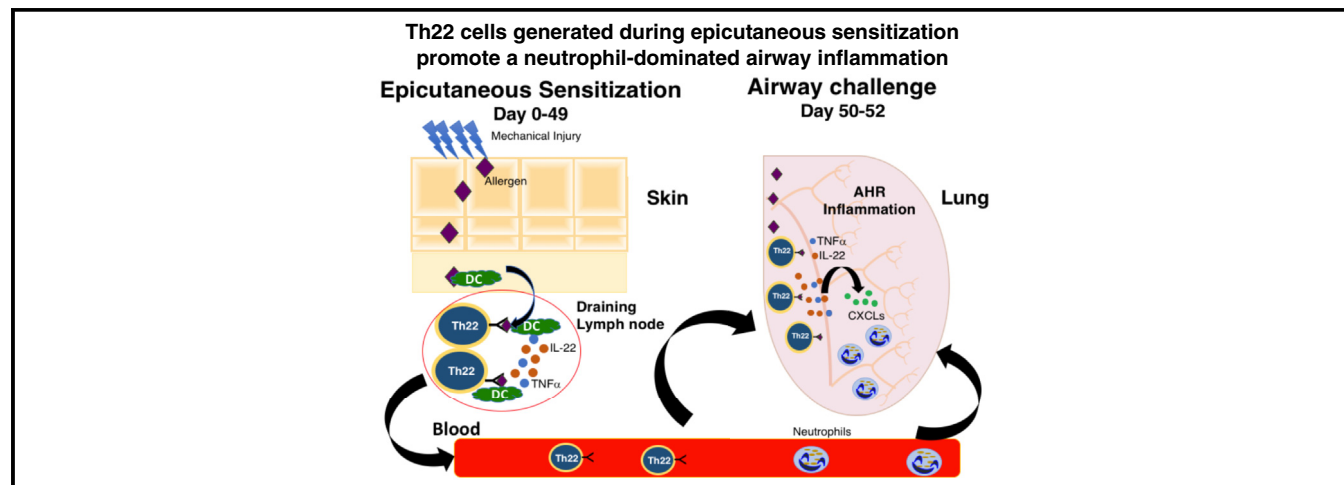


IL-22 promotes allergic airway inflammation in epicutaneously sensitized mice



Juan Manuel Leyva-Castillo, PhD,* Juhan Yoon, PhD,*[†] and Raif S. Geha, MD *Boston, Mass*

GRAPHICAL ABSTRACT



Background: Serum IL-22 levels are increased in patients with atopic dermatitis, which commonly precedes asthma in the atopic march. Epicutaneous sensitization in mice results in T_H2 -dominated skin inflammation that mimics atopic dermatitis and sensitizes the airways for antigen challenge–induced allergic inflammation characterized by the presence of both eosinophils and neutrophils. Epicutaneous sensitization results in increased serum levels of IL-22.

Objective: We sought to determine the role of IL-22 in antigen-driven airway allergic inflammation after inhalation challenge in epicutaneously sensitized mice.

Methods: Wild-type (WT) and $IL22^{-/-}$ mice were sensitized epicutaneously or immunized intraperitoneally with ovalbumin (OVA) and challenged intranasally with antigen. OVA T-cell receptor–specific T cells were T_H22 polarized *in vitro*. Airway inflammation, mRNA levels in the lungs, and airway hyperresponsiveness (AHR) were examined.

Results: Epicutaneous sensitization preferentially elicited an IL-22 response compared with intraperitoneal immunization.

Intranasal challenge of mice epicutaneously sensitized with OVA elicited in the lungs $IL22$ mRNA expression, IL-22 production, and accumulation of $CD3^+CD4^+IL-22^+$ T cells that coexpressed IL-17A and TNF- α . Epicutaneously sensitized $IL22^{-/-}$ mice exhibited diminished eosinophil and neutrophil airway infiltration and decreased AHR after intranasal OVA challenge. Production of IL-13, IL-17A, and TNF- α was normal, but IFN- γ production was increased in lung cells from airway-challenged and epicutaneously sensitized $IL22^{-/-}$ mice. Intranasal instillation of IFN- γ –neutralizing antibody partially reversed the defect in eosinophil recruitment. WT recipients of T_H22 –polarized WT, but not IL-22–deficient, T-cell receptor OVA-specific T cells, which secrete both IL-17A and TNF- α , had neutrophil-dominated airway inflammation and AHR on intranasal OVA challenge. Intranasal instillation of IL-22 with TNF- α , but not IL-17A, elicited neutrophil-dominated airway inflammation and AHR in WT mice, suggesting that loss of IL-22 synergy with TNF- α contributed to defective recruitment of neutrophils into the airways of $IL22^{-/-}$ mice. TNF- α , but not IL-22, blockade at the time of antigen inhalation challenge inhibited airway inflammation in epicutaneously sensitized mice.

Conclusion: Epicutaneous sensitization promotes generation of antigen-specific IL-22–producing T cells that promote airway inflammation and AHR after antigen challenge, suggesting that IL-22 plays an important role in the atopic march. (J Allergy Clin Immunol 2019;143:619–30.)

Key words: IL-22, atopic dermatitis, asthma, neutrophils

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Asthma is a chronic inflammatory disorder of the lungs that affects up to 15% of children and teenagers worldwide.¹ Heterogeneity of the asthma phenotype has led to multiple classifications

Abbreviations used

AD:	Atopic dermatitis
AHR:	Airway hyperresponsiveness
APC:	Allophycocyanin
BALF:	Bronchoalveolar lavage fluid
DC:	Dendritic cell
ILC:	Innate lymphoid cell
ILC1:	Type 1 innate lymphoid cell
OVA:	Ovalbumin
TCR:	T-cell receptor
WT:	Wild-type

depending on age of onset, severity or persistence of disease, allergic status, or response to treatment modalities. Despite decades of research, knowledge of the cellular and molecular mechanisms in asthmatic patients remains incomplete, constituting a barrier for optimal management of patients with the disease.

Atopic dermatitis (AD) often precedes asthma, a phenomenon known as the atopic march.² AD is a common allergic skin inflammatory disease that often starts in infancy and affects more than 17% of children in the United States. Acute AD skin lesions are characterized by epidermal and dermal thickening and by dermal infiltration of CD4⁺ T cells and eosinophils, predominant expression of the T_H2 cytokines IL-4 and IL-13, and increased expression of IL-22 and IL-17A.^{3–5} The roles of T_H2 cytokines and IL-17A have been studied extensively in mouse models of the atopic march.^{6–10} However, little is known about the role of IL-22 in this disease progression.

IL-22 is a member of the IL-10 family of cytokines produced by adaptive T_H17 and T_H22 cells, innate lymphocytes that include $\gamma\delta$ T cells and type 3 innate lymphoid cells (ILCs), as well as myeloid cells, including neutrophils.^{11–15} The IL-22 receptor is expressed on epithelial cells, but not immune cells,¹⁶ indicating an important role for IL-22 signaling in mucosal barrier function. Increased IL-22 levels have been observed in lungs, bronchoalveolar lavage fluid (BALF), and sera of asthmatic patients.^{17–19} Increased lung IL-22 levels have been observed in mouse models of asthma on airway challenge.^{17,20–22} IL-22 inhibits expression of proinflammatory chemokines and adhesion molecules induced by IFN- γ in human bronchial epithelial cells,²³ suggesting that it could play a protective role in asthmatic patients. In contrast, IL-22 enhances the proliferation and migration of human airway smooth muscle cells,^{24,25} suggesting that it could play a pathogenic role in asthma. Genetic deletion of *IL22* in mice or administration of IL-22–blocking antibody to wild-type (WT) mice aggravates the airway inflammation and airway hyperresponsiveness (AHR) elicited by intranasal challenge of intraperitoneally immunized mice.^{17,21,22} Reciprocally, intranasal instillation of rIL-22 before intranasal challenge of intraperitoneally sensitized mice reduced airway inflammation and AHR,^{17,21} suggesting a protective role of IL-22. In contrast, genetic deletion of *IL22* in mice or administration of IL-22–blocking antibody to WT mice reduced airway inflammation in mice sensitized subcutaneously with ovalbumin (OVA).¹⁷ Thus IL-22 appears to play opposing roles in antigen-driven mouse models of asthma, depending on the route of immunization.

Neither intraperitoneal nor subcutaneous immunization mimic antigen sensitization in patients. We have previously reported that

mice sensitized epicutaneously by means of application of antigen to tape-stripped skin, which mimics antigen cutaneous exposure to antigen in patients with AD, have allergic skin inflammation with features of AD.²⁶ Antigen challenge of epicutaneously sensitized mice results in airway inflammation and AHR with features of allergic asthma.²⁶ This model mimics the atopic march in patients with AD who have asthma driven by antigens that had been initially introduced through a disrupted skin barrier.

Here we demonstrate a role for IL-22 in airway inflammation and AHR in this model, suggesting that IL-22 might play a role in asthma that develops during the atopic march in patients with AD.

METHODS**Mice and sensitization**

IL22^{−/−} mice were described previously.²⁷ WT BALB/c and DO11.10 T-cell receptor (TCR)–transgenic mice were obtained from Charles River Laboratories (Wilmington, Mass) and the Jackson Laboratory (Bar Harbor, Me), respectively. All mice were housed in a specific pathogen-free environment and fed an OVA-free diet. Epicutaneous and intraperitoneal sensitization of 6- to 10-week-old mice was done, as previously described.⁷ All procedures were performed in accordance with the Animal Care and Use Committee of Boston Children's Hospital.

RNA extraction and quantitative PCR analysis

RNA was extracted from whole lungs with the Total RNA Isolation Kit (Ambion, Foster City, Calif). cDNA was prepared with the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, Calif). Quantitative real-time PCR was done with TaqMan gene expression assays, universal PCR Master Mix, and ABI Prism 7300 sequence detection system (Applied Biosystems, Foster City, Calif).

Cytokine production by spleen and lung cells

Spleen and lung single-cell suspensions were cultured at 4×10^6 /mL in the presence of OVA (200 μ g/mL) for 96 hours, as described previously.²⁸ Cytokine levels in supernatants were measured by means of ELISA with Ready-Set-Go! ELISA Kits (eBioscience, San Diego, Calif), according to the manufacturer's instructions.

Intranasal treatment and analysis of airway inflammation and AHR

Intranasal challenge with OVA (50 μ g) was performed daily for 3 days, followed by analysis of airway hyperreactivity responses. Lung resistance was measured with invasive Buxco (Buxco Electronics, Wilmington, NC) in response to increasing doses of methacholine administered by means of nebulization to anesthetized mice 24 hours after the last intranasal treatment. Immediately after death, BALF was collected, total cells were counted, and numbers of leukocytes was determined from cytospin preparations stained with the Diff-Quik Stain Set (Baxter, Miami, Fla).

Recombinant mouse cytokines were purchased from R&D Systems (Minneapolis, Minn). Mice were treated with mouse rIL-22 (100 or 1000 ng), rTNF- α (50 ng), rIL-17A (100 ng), and rIL-13 (500, 1000, or 2500 ng) alone or in combination for 3 consecutive days. For IFN- γ –blocking experiments, mice were treated with 50 μ g of mAb against IFN- γ (clone XMG1.2; Bio X Cell, West Lebanon, NH), 15 μ g of mAb against IL-22 or TNF (R&D Systems), or isotype control (Bio X Cell or R&D Systems) during intranasal OVA challenge.

Histologic analysis

Lung specimens were fixed in 4% paraformaldehyde and embedded in paraffin. Hematoxylin and eosin staining was performed in 5-mm sections.

