Resistin-like molecule-β is an allergen-induced cytokine with inflammatory and remodeling activity in the murine lung

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Submitted 16 April 2007; accepted in final form 27 May 2007

Mishra A, Wang M, Schlotman J, Nikolaidis NM, DeBrosse CW, Karow ML, Rothenberg ME. Resistin-like molecule-β is an allergen-induced cytokine with inflammatory and remodeling activity in the murine lung. Am J Physiol Lung Cell Mol Physiol 293: L305–L313, 2007. First published June 1, 2007; doi:10.1152/ajplung.00147.2007.—Resistin-like molecule (RELM)-β is a cysteine-rich cytokine implicated in insulin resistance and asthmatic responses, but its function remains an enigma. We now report that RELM-β has a role in promoting airway inflammation and lung remodeling in the mouse lung. RELM-β is strongly induced by diverse allergens and T helper type 2 (Th2) cytokines by an IL-13- and STAT6-dependent mechanism. To understand the in vivo role of RELM-β, we delivered recombinant murine RELM-β intratracheally to naïve mice. RELM-β induced dose-dependent leukocyte accumulation (most prominently involving macrophages) and goblet cell hyperplasia. The most prominent effect induced by RELM-β was increased perivascular and peribronchial collagen deposition. Mice genetically deficient in RELM-β had reduced accumulation of collagen and goblet cell hyperplasia in an experimental model of allergic airway inflammation. In vitro experiments demonstrated that RELM-β had fibroblast mitogenic activity. These results identify RELM-β as a Th2-associated cytokine with potent inflammatory and remodeling activity.

lymphocytes; asthma; fibrosis

As we enter the new millennium, the health of the Western world is threatened by immune-based diseases that are increasing in prevalence (11, 22). Experimentation in the asthma field has largely focused on analysis of the cellular and molecular events induced by allergen exposure in sensitized animals (primarily mice) and humans. These studies have identified elevated production of IgE, mucus hypersecretion, airway obstruction, eosinophilic inflammation, and enhanced bronchial reactivity to spasms in the asthmatic response (10–12, 19, 28, 43). Clinical and experimental investigations have demonstrated a strong correlation between the presence of CD4+ T helper 2 lymphocytes (Th2 cells) and disease severity, suggesting an integral role for these cells in the pathophysiology of asthma (20, 43). Th2 cells are thought to induce asthma through the secretion of an array of cytokines that activate inflammatory and resident effector pathways (42, 51). In particular, IL-4 and IL-13 are produced at elevated levels in the asthmatic lung and appear to be central regulators of many of the hallmark features of disease (8, 50, 52). They share a common receptor subunit, the IL-4 receptor α (IL-4Rα), and signaling through signal transducer and activator of transcription (STAT)6 (23, 26). Mice with the targeted deletion of IL-4, IL-13, or STAT6 develop attenuation of certain features of asthma, including inflammatory cell infiltrates and airway hyperresponsiveness (1, 24, 25, 34, 46, 49), highlighting the critical role for Th2 pathways in allergic disorders.

More recently, attention has focused on the pathogenesis of airway remodeling in the setting of chronic airway inflammation. In particular, it has been appreciated that the asthmatic airway is characterized by thickening of the reticular basement membrane, collagen deposition, and mucus metaplasia. Notably, clinical studies have supported an essential role for these processes in the clinical manifestations of disease. Recently, a critical role for mesenchymal cell signaling involving accumulation and activation of fibroblasts, induced by Th2 cytokines, has been appreciated, but the exact cytokines and mechanisms involved remain largely unknown (30, 32).

Although these studies have provided the rationale for the development of multiple therapeutic agents that interfere with specific inflammatory pathways (3, 9), the development of the asthma phenotype is likely to be related to the complex interplay of a large number of additional genes and their polymorphic variants (3, 9). Aiming to elucidate novel pathways involved in the pathogenesis of allergic asthma, we took an empiric approach using DNA microarray analysis of whole lung RNA (55). This led to the identification of 291 genes that were commonly involved in disease pathogenesis, rather than unique to a particular allergen or mode of disease induction. These “asthma signature” genes provide a valuable opportunity to define new pathways involved in the pathogenesis of allergic airway inflammation. This manuscript focuses on one such gene that encodes for a member of the resistin family of cytokines.

