IL-13 Receptor α1 Differentially Regulates Aeroallergen-Induced Lung Responses

Marc E. Rothenberg, Ting Wen, Dana Shik, Eric T. Cole, Melissa M. Mingler and Ariel Munitz

*J Immunol* 2011; 187:4873-4880; Prepublished online 28 September 2011; doi: 10.4049/jimmunol.1004159

http://www.jimmunol.org/content/187/9/4873

**References**
This article cites 47 articles, 13 of which you can access for free at:
http://www.jimmunol.org/content/187/9/4873.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2011 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
**IL-13 Receptor α1 Differentially Regulates Aeroallergen-Induced Lung Responses**

Marc E. Rothenberg,* Ting Wen,*1 Dana Shik,† Eric T. Cole, * Melissa M. Mingler,* and Ariel Munitz*†

IL-13 and IL-4 are hallmark cytokines of Th2-associated diseases including asthma. Recent studies revealed that IL-13Rα1 regulates asthma pathogenesis by mediating both IL-4– and IL-13–mediated responses. Nonetheless, the relative contribution of each cytokine in response to aeroallergen challenge and the degree of functional dichotomy between IL-4 and IL-13 in asthma remains unclear. Consistent with prior publications, we demonstrate that IL-13Rα1 regulates aeroallergen-induced airway resistance and mucus production but not IgE and Th2 cytokine production. We demonstrate that aeroallergen-induced eosinophil recruitment and chemokine production were largely dependent on IL-13Rα1 after *Aspergillus* but not house dust mite (HDM) challenges. Notably, *Aspergillus*-challenged mice displayed increased IL-13Rα1–dependent accumulation of dendritic cell subsets into lung-draining lymph nodes in comparison with HDM-challenged mice. Comparison of IL-4 and IL-13 levels in the different experimental models revealed increased IL-4/IL-13 ratios after HDM challenge, likely explaining the IL-13Rα1–independent eosinophilia and chemokine production. Consistently, eosinophil adoptive transfer experiments revealed near ablation of lung eosinophilia in response to *Aspergillus* in Il13ra1−/− mice, suggesting that *Aspergillus*-induced lung eosinophil recruitment is regulated by IL-13–independent chemokine production rather than altered IL-13 signaling in eosinophils. Furthermore, the near complete protection observed in Il13ra1−/− mice in response to *Aspergillus* challenge was dependent on mucosal sensitization, as alum/*Aspergillus*-sensitized mice that were rechallenged with *Aspergillus* developed IL-13Rα1–independent eosinophilia although other asthma parameters remained IL-13Rα1 dependent. These results establish that IL-13Rα1 is required for aeroallergen-induced airway resistance and that allergen-induced chemokine production and consequent eosinophilia is dictated by the balance between IL-4 and IL-13 production in situ. The Journal of Immunology, 2011, 187: 4873–4880.

*Division of Allergy and Immunology, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH 45229; and †Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

†T.W. and D.S. contributed equally to this work.

Received for publication December 27, 2010. Accepted for publication August 31, 2011.

This work was supported by Grant 2009222 from the US-Israel Binational Science Foundation (to A.M. and M.E.R.), the FP7 Marie-Curie Reintegration Grant (to A.M.), the Buckeye Foundation, the Food Allergy Project/Food Allergy Initiative, and the Campaign Urging Research for Eosinophilic Disease.

Address correspondence and reprint requests to Dr. Ariel Munitz or Dr. Marc E. Rothenberg, Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel (A.M.) or Division of Allergy and Immunology, Cincinnati Children’s Hospital Medical Center, 3333 Burnet Avenue, MLC 7028, Cincinnati, OH 45229 (M.E.R.). E-mail addresses: arielm@post.tau.ac.il (A.M.) and Rothenberg@cchmc.org (M.E.R.)

Abbreviations used in this article: AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; BM, bone marrow; HDM, house dust mite; PAS, periodic acid–Schiff.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11S1600 responsive/IL-13 nonresponsive (type I IL-4) or IL-4 responsive/IL-13 responsive (type II IL-4R) (1, 3). For example, airway epithelial cells do not express the common γ-chain and thus respond to IL-4 via the type II IL-4R, whereas myeloid cells express both the type I and type II IL-4R and can thus be activated by IL-4 and IL-13 (4, 5). Strategies targeting IL-13Rα1 for anti-asthma therapy are currently under way (6); however, there is incomplete data regarding the role of IL-13Rα1 in response to naturally occurring allergoallergens, which often trigger asthma.