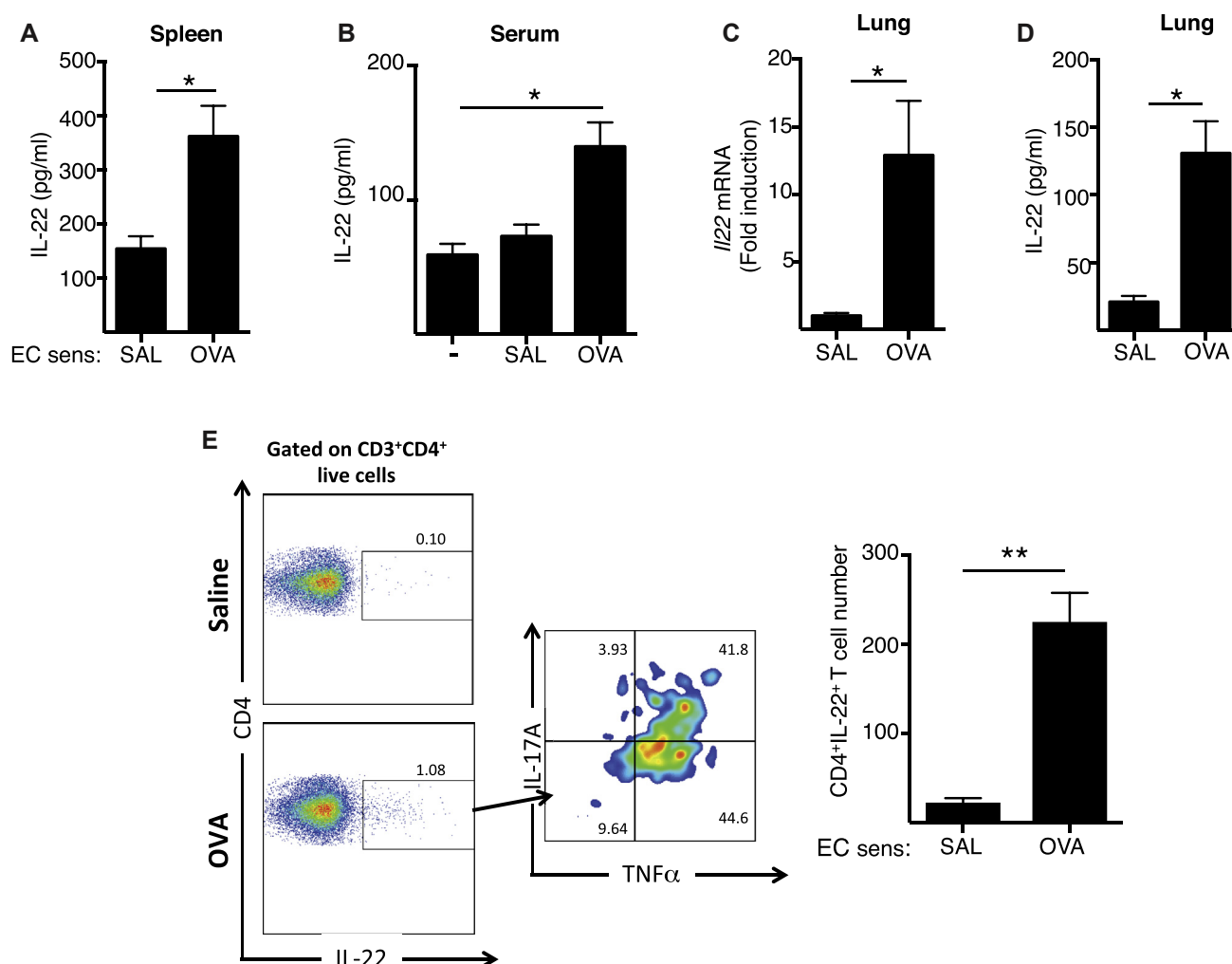


FIG 1. Epicutaneous sensitization (EC sens) elicits a systemic IL-22 response and an antigen-specific IL-22 response in the lungs. **A** and **B**, IL-22 secretion by OVA-stimulated splenocytes (Fig 1, **A**) and IL-22 serum levels (Fig 1, **B**). **C** and **D**, *Il22* mRNA expression in the lungs (Fig 1, **C**) and IL-22 secretion by OVA-stimulated lung cells (Fig 1, **D**). **E**, Representative fluorescence-activated cell sorting analysis and quantitation of intracellular expression of IL-22⁺ cells among CD3⁺CD4⁺ T cells and IL-17A⁺ and TNF-α⁺ cells among CD3⁺CD4⁺IL-22⁺ cells in the lung. Mice were sensitized epicutaneously with OVA or saline in Fig 1, **A** and **B**, followed by intranasal challenge with OVA in Fig 1, **C-F**. Bars represent means ± SEMs (n = 5-10 per group). **P* < .05 and ***P* < .005.

Antibodies and flow cytometry

Lung cells were incubated with an anti-FcγRIII/II antibody (eBioscience) on ice for 15 minutes to block Fc receptors, washed, and incubated with eF506 viability dye (eBioscience), allophycocyanin (APC)-Cy7-conjugated anti-GR1 (RB6-8C5; eBioscience), fluorescein isothiocyanate-conjugated anti-CD11b (M1/70; BioLegend, San Diego, Calif), eFluor-450-anti-CD3 (17A2; eBioscience), APC-anti-CD4 (GK1.5; eBioscience), APC-Cy7-conjugated anti-CD90 (53-2.1; eBioscience), phycoerythrin-anti-CD8 (53-6.7; eBioscience), and peridinin-chlorophyll-protein complex-eF710-conjugated TCR γδ (eBioGL3; eBioscience) for 15 minutes. For ILC staining, biotin-anti-CD11b (M1/70), biotin-anti-CD11c (N418), biotin-anti-F4-80 (BM8), biotin-anti-B220 (RA3-6B2), biotin-anti-CD19 (1D3), biotin-anti-FcεR1α (MAR-1), biotin-anti-CD49b (DX5), and biotin-anti-Gr1 (RB6-8C5) from eBioscience, followed by Streptavidin-BV605 (BD, Franklin Lakes, NJ), were used as lineage gate. ILCs were defined as CD45⁺CD3⁺Lin[−] cells. For intracellular staining, lung cells were stimulated with phorbol 12-myristate 13-acetate (50 ng/mL) and ionomycin (500 ng/mL) in the presence of GolgiStop and GolgiPlug (BD) for 4 hours in Iscove modified Dulbecco medium,

followed by surface staining for CD3, CD4, CD8, γδ TCR, and CD90. Cells were then fixed and permeabilized with the BD Cytotfix/Cytoperm Kit (BD Biosciences, San Jose, Calif) and stained with anti-IL-22 (BioLegend), anti-TNF-α (BioLegend), anti-IL-17A (eBioscience), or anti-IFN-γ (eBioscience) antibodies. Finally, cells were washed and analyzed on an LSRFortessa (BD Biosciences). Results were analyzed with FlowJo software (TreeStar, Ashland, Ore).

T_H22 *in vitro* polarization and adoptive transfer

Naive CD4⁺ T cells from DO11.10 transgenic mice were isolated from spleens by using the Naive CD4⁺ T Cell Isolation Kit, Mouse (Miltenyi Biotec, Bergisch Gladbach, Germany). Naive cells (1 × 10⁶) were cultured for 4 days in anti-CD3 antibody-coated wells (2 μg/mL) with anti-CD28 antibody (2 μg/mL), IL-6 (50 ng/mL), IL-23 (50 ng/mL), anti-IL-4 antibody (10 μg/mL), anti-IFN-γ antibody (10 μg/mL), and anti-TGF-β antibody (10 μg/mL). Then 2 × 10⁶ T_H22 *in vitro*-polarized cells were injected intravenously in WT mice.

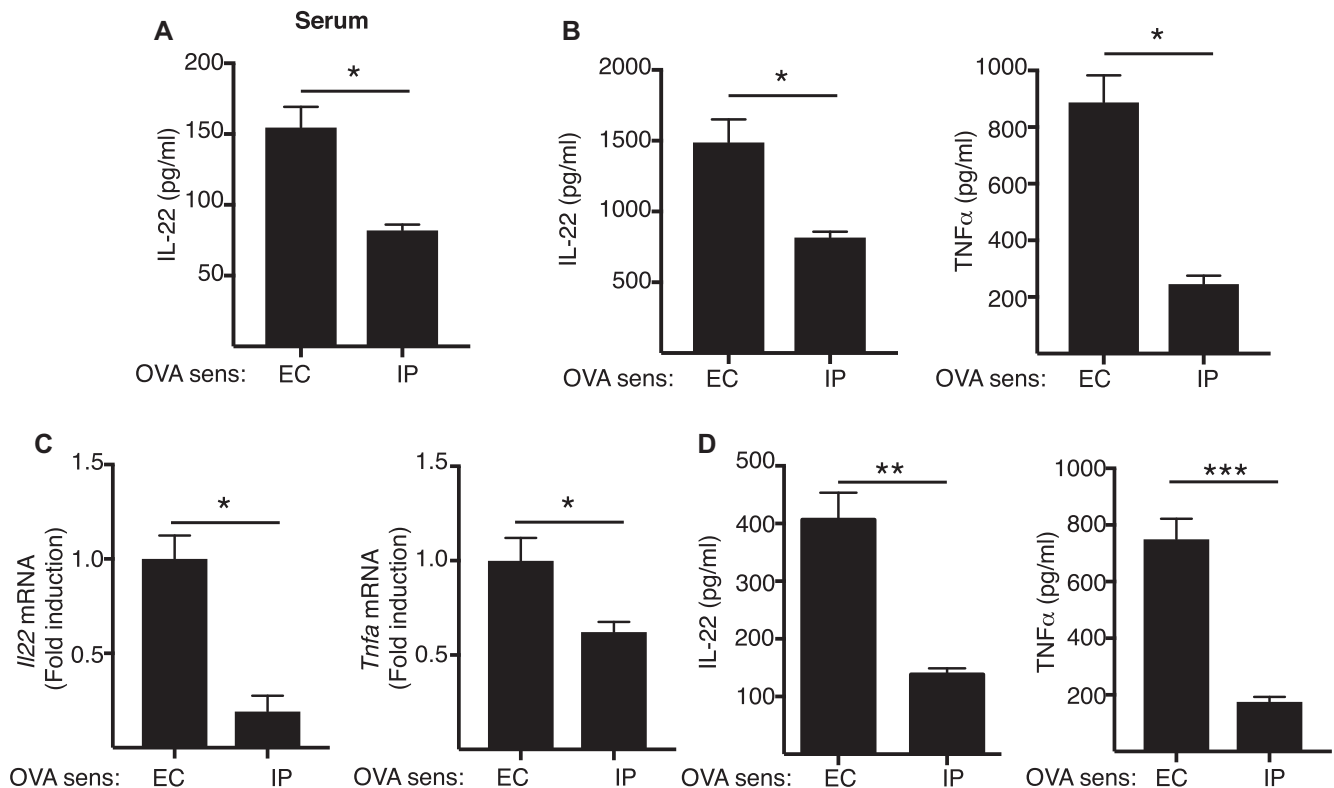


FIG 2. Epicutaneous sensitization preferentially promotes an IL-22 response. Serum IL-22 levels (**A**), IL-22 secretion by OVA-stimulated splenocytes (**B**), *Il22* and *Tnfa* mRNA expression in the lungs (**C**), and IL-22 and TNF-α secretion by OVA-stimulated lung cells (**D**) of mice sensitized epicutaneously (EC) or intraperitoneally (IP) with OVA and challenged intranasally with OVA are shown. Bars represent means $\pm$ SEMs (n = 3-5 per group). **P* < .05, ***P* < .005, and ****P* < .001.

Statistical analysis

Results were analyzed by using 2-way ANOVA or nonparametric *t* tests. A *P* value of less than .05 was considered significant.

RESULTS

Epicutaneous sensitization elicits a systemic IL-22 response and an antigen-specific IL-22 response in the lungs

Previously, we demonstrated that epicutaneous sensitization through application of antigen to tape-stripped mouse skin promotes systemic type 2 and type 17 immune responses.^{7,29} We examined whether epicutaneous sensitization also elicits a systemic IL-22 response. Splenic cells from mice sensitized epicutaneously with OVA secreted significantly more IL-22 when cultured *in vitro* with OVA compared with splenic cells from control mice sensitized epicutaneously with saline (Fig 1, A). Furthermore, as we previously described, epicutaneous sensitization with OVA, but not saline, resulted in a significant increase in serum IL-22 levels (Fig 1, B). These findings demonstrate that epicutaneous sensitization with antigen elicits a systemic IL-22 response.

We next investigated whether intranasal OVA challenge of mice sensitized epicutaneously with OVA causes *Il22* mRNA expression and IL-22 production in the lungs. After intranasal OVA challenge, *Il22* mRNA levels in the lungs were significantly increased in mice sensitized epicutaneously with OVA compared with control mice sensitized epicutaneously with saline (Fig 1, C). In addition, lungs cells from mice sensitized epicutaneously with

OVA secreted significantly higher amounts of IL-22 in response to OVA restimulation *in vitro* compared with lung cells from control mice sensitized epicutaneously with saline (Fig 1, D). These results demonstrate that epicutaneous sensitization elicits an antigen-specific IL-22 response in response to airway antigen challenge.

