筑　波　大　学

博 士 （ 医 学 ） 学 位 論 文

Skin resident memory CD8 T cells with a potential of producing IL-17A are accumulated in disease-naïve non-lesional sites of psoriasis

（乾癬非病変部に IL-17A 産生皮膚 resident memory CD8 T 細胞が蓄積される）

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Abbreviations

APCs	Antigen Presenting Cells
CLA	Cutaneous Lymphocyte Antigen
DLQI	Dermatology Life Quality Index
DN	disease-naïve non-lesional
FBS	Fetal Bovine Serum
FDE	Fixed-Drug Eruption
HLA-DR	Human Leukocyte Antigen – DR isotype
HSV	Herpes Simplex Virus
IFN-γ	interferon gamma
IL	interleukin
IMDM	Iscove's Modified Dulbecco's Media
L-CTCL	Leukemic Cutaneous T-Cell Lymphoma
MHC	Major Histocompatibility Complex
PASI	Psoriasis Area and Severity Index
PMA	Phorbol Myristate Acetate
S1P	Sphingosine 1 Phosphate
Tc	cytotoxic T cells
T_{CM}	central memory T cells
TCR	T- Cell Receptor

T_{EM}	effector memory T cells
TGF-β	Transforming Growth Factor beta
Th	helper T cells
TNF-α	Tumor Necrosis Factor-alpha
T_{RM}	resident memory T cells
2-ME	2-Mercaptoethanol

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CHAPTER 1: INTRODUCTION

1.1 Existence of T cells in human skin

While there are many reports on T cells in human disease skin, skin T cells in stable healthy condition had been overseen for a long time. In 2006, Clark et al. reported that normal adult human skin contains around 20 billion T cells, twice as many as those in whole blood circulation, demonstrating that human skin is a large pool of T cells. The majority of skin T cells are αβ T cells and show memory phenotype [1]. Skin T cells are known to be distributed both in epidermis and dermis. In steady condition, most epidermal T cells stay in the epidermal basal layers along rete ridges while dermal T cells scatter below the epidermal-dermal junction or around blood vessels [2, 3].

Memory T-cell population is constructed by differentiation of naïve T cells. Under appropriate TCR signals from MHC of APCs, naïve T cells are activated, then expand rapidly and most of them become effector T cells [4-6]. After the immune reaction is converged, many effector T cells undergo apoptosis while a small population survives and differentiates into memory T-cell subset. In addition to the classic memory T-cell subsets, central memory T cells (T_{CM}) and effector memory T cells (T_{EM}), in blood circulation [7], memory T cells have also been reported to stay in peripheral tissues such as skin [8]. Memory T cells in skin are known to express skin-addressing molecules CLA and CC-chemokine receptor 4 (CCR4) and possess potent effector function [9] [2, 10]. Activation-associated markers such as CD69, CD25 and HLA-DR were also expressed [11, 12].

1.2 Skin resident memory T cells in human

From the observation of low-dose alemtuzumab treatment on leukemic cutaneous T-cell lymphoma (L-CTCL), it turned out that human skin contains both recirculating and sessile memory T cells. Alemtuzumab depletes CD52-expressing cells including T cells only in blood circulation. Skin of the patients treated with alemtuzumab lacks $CCR7^{+}$L-$selectin^{+}$ memory T cells, which are the same phenotype as T_{CM} in mice. This result

demonstrates that CCR7$^+$L-selectin$^+$ memory T cells in human skin behave as T_{CM} and recirculate from skin. It is also proved that T cells remaining in skin after alemtuzumab treatment are skin-sessile and do not recirculate from skin [13]. These skin-sessile T cells are called resident memory T cells (T_{RM}) and recognized by the surface markers CD69 and CD103 [14] (Fig. 1). CD69 is considered to contribute to retention of T_{RM} in skin since CD69 is associated with downregulation G protein–coupled receptor for sphingosine 1 phosphate (S1P1) [15]. S1P1-expressing T cells can egress from skin into lymph nodes and from lymph nodes into blood according to the gradients of S1P [16-18]. CD103 is an α chain of $\alpha_E\beta_7$ integrin and binds to E-cadherin, which is a homotypic adhesion molecule expressed in epithelial cells [19-21]. CD103 is reported to play an important role in longevity of T_{RM} [14, 22] though the actual functional activity is yet to be known. TGF-β and IL-15 from keratinocytes and fibroblasts are reported to be necessary for the expression of T_{RM} markers and long-term survival of T_{RM} [23, 24]. CD49a, known as an α1 subunit of α1β1 integrin, is also reported to be a marker of T_{RM}, especially epidermal CD8 T_{RM}. CD49a attaches collagen IV, a component of the basement membrane between epidermis and dermis [25, 26]. It is of interest that CD49a expression in T_{RM} is related to Tc1 phenotype in human [27]. Additional studies showed the distinct cytokine production capacity of skin T_{RM} [14, 28, 29], predisposing an evidence for the strong effector function of T_{RM}.

1.3 Resident memory T cells in human skin diseases

The functions of skin T cells have been investigated mainly from the viewpoints of immune-mediated skin disorders and skin infectious diseases. As for immune-mediated skin disorders, T_{RM} are reportedly involved in at least vitiligo, fixed drug eruption (FDE) and psoriasis. Vitiligo causes depigmented skin lesions where melanocytes are destructed through T-cell mediating cytotoxicity. Two independent reports on the phenotypes and functional profiles of T_{RM} in vitiligo lesion demonstrate the production of proimflammatory cytokines such as IFN-γ and TNF-α from CD8 T_{RM}. CD49a$^+$ T_{RM} which possess IFN-γ-producing Tc1 profile are enriched in epidermis of vitiligo lesion [27, 30].

In FDE, round annular erythematous plaques or bullas occur after systemic administration of certain drugs. The plaques remain as pigmented plaques and the erythema or bullas usually recur in the same sites after rechallenge of the same drugs [31]. Histologically, the presence of IFN-γ-producing CD8 T cells are found enriched in epidermis of clinically resolved FDE lesions [32]. These T cells in epidermis of resolved FDE express T_{RM} markers CD69 and CD103 [33]. The antigen-specificity of these T cells has not yet been shown but it is supposed that these T_{RM} in FDE exert reactivity against the drug-derived antigens. The roles of T_{RM} in skin infectious disorders are also reported. In patients with genital HSV, CD8αα T_{RM} in dermal-epidermal junction of the previously HSV-infected sites are persistent and exert protective function [34]. The analyses of infectious diseases reveal that T_{RM} survive for a long time in the absence of targeted antigens at the infected sites and provide a rapid immunologic response against the re-exposure of the same pathogens without recruiting other immune cells from the blood stream [35-37].

1.4 Psoriasis

Psoriasis is a chronic inflammatory skin disorder affecting 2-3% of worldwide population [38, 39]. Subtypes exist such as guttate psoriasis and generalized psoriasis but the most frequent phenotype is plaque-type psoriasis (psoriasis vulgaris), which makes up 90% of all psoriasis cases [40]. Plaque-type psoriasis is characterized by well-demarcated, reddish and scaly plaques which typically persist or recur in the same sites (Fig. 2). Not limited to skin lesions, psoriasis is coming to be regarded as a systemic disorder and often accompanied by arthritis and metabolic disorders [41]. Increased risk of cardiovascular events possibly caused by chronic continuous inflammation should also be taken into account [42]. Even though psoriasis itself is not a lethal disease, the patients sometimes face severe social problems and the patients' burden is reported to be as high as other chronic diseases such as diabetes and heart diseases [43]. It is thus complicated for dermatologists to evaluate the disease impact on each patient. In recent consensus, several indexes are used to define the severity of psoriasis. For instance, according to the Rule of Tens [44], the patient is regarded to be in severe condition if body surface area involved >

10 % or Psoriasis Area and Severity Index (PASI) score > 10 or Dermatology Life Quality Index (DLQI) score > 10. The treatment option ranges from skin-targeted treatments such as topical cream and ultraviolet therapies to systemic treatments such as immune-suppressive medications and biologics. Dermatologists try to choose the suited treatment based on the severity of disease condition. However, it is difficult to decide when and how intensively the treatment should be started or modified. It is also controversial how to decrease the treatment intensity once the disease activity is controlled.

1.5 Pathogenesis of psoriasis

Some external factors such as infection, medications, trauma and stress and internal stimuli such as gene factors, autoantigens, immune cells and cytokines are reported to trigger psoriasis disease formation. Clinical reports indicated that a transplantation of bone marrow from a donor with psoriasis could result in development of psoriasis in the recipient without history of psoriasis [45]. This suggests that psoriasis derives from bone marrow cells. In a mouse model of human psoriasis where human non-lesional psoriatic skin was grafted to an immunodeficiency mouse strain, psoriatic lesion spontaneously developed in the grafted non-lesional skin. It is thus indicated that psoriatic disease formation can occur only by the cells existing in skin without recruitment of circulating cells [46]. In this xenograft model, neutralization of CD8 T cells by injecting anti-human CD8 antibodies prevented psoriatic disease formation in the non-lesional skin, demonstrating the importance of skin CD8 T cells in disease formation of psoriasis [47]. An efficacy of Cyclosporin A in psoriasis also confirmed the contribution of T cells in pathogenesis of psoriasis [48].

Previously, IFN-γ-producing CD4 T cells (Th1 cells) were considered especially important because skin-resident IFN-γ-producing T cells have an ability to initiate psoriatic lesions [49, 50]. After discovery of Th17 cells [49-52], many reports demonstrated the role of IL-17A in the pathogenesis of psoriasis, leading to the establishment of psoriasis as Th17 disease [51-58]. It is reported that IL-17A-producing CD4 T cells are distributed mainly in dermis and IL-17A-producing CD8 T cells are enriched in epidermis of psoriatic patients

[59-61]. The persistence of IL-17A-producing CD8 T_{RM} is demonstrated even in resolved skin of psoriasis [62]. The relative production level of IL-17A to IFN-γ from T cells of this resolved skin inversely correlated with the duration of remission. However, the role of this CD8 T_{RM} in disease initiation of psoriasis is still left unclear.

CHAPTER 2: PURPOSE OF STUDY

This study aims to elucidate the profiles of T_{RM} from lesional and disease-naïve non-lesional sites of psoriasis and investigate the involvement of T_{RM} in psoriatic disease development.

CHAPTER 3: MATERIALS AND METHODS

3.1 Sample collection

Human tissue samples were used in this study. All the protocols in this study were performed in accordance with the Declaration of Helsinki and were approved by the University of Tsukuba Hospital Institutional Review Board (Approval # H28-001). Informed consent was obtained from all the participants.

Biopsy specimens were collected at University of Tsukuba Hospital from lesional and DN sites of 17 clinically and pathologically confirmed mild psoriasis patients (<10 PASI score) who had only received topical and/or local ultraviolet treatments. Resolved sites were also excluded by thorough interview and examinations of the clinical manifestation. DN specimens were taken from the same anatomical sites as biopsied lesional sites and at least 5 cm apart from the lesion borders. Normal skin specimens from 15 healthy donors were also collected from skin grafting procedures after resection of benign tumors, malignant in situ tumors (such as Bowen's disease and basal cell carcinoma) or from the discarded edge of the resection of these tumors. The information of normal subjects is described in Table 1. These skin specimens were processed for either T-cell isolation or immunohistological evaluation. Due to sample size limitation, all experiments were not able to be conducted on each sample. The actual number of the samples analyzed in each experiment is indicated in each experimental method and figure legend.

