

Dual Role of IL-22 in Allergic Airway Inflammation and its Cross-talk with IL-17A

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Rationale: IL-22 has both proinflammatory and antiinflammatory properties. Its role in allergic lung inflammation has not been explored.

Objectives: To investigate the expression and roles of IL-22 in the onset and resolution of experimental allergic asthma and its cross-talk with IL-17A.

Methods: IL-22 expression was assessed in patient samples and in the lung of mice immunized and challenged with ovalbumin. IL-22 functions in allergic airway inflammation were evaluated using mice deficient in IL-22 or anti-IL-22 neutralizing antibodies. Moreover, the effects of recombinant IL-22 and IL-17A neutralizing antibodies were investigated.

Measurements and Main Results: Increased pulmonary IL-22 expression is found in the serum of patients with asthma and mice immunized and challenged with ovalbumin. Allergic lung inflammation is IL-22 dependent because eosinophil recruitment, Th2 cytokine including IL-13 and IL-33, chemokine production, airway hyperreactivity, and mucus production are drastically reduced in mice deficient in IL-22 or by IL-22 antibody neutralization during immunization of wild-type mice. By contrast, IL-22 neutralization during antigen challenge enhanced allergic lung inflammation with increased Th2 cytokines. Consistent with this, recombinant IL-22 given with allergen challenge protects mice from lung inflammation. Finally, IL-22 may regulate the expression and proinflammatory properties of IL-17A in allergic lung inflammation.

Conclusions: IL-22 is required for the onset of allergic asthma, but functions as a negative regulator of established allergic inflammation. Our study reveals that IL-22 contributes to the proinflammatory properties of IL-17A in experimental allergic asthma.

Keywords: allergy; lung inflammation; Th2 response; IL-22; IL-17A

Allergic asthma has increased dramatically in prevalence and severity over the last three decades. Th2 cytokines, such as IL-4, IL-5, IL-9, and IL-13, are involved in allergic response. The role of IL-17A in allergic airway inflammation has been extensively investigated in mice and human patients. IL-17A seems to

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

IL-22 is important for the regulation of tissue responses during inflammation, but its role in allergic lung inflammation is unknown.

What This Study Adds to the Field

IL-22 is required for the onset of ovalbumin-induced model of allergic asthma in mice, but inhibits the effector phase of asthma. IL-22 regulates the expression and the proinflammatory properties of IL-17A in experimental allergic asthma.

contribute to Th2-cell-mediated and eosinophilic inflammation in asthma (1–5). Th17 cells coexpress IL-17A, IL-17F, and IL-22, under the control of IL-23 (6). Apart from Th17 cells, IL-22 is also produced by other cells, such as Th22 (7, 8), Th1 (9), CD8+ T cells (9), $\gamma\delta$ T cells (10–12), classical and nonclassical natural killer (NK) cells (9, 13, 14), and CD11c+ cells (15), but it is not produced by tissue cells (7, 8, 16).

IL-22 receptor complex is composed of IL-22R and IL-10R β (17, 18), and is only expressed by nonhematopoietic cells, such as hepatocytes, keratinocytes, lung, and intestinal epithelial cells (19). IL-22 ligation to its receptor activates the STAT3 signaling pathway (20). Biologic effects of IL-22 depend on tissue localization and cytokine environment (21–23). IL-22 is protective during acute inflammation in the hepatitis model and in *Citrobacter rodentium* intestinal infection (15, 24, 25). In keratinocytes, IL-22 alone induced antibacterial proteins, such as β -defensins and S100A proteins (19). However, in experimental dermal inflammation IL-22 amplified inflammation (26, 27). The reason for this dual nature of IL-22 in the skin is probably because IL-22 alone has only minimal inflammatory properties but it enhances the inflammatory effects of tumor necrosis factor- α , IL-1 β , IL-17, and granulocyte-macrophage colony-stimulating factor (28–30).

Increased IL-22 immunoreactive cells and gene transcripts are found in the bronchial mucosa of patients with chronic obstructive pulmonary disease (31). However, IL-22 involvement to allergic lung inflammation has not yet been explored. IL-22 has been proposed to affect dendritic cells functions in an allergic model using IL-4R knockout (KO) mice (32), which do not express IL-22R (33). To investigate the role of IL-22 in allergic asthma, we compared the susceptibility of wild-type (WT) mice and mice deficient in IL-22 to develop airway inflammation after ovalbumin (OVA) immunization and challenge in the absence of adjuvant. We first found increased levels of IL-22 in the serum of patients with asthma and in the lung

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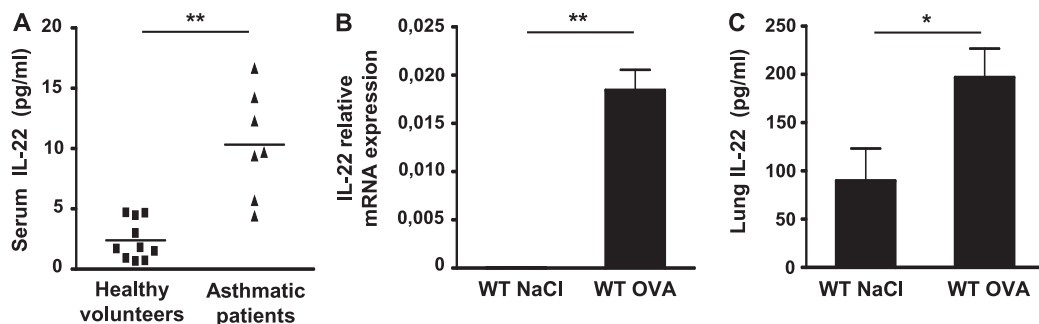


Figure 1. IL-22 is induced during allergic asthma. (A) IL-22 concentration was quantified by ELISA in serum from patients with asthma versus healthy individuals. Mice were sensitized with ovalbumin (OVA) on Days 0 and 7 and challenged three times with saline or OVA on Days 14–16. (B) Twenty-four hours after the last challenge lung are analyzed for IL22 gene transcript

by quantitative polymerase chain reaction. (C) IL-22 protein quantification was performed by ELISA on lung mononuclear cells culture supernatants after 24 hours phorbol 12-myristate 13-acetate and ionomycin stimulation. These experiments were performed twice. One representative experiment is shown. Individual values and mean are indicated for human samples; mean $\pm$ SEM of six mice per group are shown. * P less than or equal to 0.05; ** P less than or equal to 0.01. WT = wild-type.

during experimental asthma in mice. Second, we showed that lung inflammation is reduced in mice deficient in IL-22 or after IL-22 antibody neutralization during the sensitization phase. By contrast, IL-22 antibody blockade during the antigen challenge exacerbated allergic lung inflammation, whereas exogenous IL-22 inhibited allergic lung inflammation. This indicates that IL-22 is required for the onset of allergic airway inflammation,

but has inhibitory functions during the effector phase. Surprisingly, despite higher IL-17A levels, mice deficient in IL-22 were protected from the disease. Finally, we demonstrated that IL-17A antibody blockade leads to asthma exacerbation in mice deficient in IL-22, suggesting that IL-22 contributes to the proinflammatory effect of IL-17A in allergic asthma. Some of these results have been previously reported in abstract form (34).

