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The IL-17A-Producing CD8$^+$ T-Cell Population in Psoriatic Lesional Skin Comprises Mucosa-Associated Invariant T Cells and Conventional T Cells

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IL-17A is pivotal in the etiology of psoriasis, and CD8$^+$ T cells with the ability to produce this cytokine (Tc17 cells) are over-represented in psoriatic lesions. Here we demonstrate that the frequency of Tc17 cells in peripheral blood of psoriasis patients correlated with the clinical severity of the disease. Analysis of cutaneous-associated lymphocyte antigen expression showed that the blood Tc17 population contains a significantly higher proportion of cells with skin-homing potential compared with the CD8$^+$ T-cell population lacking IL-17A/IL-22 expression. IL-17A-producing CD8$^+$ T cells in blood have previously been reported to belong mainly to the mucosa-associated invariant T-cell (MAIT cell) lineage characterized by TCR Vα7.2 chain, CD161, IL-18Rα, and multidrug transporter ABCB1 expression. We demonstrate the presence of CD8$^+$ MAIT cells in the dermis and epidermis of psoriatic plaques, as well as healthy skin; however, IL-17A-producing CD8$^+$ MAIT cells were predominantly found in psoriatic skin. Notably, we observed IL-17A production in a large proportion of psoriatic plaque–derived CD8$^+$ T cells devoid of MAIT cell characteristics, likely representing conventional CD8$^+$ T cells. In conclusion, we provide supporting evidence that implicates Tc17 cells in the pathogenesis of psoriasis and describe the presence of innate CD8$^+$ MAIT cells in psoriatic lesions as an alternative source of IL-17A.

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INTRODUCTION

Recent investigations suggest an important role in the pathogenesis of psoriasis for IL-17A produced by CD4$^+$ T helper cells (Th cells) and CD8$^+$ cytotoxic T cells, termed Th17 and Tc17 cells, respectively, which may also express IL-22. The level of IL-17A message RNA is elevated in psoriatic skin, and keratinocytes stimulated *in vitro* with IL-17A share many features with keratinocytes in psoriatic plaques, such as increased proliferation and enhanced production of antimicrobial peptides and inflammatory cytokines (Teunissen *et al.*, 1998; Nograles *et al.*, 2008). IL-23, important for the activation and survival of IL-17A-producing T cells, is also expressed at increased levels by dendritic cells, macrophages, and keratinocytes in psoriatic skin (Lee *et al.*, 2004; Piskin *et al.*, 2006). Genetic polymorphisms in the *IL23A*, *IL12B*, and *IL23R* genes that code for the unique IL-23p19 chain, the common IL-12/IL-23 p40 chain, and one of the IL-23 receptor subunits, respectively, are associated with psoriasis, further implicating the importance of the IL-23/IL-17A axis in psoriasis (Capon *et al.*, 2007; Cargill *et al.*, 2007; Nair *et al.*, 2009). A neutralizing antibody against the IL-12/IL-23 p40 subunit is currently applied as an efficacious drug for treating psoriasis (Leonardi *et al.*, 2008; Strober *et al.*, 2011), and, in addition, phase 2 studies involving therapy with anti-IL-17A and anti-IL-17R showed improvement of psoriasis (Hueber *et al.*, 2010; Leonardi *et al.*, 2012; Papp *et al.*, 2012).

A number of observations indicate that CD8$^+$ T cells are key elements in the etiology of psoriasis. The major histocompatibility complex (MHC) class I allele *HLA-Cw*0602* is by far the strongest risk determinant for developing psoriasis (Mallon *et al.*, 1999; Nair *et al.*, 2006), suggesting that the corresponding MHC class I molecule may have a critical role in peptide presentation to pathogenic CD8$^+$ T cells. Approximately two-thirds of psoriasis patients carry the *HLA-Cw*0602* allele, while present in only 10–15% of the general population (Gudjonsson *et al.*, 2004). There is a close correlation between the frequency of skin-homing cutaneous-associated lymphocyte antigen (CLA)$^+$CD8$^+$ T cells in blood and the severity of psoriasis, as well as between perforin expression (a CD8$^+$ T cell-related molecule) and disease severity (Sigmundsdottir *et al.*, 2001;

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Abbreviations: CLA, cutaneous-associated lymphocyte antigen; MHC, major histocompatibility complex; MAIT cell, mucosa-associated invariant T cell; PASI, Psoriasis Area and Severity Index; Th cell, T helper cell

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Prpic *et al.*, 2007). The frequency of Tc17, but not of Th17 cells, is increased in psoriatic skin in comparison with normal skin (Ortega *et al.*, 2009; Res *et al.*, 2010), suggesting that Tc17 cells may have locally expanded in response to psoriasis-associated (auto)antigens in a MHC class I–restricted manner, as part of an uncontrolled adaptive immune response. Intriguingly and possibly in contradiction with this presumption, it has been reported that the vast majority of IL-17A-producing CD8$^+$ T cells in peripheral blood are mucosa-associated invariant T cells (MAIT cells) (Dusseaux *et al.*, 2011), which belong to the innate immune system and use a semi-invariant TCR to recognize bacterial ligands in the context of MR1 (Treiner *et al.*, 2003; Huang *et al.*, 2009; Le Bourhis L. *et al.*, 2010), a MHC class I-like antigen-presenting molecule. The typical invariant TCR α-chain on MAIT cells consists of a variable region 7.2 segment associated with a joining region 33 segment (Vα7.2–Jα33) (Tilloy *et al.*, 1999). MAIT cells further coexpress CD161 (high), IL-18Rα, and the multidrug transporter ABCB1, enabling efficient efflux of xenobiotics (Le Bourhis *et al.*, 2011). MAIT cells display an effector-memory phenotype and constitute 1–10% and less than 0.1% of human peripheral blood CD8$^+$ T cells and CD4$^+$ T cells, respectively (Dusseaux *et al.*, 2011). More than 90% of the human CD3$^+$CD161^{++}Vα7.2$^+$ MAIT cells express CD8, either as a homodimer of CD8α chains or as a heterodimer of CD8α and -β chains in approximately similar proportions (Walker *et al.*, 2012). To date, there is no information about the presence of MAIT cells in the healthy and diseased human skin and whether the Tc17 cells in psoriatic lesions represent MAIT cells.

This study was aimed to determine the possible association between clinical severity of psoriasis and the relative proportions of IL-17A-, IFN-γ-, or IL-22-producing T cells in peripheral blood. Further, we studied the skin tropism of these T-cell subsets by analyzing the expression of skin-homing molecule CLA. Finally, we investigated whether the IL-17A-producing CD8$^+$ T cells in psoriatic lesional skin belong to the MAIT cell lineage.