Recent studies have demonstrated a key role for IL-13Rα1 and the type II IL-4R in lung Th2 responses (3, 7). We have previously identified IL-13Rα1 as a fundamental receptor mediating IL-13– and IL-4–induced AHR, mucus production, and fibrosis in response to the “classical” experimental asthma model using OVA/alum sensitization followed by lung OVA challenge (3). Nevertheless, the role of IL-13α1 in experimental asthma models of naturally occurring, clinically relevant aeroallergen sensitization and mucosal challenge is unknown. This is especially noteworthy because eosinophil recruitment to the lung after adjuvant sensitization (alum) and consequent allergen exposure (OVA) is predominantly IL-13Rα1 independent even though eosinophil-selective chemokine expression (CCL11, CCL24) is entirely dependent on IL-13Rα1 (3).

Because IL-4 and IL-13 are co-upregulated in the lungs after allergen challenge (8, 9), it is likely that their differential expression and/or upregulation may determine the dependency of the asthmatic response on IL-13Rα1. Supporting this hypothesis, we have shown that IL-13Rα1 regulates both IL-4 and IL-13 signaling in the lung. Whereas IL-13–dependent responses were entirely dependent on IL-13Rα1, IL-4–induced chemokine production and inflammatory cell recruitment were IL-13Rα1 independent (3).
In the current study, we further establish the fundamental role of IL-13Rs in allergen-induced airway resistance, mucus production, and TGF-β induction. We reveal that lung chemokine expression and consequent eosinophil accumulation are differentially dependent on IL-13Rs and determined by allergen type and route of sensitization, which dictates the balance between IL-4 and IL-13. Furthermore, we demonstrate that dendritic cell accumulation in lung-draining lymph nodes is mediated by IL-13R-dependent and -independent pathways differentially regulated by specific aeroallergens.

Materials and Methods

Mice

Generation of Il13ra1−/− mice has previously been described (3, 7). Mice were back-crossed into their respective strains (BALB/c and C57BL/6) for at least 10 generations. For all experiments, BALB/c or C57BL/6 wild-type mice were obtained from Charles River (Wilmington, MA) and housed under specific pathogen-free conditions. The institutional animal experimentation ethics committee approved all of the experiments.

Allergen sensitization and challenge

Aspergillus and house dust mite (HDM) Ag-associated asthma was induced by challenging mice intranasally three times a week for 3 wk as previously described (5, 10, 11). In brief, mice were lightly anesthetized with isoflurane inhalation, and 10 μg total protein (and not dry weight) of Aspergillus or HDM extract (Bayer Pharmaceuticals, Spokane, WA) in 50 μl saline or 50 μl normal saline solution alone was applied to the nasal cavity by using a micropipette with the mouse held in the supine position. After instillation, mice were held upright until alert. Mice were euthanized 24–48 h after the last challenge. In some experiments, asthma models were induced by two i.p. injections with 100 μg Aspergillus extract and 1 mg aluminum hydroxide (alum) as adjuvant (14 d apart), followed by two intranasal challenges of 50 μg Aspergillus extract or saline (3 d apart), starting a least 10 d after the second sensitization, as previously described (10, 11). The level of LPS in the Aspergillus and HDM extracts was less than 2 pg/ml as detected by the Limulus assay. Mice were sacrificed 24–48 h after the last intranasal challenge.

Ig and mediator assessment

Serum IgE and bronchoalveolar lavage fluid (BALF) cytokines were measured with kits purchased from the following sources: IgE from BD Biosciences (lower detection limit: 15 pg/ml) and CCL1, CCL2, CCL24, CCL27, and Hprt levels in the LightCycler 480 system in conjunction with the ready-to-use LightCycler 480 SYBR Green I Master reaction kit (Roche Diagnostic Systems, Branchburg, NJ). Results were normalized to Hprt cDNA (12, 13).

Real-time PCR

RNA samples from the whole lung were subjected to reverse transcription analysis using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Real-time PCR analysis of Il13, Il4, and Hprt levels was performed using the LightCycler 480 system in conjunction with the ready-to-use LightCycler 480 SYBR Green I Master reaction kit (Roche Diagnostic Systems, Branchburg, NJ). Results were normalized to Hprt cDNA (12, 13).