Lung cell suspensions were analyzed by using flow cytometry to identify cellular sources of IL-22 in the lungs of mice that were challenged intranasally and sensitized epicutaneously. Fluorescence-activated cell sorting showed significantly increased percentages of CD3⁺CD4⁺IL-22⁺ cells in the lungs of mice sensitized epicutaneously with OVA compared with mice sensitized epicutaneously with saline (Fig 1, E). These CD3⁺CD4⁺IL-22⁺ cells coexpressed the cytokines TNF-α (90% $\pm$ 5%, n = 4) and IL-17A (50% $\pm$ 8.4% n = 4). IL-22 expression was barely detected or not detected in CD3⁺CD8⁺ cells or CD3⁺γδ TCR⁺ or CD3⁺Lin⁺CD90⁺ ILCs from lungs of mice sensitized epicutaneously with OVA or saline (see Fig E1 in this article's Online Repository at www.jacionline.org). Together, these results show that CD4⁺ T cells are the major source of IL-22 in the lungs of mice sensitized epicutaneously with OVA after intranasal challenge.

Epicutaneous sensitization preferentially promotes an IL-22 response

IL-22 serum levels (Fig 2, A), IL-22 and TNF-α secretion by splenocytes stimulated with OVA *in vitro* (Fig 2, B), *Il22* and

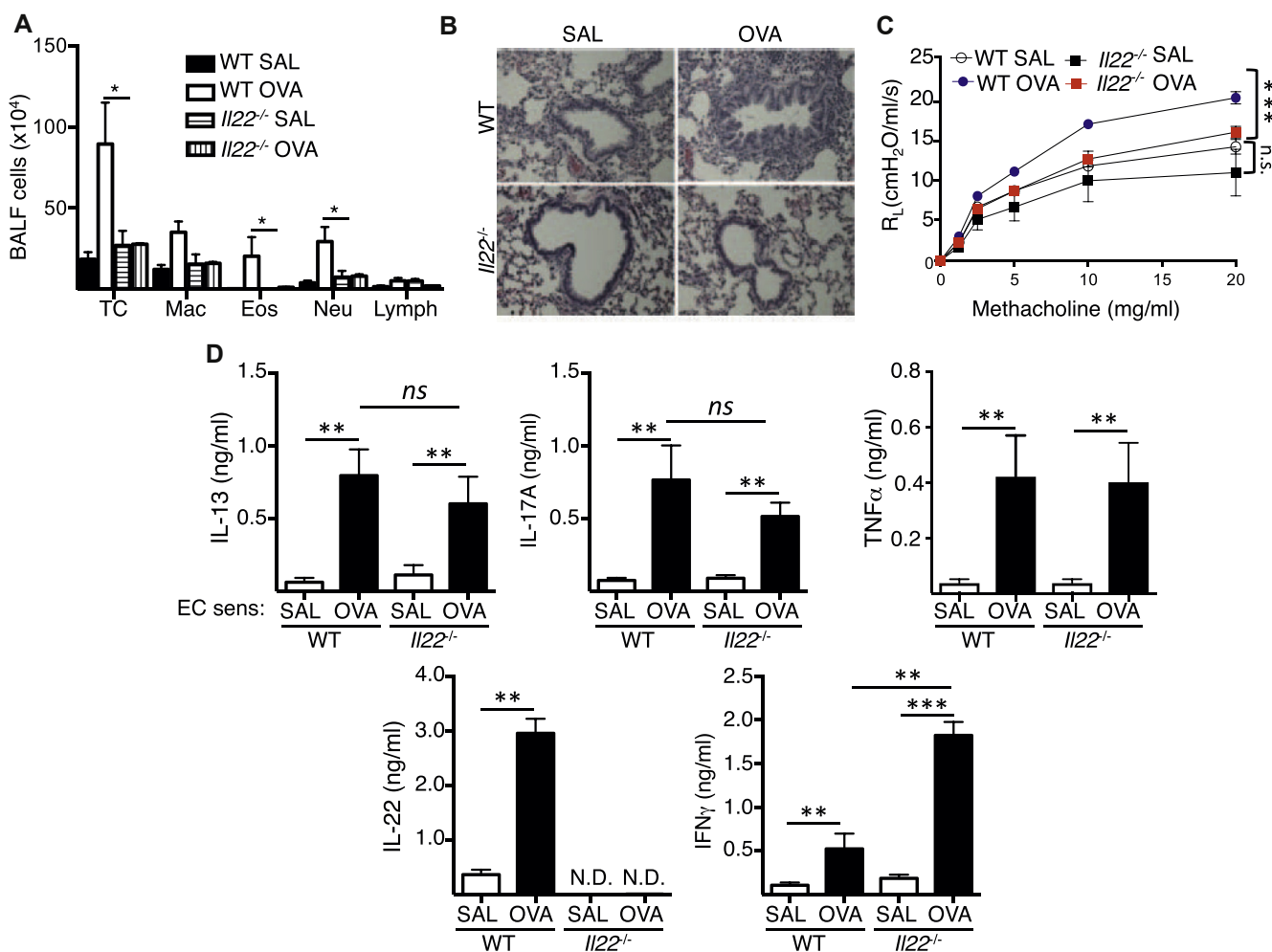


FIG 3. IL-22 is important for the development of airway inflammation and AHR after intranasal antigen challenge of epicutaneously sensitized mice. Total and differential cell counts in BALF (**A**), hematoxylin and eosin (H&E)-stained lung sections (**B**), and lung resistance in response to increasing doses of methacholine (**C**) and cytokine secretion by OVA-stimulated lung cells (**D**) in WT and *Il22*^{-/-} mice sensitized epicutaneously (EC) with OVA or saline and challenged intranasally with OVA are shown. Bars represent means $\pm$ SEMs ($n = 4$ –7 per group). * $P < .05$, ** $P < .005$, and *** $P < .001$. Eos, Eosinophils; Lymph, lymphocytes; Mac, macrophages; Neu, neutrophils; ns, not significant; TC, total cells.

Tnfa mRNA expression in the lungs after OVA airway challenge (Fig 2, C), and IL-22 and TNF- α secretion in response to OVA stimulation by lung cells from airway-challenged mice (Fig 2, D) were all significantly less in mice immunized intraperitoneally with OVA compared with mice sensitized epicutaneously with OVA. As previously reported,⁷ IL-17A production by OVA-stimulated splenocytes was greater in mice sensitized epicutaneously than in mice immunized intraperitoneally, whereas IL-4, IL-13, and IFN- γ production and serum OVA-specific IgE levels were comparable between the 2 groups (data not shown). These results show that epicutaneous sensitization preferentially promotes a systemic IL-22 response and, importantly, production of IL-22 and TNF- α in the lungs after intranasal challenge.

Previously, we had shown that TGF- β , which is richly expressed in the skin, is important for inducing an IL-17A response to epicutaneous sensitization with OVA.⁷ To examine whether TGF- β plays a role in the IL-22 response to epicutaneous sensitization, we injected a neutralizing antibody to TGF- β 6 hours before epicutaneous sensitization and then examined

dendritic cells (DCs) isolated from draining lymph nodes 24 hours after epicutaneous sensitization for their ability to drive cytokine production by naive OVA-TCR transgenic DO11.10 T cells. TGF- β blockade had no effect on the ability of DCs to drive IL-22 production by naive T cells (see Fig E2 in this article's Online Repository at www.jacionline.org). However, as we reported previously,⁷ it significantly impaired their ability to drive IL-17A production. TGF- β blockade had no effect on the ability of DCs to drive IL-13 or IFN- γ production.

IL-22 is important for development of airway inflammation and AHR after intranasal antigen challenge of mice sensitized epicutaneously

To determine the role of IL-22 in lung inflammation elicited by intranasal challenge of mice sensitized epicutaneously, we made use of *Il22*^{-/-} mice. As previously reported,^{7,26} intranasal OVA challenge of WT mice sensitized epicutaneously with OVA elicited eosinophil and neutrophil influx into the airways, as well

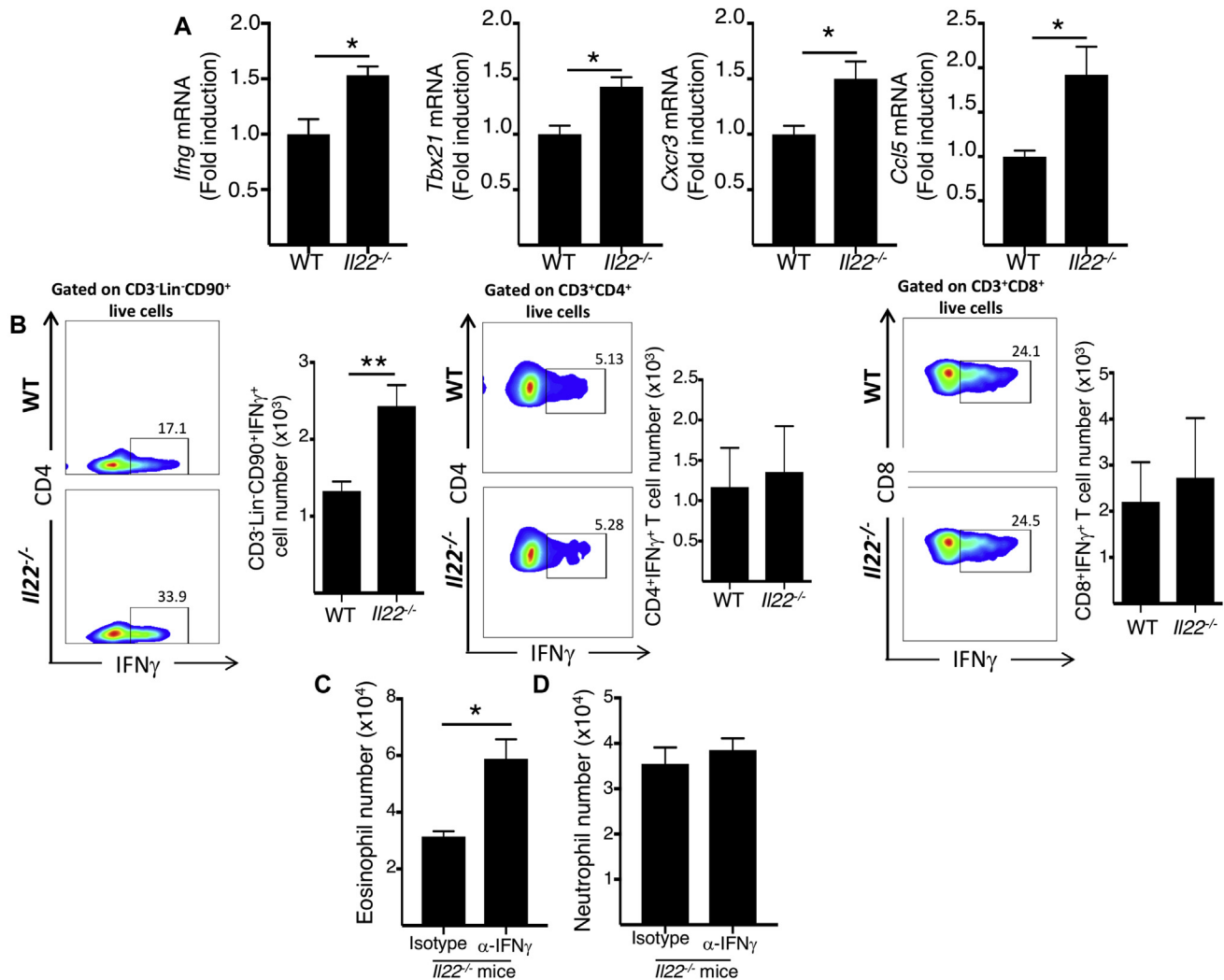


FIG 4. Increased IFN- γ production underlies the decreased airway eosinophilia in inhalation-challenged *Il22*^{-/-} mice sensitized epicutaneously with OVA. **A**, *Ifng*, *Tbx21*, *Cxcr3*, and *Ccl5* mRNA levels in the lungs. **B**, Representative fluorescence-activated cell sorting analysis and quantitation of intracellular expression of IFN- γ ⁺ cells among CD3⁺Lin⁻CD90⁺ cells, CD3⁺CD4⁺ T cells, and CD3⁺CD8⁺ T cells in the lungs of WT and *Il22*^{-/-} mice sensitized epicutaneously and challenged intranasally with OVA. **C** and **D**, Effect of intranasal administration of anti-IFN- γ antibody or an isotype control on the recruitments of eosinophils (Fig 4, C) and neutrophils (Fig 4, D) to the antigen-challenged airways of *Il22*^{-/-} mice sensitized epicutaneously with OVA. Bars represent means $\pm$ SEMs (n = 5–7 per group). *P < .05 and **P < .005.

as AHR (Fig 3, A–C). *Il22*^{-/-} mice sensitized epicutaneously with OVA accumulated significantly fewer total cells, eosinophils, and neutrophils in BALF and demonstrated reduced peribronchial cellular infiltrates in the lungs compared with OVA-sensitized WT controls (Fig 3, A and B). Importantly, after intranasal OVA challenge, *Il22*^{-/-} mice sensitized epicutaneously with OVA showed a significant decrease in airway resistance in response to methacholine compared with OVA-sensitized WT control mice (Fig 3, C).

