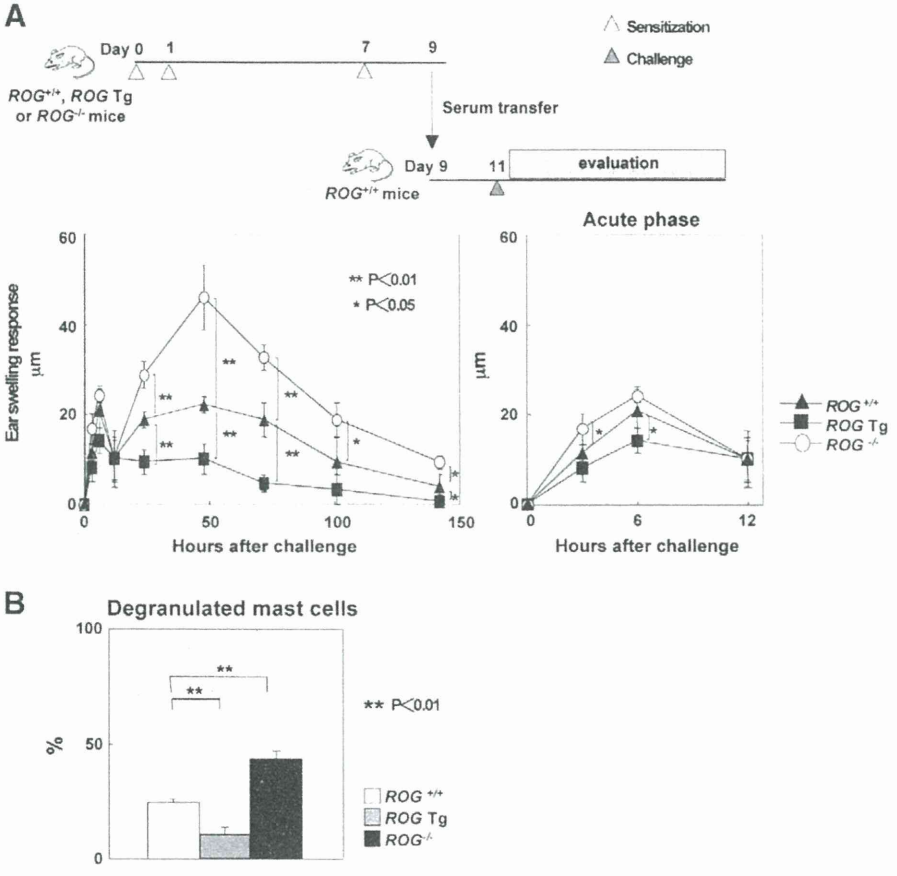

Figure 4 Replication of the contact hypersensitivity by adoptive transfer of serum. (A) An adoptive transfer experiment was carried out by transferring 300 μl of sensitized serum from the three different donor groups (the wild type $ROG^{+/+}$, ROG Tg and $ROG^{-/-}$ mice) into wild type C57BL/6 recipient mice via an intraperitoneal injection. (B) The percentages of the degranulated mast cells 48 h after challenge were assessed by May–Grunewald Giemsa staining. In each group, six microscopic fields from three mice were examined. The mean values with their SD are indicated.

ROG Tg mice showed a milder degree of dermatitis than those that received cells from wild type mice. On the other hand, the mice that received cells from $ROG^{-/-}$ donors showed an exacerbation of dermatitis. The percentage of degranulated mast cells detected by May–Grunewald Giemsa staining were lower in mice that received DNFB-sensitized $CD4^+$ T cells from the ROG Tg mice and higher in mice from the $ROG^{-/-}$ mice in comparison to that in mice which received the wild type cells (Fig. 5C). These results indicate that the attenuating effect of ROG in CHS is mediated by CD4 T cells and can be attributed to their changes in the Th2 response.

4. Discussion

This study showed that ROG expression in $CD4^+$ T cells negatively regulates the induction of CHS. Since ROG is a negative regulator of Th2 responses, this study highlights the fact that the Th2 response is critical for the pathogenesis of CHS, which is traditionally recognized as delayed type hypersensitivity.

The degree of edema in the dermis was clearly enhanced in the $ROG^{-/-}$ mice and suppressed in the ROG Tg mice (Fig. 1B). Edema is caused by an increased blood vessel permeability, which is one of the consequences of mast cell degranulation followed by the release of vasoactive amines such as histamine and serotonin together with lipid mediators such as leukotrienes or prostaglandins [38]. Mast cell degranulation is mainly triggered by the cross-linking of membrane-bound IgE by allergens [39]. In this study, the levels of serum total IgE and hapten-specific IgG1, which reflect the extent of Th2 responses, were increased in the $ROG^{-/-}$ mice and decreased in the ROG Tg mice (Figs. 3A and B). In transfer experiments with $CD4^+$ T cells, challenging immediately after the transfer of sensitized $CD4^+$ T cells without sensitizing recipient mice induced no CHS responses, but by sensitizing once after the transfer of sensitized $CD4^+$ T cells, recipient mice showed different levels of CHS responses according to the expression level of ROG in donor $CD4^+$ T cells (Figs. 5A vs. Fig. 5B). Thus, antigen stimulation of sensitized $CD4^+$ T cells and subsequent production of antigen-specific antibodies, including IgE in the recipient mice appear to be the critical process to induce the inflammation. Our data are consistent with those of previous reports showing that mast cells and Th2 reactions positively regulate the CHS reaction [12–18].

$CD8^+$ T cells have been proposed to be the main effector cells during the elicitation phase of CHS in this model [5–11].

