Journal of Dermatological Science 95 (2019) 21–27

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Original Article

Significance of IL-17A-producing $CD8^+CD103^+$ skin resident memory T cells in psoriasis lesion and their possible relationship to clinical course

Kazuo Kurihara, Toshiharu Fujiyama, Pawit Phadungsaksawasdi, Taisuke Ito, Yoshiki Tokura*

Department of Dermatology, Hamamatsu University School of Medicine, Japan

ARTICLE INFO

Article history:
Received 4 March 2019
Received in revised form 9 June 2019
Accepted 10 June 2019

Keywords:
CD103
Psoriasis
Resident memory T cell
Clinical course

ABSTRACT

Background: A number of studies have shown the relationship between the pathogenesis of psoriasis and skin resident memory T (T_{RM}) cells.
Objective: To investigate the cytokine profile of T_{RM} cells from skin lesions of psoriasis and the relationship of skin T_{RM} cells to the future clinical course of psoriasis.
Methods: We used stocked samples of T cells that were *ex vivo* expanded from skin biopsies of 10 patients with psoriasis vulgaris. A half of 4-mm punch biopsy specimens was subjected to expansion of skin-infiltrating T cells using IL-2 and anti-CD3/CD28 antibody-coated microbeads. More than 10^6 T cells per specimen were stocked at −80 °C. Defrosted cells were subjected to flow cytometric analysis. Another half of skin biopsies were subjected to immmunofluorescence staining for CD103 and other markers.
Results: The biopsied skin revealed $CD8^+CD103^+$ T_{RM} cells were present in the epidermis of psoriasis and associated with acanthosis. Sorted $CD103^+$ T cells were mostly $CD8^+$ memory T cells expressing CD69 with a skin-homing potential. A part of $CD8^+CD103^+$ T cells produced interferon-γ, IL-17A or IL-22. Notably, $CD8^+CD103^+$ T_{RM} cells more frequently produced IL-17A than did $CD8^+CD103^-$ T cells. We retrospectively divided the 10 cases into the non-advanced therapy group, and the advanced therapy group in which systemic biologics or others were initiated within one year. The frequency of $CD8^+CD103^+IL\text{-}17A^+$ T_{RM} cells tended to be higher in the advanced therapy group.
Conclusion: These results suggest that IL-17A-producing $CD8^+CD103^+$ T_{RM} cells are associated with a progressive clinical course of psoriasis.

1. Introduction

Psoriasis is one of the most common inflammatory skin diseases. Interleukin (IL)-17-producing $CD4^+$ T (Th17) cells are involved in its pathogenesis [1]. Th17 cells and their cytokines, IL-17A, IL-17F and IL-22, play an essential role for epidermal acanthosis, and they stimulate keratinocytes to produce IL-8, tumor necrosis factor (TNF)-α, CXCL10, and vascular endothelial growth factor, thereby inducing inflammation, neutrophil accumulation, and angiogenesis [2]. For maintenance of Th17 cells, IL-23 is required and representatively released from TNF-α-producing inflammatory dendritic cells (DCs) [2]. Antimicrobial peptides and plasmacytoid DCs secreting interferon (IFN)-α are also produced by keratinocytes and considered to play an initiative role for the development of psoriatic lesions. The above cytokine network in psoriasis has been proven by the therapeutic effectiveness of cytokine-blocking biologics, including antibodies against TNF-α, IL-23/IL-12p40, anti-IL-23p19, IL-17A, and IL-17 receptor [3].

While biological therapies are highly effective, some patients poorly respond to them [4]. Withdrawal of these biologics often bring about a recurrence of the disease. Notably, psoriasis plaques frequently recur at the originally affected site of the skin [5], suggesting that even after disappearance of lesions, some immune cells possibly remain. Resident memory T (T_{RM}) cells as well as Th17 cells is one of the strong candidates that evoke recurrence. T_{RM} cells are non-recirculating tissue-resident memory T cells [6] and present in lung, intestine, liver, brain, skin and other mucosal surfaces [6,7]. Skin T_{RM} cells persist for a long term in the skin surface to protect the host from pathogens [8,9]. A number of studies have revealed that the relationship between

* Corresponding author at: Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hihashi-ku, Hamamatsu, 431-3192, Japan.
E-mail address: tokura@hama-med.ac.jp (Y. Tokura).

https://doi.org/10.1016/j.jdermsci.2019.06.002
0923-1811/

psoriasis and skin T_{RM} cells [10,11]. Skin T_{RM} cells express CD103 (integrin αE subunit) [11–13], and the heterodimeric molecule (αEβ7), composed of CD103 and integrin β7 subunit [14], is a ligand for E-cadherin, which is expressed by epidermal keratinocytes [15]. The integrin αEβ7 plays a central role in $CD8^+$ T_{RM} cell sustainability by upregulating Bcl-2 molecule with an anti-apoptotic activity [12]. T_{RM} cells are involved in various inflammatory skin diseases, including fixed drug eruption [16], vitiligo [13], and psoriasis [10,11]. Particularly, T_{RM} cells in psoriasis can cause recurrence of skin lesions in the same region [17]. Furthermore, T_{RM} cells in psoriatic skin can produce cytokines and are decreased in number after improvement [11]. It has recently been reported that $CD8^+$ T_{RM} cells are accumulated in disease-naïve non-lesional sites of psoriasis possibly in correlation with disease duration [18]. However, it is an issue whether skin T_{RM} cells are related to the future clinical course of psoriasis.

In the majority of previous studies on psoriasis, skin-infiltrating T cells have been examined by immunohistochemical staining and cytokine mRNA expression of skin specimens because of the technical availability. With these methodologies, however, one cannot evaluate the cytokine profile of individual T cell populations. To evaluate the cytokine production of T cell subsets, we used T cells that were *ex vivo* expanded from skin biopsies [19]. The expanded T cells reflect the original T cell populations, as the frequency of Th17 cells was minimally affected. By using skin-derived, *ex vivo* expanded T cells, this study was conducted to characterize the cytokine profile of $CD103^+$ skin T_{RM} cells, in particular, epidermis-derived $CD8^+CD103^+$ T_{RM} cells [11,12]. In addition, we investigated whether a certain subset of skin T_{RM} cells is related to the future clinical course of psoriasis.

2. Materials and methods

2.1. Patients

In our previous study, 10 patients with psoriasis vulgaris were registered [19]. They were clinically and histopathologically diagnosed. The patients were not given any systemic medication or phototherapy for psoriasis at the study. No topical drug had been administered to the biopsied skin lesion for at least 2 weeks. The list of the patients is shown in Supp. Table S1.