RESULTS

Frequency of IL-17A-producing CD8$^+$ T cells in blood from psoriasis patients correlates with Psoriasis Area and Severity Index

In search for the possible association of clinical severity with the relative proportions of IL-17A-, IFN-γ-, or IL-22-producing T cells in peripheral blood, we assessed the Psoriasis Area and Severity Index (PASI) of fifteen psoriasis patients and determined their cytokine expression in T cells upon short-term stimulation. In the CD8$^+$ T-cell population, we found a mean of 0.26 ± 0.20% IL-17A producers, 36.1 ± 14.3% IFN-γ producers, and 0.24 ± 0.20% IL-22 producers, whereas in the CD4$^+$ T-cell population these frequencies were 1.1 ± 0.72%, 11.3 ± 5.0%, and 1.2 ± 0.70%, respectively (Figure 1). Statistical analysis revealed that only the frequencies of IL-17A-producing CD8$^+$ T cells significantly correlated with the PASI (Figure 1). Although a similar trend was found for IFN-γ-producing CD8$^+$ T cells, it did not reach significance. These results support the view that CD8$^+$ T cells, in particular the IL-17A-producing ones, may have a role in the pathogenesis of psoriasis.

IL-17A- and IL-22-producing blood T-cell populations show increased frequency of CLA expressing cells

To examine whether blood IL-17A-producing T cells can home in the skin and might be implicated in psoriasis, we determined the expression of CLA. We found that a

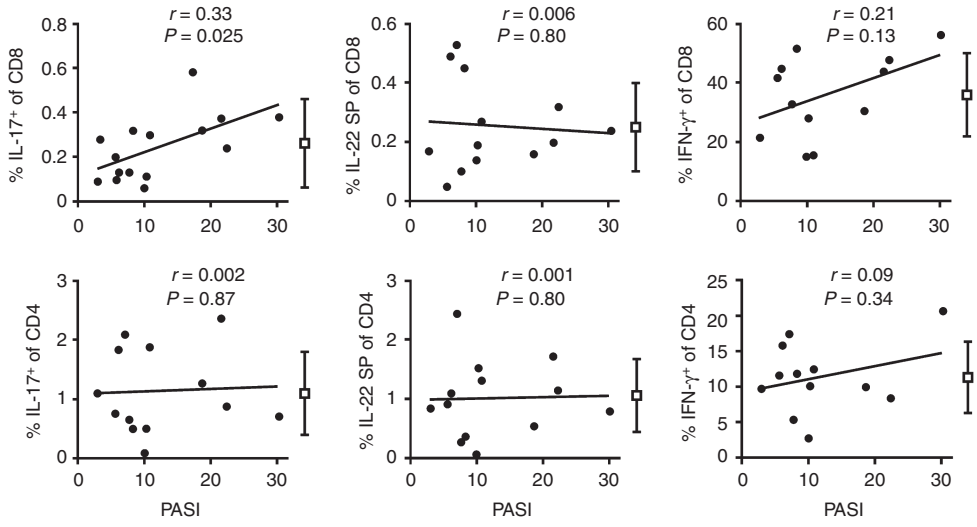


Figure 1. Frequency of IL-17A-producing CD8$^+$ T cells in blood of psoriasis patients correlates with Psoriasis Area and Severity Index (PASI). CD8$^+$ and CD4$^+$ T cells in peripheral blood of psoriasis patients were analyzed by multiparameter flow cytometry for their expression of IL-17A, IL-22, and IFN-γ upon short-term stimulation *in vitro*. The percentages of CD8$^+$ (top row) or CD4$^+$ (bottom row) T cells expressing IL-17A, IL-22, and IFN-γ, respectively, are plotted against the patient's PASI. The IL-17A and IFN-γ data shown are the total percentages irrespective of the coexpression of other cytokines, whereas the percentage of IL-22 SP (single positive) refers to cells that lacked coexpression of both IL-17A and IFN-γ. The square symbols represent the mean percentage ± SD. Data are from 12–15 independent experiments with 15 different donors.

significantly higher frequency of IL-17A-producing T cells in blood from psoriasis patients showed CLA expression (especially those with high IL-17A expression) compared with T cells without IL-17A expression, not only in the CD8$^+$ T-cell population (31.0 ± 11.5% versus 3.7 ± 3.3%; $P<0.01$), but also in the CD4$^+$ T-cell population (16.0 ± 10.1% versus 3.7 ± 2.9%; $P<0.05$) (Figure 2a). The IL-22-producing CD8$^+$ and CD4$^+$ T cells also contained significantly higher frequencies of CLA$^+$ T cells compared with those lacking IL-22 expression (CD8$^+$ T cells 27.1 ± 7.7% versus 3.1% ± 1.7, $P<0.01$ and CD4$^+$ T cells 12.4 ± 1.4% versus 3.8 ± 1.8%, $P<0.001$), whereas this feature was not observed for IFN-γ producers (data not shown). Increased levels of CLA$^+$ T cells within IL-17A- and IL-22-producing blood CD8$^+$ and CD4$^+$ T cells were not typical for psoriasis, but were also observed in healthy individuals (Figure 2b and c). Furthermore, the population of IL-17A and IL-22 double-producing T cells was also enriched for CLA$^+$ T cells (data not shown). The IL-17A- and IL-22-producing T cells represent a small proportion of the total CD4$^+$ and CD8$^+$ T-cell populations. CLA$^+$ CD8$^+$ IL-17-producing T cells constituted 0.88 ± 0.34% of the total CLA$^+$CD8$^+$ T-cell population, whereas CLA$^+$CD4$^+$ IL-17-producing T cells constituted 3.65 ± 1.44% of the total CLA$^+$CD4$^+$ T-cell population. Similarly, for the CLA$^+$ IL-22-producing CD8$^+$ and CD4$^+$ T cells, the frequencies were 1.84 ± 1.02% and 3.71 ± 1.12%, respectively.

As IL-17 and CLA positivity is almost exclusively restricted to CD45RO$^+$ memory T cells, we performed experiments ($n=3$) to find out whether the IL-17$^+$ T-cell populations are still enriched for CLA$^+$ cells when excluding the CD45RO$^-$ T cells from our analysis. As concerns the CD8$^+$ T cells, the frequencies of CLA$^+$ T cells in the IL-17A$^+$ and IL-17A$^-$ populations only marginally changed upon gating on CD45RO$^+$ cells, and as a result the CLA$^+$/CLA$^-$ ratios remained the same (data not shown). As concerns the CD4$^+$ T cells, we consistently found that the percentage of CLA$^+$ cells (and thus the CLA$^+$/CLA$^-$ ratio) increased only in the IL-17A$^-$ population (approximately 2-fold), when gating on CD45RO$^+$ T cells, yet this ratio was still markedly lower than in the IL-17A$^+$ population (data not shown). In sum, our results indicate that the population of blood T cells with the capacity to produce IL-17A and/or IL-22 shows enhanced skin tropism.

