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Mucosal-associated invariant T cells regulate Th1 response in multiple sclerosis

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Abstract

Mucosal-associated invariant T (MAIT) cells are innate T cells expressing an invariant Vα7.2-Jα33 T-cell antigen receptor α chain and are enriched in mucosal-associated lymphoid tissues. Although the regulatory role of MAIT cells in experimental autoimmune encephalomyelitis has been determined, their role in multiple sclerosis (MS) has not been elucidated. In the present study, the character of MAIT cells in the peripheral blood of MS patients was analyzed. Compared with healthy controls, the frequency of MAIT cells in peripheral blood was significantly reduced in MS patients in remission and even more profoundly reduced in those with relapse. The frequency of MAIT cells reflected the disease activity, as they were reduced significantly in patients with active disease compared with stable patients, and when blood samples from patients undergoing attack were analyzed 2–3 months later, the frequency significantly increased in parallel with clinical recovery. The frequency of MAIT cells positively correlated with the frequency of $CD4^+$ invariant NKT cells and of $CD56^{bright}$ NK cells in healthy controls but not in MS patients. This suggests the existence of an immune-regulatory link between MAIT cells and these other cell populations with disruption of this cross talk in MS. Moreover, MAIT cells showed a suppressive activity against IFN-γ production by T cells *in vitro*. This suppression required cell contact but was independent of IL-10, inducible co-stimulator or the presence of B cells. Taken together, these results suggest an immune-regulatory role of MAIT cells in MS through suppression of pathogenic T_h1 cells.

Keywords: CD161, immune regulation, IFN-γ, MR1, NKT cells

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) (1). Whereas the cause of MS is multifactorial, a central role has been suggested for autoimmune responses against the myelin component of the CNS (2). This idea is strongly supported by the results of clinical trials with altered peptide ligands. Administration of an altered peptide ligand of myelin basic protein induced MS exacerbation in some patients and this exacerbation was accompanied by an increase in IFN-γ-producing T cells cross-reactive to the original antigen (3). Furthermore, the importance of immune cell entry into the CNS was shown by treatment using a mAb against very late activation-4 (4). In addition, the immunogenetic background of MS and data from animal models also support its autoimmune nature. Currently, the pathological roles of CNS myelin-reactive helper T cells that produce IFN-γ (T_h1) and/or IL-17 (T_h17) are receiving substantial attention (1, 2). However, growing evidence has shown that the immunopathology of MS is more complicated, and the importance of a balance between pathogenic cells and immune-regulatory cells has also been suggested (1, 5). For instance, it was reported that NK cells exhibited suppressive activity against pathogenic T_h1 cells specific to myelin basic protein, but the suppressive property of NK cells was lost in MS patients with relapse (6, 7). In addition, the function of $CD4^+$ invariant natural killer T (iNKT) cells was reported to be biased toward T_h2, an immunosuppressive phenotype, in MS patients in remission but not in those with relapse (8). Moreover, a functional defect in the suppressive activity of $CD4^+CD25^{high}$ regulatory T cells in MS patients was reported (9). These findings suggest that functional defects in immune-regulatory cells are related to the development and/or relapse of MS.

Mucosal-associated invariant T (MAIT) cells are innate T cells first identified among human CD4/CD8 double-negative (DN) T cells as a novel cell population bearing an invariant T-cell antigen receptor (TCR) α chain distinct from Vα24 iNKT cells (10, 11). MAIT cells express a canonical Vα7.2-Jα33 TCR α chain in preferential combination with Vβ2 and Vβ13 in the human and are restricted by MR1, a major histocompatibility complex class Ib molecule expressed on bone marrow-derived cells (12). The

unique features of MAIT cells are that they are enriched in intestinal lamina propria and their development and peripheral expansion are dependent on the presence of B cells and commensal flora (12–14). Little is known about the role of MAIT cells in health or in disease states, but a disease-suppressive role of this cell population was reported in experimental autoimmune encephalomyelitis (EAE), an animal model of MS (15). Moreover, using PCR single-strand conformational polymorphisms, MAIT cells were found to infiltrate MS lesions (16). However, a detailed picture of the role of MAIT cells in human autoimmune diseases including MS does not yet exist.

In this study, we show that the frequency of MAIT cells in peripheral blood is reduced in MS patients and that their frequency reflects the disease activity of MS. In addition, we found that the frequency of MAIT cells positively correlates with those of $CD4^+$iNKT cells and $CD56^{bright}$ NK cells in healthy subjects but not in MS patients, suggesting that MAIT cells may regulate the immune system in concert with these cell populations to prevent MS. Moreover, we show a suppressive role of MAIT cells against IFN-γ production from T cells and suggest a disease-suppressive role for MAIT cells in MS. This is the first report to demonstrate a role for MAIT cells in human autoimmune diseases.

