

Resistin-Like Molecule- α Regulates IL-13-Induced Chemokine Production but Not Allergen-Induced Airway Responses

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Resistin-like molecule α (Relm- α) is one of the most up-regulated gene products in allergen- and parasite-associated Th2 responses. Localized to alternatively activated macrophages, Relm- α was shown to exert an anti-inflammatory effect in parasite-induced Th2 responses, but its role in experimental asthma remains unexplored. Here, we analyzed the cellular source, the IL-4 receptors required to stimulate Relm- α production, and the role of Relm- α after experimental asthma induction by IL-4, IL-13, or multiple experimental regimes, including ovalbumin and *Aspergillus fumigatus* immunization. We demonstrate that Relm- α was secreted into the airway lumen, dependent on both the IL-13 receptor- α 1 chain and likely the Type I IL-4 receptor, and differentially localized to epithelial cells and myeloid cells, depending on the specific cytokine or aeroallergen trigger. Studies performed with *Retnla* gene-targeted mice demonstrate that Relm- α was largely redundant in terms of inducing the infiltration of Th2 cytokines, mucus, and inflammatory cells into the lung. These results mirror the dispensable role that other alternatively activated macrophage products (such as arginase 1) have in allergen-induced experimental asthma and contrast with their role in the setting of parasitic infections. Taken together, our findings demonstrate the distinct utilization of IL-4/IL-13 receptors for the induction of Relm- α in the lungs. The differential regulation of Relm- α expression is likely determined by the relative expression levels of IL-4, IL-13, and their corresponding receptors, which are differentially expressed by divergent cells (i.e., epithelial cells and macrophages.) Finally, we identify a largely redundant functional role for Relm- α in acute experimental models of allergen-associated Th2 immune responses.

Keywords: resistin-like molecule- α ; asthma; IL-4; IL-13R α 1

Asthma is a chronic and complex inflammatory disease of the airways characterized by airflow obstruction, mucus production, airway hyperresponsiveness (AHR), and airway inflammation. It is the most common chronic illness of childhood, affecting up to 20% of children and 7% of adults in Western countries, with a combined prevalence of approximately 300 million people worldwide (1).

Asthmatic responses are associated with increased numbers of pulmonary inflammatory cells, including activated T-lymphocytes and eosinophils, which correlate with disease severity (2, 3). T-lymphocytes of the Th2 phenotype are thought to induce asthma

CLINICAL RELEVANCE

We demonstrate the distinct utilization of IL-4 and IL-13 receptors for the induction of resistin-like molecule- α (Relm- α) in the lungs. We also identify a largely redundant functional role for Relm- α in acute experimental models of allergen-associated Th2 immune responses.

through the secretion of an array of cytokines, and in particular, IL-4 and IL-13 (4, 5). These cytokines are produced at elevated concentrations in allergic tissue, and are central regulators of many hallmark features of the disease, such as the production of IgE, Th2 differentiation, eosinophilia, mucus hypersecretion, chemokine induction, and airway hyperresponsiveness (AHR) (6). Notably, IL-13 is considered more of an effector cytokine in the pathogenesis of allergic airway disease compared with IL-4 because AHR and mucus production are predominantly IL-13-dependent (7, 8).

Inflammation triggered by IL-4 and IL-13 is mediated by the IL-4 and IL-13 receptors expressed by multiple parenchymal cell types (including epithelial cells, smooth muscle, and vascular endothelial cells) and by infiltrative cells such as macrophages, dendritic cells, and eosinophils (9). In bone marrow-derived cells, IL-4 exerts its activities by interacting with a specific cell-surface receptor (R) comprised of IL-4R α and the common γ (γ c) chain (designated Type I IL-4R), which is shared by multiple cytokine receptors. IL-4 can also use the Type II IL-4R, comprised of IL-4R α and the IL-13 receptor- α 1 chain (IL-13R α 1) (9–11), a receptor complex that is also the cognate functional receptor for IL-13 (11). Recent analyses of *Il13ral*^{-/-} mice demonstrated an essential role for this receptor in mediating the effects of IL-13 and allergen in the lungs (12–14).

Resistin-like molecule- α (Relm- α) belongs to a family of resistin-like molecules (Relms) including Relm- α , Relm- β , and Relm- γ that are potent innate immune-modulating molecules (15). Interestingly, Relm- α and Relm- β expression is tightly regulated by IL-13, IL-4, and signal transducer and activator of transcription protein-6 (16–18). Indeed, Relm family members are implicated in Th2-associated mucosal immune responses and fibrotic diseases (15). Relm- α was originally identified in inflammatory zones associated with an experimental allergic airway disease model and was therefore also called Found in Inflammatory Zone 1 (FIZZ1) (19). Although Relm- α has not been identified in the human genome, the expression pattern of human resistin is more similar to Relm- α than to murine resistin (20), suggesting that information obtained about murine Relm- α may relate to human resistin, at least in part. Notably, Relm- α can regulate insulin resistance, particularly in the setting of intestinal inflammation (16, 21). That property may constitute a particularly important molecular link in connecting impaired metabolism (i.e., obesity) with inflammation.

Relm- α is a hallmark signature gene of murine alternatively activated macrophages (22–25). However, we (and others) recently

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showed that intestinal epithelial cells and eosinophils can also express Relm- α (19, 23, 26). The exact function of Relm- α remains unclear, in part because its receptor has not been identified. We demonstrated a major proinflammatory role for Relm- α in dextran sodium sulfate-induced colonic inflammation (21, 27), as well as the eosinophil chemoattractant activity of Relm- α (28). Notably, recent studies using Th2-associated parasite infection murine models identified a surprising anti-inflammatory role for Relm- α because *Retnla*^{-/-} mice display increased fibrosis, granuloma formation, IgE, and mucin gene expression (18, 23), consistent with the inhibition of Th2 cytokine production by Relm- α in some settings (23). Relm family members have key functions in pulmonary fibrosis (17, 28, 29). However, the function of Relm- α in the setting of experimental asthma, where it is one of the foremost induced genes (13, 30), has yet to be elucidated. In addition, the expression pattern (dependence on IL-4/IL-13 receptor components) and the cellular source of Relm- α after the induction of experimental asthma by distinct triggers remain unknown.

Here, we aimed to define the regulation and role of Relm- α in allergic lung responses. We demonstrate that IL-13R α 1 predominantly regulates increased Relm- α expression after *Aspergillus fumigatus* (Asp) allergen challenge, whereas after chicken egg ovalbumin (OVA) challenge, both IL-13R α 1 and probably Type II IL-4R contribute to the regulation of Relm- α expression. These findings were likely explained by the different sources of Relm- α expression and relatively higher IL-13/IL-4 ratio that was observed in the Asp model (14), suggesting that IL-13 directly induced Relm- α in lung epithelial cells and macrophages *in vivo* via the Type II IL-4R. Surprisingly, Relm- α was largely dispensable in the allergen-induced production of Th2 chemokines, cytokines, mucus production, and eosinophilia. Taken together, our results demonstrate that Relm- α is not essential for acute Th2-associated, allergen-driven lung responses, in contrast with its key role in helminth-induced pulmonary inflammation (18, 23).

