Possible involvement of mucosal-associated invariant T cells in the progression of inflammatory bowel diseases

Kentaro Tominaga[1], Satoshi Yamagiwa[1*], Toru Setsu[1], Naruhiro Kimura[1], Hiroki Honda[1], Hiroteru Kamimura[1], Yutaka Honda[1], Masaaki Takamura[1], Junji Yokoyama[1], Kenji Suzuki[2], Toshifumi Wakai[3], and Shuji Terai[1]

[1] Division of Gastroenterology and Hepatology, and [3] Division of Surgery, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-dori, Chuo-ku, Niigata 951-8510, Japan

2 Department of Health Informatics, Niigata University of Health and Welfare, 1398 Shimami-cho, Kita-ku, Niigata 950-3198, Japan

Address Correspondence to:

Satoshi Yamagiwa, M.D., Ph.D.

Division of Gastroenterology and Hepatology,

Niigata University Graduate School of Medical and Dental Sciences,

1-757 Asahimachi-dori, Chuo-ku, Niigata 951-8510, Japan

Tel: +81-25-227-2207

Fax: +81-25-227-0776

E-mail address: syamagi@med.niigata-u.ac.jp

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List of Abbreviations: MAIT cells, mucosal-associated invariant T cells; TCR, T cell receptor; MR1, MHC-related 1; iNKT cells, invariant natural killer T cells; PBL, peripheral blood lymphocytes; NKG2D, natural-killer group 2, member D; NKG2A, natural-killer group 2, member A; CCR6, chemokine (C-C motif) receptor 6; CCL20, chemokine (C-C motif) ligand 20; TNF, tumor necrosis factor; IFN, interferon; IL-7, interleukin-7.

ABSTRACT

Mucosal-associated invariant T (MAIT) cells are innate-like T cells involved in anti-bacterial immunity. Recent studies have demonstrated that MAIT cells might be implicated in inflammatory bowel diseases (IBDs), but their precise function in IBD remains to be elucidated. We investigated the possible involvement of MAIT cells in the immunopathogenesis of IBDs. Heparinized peripheral blood and biopsy specimens of the colon were collected from 25 patients with ulcerative colitis (UC), 15 patients with Crohn's disease (CD), and 19 heathy individuals. Lymphocytes were isolated from the blood and colon, and then MAIT cells were analyzed by flow cytometry. The frequency of MAIT cells was significantly lower in the blood of IBD patients compared to healthy donors and significantly higher in the inflamed colons compared to healthy colons (P = 0.001). Among the IBD patients, the frequency of MAIT cells in the blood and colon was correlated with disease activities. *In vitro* activated MAIT cells from IBD patients secreted significantly more tumor necrosis factor-α and interleukin-17 than those from healthy donors. These findings indicate that MAIT cells are activated in IBD patients, and their accumulation in the inflamed mucosa is correlated with disease activities.

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Inflammatory bowel diseases (IBDs), including ulcerative colitis (UC) and Crohn's disease (CD), are chronic, relapsing inflammatory conditions of the gastrointestinal tract. Although host genetic susceptibility and environmental factors have been implicated in causing the disturbed homeostasis of the intestinal immune system that results in IBD, the exact etiology of IBD is still unknown (11, 19, 20). Genetic variants clearly play a central role in conferring risk for IBD, but a wide range of environmental factors, including smoking, diet drugs, social stress, and microbial factors, are also thought to confer risk for IBD (2). Accumulating evidence has suggested that among those environmental factors, the dynamic balance between commensal flora and host defensive responses within the intestinal mucosa plays a pivotal role in both the initiation and perpetuation of IBD (2, 16). Both the host innate and adaptive immune systems work together to determine the class of a microbial threat and direct the type and degree of immune response to the exposure (25). Therefore, it is likely that both the innate and adaptive intestinal immune systems contribute to the pathology of IBD. However, our understanding of the relevant microbial factors affecting the pathogenesis of IBD, especially the importance of innate immune responses, is still quite limited.

Mucosal-associated invariant T (MAIT) cells express a semi-invariant T cell receptor (TCR) repertoire, an invariant Vα7.2-Jα33 TCRα chain, and recognize a major histocompatibility complex (MHC) class Ib molecule, MHC-related 1 (MR1) (24, 26). MAIT cells are a population of MR1-restricted innate-like T cells involved in anti-bacterial immunity

(27). In humans, contrary to invariant natural killer T (iNKT) cells, MAIT cells display a naive phenotype in the thymus as well as in the umbilical cord blood, where they exist in low numbers. After birth, MAIT cells acquire a memory phenotype and expand dramatically, comprising up to 1-4% of the total number of TCR-$\alpha\beta^+$ T cells in the blood (17). MAIT cells are also found in peripheral tissues, such as the mucosa of the intestine and the lung, and are particularly abundant in the liver (4, 30). MAIT cells are activated by cells infected with various strains of bacteria and yeast but not by cells infected with viruses. This activation required cognate interaction between the invariant TCR and MR1, which can present a bacterial-derived ligand. Thus, the development of MAIT cells depends on the presence of the microbial flora, and mature MAIT cells are activated in the presence of various bacteria and fungi in an MR1-dependent manner (14).

As many vitamin biosynthetic pathways are unique to bacteria and yeast, previous studies have suggested that MAIT cells use these metabolites to detect microbial infection. Metabolites of vitamin B2 have been identified as bacterial ligands for MR1, which may explain this broad reactivity of MAIT cells (12). In humans, MAIT cells are decreased in frequency in the blood of patients with tuberculosis, human immunodeficiency virus (HIV) infection, and IBD. It was recently reported that MAIT cells accumulate in the inflamed mucosa of patients with IBD and display increased cytokine secretion capacities (21, 29). Thus, this large T lymphocyte population is likely to have an important

role in the pathogenesis of IBD, but their role in these conditions has not been fully elucidated. Therefore, we aimed to analyze the possible involvement of MAIT cells in IBD, in particular, in the progression of these diseases.

