Mucosal-Associated Invariant T Cell Deficiency in Systemic Lupus Erythematosus

Young-Nan Cho, Seung-Jung Kee, Tae-Jong Kim, Hye Mi Jin, Moon-Ju Kim, Hyun-Ju Jung, Ki-Jeong Park, Sung-Ji Lee, Shin-Seok Lee, Yong-Soo Kwon, Hae Jin Kee, Nacksung Kim and Yong-Wook Park

*J Immunol* 2014; 193:3891-3901; Prepublished online 15 September 2014;
doi: 10.4049/jimmunol.1302701
http://www.jimmunol.org/content/193/8/3891

Supplementary Material
http://www.jimmunol.org/content/suppl/2014/09/14/jimmunol.1302701.DC1

References
This article cites 48 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/193/8/3891.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Mucosal-Associated Invariant T Cell Deficiency in Systemic Lupus Erythematosus

Young-Nan Cho,*1 Seung-Jung Kee,†,1 Tae-Jong Kim,* Hye Mi Jin,* Moon-Ju Kim,* Hyun-Ju Jung,* Ki-Jeong Park,* Sung-Ji Lee,* Shin-Seok Lee,* Yong-Soo Kwon,‡ Nacksung Kim,§ and Yong-Wook Park∗

Mucosal-associated invariant T (MAIT) cells contribute to protection against certain microorganism infections and play an important role in mucosal immunity. However, the role of MAIT cells remains enigmatic in autoimmune diseases. In this study, we examined the level and function of MAIT cells in patients with rheumatic diseases. MAIT cell, cytokine, and programmed death-1 (PD-1) levels were measured by flow cytometry. Circulating MAIT cell levels were significantly reduced in systemic lupus erythematosus (SLE) and rheumatoid arthritis patients. In particular, this MAIT cell deficiency was more prominent in CD8+ and double-negative T cell subsets, and significantly correlated with disease activity, such as SLE disease activity index and 28-joint disease activity score. Interestingly, MAIT cell frequency was significantly correlated with NKT cell frequency in SLE patients.

We also report a novel finding that this MAIT cell deficiency is associated with NKT cell deficiency and elevated PD-1 expression. These abnormalities possibly contribute to dysregulated mucosal immunity in SLE. The Journal of Immunology, 2014, 193: 3891–3901.

Received for publication October 7, 2013. Accepted for publication August 12, 2014.

This work was supported by the National Research Foundation of Korea (Grants 2011-001132 and 2013R1A2A2A01067956) and the Chonnam National University Hospital Research Institute of Clinical Medicine (Grant CRI10004-1).

Address correspondence and reprint requests to Dr. Yong-Wook Park, Department of Rheumatology, Chonnam National University Medical School and Hospital, Gwangju 501-757, Republic of Korea; and Yong-Wook Park, Department of Pulmonary and Critical Care Medicine, Chonnam National University Medical School, Gwangju 501-757, Republic of Korea.

Copyright © 2014 by The American Association of Immunologists, Inc. 0022-1767/14/$16.00

The online version of this article contains supplemental material.

Abbreviations used in this article: AS, anklylosing spondylitis; BD, Behçet’s disease; CaA, cyclosporin A; DAS28, 28-joint disease activity score; DMARD, disease-modifying antirheumatic drug; DN, double-negative; α-GalCer, α-galactosylceramide; IC, healthy control subject; IM, immunocyto; MAIT, mucosal-associated invariant T; MR1, MHC class 1b-like related protein; PD-1, programmed death-1; PD-L1, PD-L1 ligand; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1302701
and functions have not previously been investigated in rheumatic diseases. In addition, the relevance of MAIT cells to NKT cell dysfunction has not been determined. The aims of this study were to examine the level and function of MAIT cells in rheumatic diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), ankylosing spondylitis (AS), and Behcet’s disease (BD); to evaluate the clinical relevance of MAIT cell levels; to investigate the potential relationship between MAIT cells and NKT cells; and finally, to determine the mechanism responsible for MAIT cell deficiency.