Airway resistance and compliance measurements

Airway resistance was measured using the flexiVent system (Scriq Scientific Respiratory Equipment) (3). Briefly, the mice were anesthetized, a tracheostomy was performed, and a cannula inserted. A positive end-expiratory pressure of 0.2 kPa was established. Saline aerosol delivered by β-methacholine (25 and 50 mg/ml, BALB/c and C57BL/6, respectively; Sigma-Aldrich, St. Louis, MO) established control baseline. Aerosols were generated with an ultrasonic nebulizer (UltraNeb 2000; DeVilbiss, Somerset, PA) and delivered to the inspiratory line of the flexiVent. Each aerosol was delivered for 20 s during which time regular ventilation was maintained. Five measurements were made at 25-s intervals, and three peak responses were compared with the mean response of the saline aerosol.

Lung histopathology and immunohistochemistry

Histological studies were performed as follows: the right upper lobe of saline- or allergen-challenged lungs was fixed in 3.7% paraformaldehyde, embedded in paraffin, deparaffinized, and stained with H&E or with periodic acid–Schiff (PAS) reagent (14). PAS-stained slides were quantified as previously described (3, 11). Lung and esophageal eosinophils were stained and quantified by immunohistochemistry as described previously (14, 15).

Flow cytometry

Forty-eight hours after the last aeroallergen challenge, the mice were sacrificed, and lung-draining lymph nodes were harvested. Lymph nodes were delicately crushed to generate single-cell suspensions. Thereafter, single-cell suspensions were stained with the Abs CD45–650NC, CD11c–Alexa Fluor 488, CD11b–allophycocyanin, B220–PE, Gr-1–PE–Cy7 (all purchased from eBioscience) and acquired by the Gallios flow cytometer (Beckman Coulter). Data analysis was performed using Kaluza (Beckman Coulter) or FlowJo (Tree Star) on at least 50,000 events.

Adaptive transfer experiments

Eosinophils were grown from the bone marrow (BM) of wild-type mice with modifications based on a prior report (16). Briefly, BM cells were harvested and loaded on a Histopaque gradient (Sigma). Low-density BM cells were collected and cultured in the presence of stem cell factor and Flt3L for 4 d. Thereafter, the medium was replaced with IL-5 for the rest of the culture (up to day 16) (16). On days 14–16 of the BM culture, 8 × 10⁴ eosinophils were injected into the tail vein of Aspergillus-challenged mice (8 h after the fifth to sixth allergen challenge). BALF was extracted 48 h after the transfer.

Statistical analysis

Data were analyzed by ANOVA followed by Tukey post hoc test using GraphPad Prism 4 (GraphPad, San Diego, CA). Data are presented as mean ± SD, and p values < 0.05 were considered statistically significant.

Results

Regulation of Aspergillus-induced airway resistance, compliance, and mucus production in Il13ra1−/− mice

To define the role of IL-13Rs in the response to naturally occurring airborne allergens, we subjected Il13ra1−/− mice to intranasal exposure to Aspergillus, a potent inducer of allergic airway inflammation (17–19). Assessment of airway resistance in response to cholinergic stimuli revealed that Il13ra1−/− mice were entirely protected from the allergen-induced increases in airway resistance observed in wild-type mice (Fig. 1A). Furthermore, Il13ra1−/− mice had a concomitant protection from allergen-induced reductions in airway compliance (Fig. 1B). To examine the role of IL-13Rs in allergen-induced mucus production, histological sections of Aspergillus-challenged lungs were stained with PAS, and PAS+ cells were enumerated. Il13ra1−/− mice were entirely protected from allergen-induced mucus production and goblet cell hyperplasia (Fig. 1C, 1D). Assessment of active TGF-β levels in Aspergillus-challenged Il13ra1−/− mice demonstrated markedly reduced TGF-β levels in comparison with Aspergillus-challenged wild-type mice (Fig. 1E).