This study was conducted according to the Declaration of Helsinki. The study protocol was approved by the ethical committee of Hamamatsu University School of Medicine. We obtained written informed consent from all participants.

2.2. Cell preparations

Stored T cell samples obtained from the 10 psoriasis patients were used in this study. To prepare the samples, a half of 4-mm punch biopsy specimens from psoriatic plaques was subjected to expansion of skin-infiltrating T cells using IL-2 (R&D Systems, Minneapolis, MN) and anti-CD3/CD28 antibody-coated microbeads (Human T-Activator; Dynal, Copenhagen, Denmark) as previously described [19,20]. After 2-week expansion, more than 10^6 T cells per specimen were stored at −80 °C with CELLBANKER1 (Zenoaq, Kooriyama, Japan). The cells were defrosted and cultured in cRPMI supplemented with 50 U/mL human recombinant IL-2 and anti-CD3/CD28 antibody–conjugated microbeads in 6-well plates for 1 week. The culture medium was changed or added every day so that half of the total volume of medium was fresh. Another half of the punch biopsy was used for tissue immunofluorescence study.

2.3. Flow cytometric analysis and cytokine production assessments

Aliquots of 10^6 cells were washed once with FACS staining buffer, i.e. phosphate-buffered saline (PBS; pH7.4), containing 2% fetal bovine serum (FBS) and 0.03% sodium azide, with a panel of fluorescence-conjugated monoclonal antibodies (mAbs) for 30 min at 4 °C in the dark. The mAbs included PerCP-conjugated anti-CD8 (SK1; BD Biosciences, San Jose, CA), FITC-conjugated anti-CD45RO (UCHLA-1; BD), FITC-conjugated anti-CD45RA (L48; BD), PE-conjugated anti-CD49a (SR84; BD), APC-conjugated anti-CD69 (L78; BD), APC-conjugated anti-CD103 (Ber-ACT8; BD), PE-conjugated anti−CCR6 (R6H1; eBioscienes, Waltham, MA), PE-conjugated anti−CCR7 (150,503; R&D systems, Minneapolis, MN), FITC-conjugated anti-CXCR3 (49,801; R&D systems), FITC-conjugated anti-CD62L (DREG-56; BD), PE-conjugated anti-IFN-γ (B27; Biolegend, San Diego, CA), PE-conjugated anti-IL-17A (eBio64DEC17; eBioscienes), and PE-conjugated anti-IL-22 (2G12A41; Biolegend). After washing, the harvested cells were resuspended in FACS staining buffer and subjected to flow cytometric analysis. More than 1×10^6 cells per sample were analyzed on a FACSCanto II (BD Biosciences, San Jose, CA). Results were analyzed with FlowJo software (TreeStar, Ashland, OR). For the intracellular cytokine staining assay, about 2×10^6 cells/well in 24-well plates were incubated in cRPMI containing 10^{-8} mol/L phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich, St Louis, MO), 10^{-6} mol/L calcium ionophore (Sigma-Aldrich), and 1 μL/mL GolgiStop (BD) for 6 h at 37 °C. Cells were harvested and stained with fluorescence-tagged mAbs against cytokines by using the Cytofix/Cytoperm Plus Kit with GolgiStop (BD), according to the manufacturer's protocols, after staining of fluorescence-tagged antibodies against CD8 and CD103.

2.4. Isolation of t cell population

Aliquots of 10^6 cells were washed once with FACS staining buffer with a panel of fluorescence-conjugated mAbs for 30 min at 4 °C in the dark. After washing, the harvested cells were resuspended in FACS staining buffer and subjected to Moflo Astrios (Beckman Coulter, Brea, Calif) to isolate CD103-positive and -negative cells. The positive and negative cells were cultivated, respectively, for 10 days and were analyzed on a FACSCanto II.

2.5. Immunofluorescence histopathological studies

Sections of paraffin-embedded skin biopsy specimens were deparaffinized and were treated in an autoclave with 0.01 M sodium citrate buffer (pH 6.0). Slides were blocked with 1% FBS in 10 min, then stained with primary antibody (diluted 1:100 with PBS), including anti-CD3 (F7.2.38; DAKO, Les Ulis, France), anti-CD4 (4B12; LEICA, Wetzlar, Germany), anti-CD8 (C8/144B; DAKO/Agilent Pathology Solutions, Santa-Clara, CA), and anti-CD103 (EPR4166(2); Abcam, Cambrdige, UK) for 2 h, and second antibody (diluted 1:100 with 1%FBS), including Alexa Fluor 488 anti-rabbit IgG (Abcam), and Alexa Fluor 594 anti-mouse IgG (ThermoFischer Scientific Walham, MA) for 1 h. They were rinsed in PBS each time, then stained with DAPI for 1 min. The sections were scanned with a digital image scanner (Nanozoomer; Hamamatsu Photonics, Hamamatsu, Japan) and analyzed with its software.

2.6. Statistial analysis

All data were expressed as mean ± standard deviation of the mean. The Wilcoxon *t*-test, Friedman test, Mann-Whitney *U*-test, and Spearman's correlation were applied. Statistical analyses were performed using GraphPad Prism (Version 7; GraphPad Software, La Jolla, CA) and SPSS (Version 24; IBM, Armonk, NY). *P* values of less than 0.05 were considered statistically significant.

3. Result

3.1. $CD8^+CD103^+$ T_{RM} cells are present in the epidermis of psoriasis and associated with acanthosis

Skin T_{RM} cells bear CD103, a ligand for E-cadherin [7,12,13]. To investigate their CD4 or CD8 phenotype and their location in the skin, skin biopsy specimens from 10 psoriasis patients were subjected to double immunofluorescent staining for CD3, CD4, or CD8 (red) along with CD103 (green). Yellow color was yielded in the merged cells. Whole section images were captured as digital images to count the number of positive cells and to analyze localization of T_{RM} cells. As shown in a representative case, $CD3^+$ T cells infiltrated in both epidermis and dermis, and most of the cells in the epidermis co-expressed CD103 (Fig. 1a, upper left). $CD4^+$ cells mainly infiltrated in the dermis and did not express CD103 (Fig. 1a, upper right). $CD8^+$ cells infiltrating in the epidermis were positive for CD103, while those in the dermis were mostly $CD103^-$ (Fig. 1a, lower panel). All 10 cases showed virtually the same results (Fig. 1b), as most of T cells in the epidermis were $CD8^+CD103^+$ T_{RM} cells and a low number of $CD4^+CD103^+$ T_{RM} cells co-infiltrated. T cells in the dermis were negative for CD103, and a small number of $CD8^+CD103^+$ T_{RM} cells were present in the papillary and subpapillary layers. The number of $CD8^+CD103^+$ T_{RM} cells in the epidermis correlated with the thickness of the epidermis ($P = 0.016$) (Fig. 1c), suggesting their role for the formation of psoriatic lesion.