3.2 Flow cytometry

T cells were isolated from lesional and DN sites of 12 psoriasis patients and 10 normal skin specimens by two weeks of short-term explant culture as described previously [1] with Iscove's Modified Dulbecco's Media (IMDM) containing 10% Fetal Bovine Serum (FBS), penicillin/streptomycin and 2-Mercaptoethanol (2-ME) in the presence of 100 IU/ml human recombinant IL-2 (Wako, Osaka, Japan) and 20 ng/ml human recombinant IL-15 (Wako). The following directly conjugated monoclonal antibodies for the indicated

molecules and isotype controls were used for surface or intracellular staining with optimal concentrations for flow cytometry analyses: human CD3 (SK7, Thermo Fisher Scientific, MA, USA), CD4 (RPA-T14, Thermo Fisher Scientific), CD8a (RPA-T8, Thermo Fisher Scientific), CD103 (Ber-ACT8, BioLegend) for cell-surface staining and IL-17A (BL168, BioLegend), IL-22 (22URTI, Thermo Fisher Scientific), IFN-γ (4S.B3, BioLegend) and TNF-α (MAb11, BioLegend) for intracellular staining. Prior to intracellular cytokine staining, cells were stimulated with Phorbol Myristate Acetate (PMA) (50 ng/ml, Wako) and ionomycin (0.5 μg/ml, Wako) plus Golgi Plug (BD Biosciences, CA, USA) for 4 hours. Cells were surface-stained, fixed, permeabilized and stained by anti-cytokine antibodies using BD Cytofix/Cytoperm Plus (BD Biosciences) according to the manufacturer's protocol. Data collection was performed on a Beckman Coulter Gallios instrument (Beckman Coulter, IN, USA) and the data were analyzed by Kaluza Flow Analysis Software (Beckman Coulter). Lymphocyte population was determined by the intensity levels of forward scatter and side scatter. CD3 cells were determined by a histogram showing relative expression of CD3. CD4 T cells and CD8 T cells were further determined on the CD4 vs CD8 dot plot. Within these populations, the expression level of surface marker CD103 was analyzed. When cells were stimulated with PMA and ionomycin, $CD3^+$ $CD8^-$ cells on the CD3 vs CD8 dot plot were regarded as CD4 T cells and cytokine expression was analyzed within this population. The gating strategies are shown in Fig. 3.

3.3 Immunofluorescence analysis

Formalin-fixed and paraffin-embedded specimens from lesional and DN sites of 5 psoriasis patients and 5 normal donors were sliced into 3μm thickness on glass slides, de-paraffinized and rehydrated. Antigen retrieval was performed using TE buffer (10x concentrate, pH 9.0, Agilent). After blocking, sliced specimens were incubated with indicated primary antibodies with the optimized concentrations for 60 min at room temperature. Primary antibodies used in this experiment were as follows: rabbit anti-human CD3 (ab5690, Abcam, Cambridge, UK), mouse anti-human CD8a (C8/144B, Agilent) and rabbit anti-human CD103 (EPR4166, Abcam). Secondary antibodies were applied after

rinsing the slides with the optimized concentrations for 60 min at room temperature. The following secondary antibodies were used: PE-conjugated donkey anti rabbit IgG antibody (ab150074, Abcam), FITC-conjugated goat anti mouse IgG antibody (ab150117, Abcam), PE-conjugated donkey anti mouse IgG antibody (ab150106, Abcam), and FITC-conjugated goat anti rabbit IgG antibody (A-11034, Thermo Fisher Scientific). Finally, the cell nuclei were stained by DAPI (Vector Laboratories, Burlingame, CA, USA), covered with coverslips and examined by KEYENCE fluorescence microscope BZ-X700 (Keyence, Osaka, Japan).

3.4 Statistical analysis

Data were expressed as mean ± standard deviation. Comparison among three groups was performed by one-way ANOVA. Paired t-test was used for comparison between two groups. Linear regression test was applied for comparison between cytokine expression and disease duration. P value of < 0.05 was taken as statistically significant unless mentioned separately.

CHAPTER 4: RESULTS

4.1 CD8 T_{RM} with CD103 expression are enriched both in lesional and disease-naïve non-lesional sites of psoriasis skin

Isolated T cells from two-week expansion culture of psoriatic and normal skin were analyzed for surface molecules by flow cytometry. Psoriatic lesional and DN sites showed a slight tendency of CD8 T-cell enrichment compared to normal skin though the difference did not reach significance (Fig. 4A, lesion vs DN: p>0.9999, lesion vs normal: p>0.9999, DN vs normal: p>0.9999, Table 2). Interestingly, among the CD8 T cell population, lesional and DN skin from psoriasis patients included significantly more $CD103^+$ cells ($CD103^+$ CD8 T_{RM}) than normal skin (Fig. 4B, lesion vs DN: p>0.9999, lesion vs normal: p=0.0058, DN vs normal: p=0.0181, Table 3). Meanwhile, the percentage of $CD103^+$ T_{RM} in CD4 fraction was lower in lesional sites of psoriatic patients compared to normal (Fig. 4B, lesion vs DN: p= 0.0942, lesion vs normal: p<0.0007, DN vs normal: p=0.3082). These results demonstrate that T-cell profiles in normal-looking psoriatic DN sites are already skewed and enriched with $CD103^+$ CD8 T_{RM}.

4.2 $CD103^+$ CD8 T_{RM} are dominated in epidermis of both lesional and disease-naïve non-lesional sites of psoriasis skin

In order to determine the localization of CD8 T cells, especially $CD103^+$ CD8 T_{RM} in skin, first, the number of CD8 T cells in epidermis and dermis were counted from 100 μm in width of the specimens of lesional and DN sites of psoriasis patients and normal skin. Then lesional skin contained more CD8 T cells compared to normal skin, especially in epidermis. DN skin, especially epidermis, also included significantly more CD8 T cells compared to normal skin (Fig. 5A, B, in whole skin, lesion vs DN: p=0.0001, lesion vs normal: p<0.0001, DN vs normal: p=0.0255; in epidermis, lesion vs DN: p=0.0024, lesion vs normal: p<0.0001, DN vs normal: p=0.0259; in dermis, lesion vs DN: p<0.0001, lesion vs normal: p<0.0001, DN vs normal: p=0.0670, Table 3). Within each sectioned specimen, CD8 T cells localized more in epidermis than in dermis both in lesional and DN sites (Fig.

5C, lesion: p=0.0213; DN: p=0.0170; normal: p=0.0516, Table 4). These results are in concordance with the flow cytometry results and it is indicated that even DN sites from psoriasis patients contain more CD8 T cells especially in epidermis compared to normal skin.

As for T_{RM} fractions, the $CD103^+$ CD8 T_{RM} population was significantly dominant in epidermis compared to dermis among psoriasis skin including lesional and DN sites and normal skin (Fig. 6A, B, lesion: p=0.0093; DN: p=0.0349; normal: p=0.0367, Table 5). Among the three groups, within epidermis, both lesional and DN skin contained significantly more $CD103^+$ CD8 T_{RM} compared to normal skin (Fig. 6C, lesion vs DN: p=0.0119, lesion vs normal: p<0.0001, DN vs normal: p=0.0297, Table 5). On the other hand, in dermis, $CD103^+$ CD8 T_{RM} numbers were low and only slightly high in lesional skin (Fig. 6C, lesion vs DN: p=0.1953, lesion vs normal: p=0.0102, DN vs normal: p=0.2420, Table 5). These results show that $CD103^+$ CD8 T_{RM}, are enriched and localized mainly in epidermis of both lesional and DN skin of psoriasis.

4.3 CD8 T cells in disease-naïve non-lesional sites of psoriasis skin have IL-17A-generating potential

Cytokine production profiles of skin T cells isolated by short-term expansion culture were compared by flow cytometry analysis after stimulation with PMA and ionomycin. The ratios of IL-22, TNF-α- or IFN-γ-producing cells in CD4 and CD8 fractions were comparable among the three groups (Fig. 7A, B, IL-22 in CD4; lesion vs DN: p>0.9999, lesion vs normal: p>0.9999, DN vs normal: p>0.9999; IFN-γ in CD4; lesion vs DN: p>0.9999, lesion vs normal*:* p>0.9999, DN vs normal: p>0.9999; TNF-α in CD4; lesion vs DN: p=0.6966, lesion vs normal: p>0.9999, DN vs normal: p=0.2990; IL-22 in CD8; lesion vs DN: p=0.7636, lesion vs normal: p>0.9999, DN vs normal: p=0.2163; IFN-γ in CD8; lesion vs DN: p>0.9999, lesion vs normal: p>0.9999, DN vs normal: p>0.9999; TNF-α in CD8; lesion vs DN: p>0.9999, lesion vs normal: p>0.9999, DN vs normal: p>0.9999, Table 6). Notably, within the CD8 T cell population, a significantly increased ratio of IL-17A production was observed in both lesional and DN skin compared to normal skin (Fig. 7A,

B: lesion vs DN: p>0.9999, lesion vs normal: p=0.0007, DN vs normal: p=0.0012, Table 6). As for the CD4 T cell fraction, IL-17A production was comparable among three groups (Fig. 7A, B, lesion vs DN: p>0.9999, lesion vs normal: p>0.9999, DN vs normal: p>0.9999, Table 6). From these results, DN sites of psoriasis also include IL-17A-producing CD8 T cells even before disease manifestation.

4.4 $CD103^+$ CD8 T_{RM} from disease-naïve non-lesional sites of psoriasis possess a potent IL-17A-producing phenotype

When cytokine production capacity of $CD103^+$ CD8 T_{RM} were further compared, significantly more IL-17A-producing $CD103^+$ CD8 T_{RM} were detected both in psoriatic lesional and DN skin than normal skin (Fig. 8, lesion vs DN: p>0.9999, lesion vs normal: p=0.0006, DN vs normal: p=0.0020, Table 7). The production of IL-22, IFN-γ and TNF-α was not increased in psoriatic skin (Fig. 8, IL-22: lesion vs DN: p=0.7262, lesion vs normal: p>0.9999, DN vs normal: p=0.3126. IFN-γ: lesion vs DN: p>0.9999, lesion vs normal: p>0.9999, DN vs normal: p=0.8798. TNF-α: lesion vs DN: p>0.9999, lesion vs normal: p>0.9999, DN vs normal: p>0.9999, Table 7). The production profiles of these cytokines in $CD103^-$ CD8 T cells also showed a similar tendency with significantly higher IL-17A production ratio in both psoriatic lesional and DN sites than in normal skin (Fig. 8, IL-17A: lesion vs DN: p>0.9999, lesion vs normal: p=0.0053, DN vs normal: p=0.0005. IL-22: lesion vs DN: p>0.9999, lesion vs normal: p>0.9999, DN vs normal: p=0.8726. IFN-γ: lesion vs DN: p>0.9999, lesion vs normal: p>0.9999, DN vs normal: p>0.9999. TNF-α: lesion vs DN: p>0.9999, lesion vs normal: p>0.9999, DN vs normal: p>0.9999, Table 7). The production ratios of IL-17A and IL-22 in $CD103^+$ CD8 T_{RM} cells were higher than in $CD103^-$ CD8 T cells in psoriatic lesional and DN sites. Of note, IL-17A production ratio was far higher in $CD103^+$ CD8 T_{RM} cells than in $CD103^-$ CD8 T cells. IFN-γ and TNF-α production levels showed a similar tendency among these population (Figure 8A, IL-17A: p=0.0005 in lesion, p=0.0010 in DN, p=0.1250 in normal; IL-22: p=0.0059 in lesion, p=0.0391 in DN, p=0.1250 in normal; IFN-γ: p=0.2661 in lesion, p=0.0342 in DN, p=0.6406 in normal; TNF-α: p=0.1475 in lesion, p=0.6523 in DN, p=0.9375 in normal,

Table 7). $CD103^+$ CD8 T_{RM} both in psoriatic lesional and DN sites thus serve as a responsible source of IL-17A.