The resistin family of cytokines consists of several ~12.5-kDa conserved proteins with 10 or 11 cysteine residues that promote the formation of oligomeric molecular species (2, 39). The first family member, resistin, also called adipocyte-secreted factor or found in inflammatory zone 3 (FIZZ3), is a novel hormone secreted by adipocytes and has been proposed to link obesity with insulin resistance and type II diabetes (5). Resistin-like molecule (RELM)-α was originally found in inflammatory zones in a murine model of experimental asthma and subsequently designated FIZZ1 (41). The RELM family of cytokines was subsequently shown to be expressed in a number of tissues such as heart, lung, tongue, intestine, and adipose tissue (4, 29, 48). Recently, RELM-γ has been shown to be

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expressed at the highest levels in hematopoietic tissues (17). Based on the relatively stronger data emerging linking RELMs with inflammatory processes and our identification of RELM-β as a member of the asthma signature genome and an important cytokine involved in innate mucosal responses (21), we aimed to investigate the regulation and role of RELM-β in allergic airway inflammation. In this manuscript, we demonstrate that RELM-β is strongly induced in the lung by diverse allergens and Th2 cytokines (IL-4 and IL-13) by an IL-13- and STAT6-dependent mechanism. Furthermore, RELM-β induced dose-dependent leukocyte accumulation (mostly macrophages) and goblet cell hyperplasia when delivered to the lungs of naive mice. Mice genetically deficient in RELM-β had reduced allergen-induced collagen deposition and mucus cell metaplasia in a model of allergic lung disease. Notably, a prominent effect induced by RELM-β was collagen deposition. Furthermore, in vitro experiments demonstrated that RELM-β had fibroblast motogenic activity. These results identify RELM-β as a Th2-associated cytokine with inflammatory and remodeling activity.

**MATERIALS AND METHODS**

**Mice.** BALB/c mice were obtained from the National Cancer Institute (Frederick, MD) and housed under specific pathogen-free conditions. Mice deficient in STAT6 in the BALB/c background were obtained from Jackson Laboratories (Bar Harbor, ME). IL-13-deficient mice and mice deficient in both IL-4 and IL-13 were kindly provided by Dr. Andrew MacKenzie (35). All experiments were performed on age- and gender-matched mice, which were maintained in a pathogen-free facility, and animals were handled according to institutional animal care and use committee (IACUC) and National Institutes of Health guidelines. The studies reported were approved by the IACUC.

**Experimental asthma induction.** Mice were sensitized by intraperitoneal (ip) injection with 100 μg of ovalbumin (OVA) and 1 mg of aluminum hydroxide (alum) in saline on days 0 and 14. On days 24 and 27, mice were lightly anesthetized with inhaled isoflurane and challenged intranasally with 50 μg of OVA in 50 μl of saline or in saline and mice were killed 18–20 h following challenge (55). In other experiments, mice were challenged with nine doses of intranasal *Aspergillus fumigatus* antigen over the course of 3 wk (38). The allergen challenge was performed by applying 100 μg of *A. fumigatus* in 50 μl of saline or saline to the nares using a micropipette with the mouse held in a supine position. After instillation, the mice were held upright until alert. Mice were killed 18–20 h following allergen challenge.

**Intratracheal IL-4, IL-13, and RELM-β delivery.** Mice (22–25 g) were anesthetized by ip injection of 500 μg of Ketajet (Ketamine HCl; Phoenix Pharmaceutical, St. Joseph, MO). Anesthetized mice were hung upright at a 60 degree angle on a vertical platform. With the use of a flat forceps, the tongue was gently extended, and a long-loading pipette tip was directly inserted into the trachea of anesthetized mice, followed by the delivery of 20 μl of recombinant murine IL-4 in conjunction with monoclonal antibody (10 μg) directed against IL-4 (reagents kindly provided by Dr. Fred Finkelman, Univ. of Cincinnati); this allows for the half-life of IL-4 to be increased from several minutes to ~24 h. Dosing was every other day for six doses. Recombinant murine IL-13 (4 μg in 20 μl of 0.9% saline [a generous gift from Dr. Debra Donaldson, Genetics Institute, Cambridge, MA]) was administered via intratracheal delivery in ketamine-anesthetized mice for five consecutive days. Recombinant murine RELM-β (10 μg in 20 μl of 0.9% saline [a generous gift from Peprotech, Rocky Hill, NJ]) was administered via intratracheal delivery in anesthetized mice on alternate days over a 2-wk period (37).

