# IL-22 attenuates IL-25 production by lung epithelial cells and inhibits antigen-induced eosinophilic airway inflammation

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**Background: IL-22 functions as both a proinflammatory cytokine and an anti-inflammatory cytokine in various inflammations, depending on the cellular and cytokine milieu. However, the roles of IL-22 in the regulation of allergic airway inflammation are still largely unknown.**

**Objective: We sought to determine whether IL-22 is involved in the regulation of allergic airway inflammation.**

**Methods: We examined IL-22 production and its cellular source at the site of antigen-induced airway inflammation in mice. We also examined the effect of IL-22 neutralization, as well as IL-22 administration, on antigen-induced airway inflammation. We finally examined the effect of IL-22 on IL-25 production from a lung epithelial cell line (MLE-15 cells).**

**Results: Antigen inhalation induced IL-22 production in the airways of sensitized mice. $CD4^+$ T cells, but not other lymphocytes or innate cells, infiltrating in the airways produced IL-22, and one third of IL-22–producing $CD4^+$ T cells also produced IL-17A. The neutralization of IL-22 by anti–IL-22 antibody enhanced antigen-induced IL-13 production, eosinophil recruitment, and goblet cell hyperplasia in the airways. On the other hand, intranasal administration of recombinant IL-22 attenuated antigen-induced eosinophil recruitment into the airways. Moreover, anti–IL-22 antibody enhanced antigen-induced IL-25 production in the airways, and anti–IL-25 antibody reversed the enhancing effect of anti–IL-22 antibody on antigen-induced eosinophil recruitment into the airways. Finally, IL-22 inhibited IL-13–mediated enhancement of IL-25 expression in IL-1β– or LPS-stimulated MLE-15 cells.**

**Conclusion: IL-22 attenuates antigen-induced airway inflammation, possibly by inhibiting IL-25 production by lung epithelial cells. (J Allergy Clin Immunol 2011;128:1067-76.)**

***Key words:*** *Allergic inflammation, asthma, IL-22, eosinophils, IL-25*

Asthma is chronic airway inflammation characterized by eosinophil infiltration, mucus hypersecretion, and airway hyperresponsiveness (AHR) to a variety of stimuli.[1-3] These characteristics are mainly mediated by antigen-specific $T_H2$ cells and their cytokines, including IL-4, IL-5, and IL-13.[1-3] In addition, a number of studies have revealed that the airways of patients with severe asthma exhibit neutrophil infiltration accompanied by IL-17A production.[4-6] Moreover, we and others have shown that $T_H17$ cells induce neutrophilic airway inflammation in part through the production of IL-17A.[7,8] More recently, IL-22, one of the $T_H17$ cell–derived cytokines,[9] has been detected in the airways in a murine model of asthma.[10,11]

IL-22 is a member of the IL-10 cytokine family with multiple functions in various inflammatory diseases.[12,13] The fact that IL-22 markedly increases the expression of antimicrobially acting proteins in various epithelia suggests a role for this cytokine in innate immune defense.[12,13] Although previous studies have demonstrated that IL-22 is mainly produced by $T_H1$ and $T_H17$ cells,[9,12,13] recent studies have shown that skin-homing $CCR10^+$ T cells also produce IL-22 without IL-17A production and that these IL-22–producing $CD4^+$ T cells ($T_H22$ cells) show a stable and distinct phenotype from $T_H1$, $T_H2$, and $T_H17$ cells.[14-16] In addition, it has been demonstrated that a population of natural killer (NK) cells, $CD11c^+$ myeloid cells, and lymphoid tissue inducer (LTi)–like cells produce IL-22.[9,17-19]

IL-22 mediates its effects through a heterodimeric transmembrane receptor complex composed of IL-22 receptor 1 (IL-22R1) and IL-10 receptor 2 (IL-10R2) and subsequent JAK–signal transducer and activator of transcription (STAT) signaling pathways, including Jak1, Tyk2, and STAT3.[12,13] IL-10R2 has been shown to function as a receptor component not only of IL-22 but also of IL-10, IL-26, IL-28, and IL-29 and to be ubiquitously expressed in a variety of cells.[12,13,20] On the other hand, it has been shown that IL-22R1 is a receptor component of IL-22, IL-20, and IL-24, and its expression is restricted to nonimmune cells, such as epithelial cells in the intestine and lung and keratinocytes in the skin.[12,13,20,21] Indeed, it has been reported that neither resting nor activated immune cells, including T cells, B cells, NK cells, macrophages, and dendritic cells, express IL-22R1.[20] These findings suggest that IL-22 acts on nonimmune cells in the skin, intestine, and lung.

Importantly, recent studies have shown that IL-22 exhibits both proinflammatory and anti-inflammatory properties.[13,22] The

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*Abbreviations used*
AHR: Airway hyperresponsiveness
BALF: Bronchoalveolar lavage fluid
BMDC: Bone marrow–derived dendritic cell
IL-10R2: IL-10 receptor 2
IL-22R1: IL-22 receptor 1
LTi: Lymphoid tissue inducer
NK: Natural killer
OVA: Ovalbumin
PAS: Periodic acid–Schiff
qPCR: Quantitative real-time PCR
SOCS3: Suppressor of cytokine signaling 3
STAT: Signal transducer and activator of transcription
TARC: Thymus and activation-regulated chemokine
TSLP: Thymic stromal lymphopoietin

proinflammatory properties of IL-22 have been supported by the finding that IL-22–deficient mice exhibited decreased acanthosis and reduced neutrophil infiltration in the inflamed skin after repeated treatments with IL-23.[9] In addition, it has been shown that IL-22–producing T cells are involved in the pathogenesis of inflammatory skin diseases through an IL-22– and TNF-α–dependent manner.[23] On the other hand, IL-22 production by NKp46$^+$ NK cells has been shown to be involved in mucosal defense mechanisms.[24] The beneficial properties of IL-22 are further underscored by the findings that IL-22 is involved in protection against bacterial pneumonia,[21] acute liver injury,[25] and murine models of inflammatory bowel disease.[26,27] These findings suggest that the functions of IL-22 are influenced by the cellular and cytokine milieu.

