Cd8 $\alpha\alpha$ +T Cells Exert a Pro-inflammatory Role in Patients With Psoriasis

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Abstract

Psoriasis is a common chronic inflammatory disease caused by excessive activation of CD4⁺T cells, including Th17, Th1, and Th22. The role of CD8⁺T cells in psoriasis pathogenesis remains poorly understood. In this study, we aimed to identify the phenotype of CD8⁺T cells in patients with psoriasis and to investigate its role in the formation of lesions. First of all, we identify CD8αα⁺T cell as a main subset of CD8⁺T cells infiltrated in lesions of patients with psoriasis. Furthermore, we found that epidermal CD8αα⁺T cells exhibited tissue-resident memory T cells (T_{RM}) phenotypes and dermal CD8αα⁺T cells exhibited effector memory (T_{EM}) phenotypes in psoriatic lesions. Thereafter, by coculturing CD8αα⁺T cells with autogenous CD4⁺T cells to investigate the function of CD8αα⁺T cells, we found that CD8αα⁺T cells from patients with psoriasis could promote the proliferation of CD4⁺T effector cells; while CD8αα⁺T cells from healthy controls exerted immunosuppressive function. Finally, we demonstrate that CD8αα⁺T cells from patients with psoriasis did not express the markers of regulatory T cells, and produce IL-17 and IFN-γ. In conclusion, our findings demonstrate that CD8αα⁺T cells contribute to the pathogenesis of psoriasis by producing pro-inflammatory factors.

Introduction

Psoriasis is a common T cell-mediated chronic inflammatory disorder. It is generally accepted that excessive activation of CD4⁺T cells is critical for psoriasis pathogenesis via production of interleukin-17 (IL-17), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) ¹. Recent studies have shown that in addition to CD4⁺T cells, γδT cells, dendritic cells, neutrophils and CD8⁺T cells are also found to produce pro-inflammatory factors in patients with psoriasis ²⁻⁴.

CD4 and CD8 are expressed on the surface of T cells as co-receptors to enhance sensitivity to antigens. CD8 is different from CD4 in that it functions as a dimer, including homodimer CD8αα and heterodimer CD8αβ. CD8αα has been found in different immune cells, including T cells, dendritic cells, and natural killing cells ^{5,6}. CD8αβ is specifically expressed in T cells, which is called cytotoxic T cells. CD8αβ is a superior T-cell coreceptor that recruits T-cell receptors (TCRs) to lipid rafts and enhances the interaction of TCR-CD3 activation complexes with the major histocompatibility complex (MHC) class I. However, CD8αα is excluded from lipid rafts and negatively regulates TCR activation by disrupting lipid rafts and sequestering signaling molecules required for TCR-initiated downstream signaling ^{7,8}.

Previous studies have shown that CD8αα⁺T cells are found in the gut epithelium and can interact with intestinal epithelial cells by CD8αα binding to Thymus leukemia (TL, non-classical MHC class I molecules). CD8αα⁺T cells function as regulatory T cells in the intestinal mucosa, although, they do not express Foxp3 ^{9,10}. Additionally, unlike CD8αβ⁺T cells, TCR of CD8αα⁺T cells is oligoclonal and recognizes peptide fragments from T cell receptors or MHC. Thus, CD8αα⁺T cells exert a suppressive role by targeting effector T cells or antigen-presenting cells ¹¹. However, whether CD8αα⁺T cells contribute to

the pathogenesis of psoriasis has not been clarified. In our study, we investigate the phenotypes of CD8 $\alpha\alpha$ ⁺T cells in patients with psoriasis and its role in lesion formation.

Results

Increased CD8 $\alpha\alpha$ ⁺T cells located in psoriatic lesions

Previous studies have shown that CD8⁺T cells infiltrate psoriatic lesions by detecting CD8 α expression. However, CD8 α is not only expressed in CD8⁺T cells, but also in NK cells, $\gamma\delta$ T cells, and dendritic cells (DC). We performed immunofluorescence staining of CD8 α with markers for T cells, dendritic cells, and NK cells. The results showed that CD8 α co-expressed with CD3 and TCR α , but not with TCR δ , CD11c, or CD56 (Fig. S1 and 1A), which suggested that CD8⁺T cells, and not $\gamma\delta$ T cells, DC, or NK cells, infiltrated psoriatic lesions.