MATERIALS AND METHODS

Human samples

Heparinized peripheral blood and biopsy specimens of the colon were collected from 40 patients with IBD (UC, n = 25; CD, n = 15) from a remission state to a state of moderate disease activity in Niigata University Hospital between April 2013 and March 2015. The characteristics of the patients are indicated in Table 1. Nineteen healthy donors (14 male and 5 female, mean age 57 (26-80) years) were used as controls. Surgically removed colon tissues were collected from patients with colon tumors, and the colon tissues distant from the tumor were examined as controls. Biopsy specimens of normal colons were also provided by the healthy donors who underwent colonoscopies for screening purposes. Three or 4 biopsy specimens were usually collected from each patient or healthy donor. This work was conducted in accordance with the Declaration of Helsinki. Written informed consent under institutional review board-approved protocols (approval no. 1474) at Niigata University Medical and Dental Hospital was appropriately obtained from all the individuals enrolled in the study.

Cell isolation

Peripheral blood lymphocytes (PBL) were obtained by Ficoll-Paque (GE Healthcare Bio-Sciences, Uppsala, Sweden) gradient centrifugation. Biopsy specimens of the colons were shaken in Eagle’s minimum essential

medium (MEM) (GIBCO/Life Technologies, Grand Island, NY) supplemented with 5 mM HEPES (Sigma Chemical Co., St. Louis, Mo), 0.1% collagenase and 0.01% Tripsin inhibitor (Sigma) at 37°C for 30 min. Resected colon tissues were minced into pieces in Eagle's MEM supplemented with 5 mM HEPES, 0.1% collagenase and 0.01% Tripsin inhibitor. The resultant finely minced colon tissues were stirred gently in the presence of enzymes in a 37ºC water bath for 30 min. The enzymatically digested colon tissues were pressed through a 200-gauge stainless steel mesh, washed twice, and suspended in MEM containing of 5 mM HEPES and 5% heat-inactivated fetal calf serum. Colon cell suspensions were overlaid on Ficoll-Paque and centrifuged at 1500 rpm for 10 min. After centrifugation, cells were washed with phosphate-buffered saline (PBS) (GIBCO/Life Technologies) containing 0.5% heat-inactivated fetal calf serum and counted in a hemocytometer; the viability of cells was determined using trypan blue exclusion.

Reagents

Monoclonal antibodies (mAb) used for immunofluorescence assay were anti-TCR-γδ-PE (Beckman Coulter, Brea, CA, USA), anti-CD161-FITC, anti-CD3-PerCP, anti-CD8-PerCP, anti-CD69-PE, anti-NKG2D-PE, anti-NKG2A-PerCP, anti-IL7R-PE, anti-CD195-PE, anti-CD196-PerCP, anti-IFNγ-PE (Becton Dickinson), anti-TCR-Vα7.2-APC, anti-TNFα-PerCP-Cy5.5 (Biolegend, San Diego, CA), anti-IL-17-PerCP, and anti-IL-22-PerCP (R&D systems Inc., Minneapolis, MN). Monoclonal antibodies used

for immune histochemical staining was anti-Vα7.2 (Biolegend) and anti-CD161 (Becton Dickinson, San Jose, CA).

Flow cytometry

Cells (10^5) were labeled with several mAbs at 4°C for 30 min in darkness for the surface antigens, and then washed 2 times and acquired by FACS Calibur flow cytometer (Becton Dickinson). Data were analyzed using Flow Jo software (Tree Star Inc., Ashland, OR) and Cell quest pro (Becton Dickinson). MAIT cells in total blood/colon mononuclear populations were gated based on $CD161^{high}$ $V\alpha7.2^{+}$ expressions (24, 26, 27). The percentage of MAIT cells was calculated as follows: % MAIT (number of $CD3^{+}$ TCR-$\gamma\delta^{-}$ $CD161^{high}$ $V\alpha7.2^{+}$ cells) / (number of $CD3^{+}$TCR-$\gamma\delta^{-}$ cells).

Immunohistochemistry

Cryostat sections 8-μm thick were cut and fixed in cold acetone for 5 min. After immersion in blocking serum, sections were incubated with mouse anti-human CD161 (Becton Dickinson) or anti-human TCR-Vα7.2 (Biolegend) at a 1:200 or 1:100 dilution, respectively, in PBS supplemented with 3% BSA at 4°C overnight. After successive washing in PBS, sections were incubated with biotinylated anti-mouse immunoglobulin at a 1:100 dilution in PBS supplemented 5% BSA.

Immunohistochemical detection was performed according to the avidin-biotin-peroxidase complex method using the Vectastain Elite ABC kit (Vector Laboratories, Inc., Burlingame, CA). Sections were final

developed with diaminobenzidine substrate (Muto Pure Chemicals, Tokyo, Japan). Specimens were then counterstained with Haematoxylin-eosin and mounted.

Cell culture and in vitro cytokine production assay

Peripheral blood mononuclear cells were left untreated or were treated with interleukin (IL)-7 (R&D) at 10 ng/mL and cultured for 2 days as described previously (23). Cells were subsequently stimulated with or without anti-CD3/CD28-coupled beads (Invitrogen, 1:1 bead/cell ratio) overnight. Cells were then stained for respective surface and intracellular molecules. For intracellular staining, the Cytofix/Cytoperm™ Fixation/Permeabilization kit was used according to the manufacturer's specification (BD Biosciences).

