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Loss of Circulating Mucosal-Associated Invariant T Cells in Common Variable Immunodeficiency Is Associated with Immune Activation and Loss of Eomes and PLZF

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ABSTRACT

Common variable immunodeficiency (CVID) is characterized by low levels of IgGs leading to increased risk of infections. Mucosal-associated invariant T (MAIT) cells are a recently identified population of innate T cells with potent antibacterial activity. We hypothesized that CVID is associated with alterations in MAIT cells. Cryopreserved PBMC from CVID patients and healthy controls were used to study the frequency, phenotype, and response to Escherichia coli stimulation of MAIT cells by flow cytometry. MAIT cell frequency and absolute counts were depressed in CVID. Residual MAIT presented elevated coexpression of CD38 and HLA-DR, and reduced expression of CCR6, whereas levels of CD127 (IL-7 receptor) were unchanged. CVID patients also had an accumulation of MAIT cells lacking the critical transcription factors eomesodermin and promyelocytic leukemia zinc finger protein. MAIT cell frequency was inversely associated with levels of soluble CD14, with coexpression of CD38 and HLA-DR, and accumulation of MAIT cells lacking eomesodermin or promyelocytic leukemia zinc finger protein expression. None of these changes were normalized by IgG replacement therapy. Finally, MAIT cells from CVID patients displayed poor IFN-γ responses to E. coli stimulation, in part due to defective Ag presentation, and these responses were increased by pretreatment with IL-7. Defective MAIT cell response may contribute to the increased incidence of microbial infections seen in CVID patients on IgG replacement therapy. ImmunoHorizons, 2017, 1: 142–155.

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1E.G.K. and D.F.N. contributed equally to this work.

D.P.-P., J.K.S., D.F.N., and E.G.K. conceived and designed the experiments; D.P.-P., B.A.N.S., and N.S.B. performed the experiments; D.P.-P., B.A.N.S., and N.S.B. analyzed the data; A.K.B.B.M., C.M.K., M.T.B., K.I.C., M.E., J.K., D.F.N., and E.G.K. contributed reagents, materials, and analysis tools; and D.P.-P., D.F.N., and E.G.K. wrote the paper.

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Abbreviations used in this article: CVID, common variable immunodeficiency; Eomes, eomesodermin; IVIg, intravenous IgG; MAIT, mucosal-associated invariant T; MOI, multiplicity of infection; PLZF, promyelocytic leukemia zinc finger; sCD14, soluble CD14.

The online version of this article contains supplemental material.

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**INTRODUCTION**

Common variable immunodeficiency (CVID) is characterized by low levels of IgG and IgA, and/or IgM (1, 2). A number of genetic mutations causing CVID have been identified, but in the majority of patients, the exact genetic basis of disease is unknown (3, 4). CVID patients have poor humoral immunity, resulting in recurrent infections of the gastrointestinal and upper respiratory tracts. The treatment for CVID consists of s.c. or intravenous IgG (IVIg) replacement therapy. IVIg is obtained from pooled plasma from healthy donors and is also used to treat inflammatory and autoimmune diseases (5). IVIg therapy reduces the occurrence of clinically significant infections in CVID (6, 7). However, infection-related complications remain the most frequent problem seen in CVID patients (8). Furthermore, up to 50% of CVID patients will also have inflammatory or autoimmune complications (8–10).

CVID patients have several changes in both the innate and adaptive arms of the immune system, including persistent immune activation and exhaustion (11, 12). IgG replacement therapy reduces immune activation in CVID patients (13), but invariant NK T cells and regulatory T cells remain low in CVID patients on treatment (14–16). Chronic upregulation of the IFN pathway, due to expansion of proinflammatory group 3 innate lymphoid cells, has been reported in a subset of CVID patients with inflammatory complications (17, 18).