3.2. Characterization of T_{RM} phenotype and culture-independent expression of CD103

We previously established a method for expansion of skin-infiltrating T cells, which reflects the original Th1/Th2/Th17 balance and allows us to evaluate intracellular cytokines by flow cytometry [19,20]. We used the same method to investigate the phenotype and cytokine profile of T_{RM} cells. It has been reported that skin T_{RM} cells are positive for CD103 and CD69, but negative for CD62L and CCR7 [7]. We first examined the changes in the expression of CD103 and CD69 during cultivation of T_{RM} cells. We isolated $CD103^+$, $CD103^-$, $CD69^+$, and $CD69^-$ T cells (Fig. 2a) and expanded each population with anti-CD3/CD28 Ab-conjugated microbeads and IL-2 for 10 days. In $CD103^+$ and $CD103^-$ T cells, the positive and negative expression of CD103 was unchanged (Fig. 2b, upper panel). While a part of the isolated $CD69^-$ T cells were converted to $CD69^+$ T cells during culture, the isolated $CD69^+$ T cells were altered to $CD69^-$ T cells (Fig. 2b, lower panel), suggesting that CD69 expression can be changed bidirectionally by cultivation. When isolated $CD103^+CD69^+$ cells were monitored, some of $CD69^+$ T cells were converted to $CD69^-$ T cells, while they kept CD103 expression (Suppl. Fig. S1b). Meanwhile, some of $CD69^-$ T cells were converted to $CD69^+$ T cells in the isolated $CD69^-$ $CD103^+$ T cells (Suppl. Fig. S1d). Thus, CD69 expression were changeable during cultivation. We therefore did not use CD69 as T_{RM} marker in this study.

In the stored T cell samples expanded from psoriasis lesional skin, a part of $CD8^+$ T cells expressed CD103 (Fig. 3a), while much lower frequencies of $CD4^+$ T cells bore CD103 (Fig. 3b). $CD103^+$ T cells sorted from the stored samples were mostly $CD8^+$ (Fig. 3c) memory T cells ($CD45RO^+CD45RA^-$; Fig. 3d, e) expressing CD69 (Fig. 3f) with a skin-homing potential (partially $CCR6^+$ and mostly $CCR7^-$ $CD62L^-$; Fig. 3g–i). They contained approximately 28% of $CXCR3^+$ or $CD49a^+$ cells.

Because of the unchange of CD103 positivity/negativity during cultivation, we used $CD103^+$ T cells as T_{RM} cells in the following studies.

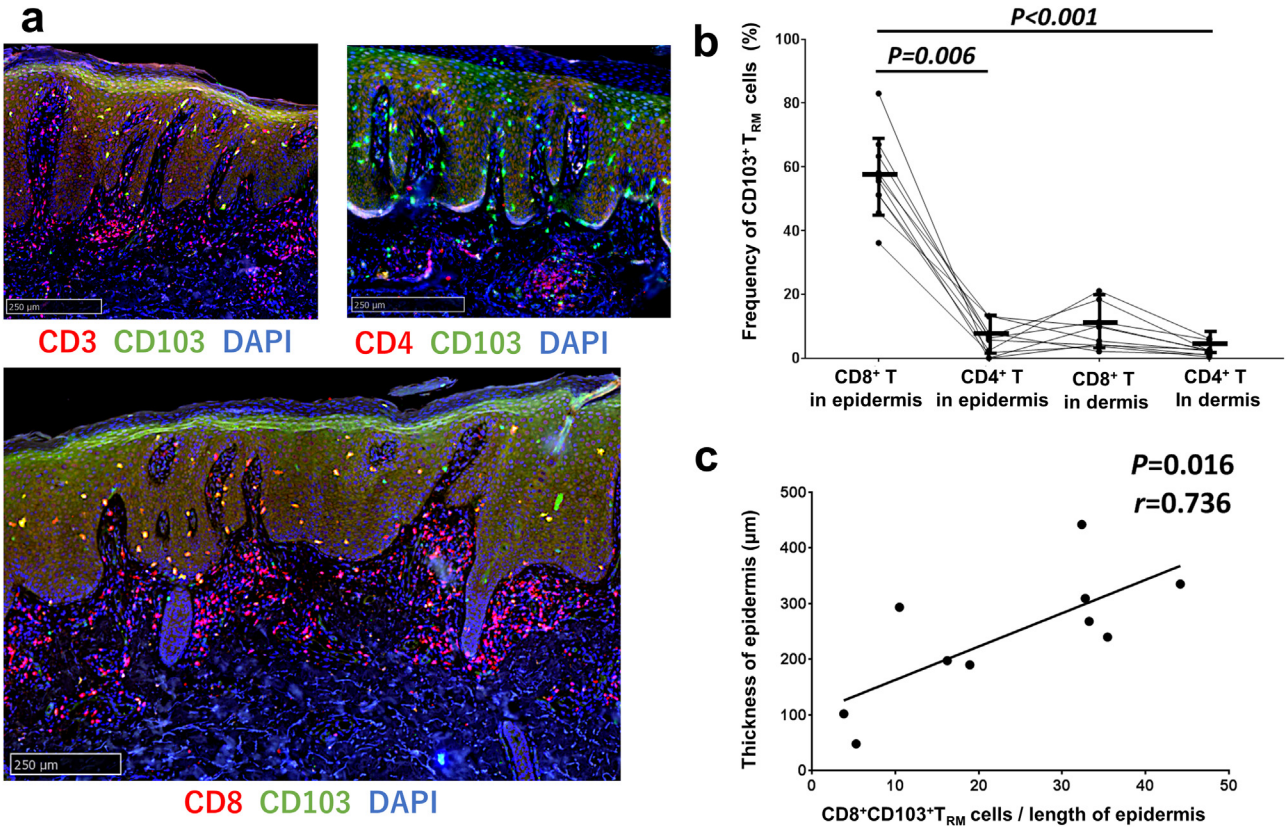

Fig. 1. Fluorescence immunostaining of T_{RM} cells in psoriasis lesions. (a) Double immunofluorescent staining for CD3, CD4 or CD8 (red), and CD103 (green). The merged cells exhibit yellow. (b) Frequency of $CD103^+$ cells in $CD8^+$ or $CD4^+$ T cells infiltrating in the epidermis or dermis. *P* values were calculated using the Friedman test (n = 10). (c) Correlation of the number of $CD8^+CD103^+$ T_{RM} cells per 1-μm horizontal length of the epidermis and the thickness of the epidermis (μm). The epidermal thickness was calculated by dividing the area of the epidermis by the length of the epidermis with a digital image scanner. *P* value was calculated by the Spearman's correlation (n = 10).