Statistical analysis

The significance of differences was analyzed statistically by the compared *t* test with Welch's correction or Mann-Whitney *U* test, using SPSS software (Ver.18, SPSS Inc., Chicago, IL). In all cases, the level of significance was set at $P < 0.05$.

RESULTS

Peripheral blood MAIT cells are specifically reduced in IBD patients

MAIT cells can be identified as $CD3^+TCR\text{-}\gamma\delta^-CD161^{hi}V\alpha7.2^+$ lymphocytes (Fig. 1) (24, 26, 27). To examine their potential involvement in IBD, we measured and compared their frequency among $CD3^+TCR\text{-}\gamma\delta^-$ lymphocytes in the peripheral blood of IBD patients and healthy donors. We also analyzed whether the extent of MAIT cells in the blood might reflect the disease activity of IBD. As we could not find any significant difference in the frequency of MAIT cells between UC and CD patients (data not shown), we separated the IBD patients into three groups according to their clinical activities, as evaluated by the Partial Mayo Clinic score and the Crohn's Disease Activity Index (CDAI) (Table 1, Fig. 2A). The activities of 10 patients in remission were mild in the 5 patients (UC, n=2; CD, n=3) and moderate in the 5 patients (UC, n=3; CD, n=2) at the onset. We observed that the frequency of MAIT cells decreased significantly in the blood of IBD patients with both mild disease activity (1.21 ± 0.64%) (mean ± SD) and moderate disease activity (0.50 ± 0.29%) compared with that of the controls (3.15 ± 1.58%, both P <0.001). We also found that the frequency of MAIT cells in IBD patients with moderate disease activity (0.50 ± 0.29%) was significantly decreased compared with that of the remission group (2.33 ± 1.67%, P = 0.021) (Fig. 2B). To confirm the changes in the frequencies of MAIT cells, we calculated their absolute numbers based on total mononuclear cells in the blood. We found that the absolute cell

numbers of MAIT cells in the blood were significantly decreased in IBD patients with both mild disease activity and moderate disease activity compared with that of the controls (P = 0.033 and P = 0.026, respectively) (Table 2). Although all the examined patients underwent treatments, we could not find any correlations between the proportion of MAIT cells and the types of ongoing treatments (data not shown). Irrespective of the treatments, the frequency of MAIT cells in the blood were inversely correlated with the disease activity of IBD.

In vivo activation of MAIT cells in IBD

Most MAIT cells display either a $CD8\alpha^+$ or $CD4^-CD8^-$ double-negative phenotype, whereas a small proportion express the CD4 molecule (17). We observed that $CD8\alpha^+$ MAIT cells were significantly decreased in the blood of IBD patients with moderate disease activity (83.7 ± 10.4%) compared to the healthy controls (92.0 ± 5.1%, P <0.01) (Fig. 2C). As shown in Fig. 2C, the frequency of MAIT cells expressing an activation marker, CD69, was significantly higher in the IBD patients with moderate disease activity (53.3 ± 24.2%) compared to the remission group (25.2 ± 22.7%, P = 0.016) and the controls (31.0 ± 19.4%, P = 0.021). This might suggest that MAIT cells were more activated *in vivo* in the IBD patients. Although a previous study has shown that MAIT cells exhibit an increased NKG2D expression in IBD (21), we could not find a higher expression of NKG2D in the IBD patients compared to the controls. We also analyzed the expressions of NKG2A, IL7R, CCR6 and CCR5 on the MAIT cells in the blood, but we

found no significant difference between IBD patients and controls (data not shown).

MAIT cells accumulate in the inflamed colons of IBD patients

Previous studies have shown that MAIT cells accumulate in inflamed or infected tissues, such as the lung in tuberculosis (10). Therefore, we used immunohistochemistry to examine MAIT cells in the inflamed mucosa of IBD patients and the normal mucosa of colon cancer patients. Surgically resected colon specimens of patients with UC and colon cancer were stained with anti-CD161 or anti-TCR-Vα7.2 antibodies. As shown in Fig. 3, we detected several $CD161^{+}$ or TCR-$V\alpha7.2^{+}$ cells in healthy colon tissues. Interestingly, we also found a strong accumulation of those cells in the inflamed mucosa of UC patients (Fig. 3). We then analyzed the distribution of $CD3^{+}$TCR-$\gamma\delta^{-}$ $CD161^{hi}$TCR-$V\alpha7.2^{+}$ MAIT cells in the colon biopsy tissues of 11 healthy individuals and the inflamed colon biopsy tissues of 11 patients with UC and 6 patients with CD by flow cytometry (Fig. 4A). Because we could not obtain enough number of biopsy samples from the patients with moderate disease activity, we determined the frequency of MAIT cells in the colon biopsy tissues of patients with remission and mild disease activity. In the healthy colon tissues, MAIT cells represented, on average, 0.92 ± 0.47% of the total number of $CD3^{+}$TCR-$\gamma\delta^{-}$ T cells. The frequency of MAIT cells was significantly increased in the IBD patients with mild disease activity (3.00 ± 0.17%, $P = 0.001$) (Fig. 4B). However, we could not find any difference in the

proportion of MAIT cells between the IBD patients in remission and the controls (Fig. 4B). Taken together with the blood results, an accumulation of MAIT cells in the inflamed mucosa was correlated with their decreased frequency in the peripheral blood of IBD patients with higher disease activity. We also analyzed the expression of CD69, NKG2D, NKG2A, IL7R, CCR6 and CCR5 on the colon MAIT cells of the IBD patients. As shown in Fig. 4C, the proportion of colon MAIT cells expressing CCR6 was markedly higher in the IBD patients with mild disease activity (71.1 ± 10.8%) compared to the controls (38.4 ± 14.7%, $P = 0.02$). The frequency of colon MAIT cells expressing CD69 or NKG2D was not significantly different between the IBD patients and the controls, but the frequency of MAIT cells expressing CD69 was remarkably higher in the colon than in the blood (Fig. 2C and 4C).