Regulation of Aspergillus-induced lung chemokine production and leukocytosis in Il13ra1−/− mice

Interleukin-13 and IL-4 are potent inducers of various chemokines including CCL1, CCL24, and CCL17. To define the role of IL-13Rs in aeroallergen-induced chemokine production, BALF from Aspergillus-challenged wild-type and Il13ra1−/− mice was examined for the aforementioned chemokines. Il13ra1−/− mice displayed nearly complete protection from Aspergillus-induced CCL11 and CCL24 expression (93 and 91% reduction, respectively; Fig. 2A, 2B). Moreover, Aspergillus-induced CCL17 was undetectable in BALF samples obtained from Il13ra1−/− mice (Fig. 2C). Consistent with the substantial decrease in chemokine expression, cellular recruitment of eosinophils into the BALF and lungs was dramatically attenuated in allergen-challenged Il13ra1−/− mice (Fig. 2D); however, no changes were observed in neutrophil...
FIGURE 1. Regulation of Aspergillus-induced airway resistance, compliance, and mucus production in Il13ra1−/− mice. Forty-eight hours after the last Aspergillus challenge, wild-type and Il13ra1−/− mice were examined for airway resistance (A), lung compliance (B), mucus production (C, D), and active TGF-β production (E). Data are representative of one of three experiments (6–17 mice per experiment per group). In A, a representative photomicrograph of PAS staining is depicted (original magnification ×200). *p < 0.05, **p < 0.01, ***p < 0.001. Asp, Aspergillus; WT, wild-type.

and lymphocyte BALF levels. Lung tissue eosinophilia was decreased by ~80% in Aspergillus-challenged Il13ra1−/− mice (Fig. 2E). To determine whether IL-13Rα1 regulates eosinophilia in a tissue-specific fashion, we assessed Aspergillus-induced eosinophilia in the esophagus (20). Notably, aeroallergen-challenged Il13ra1−/− mice displayed near complete protection from eosinophil accumulation into the esophagus as well (Fig. 2F). To demonstrate definitively that decreased eosinophil migration into the lungs of Aspergillus-challenged Il13ra1−/− mice was not due to an intrinsic defect of IL-13 signaling in eosinophils, an adoptive transfer approach was used. Wild-type eosinophils, generated from low-density BM cells, were adoptively transferred intravenously into Aspergillus-challenged wild-type and Il13ra1−/− mice. Indeed, donor wild-type eosinophils that were adoptively transferred into Aspergillus-challenged wild-type mice were readily detectable in the BALF (Fig. 2G). In sharp contrast, wild-type eosinophils that were transferred into Aspergillus-challenged Il13ra1−/− mice were hardly detectable in the BALF and markedly reduced (100- to 1000-fold lower than in wild-type mice) (Fig. 2G).

Assessment of Aspergillus-induced IgE and Th2 cytokine induction in Il13ra1−/− mice

The striking protection of Il13ra1−/− mice from the local effects of Aspergillus suggested that Il13ra1−/− might not be able to mount a typical Th2 response, which is characterized by increased IgE production and expression of hallmark Th2 cytokines such as IL-4, IL-13, and IL-5 (21, 22). To examine this possibility, Aspergillus-challenged wild-type and Il13ra1−/− mice were assessed for total serum IgE. No difference was observed in allergen-induced total serum IgE levels between wild-type and Il13ra1−/− mice (Fig. 3A). Consistent with this observation, Aspergillus-challenged Il13ra1−/− mice displayed a significant increase in IL-4, IL-13, and IL-5 levels (Fig. 3B–D). Notably, Il13ra1−/− mice displayed a minor, but statistically significant, increase in IL-13 levels (Fig. 3C) in comparison with wild-type mice but had similar IL-4 and IL-5 levels (Fig. 3B, 3D).

Regulation of HDM-induced airway resistance, compliance, and mucus production in Il13ra1−/− mice

Various studies have demonstrated different mechanisms for allergenicity to airborne allergens (23–25). Thus, we were interested to examine whether the roles of IL-13Rα1 in the regulation of allergen-induced lung responses were allergen specific or a shared phenomenon between allergens. To address this question, we used an additional model of mucosal sensitization after repetitive HDM intranasal exposures. Similar to our findings with Aspergillus-challenged Il13ra1−/− mice (Fig. 1), HDM-challenged Il13ra1−/− mice were entirely protected from increased allergen-induced airway resistance and decreased compliance (Fig. 4A, 4B). Assessment of PAS+ cells in Il13ra1−/− mice after HDM challenge revealed that allergen-challenged Il13ra1−/− mice displayed nearly complete protection from allergen-induced mucus production (Fig. 4C, 4D). Furthermore, HDM-challenged mice were also protected from allergen-induced elevation in TGF-β (Fig. 4E).