Lungs cells from WT mice sensitized epicutaneously with OVA secreted significantly more IL-13, IL-17A, IFN- γ , IL-22, and TNF- α in response to restimulation *in vitro* with OVA compared with mice sensitized epicutaneously with saline. Lung cells from *Il22*^{-/-} mice sensitized epicutaneously with OVA and subjected to airway antigen challenge secreted

comparable amounts of IL-13, IL-17A, and TNF- α but significantly greater levels of IFN- γ in response to OVA restimulation *in vitro* compared with lung cells from OVA-sensitized WT control mice (Fig 3, D). The increased IFN- γ production was selective to lung cells because we previously reported normal production of IFN- γ by OVA-stimulated splenocytes of epicutaneously sensitized *Il22*^{-/-} mice, as well as normal levels of OVA-specific IgG_{2a} antibody, in addition to normal production of IL-4 and IL-13 by OVA-stimulated splenocytes and normal levels of OVA-specific IgG₁ and IgE antibody.³⁰ The increased IFN- γ production by lung cells in *Il22*^{-/-} mice is consistent with a previous report that IL-22 downregulates TH1 responses.³¹ As expected, IL-22 secretion was not detected in lung cells from *Il22*^{-/-} mice sensitized epicutaneously with OVA (Fig 3, D). These results demonstrate that IL-22 plays

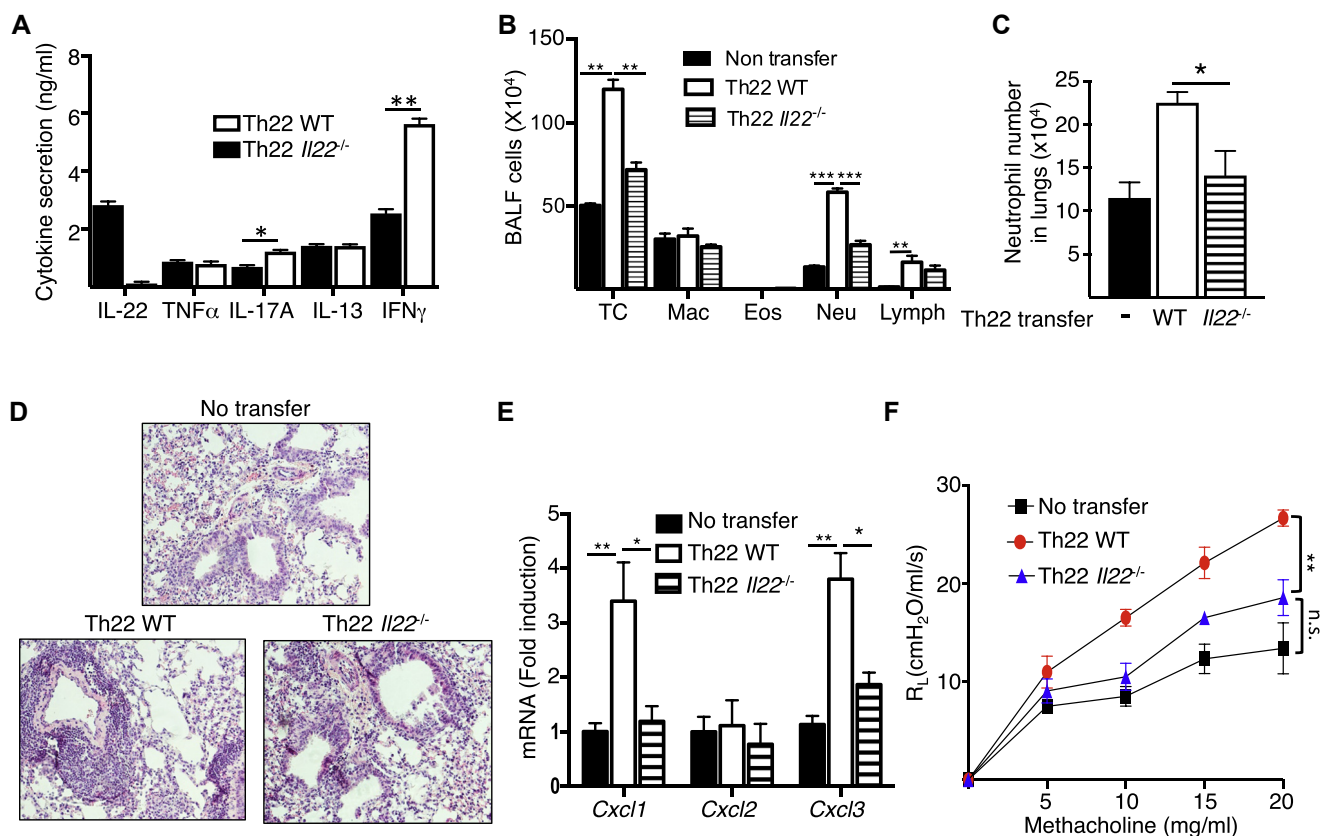


FIG 5. T_H22 antigen-specific CD4⁺ T cells drive neutrophil-dominated airway inflammation and AHR in response to intranasal antigen challenge. **A**, Cytokine secretion by *in vitro*-polarized T_H22 cells from in WT and //22^{-/-} mice. **B–F**, Total and differential cell counts in BALF (Fig 5, B), frequency of neutrophils in lungs (Fig 5, C), hematoxylin and eosin (H&E)-stained lung sections (Fig 5, D), chemokine mRNA levels in lungs (Fig 5, E), and lung resistance in response to increasing doses of methacholine (Fig 5, F) in WT recipients of T_H22-polarized OVA-specific CD4⁺ T cells from DO11.10 and DO11.10//22^{-/-} mice after intranasal challenge with OVA. Mice that received no T cells were used as controls. Bars represent means $\pm$ SEMs (n = 4–6 per group). Eos, Eosinophils; Lymph, lymphocytes; Mac, macrophages; Neu, neutrophils; n.s., not significant; TC, total cells. *P < .05, **P < .005, and ***P < .001.

an important role for antigen-driven airway inflammation and AHR in mice sensitized epicutaneously after intranasal challenge.

Increased IFN- γ production underlies decreased airway eosinophilia in inhalation-challenged //22^{-/-} mice sensitized epicutaneously with OVA

IL-13 plays an important role in airway eosinophilia elicited by inhalation challenge in mouse models of allergic airway inflammation.³² Furthermore, instillation of IL-13 in the airways elicits airway eosinophilia.³³ The observation that eosinophil recruitment to antigen-challenged airways was virtually absent in //22^{-/-} mice sensitized epicutaneously with OVA prompted us to examine whether IL-22 synergizes with IL-13 in driving airway eosinophilia. We first determined the dose of IL-13 that resulted in suboptimal airway eosinophilia (approximately 70% of maximum) when instilled in the airway by means of intranasal inhalation (see Fig E3, A, in this article's Online Repository at www.jacionline.org). We then examined whether addition of IL-22 enhances IL-13-driven eosinophilia. Nasal instillation of IL-22 by itself caused no

airway eosinophilia (see Fig E3, B). Coadministration of up to 1 μ g of IL-22 by means of nasal instillation did not enhance IL-13-driven airway eosinophilia (see Fig E3, B). Thus impaired eosinophilia in the antigen-challenged airways of //22^{-/-} mice sensitized epicutaneously with OVA is not due to loss of a potential synergistic effect of IL-22 on IL-13-driven airway eosinophilia.

IFN- γ has been reported to inhibit the ability of IL-13 to cause airway eosinophilia.³⁴ We investigated whether //22^{-/-} mice sensitized epicutaneously with OVA exhibit increased *Ifng* mRNA levels and enhanced recruitment of IFN- γ -producing cells in the lungs. Both CD3⁺CD4⁺ T cells and type 1 innate lymphoid cells (ILC1s) are a source of IFN- γ .³⁵ *Ifng* expression is dependent on the transcription factor T-bet encoded by *Tbx21*,³⁶ and both T_H1 cells and ILC1s are recruited to tissues by the chemokine CCL5, which binds to the chemokine receptor CXCR3 expressed on these cells.³⁵ mRNA levels of *Ifng*, *Tbx21*, *Cxcr3*, and *Ccl5* were increased significantly in lungs of //22^{-/-} mice sensitized epicutaneously with OVA after intranasal OVA challenge compared with their WT counterparts (Fig 4, A). In addition, numbers of CD3⁺Lin⁻CD90⁺IFN- γ ⁺ ILC1s, but not CD3⁺CD4⁺IFN- γ ⁺ T_H1 cells or CD3⁺CD8⁺IFN- γ ⁺ cells,

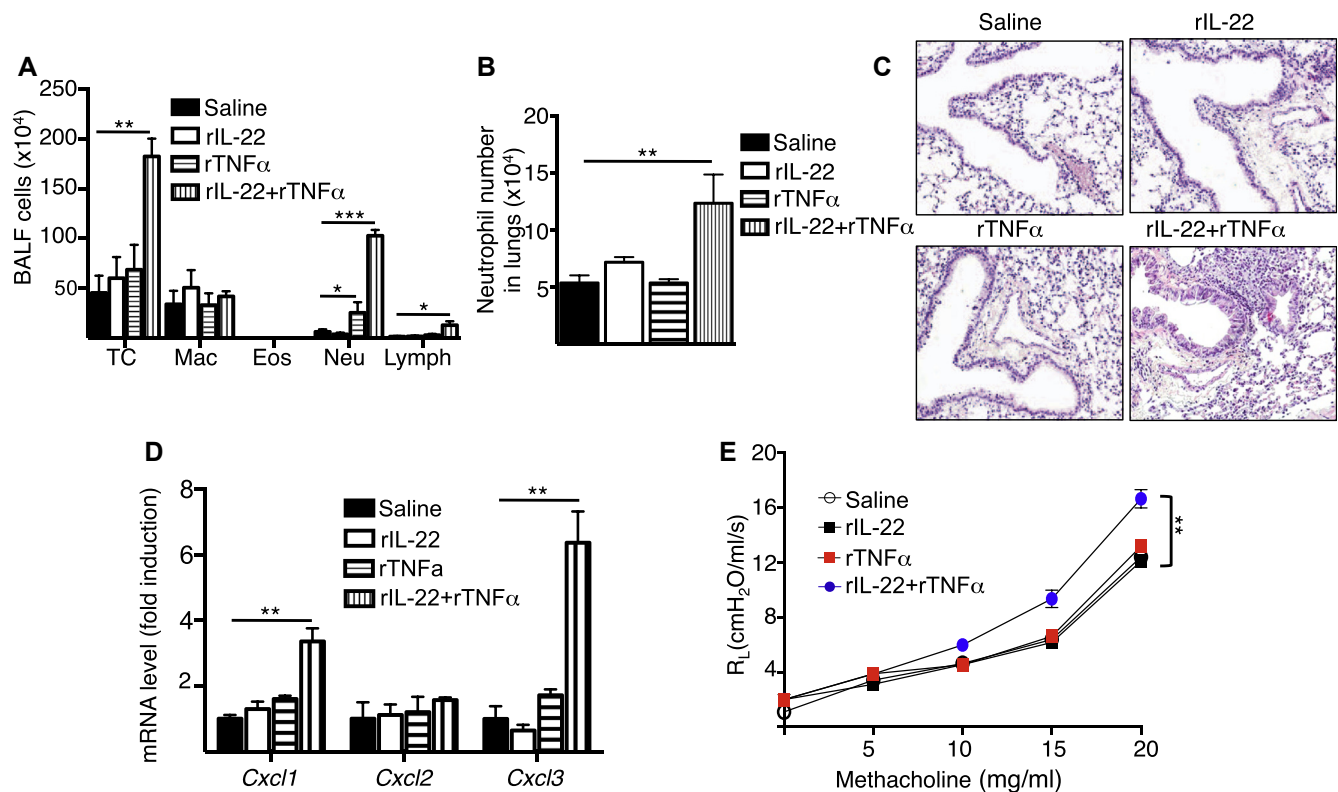


FIG 6. IL-22 synergizes with TNF- α to promote neutrophil airway inflammation. Total and differential counts in BALF (A), frequency of neutrophils in lungs (B), hematoxylin and eosin (H&E)-stained lung sections (C), chemokine mRNA levels in lungs (D), and lung resistance in response to increasing doses of methacholine (E) in WT mice treated intranasally with saline, rIL-22, rTNF- α , or rIL-22 plus rTNF- α are shown. Bars represent means $\pm$ SEMs ($n = 4$ -6 per group). Eos, Eosinophils; Lymph, lymphocytes; Mac, macrophages; Neu, neutrophils; TC, total cells. * $P < .05$, ** $P < .005$, and *** $P < .001$.

were increased in *IL22*^{-/-} mice sensitized epicutaneously after intranasal OVA challenge (Fig 4, B), suggesting that IL-22 controls recruitment of ILC1s to the lungs after intranasal challenge.

