

Differential expression of alarmins: IL-33 as a candidate marker for early diagnosis of toxic epidermal necrolysis

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Clinical Implications

- Serum level of IL-33, which might be released from epidermal keratinocytes, was elevated in patients with toxic epidermal necrolysis (TEN) in the early stage. Serum level of IL-33 might be useful for the early diagnosis of TEN.

TO THE EDITOR:

IL-33 and high mobility group box-1 protein (HMGB1) are known as alarmins and initiate rapid innate immune responses when released from necrotic cells.^{1,2} Soluble ST2 (sST2) acts as

a decoy receptor for IL-33 and reduces the effect of T_H2-mediated responses.³ Several biomarkers, such as granulysin,⁴ FasL,⁵ and HMGB1,⁶ have been reported to be useful in diagnosing Stevens Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) in the early stage but IL-33/sST2 have not been reported. We compared their serum levels with those of HMGB1 and examined the histopathological localization of IL-33 and HMGB1.

Nineteen adult patients (9 men, 10 women; 61.8 ± 17.4 years old) and 14 healthy controls (HCs; 6 men, 8 women; 56.4 ± 16.1 years old) were enrolled in the study. Of the patients, 4 had TEN, 4 had SJS, 3 had drug-induced hypersensitivity syndrome/drug reaction with eosinophilia systemic symptoms, and 8 had other types of drug eruptions. Day 0 in patients with SJS/TEN was defined as the day when mucocutaneous involvements occurred. The early and late stages were defined as days 0 to 3 and days 4 to 14, respectively. All subjects provided written consent, and the protocols of this study were approved by the Ethical Committee of Jichi Medical University Graduate School of Medicine and Kyoto University Graduate School of Medicine. The serum levels of IL-33, sST2, and HMGB1 were evaluated using an IL-33 ELISA kit (R&D Systems, Minneapolis, Minn), sST2 ELISA kit (MBL, Woburn, Mass), and HMGB1 ELISA

