

Background and Aim: Ulcerative colitis (UC) is a chronic, relapsing and remitting, inflammatory disorder of the large intestine. Mucosal associated invariant T (MAIT) cells are a member of innate-like lymphocytes found abundantly in the mucosal tissue. The contribution of MAIT cells in the pathogenesis of UC is still unclear; therefore, this study aimed at investigating the role of these cells in patients with UC.

Methods: The frequency of MAIT cells, as well as the production of cytokines and expression levels of activation markers by these cells in the peripheral blood of UC patients and healthy controls were analyzed by flow cytometry. MAIT cells were also quantified in colon biopsies of UC patients using a confocal microscope.

Results: There was a significant reduction in MAIT cell frequency in the peripheral blood ($P < 0.0001$). MAIT cells from UC patients secreted more interleukin (IL)-17 than healthy controls ($P < 0.05$). The expression levels of CD69 on these cells were correlated with disease activity and endoscopic scores as well as plasma levels of IL-18. Furthermore, MAIT cells increased in the inflamed mucosa and their frequency was correlated with clinical and endoscopic disease activity in UC patients.

Conclusions: The findings from this study indicate that MAIT cells could be involved

in the pathogenesis of UC, and may serve as potential biomarkers or therapeutic targets in UC.

Key words: MAIT cells, ulcerative colitis, activation marker, inflammatory bowel diseases.

Introduction

Ulcerative colitis (UC) is a chronic, relapsing and remitting, inflammatory disorder of the large intestine. Inflammatory bowel diseases (IBD) such as Cohn's disease (CD) and UC result from a dysregulated response of the mucosal immune system to components of the luminal micro biota, and the breakdown of immune tolerance in individuals who are genetically predisposed to the disease.[1,2] In recent years, several studies have improved our knowledge and understanding of the mucosal immune system by providing novel insights into the environmental influences of diet and the micro biota. The mucosal immune system has evolved in order to regulate the delicate and dynamic balance between tolerance and vigilance.[3] Recent advances in drug development involving biological agents that target tumor necrosis factor (TNF), integrin's, and other monoclonal antibodies, have contributed to the remarkable progress towards treatments against IBDs such as CD and UC. This changed the way to treat IBD refractory to standard medications and allowed us to reach new therapeutic goals such as mucosal healing and deep remission.[4,5] However, further understanding of the pathogenesis of UC will aid in comprehending the patient's condition, leading to the development of more effective treatment methods.

Innate-like lymphocytes reside in unique locations such as the epithelial and mucosal tissues, and rapidly exert effector functions in the absence of clonal expansion. Therefore, these innate-like lymphocytes are thought to play important roles in immune responses againt exogenous stimuli within the mucosal tissues. As mucosal associated invariant T (MAIT) cells are preferentially located in the gut lamina propria,[6,7] there is a growing interest in the role of these cells in various types of immune responses. MAIT cells are restricted by a nonpolymorphic MHC class 1b molecule, the MHC-related molecule-1 (MR1), and express an invariant TCRα chain paired with a limited set of Vβ chains.[7,8] They are selected in the thymus, and require B cells and commensal flora for their peripheral expansion.[7,9,10] MAIT cells are abundant in human peripheral blood, where they usually represent 1-10% of total αβ T cells.[9,11,12] In addition to the gut mucosa and peripheral blood, MAIT cells are also found in the lung and liver.[11,13,14,15] An important functional feature of MAIT cells is their ability to produce large amounts of cytokines including, interleukin (IL)-17, interferon (IFN)-γ, and TNF-α, very rapidly upon activation. Taken together, these findings suggest that MAIT cells play an important role in immune regulation. Both human and mouse MAIT cells have been shown to be activated by *Escherichia coli*-infected antigen presenting cells in a

MR1-dependent manner, and MAIT cells exhibit antimicrobial functions under infectious conditions.[13,14,16,17] They have been reported to play a protective role against autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), and 2,4,6-trinitrobenzene sulfonic acid (TNBS) induced colitis, an animal model of Cohn's disease (CD); however, they are also known to have pathogenic roles in murine models of arthritis.[18,19,20]

Recently the frequency of MAIT cells in peripheral blood of IBD patients has been reported, although the cause of the decrease of MAIT cells remains controversial.[21,22] Serriari et al. showed the accumulation of MAIT cells in the inflamed ileum of CD patients.[21] In contrast, Hiejima et al. demonstrated the decrease of MAIT cells in the colon in patients with CD and UC and further showed the enhanced apoptosis of these cells in peripheral blood, speculating that the decrease of MAIT cells might contribute to the enhanced inflammation in mucosal tissues.[22] In the present study, we demonstrate for the first time, the accumulation of MAIT cells within the colonic tissues in active UC patients. Their frequency in the inflamed colon was correlated with clinical disease activity and endoscopic scores. Conversely, their proportion in peripheral blood was decreased in UC patients, although apoptosis of these cells was

not increased. The MAIT cells were found to be activated in association with enhanced production of inflammatory cytokines in the peripheral blood. Interestingly the expression levels of CD69 on these cells were correlated with clinical and endoscopic scores as well as plasma levels of IL-18. Taken together, MAIT cells were activated and were recruited to the colonic tissues and could be involved in the pathogenesis of UC, suggesting the potential of these cells as biomarkers, or therapeutic targets in UC.