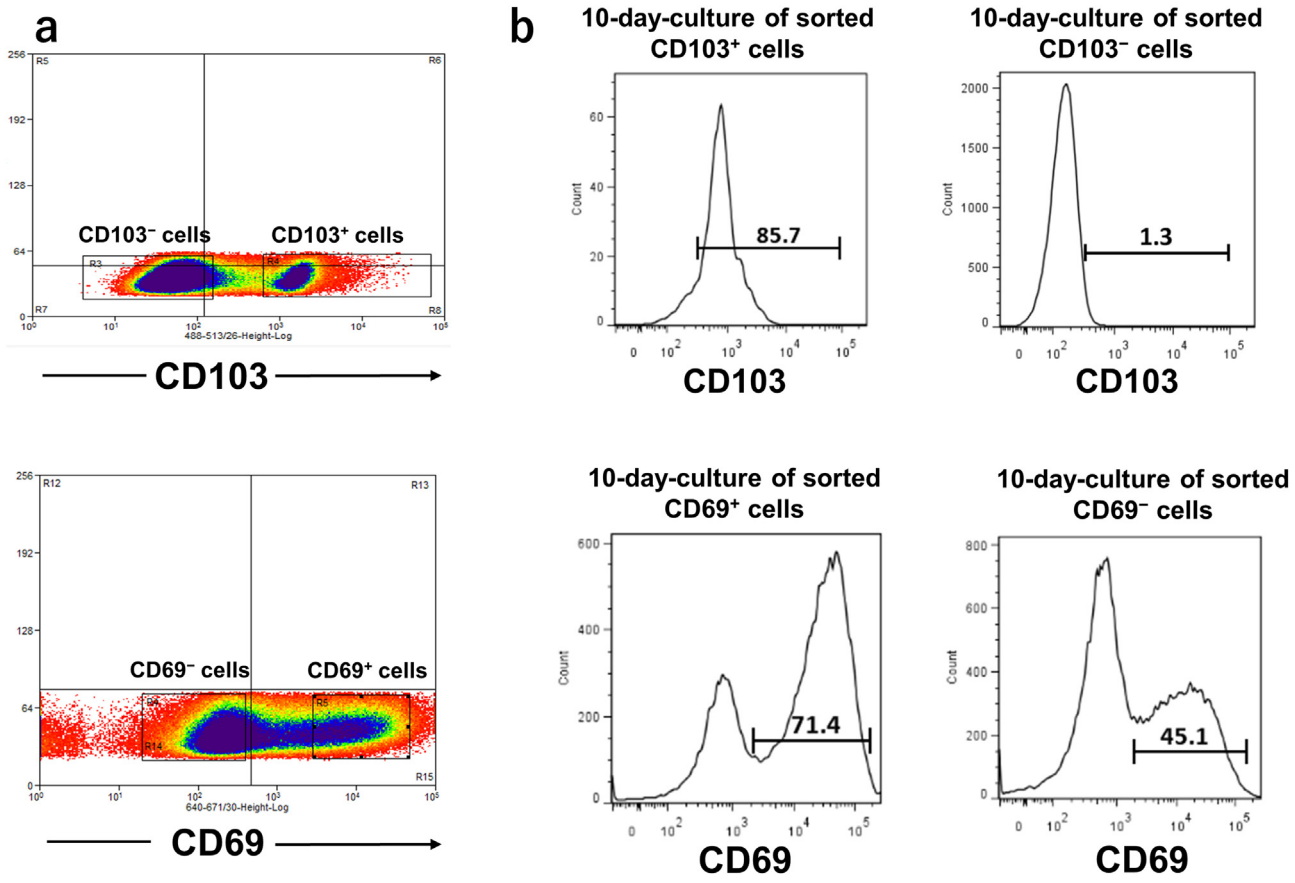

Fig. 2. No change in the expression of CD103, but increased expression of CD69 during culture of CD103 or CD69-sorted cells. (a) CD103$^+$ or CD69$^+$ cells were sorted from the *ex vivo* expanded lesional skin-infiltrating cells. The negative cells for these markers were also collected. (b) The positive and negative cells were cultured for 10 days, and their CD103 or CD69 expression was monitored by flow cytometry. The data show a representative case.