Materials and Methods

Patients and healthy control subjects

The study cohort included 54 patients diagnosed as having SLE (51 women and 3 men; mean age ± SD, 34.7 ± 11.2 y) according to the 1997 American College of Rheumatology revised criteria for the classification criteria of SLE (27), 60 patients diagnosed as having RA (54 women and 12 men; mean age ± SD, 56.6 ± 14.3 y) according to the American College of Rheumatology/European League Against Rheumatism 2010 classification criteria for RA (28), 21 patients diagnosed as having AS (2 women and 19 men; mean age ± SD, 35.3 ± 10.8 y) according to the modified New York criteria for AS (29), 9 patients diagnosed as having BD (2 women and 7 men; mean age ± SD, 49.9 ± 8.4 y) according to the International Study Group for BD criteria (30), and 136 healthy control subjects (HCs; 67 women and 69 men; mean age ± SD, 50.0 ± 18.4 y) who had no history of autoimmune disease, infectious disease, malignancy, chronic liver or renal disease, or diabetes mellitus. None of the control subjects had ever received immunosuppressive therapy, and none had fever during the 72 h before enrollment. The clinical and laboratory characteristics of the patients and controls are summarized in Table I. The study protocol was approved by the Institutional Review Board of Chonnam National University Hospital, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

mAbs and flow cytometry

The following mAbs and reagents were used in this study: FITC-conjugated anti-CD3, PerCP-conjugated anti-CD4, allophycocyanin- or PE-Cy7–conjugated anti-CD127, FITC–conjugated anti-TCR Vα7.2, PE-conjugated HLA-DR, PE-conjugated anti-IFN-γ, PE-conjugated anti–IL-4, PE-conjugated anti–IL-17, and PE-conjugated mouse IgG isotype control (all from Becton Dickinson, San Diego, CA); allophycocyanin-conjugated anti-TCR Vα7.2 (BioLegend, San Diego, CA); allospecific antimouse Fluor 700-conjugated anti-CD3 and Pacific Blue-conjugated anti-CD4 (Beckman Coulter, Marseille, France); and PE-conjugated anti–programmed death-1 (anti–PD-1; BioRad, Hercules, CA). Cells were stained with combinations of appropriate mAbs for 20 min at 4˚C. Stained cells were analyzed and sorted into a Navios flow cytometer using Kaluza software (Beckman Coulter, Brea, CA).

Isolation of PBMCs and the identification of MAIT and NKT cells

Peripheral venous blood samples were collected in heparin-containing tubes, and PBMCs were isolated by density-gradient centrifugation using Ficoll–Paque Plus solution (Amersham Biosciences, Uppsala, Sweden). MAIT cells and NKT cells were identified phenotypically as CD3+ TCRγδ+ Vα7.2+CD161+ and CD3+ CD8+ NKT cells, respectively, by flow cytometry as previously described (4, 5, 17, 19). To confirm whether the reported proportions of MAIT cells in PBMCs contain the MAIT cell rearrangement, we performed TCR genotyping in sorted CD3+ TCRγδ+ Vα7.2+CD161+ cells. In brief, CD3+ TCRγδ+ Vα7.2+CD161+ cells were sorted to the purity of >95% from PBMCs of HCs by FACS sorter. After DNA isolation, TCR Vα7.2 T-cell region was amplified with a pair of Vα7.2 and Cα primers by RT-PCR. The amplified cDNA were cloned and >10 clones from each individual were submitted to sequencing.

Statistical analysis

All comparisons of MAIT cells and their subset levels, cytokine levels of MAIT cells, and expression levels of CD69 and PD-1 were performed by analysis of covariance after adjusting for age and sex using the Bonferroni correction for multiple comparisons. Changes in IFN-γ expression in MAIT cells after treatment with IM or inhibitor were examined using paired t test. Linear regression analysis was used to test associations between MAIT cell levels and clinical variables. Relationships between MAIT and NKT cell levels were examined using Spearman’s correlation coefficient. Wilcoxon signed rank test was used for the comparison of MAIT cell levels between peripheral blood and synovial fluid. The p values <0.05 were considered statistically significant. All statistical analyses were performed using SPSS version 17.0 software (SPSS, Chicago, IL).

Results

Reduced numbers of circulating MAIT cells in SLE and RA patients

The percentages and absolute numbers of MAIT cells in the peripheral blood samples from 54 SLE patients, 66 RA patients, 21 AS patients, 9 BD patients, and 136 HCs were determined by flow cytometry (Table I). All comparisons of percentages and absolute
numbers of MAIT cells were performed by analysis of covariance after adjusting for age and sex using the Bonferroni correction for multiple comparisons, as described in Materials and Methods. MAIT cells were defined as CD3⁺γδ T cells expressing TCR Vα7.2 and CD161bd (Fig. 1A). Percentages of MAIT cells were significantly lower in SLE patients and in RA patients than in HCs (median: 0.29 versus 2.13% [p < 0.001] and 0.73 versus 2.13% [p < 0.01], respectively; Fig. 1B). Absolute numbers of MAIT cells were calculated by multiplying the MAIT cell percentages by the CD3⁺γδ T cell percentages and the total lymphocyte numbers (per microliter). SLE patients and RA patients had significantly lower absolute numbers of MAIT cells as compared with HCs (median: 0.9 versus 16.2 cells/μl [p < 0.001] and 3.1 versus 16.2 cells/μl [p < 0.05], respectively; Fig. 1C). However, no significant differences were observed in the percentages and absolute numbers of MAIT cells between AS or BD patients and HCs.