Methods

Human samples

A total of 34 UC patients with varying disease activity, and 34 age and gender matched healthy controls (HCs) were included in this study. The healthy control subjects had no history of autoimmune disease, malignancy, infections, chronic liver disease, or diabetes mellitus. The clinical disease activity in the patients with UC was determined using the Mayo score, and endoscopic severity was determined using the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). Informed consent was obtained from all patients and healthy individuals according to ethical guidelines on Good Clinical Practice of Juntendo University Hospital. Table1 illustrates the characteristics of the subjects included in this study.

Flow cytometry

Peripheral venous blood samples were collected in heparin-containing tubes. Peripheral blood mononuclear cells (PBMCs) from healthy volunteers and UC patients were purified by density-gradient centrifugation using Ficoll-Paque Plus solution (Bio-Sciences, Uppsala, Sweden). The cells were stained with combinations of appropriate antibodies. The following surface marker antibodies and a tetramer were

used in this study: anti-CD3-V500 (BD Bioscience, San Diego, California), anti-CD4-APC-H7 (BD Bioscience), anti-CD8β-ECD (Beckman Coulter, Villepinte, France), anti-CD161-Brilliant violet-421 (BioLegend, San Diego, California), anti-TCR-γδ-FITC (Beckman Coulter), anti-Vα7.2-PE (BioLegend), anti-CD19-V500 (BD Bioscience), anti-CD3-APC-H7 (BD Bioscience), anti-CD161-PerCPCy5.5 (BioLegend), anti-CD56-Alexa700 (BD Bioscience), anti-CD14- Brilliant violet 570 (BioLegend), and anti-CD4- Brilliant violet 421 (BioLegend), hCD1d tetramer loaded with PBS-57-APC (NIH tetramer core facility at Emory University, Atlanta, Georgia), and anti-CD69- Brilliant violet 605 (BioLegend). MAIT cells were identified as $CD3^{+}\gamma\delta TCR^{-}V\alpha7.2TCR^{+}CD161^{high}$ cells, γδT cells as $CD3^{+}\gamma\delta TCR^{+}$ cells, iNKT cells as $CD1d/PBS\text{-}57tetramer^{+}CD3^{+}\gamma\delta TCR^{-}$cells, NK cells as $CD3^{-}CD56^{+}$cells, and B-1cells as $CD19^{+}CD20^{+}CD27^{+}CD43^{+}$cells. For intracellular active caspase-3 staining, PBMC were stained with surface markers followed by staining of intracellular active caspase-3 using Active Caspase-3 Apoptosis Kit (BD Bioscience). Cells were analyzed by FACS LSR Fortessa (BD, San Diego, California), and FACS data were analyzed using Flowjo software (version 7.6.5, Tree Star, Oregon).

Intracellular cytokine staining

PBMCs (2×10^6/well) were stimulated with PMA (50ng/ml, Sigma-Aldrich, St. Louis, Missouri) and ionomycin (500ng/ml, Sigma-Aldrich) for 1h, followed by the addition of brefeldin A (GolgiPlug; BD). Two hours later, the cells were stained for surface antigens and then fixed and permeabilized with Perm/Wash solution (BD Biosciences). After fixation, the cells were stained with anti-IL-17A-Alexa 647 (BioLegend), anti-IFN-γ-PEcy7 (eBioscience), anti-IL-6-APC (BioLegend), and anti- TNF-α- PEcy7 (BD Bioscience) and analyzed by flow cytometry.

Confocal microscopy

The detection of MAIT cells in colon biopsy samples was performed on acetone-fixed snap-frozen sections. The antibody panel included anti-CD3 (rabbit pAb to human CD3; Abcam, Cambridge, United Kingdom), anti-IL-18Rα (anti-human-IL-18Rα–Polyclonal Goat IgG; R&D Systems, Minneapolis, Minnesota), and anti-Vα7.2 (mouse anti-human-Vα7.2; BioLegend) which were detected by their respective secondary antibodies (anti-rabbit-IgG-Alexa647; Molecular Probes, donkey anti-mouse-IgG-Alexa488; Molecular Probes, Minneapolis, Minnesota, and donkey anti-goat-IgG-Alexa594; Jackson Immunoresearch, West Grove, Pennsylvania, respectively). The frequency of MAIT cells was calculated as follows: percentage of

MAIT cells = (number of $CD3^+IL\text{-}18R\alpha^+V\alpha7.2^+$cells) / (number of $CD3^+$ cells) ×100.