MATERIALS AND METHODS

Mice

The generation of *Retnla*^{-/-}, *Retnlb*^{-/-}, and *Il13ra1*^{-/-} mice was previously described (13, 27). All experiments involving animals were approved by the Animal Experimentation Ethics Committees of the Cincinnati Children's Hospital Medical Center and Tel-Aviv University. BALB/c or C57BL/6 wild-type mice were obtained from Charles River (Wilmington, MA) or Harlan (Rehovot, Israel), and housed under specific pathogen-free conditions.

Real-Time PCR

Lung RNA samples (0.5–1 μ g) were subjected to reverse transcription analysis with iScript reverse transcriptase (Bio-Rad Laboratories, Hercules, CA), according to the manufacturer's instructions. Murine *Retnla* (forward, CCCTCCACTGTAAACGAAGACTC; reverse, CACACCAGTAGCAGTCATCC) and *Hprt* (forward, CAGACTGAAGAGC TATTGTAATG; reverse, CCAGTGTCAATTATATCTTCCAC) were quantified by means of real-time PCR with the iQ5 instrument or CFX96 and SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA). Results were then normalized to *Hprt* amplified from the same cDNA mix and expressed as fold induction relative to *Hprt* (31).

Cytokine-Induced Airway Inflammation

Three doses (10 μ g/mouse) of IL-13 (Peprotech, Rocky Hill, NJ) were administered intratracheally every other day for 6 days. Mice were killed 48 hours after the final cytokine administration.

Allergen Sensitization and Challenges

Experimental asthma was induced by challenging mice with Asp or by sensitizing the mice to chicken egg OVA, followed by two intranasal challenges as previously described (13, 30, 32). In all experiments, mice were killed 24 to 48 hours after the final intranasal challenge.

Bronchoalveolar Lavage Collection and Analysis

Bronchoalveolar lavage collection was performed, and cytospin preparations were stained and determined, as described elsewhere (13, 32).

ELISA

Bronchoalveolar lavage fluid (BALF) cytokines and chemokines were measured with Duo-Set kits (R&D Systems, Minneapolis, MN). The lower detection limits for CCL11, CCL24, CCL2, CCL17, IL-4, IL-13, and IL-5 were 32.5, 15.6, 15.6, 3.9, 31.2, 6.25, and 31.2 pg/mL, respectively. The protein concentrations of Relm- α were determined by ELISA, using anti-Relm- α antibodies (Peprotech) according to the manufacturer's instructions, as detailed elsewhere (21, 27).

Immunofluorescence

Fixed, frozen sections were treated with 100% acetone and blocked with 3% goat serum in PBS. Slides were incubated with isotype controls (rat IgG1 and rabbit IgG; Vector, Burlingame, CA), anti-Mac-3 (BD Pharmingen, San Jose, CA), anti-MBP (kindly provided by James Lee, Mayo Clinic, Rochester, MN), and/or anti-Relm- α (Peprotech) (overnight at 4°C) followed by goat anti-rabbit Alexa 488 and donkey anti-rat Alexa 594 (Invitrogen, Grand Island, NY) and counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI)/Supermount G solution (Southern Biotech, Birmingham, AL). Images were captured using a Zeiss microscope and Axi-viewer image analysis software (Deutschland; Carl Zeiss Corp., Jena, Germany) (21).

Flow Cytometry

BALF cells were washed and incubated with anti-Gr-1-FITC and anti-F4/80-Alexa fluor 647 (eBioscience, San Diego, CA). At least 10,000 events were acquired using FACSCalibur (BD Biosciences, San Jose, CA) and analyzed with FlowJo version 8.8.6 (Tree Star, Ashland, OR).

Lung Histopathology

Hematoxylin and eosin or periodic acid-Schiff staining were performed and quantified as previously described (13).

Microarray Data Analysis

Whole-lung RNA was extracted and subjected to Affymetrix Gene Chip microarray analysis (Affymetrix, Santa Clara, CA), as previously reported (30).

Statistical Analysis

Data were analyzed by ANOVA followed by the Tukey *post hoc* test, using GraphPad Prism 4 (GraphPad, San Diego, CA). Data are presented as mean $\pm$ SD, and $P < 0.05$ was considered statistically significant.

RESULTS

Regulation of Relm- α Expression by IL-13R α 1

Previous reports demonstrated that Relm- α is highly induced in settings of experimental asthma (33). Given the critical role of the IL-13–IL-13R α 1 pathway in experimental asthma, we aimed to identify allergen-induced genes that were differentially regulated by IL-13R α 1 (13). Consistent with previous reports (13, 19, 29), Relm- α was highly induced after allergen (i.e., OVA) challenge (Figure 1A). Interestingly, although Relm- α was highly induced by the direct administration of IL-13, the expression of Relm- α in OVA-induced experimental asthma was mostly IL-13R α 1-independent. In contrast, the induction of Relm- β after OVA allergen challenge was entirely dependent on IL-13R α 1 (Figure 1B).

Given that IL-4 can also use IL-13R α 1 to elicit some of its effects (13, 34), we examined whether IL-4-induced Relm- α and Relm- β were IL-13R α 1-dependent. Indeed, IL-4 delivery substantially increased the expression of Relm- α and Relm- β in the lungs (Figures 1A and 1B). Similar to our findings with OVA-induced