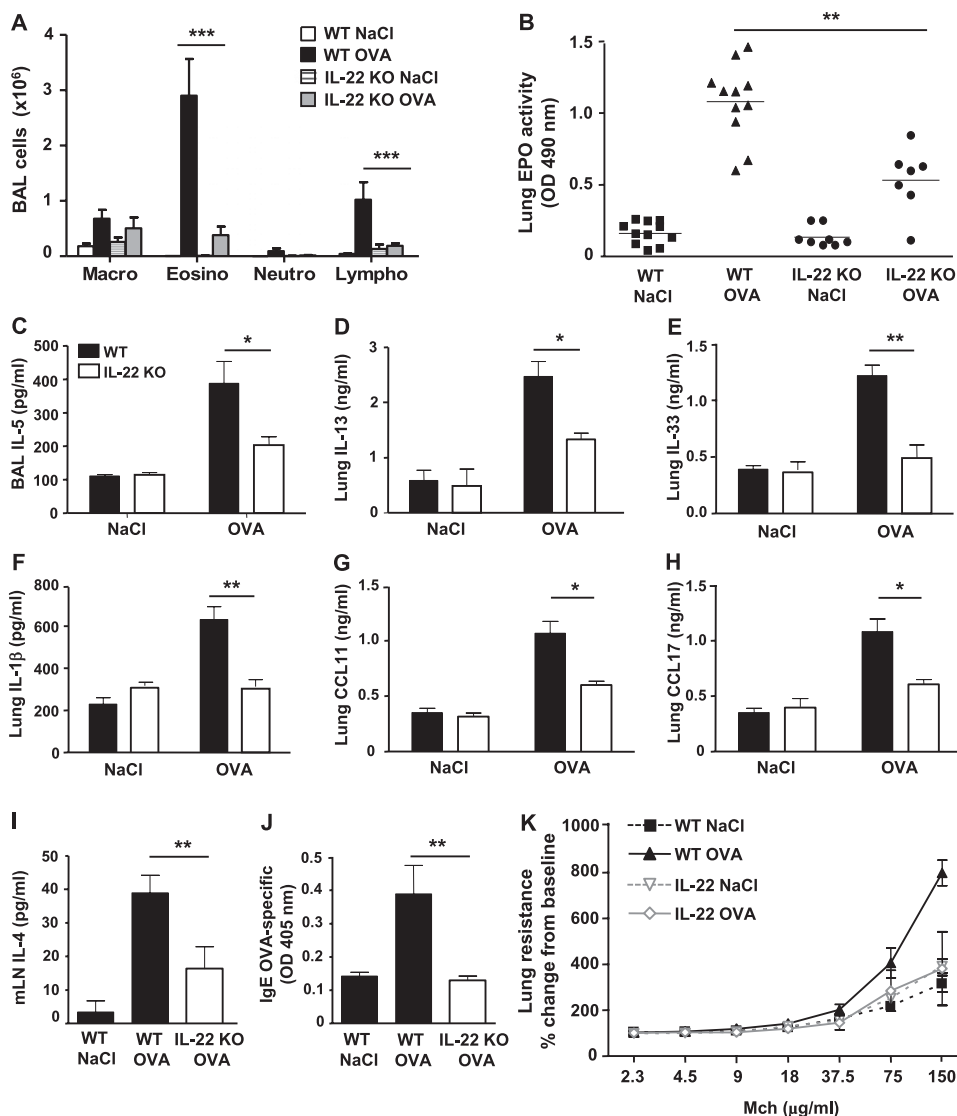


Figure 2. Reduced allergic asthma in mice deficient in IL-22. Ovalbumin (OVA)-sensitized wild-type (WT) and IL-22 knockout (KO) mice were challenged three times with OVA instillation. (A) Twenty-four hours after the third challenge, eosinophil numbers were determined in the bronchoalveolar lavage (BAL). Eosinophil peroxidase (EPO) activity, IL-13, IL-33, IL-1 β , CCL11, and CCL17 in the lung homogenate (B, D–H) and BAL IL-5 level (C) were measured by ELISA. (I) IL-4 levels in the cell culture supernatants of mediastinal lymph nodes restimulated *in vitro* for 5 days with OVA were accessed by ELISA. (J) OVA-specific IgE concentrations were assayed in serum from OVA-treated WT and IL-22 KO mice by ELISA. (K) Airway hyperresponsiveness to increasing doses of metacholine (Mch) was measured 24 hours after the last saline or OVA challenge on WT and IL-22 KO mice by recording changes in lung resistance. (L) The formalin-fixed lung sections were stained with hematoxylin and eosin (HE) to visualize cell recruitment and with periodic acid–Schiff reagent (PAS) to visualize mucus, as shown by the arrows. Representative sections from saline control and OVA-treated WT and IL-22 KO mice are shown (original magnification $\times 400$). B = mean bronchus; V = mean vessel. (M) Semiquantitative histologic assessment of cell infiltration and mucus hypersecretion was performed by two independent observers. A scale from 0–3 is given on the axis. These experiments were performed four times ($n = 6$ mice per group). Values are the mean $\pm$ SEM of 6–11 mice per group. * P less than or equal to 0.05; ** P less than or equal to 0.01; *** P less than or equal to 0.001.

METHODS

IL-22 Levels in Patients with Allergic Asthma

Serum was taken from healthy volunteers and patients with allergic asthma and tested for IL-22 levels by ELISA (*see online supplement*).

Mice

C57BL/6 WT mice and IL-22 KO were bred in a specific pathogen-free animal facility at CNRS (Orléans, France). IL-22 KO mice (on C57BL/6 genetic background) were from the Ludwig Institute for Cancer Research (Brussels, Belgium) (35). Mice were maintained in a temperature-controlled (23°C) facility with a strict 12-hour light–dark cycle and were given free access to food and water. All protocols were approved by the CNRS institutional animal research committee and complied with the French Government's ethical and animal experiment regulations.

Allergic Airway Inflammation Induction

OVA-induced allergic lung inflammation model was performed (*see online supplement*).

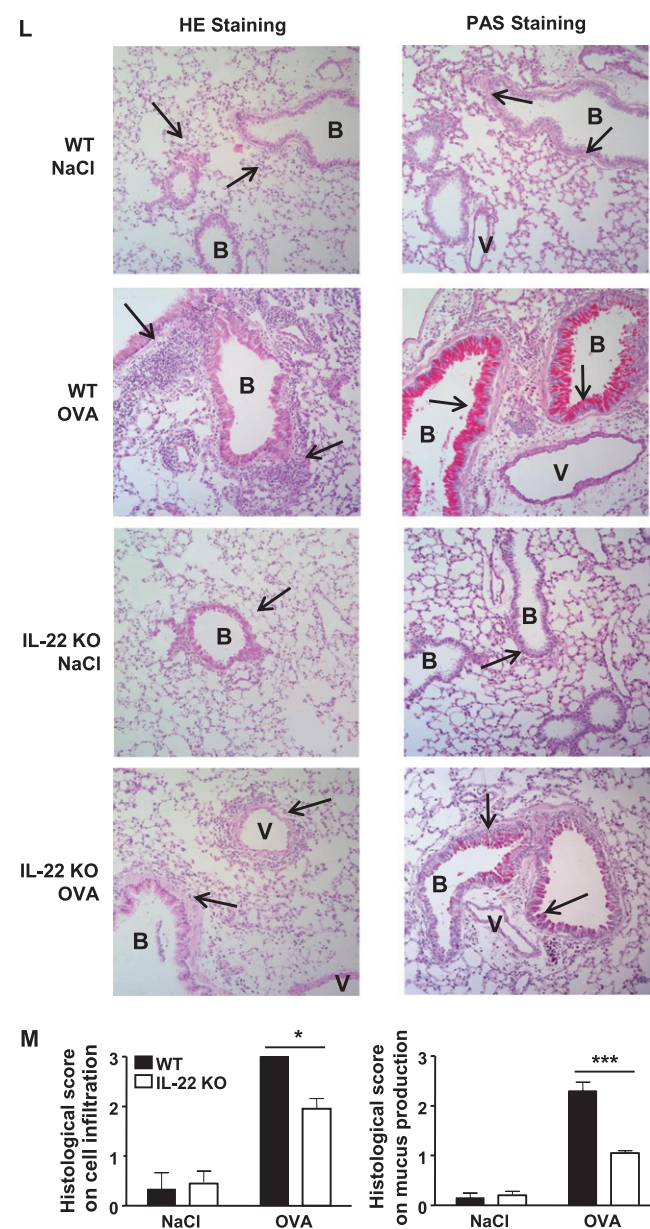


Figure 2. (Continued).

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was performed, as previously described (36).

Lung Histology

Three-micrometer sections of formalin-fixed, paraffin-embedded lung were stained with hematoxylin and eosin or with periodic acid–Schiff reagent and examined with a Leica microscope ($\times 400$ magnification; Leica Microsystems, Nanterre, France), as described previously (37). Peribronchial infiltrates and mucus hypersecretion were assessed by a semiquantitative score (0–3) by two independent observers.

