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Concise report

Persistent deficiency of mucosal-associated invariant T cells during dermatomyositis

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Abstract

Objectives. Mucosal-associated invariant T (MAIT) cells are innate-like lymphocytes that are important for antibacterial immunity and may have regulatory roles. MAIT cells are decreased during SLE. However, their frequencies and phenotype have not been investigated in DM. We studied MAIT cell frequencies and phenotype in DM patients with active and inactive disease (after treatment).

Methods. Peripheral blood flow cytometry analysis of MAIT cells was compared between DM ($n = 22$), SLE ($n = 10$), psoriasis ($n = 7$) and atopic dermatitis ($n = 5$) patients, and healthy controls ($n = 19$).

Results. A dramatic decrease of circulating MAIT cell frequency was observed in active DM and SLE patients compared with healthy controls and other inflammatory skin diseases [active DM: median = 0.25% (interquartile range 0.19–0.6%), $P < 0.0001$; active SLE: median = 0.61 (0.55–0.77), $P < 0.0001$ *vs* healthy controls: 2.32% (1.18–4.45%)]. MAIT cells from active DM patients had an abnormal phenotype including increased expression of CD25 and cytotoxic T-lymphocyte-associated protein 4 that correlated with their low frequency in the blood.

Conclusion. In DM, peripheral blood MAIT cells are dramatically reduced and have an activated/exhausted phenotype that may be linked to increased activation-induced cell death.

Key words: dermatomyositis, mucosal-associated invariant T cells, immunology, T-lymphocytes, autoimmunity

Rheumatology key messages

- Mucosal-associated invariant T cells are deficient in active DM patients.
- During DM, mucosal-associated invariant T cells exhibit an abnormal phenotype, suggesting activation-induced cell death.
- These abnormalities may contribute to dysregulated cutaneous and systemic immunity in DM patients.

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CLINICAL SCIENCE

Introduction

DM is a chronic inflammatory and autoimmune disorder belonging to the idiopathic inflammatory myopathies affecting primarily skin and muscle with variable extent. Comprehension of the disease mechanisms of DM is growing, with a pivotal role of both innate and adaptive immune system and involvement of both cellular and humoral actors [1]. Mucosal-associated invariant T (MAIT) cells are innate-like lymphocytes that can be activated either through their semi-invariant T cell receptors [mainly riboflavin derivative presented by MR1 (major histocompatibility complex class I-related gene protein)] or through unspecific stimulation (by IL-12 and/or IL-18 and/or IFN-α) [2–4]. They are characterized by high-level expression of CD161, CD26 and IL-18R [5], and play a role in anti-bacterial responses, but they have also been implicated in autoimmune disorders [6].

We investigated MAIT cell frequencies and phenotype in DM patients with active and inactive disease (after treatment) in comparison with healthy controls (HC) and patients with other inflammatory skin disorders (psoriasis, atopic dermatitis) or systemic inflammatory disorders (SLE).

Methods

Patients

Twenty-two DM patients (Dermatology Department, Saint-Louis Hospital, Paris, France), from 2014 to 2018, were included (Table 1). DM was diagnosed according to European Neuromuscular Centre and/or ACR/EULAR criteria [7, 8]. All DM patients were free of therapy at first analysis. Ten patients with active SLE (SLEDAI >14), five patients with severe atopic dermatitis (Eczema Area and Severity Index score ≥16) (as Th2 controls) and seven patients with severe psoriasis [Psoriasis Area and Severity Index score ≥10; median: 16.3 (interquartile range 14.1–20)] (as Th17 controls) were included. All the patients and HC were fully informed and gave their consent to participate. The study was performed according to the Helsinki declaration, and the study protocol was reviewed and approved by the local Ethics Committee.