4.5 Relative expression levels of IL-17A to IFN-γ in $CD103^+$ CD8 T_{RM} from disease-naïve non-lesional sites correlate with disease duration

In our study, we used PASI score to evaluate disease severity. However, PASI score did not correlate with disease duration (Fig. 9A). Correlation between PASI score or disease duration and $CD103^+$ CD4 T_{RM} or $CD103^+$ CD8 T_{RM} both in psoriatic lesional and DN sites was not observed, either (Fig. 9B, C). In addition, from aspect of cytokines, we found that the ratio of IL-17A-producing cells in whole CD8 fraction in DN sites tended to be higher in the patients with longer disease duration (Fig. 10A, p=0.0813) although the tendency did not show significant difference. However, the ratio of % IL-17A-producing cells to % IFN-γ-producing cells in whole CD8 in DN sites correlated with disease duration (Fig. 10B, p=0.0270). Within CD8 T cells, %IL-17A/% IFN-γ-producing cells correlated with disease duration in $CD103^+$ CD8 T_{RM} but not in $CD103^-$ CD8 fraction (Fig. 10B, p=0.0343 in $CD103^+$CD8 T_{RM}, p=0.1119 in $CD103^-$CD8).

CHAPTER 5: DISCUSSION

In addition to previous reports showing IL-17A production from CD8 T_{RM} in lesional and resolved sites [60, 62], our results demonstrate that $CD103^+$ CD8 T_{RM} residing in psoriatic DN sites already possess IL-17A-generating potential. These cells are confined in epidermis. On the other hand, the production levels of IL-17A in $CD103^-$ CD8 T cells are much lower than those in $CD103^+$ CD8 T_{RM}. These results may implicate the role of $CD103^+$ CD8 T_{RM} within DN sites in the pathogenesis of psoriasis. Recent studies suggested the relationship of cells resident in skin to the pathogenesis of psoriasis as the grafted psoriatic non-lesional skin in an immunodeficient mouse strain spontaneously developed psoriatic lesion without grafting any human circulating cells [63]. Other experiments demonstrated that the blockage of E-selectin, which plugs the trafficking of memory T cells into skin from blood, is not effective in the treatment of psoriasis even though its importance in psoriatic pathogenesis was previously suggested [64, 65]. Based on these reports, psoriatic disease formation does not apparently require recruitment of cells from circulation [66, 67].

Psoriasis is regarded as an IL-17-mediated disorder [68-70] as both active and resolved psoriatic lesions contain CD4 and CD8 T cells that produce IL-17 and IL-22 [62, 71]. Notably, CD8 T cells from DN sites also showed a higher IL-17A production profile than those from normal skin. Furthermore, among these CD8 T cells, epidermal $CD103^+$ CD8 T_{RM} cells in both psoriatic lesional and DN sites demonstrated a significantly higher IL-17A-generating potential compared to normal skin. On the other hand, IL-17A-producing potential was far higher in $CD103^+$ CD8 T_{RM} cells than in $CD103^-$ CD8 T cells, even though $CD103^-$ CD8 T cells from psoriatic lesional and DN sites also showed significantly more IL-17A production than normal skin.

From the aspect of clinical manifestation, our results demonstrate that the production ratio of IL-17A to IFN-γ increases along with disease duration in whole CD8 and $CD103^+$ CD8 T_{RM} fraction, but not in $CD103^-$ CD8 T-cell fraction, residing in psoriatic DN, suggesting that the relative accumulation of IL-17A-generating T_{RM} in DN according

to chronic disease activity makes the prepared condition for disease formation. This result aligns with the negative correlation of IL-17A/IFN-γ and IL-17A/IL-10 ratios in the resolved psoriatic skin with the duration of remission [72]. Relative accumulation of IL-17A-producing cells will thus make the foundation for further lesion development. CD4 T cells have been indicated in many reports to serve as a source of IL-17A in the pathogenesis of psoriasis. Our observation corresponds with the previous indication that CD4 T cells maintain psoriatic lesions [73-75] and may also support the idea that IL-17A-producing CD8 T_{RM} cells are involved in disease staging and pathogenesis [47]. We were limited in observations of this development as we were not able to follow up to see if the biopsied non-lesional sites actually develop psoriatic disease. At least since these sites are not developing psoriatic lesions at the time of biopsy even with plenty amount of IL-17A-producing CD8 T_{RM}, other factors would be needed for disease formation. Investigating the direct factors for lesion initiation would be required for revealing the detailed contribution of this fraction to disease pathogenesis.

In clinical settings, some patients experience unexpectedly long remission periods after intermitting treatments including biologics. Clinical trials of 2 distinct biologics demonstrated that 5 to 10 % of the participants maintain the remission status with over 75 % of PASI improvement [76-78]. These results raise the argument on intermission of biologics and on the evaluation of deep remission. Evaluating IL-17A profiles of T_{RM} in DN may have a potentiality of serving as an index not only for future disease manifestation but also for required treatment intensity from the viewpoint of treatment modality and treatment duration.

CHAPTER 6: OVERVIEW AND PROSPECTS

Psoriasis is a chronic inflammatory skin disease which is characterized by hyperproliferation of keratinocytes and infiltration of immune competent cells. After discovery of Th17 cells, many reports demonstrated the role of IL-17A-producing T cells in the pathogenesis of psoriasis, leading to its establishment as an IL-17-mediated disease. From clinical aspects, psoriatic lesion is well-demarcated and recurs in the same sites, suggesting that disease formation is mediated by skin-sessile cells. However, the detailed roles of T_{RM} in the pathogenesis of psoriasis are still left unclear.

In this study, we found a dominance of $CD103^+$ CD8 T_{RM} in psoriatic DN sites, which have never experienced disease formation. Thus, normal-looking sites in psoriasis patients already have skewed distribution of T cells before disease manifestation. Previous reports showed both active and resolved psoriatic lesions contain CD4 and CD8 T cells with a potential of producing IL-17A. Keratinocytes of DN sites were also reported to upregulate CCL20 expression, which recruit $CCR6^+$ IL-17A-producing T cells. In this research, we demonstrated the IL-17A-generating phenotype of CD8 T cells in psoriatic DN sites. Meanwhile, in CD4 fraction, IL-17A-producing potential was comparable among lesion, DN and normal skin. It is assumed that CD8 T cells are strikingly involved in preparation and initiation of disease formation. In comparison with $CD103^-$ CD8 T cells, $CD103^+$ CD8 T_{RM} demonstrated a significantly stronger ability of IL-17A production in both lesional and DN sites. These results implicate a contribution of $CD103^+$ CD8 T_{RM} to the pathogenesis of lesion formation in DN sites.

We revealed that IL-17A-producing CD8 T_{RM} are accumulated in epidermis of psoriatic DN sites according to disease course. IL-17A-producing $CD103^+$ CD8 T_{RM} may be considered not only as an index for baseline disease activity but also as a predictor of suited treatment intensity. In addition, IL-17A-producing CD8 T_{RM} may also be an attractive treatment target for preventing disease progress. On the other hand, since these DN sites do not immediately develop psoriatic lesions even in the presence of IL-17A-

producing CD8 T_{RM}, finding other factors eliciting disease formation cascade will be of great importance in the treatment strategy of psoriasis.

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REFERENCES

(1) Clark RA, Chong BF, Mirchandani N *et al.* A novel method for the isolation of skin resident T cells from normal and diseased human skin. *J Invest Dermatol* 2006; **126**: 1059-1070.

(2) Bos JD, Zonneveld I, Das PK *et al*. The skin immune system (SIS): distribution and immunophenotype of lymphocyte subpopulations in normal human skin. *The Journal of investigative dermatology* 1987; **88**: 569-73.

(3) Nestle FO, Di Meglio P, Qin J-Z *et al*. Skin immune sentinels in health and disease. *Nat Rev Immunol* 2009; **9**: 679-91.

(4) Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol* 2013; **13**: 227-42.

(5) Gutcher I, Becher B. APC-derived cytokines and T cell polarization in autoimmune inflammation. *J Clin Invest* 2007; **117**: 1119-27.

(6) Curtsinger JM, Mescher MF. Inflammatory cytokines as a third signal for T cell activation. *Current Opinion in Immunology* 2010; **22**: 333-40.

(7) Sallusto F, Lenig D, Forster R*et al*. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999; **401**: 6754.

(8) Carbone FR, Mackay LK, Heath WR *et al.* Distinct resident and recirculating memory T cell subsets in non-lymphoid tissues. *Curr Opin Immunol* 2013; **25**: 329-33.

(9) Picker LJ, Terstappen LW, Rott LS *et al.* Differential expression of homing-associated adhesion molecules by T cell subsets in man. *J Immunol* 1990; **145(10)**: 3247–3255.

(10) Clark RA, Chong B, Mirchandani N *et al*. The vast majority of CLA+ T cells are resident in normal skin. *J Immunol* 2006; **176**: 4431-4439.

(11) Testi R, Phillips JH, Lanier LL. T cell activation via Leu-23 (CD69). *J Immunol* 1989; **143**: 1123-1128.

(12) Caruso A, Licenziati S, Corulli M *et al*. Flow cytometric analysis of activation markers on stimulated T cells and their correlation with cell proliferation. *Cytometry* 1999; **27**: 71–76.

(13) Clark RA, Watanabe R, Teague JE *et al*. Skin effector memory T cells do not recirculate and provide immune protection in alemtuzumab-treated CTCL patients. *Science Translational Medicine* 2012; **4**: 117ra7.

(14) Watanabe R, Gehad A, Yang C *et al*. Human skin is protected by four functionally and phenotypically discrete populations of resident and recirculating memory T cells. *Sci Transl Med* 2015; **7**: 279ra39.

(15) Mackay LK, Braun A, Macleod BL *et al*. Cutting Edge: CD69 interference with sphingosine-1-phosphate receptor function regulates peripheral T cell retention. *J Immunol* 2015; **194**: 2059-63.