To investigate the subset of CD8⁺T cells in psoriatic lesions, we detected CD8 α and CD8 β expression by immunofluorescence staining. We found that the proportion of CD8 $\alpha\alpha$ ⁺T cells markedly increased in psoriatic lesions ($88.48\% \pm 7.39\%$), and infiltrated both the dermis and epidermis. Whereas, in normal skin, we found that CD8 $\alpha\beta$ ⁺T cells were predominantly scattered throughout the derma-epidermal junction (Fig. 1B-1C).

Next, we investigated the frequency of circulating CD8 $\alpha\alpha$ ⁺T cells in patients with psoriasis using flow cytometry. As shown in Figure 1D, the frequency of CD8 $\alpha\alpha$ ⁺T cells were comparable in patients with psoriasis and healthy controls (Figure 1E, $6.54\% \pm 1.81\%$ vs. $5.48\% \pm 1.04\%$, $P > 0.05$). There was no correlation between frequency of psoriatic circulating CD8 $\alpha\alpha$ ⁺T cells and the PASI score (Fig. 1F, $R^2 = 0.008$, $P = 0.9$). Thus, these data indicated that lesional CD8 $\alpha\alpha$ ⁺T cells, but not CD8 $\alpha\beta$ ⁺T cells, contribute to pathogenesis of psoriasis.

Epidermal CD8 $\alpha\alpha$ ⁺T cells exhibit tissue-resident memory T cells (T_{RM}) phenotypes and dermal CD8 $\alpha\alpha$ ⁺T cells exhibit effector memory (T_{EM}) phenotypes in psoriatic lesions

We investigated CD8 $\alpha\alpha$ ⁺T cell phenotypes in patients with psoriasis using the immunofluorescence assay. We found that approximate 90% of the CD8 $\alpha\alpha$ ⁺T cells did not express CD45RA and CCR7 in psoriatic lesions. In normal skin, CD8⁺T cells did not express CCR7, but partially expressed CD45RA (Fig. S2A-S2B). CD103 and CD69, identified as tissue-resident memory markers, were expressed in T_{RM} cells. Notably, we found that more than 95% of the CD8 $\alpha\alpha$ ⁺T cells expressed CD103 in the epidermis of patients with psoriasis, but approximate 1.7% of the CD8 $\alpha\alpha$ ⁺T cells expressed CD103 in the dermis (Fig. 2A-2B). Unexpectedly, different from CD103 expression, approximate 17.2% of the CD8 $\alpha\alpha$ ⁺T cells expressed CD69 in the epidermis of patients with psoriasis, but approximate 38.3% of the CD8 $\alpha\alpha$ ⁺T cells expressed CD103 in the dermis (Fig. 2C-2D).

Thus, these results indicate that psoriatic epidermal CD8 $\alpha\alpha$ ⁺T cells were predominantly T_{RM} cells which exhibited the CD69⁺/CD103⁺ phenotype and psoriatic dermal CD8 $\alpha\alpha$ ⁺T cells were T_{EM} cells which exhibited the CD69⁺/CD103⁻CCR7⁻CD45RA⁻ phenotype.

CD8 $\alpha\alpha$ ⁺T cells do not exhibit a regulatory role in psoriasis

CD8 $\alpha\alpha$ ⁺T cells expressed Treg cell markers and exhibited a regulatory role in intestinal mucosa. To determine whether CD8 $\alpha\alpha$ ⁺T cells have a suppressive immune function in psoriasis, we detected the expression of Treg cell markers in psoriatic CD8 $\alpha\alpha$ ⁺T cells. Immunofluorescence showed that CD8 $\alpha\alpha$ ⁺T cells expressed little CD122, and did not express Foxp3 and CD25 in psoriatic lesions (Fig. 3).