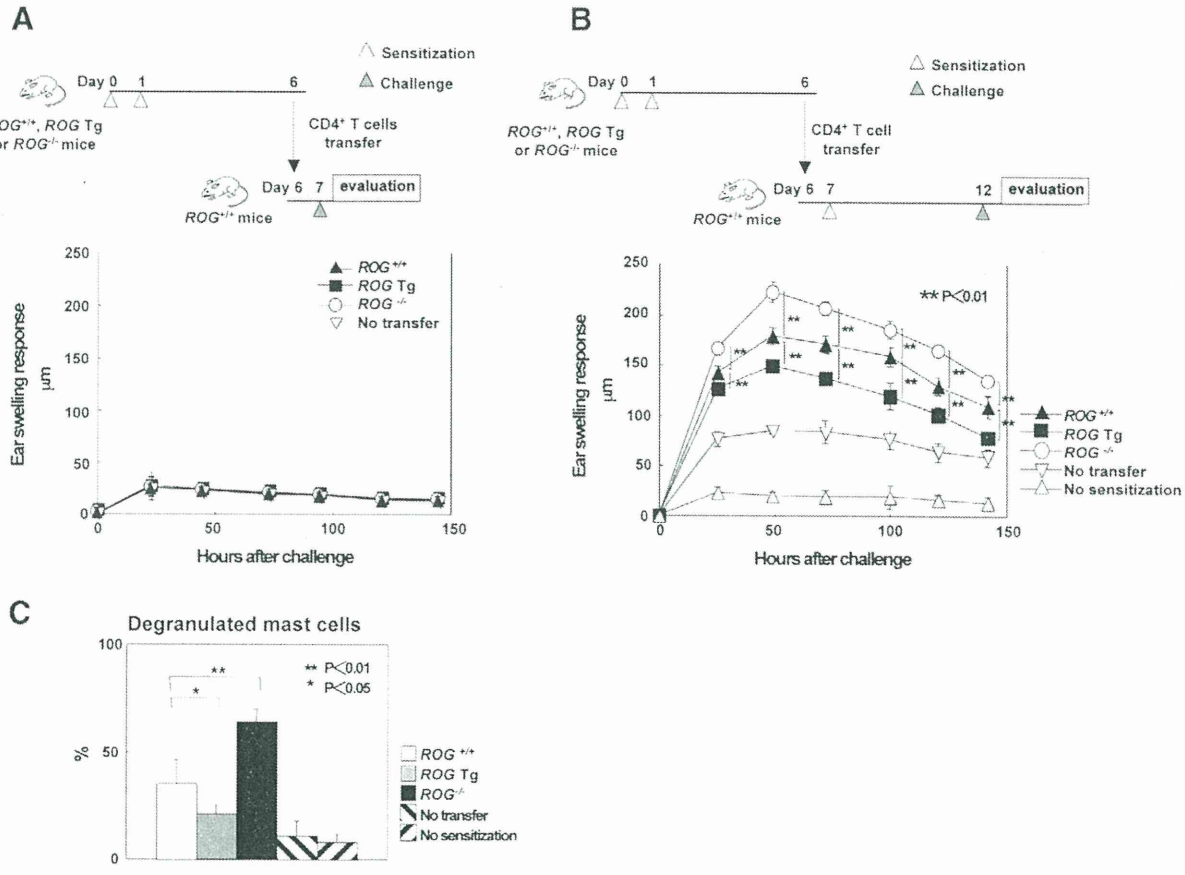

Figure 5 Replication of the contact hypersensitivity by adoptive transfer of $CD4^+$ T cells. (A–B) The adoptive transfer experiment was carried out by transferring sensitized splenic $CD4^+$ T cells from the three different donor groups (the $ROG^{+/+}$, ROG Tg and $ROG^{-/-}$ mice), into the wild type syngeneic C57BL/6 recipient mice via a tail vein injection. In one experiment, the recipient mice were challenged on the following day, and the ear swelling responses were assessed (A). In another experiment, the recipient mice were sensitized once on day 7 and challenged on day 12. Then the ear swelling responses were assessed (B). (C) The percentages of the degranulated mast cells 48 h after challenge was assessed by May–Grunewald Giemsa staining. In each group, six microscopic fields from three mice were examined. The mean values with their SD are indicated.

Hapten-specific $CD8^+$ T cells induce keratinocyte apoptosis via Fas/Fas-L and perforin/granzyme cytotoxic activities [9] resulting in a thickened epidermis. The thickened epidermis resulting after the hapten challenge was similar in the $ROG^{+/+}$, ROG Tg and $ROG^{-/-}$ mice (Fig. 1B). The number of infiltrated $CD8^+$ T cells was also similar in the inflammatory ear lesions of the three groups (Fig. 1E). In addition, the ability to produce cytokines and the cytotoxic killing of hapten-specific $CD8^+$ T cells was not altered between the three groups (Fig. 2C). Furthermore, the adoptively transferred $CD8^+$ T cells from the $ROG^{+/+}$, ROG Tg and $ROG^{-/-}$ mice elicited a similar level of ear swelling (Figs. 2D and E). Taken together, our data indicate that ROG molecules in $CD4^+$, but not in $CD8^+$ T cells, positively contribute to the modulation of the CHS responses. Several investigators have reported that the CHS could be induced by the application of hapten, even in CD8-depleted or -KO mice [6,40]. Therefore, based on both current study and these previous reports, it appears that CD8 T^+ cells may not play an important role in this CHS reaction setting where the exacerbation and the attenuation of edema in $ROG^{-/-}$ mice and ROG Tg, respectively, were observed 24–48 h after the hapten-challenge.

IL-17 and Th17 also play an important role in CHS [41–43]. The effect of ROG expression on Th17 differentiation was assessed in an *in vitro* culture system, and showed no obvious difference in Th17 differentiation among the wild type, ROG Tg and $ROG^{-/-}$ mice (unpublished observation).

This study demonstrates that ROG expression in $CD4^+$ T cells can down-regulate the severity of CHS in mice and suggests that the targeting of ROG may help to design innovative strategies to treat contact dermatitis in humans. In addition, Th2 responses are involved in the pathogenesis of atopic dermatitis, where the prolonged use of steroid and/or calcineurin inhibitors suppress cellular immunity and often cause vulnerability to herpes virus infection and bacterial infections [44–46]. Specific and selective therapy that inhibits Th2 function will help avoid these complications. Therefore, the results of the current study may also lead to the design of innovative strategies to treat atopic dermatitis.

5. Conclusions

ROG negatively regulates the induction of CHS through the control of the Th2 cell-mediated allergic responses,

including IgE generation and mast cell degranulation, and thus ROG may represent another potential therapeutic target for the treatment of allergic skin disorders where type 2 responses are involved in the pathogenesis.

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

Kentaro Takahashi, MD,[a] Koichi Hirose, MD, PhD,[a] Saki Kawashima, MSc,[a] Yusuke Niwa,[a] Hidefumi Wakashin, MD, PhD,[a] Arifumi Iwata, MD, PhD,[a] Koji Tokoyoda, PhD,[b] Jean-Christophe Renauld, PhD,[c] Itsuo Iwamoto, MD, PhD,[d] Toshinori Nakayama, MD, PhD,[b] and Hiroshi Nakajima, MD, PhD[a] *Chiba, Japan, and Brussels, Belgium*

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

From the Departments of [a]Molecular Genetics and [b]Immunology, Graduate School of Medicine, Chiba University; [c]the Ludwig Institute for Cancer Research, Brussels Branch, de Duve Institute, Universite Catholique de Louvain, Brussels; and, [d]the Research Center for Allergy and Clinical Immunology, Asahi General Hospital, Chiba.

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Corresponding author: Hiroshi Nakajima, MD, PhD, or Koichi Hirose, MD, PhD, Department of Molecular Genetics, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chiba City, Chiba 260-8670, Japan. E-mail: hirose-kh@faculty.chiba-u.jp or nakajimh@faculty.chiba-u.jp.

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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]

Culture of MLE-15 cells

A murine lung epithelial cell line (MLE-15 cells) was grown in HITES medium, as described previously.[34] MLE-15 cells were stimulated with IL-1β (20 ng/mL) or LPS (100 ng/mL), IL-13 (20 ng/mL), or both in the presence or absence of IL-22 (20 ng/mL) in HITES medium for 6 hours.

Quantitative real-time PCR analysis

Quantitative real-time PCR (qPCR) was performed with a standard protocol on an ABI PRISM 7300 instrument (Applied Biosystems, Foster City, Calif; see the Methods section in this article's Online Repository for further details).

Western blotting

Western blotting was performed as described previously[38] by using anti-STAT3 antibody, anti–phospho-STAT3 antibody, and anti–phospho-STAT6 antibody (Cell Signaling Technology, Boston, Mass).

Preparation of bone marrow–derived dendritic cells

Bone marrow–derived dendritic cells (BMDCs) were prepared as described previously (see the Methods section in this article's Online Repository for further details).[39]

Data analysis

Data are summarized as means ± SDs. The statistical analysis of the results was performed by using the unpaired *t* test. *P* values of less than .05 were considered significant.

RESULTS

Lung-infiltrating $CD4^+$ T cells produce IL-22 in a murine model of asthma

To determine whether IL-22 is involved in the regulation of antigen-induced airway inflammation, we first examined the expression of IL-22 at the site of allergic airway inflammation. Consistent with recent reports,[10,29] levels of IL-22 were increased in the BALF of OVA-sensitized mice at 48 hours after OVA inhalation (n = 5 mice in each group, $P < .05$; Fig 1, *A*). Even in the absence of OVA inhalation (saline inhalation), IL-22 was detected at low levels in BALF (Fig 1, *A*). The induction of IL-22 expression in the lungs of OVA-sensitized mice by OVA inhalation was confirmed at mRNA levels by means of qPCR analysis (data not shown).

$CD4^+$ T cells in BALF were isolated from OVA-sensitized mice at 48 hours after OVA inhalation, stimulated with phorbol 12-myristate 13-acetate plus ionomycin, and analyzed by means of intracellular cytokine staining to identify the cellular source of IL-22 expressed at the site of allergic airway inflammation. As shown in Fig 1, *B*, 1.6% ± 0.5% of $CD4^+CD3^+$ T cells in BALF were positive for intracellular IL-22 staining, whereas only 0.2% ± 0.3% of $CD4^+$ T cells from inguinal lymph nodes in the same mice were positive for intracellular IL-22 staining (means ± SDs, n = 4, $P < .05$; Fig 1, *B*). Among IL-22–producing $CD4^+$ T cells in BALF, 33.2% ± 8.5% of cells were also positive for IL-17A staining, whereas few IL-22–producing $CD4^+$ T cells were positive for IFN-γ or IL-4 staining (Fig 1, *C*). Further analysis revealed that 21.6% ± 6.3% of IL-22–producing $CD4^+$ T cells expressed CCR6, a representative chemokine receptor expressed on T_H17 cells and LTi cells (Fig 1, *D*). In contrast, IL-22–producing $CD4^+$ T cells did not express CCR10 (Fig 1, *D*), which has been shown to be expressed on skin-homing IL-22–producing T cells in human subjects.[14,16] Moreover, IL-22–producing $CD4^+$ T cells lacked the expression of CCR5 and CCR3 and representative chemokine receptors expressed on T_H1 cells and T_H2 cells, respectively.