Materials and methods

Subjects

Thirty-two patients with relapsing–remitting MS [age: 40.2 ± 1.7 (mean ± SE)] and 16 age- and sex-matched healthy controls (HC) (age: 40.4 ± 2.6) were examined. The diagnosis of MS was established according to the 2005 version of the McDonald's criteria (17, 18), and patients with neuromyelitis optica were not included. Among the 32 MS patients, 25 (age: 40.7 ± 2.0) were in remission and 7 (age: 38.1 ± 3.2) were in an acute phase of relapse. None of the patients in remission was taking any medications affecting the immune system, such as IFN-β, corticosteroids or immunosuppressants. Among the seven patients in relapse, four were free of medication, two were being treated with oral prednisolone and one was being treated with mizoribine. This study was approved by the Ethical Committee of the National Institute of Neuroscience and written consent was obtained from all subjects.

Flow cytometry

Fresh peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by Ficoll-Paque (GE Healthcare, Buckinghamshire, UK) separation and then stained with combinations of the following mAb against human cell surface antigens: FITC-anti-TCR-γδ mAb, FITC-anti-TCR-Vα24 mAb, phycoerythrin (PE)-anti-CD161 mAb, PE-anti-TCR-Vβ11 mAb, PE-Texas Red-anti-CD3 mAb, PE-anti-CD4 mAb, allophycocyanin (APC)-anti-CD56 mAb (all from BeckmanCoulter, Brea, CA, USA), FITC-anti-CD19 mAb, Peridinin chlorophyll protein-anti-CD5 mAb, Peridinin chlorophyll protein-anti-CD8α mAb, APC-anti-CC chemokine receptor (CCR) 5 mAb, -mouse IgG1, APC-Cy7-anti-CD4 mAb and APC-Cy7-anti-CD3 mAb (all from BD Biosciences, Franklin Lakes, NJ, USA). FITC-anti-CCR6 mAb and -mouse IgG1 were purchased from R&D Systems (Minneapolis, MN, USA). Staining of biotin-conjugated 3C10 mAb (13) was visualized using streptavidin-PE-Cy7 (BD Biosciences). Cells were analyzed on an FACS Aria flow cytometer (BD Biosciences) with FloJo software (Tree Star, Ashland, OR, USA).

Intracellular cytokine staining

PBMC (5×10^5 cells per well in 24-well culture plates) were stimulated with 50 ng ml^{-1} phorbol-myristate-acetate (PMA) (Sigma, St Louis, MO, USA) and 500 ng ml^{-1} ionomycin (IM) (Sigma) for 4 h at 37°C in 5% CO_2. Monensin (BD Bioscience) was added in the last 2 h of culture at a concentration of 2 μM. After staining the cell surface antigens, intracellular cytokines were stained using the BD Cytofix/Cytoperm Fixation/Permeabilization Solution Kit (BD Bioscience) and FITC-anti-IFN-γ (BeckmanCoulter), FITC-anti-IL-17, APC-anti-IL-4 or APC-anti-IL-10 mAbs or their isotype control antibodies (all from eBiosciences, San Diego, CA, USA). Cells were analyzed on an FACS Aria flow cytometer with FloJo software.

Cell culture

To analyze cytokine production from MAIT cells *in vitro*, MAIT cells ($CD5^+CD19^-$TCR-γδ$^-CD161^{high}3C10^+$) or other T cells ($CD5^+CD19^-$TCR-γδ$^-CD161^-3C10^-$) were isolated from the PBMC of HC using a FACS Aria cell sorter and cultured in 96-well flat-bottom plates at 1×10^5 cells per well with RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, 0.05 mM 2-mercaptoethanol, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Cells were stimulated with immobilized anti-CD3 mAb (5 μg ml^{-1}, clone OKT3; American Type Culture Collection, Manassas, VA, USA) and soluble anti-CD28 mAb (1 μg ml^{-1}, clone CD28.2; BeckmanCoulter) or PMA (50 ng ml^{-1}) and IM (500 ng ml^{-1}) for 48 h. IFN-γ, IL-4 and IL-10 in the supernatant were quantified using an ELISA Kit (BD Bioscience). IL-17 was measured using an ELISA Kit purchased from R&D Systems.