To examine whether increased IFN- γ production contributed to the impaired recruitment of eosinophils to the antigen-challenged airways of *IL22*^{-/-} mice sensitized epicutaneously with OVA, we treated the mice intranasally with neutralizing antibody to IFN- γ during OVA airway challenge. Administration of anti-IFN- γ antibody, but not isotype control, significantly increased airway eosinophilia after antigen inhalation in *IL22*^{-/-} mice sensitized epicutaneously with OVA (Fig 4, C). This effect was specific to eosinophils because administration of IFN- γ antibody did not restore neutrophil recruitment to the airways in these mice (Fig 4, D). Reconstitution of airway eosinophilia in the challenged airways of *IL22*^{-/-} mice sensitized epicutaneously through administration of anti-IFN- γ antibody was partial because the numbers of eosinophils in the airways of these mice remained lower than in the challenged airways of WT mice sensitized epicutaneously ($5.9 \pm 0.52 \times 10^4$ vs $2.04 \pm 0.32 \times 10^5$). These results suggest that the impaired eosinophilia in antigen-challenged airways of *IL22*^{-/-} mice sensitized epicutaneously is in part secondary to the increased production of IFN- γ in the lungs of these mice.

T_H22 antigen-specific CD4⁺ T cells drive neutrophil-dominated airway inflammation and AHR in response to intranasal antigen challenge

We next addressed the mechanisms by which lack of IL-22 impairs neutrophil recruitment into the antigen-challenged airways of mice sensitized epicutaneously. We first investigated the ability of CD4⁺ T_H cells that produce IL-22 to elicit airway inflammation and AHR. We examined the response to intranasal antigen challenge of WT recipients of antigen-specific CD4⁺ T cells polarized *in vitro* into IL-22-producing cells. Naive splenic CD4⁺CD62L⁺ T cells from TCR-transgenic DO11.10 mice were activated with anti-CD3 plus anti-CD28 and IL-23 in the presence of neutralizing antibodies to IL-4, IFN- γ , and TGF- β , conditions known to drive the differentiation of naive CD4⁺ cells into IL-22-producing cells.^{27,37} T cells polarized *in vitro* under these conditions are henceforth designated T_H22 cells. As expected, T_H22 cells from DO11.10 mice, but not DO11.10/*IL-22*^{-/-} mice, secreted IL-22 (Fig 5, A). The 2 types of cells secreted comparable levels of TNF- α and IL-13 and no detectable levels of IL-4 (Fig 5, A, and data not shown). T_H22 cells from DO11.10/*IL-22*^{-/-} mice secreted modestly but significantly more IL-17A and IFN- γ than T_H22 cells from WT control mice (Fig 5, A).

After antigen inhalation challenge, recipients of T_H22-polarized DO11.10 CD4⁺ T cells, but not DO11.10/*IL-22*^{-/-}

CD4⁺ T cells, exhibited a significant increase in the number of total cells and neutrophils in BALF and lung tissue (Fig 5, B and C) and increased peribronchial cellular infiltrates (Fig 5, D) compared with control mice that received no T cells. In line with the increase in neutrophil counts in the airways and lungs, recipients of T_H22-polarized DO11.10 CD4⁺ T cells showed significantly increased mRNA levels of *Cxcl1* and *Cxcl3*, but not *Cxcl2*, in their lungs compared with recipients of DO11.10/IL-22^{-/-} CD4⁺ T cells and control mice that received no T cells (Fig 5, E). Comparable *Il17a* and *Ifng* mRNA levels were observed in airway-challenged lungs of recipients of T_H22-polarized WT and IL-22-deficient OVA TCR-transgenic T cells (see Fig E4 in this article's Online Repository at www.jacionline.org). Moreover, intranasally challenged recipients of T_H22-polarized DO11.10 CD4⁺ T cells demonstrated significantly increased airway resistance in response to methacholine compared with recipients of DO11.10/IL-22^{-/-} CD4⁺ T cells or control mice that received no T cells (Fig 5, F). Together, these results demonstrate that IL-22 produced by CD4⁺ T cells can drive neutrophil-dominated airway inflammation and AHR.

IL-22 synergizes with TNF- α to drive neutrophil-dominated airway inflammation and AHR

We investigated whether IL-22 is sufficient to drive airway inflammation and AHR. rIL-22 was administered intranasally in 2 different doses (100 and 1000 ng) daily for 3 days to WT mice. Intranasal administration of rIL-22 caused no significant increase in the number of total cells or neutrophils in BALF; no significant increase in the number of neutrophils in the lungs; no increase in peribronchial cellular infiltrates; no significant increase in mRNA levels of *Cxcl1*, *Cxcl2*, and *Cxcl3* in the lungs; and no significant AHR to methacholine compared with intranasal administration of saline (Fig 6 and see Fig E5 in this article's Online Repository at www.jacionline.org).

Because IL-17A and TNF- α were coexpressed with by CD3⁺CD4⁺IL-22⁺ lung cells and IL-17A and TNF- α were secreted by *in vitro*-polarized T_H22 cells, which, when adoptively transferred, resulted in development of neutrophil-dominated airway inflammation and AHR in response to antigen inhalation challenge, we examined whether IL-17A and TNF- α might synergize with IL-22 to drive this response. We treated WT mice intranasally with rIL-22 (1000 ng) in combination with rIL-17A (100 ng) or rTNF- α (50 ng). Mice treated with TNF- α alone exhibited a modest but significant increase in neutrophil numbers in BALF (Fig 6, A); no significant increase in total lung neutrophil counts (Fig 6, B); a minimal increase in peribronchial cellular infiltrates (Fig 6, C); no significant increase in *Cxcl1*, *Cxcl2*, and *Cxcl3* mRNA levels in the lungs (Fig 6, D); and no increase in airway resistance compared with control mice treated with saline (Fig 6, E). Importantly, mice treated with rIL-22 plus rTNF- α exhibited significantly increased numbers of total cells, neutrophils, and lymphocytes in BALF (Fig 6, A); increased numbers of neutrophils in the lungs (Fig 6, B); increased peribronchial cellular infiltrates (Fig 6, C); significantly increased mRNA levels of *Cxcl1* and *Cxcl3* in the lungs (Fig 6, D); and significantly enhanced airway resistance (Fig 6, E) compared to controls. There was no significant increase in *Il13*, *Il33*, *Il25*, or *Il17a* mRNA in the lungs of mice that received rIL-22 plus rTNF- α (see Fig E6 in this article's Online Repository at www.jacionline.org). Numbers of total cells, macrophages, neutrophils, and lymphocytes in BALF;

numbers of neutrophils in the lungs; levels of peribronchial cellular infiltrates; mRNA levels of *Cxcl1*, *Cxcl2*, and *Cxcl3* in the lungs; and airway resistance in mice treated with IL-17A alone or in combination with IL-22 were comparable with those in control mice treated with saline (see Fig E7 in this article's Online Repository at www.jacionline.org). These results demonstrate that IL-22 synergizes with TNF- α , but not with IL-17A, to promote neutrophil airway inflammation and suggest that IL-22 and TNF- α produced by T_H22 cells synergize to drive neutrophil-dominated airway inflammation and AHR.

Blockade of TNF- α , but not IL-22, during OVA intranasal challenge decrease airway inflammation in mice epicutaneously sensitized with OVA

We investigated whether blockade of IL-22 or TNF- α during intranasal challenge decreased airway inflammation in mice sensitized epicutaneously. Intranasal administration of TNF- α blocking antibody during OVA challenge caused a significant decrease in numbers of total cells, eosinophils, and neutrophils in BALF; a significant decrease in the number of neutrophils in the lungs; a decrease in levels of peribronchial cellular infiltrates; and a significant decrease in mRNA levels of *Cxcl1*, *Cxcl2*, and *Cxcl3* in the lungs compared with intranasal administration of isotype control antibody (Fig 7). Intranasal administration of IL-22 blocking antibody had no apparent effect on airway inflammation induced by intranasal OVA challenge of mice sensitized epicutaneously with OVA (Fig 7). These results demonstrate that TNF- α plays an important role in mediating airway inflammation in response to intranasal challenge of mice sensitized epicutaneously. In contrast, although important for the induction of airway inflammation in mice challenged intranasally and sensitized epicutaneously, IL-22 plays no detectable role in the effector phase of the response to intranasal challenge (Fig 7).

DISCUSSION

We demonstrate that IL-22 is produced by CD4⁺ T cells in the lungs of mice sensitized epicutaneously and plays a critical role in airway inflammation and AHR elicited by antigen inhalation challenge of these mice. Moreover, we show that synergistic action of IL-22 with TNF- α produced by allergen-specific T_H22 cells can drive neutrophil-dominated airway inflammation.

Serum IL-22 levels, IL-22 production by antigen-stimulated splenocytes, and *Il22* mRNA expression and IL-22 production in the lungs after intranasal antigen challenge all increased in WT mice sensitized epicutaneously with OVA. Epicutaneous sensitization preferentially elicited an IL-22 response compared with intraperitoneal immunization. We previously showed that epicutaneous sensitization with OVA also preferentially elicits an IL-17A response compared with evidence of increased serum IL-17A levels, IL-17A production by antigen-stimulated splenocytes, and *Il17a* mRNA expression and IL-17A production in the lungs after intranasal antigen challenge.⁷ Induction of both IL-22 and IL-17A after epicutaneous sensitization is consistent with the observation that the 2 cytokines are often produced by overlapping populations of T_H cells that require different factors for their induction.²⁷ Previously, we demonstrated that IL-22 production and, to a lesser extent, IL-17A production after epicutaneous sensitization is dependent on IL-23, a cytokine critical for induction of IL-22-producing CD4⁺ T_H cells.³⁰

