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Original article

Distinct effects of endogenous interleukin-23 on eosinophilic airway inflammation in response to different antigens

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Abbreviations:
BALF, bronchoalveolar lavage fluid; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceradehyde-3-phosphate dehydrogenase; HDM, house dust mite; IFN-γ, interferon gamma; Ig, immunoglobulin; IL, interleukin; OVA, ovalbumin; PBS, phosphate buffered saline; (RT-) PCR, (reverse transcription) polymerase chain reaction; SEM, standard error of the mean; Th, helper T; TLR, toll-like receptor

ABSTRACT

Background: The role of interleukin (IL)-23 in asthma pathophysiology is still controversial. We examined its role in allergic airway inflammation in response to two distinct antigens using IL-23-deficient mice.

Methods: Allergic airway inflammation was evaluated in wild-type and *IL-23p19*$^{-/-}$ mice. Mice were sensitized to ovalbumin (OVA) or house dust mite (HDM) by intraperitoneal injection of antigen and their airways were then exposed to the same antigen. Levels of antigen-specific immunoglobulins in serum as well as cytokines in bronchoalveolar or peritoneal lavage fluid and lung tissue were determined by enzyme-linked immunosorbent assay and/or quantitative polymerase chain reaction.

Results: Deficiency of *IL-23p19* decreased eosinophils and Th2 cytokines in bronchoalveolar lavage fluid (BALF) of OVA-treated mice, while it increased BALF eosinophils of HDM-treated mice. Peritoneal injection of OVA with alum, but not of HDM, induced local synthesis of IL-6, IL-10, and IL-23. Systemic production of antigen-specific IgG_1 was partially dependent on IL-23. In contrast, airway exposure to HDM, but not to OVA, induced *IL-23p19* mRNA expression in the lungs. In *IL-23p19*-deficient mice, HDM-exposed lungs did not exhibit the induction of *IL-17A*, which negatively regulates eosinophilic inflammation.

Conclusions: Different antigens induced IL-23 at different part of the body in our similar asthma models. Endogenous IL-23 production at the site of antigen sensitization facilitates type-2 immune responses, whereas IL-23 production and subsequent IL-17A synthesis in the airways suppresses allergic inflammation.

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Introduction

Bronchial asthma is a chronic inflammatory disease of the airways characterized by eosinophilic infiltrates, mucus hypersecretion, airway remodeling, and bronchial hyperresponsiveness.[1] Allergic airway inflammation is primarily driven by allergen-specific $CD4^+$ T cells. Upon allergen presentation by dendritic cells, naïve $CD4^+$ T cells differentiate into four distinct populations depending on the cytokine environment: T helper cell type 1 (Th1), Th2, Th17, and regulatory T cells. Asthma is accompanied by the

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induction of antigen-specific Th2 cells and related cytokines such as interleukin (IL)-4, IL-5, and IL-13.[2–4] Recent studies have shown that Th17 cells and related cytokines, including IL-17A, IL-17F, and IL-22, are also associated with allergic inflammation.[5] In asthmatic patients, IL-17A concentration is increased in peripheral blood, sputum, and bronchoalveolar lavage fluid (BALF).[6–8] In experimental models of asthma, Th17 cells enhance not only neutrophilic airway inflammation[8] but also Th2 cell-mediated eosinophilic airway inflammation.[9]

IL-23, secreted from antigen-presenting cells such as activated macrophages and dendritic cells, is an essential cytokine in the maintenance of Th17 cells, secretion of IL-17A, and memory T-cell proliferation.[5,9–14] Recently, Wakashin *et al.*[9] found that IL-23 enhances antigen-induced Th2 cytokine production and eosinophil recruitment in airways. Peng *et al.*[15] reported the activation of IL-23 receptors expressed on Th2 cells promotes Th2 polarization and cytokine production. These results suggest important roles for IL-23 in the development of asthma. Conversely, there are some reports that provide evidence for IL-23 and/or IL-17A as negative regulators of allergic inflammation.[16,17] IL-23 suppresses inflammatory responses to fungi in the airways in a toll-like receptor (TLR) 6-dependent manner, which includes the activation of T cells, Th2 cytokine production, and granulocyte recruitment.[17]

The precise roles of the IL-23/Th17 axis in the regulation of allergic airway inflammation are still controversial. Thus, we performed this study using IL-23 deficient (*IL-23p19*$^{-/-}$) mice in two different models of asthma.