To deplete MAIT cells from the PBMC of HC and MS patients, $CD5^+CD19^-$TCRγδ$^-CD161^{high}3C10^+$ cells were depleted using a FACS Aria cell sorter. Control PBMC were also stained with the same combination of mAbs and passed through the cell sorter without depletion of MAIT cells. Cells were cultured in 96-well flat-bottom plates at 2×10^5 cells per well and stimulated with 2 μg ml^{-1} of PHA (Sigma). Cytokine concentrations in the supernatant at 48 h of culture were measured using the ELISA kits described above. In some experiments, MAIT cells (1×10^5 cells per well) isolated using the cell sorter were added back into PBMC before stimulation, from which MAIT cells had been depleted. In this add-back experiments, MAIT cells were depleted from PBMC using anti-PE-CD161 mAb and anti-PE-magnetic beads (Miltenyi Biotec, Gladbach, Germany). To block cell contact between MAIT cells and other cells, transwell inserts (Corning, Corning, NY, USA) were used. For blocking experiments, anti-IL-10 mAb (10 μg ml^{-1}), anti-inducible co-stimulator (ICOS)-ligand (L) mAb (10 μg ml^{-1}) (both from eBioscience), anti-transforming growth factor (TGF)-β mAb (10 μg ml^{-1}; R&D systems) or their isotype control antibodies were added to the culture. Anti-CD19-magnetic beads (Miltenyi Biotec) were used to deplete B cells from PBMC.

Quantification of cytokine mRNA

Total RNA was extracted from isolated MAIT cells or control T cells using RNeasy spin columns (QIAGEN, Germantown, MD, USA) and reverse transcribed into complementary DNA using Primescript reverse transcriptase (Takara, Ohtsu, Japan). Levels of IFN-γ and IL-17 mRNA were measured by quantitative PCR using an SYBR Premix Ex Taq Kit (Takara) on a LightCycler1.5 (Roche, Basel, Switzerland). Expression levels relative to those of β-actin are presented. The primer pairs used were as follows: IFN-γ forward, 5′-ACAGGGAAGCGAAAAAGGAGTCAG-3′ and IFN-γ reverse, 5′-CATGGGATCTTGCTTAGGTTGG-3′; IL-17 forward, 5′-CCAGGATGCCCAAATTCTGAGGAC-3′ and IL-17 reverse, 5′-CAAGGTGAGGTGGATCGGTTGTAG-3′ and β-actin forward, 5′-CACTCTTCCAGCCTTCCTTCC-3′ and β-actin reverse, 5′-GCGTACAGGTCTTTGCGGATG-3′.

Results

MAIT cells are reduced in the peripheral blood of MS patients and reflect disease activity

Previously, CD161^{high} 3C10 (Vα7.2)-positive cells have been reported to represent MAIT cells in adult human peripheral blood (13, 19, 20). Therefore, we used this definition of MAIT cells to analyze the frequency of MAIT cells in peripheral blood by flow cytometry in HC and MS patients in remission (MS remission) or in relapse (MS relapse). MAIT cells could be identified as a distinct cell population bearing a Vα7.2^{+}CD161^{high} phenotype in all subjects (Supplementary Figure 1A is available at *International Immunology* Online). Representative profiles of a HC and an MS relapse are shown in Fig. 1(A). In HC, the frequency of MAIT cells among total αβT cells was 3.79 ± 0.52% (mean ±SEM). In MS remission, the frequency of MAIT cells was 2.33 ± 0.39%, which was significantly lower than that in HC (Fig. 1B). The frequencies of Vα7.2^{+}CD161^{low} and Vα7.2^{+}CD161^{-} or Vα7.2^{-}CD161^{high} populations were not different between HC and MS patients, suggesting that the reduced frequency of MAIT cells in MS patients was not simply due to down-modulation of the Vα7.2 TCR or CD161 molecules in MAIT cells in MS patients. In addition to the DN population, within which MAIT cells were first identified, MAIT cells include also CD4, CD8αα and CD8αβ populations (13). Since all the CD4, CD8αβ, CD8αα and the DN MAIT cell sub-populations were reduced in MS patients compared with HC, the decrease in MAIT cell frequency was not attributed to reduction of a certain sub-population of MAIT cells (Fig. 1C). The frequency of total αβT cells among PBMC was not different between HC, MS remission and MS relapse (61.7 ± 4.7, 65.8 ± 1.9 and 67.85 ± 3.2%, respectively).

The decrease in the frequency of MAIT cells was more profound in MS relapse (0.87 ± 0.24%) (Fig. 1B). MS patients who had at least one attack or had been found to have an active lesion by magnetic resonance imaging within 1 year had significantly lower numbers of MAIT cells compared with patients stable for more than a year (Fig. 1D). Furthermore, when MS relapse patients were followed up for 2–3 months after the attack, the frequency of MAIT cells significantly increased along with the clinical recovery