Previous studies have shown that not only $CD4^+$ T cells but also alveolar macrophages from patients with inflammatory lung disease and a population of NK cells in secondary lymphoid tissues produce IL-22.[40,41] To confirm IL-22 production by $CD4^+$ T cells, we performed immunostaining on BALF cells recovered from OVA-sensitized, OVA-inhaled mice. Although the frequency of cells showing a positive signal for anti–IL-22 staining was less than 1% of cells in BALF, approximately 80% of signals with anti–IL-22 staining were colocalized with $CD4^+$ cells (Fig 1, *E*). In addition, IL-22 mRNA was detected in $CD4^+$ cells but not in $CD8^+$ cells, $B220^+$ cells, $DX5^+$ cells, or $CD11c^+$ cells recovered from the lung of OVA-sensitized, OVA-inhaled mice (see Fig E1 in this article's Online Repository at www.jacionline.org). Moreover, IL-22 mRNA was not detected in $CD4^+CD3\epsilon^-$ cells in the lung (see Fig E1), suggesting that LTi-like cells are not responsible for IL-22 production in this asthma model. These results suggest that $CD4^+$ T cells, including T_H17 cells, are a major source for IL-22 in patients with allergic airway inflammation.

Anti–IL-22 antibody enhances antigen-induced eosinophil recruitment into the airways and AHR

To address the role of IL-22 in antigen-induced airway inflammation, we next examined the effects of IL-22 neutralization on the development of antigen-induced airway inflammation. Anti–IL-22 polyclonal antibody (20 μg per mouse), which neutralizes the activity of IL-22,[42] or control antibody was injected intraperitoneally into OVA-sensitized mice, and 24 hours later, the mice were challenged with OVA inhalation. As shown in Fig 2, *A*, anti–IL-22 antibody significantly enhanced antigen-induced eosinophil and $CD4^+$ T-cell recruitment into the airways (n = 10 mice in each group, $P < .05$). Similarly, anti–IL-22 mAb (clone MH22B2), which exhibits IL-22–neutralizing capacity equivalent to polyclonal anti–IL-22 antibody, enhanced antigen-induced eosinophil and $CD4^+$ T-cell recruitment into the airways (data not shown). In addition, anti–IL-22 antibody significantly enhanced the levels of IL-13 in BALF (n = 10 mice in each group, $P < .05$; Fig 2, *B*). On the other hand, IL-5 levels in BALF in anti–IL-22 antibody-treated mice were similar to those seen in control antibody-treated mice (Fig 2, *B*). The expression of IL-23 p19 and IL-12/IL-23 p40 mRNA in the lungs in anti–IL-22 antibody–treated mice was also similar to that seen in control antibody–treated mice (data not shown).

Histologic analysis revealed that increased eosinophil counts in BALF in anti–IL-22 antibody–treated mice were associated with enhanced peribronchial and perivascular inflammatory cell infiltration, which consists of lymphocytes, eosinophils, and some neutrophils, compared with that seen in control mice (Fig 3, *A*). PAS staining of lung sections revealed that goblet cell hyperplasia was also enhanced in anti–IL-22 antibody–treated mice (n = 5, $P < .05$; Fig 3, *A*). To determine whether IL-22 is involved in antigen-induced AHR, we examined the effects of anti–IL-22 antibody on airway responsiveness to acetylcholine in OVA-sensitized mice after OVA inhalation. Consistent with the enhanced eosinophil recruitment and IL-13 production in anti–IL-22 antibody–treated mice (Fig 2), airway responsiveness to acetylcholine was significantly enhanced in anti–IL-22 antibody–treated mice (n = 10, $P < .05$; Fig 3, *B*). These results

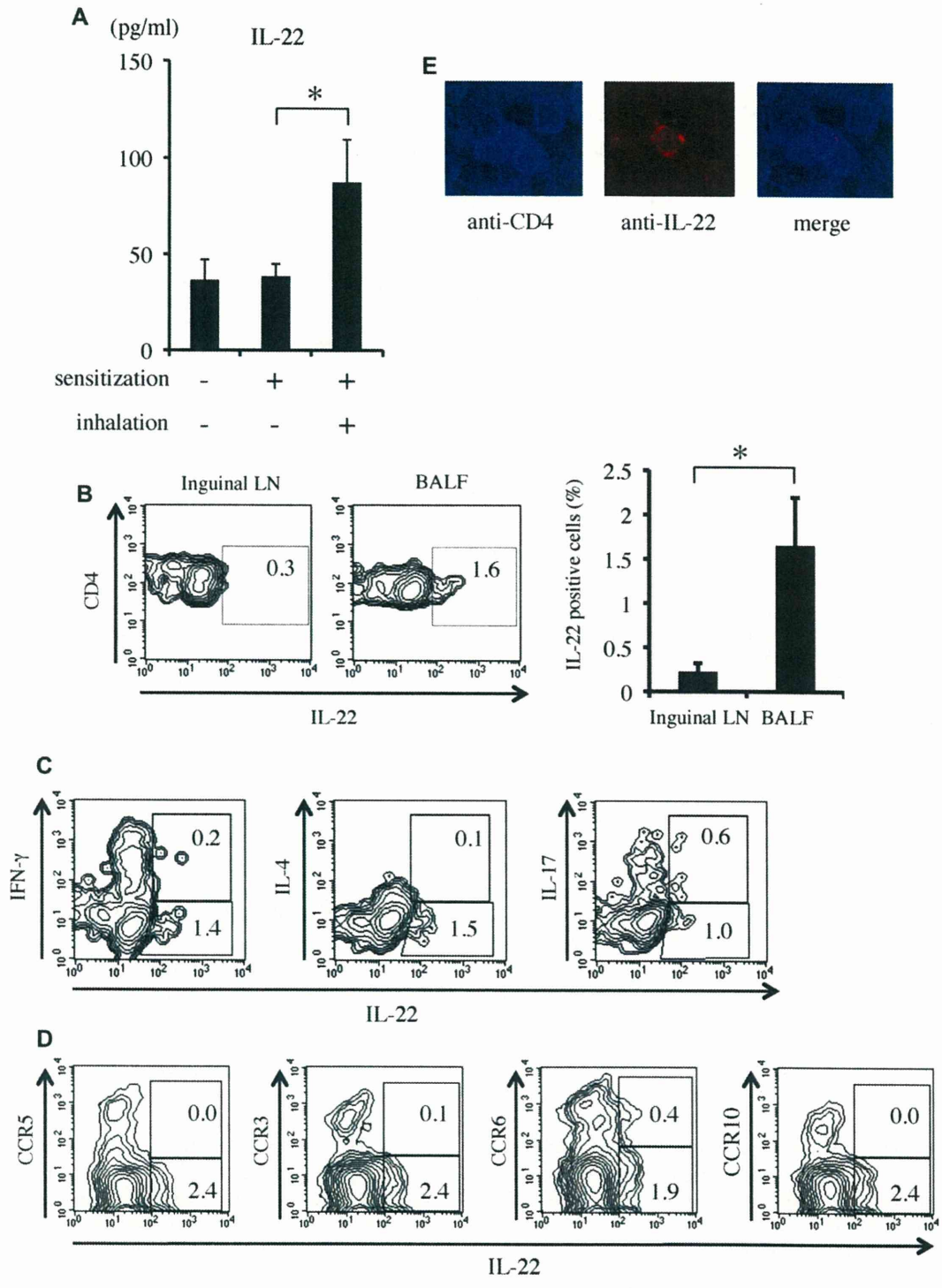

FIG 1. Lung-infiltrating $CD4^+$ T cells produce IL-22 in allergic airway inflammation. OVA-sensitized BALB/c mice were challenged with inhaled OVA or saline (control). IL-22 levels and $CD4^+$ T-cell numbers in BALF were then analyzed at 48 hours after OVA inhalation. **A**, IL-22 levels are increased in the lungs of sensitized mice on antigen inhalation. The amounts of IL-22 in BALF were determined by means of ELISA. Data are presented as means ± SDs for 5 mice in each group. $*P < .05$. **B**, IL-22–producing $CD4^+$ T-cell numbers are increased in allergic airway inflammation. $CD4^+$ T cells in BALF or inguinal lymph nodes (control) were collected by means of magnetic cell sorting and analyzed for the expression of CD4 and intracellular IL-22. Representative CD4 versus IL-22 staining of cells from BALF and inguinal lymph nodes *(LN)* and means ± SDs of the percentage of $IL\text{-}22^+$ cells among $CD4^+$ T cells are shown (n = 4 each). $*P < .05$. **C**, $CD4^+$ T cells in BALF were analyzed for intracellular IL-22 together with IFN-γ, IL-4, and IL-17. **D**, $CD4^+$ T cells in BALF were analyzed for intracellular IL-22 together with CCR5, CCR3, CCR6, and CCR10. **E**, Cells in BALF were stained with anti-CD4 antibody and anti–IL-22 antibody. Representative photomicrographs from 4 mice are shown.