For verification, we used an *in vitro* lymphocyte co-culture model of CD8 $\alpha\alpha$ ⁺T cells and CD4⁺T cells. We analyzed the suppressive function of CD8 $\alpha\alpha$ ⁺T cells by assessing the proliferation of CD4⁺T cells using flow cytometry. We found that CD8 $\alpha\alpha$ ⁺T cells enhanced the proliferation of CD4⁺T cells in patients with psoriasis but inhibited its proliferation in healthy controls (Fig. 4A-4C). These results suggest that CD8 $\alpha\alpha$ ⁺T cells exhibit a pro-inflammatory role in psoriasis, but not a suppressive function.

CD8 $\alpha\alpha$ ⁺T cells exhibit pro-inflammatory activity in psoriasis

To further investigate the mechanism of how CD8 $\alpha\alpha$ ⁺T cells exhibited a pro-inflammatory function, we detected cytokines produced by CD8 $\alpha\alpha$ ⁺T cells in psoriasis. We found that CD8 $\alpha\alpha$ ⁺T cells could produce IL-17A and IFN- γ in lesions of patients with psoriasis but did not express these cytokines in normal skin (Fig. 5A-5B). Additionally, CD8 $\alpha\alpha$ ⁺T cells from psoriatic peripheral blood produced significantly higher levels of IL-17A and IFN- γ than those from healthy controls (Fig. 5C-5F). Moreover, CD8 $\alpha\alpha$ ⁺T cells produce higher levels of IL-17A and IFN- γ than CD8 $\alpha\beta$ ⁺T cells, both in peripheral blood of patients with psoriasis and healthy controls. Thus, these results suggest that CD8 $\alpha\alpha$ ⁺T cells exhibit a pro-inflammatory function by producing IL-17A and IFN- γ in psoriasis.

Discussion

The current study investigated the function of CD8 $\alpha\alpha$ ⁺T cells in psoriasis. We found that the proportion of CD8 $\alpha\alpha$ ⁺T cells were markedly increased in psoriatic lesions. In addition, psoriatic epidermal CD8 $\alpha\alpha$ ⁺T cells were predominantly CD69⁺/CD103⁺CCR7⁻CD45RA⁻T_{RM} cells, whereas psoriatic dermal CD8 $\alpha\alpha$ ⁺T cells were CD69⁺/CD103⁻CCR7⁻CD45RA⁻T_{EM} cells. Moreover, CD8 $\alpha\alpha$ ⁺T cells exhibited a pro-inflammatory role in psoriasis by producing IL-17A and IFN- γ . Therefore, our results indicate that CD8 $\alpha\alpha$ ⁺T_{RM} cells contribute to psoriasis by producing pro-inflammatory cytokines.

Previous studies have shown that dense infiltration of CD8⁺T cells are found in the psoriatic lesions. CD8⁺T cells have been identified as a potent source of pro-inflammatory cytokines in psoriasis^{2,12}. However, these studies solely identify CD8⁺T cells by detecting CD8 α expression. CD8 α is not only

expressed on T cells, but also on the surface of NK cells, DC and $\gamma\delta$ T cells. Thus, by performing immunofluorescence, we found that the identity of infiltrating CD8⁺ cells was not NK cells, DC, or $\gamma\delta$ T cells, but TCR $\alpha\beta$ ⁺T cells. Furthermore, CD8 $\alpha\alpha$ ⁺T cells primarily infiltrated psoriatic lesions, and not CD8 $\alpha\beta$ ⁺T cells.

Like a subpopulation of Th cells, according to different kinds of cytokine production, CD8⁺T cells can be divided into Tc1, Tc2, Tc17, and Tc22. Additionally, unlike the CD4 oligomer, CD8 can be expressed as a CD8 $\alpha\alpha$ homodimer and CD8 $\alpha\beta$ heterodimer on the surface of CD8⁺T cells⁶. Although CD8 $\alpha\alpha$ and CD8 $\alpha\beta$ bind similarly to MHC class I molecules, CD8 β endows CD8 with coreceptor function. Indeed, CD8 $\alpha\beta$, but not CD8 $\alpha\alpha$, associated with TCR/CD3, strengthens pMHC binding, and promotes CD8 association with lipid rafts and p56^{lck} and hence TCR signaling. CD8 $\alpha\alpha$ excluded from lipid rafts, and negatively regulates TCR activation¹³. As we know, CD8 $\alpha\beta$ expressed on the surface of T cells is also called cytotoxic T cells. However, CD8 $\alpha\alpha$ is expressed on T cells, NK cells, DC, and $\gamma\delta$ T cells. CD8 $\alpha\alpha$ ⁺T cells were first found to be infiltrating the intestine of mouse, and contributed to the pathogenesis of intestinal diseases associated with infection and inflammation¹¹. Little is known about its role in human bodies. Jia Zhu et.al. found that CD8 $\alpha\alpha$ ⁺T cells persisted in the genital skin and mucosa at the derma-epidermal junction to exhibit an immune surveillance role in HSV infection¹⁴. Consistent with this study, we found markedly increased number of CD8 $\alpha\alpha$ ⁺T cells in the lesions of psoriasis, and they have a pro-inflammatory function.