Analyses were performed using the ×40 objective of a TCS SP5 confocal microscope (Leica, Wetzlar, Germany).

Quantification of IL-18 plasma levels

Blood plasma of healthy volunteers and UC patients were collected by density-gradient centrifugation of blood samples and frozen at -80°C. IL-18 was quantified in simultaneously thawed plasma using the human IL-18 enzyme-linked immunosorbent assay (ELISA) Kit (MBL, Nagoya, Japan).

Statistical Analysis

All data were analyzed using GraphPad Prism (version 6, GraphPad, La Jolla, California). Differences between groups were analyzed using Mann-Whitney's U test, whereas associations between the variables were analyzed using Spearman's correlation. The significance was defined at $P<0.05$.

Results

MAIT cells are reduced in the peripheral blood of UC patients.

We first examined the frequency of innate-like lymphocytes including MAIT cells, γδ T cells, NK cells, iNKT cells, and B-1 cells in the peripheral blood of UC patients and HCs using flow cytometry. MAIT cells were identified as $CD3^{+}\gamma\delta TCR^{-}V\alpha7.2TCR^{+}CD161^{high}$ cells, γδ T cells as $CD3^{+}\gamma\delta TCR^{+}$ cells, iNKT cells as CD1d/PBS-57tetramer$^{+}CD3^{+}\gamma\delta TCR^{-}$cells, NK cells as $CD3^{-}CD56^{+}$cells, and B-1cells as $CD19^{+}CD20^{+}CD27^{+}CD43^{+}$cells. The frequency of MAIT cells and NK cells was significantly reduced in the peripheral blood of UC patients (mean, 1.86% ± 1.59; 8.02% ± 4.74, respectively) when compared with the HCs (mean, 5.65% ± 3.26; 14.0% ± 6.53, respectively) (Figure 1, Table 2). The frequencies of other innate-like lymphocytes such as γδ T cells, iNKT cells, and B-1cells were not significantly different between UC patients and HCs (Table 2). The frequency of MAIT cells was not correlated with any of the specific treatments such as corticoids, azathioprine or TNF inhibitors. Furthermore, although the frequency of MAIT cells tended to be low when the Mayo scores were high, the correlation between the frequency of MAIT cells and UCEIS did not reach statistical significance.

Enhanced cytokine production by MAIT cells in UC patients.

Since MAIT cells are highly capable of producing a variety of cytokines, we assessed the alterations in cytokine production from these cells in UC patients. Previous studies have shown that human MAIT cells produce inflammatory cytokines, but not Th2 cytokines such as IL-4 and IL-10. Therefore we performed intracellular staining of cytokines including IL-17, IL-6, TNF-α, and IFN-γ in MAIT cells upon activation with PMA and ionomycin. IL-17 producing MAIT cells were significantly abundant in UC patients than in the HCs (mean, 2.87% ± 0.57; 1.39% ± 0.28, respectively; Figure 2). IL-6 or TNF-α producing MAIT cells also exhibited a trend towards increase in UC patients when compared to the HCs; however, the differences did not reach to statistical significance.

Activation of MAIT cells reflects disease activity in UC patients.

Enhanced cytokine production in MAIT cells suggests that the cells were activated in vivo in UC patients. Hence, we examined the surface expression of CD69 on MAIT cells, and observed increased frequency of CD69 positive MAIT cells in UC patients when compared with HCs (Figure 3a). Interestingly, the expression levels of CD69 were negatively correlated with the frequency of MAIT cells in UC

patients.(r_s=0.425, p=0.027) (Figure 3b). The expression levels of CD69 on MAIT cells were correlated with disease activity as assessed by both Mayo score (r_s=0.450, p=0.013: Figure 3c) as well as UCEIS (r_s=0.539, p=0.040: Figure 3d). These results indicate that MAIT cells are activated in vivo, and the activation status reflects disease activity in UC patients. We also examined the surface expression of HLA-DR on MAIT cells; no significant differences in HLA-DR expression between HC and UC MAIT cells.

MAIT cells are activated by IL-18 in UC patients.

IL-18 is a cytokine that belongs to the IL-1 super family, and is involved in infection, inflammation and autoimmune diseases including IBD. It is reported to be increased in the serum of IBD patients. In addition, exclusively high levels of IL-18Rα have been reported on MAIT cells[11,14] and IL-18 activated MAIT cells in vitro.[16,23,24] Therefore, we examined plasma levels of IL-18 in UC patients. Plasma IL-18 levels were elevated in UC patients (Figure 4a), and IL-18 levels were found to be correlated with the expression levels of CD69 on the MAIT cells (r_s=0.305, p=0.027: Figure 4b), (r_s=0.625, p=0.0003: Figure 4c), implying that IL-18 might be an activator of MAIT cells in UC patients.