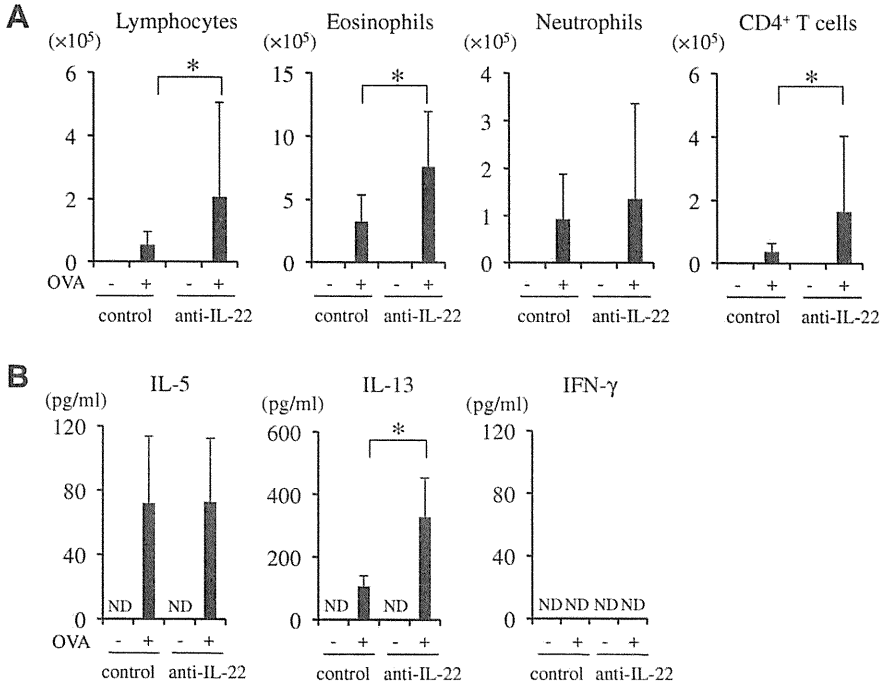

FIG 2. Anti–IL-22 antibody enhances antigen-induced eosinophil and $CD4^+$ T-cell recruitment into the airways. OVA-sensitized BALB/c mice were injected intraperitoneally with polyclonal anti–IL-22 antibody (20 μg per mouse) or control antibody. Twenty-four hours later, the mice were challenged with inhaled OVA or saline. **A**, Numbers of lymphocytes, eosinophils, neutrophils, and $CD4^+$ T cells in BALF were evaluated at 48 hours after OVA inhalation. Data are presented as means ± SDs for 10 mice in each group. $*P < .05$. **B**, Levels of IL-5, IL-13, and IFN-γ in BALF were evaluated by means of ELISA at 48 hours after OVA inhalation. Data are presented as means ± SDs for 10 mice in each group. *ND*, Not determined. $*P < .05$.

indicate that endogenously produced IL-22 inhibits the development of antigen-induced eosinophil and $CD4^+$ T-cell recruitment into the airways, goblet cell hyperplasia, and AHR.

Recombinant IL-22 inhibits antigen-induced eosinophil recruitment into the airways

We next examined the effect of IL-22 on the development of antigen-induced airway inflammation. Recombinant IL-22 (0.1 μg per mouse) or saline was administrated to OVA-sensitized mice intranasally at 48 hours and 2 hours before OVA inhalation, and airway inflammation was evaluated at 48 hours after OVA inhalation. As shown in Fig 4, *A*, the intranasal administration of IL-22 inhibited antigen-induced recruitment of eosinophils and $CD4^+$ T cells but not that of neutrophils into the airways. Although the difference did not reach statistical significance, intranasal administration of IL-22 also tended to inhibit antigen-induced IL-5 and IL-13 production in the airways (Fig 4, *B*) and airway responsiveness to acetylcholine (see Fig E2 in this article's Online Repository at www.jacionline.org). These results further indicate the inhibitory effects of IL-22 on the development of antigen-induced airway inflammation.

We also examined the effect of IL-22 on the resolution phase of allergic airway inflammation. In this experiment OVA-sensitized mice were challenged with OVA inhalation 3 times at a 48-hour interval, and IL-22 or saline was administered intranasally to the mice 3 times at 24, 48, and 72 hours after the last OVA inhalation. IL-22–treated mice exhibited significantly low numbers of eosinophils and lymphocytes and low levels of IL-13 in BALF (see Fig E3 in this article's Online Repository at www.jacionline.org). These results suggest that IL-22 is able to accelerate the resolution of allergic airway inflammation in mice.

Lung epithelial cells express functional IL-22 receptors

To address the mechanisms by which IL-22 attenuates antigen-induced airway inflammation, we searched possible IL-22–responding cells in patients with allergic airway inflammation. The functional IL-22 receptor is a heterodimer of IL-22R1 and IL-10R2,[12,13] and previous studies have shown that IL-10R2 is ubiquitously expressed in various cells but that the expression of IL-22R1 is restricted to nonimmune cells.[12,13,20] We first examined the expression of IL-22R1 in the lung by means of immunostaining with anti–IL-22R1 antibody. The expression of IL-22R1 was restricted to the luminal side of the epithelial cells in the lung (Fig 5, *A*). We also examined the expression of IL-22R1 and IL-10R2 mRNA in several cell types by means of qPCR analysis. IL-10R2 was expressed at moderate levels in $CD4^+$ T cells, $CD8^+$ T cells, and alveolar macrophages in the BALF and a lung epithelial cell line (MLE-15 cell), which shows the characteristics of the distal bronchiolar and alveolar epithelium,[34] and at high levels in BMDCs (Fig 5, *B*). In contrast, IL-22R1 was expressed in MLE-15 cells but not in $CD4^+$ T cells, $CD8^+$ T cells, or alveolar macrophages in BALF or BMDCs (Fig 5, *B*).

We further examined whether IL-22 transduced its signal in MLE-15 cells and found that IL-22, as well as IL-6, phosphorylated STAT3, a major signal transducer of IL-22,[43] in MLE-15 cells (Fig 5, *C*). IL-22 also induced the expression of suppressor of cytokine signaling 3 (SOCS3), one of the target genes of