Mucosal-associated invariant T (MAIT) cells are a recently described innate population that represents 1–10% of T cells in the blood of healthy individuals. They express a semi-invariant TCR using Vα7.2 coupled with Jα33 and a limited Vβ repertoire (19). MAIT cells can be identified by the expression of Vα7.2 in combination with CD161 or the IL-18 receptor. They have been shown to recognize microbial vitamin B2 (riboflavin) metabolites presented by the MHC class I-like protein MR1 (20). This allows MAIT cells to respond to a range of bacteria and mycobacteria. MAIT cells can also be activated indirectly by IL-12 and IL-18 (21), allowing them to respond to pathogens not producing riboflavin, such as viruses (22). MAIT cells have high expression of granzyme A and K, and are cytotoxic against bacterially infected cells (23, 24). In the mouse, MAIT cells have been reported to require B cells and commensal gut flora for expansion and acquisition of an effector phenotype (25), whereas in humans, MAIT cells gain some of their innate-like antimicrobial properties before birth in the mucosa (26). In HIV type 1 and human T cell leukemia virus type 1 infections, MAIT cells are reduced in number and display impaired functionality in response to bacterial stimulation (27–29). Microbial translocation is now recognized to have a major role in HIV immuno-pathogenesis, and recent evidence suggests that microbial translocation could be responsible for CD4+ T cell exhaustion in CVID patients (30). Elevated levels of LPS in CVID are also associated with dysbiosis of the gut microbiota (31).

This present study aimed to investigate if MAIT cell frequency, phenotype, and function are affected in CVID patients. We found that MAIT cells were reduced in CVID, and did not recover after IVIg treatment. MAIT cells from CVID patients had an activated phenotype and decreased levels of the transcription factors eomesodermin (Eomes) and promyelocytic leukemia zinc finger protein (PLZF). Finally, MAIT cells from CVID patients had a lower IFN-γ response after stimulation with bacteria, and IL-7 pretreatment alleviated this functional deficiency.

**MATERIALS AND METHODS**

**Study cohort and samples**

CVID patients and healthy controls were enrolled at the Hospital das Clínicas, Medical School, University of São Paulo, and their demographics have been previously described (32), or at the Children’s National Medical Center (Washington, DC) (Table I). The study was approved by the Hospital das Clínicas, University of São Paulo Medical School Ethics Committee, and written informed consent was provided by all participants or their legal guardians in accordance with the Declaration of Helsinki. None of the patients suffered from active infection at the time of enrollment and all patients were HIV negative. PBMCs were isolated by density-gradient sedimentation using Ficoll-Paque (Lymphoprep; Nycomed Pharma, Oslo, Norway). Isolated PBMCs were washed twice in HBSS (Life Technologies, Grand Island, NY), and cryopreserved in RPMI 1640 (Life Technologies), supplemented with 20% heat inactivated FBS (HyClone Laboratories, Logan, UT), 50 U/ml of penicillin (Life Technologies), 50 μg/ml of streptomycin (Life Technologies), 10 mM glutamine (Life Technologies), and 7.5% DMSO (Sigma, St. Louis, MO). Cryopreserved cells from all subjects were stored in liquid nitrogen until used in the assays. For CVID patients, samples were collected before and 9–15 mo after initiation of IVIg treatment.

**Flow cytometry and mAbs**

Cryopreserved specimens were thawed and washed, and counts and viability were assessed using the Countess Automated Cell Counter system (Invitrogen, Carlsbad, CA). Cells were washed and stained in Brilliant Violet Stain Buffer (BD Biosciences, San Jose, CA) at room temperature for 15 min in 96-well V-bottom plates in the dark. Samples were then washed and fixed using Cytofix/ Cytoperm (BD Biosciences) before flow cytometry data acquisition. Intracellular staining was performed in Perm/Wash (BD Biosciences). The mAbs used in flow cytometry were: CD3 AF700, CD3 PerCP/Cy5.5 (both clone UCHT1), CD4 BV605 (clone RPA-T4), CD8 BV711 (clone RPA-T8), CD38 APC-H7 (clone HB7), CD127 FITC (clone HIL-7R–M2), CD161 BV421, CD161 FITC (both clone DX12), CCR6 BV786 (clone 11A9), HLA-DR APC (clone L243), IFN-γ APC (clone B27), and PD-1 PE-Cy7 (clone EH12.1), all from BD Biosciences; PLZF APC from R&D Systems (Minneapolis, MN); EOMES FITC (clone W9298) from eBio-science; and TCR Vα7.2 PE and TCR Vα7.2 PerCP-Cy5.5 (both clone 3C10) from BioLegend (San Diego, CA). Live/dead aqua fixable cell stain was from Life Technologies (Eugene, OR). In some experiments, cells were stained with the MR1 tetramer (33) PE (National Institutes of Health Tetramer Core Facility) for 30 min at room temperature before staining for other surface and
Intracellular markers. Data were acquired on a BD LSRFortessa instrument (BD Biosciences), and analyzed using FlowJo version 9.8.5 software (Tree Star, Ashland, OR).