Pulmonary Eosinophil Peroxidase Activity

Pulmonary eosinophil peroxidase (EPO) activity was determined to estimate eosinophil recruitment into the lung parenchyma as described (37).

Determination of Airway Hyperresponsiveness

Airway hyperresponsiveness was determined as described (*see online supplement*).

Quantification of IL-4, IL-5, IL-1 β , IL-13, IL-17A, IL-22, IL-33, IgE, CCL5, CCL11, and CCL17

Cytokine and chemokine in BAL or lung homogenate were determined by ELISA, using commercial kits from R&D Systems (Abingdon, UK) according to the manufacturer's recommendation (*see online supplement*).

Lung Mononuclear Cell Isolation

Lung mononuclear cells were isolated from mice 24 hours after the last challenge, as described (38). Lymphoid cells were plated in round-bottom 96-well plates (2×10^6 /ml) and restimulated for 4 hours *in vitro* with phorbol 12-myristate 13-acetate (50 ng/ml) and ionomycin (750 ng/ml) in complete medium (Iscove's modified Dulbecco's medium [IMDM] supplemented with 5% [vol/vol] fetal calf serum, L-glutamine [2 μ M], penicillin [100 U/ml], streptomycin [100 μ g/ml], and β -mercaptoethanol [50 nM]) (all from Invitrogen, Cergy Pontoise, France).

Flow Cytometry Analysis on Lung Mononuclear Cells and Mediastinal Lymph Nodes

Fluorescence-activated cell sorter analysis on lung mononuclear cells is described in the online supplement.

Gene Expression Analysis

Lung tissues were processed for gene expression analysis, as described in the online supplement.

Mediastinal Lymph Node Restimulation

Isolated mLN cells from OVA and saline-treated WT and IL-22 KO mice were plated in round-bottom 96-well plates at 5.10^6 cells/ml in complete medium and restimulated with OVA (10 μ g/ml) for 5 days.

Statistical Analysis

The data are presented as the mean $\pm$ SEM with 6–10 animals per condition. The significance of differences between two groups was determined by one-way analysis of variance (nonparametric test), followed by Bonferroni comparison test using Prism software (GraphPad Software, La Jolla, CA). Statistical significance was reported if P less than 0.05 was achieved.

RESULTS

Increased IL-22 Expression on Allergic Airway Inflammation

We first examined whether IL-22 is modulated in patients with allergy. We found increased IL-22 protein levels in serum from patients with asthma compared with healthy volunteers (Figure 1A), suggesting that IL-22 might contribute to an allergic response in humans. Second, using an OVA antigen-induced

allergic lung inflammation model, we showed a significant induction of *IL22* transcript in the lung of OVA-sensitized and challenged mice (Figure 1B). However, we were unable to detect IL-22 protein by ELISA in the supernatant of lung homogenates. We then determined IL-22 produced by isolated lung infiltrating mononuclear cells from OVA-treated mice after phorbol 12-myristate 13-acetate and ionomycin restimulation. Production of IL-22 was increased in OVA-treated mice compared with saline control 24 hours after *ex vivo* restimulation

(Figure 1C). No IL-22 was detectable in nonstimulated cells, or after antigen restimulation (data not shown). Therefore, IL-22 up-regulation in patients with asthma and in the mice model of asthma suggests a possible involvement of this cytokine in allergic airway inflammation.

IL-22 Is Required for Allergic Airway Inflammation

To test directly the functional role of IL-22 in allergic lung inflammation model, we immunized WT mice and mice de-

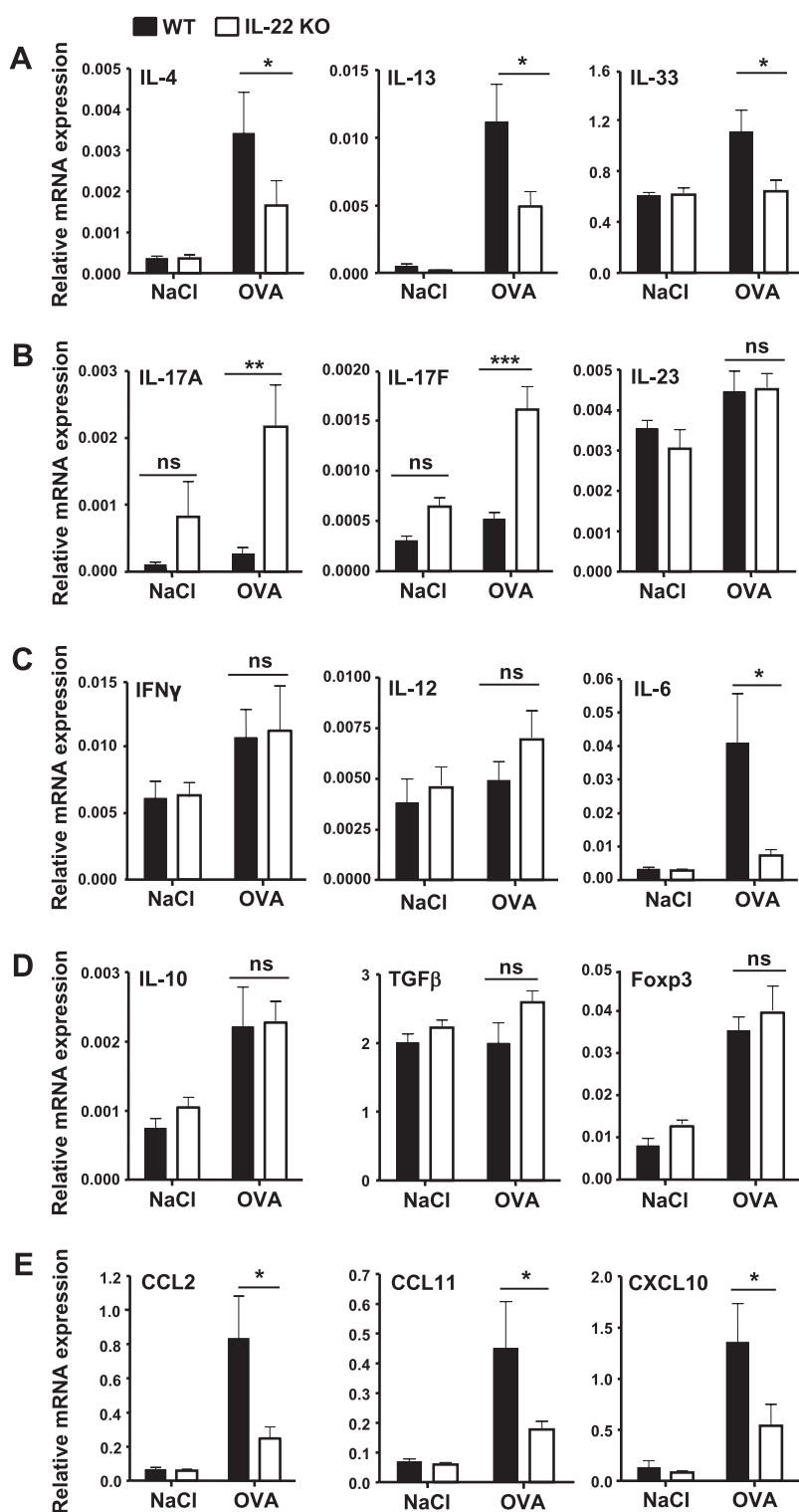


Figure 3. Mice deficient in IL-22 develop impaired Th2 response but up-regulate IL-17A and IL-17F. Ovalbumin (OVA)-sensitized wild-type (WT) and IL-22 knockout (KO) mice were challenged three times with saline or OVA. Twenty-four hours after the third challenge, RNA was extracted from lung tissue and analyzed for mRNA transcripts. (A–E) IL-4, IL-13, IL-33, IL-17A, IL-17F, IL-23(p19), IFN γ , IL-12(p35), IL-6, IL-10, TGF β , Foxp3, CCL2, CCL11, and CXCL10 transcript levels were quantified by real-time quantitative PCR. These experiments were performed twice (n = 6 mice per group). Mean values $\pm$ SEM are shown. **P* less than or equal to 0.05; ***P* less than or equal to 0.01; ****P* less than or equal to 0.001; ns = not significant; TGF = transforming growth factor.