(16) Turner DL, Farber DL. Mucosal resident memory CD4 T cells in protection and immunopathology. *Front Immunol* 2014; **5**: 331.

(17) Skon CN, Lee JY, Anderson KG *et al*. Transcriptional downregulation of S1pr1 is required for the establishment of resident memory CD8+ T cells. *Nat Immunol* 2013; **14**: 1285-93.

(18) Cyster JG, Schwab SR. Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu Rev Immunol* 2012; **30**: 69-94.

(19) Laidlaw BJ, Zhang N, Marshall HD *et al*. CD4+ T cell help guides formation of CD103+ lung-resident memory CD8+ T cells during influenza viral infection. *Immunity* 2014; **41**: 633-45.

(20) Sheridan BS, Pham QM, Lee YT *et al*. Oral infection drives a distinct population of intestinal resident memory CD8+ T cells with enhanced protective function. *Immunity* 2014; **40**: 747-57.

(21) Piet B, Godelieve J. de Bree G, Dierdorp B *et al*. CD8+ T cells with an intraepithelial phenotype upregulate cytotoxic function upon influenza infection in human lung. *J Clin Invest* 2011; **121**: 2254-2263.

(22) Mackay LK, Rahimpour A, Ma JZ *et al*. The developmental pathway for CD103+CD8+ tissue-resident memory T cells of skin. *Nat Immunol* 2013; **14**: 1294-301.

(23) Casey KA, Fraser KA, Schenkel JM *et al.* Antigen-independent differentiation and maintenance of effectorlike resident memory T cells in tissues. *J Immunol* 2012; **188**: 4866–4875.

(24) Lee YT, Suarez-Ramirez JE, Wu T *et al*. Environmental and antigen receptor-derived signals support sustained surveillance of the lungs by pathogen-specific cytotoxic T lymphocytes. *J. Virol* 2011; **85**: 4085–4094.

(25) Hemler ME. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. Annual Review of Immunology 1990; **8**: 365-400.

(26) RO Hynes. Integrins: bidirectional, allosteric signaling machines. Cell 2002; 110: 673-87.

(27) Cheuk S, Schlums H, Gallais Serezal I *et al*. CD49a Expression Defines Tissue-Resident CD8(+) T Cells Poised for Cytotoxic Function in Human Skin. *Immunity* 2017; **46**: 2.

(28) Naik S, Bouladoux N, Linehan JL *et al.* Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. *Nature* 2015; **520**: 104-8.

(29) Sanchez RR, Pauli ML, Neuhaus IM *et al.* Memory regulatory T cells reside in human skin. *J Clin Invest.* 2014; **124**: 1027-36.

(30) Boniface K, Jacquemin C, Darrigade AS *et al.* Vitiligo Skin Is Imprinted with Resident Memory CD8 T Cells Expressing CXCR3. *The Journal of investigative dermatology* 2018; **138**: 355-364.

(31) Shiohara T. Fixed drug eruption: pathogenesis and diagnostic tests. *Current opinion in allergy and clinical immunology* 2009; **9**: 316-21.

(32) Teraki Y, Shiohara T. IFN-gamma-producing effector CD8+ T cells and IL-10-producing regulatory CD4+ T cells in fixed drug eruption. *The Journal of allergy and clinical immunology* 2003; **112**: 609-15.

(33) Mizukawa Y, Yamazaki Y, Teraki Y *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.* 2002; **161**: 1337-1347.

(34) Zhu J, Koelle DM, Cao J *et al*. Virus-specific CD8+ T cells accumulate near sensory nerve endings in genital skin during subclinical HSV-2 reactivation. *The Journal of experimental medicine* 2007; **19**: 204:595.

(35) Gebhardt T, Wakim ML, Eidsmo L *et al*. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nature Immunol* 2009; **10**: 524-530.

(36) Jiang X, Clark RA, Liu L *et al*. Skin infection generates non-migratory memory CD8+ T(RM) cells providing global skin immunity. *Nature* 2012; **483**: 7388.

(37) Williams MA, Bevan MJ. Effector and memory CTL differentiation. *Annu Rev Immunol* 2007; **25**: 171-192.

(38) Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. *Nature* 2007; **445**: 866-73.

(39) Nestle FO, Kaplan DH, Barker J. Psoriasis. *New England Journal of Medicine* 2009b; **361**: 496-509.

(40) Boehncke WH, Schön MP. Psoriasis. *The Lancet* 2015; **386**: 9997, p983-994.

(41) Henseler T, Christophers E. Disease concomitance in psoriasis. *Journal of the American Academy of Dermatology* 1995; **32**: 982-6.

(42) Shahwan KT, Kimball AB. Psoriasis and Cardiovascular Disease. *Med Clin North Am* 2015; **99**: 1227-42.

(43) Rapp SR, Feldman SR, Exum ML *et al.* Psoriasis causes as much disability as other major me dical diseases. *J Am Acad Dermatol* 1999; **41**: 401-7.

(44) Finlay AY. Current severe psoriasis and the rule of tens. *Br J Dermatol* 2005; **152**: 861-7.

(45) Gardembas-Pain M, Ifrah N, Foussard C *et al*. Psoriasis after allogeneic bone marrow transplantation. *Archives of dermatology* 1990; **126**: 1523.

(46) Conrad C, Boyman O, Tonel G, *et al*. Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. *Nat Med* 2007; **13**: 836-42.

(47) Di Meglio P, Villanova F, Navarini AA *et al*. Targeting CD8(+) T cells prevents psoriasis development. *J Allergy Clin Immunol* 2016; **138**: 274-276.

(48) Gottlieb SL, Gilleaudeau P, Johnson R *et al* .Response of psoriasis to a lymphocyte-selective toxin (DAB389IL-2) suggests a primary immune, but not keratinocyte, pathogenic basis. *Nat Med* 1995; **1**: 442–447.

(49) Szabo SK, Hammerberg C, Yoshida Y *et al*. Identification and quantitation of interferon-gamma producing T cells in psoriatic lesions: localization to both CD4+ and CD8+ subsets. *J Invest Dermatol* 1998; **111**: 6.

(50) Bata-Csorgo Z, Hammerberg C, Voorhees JJ *et al*. Kinetics and regulation of human keratinocyte stem cell growth in short-term primary ex vivo culture. Cooperative growth factors from psoriatic lesional T lymphocytes stimulate proliferation among psoriatic uninvolved, but not normal, stem keratinocytes. *J Clin Invest* 1995; **95**: 1.

(51) Langrish CL, Chen Y, Blumenschein WM *et al*. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 2005; **201**: 2.

(52) Infante-Duarte C, Horton HF, Byrne MC *et al*. Microbial lipopeptides induce the production of IL-17 in Th cells. *J Immunol* 2000; **165**: 11.

(53) Harrington LE, Hatton RD, Mangan PR *et al*. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005; **6**: 11.

(54) Park H, Li Z, Yang XO *et al*. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005; **6**: 11.

(55) Kryczek I, Bruce AT, Gudjonsson JE *et al*. Induction of IL-17+ T cell trafficking and development by IFN-gamma: mechanism and pathological relevance in psoriasis. *J Immunol* 2008; **181**: 7.

(56) Lowes MA, Kikuchi T, Fuentes-Duculan J *et al*. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J Invest Dermatol* 2008; **128**: 5.

(57) Guttman-Yassky E, Lowes MA, Fuentes-Duculan J *et al*. Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. *J Immunol* 2008; **181**: 10.

(58) Harper EG, Guo C, Rizzo H *et al*. Th17 cytokines stimulate CCL20 expression in keratinocytes in vitro and in vivo: implications for psoriasis pathogenesis. *J Invest Dermatol* 2009; **129**: 9.

(59) Conrad C, Boyman O, Tonel G *et al*. Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. *Nat Med* 2007; **13**: 7.

(60) Res PC, Piskin G, de Boer OJ *et al*. Overrepresentation of IL-17A and IL-22 producing CD8 T cells in lesional skin suggests their involvement in the pathogenesis of psoriasis. *PLoS One* 2010; **5**: 11.

(61) Hijnen D, Knol EF, Gent YY *et al*. CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-gamma, IL-13, IL-17, and IL-22. *J Invest Dermatol* 2013; **133**: 4.

(62) Cheuk S, Wiken M, Blomqvist L *et al*. Epidermal Th22 and Tc17 cells form a localized disease memory in clinically healed psoriasis. *J Immunol* 2014; **192**: 7.

(63) Boyman O, Hefti HP, Conrad C *et al*. Spontaneous development of psoriasis in a new animal model shows an essential role for resident T cells and tumor necrosis factor-alpha. *J Exp Med* 2004; **199**: 5.

(64) Bhushan M, Bleiker TO, Ballsdon AE *et al*. Anti-E-selectin is ineffective in the treatment of psoriasis: a randomized trial. *Br J Dermatol* 2002; **146**: 5.

(65) Szepietowski J, Wasik F, Bielicka E *et al*. Soluble E-selectin serum levels correlate with disease activity in psoriatic patients. *Clin Exp Dermatol* 1999; **24**: 1.

(66) Park CO, Kupper TS. The emerging role of resident memory T cells in protective immunity and inflammatory disease. *Nat Med* 2015; **21**: 7.

(67) Jiang X, Clark RA, Liu L *et al*. Skin infection generates non-migratory memory CD8+ T(RM) cells providing global skin immunity. *Nature* 2012; **483**: 7388.

(68) Krueger JG, Fretzin S, Suarez-Farinas M *et al*. IL-17A is essential for cell activation and inflammatory gene circuits in subjects with psoriasis. *J Allergy Clin Immunol* 2012; **130**: 1.

(69) Fitch E, Harper E, Skorcheva I *et al*. Pathophysiology of psoriasis: recent advances on IL-23 and Th17 cytokines. *Curr Rheumatol Rep* 2007; **9**: 6.

(70) Cai Y, Fleming C, Yan J. New insights of T cells in the pathogenesis of psoriasis. *Cell Mol Immunol* 2012; **9**: 4.

(71) Suarez-Farinas M, Fuentes-Duculan J, Lowes MA *et al*. Resolved psoriasis lesions retain expression of a subset of disease-related genes. *J Invest Dermatol* 2011; **131**: 2.

(72) Gallais Serezal I, Classon C, Cheuk S *et al*. Resident T Cells in Resolved Psoriasis Steer Tissue Responses that Stratify Clinical Outcome. *J Invest Dermatol* 2018; **138**: 8.

(73) Bata-Csorgo Z, Hammerberg C, Voorhees JJ *et al*. Intralesional T-lymphocyte activation as a mediator of psoriatic epidermal hyperplasia. *J Invest Dermatol* 1995; **105**: 1 Suppl.

(74) Wrone-Smith T, Nickoloff BJ. Dermal injection of immunocytes induces psoriasis. *J Clin Invest* 1996; **98**: 8.

(75) Nickoloff BJ, Wrone-Smith T. Injection of pre-psoriatic skin with CD4+ T cells induces psoriasis. *Am J Pathol* 1999; **155**: 1.