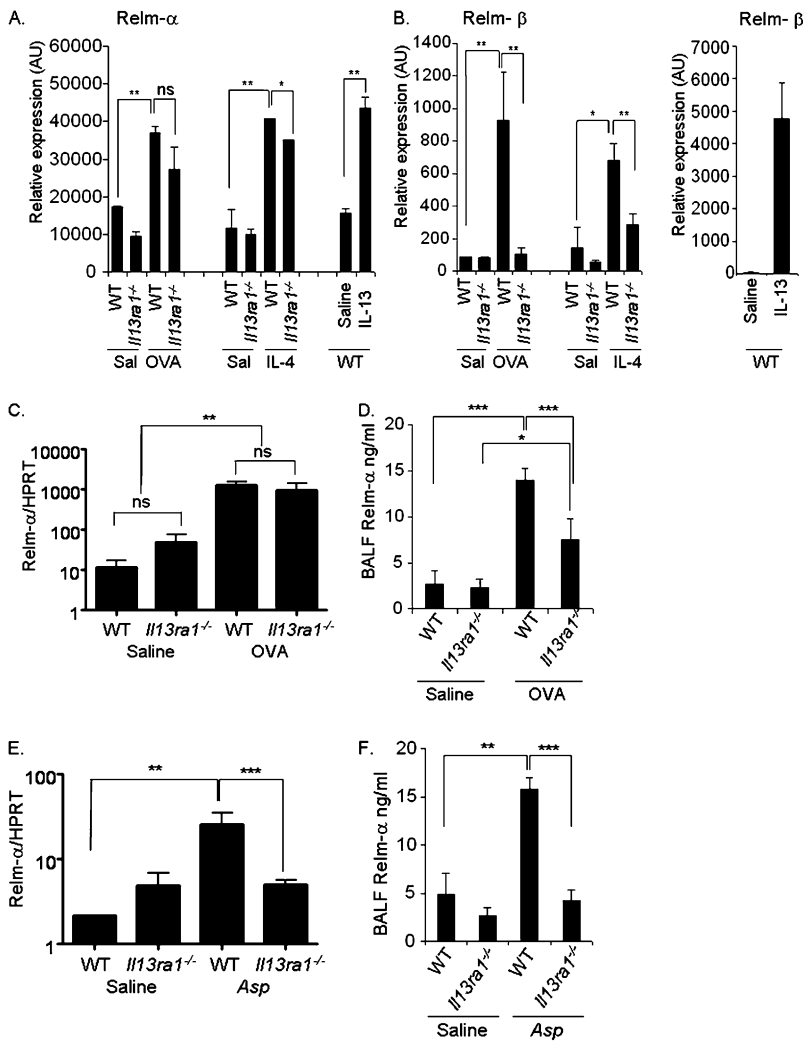


Figure 1. Regulation of resistin-like molecule- α (Relm- α) expression by IL-13 receptor- α 1 chain (IL-13R α 1). Microarray analysis of whole-lung (A) Relm- α and (B) Relm- β expression after chicken egg ovalbumin allergen (OVA), IL-4, or IL-13 treatment of wild-type (WT) and *Il13ra1*^{-/-} mice. Real-time PCR assessment of Relm- α expression was performed after (C) OVA and (E) *Aspergillus fumigatus* (Asp) challenges in WT and *Il13ra1*^{-/-} mice. Assessment of Relm- α protein expression in bronchoalveolar lavage fluid (BALF) was performed after (D) OVA and (F) Asp challenges in WT and *Il13ra1*^{-/-} mice. Data are representative of $n = 3$ (6–8 mice per group). ns, not significant; Sal, saline. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

experimental asthma, the expression of IL-4-induced Relm- α was predominantly independent of IL-13R α 1, whereas Relm- β expression was partly IL-13R α 1-dependent (Figures 1A and 1B).

The expression and dependence of Relm- α on IL-13R α 1 was also assessed by real-time PCR and ELISA. After OVA challenge, the increased mRNA expression of Relm- α was predominantly independent of IL-13R α 1 (Figure 1C), and the expression of Relm- α protein was partly IL-13R α 1-dependent (Figure 1D). To define whether the IL-13R α 1-independent regulation of Relm- α expression was allergen-specific, wild-type and *Il13ra1*^{-/-} mice were subjected to experimental asthma, using a naturally occurring aeroallergen (Asp). Asp-challenged wild-type mice displayed a significant up-regulation in Relm- α expression, whereas Asp-challenged *Il13ra1*^{-/-} mice showed no increase in Relm- α expression (Figure 1E). Notably, the IL-13R α 1-dependent expression of Relm- α was also observed at the protein level, as Asp-challenged *Il13ra1*^{-/-} mice displayed no increase in expression of Relm- α protein (Figure 1F). Collectively, these results indicate that Relm- α expression is largely IL-13R α 1-independent after OVA challenge (and IL-4 delivery), but it is IL-13R α 1-dependent after Asp challenge. These results likely reflect the predominant utilization of Type I and Type II IL-4R by IL-4 and IL-13, respectively, for the induction of Relm- α . Further, our results identify a differential regulation of Relm- α compared with Relm- β because Relm- β remains critically dependent on IL-13R α 1 (e.g., the Type II IL-4 receptor), even after OVA challenge.

Assessment of Relm- α Cellular Expression after IL-13 and Allergen Challenge

Recent data demonstrate the expression of Relm- α in additional cells besides alternatively activated macrophages, including epithelial cells and eosinophils (18, 21, 23, 27). Thus, we aimed to define the cellular source of Relm- α expression after IL-13 or allergen challenge. Under normal conditions, Relm- α protein is expressed in epithelial cells, but not in alveolar macrophages or cells in the lung parenchyma (Figures 2A and 2B). After the administration of IL-13, Relm- α expression was highly up-regulated in lung epithelial cells and was often detected in the airway lumen (Figures 2D and 2F). In addition, Relm- α was also expressed in alveolar macrophages (Figure 2E). Similarly, after allergen challenge, Relm- α was up-regulated in airway epithelial cells and macrophages (Figures 2G–2L), but not in infiltrating eosinophils, although those eosinophils displayed substantial mRNA concentrations of Relm- α (Figures 2G–2L, and data not shown). Importantly, the attempt to stain lung tissue with an isotype control produced no staining (Figure 2C).

The analysis of Relm- α expression in allergen-challenged *Il13ra1*^{-/-} mice revealed that allergen-induced Relm- α expression in epithelial cells was entirely dependent on IL-13R α 1, because airway epithelial cells in allergen-challenged *Il13ra1*^{-/-} mice showed expression similar to that of airway epithelial cells in saline-treated mice (Figures 3A, 3B, 3D, and 3E; magnification, $\times 100$).