The presence of MAIT cells in psoriatic lesional skin and healthy human skin

Because the majority of the IL-17A-producing CD8$^+$ T cells in blood are MAIT cells (Dusseaux *et al.*, 2011) and IL-17A-producing CD8$^+$ T cells are over-represented in psoriatic lesional skin (Res *et al.*, 2010), we wondered whether IL-17A-producing CD8$^+$ T cells in psoriatic lesions belong to the MAIT cell lineage. We found that 13.4 ± 12.7% (range 1.9–27.9%) of the CD8$^+$ T cells from psoriatic dermis showed a high expression of CD161, carried the IL-18Rα subunit, and displayed the invariant TCR Vα7.2 chain (detected by the monoclonal antibody 3C10), altogether typifying MAIT cells (Figure 3a). Similarly, 5.1 ± 3.0% (range 1.5–9.3%) of the CD8$^+$ T cells from psoriatic epidermis

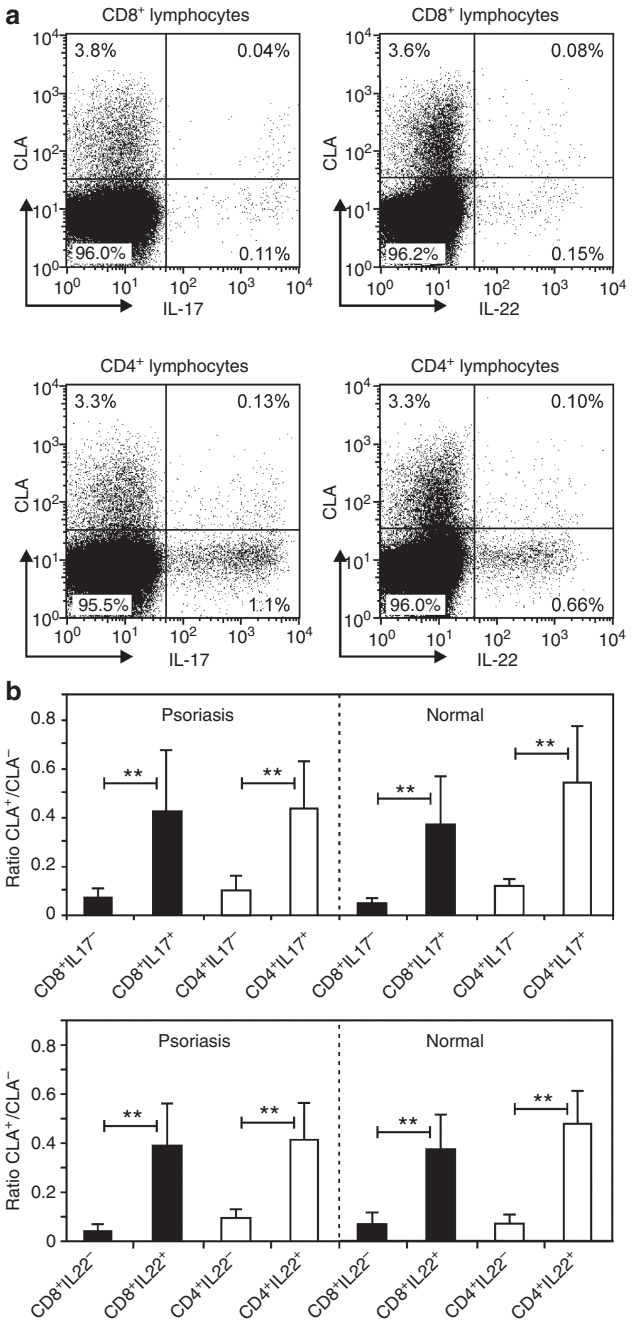


Figure 2. IL-17A$^+$ and IL-22$^+$ T cells in blood from psoriasis patients and healthy individuals show increased frequency of cutaneous-associated lymphocyte antigen (CLA) compared with IL-17A$^-$ and IL-22$^-$ T cells. Peripheral blood T cells were short-term stimulated, after which the CD8$^+$ and CD4$^+$ T cells were analyzed for IL-17A and IL-22 production and CLA expression by six-color flow cytometry. The populations of IL-17A- and IL-22-producing CD8$^+$ and CD4$^+$ T cells contain a considerable higher frequency of CLA-expressing cells as compared with the populations that lack production of these cytokines. (**a**) Analysis of skin-homing receptor CLA expression and IL-17A and IL-22 production in T cells of a representative psoriasis patient. Summary of the CLA$^+$/CLA$^-$ ratios in (**b**) IL-17A- and IL-22-producing CD8$^+$ and CD4$^+$ T cells (ratio of the percentage in the upper right quadrant versus percentage in bottom right quadrant) or nonproducing T cells (ratio of the percentage in the upper left quadrant versus percentage in bottom left quadrant) from five different psoriasis patients and five healthy donors. $^{**}P<0.01$.

appeared to be MAIT cells (Figure 3a). We were able to expand the MAIT cells along with the total T-cell population by nonspecific polyclonal stimulation (Figure 3a). The presence of MAIT cells in skin is not unique to psoriatic skin, as part of the CD8$^+$ T cells from healthy dermis (4.6 ± 4.0%, range 0.4–10.5%) and epidermis (11.6 ± 11.0%, range 2.2–19.6%) also expressed the MAIT cell phenotype CD161^{hi}TCR-Vα7.2$^+$IL-18Rα$^+$ (Figure 3b). The percentages of MAIT cells in psoriatic and healthy skin were not significantly different. Of note, the skin T cells analyzed in this study were obtained by spontaneous migration from skin explants, but the composition of the skin cell suspension might vary to some extent when applying other processing methods (e.g., enzyme digestion of skin tissue). Detection of the invariant TCR Vα7.2–JVα33 transcripts in the bulk T-cell cultures provided further proof for the presence of MAIT cells in psoriatic skin (Figure 3c). CD8$^+$ MAIT cells derived from psoriatic plaque skin were capable of effluxing the dye rhodamine, showing their functional expression of the multidrug transporter ABCB1 (Figure 3d).