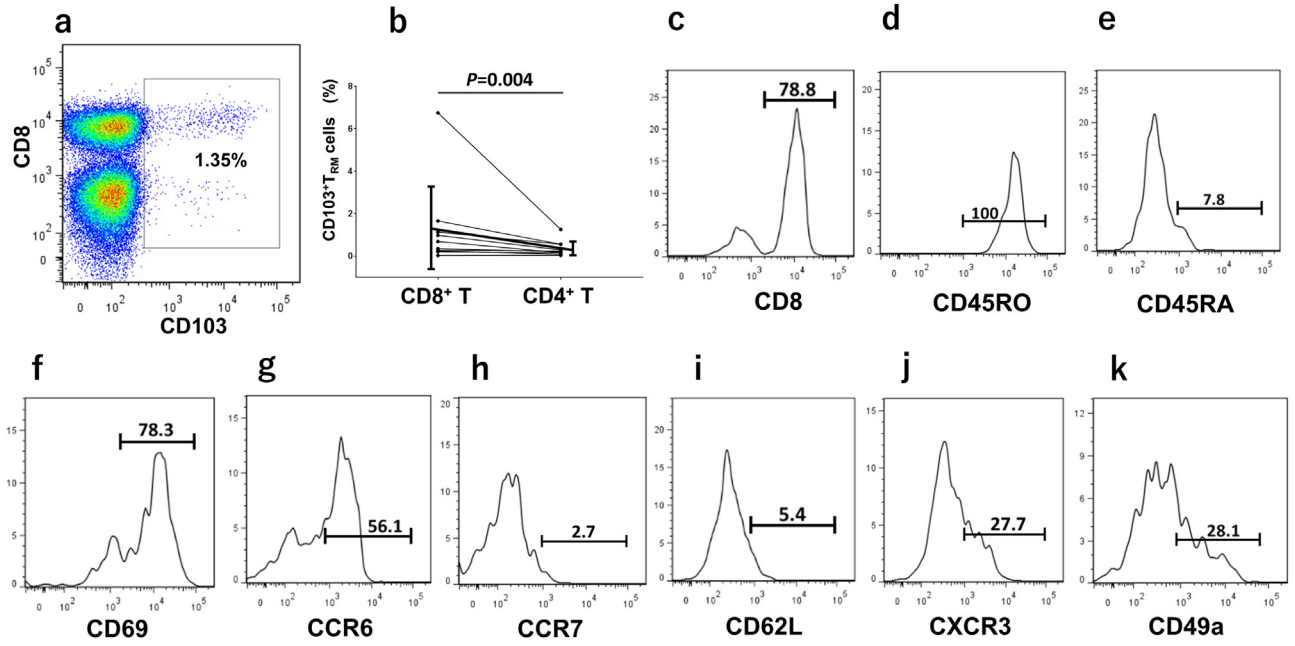

Fig. 3. Phenotype of CD8$^+$CD103$^+$ skin T_{RM} cells. (a) The *ex vivo* expanded skin-infiltrating T cells were stained for CD103 and CD8. CD103$^+$ cells were gated in the *ex vivo* expanded skin-infiltrating T cells. (b) In the 10 cases, CD8$^+$ cells contained higher frequencies CD103$^+$ cells than CD4$^+$ cells. P value was calculated by the Wilcoxon *t*-test. (c–k) Flow cytometric analysis of the indicated surface molecules in the sorted CD103$^+$ cells.

3.3. High production of IL-17A by CD8$^+$CD103$^+$ T_{RM} cells

Previous studies have shown that CD103$^+$ T_{RM} cells produce IFN-γ, IL-17A and IL-22 [11,12,21]. We tested the cytokine production by the *ex vivo* expanded T cells. As shown in a representative case, a part of CD8$^+$CD103$^+$ T cells produced IFN-γ, IL-17A or IL-22 (Fig. 4a), while CD4$^+$CD103$^+$ T cells scarcely elaborated these cytokines (Fig. 4b). In the expanded T cells from the 10 patients, CD8$^+$CD103$^+$ T_{RM} cells produced IFN-γ and IL-17A at significantly higher frequencies (both $P = 0.027$) than CD4$^+$CD103$^+$ T_{RM} cells.

The cytokine profile of CD103$^+$ T_{RM} cells was compared with CD103$^-$ T cells in the 10 patients. In CD8$^+$ T cells, CD103$^+$ T_{RM} cells more frequently produced IL-17A than did CD103$^-$ T cells (Fig. 5a). There were no significant differences between CD103$^+$ and CD103$^-$ T cells in the production of IFN-γ or IL-22. In CD4$^+$ T cells, CD103$^+$ and CD103$^-$ cells had comparable frequencies of cytokine-producing T cells (Fig. 5b). These results suggest that CD8$^+$CD103$^+$ T_{RM} cells efficiently produce IL-17A.

3.4. Possible association of IL-17A-producing CD103$^+$ T_{RM} cells with the future clinical course of psoriasis

Although skin T_{RM} cells are associated with the pathophysiology of psoriasis [7,12,18], it remains unclear whether they are related to the future clinical course of psoriasis. To address this issue, we retrospectively divided the 10 cases into two groups by the follow-up treatments. One was the non-advanced therapy group, which represented the patients treated with topical therapies alone even one year after the skin biopsy for T cell expansion. The other one was the advanced therapy group, in which systemic biologics, oral phosphodiesterase 4 (PDE4) inhibitor, or oral cyclosporine was initiated within one year after the biopsy. Between these two groups, we compared the frequency of each T cell population in the *ex vivo* expanded T cells. In this analysis, T cells were first divided into CD103$^+$ and CD103$^-$ cells, and then, further subdivided into CD4$^+$ or CD8$^+$ phenotype and intracellular IFN-γ, IL-17A or IL-22 positivity.

Among CD103$^+$ T cells, the frequencies of CD8$^+$CD103$^+$IL-17A$^+$ and CD4$^+$CD103$^+$IL-17A$^+$ T_{RM} cells tended to be higher in the advanced therapy group than in the non-advanced therapy group (Table 1). The CD8$^+$ T_{RM} cells showed a high frequency compared with the CD4$^+$ T_{RM} cells. There was no difference in the CD103$^-$ T cell populations between the two groups (Suppl. Table S2). In the 10 cases, we examined the correlation of CD8$^+$CD103$^+$IL-17A$^+$ or CD4$^+$CD103$^+$IL-17A$^+$ T_{RM} cells with the severity of the original psoriasis plaques from which skin biopsy specimens were taken. The clinical severity of individual lesions was assessed by SUM score, which include severity (0–4) of erythema, induration, and scaling (maximum score, 12) [19]. The severity in the 10 cases ranged from 4 to 12. Neither severity, disease duration, nor age correlated with the expanded T cell frequencies. There was no significant difference in the number of expanded T cells producing IL-17A between the two groups. These results suggest that IL-17A-producing CD8$^+$CD103$^+$ T_{RM} cells may be associated with a progressive clinical course of psoriasis rather than the severity of skin lesions.

4. Discussion

In this study, we used *ex vivo* expanded T cells from lesional skin of psoriasis to directly show the cytokine profile of skin T_{RM} cells. In advance of testing cultured T cells, we performed an immunohistochemical study and demonstrated that most of the CD103$^+$ cells in the epidermis were CD8$^+$CD103$^+$ T_{RM} cells with a small number of CD4$^+$CD103$^+$ T_{RM} cells. Correlation of the CD8$^+$CD103$^+$ T_{RM} cell