Altered patterns of cytokine secretion by MAIT cells in IBD

Several studies have shown that MAIT cells produce mainly tumor necrosis factor (TNF)-α, IL-17, interferon (IFN)-γ and IL-22 when stimulated *in vitro* (18, 22). We speculated that when activated *in vivo*, the MAIT cells in IBD patients might produce more of these pro-inflammatory cytokines. To examine this, we analyzed their cytokine secretion patterns after *in vitro* stimulation with anti-CD3/CD28-coupled beads with or without IL-7 using intracellular cytokine staining. We found no difference between the MAIT cell secretion patterns in the UC patients compared to the CD patients (data not shown). Therefore, we analyzed the differences

in the cytokine secretion patterns between IBD patients and controls. MAIT cells from the IBD patients secreted significantly more TNF-α and IL-17 compared to the healthy controls (Fig. 5A). Interestingly, MAIT cells from the IBD patients secreted significantly higher amount of those cytokines without any stimulation. This might result from the *in vivo* activation of MAIT cells in patients with IBD. We also analyzed the cytokine secretion of INF-γ and IL-22 and the expression of CCR6 by MAIT cells in the blood, but we found no differences in the amount of cytokines or the expression of CCR6 between the IBD patients and the controls (Fig. 5B). These results suggested that MAIT cells secreted significantly higher amounts of TNF-α and IL-17 and might be involved in the disease progression of IBD.

DISCUSSION

In the present study, we found a significant decrease of MAIT cells, especially CD8α$^+$ MAIT cells, in the blood of patients with IBD compared to the healthy controls. On the other hand, the frequency of tissue MAIT cells was significantly higher in the inflamed colons compared to the normal colons. When the IBD patients were classified according to clinical activity, we observed that the proportion of MAIT cells in the colon increased with more severe disease activity. To the best of our knowledge, this is the first study demonstrating the association between the proportion of MAIT cells in the colon and the clinical activity of IBD patients, as determined by flow cytometry. Compared to the healthy controls, MAIT cells in the patients with higher levels of inflammation showed a greater number of activated phenotypes, and MAIT cells in the patients with IBD produced significantly higher amount of TNF-α and IL-17 spontaneously and after stimulation with CD3/CD28 and IL-7. These results suggest that activated MAIT cells accumulated in the inflamed colon in IBD patients and may be involved in the immunopathogenesis of the disease through the production of cytokines, including TNF-α and IL-17.

There are a number of reports describing a notable association between the frequency of MAIT cells in the peripheral blood and inflammatory diseases. In active tuberculosis, HIV infection and multiple sclerosis, the numbers of MAIT cells are significantly reduced in the

peripheral blood compared to healthy controls (3, 5, 15, 10, 31). Several studies have also shown an accumulation of MAIT cells in infected or inflamed tissues, such as the lung in tuberculosis and brain lesions in multiple sclerosis (6, 31). Willing *et al.* demonstrated that a reduction of MAIT cells in the blood of patient with multiple sclerosis was significantly correlated with serum IL-18 levels in the patients and suggested that an IL-18-driven activation of MAIT cells might contribute to their infiltration to the central nervous system in multiple sclerosis (31). Moreover, Cosgrove *et al.* used immunohistochemistry in biopsy specimens to investigate MAIT cells in the colons of patients with HIV infection, suggesting that gut sequestration and apoptosis in response to bacterial signals might be mechanisms that contribute to a specific decrease of MAIT cells in the blood of patients with HIV infection (3).

Although MAIT cells are found in normal tissues, such as the lung and the intestine, and are particularly abundant in the human liver (4), only a few reports are available on the proportions of MAIT cells in the intestinal mucosa of patients with IBD. Serriari *et al.* reported that the frequency of MAIT cells was specifically reduced in the blood of IBD patients compared to healthy donors and that it was dramatically greater in the inflamed mucosa of ileum in CD patients compared with the normal mucosa (21). Haga *et al.* recently reported that MAIT cells increased in the inflamed mucosa, and their frequency was correlated with clinical and endoscopic disease activities in UC patients (7). On the other hand, Hiejima *et al.* reported that the number of MAIT cells was lower in both

the blood and inflamed mucosa of patients with UC and CD compared to non-IBD controls (9). Although those studies mainly employed immunohistochemistry to analyze the frequency of MAIT cells, we examined MAIT cells in the colon using flow cytometry. We clearly found a strong accumulation of MAIT cells in the inflamed mucosa of the colon in IBD patients, which was consistent with previous reports (6, 21). Moreover, we could also examine the expression of several markers, including CD69, NKG2D, NKG2A, IL7R, CCR5 and CCR6, on MAIT cells in the mucosa of colon. Interestingly, almost all the MAIT cells in the colon from both the IBD patients and controls expressed CD69, and there was no significant difference in the expression of CD69 on MAIT cells in the colon between the patients with IBD and the controls, although CD69 expression was markedly higher in the colon than in the blood. MAIT cells also expressed a high level of CD161, which has been shown to be associated with a high level of IL-18Rα expression (28). We also observed that MAIT cells expressed a high level of IL-18Rα both in the blood and colon (data not shown). In the patients with multiple sclerosis, it has been suggested that an IL-18-driven activation of MAIT cells might contribute to their infiltration to the central nervous system (31). Elevated levels of IL-18 are found in many chronic inflammatory disorders, including IBD, and polymorphisms in the IL-18R1-IL-18RAP locus are associated with IBD susceptibility (8). Therefore, the IL-18-driven activation of MAIT cells might also contribute to their accumulation in the intestine. Furthermore, we found that the frequency of colon MAIT cells expressing

CCR6 was markedly higher in the IBD patients in an active disease state compared with the controls. Because CCL20, the ligand for CCR6, is expressed by healthy and inflamed intestinal mucosa in humans (1, 13), these findings might indicate that in IBD patients, circulating MAIT cells were activated *in vivo* and then accumulated in the inflamed colon tissues as they expressed greater levels of the tissue-targeting molecule CCR6.