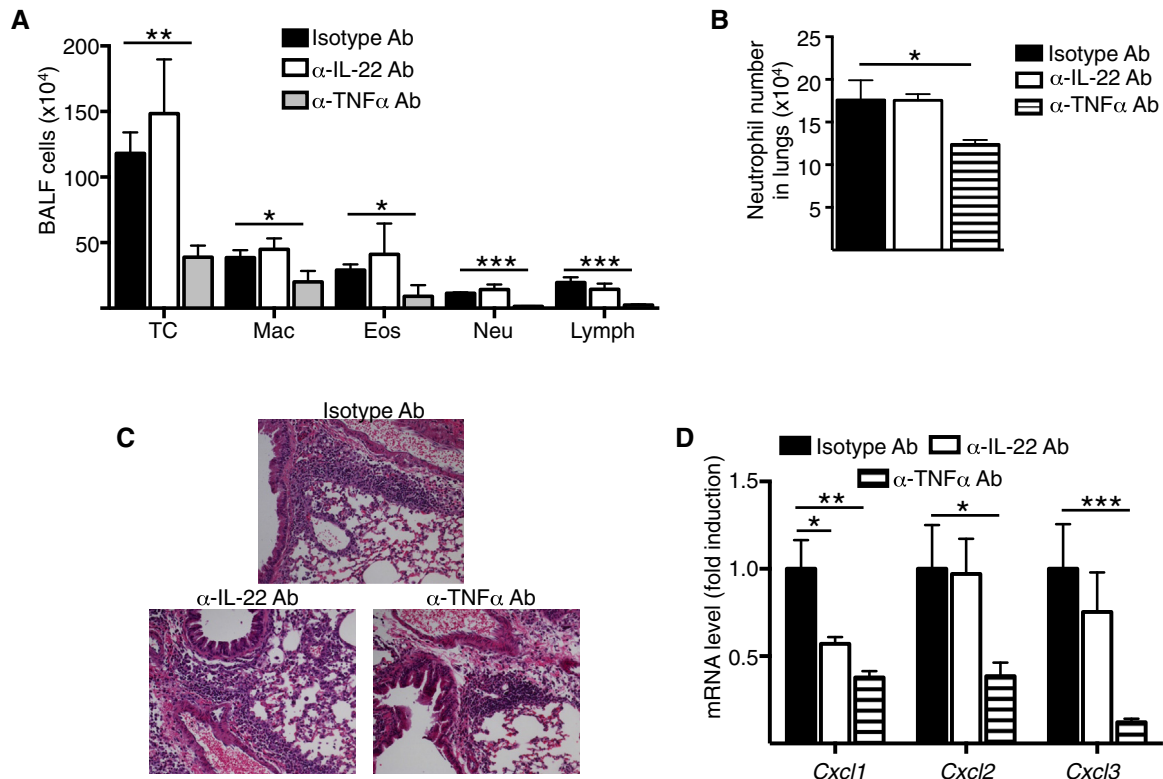


FIG 7. TNF- α , but not IL-22, blockade during the challenge phase inhibits airway inflammation. Total and differential counts in BALF (**A**), numbers of neutrophils in lungs (**B**), hematoxylin and eosin (H&E) staining of lung sections (**C**), and chemokine mRNA levels in the lungs (**D**) in WT mice epicutaneously sensitized with OVA and then subjected to intranasal instillation of OVA together with neutralizing anti-IL-22 IgG antibody, neutralizing anti-TNF- α IgG antibody, or IgG isotype controls are shown. Bars represent means $\pm$ SEMs ($n = 4$ -6 per group). Ab, Antibody; Eos, eosinophils; Lymph, lymphocytes; Mac, macrophages; Neu, neutrophils; ns, not significant; TC, total cells. * $P < .05$, ** $P < .005$, and *** $P < .001$.

Although TGF- β is important for IL-17A induction after epicutaneous sensitization,⁷ it had no detectable role in IL-22 induction in epicutaneously sensitized mice. We also showed that IL-23 is primarily induced in and released by keratinocytes after tape stripping, which is used for epicutaneous sensitization.³⁰ *Il23* mRNA expression and IL-23 production are also induced in normal human skin subjected to mechanical injury by tape stripping.³⁰ Furthermore, *Il23* mRNA expression is increased in the skin of patients with AD.³⁸ Together, these findings suggest that our examination of the role of IL-22 in airway inflammation elicited by antigen inhalation in epicutaneously sensitized mice is relevant to asthma that develops in patients with AD.

Our results demonstrate that IL-22 plays a proinflammatory role in airway inflammation in epicutaneously sensitized mice. *Il22*^{-/-} mice sensitized epicutaneously with OVA exhibited decreased eosinophil and neutrophil influx in the BALF, attenuated lung inflammation, and diminished AHR after intranasal challenge. In line with these results, previous reports have been shown that IL-22 deficiency or blockade by antibody reduces airway inflammation in mice sensitized subcutaneously with OVA¹⁷ or mice treated intratracheally with a high dose of bleomycin.³⁹ In contrast, several reports indicate a protective role of IL-22 in the lungs of intraperitoneally sensitized mice because intraperitoneally sensitized *Il22*^{-/-} mice exhibited enhanced airway inflammation after inhalation challenge and

intranasal instillation of rIL-22. These results suggest that the route of allergen sensitization might determine whether IL-22 plays a pathogenic role or a protective role in airway inflammation elicited by antigen inhalation.

Deficiency of IL-22 increased the expression of *Ifng* and its transcriptional regulator, *Tbx21*, as well as of the chemokine *Ccl5* and its receptor, *Cxcr3*, expressed by T_H1 cells and ILC1s in lungs of mice sensitized epicutaneously with OVA and challenged intranasally with antigen. It also increased, the number of IFN- γ -producing lung ILCs and IFN- γ production by OVA-stimulated lung cells in these mice. These results suggest that IL-22 inhibits local IFN- γ production during antigen-driven airway inflammation. IL-22 production in BALF of asthmatic patients inversely correlates with IFN- γ -regulated proinflammatory mediators.²³ Furthermore, IL-22 blockade⁴⁰ or selective ablation in CD4⁺ T cells of *Ahr*, a transcription factor required for T_H22 polarization,³¹ increases the number of T_H1 cells in a model of collagen-induced arthritis.⁴¹ IFN- γ inhibits airway eosinophilia by counteracting the effects of IL-4 and IL-13.⁴² Importantly, IFN- γ blockade was restored in part in airway eosinophilia in the antigen-challenged lungs of *Il22*^{-/-} mice sensitized epicutaneously with OVA. This suggests that IL-22 promotes airway eosinophilia in our model by inhibiting local production of IFN- γ . Partial restoration of airway eosinophilia in the antigen-challenged airways of *Il22*^{-/-} mice sensitized epicutaneously

by IFN- γ blockade suggests the presence of additional mechanisms by which IL-22 promotes eosinophil recruitment.

CD4⁺ T cells are the major source of IL-22 during airway inflammation.³⁶ We demonstrated that IL-22 was expressed in CD4⁺ T cells but was not detectable in ILCs, CD8⁺ T cells, or TCR $\gamma\delta$ T cells in the lungs of WT mice sensitized epicutaneously with OVA and challenged with antigen. DCs and natural killer cells have been reported as potential sources of IL-22.^{41,42} We cannot exclude these cell types as sources of IL-22 in the lungs in our model of airway inflammation. Importantly, antigen-specific CD4⁺ T cells polarized *in vitro* to T_H22 cells promoted neutrophil-dominated airway inflammation and AHR in WT recipients intranasally challenged with antigen. IL-22 production by adoptively transferred T_H22 cells was essential because adoptive transfer of T_H22-polarized CD4⁺ T cells derived from *Il22*^{-/-} mice did not drive airway inflammation and AHR in WT recipients challenged intranasally with antigen.

Although IL-22 expression by T_H22-polarized CD4⁺ T cells was essential for driving airway inflammation and AHR, IL-22 by itself was not sufficient. As previously reported,^{39,43} intranasal installation of rIL-22 did not result in either neutrophil recruitment to the airways or AHR. The pathologic or protective functions of IL-22 are often mediated by its combined action with other cytokines.^{39,44-48} IL-22 synergized with TNF- α , a cytokine expressed by T_H22 cells, in driving airway inflammation and AHR. Synergy between IL-22 and TNF- α is important for the control of *Chlamydia trachomatis*⁴⁹ and *Candida albicans*⁴⁶ infections and is thought to be important in driving tissue inflammation in patients with scleroderma⁴⁴ and Behçet disease.⁴⁸ In contrast, intranasal installation of IL-22 together with IL-17A, another cytokine expressed by T_H22 cells, had no detectable effect, although IL-22 and IL-17A have been reported to synergize to promote airway inflammation³⁹ and in inducing the production of antimicrobial peptides by keratinocytes.⁴³ Given the normal production of TNF- α and IL-17A by lung cells from airway-challenged and epicutaneously sensitized *Il22*^{-/-} mice, our data suggest that loss of IL-22 synergy with TNF- α contributed to defective recruitment of neutrophils into the airways of *Il22*^{-/-} mice.

Consistent with the results obtained in the subcutaneous immunization model,¹⁷ IL-22 blockade before inhaled antigen challenge had no effect on airway inflammation, whereas TNF- α blockade inhibited it. This suggests that although IL-22 is important in eliciting an immune response that promotes airway inflammation, it is not important in the effector phase of the response. Thus IL-22 blockade could be beneficial during priming of the response to epicutaneous sensitization, whereas TNF- α blockade could be beneficial in protecting from inhaled antigen challenge subsequent to epicutaneous sensitization.

In summary, our results suggest that cutaneous sensitization through mechanically injured skin, as occurs in patients with AD, elicits antigen-specific T_H22 cells that produce IL-22 and TNF- α in response to allergen exposure through the airways and thereby promote eosinophil- and neutrophil-mediated lung inflammation and AHR. Our findings suggest that blockade of IL-22 might be beneficial in prevention of allergic asthma in patients with AD.

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Key messages

- Intranasal antigen challenge of epicutaneously sensitized mice elicits accumulation in the lungs of T_H22 cells that coexpress IL-17A and TNF- α .
- IL-22 is important for airway inflammation and AHR in epicutaneously sensitized mice challenged intranasally with antigen.
- TNF- α and IL-22 produced by T_H22 cells synergize to drive neutrophil-dominated airway inflammation and AHR.

REFERENCES

- Pearce N, Ait-Khaled N, Beasley R, Mallol J, Keil U, Mitchell E, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax* 2007;62:758-66.
- Spergel JM, Paller AS. Atopic dermatitis and the atopic march. *J Allergy Clin Immunol* 2003;112(suppl):S118-27.
- Bieber T. Atopic dermatitis. *N Engl J Med* 2008;358:1483-94.
- Di Cesare A, Di Meglio P, Nestle FO. A role for Th17 cells in the immunopathogenesis of atopic dermatitis? *J Invest Dermatol* 2008;128:2569-71.
- Nograla KE, Zaba LC, Shemer A, Fuentes-Duculan J, Cardinale I, Kikuchi T, et al. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. *J Allergy Clin Immunol* 2009;123:1244-52.e2.
- He R, Kim HY, Yoon J, Oyoshi MK, MacGinnitie A, Goya S, et al. Exaggerated IL-17 response to epicutaneous sensitization mediates airway inflammation in the absence of IL-4 and IL-13. *J Allergy Clin Immunol* 2009;124:761-70.e1.
- He R, Oyoshi MK, Jin H, Geha RS. Epicutaneous antigen exposure induces a Th17 response that drives airway inflammation after inhalation challenge. *Proc Natl Acad Sci U S A* 2007;104:15817-22.
- Leyva-Castillo JM, Hener P, Jiang H, Li M. TSLP produced by keratinocytes promotes allergen sensitization through skin and thereby triggers atopic march in mice. *J Invest Dermatol* 2013;133:154-63.
- Savinko T, Karisola P, Lehtimäki S, Lappeteläinen AM, Haapakoski R, Wolff H, et al. ST2 regulates allergic airway inflammation and T-cell polarization in epicutaneously sensitized mice. *J Invest Dermatol* 2013;133:2522-9.
- Yu J, Oh MH, Park JU, Myers AC, Dong C, Zhu Z, et al. Epicutaneous exposure to staphylococcal superantigen enterotoxin B enhances allergic lung inflammation via an IL-17A dependent mechanism. *PLoS One* 2012;7:e39032.
- Duhen T, Geiger R, Jarrossay D, Lanzavecchia A, Sallusto F. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat Immunol* 2009;10:857-63.
- Kreymborg K, Etzensperger R, Dumoutier L, Haak S, Rebollo A, Buch T, et al. IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. *J Immunol* 2007;179:8098-104.
- Mielke LA, Jones SA, Raverdeau M, Higgs R, Stefanska A, Groom JR, et al. Retinoic acid expression associates with enhanced IL-22 production by gammadelta T cells and innate lymphoid cells and attenuation of intestinal inflammation. *J Exp Med* 2013;210:1117-24.
- Xie MH, Aggarwal S, Ho WH, Foster J, Zhang Z, Stinson J, et al. Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. *J Biol Chem* 2000;275:31335-9.
- Zindl CL, Lai JF, Lee YK, Maynard CL, Harbour SN, Ouyang W, et al. IL-22-producing neutrophils contribute to antimicrobial defense and restitution of colonic epithelial integrity during colitis. *Proc Natl Acad Sci U S A* 2013;110:12768-73.
- Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. *Immunity* 2004;21:241-54.
- Besnard AG, Sabat R, Dumoutier L, Renaud JC, Willart M, Lambrecht B, et al. Dual Role of IL-22 in allergic airway inflammation and its cross-talk with IL-17A. *Am J Respir Crit Care Med* 2011;183:1153-63.
- Zhao Y, Yang J, Gao YD, Guo W. Th17 immunity in patients with allergic asthma. *Int Arch Allergy Immunol* 2010;151:297-307.
- Zhu J, Cao Y, Li K, Wang Z, Zuo P, Xiong W, et al. Increased expression of aryl hydrocarbon receptor and interleukin 22 in patients with allergic asthma. *Asian Pac J Allergy Immunol* 2011;29:266-72.