Cell death of MAIT cells was not enhanced in the peripheral blood of UC patients.

Peripheral blood MAIT cells were stained with intracellular active caspase-3 in order to examine whether the cells undergo cell death. Active caspase-3 positive MAIT cells were not increased in UC patients (3.40% ± 0.89) when compared with HCs (3.36% ± 0.98). In addition, although MAIT cells were activated in UC, the expression of FAS was not increased in MAIT cells in UC patients (MFI; 1382 ± 175.9) compared to healthy individuals (MFI; 1326 ± 162.6), indicating MAIT cell death was not enhanced in the peripheral blood.

MAIT cells accumulate in the inflamed mucosa of UC patients.

Since several studies have shown an accumulation of MAIT cells in inflamed or infected tissues such as the lung in tuberculosis patients or ileum in CD patients, we investigated whether MAIT cells accumulate in the inflamed mucosa of UC patients. Immunohistochemical analysis was used to assess this feature because we were unable to obtain sufficient numbers of cells for flow cytometry analysis from the biopsy specimens. MAIT cells were identified as $CD3^{+}V\alpha7.2TCR^{+}IL\text{-}18R\alpha^{+}$cells in the colon tissue of UC patients (Figure 5a). We compared the number of MAIT cells in colon biopsies from active and non-active UC patients. Active disease patients were defined

as those with moderate to severe UC; Mayo score ≧6. We found that colon MAIT cells were increased in active UC patients when compared to the non-active patients (19.08% ± 0.76 vs 11.86% ± 1.36, respectively; Figure 5b). Furthermore, the frequency of MAIT cells in the colon was correlated with disease activity as assessed by both Mayo score (r_s=0.625, p=0.022: Figure 5c) and UCEIS (r_s=0.332, p=0.039: Figure 5d). These results implicate that the decrease in the number of MAIT cells in the peripheral blood could be due to their recruitment to the inflamed mucosa in UC patients.

Discussion

This study demonstrated a significant increase in the number of MAIT cells in the colon tissues of active UC patients when compared to non-active patients. The frequency of MAIT cells in the colon was correlated with clinical and endoscopic disease activity. In peripheral blood of UC patients, MAIT cells decreased in the number, exhibited enhanced IL-17 production and increased CD69 surface expression. Additionally, the expression levels of CD69 reflected disease activity.

The reduction in the number of MAIT cells in the peripheral blood of UC patients in this study is consistent with previous studies.[21,22] Decreased frequency of MAIT cells was also reported in patients with autoimmune disorders such as MS, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), as well as metabolic or infectious diseases such as obesity, type 2 diabetes, HIV, and tuberculosis.[12,14,25,26,27,28] In contrast to the reduced number of MAIT cells in the peripheral blood of UC patients, the frequency of MAIT cells in the colon was increased in active UC lesions, and their frequency was correlated with both disease activity and endoscopic scores. Similarly, Serriari et al. reported that the percentage of MAIT cells among T cells was higher in the inflamed ileum than in the non-inflamed ileum of CD

patients.[21] However, more recently, Hiejima et al showed that the frequency of MAIT cells was lower in the colon of UC patients and small intestine of CD patients when compared to those of surgical specimens obtained from patients with gastrointestinal cancer.[22] In addition, they did not find any difference in the frequency of MAIT cells between inflamed and non-inflamed tissues in IBD patients, and concluded that the reduction of these cells in the peripheral blood and tissues was caused by their augmented apoptosis based on the increased expression of activated caspase by the MAIT cells, which was not observed in the present study. Inconsistent findings among studies may be attributed to the stage of the disease as Hiejima et al examined surgically treated samples from CD patients. Another possible reason of the differences may be due to the detection method of MAIT cells by immunohistochemistry. As Vα7.2TCR-positive T cells with low CD161 expression include non-MAIT cells bearing variant Vα7.2 TCR,[9] we distinguished MAIT cells as $CD3^{+}V\alpha7.2TCR^{+}IL\text{-}18R\alpha^{+}$ cells by immunohistochemical staining, in accordance with previous studies,[11,14,21] and found that the cells are accumulated in inflamed tissues of the colon. The accumulation of MAIT cells in inflamed tissue has also been reported in other diseases such as multiple sclerosis and tuberculosis.[14,23,29] These findings suggest

that peripheral blood MAIT cells are recruited into the inflamed tissues and contribute to the pathogenesis of UC.