Relation between circulating MAIT cell levels and clinical parameters in SLE and RA patients

To evaluate the clinical relevance of MAIT cell levels in SLE and RA patients, we investigated relationships between absolute MAIT cell numbers in peripheral blood and clinical parameters by regression analysis (Tables II and III). Univariate linear regression analysis showed that log-transformed absolute MAIT cell numbers were significantly correlated with age, lymphocyte count, and SLE disease activity index (SLEDAI; p = 0.026, p = 0.020, and p < 0.001, respectively) in SLE patients. After multivariate analysis, age and SLEDAI were found to be significantly correlated with log-transformed absolute MAIT cell numbers (p = 0.002 and p < 0.001, respectively; Table II). In RA patients, univariate linear regression analysis showed that log-transformed absolute MAIT cell numbers were significantly correlated with age, lymphocyte count, and 28-joint disease activity score (DAS28; p = 0.031, p = 0.002, and p < 0.001, respectively), and after adjusting for these variables, the correlation with lymphocyte count and DAS28 remained statistically significant (p = 0.002 and p = 0.002, respectively; Table III).

Selective reduction of the CD8⁺ and double-negative MAIT cell subsets in the peripheral blood of SLE and RA patients

Based on the expression of CD4 and CD8, MAIT cells were subdivided into CD8⁺, CD8⁻, and double-negative (DN) subsets (Fig. 2A). As previously observed (2, 4, 5, 26, 34), the majority of MAIT cells in blood consisted of CD8⁺ and DN subsets. Percentages of CD8⁺ MAIT cell subsets in CD8⁺ T cells were significantly lower in SLE patients and in RA patients than in HCs (median: 0.2 versus 1.7% [p < 0.001] and 0.56 versus 1.7% [p < 0.005], respectively). SLE and RA patients had significantly lower percentages of DN subsets as compared with HCs (median: 0.02 versus 0.14% [p < 0.005] and 0.05 versus 0.14% [p < 0.01], respectively; Fig. 2B).

Reduced numbers of circulating NKT cells in SLE patients

The percentages and absolute numbers of NKT cells in the peripheral blood samples from 42 SLE patients, 54 RA patients, 17 AS patients, 7 BD patients, and 100 HCs were determined by flow cytometry. Because age and sex can affect the NKT cell numbers, all comparisons of the percentages and absolute numbers of the NKT cells in HCs and patients were analyzed after adjusting for age and sex, as described in Materials and Methods. NKT cells were defined as lymphocytes coexpressing CD3 and 6B11 (Fig. 3A). Percentages of NKT cells were significantly lower in SLE patients than in HCs (median 0.01 versus 0.07%, p < 0.05; Fig. 3B). Absolute numbers of NKT cells were calculated by multiplying the NKT cell percentages by the total lymphocyte numbers (per microliter). SLE patients had significantly lower absolute numbers of NKT cells as compared with HCs (median 0.2 versus 1.5 cells/μl, p < 0.005; Fig. 3C). However, no significant differences were observed in the percentages and absolute numbers of NKT cells between RA, AS, or BD patients and HCs.

Correlation of MAIT cell deficiency with a lack of NKT cells in SLE patients

To evaluate the relationship between MAIT cells and NKT cells in SLE and RA patients, we investigated the association between MAIT and NKT cell levels in peripheral blood using Spearman’s correlation analysis (Table IV). The analysis revealed that total MAIT cell percentages were significantly correlated with total NKT cell percentages (r = 0.488, p = 0.001; n = 42) in SLE patients, but no significant correlation was found between MAIT and NKT cell levels in RA patients. In addition, CD8⁺ and DN MAIT cell subset percentages were found to be significantly correlated with CD8⁺ and DN NKT cell subset percentages in SLE patients (p = 0.021 and p = 0.004, respectively). These results suggest that MAIT cell deficiency is correlated with NKT cell deficiency in SLE, but not in RA.