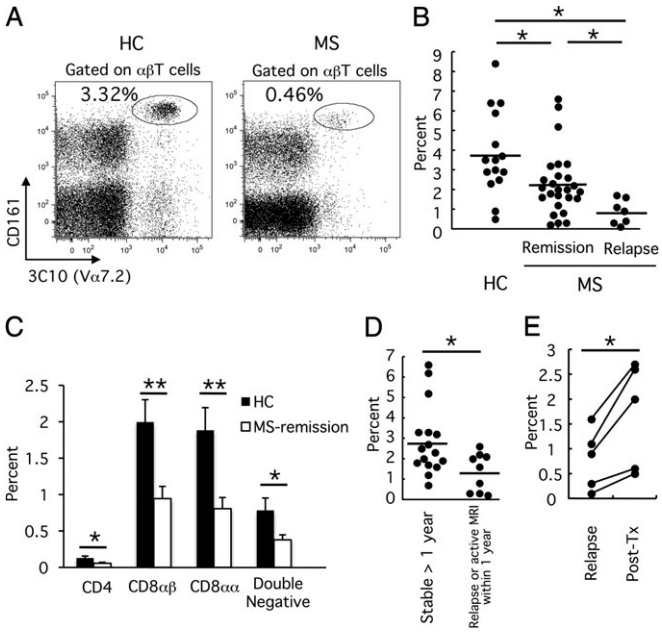

Fig. 1. Frequency of MAIT cells among αβT cells in peripheral blood. (A) Representative flow cytometry profiles of CD3^{+}TCR-γδ^{-} cells in a HC (left) and in an MS relapse (right). (B) Frequency of MAIT cells among αβT cells in HC (N = 16), MS remission (N = 25) and MS relapse (N = 7). Each symbol represents the value of one individual. Horizontal bars indicate the means. *P < 0.05 (Kruskal–Wallis H-test followed by Mann–Whitney U-test with Bonferroni correction). (C) Frequency of CD4, CD8αβ, CD8αα and DN MAIT cell sub-population among total αβT cells in HC and MS remission. Error bars represent the SEM. *P < 0.05, **P < 0.01 (Mann–Whitney U-test). Since the proportion of MAIT cells in some patients with MS remission and most of those with MS relapse was too low to assess precisely the frequency of each MAIT cell sub-population, they were not included in the analysis. (D) Frequency of MAIT cells in MS patients stable for >1 year and those who had at least one clinical attack or had been found to have active magnetic resonance imaging lesions within 1 year. *P < 0.05 (Mann–Whitney U-test). (E) Frequency of MAIT cells in five patients analyzed at an acute phase of relapse and 2–3 months after steroid therapy (Post-Tx). *P < 0.05 (Wilcoxon t-test).

(Fig. 1E). These results indicate that the frequency of MAIT cells in peripheral blood is reduced in MS remission and reduced even more profoundly in MS relapse, and the frequency reflected disease activity.

The positive correlations in the frequency of MAIT cells with those of CD4^{+}iNKT cells and CD56^{bright} NK cells are lost in MS.

Since several other innate lymphocytes such as CD4^{+}iNKT cells and CD56^{bright} NK cells are believed to participate in the regulation of MS (8, 21), we next examined the correlations of the frequency of MAIT cells with the frequencies of those innate lymphocytes. As shown in Fig. 2 (upper panels), positive correlations between the frequencies of MAIT cells and those of CD4^{+}iNKT cells and CD56^{bright} NK cells were observed in HC. In MS patients, however, the frequency of CD56^{bright} NK cells was decreased along with MAIT cells (Fig. 2, lower right panel). In the case of CD4^{+}iNKT cells, the positive correlation with MAIT cells that was observed in HC was disrupted in MS (Fig. 2, lower left panel).

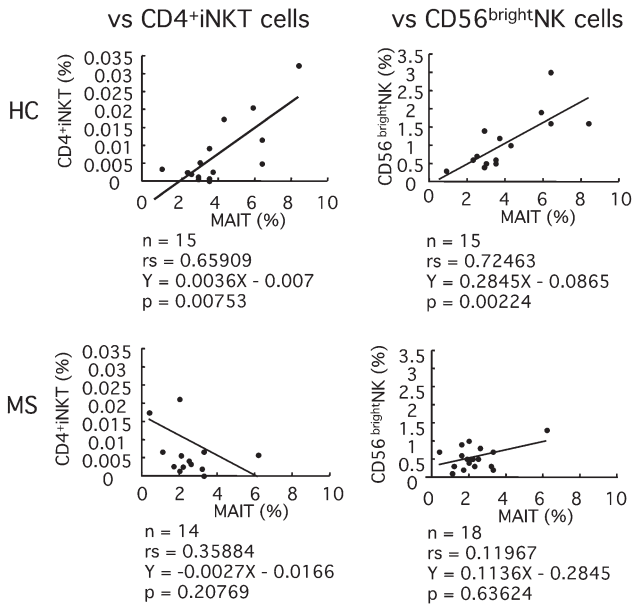

Fig. 2. Correlation of the frequency of MAIT cells with the frequency of $CD4^+$iNKT cells and $CD56^{bright}$ NK cells. The frequency of MAIT cells was plotted against the frequency of $CD4^+$iNKT cells ($CD3^+CD4^+$TCR-$V\alpha24^+$-$V\beta11^+$) among total T cells (left panels) or that of $CD56^{bright}$ NK cells ($CD3^-CD56^{bright}$) among total lymphocytes (right panels) in HC (upper panels) or in MS remission (lower panels). Correlations were analyzed using Spearman's correlation.