As we and other groups have shown that human MAIT cells produce inflammatory cytokines, but not Th2 cytokines, we investigated the inflammatory cytokine production by MAIT cells in UC patients.[11,12,13,21] IL-17 producing cells were significantly increased in UC patients, with a trend towards increased TNF-α and IL-6 producing cells. The enhanced production of IL-17 was also observed in CD and RA, indicating that augmentation of IL-17 production is a common feature when MAIT cells are activated in inflammatory conditions.[21,25] Consistent with the enhanced cytokine production, the frequency of MAIT cells expressing the activation marker CD69 was also increased in UC patients when compared with HCs, suggesting that MAIT cells are activated in the peripheral blood. Interestingly, IL-18 plasma levels were elevated and correlated with the CD69 levels on the MAIT cells. Considering the fact that MAIT cells constitutively express IL-18 receptor and are activated by IL-18 in vitro, IL-18 might be an activator of these cells under inflammatory conditions such as UC.

MAIT cells are potent producers of inflammatory cytokines, including TNF-α, which is an established therapeutic target of UC. They comprise around 10% of

T cells in the gut mucosa, which can increase to 20% in inflamed tissues in UC patients. The accumulation of activated MAIT cells would lead to tissue inflammation and contribute to the pathogenesis of UC. Recent therapeutic options for UC involve evaluation of mucosal inflammatory conditions in order to choose an effective treatment strategy. Although CRP is used as a biomarker for disease activity in UC, it does not always reflect the mucosal inflammation.[30,31] In the present study, although most patients presented with normal CRP levels, the activation status of MAIT cells in the peripheral blood, such as the expression levels of CD69, correlated with clinical disease activity and endoscopic scores. Our findings suggest that MAIT cells may serve as a biomarker for mucosal inflammation in UC.

This is the first study to demonstrate the accumulation of MAIT cells in the inflamed mucosa in UC patients. We showed that MAIT cells were increased in the colon tissue of active UC patients and were activated in association with enhanced production of inflammatory cytokines in peripheral blood. Positive correlations between the activation as well as frequency of MAIT cells in the inflamed colon and disease activity indicate the potential of MAIT cells as new biomarkers of disease activity, and therapeutic targets in UC.

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Table 1. Characteristics of the patients included in the study.

	Patients with UC (n=34)
Age (years) †	37.2±10.3
Sex (Male/Female)	21/13
Duration of disease (years) †	8.8±5.9
Disease location (cases)	
Proctitis	11
Left-sided colitis	15
Extensive colitis	8
Ongoing treatments (cases)	
5-ASA	34
Corticosteroids	3
Cytapheresis	6
Azathioprine, 6-MP	7
Anti-TNF therapy	6
Tacrolims	4
Mayo score (median , range)	3 (0-12)
UCEIS (median , range)	4 (3-9)
CRP (mg/dl) †	0.12±0.21
Endoscopic Examination (cases)	25

†Mean ± standard deviation

UC, ulcerative colitis; 5-ASA, 5-aminosalicylic acid; 6-MP, mercaptopurine;

TNF, tumor necrosis factor; UCEIS, the ulcerative colitis endoscopic index of severity.

Table 2. Frequency of innate cells and innate-like lymphocytes in peripheral blood of UC patients and healthy controls.

	HC	UC
MAIT cell (% among αβTcells)	5.65±3.26	1.86±1.59 ****
iNK cell (% among αβTcells)	0.057±0.06	0.044±0.08
γδTcell (% among αβTcells)	3.45±1.90	3.53±2.96
NK cell (% among lymphocytes)	14.0±6.53	8.02±4.74 ****
B-1 cell (% among B cells)	1.36±1.24	1.58±1.54

P values are only shown when significant. **** = P<0.0001 (Mann-Whitney test)

Values are expressed as the mean ± standard deviation.

MAIT cell, mucosal associated invariant T cell; HC, healthy control; UC, ulcerative colitis.

Figure legends

Fig. 1. MAIT cells are reduced in the peripheral blood of UC patients.

The proportion of mucosal associated invariant T (MAIT) cells in the peripheral blood was analyzed by flow cytometory. The percentage of MAIT cells among αβ T cells in healthy controls (HCs) (n=34), and ulcerative colitis (UC) patients (n=34). Symbols represent individual subjects; horizontal bars show the mean values. ****P < 0.0001 (analyzed by Mann-Whitney test).

Fig. 2. Enhanced cytokine production by MAIT cells in UC patients.

The cells were stimulated with phorbol myristate acetate (PMA) and ionomycin. The proportion of cytokine producing MAIT cells from HCs (n=19) and UC patients (n=19) analyzed by intracellular staining with Interleukin (IL)-17, IL-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ. Symbols represent individual subjects; horizontal bars show the mean values. *P < 0.05 (analyzed by Mann-Whitney test).

Fig. 3. Activation of MAIT cells reflects disease activity in UC patients.

The percentage of CD69-positive cells in the total MAIT cells obtained from HCs (n=26) and UC patients (n=32) were determined by flow cytometry (a). The correlation between mean fluorescence intensity (MFI) of CD69 on the MAIT cells, and the

frequency of MAIT cells among the αβ T cells in UC patients (n=32) (b). Symbols represent individual subjects; horizontal bars show the mean values. *P < 0.05 (analyzed by Mann-Whitney test). Correlations were analyzed using Spearman's correlation.