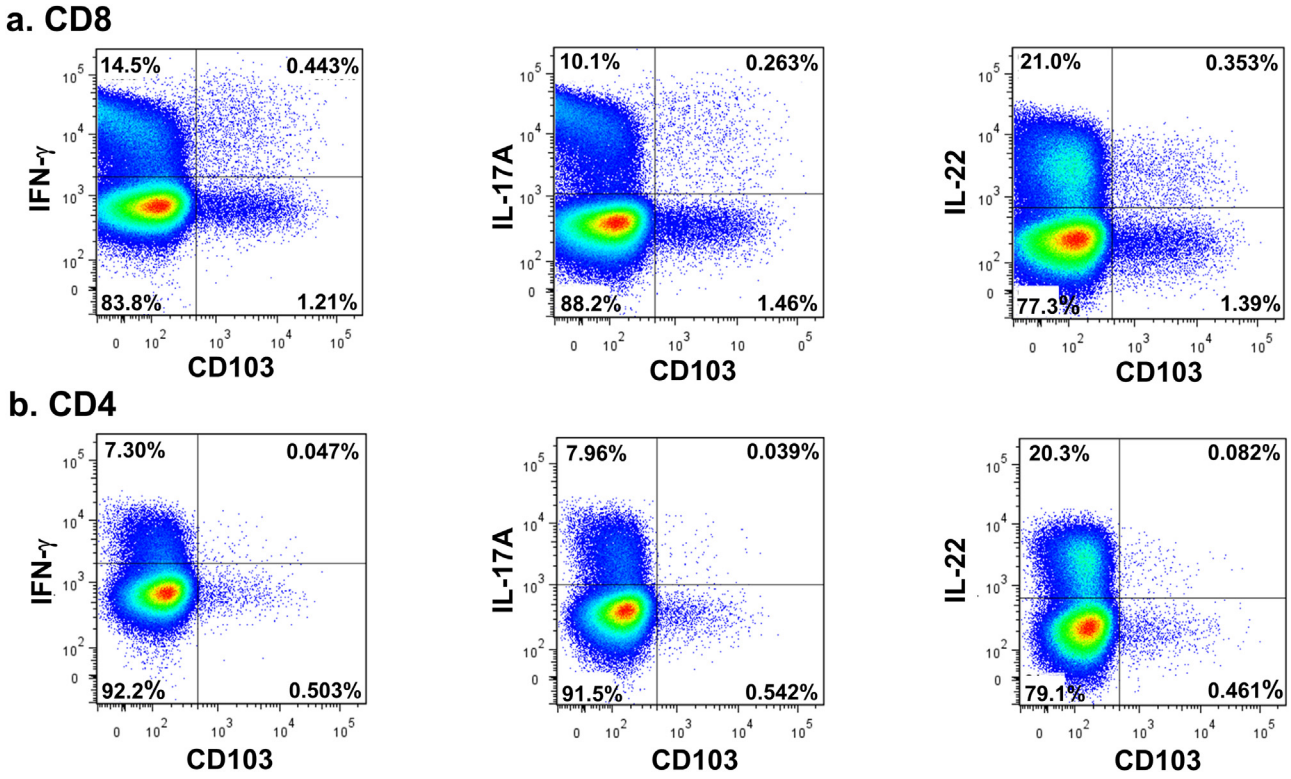

Fig. 4. Flow cytometric analysis of cytokine production in CD103$^+$ skin T_{RM} cells.
(a, b) In a representative case, the *ex vivo* expanded skin-infiltrating T cells were intracellularly stained for IFN-γ, IL-17A or IL-22 and subsequently stained for CD8 and CD103. CD8$^+$ or CD4$^+$ (corresponding to CD8$^-$) cells were gated, and their CD103$^+$ population was analyzed for the expression of IFN-γ, IL-17A or IL-22.

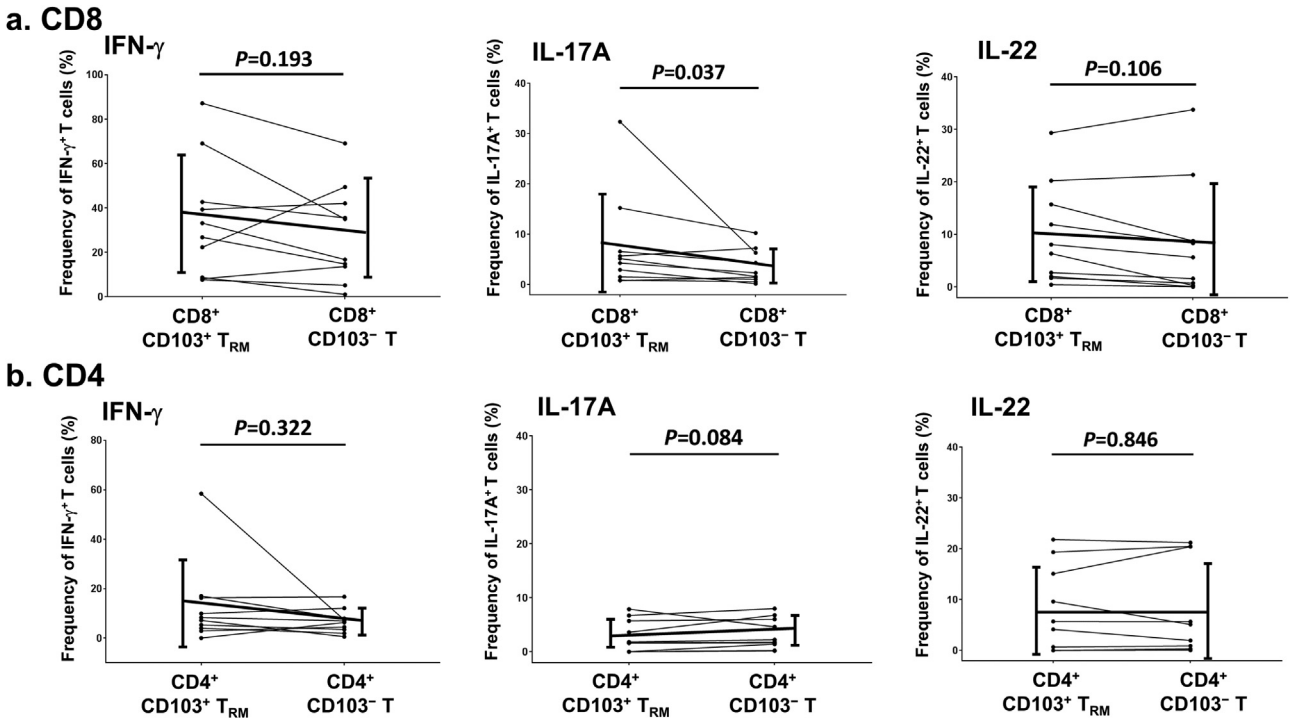

Fig. 5. Frequencies of individual cytokine-producing cells in $CD8^+$ or $CD4^+$, $CD103^+$ or $CD103^-$ *ex vivo* expanded T cells in 10 cases. (a, b) In the 10 cases, the *ex vivo* expanded skin-infiltrating T cells were intracellularly stained for IFN-γ, IL-17A or IL-22 and subsequently stained for CD8 and CD103. $CD8^+$ or $CD4^+$ (corresponding to $CD8^-$) cells were gated, and their $CD103^+$ population was analyzed for the expression of IFN-γ, IL-17A or IL-22. P values were calculated by the Wilcoxon *t*-test.

