Blockade of Interleukin-33 Attenuates Allergic Contact Dermatitis in Model Mice: Possible Mechanism via Eosinophil Infiltration

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Abstract

Background: Interleukin (IL)-33, a novel member of the IL-1 family, is mainly produced by epithelial cells and endothelial cells. Effects of IL-33 on allergic diseases have been reported. Allergic contact dermatitis is a clinical form of contact hypersensitivity that involves a delayed-type hypersensitivity reaction. We previously reported that IL-33 is induced by tumor necrosis factor-alpha and interferon-gamma in keratinocytes and plays a critical role in allergic contact dermatitis. However, the mechanism underlying how IL-33 is involved in the pathogenesis of allergic contact dermatitis is not fully understood. We investigated the role of IL-33 in allergic contact dermatitis using model mice.

Methods: Allergic contact dermatitis model mice were generated. Epidermal thickness and eosinophil infiltration in the dermis were evaluated by histology. The function of IL-33 was investigated by in vivo administration of an anti-IL-33 antibody.

Results: Epidermal thickness was increased in the ear lesions of allergic contact dermatitis model mice. We showed that eosinophil infiltration in the dermis was increased in the ear lesions. We further found that blockade of IL-33 attenuated not only the epidermal thickness but also the eosinophil infiltration in the dermis in the ear lesions.

Conclusions: IL-33 may promote inflammation via eosinophil infiltration in allergic contact dermatitis. Blockade of IL-33 may represent a novel and potent therapeutic strategy for allergic contact dermatitis.

Keywords: Interleukin-33; Allergic contact dermatitis; Epidermal thickness; Eosinophil

Abbreviations: Oxa: 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one; IL: Interleukin; TNF-a: Tumor Necrosis Factor-alpha; IFN-y: Interferon-gamma

Introduction

Interleukin (IL)-33 was identified as a novel member of the IL-1 family, IL-1F11 [1]. IL-33 was reported as DVS 27 in 1999 and also reported as nuclear factor from high endothelial venules (NF-HEV) in 2003 [2,3]. Epithelial cells and endothelial cells are the main cellular sources of IL-33 [1,4,5]. IL-33 is released by cells undergoing necrotic cell death and functions as a Damage-Associated Molecular Pattern (DAMP) and alarming [5]. The receptor for IL-33, ST2, is expressed on various immune cells including T-helper type 2 (Th2) cells CD8+ T cells mast cells eosinophils basophils NK cells, NKT cells and dendritic cells [6-12]. Natural helper cells and nuocytes produce abundant Th2 cytokines when stimulated by IL-33 [13,14].

IL-33 is closely related to type 2 immune responses. It has been reported that IL-33 is involved in various allergic diseases, including bronchial asthma allergic rhinitis allergic conjunctivitis atopic dermatitis and allergic contact dermatitis [15-19]. Allergic contact dermatitis is a clinical form of contact hypersensitivity that involves a delayed-type hypersensitivity reaction, and its mechanism is complex.

In the previous study, we found that IL-33 plays a critical role in allergic contact dermatitis model mice [20]. However, we could not fully reveal the mechanism underlying how IL-33 is involved in the pathogenesis of allergic contact dermatitis. In the present study, we further investigated the role of IL-33 in allergic contact dermatitis using model mice. Here, we show that not only epidermal thickness but also eosinophil infiltration in the dermis are increased in the ear lesions of allergic contact dermatitis model mice. We further demonstrate that blockade of IL-33 attenuates the epidermal thickness and eosinophil infiltration in the dermis in the ear lesions. These findings suggest that IL-33 functions like a chemoattractant for eosinophils in allergic contact dermatitis model mice, and that IL-33 is a novel therapeutic target for allergic contact dermatitis.

Materials and Methods

Generation of allergic contact dermatitis model mice

All animal experiments were undertaken in accordance with the guidelines for the care and use of experimental animals of the Japanese Association for Laboratory Animal Science (1987). Six-week-old female C57BL/6 mice were purchased from Kyudo (Saga, Japan). The protocol for preparing allergic contact dermatitis model mice was previously reported [20,21]. Briefly, mice were sensitized with 100 μl of 5% 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxa) in ethanol on their shaved abdomen on day 0. Oxa challenges were performed on days 5, 7, 9, 11, and 13 after sensitization. The right ears of the mice were applied with 0.1% oxa, while the left ears were applied with ethanol alone. An anti-mouse IL-33 antibody (Functional Grade Purified; Medical & Biological Laboratory, Research Triangle Park, NC) was

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subcutaneously injected into the ears on day 9. An IgG1 isotype control antibody (Abcam, Cambridge, MA) was also evaluated.

**Histological analysis**

For histological examination, specimens were obtained from the ears at 48 hours after the last oxa challenge. The specimens were fixed in formalin and embedded in paraffin. Cross-sections were prepared and subjected to H&E staining. To evaluate eosinophil infiltration, the total numbers of eosinophils in the dermis in five high power fields (magnification, ×400) were counted.

**Statistical analysis**

Statistical analyses were performed using Prism 5.0 (GraphPad Software, La Jolla, CA). Data are presented as means ± SD. The significance of differences between values was assessed using an unpaired Student’s t-test. Values of *P*<0.05 were considered statistically significant.

**Results**

**Generation of allergic contact dermatitis model mice**

To investigate the function of IL-33 in allergic contact dermatitis, we initially generated allergic contact dermatitis model mice using oxa (Figure 1a). The epidermal thickness of the ear tissue was increased in allergic contact dermatitis model mice compared with control mice that were not sensitized with oxa (Figure 1b). This finding indicated that the preparation of allergic contact dermatitis model mice was successful. There was no difference of the ears between allergic contact dermatitis model mice and control mice in appearance (data not shown).