The correlation between MFI of CD69 expression on the MAIT cells and the disease activity assessed using the Mayo score (c). The correlation between MFI of CD69 among MAIT cells and the Ulcerative Colitis Endoscopic Index of Severity (UCEIS) (d). Correlations were analyzed using Spearman's correlation.

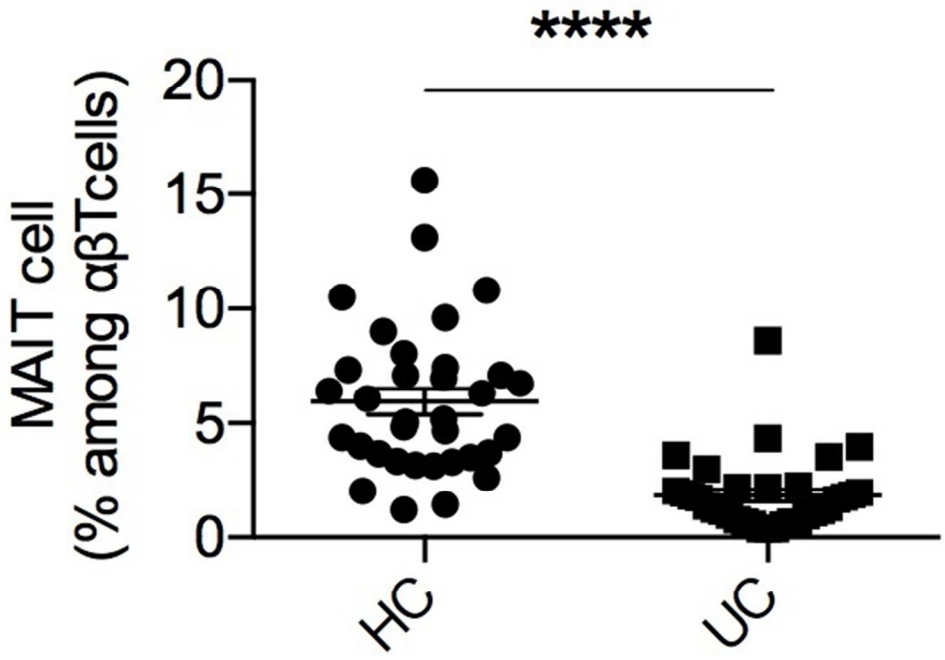
Fig.4. The concentrations of IL-18 in the plasma were elevated in UC patients.

Plasma levels of Interleukin (IL)-18 in HCs (n=14) and UC patients (n=18) were assessed by Enzyme-linked immunosorbent assay (ELISA) (a). Correlation between frequency of CD69-positive cells among total MAIT cells and plasma levels of IL-18 in UC patients (n=16) (b). Correlation between MFI of CD69 expression on MAIT cells and plasma levels of IL-18 in UC patients (n=16) (c). Symbols represent individual subjects; horizontal bars show the mean. *P < 0.05 (analyzed by Mann-Whitney test). Correlations were analyzed using Spearman's correlation.

Fig.5. Quantification of MAIT cells in colon biopsies from patients with UC.

Snap-frozen colon tissues from UC patients were triple-stained with IL-18Rα (red), anti-Vα7.2 (green), anti-CD3 (white) antibodies, allowing for the identification of $CD3^{+}V\alpha7.2^{+}IL\text{-}18R\alpha^{+}$ mucosal associated invariant T (MAIT) cells using confocal microscopy. The arrow denotes MAIT cell, and arrowheads denote $CD3^{+}V\alpha7.2TCR^{-}IL\text{-}18R\alpha^{-}$ Tcells (a). Percentage of MAIT cells among T cells in the colon mucosa of active versus non-active UC patients (b). Correlation between frequency of MAIT cells among T cells in the colon mucosa, and Mayo score (c), and UCEIS (d) in UC patients (n=13). Analyses were performed using the ×40 objective of a TCS SP5 confocal microscope (Leica). Symbols represent individual subjects; horizontal bars show the mean values. *P < 0.05 (analyzed by Mann-Whitney test). Correlations were analyzed using Spearman's correlation.