Characterization of MAIT cells in HC and MS

To further characterize MAIT cells, we analyzed the expression of chemokine receptors important for CNS invasion. Compared with other T cells, MAIT cells exhibited higher expression of CCR5 and CCR6 (Fig. 3A, top panels), although the expression levels were not different between HC and MS (Fig. 3A, lower panels). We next assessed cytokine production from MAIT cells purified from the PBMC of HC. In response to PMA and IM stimulation, MAIT cells produced substantial levels of IFN-γ and IL-17 (Fig. 3B) but not IL-4 or IL-10 (data not shown). However, none of these responses was observed when MAIT cells were stimulated through CD3 and CD28 (Fig. 3B). These results suggest that the activation of MAIT cells is differently regulated from that of conventional T cells. Intracellular cytokine staining also revealed that MAIT cells produced high levels of IFN-γ in response to PMA and IM (Fig. 3C, upper panels). However, interestingly, the proportion of IFN-γ^+ MAIT cells was not different between HC and MS patients (Fig. 3C, middle left panel). We could also detect intracellular IL-17 in response to PMA and IM, but the frequency was substantially lower compared with the frequency of MAIT cells positive for IFN-γ and was not different between HC and MS patients (Fig. 3C, middle right panel). In contrast to these two cytokines, the frequencies of IL-4^+ and IL-10^+ MAIT cells were lower than those of other T cells positive for these cytokines and were not different between HC and MS patients (Fig. 3C, lower panels).

To evaluate the *in vivo* status of MAIT cells, we next measured cytokine mRNA expression in MAIT cells isolated from HC or MS without additional stimulation. As shown in Fig. 3(D), expression levels of IFN-γ and IL-17 in MAIT cells were not different from control T cells, and the values were comparable between HC and MS patients.

MAIT cells suppress IFN-γ production from T cells in a cell contact-dependent manner

To address the function of MAIT cells in peripheral blood, we evaluated whether depletion of MAIT cells from PBMC might affect cytokine production from T cells. As shown in Fig. 4(A), IFN-γ production in response to PHA stimulation was increased by depletion of MAIT cells from PBMC derived from both HC and MS patients. The enhanced production of IFN-γ by MAIT cell depletion was also observed when PBMC were stimulated through CD3 or CD3 and CD28 (Supplementary Figure 2 is available at *International Immunology* Online). The enhancement of the production was specific to IFN-γ since other cytokines including IL-4, IL-10 and IL-17 were not altered by depletion of MAIT cells from PBMC (Supplementary Figure 3 is available at *International Immunology* Online). These findings suggested that MAIT cells suppress IFN-γ production from T cells in peripheral blood. This IFN-γ suppression by MAIT cells was confirmed by adding purified MAIT cells back into PBMC from which MAIT cells had been depleted (Fig. 4B).

To further elucidate the mechanism of IFN-γ suppression by MAIT cells, we first examined the involvement of suppressive cytokines such as IL-10 and TGF-β by adding their specific mAbs to the culture. MAIT cell-mediated suppression of IFN-γ production was not altered in the presence of these mAbs (Fig. 4C). We next examined whether MAIT cell-mediated IFN-γ production requires cell contact. As shown in Fig. 4(B), the IFN-γ suppression by MAIT cells could not be observed when the cell contact between MAIT cells and other cells was blocked using transwell inserts. Since we previously showed that ICOS/ICOS-L interaction is involved in the suppression of EAE (15), we next examined the effect of anti-ICOS-L mAb in this culture system. The inhibition of IFN-γ production was similar in the presence of anti-ICOS-L mAb compared with that in the presence of control immunoglobulin (Fig. 4C). We next assessed the requirement for B cells in MAIT cell suppression of IFN-γ since we have previously shown that MAIT cell suppression of EAE was dependent on the presence of B cells in this model (15). However, as shown in Fig. 4(D), B-cell depletion had no effect on MAIT cell-dependent suppression of IFN-γ production. These findings indicate that MAIT cell-mediated suppression of IFN-γ production from T cells in peripheral blood required cell contact but not IL-10, TGF-β, ICOS or B cells.

