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Production of interleukin (IL)-33 in the lungs during multiple antigen challenge-induced airway inflammation in mice, and its modulation by a glucocorticoid

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ABSTRACT

Although interleukin (IL)-33 is a candidate aggravator of asthma, the cellular sources of IL-33 in the lungs during the progression of antigen-induced airway inflammation remain unclear. Furthermore, it has not been determined whether the antigen-induced production of IL-33 can be pharmacologically modulated *in vivo*. In this study, we examined the production of IL-33 in the lungs of sensitized mice during multiple intratracheal challenges with the antigen, ovalbumin. The 1st challenge clearly induced the IL-33 production in the lungs, and it was enhanced by the 2nd–4th challenges. IL-33 mRNA transcription was also induced after these challenges. An immunohistochemical analysis revealed that the cellular sources of IL-33 after the 1st challenge were mainly bronchial epithelial cells, while those after the 3rd challenge were not only the epithelial cells, but also inflammatory cells that infiltrated the lungs. Flow cytometric analyses indicated that approximately 20% and 10% of the IL-33-producing cells in the lungs were M2 macrophages and conventional dendritic cells, respectively. A systemic treatment with dexamethasone before the 1st challenge potently suppressed the IL-33 production. When dexamethasone was administered before the respective challenges, production of the IL-33 protein and the infiltration of IL-33-producing M2 macrophages and dendritic cells into the lungs in the 3rd challenge were also suppressed. In conclusion, the cellular sources of IL-33 in the lungs were dynamically altered during multiple challenges: not only bronchial epithelial cells, but also the M2 macrophages and dendritic cells that infiltrated the lungs produced IL-33. The production of IL-33 was susceptible to the glucocorticoid treatment.

1. Introduction

Interleukin (IL)-33, a member of the IL-1 family of cytokines, is released when the airway epithelial cells are exposed to exogenous stimuli such as allergens and microbes (Schmitz et al., 2005). IL-33 is considered to function as an 'alarmin', which belongs to the larger family of damage-associated molecular pattern molecules. This activates various immune cells including mast cells, basophils, macrophages, dendritic cells, Th2 cells, and type 2 innate lymphoid cells (ILC2) through the cell surface IL-33 receptors, ST2, leading to the production of various pro-inflammatory molecules (Schmitz et al., 2005; Oboki et al., 2010; Nakae et al., 2013; Nabe, 2014). IL-33 is expressed by various organs including the lungs (Schmitz et al., 2005). Epithelial cells, endothelial cells, smooth muscle cells, and fibroblasts in the lung tissues are capable of producing IL-33 (Schmitz et al., 2005). In addition to structural cells, hematopoietic cells, especially macrophages, have been reported to produce IL-33 (Schmitz et al., 2005; Oboki et al., 2010; Nakae et al., 2013; Nabe, 2014). In *in vivo* models of murine pollenosis, the IL-33 protein was shown to be constitutively expressed in the conjunctival and nasal epithelial cells, and an allergen challenge induced the transcription

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of IL-33 mRNA (Matsuba-Kitamura et al., 2010; Haenuki et al., 2012). However, the mechanisms underlying the production of IL-33 and its cellular sources in atopic diseases have not been elucidated in detail.

Th2 cells orchestrate atopic asthma through the production of IL-4, IL-5, and IL-13, which contribute to establishing airway inflammation and remodeling. ILC2 are also capable of producing IL-5 and IL-13 in response to IL-33 *via* Th2 cell-independent mechanisms (Klein Wolterink et al., 2012; Licona-Limón et al., 2013; Li and Hendriks, 2013; Halim et al., 2014). We previously reported that the almost complete depletion of $CD4^+$ Th2 cells did not significantly reduce the production of IL-5 and IL-13 in the lungs in ovalbumin (OVA)-induced asthma model of mice showing airway remodeling (Nabe et al., 2011), indicating that other cellular sources of Th2 cytokines exist. Considering the nature of IL-33 (Schmitz et al., 2005), a foreign protein, OVA can induce IL-33 generation from the airway epithelial cells even at the initial exposure. Thus, we speculated that IL-33 was produced in the lungs, and the IL-33 subsequently induced the generation of IL-5 and IL-13. However, it has been unclear what amount of IL-33 is produced in the lung, and the exact cellular sources of IL-33 in the lungs have not been identified. It also remains unclear whether cellular sources of IL-33 are altered during repeated antigen exposure. Additionally, another murine study revealed that exogenous IL-33-induced airway inflammation was resistant to a systemic treatment with a glucocorticoid (Kabata et al., 2013). However, it has yet to be determined whether allergic IL-33 production in the lungs can be modulated by glucocorticoid treatments.

In the present study, time-course changes in IL-33 protein and IL-33 mRNA levels were observed during multiple challenges with an antigen, OVA in actively sensitized mice, and the cellular sources of IL-33 were detected by immunohistochemistry and flow cytometry. We also investigated whether OVA-induced IL-33 production was susceptible or resistant to a systemic treatment with the glucocorticoid, dexamethasone.