As the expression of skin-tropic chemokine receptors is essential to enable skin accumulation by cells from the peripheral blood, we determined the expression of several skin-homing receptors on peripheral blood CD8$^+$ MAIT cells in comparison with conventional CD8$^+$ T cells. As shown in Figure 3e, we found that the MAIT cell population contained a slightly, but not significantly, higher frequency of CLA$^+$ T cells than the CD8$^+$ T-cell population (a mean of 6.6 ± 2.6% versus 4.7 ± 3.4%). Remarkably, a significantly higher portion of MAIT cells displayed the expression of CCR6 (28.8 ± 7.9% versus 2.4 ± 1.3%), whereas the expression of CCR7 was virtually absent (0.3 ± 0.1% versus 25.3 ± 16.6%). Many MAIT cells expressed CD49a (25.4 ± 24.4%) and to a lesser extent CCR10 (5.5 ± 5.3%) and CD103 (4.3 ± 1.1%), but these frequencies were very similar for conventional CD8$^+$ T cells (21.7 ± 12.5%; 5.6 ± 6.0% and 4.0 ± 1.6%). In sum, these data indicate that MAIT cells are common residents in human skin and may originate from blood CD8$^+$ MAIT cell populations, which express receptors related to skin homing.

IL-17A-producing CD8$^+$ T cells in psoriatic lesions comprises MAIT cells and conventional T cells

Next we examined whether the IL-17A-producing CD8$^+$ T cells from psoriatic lesions are part of the MAIT cell lineage or represent conventional T cells. Dependent on the number of cells that had migrated out of skin fragments, cytokine production was either directly assayed or cells were expanded one cycle to yield sufficient cells for cytokine expression analysis. The T cells were either directly stimulated (Figure 4a) or first sorted into phenotypic distinct subsets before stimulation (Figure 4b). With both approaches, we observed IL-17A expression in TCR-Vα7.2$^+$CD161$^+$ MAIT cells, as well as in conventional T-cell subsets within the population of psoriatic plaque–derived CD8$^+$ lymphocytes. Within both TCR-Vα7.2$^+$ and TCR-Vα7.2$^-$ T-cell populations, the highest percentages of IL-17A producers were consistently found in CD161$^+$ T cells. The MAIT cells contained a relatively lower percentage of IL-22 producers than other CD8$^+$ T-cell subsets. In summary, the IL-17A-producing CD8$^+$ T-cell population in psoriatic lesions comprises MAIT cells and conventional T cells, whereas IL-17A-producing CD8$^+$ T cells are almost absent in normal skin (data not shown and Res *et al.*, 2010).

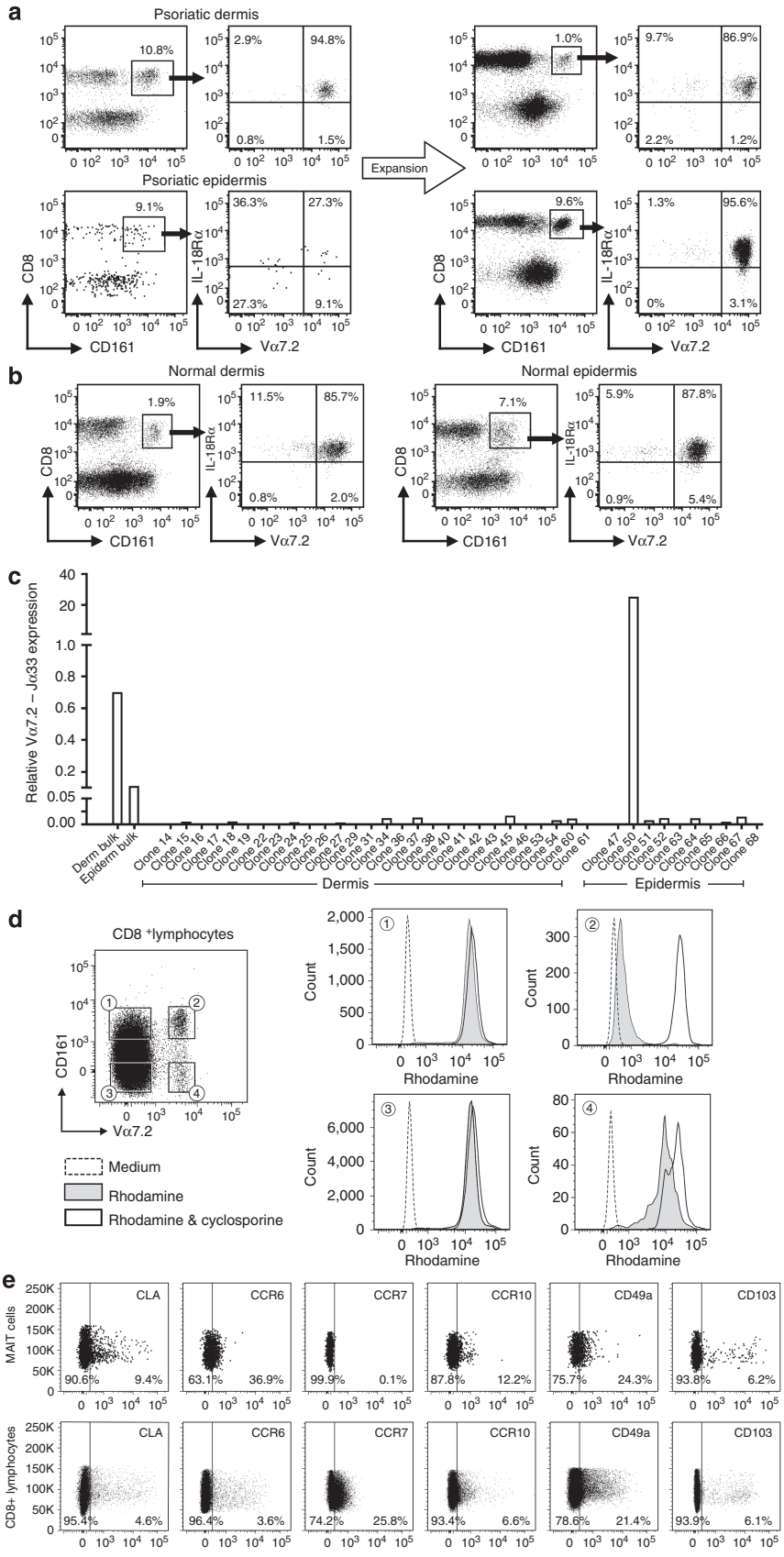
Tc17 cells within peripheral blood encompass almost all MAIT cells (Dusseaux *et al.*, 2011), which is clearly different from lesional psoriatic skin where Tc17 cells are more evenly distributed among MAIT cells and conventional CD8$^+$ T cells (Figure 4a and b).