**Northern blot analysis.** RNA was extracted from the saline and allergen-challenged lungs of mice by TRIzol reagent (GIBCO-BRL, Grand-Island, NY) following the manufacturer’s protocol. Twenty micrograms of total RNA was used for Northern blot analysis, as previously described (36).

**Real-time PCR analysis.** The RNA samples (500 ng) were subjected to reverse transcription analysis using Biorscript reverse transcriptase (Bio-Rad, Hercules, CA) according to the manufacturer’s instructions. RELM-β was quantified by real-time PCR using the LightCycler instrument and LightCycler FastStart DNA master SYBR green I as a ready-to-use reaction mix (Roche, Indianapolis, IN). Results were then normalized to GAPDH amplified from the same cDNA mix and expressed as fold induction compared with the controls. cDNA were amplified using the following primers:

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**Fig. 1.** Expression of resistin-like molecule (RELM)-β during induction of experimental asthma. The experimental asthma was induced by *Aspergillus* and ovalbumin (OVA) allergen challenge in mice following the protocols outlined in A and B. Northern blot analysis of RELM-β mRNA expression after 9 repeated intranasal (i.n.) allergen challenges with *Aspergillus fumigatus* (C) or following 2 OVA challenges in OVA-alum-sensitized mice (D). RNA was obtained 18–20 h after the last allergen challenge. The ethidium bromide (EtBr)-stained gels are shown. Each lane represents a separate animal. The quantitative real-time PCR analyses of RELM-β mRNA expression in the *Aspergillus* (E, left) and OVA (E, right) models are shown. The data are expressed as means ± SD (n = 2, 4–5 mice in each group per experiment).
mRELM-β (80 bp): gctctcctctctctctctctctctct and aacacagttaagctcagtct; GAPDH (400 bp): ttggaatccataatcgt and gctcgggagccaggat.

In situ hybridization. Paraffin-embedded saline or OVA-challenged mice lung sections were deparaffinized and submerged in 30% sucrose, and in situ hybridization was performed with radiolabeled [α-35SthioUTP] sense and antisense probes as described earlier (55).

In brief, murine RELM-β cDNA in plasmid pT7T3 (American Type Culture Collection; ATCC) was linearized by EcoRI or NolI digestion, and antisense and sense RNA probes, respectively, were generated by T3 and T7 RNA polymerase (Riboprobe Gemini Core System II transcription kit; Promega, Madison, WI). The radiolabeled probes were hybridized and washed under high-stringency conditions.

Bronchoalveolar lavage fluid collection and analysis. The mice were euthanized by CO2 inhalation. Immediately thereafter, a midline neck incision was made, and the trachea was cannulated. The lungs were lavaged, and differentiated leukocyte counts were determined as previously described (38).

Goblet cell analysis. Lung tissue samples were fixed with 4% paraformaldehyde, embedded in paraffin, and cut into 5-μm sections, and periodic acid-Schiff reaction staining (Poly Scientific R&D, Bay Shore, NY) was then performed on the tissue sections according to the manufacturer’s recommendations, as previously reported (40). The lung sections were taken from the same location in each set of mice, and at least four to five random sections/mouse were analyzed. With the use of light microscopy, tissue regions associated with the entire bronchial region in the lung were quantified for percent of total mucus-producing cells relative to total number of epithelial cells.

Analysis of lung collagen. Lung tissue samples from the control and experimental mice were taken, and total collagen content was quantified in 0.5 M acetic acid homogenate by the Sircol reagent (Biocolor) using the manufacturer’s protocol. Total lung protein was determined by BCA reagents (Pierce, Rockford, IL) following the manufacturer’s protocol to express microgram of collagen per milligram of tissue protein.

Cell lines. The NIH/3T3 cell line (ATCC, Rockville, MD) was grown in DMEM (GIBCO-BRL) supplemented with 10% FCS, 50 U/ml penicillin G, and 50 μg/ml streptomycin sulfate (penicillin-streptomycin, GIBCO-BRL). Primary normal human lung fibroblasts were procured from Cambrex Bioscience (Walkersville, MD) and grown in fibroblast basal medium (Clonetics-BioWhittaker, Walkersville, MD) at 37°C and 5% CO2-95% air. Fibroblast basal media was supplemented with 2% fetal bovine serum, human fibroblast growth...
factor-β (1 μg/ml), insulin (5 mg/ml), gentamicin, and amphoteri-
cin B.