We analyzed the frequencies of MAIT cells in the patients with both UC and CD, but at first, we could not find any significant differences between UC and CD. Although UC and CD are two distinct diseases with different immunopathological mechanisms, our findings were consistent with those of a previous report (21). However, when we classified the IBD patients according to clinical activity, we revealed that the frequencies of MAIT cells were correlated with the disease activities. This may have resulted from a migration of MAIT cells into the inflamed mucosa that produced such mediators as chemokines, including CCL20. Another speculation is that dysbiosis and the alterations of the mucosal barrier in IBD cause a presentation of present commensal bacterial antigens by MR1, which do not take place under physiological situations (21). The severity of alteration of mucosal barrier might be affected mainly by the inflammation activity; therefore, the MAIT cells accumulated in the inflamed mucosa in both UC and CD. As we revealed that the frequencies of MAIT cells both in the blood and the colon were correlated with the clinical activity of IBD, the frequency of MAIT cells in the blood might serve as a potential biomarker in IBD.

Several studies have shown that MAIT cells primarily produce TNF-α, IL-17, IFN-γ, and IL-22 when stimulated *in vitro* (18, 22). In this study, we analyzed the cytokine secretion patterns of MAIT cells in the blood of IBD patients after *in vitro* stimulation with anti-CD3/CD28-coupled beads in the presence or absence of IL-7. MAIT cells also expressed high levels of IL-7R. Tang *et al.* showed that IL-7, a cytokine produced by hepatocytes during inflammation, regulates the TCR-mediated activation of MAIT cells, promoting their dramatically increased production of Th1 cytokines and IL-17A (23). We observed that MAIT cells from IBD patients secreted significantly more TNF-α and IL-17 than those from healthy donors. Interestingly, MAIT cells from the IBD patients spontaneously secreted TNF-α and IL-17 without any *in vitro* stimulation. Therefore, it is suggested that MAIT cells are activated *in vivo* in the patients with IBD, and IL-17 secreted by the MAIT cells accumulated in the mucosa might contribute to the intestinal inflammation. However, because IL-17 is not always pathogenic and has been shown to play protective functions in several models of intestinal inflammation (18), the role of MAIT cells during intestinal inflammation in IBD remains to be elucidated.

In conclusion, the present study confirms and extends previous studies and suggests that MAIT cells are activated in patients with IBD, accumulate in the inflamed mucosa, and display increased cytokine secretion capacities, especially TNF-α and IL-17. However, any possible biases can be enumerated. First, the low number of patients studied,

especially in the CD group, might also explain the low statistical power of these data. Second, most of our patients showed only mild disease activity, and therefore, their results might not accurately reflect the conditions in strongly active disease state. Third, we could not obtain endoscopic data from all the patients in this study. Furthermore, because of the sample sizes of the biopsies, we could not examine intraepithelial lymphocytes and lamina propria lymphocytes separately. Therefore, further studies are required to clarify the role of MAIT cells in IBD.

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Table 1 Patient Characteristics

	UC	CD	HI
n	25	15	19
Age (Median) (range)	45 (22-72)	39 (26-60)	57 (26-80)
Gender (Male / Female)	17 / 8	13 / 2	14 / 5
Ongoing treatments			
5-ASA	22	10	
Corticosteroids	10	1	
Immunosuppressors	5	4	
Anti-TNFalpha	0	1	
Surgery (yes / no)	1 / 24	4 / 11	
Clinical activity			
Partial Mayo Clinic score / Crohn's Disease Activity Index			
moderate	10	3	
mild	9	8	

remission	6	4

Abbreviations: UC, ulcerative colitis; CD, Crohn's disease; HI, healthy individuals; 5-ASA, 5-aminosalicylic acid; TNF, tumor necrosis factor.

Table 2 Absolute cell numbers of MAIT cells in the blood

	n	Total MNCs ($x10^6$ / mL blood)	MAIT cells ($x10^4$ / mL blood)
Healthy individuals	7	1.13 ± 0.39	2.38 ± 1.32
IBD (Clinical activity)			
remission	5	1.23 ± 0.41	1.63 ± 0.08
mild	5	1.12 ± 0.63	0.99 ± 0.39*
moderate	5	1.19 ± 0.55	0.90 ± 0.50**

MNCs, mononuclear cells; MAIT cells, mucosal-associated invariant T cells; IBD, inflammatory bowel disease. *P = 0.033, **P = 0.026 (compared to healthy individuals). The mean ± SD is shown.

Figure legends

Fig. 1 Identification and analysis of mucosal-associated invariant T (MAIT) cells in the blood and colons of patients. (A) Representative results of flow cytometry gating on $CD3^{+}TCR\text{-}\gamma\delta^{-}$ cells in the peripheral blood and colon from a patient are shown. (B) Representative results of flow cytometry gating on TCR-Vα7.2^{+}CD161^{high} MAIT cells in the peripheral blood and colon from a patient are shown.