Impaired IFN-γ production in MAIT cells of SLE patients

To examine the MR1-independent cytokine expression in MAIT cells, we incubated PBMCs from 10 SLE patients, 10 RA

Table I. Clinical and laboratory characteristics of the patients and HC subjects

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>SLE</th>
<th>RA</th>
<th>AS</th>
<th>BD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y), mean ± SD</td>
<td>50.0 ± 18.4</td>
<td>34.7 ± 11.2</td>
<td>56.6 ± 14.3</td>
<td>35.3 ± 10.8</td>
<td>49.9 ± 8.4</td>
</tr>
<tr>
<td>Disease duration (y), mean ± SD</td>
<td>4.1 ± 2.7</td>
<td>6.0 ± 5.6</td>
<td>3 ± 2.6</td>
<td>4.8 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Leukocytes (cells/μl), mean ± SD</td>
<td>6385 ± 1429</td>
<td>5526 ± 2084</td>
<td>7595 ± 2251</td>
<td>7490 ± 1797</td>
<td>6711 ± 1822</td>
</tr>
<tr>
<td>Lymphocytes (cells/μl), mean ± SD</td>
<td>2317 ± 632.0</td>
<td>1246 ± 625.6</td>
<td>1747 ± 563.5</td>
<td>2171 ± 820.5</td>
<td>1833 ± 406.2</td>
</tr>
<tr>
<td>CRP (mg/dl), mean ± SD</td>
<td>0.2 ± 0.08</td>
<td>0.5 ± 0.9</td>
<td>2.0 ± 2.7</td>
<td>1.2 ± 1.2</td>
<td>0.6 ± 0.6</td>
</tr>
<tr>
<td>Complement 3 (mg/dl), mean ± SD</td>
<td>ND</td>
<td>73 ± 21.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Complement 4 (mg/dl), mean ± SD</td>
<td>ND</td>
<td>14 ± 6.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CH50 (Um/l), mean ± SD</td>
<td>43 ± 13.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Anti-dsDNA (Um/l), mean ± SD</td>
<td>251.3 ± 1037</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SLEDAI, mean ± SD</td>
<td>ND</td>
<td>5.5 ± 4.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DAS28, mean ± SD</td>
<td>ND</td>
<td>3.1 ± 1.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Medication naive, n (%)</td>
<td>ND</td>
<td>6 (11)</td>
<td>6 (9.1)</td>
<td>2 (9.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Current user of steroid, n (%)</td>
<td>ND</td>
<td>48 (89)</td>
<td>60 (90.9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Current user of immunosuppressive agent,&quot; n (%)</td>
<td>ND</td>
<td>17 (31.5)</td>
<td>46 (69.7)</td>
<td>0 (0)</td>
<td>1 (11.1)</td>
</tr>
</tbody>
</table>

*Indicates current use of cyclophosphamide, methotrexate, azathioprine, cyclosporine, or mycophenolate mofetil.

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ND, not done.
patients, and 10 HCs for 4 h in the presence of PMA and IM; then the expression of IFN-γ, IL-17, and IL-4 in the MAIT cell population was examined at the single-cell level by intracellular flow cytometry. Percentages of MAIT cells were calculated within an αβ T cell gate. (A) Representative MAIT cell percentages as determined by flow cytometry. (B) MAIT cell percentages among peripheral blood αβ T cells. (C) Absolute MAIT cell numbers (per microliter of blood). Symbols (●) represent individual subjects; horizontal bars show the median. *p < 0.05, **p < 0.01, ***p < 0.001.