(76) Deodhar A, Gottlieb AB, Boehncke WH *et al.* Efficacy and safety of guselkumab in patients with active psoriatic arthritis: a randomised, double-blind, placebo-controlled, phase 2 study. *Lancet.* 2018; **391**: 2213-2224.

(77) Gordon KB, Colombel JF, Hardin DS. Phase 3 Trials of Ixekizumab in Moderate-to-Severe Plaque Psoriasis. *N Engl J Med*. 2016; **375**:2102.

(78) Langley RG, Elewski BE, Lebwohl M *et al*. Secukinumab in plaque psoriasis-results of two phase 3 trials. *N Engl J Med*. 2014; **371**:326-38.

FIGURES AND LEGENDS

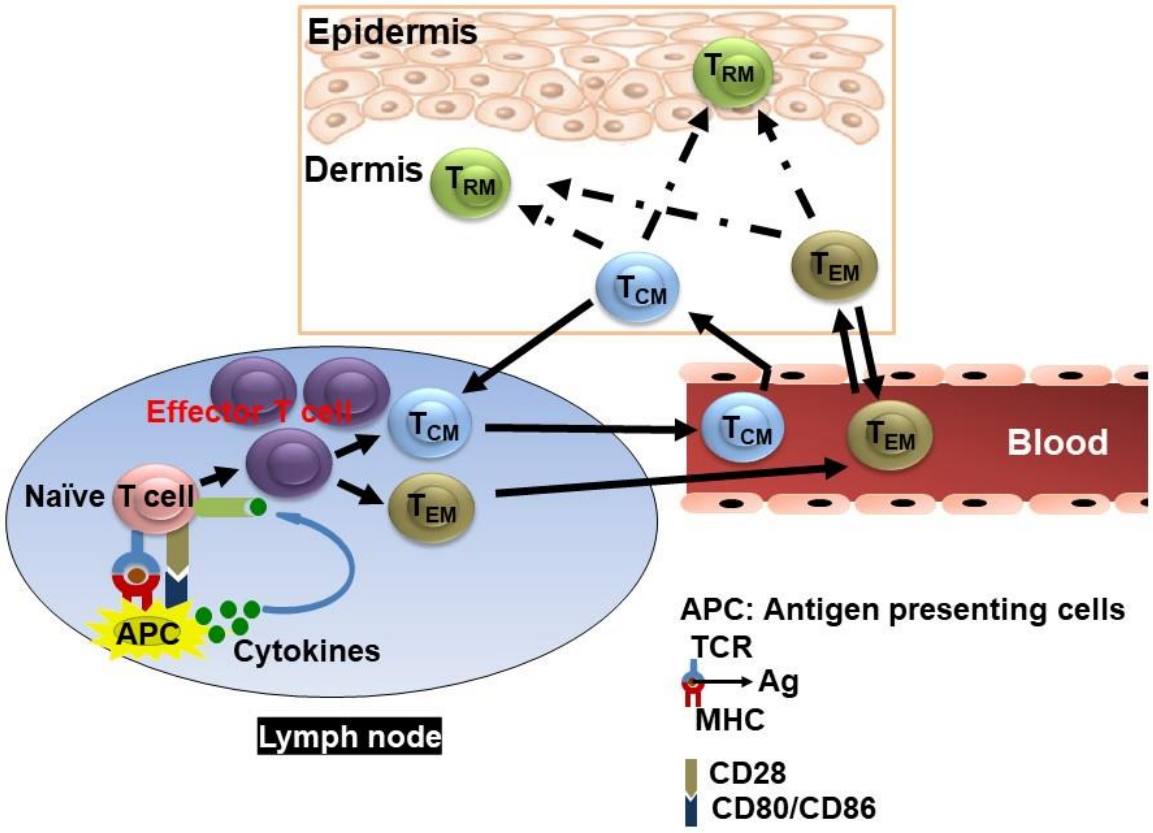

Figure 1: Memory T cell subsets were found in skin. Upon the appropriate interaction of the signals, naïve T cells are activated by antigen presenting cells. Then, memory T cells fractions are constructed from the naïve T cells. T_{CM} expressing CCR7 and L-selectin are circulating between blood stream, skin and lymph nodes. T_{EM} are found both in blood stream and skin. After migrating into skin, a certain subset of memory T cells is regarded to be differentiated into T_{RM} and stay in skin for a long time even in the absence of antigens and provide a rapid response against the re-exposure of the same pathogens without recruiting the cells from the circulation. Ag: antigen, APC: Antigen Presenting Cell, MHC: Major Histocompatibility Complex, T_{CM}: central memory T cells, TCR: T- Cell Receptor, T_{EM}: effector memory T cells, T_{RM}: resident memory T cells.

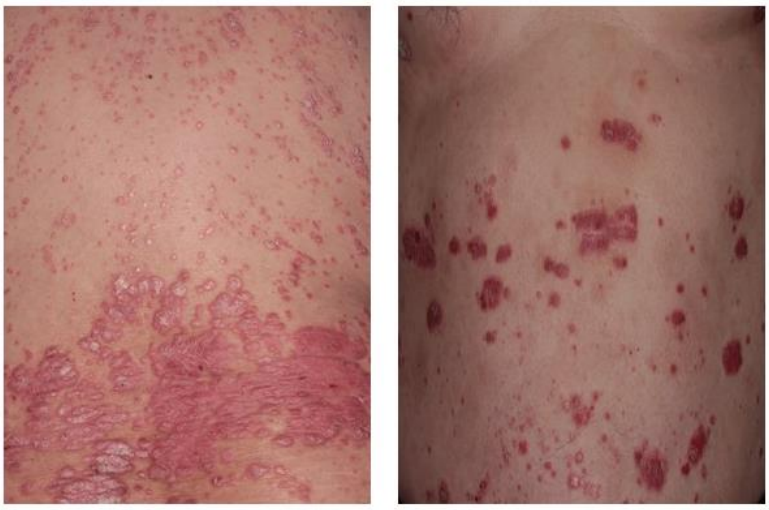

Figure 2: Clinical manifestation of plaque-type psoriasis. Courtesy of Dr. Rei Watanabe. The lesion of psoriasis is a well-demarcated, raised, red plaque with a white scaly surface. The new psoriatic lesions recur on pigmented plaques where are the previous psoriatic lesions (on the right picture).

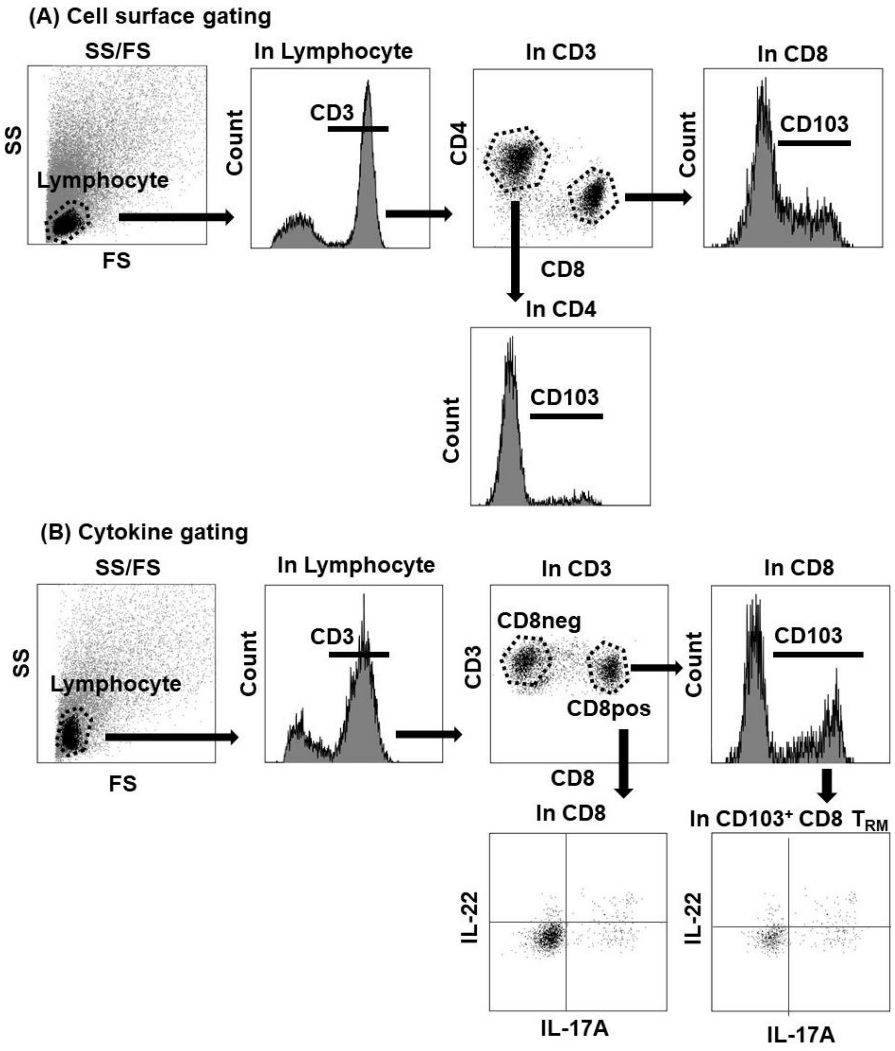

Figure 3: Gating strategy. Lymphocyte were gated by the intensity levels of forward scatter (FS) and side scatter (SS). Then, CD3 T cells were determined by a histogram indicating relative expression of CD3. (A) In cell surface gating, without stimulation, CD4 T cells and CD8 T cells were performed on the CD4 vs CD8 dot plot. Within these populations, the expression level of surface marker CD103 was further analyzed. (B) In cytokine gating, after stimulating the cells with PMA and ionomycin, $CD3^+$ $CD8^-$ cells on the CD3 vs CD8 dot plot were regarded as CD4 T cells. Then, the expression level of surface marker CD103 was analyzed. Next, cytokine production was analyzed in these populations. The demonstration of analyzing IL-17 and IL-22 in CD8, $CD103^+$ CD8 T_{RM} was example for gating cytokines.