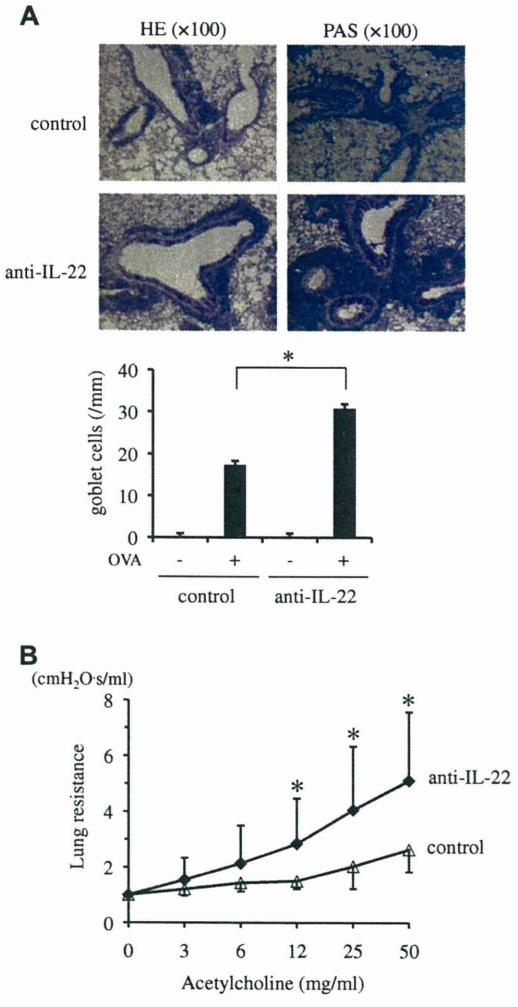

FIG 3. Anti–IL-22 antibody enhances antigen-induced goblet cell hyperplasia and AHR. OVA-sensitized BALB/c mice were injected intraperitoneally with polyclonal anti–IL-22 antibody or control antibody. Twenty-four hours later, the mice were challenged with inhaled OVA 3 times at a 48-hour interval. **A**, Histologic analysis of the lung was performed with hematoxylin and eosin *(HE)* and PAS staining at 48 hours after the final OVA inhalation. Representative photomicrographs of lung sections and the number of goblet cells in the airways are shown. Data are presented as means ± SDs for 5 mice in each group. $*P < .05$. **B**, Airway resistance to acetylcholine was measured at 24 hours after the final OVA inhalation by using the flexiVent system, as described in the Methods section. Data are presented as means ± SDs for 10 mice in each group. $*P < .05$.

STAT3 signaling, in MLE-15 cells (see Fig E4 in this article's Online Repository at www.jacionline.org), indicating that MLE-15 cells express functional IL-22 receptor complexes and signaling molecules. Although it is still possible that other cell types could respond to IL-22, these results suggest that the inhibitory effects of IL-22 on the development of antigen-induced allergic airway inflammation seem to be mediated by lung epithelial cells.

Anti–IL-22 antibody enhances antigen-induced IL-25 production in the airways

To further address the mechanisms of IL-22–mediated inhibition of antigen-induced airway inflammation, we examined the expression levels of chemokines in the lungs of OVA-sensitized mice after OVA or saline inhalation with or without IL-22 neutralization. However, the expression levels of eotaxin-1 and thymus and activation-regulated chemokine (TARC) in lung tissue, which seem to play important roles in allergic airway inflammation,[44,45] were not significantly enhanced in mice treated with anti–IL-22 antibody (Fig 6, *A*).

We then examined levels of epithelial cell–derived cytokines that promote T_H2-type immune responses, such as TSLP, IL-25, and IL-33,[46,47] in BALF of OVA-sensitized mice after OVA or saline inhalation with or without IL-22 neutralization. Importantly, the levels of IL-25 in BALF were significantly enhanced in mice treated with anti–IL-22 antibody ($n = 5$, $P < .05$; Fig 6, *B*). In contrast, the levels of TLSP and IL-33 were very low, even after OVA inhalation, and anti–IL-22 antibody did not significantly affect the levels of TLSP and IL-33 (Fig 6, *B*).

IL-22 inhibits IL-25 expression in MLE-15 cells

We also examined the effect of IL-22 on the expression of IL-25 in MLE-15 cells at mRNA levels. As shown in Fig 6, *C*, both IL-1β and LPS induced the expression of IL-25 in MLE-15 cells. IL-13 itself did not induce the expression of IL-25 but enhanced the expression of IL-25 in IL-1β– or LPS-stimulated MLE-15 cells (Fig 6, *C*). Importantly, although IL-22 did not significantly inhibit IL-1β– or LPS-induced expression of IL-25 in MLE-15 cells, IL-22 inhibited IL-13–mediated enhancement of IL-25 expression in IL-1β– or LPS-stimulated MLE-15 cells (Fig 6, *C*). On the other hand, although a previous report has shown that IL-17A reverses the protective effects of IL-22 on lung epithelial cells,[48] IL-17A did not significantly affect the inhibitory effect of IL-22 on the expression of IL-25 in MLE-15 cells (data not shown).

Anti–IL-25 antibody suppresses anti–IL-22 antibody–mediated enhancement of antigen-induced eosinophil recruitment into the airways

To determine whether IL-25 is involved in the enhancement of allergic airway inflammation in anti–IL-22 antibody–treated mice, we finally examined the effect of anti–IL-25 antibody on antigen-induced airway inflammation with or without administration of anti–IL-22 antibody. As shown in Fig 7, administration of anti–IL-25 antibody (20 μg per mouse) suppressed anti–IL-22 antibody–mediated enhancement of antigen-induced eosinophil and lymphocyte recruitment into the airways ($n = 5$, $P < .05$). Furthermore, although the difference did not reach significance, anti–IL-25 antibody tended to decrease anti–IL-22 antibody–mediated enhancement of IL-13 production in the airways (see Fig E5 in this article's Online Repository at www.jacionline.org). Taken together, these results suggest that IL-22 attenuates the development of antigen-induced allergic inflammation by inhibiting IL-25 production from lung epithelial cells in the T_H2 environment.

DISCUSSION

In this study we show that IL-22 is produced by $CD4^+$ T cells in the effector phase of allergic airway inflammation and attenuates T_H2 cell–mediated airway inflammation, possibly by inhibiting IL-25 production from lung epithelial cells.

We show that $CD4^+$ T cells are major IL-22–producing cells at the site of allergic airway inflammation (Fig 1). Consistent with previous studies showing that IL-22 is detected in lungs on

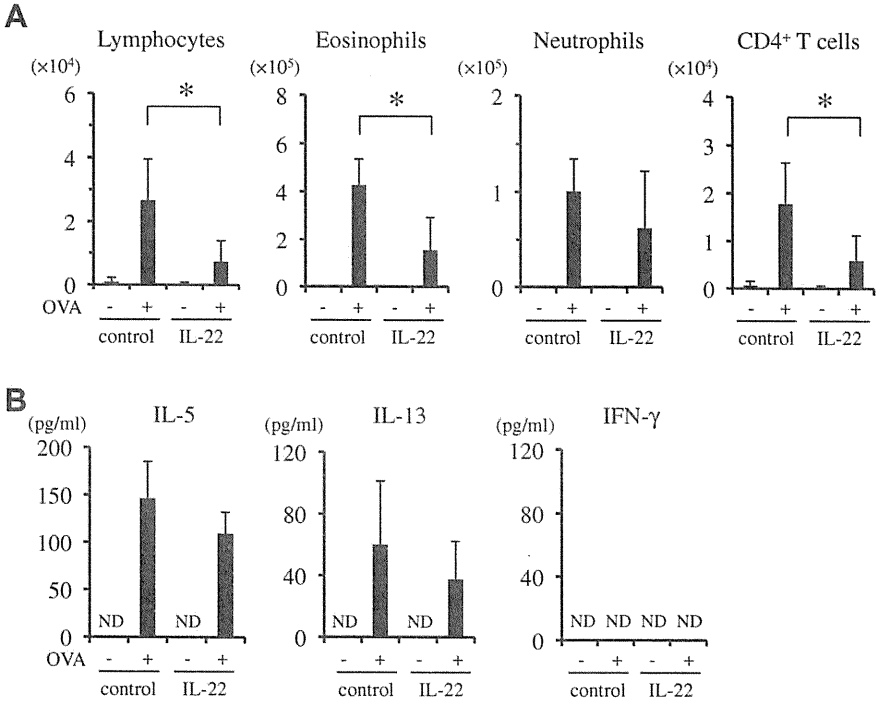

FIG 4. Intranasal administration of IL-22 attenuates antigen-induced eosinophil recruitment into the airways. Recombinant IL-22 (0.1 μg per mouse) or saline was administrated intranasally to OVA-sensitized BALB/c mice twice at 48 hours and 2 hours before the inhaled OVA challenge. Mice were challenged with OVA or saline inhalation, and at 48 hours after inhalation, numbers of lymphocytes, eosinophils, neutrophils, and $CD4^+$ T cells **(A)** and levels of IL-5, IL-13, and IFN-γ **(B)** in BALF were evaluated. Data are presented as means ± SDs for 4 mice in each group. *ND*, Not determined. $*P < .05$.

antigen challenge,[10,11,29] we found that $CD4^+$ T cells recovered from the airways of sensitized mice after antigen inhalation produced IL-22 on stimulation (Fig 1, *B*). In addition, we found that although the frequency of cells expressing IL-22 was very low in the airways, approximately 80% of signals with anti–IL-22 staining were colocalized with $CD4^+$ cells (Fig 1, *E*). On the other hand, although previous reports have shown that NK,[17,26,41] $CD11c^+$ myeloid,[40] and LTi-like cells[19] produce IL-22, we found that $DX5^+$, $CD11c^+$, or $CD4^+CD3\epsilon^-$ cells in the lung did not express IL-22 in allergic airway inflammation (see Fig E1). Taken together, these results suggest that $CD4^+$ T cells are a major source for IL-22 in patients with allergic airway inflammation.

