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Epidermal $CD8^+CD103^+$ skin resident memory T cells in psoriasis plaques are reduced in number but remain in the basement membrane zone after topical application of corticosteroid and vitamin D3

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To the editor

Psoriasis is a common chronic inflammatory skin disease with a mutually connected cytokine network [1]. The biologic antibodies for various cytokines are highly effective, but withdrawal of the biologics often results in the recurrence. Since psoriasis plaques often recur at the same sites [2], some immunocompetent cells remain in the previously affected, currently normal-appearing skin.

Skin resident memory T cells (T_{RM}) persist for a long term in the skin without recirculation. Various studies have suggested the pathogenetic role of skin T_{RM} in psoriasis [3,4]. Skin T_{RM} are also considered as a strong candidate that evokes recurrence [5]. Skin T_{RM} express tissue-retention markers, CD103 and CD69 [6]. The majority of T cells in the lesional epidermis in psoriasis are $CD8^+CD103^+$ T_{RM}, which have an intriguing cytokine production capacity for psoriasis [7]. The effects of biologics on T_{RM} have been studied [8] with their suppressive role on cytokines [9]. However, modulation of epidermal $CD8^+CD103^+$ T_{RM} by topical treatments remains unclear. In this study, we investigated the numerical changes of $CD8^+CD103^+$ T_{RM}, focusing on their epidermal distribution, in psoriasis lesions after topical application of corticosteroid, vitamin D3, and combination of these agents. We defined $CD8^+CD103^+$ T cells in the epidermis as $CD8^+CD103^+$ $T_{RM,}$ because most of the $CD8^+CD103^+$ T cells were also positive for CD69

(Supplemental Fig. S1). We also divided $CD4^+$ T cells in epidermis into two populations ($CD103^+$ or $CD103^-$), and examined their number and distribution in epidermis.

In our previous study, 10 patients with psoriasis vulgaris were registered [10]. All patients didn't receive any systemic therapy or ultraviolet therapy. Four psoriasis plaque sites that hadn't been topically treated for 2 weeks were selected, and betamethasone dipropionate ointment (Bet; Rinderon DP, Shionogi, Japan), calcipotriol ointment (Cal; Dovonex, LEO pharma, Ballerup, Denmark), or calcipotriol and betamethasone dipropionate 2-compound formulation (CB; Dovobet, LEO pharma) was applied once a day to 3 different areas with similar severity of psoriatic plaques for 2 weeks. The remaining unapplied lesion was used as a control. After 2 weeks treatment, 4-mm punch skin biopsies were performed from the four sites. The study protocol was approved by the ethical committee of Hamamatsu University School of Medicine (research number: 18-191). We obtained written informed consent from all participants.

Topical application of Bet, Cal, and CB cleared the psoriatic lesions to the similar extent, while the lesions in the control area were unchanged (Fig 1a). To investigate the alteration of $CD8^+CD103^+$ T_{RM} by each topical application, immunofluorescent staining was performed in biopsy sections as previously described [7] (Fig 1b, c), and the results were statistically analyzed (Supplemental Methods). We found that the numbers of total

$CD8^+$ and/or $CD4^+$ T cells in the epidermis were significantly reduced by each treatment, especially CB (Supplemental Fig S2a, b). More importantly, the numbers of $CD8^+CD103^+$ T_{RM} and $CD4^+CD103^+$ T cells in the epidermis were also significantly reduced in CB group (Fig 2a, c). We also examined the localization of $CD8^+CD103^+$ T_{RM}. After any of the three treatments, a considerable number of cells were localized in the epidermal basal layer (Supplemental Fig S3). We defined $CD8^+CD103^+T_{RM}$ attaching the epidermal basal cells as $CD8^+CD103^+T_{RM}$ in the basement membrane zone (BMZ) and calculated the percentage of this T_{RM} population among whole number of epidermal T_{RM}. Notably, the frequency of $CD8^+CD103^+$ T_{RM} in BMZ was significantly increased in CB-treated skin and tended to increase in Cal and Bet-applied skin (Fig 2b), despite the decreased number of $CD8^+CD103^+$ T_{RM} in BMZ in Bet- and CB-treated skin (Supplemental Fig S4). This tendency was not observed in $CD4^+CD103^+$ T cells (Fig 2c, d). The absolute numbers of $CD8^+CD103^-$ T cells and $CD4^+CD103^-$T cells were also reduced by CB treatment (Fig 2e, g), but their frequencies in BMZ was not affected (Fig 2f, h). These results suggest that $CD8^+CD103^+$ T_{RM} were relatively resistant to topical treatment, and the preferential change of T cell distribution toward BMZ by CB treatment specifically occur in $CD8^+CD103^+$ T_{RM}. Moreover, most of the $CD103^+$ T_{RM} in BMZ were CD49a negative by immunohistochemistry (Supplemental Fig S5), suggesting that

