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IL-13 Receptor Alpha 2 Contributes to Development of Experimental Allergic Asthma

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Abstract

Background—IL-13 receptor alpha2 (IL-13Rα2) binds IL-13 with high affinity and modulates IL-13 responses. There are soluble and membrane forms of IL-13Rα2 generated by alternative splicing in mice but humans express only the membrane form (memIL-13Rα2).

Objective—We determined the role of memIL-13Rα2 in development of allergic inflammation in mouse models of asthma.

Methods—IL-13Rα2-deficient and memIL-13Rα2 lung epithelium-specific transgenic mice were challenged with house dust mite (HDM). Airway hyperresponsiveness (AHR) and inflammation were assessed by airway pressure time index, bronchoalveolar lavage (BAL) cell counts and lung histology. The mucus production was determined by periodic acid-Schiff (PAS) staining of lung sections, western blot analysis of chloride channel calcium activated 3 (CLCA3) expression in lung homogenates, and ELISA of Muc5ac in BAL fluid (BALF). The expression of cytokines and chemokines was determined by RT-quantitative PCR.

Results—In IL-13Rα2-deficient mice, AHR and airway inflammation were attenuated compared to wild type mice following HDM challenge. Lung epithelium overexpression of memIL-13Rα2 in the IL-13Rα2-deficient mice reconstituted AHR and inflammation to levels similar to those observed in HDM-challenged wild type mice. Mucus production was attenuated in lungs from HDM-treated IL-13Rα2-deficient mice while lung epithelium overexpression of memIL-13Rα2 increased mucus production. Lung epithelium overexpression of memIL-13Rα2 had no effect on

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the levels of sIL-13Rα2 in the serum or BALF and did not affect IL-13-dependent STAT6 activation in the lungs.

Conclusion—These data collectively support a distinct role for memIL-13Rα2 in lung, and suggest that memIL-13Rα2 may contribute to allergic inflammation.

Keywords

IL-13; IL-13 receptor; lung; airway hyperresponsiveness; airway inflammation

Introduction

IL-13 is a central mediator of allergic inflammation and asthma, which is necessary and sufficient for development of airway hyperresponsiveness (AHR) in mouse models [1, 2]. IL-13 mediates its effects through receptors including IL-4 receptor alpha (IL-4Rα), IL-13 receptor alpha 1 (IL-13Rα1) and IL-13Rα2 [3-6]. IL-13Rα1 binds IL-13 with low affinity by itself but it binds IL-13 with high affinity when it is paired with IL-4Rα to form a functional IL-13 receptor [7]. In contrast, IL-13Rα2 has high affinity for IL-13 but its expression is insufficient to render cells responsive to IL-13, suggesting that IL-13Rα2 acts as a decoy receptor that does not signal [8]. This may result from the short cytoplasmic tail of IL-13Rα2, which lacks box 1 or box 2 signaling motifs [5, 8].

The precise role of IL-13Rα2 in IL-13 signaling and responses is unclear. Characterization of IL-13Rα2-deficient mice revealed that these mice have elevated IgE levels, consistent with a role of IL-13Rα2 as a decoy receptor [9]. The decoy role for IL-13Rα2 is further supported by the finding that a soluble form of IL-13Rα2 (sIL-13Rα2) exists *in vivo* and administration of sIL-13Rα2 has been shown to inhibit IL-13-mediated airway inflammation in mouse models of asthma [1, 2, 6, 10, 11]. Other studies showed that IL-13Rα2 down-regulates granulomatous inflammatory responses after *Schistosoma mansoni* infection and regulates immune and biological effects of IL-13 by limiting IL-13 availability during *Nippostrongylus brasiliensis* infection [12, 13]. In a mouse model of cutaneous inflammation, transepidermal water loss, cutaneous inflammation, peripheral eosinophilia, and IgG1 and IgE levels are significantly increased in IL-13Rα2-deficient mice compared to wild-type mice [14]. IL-13 activity is enhanced in IL-13Rα2/IL-10 double deficient mice compared to IL-10-deficient mice [15]. These observations indicate that IL-13Rα2 functions as a decoy receptor in IL-13 signaling pathways in mice.

Although IL-13Rα2 can act as a decoy receptor, it may also contribute to IL-13 responses. Studies have shown that IL-13-induced TGFβ-dependent fibrosis is dependent on IL-13Rα2 through activation of transcription factor AP-1 [16, 17]. In humans, studies have also supported a contributory signaling role for IL-13Rα2 [18].