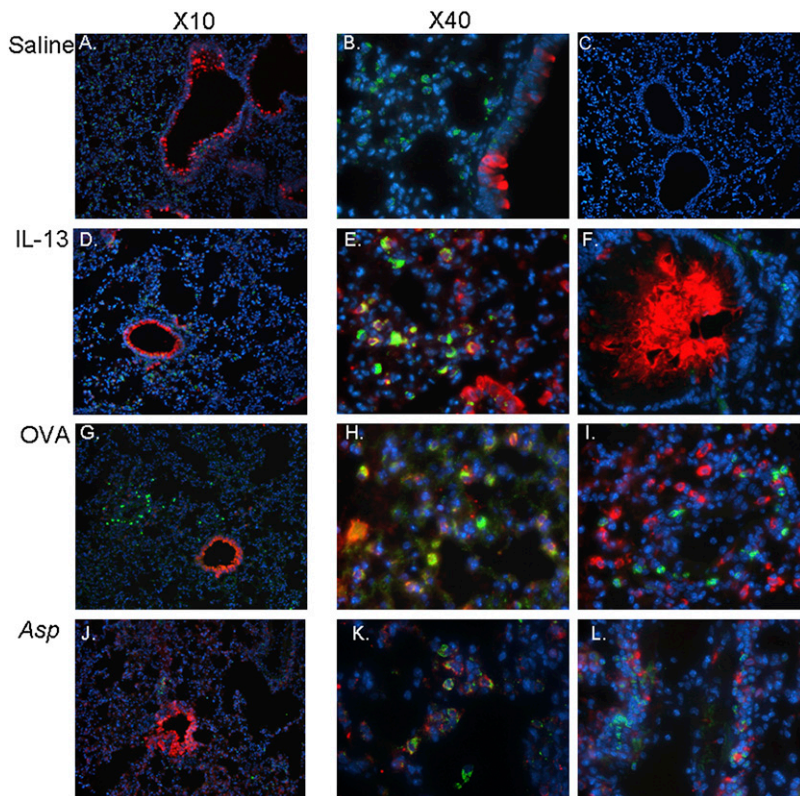


Figure 2. Relm- α cellular expression after allergen challenge. Assessment of Relm- α cellular expression in a model of IL-13- and allergen-induced airway inflammation. Forty-eight hours after the final administration of saline (A, B), IL-13 (D–F), OVA (G–I), or *Aspergillus fumigatus* (Asp) (J–L) allergen challenge, the mice were killed, and Relm- α (red) expression was assessed in Mac-1-positive cells (green; B, E, H, and K) or eosinophil major basic protein-positive cells (green; I and L), using immunofluorescent analysis. Antibody control-stained slides are shown in C. Data are representative photomicrographs of $n = 3$ (6–8 mice per group).

Nonetheless, after OVA challenge, Relm- α expression in alveolar macrophages was IL-13R α 1-independent (Figure 3C; magnification, $\times 400$), whereas its expression in macrophages after Asp challenge was IL-13R α 1-dependent (Figure 3F; magnification, $\times 400$).

Roles of Relm- α in IL-13-Induced Lung Inflammation

Our findings defined a marked induction of Relm- α by IL-13 as well as by two distinct allergens (OVA and Asp), raising the question of the role of Relm- α in Th2-associated lung responses. We aimed to define the role of Relm- α in IL-13-induced lung pathology (9, 35). In response to IL-13 challenge, *Retnla*^{−/−} mice displayed elevated concentrations of CCL24, CCL2, and CCL11 (Figures 4B and 4C, and data not shown), decreased CCL17, and a trend toward decreased CCL22 expression (Figures 4A and 4D) compared with IL-13-challenged wild-type (WT) mice. Given the key role of chemokines in the recruitment of inflammatory cells to

the lung (36), we then assessed the role of Relm- α in inflammatory cell recruitment. No difference was evident in inflammatory cell recruitment in *Retnla*^{−/−} mice in response to IL-13 (Figure 4E). Similarly, increased mucus production in response to IL-13 was similar in cytokine-challenged wild-type and *Retnla*^{−/−} mice (data not shown).

For comparison, we examined the role of Relm- β in IL-13-induced lung inflammation. Indeed, the regulation of IL-13-induced CCL17 and CCL24 was similar in *Retnla*^{−/−} and *Retnlb*^{−/−} mice, as both gene-targeted mice displayed decreased and augmented concentrations of CCL17 and CCL24, respectively, in response to IL-13 challenge (Figures 5A and 5B). Interestingly, Relm- β (and to a lesser extent, Relm- α) enhanced IL-13-induced CCL22, as IL-13-induced CCL22 expression was significantly reduced in IL-13-challenged *Retnlb*^{−/−} mice in comparison with IL-13-challenged *Retnla*^{−/−} and WT mice (Figure 5C). In contrast, the production of CCL2 was significantly increased in IL-13-challenged

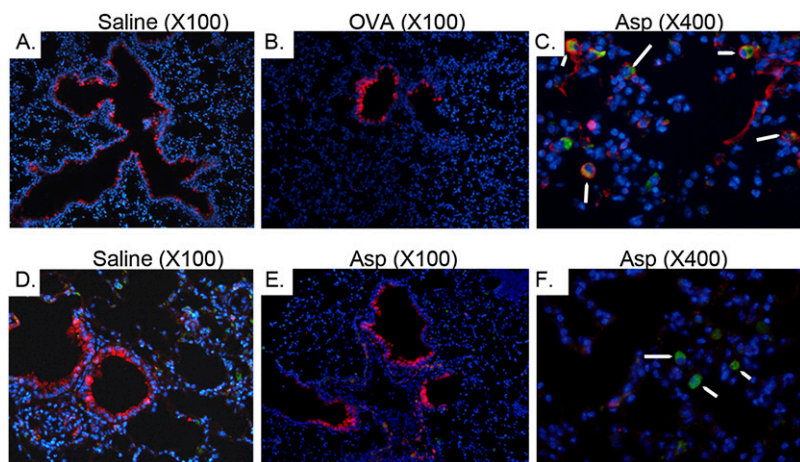


Figure 3. Assessment of Relm- α cellular source in allergen-challenged *Il13ra1*^{−/−} mice. Frozen lung sections obtained from allergen-challenged *Il13ra1*^{−/−} mice were stained for Relm- α (red) and Mac-1 (green) expression after OVA (A–C) or *Aspergillus fumigatus* (Asp) challenges (D–F) and analyzed by immunofluorescence. Arrows indicate Mac-1⁺ cells in the lung parenchyma, which are either positive (C) or negative (F) for Relm- α expression. Data are representative photomicrographs of $n = 3$ (4–6 mice per group). Numbers in parentheses indicate extent of magnification.

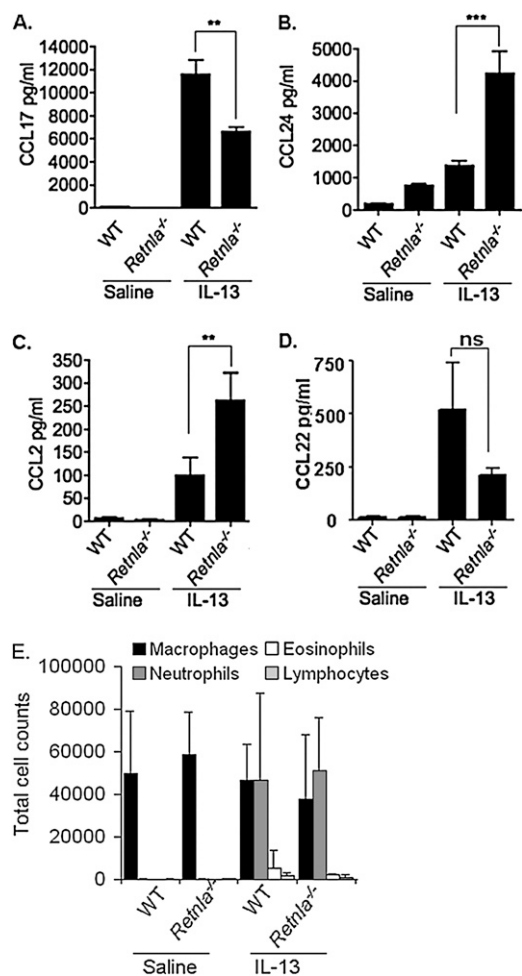


Figure 4. Effects of Relm- α on IL-13-induced lung responses. Relm- α -mediated responses were assessed in a model of IL-13-induced airway inflammation. Forty-eight hours after the final IL-13 challenge, mice were assessed for BALF chemokine expression (A–D) and BALF cellular infiltration (E). Data are representative of three experiments (6–8 mice per experimental group). ns, not significant; WT, wild-type. ** $P < 0.01$. *** $P < 0.001$.