Recently, it has been shown that IL-22 is detected at the site of allergic airway inflammation.[10,11] In addition, Zhao et al[28] have reported that serum levels of IL-22 are higher in patients with severe asthma than those seen in patients with mild asthma and healthy control subjects. Moreover, it has been reported that IL-22 inhibits inflammatory responses in a murine model of asthma by modulating the function of dendritic cells.[10] Furthermore, Besnard et al[29] have recently shown that IL-22 is required for the sensitization phase of allergic inflammation but exerts inhibitory functions in the effector phase. However, the mechanisms by which IL-22 regulates allergic airway inflammation remain largely unknown.

In this study we sought to determine whether IL-22 regulates allergic airway inflammation in a murine model of asthma and, if so, to determine the mechanism by which this occurs. We found that IL-22 was produced by CD4$^+$ T cells infiltrating the airways on antigen challenge, that the neutralization of IL-22 by anti–IL-22 antibody in the effector phase enhanced antigen-induced eosinophil recruitment in the airways, and that intranasal administration of recombinant IL-22 inhibited antigen-induced eosinophil recruitment in the airways. We also found that anti–IL-22 antibody enhanced antigen-induced IL-25 production in the airways, which is known to enhance $T_H2$-type immune responses in the airways,[30-32] and indeed, coinjection of anti–IL-25 antibody reversed the enhancing effect of anti–IL-22 antibody on antigen-induced eosinophil recruitment into the airways. Finally, we found that IL-22 inhibited IL-13–mediated enhancement of IL-25 expression in an IL-1β– or LPS-stimulated lung epithelial cell line (MLE-15 cells). Our results suggest that IL-22 attenuates antigen-induced airway inflammation in part by inhibiting the expression of IL-25 in lung epithelial cells.

# METHODS

## Mice

BALB/c mice (Charles River Laboratories, Atsugi, Japan) were housed in microisolator cages under pathogen-free conditions. The Chiba University Animal Care and Use Committee approved the animal procedures used in this study.

## Reagents

Polyclonal anti–IL-22 antibody and anti–IL-25 (IL-17E) antibody were obtained from R&D Systems (Minneapolis, Minn) and BioLegend (San Diego, Calif), respectively. The anti–IL-22 mAb (clone MH22B2) was described previously.[33] Recombinant cytokines were purchased from PeproTech (Rocky Hill, NJ). A murine lung epithelial cell line (MLE-15 cell) was a kind gift from Dr Jeffrey Whitsett (University of Cincinnati).[34] See the Methods section in this article's Online Repository at www.jacionline.org for further details.

## Antigen-induced allergic inflammation in the airways

BALB/c mice (aged 6-8 weeks) were immunized intraperitoneally with ovalbumin (OVA) and challenged once with inhaled OVA, as described previously (see the Methods section in this article's Online Repository for further details).[35] For the analysis of goblet cell hyperplasia and AHR, OVA-sensitized mice were challenged with inhaled OVA 3 times at a 48-hour interval.[7] Where indicated, mice were injected intraperitoneally with anti–IL-22 antibody (20 μg per mouse), anti–IL-25 antibody (20 μg per mouse), or control antibody (BD Biosciences, San Diego, Calif) at 24 hours before the inhaled OVA challenge. In other experiments recombinant IL-22 (0.1 μg per mouse) or saline (as a control) was administered intranasally twice at 48 and 2 hours before the inhaled OVA challenge, respectively. The numbers of eosinophils, neutrophils, lymphocytes, and CD4$^+$ T cells recovered in bronchoalveolar lavage fluid (BALF) were evaluated at 48 hours after OVA inhalation, as described previously.[7]

## Cytokine assay

The amounts of IL-5, IL-13, IL-22, IL-25, IL-33, IFN-γ, and thymic stromal lymphopoietin (TSLP) in BALF were determined by mean of ELISA, according to the manufactures' instructions (see the Methods section in this article's Online Repository for further details).

## Measurement of airway responsiveness

Airway responsiveness to aerosolized acetylcholine was assessed by using a computer-controlled small animal ventilator system (flexiVent; SCIREQ, Inc, Montreal, Quebec, Canada), as described elsewhere.[36]

## Cytokine production and chemokine receptors of CD4$^+$ T cells

CD4$^+$ T cells were isolated from BALF cells, inguinal lymph node cells, or lung homogenates by means of magnetic cell sorting.[7] For intracellular cytokine analysis, CD4$^+$ T cells were stimulated with phorbol 12-myristate 13-acetate (20 ng/mL; Calbiochem, San Diego, Calif) plus ionomycin (1 μg/mL, Calbiochem) at 37°C for 4 hours in the presence of brefeldin A (10 μmol/L, BD Bioscience). Cytokine profiles (IFN-γ, IL-4, IL-17A, and IL-22) and the expression of chemokine receptors (CCR3, CCR5, CCR6, and CCR10) of CD4$^+$ T cells were evaluated by means of flow cytometry (see the Methods section in this article's Online Repository for further details).

## Histologic and immunohistologic analysis

The number of goblet cells was counted on periodic acid–Schiff (PAS)–stained lung sections, as described elsewhere.[31] Immunostaining of cryosections was performed as described previously (see the Methods section in this article's Online Repository for further details).[37]