The frequency of mucosal-associated invariant T (MAIT) cells is selectively increased in dermatitis herpetiformis

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Structured Abstract

Background/ Objectives:

Mucosal-associated invariant T (MAIT) cells are a novel subset of innate-like T cells that are enriched in mucosal tissues. Their presence in human skin has only recently been recognized. We describe the expression of skin-tropic molecules on human skin MAIT cells at steady state and investigate their contribution to various dermatoses with known T cell involvement.

Methods:

To examine the expression of skin-tropic molecules by MAIT cells at steady state, we performed flow cytometric analysis of blood and skin samples from healthy donors. To investigate any potential wider contribution of MAIT cells to skin disease, we examined psoriasis, alopecia areata and dermatitis herpetiformis biopsies using immunofluorescent staining to identify the proportion of T cells expressing MAIT cell surface markers.

Results:

We found that MAIT cells constituted a small population of T cells in normal human skin, similar to the percentage found in peripheral blood. Like other skin T cells, skin MAIT cells expressed high levels of the skin-associated markers cutaneous lymphocyte antigen and CD103. In psoriasis and alopecia areata the proportion of MAIT cells was similar to that found in normal skin,

but in dermatitis herpetiformis the proportion of MAIT cells was significantly elevated.

Conclusions:

The expression of skin-tropic molecules by skin MAIT cells is consistent with their resident status in normal human skin. Our results suggest that MAIT cells may play a role in the pathogenesis of dermatitis herpetiformis.

Introduction

Mucosal-associated invariant T (MAIT) cells are a novel subset of innate-like T cells that comprise 1-10% of human peripheral blood T cells and are enriched in mucosal tissues, particularly the gastrointestinal tract^{1, 2}.

MAIT cells are characterized by expression of the semi-invariant T cell receptor V α 7.2 and restriction by the non-classical major histocompatibility complex (MHC) class I-like molecule MR1¹. MAIT cells can also be readily identified by high surface expression of CD161 and interleukin-18 receptor α chain (IL-18R α)³. MR1 is capable of binding microbial vitamin B metabolites for presentation to MAIT cells². MAIT cells also exhibit reactivity to uninfected cells⁴, suggesting that endogenous ligands for MR1 may exist as well.

While MAIT cells have been recently found in normal and psoriatic human skin⁵, their involvement in other cutaneous pathologies has been unexplored. T cells in human skin are known to express several skin-associated markers^{6, 7}, but whether skin MAIT cells similarly express these markers is unknown. Furthermore, although MAIT cells may variably express CD8 or CD4⁸, the previous report on skin MAIT cells considered only CD8⁺ MAIT cells⁵.

Materials and Methods

Patients and controls

Lesional skin biopsies were collected from patients with alopecia areata (6 donors), psoriasis (9 donors) and dermatitis herpetiformis (5 donors).

Diagnoses were confirmed histologically or, for a subset of alopecia areata and psoriasis donors, based on a characteristic clinical appearance. From healthy volunteers samples were obtained of seborrhoeic keratoses (2 donors), normal skin (10 donors), and peripheral blood mononuclear cells (PBMC; 6 donors). Ethics approval was obtained from the relevant human ethics committees and all patients provided written informed consent. Skin tissue samples were either frozen immediately in optimum cutting temperature compound for cryosectioning or enzymatically digested to release T cells for flow cytometric analysis.

Enzymatic tissue digestion and flow cytometry

Normal skin samples were incubated in dispase 1U/ml (Roche, Basel, Switzerland) overnight at 4°C, then forceps were used to separate the epidermis from the dermis. Samples were incubated in 3mg/ml collagenase type 3 (Worthington, Lakewood, USA) with 5µg/ml deoxyribonuclease in RPMI medium containing 2% (v/v) foetal calf serum at 37°C for 90 minutes. Epidermal samples were manually minced into a cell suspension while dermal samples were processed to suspension using the Beckton Dickinson Medimachine tissue dissociator (BD, Franklin Lakes, USA).

Biotinylated MR1-5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (MR1-5-OP-RU) monomers were tetramerized with streptavidin conjugated to

phycoerythrin as previously described^{8,9}. Cell suspensions were stained with MR1-5-OP-RU tetramer as well as fluorophore-conjugated antibodies to CD45 (BD), CD3 (Biolegend, San Diego, USA), cutaneous lymphocyte antigen (BD), and CD161 (Biolegend). Propidium iodide was used to exclude non-viable cells. LSRFortessa (BD) and Flowjo software (Treestar, Ashland, USA) were used for analysis.