Table 1 Clinical and biological characteristics of DM patients

	HC	DM
Total, *n*	19	22
Female/male, *n*	13/6	14/7
Age, mean (S.D.), years	46 (8.48)	55 (15.42)
Disease duration (months), mean (S.D.)	NA	19 (37.7)
Myopathic/amyopathic, *n*	NA	13/8
CDASI, mean (S.D.)	NA	21.6 (10.7)
MMT (/80), mean (S.D.)	NA	75.5 (17.7)
MSA (MDA5/Mi2/TIF1/JO1/none), *n*	NA	5/1/3/1/12
CRP, mean (S.D.)	NA	6.1 (5.85)
CK, mean (S.D.)	NA	306 (443)
Lymphocytes (cells/μl), mean (S.D.)	NA	1659 (881)
Neutrophils (cells/μl), mean (S.D.)	NA	4665 (2453)

HC: healthy controls; NA: not applicable; CDASI: Cutaneous Dermatomyositis Disease Area and Severity Index; MMT: manual muscle testing; MSA: myositis-specific antibody; CK: creatine kinase.

Cell preparation

Peripheral venous blood of patients and HC was diluted with PBS, and peripheral blood mononuclear cells were isolated by density-gradient centrifugation on Ficoll-Hypaque (Pharmacia, St Quentin en Yvelines, France). Peripheral blood mononuclear cells were stored liquid-frozen until further use.

Staining and flow cytometric analysis

A multicolour immunophenotyping approach was used for the identification and analysis of lymphocyte subpopulations. Immunophenotypic studies were performed on frozen samples, using 18-colour flow cytometry as follows: CD45RA-BV650 HI100, CD8-BUV395 RPA-T8, CD161-BV605 DX12, CD14-BV510 MPhiP9, CD3-BUV737 UCHT1, CD25-BV785 M-A251, CD94-BV711 HP-3D9, CD4-BB700 Sk3, CD28-APC R700 CD28.2, FOXP3-PECF594 236 A/E7 and cytotoxic T-lymphocyte-associated protein 4 (CTLA4)-PC5 BNI3 were obtained from BD (BD Bioscience, Le Pont de Claix, France), TCRγδ-Pc5.5 was obtained from Beckman Coulter (Beckman Coulter France S.A.S., Villepinte, France), NKG2A-PE REA110, CD158d-APC REA768 and CD56-APC vio770 REA196 were obtained from Miltenyi (Miltenyi Biotec, Bergisch-Gladbach, Germany), and CD26-FITC BA5b and CD39-PC7 A1 were obtained from BioLegend (BioLegend France, Paris, France).

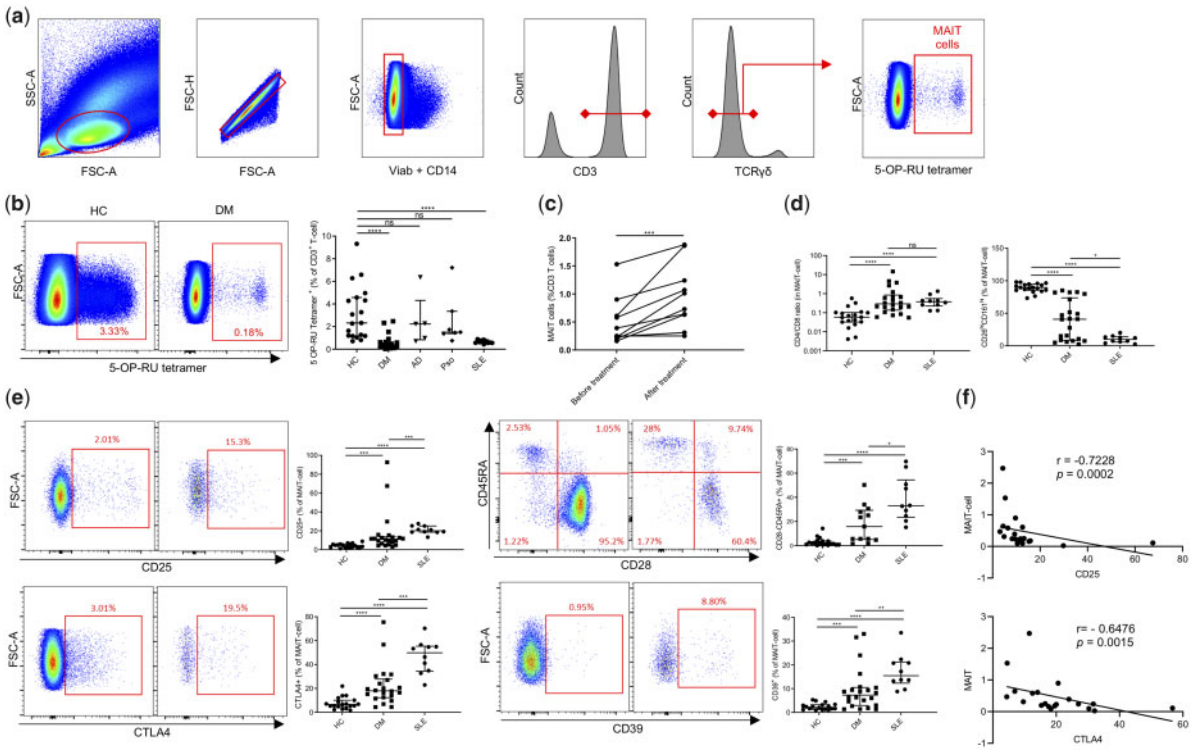
MAIT cells were identified using the 5-OP-RU tetramer BV421, which was obtained from the National Institutes of Health tetramer core facility.