Retnlb^{-/-} mice compared with IL-13-challenged *Retnlb*^{-/-} mice (Figure 5D). An assessment of IL-13-induced cellular recruitment into the lungs revealed that IL-13-challenged *Retnlb*^{-/-} mice displayed a significant decrease in such neutrophil and eosinophil recruitment (Figure 5E). These data were confirmed by flow cytometric analysis using the F4/80 and Gr-1 markers, inasmuch as F4/80⁺/Gr-1^{high} cells (i.e., neutrophils) and F4/80^{dim}/Gr-1^{dim} cells were significantly reduced in the BALF of IL-13-challenged *Retnlb*^{-/-} but not IL-13-challenged *Retnlb*^{-/-} and WT mice (Figures 5F and 5G).

Role of Relm- α in OVA-Induced Experimental Asthma

OVA-challenged WT mice displayed significantly increased CCL11, CCL24, CCL2, and CCL17 concentrations. Interestingly, OVA-challenged *Retnlb*^{-/-} mice displayed significantly reduced CCL17 concentrations but comparable concentrations of CCL11, CCL22, and CCL24 in comparison with OVA-challenged WT mice (Figures 6A–6D). Consistent with these data, *Retnlb*^{-/-} mice displayed similar inflammatory cellular recruitment into the BALF as did WT mice (Figure 6E).

An assessment of the major Th2 cytokines in the BALF of OVA-challenged mice indicated that *Retnlb*^{-/-} mice displayed similar concentrations of IL-4, IL-13, and IL-5 as WT mice

(Figures 6F–6H). Moreover, OVA-challenged WT and *Retnlb*^{-/-} mice showed similar concentrations of IL-10 (Figure 6I).

Finally, an assessment of the OVA-induced epithelial-cell pathology that is associated with asthma showed that OVA-challenged *Retnlb*^{-/-} mice displayed similar levels of goblet cell hyperplasia and mucus production as did WT mice (Figures 6J and 6K).

Role of Relm- α in Asp-Induced Experimental Asthma

We hypothesized that the lack of activity for Relm- α in the “classic” OVA/alum experimental asthma model may be attributable to the effects of artificial adjuvant sensitization, which may override many features of natural mucosal sensitization (especially those driven by IL-13, which is more prominent in the Asp experimental asthma model). Thus, *Retnlb*^{-/-} mice were subjected to an additional experimental asthma model using Asp. However, intranasal Asp delivery to *Retnlb*^{-/-} mice resulted in no significant alterations in the Asp-induced production of CCL17 and CCL22 (Figures 7A–7B). Similarly, concentrations of CCL11 and CCL24 were found to be similar in Asp-challenged *Retnlb*^{-/-} mice (Figures 7C and 7D). The recruitment of eosinophils into the BALF was only modestly but statistically significantly reduced (35% $\pm$ 8.2%) in *Retnlb*^{-/-} mice (Figures 7E and 7F). The assessment of BALF and the lung Th2 cytokine profile demonstrated no change in IL-4 and IL-13 concentrations (Figures 7G and 7H). In addition, Asp-challenged *Retnlb*^{-/-} mice revealed similar levels of goblet cell hyperplasia and mucus production compared with WT mice (data not shown).

DISCUSSION

The Relm family of proteins was originally identified in the lung and gastrointestinal tract and is strongly linked with the induction of Th2 immune responses and mucosal immunity, involving asthma, helminthic parasites, and inflammatory bowel disease (15–17, 26, 37–39). Relm- α is a hallmark signature gene of murine alternatively activated macrophages (25). However, epithelial cells, eosinophils, and adipose tissue may also express Relm- α (16, 27, 33, 37). Surprisingly, despite intensive research into Relm- α , its role in models of asthma remains unknown.

In this study, we demonstrate several key and unexpected results regarding the regulation and roles of Relm- α , using acute models of experimental asthma. First, we demonstrate the differential regulation of Relm- α by IL-13R α 1, and likely the Type I IL-4R, depending on the experimental asthma model. In particular, we demonstrate that IL-13R α 1 critically regulates Relm- α expression after Asp challenge. However, after OVA + alum-induced experimental asthma, Relm- α expression is regulated by both IL-13R α 1 and most likely the Type I IL-4R. Second, we demonstrate that baseline Relm- α expression is restricted to airway epithelial cells, whereas after the induction of experimental asthma, airway epithelial cells and macrophages express Relm- α , consistent with the presence of alternatively activated macrophages only after the induction of Th2-associated lung disease. Third, we demonstrate that Relm- α partly regulates IL-13-induced lung chemokine production. In particular, Relm- α -deficient mice display elevated concentrations of CCL24, CCL2, and CCL11 and decreased concentrations of CCL17 and CCL22. Notably, IL-13-challenged *Retnlb*^{-/-} mice displayed significantly more CCL2 and less CCL22 than IL-13-challenged *Retnlb*^{-/-} mice. Finally, using two models of experimental asthma, we demonstrate that Relm- α does not have a marked role in the overall Th2 response in the lung, as assessed by the production of lung Th2 cytokine, chemokine, cellular recruitment, and mucus production.

Our results demonstrate that IL-13R α 1 differentially regulates Relm- α expression. After OVA + alum sensitization