In the mouse model of inflammatory bowel disease, CD8 $\alpha\alpha$ ⁺T cells were found to play an immunosuppressive role. As mentioned above, CD8 $\alpha\alpha$ can negatively regulate TCR activation by interacting with its ligand, TL, which is abundantly expressed by intestinal epithelial cells. Although Foxp3 and CD25 (IL-2 receptor subunit α) were identified as markers of Treg cells, they are not expressed in CD8 $\alpha\alpha$ ⁺T cells^{9,10}. However, in the intestine of mice, CD122 (IL-2/15 receptor subunit β) is found on the surface of CD8 $\alpha\alpha$ ⁺T cells and is necessary for cell survival. Consistent with previous studies, we found that Foxp3 and CD25 were negatively expressed in CD8 $\alpha\alpha$ ⁺T cells of patients with psoriasis, and unexpectedly, CD122 was also expressed little.

Furthermore, previous studies have shown the immunoregulatory role of CD8 $\alpha\alpha$ ⁺T cells in some animal models. Non-obese diabetic (NOD) mice are defective in CD8 $\alpha\alpha$ ⁺T cell generation¹⁵. Additionally, transfer of CD8 $\alpha\alpha$ ⁺T cells can inhibit colitis, induced by the adoptive transfer of CD4 T cells into severe combined immunodeficient (SCID) mice¹⁶. Unlike CD4⁺Treg cells, the mechanisms underlying the immunosuppressive function of CD8 $\alpha\alpha$ ⁺T cells were that they recognized pathogenic TCR-derived peptides, which is presented on the MHC-I Qa-1 molecule of pathogenic T cells in an activation-dependent manner^{8,17,18}. Therefore, to further determine whether CD8 $\alpha\alpha$ ⁺T cells have an immunosuppressive function in psoriasis, we cocultured CD8 $\alpha\alpha$ ⁺T cells with auto-CD4⁺T cells. Consistent with previous studies, CD8 $\alpha\alpha$ ⁺T cells from healthy subjects suppressed the proliferation of auto-CD4⁺T cells, but promoted its proliferation in patients with psoriasis. Thus, our results indicate that CD8 $\alpha\alpha$ ⁺T cells play a pro-inflammatory role in psoriasis, instead of an immunosuppressive function.

In mice, CD8 $\alpha\alpha$ ⁺T cells are identified as T_{RM} cells, which persist in the same sites of intestinal epithelium for a long time, noncirculating and largely maintained independently of circulating populations¹¹. These T_{RM} cells are phenotypically heterogeneous, and there are no perfect markers of tissue residency; the most commonly associated markers are CD69 (c-type lectin), CD103 (an E-cadherin receptor), CD49a, CXCR3/6. Additionally, unlike central memory (T_{CM}) and T_{EM} cells, T_{RM} cells do not express CCR7 (a lymph node homing receptor) and CD62L (L-selectin)¹⁹⁻²¹. Previous studies have shown that CD103⁺CD8⁺T_{RM} cells and CD69⁺CD8⁺T_{RM} cells infiltrated in psoriatic lesions and non-lesional psoriatic skin; they are even found in the cleared skin after treatment and could produce IL-17, IFN- γ and IL-22 persistently^{22,23}. Cheuk et al. reported that CD49a⁺CD8⁺T_{RM} cells from psoriatic lesions predominantly generated the IL-17 response, whereas CD49a⁺CD8⁺T_{RM} cells preferentially produced IFN- γ , and displayed high cytotoxic capacity contributing to vitiligo²⁴. In our study, we found that more than 95% of the CD8⁺T cells in psoriatic lesions are of the CD8 $\alpha\alpha$ ⁺ phenotype, and identified a new phenotype of T_{RM} cells, CD103⁺CD8 $\alpha\alpha$ ⁺, in the epidermis of psoriatic lesions. Additionally, CD103⁺CD8 $\alpha\alpha$ ⁺T cells in the dermis may be not T_{RM} cells. Although CD69 be considered to be marker of T_{RM} cells, it can also be expressed in T effector cells instantaneously. We found that CD69 partially expressed both in CD103⁺CD8 $\alpha\alpha$ ⁺ T_{RM} cells in epidermis and CD103⁺CD8 $\alpha\alpha$ ⁺T_{EM} cells in psoriasis lesions. Moreover, CD8 $\alpha\alpha$ ⁺T cells from both epidermis and dermis do not express CCR7 and CD45RA (a naïve T cell marker) in psoriatic lesions. Thus, our results indicate that CD8 $\alpha\alpha$ ⁺T cells, as a subset of T_{RM} cells in epidermis, contribute to the pathogenesis of psoriasis.