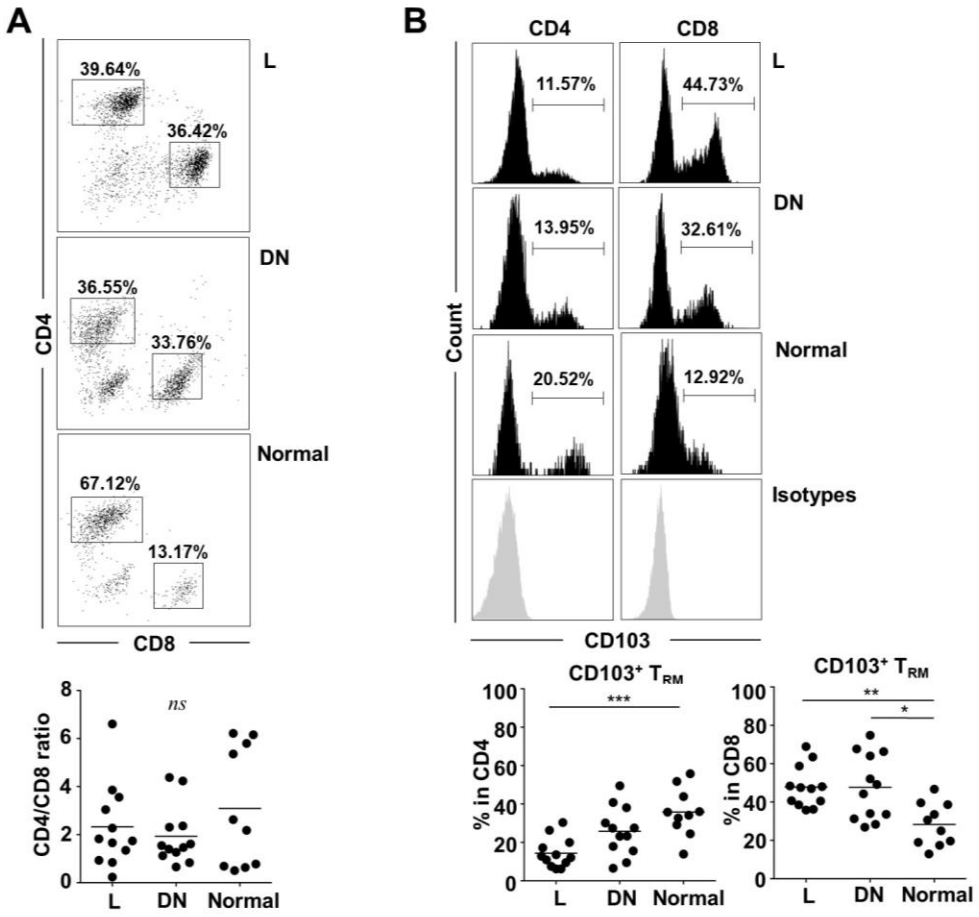

Figure 4: Both lesional and disease-naïve non-lesional sites from psoriasis patients are enriched with CD103$^+$ CD8 T_{RM} cells. T cells were harvested from 12 psoriasis patients at both lesional and DN sites as well as skin from 10 normal subjects after two weeks of explant culture, then analyzed by flow cytometry. (A) Representative flow cytometry dot plots of CD4 and CD8 populations and a cumulative graph showing CD4/CD8 ratio from lesional and DN sites of psoriasis patients and normal skin specimens. (B) Representative flow cytometry histograms of CD103 expression in CD4 and CD8 T cells and cumulative graphs from lesional and DN sites of the same psoriasis patient and a normal control showing the percentage of CD103$^+$ (CD103$^+$ T_{RM}) in CD4 and CD8 fractions. L: lesion; DN: disease-naïve non-lesion. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Error bar: mean ± SD.

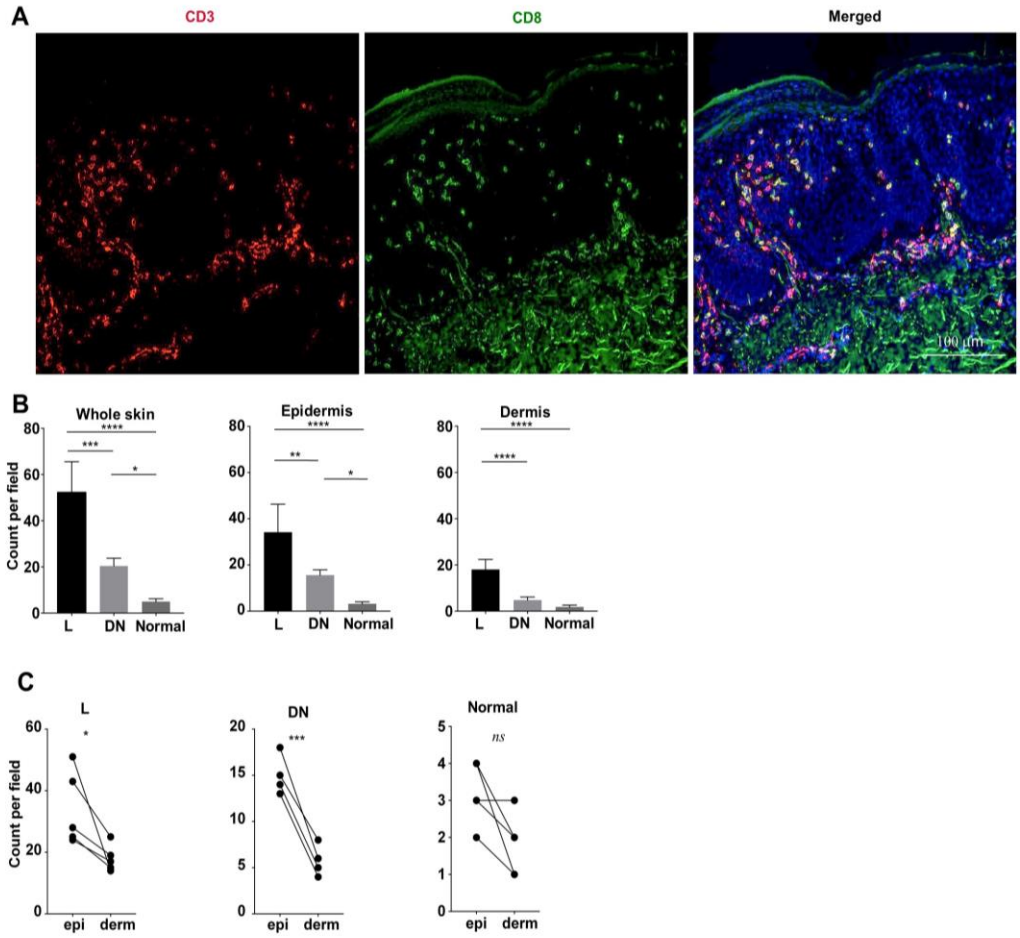

Figure 5: CD8 T cells are predominantly localized to epidermis of lesional psoriatic skin. (A) Immunofluorescence staining of CD3 (red) and CD8 (green) expression was performed on formalin-fixed, paraffin-embedded skin specimens in lesional psoriatic skin. (B) The actual number of CD8 T cells was counted in whole skin, epidermis and dermis from psoriatic lesional and DN sites, and normal skin specimens. (C) A comparison of CD8 T-cell numbers in epidermis and dermis of the same individuals from the stained sections in (A). Cell counts within 100 μm of epidermis and dermis of the stained sections in (A). Five cases are included in each group. L: lesion; DN: disease-naïve non-lesion. Epi: Epidermis. Derm: Dermis. Scale bar = 100 μm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p<0.0001$. Error bar: mean ± SD.

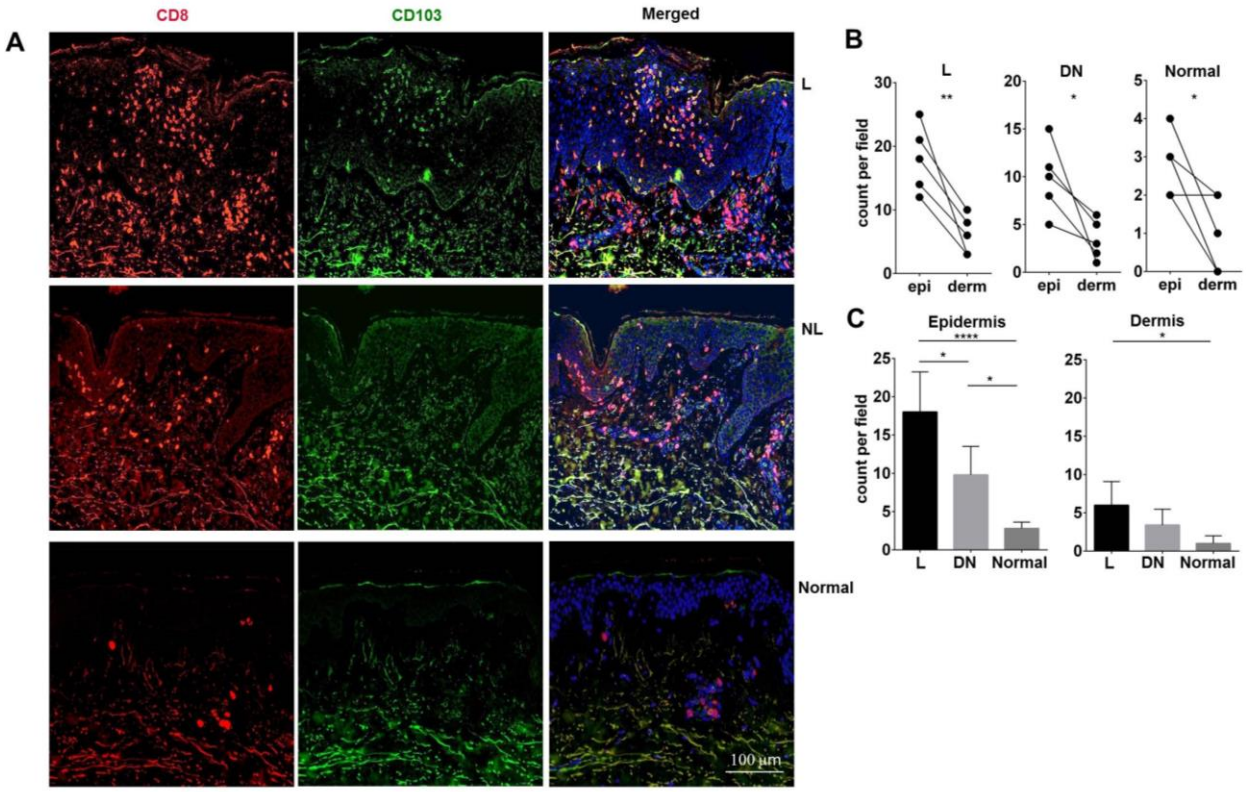

Figure 6: CD103$^+$ CD8 T_{RM} cells predominately localize in epidermis of both lesional and DN psoriatic skin. (A) Immunofluorescence staining of CD8 (red) and CD103 (green) expression was performed on formalin-fixed, paraffin-embedded skin in lesional and DN sites from psoriasis patients and in normal skin. (B) The number of CD103$^+$ CD8 T_{RM} in epidermis and dermis of the same individuals from psoriatic lesional and DN specimens and normal skin were compared from 100 µm widths of the stained specimens. The same sections as in (A) were used. (C) Number of CD103$^+$ CD8 T_{RM} cells in epidermis and dermis were compared among lesional and DN sites of psoriatic and normal skin specimens. Cells were counted within a 100 µm of epidermis and dermis from the stained sections in (A). Five cases are included in each group. L: psoriasis lesion; DN: disease-naïve non-lesion. Scale bar = 100 µm. *p < 0.05, **p < 0.01, ****p<0.0001. Error bar: mean ± SD.