One potential reason for the confusing findings is the data from our group and others, which clearly demonstrate that human and murine IL-13Rα2 biology are markedly different [19, 20]. There are distinct membrane and soluble isoforms of IL-13Rα2 generated by alternative splicing in mice [21]. Mice have nanogram quantities of soluble IL-13Rα2 in their serum even at baseline, while humans lack soluble IL-13Rα2 [19, 20]. Thus, conclusions from IL-13 studies done in mice may not be fully applicable to humans. Humans express only memIL-13Rα2, which may have unique roles independent of sIL-13Rα2. In current studies, we utilized IL-13Rα2-deficient mice and memIL-13Rα2 lung transgenic mice to elucidate the biological functions of IL-13Rα2 in development of allergic asthma. Our data demonstrate that memIL-13Rα2 contributes to allergen-induced AHR and airway inflammation.

Methods

Mice

8-12 week old FVB/N or BALB/c wild type mice were purchased from Jackson Laboratory (Bar Harbor, Maine). All mice were maintained in barrier facility at Cincinnati Children's Hospital Medical Center and handled under Institutional Animal Care and Use Committee approved procedures.

Generation of memIL-13Rα2 transgenic mice

The pBluescript II KS+/rCC10/mIL-13Rα2/hGH transgenic construct was generated from mouse membrane form IL-13Rα2 (memIL-13Rα2) cDNA and human growth hormone (hGH) intronic and polyadenylation sequences using the rat Clara cell 10-kDa (rCC10) promoter for lung epithelial cell-specific expression [22]. The rat CC10 promoter was kindly provided by Dr. Jeffrey Whitsett (Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio) [23]. FVB/N IL-13Rα2 transgenic mice were generated using standard microinjection procedures by the transgenic core facility at the Cincinnati Children's Hospital Medical Center. IL-13Rα2-deficient mice were kindly provided by Dr. Michael Grusby (Harvard School of Public Health, Boston, Massachusetts) [9]. The FVB/N IL-13Rα2 lung transgenic mice were crossed to BALB/c IL-13Rα2-deficient mice to generate memIL-13Rα2 transgenic/IL-13Rα2-deficient mice.

Genotyping and Northern blot

The memIL-13Rα2 transgenic mice were genotyped by both Southern blot and PCR. The Southern blot was performed using an hGH gene probe with AatII-digested genomic tail DNA from the mice. The memIL-13Rα2 transgenic mice were genotyped using forward primer 5'-AGAGGACACAGGTGCCTACAGTTC-3' (in the 3' end of the rCC10 promoter) and reverse primer 5'-AGCCTGGATAAGGGAATGGTTG-3' (in the 5' end of hGH gene). Total RNA was isolated from mouse lung tissue using Trizol reagent (Invitrogen) and Northern blot analysis was performed using a 1 kb IL-13Rα2 cDNA probe. The IL-13Rα2-deficient mice were genotyped by PCR using IL-13Rα2 forward primer 5'-CTGGGGTGTTCATGTAGACTT-3', IL-13Rα2 reverse primer 5'-CTTCAAATAATCATGTGGGTAAAC-3' and neomycin cassette reverse primer 5'-CTCGTCAAGGCGATAGAAGGCGAT-3'.

Immunization protocol

For an acute asthma model, on day 0 and day 7, mice were sensitized intraperitoneally with 10 μg of HDM (Greer Laboratories) in 100 μl PBS or equivalent amount of PBS alone. On day 14 and day 21, mice were challenged intratracheally with 100 μg HDM in 50 μl PBS or PBS alone [21]. On day 22 or day 23, AHR was analyzed.

Measurement of AHR

For AHR measurement, the mice were anesthetized (80–90 mg/kg sodium pentobarbital), cannulated, ventilated and then paralyzed with decamethonium bromide (25mg/kg). Acetylcholine (50μg/kg) was injected into the inferior vena cava and changes in airway pressure were recorded and expressed as airway pressure time index (APTI) [24, 25].

Bronchoalveolar lavage (BAL) and lung histology

After AHR measurement, blood samples were collected and BAL was performed. The total cell number and cell differential percentage were determined as previously described [25, 26]. After completion of BAL, the lungs were removed, fixed with 10% neutral formalin, embedded in paraffin and 5 μm lung sections were cut. Hematoxylin and eosin (H&E) and

periodic acid-Schiff (PAS) staining were performed as previously described [26]. The collagen staining was done using Accustain Trichrome Stains (Masson) from Sigma. Histological analysis was done using Nikon Eclipse 90i Microscope System (Nikon).