**Functional assay**

MAIT cell function was determined in vitro using paraformaldehyde-fixed *Escherichia coli* stimulation [One Shot TOP10, multiplicity of infection (MOI) 10; Life Technologies] in the presence of 1.25 mg/ml anti-CD28 mAb (clone L293, BD Biosciences) (34). PBMCs were further cultured for 24 h at 37°C and 5% CO2 in RPMI 1640 medium supplemented with 10% FBS. Monensin (Golgi Stop; BD Biosciences) was added during the last 6 h of the stimulation. In some experiments, PBMCs were pretreated for 24 h with 10 ng/ml of IL-10 (R&D Systems) or with anti-MR1 (5 μg/ml; BioLegend) before the addition of *E. coli*.

**Cell sorting**

PBMC samples were thawed and stained with anti-CD3, anti-CD161, and anti-Vα7.2 as previously described. MAIT cells were then sorted on an SH800Z (Sony Biotechnology, San Jose, CA). Purity was typically over 90%. Sorted cells were then labeled with cell trace violet (Invitrogen) following the manufacturer’s instructions, and mixed together with PBMCs at a 10:1 (PBMCs/sorted MAIT) ratio.

**IL-7, MIP-3, and soluble CD14 measurement**

IL-7, MIP-3, (both from RayBiotech, Norcross, GA), and soluble CD14 (sCD14) (R&D Systems) were measured in plasma by ELISA following the manufacturer’s instruction.

**Statistics**

All statistical analysis was performed using Graph Pad Prism version 6.0f for Mac OSX (GraphPad Software, La Jolla, CA). The comparison between healthy controls and CVID patients was analyzed using Kruskal–Wallis test and changes after IVIg initiation in CVID patients were analyzed with Wilcoxon matched-pairs signed rank test. Associations between groups were determined by Spearman rank correlation. A p value < 0.05 was considered statistically significant.

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**TABLE I. Subject demographics**

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CTRL, control; F, female; M, male; NA, not available.

https://doi.org/10.4049/immunohorizons.1700039
FIGURE 1. Persistent loss of MAIT cells in CVID. Representative gating strategy for MAIT cells (A). Frequency (left panel) and absolute count (right panel) of MAIT cells (B), CD8+ MAIT cells (C), CD4+CD8− MAIT cells (D), and CD4+ MAIT cells (E) in healthy controls (n = 22), treatment-naive (n = 15), and IVig-treated CVID patients (frequency n = 11, cell count n = 7). The lines and whiskers represent the median and interquartile range respectively. *p < 0.05, **p < 0.01, ***p < 0.001.
Study approval
The study was approved by the University of São Paulo institutional review board and the George Washington University institutional review board, and written informed consent was provided by all participants, or their legal tutors, according to the Declaration of Helsinki.

RESULTS

Persistent loss of MAIT cells in CVID patients
A total of 15 newly diagnosed CVID patients and 22 healthy controls were enrolled in this study; patient and control demographics have been previously described (32) (Table I). For 11 of the CVID patients, follow-up samples were obtained 9–15 mo after the patients started on IVIg treatment. MAIT cells in treatment-naive CVID patients were reduced both as a percentage of T cells and in absolute count, and did not recover after initiation of IVIg treatment (Fig. 1A, 1B). Next, we investigated the distribution of MAIT cell CD8+, CD4+CD8−, and CD4+ subsets in CVID. The majority of MAIT cells were CD8+, similar to healthy controls. However, the proportion of CD8+ MAIT cells was reduced in CVID patients together with their absolute count (Fig. 1C). The proportion of CD4+CD8− MAIT cells was not different in patients compared with controls, but their absolute count was reduced (Fig. 1D). Interestingly, the proportion of CD4+ MAIT cells was increased in CVID patients, but their absolute count was normal (Fig. 1E). We used the MR1 tetramer to confirm that CD4+ MAIT cells are truly MAIT cells (Supplemental Fig. 1A, 1B). This suggests that this subset of MAIT cells is preserved in CVID patients. There was no significant change in the proportion or absolute count of any MAIT cell subsets after replacement therapy (Supplemental Fig. 1C–F). CVID patients are a heterogeneous group with B cell frequencies ranging from undetectable to normal. Because of the previously reported requirement of B cells for MAIT cell expansion in mouse models (25), we investigated if there was any association between B cell frequency and MAIT cell frequency in CVID patients, and found no significant association (Fig. 2A). We then enrolled a CVID patient known to have undetectable B cells and evaluated his frequency of MAIT cells [CVID-4 in Paquin-Proulx et al. (13)]. The MAIT cell frequency in this patient was 2.26%, well within the normal range (Fig. 2B). Our results show that the loss of MAIT cells in CVID patients is not restored by IVIg therapy and is independent of the loss of B cells.