Table II. Regression coefficients of log-transformed absolute MAIT cell numbers with respect to clinical features and laboratory findings in SLE patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th></th>
<th></th>
<th>Multivariate*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>p</td>
<td>β</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.467</td>
<td>0.549</td>
<td>0.398</td>
<td>-0.028</td>
</tr>
<tr>
<td>Age (y)</td>
<td>-0.025</td>
<td>0.011</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Disease duration (mo)</td>
<td>0.000</td>
<td>0.008</td>
<td>0.976</td>
<td></td>
</tr>
<tr>
<td>Malar rash</td>
<td>-0.38</td>
<td>0.248</td>
<td>0.131</td>
<td></td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>-0.134</td>
<td>0.277</td>
<td>0.631</td>
<td></td>
</tr>
<tr>
<td>Oral ulcer</td>
<td>0.411</td>
<td>0.335</td>
<td>0.225</td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td>-0.012</td>
<td>0.256</td>
<td>0.963</td>
<td></td>
</tr>
<tr>
<td>Serositis</td>
<td>-0.262</td>
<td>0.275</td>
<td>0.345</td>
<td></td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>0.538</td>
<td>0.324</td>
<td>0.108</td>
<td></td>
</tr>
<tr>
<td>Neurologic disorder</td>
<td>0.641</td>
<td>0.545</td>
<td>0.245</td>
<td></td>
</tr>
<tr>
<td>Hematologic disorder</td>
<td>0.331</td>
<td>0.269</td>
<td>0.223</td>
<td></td>
</tr>
<tr>
<td>Immunologic disorder</td>
<td>0.049</td>
<td>0.670</td>
<td>0.942</td>
<td></td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>0.000</td>
<td>0.000</td>
<td>0.907</td>
<td></td>
</tr>
<tr>
<td>Complement 3</td>
<td>0.013</td>
<td>0.063</td>
<td>0.831</td>
<td></td>
</tr>
<tr>
<td>Complement 4</td>
<td>0.013</td>
<td>0.021</td>
<td>0.544</td>
<td></td>
</tr>
<tr>
<td>CH50</td>
<td>0.002</td>
<td>0.011</td>
<td>0.858</td>
<td></td>
</tr>
<tr>
<td>Leukocyte count (cells/μl)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.479</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte count (cells/μl)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.020</td>
<td>0.000</td>
</tr>
<tr>
<td>Platelet count (cells/μl)</td>
<td>-0.002</td>
<td>0.002</td>
<td>0.324</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>0.013</td>
<td>0.063</td>
<td>0.831</td>
<td></td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>-0.005</td>
<td>0.006</td>
<td>0.382</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>-0.207</td>
<td>0.155</td>
<td>0.189</td>
<td></td>
</tr>
<tr>
<td>SLEDAI</td>
<td>-0.119</td>
<td>0.026</td>
<td>&lt;0.001</td>
<td>-0.118</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>-0.032</td>
<td>0.093</td>
<td>0.735</td>
<td></td>
</tr>
<tr>
<td>Current use of steroid</td>
<td>-0.238</td>
<td>0.401</td>
<td>0.555</td>
<td></td>
</tr>
<tr>
<td>Current use of immunosuppressive drug</td>
<td>0.084</td>
<td>0.272</td>
<td>0.758</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age, lymphocyte count, and SLEDAI score.
β, regression coefficient. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.
tended to have lower percentages of IFN-γ⁺ MAIT cells as compared with HCs, the difference was not significant (median 44.2 versus 53.0%, p = 0.431). IL-17⁺ or IL-4⁺ MAIT cell levels were comparable between the patients and HCs (Fig. 4B). Next, we examined MR1-dependent cytokine production by MAIT cells. PBMCs from 5 SLE patients, 7 RA patients, and 6 HCs were stimulated for 24 h with *E. coli*-infected THP-1 cells; then the production of IFN-γ, IL-17, and IL-4 in MAIT cells was examined by intracellular cytokine flow cytometry. Percentages of IFN-γ⁺ MAIT cells were found to be significantly lower in SLE patients than in HCs (mean ± SEM: 2.97 ± 0.55 versus 21.4 ± 4.86%, p < 0.05). RA patients tended to have lower IFN-γ⁺ MAIT cell levels as compared with HCs (mean ± SEM: 10.9 ± 1.95 versus 21.4 ± 4.86%), but the difference was not significant (p = 0.182). IL-17⁺ or IL-4⁺ MAIT cell levels were similar between the patients and HCs (Fig. 4C).

**Defect in NFAT1 nuclear translocation in MAIT cells of SLE patients**

Our data showed that IFN-γ production by MAIT cells was defective in SLE patients. To determine which signaling pathway is the main regulator of IFN-γ in MAIT cells, ERK (PD98059), p38 MAPK (SB220025), or calcineurin (CsA) inhibitors were added to the cells before PMA and IM stimulation. Pretreatment with CsA and PD98059 significantly reduced IFN-γ production by MAIT cells in both HCs and SLE patients. Moreover, CsA had a more suppressive effect than PD98059. However, no suppressive effect of SB220025 was found in both HCs and SLE patients (Fig. 4D).

The activation of NFAT transcription factors requires sustained intracellular Ca²⁺ levels induced by TCR engagement and calcium influx and then activates the Ca²⁺-dependent phosphatase calcineurin, which is blocked by CsA (35). Considering that IFN-γ production by MAIT cells was blocked in a CsA-sensitive manner, we next investigated the NFAT1 protein levels of MAIT cells in HCs and SLE patients. In MAIT cells isolated from HCs, IM treatment resulted in NFAT1 nuclear translocation, which was again blocked by CsA, reaching the statistical significance. However, the NFAT1 nuclear translocation by IM treatment was not marked between the patients and HCs (Fig. 4C).