ficient in IL-22 (IL-22 KO) on Days 0 and 7 with OVA (subcutaneous injections of 10 μ g OVA without alum) followed by three intranasal challenges on Days 14, 15, and 16. OVA-sensitized and -challenged WT mice showed a robust cell infiltration into the lung compared with saline-treated mice (Figure 2A). By contrast, we found a significant reduction of eosinophil recruitment in BAL and in lung (EPO activity) in OVA-challenged mice deficient in IL-22 compared with WT mice (Figures 2A and 2B). Moreover, infiltrating lymphocytes were also significantly decreased in the airways of IL-22 KO mice (Figure 2A). The reduced leukocyte infiltration seen in mice deficient in IL-22 correlated with diminished Th2 cytokines including IL-5 in BAL (Figure 2C), IL-13, and IL-33 in lung (Figures 2D and 2E), and diminished IL-4 level in the culture supernatants of mediastinal lymph nodes after OVA restimulation (Figure 2I). The expression of IL-1 β and of chemokines CCL11 (eotaxin), known to play a role in eosinophil influx into the lung, and CCL17 (TARC), which is a Th2 lymphocyte selective chemokine, were also drastically reduced in lung of OVA-treated IL-22 KO mice compared with WT mice (Figures 2F–2H). Serum levels of OVA-specific IgE in mice deficient in IL-22 were significantly lower than in WT mice, indicating a systemic impairment of the adaptive Th2 response in IL-22 KO mice (Figure 2J). Airway hyperresponsiveness, a characteristic feature of allergic asthma, was

assessed in WT mice and mice deficient in IL-22. OVA-challenged and -sensitized WT mice exhibited a significant change of lung resistance in response to intravenous methacholine, compared with saline-treated mice (Figure 2K). By contrast, lung resistance of OVA-treated IL-22 KO mice was significantly reduced compared with WT mice (Figure 2K). Microscopically, lung tissue of OVA-challenged WT mice showed peribronchial mononuclear cell recruitment together with mucus overproduction and hyperplasia of the epithelium with hyperplasia of smooth muscle and goblet cells, parameters that were significantly reduced in IL-22 KO mice (Figures 2L and 2M). Thus, IL-22 is required to develop a robust allergic response because lung function, inflammatory cell infiltration, cytokines, and chemokines production and specific IgE response were impaired in mice deficient in IL-22.

Reduced Allergic Lung Inflammation in Mice Deficient in IL-22 Correlates with Impaired Th2 Cytokine and Chemokine Expression and Up-regulation of IL-17A and IL-17F Expression

To investigate further how IL-22 regulates allergic lung inflammation, we analyzed lung tissues from OVA-challenged and -sensitized WT mice and mice deficient in IL-22 by

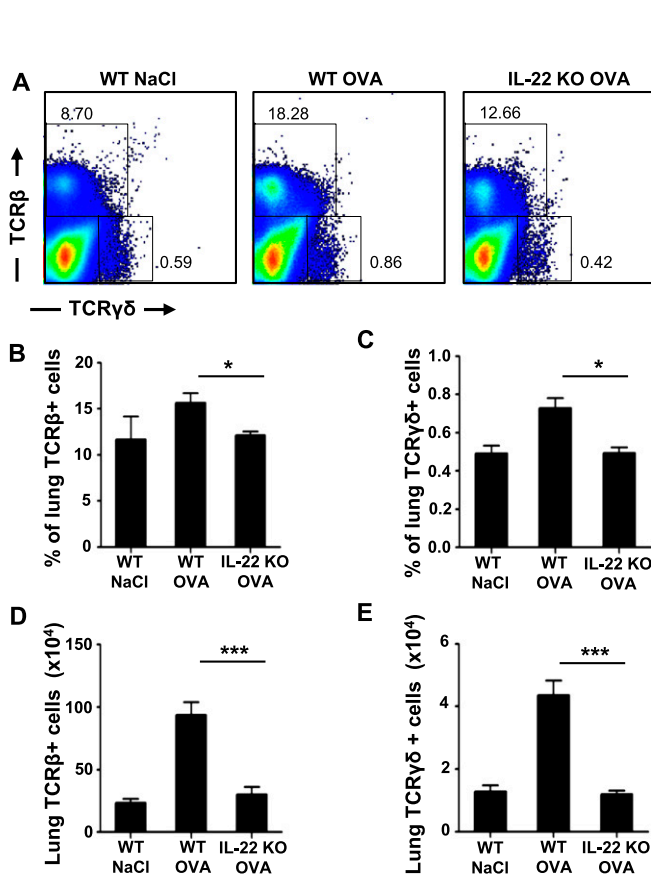


Figure 4. Reduced pulmonary recruitment of T cells in the absence of IL-22. Lung mononuclear cells from ovalbumin (OVA)-sensitized and -challenged wild-type (WT) and IL-22 knockout (KO) mice were isolated and restimulated for 4 hours with phorbol 12-myristate 13-acetate and ionomycin followed by TCR $\alpha\beta$ and TCR $\gamma\delta$ antibody staining. (A–E) The frequency and the absolute numbers of TCR $\alpha\beta$ ⁺ and TCR $\gamma\delta$ ⁺ cells are shown. These experiments were performed three times. (B–E) Values are the mean $\pm$ SEM of six mice per group. **P* less than or equal to 0.05; ****P* less than or equal to 0.001.

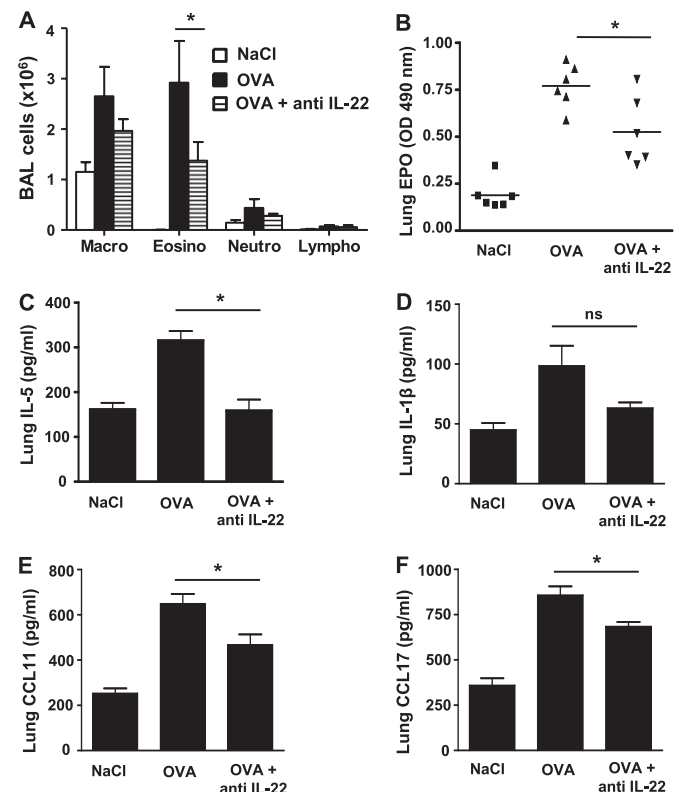


Figure 5. IL-22 neutralization during sensitization phase reduces airway allergic inflammation. Wild-type (WT) mice were sensitized with ovalbumin (OVA) on Days 0 and 7 with or without neutralizing IL-22 antibody or isotype control treatment (100 μ g by i.p. injection). Mice were then challenged three times with OVA instillation. (A, B) Twenty-four hours after the third challenge, eosinophil numbers and eosinophil peroxidase (EPO) activity were determined in the bronchoalveolar lavage (BAL) and in the lung, respectively. (C–F) IL-5, IL-1 β , CCL11, and CCL17 were measured by ELISA in the lung homogenates. These experiments were performed three times. One representative experiment is shown. Values are the mean $\pm$ SEM of six mice per group. **P* less than or equal to 0.05; ns = not significant.

quantitative real-time polymerase chain reaction. IL-4, IL-13, and IL-33 expression were significantly reduced in IL-22 KO mice compared with WT control mice after OVA challenge (Figure 3A). Because adaptive IL-22 is produced by Th17 T cells, we analyzed Th17-related cytokines expression. IL-17A and IL-17F expression, but not IL-23, were significantly increased in the lung of OVA-treated mice deficient in IL-22 compared with WT mice (Figure 3B). This indicates an up-regulation of IL-17A and IL-17F in the absence of IL-22. The expression of Th1 cytokines IFN γ and IL-12 was not different in allergic WT mice and mice deficient in IL-22, whereas IL-6 expression was reduced (Figure 3C). Furthermore, the expression of the regulatory factors IL-10, transforming growth factor- β , and Foxp3 was maintained in immunized mice deficient in IL-22 (Figure 3D). In addition, CCL2, CCL11, and CXCL10 chemokines expression were significantly decreased in mice deficient in IL-22 (Figure 3E). Taken together, our data suggest that the Th2 response is reduced, whereas the Th17 response is augmented in the absence of IL-22.