Immunofluorescent staining and confocal microscopy

Cryosections of 12 µm thickness were permeabilized in ice-cold acetone for 10 minutes. Slides were incubated in serum-free protein block (Dako, Glostrup, Denmark) for 10 minutes at room temperature then the primary antibody cocktail was applied overnight at 4°C. The slides were washed in phosphate-buffered saline (PBS) then secondary antibodies conjugated to appropriate fluorophores (Thermo Scientific, Waltham, USA) were applied for 1 hour at room temperature. The slides were washed again in PBS and counterstained with Hoechst nuclear dye. Prolong Gold Antifade medium (Thermo Scientific) was added prior to mounting.

Tile scan images covering dimensions of at least 500 by 500 µm were captured using a Zeiss LSM 700 confocal microscope (Zeiss, Oberkochen, Germany) at the Biological Optical Microscopy Platform (The University of Melbourne, <http://www.microscopy.unimelb.edu.au>).

Statistical analysis

Two-tailed Mann-Whitney U test was used in 2-group comparisons; Kruskal-Wallis test used for analysis of multiple groups, followed by Dunn's post hoc multiple comparisons test. Percentages in text are expressed as mean ± s.e.m.

Results

First, we compared the frequency and characteristics of MAIT cells in normal skin to those in healthy PBMC by flow cytometry (Fig. 1a). MAIT cells were identified by positive binding to CD161 antibody and MR1 tetramer^{8,9}. We found that MAIT cells constituted a small percentage of the total T cell population in both skin and PBMC, with no significant difference in frequency (Fig. 1b).

Teunissen *et al.* reported that a minor population of MAIT cells in PBMC expressed skin-homing receptors, and suggested that these cells selectively migrate to skin⁵. Similarly, in our PBMC samples, we found that a minority ($7.97 \pm 1.25\%$) of MAIT cells expressed the skin-homing marker cutaneous lymphocyte antigen (CLA; Fig. 1c-d). In contrast, in the normal skin samples, the vast majority of MAIT cells were CLA⁺ (mean 87.5%, range 75-100%; Fig. 1c-d). In addition, the skin MAIT cells preferentially expressed the α -chain (CD103) of the integrin $\alpha\text{E}\beta 7$ (Fig. 1e), which is a known surface marker for lymphocytes embedded in non-lymphoid tissues, including skin⁶. Collectively, these results identify a population of MAIT cells that are resident in normal human skin.

Next, we studied the frequency of MAIT cells in different inflammatory skin conditions involving T cells: psoriasis, alopecia areata and dermatitis herpetiformis. As controls we examined a benign proliferative condition, seborrhoeic keratosis, and normal skin from healthy donors.

Immunofluorescent staining was performed on tissue cryosections using primary antibodies directed against CD3 (Abcam, Cambridge, UK), IL-18R α (R&D Systems, Minneapolis, USA) and the V α 7.2 receptor (D5 monoclonal antibody^{2, 8}). This staining combination has previously been used to demonstrate MAIT cells in human liver, intestine and lymph node tissue sections¹⁰. Confocal microscopy was used to capture images with dimensions of at least 500 $\times$ 500 μ m. Triple-positive cells representing MAIT cells were manually identified and recorded as a proportion of all CD3⁺ cells present in each image (Fig. 2-3).

With this method, we saw that in normal skin, sparse cells with the MAIT phenotype could be demonstrated in the papillary dermis and adjacent to the superior vascular plexus (Fig. 2a). As the normal epidermis is very thin relative to the dermis, epidermal T cells, MAIT or non-MAIT, were overall rarely seen in normal skin. Epidermal MAIT cells could however be found on examination of the seborrhoeic keratosis biopsies (Fig. 2b), likely a reflection of the increased bulk of the epidermis.