$CD103^+$ T_{RM} in BMZ were IL-17-producing T_{RM} [4]. To examine the molecular mechanisms by which $CD8^+CD103^+$ T_{RM} preferentially localize to BMZ, we performed an immunohistochemistry of E-cadherin, a ligand for CD103, in psoriatic lesions, and compared the expression levels between basal layer and the upper layer (spinous layer and granular layer) in epidermis. However, no apparent differences were observed in its expression levels between them (Supplemental Fig S6), suggesting that E-cadherin is not involved in the mechanisms of preferential localization of $CD8^+CD103^+$ T_{RM} in BMZ.

$CD8^+CD103^+T_{RM}$ produce essential cytokines for psoriasis pathogenesis and are deeply involved in relapse and treatment resistance [7]. It is an issue whether the topical therapies exert numerical and distributional effects on epidermal $CD8^+CD103^+$ T_{RM}. In our present study, $CD8^+CD103^+$ T_{RM} were reduced in number, but still present after the topical application, even though the skin lesions were clinically improved. It should be stressed that $CD8^+CD103^+$ T_{RM} preferentially remained in BMZ, especially after treatment with CB, which is one of the most effective topical therapies for psoriasis. Although the molecular mechanisms of preferential localization of $CD8^+CD103^+$ T_{RM} in BMZ remain unclear, $CD8^+CD103^+T_{RM}$ in BMZ may acquire chemotactic activities during psoriatic inflammation, leading to the appearance in upper epidermis. Thus, preferential localization of T_{RM} after treatment may not be specific phenomenon in CB.

Nevertheless, our results suggest that $CD8^+CD103^+$ T_{RM} remaining in BMZ are involved in the recurrence of psoriasis lesions.

The number of patients in this study was not large enough to compare the effect of each treatment, but there has been no study that evaluates the effect of each topical therapy on T_{RM}. Our results suggest that CB was most potent in reducing the number of $CD8^+CD103^+$ T_{RM} in epidermis, although $CD8^+CD103^+$ T_{RM} remained in BMZ even after the treatment. It is an interesting issue whether some highly permeable CB formulation with a capacity to penetrate the epidermis efficaciously reduces $CD8^+CD103^+$ T_{RM} in BMZ. Such a preparation will be a therapeutically important strategy in future.

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Figure Legends

Fig. 1. Clinical appearance and immunofluorescence study of skin-infiltrating T cells.

(a) Representative clinical pictures of psoriatic plaques before and 2 weeks after topical treatment in a patient. Bet: betamethasone dipropionate ointment, Cal: calcipotriol ointment, CB: calcipotriol and betamethasone dipropionate 2-compound formulation.

(b, c) Representative fluorescence immunostaining of T_{RM} in psoriasis lesions. (b) Double immunofluorescent staining for CD8 (red) and CD103 (green). (c) Double immunofluorescent staining for CD4 (red) and CD103 (green). The merged cells exhibit yellow.

Fig. 2. Numbers and frequencies of T cell populations in the epidermis and basement membrane zone (BMZ). The number of the cell per 1-mm horizontal length of the epidermal skin is indicated.

(a) Number of $CD8^+CD103^+$ T_{RM} in the epidermis. (b) Frequency of $CD8^+CD103^+$ T_{RM} in BMZ for total $CD8^+CD103^+$ T_{RM} in the epidermis. (c) Number of $CD4^+CD103^+$ T cells in the epidermis. (d) Frequency of $CD4^+CD103^+$ T cells in BMZ for total $CD4^+CD103^+$ T cells in the epidermis. (e) Number of $CD8^+CD103^-$ T cells in the epidermis. (f) Frequency of $CD8^+CD103^-$ T cells in BMZ for total $CD8^+CD103^-$ T cells in the epidermis.

(g) Number of $CD4^+CD103^-$ T cells in the epidermis. (h) Frequency of $CD4^+CD103^-$ T cells in BMZ for total $CD4^+CD103^-$ T cells in the epidermis.

P values were calculated using the Friedman test (n=10). *P < 0.05, **P < 0.01.