Regulation of HDM-induced lung chemokine production and leukocytosis in Il13ra1−/− mice

Notably, and in contrast to our findings in Il13ra1−/− mice after Aspergillus challenge, HDM-challenged Il13ra1−/− mice displayed elevated CCL11, CCL24, and CCL17 levels in the BALF, albeit decreased compared with those of wild-type mice (Fig. 5A–C). These results were confirmed by real-time quantitative PCR analysis (data not shown) demonstrating only partial regulation of CCL11, CCL24, and CCL17 production by IL-13Rα1 in response to HDM challenge. Consistent with these findings, HDM-challenged Il13ra1−/− mice revealed substantial eosinophil infiltration into the lungs and BALF of HDM-challenged mice, which was predominantly IL-13Rα1 independent (Fig. 5D, 5E). Similar to our findings with Aspergillus, HDM-challenged Il13ra1−/− mice displayed similar IgE and Th2 cytokines compared with those of HDM-challenged wild-type mice (Fig. 6).

Differential IL-4 and IL-13 production in response to Aspergillus and HDM

We have previously shown that IL-13Rα1 differentially regulates IL-4- and IL-13-induced responses in the lung (3). Thus, we hypothesized that the role of IL-13Rα1 in response to allergen challenge may be dictated by the net ratio between allergen-induced IL-4 and IL-13. Comparing the key roles of IL-13Rα1 in Aspergillus-induced chemokine production and eosinophil recruitment (Figs. 1, 2) with its partial role in HDM-induced chemokine production and eosinophil recruitment suggested that HDM may preferentially use the type I IL-4R as the ratio of IL-4 to IL-13 should be higher after HDM challenge than after Aspergillus challenge. To investigate this possibility, real-time quantitative PCR analysis of saline- and allergen-challenged (Aspergillus and HDM) lungs obtained from wild-type mice was performed. Indeed, both Aspergillus and HDM were capable of significantly increasing IL-4 and IL-13 mRNA expression (Fig. 7A–D). To determine relative IL-4 and IL-13 levels in the different models, allergen-induced IL-4 and IL-13 mRNA levels were normalized to IL-4 and IL-13 baseline levels in saline-treated mice (Fig. 7E). Notably, IL-4/IL-13 mRNA ratios in HDM-induced responses were...
higher than those observed in response to *Aspergillus* challenge (Fig. 7F).

**Differential regulation of dendritic cell accumulation in lung-draining lymph nodes by IL-13Rα1**

Dendritic cells have key roles in the initiation of Th2 responses by regulating the polarization of Th2 cells and thus IL-4 and IL-13 cytokine production. Hence, we next hypothesized that IL-13Rα1 may differentially regulate recruitment of dendritic cells into lung-draining lymph nodes in response to allergen challenge. Assessment of B220+/CD11b−/Gr-1+ and B220+/CD11b+/Gr-1− dendritic cell subsets after *Aspergillus* and HDM challenge revealed significantly increased dendritic cell accumulation into the lung-draining lymph nodes (Fig. 8A, 8B). Notably, IL-13Rα1 predominantly regulated dendritic cell accumulation in lung-draining lymph nodes in response to *Aspergillus* challenge and to a significantly lesser extent after HDM challenge (Fig. 8C, 8D).

**FIGURE 2.** Regulation of *Aspergillus*-induced lung chemokine production and leukocytosis in IL13ra1−/− mice. Forty-eight hours after the last *Aspergillus* challenge, wild-type and IL13ra1−/− mice were examined for chemokine production (A–C), BALF differential cell counts (D), and lung (E) and esophageal (F) eosinophils (as assessed by anti-MBP stain). Data are representative of one of three experiments (6–17 mice per group per experiment). ***p < 0.001. Forty-eight hours after adoptive transfer of wild-type eosinophils into *Aspergillus*-challenged wild-type and IL13ra1−/− mice (G), the BALF was collected, and eosinophils were assessed by flow cytometry. Data are representative of two experiments (four to eight mice per group per experiment). ***p < 0.001. Asp, *Aspergillus*; WT, wild-type.

**FIGURE 3.** Assessment of IgE production and Th2 cytokines in *Aspergillus*-challenged IL13ra1−/− mice. Forty-eight hours after the last *Aspergillus* challenge, wild-type and IL13ra1−/− mice were examined for total IgE (A) and Th2 cytokines in the BALF (B–D). Data are representative of one of three experiments (6–17 mice per group per experiment). *p < 0.05, **p < 0.01, ***p < 0.001. Asp, *Aspergillus*; ns, non-significant; WT, wild-type.