Cell migration assay. Transwell units (24 wells) of 5-μm porosity polycarbonate filters (Corning, Corning, NY) coated with 1% gelatin were used for monitoring in vitro cell migration assay. 3T3 cells (5 × 10⁵ cells/well) were placed in the upper chamber, and different concentrations (10 nM to 500 nM) of RELM-β or transforming growth factor (TGF)-β were added to the upper and/or lower chamber. The transwell unit was kept for 2 h at 37°C in a humidified 95% air-5% CO₂ atmosphere. After 2 h, trypsin (Clonetics, Nashville, TN) was added to the upper chamber to release adherent cells below the chamber. The media from the lower chamber was then centrifuged 250 g, and cells were resuspended in 0.1 ml of PBS. The number of migrated cells in the lower chamber was counted with a hemocytometer. TGF-β (40 nM) was used as a positive control. Each assay was set up in duplicate and repeated at least four times.

Generation of RELM-β gene-targeted mice. RELM-β-deficient mice were designed and developed by VelociGene Technology, as previously described (21, 33). All experiments were performed on 6- to 8-wk-old RELM-β gene-deficient and background-matched wild-type mice.

RESULTS

Identification of RELM-β as an allergen-induced gene in experimental asthma. We previously reported that RELM-β mRNA expression was significantly increased in two distinct models of experimental asthma, induced by OVA or A. fumigatus based on global quantitative microarray analysis of the lung (55). We subsequently determined by Northern blot analysis that RELM-β was indeed induced by allergen challenge in both Aspergillus- and OVA-induced models (Fig. 1, A and B) compared with low baseline expression in the saline-challenged lung (Fig. 1, C and D). As a control, resistin was not induced by both allergen challenges (data not shown). Furthermore, by using quantitative real-time PCR analysis (LightCycler), we found a 6- to 20-fold increase in RELM-β mRNA in the lungs of Aspergillus- and OVA-challenged mice compared with saline-challenged mice (Fig. 1E, and left and right)

Fig. 3. Regulation of RELM-β by IL-4, IL-13, and STAT6. The quantitative real-time PCR analyses of RELM-β mRNA following IL-4 (A) or IL-13 (B) delivery to wild-type and STAT6-deficient (-/-) mice. RELM-β mRNA levels in OVA- and Aspergillus-challenged wild-type, IL-13-deficient (-/-) (C and D), and IL-4/IL-13 double gene-deficient mice (E and F) are shown. The OVA-induced RELM-β mRNA level in STAT6-deficient mice (G) is shown. The data are expressed as means ± SD (n = 2, 4–5 mice in each group per experiment).

Statistical analysis. Data are expressed as means ± SD. Statistical significance comparing different sets of mice was determined by unpaired InStat GraphPad t-test.
**RELM-β is expressed in the airway epithelium.** We next examined the expression pattern of RELM-β in the allergen-challenged lung by performing mRNA in situ hybridization with RELM-β probes under conditions of high stringency. As shown in Fig. 2, saline-challenged lungs showed no lung inflammation (Fig. 2A) and non-detectable RELM-β in the airway epithelium (Fig. 2B). Following allergen challenge, RELM-β mRNA (Fig. 2D) induction was seen in the airway epithelium, as well as infiltrative cells (primarily mononuclear cells) surrounding blood vessels and airways (Fig. 2, C and D, and data not shown). The control RELM-β sense probe had very little background expression (Fig. 2, E and F). Together, and consistent with prior studies, these data show that RELM-β is induced by allergen challenge in the lung.

**RELM-β is induced by IL-4, IL-13, and allergens via STAT6.** Because asthma is a Th2-associated process, we were interested in testing the hypothesis that pharmacological delivery of IL-4 and IL-13 was sufficient for induction of RELM-β. To test this hypothesis, we administered IL-4 or IL-13 by repeated delivery to the respiratory tract of anesthetized mice. These protocols induce several features of experimental asthma, including eosinophilic inflammation, chemokine induction, mucous production, and airway hyperactivity (15, 46, 47, 53, 54). Administration of either cytokine induces significant levels of RELM-β mRNA compared with saline-treated control mice (Fig. 3). To test the role of STAT6 in the induction of RELM-β in vivo, we delivered IL-4 to wild-type and STAT6-deficient mice. The RELM-β mRNA levels were greatly reduced in STAT6-deficient mice following IL-4 delivery (Fig. 3A). Similarly, IL-13-induced RELM-β in STAT6-deficient mice was also found reduced compared with wild-type mice (Fig. 3B).