20. Schnyder B, Lima C, Schnyder-Candrian S. Interleukin-22 is a negative regulator of the allergic response. *Cytokine* 2010;50:220-7.
21. Takahashi K, Hirose K, Kawashima S, Niwa Y, Wakashin H, Iwata A, et al. IL-22 attenuates IL-25 production by lung epithelial cells and inhibits antigen-induced eosinophilic airway inflammation. *J Allergy Clin Immunol* 2011;128:1067-76.e1-6.
22. Taube C, Tertilt C, Gyulveszi G, Dehzad N, Kreymborg K, Schneeweiss K, et al. IL-22 is produced by innate lymphoid cells and limits inflammation in allergic airway disease. *PLoS One* 2011;6:e21799.
23. Pennino D, Bhavsar PK, Effner R, Avitabile S, Venn P, Quaranta M, et al. IL-22 suppresses IFN-gamma-mediated lung inflammation in asthmatic patients. *J Allergy Clin Immunol* 2013;131:562-70.
24. Chang Y, Al-Alwan L, Risse PA, Halayko AJ, Martin JG, Bagloli CJ, et al. Th17-associated cytokines promote human airway smooth muscle cell proliferation. *FASEB J* 2012;26:5152-60.
25. Chang Y, Al-Alwan L, Risse PA, Roussel L, Rousseau S, Halayko AJ, et al. TH17 cytokines induce human airway smooth muscle cell migration. *J Allergy Clin Immunol* 2011;127:1046-53.e1-2.
26. Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, Geha RS. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *J Clin Invest* 1998;101:1614-22.
27. Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, et al. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* 2007;445:648-51.
28. Jin H, Oyoshi MK, Le Y, Bianchi T, Koduru S, Mathias CB, et al. IL-21R is essential for epicutaneous sensitization and allergic skin inflammation in humans and mice. *J Clin Invest* 2009;119:47-60.
29. Ma W, Bryce PJ, Humbles AA, Laouini D, Yalcindag A, Alenius H, et al. CCR3 is essential for skin eosinophilia and airway hyperresponsiveness in a murine model of allergic skin inflammation. *J Clin Invest* 2002;109:621-8.
30. Yoon J, Leyva-Castillo JM, Wang G, Galand C, Oyoshi MK, Kumar L, et al. IL-23 induced in keratinocytes by endogenous TLR4 ligands polarizes dendritic cells to drive IL-22 responses to skin immunization. *J Exp Med* 2016;213:2147-66.
31. Basu R, O'Quinn DB, Silberger DJ, Schoeb TR, Fouser L, Ouyang W, et al. Th22 cells are an important source of IL-22 for host protection against enteropathogenic bacteria. *Immunity* 2012;37:1061-75.
32. Ingram JL, Kraft M. IL-13 in asthma and allergic disease: asthma phenotypes and targeted therapies. *J Allergy Clin Immunol* 2012;130:829-44.
33. Pope SM, Brandt EB, Mishra A, Hogan SP, Zimmermann N, Matthaei KI, et al. IL-13 induces eosinophil recruitment into the lung by an IL-5- and eotaxin-dependent mechanism. *J Allergy Clin Immunol* 2001;108:594-601.
34. Ford JG, Rennick D, Donaldson DD, Venkayya R, McArthur C, Hansell E, et al. IL-13 and IFN-gamma: interactions in lung inflammation. *J Immunol* 2001;167:1769-77.
35. Bernink J, Mjosberg J, Spits H. Th1- and Th2-like subsets of innate lymphoid cells. *Immunol Rev* 2013;252:133-8.
36. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000;100:655-69.
37. Yeste A, Mascanfroni ID, Nadeau M, Burns EJ, Tupah AM, Santiago A, et al. IL-21 induces IL-22 production in CD4+ T cells. *Nat Commun* 2014;5:3753.
38. Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Zaba LC, Cardinale I, Nogales KE, et al. Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. *J Immunol* 2008;181:7420-7.
39. Sonnenberg GF, Nair MG, Kim TJ, Zaph C, Fouser LA, Artis D. Pathological versus protective functions of IL-22 in airway inflammation are regulated by IL-17A. *J Exp Med* 2010;207:1293-305.
40. Justa S, Zhou X, Sarkar S. Endogenous IL-22 plays a dual role in arthritis: regulation of established arthritis via IFN-gamma responses. *PLoS One* 2014;9:e93279.
41. Nakahama T, Kimura A, Nguyen NT, Chinen I, Hanieh H, Nohara K, et al. Aryl hydrocarbon receptor deficiency in T cells suppresses the development of collagen-induced arthritis. *Proc Natl Acad Sci U S A* 2011;108:14222-7.
42. Mitchell C, Provost K, Niu N, Homer R, Cohn L. IFN-gamma acts on the airway epithelium to inhibit local and systemic pathology in allergic airway disease. *J Immunol* 2011;187:3815-20.
43. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006;203:2271-9.
44. Brembilla NC, Dufour AM, Alvarez M, Hugues S, Montanari E, Truchetet ME, et al. IL-22 capacitates dermal fibroblast responses to TNF in scleroderma. *Ann Rheum Dis* 2016;75:1697-705.
45. Eyerich S, Eyerich K, Pennino D, Carbone T, Nasorri F, Pallotta S, et al. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J Clin Invest* 2009;119:3573-85.
46. Eyerich S, Wagener J, Wenzel V, Scarponi C, Pennino D, Albanesi C, et al. IL-22 and TNF-alpha represent a key cytokine combination for epidermal integrity during infection with *Candida albicans*. *Eur J Immunol* 2011;41:1894-901.
47. Liang SC, Long AJ, Bennett F, Whitters MJ, Karim R, Collins M, et al. An IL-17F/A heterodimer protein is produced by mouse Th17 cells and induces airway neutrophil recruitment. *J Immunol* 2007;179:7791-9.
48. Sugita S, Kawazoe Y, Imai A, Kawaguchi T, Horie S, Keino H, et al. Role of IL-22 and TNF-alpha-producing Th22 cells in uveitis patients with Behcet's disease. *J Immunol* 2013;190:5799-808.
49. Zhao X, Zhu D, Ye J, Li X, Wang Z, Zhang L, et al. The potential protective role of the combination of IL-22 and TNF-alpha against genital tract *Chlamydia trachomatis* infection. *Cytokine* 2015;73:66-73.

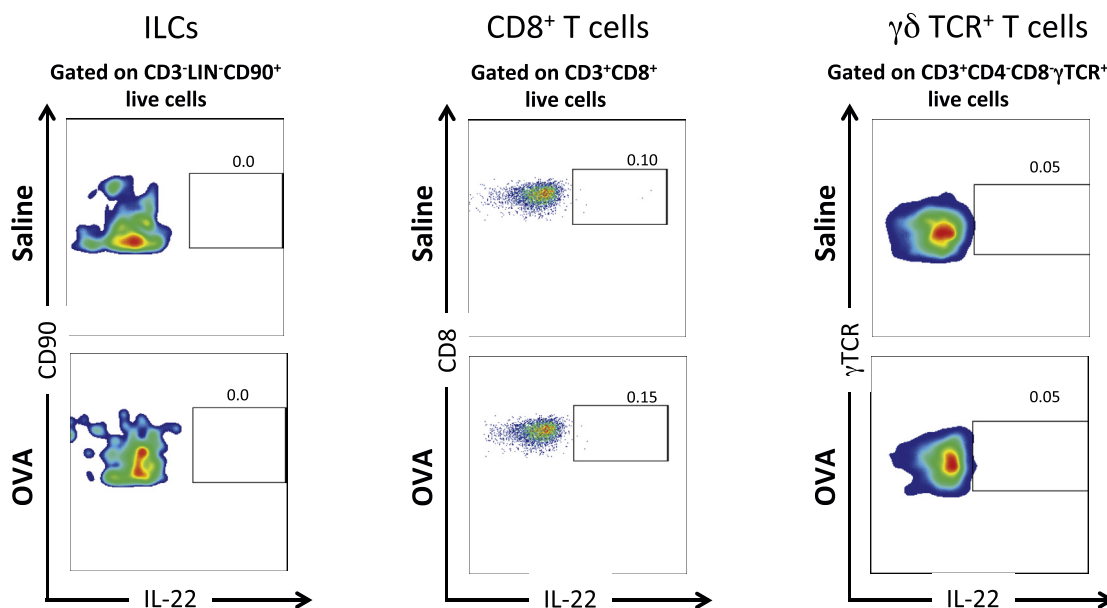


FIG E1. IL-22 is not produced in lung ILCs, CD3⁺CD8⁺ T cells, or CD3⁺TCRγδ⁺ T cells. Representative fluorescence-activated cell sorting analysis of intracellular expression of IL-22⁺ cells among CD3⁺Lin⁻CD90⁺, CD3⁺CD8⁺, and CD3⁺γδTCR⁺ T cells.

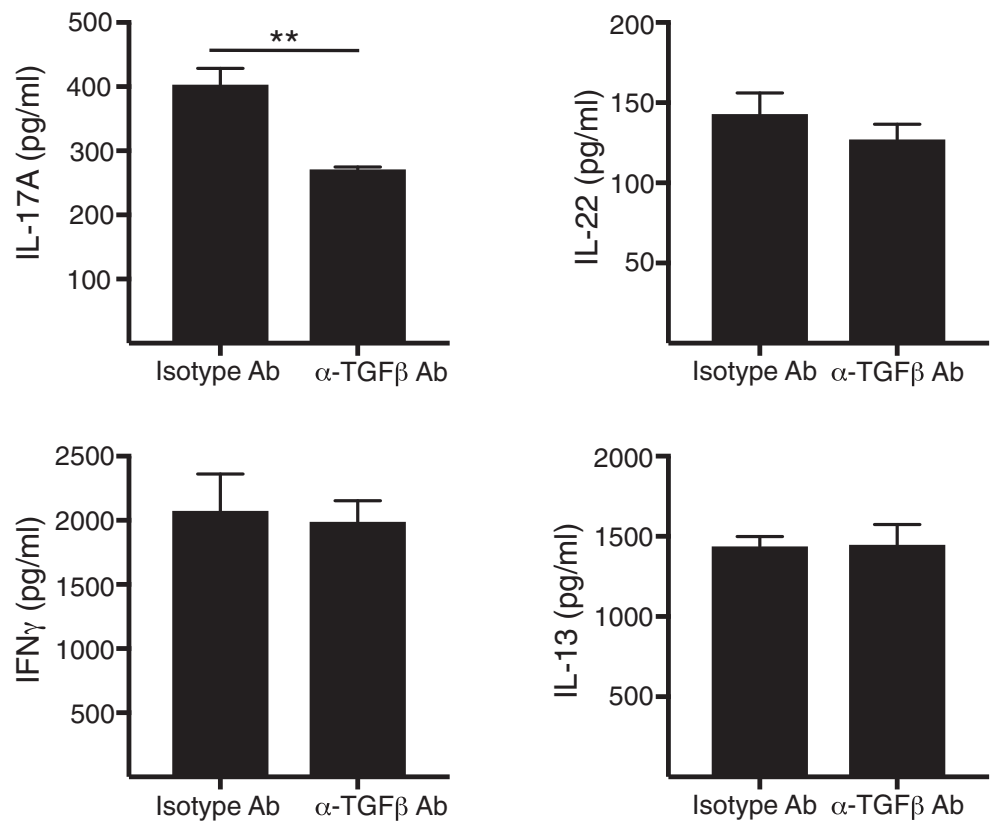


FIG E2. Skin TGF- β is important for the ability of skin DC emigrants to induce the production of IL-17A, but not IL-22, IFN- γ , or IL-13, by naive T cells. Cytokine secretion by naive CD4⁺ DO11.10T cells cultured with DCs isolated from skin draining lymph nodes 24 hours after tape stripping and OVA application in WT mice subjected to intradermal injection of TGF- β -neutralizing antibody or isotype control. No exogenous OVA was added to the cultures. Graphs represent means $\pm$ SEMs (n = 5 per group). Ab, Antibody. **P < .005.