**Interactions between MAIT cells and NKT cells in SLE and RA patients**

To assess whether NKT cells have the potential to activate MAIT cells, we preincubated PBMCs from 9 SLE patients, 9 RA patients, and 8 HCs for 3 d in the presence or absence of α-GalCer, and CD69 expression in the MAIT cell population was determined by flow cytometry. The percentages of CD69⁺ MAIT cells in control subjects were markedly increased in the presence of α-GalCer as compared with the absence of α-GalCer (14.8 versus

### Table III. Regression coefficients of log-transformed absolute MAIT cell numbers with respect to clinical features and laboratory findings in RA patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.099</td>
<td>0.179</td>
</tr>
<tr>
<td>Age (y)</td>
<td>-0.010</td>
<td>0.005</td>
</tr>
<tr>
<td>RF</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Leukocyte count (cells/μl)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Lymphocyte count (cells/μl)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Platelet count (cells/μl)</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>0.073</td>
<td>0.044</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>-0.003</td>
<td>0.002</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>-0.048</td>
<td>0.026</td>
</tr>
<tr>
<td>DAS28</td>
<td>-0.195</td>
<td>0.051</td>
</tr>
<tr>
<td>Current use of steroid</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Current use of DMARD</td>
<td>0.286</td>
<td>0.259</td>
</tr>
</tbody>
</table>

*Adjusted for age, lymphocyte count, and DAS28 score.

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.
2.83% on day 3 in a representative subject), whereas the percentages of CD69+ MAIT cells in RA patients were increased ∼2-fold in response to α-GalCer (from 8.55 to 17.3% on day 3 in a representative subject). In contrast, the percentages of CD69+ MAIT cells in SLE patients were not increased or were increased only slightly in response to α-GalCer (from 2.79 to 3.12% on day 3 in a representative subject; Fig. 5A). Overall changes in expression levels of CD69 were lesser in SLE patients than in HCs (mean SEM fold increase 1.31 ± 0.19 versus 4.78 ± 1.25, p = 0.05). However, no significant differences were observed in the changes in expression levels of CD69 between RA patients and HCs (mean ± SEM fold increase 1.87 ± 0.25 versus 4.78 ± 1.25, p = 0.20; Fig. 5B). These findings suggest that the reduced indirect activation could be caused by dysfunction in NKT cells, MAIT cells, or a combination of these in SLE.

Elevated PD-1 expression in MAIT cells, NKT cells, and T cells in SLE patients

PD-1 and its ligands, PD-1 ligand 1 [PD-L1] and PD-L2, deliver inhibitory signals that regulate the balance among T cell activation, tolerance, and immunopathology (36). Moreover, recent reports have shown that PD-1–PD-L interaction is involved in the induction and maintenance of NKT cell anergy (37, 38). To determine whether MAIT and NKT cell dysfunctions are related to PD-1, we examined the expression levels of PD-1 in T cells, NKT cells, and MAIT cells in SLE patients (Fig. 6A). Interestingly, the percentages of PD-1–expressing T cells, PD-1–expressing NKT cells, and PD-1–expressing MAIT cells were found to be significantly higher in SLE patients than in HCs (median 13.1 versus 4.94%, p < 0.01; median 24.0 versus 8.60%, p < 0.001; and median 16.4 versus 4.32%, p < 0.05, respectively). However, the expression levels of PD-1 in T cells, NKT cells, and MAIT cells were similar between RA patients and HCs (Fig. 6B). These findings suggest that the reduced indirect activation could be caused by dysfunction in NKT cells, MAIT cells, or a combination of these in SLE.

Table IV. Spearman’s correlation analysis between MAIT cell levels and NKT cell levels in SLE and RA patients

<table>
<thead>
<tr>
<th>Disease</th>
<th>MAIT Cell Subsets (%)</th>
<th>NKT Cell Subsets (%)</th>
<th>Total</th>
<th>DN</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>r_s</td>
<td>0.488</td>
<td>0.474</td>
<td>0.415</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.001</td>
<td>0.007</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>DN</td>
<td>r_s</td>
<td>0.514</td>
<td>0.507</td>
<td>0.370</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>CD8</td>
<td>r_s</td>
<td>0.492</td>
<td>0.490</td>
<td>0.413</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>r_s</td>
<td>0.187</td>
<td>0.256</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.181</td>
<td>0.070</td>
<td>0.066</td>
<td></td>
</tr>
<tr>
<td>DN</td>
<td>r_s</td>
<td>0.180</td>
<td>0.293</td>
<td>0.135</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.201</td>
<td>0.039</td>
<td>0.351</td>
<td></td>
</tr>
<tr>
<td>CD8</td>
<td>r_s</td>
<td>0.182</td>
<td>0.256</td>
<td>0.256</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.193</td>
<td>0.069</td>
<td>0.069</td>
<td></td>
</tr>
</tbody>
</table>

