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Letter to the Editor**Disturbed balance in three subpopulations of CD4⁺Foxp3⁺ regulatory T cells in Stevens-Johnson syndrome and toxic epidermal necrolysis patients**

Naoya Yoshioka*, Asuka Suto*, Riichiro Abe, Nao Saito, Junko Murata, Inkin Hayashi-Ujiie, Daichi Hoshina, Yasuyuki Fujita, Hiroshi Shimizu

Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

*Authorship note: Naoya Yoshioka and Asuka Suto contributed equally to this work.

Key words: Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), regulatory T cell, Treg-subpopulations

Correspondence to:

Riichiro Abe, M.D., Ph.D.

or

Hiroshi Shimizu, M.D., Ph.D.

Department of Dermatology,

Hokkaido University Graduate School of Medicine

N15 W7, Kita-ku, Sapporo 060-8638, Japan.

Tel: +81-11-706-7387

Fax: +81-11-706-7820

e-mail: aberi@med.hokudai.ac.jp

or

shimizu@med.hokudai.ac.jp

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening diseases that are two of the most severe cutaneous adverse reactions[1]. The regulatory T cell (Treg) has been reported to be involved in the pathogenesis of SJS/TEN [2, 3]. Treg maintains self-tolerance and suppresses immune responses. It was reported that even though the relative frequency of Treg is normal in TEN, Treg function is profoundly impaired, which may result in epidermal damage by excessive activation of effector T cells [3]. Recent reports have shown that CD4⁺Foxp3⁺ Treg exist in heterogeneous subpopulations divided by the level of CD45RA expression [4]. Human CD4⁺Foxp3⁺ Treg is divisible into three functionally and phenotypically distinct subsets: CD4⁺CD45RA⁺ Foxp3^{low} resting Treg (rTreg), CD4⁺CD45RA⁻Foxp3^{high} activated Treg (aTreg) and cytokine-secreting CD4⁺CD45RA⁻Foxp3^{low} nonsuppressive T cells (non-Treg). rTreg and aTreg have potently immunosuppressive activity, whereas non-Treg lacks such activity and has the potential to secrete proinflammatory cytokines such as IFN- γ and IL-17[4]. Furthermore, the relative frequencies of the three CD4⁺Foxp3⁺ Treg subpopulations differ with various disease conditions [5-7]: In active systemic lupus erythematosus, for example, the number of aTreg cells is lower and that of non-Treg cells is higher than those of healthy controls, allowing an autoimmune reaction to develop [4]. SJS/TEN might also be considered susceptible to development under the imbalance of CD4⁺Foxp3⁺ Treg subpopulations. This study investigates the relative frequencies and cytokine production characteristics of CD4⁺Foxp3⁺ Treg subpopulations in SJS/TEN patients.

The investigations were performed in accordance with the Declaration of

Helsinki. Written informed consent was obtained from the patients' parents. All control subjects gave informed consent in accordance with the Declaration of Helsinki. Peripheral blood mononuclear cells (PBMCs) from SJS/TEN patients (acute stage: n=3, resolution stage: n=7) (Supplemental Table 1) and 24 healthy controls were prepared by Ficoll gradient centrifugation. PBMCs were stained with anti-CD4, anti-CD45RA and anti-Foxp3, and then analyzed by flow cytometry (materials and methods are detailed in Supplemental data). The three subpopulations of Treg were defined by the expression of CD45RA and Foxp3 (Fig. 1a). In these three subpopulations, the frequency of non-Treg, which has no suppressive potential, in the acute stage of SJS/TEN (2.04 ± 0.89 %) was significantly lower, but that in the resolution stage of SJS/TEN (4.50 ± 0.87 %) was significantly higher than that of the healthy controls (3.61 ± 0.87 %) (Fig. 1b). In contrast, rTreg and aTreg did not differ among these groups. Over 95% of CD4⁺CD25⁺Foxp3⁺ cells showed low CD127 expression (Supplemental Fig. 1). From this data, in our experiments, CD4⁺CD25⁺Foxp3⁺ cells were defined as regulatory T cells.

Furthermore, the three CD4⁺Foxp3⁺ Treg subpopulations were analyzed for the expression of cytokines in patients in the resolution stage of SJS/TEN by intracellular cytokine staining assay. Interestingly, the percentage of IFN- γ -producing aTreg in patients in the resolution stage of SJS/TEN was significantly lower than that of healthy controls, whereas IFN- γ -secreting non-Treg and rTreg showed no differences between SJS/TEN patients and healthy controls. There were no significant differences in frequency of IL-17A-producing cells among the three Treg subpopulations (Supplemental

Table 2).

The present study shows that the frequency of non-Treg in the acute stage of SJS/TEN was significantly lower, but that the frequency of non-Treg in the resolution stage of SJS/TEN was significantly higher than that of healthy controls. Among Treg subpopulations, only the frequency of non-Treg drastically changed during the disease course. Although it is not known why the relative frequency of non-Treg is equal before and after disease, the relative frequency in the resolution stage of SJS/TEN is higher than that of healthy controls, indicating that non-Treg might influence susceptibility to SJS/TEN.

Furthermore, the frequency of IFN- γ -producing aTreg in SJS/TEN patients was significantly lower than that of the healthy controls. With respect to plasticity, Treg was found to differentiate into helper T cells and to express T-bet, which is a master transcription factor of Th1 effector cells which produce IFN- γ . T-bet⁺Foxp3⁺ Treg inhibits the Th1-dependent response[8]. We demonstrated that the frequency of IFN- γ -secreting aTreg cells, namely Th1-like aTreg, was significantly lower in SJS/TEN patients than in healthy controls. There are several reports about Th1/Th2 polarization in SJS/TEN. The SJS/TEN lesions showed a mixed Th1/Th2 pattern [9]. Quaglino P., et al. showed the coexistence of a Th2 response in addition to the predominant Th1 profile in blood [10]. In addition, Th2 cells are known to outnumber Th1 cells. On the other hand, the percentage of T-bet⁺ was 56.7% in aTreg. IFN- γ production is an indirect indication of Th1-controlling Treg cells [8]. Our results suggest that the Th1-like aTreg subpopulation in SJS/TEN patients reduces the inhibitory capacity under immune active conditions, such as drug-mediated allergic reaction.

To date, no reports have identified a correlation between IL-17 production of CD4⁺ T cells (Th17) and SJS/TEN development. In our study, we also investigated the frequency of Th17. That frequency did not differ significantly between SJS/TEN patients and healthy controls (SJS/TEN; 1.92 ± 1.01 %, healthy controls; 1.80 ± 0.72 %, $P=0.80$).

In conclusion, the relative frequencies of the three Treg subpopulations and cytokine-secreting activity are found to differ between SJS/TEN patients and healthy controls. These results indicate that the disturbed balance of Treg subpopulations is involved in the pathogenesis of SJS/TEN.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Figure Legends

Fig 1. The relative frequencies and cytokine production characteristics of the three CD4⁺Foxp3⁺ Treg subpopulations in patients who had recovered from SJS/TEN and in healthy controls.

(a) Three subpopulations of CD4⁺ T cells defined by the expression of CD45RA and Foxp3: CD45RA⁺Foxp3^{low} rTreg, CD45RA⁺Foxp3^{high} aTreg, CD45RA⁻Foxp3^{low} non-Treg. The percentage of each area in CD45RA and Foxp3 is shown. (b) Percentages of each Foxp3⁺ subpopulation among CD4⁺ T cells in patients with SJS/TEN (open circles, n=7) and healthy controls (closed circles, n=24) (*; p<0.05; NS: not significant).

Figure 1