**FIGURE 4.** Regulation of HDM-induced airway resistance, compliance, and mucus production in IL13ra1−/− mice. Forty-eight hours following the last HDM challenge, wild-type and IL13ra1−/− mice were examined for airway resistance (A), lung compliance (B), mucus production (C, D), and active TGF-β production (E). Data are representative of one of three experiments (9–14 mice per group per experiment). In C, a representative photomicrograph of PAS staining is depicted (original magnification ×100). *p < 0.05, **p < 0.01, ***p < 0.001. WT, wild-type.
Alum sensitization leads to IL-13Rα1-independent allergen-induced eosinophilic inflammation in the lung

Given the striking IL-13Rα1 dependency of eosinophilia after Aspergillus challenge, we aimed to define whether this phenomenon was attributed to the mode of allergic sensitization or an inherent trait of the allergen itself. Therefore, we established a model of experimental airway inflammation using alum and Aspergillus similar to the conventional alum and OVA model (10, 11) and assessed allergen-induced lung inflammation. As expected, Il13ra1−/− mice were entirely protected from increased allergen-induced airway resistance and decreased allergen-induced compliance (Fig. 9A, 9B), mucus production, and TGF-β expression (Fig. 9C, 9D). Furthermore, allergen-induced chemokine (e.g., CCL11, CCL24, and CCL17) production was entirely dependent on IL-13Rα1 (Fig. 9E–G). However, lung eosinophilia was predominantly independent of IL-13α1, as Il13ra1−/− mice displayed eosinophil levels similar to those of wild-type mice (Fig. 9H, 9I). A full summary of IL-13Rα1–dependent and –independent pathways in response to the various experimental asthma models is shown in Table I.

Discussion

The pathological effects of IL-4 and IL-13 in Th2 immunity have been a focus of intense research in the past decade (1, 4, 10, 26).

FIGURE 5. Regulation of HDM-induced lung chemokine production and leukocytosis in Il13ra1−/− mice. Forty-eight hours after the last HDM challenge, wild-type and Il13ra1−/− mice were examined for chemokine production (A–C), BALF differential cell counts (D), and lung eosinophils (as assessed by anti-MBP stain) (E). Data are representative of one of three experiments (9–14 mice per group per experiment). *p < 0.05, **p < 0.01, ***p < 0.001. ns, non-significant; WT, wild-type.

FIGURE 6. Assessment of IgE production and Th2 cytokines in HDM-challenged Il13ra1−/− mice. Forty-eight hours after the last HDM challenge, wild-type and Il13ra1−/− mice were examined for total IgE (A) and Th2 cytokines in the BALF (B–D). Data are representative of one of three experiments (6–17 mice per group per experiment). **p < 0.01. ns, non-significant; WT, wild-type.

FIGURE 7. Differential IL-4 and IL-13 production in response to Aspergillus and HDM. Forty-eight hours after the last Aspergillus and HDM challenge, whole lung RNA was isolated from wild-type and Il13ra1−/− mice, and cDNA was generated. Il4, Il13, and Hprt levels were assessed using real-time quantitative PCR analysis (A–D). Next, IL-4 and IL-13 protein levels after Aspergillus and HDM challenges were normalized to fold increase over saline protein levels (E) and expressed as IL-4/IL-13 ratios (F). Data represent three repetitions of real-time quantitative PCR on cDNA from three experiments (six mice per group per experiment). *p < 0.05. **p < 0.01. ***p < 0.001. Asp, Aspergillus; ns, non-significant; WT, wild-type.
Both cytokines are capable of driving major features of allergic asthma; namely, airway resistance, mucus production, and fibrosis. Thorough examination of the IL-4/IL-13–IL-13R signaling axis in asthma requires further attention as agents that target these cytokines, receptors, and subsequent signaling responses are being actively developed for the treatment of Th2-associated diseases, especially asthma. To dissect fully the involvement of IL-13Rα1 in the lung, we examined divergent Th2 responses in Il13ra1−/− mice after mucosal sensitization and challenge of naturally occurring, clinically relevant allergoagents; namely, Aspergillus and HDM. We report that 1) IL-13Rα1 is the key receptor mediating AHR, mucus production, and TGF-β induction in response to aeroallergens; 2) decreased eosinophilia in Aspergillus-challenged Il13ra1−/− mice is not due to a defect in IL-13–mediated eosinophils but due to extrinsic activity likely mediated by IL-13Rα1 and HDM; 3) decreased eosinophilia in HDM/challenged Il13ra1−/− mice was determined (A, B). Next, average levels of Aspergillus- and HDM-induced dendritic cells in wild-type mice was assigned the value of 100% and the percentage of inhibition in allergen-challenged Il13ra1−/− mice was determined (C, D). Data are representative experiments (four to eight mice per group per experiment). *p < 0.05, **p < 0.01. Asp, Aspergillus; DCs, dendritic cells.