Cells were stained for membrane markers (at 4°C in the dark for 30 min) using cocktails of Ab diluted in PBS containing BSA/NaN_3 (0.5% BSA, 0.01% NaN_3) (FACS buffer). Appropriate isotype control Abs were used for each staining combination. Samples were acquired on BD LSR-Fortessa flow cytometer using FACSDiva software (Beckton Dickinson, BD Bioscience, San Jose, CA, USA). Flow data were analysed using FlowJo software (FlowJo, LLC, Ashland, Oregon, USA).

Statistical analysis data

Data were expressed as median with interquartile range. Data were analysed using a statistical software package (GraphPad Prism, GraphPad Software, San Diego, California, USA), the Mann–Whitney *U* test for non-paired data, Wilcoxon ranked *t*-test for paired data, Kruskal–Wallis with Dunn's correction for multiples comparison and Spearman's rank correlation. Differences at $P < 0.05$ were considered to be statistically significant.

Fig. 1 Peripheral blood MAIT cell deficiency in active DM patients



(**a**) Gating strategy to identify MAIT cells in peripheral blood mononuclear cells. (**b**–**d**) Representative flow cytometry dot plots and scatter dot plots are depicted. Horizontal bar represents median and whiskers interquartile range of cell populations. (b) Decreased frequency of circulating MAIT cells in active DM patients. Frequency of MAIT cells among $CD3^+$ T cells in active DM patients ($n = 21$) compared with HC ($n = 19$), SLE ($n = 10$), psoriasis ($n = 7$) and atopic dermatitis patients (AD) ($n = 5$). (c) MAIT cell frequencies significantly increase after DM treatment. MAIT cell frequency before and after DM treatment is shown. (d) Abnormal MAIT cells phenotype in active DM patients with higher CD4/CD8 ratio and decreased expression of CD26 and CD161. CD4/CD8 ratio and frequency of $CD26^{hi}CD161^{hi}$ population in MAIT cells in active DM patients compared with SLE and HC are shown. (**e**) MAIT cells from active DM patients are activated and exhausted, as depicted by higher frequency of activation and exhaustion markers. Frequency of CD25, CD28, CD45RA, CTLA4 and CD39 expression in MAIT cells in DM patients compared with SLE and HC. (**f**) MAIT cell frequency is inversely correlated with cell surface expression of activation and exhaustion markers. Correlation of MAIT cell frequency with CD25 and CTLA4 in DM patients. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. MAIT cell: mucosal-associated invariant T cell; HC: healthy controls.

Results

The gating strategy is illustrated in Fig. 1a. Using the specific staining with the 5-OP-RU tetramer (specific for MAIT cells in humans [9]), we found that circulating MAIT cells were dramatically decreased in active DM patients in comparison with HC [median: 0.25 (0.19–0.6%) *vs* 2.32% (1.18–4.45%), $P < 0.0001$] (Fig. 1b). This decrease was also observed in SLE in comparison with HC [median: 0.61 (0.55–0.77), $P < 0.0001$], but MAIT cell frequencies were unaffected in atopic dermatitis and psoriasis patients (Fig. 1b). After a median follow-up of 15.4 months (12.04–23.31), nine DM patients were in complete remission and three in partial remission. MAIT cell frequencies increased after DM treatment [median: 0.25% (0.24–0.60%) at diagnosis *vs* 0.73% (0.47–1.16%) at last follow-up, $P = 0.002$] (Fig. 1c), but did not return to normal HC frequencies. Residual MAIT cells from both active DM and SLE patients had a peculiar phenotype associating a great increase in the CD4/CD8 ratio [median: DM = 0.29 (0.16–0.7), $P < 0.0001$, SLE = 0.37 (0.26–0.43), $P < 0.0001$ *vs* HC = 0.06 (0.03–0.1)] and a decrease of the median percentage of CD26 and CD161 co-expression [median: DM = 40.8% (9.1–66.6%), $P < 0.0001$, SLE = 9.8 (6.59–13.2), $P < 0.0001$ *vs* HC = 89.6% (85.5–94.1%)] (Fig. 1d).