Fig. 2 Comparison of the frequency of MAIT cells in the peripheral blood from patients with inflammatory bowel diseases (IBDs) and healthy donors. (A) The proportion of MAIT cells in the blood of IBD patients and healthy donors was analyzed by flow cytometry. We separated the IBD patients into three groups according to clinical activity. One representative example for each group is shown. (B) The proportion of MAIT cells among $CD3^{+}TCR\text{-}\gamma\delta^{-}$ T cells in healthy individuals (HI) (n = 19), UC [n = 25: remission (n = 6), mild disease activity (n = 9), and moderate disease activity (n = 10)] and CD [n = 15: remission (n = 4), mild disease activity (n = 3), and moderate disease activity (n = 8)] patients. MAIT cells are specifically decreased in the blood of IBD patients. (C) Bar graphs representing the levels of CD8α, CD69 and NKG2D expression on MAIT cells in the peripheral blood from patients. The data are presented as the means + standard errors. *P <0.001; **P <0.05.

Fig. 3 $CD161^{+}V\alpha7.2^{+}$ cells accumulate in the inflamed mucosa of UC patients. Surgically removed colon tissues were collected from patients with colon tumors, and the colon tissues distant from the tumor were examined as controls. Colon surgical specimens of patients with colon cancer (A) and UC (B) were stained with anti-CD161 or anti-TCR-Vα7.2 antibodies. This staining strategy allowed for the identification of $CD161^{+}$ cells or TCR-$V\alpha7.2^{+}$ cells. Representative results from 12 healthy individuals and 3 patients with UC are shown. Scale bar represents 100 μm. (Original magnification, x100)

Fig. 4 Comparison of the frequency of MAIT cells in the peripheral blood and colons from healthy donors and IBD patients. (A) One representative example for each group is shown. (B) The percentage of MAIT cells among $CD3^{+}$TCR-$\gamma\delta^{-}$ T cells in healthy individuals (HI) (n = 11) and in UC (n = 15) and CD (n = 6) patients. We separated the IBD patients into two groups according to clinical activity: remission (n = 10) and mild disease activity (n = 11). The frequency of MAIT cells are specifically increased in the colons of IBD patients with mild disease activity. (C) Bar graphs representing the levels of CD69, NKG2D and CCR6 expression in MAIT cells in the colons of patients. The data are presented as the means + standard errors. $*P < 0.01$; $**P < 0.05$.

Fig. 5 Cytokine secretion patterns and chemokine receptor expression of MAIT cells in the blood of IBD patients. The proportion of positive for

cytokines and receptors on MAIT cells from the blood of healthy individuals (HI) (n = 14) and UC (n = 15) and CD (n = 10) patients were analyzed after stimulation with anti-CD3/CD28-coupled beads in the presence or absence of IL-7 for 72h. The results for TNFα, IL-17, IFN-γ, IL-22 and CCR6 are shown. The data are presented as the means + standard errors. *P <0.05