Reduced Pulmonary T Cells Recruitment in the Absence of IL-22

We then asked whether the number of T lymphocytes in lung was altered in mice deficient in IL-22. T lymphocyte recruitment in the lung was increased on OVA sensitization and challenge in WT mice, but was reduced in mice deficient in IL-22 (Figure 4A). The frequency and absolute number of T cell receptors (TCR) β^+ and TCR $\gamma\delta^+$ cells recruited in the lung on OVA challenge were diminished in IL-22 KO mice compared with WT mice (Figures 4B–4E). These results together with the reduced Th2 cytokines and chemokine levels might suggest that in the absence of IL-22, effector T cells are not efficiently recruited into

the lung to mount a full adaptive response to OVA antigen during allergic inflammation.

IL-22 Neutralization during the Sensitization Phase Reduces Allergic Lung Inflammation

To ascertain further that IL-22 produced during antigen immunization was required for the allergic response, and to exclude an unrelated effect in IL-22 KO mice, we tested the effects of IL-22 antibody neutralization during the sensitization phase. First, we confirmed that the IL-22 antibody has neutralizing property. In fact, IL-22 antibody injection (100 μ g i.p.) inhibited rmIL-22 induced STAT3 phosphorylation in the lung (data not shown). Blocking endogenous IL-22 by the neutralizing IL-22 antibody during antigen sensitization decreased significantly eosinophil recruitment both into the airways and lungs (Figures 5A and 5B), which was not found with the isotype control antibody (data not shown). Furthermore, IL-5, CCL11, CCL17, and IL-1 β levels (Figures 5C–5F) were reduced in the lung after IL-22 antibody neutralization. However, as expected the extent of inhibition by injection of IL-22 neutralizing antibodies is less than that observed in mice gene-deficient in IL-22. Thus, antibody blockade during sensitization confirms the data that IL-22 is required during immunization for the onset of allergic inflammation.

IL-22 Neutralization during Antigen Challenge Phase Augments Allergic Lung Inflammation

We then asked whether IL-22 neutralization during antigen challenge would have a comparable inhibitory effect. Surprisingly, IL-22 antibody blockade during the antigen challenge enhanced airway inflammation with increased accumulation of eosinophils and neutrophils (Figure 6A) and augmented lung EPO activity (Figure 6B). IL-22 antibody administration aug-

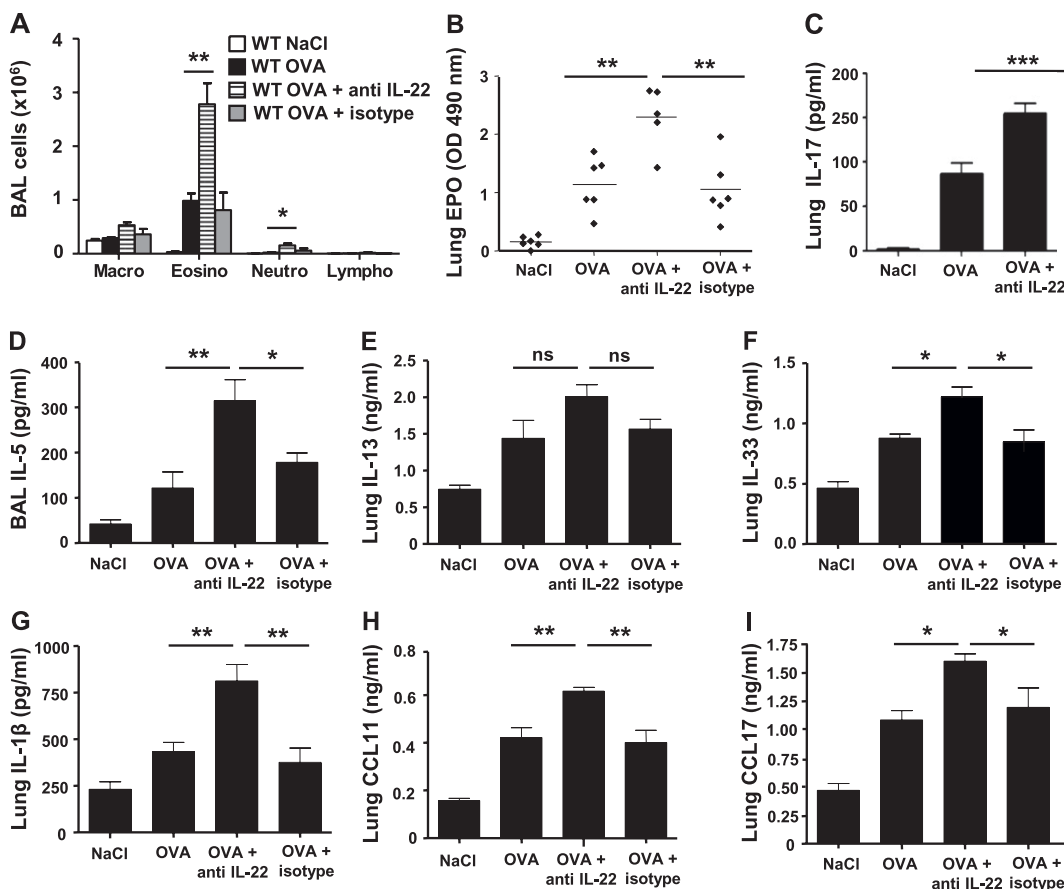


Figure 6. IL-22 neutralization during effector phase increases airway allergic inflammation. Ovalbumin (OVA)-sensitized wild-type (WT) mice were challenged three times with OVA instillation with or without neutralizing IL-22 antibody or isotype control treatment (100 μ g by intranasal on Days 14–16) during challenge phase. (A, D) Twenty-four hours after the third challenge, eosinophil, lymphocyte, neutrophil counts, and IL-5 concentration were determined in the bronchoalveolar lavage (BAL). (B, C, E–I) Eosinophil peroxidase (EPO) activity, IL-17A, IL-13, IL-33, IL-1 β , CCL11, and CCL17 concentrations were measured in the lung homogenates by ELISA. These experiments were performed three times. Values are the mean $\pm$ SEM of 6–10 mice per group. **P* less than or equal to 0.05; ***P* less than or equal to 0.01; ****P* less than or equal to 0.001; ns = not significant.