CD4+ T cells expressing CD161 are preserved in CVID
We next investigated if the loss of MAIT cells in CVID patients was reflective of a more generalized loss of CD161-expressing T cells or a specific process. We studied CD4+ T cells expressing CD161 (Fig. 3A) and observed that their frequency was increased in CVID compared with controls (Fig. 3B), whereas their absolute count was normal (Fig. 3C). The loss of overall CD4+ T cells in CVID patients is well documented (13, 35–37), and we found that CD4+ T cells were reduced also in this cohort (data not shown). This suggests that the subset of CD4+ T cells expressing CD161 is specifically preserved in CVID patients. Finally, we studied the frequency and absolute count of CD8+ T cells with intermediate expression of CD161 (CD161int) (Fig. 3A), a subset that has recently been described as highly functional memory cells (38). CD8+ CD161int T cells were present at normal frequency and number in CVID patients (Fig. 3D, 3E), suggesting that there is no generalized loss of CD161-expressing T cells in CVID.

MAIT cells from CVID patients are activated and have low expression of CCR6
We then characterized the phenotype of the residual total MAIT cell population in treatment-naive CVID patients and observed elevated levels of coexpression of CD38 and HLA-DR (Fig. 4A, Supplemental Fig. 2A), suggesting higher levels of activation of MAIT cells in CVID. In contrast, levels of CCR6 were low on MAIT cells of CVID patients compared with controls (Fig. 4B, Supplemental Fig. 2B). MAIT cells from CVID patients expressed high levels of PD-1, similar to healthy controls (Fig. 4C,

FIGURE 2. MAIT cell loss in CVID is independent of B cell loss.
Association between B cell frequency and MAIT cell frequency in treatment-naive CVID patients (A). Flow cytometry plot showing the MAIT cell population in a CVID patient with undetectable B cells (B).

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FIGURE 3. CD4 expressing CD161 and CD8 expressing CD161int are preserved in CVID.

Gating strategy for CD161+ CD4 T cells and CD161int CD8 T cells (A). Frequency (B) and absolute count (C) of CD4 T cells expressing CD161, and frequency (D) and absolute count (E) of CD8 T cells expressing intermediate levels of CD161 in healthy controls (n = 22), treatment-naive (n = 15), and IVIG-treated CVID patients (n = 7). The lines and whiskers represent the median and interquartile range respectively. *p < 0.05, **p < 0.01.

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Supplemental Fig. 2C). Although some CVID patients had low levels of CD127 (IL-7 receptor), the overall levels of CD127 were not different from controls (Fig. 4D, Supplemental Fig. 2D). Finally, the expression of all the markers studied was unchanged after initiation of replacement therapy (Supplemental Fig. 2E–H). These results indicate that MAIT cells from CVID patients have elevated levels of immune activation; however, unlike for conventional CD8 T cells (13) and γδ T cells (32), MAIT cell activation was not reduced by IVIg treatment.

Loss of Eomes and PLZF expression by MAIT cells in CVID is associated with their reduced frequency

Partial loss of Eomes and PLZF expression by MAIT cells has been reported in HIV and hepatitis C virus infections (39, 40). This aberrant transcription factor profile was also associated with reduced MAIT cell frequency and functionality. Thus, we investigated if MAIT cells from CVID patients had a similarly reduced expression of these key transcription factors. Indeed, total MAIT cells from CVID patients had an accumulation of Eomes− and PLZF− cells (Fig. 5A). There was a trend toward elevated levels of MAIT cells lacking Eomes (p = 0.08, Fig. 5B) and a significant increase in PLZF−MAIT cells (Fig. 5C) in treatment-naive CVID patients. IgG replacement therapy did not restore Eomes and PLZF expression in MAIT cells (Supplemental Fig. 3A, 3C). We used the MRI tetramer to confirm that Eomes− and PLZF-negative cells were in fact MAIT cells rather than some contaminating cell population (Supplemental Fig. 3B, 3D). We observed significant inverse associations between MAIT cell frequency and the accumulation of Eomes− (Fig. 5D) and PLZF− (Fig. 5E) MAIT cells. To investigate if the loss of Eomes and PLZF expression by MAIT cells may be a consequence of chronic activation, we investigated possible associations between coexpression of CD38 and HLA-DR and Eomes− or PLZF− MAIT cell frequency. There were positive associations in both cases (Fig. 5F, 5G respectively), suggesting a role for chronic activation in MAIT cell dysregulation.