Figure 1

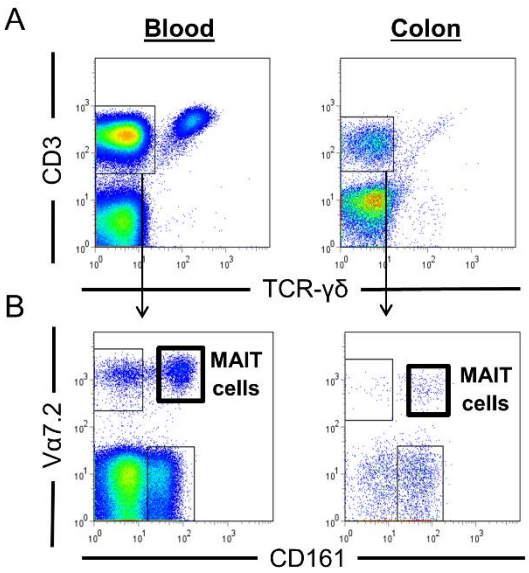


Figure 2

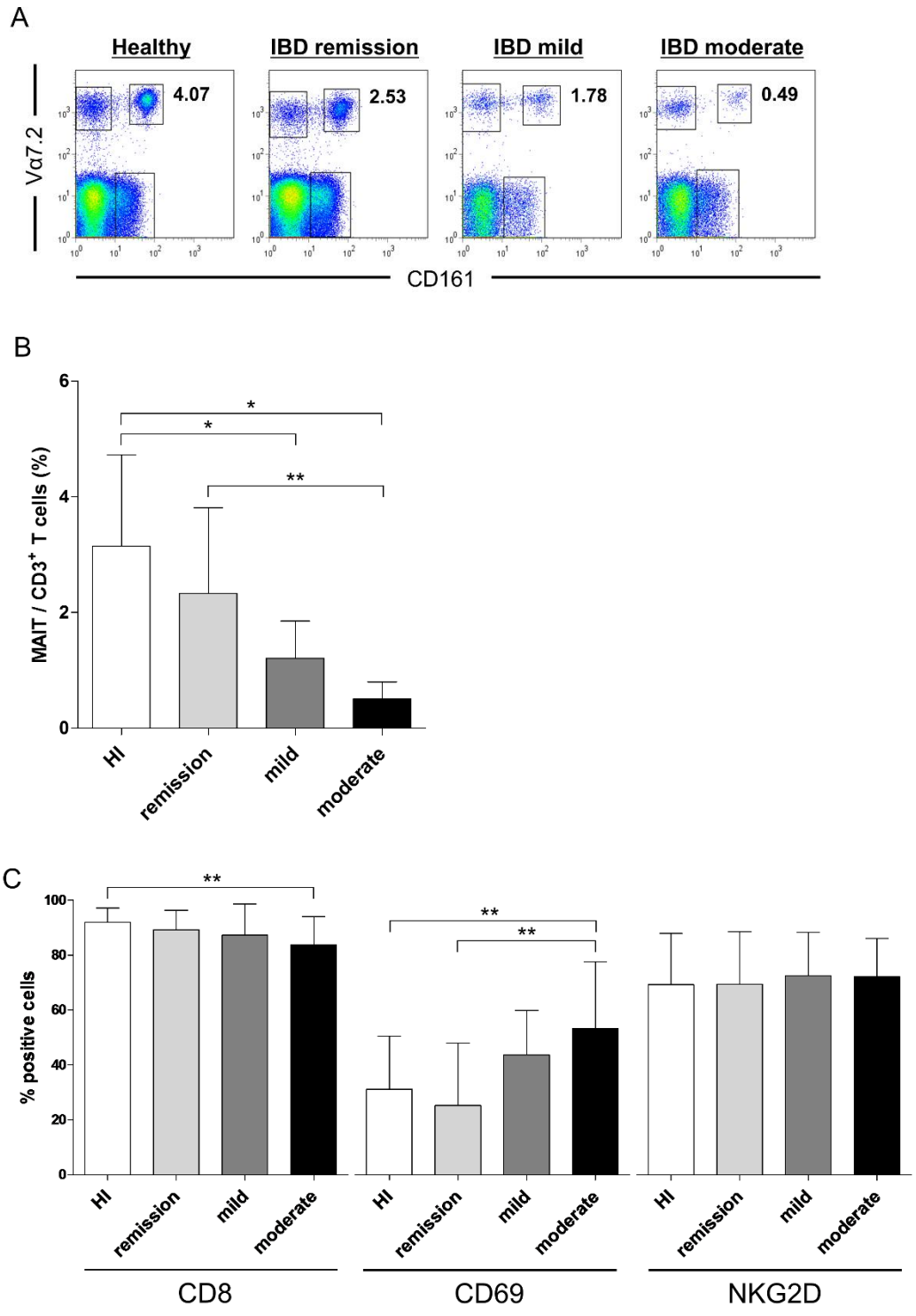

A
Healthy
IBD remission
IBD mild
IBD moderate
4.07
2.53
1.78
0.49
Vα7.2
CD161
B
MAIT / CD3+ T cells (%)
HI
remission
mild
moderate
C
% positive cells
CD8
CD69
NKG2D