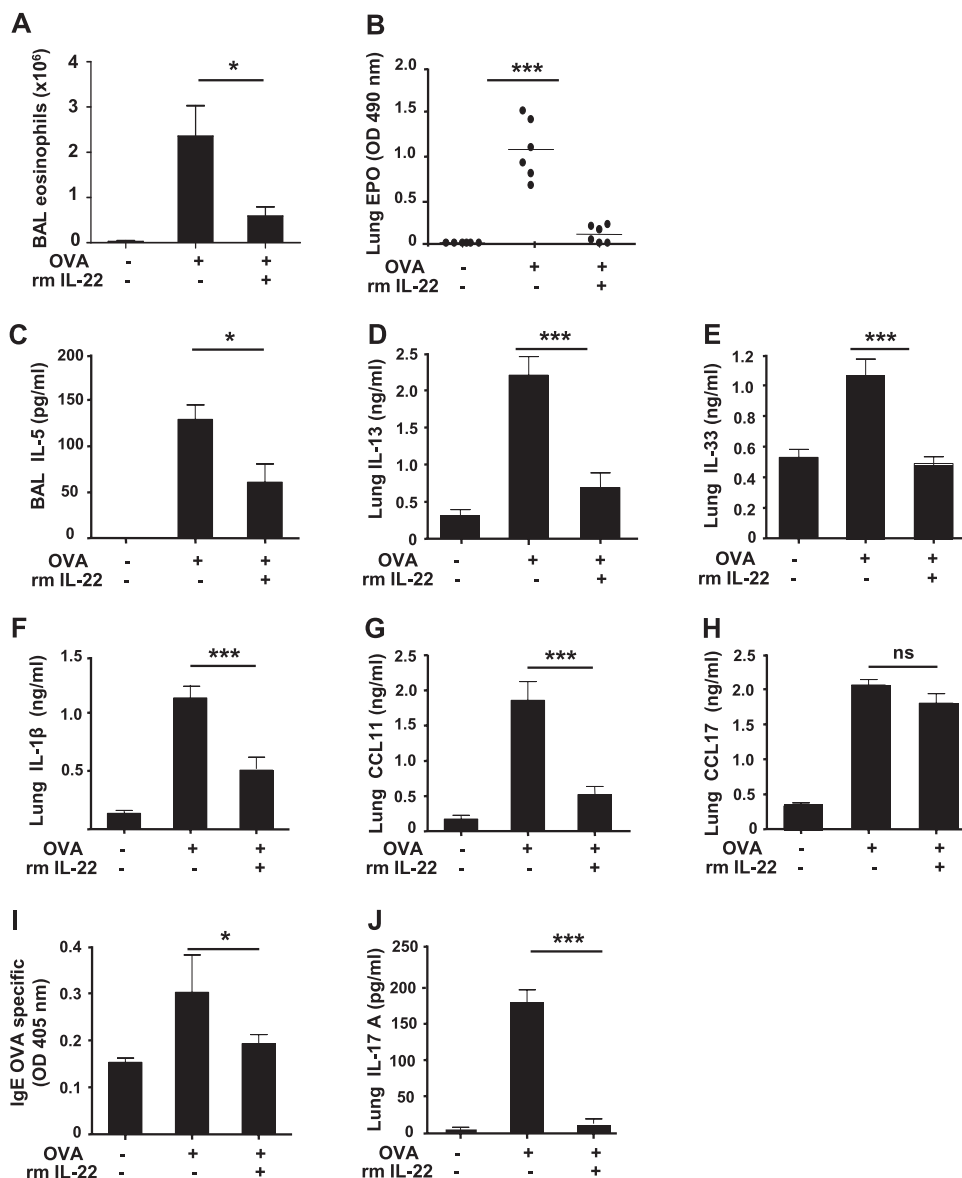


Figure 7. Exogenous IL-22 prevents established allergic airway inflammation. Ovalbumin (OVA)-sensitized wild-type (WT) mice were challenged three times with OVA instillation with and without recombinant murine IL-22 treatment (1 μ g intranasally on Days 14, 15, and 16) during the effector phase. (A, C) Twenty-four hours after the third challenge, eosinophil counts and IL-5 levels were determined in the bronchoalveolar lavage (BAL). (B, D–H, J) Eosinophil peroxidase (EPO) activity, IL-13, IL-33, IL-1 β , CCL11, CCL17, and IL-17A concentrations were measured by ELISA in the lung homogenates. (I) OVA-specific IgE concentrations were assayed in serum from WT mice treated with OVA rmIL-22 by ELISA. These experiments were performed three times. Values are the mean $\pm$ SEM of six mice per group. **P* less than or equal to 0.05; ****P* less than or equal to 0.001; ns = not significant.

mented significantly IL-17A in lung homogenate of immunized control mice (Figure 6C). IL-5 in BAL, IL-13, IL-33, and IL-1 β in lung were increased in IL-22 antibody-treated mice compared with untreated mice and isotype control antibody-treated mice (Figures 6D–6G). Furthermore, the levels of the chemokine CCL11 and CCL17 in the lung were also increased by IL-22 neutralization (Figures 6H and 6I). Histologic lung sections showed that the robust pulmonary cellular infiltration and mucus production seen after OVA challenge were exacerbated by IL-22 antibody neutralization (*see* Figure E1 in the online supplement). Therefore, IL-22 antibody neutralization during the challenge phase enhanced the allergic response, Th2 cytokines, and IL-17A production suggesting that endogenous IL-22 produced in the challenge phase regulate negatively allergic asthma.

Exogenous IL-22 during Antigen Challenge Reduces Allergic Inflammation

We therefore asked whether addition of exogenous recombinant murine IL-22 (rmIL-22) during the antigen challenge might inhibit experimental asthma. Consistent with the hypothesis that IL-22 may function as a negative regulator in the effector phase,

exogenous IL-22 given with the antigen challenge significantly reduced eosinophil recruitment in BAL and lung (Figures 7A and 7B). Furthermore, the concentrations of IL-5 in BAL, IL-13, IL-33, IL-1 β , CCL11, and CCL17 in lung and OVA-specific IgE in serum were reduced on rmIL-22 administration (Figures 7C–7I). Interestingly, exogenous IL-22 treatment reduced IL-17A production in lung of OVA-challenged mice (Figure 7J). Microscopically, the peribronchial eosinophil and lymphocyte recruitment and hyperplasia of goblet cell and of smooth muscle were reduced after treatment with exogenous IL-22 protein (Figure E2).

The fact that IL-22 antibody neutralization augments, and exogenous IL-22 treatment inhibits, eosinophil influx into the lung and the airways, chemokines, and cytokines production during the antigen challenge phase, indicates that IL-22 may function as a negative regulator, which contributes to the resolution of established allergic asthma.

IL-17A Neutralization during Antigen Challenge Restores Allergic Asthma in the Absence of IL-22

To address the contribution of IL-22 and its relation to IL-17A response in allergic lung inflammation further, we analyzed the

