double knockout of Kcnk4 and Kcnk5, however, suggested that there may be some redundancy of K^+ channels in sweat glands, and sufficient activity remains or compensates when only one is lost. As a less likely speculative alternative, different channels might function differentially in different cells, with one cell type(s) able to increase its activity to compensate for the loss of function in another type.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2015.11.001.

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Letter to the Editor

Reciprocal contribution of Th17 and regulatory T cells in severe drug allergy

Keywords
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Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) are pathogenetically classified into the same entity characterized by epidermal necrosis with diversity of the affected area. Drug-induced hypersensitivity syndrome (DIHS), also called as drug rash with eosinophilia and systemic symptoms, is clinically distinct from SJS/TEN by a delayed onset after taking the inducing drug, severe cutaneous and extracutaneous organ involvement and the reactivation of human herpesviruses during disease course. Although cytotoxic $CD8^+$ T cells play a crucial role in the pathogenesis of both disease spectrums, the roles of $CD4^+$ T cells that collocate with $CD8^+$ T cells in skin lesions remain poorly understood. To clarify this issue, we immunologically investigated pathogenetic $CD4^+$ T cells from the skin lesions of these diseases and delineated their characteristics.

All patients (SJS/TEN, $n = 6$; DIHS, $n = 10$, supplementary Table E1) enrolled in this study were informed and agreed to participate. The skin-infiltrating T cell analysis was approved by the ethical committee of Hamamatsu Univirsity School of Medicine. We first expanded infiltrating (for SJS and DIHS) and blister-containing (for TEN) T cells from skin lesions using our previously established method [1] and generated drug-specific $CD4^+$ T cell clones (TCCs) from these cells. After stimulation with phorbol 12-myristate 13-acetate and ionomycin an intracellular interleukin (IL)-17 was stained with fluorescent-tagged antibody (R&D Systems, Minneapolis, MN, USA) in skin-infiltrating cells, and after stimulation with immobilized anti-CD3 mAb for 72 h, concentrations of IL-2, IL-4, IL5, IL-6, IL-8, IL-10, IL-17, interferon (IFN)-γ, and tissue necrosis factor (TNF)-α were measured in culture supernatants of $CD4^+$ TCCs by cytometric bead array assay (Th1/Th2/Th17 cytokine CBA and inflammation cytokine CBA kits; BD Biosciences).

Abbreviations: DIHS, drug-induced hypersensitivity syndrome; DRESS, drug rash with eosinophilia and systemic symptoms; SJS, Stevens–Johnson syndrome; TCCs, T cell clones; TEN, toxic epidermal necrolysis.