MAIT cell and conventional Tc17 clones from psoriatic lesional skin

To find further support for our observation that Tc17 cells from lesional psoriatic skin comprise both MAIT cells and conventional CD8$^+$ T cells, we generated T-cell clones from CD8$^+$ T cells expressing CD161, which has been put forward as a marker that identifies most closely those T cells with IL-17A production (Maggi *et al.*, 2010). We generated from one psoriasis patient 28 dermal and 10 epidermal TCRαβ CD8$^+$ T-cell clones, of which 25 dermal and 7 epidermal T-cell clones produced IL-17A and IL-22 and in many cases also IFN-γ (data not shown). Only few T-cell clones of this panel produced IFN-γ in the absence of IL-17A and IL-22, indicating that the CD8$^+$CD161$^+$ T cells were indeed enriched for Tc17 cells. Only one Tc17 cell clone displayed a MAIT cell phenotype, whereas all other Tc17 clones represented conventional CD8$^+$ T cells. This MAIT cell clone was the only clone that expressed TCR Vα7.2-Jα33 mRNA (Figure 3c) and was recognized by the TCRVα7.2 antibody (Figure 5). In comparison with a second MAIT cell clone generated from blood of another psoriasis patient, both MAIT cell clones displayed IL-17 expression and the ability to efflux rhodamine dye (Figure 5), but showed differential expression of IL-22 and IFN-γ. Our analysis at the clonal level also clearly demonstrates the coexistence of MAIT cells and conventional Tc17 cells in psoriatic lesional skin.

DISCUSSION

In this study, we provide support for the involvement of IL-17A-producing CD8$^+$ T cells in the pathogenesis of psoriasis. First, we demonstrated that the Tc17 cell frequency in blood of psoriasis patients significantly correlates with the clinical severity. Remarkably, and perhaps unexpectedly, there was no correlation between clinical severity and the percentage of Th17 cells, which are generally believed to have a major role in psoriasis (Elloso *et al.*, 2012). Second, based on the expression of CLA, we show that the population of Tc17 cells in blood has a relatively increased tropism for skin, in particular those cells with a high level of IL-17A production. Our observations fit with earlier reports showing correlation between the frequency of blood CLA$^+$CD8$^+$ T cells and severity of psoriasis (Sigmundsdottir *et al.*, 2001) and showing overrepresentation of Tc17 cells in lesional psoriatic skin (Res *et al.*, 2010). CD8$^+$ MAIT cells also express CLA and several other receptors that have been implicated in the migration of CD8$^+$ T cells into the skin (Conrad *et al.*, 2007; Gunther *et al.*, 2012), such as CD49a, CCR6, CCR10, CD103 (present


a
Psoriatic dermis
10.8%
2.9%
94.8%
0.8%
1.5%
Expansion
1.0%
9.7%
86.9%
2.2%
1.2%
Psoriatic epidermis
9.1%
36.3%
27.3%
27.3%
9.1%
9.6%
1.3%
95.6%
0%
3.1%
CD8
CD161
IL-18Rα
Vα7.2
b
Normal dermis
1.9%
11.5%
85.7%
0.8%
2.0%
Normal epidermis
7.1%
5.9%
87.8%
0.9%
5.4%
c
Relative Vα7.2 – Jα33 expression
Derm bulk
Epiderm bulk
Dermis
Epidermis
d
CD8 +lymphocytes
CD161
Vα7.2
Medium
Rhodamine
Rhodamine & cyclosporine
Count
Rhodamine
e
MAIT cells
CD8+ lymphocytes
CLA
CCR6
CCR7
CCR10
CD49a
CD103
90.6%
9.4%
63.1%
36.9%
99.9%
0.1%
87.8%
12.2%
75.7%
24.3%
93.8%
6.2%
95.4%
4.6%
96.4%
3.6%
74.2%
25.8%
93.4%
6.6%
78.6%
21.4%
93.9%
6.1%

study), and CXCR6 (Dusseaux *et al.*, 2011), indicative of the potential of $CD8^+$ MAIT cells to accumulate in the skin. Third, although we describe MAIT cells as common residents in normal skin, IL-17A-producing $CD8^+$ MAIT cells were found predominantly in psoriatic lesional skin. Finally, we show that, in contrast to the situation in blood, IL-17A-producing $CD8^+$ T cells from lesional skin include a large portion of conventional $CD8^+$ T cells, which may represent disease-related $CD8^+$ T cells. In addition, our results confirm our earlier observation that psoriatic plaque epidermis contains a higher proportion of IL-17A-producing $CD8^+$ T cells than plaque dermis, whereas normal skin from healthy donors is almost devoid of Tc17 cells (Res *et al.*, 2010).

The increased numbers of Tc17 cells in psoriatic plaques may be explained by the selective advantage these cells have to enter the skin. On the other hand, part of the Tc17 cells in the lesional infiltrate may have expanded upon local activation, after recognition of (auto)antigens in the context of MHC class I, with HLA-Cw*0602 as the most likely restriction element in a large part of the psoriasis patients. In this view, the Tc17 cell response within the lesion would represent an adaptive immune response by conventional $CD8^+$ T cells—rather than an innate immune response of $CD8^+$ MAIT cells, which recognize antigens presented by MR1. This proposition would be in concordance with our finding that many IL-17A-producing $CD8^+$ T cells present in psoriatic plaques do not express the invariant TCR Vα7.2-Jα33 chain that marks MAIT cells. Although blood Tc17 cells almost all belong to the MAIT cell lineage (Dusseaux *et al.*, 2011), we observed that psoriatic lesions contained considerable proportions of conventional Tc17 cells. Further, all IL-17A-producing $TCR\alpha\beta^+$ T-cell clones turned out to be conventional Tc17 cells, except for one MAIT cell clone. However, it cannot be ruled out that this low yield of MAIT cell clones just reflects possible difference in the potential to expand $CD8^+$ MAIT cells and conventional $CD8^+$ T cells at the single-cell level.

Patients with hepatitis C infection have Tc17 cells that recognize self-antigens from apoptotic cells presented by HLA molecules (Franceschini *et al.*, 2012), clearly showing that IL-17A production is present in conventional $TCR\alpha\beta^+CD8^+$ T cells. If autoreactive HLA-restricted Tc17 cells exist in psoriasis, they are presumably present predominantly in the inflamed sites. We have shown that IL-17A-producing MAIT cells coexist with conventional Tc17 cells. Although MAIT cells appear to recognize only bacterial and yeast antigens in the context of MR1, it cannot be excluded that some MAIT cells also respond to peptides presented by HLA peptides and thus behave as conventional T cells. Indeed, Havenith *et al.* (2012) have shown that tetramers, consisting of influenza virus or cytomegalovirus-derived peptides coupled to HLA-A2 molecules, bind to a minor fraction of $CD8^+CD161^{hi}IL\text{-}18R\alpha^+$ T cells, which could be expanded through stimulation with antigen-presenting cells and relevant peptides. However, as Vα7.2 expression on the Tet^+ cells was not determined in these experiments, the relationship of the antiviral $CD161^{hi}$ $IL\text{-}18R\alpha^+$ CD8 T cells with MAIT cells is not clear. The presence of MAIT cells in psoriatic lesions could be instrumental in the disease or a nonspecific migration to an inflamed tissue. Study of other skin diseases with similar inflammation would help decipher this issue.