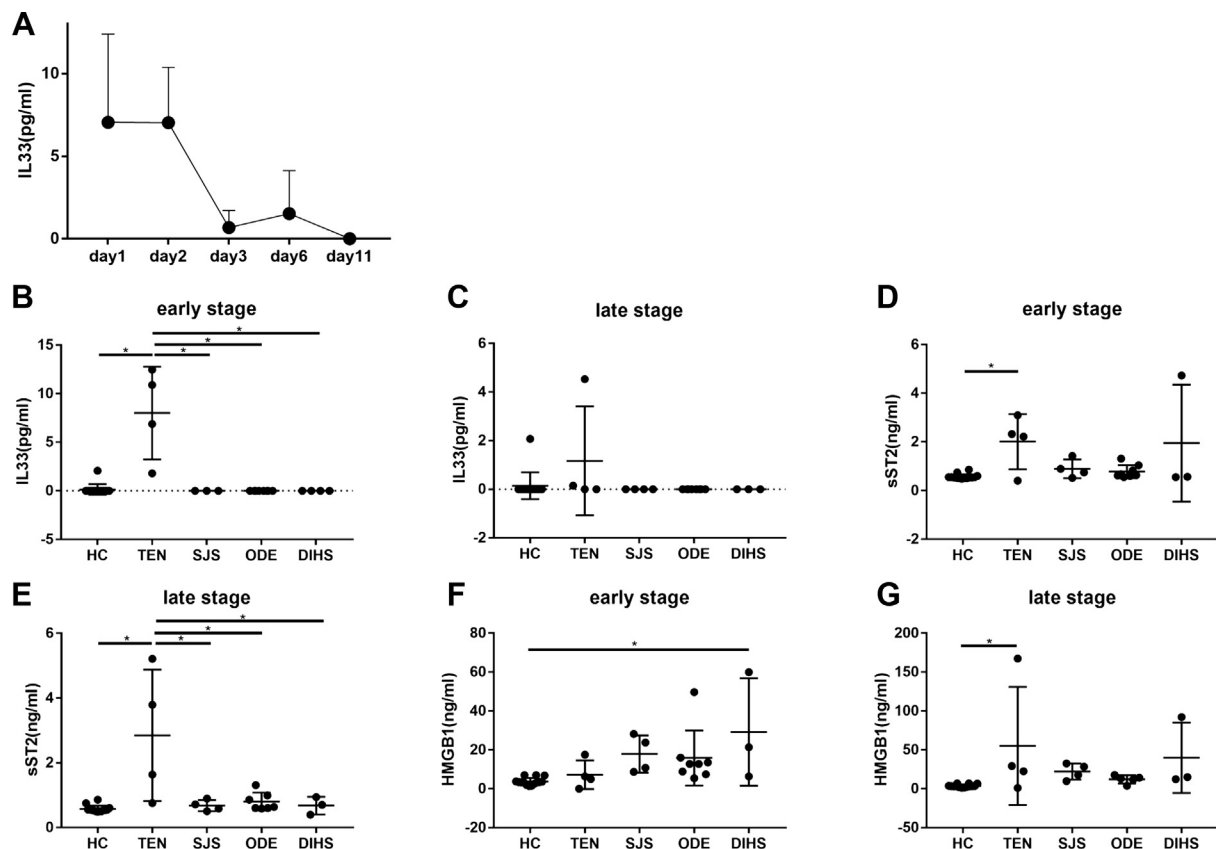


FIGURE 1. A, Average serum IL-33 level over time in patients with TEN. Serum levels of (B) IL-33, (D) sST2, and (F) HMGB1, in the early stage, and (C) IL-33, (E) sST2, and (G) HMGB1, in the late stage. One-way ANOVA with Tukey multiple comparison test was used to compare serum levels. DIHS, drug-induced hypersensitivity syndrome; ODE, other types of drug eruptions.

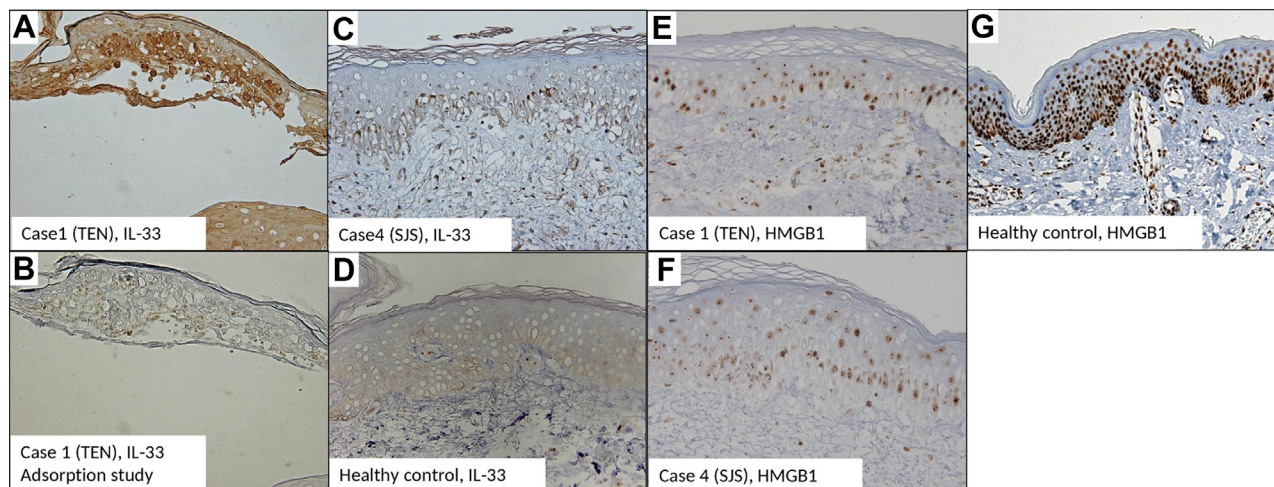


FIGURE 2. Immunohistochemical analysis of (A) IL-33 in case 1 (patient with TEN) (400 $\times$), (B) adsorption study of IL-33 in case 1 (400 $\times$), (C) IL-33 in case 4 (patient with SJS) (400 $\times$), (D) IL-33 in the healthy control (400 $\times$), (E) HMGB1 in case 1 (400 $\times$), (F) HMGB1 in case 4 (400 $\times$), and (G) HMGB1 in the healthy control (400 $\times$).

kit (Shino-Test Co., Tokyo, Japan), according to the manufacturers' protocols.