Accumulation of MAIT cells in the synovial fluid of RA patients

To examine whether circulating MAIT cell deficiency in RA might be associated with the accumulation of MAIT cells in the synovial fluid, we obtained paired samples of peripheral blood and synovial fluid from eight RA patients with knee effusion, and MAIT cell levels were determined by flow cytometry (Fig. 7A). Percentages of MAIT cells were significantly higher in synovial fluid than in peripheral blood (mean ± SD: 2.21 ± 2.00 versus 0.69 ± 0.68%, p < 0.01; Fig. 7B).
This study represents the first attempt, to our knowledge, to investigate the numerical and functional deficiencies of MAIT cells in SLE patients. We also demonstrated that MAIT cell deficiency reflects disease activity. In particular, MAIT cell frequency was found to be correlated with NKT cell frequency in SLE. In vitro experiments using α-GalCer–stimulated NKT cells showed poor activation of MAIT cells in SLE patients, suggesting that NKT cell dysfunction influences MAIT cell dysfunction. Finally, our study showed that MAIT cell deficiency was associated with elevated PD-1 expression in SLE.

The observation that circulating MAIT cell numbers are reduced has also been reported in some human diseases, including human HIV infection, multiple sclerosis, and tuberculosis (4, 26, 34, 39). In this study, we found that circulating MAIT cell numbers were reduced in SLE and RA patients. In particular, this MAIT cell deficiency was more prominent in CD8+ and DN MAIT cell subsets. These findings have also been previously demonstrated in patients with multiple sclerosis or HIV infection, in whom all the MAIT cell subset levels were reduced (26, 34). Thus, reductions in CD8+ and DN subset levels might contribute to circulating MAIT cell deficiencies in SLE and RA patients.
Our data also indicate that MAIT cell levels reflect disease activity in SLE and RA. Our univariate analysis showed that age, lymphocyte count, SLEDAI, and DAS28 were correlated with MAIT cell deficiency. After adjusting for these factors, multivariate analysis identified two independent determinants of MAIT deficiency: SLEDAI and DAS28. To exclude the effect of medication on MAIT cell levels, we investigated MAIT cell levels in patients receiving medication. However, no significant correlations were found between MAIT cell levels and use of steroid, immunosuppressive drug, or disease-modifying antirheumatic drug. These findings suggest that chronic inflammatory activity rather than medication effect may lead to depletion of MAIT cells in blood during the disease process.

Numerical deficiencies of immune cells in the peripheral blood have often been linked to functional deficiencies of these cells in autoimmune diseases (17, 40). Because the same abnormalities might exist in MAIT cells of SLE and RA patients, we considered performing functional studies that assess the relationship between

![Figure 5](http://www.jimmunol.org/download/3898_MAIT CELLS IN SYSTEMIC LUPUS ERYTHEMATOSUS)

**Figure 5.** Effect of α-GalCer on MAIT cells in SLE and RA patients. (A) Freshly isolated PBMCs (1 × 10⁶/well) were stimulated with α-GalCer (100 ng/ml) or 0.1% DMSO as a control for 3 d in the presence of IL-2 (100 IU/ml), and CD69 expression in the MAIT cell population was determined by flow cytometry. (B) Data were obtained from eight HCs, nine patients with SLE, and nine patients with RA. Changes in expression levels of CD69 are expressed as fold increases, that is, the ratio of CD69 levels on day 3 in the presence of α-GalCer to CD69 levels on day 3 in the absence of α-GalCer. *p < 0.05.

![Figure 6](http://www.jimmunol.org/download/3898_MAIT CELLS IN SYSTEMIC LUPUS ERYTHEMATOSUS)

**Figure 6.** Elevated PD-1 expression in SLE patients. (A) Percentages of PD-1–expressing cells in T cells, NKT cells, and MAIT cells were determined by flow cytometry. (B) Data were obtained from 10 HCs, 11 patients with SLE, and 10 patients with RA. Symbols represent individual subjects; horizontal bars indicate the median. *p < 0.05, **p < 0.01, ***p < 0.001.
MAIT cell level and function. Therefore, we next examined the secretion levels of several cytokines produced by MAIT cells in response to stimulation with PMA/IM or E. coli–infected APCs. Our study demonstrated that the production of IFN-γ by MAIT cells was significantly diminished in SLE patients, irrespective of the mode of stimulation (i.e., in an MR1-dependent or MR1-independent manner). Notably, functional deficiencies of MAIT cells were prominent in patients with lower frequencies of MAIT cells (<0.7%) than in patients with higher frequencies (>0.7%; Supplemental Fig. 1). In contrast, the production of IFN-γ was found to be preserved in RA patients. In our in vitro culture system, however, no significant differences were found in IL-17 or IL-4 levels between the patients and HCs because of a relatively weak production of these cytokines, which has also been reported in other studies (26, 34, 39). Similar findings have also been reported in chronically HIV-infected patients. They displayed preserved MAIT cell responses following PMA/IM stimulation, but MAIT cell responses to E. coli were impaired, which were at least partly restored by effective antiretroviral therapy (34).