Our observation that the blood Tc17 cell frequency is correlated with disease activity implicates that Tc17 cells are relatively more abundant in blood of patients with severe psoriasis. The cause of this increase in blood Tc17 cells is unknown. On one hand, severe psoriasis may be accompanied by excessive local proliferation of Tc17 cells, which subsequently leave the skin through the lymphatic system and finally end up in the blood circulation. However, according to a recently proposed concept, a population of long-lived nonrecirculating $CD8^+$ T cells with self-renewal capacity would reside in the skin, providing long-term protective immunity (Gebhardt *et al.*, 2009; Gebhardt *et al.*, 2011; Jiang *et al.*, 2012). At present, it remains unclear whether this skin-resident $CD8^+$ T-cell population comprises Tc17 cells and/or $CD8^+$ MAIT cells. On the other hand, many blood Tc17 cells may have originated from lymphoid organs, in particular the tonsils, as throat infection with *Streptococci* often precedes or exacerbates psoriasis (Sigurdardottir *et al.*, 2013). Tc17 cells expanding in response to streptococcal antigens in the inflamed tonsils represent perhaps the principal source of the blood Tc17 cells observed in severe psoriasis. Consistent with this, it has been shown that there is a correlation in psoriasis patients between the percentages of CLA^+CD8^+ T cells in blood and tonsils, which produce IL-17A upon stimulation with streptococcal antigens (Thorleifsdottir *et al.*, 2012). Interestingly, psoriasis patient blood–derived CLA^+ T cells, but not CLA^- T cells, when cocultured with autologous epidermal cells, showed IL-17A and IL-22 production, only if streptococcal extract was added to the culture (Ferran *et al.*, 2013). Furthermore, stimulation of

Figure 3. The presence of mucosa-associated invariant T (MAIT) cells in psoriatic lesional skin and healthy skin. Migratory cells from dermal and epidermal tissue from psoriasis patients or normal individuals were collected after culture, stimulated, and analyzed by multiparameter flow cytometry. (**a**) A clear population of MAIT cells, identified as TCR $V\alpha7.2^+IL\text{-}18R^+$ cells within the $CD8^+CD161^+$ fraction of lymphocytes, is present in both dermal and epidermal cell suspensions (panels left) and could be maintained and propagated *in vitro* (panels right). Shown data are from one psoriasis patient and representative of five independent experiments. (**b**) MAIT cells are present in the dermal and epidermal cell suspensions from one normal individual, being representative of three healthy donors. (**c**) Detection of MAIT cell-marker Vα7.2-Jα33 mRNA in bulk T-cell populations and $CD8^+$ T-cell clones derived from the dermis and epidermis of one psoriasis patient. Data are depicted as arbitrary units relative to the expression of housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (**d**) Capacity of psoriatic plaque–derived $CD8^+$ epidermal MAIT cells (TCR-$V\alpha7.2^+CD161^+$) and other indicated $CD8^+$ T-cell subsets to efflux rhodamine by the multidrug transporter ABCB1. Expression of a functional transporter can be appreciated as a drop of the rhodamine signal in preloaded cells toward the unloaded medium control as compared with preloaded cells incubated in the presence of cyclosporine A, which blocks the multidrug transporter. (**e**) Expression of skin tropism–related molecules by peripheral blood $CD8^+$ MAIT cells and conventional $CD8^+$ T cells. Data are representative of seven independent experiments with different donors.

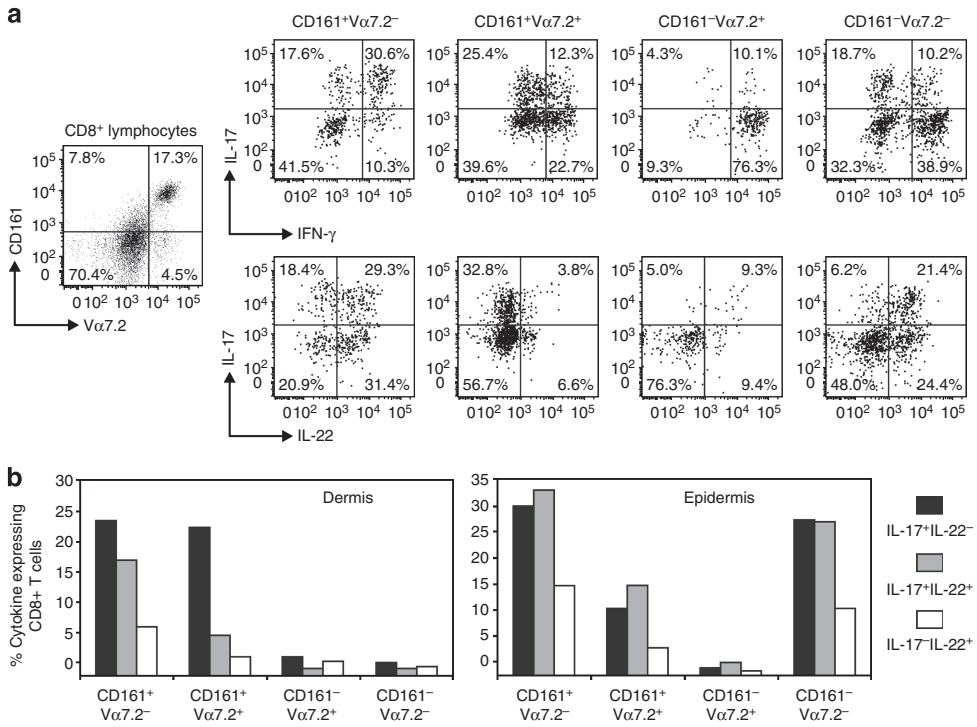


Figure 4. IL-17A production in psoriatic lesional skin by CD8$^+$ mucosa-associated invariant T (MAIT) cells and CD8$^+$ conventional T cells. Cells derived from psoriatic skin were polyclonally expanded and either (**a**) first stimulated with phorbol 12-myristate 13-acetate (PMA) plus ionomycin and then analyzed for cell membrane phenotype and cytokine expression or (**b**) first sorted on the basis of the indicated cell surface phenotypes and then stimulated with PMA plus ionomycin followed by analysis of cytokine expression. IL-17A production was observed in both CD8$^+$ MAIT cell (TCR-Vα7.2$^+$CD161$^+$) and conventional CD8 T-cell populations (TCR-Vα7.2$^+$CD161$^-$, TCR-Vα7.2$^-$CD161$^+$, and TCR-Vα7.2$^-$CD161$^-$) in lesional epidermis (**a** and **b**)- and dermis (**b**)-derived T-cell lines. Data in **a** and in **b** show one representative experiment out of four independent experiments with different donors.