To examine the role of IL-4 and IL-13 in allergen-induced RELM-β expression, we assessed OVA- and Aspergillus-induced experimental asthma in mice genetically deficient in IL-13 or double-deficient in IL-4 and IL-13. Notably, IL-13 gene-targeted mice had low levels of RELM-β mRNA compared with wild-type mice following allergen challenge (Fig. 3, C and D). Furthermore, IL-4Rx gene-targeted mice showed similar RELM-β mRNA expression compared with wild-type mice following allergen challenge (data not shown). Mice deficient in both IL-4 and IL-13 also showed a reduction in Aspergillus- and OVA-induced RELM-β mRNA in the lung (Fig. 3, E and F). Last, mice deficient in STAT6 had significantly reduced levels of RELM-β mRNA following OVA challenge compared with OVA-challenged wild-type mice, indicating that RELM-β was largely STAT6 dependent (Fig. 3G). Collectively, these data suggest that RELM-β is a Th2-associated cytokine. In particular, RELM-β is induced by IL-4 and IL-13, and allergen-induced RELM-β is largely mediated by IL-13.

**Intratracheal administration of RELM-β induces leukocyte accumulation in the bronchoalveolar lavage fluid.** We hypothesized that overexpression of RELM-β in the lung would induce an asthma-like phenotype, at least in part. To begin to address this hypothesis, we administered recombinant murine RELM-β via intratracheal delivery and examined the effect on the level of cells in the bronchoalveolar lavage fluid (BALF). In the initial experiments, we administered seven doses of RELM-β (10 μg each/dose) and performed quantitative analysis of BALF cells 18 h following the seventh dose. As shown in Fig. 4, RELM-β induced substantial increases in total BALF cells, and differential analysis revealed that macrophages were the predominant cell type affected. Seven doses of intratracheal RELM-β resulted in an approximate sixfold increase in the total cell count as well as total macrophages in BALF (Fig. 4A). As a control, when RELM-β protein was heat inactivated
for 15 min, this completely eliminated all of its activity (data not shown), suggesting that proper protein folding was required for RELM-β activity.

**Intratracheal administration of RELM-β induces collagen deposition.** Lung sections from mice treated with seven doses of intratracheal RELM-β were stained with Masson’s trichrome for collagen. This analysis revealed an impressive perivascular and peribronchial thickening of the airway reticular basement membrane composed of trichrome-positive material (Fig. 4, B and C). To independently show that RELM-β administration induced collagen deposition in the lung, we quantified whole lung collagen levels by specific binding of collagen to the dye Sirius Red. The total lung collagen in saline-challenged mice was 54.2 ± 9.4 μg/mg protein compared with 105.1 ± 30.2 μg/mg protein in RELM-β-challenged mice (means ± SD, n = 12, P < 0.001). The specificity of RELM-β-induced lung collagen was tested by exposing the mice to heat-activated RELM-β, which induced comparable collagen as saline-exposed mice (Fig. 4D). Furthermore, we also observed a 10 ± 4 percent increase of PAS+ goblet cells in the airways following seven doses of intratracheal RELM-β compared with none in saline-challenged mice.

**The role of RELM-β in lung remodeling.** Induction of experimental asthma in RELM-β gene-deficient mice revealed no significant change in the number of infiltrating eosinophils compared with wild-type mice following Aspergillus challenge; however, a significant reduction in collagen deposition was observed. The eosinophil numbers in the airway of Aspergillus-challenged wild-type and RELM-β gene-deficient mice were 126.3 ± 69.4 × 10⁴ and 99.4 ± 49.7 × 10⁴ compared with 0.01 ± 0.01 × 10⁴ and 0.02 ± 0.02 × 10⁴ in saline-challenged mice. In contrast, RELM-β gene-deficient mice displayed significantly reduced collagen deposition in both perivascular and peribronchial areas compared with wild-type Aspergillus-challenged mice; however, both allergen-challenged groups showed increased collagen compared with saline-challenged mice (Fig. 5, A–C). No significant difference in the baseline lung collagen deposition pattern was observed in the saline-challenged wild-type and RELM-β gene-deficient mice (data not shown). The collagen production in the lungs of RELM-β gene-deficient mice and their littermate controls following Aspergillus antigen challenge was quantified by Sircol binding; allergen-treated RELM-β gene-deficient mice had decreased collagen compared with wild-type allergen-treated mice (Fig. 5D). The total lung collagen levels in allergen-challenged wild-type and RELM-β gene-deficient mice was 167.8 ± 20.8 μg/mg protein and 113.9 ± 26.7 μg/mg protein, respectively (means ± SD, n = 12, P < 0.05), whereas saline-challenged wild-type and RELM-β gene-deficient mice showed 33.9 ± 9.4 μg/mg protein and 44.3 ± 4.7 μg/mg protein, respectively (means ± SD, n = 12). In addition, we observed a reduced number of goblet cells in the inflamed lungs of RELM-β gene-deficient mice compared with wild-type mice (Fig. 5E).