Discussion

In this study, we show that MAIT cells, which comprise a large cell population in human peripheral blood, are reduced in MS patients, especially in those with active disease. Although the precise mechanism of this reduction of MAIT cells in the peripheral blood of MS patients could not be addressed in our present study, the trafficking of MAIT cells from blood into MS lesions is a possible explanation, especially in patients with active disease and those in relapse since we previously showed that MAIT cells invade MS lesions (16). In support of this idea, we found in this

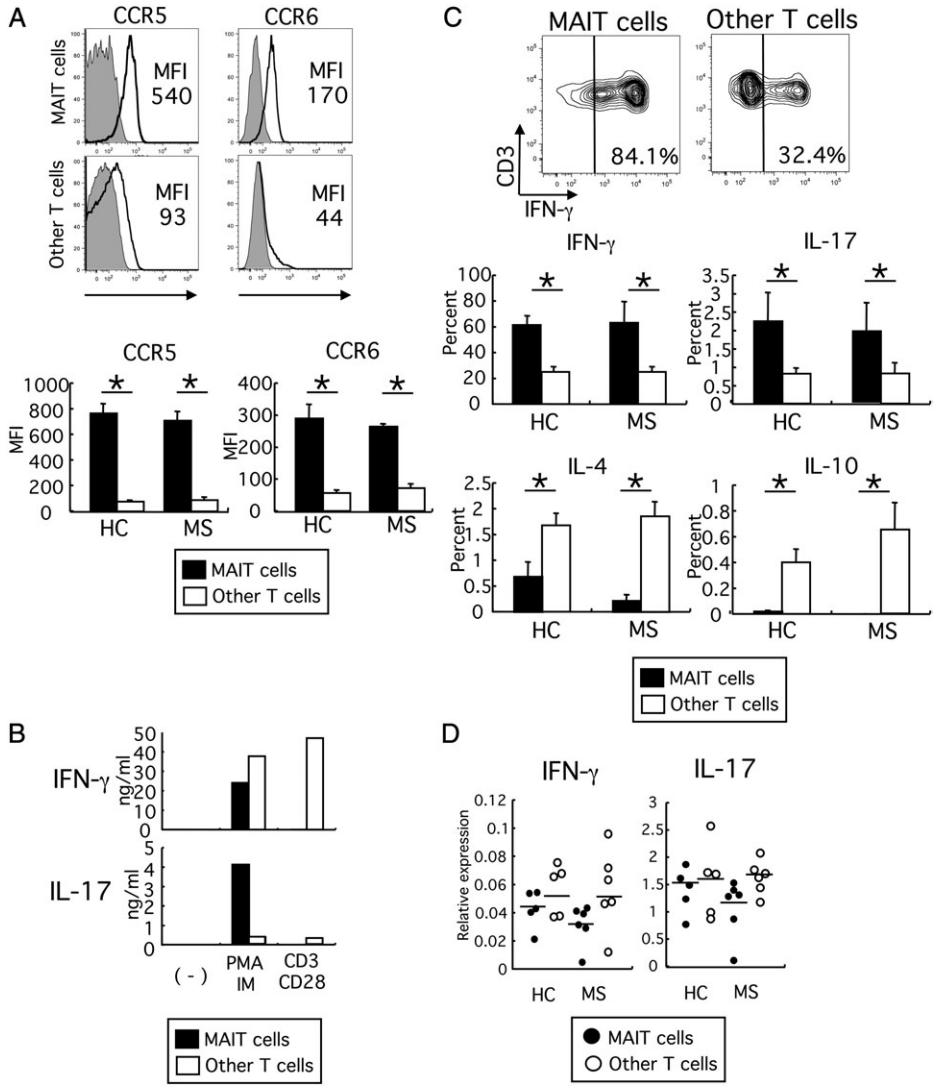

Fig. 3. Phenotype, activation properties and cytokine profile of MAIT cells. (A) Representative histograms of CCR5 and CCR6 expression on MAIT cells ($CD3^+TCR\text{-}\gamma\delta^-CD161^{high}3C10^+$) and other T cells ($CD3^+TCR\text{-}\gamma\delta^-3C10^-$) from an MS remission (upper panels). Bold lines indicate staining of the specific mAb, and shaded histograms indicate background staining of their isotype control antibodies. Mean fluorescence intensity is indicated in each histogram. Results from HC (n = 9) and MS remission (n = 6) are summarized in the lower panels. Error bars represent the SEM. *P < 0.05 (Wilcoxon t-test). (B) IFN-γ (upper panel) and IL-17 (lower panel) production from isolated MAIT cells ($CD5^+CD19^-TCR\text{-}\gamma\delta^-CD161^{high}3C10^+$) and other T cells ($CD5^+CD19^-TCR\text{-}\gamma\delta^-CD161^-3C10^-$) stimulated with PMA and IM or anti-CD3- and -CD28-mAb. Representative results from four independent experiments using cells from three HCs are shown. (C) Intracellular cytokine staining of MAIT cells ($CD3^+TCR\text{-}\gamma\delta^-CD161^{high}3C10^+$) and other T cells ($CD3^+TCR\text{-}\gamma\delta^-3C10^-$). Representative staining profiles of IFN-γ from an MS remission are shown (upper panels), and results of IFN-γ, IL-17, IL-4 and IL-10 staining from HC (n = 8) and MS remission (n = 5) are summarized (lower panels). Error bars represent the SEM. *P < 0.05 (Wilcoxon t-test). (D) IFN-γ (left) and IL-17 (right) mRNA expression in MAIT cells ($CD3^+TCR\text{-}\gamma\delta^-CD161^{high}3C10^+$) and other T cells ($CD3^+TCR\text{-}\gamma\delta^-3C10^-$) isolated from HC ($n$ = 5) and MS remission (n = 6). Each symbol represents the value of one individual. Horizontal bars indicate the means.