Immune activation is associated with MAIT cell loss

sCD14 is a marker of monocyte activation and is considered an indirect marker of microbial translocation (41). sCD14 has been
FIGURE 5. Loss of Eomes and PLZF are associated with loss of MAIT cells.

Representative flow plots showing Eomes and PLZF expression by total MAIT cells in healthy controls and CVID patients (A). Frequency of Eomes\(^{-}\) (B) and PLZF\(^{-}\) (C) MAIT cells in healthy controls (n = 18), treatment-naive (n = 11), and IVIg-treated CVID patients (n = 9). Association between Eomes\(^{-}\) (D) or PLZF\(^{-}\) (E) and MAIT cell frequency in treatment-naive CVID patients. Association between Eomes\(^{-}\) (F) or PLZF\(^{-}\) (G) and coexpression of CD38 and HLA-DR by MAIT cells in treatment-naive CVID patients. The lines and whiskers represent the median and interquartile range respectively. *p < 0.05, **p < 0.01.
reported to be elevated in CVID patients (13, 42). A possible explanation for the low levels of MAIT cells in the blood is their recruitment to tissues. The lower levels of the chemokine receptor CCR6 we observed on MAIT cells in CVID patients may possibly represent internalization of this receptor following interaction with its ligand MIP-3 (43). In HIV-infected subjects, the levels of IL-7 are associated with MAIT cell frequency (39). Therefore, we measured the plasma levels of sCD14, MIP-3, and IL-7 in our cohort. Levels of sCD14 were not elevated in treatment-naive CVID patients, but were significantly increased after patients initiated IVIg treatment ($p = 0.01$, Fig. 6A). MIP-3 was undetectable in all healthy controls but was detected in 5 of 12 treatment-naive, and 3 of 10 IVIg-treated CVID patients for whom plasma samples were available (Fig. 6B). IL-7 was significantly reduced in treatment-naive CVID patients, with the majority having levels below the limit of detection (Fig. 6C). The levels of IL-7 became detectable in the majority of CVID patients under replacement therapy, although they remained significantly lower than in healthy controls. There was no significant association between the levels of IL-7 and MAIT cell frequency in CVID patients. However, there was a trend toward an inverse association between the levels of sCD14 and the frequency of MAIT cells in CVID patients ($p = 0.0794$, Fig. 6D) but not in healthy controls ($p = 0.2767$), suggesting a possible role for microbial translocation and monocyte activation in MAIT cell loss. Treatment-naive CVID patients with detectable MIP-3 were grouped and we compared their MAIT cell frequency to those with undetectable MIP-3. Patients with undetectable MIP-3 had MAIT cell frequencies ranging from normal to low, whereas patients with detectable MIP-3 all had low MAIT cell frequency, although this difference between the groups did not reach significance (Fig. 6E). Immune activation has also been reported to be associated with MAIT cell loss in HIV patients (27). We observed a similar negative association between levels of activation in MAIT cells and their frequency in untreated CVID patients (Fig. 6F). This association remained significant after removing the two patients with higher levels of immune activation ($p = 0.0368$, $r = -0.6455$). Overall, these results indicate that immune activation is associated with a loss of MAIT cells in CVID patients.

Reduced IFN-$\gamma$ response to E. coli by MAIT cells from CVID patients

To evaluate the functionality of the residual MAIT cell population in CVID patients, we interrogated MAIT cell responses to mildly fixed E. coli. Except for two outliers, MAIT cells from treatment-naive CVID patients had a lower capacity to produce IFN-$\gamma$ compared with healthy controls (Fig. 7A, 7B). The IFN-$\gamma$ response of CVID patients under replacement therapy was significantly reduced compared with healthy controls, but there was no significant change compared with treatment-naive patients (Fig. 7B, Supplemental Fig. 4). IL-7 has been shown to increase MAIT cell effector functions in vitro following TCR stimulation by
FIGURE 7. Reduced IFN-γ production by MAIT cells in CVID.