**Blockade of IL-33 attenuates the epidermal thickness in allergic contact dermatitis model mice**

To evaluate the function of IL-33, we administered the anti-IL-33 antibody into the lesions of allergic contact dermatitis model mice (Figure 2a). Administration of the anti-IL-33 antibody suppressed the epidermal thickness in allergic contact dermatitis model mice compared with administration of the IgG1 isotype control antibody (Figure 2b). This finding suggested that blockade of IL-33 attenuated allergic contact dermatitis.

**Eosinophil infiltration in the dermis is suppressed by blockade of IL-33 in allergic contact dermatitis model mice**

We confirmed that IL-33 was functionally involved in the pathogenesis of allergic contact dermatitis. IL-33 activates the eosinophil, a potent mediator of allergic inflammation, and also increases eosinophil survival in humans [22]. We investigated the relationship between IL-33 and eosinophils in our allergic contact dermatitis model mice. We found that the eosinophil infiltration in the dermis was significantly increased in allergic contact dermatitis model mice compared with control mice that were not sensitized with oxa (Figure 3). On the other hand, administration of the anti-IL-33 antibody significantly suppressed the eosinophil infiltration in the dermis compared with administration of the IgG1 isotype control antibody (Figure 3). Very few lymphocytes, neutrophils, basophils, and macrophages appeared in the skin lesions in our allergic contact dermatitis model mice. These data suggest that IL-33 functions like a chemoattractant for eosinophils, which facilitate allergic inflammation.

**Discussion**

IL-33 is involved in various allergic diseases [15-19]. We previously
reported that IL-33 contributes to allergic contact dermatitis, as do tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) [20]. However, the mechanism underlying how IL-33 is involved in the pathogenesis of allergic contact dermatitis is not fully understood. To investigate the role of IL-33, we generated allergic contact dermatitis model mice under the condition that relatively mild inflammation was sustained to omit the involvement of excessive immune cells. Although a difference of epidermal thickness between the control and experimental mice appeared, edema and hypertrophy of the dermis, the characteristics of severe dermatitis, did not appear in our model mice. In addition, the serum IgE concentrations were increased in our model mice. This mouse model is suitable for analyses of the early stage of skin allergic inflammation. We previously reported that IL-33 protein was upregulated in keratinocytes in the lesions of allergic contact dermatitis model mice [20].

IL-33 and its receptor ST2 appear to contribute to allergic contact dermatitis. For example, ST2 was upregulated in the lesions of contact dermatitis model rats by a representative hapten, 2,4-dinitrochlorobenzene [23]. In oxazolone-induced allergic contact dermatitis model mice, inflammation was attenuated in ST2-deficient mice compared with wild-type mice, because activation of B-1 cells via IL-33 and ST2 interactions could not occur [19]. Allergic contact dermatitis, which is a clinical form of contact hypersensitivity, has properties both of type 1 and type 2 immune responses, especially in chronic inflammation. IL-33 closely relates to type 2 immune responses. On the other hand, TNF-α and IFN-γ closely relate to type 1 immune responses. We have reported that TNF-α and IFN-γ induce IL-33 production in KERTr cells, normal human keratinocyte cell line [20]. These are supporting data for the coexistence of the properties of type 1 and type 2 immune responses.

Effects of IL-33 on various immune cells have been reported [6-14]. We paid the attention to eosinophils, which express ST2 and are involved in the pathogenesis of various allergic diseases, including contact dermatitis. IL-33 induces eosinophil superoxide anion production and degranulation, and also increases eosinophil survival in humans [21]. In the present study, we showed a relationship between IL-33 and eosinophils in allergic contact dermatitis model mice. We further showed that blockade of IL-33 attenuated epidermal thickness in allergic contact dermatitis model mice, together with decreased eosinophil infiltration in the dermis. These data suggest that IL-33 is functionally involved in the pathogenesis of allergic contact dermatitis model mice as if it were a chemoattractant for eosinophils. IL-33 does not influence the eosinophil migration directly. It has reported that IL-33 induced the production of eotaxin, a potent chemoattractant for eosinophils, in fibroblasts in allergic bronchial asthma model mice [24]. Accordingly, IL-33 influences the eosinophil migration indirectly. Lymphocytes, neutrophils, basophils, and macrophages seldom appeared in the skin lesions at least in our allergic contact dermatitis model mice. IL-33 may be initially upregulated in keratinocytes, epithelial cells in the epidermis, and secreted as an alarm when keratinocytes are damaged and exposed to TNF-α and IFN-γ [5,20]. We suggest that IL-33 subsequently attracts eosinophils that mediate allergic responses in the skin lesions (Figure 4). Thus, inflammation may be facilitated and sustained in allergic contact dermatitis. Blockade of IL-33 may attenuate allergic contact dermatitis by suppressing eosinophil infiltration into the skin lesions.

A corticosteroid and antihistamine drugs with external application and systemic administration are currently the main treatments for contact dermatitis. These treatments successfully inhibit the allergic inflammation [25]. However, the corticosteroid therapy and antihistamine therapy are symptomatic therapies, rather than curative therapies [25]. The data obtained in the present study suggest that blockade of IL-33 could become a novel strategy for allergic contact dermatitis as a molecularly targeted therapy.

In Conclusion, blockade of IL-33 attenuated allergic contact dermatitis in our model mice. This process may be mediated by suppression of eosinophil infiltration in the dermis. These findings could pave the way for successful treatment of allergic contact dermatitis.

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