However, there are still some limitations in our study. Because it is difficult to obtain enough skin tissue from patients with psoriasis, we just performed immunofluorescence to identify the phenotype of CD8 $\alpha\alpha$ ⁺ T cells in lesions. Flow cytometry will be better to identify the phenotype of lesional CD8 $\alpha\alpha$ ⁺ T cells. In addition, although we found CD103⁺CD8 $\alpha\alpha$ ⁺ T_{RM} cells infiltrated the epidermis of psoriasis, some of them did not express CD69. Difference of CD69⁺CD103⁺CD8 $\alpha\alpha$ ⁺T_{RM} cells from CD69⁺CD103⁺CD8 $\alpha\alpha$ ⁺ T_{RM} cells should be further investigated.

In conclusion, our results suggested that CD8 $\alpha\alpha$ ⁺T cells exhibited an immunosuppressive function in healthy controls and exhibited the tissue-resident memory phenotype in psoriatic epidermis, playing a pro-inflammatory role by producing elevated levels of IL-17A and IFN- γ . CD8 $\alpha\alpha$ ⁺T cells in the dermis may redirect from peripheral blood exhibiting the phenotype of T_{EM} cells to contribute to the pathogenesis of psoriasis.

Methods

Patients and samples

This study was approved by the ethical committee of Xijing Hospital of the Fourth Military Medical University. All healthy volunteers and patients with psoriasis agreed to participate in this study and

provided written informed consent. Twenty-milliliter blood samples were collected from each patient in the progressive stage with mild-to-moderate psoriasis (psoriasis area and severity index, PASI $\geq$ 5), or severe psoriasis (PASI $\geq$ 12), or who was a healthy control (Supplementary Table S1). Normal skin and psoriatic lesions were obtained from patients via biopsy. All patients were diagnosed clinically and pathologically. Patients did not receive any topical treatment for a minimum of 1 month and were naïve to photo(chemo)therapy or systemic treatments for psoriasis. All patients and controls were otherwise healthy and did not use any other systemic medications.

Immunofluorescence analysis

Immunofluorescence was performed on paraffin-embedded 5- μ m sections of skin samples. Following paraffin removal, epitope retrieval, and blocking in phosphate buffered saline/4% bovine serum albumin, sections were incubated with primary antibodies for 16 hours at 4°C. Thereafter, secondary antibodies were added and incubated for 1 hour at room temperature in the dark. Nuclei were stained with Hoechst (Beyotime, Shanghai, China, at a dilution of 1:1000) for 10 minutes at room temperature in the dark. Immunofluorescence staining was analyzed using an OLYMPUS FV10 confocal laser scanning microscope (CLSM) 510 system (Carl Zeiss AB, Jena, Germany). Primary and secondary antibodies are listed in Supplementary Table S2.