ELISA

IL-13Rα2 from BALF, serum and lung lysate was determined by ELISA as previously described [21]. The IL-13 binding assay for IL-13Rα2 was performed as previously described [25]. To prepare lung lysate, the lung tissue was minced in lysis buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1.5 mM $MgCl_2$, 0.2% Nonidet P-40, 1.0 mM DTT, 0.5 mM PMSF) and incubated on ice for 5 min, centrifuged 10,000 × g for 5 min, and the supernatant was collected as lung lysate. The protein concentration of lung lysate was determined by Protein Assay Kit (Bio-Rad Laboratories). For free and IL-13-bound IL-13Rα2 assay, the standards and samples were preincubated with 10 ng/ml of mouse IL-13 (PeproTech) at 37°C for 1 hour. Anti-mouse IL-13Rα2 polyclonal antibody (1 μg/ml) and biotinylated anti-mouse IL-13 polyclonal antibody (0.5 μg/ml) were used for capture and detection respectively. Serum total and HDM-specific IgE ELISA was done as previously described [14]. For Muc5ac ELISA, goat anti-Muc5ac (K20) polyclonal antibody (Santa Cruz Biotechnology) and biotinylated wheat germ agglutinin (Sigma) were used for capture and detection.

RT-PCR

The total RNA from mouse lung was digested with RNase-free DNase and purified using RNeasy MinElute Kit (QIAGEN). The reverse transcription was done with the Superscript II First-strand cDNA Synthesis Kit (Invitrogen) using random hexamers and the purified RNA. qPCR was performed using LightCycler 480 SYBR Green I Master according to the manufacturer's instructions (Roche Diagnostics) and the values were normalized to HPRT [19]. The primers used were listed in Table 1.

Western blot analysis

Western blot analysis was performed on lung homogenates using the following antibodies: CLCA3 (Abcam) and β-actin (Sigma). HRP-conjugated secondary antibodies were goat anti-rabbit and goat anti-mouse (Santa Cruz Biotechnology). Data were imaged using an LAS4000 imaging system (Fujifilm) and quantitated with Quantity One Software (Bio-Rad Laboratories).

EMSA

FVB/N nontransgenic and memIL-13Rα2 transgenic mice were given 1 μg of IL-13 (PeproTech) in 50 μl PBS intratracheally for 10 consecutive days. On day 15, the mice were given 50 μl of PBS or 1 μg of IL-13 in 50 μl PBS intratracheally and lungs were harvested at 1, 2, 4, 8, 16 and 24h after the treatment. Nuclear extract was isolated from lung tissue and EMSA was performed as previously described [11]. Five μg of nuclear extract was used for EMSA using ^{32}P-labeled STAT6 probe (Santa Cruz Biotechnology). The acquired digital images of EMSA were quantified using Quantity One (Bio-Rad Laboratories).

Statistical analysis

All values are expressed as mean ± SD. The data were analyzed with 2-tailed nonparametric Mann-Whitney test or 1-way ANOVA with Newman-Keuls' post test using Prism 5.0c for Mac OS X from GraphPad Software (San Diego, CA). A p value of <0.05 was considered statistically significant.

Results

HDM-induced AHR and airway inflammation were attenuated in IL-13Rα2-deficient mice

To assess the role of IL-13Rα2 in allergen-induced AHR and airway inflammation, we examined IL-13Rα2-deficient mice in an HDM-challenged model of asthma. BALB/c wild type and IL-13Rα2-deficient mice were sensitized twice intraperitoneally with 10 μg of HDM in PBS or PBS alone and then challenged twice intratracheally with 100 μg of HDM in PBS or PBS alone. Airway responsiveness was assessed by APTI 48 hours after the second challenge. HDM-treated IL-13Rα2-deficient mice showed decreased AHR, bronchoalveolar lavage (BAL) total cells and eosinophils compared to HDM-treated wild type mice (Fig.1A, 1B), suggesting that IL-13Rα2 contributes to allergen-induced AHR and airway inflammation in this model of allergic asthma.

AHR and airway inflammation were enhanced in HDM-treated memIL-13Rα2 lung transgenic mice

To elucidate the role of memIL-13Rα2 in allergen-induced AHR and airway inflammation, we generated memIL-13Rα2 lung epithelial cell-specific transgenic mice using rat CC10 promoter followed by memIL-13Rα2 cDNA and human GH intronic and polyadenylation sequences (Fig. E1A of Online Repository Materials). Several founder lines of FVB/N memIL-13Rα2 transgenic mice were obtained, which had various transgene copy numbers and mRNA expression levels. One founder line selected for our studies displayed high expression level with ~50 copy number of IL-13Rα2 transgene as estimated by the southern blot analysis (Fig. E1B of Online Repository Materials). Northern blot analysis of total RNA isolated from mouse lung tissue using a mouse IL-13Rα2 cDNA probe confirmed the lung expression of the memIL-13Rα2 transgene since the expression of endogenous IL-13α2 was not detected in nontransgenic mice (Fig. E1C of Online Repository Materials). RT-qPCR and ELISA showed that higher mRNA and protein expression levels of IL-13Rα2 in lungs from transgenic mice (Fig. E1D, E1E of Online Repository Materials).