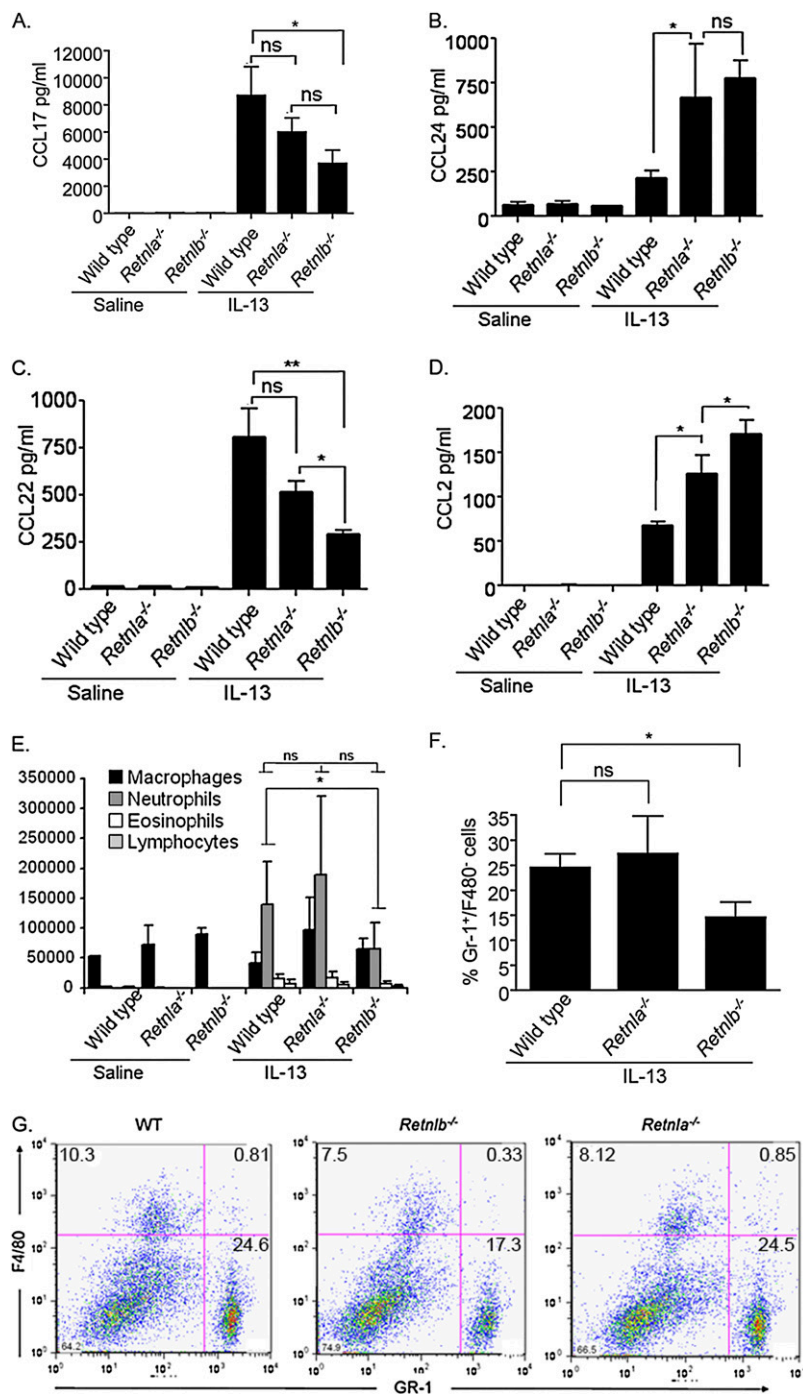


Figure 5. Effects of Relm- β on IL-13-induced lung responses. Relm- α -mediated and Relm- β -mediated responses were assessed in a model of IL-13-induced airway inflammation. Forty-eight hours after the final IL-13 challenge, mice were assessed for BALF chemokine expression (A–D), BALF cellular infiltration by differential cell counts (E), and cytometric analysis using anti-Gr-1 and F4/80 staining (F and G). Data are representative of three experiments (6–8 mice per experimental group). Flow cytometric dot plots are representative of $n = 7$ mice per group. ns, not significant. * $P < 0.05$. ** $P < 0.01$.

and consequent intranasal OVA challenge, Relm- α expression is predominantly IL-13R α 1-independent, whereas after mucosal sensitization and challenge (using Asp extract), the expression of Relm- α was IL-13R α 1-dependent. Whereas airway epithelial cells predominantly express Type II IL-4R, which mediates IL-4 and IL-13 signaling, infiltrating hematopoietic cells predominantly express Type I IL-4R (40). Indeed, we demonstrate that after allergen challenge, Relm- α is expressed by both airway epithelial cells and macrophages. Therefore, the differential regulation and expression of Relm- α are likely driven by the marked differences between the OVA + alum and Asp models and may result from differential IL-4 versus IL-13 production (14), as the ratio between IL-13 and IL-4 is substantially higher after Asp inoculation than after OVA inoculation (13, 14). Despite our finding that lung

eosinophils express Relm- α mRNA and that gastrointestinal eosinophils express Relm- α protein, murine eosinophils did not express Relm- α protein after IL-13 challenge or allergen challenge. These results may indicate different roles for eosinophils or eosinophil-derived Relm- α in innate-immune gastrointestinal inflammatory settings compared with allergic airway inflammation.

Given the strong association between IL-13 and Relm- α induction and the ability of IL-13 to induce Relm- α directly, we hypothesized that Relm- α would regulate IL-13-induced lung responses. In particular, IL-13-treated *Retnla*^{-/-} mice displayed altered chemokine induction. CCL17 and CCL22 were decreased in *Retnla*^{-/-} mice, indicating a role for Relm- α in the induction of these chemokines. CCL17 and CCL22 are mainly implicated in the recruitment of Th2 T-cells (41). Although we