Flow cytometry

To detect cytokine production, peripheral blood mononuclear cells (PBMCs) were incubated with lipopolysaccharides for 12 hours to activate monocytes exhibiting antigen presentation, followed by a cell stimulation cocktail (eBioscience, CA, USA) for 4 hours to amplify the immune response and maintain cytokines intracellularly. PBMCs were incubated with anti-human CD3-PE/CY7, anti-human CD8 α -PE/CY5 and anti-human CD8 β -super bright 436 (eBioscience, CA, USA) at 4°C for 30 minutes. After fixation and permeabilization using fixation/permeabilization solution and permeabilization buffer (all from eBioscience, CA, USA), cells were incubated with anti-human IL-17A-PE or anti-human IFN- γ -PE at 4°C for 30 minutes. Then, flow cytometric analysis using FACS Calibur (BD Biosciences, USA). Data were analyzed with the BD FACS Diva software (BD Biosciences). Primary and secondary antibodies are listed in Supplementary Table S2.

T cell isolation and sorting

Peripheral blood mononuclear cells were separated using Ficoll-Hypaque (Dakewei, Shenzhen, China). Cells were labeled with anti-human CD3-PECY7, anti-human CD4-APC, anti-human CD8 α -PE, and anti-human CD8 β -super bright 436. Thereafter, CD3⁺CD4⁺Tresp cells, CD3⁺CD8 α ⁺CD8 β ⁺T cells, and CD3⁺CD8 α ⁺CD8 β ⁺T cells were purified using fluorescence-activated cell sorting (FACS; Moflo XDP, Beckman, CA, USA) at purity >95%. Isolated cells were subsequently cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; Gibco, CA, USA).

In vitro function assay of CD8 $\alpha\alpha$ ⁺T cells

To investigate the suppressive function of CD8 $\alpha\alpha$ ⁺T cells, FACS-sorted CD4⁺T cells from healthy subjects and patients with psoriasis (5×10^4 per well) were labeled with carboxyfluorescein succinimidyl ester (CFSE), and subsequently cocultured separately with CD8 $\alpha\alpha$ ⁺T cells (5×10^4 per well) isolated from the same subject in 96-well U-bottom plates. Thereafter, dynabeads human T-activator CD3/CD28 at a bead-to-cell ratio of 1:1 and rIL-2 (3 μ g/mL) were added to cocultures and incubated for 96 hours at 37°C. The proliferation of CD4⁺T cells was then analyzed using flow cytometry based on the CFSE dilution.

Statistical analysis

Data analysis was performed using the GraphPad Prism version 7.0 software (GraphPad Software, San Diego, CA). Statistically significant differences between two groups were determined by two-tailed t-tests. P-values of less than 0.05 were considered statistically significant. Data are expressed as the mean standard error.

Abbreviations

T_{RM} cells, tissue-resident memory T cells; T_{EM} cells, effector memory; IL-17, interleukin-17; IFN- γ , interferon- γ ; TNF- α , tumor necrosis factor- α ; TCRs, T-cell receptors; MHC, major histocompatibility complex; TL, Thymus leukemia; PASI, psoriasis area and severity index; DC, dendritic cells;

Declarations

Ethics and consent

We declare that all methods were performed in accordance with relevant guidelines and regulations.

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Author Contributions

Yangyang Zhang, Yiting Lin and Lei Wang performed the research, and analyzed the data and wrote the manuscript; Xiuwen Sun performed the research; Erle Dang and Ke Xue analyzed the data; Weigang Zhang, Qian Zhang, Junqin Li and Kaiming Zhang contribute essential reagents or tools; Gang Wang and Bing Li designed the research study, analyzed the data and wrote the manuscript.

Conflicts of interest

The authors have no potential conflict of interest to declare.