We also show that T_H17 cells could produce IL-22 in allergic airway inflammation (Fig 1, *C*). Among $CD4^+$ T-cell subsets, T_H1 and T_H17 cells have been shown to be potent IL-22–producing cells.[9,12,13] In addition, recent studies have shown that skin-homing $CCR10^+$ IL-22–producing T cells (T_H22 cells) show a stable and distinct phenotype from T_H1, T_H2, and T_H17 cells and play a role in chronic inflammatory skin disease in human subjects.[14-16,23] In a murine model of asthma we found that one third of IL-22–producing $CD4^+$ T cells in the airways were also positive for intracellular IL-17A staining (Fig 1, *C*) and that approximately 20% of IL-22–producing $CD4^+$ T cells expressed CCR6 (Fig 1, *D*), suggesting that part of lung-infiltrating T_H17 cells have a potential to produce IL-22 on stimulation. In contrast, IL-22–producing $CD4^+$ T cells did not express IFN-γ (Fig 1, *C*), CCR5, or CCR10 (Fig 1, *D*), suggesting that T_H1 cells and T_H22 cells are not responsible for IL-22 production in patients with allergic airway inflammation.

We show that IL-22 inhibits allergic airway inflammation in the effector phase. We found that intranasal administration of IL-22 attenuated antigen-induced eosinophil and $CD4^+$ T-cell recruitment into the airways (Fig 4). We also found that the neutralization of IL-22 in the effector phase enhanced antigen-induced eosinophil and $CD4^+$ T-cell recruitment and IL-13 production in the airways (Fig 2) and AHR (Fig 3), which is consistent with the results of recent studies.[10,29] These results indicate that endogenously produced IL-22 inhibits antigen-induced allergic inflammation in the effector phase. Importantly, we also found that the administration of IL-22 accelerated the resolution of antigen-induced eosinophilic inflammation in the airways (see Fig E3), suggesting that inhaled IL-22 might have the therapeutic potential for asthma.

Regarding the mechanisms by which IL-22 inhibits allergic airway inflammation, it has been shown that the alteration of dendritic cell function by IL-22 is responsible for the inhibition of allergic inflammation.[10] On the other hand, consistent with a previous report,[20] we found that IL-22R1 was expressed on lung epithelial cells (Fig 5, *A* and *B*) but not on immune cells, including BMDCs (Fig 5, *B*). We also found that IL-22 phosphorylated STAT3 in a lung epithelial cell line (MLE-15 cells; Fig 5, *C*) but not in BMDCs (data not shown), suggesting that dendritic cells might not be a direct target of IL-22 in allergic airway inflammation.

Importantly, we found that anti–IL-22 antibody enhanced antigen-induced production of IL-25, an epithelial cell–derived cytokine that enhances T_H2-type immune responses,[30-32,46,47] in the airways (Fig 6, *B*). In addition, we found that IL-22 inhibited

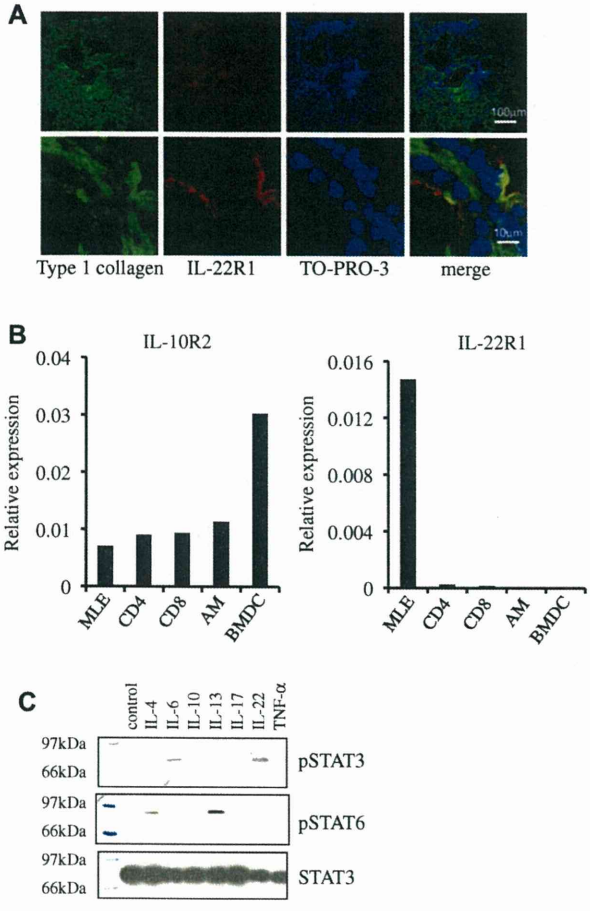

FIG 5. Lung epithelial cells express functional IL-22 receptors. **A**, Lung sections of OVA-sensitized, OVA-inhaled BALB/c mice were stained with anti–IL-22R1 antibody and anti–type 1 collagen antibody. The nuclei were counterstained with TO-PRO-3 iodide. Representative photomicrographs from 3 independent experiments are shown. **B**, $CD4^+$ T cells, $CD8^+$ T cells, and alveolar macrophages *(AM)* were purified from BALF at 48 hours after OVA inhalation. BMDCs were prepared as described in the Methods section. cDNA was prepared from $CD4^+$ T cells, $CD8^+$ T cells, alveolar macrophages, BMDCs, and MLE-15 cells, and expression levels of IL-10R2, and IL-22R1 mRNA were analyzed by means of qPCR analysis. Shown are representative data of 4 independent experiments. **C**, MLE-15 cells were stimulated with indicated cytokines (20 ng/mL) for 15 minutes, and cell lysates were subjected to immunoblotting with anti-phospho-STAT3 *(pSTAT3)* antibody, anti-phospho-STAT6 *(pSTAT6)* antibody, or anti-STAT3 antibody. Shown are representative data of 3 independent experiments.

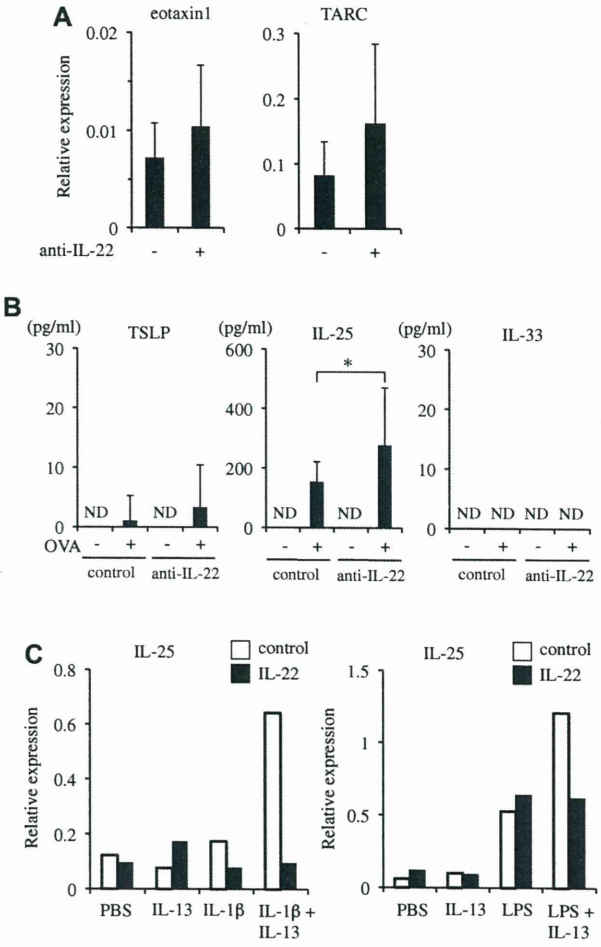

FIG 6. Anti–IL-22 antibody enhances antigen-induced IL-25 production in the airways. **A** and **B**, OVA-sensitized BALB/c mice were injected intraperitoneally with anti–IL-22 antibody or control antibody. Twenty-four hours later, the mice were challenged with inhaled OVA or saline. Fig 6, *A*, Expression of eotaxin-1 and TARC mRNA in the lung tissue was evaluated at 48 hours after OVA inhalation determined by mean of qPCR analysis. Data are presented as means ± SDs for 5 mice in each group. Fig 6, *B*, Levels of TSLP, IL-25, and IL-33 in BALF were evaluated by means of ELISA at 48 hours after OVA inhalation. Data are presented as means ± SDs for 5 mice in each group. *ND*, Not determined. $*P < .05$. **C**, MLE-15 cells were stimulated with IL-1β (20 ng/mL), LPS (100 ng/mL), and/or IL-13 (20 ng/mL) in the presence or absence of IL-22 (20 ng/mL) for 6 hours. The expression of IL-25 mRNA was evaluated by means of qPCR. Data shown are representative of 4 independent experiments.