PBMCs were stimulated with *E. coli* for 24 h at an MOI of 10 and Monensin was added during the last 6 h before staining for surface Ags and intracellular staining for IFN-γ. Representative flow plots of IFN-γ production by MAIT cells from healthy controls and treatment-naive CVID patients (A). IFN-γ production by MAIT cells in healthy controls (*n* = 21), treatment-naive (*n* = 15), and IVIg-treated CVID patients (*n* = 11) (B). PBMCs from CVID patients (*n* = 9) were pretreated with IL-7 at 10 ng/ml for 24 h before stimulation with *E. coli* for 24 h at an MOI of 10 (C). PBMCs from healthy controls (*n* = 7) or CVID patients (*n* = 6) were stimulated with *E. coli* at an MOI of 10 in the presence or absence of MR-1 blocking (Continued)
upregulating the expression of TCR-signaling components and of the transcription factors PLZF, RORγt, and Eomes (39, 44). Therefore, we pretreated PBMCs from CVID patients with IL-7 for 24 h before stimulation with fixed E. coli. All patients but one showed an increase in IFN-γ production when the PBMCs were pretreated with IL-7 (Fig. 7C). These results indicate that IL-7 can restore the IFN-γ response of MAIT cells in CVID. Next, we investigated the relative importance of MR1 in MAIT cell activation in CVID patients. MAIT cell IFN-γ production following E. coli stimulation was approximately ~80% dependent on MR1, which was similar to healthy controls (Fig. 6D). Finally, we tested the capability of PBMCs from CVID patients to stimulate MAIT cells from healthy donors. MAIT cells from healthy controls were sorted, labeled with cell trace violet, and mixed together with PBMCs from CVID patients or healthy controls before E. coli stimulation. Exogenously added MAIT cells were identified at the end of the assay as cell trace violet positive and we evaluated their IFN-γ production. We found that PBMCs from CVID patients were less efficient at inducing IFN-γ production by healthy donor MAIT cells as compared with healthy donor PBMCs (Fig. 6E). These results suggest that CVID might also be associated with a defect in APCs, which contribute to the impairment of MAIT cells in this disease.

**DISCUSSION**

In this study, we found that CVID is associated with a loss of MAIT cells. The remaining MAIT cells had an elevated expression of activation markers, low expression of CCR6, and showed a poor IFN-γ response following in vitro stimulation with E. coli. Activation and loss of MAIT cells in CVID is similar to what has been described for chronic viral infections such as HIV (27, 28), human T cell leukemia virus type 1 (29), and hepatitis C virus (40). Loss of MAIT cells has also been reported in cystic fibrosis (45), a genetic condition associated with chronic bacterial infections. Similarly, individuals carrying Helicobacter pylori, with mycobacterial infections, or patients with severe bacterial infection have lower blood MAIT cells (46–48). Overall, this suggests that the loss of MAIT cells in CVID and other diseases could reflect the infection burden.

Microbial translocation is now recognized as a driver of immune activation in HIV infection (49) and is associated with an abnormal distribution of 61 and 82 γδ T cells in SIV infection (50). We have recently reported that similar perturbations in γδ T cells also occur in CVID (32), and in the current study we observed a trend toward an association between the levels of sCD14, an indirect marker of microbial translocation, and MAIT cell loss in CVID. Two studies have reported elevated levels of LPS in CVID (30, 31) and elevated LPS levels were associated with CD4+ T cell exhaustion. This suggests that microbial translocation may underlie several perturbations in CVID and possibly explain some of the similarities in T cell anomalies between CVID and HIV infection (11). Further support for this is the accumulation of Eomes- and PLZF-negative MAIT cells in CVID patients. Lack of expression of those transcription factors was associated with MAIT cell loss, and Eomes- and PLZF-negative MAIT cells can be generated in vitro by exposing healthy PBMCs to E. coli (39), re-enforcing the idea that microbial translocation may be involved in MAIT cell perturbations in CVID. Reduced PLZF expression by MAIT cells in chronic inflammatory conditions may be a mechanism to protect from PLZF-dependent apoptosis (51). MAIT cells are present in the gut, lung, and genital mucosa (27, 52, 53). Therefore, it is possible that progressive loss and exhaustion of MAIT cells at the mucosal barriers could feed a progressive destruction loop with increasing microbial translocation. Future studies will need to address the frequency and functionality of MAIT cells at mucosal surface in CVID.