References

- 1 Lowes, M. A., Suarez-Farinas, M. & Krueger, J. G. Immunology of psoriasis. *Annu Rev Immunol***32**, 227-255, doi:10.1146/annurev-immunol-032713-120225 (2014).
- 2 Hijnen, D. *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***133**, 973-979, doi:10.1038/jid.2012.456 (2013).
- 3 Di Cesare, A., Di Meglio, P. & Nestle, F. O. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. *J Invest Dermatol***129**, 1339-1350, doi:10.1038/jid.2009.59 (2009).
- 4 Jeschke, J. C. & Williams, C. B. Editorial: Crossing the divide: a novel Cd8 enhancer with activity in CTLs and CD8alphaalpha+ dendritic cells. *J Leukoc Biol***97**, 623-625, doi:10.1189/jlb.2CE1214-606R (2015).
- 5 Wu, J. *et al.* Identification of a bone marrow-derived CD8alphaalpha+ dendritic cell-like population in inflamed autoimmune target tissue with capability of inducing T cell apoptosis. *J Leukoc Biol***88**, 849-861, doi:10.1189/jlb.0310133 (2010).
- 6 Cheroutre, H. & Lambolez, F. Doubting the TCR coreceptor function of CD8alphaalpha. *Immunity***28**, 149-159, doi:10.1016/j.immuni.2008.01.005 (2008).
- 7 Rybakina, V., Clamme, J. P., Ampudia, J., Yachi, P. P. & Gascoigne, N. R. CD8alphaalpha and - alphabeta isotypes are equally recruited to the immunological synapse through their ability to bind to MHC class I. *EMBO Rep***12**, 1251-1256, doi:10.1038/embor.2011.209 (2011).
- 8 Fanchiang, S. S. *et al.* Global expression profiling of peripheral Qa-1-restricted CD8alphaalpha+TCRalphabeta+ regulatory T cells reveals innate-like features: implications for immune-regulatory repertoire. *Hum Immunol***73**, 214-222, doi:10.1016/j.humimm.2011.07.306 (2012).
- 9 Qiu, Y., Peng, K., Liu, M., Xiao, W. & Yang, H. CD8alphaalpha TCRalphabeta Intraepithelial Lymphocytes in the Mouse Gut. *Dig Dis Sci***61**, 1451-1460, doi:10.1007/s10620-015-4016-y (2016).
- 10 Olivares-Villagomez, D. & Van Kaer, L. TL and CD8alphaalpha: Enigmatic partners in mucosal immunity. *Immunol Lett***134**, 1-6, doi:10.1016/j.imlet.2010.09.004 (2010).
- 11 Ruscher, R. & Hogquist, K. A. Development, ontogeny, and maintenance of TCRalphabeta(+) CD8alphaalpha IEL. *Curr Opin Immunol***58**, 83-88, doi:10.1016/j.coi.2019.04.010 (2019).
- 12 Di Meglio, P. *et al.* Targeting CD8(+) T cells prevents psoriasis development. *J Allergy Clin Immunol***138**, 274-276 e276, doi:10.1016/j.jaci.2015.10.046 (2016).

- 13 Bai, L. & Peng, H. Generating CD8alphaalpha IELs from two sources of thymic precursors. *Cell Mol Immunol***15**, 640-641, doi:10.1038/cmi.2017.93 (2018).
- 14 Zhu, J. *et al.* Immune surveillance by CD8alphaalpha+ skin-resident T cells in human herpes virus infection. *Nature***497**, 494-497, doi:10.1038/nature12110 (2013).
- 15 Holler, P. D. *et al.* The same genomic region conditions clonal deletion and clonal deviation to the CD8alphaalpha and regulatory T cell lineages in NOD versus C57BL/6 mice. *Proc Natl Acad Sci U S A***104**, 7187-7192, doi:10.1073/pnas.0701777104 (2007).
- 16 Poussier, P., Ning, T., Banerjee, D. & Julius, M. A unique subset of self-specific intrainestinal T cells maintains gut integrity. *J Exp Med***195**, 1491-1497, doi:10.1084/jem.20011793 (2002).
- 17 Tang, X. *et al.* Regulation of immunity by a novel population of Qa-1-restricted CD8alphaalpha+TCRalphabeta+ T cells. *J Immunol***177**, 7645-7655, doi:10.4049/jimmunol.177.11.7645 (2006).
- 18 Sheng, H. *et al.* Distinct PLZF(+)CD8alphaalpha(+) Unconventional T Cells Enriched in Liver Use a Cytotoxic Mechanism to Limit Autoimmunity. *J Immunol***203**, 2150-2162, doi:10.4049/jimmunol.1900832 (2019).
- 19 Sasson, S. C., Gordon, C. L., Christo, S. N., Klenerman, P. & Mackay, L. K. Local heroes or villains: tissue-resident memory T cells in human health and disease. *Cell Mol Immunol***17**, 113-122, doi:10.1038/s41423-019-0359-1 (2020).
- 20 Wang, X., Tian, Z. & Peng, H. Tissue-resident memory-like ILCs: innate counterparts of TRM cells. *Protein Cell***11**, 85-96, doi:10.1007/s13238-019-0647-7 (2020).
- 21 Dijkgraaf, F. E. *et al.* Tissue patrol by resident memory CD8(+) T cells in human skin. *Nat Immunol***20**, 756-764, doi:10.1038/s41590-019-0404-3 (2019).
- 22 Vo, S. *et al.* CD8 resident memory T cells with interleukin 17A-producing potential are accumulated in disease-naïve nonlesional sites of psoriasis possibly in correlation with disease duration. *Br J Dermatol***181**, 410-412, doi:10.1111/bjd.17748 (2019).
- 23 Kurihara, K., Fujiyama, T., Phadungsaksawasdi, P., Ito, T. & Tokura, Y. Significance of IL-17A-producing CD8(+)CD103(+) skin resident memory T cells in psoriasis lesion and their possible relationship to clinical course. *J Dermatol Sci***95**, 21-27, doi:10.1016/j.jdermsci.2019.06.002 (2019).
- 24 Cheuk, S. *et al.* CD49a Expression Defines Tissue-Resident CD8(+) T Cells Poised for Cytotoxic Function in Human Skin. *Immunity***46**, 287-300, doi:10.1016/j.immuni.2017.01.009 (2017).