The quantitative image analysis (Fig. 3) showed that the percentages of MAIT cells in normal skin ($3.83 \pm 0.32\%$) and seborrhoeic keratosis ($5.55 \pm 1.26\%$) were statistically similar to the percentages found in the T cell infiltrates of psoriasis (Fig. 2c; $6.25 \pm 0.67\%$) and alopecia areata (Fig. 2d; $4.05 \pm 0.82\%$). In sharp contrast to these conditions, the percentage of MAIT cells in dermatitis herpetiformis (DH) was significantly elevated (Fig. 2e; $20.3 \pm 1.74\%$) compared to all other groups (Fig. 3). These MAIT cells were located together with other T cells within infiltrates in the upper dermis of the DH biopsies (Fig. 2e).

Discussion

Teunissen *et al.* found that a population of MAIT cells exists in lesional psoriasis⁵, although their relatively low abundance, as echoed in our study, argued against a dominant contribution to pathology¹¹. We, however, show that DH is uniquely associated with an increase in MAIT cell skin content, consistent with their local contribution to disease progression.

DH, like coeliac disease, is a clinical manifestation of gluten hypersensitivity¹². The mechanism of action of MAIT cells in DH remains undefined, although activated MAIT cells are capable of producing the cytokines tumour necrosis factor- α , interferon- γ , and interleukin-17. As these cytokines have all been previously associated with DH¹²⁻¹⁴, we speculate that cytokine production by MAIT cells could contribute to the disease, although we were unable to assess this using the confocal microscopy technique. Interestingly, a previous study showed that the frequency of MAIT cells is reduced in the blood and intestinal lamina propria of patients with coeliac disease, presumably without skin involvement¹⁵.

There are several limitations to our study. The MAIT cell enumeration was not performed in a blinded fashion as each condition being studied had a characteristic histological appearance. Also, due to limited availability of patient specimens, the samples obtained were not matched to age, gender or anatomical location. For example, normal skin samples were obtained from the inframammary or abdominal regions, but alopecia areata samples were obtained from the scalp and DH samples from the limbs. Each of these factors could have impacted the results of the quantitative image analysis. Further studies, ideally with matched samples, are required to confirm these preliminary results.

In conclusion, we describe the presence of a population of CLA⁺ MAIT cells present in human skin at steady state. The proportion of MAIT cells infiltrating the skin appears to be selectively increased in DH, suggesting a role for MAIT cells in the pathogenesis of this disease.

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Figure 1. Mucosal-associated invariant T (MAIT) cells in human skin express CLA and CD103. (a) Flow cytometric analysis of peripheral blood mononuclear cells (PBMC), dermal and epidermal cell suspensions from healthy donors. Epidermal and dermal staining shown are from the same skin donor. MAIT cells are CD161⁺ and bind to MR1 tetramer. Representative images shown from >3 independent experiments. (b) Frequency of MAIT cells in PBMC and normal skin. N.s. = not significant. (c) Relative expression of the skin-tropic markers cutaneous lymphocyte antigen (CLA) and CD103 by MAIT cells and non-MAIT T cells from PBMC (red histograms), dermis (orange histograms) and epidermis (green histograms). Representative graphs shown from >3 independent experiments. Percentages of MAIT cells expressing (d) CLA and (e) CD103 in PBMC, dermis and epidermis. Kruskal-Wallis test with Dunn's post-test, *P<0.05; **P<0.01. Error bars show mean $\pm$ s.e.m.

Figure 2. Demonstration of mucosal-associated invariant T (MAIT) cells in human skin by immunofluorescent staining. Immunofluorescent staining performed on biopsies of (a) normal skin, (b) seborrhoeic keratosis, (c) psoriasis, (d) alopecia areata (peribulbar area shown), and (e) dermatitis herpetiformis, with single-colour and merged images of boxed region. Arrowheads show MAIT cells triple-positive for CD3 (grey), IL-18R α (green) and V α 7.2 (red). Representative images shown from >3 independent experiments. Dotted lines indicate dermo-epidermal junction. Hoechst nuclear stain = blue. Scale bars = 50 μ m.

Figure 3. The frequency of mucosal-associated invariant T (MAIT) cells is selectively increased in dermatitis herpetiformis. Results of quantitative image analysis showing the frequency of MAIT cells in the different skin conditions. Pooled data from >3 independent experiments; symbols indicate individual images analysed. Kruskal-Wallis test with Dunn's post-test, **P<0.01; ***P<0.001. Error bars show mean $\pm$ s.e.m.

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