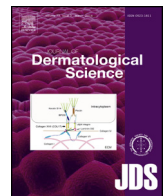




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Letter to the Editor

Immediate-type contact hypersensitivity is reduced in interleukin-33 knockout mice

Interleukin-33 (IL-33) is a member of the IL-1 cytokine family, along with IL-1 β and IL-18. IL-33 binds to its receptor ST2 (IL-1RL1), which is expressed by Th2 cells and by various innate immune cells including mast cells and basophils, in which ST2 and the IL-18 receptor α chain, respectively, are predominant [1]. IL-33 stimulates the production of Th2 cytokines by those cells and is involved in various types of inflammation [2]. Recently, we have found that ragweed-induced allergic rhinitis is dependent on IL-33 [3] and mast cells are abundant in the dermis of skin-specific IL-33 transgenic mice [4]. Those prompted us to elucidate the role of IL-33 in cutaneous immediate-type contact hypersensitivity. In this study, we examined hapten-induced immediate-type contact hypersensitivity in IL-33 knockout (KO) mice.

All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care Committee of the Hyogo College of Medicine. WT BALB/c mice (Charles River Laboratories, Yokohama, Japan) and IL-33 KO mice [2] were sensitized on their shaved dorsal skins with 0.5% (wt/vol) 2,4-dinitro-fluorobenzene (DNFB; Sigma, St. Louis, MO), and were repeatedly challenged with 0.2% (wt/vol) DNFB on their left ears and with vehicle only on their right ears once a week (Fig. 1a), as described previously [5]. The ear thickness was measured before and after each challenge using a dial thickness gauge (Ozaki, Tokyo, Japan). No immediate reaction occurred after the first challenge. However, WT mice showed both immediate and delayed-type reactions after the third challenge (Fig. 1b, open squares). In contrast, in IL-33 KO mice the delayed-type reaction remained as reported that IL-33 is unnecessary for T cell-dependent acquired immune responses [6], but the immediate reaction was significantly suppressed (Fig. 1b, closed squares). When only vehicle was applied, no immediate reactions occurred in WT or in IL-33 KO mice, and therefore those reactions were hapten-specific. To determine whether the difference of ear swelling in those mice is related with IgE, we measured serum levels of hapten-specific IgE by ELISA as described previously [7]. However, both IL-33 KO and WT mice showed a strong hapten-specific IgE response (Fig. 1c). Plasma histamine levels, which were measured using an ELISA kit (Immunotech, Marseille, France),

were significantly lower in IL-33 KO mice than in WT mice (Fig. 1d). This result might coincide with the poor immediate hypersensitivity reaction in IL-33 KO mice. Because both mast cells and basophils could be a major source of histamine, we examined the numbers of those cells in the skin just before the third challenge. Toluidine blue staining showed that the number of mast cells in the skin was increased in WT mice, but not in IL-33 KO mice (Fig. 1e and f). In contrast, immunohistochemistry using a murine basophil-specific anti-mMCP-8 antibody (clone TUG8, Biolegend, San Diego, CA) [3] revealed that the number of basophils in the skin was not different between WT and IL-33 KO mice (Fig. 1g and h). Therefore, DNFB-induced mast cell recruitment might be mediated by IL-33 and the difference of ear swelling in the immediate hypersensitivity reaction might be dependent on the number of mast cells in the skin.

Next, the effect of the hapten DNFB on the localization and/or expression of IL-33 was examined by immunofluorescence (Fig. 2) as described previously [7]. IL-33 was localized in the nuclei of keratinocytes in naïve mouse skin, but the epidermal expression of IL-33 was down-regulated 6 h after the DNFB challenge, and it recovered at 24 h (Fig. 2b). DNFB-induced IL-33 up-regulation was only seen in keratinocytes, not in endothelial cells. Consequently, DNFB may cause the production of IL-33 from keratinocytes, instead of other IL-33 producing cells. The time-course of IL-33 expression suggests that exposure of the epidermis to DNFB may cause the secretion of IL-33 from keratinocytes and thereby elicits a transient decrease in the nuclear IL-33 protein. In turn, real-time PCR revealed that IL-33 mRNA expression increased 6 h after the application of DNFB and returned toward the baseline level at 24 h (Fig. 2c). Such a loss of IL-33 protein with an increase in IL-33 mRNA has been found in nasal epithelial cells in patients with allergic rhinitis [3].