Figure 3

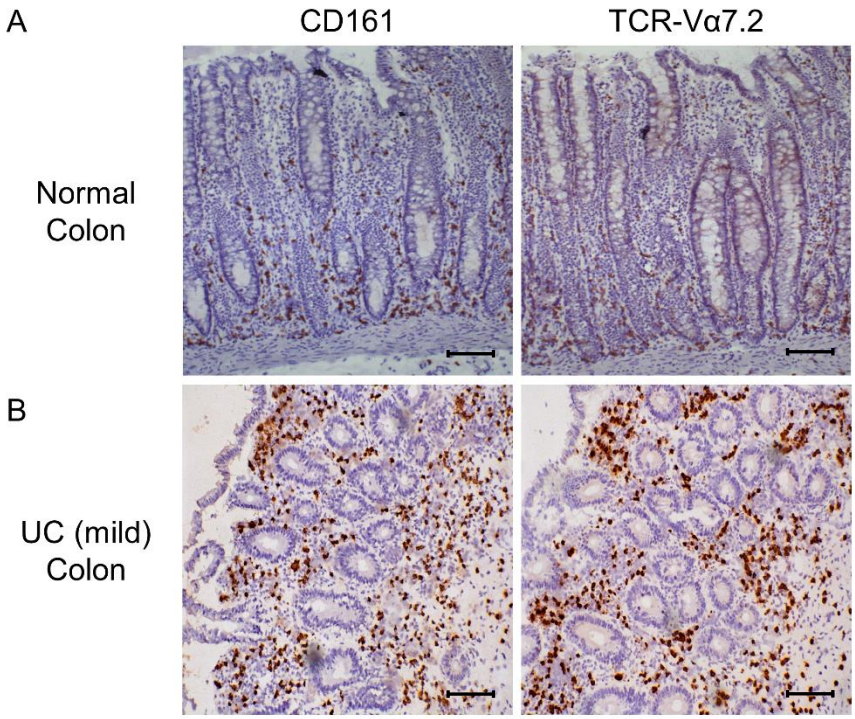


Figure 4

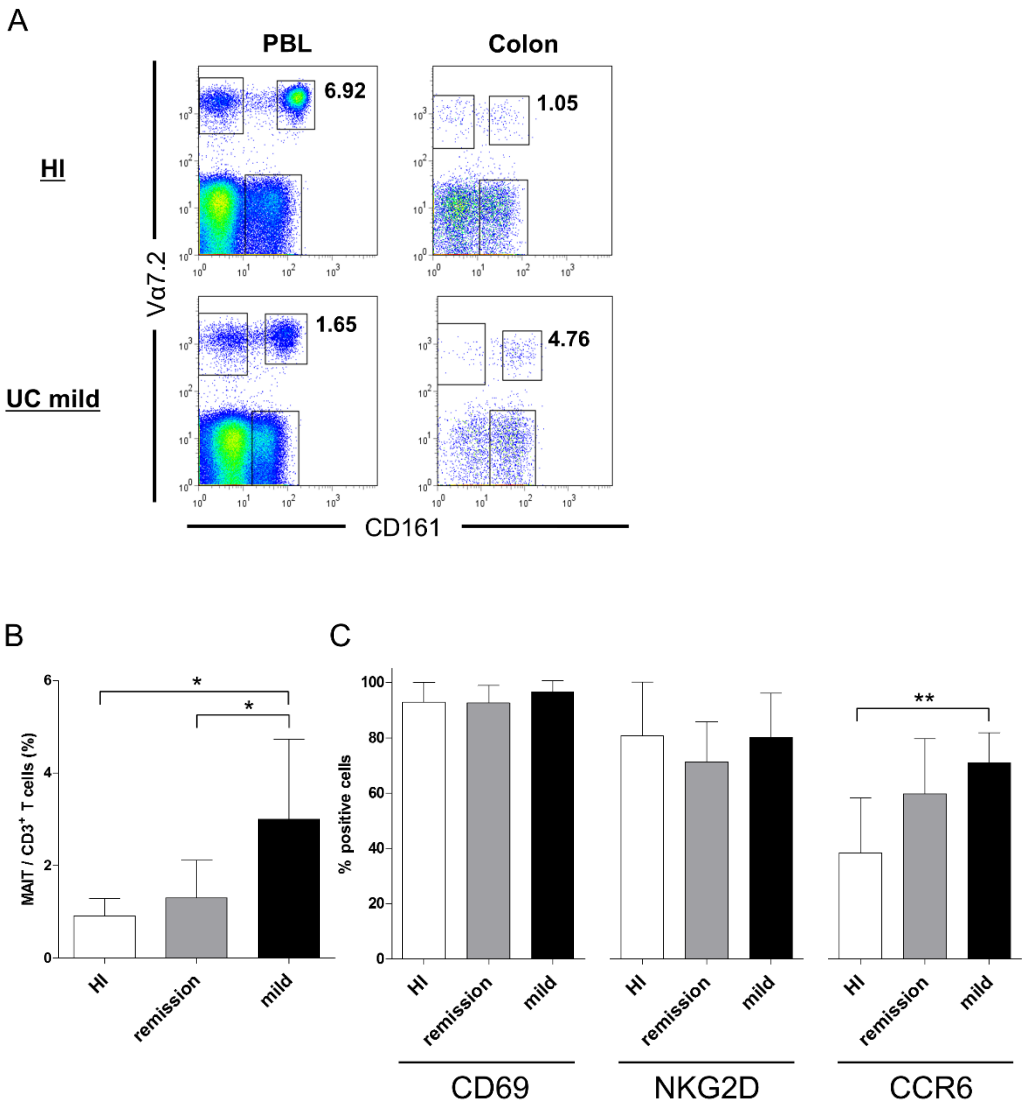
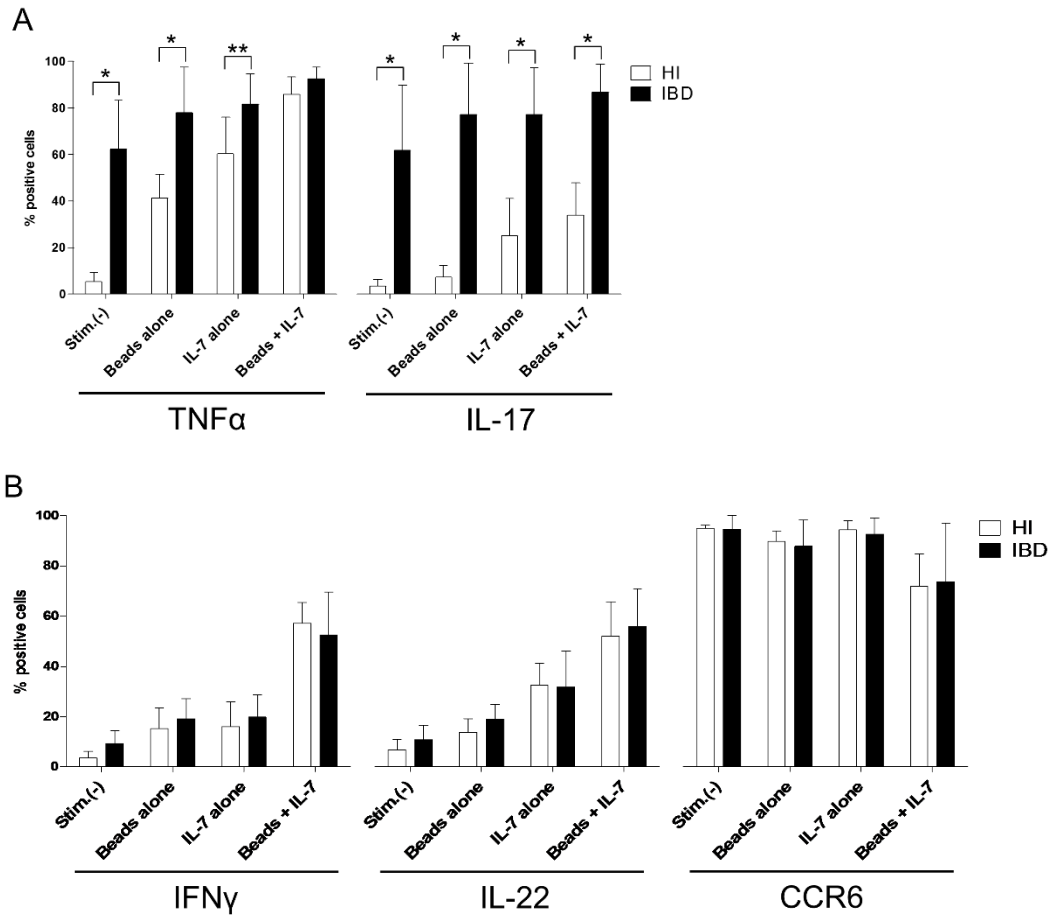

A
PBL
Colon
HI
UC mild
6.92
1.05
1.65
4.76
Vα7.2
CD161
B
MAIT / CD3+ T cells (%)
HI
remission
mild
C
% positive cells
CD69
NKG2D
CCR6

Figure 5


A
% positive cells
HI
IBD
Stim.(-)
Beads alone
IL-7 alone
Beads + IL-7
TNFα
IL-17
B
IFNγ
IL-22
CCR6