IL-13–mediated enhancement of IL-25 expression in IL-1β– or LPS-stimulated MLE-15 cells (Fig 6, *C*). Moreover, we found that IL-22 induced SOCS3, which could inhibit IL-13 signaling,[49] in MLE-15 cells (see Fig E4). Furthermore, we found that anti–IL-25 antibody suppressed anti–IL-22 antibody–mediated enhancement of antigen-induced eosinophil recruitment into the airways (Fig 7). Therefore it is suggested that IL-22 attenuates T_H2 cell–mediated airway inflammation by inhibiting IL-25 production from lung epithelial cells.

Our results indicate that T_H17 cells play both promotional and inhibitory roles in the induction of allergic airway inflammation. We show here that part of T_H17 cells produce IL-22 in the airways and that IL-22 attenuates allergic airway inflammation (Figs 1 and 4 and see Fig E3). On the other hand, we have previously shown that by using adoptive transfer experiments, T_H17 cells enhance T_H2 cell–mediated allergic airway inflammation.[7] In relation to these observations, it has recently been demonstrated that IL-22 is pathological in bleomycin-induced airway inflammation in the presence of IL-17A but is tissue protective in the absence of IL-17A.[48] These findings suggest that although an overall effect of T_H17 cells on T_H2 cell–mediated allergic airway inflammation seems to be enhancing,[7] T_H17 cell–derived IL-22 might play a role in fine tuning of allergic airway inflammation.

It is also possible that the role of IL-22 in patients with allergic airway inflammation depends on the phase of immune responses. Interestingly, we found that when anti–IL-22 antibody was given to sensitized mice at 48 hours after inhaled antigen challenge, anti–IL-22 antibody did not significantly enhance allergic airway inflammation (data not shown), suggesting that endogenously produced IL-22 functions as a negative regulator of allergic

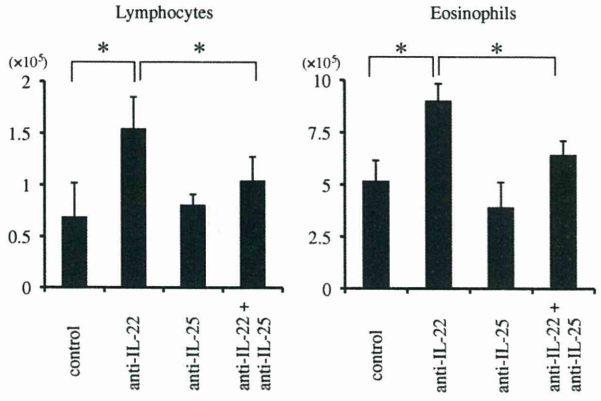

FIG 7. Anti–IL-25 antibody reverses the enhancing effect of anti–IL-22 antibody on antigen-induced airway inflammation. OVA-sensitized BALB/c mice were injected intraperitoneally with anti–IL-22 antibody, anti–IL-25 antibody, or both (20 μg per mouse) at 24 hours before OVA inhalation. Numbers of eosinophils and lymphocytes in BALF were evaluated at 48 hours after OVA inhalation. Data are presented as means ± SD for 5 mice in each group. $*P < .05$.

airway inflammation at the early stage but not at the late stage of the effector phase. On the other hand, it has recently been demonstrated that IL-22 is required for mounting allergic airway inflammation during the sensitization phase by using IL-22 knockout mice.[29] Further studies with conditional IL-22 knockout mice and IL-22 knock-in mice seem beneficial to address the complicated role of IL-22 in allergic airway inflammation.

In summary, we have shown that IL-22 is expressed by $CD4^+$ T cells at the site of allergic airway inflammation and attenuates eosinophilic airway inflammation, possibly by inhibiting IL-25 production from lung epithelial cells. Although additional studies are required for further elucidation of molecular mechanisms, our results raise the possibility that IL-22 could be used as a novel therapeutic approach for asthma.

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Key message

- Endogenously produced IL-22 inhibits T_H2 cell–mediated allergic airway inflammation by acting on lung epithelial cells.

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METHODS

Reagents

A murine lung epithelial cell line (MLE-15 cells, a kind gift from Dr Jeffrey Whitsett, University of Cincinnati), which was produced from lung tumors generated in transgenic mice expressing the viral oncogene SV40 large T antigen under the control of a promoter region from the human surfactant protein C gene, was grown in HITES medium, as described previously.[E1] MLE-15 cells show the characteristics of the distal bronchiolar and alveolar epithelium.[E1]

Antigen-induced allergic inflammation in the airways

BALB/c mice (aged 6-8 weeks) were immunized intraperitoneally twice with 10 μg of OVA in 4 mg of aluminum hydroxide (alum) at a 2-week interval, and 2 weeks after the second immunization, the sensitized mice were administered aerosolized OVA (50 mg/mL) dissolved in 0.9% saline for 20 minutes through a DeVilbiss 646 nebulizer (DeVilbiss Corp, Somerset, Pa).[E2]

Effect of IL-22 on antigen-induced allergic inflammation in the resolution phase

OVA-sensitized BALB/c mice were challenged with inhaled OVA 3 times at a 48-hour interval. Recombinant IL-22 (0.1 μg per mouse) was administered intranasally to the mice 3 times at 24, 48, and 72 hours after the last OVA inhalation. Numbers of eosinophils and lymphocytes and cytokine levels in BALF were evaluated at 4 hours after the last IL-22 administration.

Cytokine assay

Amounts of IL-13, IL-22, IL-33, IFN-γ, and TSLP in BALF were determined by means of ELISA, according to the manufacturer's instructions (R&D Systems). Levels of IL-5 and IL-25 in BALF were determined by using ELISA kits from BD Biosciences and BioLegend, respectively. The detection limits of these assays were 15 pg/mL for IL-5 and TSLP; 30 pg/mL for IFN-γ, IL-22, IL-25, and IL-33; and 60 pg/mL for IL-13.

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,[E3] according to the manufacturer's instructions (Miltenyi Biotec, Auburn, Calif). For intracellular cytokine analysis, $CD4^+$ T cells were stimulated with phorbol 12-myristate 13-acetate (20 ng/mL, Calbiochem) plus ionomycin (1 μg/mL, Calbiochem) at 37°C for 4 hours in the presence of brefeldin A (10 μmol/L, BD Bioscience). Cells were then stained with antibodies to CD4 and either CCR3 (BioLegend), CCR5 (eBioscience, San Diego, Calif), CCR6 (BioLegend), or CCR10 (eBioscience). After surface staining, cells were fixed, permeabilized, and stained by Alexa Fluor 488–conjugated anti–IL-22 antibody together with allophycocyanin-conjugated anti–IFN-γ, anti–IL-4, or anti–IL-17A antibody, as described previously.[E4] Anti–IL-22 antibody (clone MH22B2) was labeled with Monoclonal Antibody Labeling Kit (Invitrogen, Carlsbad, Calif), according to the manufacturer's instructions.