**RELM-β has fibroblast motogenic activity in vitro.** The impressive deposition of collagen in the lung of mice treated with RELM-β prompted us to examine the direct effect of recombinant RELM-β on fibroblasts in vitro. We hypothesized

Fig. 5. Experimental allergic lung inflammation in RELM-β gene-targeted mice. The collagen and goblet cell analysis in the lung sections of wild-type (+/+ ) and RELM-β gene-deleted (−/−) mice was assessed following saline and Aspergillus challenge. The representative photomicrograph (original magnification ×100) of wild-type saline (A) and wild-type Aspergillus (B) and RELM-β gene-deficient Aspergillus- (C) challenged mice is shown. The quantification of whole lung total collagen and the percent periodic acid-Schiff (PAS)-positive goblet cells in the airway of wild-type (+/+ ) and RELM-β gene-deleted (−/−) mice are shown (D and E). The data are expressed as means ± SD and are representative of 3 experiments. The parameters were determined 18–20 h after the 9 saline or allergen intranasal challenges.
that RELM-β treatment would induce fibroblast proliferation. To test this hypothesis, murine 3T3 fibroblasts were exposed to a full dose range of RELM-β for 24–72 h, and their proliferative response was measured. RELM-β treatment failed to induce 3T3 cell proliferation even though control TGF-α treatment induced proliferation (data not shown). We next hypothesized that RELM-β might be inducing fibroblast motility. To test this hypothesis, the ability of RELM-β to induce 3T3 cell chemotraction in vitro was analyzed (Fig. 6). Notably, RELM-β induced dose-dependent 3T3 cell movement through a transwell membrane; activity was seen with doses as low as 10 nM. As a positive control, exposure to TGF-β (4 nM), a known fibroblast motogen, induced an eightfold increase in fibroblast movement (Fig. 6A). Finally, we were interested in determining if the ability of RELM-β to induce fibroblast movement in a murine system was applicable to a human system. To examine this hypothesis, we looked at the motogenic activity of human recombinant RELM-β on human primary lung fibroblasts. This analysis revealed that human RELM-β had similar activity on human lung fibroblasts as in the murine system (Fig. 6B).

**DISCUSSION**

To understand the complex mechanisms involved in the pathogenesis of allergic lung inflammation, we employed transcript expression profile analysis to define a set of asthma signature genes. We chose to focus on the RELM-β gene because of the prior association of this family of molecules with inflammatory processes, because of our identification of RELM-β as a member of the asthma signature genome, and because it is an important cytokine involved in innate mucosal responses (5, 21, 31, 45). Our analysis demonstrated that RELM-β was induced by allergen and Th2 cytokines (IL-4 and IL-13) and regulated by STAT6. Mice deficient in the shared subunit of the IL-4 and IL-13 receptor (IL-4Rα) had a large attenuation of allergen-induced RELM-β mRNA accumulation (data not shown). Furthermore, using in situ hybridization, RELM-β was shown to be expressed by airway epithelium and infiltrating mononuclear cells in the inflamed lungs.