In the current study, IFN-γ production by MAIT cells was found to be mainly regulated by NFAT1 transcription factor, which is consistent with the previous study showing NFAT1-dependent IFN-γ production by CD8+ T cells (33). Such similarities between two cell populations can be explained in part by the fact that the majority of MAIT cells are CD8+ T cells. Notably, the nuclear translocation of NFAT1 in MAIT cells upon IFN-γ stimulation was not marked in SLE patients. These findings suggest that impaired IFN-γ production in MAIT cells of SLE patients is due to an intrinsic defect in the Ca2+/calcineurin/NFAT1 signaling pathway. To our knowledge, this is the first study to show that MAIT cells in SLE have a defect in MAIT cell signaling from the early event of TCR triggering to the final event of cytokine production.

MAIT and NKT cells represent peculiar T cell subpopulations with innate-like properties that differ from conventional T cells (1, 12). Recent studies provided evidence that MAIT and NKT cells have a close lineage relationship and are highly susceptible to apoptosis, a feature not shared with conventional T cells (21, 22, 41). We next investigated the numerical and functional relationships between MAIT cells and NKT cells. Correlation analysis showed that circulating MAIT cell levels were significantly correlated with peripheral blood NKT cell levels in SLE patients. Interestingly, no significant correlations were found between circulating MAIT cell levels and NKT cell levels in RA patients. In addition, in vitro experiments using α-GalCer–stimulated NKT cells showed the dysfunction between MAIT cells and NKT cells in SLE patients. Moreover, poor activation of MAIT cells in SLE patients was not rescued even in initial NKT cell levels similar with healthy controls (HCs; Supplemental Fig. 2). These findings imply that the mechanism responsible for circulating MAIT cell deficiency is different in SLE and RA; that is, it appears likely that in SLE, the numerical and functional deficiencies of MAIT cells are correlated with the numerical and functional deficiencies of NKT cells, whereas the MAIT cell deficiency in RA is likely to occur regardless of NKT cell level or function. To investigate the possibility that only MAIT cells are uniquely affected by NKT cells, we measured CD69 expression levels in other cells, such as T cells or non-T cells, after stimulation with α-GalCer. As shown in Supplemental Fig. 3, changes in expression levels of CD69 after NKT cell activation were not prominent in CD3+ T cells or CD3- cells (i.e., <2-fold change in HCs) as compared with MAIT cells (i.e., >4-fold change in MAIT cells in HCs). Moreover, no significant differences were observed in the changes in expression levels of CD69 of CD3+ T cells or CD3- cells between the patients and HCs. Thus, this activation would be more limited to MAIT cells rather than to other cells.

PD-1 is a well-known coinhibitory molecule that is expressed on T cells. In conventional T cells, PD-1 is not expressed on naive T cells, but it is inducibly expressed after T cell activation (36). In recent reports, PD-1 and its ligands have been implicated in the induction and maintenance of T cell and NKT cell anergy (37, 38, 42, 43). In our previous report, we demonstrated that NKT cell dysfunction was associated with upregulation of inhibitory receptor PD-1 in active tuberculosis, and it was partially recovered by blockade of PD-1 (44). In this study, we investigated the relevance of PD-1 expression to MAIT cell dysfunction in SLE and RA. Contrary to RA patients, SLE patients were found to have elevated PD-1 expression levels in MAIT cells, NKT cells, and conventional T cells. However, MAIT cell dysfunction was not fully recovered by blockade of PD-1 (data not shown). Blackburn et al. (45) reported that the coexpression of multiple distinct inhibitory receptors was correlated with more severe T cell exhaustion. Accordingly, these findings suggest that persistent functional impairment of MAIT cells may be associated with the complex negative regulation resulting from the coexpression of multiple inhibitory receptors rather than the expression of only a single inhibitory receptor, PD-1.

The results of our study showed numerical deficiency and preserved cytokine production in MAIT cells from RA patients with low levels of PD-1 expression, suggesting that MAIT cell deficiency may be related to other factors rather than MAIT cell anergy or exhaustion. In this study, MAIT cell levels were found to be increased in the synovial fluid as compared with the peripheral blood. A previous study revealed that MAIT cells displayed high levels of CCR5, CCR6, and CXCR6 (3). These chemokine receptors are known to be involved in trafficking of T cells to synovial tissues (46–48). Furthermore, it has been recently demonstrated that MAIT cells promote inflammation and

FIGURE 7. Increased MAIT cell levels in the synovial fluid of RA patients. (A) Representative paired sample was obtained from a RA patient. (B) Data were obtained from eight patients with RA. **p < 0.01.
The authors have no financial conflicts of interest.

References