Methods

Animal preparations

Specific pathogen- and viral antibody-free, 6-week-old, female C57BL/6J (WT) mice weighing between 20 and 25 g were purchased from Charles River Laboratories (Yokohama, Japan). IL-23-deficient (*IL-23p19*$^{-/-}$) and IL-17A-deficient (*IL-17A*$^{-/-}$) mice with a C57BL/6 genetic background were developed as previously reported.[18,19] All animals were housed in a facility in bioBubble® barrier units (bioBubble Inc., Fort Collins, CO) under positive pressure. The experimental protocol was reviewed and approved by the Laboratory Animal Care and Use Committee of Keio University School of Medicine.

Allergen sensitization and exposure

In the ovalbumin (OVA)-induced airway inflammation model, sensitization to OVA was achieved by intraperitoneal injection of 15 μg OVA in an alum solution (Imject®Alum, Pierce Chemical, Rockford, IL) on days 0, 7, and 14. Airway challenge was carried out in a dedicated chamber with aerosolized 1% (w/v) OVA diluted in phosphate buffered saline (PBS) for 20 min on days 21, 22, 23, and 24.

In the house dust mite antigen (HDM)-induced airway inflammation model, mice were actively sensitized against *Dermatophagoides pteronyssinus* antigen (Biostir, Kobe, Japan) by intraperitoneal injection of 10 μg HDM on days 0, 7, and 14. On days 21, 22, and 23, the mice were exposed to HDM (100 μg/body) via the nares. Mice treated with PBS were used as controls.

Bronchoalveolar and peritoneal cavity lavage fluid

Mice were sacrificed by giving an intraperitoneal overdose of pentobarbital 24 h after the final OVA or HDM airway exposure. The trachea was cannulated and the lungs were lavaged with 1.4 ml of cold PBS containing 0.6 mM ethylenediamine tetraacetic acid (EDTA) to collect bronchoalveolar lavage fluid (BALF). In some animals, the peritoneal cavity was lavaged with 5 ml of PBS/EDTA 3, 6, 12, and 24 h after an OVA or HDM peritoneal injection.

The cells in BALF and the abdominal cavity lavage fluid were counted using a hemocytometer, and white blood cell differential counts were determined on Diff-Quik-stained cytospin slides (Symex, Kobe, Japan).

Cytokine analysis

The concentrations of cytokines in BALF and peritoneal lavage fluid were measured with an ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Measurement of serum ova- and hdm-specific IgG_1 antibodies in serum

Blood was collected from the inferior vena cava 24 h after the last challenge of antigens in the airways. OVA IgG_1 was measured using a commercially available ELISA kit (Shibayagi, Gunma, Japan), according to the manufacturer's instructions. HDM-specific IgG_1 antibody in serum was measured by direct ELISA using target antigen-coated plates, biotinylated anti-mouse IgG_1 rat antibody (BD Biosciences, San Jose, CA, USA) and avidin-peroxidase (Sigma–Aldrich, St. Louis, MO, USA). Protein concentration was determined by measuring the optical density at 450 nm after color development with 3,3′,5,5'-tetramethylbenzidine Substrate Reagent Set (BD Biosciences), which was stopped by the addition of 1 M H_2SO_4.

Quantitative real-time RT-PCR

Tissue samples were homogenized and total RNA was extracted using the RNeasy Mini kit (QIAGEN, Hilden, Germany). Complementary DNA was generated with SuperScript reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The expression level of mRNA was measured by real-time quantitative PCR using SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) and an ABI 7500 Real-Time PCR System (Applied Biosystems). We used the ΔΔ threshold cycle (ddCt) technique to calculate relative mRNA expression of target genes normalized to levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene.

Statistical analysis

Data are presented as means ± SEM. Student's *t*-test and one-way analysis of variance were performed for comparisons of two and three or more groups, respectively. Tukey's tests for comparison with control group and Dunett's multiple comparison tests for analysis among groups without control group were used as post-hoc tests. Data were analyzed with GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA). A p value less than 0.05 was regarded as significant.

Results

IL-23 promotes ova-induced asthmatic responses

In order to examine the roles of IL-23, we compared OVA-induced airway inflammation in *IL-23p19*$^{-/-}$ mice with that of wild-type mice (Fig. 1). Although both types of animals exhibited eosinophilic inflammation in response to OVA exposure in the airways, *IL-23p19*$^{-/-}$ mice had less eosinophils in the BALF than wild-type mice (Fig. 1a, b). The amounts of IL-5 and IL-13 in BALF were also reduced by half in *IL-23p19*$^{-/-}$ mice (Fig. 1c, d, $P < 0.01$).

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