Figure 1



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****P < 0.0001 (analyzed by Mann-Whitney test).
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Figure 2

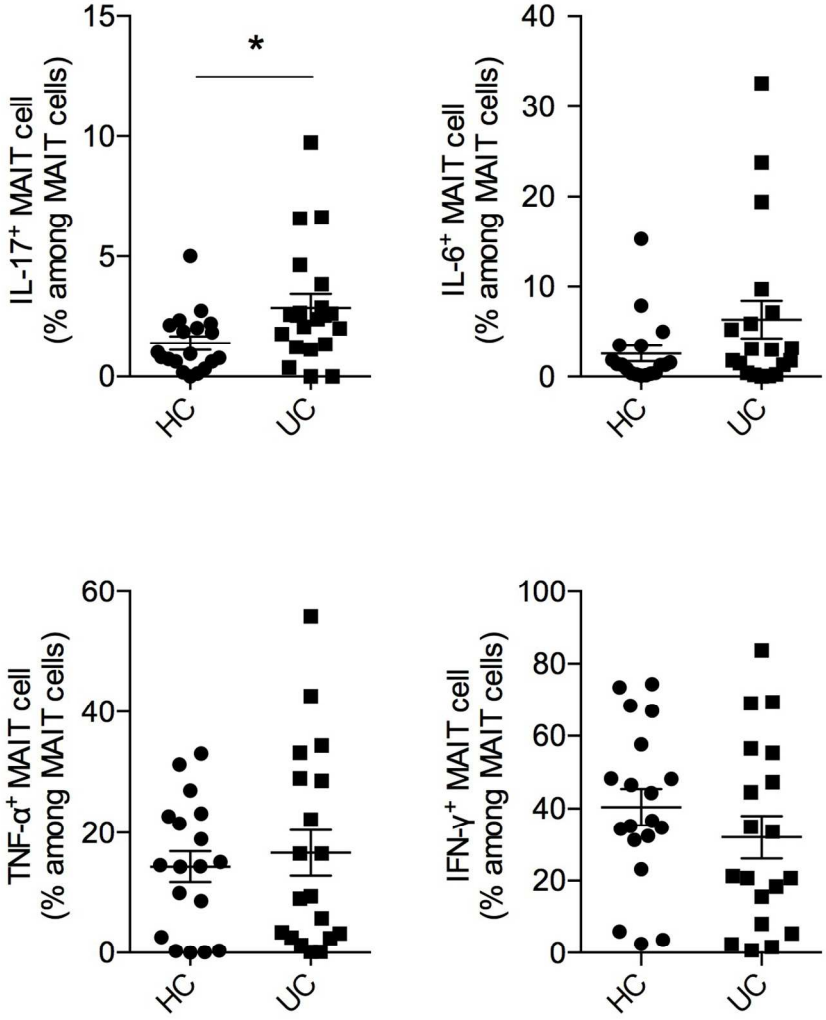

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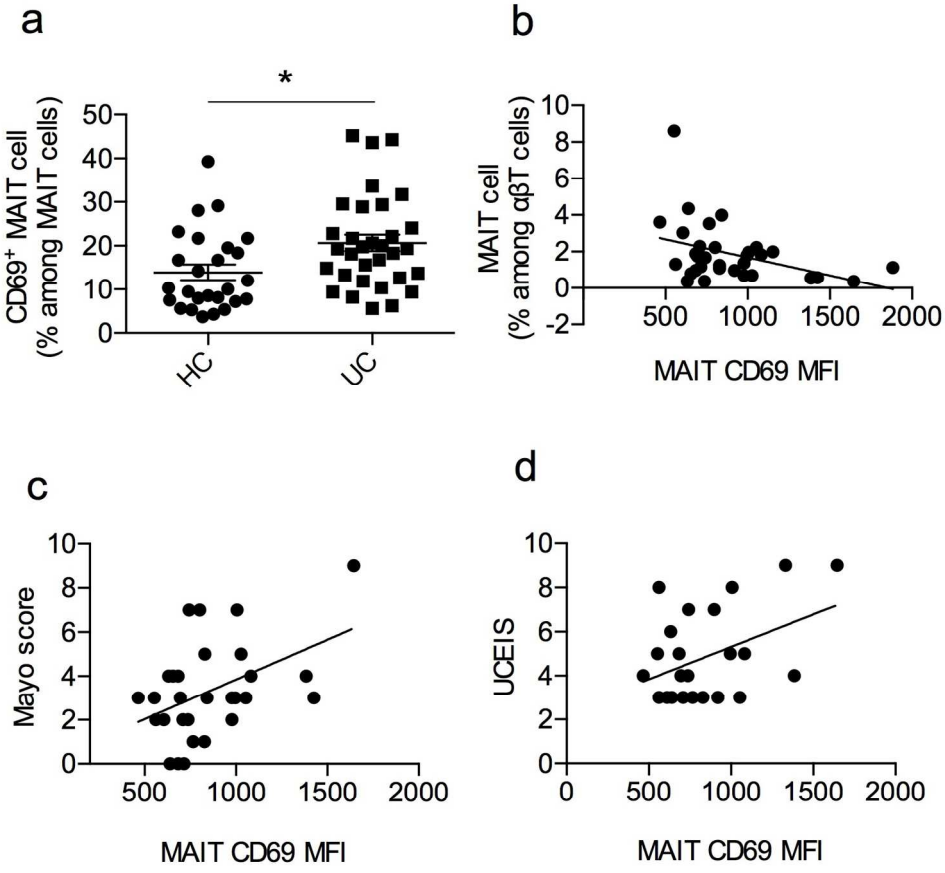
Figure 3