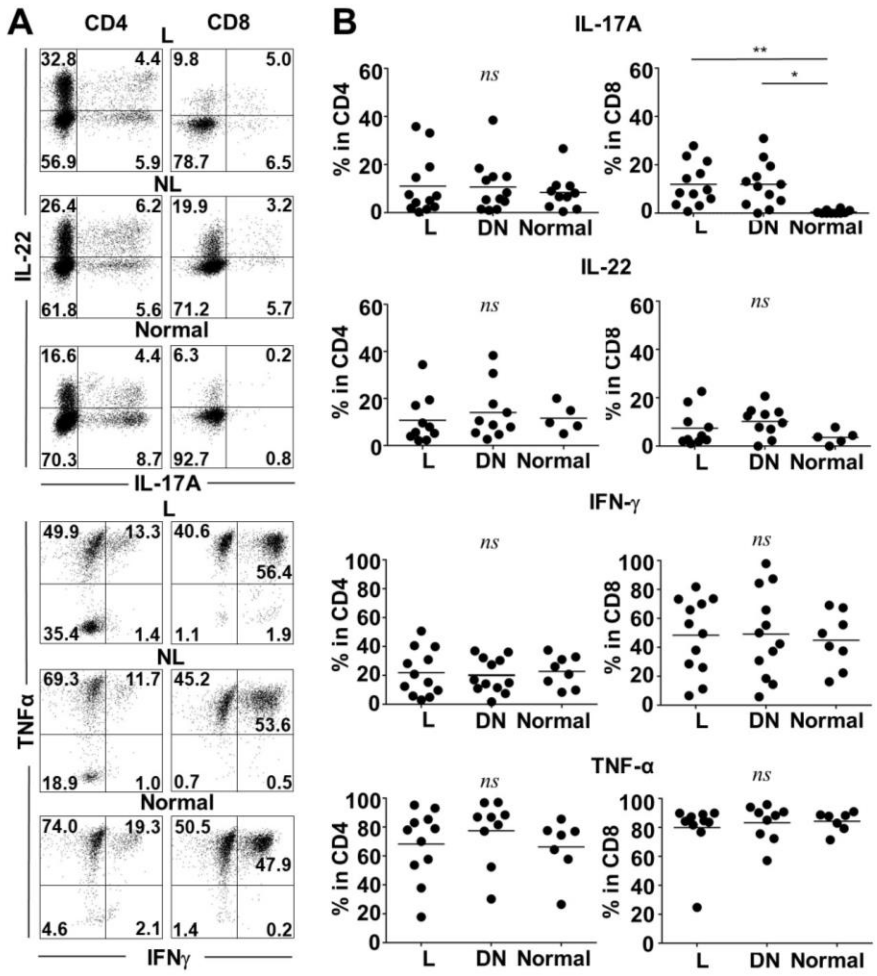

Figure 7: CD8 T cells with IL-17A-generating potential are enriched both in psoriatic lesional and DN skin. (A) Representative flow cytometry dot plots showing the profiles of IL-17A, IL-22, IFN-γ and TNF-α production from CD4 and CD8 fractions of psoriatic lesional and DN skin from the same patients as well as normal skin. Isolated T cells from two weeks of explant culture were stimulated with PMA and ionomycin for four hours and then stained for surface markers and intracellular cytokines. (B) Cumulative graphs showing production of each cytokine in CD4 and CD8 T cells. A total of 12 L, 12 DN and 10 Normal were analyzed for IL-17A. 10 L, 10 DN and 5 Normal for IL-22, 12 L, 12 DN and 8 Normal for IFN-γ and 11 L, 9 DN and 7 Normal for TNF-α. L: lesion; DN: disease-naïve non-lesion. *$p < 0.05$, **$p < 0.01$. Error bar: mean ± SD.

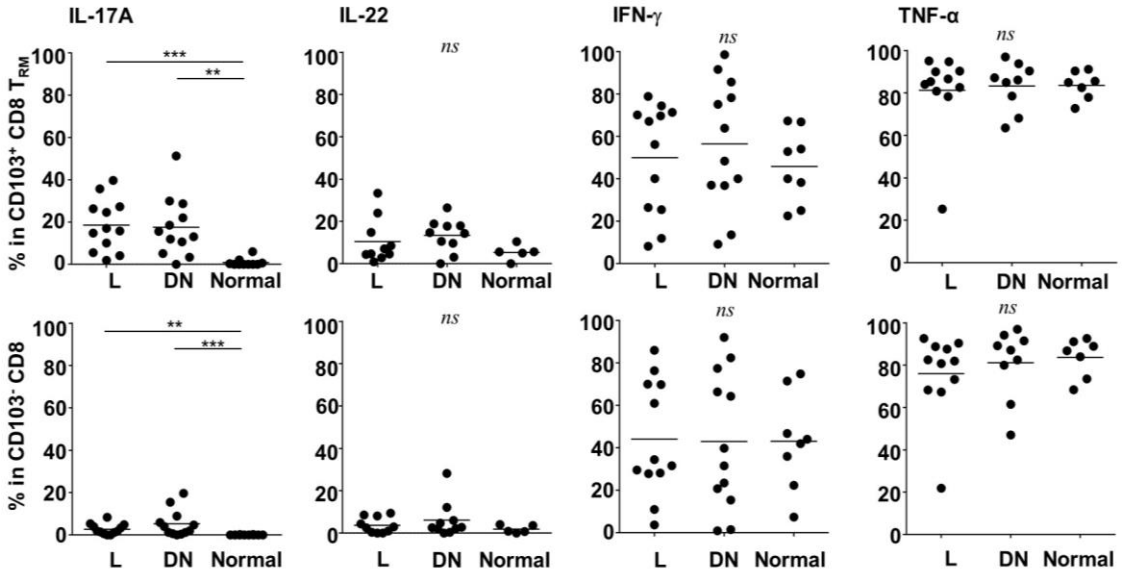

Figure 8: CD103$^+$ CD8 T_{RM} cells from psoriatic disease-naïve, non-lesional skin have an IL-17A-generating potential. The cells were harvested after 2 weeks of explant culture, and then stimulated with PMA and ionomycin for four hours before cell-staining and analysis by flow cytometry. The percentage of the cells producing the indicated cytokines in CD103$^+$ CD8 T_{RM} (upper panels) and CD103$^-$ CD8 T cells (lower panels). 12 L, 12 DN and 10 Normal were analyzed for IL-17A, 10 L, 10 DN and 5 Normal for IL-22, 12 L, 12 DN and 8 Normal for IFN-γ and 11 L, 9 DN and 7 Normal for TNF-α. L: lesion; DN: disease-naïve non-lesion. $^{**}p < 0.01$, $^{***}p < 0.001$. Error bar: mean ± SD.

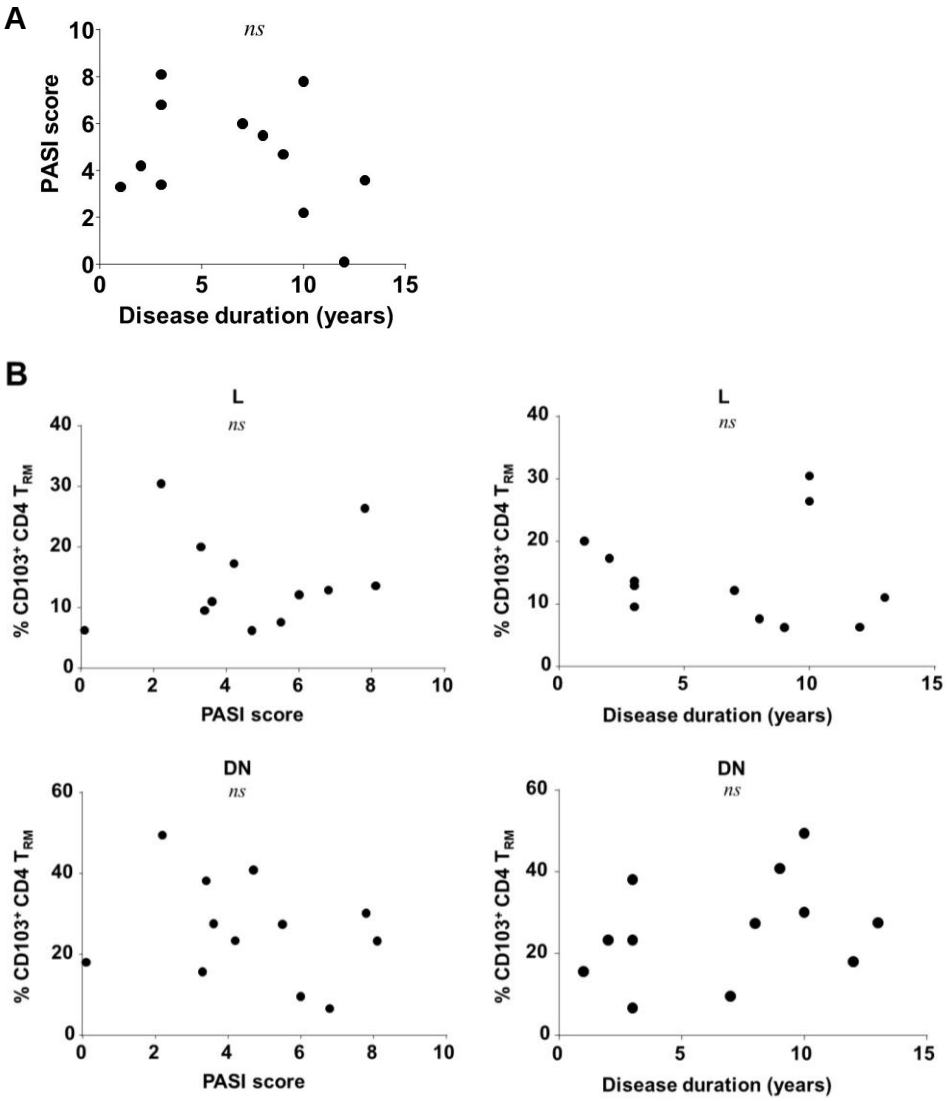

Figure 9A, B: No correlation between PASI score or disease duration and CD103$^+$ CD4 T_{RM} or CD103$^+$ CD8 T_{RM}. (A) PASI score did not correlate with disease duration (top left). (B) The middle panels showed no correlation between PASI score or disease duration and CD103$^+$ CD4 T_{RM} in psoriatic lesional skin. In addition, correlation between PASI score or disease duration and CD103$^+$ CD4 T_{RM} in DN sites was not observed (the lower panels). L: lesion; DN: disease-naïve non-lesion. N=12.