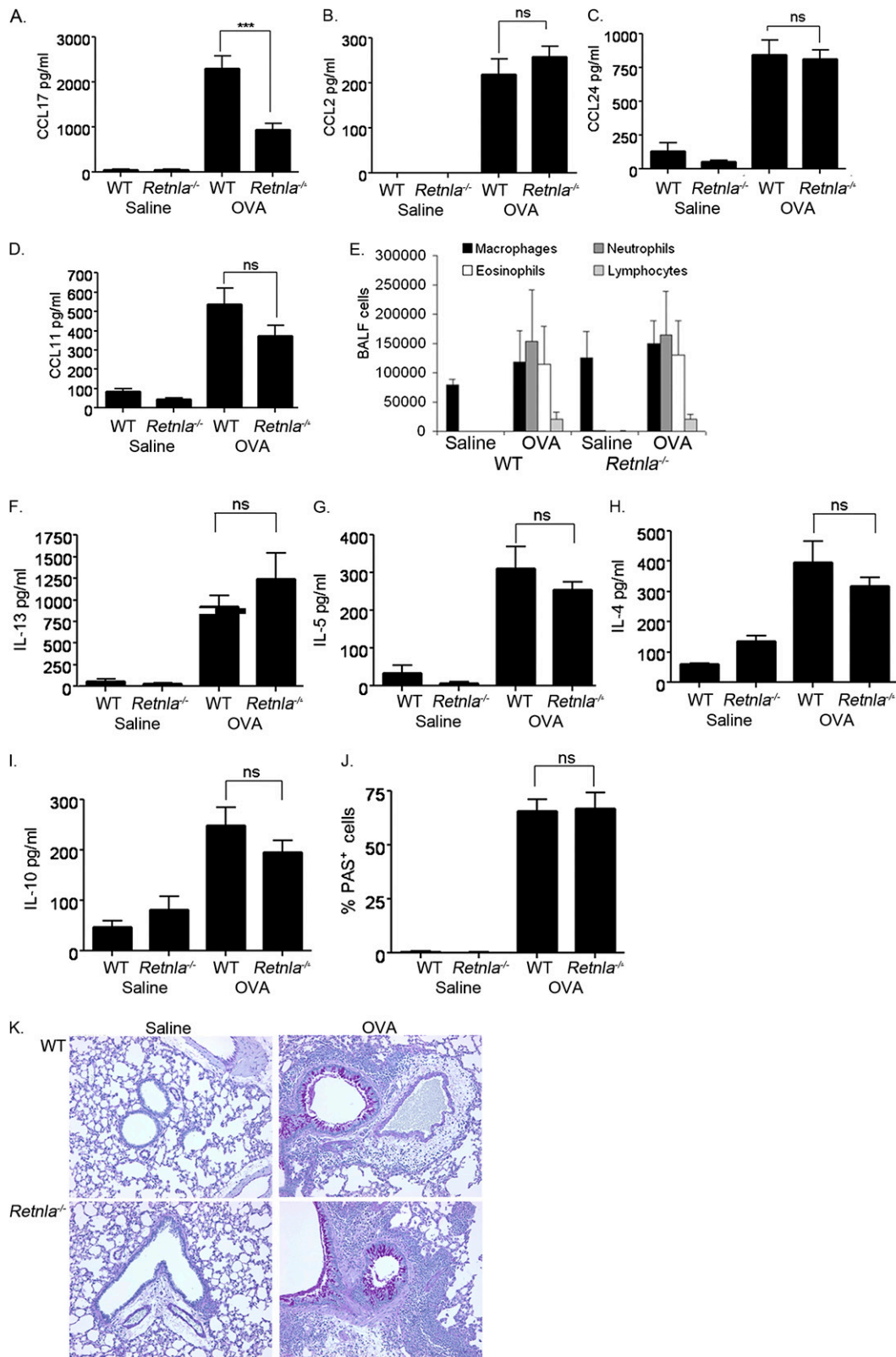


Figure 6. Effects of Relm- α on OVA-induced allergic airway inflammation. Twenty-four hours after the final allergen challenge, mice were examined for BALF chemokine production (A–D) as well as cellular infiltration (E), cytokine production (F–I), and mucus production (J and K). Data are representative of one of three experiments (6–17 mice per experimental group). ns, not significant. *** $p < 0.001$.

could not detect any alterations in IL-13-induced T-cell recruitment into the lung, in different settings, Relm- α may be able to modulate T-cell responses by governing their chemotactic signals. Moreover, CCL24 (a hallmark eosinophil chemokine) (42, 43) and CCL2 (which recruits monocytes and dendritic cells) (44) were increased in IL-13-challenged *Retnla*^{-/-} mice, indicating a suppressive role for Relm- α . Collectively, these data

suggest that the effect of Relm- α was predominantly attributable to the effects of Relm- α on airway structural cells (such as epithelial cells), as these are responsive to IL-13 in the induction of chemokine production (40). Furthermore, given the structural similarities between Relm- α and Relm- β (15, 39), we were interested in determining whether Relm-regulated IL-13-induced lung chemokine production was Relm- α -specific. The regulatory effects of

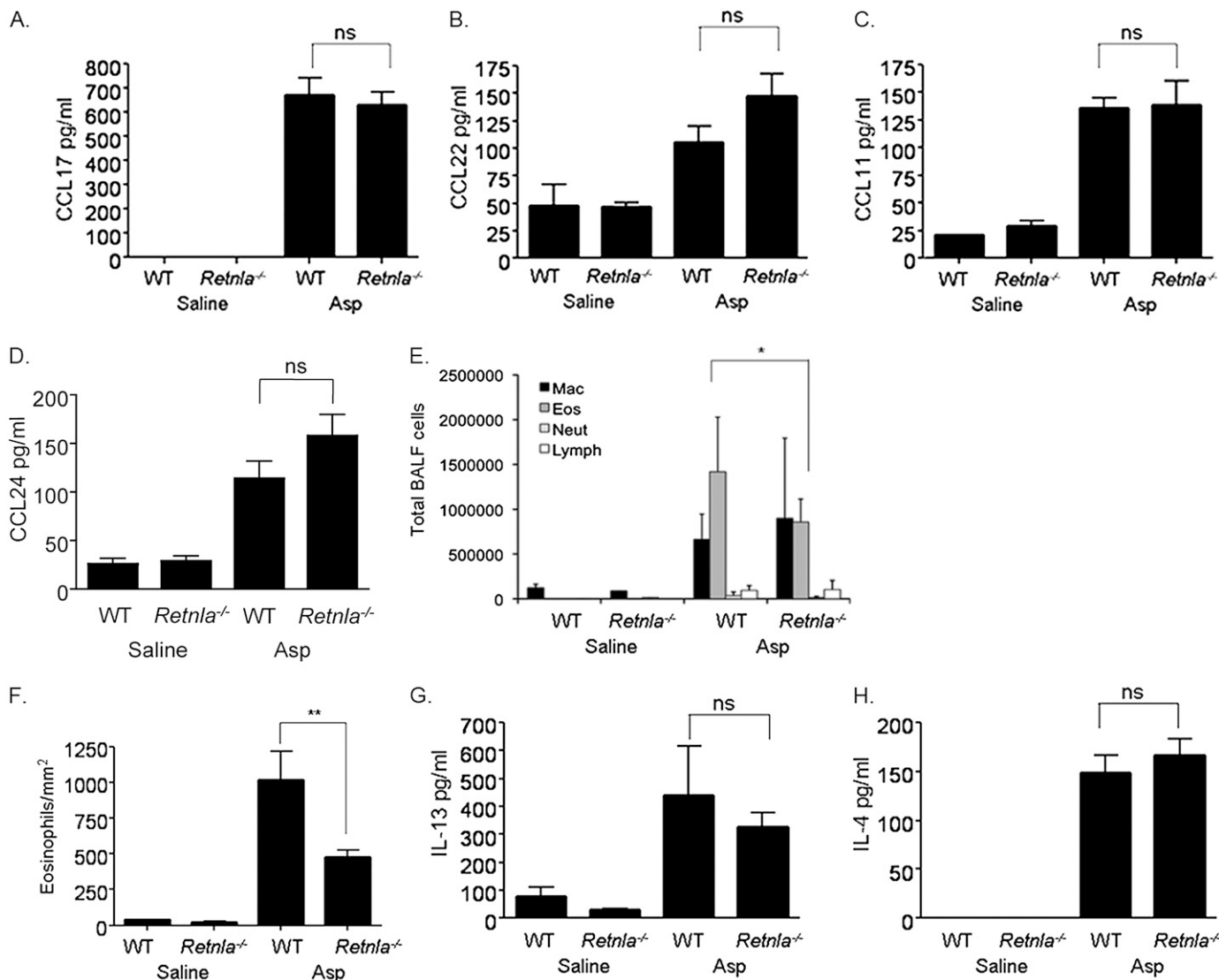


Figure 7. The effects of Relm- α on *Aspergillus fumigatus* (Asp)-induced allergic airway inflammation. Forty-eight hours after the final allergen challenge, mice were examined for BALF chemokine production (A–D) and BALF cells (E). (F) In addition, paraffin-embedded, allergen-challenged lungs were analyzed for eosinophilic infiltration, using anti-eosinophil major basic protein immunohistochemistry. (G and H) BALF samples were analyzed for IL-13 and IL-4 expression. Data are representative of one of three experiments (6–12 mice per experimental group). ns, not significant. * $P < 0.05$. ** $P < 0.01$.