The elevated levels of MIP-3 suggest a possible role for this chemokine in recruitment of MAIT cells to tissues, which may contribute to low levels of MAIT cells in circulation. Interestingly, all CVID patients with MAIT cell frequency within the normal range had undetectable MIP-3, whereas all those with detectable MIP-3 had a low frequency of MAIT cells. Furthermore, it is possible that the low levels of CCR6 on MAIT cells may result from internalization of CCR6 following binding of its ligand (43). However, some CVID patients had a low frequency of circulating MAIT cells but undetectable MIP-3 levels, suggesting that other mechanisms are also contributing to the reduced frequency of peripheral MAIT cells.

MAIT cells constitutively express the IL-7 receptor (CD127) and we found that its expression was not altered in CVID. However, the levels of IL-7 in plasma were lower in CVID, with the majority of the treatment-naive patients having undetectable levels, and IL-7 levels remained low in patients after IVIg initiation. Similar to a previous report on MAIT cells in HIV-infection (39), we found that pretreatment of PBMCs with IL-7 increased the IFN-γ response of MAIT cells in CVID. Therefore, one possible explanation for the low functionality of MAIT cells in CVID could be the low levels of IL-7 in the blood of those patients. PBMCs from CVID patients had a poor capacity to activate control MAIT cells, supporting the notion that APCs are also dysfunctional in CVID. This opens the possibility that IL-7 may act on APCs to support enhanced MAIT cell activation. It was recently reported that CVID patients with complications have lower MAIT cell numbers compared with CVID patients without

Ab (5 μg/ml) for 24 h, and IFN-γ production by MAIT cells was evaluated (D). Sorted MAIT cells were labeled with cell trace violet and mixed with PBMCs from controls (n = 7) or CVID patients (n = 7) (ratio 10:1 PBMC/MAIT cells) before stimulation with E. coli for 24 h at an MOI of 10. IFN-γ production was evaluated on the cell trace violet−positive MAIT cells (E). The lines and whiskers represent the median and interquartile range respectively. *p < 0.05, **p < 0.01.

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complications (54), suggesting that boosting MAIT cell frequency or functionality might be clinically beneficial. The same study found no difference in MAIT cell IFN-γ response following whole blood stimulation with anti-CD3/CD28 and PMA/ionomycin. Using a more physiological assay, we found that APCs from CVID had a reduced capacity to stimulate MAIT cells to produce IFN-γ. Because in studies based on mouse models MAIT cell expansion has been reported to depend on B cells (25), and a subset of CVID patients present very low or absent B cells, we considered the possibility that the low levels of MAIT cells in CVID may not represent a loss of those cells, but rather an impaired expansion. However, we did not find any association between the frequencies of B cells and MAIT cells in CVID. Furthermore, we described a CVID patient with no detectable B cells and a frequency of MAIT well within the normal range. Together, these results suggest that loss of B cells in CVID does not directly lead to the loss of MAIT cells.

We found that CD4+ MAIT cells were preserved in CVID together with CD4+ T cells expressing CD161 and CD8+ T cells with a CD161int phenotype. Preservation of CD4+ but loss of CD8+ and CD4+CD8− MAIT cells in CVID has also been observed in another cohort (54). It is unknown why CD161+ CD4+ T cells were preserved in CVID, unlike the general loss of CD4+ T cells, which has been well documented (13, 35–37). This would suggest that CD161 expression identifies a subset of CD4+ T cells that is preserved in CVID. Human Th17 cells are believed to originate from CD161− CD4+ precursors (55), but Th17 cells have also been reported to be reduced in CVID (56) as well as IL-17 transcripts (57). CD8+ CD161int T cells have been described has highly functional memory cells (38) and were preserved in our cohort of CVID patients. The maintenance of these subsets suggests that a MAIT cell–specific pathway is responsible for the loss of these cells.

Based on our results, we propose a model where chronic immune activation, possibly caused by microbial translocation, together with low levels of IL-7 leads to a loss of MAIT cells and to an altered phenotype with poor IFN-γ response of the residual MAIT cells in CVID. Given their important antimicrobial role, low MAIT cell frequency and function could explain the increased incidence of infections in CVID, even after the initiation of replacement therapy. Our results suggest that IL-7 could be considered as an augmentative therapy to rescue MAIT cell function in these patients.

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DISCLOSURES

The authors have no financial conflicts of interest.

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