study that MAIT cells express high levels of CCR5, CCR6 and α4β1 integrin (data not shown), molecules that are important in the infiltration of T cells into MS lesions, although expression level of these molecules were not different between HC and MS patients. In addition to these findings in MS, it was recently shown that MAIT cells express specific pattern of chemokine receptor (14) and infiltrate lesions resulting from bacterial infection (19, 20), chronic inflammatory demyelinating polyneuropathy (16) and kidney and brain tumors (22). These findings suggest that it is the MAIT cells' character to infiltrate inflammatory lesions.

A second possible explanation for the reduced MAIT cell frequency in the PBMC of MS patients is developmental impairment of MAIT cells in patients. It was previously shown that the development and peripheral expansion of MAIT cells were dependent on the host's microbiological environment (12, 13). In addition, recent epidemiological studies pointed out a universal increase in the prevalence of MS over time (23) and emphasized the importance of changes in environmental factors including sanitation and food quality, factors that affect the profile of intestinal microflora. In this context, our hypothesis is that the change in sanitation status and

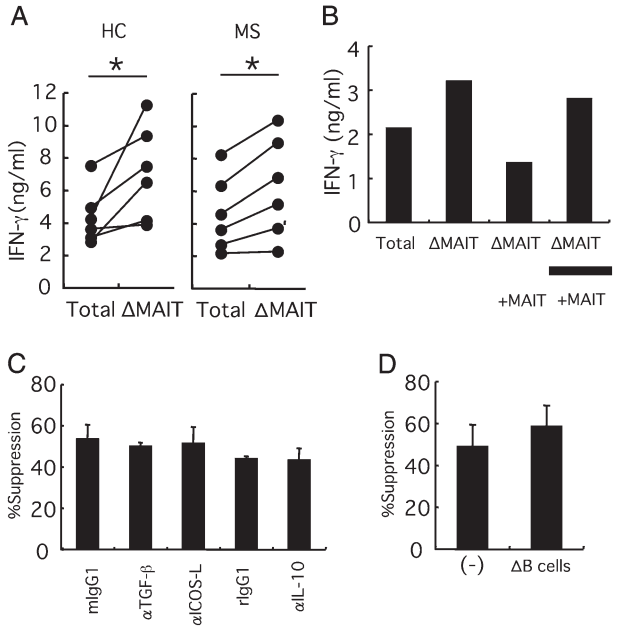

Fig. 4. Suppression of IFN-γ production from T cells by MAIT cells. (A) IFN-γ production from PBMC stimulated with PHA with (ΔMAIT) or without (total) depletion of MAIT cells in HC and MS remission ($n = 6$ each). *$P < 0.05$ (Wilcoxon *t*-test). (B) IFN-γ production from MAIT cell-depleted PBMC (ΔMAIT) with (+MAIT) or without addition of MAIT cells. Horizontal bar indicates the presence of transwell inserts between MAIT cells and other cells. A representative result from four independent experiments using PBMC from three HCs is shown. (C and D) Efficiency of IFN-γ suppression (%suppression) by MAIT cells in the presence of mAbs against TGF-β, ICOS-L, IL-10 (C) or when B cells were depleted from the culture (ΔB cells) (D). Mean ± SEM of three independent experiments using PBMC from three HCs are shown.

quality of food intake has induced alterations in the profile of gut microflora and impaired the development of MAIT cells and, consequently, resulted in the increased prevalence of MS. On the other hand, genetic factors may also be relevant to an impairment in MAIT cell development in MS. In this regard, it is interesting to note that a single-nucleotide polymorphism in the CD161 molecule, which is expressed at high levels in MAIT cells, has been correlated with MS susceptibility (24); however, the role of this molecule in MAIT cell function and development has not been elucidated.