2. Materials and methods

2.1. Sensitization and challenge

Six-week-old BALB/c mice were purchased from Japan SLC (Hamamatsu). As shown in Fig. 1, mice were sensitized by intraperitoneal (i.p.) injections with OVA (Grade V; Sigma Chem., St. Louis, MO, USA) adsorbed to $Al(OH)_3$, which was made according to our previously reported method (Nabe et al., 2005, 2011, 2013), at a dose of 50 μg OVA adsorbed to 2 mg $Al(OH)_3$/0.5 ml PBS/animal on days 0, 14, and 28. The sensitized mice were challenged on days 35, 36, 37 and 40 under inhalation anesthesia with isoflurane (Abbott Japan, Tokyo) with 2% OVA at a volume of 25 μl by intratracheal administration, as reported previously (19). Dexamethasone (0.3, 1 and 3 mg/kg) was administered i.p. once 3 h before the 1st OVA challenge (DEX-Exp. 1) or repeatedly 3 h before the respective 1st–3rd challenges (DEX-Exp. 2).

This animal study was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

2.2. Measurement of the amount of IL-33 in the lungs

The right lobes of the lungs were isolated under anesthesia with ketamine and xylazine before or after the 1st–4th OVA challenges, and then homogenized in 1 ml/right lobe of protease inhibitor cocktail tablet (Complete, Mini®, Roche, Mannheim, Germany)—containing tissue protein extraction reagent (T-PER, Thermo Scientific, Rockford, IL, USA). Following centrifugation, the amount of IL-33 in the supernatant was assayed by the ELISA kit (R&D Systems, Minneapolis, MN, USA).

2.3. Immunohistochemistry

The left lobes of the lungs were isolated under anesthesia with ketamine and xylazine before and 4 h after the 1st and 3rd OVA challenges, and then fixed with 10% buffered formalin. Tissues were embedded in paraffin, and 4-μm-thick sections were prepared. IL-33 was detected in the lung tissue by immunohistochemistry. Antigenicity in the tissue sections was generally activated by immersion of the sections on slides in Histo VT one (Nacalai Tesque, Kyoto, Japan). After washing, the sections were treated with 3% hydrogen peroxide–methanol solution. Following washing, the sections were treated with 1.5% normal rabbit serum for 20 min in order to prevent non-specific binding of the secondary antibody. After washing, sections were stained with an anti-murine IL-33 antibody (AF3626, 5 μg/ml, R&D Systems), anti-murine ST2/IL-1R4 antibody (AF1004, 20 μg/ml, R&D Systems), or goat IgG for 1 h. After washing with PBS, sections were stained with a biotinylated rabbit anti-goat IgG antibody (Jackson ImmunoResearch Lab., West Grove, PA, USA) for 30 min. After washing, sections were stained with streptavidin–horseradish peroxidase (Vectastain Elite ABC standard kit, Vector Lab., Burlingame, CA, USA), followed by color development using a 3,3′-diaminobenzidine tablet (Sigma-Aldrich, St. Louis, MO, USA), and then counterstaining with hematoxylin solution.

2.4. Flow cytometric analyses of IL-33-producing cells in the lungs

The whole lobes of the lungs were isolated under anesthesia with ketamine and xylazine before and 4 h after the 1st and 3rd OVA challenges. This tissue was cut into 1 × 1 × 1-mm^3 pieces, followed by incubation with 3 mg/ml collagenase type-I (220 U/mg, Gibco, Grand Island, NY, USA) at 37 °C for 1 h. Cells were dispersed using a syringe, and then filtrated through a 70-μm nylon mesh. After centrifugation,

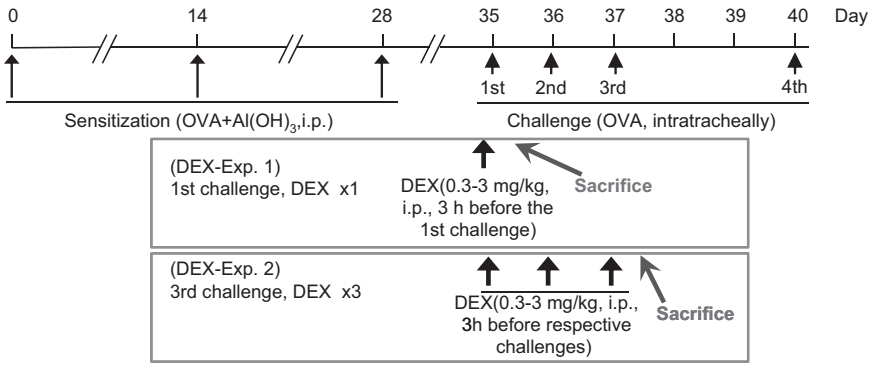

Fig. 1. Schedules for sensitization and challenges, and the administration of dexamethasone.