To address possible phenotype changes in MAIT cells from active DM patients, we studied MAIT cells surface expression of CD25, CD28 and CD45RA as activation markers and CTLA-4 and CD39 as exhaustion markers [10]. A higher frequency of MAIT cells expressing both activation and exhaustion markers was found in both active DM and SLE patients compared with HC, as shown in Fig. 1e. Notably, residual MAIT cells from active DM

patients expressed higher levels of CD25 [median: 10.05% (7.5–12%) *vs* 4.39% (2.43–5.76%), $P = 0.0006$] and had an antigen-experienced phenotype, i.e. $CD45RA^{hi}CD28^{-}$ [11] [median: 15.8% (5.61–28%) *vs* HC: 2.09% (0.825–3.79%), $P = 0.004$]; this subset classically expresses hallmarks of highly differentiated and senescent cells (CD57, KLRG1 and PD-1) [12]. SLE patients exhibited even stronger signs of activation and exhaustion in comparison with HC [$CD25^{+}$ median: 20.05% (18.7–24.15%), $P < 0.0001$, $CD45RA^{hi}CD28^{-}$ median: 33% (25.73–49.9%), $P < 0.0001$].

We next investigated the correlation between MAIT cell frequencies and the expression of cell surface immune activation markers in active DM patients. MAIT cell frequencies inversely correlated with cell surface expression of CD25 ($r = -0.7228$, $P = 0.0002$) and CTLA4 ($r = -0.6476$, $P = 0.0015$; Fig. 1f). We did not observe any correlation between MAIT cell frequencies and DM clinical scores (Cutaneous Dermatomyositis Disease Area and Severity Index) or biological markers (data not shown).

Discussion

Decreased frequency of circulating MAIT cells has been reported in HIV infection, human T-lymphotropic virus 1 infection, multiple sclerosis and SLE [13–15]. In this study, we found that circulating MAIT cell frequencies were reduced in active DM patients. Reduced MAIT cell frequency was associated with an increased expression of activation and exhaustion markers such as CD25, CD39 and CTLA4. This MAIT cell deficiency was more prominent within the $CD8^{+}$ T cell subset. The cytokine microenvironment found in DM (high level of IL-12, IL-18 and IFN-α) could preferentially activate $CD8^{+}$ MAIT cells, leading to activation-induced cell death as $CD4^{+}$ MAIT cells express less IL-12R and IL-18R compared with $CD8^{+}$ MAIT cells [16, 17]. Interestingly, and in contradiction to previous reports [18], this decrease was not due to immunosuppressive treatments as the blood of our patients was collected before any steroid or any immunosuppressive drug. Previous functional studies have shown that during SLE, MAIT cells are more prone to activation in both a TCR-dependent and -independent manner. First, monocytes from SLE patients, when compared with monocytes from HC, were potent MAIT cell activators at least in part through IL-12 secretion; secondly, MAIT cells are sensitive to type I IFN alone or in association with IL-12 and IL-18, in both SLE and viral infections, cytokines that are known to be elevated in SLE and DM [4, 19]. These results suggest that chronic inflammation rather than immunosuppressive treatment may explain the MAIT cell abnormalities seen in active DM patients. Regulatory roles have been described for MAIT cells [20], and their persistent deficiency could potentially participate in chronic inflammation.

In summary, this study demonstrates that MAIT cells are deficient in active DM patients, with an abnormal phenotype, suggesting activation-induced cell death. These abnormalities may contribute to dysregulated cutaneous and systemic immunity in DM patients.

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