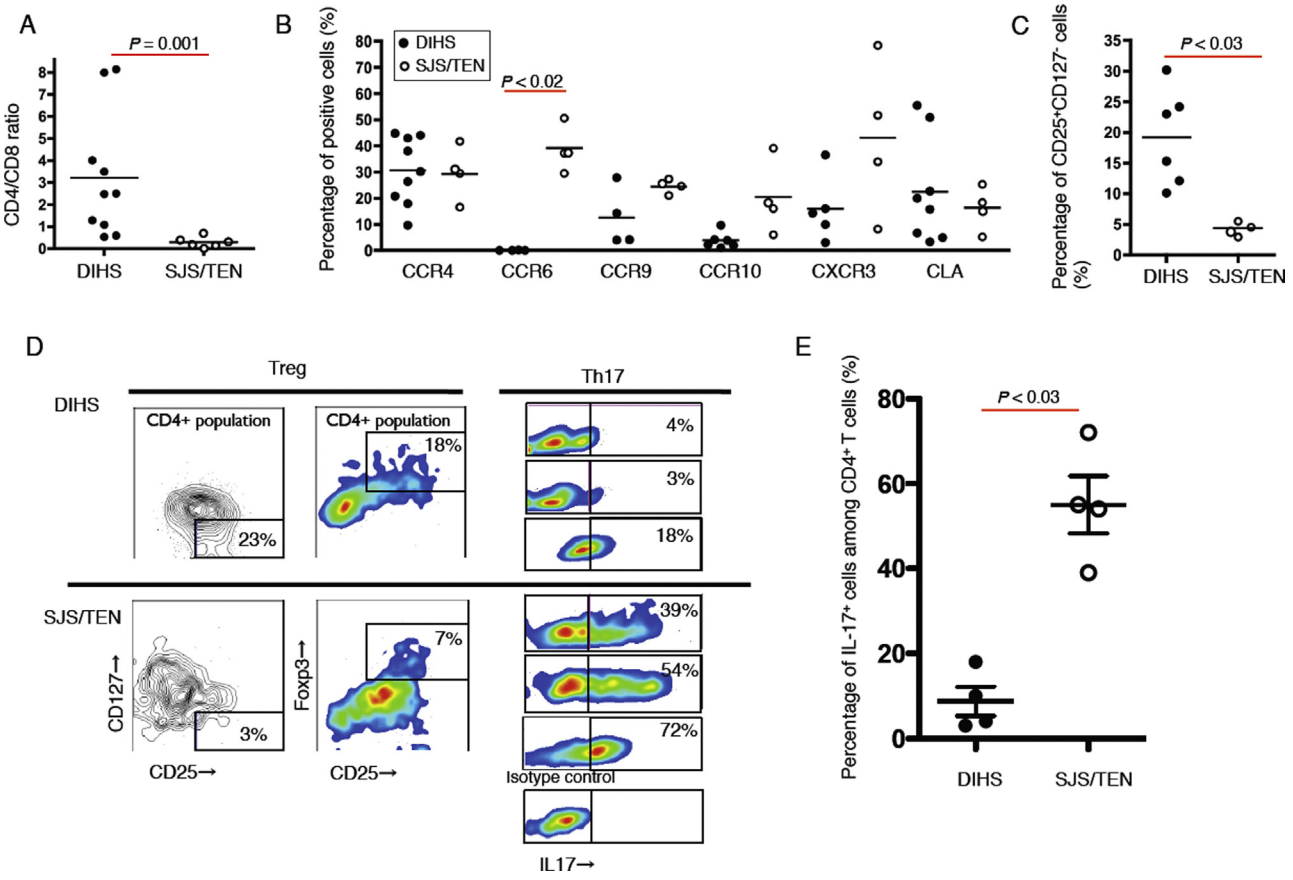

Fig. 1. Different characteristics of $CD4^+$ T cells from skin lesions between SJS/TEN and DIHS. (A) CD4/CD8 ratio. (B) Chemokine receptor expressions. (C) Percentage of T cells indicating Treg phenotype ($CD25^+CD127^-$) in $CD4^+$ cells. (D) Foxp3 and IL-17 expression. IL-17 expression was measured after stimulation with phorbol myristate acetate (10 ng/ml) for 3 h. Numbers indicate percentage of gated cells among the $CD4^+$ cells. Representative data of $CD4^+$ $CD25^+$ CD127- cells/$CD4^+$ $CD25^+$ $Foxp3^+$ cells from a DIHS and a SJS patients (left), and IL-17 expression of 3 DIHS patients (Pt#1-3) and 3 SJS/TEN patients (Pt#4-6) (right). (E) Percentage of T cells expressing IL-17 in $CD4^+$ cells.

The ratio of $CD4^+$ T cells/$CD8^+$ T cells in SJS/TEN skin lesions ($n = 6$) was significantly lower than in DIHS/DRESS skin lesions ($n = 10$) (Fig. 1A, $P < 0.001$, Mann–Whitney test). The percentage of $CCR6^+$ cells among total $CD4^+$ T cells was significantly greater in SJS/TEN ($n = 4$) than DIHS ($n = 4$) (Fig. 1B, $P < 0.02$, Mann–Whitney test). Higher levels of IL-17 production were observed in $CD4^+$ T cells from SJS/TEN skin lesions ($n = 4$) than in DIHS skin lesions ($n = 4$) after phorbol myristate acetate stimulation (Fig. 1D, right and Fig. 1E). Consistent with this, immunofluorescence analysis revealed that IL-17^+ $CD4^+$ cells infiltrated in the SJS/TEN skin lesions but not in the DIHS skin lesions (Supplementary Fig. E1). On the other hand, the percentage of $CD4^+CD25^+CD127^-$ cells, likely Treg cells, increased in T cells from DIHS skin lesions compared with those from SJS/TEN (Fig. 1C, $P < 0.03$, Mann–Whitney test, and Fig. 1D, left in Treg). We also confirm an increase of Treg cells by comparison of $CD4^+CD25^+Foxp3^+$ cells in expanded T cells between DIHS and SJS/TEN skin lesions after a more 4 days' culture with low dosage of IL-2 in several cases (Fig. 1D, right in Treg). We also found higher production of IL-17 in $CD4^+$ T cells expanded from SJS/TEN lesions than those from DIHS/DRESS lesions (Fig. 1D). These observations suggest that Th17 and Treg cells dominated in skin lesions of SJS/TEN and DIHS, respectively, although we could not convince that these cells were pathogenetic. To confirm these findings, we further investigated cytokine production of drug-reactive $CD4^+$ T cells; generated 15 drug-reactive $CD4^+$ TCCs from skin lesions including acetaminophen-induced TEN (4 clones), ibuprofen- and phenobarbital-induced SJS (3 clones and 2 clones), and carbamazepine-induced hypersensitivity syndrome (DIHS) (6 clones). TCCs of SJS/TEN released significantly higher IL-17 (mean ± SD, 15,680 pg/ml ± 13,990 pg/ml; $p = 0.026$, Mann–Whitney test) compared with DIHS, in which TCCs produced marginal levels (58.7 pg/ml ± 21.2 pg/ml) (Fig. 2A). Furthermore, IL-17 concentration had tendency to be greater in a severer clinical type, TEN than SJS. High amounts of IFN-γ and TNF-α were detected in SJS/TEN and DIHS groups, however, production levels were significantly higher in the latter than the former ($p = 0.008$, Mann–Whitney test). Interestingly, we found that two drug-reactive IL-17^+ $CD4^+$ TCCs, E11 and C5 from SJS skin lesions had significant expression of granulysin as well as a drug-reactive IL-17^-CD8^+ TCC, C6 (Fig. 2B). Furthermore, we established one TCC that expressed the molecules specific to Tregs and showed an inhibitory effect on autologous lymphocyte proliferation form DIHS skin lesions (supplementary Fig. E2) despite hyporesponsiveness of Tregs to IL-2. Surprisingly, this clone expressed the human herpes virus (HHV)-6 antigen (Fig. 2C).

Th17/Treg cells are reciprocally generated from naïve $CD4^+$ T cells depending on their surrounding cytokine milieu. IL-17-producing $CD4^+$ T cells, designated Th17, express CCR6 and high levels of transcription factor RORγt, and contribute to defense against microorganisms and enhance inflammation and regeneration. On the other hand, Treg cells are $CD4^+CD25^{high}CD127^-$ and the forkhead family transcription factor $Foxp3^+$ and have regulatory function against inflammatory responses.