Table 1
Higher frequency of $IL\text{-}17A^+$ skin T_{RM} in the advanced therapy group. P values were calculated by the Mann-Whitney *U* test

T cell population	Non-advanced therapy (n = 7)	Advanced therapy (n = 3)	*P* value
$CD8^+IFN\text{-}\gamma^+CD103^+T_{RM}$ (%)	30.95 ± 21.58	42.83 ± 40.42	0.833
$CD8^+IL\text{-}17A^+CD103^+T_{RM}$ (%)	4.41 ± 5.07	14.88 ± 15.17	0.067
$CD8^+IL\text{-}22^+CD103^+T_{RM}$ (%)	8.98 ± 11.27	11.91 ± 3.83	0.383
$CD4^+IFN\text{-}\gamma^+CD103^+T_{RM}$ (%)	15.72 ± 19.31	6.38 ± 8.69	0.267
$CD4^+IL\text{-}17A^+CD103^+T_{RM}$ (%)	1.68 ± 2.37	5.73 ± 2.15	0.067
$CD4^+IL\text{-}22^+CD103^+T_{RM}$ (%)	5.83 ± 8.13	11.86 ± 9.05	0.258

number with the epidermal thickness suggests that the T_{RM} cells are numerically changed in parallel with keratinocyte proliferation. The importance of $CD8^+$ T cells in the pathogenesis of psoriasis has been noted [13,18,22,23], and their cytokines are essential for disease deterioration. Our finding is in accordance with these previous observations and further suggests that some part of $CD8^+$ cells serve as T_{RM} cells.

The sorted $CD103^+$ cells had a memory skin-homing phenotype. They also expressed CXCR3 or CD49a at a frequency of 28%, sharing the feature with Tc1 or reported IFN-γ-producing T cells [11,12]. The rest cells were CD49a negative or low, presumably corresponding to IL-17A-producing T cells [11,12]. In fact, $CD8^+CD103^+$ T_{RM} cells had intracellular IL-17A at a higher frequency than did $CD8^+CD103^-$ cells or $CD4^+CD103^+$ cells.

Skin T_{RM} cells remain longer in the same position than effector memory T cells [24] and produce certain cytokines in psoriasis [11,12,14]. In accordance with the previous studies [11,12,21], we demonstrated that psoriatic skin-derived $CD103^+$ T_{RM} cells produced IFN-γ, IL-17A and IL-22. In $CD103^+$ T_{RM} cells, $CD8^+$ cells outnumbered $CD4^+$ cells and more frequently produced IL-17A and IFN-γ, further supporting the notion that $CD8^+CD103^+$ T_{RM} cells are the largest T cell population in the epidermis. Considering the importance of IL-17 in the pathogenesis of psoriasis [1,25], $CD8^+CD103^+$ T_{RM} cells may contribute to the occurrence of psoriasis lesions as well as Th17 cells.

Recent studies showed that IL-17A production by T cells in resolved psoriasis epidermis could be associated with early relapse [26], and $CD8^+$ T_{RM} cells with IL-17A-producing potential in disease-naïve non-lesional sites possibly correlate with disease duration [18]. However, it remains unknown whether skin T_{RM} cells are related to the future clinical course of psoriasis and whether the frequency of skin T_{RM} cells predicts the resistance to treatments. We surveyed the 10 patients as to whether systemic biologics, oral phosphodiesterase 4 (PDE4) inhibitor, or oral cyclosporine was initiated within one year after the biopsy. We found that the patients having entered these advanced therapies possessed higher frequencies of $CD8^+CD103^+IL\text{-}17A^+$ T_{RM} cells. Although our study is a retrospective observation, this suggests that the presence of T_{RM} cells in the epidermis contributes to the future prognosis of psoriasis. One can speculate that upon stimulation of the skin of psoriasis patients, reactivated $CD8^+CD103^+$ T_{RM} cells initiate the condition with IL-17A and/or IFN-γ.

We acknowledge there are several limitations in this study. First, T cells from the specimen were cultured for totally 3 weeks *ex vivo*, possibly yielding alteration of the T cell phenotype and function. In our previous study, however, we found no considerable changes in the frequencies of T cells producing each cytokine after the cultivation [19]. Second, CD69 is one of the major markers of T_{RM} cells, but we were unable to use this marker, because $CD69^-$ T cells became CD69-positive ones during cultivation, presumably due to early activation [27]. According to many studies using $CD103^+$ T cells as T_{RM} cells [12,13,28,29], we therefore evaluated $CD103^+$ T cells as T_{RM} cells. Finally, the number of patients was small and the study was retrospective. A further study should be conducted.

In conclusion, our study provides an important possibility that the frequency of IL-17A-producing $CD8^+CD103^+$ T_{RM} cells is associated with the future clinical course of psoriasis. With the use of this notion, the development of a biomarker of prospective

psoriasis condition could be expected and might lead to a useful therapy choice for individual patients.

Funding sources

This study was funded by Eli Lilly and Company.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.06.002.