One of the major findings presented in this study is that after Aspergillus challenge, eosinophil recruitment and chemokine production are largely IL-13Rα1 dependent. Indeed, our adoptive transfer experiments indicate that eosinophil recruitment to the lungs after Aspergillus challenge is likely regulated by IL-13–induced chemokine production, likely by epithelial cells rather than an inherent defect in IL-13–induced responses of eosinophils. In fact, we cannot demonstrate direct signaling induced by IL-13 on murine eosinophils, even though IL-4 is very potent (C. Bouaffi and M.E. Rothenberg, manuscript in preparation). In contrast, after HDM challenges IL-13Rα1–independent pathways exist, which regulate eosinophil recruitment and chemokine production. The finding that alum/Aspergillus-sensitized Il13ra1−/− mice developed substantial lung eosinophilia independent of IL-13Rα1 indicates that the mode of allergen sensitization is a key determinant for IL-13Rα1 dependency. Moreover, we show that allergen-induced esophageal eosinophilia is IL-13Rα1 dependent. This finding is particularly important because IL-13 has been shown to be sufficient to induce eosinophilic esophagitis in mice (28) and likely man (29, 30). Yet, the receptor requirement has not been elucidated even though Il13ra2−/− mice display increased esophagitis (31). Because anti–IL-13 reagents are now in clinical trials for asthma and eosinophilic esophagitis, these preclinical findings have broad implications. Mechanistically, we demonstrate increased IL-4/IL-13 ratios after HDM challenge; this may explain the IL-13Rα1–independent eosinophilia and chemokine production, as IL-4 likely becomes the more dominant signaling pathway under these conditions. This suggests that in allergic settings where IL-13 production is relatively higher than IL-4, blockade of IL-13Rα1 will have better therapeutic value than in allergic settings displaying higher IL-4 to IL-13 ratios. In low IL-4/IL-13 ratios, observation of IL-4–driven chemokine production and tissue eosinophilia may be likely. It is notable that Aspergillus and HDM use distinct mechanisms to induce allergic lung inflammation; HDM exerts its effects via functional mimicry of TLR signaling (24, 32, 33), whereas Aspergillus uses protease-dependent pathways (23, 34, 35). Exposure of airway epithelium to HDM results in upregulation of CCL20, which attracts immature dendritic cells. Notably, CCL20 induction is HDM specific as ragweed pollen and cockroach Ag do not induce CCL20 secretion and depend upon β-glucan recognition rather than protease activity (25). Although not much is known regarding the effects of Aspergillus on dendritic cell recruitment in allergic settings, recent data indicate that CCR7 and its ligands CCL19 and CCL21, which are upregulated in asthma (36, 37), are involved in response to invasive aspergillosis (38). Notably, we show that both HDM and Aspergillus induce significant recruitment of dendritic cells to the lung-draining lymph nodes. However, Aspergillus induces greater dendritic cell accumulation, which is predominantly regulated by IL-13Rα1. Thus, differential recruitment of dendritic cells in response to allergen challenge may determine the functional consequence of differential IL-4/IL-13 ratios in the lung and consequent eosinophilia (39). Directly related and supporting this hypothesis, we demonstrate that systemic sensitization of Il13ra1−/− mice using Aspergillus and alum and consequent local Aspergillus challenge was capable of inducing IL-13Rα1–independent eosinophil lung accumulation. This result is consistent with a previous report that OVA- and alum-sensitized Il13ra1−/− mice develop pulmonary eosinophilia (3). Yet, two differences were observed between these models: 1) in the OVA/alum model, lung eosinophilia was significantly decreased, whereas in the Aspergillus/alum model, eosinophil numbers in wild-type and Il13ra1−/− mice were similar (3); and 2) in response to OVA/alum, Il13ra1−/− mice displayed a concomitant upregulation in neutrophil accumulation (3), whereas neutrophil levels remained similar to allergen-challenged wild-type mice in response to Aspergillus/alum. It is important to note that our overall findings are consistent with observations that STAT6-independent lung eosinophilia can occur after Aspergillus (40). The finding that IL-13Rα1–independent eosinophilia can occur [as observed in the
OVA/alum-sensitized mice (3) or in the lung tissue of Aspergillus-challenged mice identifies a pathway for eosinophil recruitment to the lung that appears to be primarily independent of classic eosinophil chemokines such as the eotaxins (15, 41, 42). A comprehensive summary of IL-13Rα1–dependent and –independent pathways in response to the various experimental asthma models is shown in Table I.