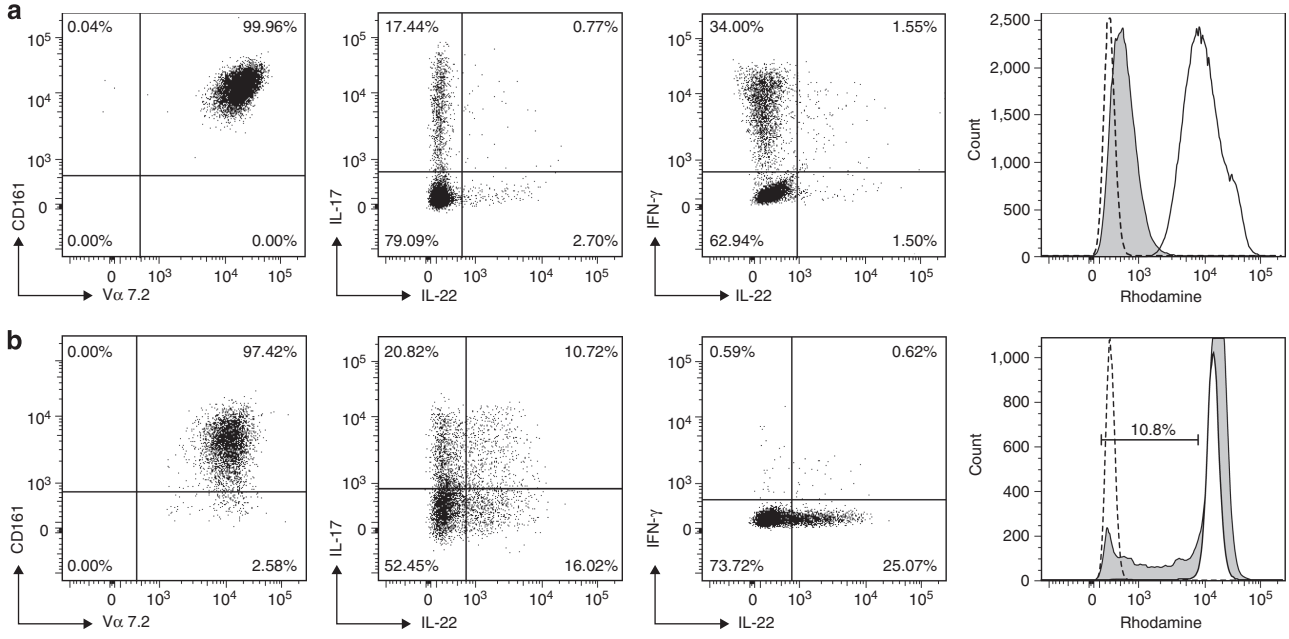


Figure 5. Cytokine production profiles of CD8$^+$ mucosa-associated invariant T (MAIT) cell clones derived from blood and lesional psoriatic skin. MAIT cell clones derived from (**a**) peripheral blood from one psoriasis patient and (**b**) from psoriatic plaque epidermis of another psoriasis patient. Both MAIT cell clones express IL-17, whereas they display differential IL-22 and IFN-γ expression. One clone clearly showed rhodamine efflux activity, whereas the other clone showed only partial efflux activity, which may represent loss of function in some cells owing to long-term *in vitro* expansion, including several cycles of reactivation with phytohemagglutinin (PHA), cytokines, and feeder cells. For explanation of the lines in the rhodamine graphs, see Figure 3d.

blood mononuclear cells with streptococcal antigens also induces the expression of CLA (Leung *et al.*, 1995), thereby facilitating streptococci-specific T cells to enter the skin. Preactivated T cells have a lower threshold of reactivation, and therefore, once CLA$^+$ streptococci-primed Tc17 cells have entered the skin, they may be more prone to cross-react with local self-antigens, such as keratinocyte-derived keratins that have been shown to share homologous epitopes with streptococcal antigens (Sigmundsdottir *et al.*, 1997; Gudmundsdottir *et al.*, 1999; Johnston *et al.*, 2004; Shen *et al.*, 2005).

IL-17A is a key cytokine in psoriasis, as therapy by biologically targeting IL-17A or its receptor, IL-17RA, has a significant clinical effect (Hueber *et al.*, 2010; Leonardi *et al.*, 2012; Papp *et al.*, 2012). Hence, it is important to know all potential IL-17A sources in psoriatic skin as they might be involved in the pathological mechanism of the disease. Here, we identify that MAIT cells in psoriatic skin represent an additional source of IL-17A. MAIT cells belong to the group of innate T cells that, upon activation, are able to rapidly secrete effector molecules, including IL-17A, but also IFN-γ and tumor necrosis factor-α (Dusseaux *et al.*, 2011), leading to strong inflammatory responses. MAIT cells respond to compounds of a large variety of microorganisms that are presented by the MR1 molecule, which is ubiquitously expressed—among others by dendritic cells (DCs) and epithelial cells, such as keratinocytes. DCs and keratinocytes in psoriatic skin show increased levels of IL-23 (Lee *et al.*, 2004; Piskin *et al.*, 2006), which promotes IL-17A production. In addition, MAIT cell activation is modulated by CD161 triggering via its ligand LLT1, which is present on TLR-activated DCs (Germain *et al.*, 2011), and increased numbers of activated CD11c$^+$ DCs are present in psoriasis lesions (Zaba *et al.*, 2009; Teunissen *et al.*, 2012). Although elements (such as MR1, IL-23, LLT1) required to stimulate MAIT cells are locally present, it is currently unclear whether any MAIT cells become activated within psoriatic skin and whether microbial agent(s) are involved in their activation. An interesting candidate would be streptococcal antigens, as outlined above; however, it has been reported that MAIT cells do not respond to *Streptococci* (Le Bourhis *et al.*, 2010). As there is no clear candidate microbial antigen yet, it is difficult to indicate whether MAIT cells become activated and are involved in the pathogenesis of psoriasis. Their activation may also just be a consequence of the ongoing inflammation (for example, MAIT cells can be activated by a cytokine combination of IL-12 plus IL-18 in a TCR-independent manner, (Ussher *et al.*, 2014)). Irrespective of the answer, by means of secretion of large quantities of effector molecules, MAIT cells may contribute to the perpetuation of the inflammation.