We performed immunohistochemical staining using the avidin-biotin-horseradish peroxidase method with mAbs to human IL-33 (mouse IgG1 κ , Nessler-1; Enzo Life Sciences, Farmingdale, NY) and human HMGB1 (rabbit IgG, EPR3507; Abcam, Cambridge, Mass). To confirm the specificity of staining with anti-IL-33 antibody, we performed an adsorption study using a recombinant human IL-33 N-terminal peptide (*Escherichia coli*-derived, Ser112-Thr270; R&D Systems). The IL-33 mAb was adsorbed with recombinant human IL-33 peptide for 1 hour at 37°C.

The serum level of IL-33 in the early stage in patients with TEN was 8.00 ± 4.76 pg/mL, which gradually decreased during their clinical course (Figure 1, A and C), whereas that in other patients as well as 13 HCs was not detected except in 1 HC (Figure 1, B). The serum level of sST2 in patients with TEN was significantly elevated compared with that in HCs but was not elevated compared with that in other patients in the early stage (Figure 1, D). In contrast, the serum level of sST2 was significantly elevated in the late stage compared with that in other patients and HCs (Figure 1, E). The serum level of HMGB1 in patients with TEN was not significantly elevated in the early or late stage, compared with that in other patients (Figure 1, F and G). IL-33 staining was positive in the nucleus and cytoplasm of keratinocytes in patients with TEN, which was abolished in the adsorption study (Figure 2, A and B). However, in other types of drug eruption and HCs, IL-33 staining was positive in only a small number of nucleus of keratinocytes (Figure 2, C and D). HMGB1 staining was positive in the nucleus of keratinocytes in all patients (Figure 2, E and F), whereas a greater number of positive cells were observed in HCs (Figure 2, G).

We demonstrated that, in patients with TEN, the serum level of IL-33 was elevated in the early stage and IL-33 staining was positive in the nucleus and cytoplasm of keratinocytes, whereas IL-33 is typically localized in the nuclei of keratinocytes in the steady states and IL-33 signal is generally low with

immunohistochemical analysis.⁷ In the late stage, the serum level of sST2 was elevated only in patients with TEN, and this result corresponds to the action of sST2 as a decoy receptor for IL-33. TEN and SJS are thought to be due to the same pathogenesis; however, serum level of IL-33 was not detected in patients with SJS in our system. We assume that the elevation of serum level of IL-33 depends on the area of epidermal necrosis. In contrast, serum level of HMGB1 was not elevated in the early stage in patients with TEN, and HMGB1 was stained with fewer keratinocytes than in HCs. These results suggest that HMGB1 expression might vary on a case-to-case basis, according to the previous report.⁶ Collectively, the serum level of IL-33 may be a good marker of the early stage of TEN; however, this study included only a small number of patients and a larger sample size would be needed in future studies.

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Conflicts of interest: The authors declare that they have no relevant conflicts of interest.

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REFERENCES

- Martin NT, Martin MU. Interleukin 33 is a guardian of barriers and a local alarmin. *Nat Immunol* 2016;17:122-31.
- Harris HE, Andersson U, Pisetsky DS. HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease. *Nat Rev Rheumatol* 2012;8:195-202.
- Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem* 2007;282:26369-80.

4. Chung WH, Hung SI, Yang JY, Su SC, Huang SP, Wei CY, et al. Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis. *Nat Med* 2008;14:1343-50.
5. Murata J, Abe R, Shimizu H. Increased soluble Fas ligand levels in patients with Stevens-Johnson syndrome and toxic epidermal necrolysis preceding skin detachment. *J Allergy Clin Immunol* 2008;122:992-1000.
6. Nakajima S, Watanabe H, Tohyama M, Sugita K, Iijima M, Hashimoto K, et al. High-mobility group box 1 protein (HMGB1) as a novel diagnostic tool for toxic epidermal necrolysis and Stevens-Johnson syndrome. *Arch Dermatol* 2011;147:1110-2.
7. Meephansan J, Komine M, Tsuda H, Karakawa M, Tominaga S, Ohtsuki M. Expression of IL-33 in the epidermis: the mechanism of induction by IL-17. *J Dermatol Sci* 2013;71:107-14.