From these results, we propose the following mechanism for immediate contact hypersensitivity. IL-33 is secreted from keratinocytes in response to stimuli such as allergens. IL-33 acts as an alarmin and recruits ST2⁺ mast cells into the skin. Antigen stimulation then releases a large amount of histamine from the abundant mast cells and triggers immediate contact hypersensitivity. However, further study is necessary to elucidate how IL-33 recruits mast cells into the skin [4,8].

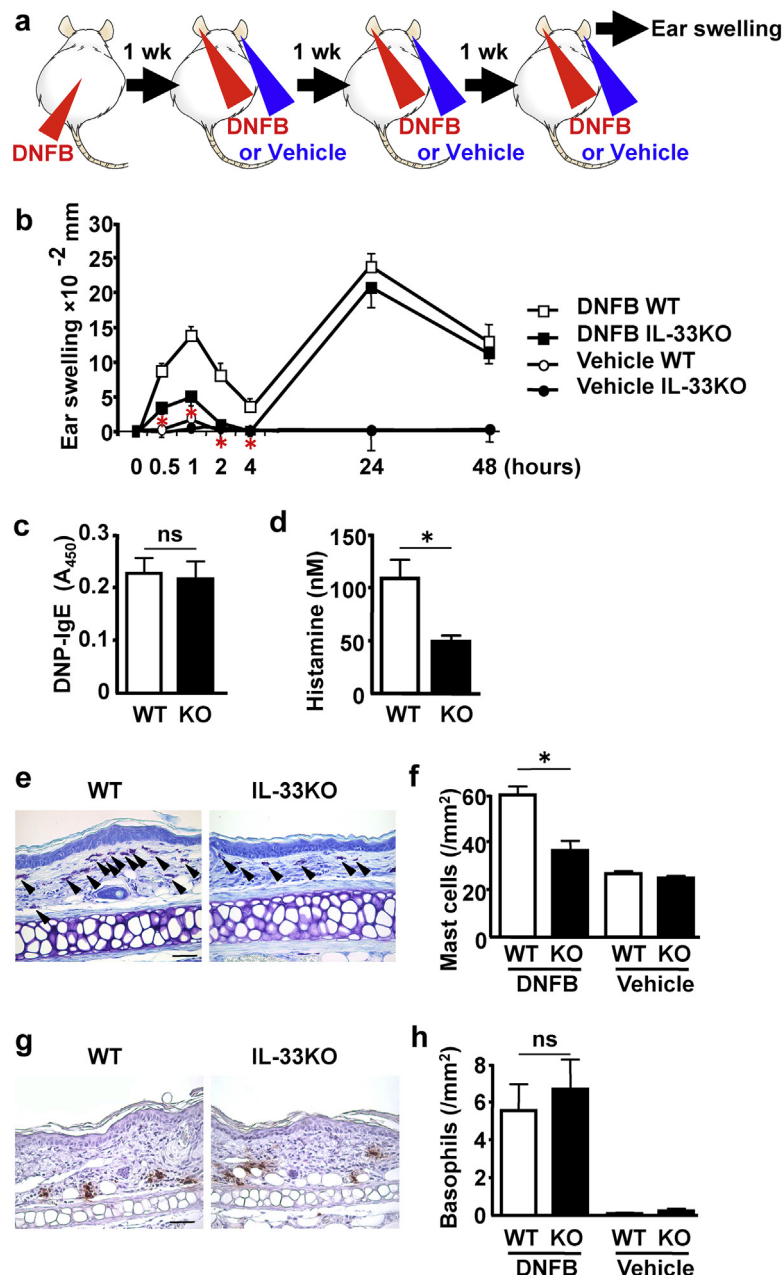


Fig. 1. Immediate contact hypersensitivity is suppressed in IL-33 KO mice. (a) Induction of contact hypersensitivity. WT BALB/c mice or IL-33 KO mice were sensitized on their shaved dorsal skins with 25 μ l 0.5% DNFB in a 4:1 acetone/olive oil solution (A/O) in week 0, and were repeatedly challenged with 20 μ l 0.2% DNFB in A/O on their left ears or vehicle only on their right ears once a week. wk, week. (b) Time-course of ear thickness in WT and IL-33KO mice after the third challenge. (c) Sera levels of hapten-specific IgE. Sera were sampled 48 h after the third challenge and anti-2,4-dinitrophenol (DNP)-IgE was measured by ELISA. Absorbance at 450 nm (A₄₅₀) was measured using a microplate spectrophotometer (Benchmark Plus; Bio-Rad, Hercules, CA). WT mouse serum was used as a negative control (blank absorbance). (d) Histamine concentration in plasma measured by ELISA. (e) Toluidine blue staining of WT and IL-33 KO skins. Arrowheads indicate mast cells. Bar, 50 μ m. (f) The number of mast cells in the skin was calculated as the mean of 6 microscopic fields. (g) Immunohistochemistry of basophils in the skin from WT and from IL-33 KO mice. Bar, 50 μ m. (h) The number of basophils in the skin was calculated as the mean of 6 microscopic fields. All data are expressed as means $\pm$ SEM ($n = 8$) and representative data of two independent experiments are shown. * $P < 0.05$; ns, not significant (unpaired t -test, calculated using PRISM6; GraphPad Software, San Diego, CA).