qPCR analysis

Total RNA was prepared with ISOGEN solution (Nippon GENE, Tokyo, Japan), and reverse transcription was carried out with an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, Calif). Expression of IL-10R2, IL-22R1, SOCS3, eotaxin-1, TARC, IL-25, IL-23p19, and IL-12/IL-23p40 was determined by means of qPCR with a standard protocol on an ABI PRISM7300 instruments (Applied Biosystems, Foster City, Calif) by using a SYBER green reagent (Power SYBER Green PCR Master Mix, Applied Bioscience). Expression of IL-22 was determined by means of quantitative Taqman PCR with a standard protocol on the ABI PRISM 7300 instrument. The levels of each expression were normalized to the levels of β-actin. The sequences of PCR primers and fluorogenic probes are as follows: IL-23 p19—forward primer, ATCCAGTGTGAAGATGGTTGTGA; reverse primer, GCAAGCAGAAC TGGCTGTTG; IL-12/IL-23 p40—forward primer, TGGTTTGCCATCGTT TTGCTG; reverse primer, ACAGGTGAGGTTCACTGTTTCT; IL-10R2—forward primer, GGACGTCTCTTCCACAGCAC; reverse primer, CTGCT TGCTGCCTTCAGACT; IL-22R1—forward primer, GCTCGCTGCAGCAC ACTACCAT; reverse primer, TGAGTGTGGGGTGGACCAGCAT; SOCS3—forward primer, CCTTCAGCTCCAAAAGCCAG; reverse primer, GCTCT CCTGCAGCTTGCG; eotaxin-1—forward primer; TGTCTCCCTCCAC CATGCA; reverse primer, GATCTTCTTACTGGTCATGATAAAGCA; TARC—forward primer, TTGTGTTCGCCTGTAGTGCATA; reverse primer, CAGGAAGTTGGTGAGCTGGTATA; IL-25—forward primer, CGGAGG AGTGGCTGAAGTGGAG; reverse primer, ATGGGTACCTTCCTCGC CATG; IL-22—forward primer, TCCGAGGAGTCAGTGCTAAA; reverse primer, AGAACGTCTTCCAGGGTGAA; probe, TGAGCACCTGCTTCAT CAGGTAGCA; β-actin—forward primer, GCTCTGGCTCCTAGCACCAT; reverse primer, GCCACCGATCCACACAGAGT; probe, TCAAGATCAT TGCTCCTCCTGAGCGC.

Histologic and immunohistologic analysis

Lung sections (3 μm thick) were stained with hematoxylin and eosin and PAS according to standard protocols. The number of goblet cells was counted on PAS-stained lung sections, as described elsewhere.[E5] Immunostaining of cryosections was performed as described previously.[E6] The following antibodies were used: anti-CD4 antibody (Clone GK1.5, eBioscience), anti–IL-22 antibody (Clone 140301, R&D Systems), anti–type 1 collagen antibody (COSMO Bio, Tokyo, Japan), and anti–IL-22 receptor 1 antibody (Clone 496514, R&D Systems). The nuclei were counterstained with TO-PRO-3 iodide (Invitrogen).

Preparation of BMDCs

BMDCs were prepared as described previously.[E7] In brief, a single-cell suspension of bone marrow cells was obtained from 8-week-old BALB/c mice and cultured in complete DMEM medium containing GM-CSF (50 ng/mL) and IL-4 (20 ng/mL) for 9 days, with replacement of the medium containing cytokines every 3 days. $CD11c^+$ cells were purified by using an isolation kit from Miltenyi Biotech. The purity of collected cells was determined by means of flow cytometry, and these were routinely greater than 95% $CD11c^+$ cells.

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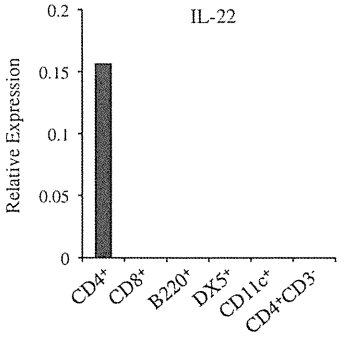

FIG E1. Lung-infiltrating $CD4^+$ cells but not $CD4^+CD3\epsilon^-$ cells express IL-22 mRNA in allergic airway inflammation. OVA-sensitized BALB/c mice were challenged with inhaled OVA. Forty-eight hours after inhalation, $CD4^+$, $CD8^+$, $B220^+$, $DX5^+$, $CD11c^+$, and $CD4^+CD3\epsilon^-$ cells were isolated from lung homogenates by means of magnetic cell sorting, and qPCR analysis for IL-22 was performed. Representative data from 4 independent experiments are shown.

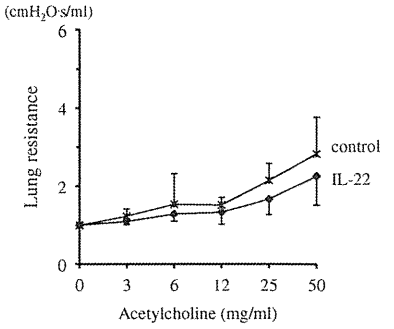

FIG E2. Intranasal administration of IL-22 tends to attenuate airway hyperreactivity. OVA-sensitized BALB/c mice were challenged with inhaled OVA 3 times at a 48-hour interval. Recombinant IL-22 (0.1 μg per mouse) or saline was administrated intranasally to the mice 2 hours before each OVA inhalation. Twenty-four hours after the last OVA inhalation, airway resistance to acetylcholine was evaluated. Data are presented as means ± SDs for 4 mice in each group.

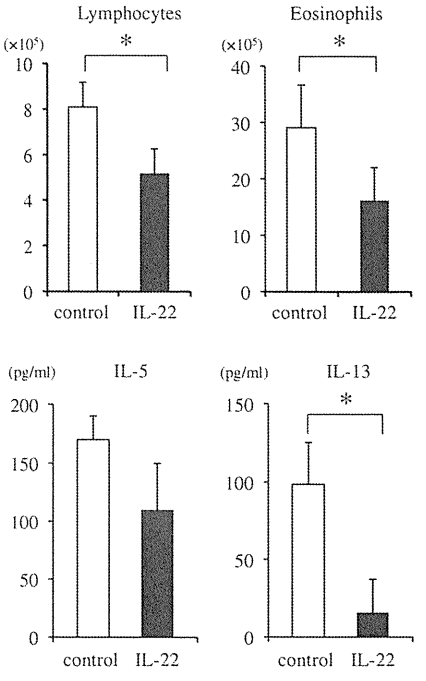

FIG E3. Intranasal administration of IL-22 accelerates the resolution of allergic airway inflammation. OVA-sensitized BALB/c mice were challenged with inhaled OVA 3 times at a 48-hour interval. IL-22 (0.1 μg per mouse) or saline was administered intranasally to the mice 3 times at 24, 48, and 72 hours after the last OVA inhalation. Four hours alter the last IL-22 administration, numbers of eosinophils and lymphocytes and levels of IL-5 and IL-13 in BALF were evaluated. Data are presented as means ± SDs (n = 5 each). *$P < .05$.

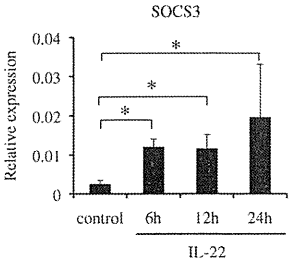

FIG E4. IL-22 induces SOCS3 mRNA in MLE-15 cells. MLE-15 cells were stimulated with IL-22 (20 ng/mL) for indicated time periods, and the expression of SOCS3 mRNA was evaluated by means of qPCR analysis. Data are presented as means ± SDs (n = 4 in each group). *$P < .05$.

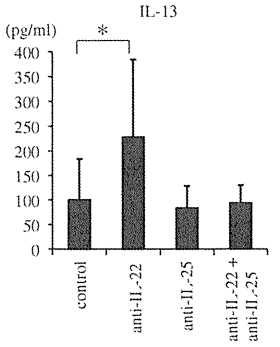

FIG E5. Anti–IL-25 antibody tends to reverse the enhancing effect of anti–IL-22 antibody on antigen-induced IL-13 production in the airways. OVA-sensitized BALB/c mice were injected intraperitoneally with anti–IL-22 antibody (20 μg per mouse), anti–IL-25 antibody (20 μg per mouse), or both at 24 hours before OVA inhalation. Levels of IL-13 in BALF were evaluated at 48 hours after OVA inhalation. Data are presented as means ± SDs (n = 5 mice per group). *$P < .05$.