Figures

Figure 1

CD8α⁺T cells increasingly infiltrate into psoriatic lesions. (A, B) Representative immunofluorescence staining of CD8α co-expressed with TCRα, TCRδ and CD8β in psoriatic lesions and normal skin. (C) Proportion of CD8αβ⁺T and CD8α⁺T cells determined by analysis of immunofluorescence staining (n = 8). (D, E) Frequency of circulating CD8αβ⁺T and CD8α⁺T cells (bottom) in gated CD3⁺CD8⁺T cells (up) from patients with psoriasis (n = 32) and healthy controls (n = 16). (F) Frequency correlation of circulating CD8α⁺T cells with PASI scores of patients with psoriasis (n = 32). **P < 0.01.

Figure 2

Epidermal CD8 α +T cells exhibit TRM phenotypes. (A, C) Representative immunofluorescence staining of CD103 and CD69 expressed in CD8 α +T cells of psoriatic lesions and normal skin. (B, D) Proportion of CD103+CD8+TRM and CD69+CD8+TRM cells determined by analysis of immunofluorescence staining (n = 8). **P < 0.01.

Figure 3

CD8 α +T cells do not express markers of Treg cells in psoriatic lesions. Representative immunofluorescence staining of Foxp3, CD25, and CD122 expressed in CD8 α +T cells of psoriatic lesions and normal skin.

Figure 4

CD8αα⁺T cells exhibit a pro-inflammatory role in patients with psoriasis. (A) CD8αα⁺T cells from patients with psoriasis or healthy controls were cocultured with autogenous CD4⁺T cells. Proliferation of CD4⁺T cells were detected by flow cytometry, which could reflect the function of CD8αα⁺T cells. (B) Statistical analysis of flow cytometry. (C) Mean difference of CD4⁺T cell proliferation (proportion of proliferated CD4⁺T cells in the coculture group minus that in the group of autogenous CD4⁺T cells alone, n = 5). **P < 0.01.

Figure 5

CD8 α α ⁺T cells produce pro-inflammatory factors in patients with psoriasis. (A) Representative immunofluorescence staining of IL-17A and IFN- γ expressed in CD8 α α ⁺T cells of psoriatic lesions and normal skin. (B) Proportion of IL-17A⁺ CD8 α α ⁺T and IFN- γ ⁺CD8 α α ⁺T cells determined by analysis of immunofluorescence staining (n = 8). (C, D) The expression of IL-17A and IFN- γ in circulating CD8 α α ⁺T cells from patients with psoriasis (n = 32) and healthy controls (n = 16) by flow cytometry. (E, F) Statistical analysis of flow cytometry. *P < 0.05, **P < 0.01 and ***P < 0.001.

Supplementary Files

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