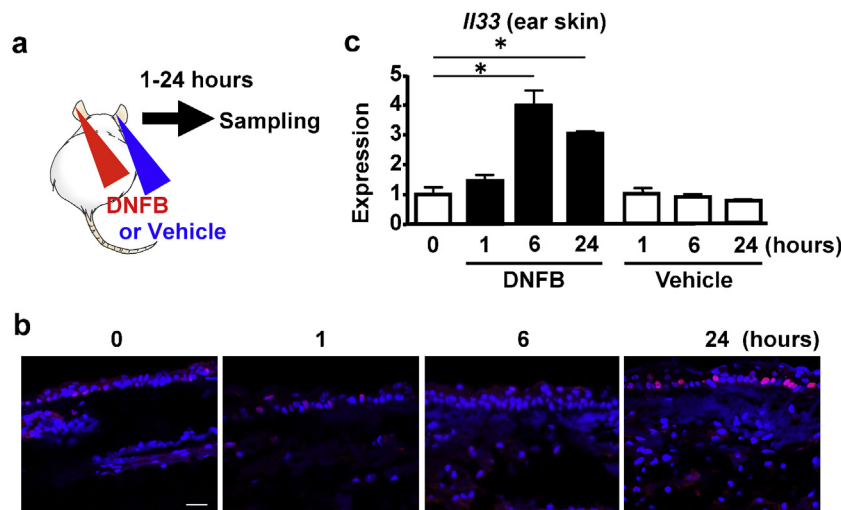


Fig. 2. Expression of IL-33 in the epidermis after DNFB challenge. (a) Naïve WT BALB/c mice were challenged with 20 μ l 0.5% DNFB in a 4:1 acetone/olive oil solution on their left ears or with vehicle only on their right ears. (b) Immunofluorescence of IL-33 in the epidermis of WT ear skins. Deparaffinized sections were incubated with an affinity-purified rabbit anti-mouse IL-33 polyclonal antibody [7]. Bound antibodies were visualized with a biotinylated goat anti-rabbit IgG antibody (Vector, Burlingame, CA) and streptavidin-Alexa-594 (Invitrogen, Carlsbad, CA). After mounting with Prolong GOLD Antifade with DAPI (Life Technologies, Gaithersburg, MD), fluorescent images were recorded using a confocal laser-scanning microscope LSM510 (Carl Zeiss, Thornwood, NY). Intense staining of IL-33 is evident in nuclei of the epidermis. Bar, 50 μ m. Representative images of 3 independent experiments are shown. (c) Expression of the *Il33* gene in the DNFB-treated ear skin. Total RNAs of mouse ears were prepared using an RNeasy Fibrous Tissue Kit (Qiagen, Hilden, Germany). For quantitative real-time PCR, a TaqMan[®] RNA-to-Ct Kit (Applied Biosystems, Foster City, CA) was used; the product number of the TaqMan[®] probe for *Il33* was Mm00505403_m1. Each bar shows the expression of the *Il33* gene in DNFB-treated or vehicle-treated mouse ears (1 h, 6 h, and 24 h) relative to non-treated mice (0 h). Data are expressed as means $\pm$ SEM ($n = 4$) and representative data of two independent experiments are shown. * $P < 0.05$ (Dunn's multiple comparison test).

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