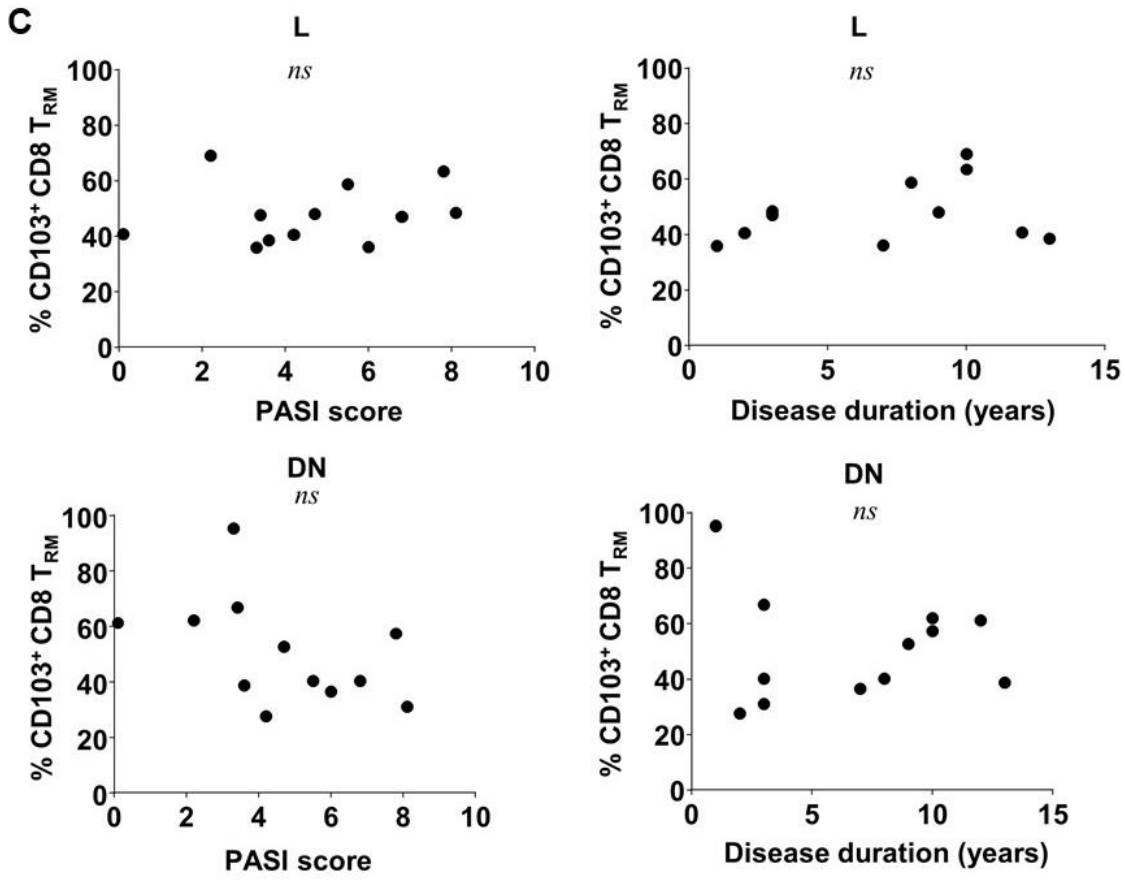

Figure 9C: No correlation between PASI score or disease duration and CD103$^+$ CD4 T_{RM} or CD103$^+$ CD8 T_{RM}. (C) The upper panels showed no correlation between PASI score or disease duration and CD103$^+$ CD8 T_{RM} in psoriatic lesional skin. In addition, correlation between PASI score or disease duration and CD103$^+$ CD8 T_{RM} in DN sites was not observed (the lower panels). L: lesion; DN: disease-naïve non-lesion. N=12.

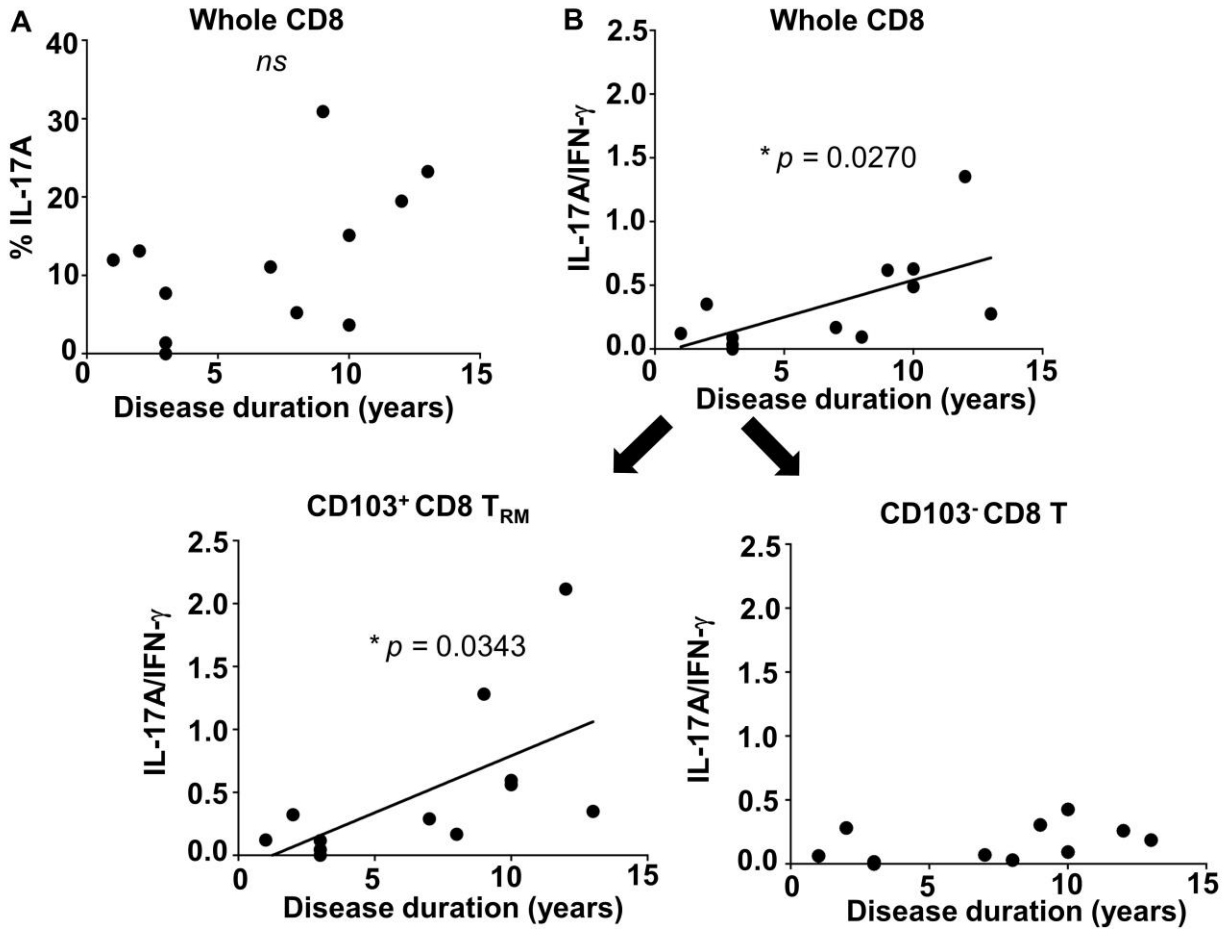

Figure 10: The ratio of IL-17A/IFN-γ in CD103$^+$ CD8 T_{RM} was correlated with disease duration. (A) Top left: The relation of disease duration and %IL-17A production in whole CD8 T cells from DN. (B) Top right: The ratio of %IL-17A-producing cells/%IFN-γ-producing cells in whole CD8 T cells from DN. In CD8 T cells, this IL-17A/IFN-γ production ratio in CD103$^+$ CD8 T_{RM} (left) and CD103$^-$ CD8 T cells (right) from DN sites of 12 psoriasis patients are shown according to disease duration.

TABLES

Table 1: Description of the normal subjects

Normal	
Age	Gender
84	M
49	F
68	M
72	M
66	F
72	M
56	M
87	F
64	F
96	F
72	F
57	F
54	M
37	M
79	M

Table 2: The ratio of CD4/CD8 T cells in psoriatic lesional, DN sites and normal skin

Skin specimens	The ratio of CD4/CD8 T cells (Mean ± SD)
Lesional skin	2.3 ± 1.7
DN skin	1.9 ± 1.2
Normal skin	3.1 ± 2.5

Table 3: Comparative data of the expression of $CD103^+$ T_{RM} in CD4 and CD8 T cells

	Percentage of $CD103^+$ in difference groups (Mean ± SD)	
	In CD4 T cells	In CD8 T Cells
Lesional skin	14.4 ± 7.8	47.8 ± 10.8
DN skin	25.9 ± 12.7	50.8 ± 19.1
Normal skin	35.8 ± 12.4	28.3 ± 11.2

Table 4: CD8 T cells counted within 100 μm from immunofluorescence-stained pictures

Skin specimens	Number of CD8 T cells in different groups (Mean ± SD)		
	Whole skin	Epidermis	Dermis
Lesional skin	52.2 ± 13.4	34.2 ± 12.1	18.0± 4.4
DN skin	20.4 ± 3.4	15.6 ± 2.3	4.8 ± 1.3
Normal skin	5.0 ± 1.2	3.2 ± 0.8	1.8 ± 0.8

Table 5: $CD103^+$ CD8 T_{RM} cells counted within 100 μm from immunofluorescence-stained pictures

Skin specimens	Number of $CD103^+$ CD8 T_{RM} cells in different groups (Mean ± SD)	
	Epidermis	Dermis
Lesional skin	18.0 ± 5.2	6.0 ± 3.1
DN skin	9.8 ± 3.7	3.4 ± 2.1
Normal skin	2.8 ± 0.8	1.0 ± 1.0

Table 6: Comparative data of IL-17A, IL-22, TNF-α, and IFN-γ-producing cells in CD4 and CD8 fractions

Skin specimens	Percentage of CD4 or CD8 in difference groups (Mean ± SD)							
	IL-17A		IL-22		IFN-γ		TNF-α	
	CD4 cells	CD8 cells	CD4 cells	CD8 cells	CD4 cells	CD8 cells	CD4 cells	CD8 cells
Lesional skin	11.0 ± 12.3	11.9 ± 8.8	10.7 ± 10.2	7.4 ± 7.5	21.9 ± 16.0	48.5 ± 25.9	68.3 ± 24.2	80.0 ± 19.0
DN skin	10.7 ± 10.6	11.9 ± 9.3	14.0 ± 11.8	10.2 ± 6.2	20.1 ± 12.0	49.2 ± 30.1	77.6 ± 22.2	83.3 ± 12.6
Normal skin	8.4 ± 7.4	0.5 ± 0.8	11.6 ± 6.0	3.6 ± 2.9	22.9 ± 11.0	44.9 ± 19.4	66.2 ± 20.0	84.3 ± 6.9

Table 7: Percentage of cytokine production capacity of $CD103^+$ CD8 T_{RM} and $CD103^-$ CD8 T cells (Mean ± SD)

	Skin specimens	IL-17A	IL-22	IFN-γ	TNF-α
In $CD103^+$ CD8 T_{RM}	Lesional skin	18.6 ± 12.3	10.5 ± 10.5	50.0 ± 26.0	81.2 ± 19.3
	DN skin	17.5 ± 14.1	13.3 ± 7.8	54.5± 30	83.3 ± 11.3
	Normal skin	0.9 ± 1.9	5.3 ± 3.7	45.9 ± 17.3	83.6 ± 6.6
In $CD103^-$ CD8 T cells	Lesional skin	2.7 ± 2.5	3.7 ± 3.7	44.1 ± 27.2	76.0 ± 20.0
	DN skin	5.3 ± 6.3	6.1 ± 8.5	43.0 ± 32.2	81.1 ± 16.5
	Normal skin	0.05 ± 0.1	1.8 ± 1.8	43.1 ± 22.6	83.7 ± 9.2

SOURCE

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