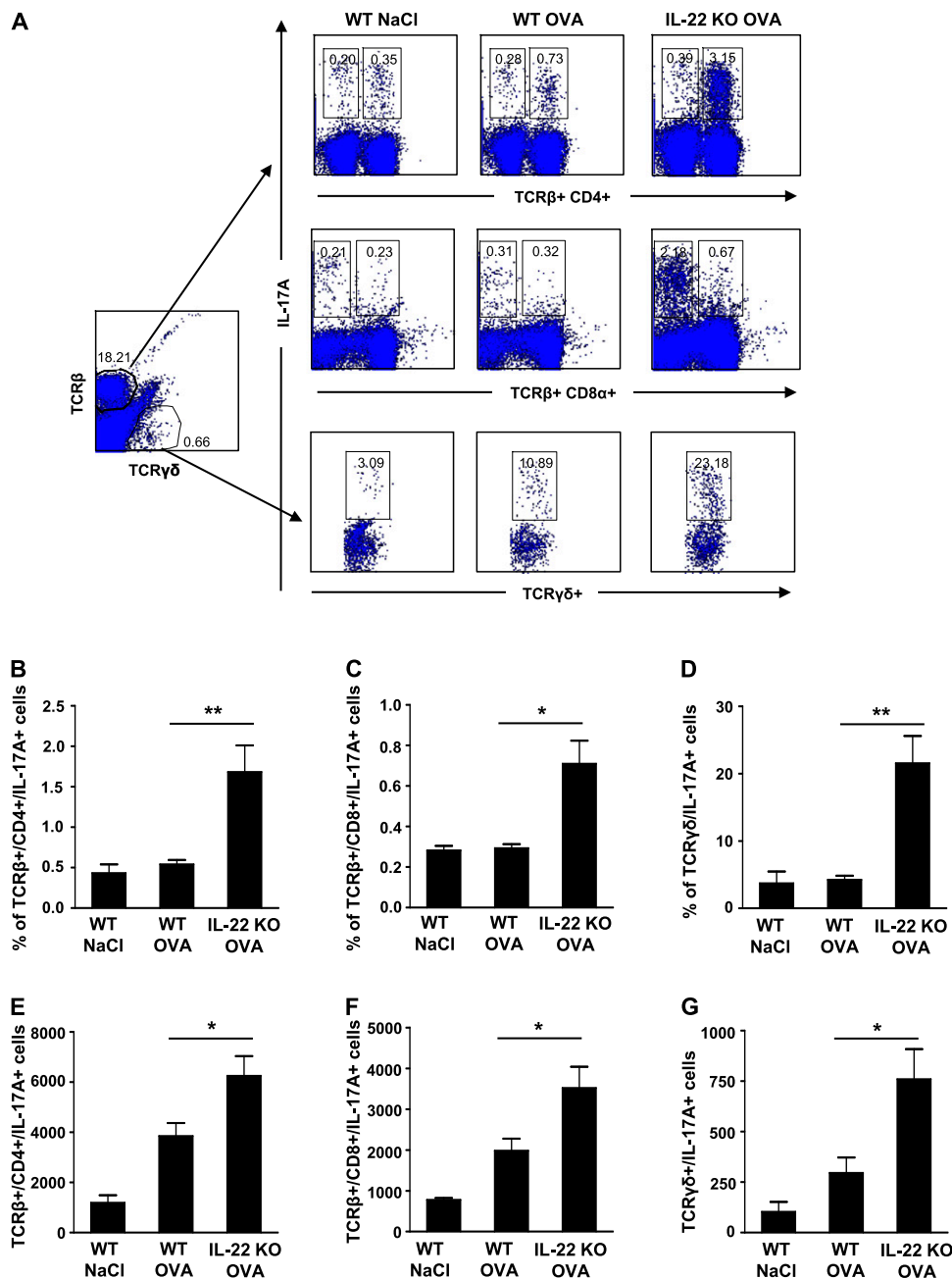


Figure 8. IL-17A is up-regulated in T lymphocytes of mice deficient in IL-22. Ovalbumin (OVA)-sensitized wild-type (WT) and IL-22 knockout (KO) mice were challenged three times with OVA. Twenty-four hours after the third challenge, lung mononuclear cells were isolated and restimulated with phorbol 12-myristate 13-acetate and ionomycin in presence of brefeldin A followed by extracellular staining of TCRβ, TCRγδ, CD4, and CD8α, and intracellular staining of IL-17A. Representative dot plots (A), the frequency and cell number of TCRβ⁺ CD4⁺ IL-17A⁺ (B, E), TCRβ⁺ CD8α⁺ IL-17A⁺ (C, F), and TCRγδ⁺ IL-17A⁺ (D, G) cell populations are given. These experiments were performed three times. Values are the mean ± SEM of six mice per group. **P* less than or equal to 0.05; ***P* less than or equal to 0.01.

frequency of the different T lymphocyte populations including Th17 cell populations in WT mice and mice deficient in IL-22. Similar numbers of TCRβ⁺CD4⁺, TCRβ⁺CD8α⁺, and TCRγδ⁺ expressing IL-17A cells were observed in saline-treated WT mice and mice deficient in IL-22 (data not shown). We found an increased expression of IL-17A in TCRβ⁺ cells, which were mostly CD4⁺ cells (Figures 8A–8C, 8E, and 8F), and to a lesser extent in TCRγδ⁺ cells (Figures 8A, 8D, and 8G) in IL-22 KO mice compared with WT mice after OVA sensitization and challenge. However, TCRγδ⁺ T cells do not seem to play a major role because mice deficient in TCRγδ had the same phenotype as WT mice in our model of OVA-induced allergic lung inflammation (data not shown).

IL-17A was described to exacerbate airway inflammation (1, 3, 4, 39), but we observed that despite IL-17A up-regulation in the lung of mice deficient in IL-22, these mice are protected from allergic airway inflammation. We next asked whether IL-

22 may contribute to the proinflammatory effect of IL-17A in allergic airway inflammation.

IL-17A antibody neutralization in WT mice during antigen challenge decreased allergic airway inflammation included eosinophil recruitment in BAL and lung (Figures 9A and 9B), IL-5 level in BAL and IL-13, IL-33, IL-1β, CCL5, and CCL11 levels in lung (Figures 9C–9H). By contrast, in the absence of IL-22, IL-17A antibody blockade during the antigen challenges partially restored eosinophil recruitment, IL-5 level in BAL, and Th2 cytokine and chemokine levels in the lung (Figures 9A–9H). Histologically, cell infiltration and mucus overproduction that were seen in WT mice were largely absent in mice deficient in IL-22 on OVA challenge, but were fully restored after IL-17A neutralizing antibody treatment in IL-22 KO mice (Figure E3).

Therefore, the proinflammatory function of IL-17A during the challenge phase is partially dependent of IL-22, because IL-

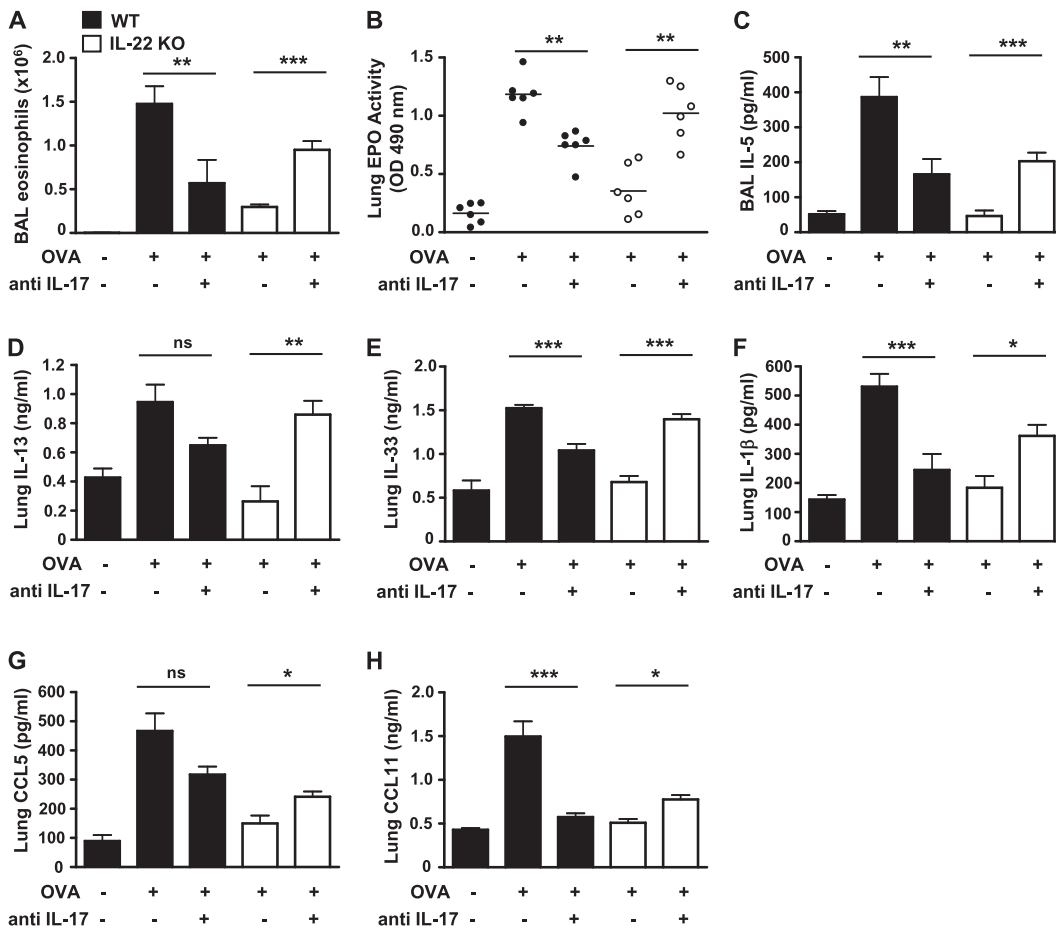


Figure 9. IL-17A neutralization restores allergic airway inflammation in mice deficient in IL-22. Ovalbumin (OVA)-sensitized wild-type (WT) or mice deficient in IL-22 were OVA challenged three times with or without treatment with neutralizing IL-17A antibody. (A, C) Twenty-four hours after the last challenge, eosinophil counts and IL-5 concentrations were determined in the bronchoalveolar lavage (BAL). (B, D–H) Eosinophil peroxidase (EPO) activity, IL-13, IL-33, IL-1 β , CCL5, and CCL11 concentrations were measured by ELISA in the lung homogenates. These experiments were performed three times. Values are the mean $\pm$ SEM of six mice per group. **P* less than or equal to 0.05; ***P* less than or equal to 0.01; ****P* less than or equal to 0.001; ns = not significant.