Asthma has been considered an essentially reversible disorder, but more recently it has been recognized that chronic inflammation may lead to structural alterations resulting in lung remodeling (14) involving collagen deposition (16). Notably, remodeling of lung architecture (including subepithelial thickening and collagen deposition) is increased in severe asthma, and recent evidence suggests that glucocorticoid therapy may not reverse these changes (27), highlighting the need to elucidate the mechanisms of lung remodeling. Recently, a critical role for mesenchymal cell signaling involving accumulation and activation of fibroblasts, induced by Th2 cytokines (14), has been appreciated, but the exact cytokines and mechanisms involved remain largely unknown. RELM-α has been implicated in lung fibrogenesis (30) that is regulated by Th2 cytokines (IL-4 and IL-13) (32, 45). In the present study, we demonstrate that RELM-β is potently and selectively induced during Th2-mediated lung inflammation by an IL-13-dependent pathway. The finding that pharmacological delivery of RELM-β promotes a variety of proinflammatory effects on the murine lung strongly implicates RELM-β in lung remodeling. The induction of collagen by pharmacological delivery and its attenuation in allergen-challenged RELM-β gene-deficient mice further revealed that RELM-β was involved in collagen accumulation. The specificity of RELM-β to induce collagen was supported by the finding that heat-inactivated RELM-β did not induce collagen accumulation. Together, our findings compel the analysis of RELM-β in human lung diseases involving allergic airway inflammation and fibrogenesis.

Despite the growing association of RELM family members with inflammatory conditions (17, 18, 29), there is a paucity of information concerning the function of this family of cytokines. Recently, RELM-β has been shown to be produced by intestinal goblet cells and has an immunoregulatory function (18, 21). Therefore, we induced experimental asthma in RELM-β gene-targeted mice along with wild-type mice to examine the consequences of RELM-β deficiency in lung remodeling activity. RELM-β gene-deficient mice had decreased collagen deposition and goblet cell development compared with wild-type mice following Aspergillus challenge. The reduction of mucus-producing cells in RELM-β gene-deficient mice following allergen challenge is likely to be explained by direct signaling of IL-13 in respiratory epithelial cells via the IL-4/IL-13Rα1 molecule known to be expressed by these cells (13). Consistent with this, we and others have previously shown that goblet cell hyperplasia is dependent on IL-13 (6, 40, 44); we now extend these findings by showing that in the absence of RELM-β, IL-4/IL-13-induced mucus cell production does not fully develop. Although RELM-β delivery induced lung BALF leukocytosis we did not observe any

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Fig. 6. Fibroblast motility in response to RELM-β. The ability of RELM-β to induce motility of murine 3T3 fibroblasts in vitro was measured in response to various concentrations of RELM-β (A). The effect of transforming growth factor (TGF)-β is shown for comparison. Similarly, the motility of primary human lung fibroblasts in response to various concentrations of RELM-β was measured (B). The net cell numbers after 2 h is shown. The data are expressed as means ± SD of n = 3.
difference in airway leukocyte numbers in wild-type and RELM-β gene-deficient mice following Aspergillus antigen challenge (data not shown). Perhaps these differences may be explained by the unique nature of Aspergillus antigen that induces pulmonary inflammation by STAT6-dependent and -independent pathways (7, 56). Alternatively, the RELM family members may compensate for RELM-β dependency at least with regard to its role in regulating allergen-induced leukocyte recruitment. Furthermore, our in vitro data also indicate that RELM-β had fibroblast motogenic activity, suggesting an important role for this cytokine in lung remodeling. These data suggest that motogenic activity results in increased levels of collagen-producing fibroblasts in the lung following RELM exposure. These studies extend the recent in vitro observation that expression of an exogenous RELM gene in fibroblasts promotes the transcription of collagen and α-smooth muscle actin (30). Because RELM-β has fibroblast motogenic activity, it will be interesting to analyze its synergy or additive effects with known mediators of lung remodeling, including TGF-α and TGF-β.

Together, these findings add to our understanding of the pathogenesis of Th2 inflammation in a number of ways. First, because allergic lung pathology is dependent on IL-13, these results suggest that IL-13-induced pathology is mediated by RELM-β, at least in part. Second, our results identify RELM-β as a proinflammatory cytokine with activity on fibroblasts. Third, our results identify RELM-β as a mediator linked with lung remodeling activity, at least in the setting of Th2 immune responses; therefore, RELM-β may have an important role in allergic lung disease.

ACKNOWLEDGMENTS

We thank Drs. Simon Hogan, Nives Zimmermann, Keith Stringer, and Carine Blanchard, for their help. We also thank Melissa McBride for technical assistance and Andrea Lippelman for editorial assistance. In addition, we are grateful to Drs. Andrew MacKenzie and Debra Donaldson, Wyeth Laboratory, for critical reagents.

GRANTS

This work was supported in part by National Institutes of Health Grants R01-AI-45898 (M. E. Rothenberg), AI-42242 (M. E. Rothenberg), and R01-DK-067255 (A. Mishra).

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