Various studies have shown IL-13Rα2–dependent TGF-β induction (43, 44). Our findings demonstrate that allergen-induced TGF-β production was completely dependent on IL-13Rα1. Similarly, TGF-β production in liver fibrosis after Schistosoma mansoni infection has been proposed to be independent of IL-13Rα1 (27). Despite this, we cannot exclude the possibility that IL-13–IL-13Rα1 interactions upregulate IL-13Rα2 expression, which mediates TGF-β production. Nonetheless, the finding that IL-13Rα1 is upstream of allergen-induced TGF-β production has significant implications for asthma-related fibrosis. Although eosinophils may be a significant source for TGF-β expression in settings of allergic inflammation (45, 46), decreased allergen-induced TGF-β production is not likely due to eosinophil-derived TGF-β as TGF-β levels were abrogated even in the presence of eosinophilia (as in the HDM model). Nevertheless, it is still possible that IL-13Rα1 mediates TGF-β production in eosinophils and, therefore, that Il13ra1-/- eosinophils may not be capable of producing TGF-β in the allergic lung.

Our results establish a specific and key role for IL-13 in driving the effector arm of allergic lung responses, as allergen-induced IgE and Th2 cytokine production occurred independent of IL-13Rα1. Notably, whereas IL-13–IL-13Rα1 interactions are not involved in Th2 immune polarization in the lungs, they may have a role in Th2 polarization in mouse models of epicutaneous sensitization as Il13ra1--/--, Il4--/-- and Stat6--/-- mice display defective Th2 cytokine production in skin draining lymph node cells after epicutaneous OVA sensitization (47).

In summary, our results establish the critical role for IL-13Rα1 in experimental asthma pathogenesis mediated by natural allergens after mucosal sensitization, conditions that may better mimic human asthma compared with experimental models that rely on i.p. sensitization with adjuvants (e.g., alum). The finding that IL-

### Table I. Summary of IL-13Rα1–dependent and –independent pathways in experimental asthma models

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OVA/Alum</th>
<th>Asp</th>
<th>Asp/Alum</th>
<th>HDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th2 cytokines</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>IgE</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Airway resistance</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Mucus production</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Th2 chemokines</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Asp, Aspergillus; --, IL-13Rα1 independent; +, partially IL-13Rα1 dependent; ++, strongly IL-13Rα1 dependent.
13Rα1 regulates the key effector features of allergic asthma, independent of regulating adaptive immunity (as evidenced by sustained production of IgE and Th2 cytokines in IL13Rα1−/− mice), position IL-13Rα1 as a potent and promising target for asthma treatment. Furthermore, our data highlight that IL-13Rα1 mechanistically regulates aeroallergen-induced eosinophil recruitment by an extrinsic mechanism (likely dependent upon chemokine production) and aeroallergen-induced dendritic cell homing to draining lymph nodes. Finally, our results suggest that outcomes of future IL-13Rα1-targeted asthma therapy may vary in individuals according to the levels of allergen-induced IL-4.

Acknowledgments

We thank Drs. Jamie Lee and Nancy Lee (Mayo Clinic, Scottsdale, AZ) for the anti-MBP Ab, Dr. Patty Fullkerson for developing the bone marrow-derived eosinophil culture, and Shawna Hottinger for final edits to the manuscript.

Disclosures

M.E.R. has an equity interest in reslizumab, a drug developed by Cephalon, Inc. The other authors have no financial conflicts of interest.

References