In conclusion, evidence for a central role of CD8$^+$ T cells in psoriasis is accumulating and nicely fits with the fact that *HLA-Cw*0602* is the strongest risk allele for developing this inflammatory skin disease. Here we provide additional evidence that implicates IL-17A-producing CD8$^+$ T cells in the pathogenesis of psoriasis. Remarkably, although normal skin is almost devoid of these T cells, psoriatic lesions (in particular in the epidermis) contains large proportions of Tc17 cells consisting of innate CD8$^+$ MAIT cells and conventional CD8$^+$ T cells. Combined analysis of the antigen recognition, HLA restriction, and TCR usage of MAIT cells and conventional Tc17 cells derived from skin and peripheral blood may help unravel the respective involvement of these two cell types in the disease. Whether and how both populations of Tc17 cells become activated inside plaque skin to release their pathogenic cytokine content remains the topic of future investigations.

MATERIALS AND METHODS

Patients

Adult patients (10 female/19 male, mean age 46.5 years; range 20–69) with active psoriasis vulgaris lesions who visited the Department of Dermatology at the Academic Medical Center in Amsterdam and fulfilled the inclusion criteria were asked to participate in this study. The study was reviewed and approved by the institutional Medical Ethical Committee and was conducted according to the Declaration of Helsinki principles. All patients gave their written informed consent before enrollment. All patients refrained from any systemic therapy or phototherapy for at least 4 weeks before participation, and topical treatments for psoriasis were not allowed 14 days before baseline. After determination of their PASI (mean 13.1; range 2.3–25.2), the patients donated 4-mm punch biopsies from an active plaque, and blood was sampled via venipuncture. Normal adult skin and blood samples of the same donor were obtained from healthy subjects undergoing plastic surgery of the breast or abdomen after informed consent.

Isolation of cells from peripheral blood and skin

Blood mononuclear cells were isolated with lymphocyte separation medium LSM 1077 (PAA Laboratories, Pasching, Austria). Skin samples were incubated overnight at 4 °C in phosphate-buffered saline with 0.3% dispase II (Roche Diagnostics, Almere, the Netherlands). Next, the dermis and epidermis of the biopsies were separated and cultured for 6–7 days in Iscove's modified dulbecco's media (PAA Laboratories) with 10% normal human AB serum (NHS; Lonza, Breda, the Netherlands) to allow spontaneous migration of cells from the tissue fragments. Dermal and epidermal tissues from normal skin were cultured in Iscove's modified dulbecco's media with 10% fetal calf serum (Gibco, Life Technologies, Bleiswijk, the Netherlands).

Generation of T-cell lines and clones

T-cell lines and clones were generated by stimulation with PHA, IL-2, and feeder-cell mix, as described before (Res *et al.*, 2010).

Flow cytometry analysis

The following antibodies were used: FITC-conjugated anti-CLA, APC-conjugated anti-CD8, APC-conjugated anti-CD161, FITC-conjugated anti-CD45RO, PE-conjugated anti-CCR7 (all from BD Biosciences, Mountain View, CA); and PE-conjugated anti-IL-18Rα, PerCP-conjugated anti-CD3, APC-Cy7 or PE-Cy7-conjugated anti-CD4, FITC or APC-Cy7-conjugated anti-CD8, PE-conjugated anti-CD49a, PE-conjugated anti-CD103 (all from BioLegend, San Diego, CA), PE-conjugated anti-CCR6 and PE-conjugated anti-CCR10 (R&D Systems, Abingdon, UK), biotin-conjugated anti-TCR Vα7.2 chain (clone 3C10) (Martin *et al.*, 2009) using PE-Cy7-conjugated streptavidin (BioLegend) as second step.

Cytokine production was induced in T cells by stimulation for 5 hours with 100 ng ml^{-1} PMA (Sigma-Aldrich, St Louis, MO) and 1 μg ml^{-1} ionomycin (Sigma-Aldrich), and for the last 4 hours in the presence of GolgiPlug (BD Biosciences). After fixation with 4% formaldehyde in phosphate-buffered saline for 10 minutes, cells were stained for cell surface markers and intracellular cytokines, using PE, PerCP5.5, or Alexa Fluor 647-conjugated anti-IL-17A (eBioscience, San Diego, CA), PE-conjugated anti-IL-22 (R&D Systems Europe, Abingdon, UK), and FITC-conjugated anti-IFN-γ (BD Biosciences) for 30 minutes (Res *et al.*, 2010). Acquisition was performed with a FACS Aria and analysis with the FlowJo software (TreeStar, Ashland, OR).

Rhodamine efflux assay

Cells were loaded in RPMI 1640 (PAA Laboratories) with 10% fetal calf serum, containing 0.1 μg ml^{-1} rhodamine (Rh123; Sigma-Aldrich), in the presence or absence of 3 μg ml^{-1} cyclosporine A (Sigma-Aldrich), for 30 minutes at 37 °C. After extensive washing, efflux of rhodamine was allowed for 30 minutes at 37 °C in the presence or absence of cyclosporine A and analyzed by flow cytometry.

Real-time quantitative PCR

PCR was performed as described before (Yeremenko *et al.*, 2013). In short, total RNA was extracted with a MiniPrep RNA kit (Sigma-Aldrich) and reverse-transcribed using the RevertAid H Minus First-Strand cDNA synthesis kit (Fermentas, Fisher Scientific, Landsmeer, The Netherlands). Specific PCR primers and TagMan probe for human TCR Vα7.2–JVα33 were designed by Custom TaqMan Assay Design Tool (Applied Biosystems, Life Technologies Europe, Bleiswijk, The Netherlands): sense primer, 5′-TTCCTTAGTCGGTCTAAAGGGT ACA-3′, antisense primer, 5′-ACAGAGGTAAGAGGCAGAGTCTTTC A-3′ probe, 5′-CCTTTTGAAGGAGCTCC-3′. PCR was performed using glyceraldehyde-3-phosphate dehydrogenase (4310884E, Applied Biosystems) in duplex assay format (duplex Vα7.2–JVα33-FAM and GAPDH-VIC probes) according to the manufacturer's protocol (Applied Biosystems). All samples were normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase and results are presented in arbitrary units.

Statistical analysis

Statistical analysis was done with GraphPad Prism (GraphPad Software, La Jolla, CA) using the paired Student's *t*-test and Spearman correlation test, taking $P<0.05$ as significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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