References

[1] W.H. Boehncke, M.P. Schon, Psoriasis, Lancet 386 (2015) 983–994.
[2] Y. Tokura, T. Mori, R. Hino, Psoriasis and other Th17-mediated skin diseases, J. UOEH 32 (2010) 317–328.
[3] J.E. Hawkes, B.Y. Yan, T.C. Chane, J.G. Krueger, Discovery of the IL-23/IL-17 signaling pathway and the treatment of psoriasis, J. Immunol. 201 (2018) 1605–1613.
[4] T. Shimauchi, S. Hirakawa, T. Suzuki, A. Yasuma, Y. Majima, K. Tatsuno, et al., Serum interleukin-22 and vascular endothelial growth factor serve as sensitive biomarkers but not as predictors of therapeutic response to biologics in patients with psoriasis, J. Dermatol. 40 (2013) 805–812.
[5] M. Masson Regnault, M.P. Konstantinou, A. Khemis, Y. Poulin, M. Bourcier, F. Amelot, et al., Early relapse of psoriasis after stopping brodalumab: a retrospective cohort study in 77 patients, J. Eur. Acad. Dermatol. Venereol. 31 (2017) 1491–1496.
[6] R.A. Clark, Resident memory T cells in human health and disease, Sci. Transl. Med. 7 (2015) 269rv1.
[7] J.J. Thome, D.L. Farber, Emerging concepts in tissue-resident T cells: lessons from humans, Trends Immunol. 36 (2015) 428–435.
[8] P.C. Rosato, L.K. Beura, D. Masopust, Tissue resident memory T cells and viral immunity, Curr. Opin. Virol. 22 (2017) 44–50.
[9] C.O. Park, X. Fu, X. Jiang, Y. Pan, J.E. Teague, N. Collins, et al., Staged development of long-lived T-cell receptor alphabeta Th17 resident memory T-cell population to Candida albicans after skin infection, J. Allergy Clin. Immunol. 142 (2018) 647–662.
[10] S. Cheuk, M. Wiken, L. Blomqvist, S. Nylen, T. Talme, M. Stahle, et al., Epidermal Th22 and Tc17 cells form a localized disease memory in clinically healed psoriasis, J. Immunol. 192 (2014) 3111–3120.
[11] S. Cheuk, H. Schlums, I. Gallais Serezal, E. Martini, S.C. Chiang, N. Marquardt, et al., CD49a expression defines tissue-resident CD8+ T cells poised for cytotoxic function in human skin, Immunity 46 (2017) 287–300.
[12] L.K. Mackay, A. Rahimpour, J.Z. Ma, N. Collins, A.T. Stock, M.L. Hafon, et al., The developmental pathway for CD103(+)CD8+ tissue-resident memory T cells of skin, Nat. Immunol. 14 (2013) 1294–1301.
[13] K. Boniface, C. Jacquemin, A.S. Darrigade, B. Dessarthe, C. Martins, N. Boukhedouni, et al., Vitiligo skin is imprinted with resident memory CD8 T cells expressing CXCR3, J. Invest. Dermatol. 138 (2018) 355–364.
[14] G.A. Hadley, S.T. Bartlett, C.S. Via, E.A. Rostapshova, S. Moainie, The epithelial cell-specific integrin, CD103 (alpha E integrin), defines a novel subset of alloreactive CD8+ CTL, J. Immunol. 159 (1997) 3748–3756.
[15] K.L. Cepek, S.K. Shaw, C.M. Parker, G.J. Russell, J.S. Morrow, D.L. Rimm, et al., Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin, Nature 372 (1994) 190–193.
[16] Y. Mizukawa, Y. Yamazaki, Y. Teraki, J. Hayakawa, K. Hayakawa, H. Nuriya, et al., Direct evidence for interferon-gamma production by effector-memory-type intraepidermal T cells residing at an effector site of immunopathology in fixed drug eruption, Am. J. Pathol. 161 (2002) 1337–1347.
[17] T.R. Matos, J.T. O'Malley, E.L. Lowry, D. Hamm, I.R. Kirsch, H.S. Robins, et al., Clinically resolved psoriatic lesions contain psoriasis-specific IL-17-producing αβ T cell clones, J. Clin. Invest. 127 (2017) 4031–4041.
[18] S. Vo, R. Watanabe, H. Koguchi-Yoshioka, Y. Matsumura, Y. Ishitsuka, Y. Nakamura, et al., CD8 resident memory T cells with IL-17A-producing potential are accumulated in disease-naïve non-lesional sites of psoriasis possibly in correlation with disease duration, Br. J. Dermatol. (in press).
[19] T. Fujiyama, T. Ito, T. Umayahara, S. Ikeya, K. Tatsuno, A. Funakoshi, et al., Topical application of a vitamin D3 analogue and corticosteroid to psoriasis plaques decreases skin infiltration of TH17 cells and their ex vivo expansion, J. Allergy Clin. Immunol. 138 (2016) 517–528 e5.
[20] H. Hashizume, A. Hansen, L.K. Poulsen, A.R. Thomsen, M. Takigawa, K. Thestrup-Pedersen, In vitro propagation and dynamics of T cells from skin biopsies by methods using interleukins-2 and -4 or anti-CD3/CD28 antibody-coated microbeads, Acta Derm. Venereol. 90 (2010) 468–473.
[21] I. Gallais Serezal, E. Hoffer, B. Ignatov, E. Martini, B. Zitti, M. Ehrstrom, et al., A skewed pool of resident T cells triggers psoriasis-associated tissue responses in never-lesional psoriasis skin, J. Allergy Clin. Immunol. 143 (2019) 1444–1454.
[22] P. Di Meglio, F. Villanova, A.A. Navarini, A. Mylonas, I. Tosi, F.O. Nestle, et al., Targeting CD8(+) T cells prevents psoriasis development, J. Allergy Clin. Immunol. 138 (2016) 274–276 e6.
[23] M.B.M. Teunissen, N.G. Yeremenko, D.L.P. Baeten, S. Chielie, P.I. Spuls, M.A. de Rie, et al., The IL-17A-producing CD8+ T-cell population in psoriatic lesional skin comprises mucosa-associated invariant T cells and conventional T cells, J. Invest. Dermatol. 134 (2014) 2898–2907.
[24] B.V. Kumar, W. Ma, M. Miron, T. Granot, R.S. Guyer, D.J. Carpenter, et al., Human tissue-resident memory T cells are defined by core transcriptional and functional signatures in lymphoid and mucosal sites, Cell Rep. 20 (2017) 2921–2934.
[25] M.P. Schon, L. Erpenbeck, The interleukin-23/interleukin-17 axis links adaptive and innate immunity in psoriasis, Front. Immunol. 9 (2018) 1323.
[26] I. Gallais Serezal, C. Classon, S. Cheuk, M. Barrientos-Somarribas, E. Wadman, E. Martini, et al., Resident T cells in resolved psoriasis steer tissue responses that stratify clinical outcome, J. Invest. Dermatol. 138 (2018) 1754–1763.
[27] L.K. Mackay, A. Braun, B.L. Macleode, N. Collins, C. Tebartz, S. Bedoui, et al., Cutting edge: CD69 interference with sphingosine-1-phosphate receptor function regulates peripheral T cell retention, J. Immunol. 194 (2015) 2059–2063.
[28] D.J. Topham, E.C. Reilly, Tissue-resident memory CD8(+) T cells: from phenotype to function, Front. Immunol. 9 (2018) 515.
[29] P. Hombrink, C. Helbig, R.A. Backer, B. Piet, A.E. Oja, R. Stark, et al., Programs for the persistence, vigilance and control of human CD8(+) lung-resident memory T cells, Nat. Immunol. 17 (2016) 1467–1478.