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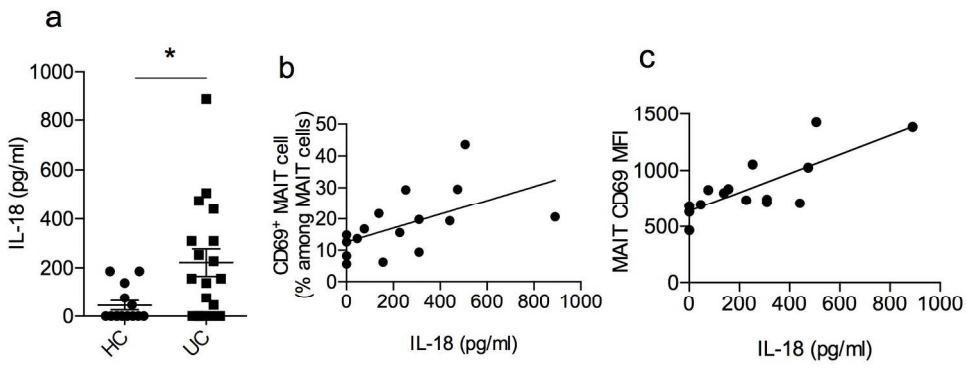
153x157mm (300 x 300 DPI)

Figure 4



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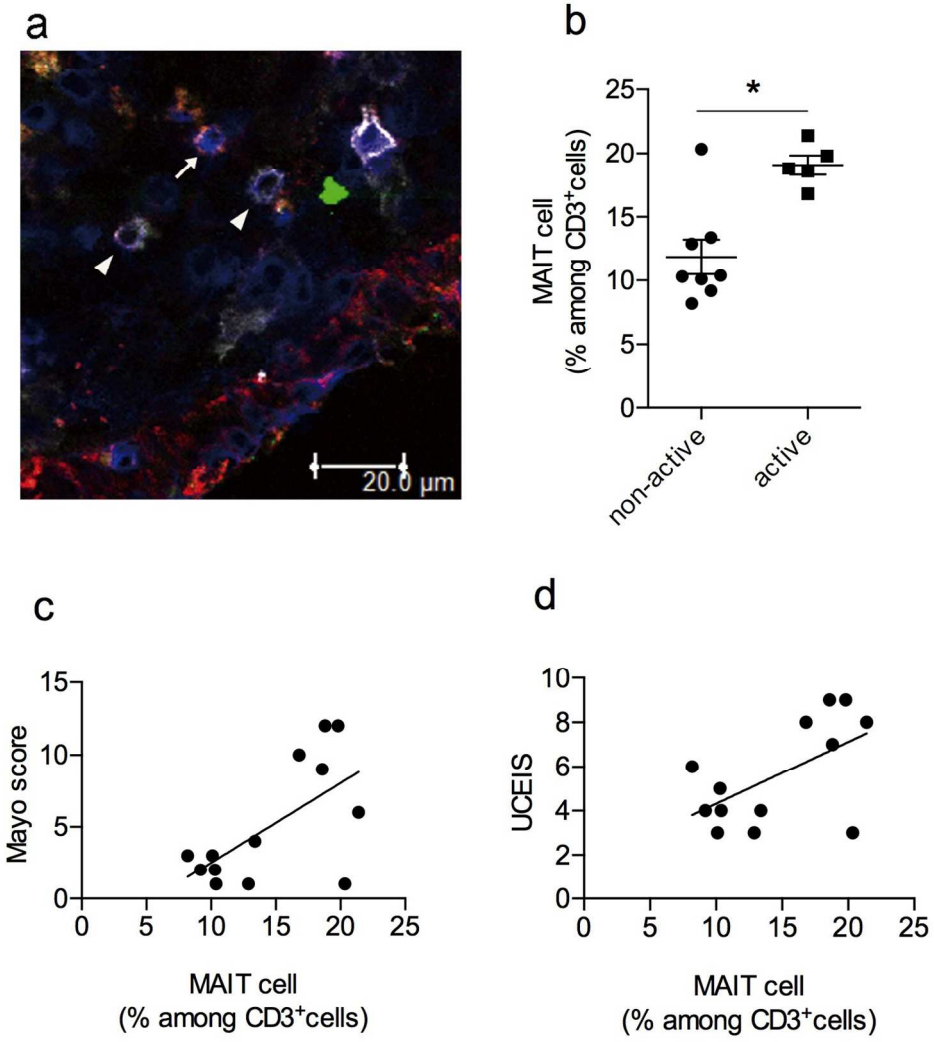
189x87mm (300 x 300 DPI)

Figure 5



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133x159mm (300 x 300 DPI)