17A up-regulation in mice deficient in IL-22 is not sufficient to induce allergic airway inflammation. These data collectively indicate that the proinflammatory function of IL-17A depends on IL-22 availability.

DISCUSSION

IL-22 is known to have different functions depending on the nature of the affected tissue and the local cytokine milieu. We examined here the role of IL-22 in a mouse model of allergic asthma and report distinct roles in the onset and resolution of allergic asthma. First, we found augmented pulmonary expression of IL-22 in OVA-sensitized and -challenged mice, which may be relevant because we also found increased IL-22 levels in the serum of human patients with allergic asthma. Functionally, IL-22 expressed in allergic asthma may play a proinflammatory role because we report a significant reduction of allergic airway inflammation in mice deficient in IL-22 with an important decrease in eosinophil recruitment and in inflammatory cytokine levels compared with WT mice. Quantitative polymerase chain reaction analysis confirmed a reduction of the Th2 cytokines including IL-13 and IL-33 and chemoattractant chemokines in the lung of sensitized and challenged mice deficient in IL-22. Importantly, no significant difference was observed in IL-12, IFN γ , IL-10, transforming growth factor- β , and Foxp3 gene expression, suggesting that the decrease in airway inflammation in IL-22 KO mice is likely not caused by reduction of Th1 response and augmented regulatory factors. In addition, IL-22 antibody neutralization in WT mice during the sensitization phase diminished allergic inflammation. These data indicate that IL-22 is required to develop allergic airway inflammation in mice.

Reduced allergic inflammation is associated further with diminished IL-33 production and T cell recruitment into the lung and Th2 polarization and specific IgE production. Furthermore, we also found a decrease of IL-13 levels in the lung of mice deficient in IL-22, which correlates with diminished airway hyperresponsiveness. Our data suggest that IL-22–IL-22R signaling is critical for allergic airway inflammation. In the lung, the major source of IL-22 seems to be T cells, likely Th17 or $\gamma\delta$ T cells (23, 40). Furthermore, a distinct NK cell population, which expresses IL-22 but not IL-17, was newly discovered in humans and mice in the mucosa-associated lymphoid tissues (13, 14, 41). These cells, called NK-22, provide protection and regulate the mucosal homeostasis (41, 42). However, we could not detect NK-22 cells in the allergic lung response.

Despite the lack of its receptor expression on immune cells, IL-22 could have considerable indirect effects on immunity and leukocyte functions. Intraperitoneal injection of IL-22 in mice leads to hepatic gene expression of acute-phase protein, such as serum-amyloid protein A (43). Serum-amyloid protein A increases bacteria and virus opsonization, cell trafficking, and chemotaxis, and cytokine and chemokine expression (44). On respiratory epithelial cells, IL-22 up-regulates granulocyte-colony stimulating factor; antimicrobial proteins (β -defensin, S100 protein family, and lipocalin); the neutrophilic granulocyte-attracting chemokines CXCL1 and CXCL5; and the mucus-associated protein MUC1 (45). Therefore, these data indicate that IL-22 can contribute to airway inflammation by inducing chemoattractant chemokine and serum-amyloid protein A, recruiting and activating lymphocytes, and enhancing the effect of the Th2 cytokines including IL-13 and IL-33.

We investigated the role of IL-22 during established allergic inflammation and we showed that IL-22 antibody neutralization during the challenge phase exacerbates the disease indicating a tissue protective role for IL-22. Consistent with this hypothesis, we found that recombinant IL-22 given with the OVA challenge protects from lung inflammation. These results demonstrate that IL-22 functions as a negative regulator in established allergic inflammation. Our data are consistent with a recent report suggesting that IL-22 antibody neutralization in IL-4R KO mice during established asthma enhanced eosinophils recruitment and allergic lung inflammation (32). However, the authors ascribe the negative regulatory function of IL-22 to a direct effect of this cytokine on dendritic cells, although IL-22 receptor expression has never been reported on these cells (19, 33, 46). To address the possibility that IL-22 may affect dendritic cells functions, we investigated OVA uptake and processing by myeloid dendritic cells and OVA-induced up-regulation of costimulatory molecules, but we were unable to show any effects of IL-22 on these cells. In line with our assumption, IL-22 may act on the respiratory epithelium modulating chemokine expression and epithelial repair (19, 45, 47). Importantly, it has recently been shown that IL-22 may protect mouse pulmonary epithelial cells from bleomycin-induced apoptosis, and that IL-22-mediated protection was associated with increased expression of the antiapoptotic genes Bcl2 and Bcl2l1 (23). In the context of allergic asthma, "danger signals" released by cellular stress or cell death contribute to airway inflammation through inflammasome activation and IL-1 β production (48). Therefore, IL-22 may exert its protective effect during the effector phase by reducing airway epithelial cell death.

Our study also highlights a role of IL-22 in IL-17A expression. First, we observed an increase of IL-17A and IL-17F gene expression in mice deficient in IL-22 after allergen sensitization and challenge. Flow cytometric analysis revealed that IL-17A is mainly produced by TCR β +CD4+ and to a lesser extent by TCR $\gamma\delta$ + cells in the lung of allergic mice. Second, antibody blockade of IL-22 during OVA challenge up-regulates IL-17A expression in the lung leading to exacerbation of allergic airway inflammation. Finally, we showed that IL-17A production is abolished in the lung by exogenous IL-22 treatment during antigen challenge, which protects mice from lung inflammation. These data suggest that the regulation of IL-17A production by IL-22 is critical during effector phase of allergic asthma. Previous studies had already shown a cross-talk between IL-17A and IL-22 production (49, 50). Sonnenberg and coworkers (23) showed *in vitro* that IL-17A could suppress IL-22 expression from Th17 cells in a dose-dependent manner. *In vivo*, they found an increase of IL-22 in the lung of mice deficient in IL-17A after bleomycin injury. Collectively, these observations suggest a reciprocal regulation of IL-17A and IL-22. However, the underlying molecular mechanisms are unknown.

Surprisingly, despite increased IL-17A production, mice deficient in IL-22 were protected from allergic lung inflammation, indicating that IL-17A acts in synergy with IL-22 to promote airway inflammation. Consistent with this, we observed a partial loss of protection after IL-17A antibody treatment in mice deficient in IL-22. These data indicate that IL-22 is required for IL-17A proinflammatory properties. Other studies suggest that IL-17A and IL-22 act synergistically in the airways, and that IL-22 requires the presence of IL-17A to promote expression of inflammatory cytokines and chemokines leading to the recruitment of neutrophils (23, 43).

In conclusion, we report distinct functions of IL-22 in murine allergic asthma. OVA-induced airway inflammation was diminished in mice deficient in IL-22 or by anti-IL-22 antibody

treatment during the sensitization of WT mice, indicating a proinflammatory role for IL-22 in the onset of the disease. In contrast, IL-22 plays a regulatory role in established asthma and participates to the resolution of inflammation. Our study also revealed that IL-22 may regulate the expression of the proinflammatory IL-17A in allergic mice. A better understanding of IL-22 effect on pulmonary epithelial cells and its cross-talk with IL-17A in allergic airway inflammation may lead to novel targets to treat allergic asthma.

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