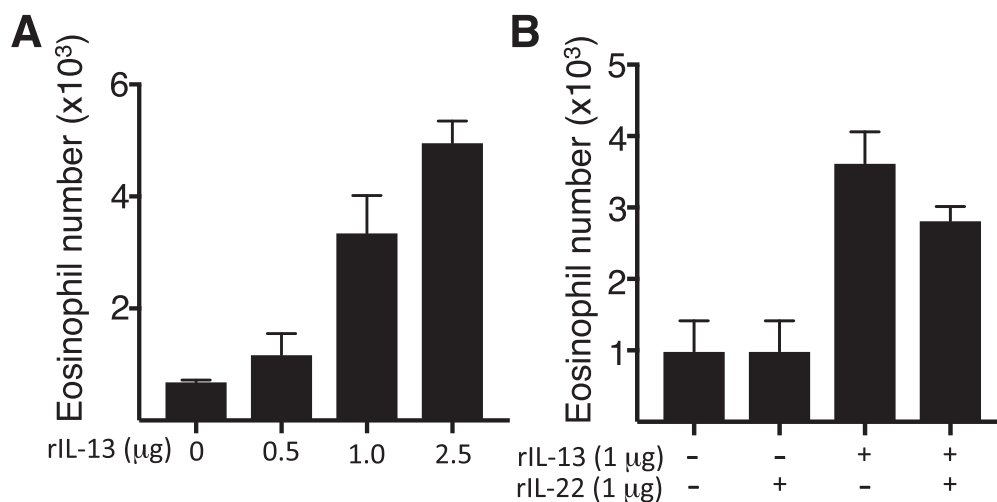


FIG E3. IL-22 does not enhance IL-13-mediated lung eosinophilia. **A**, Dose-response analysis of induction of airway eosinophilia by means of intranasal installation of rIL-13. **B**, Effect of intranasal coadministration of rIL-22 with rIL-13 on airway eosinophilia. Bars represent means $\pm$ SEMs (n = 4-6 per group).

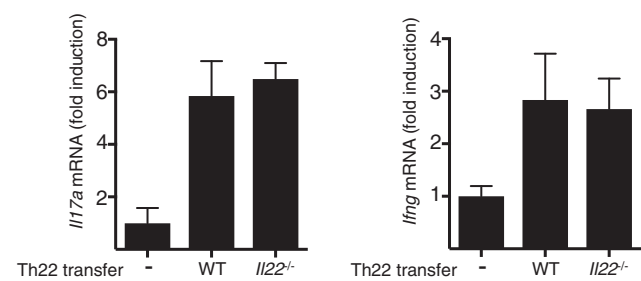


FIG E4. IL-22 deficiency does not affect *Il17a* or *Ifng* mRNA levels after OVA intranasal challenge in mice receiving OVA TCR-specific T_H22 cells. *Il17a* and *Ifng* mRNA levels in the lungs of WT recipients of T_H22-polarized OVA-specific CD4⁺ T cells from DO11.10 and DO11.10/*Il22*^{-/-} mice after intranasal challenge with OVA. Mice that received no T cells were used as controls. Bars represent means ± SEMs (n = 4-6 per group).

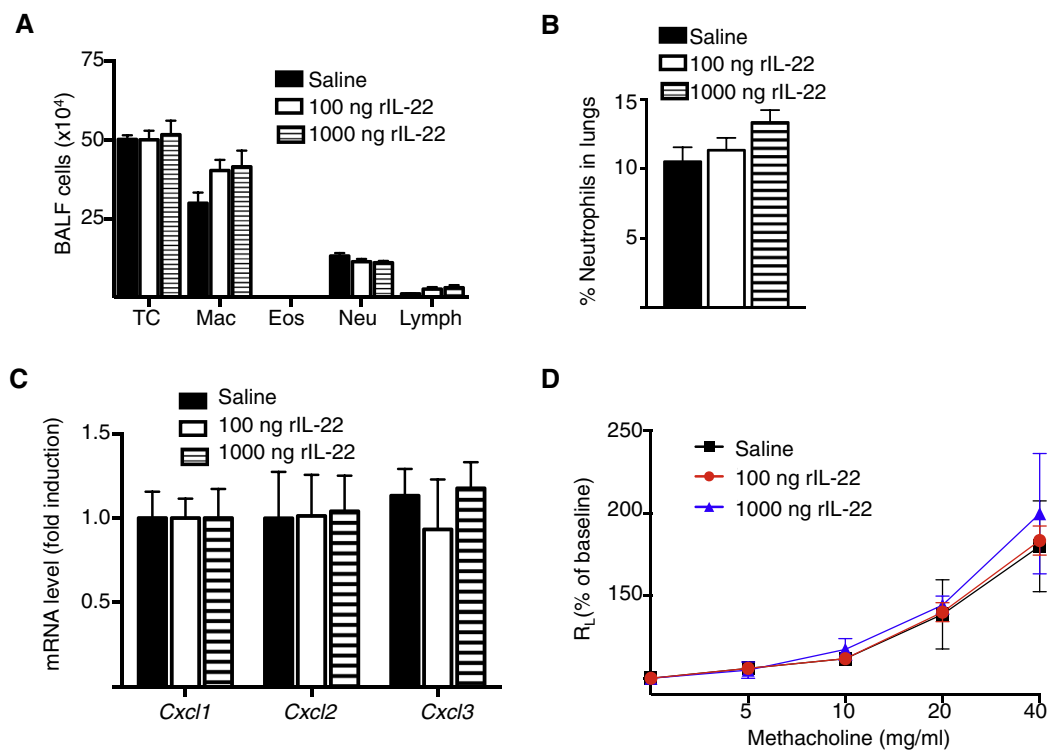


FIG E5. IL-22 is not sufficient to promote neutrophil airway inflammation. Total and differential counts in BALF (**A**), frequency of neutrophils in lungs (**B**), chemokine mRNA levels in lungs (**C**), and lung resistance in response to increasing doses of methacholine (**D**) in WT mice treated intranasally with saline or different rIL-22 doses are shown. Graphs represent means $\pm$ SEMs ($n = 4-6$ per group). *Eos*, Eosinophils; *Lymph*, lymphocytes; *Mac*, macrophages; *Neu*, neutrophils; *TC*, total cells.

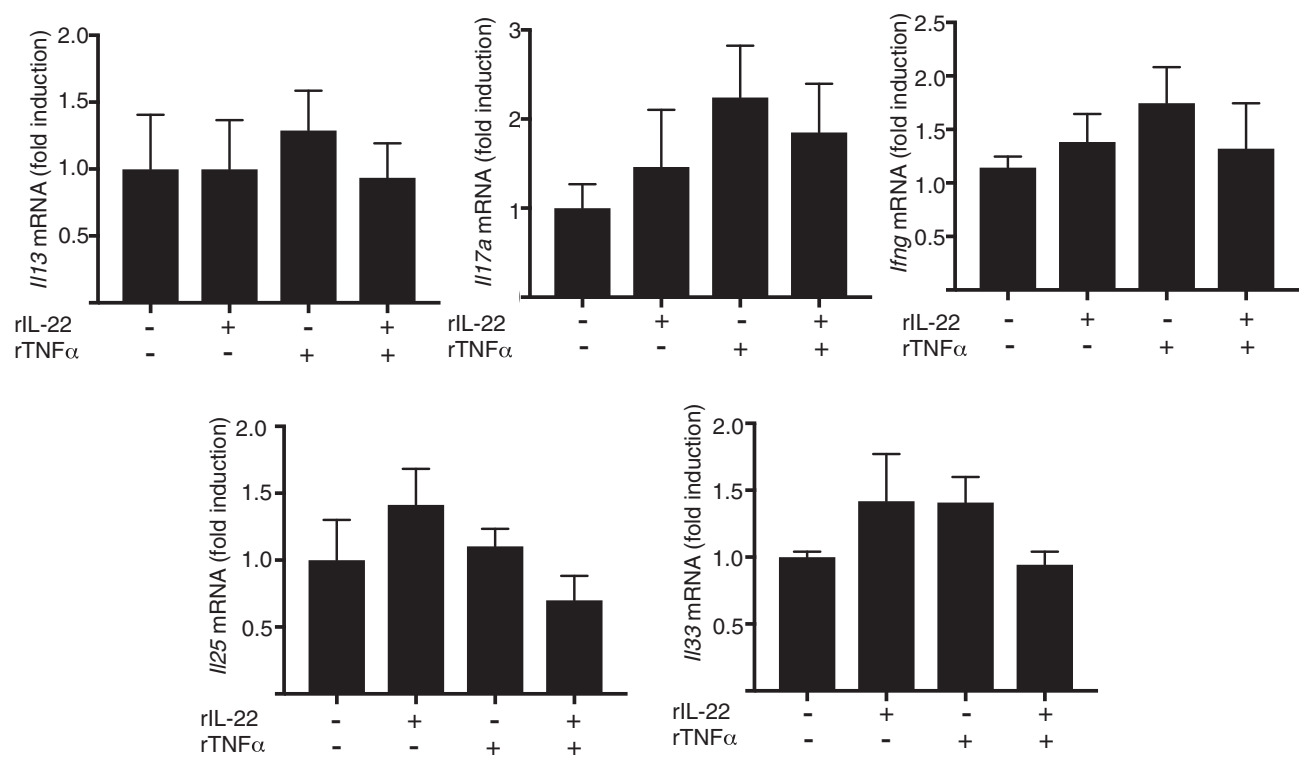


FIG E6. Intranasal instillation of rIL-22, rTNF- α , or its combination does not affect expression of *Il13*, *Il17a*, *Ifng*, *Il25*, or *Il33* in the lungs. *Il13*, *Il17a*, *Ifng*, *Il25*, and *Il33* mRNA levels in lungs of WT mice treated intranasally with saline, rIL-22, rTNF- α , or rIL-22 plus rTNF- α . Bars represent means $\pm$ SEMs (n = 4-6 per group).

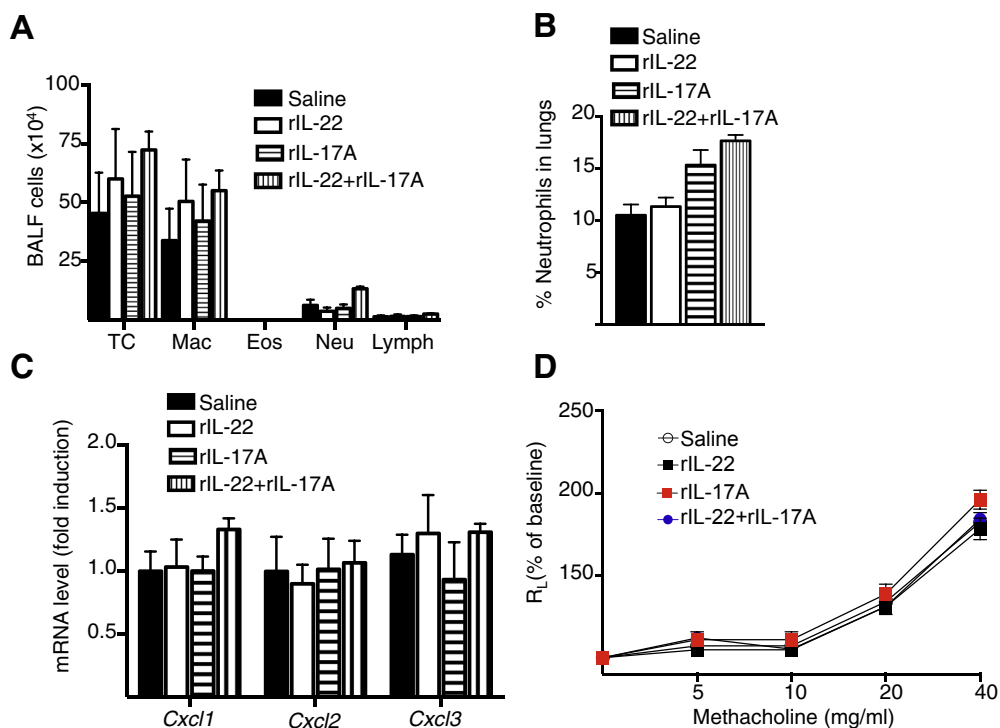


FIG E7. The combination of IL-22 and IL-17A is not sufficient to promote neutrophil airway inflammation and AHR. Total and differential counts in BALF (**A**), frequency of neutrophils in lungs (**B**), chemokine mRNA levels in lungs (**C**), and lung resistance in response to increasing doses of methacholine (**D**) in WT mice treated intranasally with saline, rIL-22, rIL-17A, or rIL-22 plus rIL-17A are shown. Graphs represent means $\pm$ SEMs (n = 4-6 per group).