We observed positive correlations between the frequency of MAIT cells and those of $CD4^+$iNKT and $CD56^{bright}$ NK cells in HC. In contrast, these correlations were not observed in MS patients. These findings suggest the existence of an immune-regulatory link among these innate lymphocyte populations wherein they cooperate to regulate autoimmune responses and imply that the immune pathology of MS is related to a disruption in this regulatory link. In addition, these findings suggest that studies on the immune pathology of MS should not focus only on a single cell population but should also take notice of the system within which the immune cells exist. On the other hand, we cannot deny that these cell populations in peripheral blood are regulated independently. Indeed, while MAIT cells have a propensity to infiltrate inflammatory tissues, $CD56^{bright}$ NK cells are known to migrate into lymph nodes.

An interesting property of human MAIT cells identified in this study is their non-responsiveness to CD3 and CD28 stimulation *in vitro*. This is consistent with a previous report that the $CD8^+CD161^{high}$ T-cell population was not responsive to CD3 and CD28 stimulation even in the presence of exogenous IL-2 (25). We confirmed that most of this $CD8^+CD161^{high}$T cells express Vα7.2 TCR and correspond to MAIT cells. On the other hand, it was recently shown that MAIT cells respond to antigen-presenting cells by producing IFN-γ in an MR1-dependent manner only when the antigen-presenting cells are infected with bacteria (20). These observations suggest a unique activation property of MAIT cells, although the precise mechanism of activation and the cognate antigen are unknown.

We demonstrated in this study that MAIT cells suppress IFN-γ production from T cells and suggest a disease-suppressive role for MAIT cells in MS via suppression of autoreactive T_h1 cells. A suppressive role for MAIT cells was similarly seen in the mouse EAE model and preferential suppression of IFN-γ over other cytokines was also observed in this system (15). With regard to the mechanism of suppression, however, factors other than the requirement for cell contact were quite different between these two species. In mice, the interaction of MAIT cells with B cells through ICOS induced IL-10 production from both cell populations, and this IL-10 up-regulation was associated with EAE amelioration (15). In contrast, we could not detect ICOS expression (data not shown) or IL-10 production by human MAIT cells and suppression of IFN-γ did not require IL-10, ICOS or B cells. In addition, other MAIT cell differences between these species have been reported: human MAIT cells express zinc finger-and BTB domain-containing protein 16 (ZBTB16) transcription factor (26), show a memory phenotype (13, 14), are anergic to CD3 and CD28 stimulation and produce mainly pro-inflammatory cytokines in response to PMA and IM. In contrast, mouse MAIT cells are negative for ZBTB16 (13), show a naive phenotype (13) and respond to TCR stimulation by producing both pro- and anti-inflammatory cytokines (27, 28). The reason for these differences is not clear, but one possibility is the difference in commensal flora that these species are exposed to during their evolution.

In contrast to the present findings, a pro-inflammatory role for MAIT cells in MS cannot formally be ruled out since MAIT cells produced IFN-γ and IL-17 in response to PMA and IM stimulation in this study. Similar finding was reported recently by Dusseaux *et al.* (14). However, similar to us, they could not detect IL-17 production in response to CD3 and CD28 stimulation even in the presence of IL-18 or IL-23, in contrast to the high level of IL-17 and IFN-γ production from MAIT cells stimulated with PMA and IM. Therefore, as the activation properties of MAIT cells are quite unique and as the signal(s) required for MAIT cell activation in MS is largely unknown, conclusions from studies using only PMA and IM do not necessarily reflect the *in vivo* cytokine profile of this cell population. This question requires further studies analyzing the cytokine profile of MAIT cells in MS lesions without exogenous stimulation. In this regard, the results of our cytokine mRNA quantification in unstimulated MAIT cells from peripheral blood are in contradiction to the inflammatory nature of this cell population in MS.

In summary, we show that MAIT cells are reduced in the peripheral blood of MS patients and that their frequency reflects the disease activity of MS. Moreover, we found that MAIT cells, consistent with an immune-regulatory link with other innate immune cell populations, provide a disease-suppressive role in MS by repressing IFN-γ production from T cells. We hypothesize that MAIT cells act as a sensor for environmental changes by responding to alterations in gut microflora by modulating the host's immune system. This property of MAIT cells should be favorable for host defense in most cases but may be disadvantageous in some case including MS. It is possible, however, that a novel treatment for MS might be established by enhancing the immunosuppressive property of MAIT cells through modulation of the host's gut microflora.

Supplementary data

Supplementary data are available at *International Immunology* Online.

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