Relm- α and Relm- β on IL-13-induced chemokine production were similar, although Relm- β was more potent at increasing CCL24 and CCL2 and decreasing CCL17 and CCL22. Although the receptors for Relm- α and Relm- β remain unknown, the different potencies of Relm- α and Relm- β in regard to IL-13-induced chemokine production may partly attributable to their respective receptor expression or the induction of intracellular signaling.

Recent studies indicate a key role for Relm- α in helminth-induced Th2 responses (Table 1) (18). Indeed, *Nippostrongylus brasiliensis*-infected *Retnla*^{-/-} mice display significantly increased Th2 cytokine production (including IL-4, IL-5, and IL-13) 7 days after infection. Furthermore, *Schistosoma mansoni*-infected *Retnla*^{-/-} mice displayed increased lung pathology (increased size of egg-induced granulomas, and elevated fibrosis), which was associated with elevated Th2 cytokines and IgE production (23). In fact, macrophage-derived Relm- α was shown to negatively regulate Th2 cytokine production from anti-CD3/anti-CD28-stimulated splenocytes. Collectively, these data suggest that Relm- α would also be a negative regulator of allergen-induced allergic airway inflammation. Nevertheless, in response

to allergen challenge, using two distinct experimental asthma models, *Retnla*^{-/-} mice displayed similar Th2 cytokine production compared with WT mice. Furthermore, mucus production and chemokine induction were similar in allergen-exposed *Retnla*^{-/-} and WT mice.

Interestingly, after exposure to Asp, *Retnla*^{-/-} mice displayed a minor (but statistically significant) decrease in lung eosinophilia. Similar to Relm- α , arginase I is another hallmark gene of alternatively activated macrophages and is induced after allergen challenge and helminth infection. Although the cationic amino-acid transporter-2 and arginase I were shown to play key roles in response to helminth infection (45, 46), arginase I is not required for allergen-induced inflammation, AHR, or collagen deposition (47). Furthermore (and similar to our data demonstrating a role for Relm- α only in IL-13-induced responses but not allergen challenge), RNA interference targeting arginase 1 abrogated the development of IL-13-induced AHR (48), but not allergen-induced AHR (47).

Collectively, these data suggest that the classic and major products of alternatively activated macrophages (Relm- α and arginase I)

TABLE 1. COMPREHENSIVE SUMMARY COMPARING EFFECTS REGULATED BY RELM- α AND ARGINASE 1 IN EXPERIMENTAL ASTHMA AND HELMINTH INFECTIONS

Property	Relm- α		Arginase 1	
	Asthma	Parasites	Asthma	Parasites
Expression	Up-regulated: Epi Cells, Mac, and Eos (RNA only)	Up-regulated: Epi Cells, Mac, and Eos (protein + RNA)	Up-regulated: bone marrow-derived cells	Up-regulated: bone marrow-derived cells
Urea concentrations	NA	NA	No	Yes
Antibody production	No	Yes	No	Yes
Eosinophilia	Model-dependent	Yes	No	Unknown
AHR	No	Unknown	Model-dependent (47, 48)	Unknown
Fibrosis	No	Yes	No	Yes
Granuloma	NR	Yes	NR	Yes
Mucus	No	Yes	No	Yes
Th1 cytokines	No	Unknown	Unknown	Unknown
Th2 cytokines	No	Inhibitory	No	Dual activating (60) and inhibitory (46)
Chemokines	No	Unknown	Unknown	Unknown

Definition of abbreviations: AHR, airway hyperresponsiveness; Eos, eosinophils; Epi, epithelial; Mac, macrophages; NA, not assessed; NR, not relevant; Relm- α , resistin-like molecule- α .

play key roles in helminth-induced immune responses, but limited roles in allergen-induced airway allergic inflammation (Table 1). This effect could be attributable to the lack of chronicity of allergen or antigen exposure. Although allergen exposure is rather limited, the exposure to helminth antigens driving the Th2 response may be more chronic. Because our experimental regimes were mainly conducted in models that mimic acute allergic airway inflammation, we cannot exclude the possibility that Relm- α may play a more important role after chronic exposure to allergens. In addition, the role of Relm- α may be dependent on its cellular source and its synergy with other secreted molecules that may be present in the inflammatory milieu, which may differ in allergic and helminth infection. For example, we recently established a proinflammatory and synergistic role for Relm- α in LPS-induced macrophage activation (27). In the presence of LPS, Relm- α induced macrophage IL-6 and TNF- α secretion, while decreasing IL-10 secretion (27). Thus, the role of Relm- α may be dependent on its synergism with LPS or other pattern recognition-dependent pathways, which may largely vary between allergic settings and parasitic infections. For example, recent studies highlighted key roles for low doses of LPS and Toll-like receptor 4 in allergenicity. In contrast to allergens, which require narrow (but necessary) innate immune activation (49, 50), helminth infections may activate numerous innate immune pathways, including various pattern-recognition receptors (e.g., Toll-like receptors, C type lectin receptors, protease-activated receptors, and nod-like receptors) (51). In fact, parasites can activate multiple innate immune components via chitin, proteases, lectins, and secretory components (e.g., lacto-*N*-fucopentaose III, schistosome-derived lysophosphatidylserine, and phosphorylcholine-rich glycoprotein), whereas the repertoire of innate immune activation by allergens is more limited (49–58). It would be interesting to determine whether any of the aforementioned components up-regulate Relm- α expression and whether Relm- α regulates LPS-induced effects in the lungs. Moreover, given the proposed role of Relm- α in innate immune responses and in helminth-induced Th2 responses (23, 26), to assess its function in infection-associated asthma would be intriguing. For example, rats infected with localized pulmonary cryptococcal infection display increased IL-13 expression and consequent disease pathology (including AHR and mucus production) (60). Thus, Relm- α may play a more significant role in disease settings that involve increased IL-13 responsiveness.

In conclusion, we demonstrate that Relm- α is secreted into the airway lumen and is differentially dependent on IL-13R α 1 and Type I IL-4 receptors after OVA and Asp challenges, respectively. The differing cellular sources of Relm- α after exposure to these

two allergens are likely explained by the differential IL-4R requirement and the relative induction of IL-4 and IL-13 in response to distinct allergens (14). Indeed, increased IL-13 concentrations will promote a predominant Type II IL-4R-dependent response pathway because IL-13-induced Relm- α expression mainly occurs in epithelial cells. Functionally, using Relm- α gene-targeted mice, we showed that Relm- α was largely redundant in terms of inducing Th2 cytokines, mucus, and inflammatory cell infiltration into the lung. These results mirror the dispensable role that other alternatively activated macrophage products (such as arginase 1) play in acute models of allergen-induced experimental asthma and contrast with their role in the setting of parasitic infections. These data suggest a divergence between allergen-induced responses and helminth-induced Th2-type immunity, based on our collective data concerning Relm- α .

Author disclosures are available with the text of this article at www.atsjournals.org.

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