The memIL-13Rα2 transgenic and nontransgenic mice were sensitized and challenged with PBS or HDM as described above. Airway responsiveness was assessed by APTI 24 hours after the second challenge. HDM-treated transgenic mice displayed increased AHR compared to HDM-treated nontransgenic mice (Fig. 2A). HDM-treated transgenic mice also showed increased BAL total cells and eosinophils compared to the HDM-treated nontransgenic mice but no difference in numbers of macrophages, neutrophils and lymphocytes (Fig. 2B). No significant difference was observed in lung mucus production and collagen deposition between HDM-treated transgenic mice and HDM-treated nontransgenic mice assessed by PAS staining and Masson's Trichrome staining (data not shown). We determine the expression of several cytokines and chemokines (including IL-4, Eotaxin-1, MCP-1, MCP-2, MCP-3, MCP-5, MIP-1β, MIP-2 and TARC) by RT-qPCR, but did not observe difference in expression of genes tested (data not shown).

To further study the function of memIL-13Rα2, we crossed the memIL-13Rα2 lung transgenic mice with the IL-13Rα2-deficient mice to generate mice that expressed exclusively memIL-13Rα2 only in the lung epithelium. Mice were then studied in the asthma model outlined above. Twenty-four hours after the second challenge, airway responsiveness was measured by APTI. The IL-13Rα2-deficient mice displayed decreased AHR compared to the wild type mice. Lung overexpression of memIL-13Rα2 in the IL-13Rα2-deficient mice restored allergen-induced AHR to levels observed in wild type mice (Fig. 2C). Serum total IgE level was increased in IL-13Rα2-deficient mice but overexpression of memIL-13Rα2 had no effect on the total IgE level before and after HDM treatment (Fig. 2D). IL-13Rα2 deficiency had no effect on serum HDM-specific IgE levels

(Fig. E2 of Online Repository Materials). Lung H&E staining showed decreased cellular infiltration around airways in HDM-treated IL-13Rα2-deficient mice and overexpression of memIL-13Rα2 increased the cellular infiltration (Fig. 3). PAS staining showed increased mucus production in airways after HDM treatment (Fig. 4A). Quantification of mucus production using western blot analysis for CLCA3 production showed decreased expression of CLCA3 in lungs of HDM-treated IL-13Rα2-deficient mice compared to HDM-treated wild type mice. Overexpression of memIL-13Rα2 increased the expression of CLCA3 (Fig. 4B, 4C). RT-qPCR and ELISA showed decreased expression of Muc5ac in lungs of HDM-treated IL-13Rα2-deficient mice compared to HDM-treated wild type mice and overexpression of memIL-13Rα2 increased the expression of Muc5ac (Fig. 4D, 4E). We determined the expression of several cytokines and chemokines in the lungs. As shown in Fig 5, there was no difference in expression of Th2 cytokines IL-13 and IL-4. The expression of IFNγ and eotaxin was increased in HDM-treated TG/KO mice. No difference in expression of MDC and TARC was observed among the HDM-treated groups. Thus, memIL-13Rα2 contributes to the development of AHR in allergen-exposed mice, suggesting a distinct role of membrane form of IL-13Rα2 in the development of AHR.

Levels of sIL-13Rα2 were unchanged in memIL-13Rα2 lung transgenic mice

Since sIL-13Rα2 inhibits IL-13 responses and it can be generated from cleavage of memIL-13Rα2, we determined the potential effect of memIL-13Rα2 overexpression on the levels of sIL-13Rα2. As shown in Fig E3 of Online Repository Materials, sIL-13Rα2 was absent in the serum from memIL-13Rα2 lung transgenic/IL-13Rα2-deficient mice and a significantly increased level of sIL-13Rα2 in BALF was only observed in HDM-treated wild type mice, suggesting that sIL-13Rα2 in BALF was derived exclusively from the sIL-13Rα2 transcript and overexpression of memIL-13Rα2 did not alter the level of sIL-13Rα2 in mouse lung and serum. The levels of total sIL-13Rα2 and IL-13-bound sIL-13Rα2 in serum and BALF from nontransgenic mice and memIL-13Rα2 transgenic mice were similar at baseline and after HDM challenge (Fig. E4 of Online Repository Materials). These data suggest that overexpression of memIL-13Rα2 had no effect on the levels of sIL-13Rα2.

Expression of IL-13Rα1 and IL-4Rα was unaffected in memIL-13Rα2 transgenic mice

Since IL-13 responses are also affected by IL-4Rα and IL-13Rα1, we next determined if alterations in the expression of IL-13Rα1 or IL-4Rα were occurring secondary to overexpression of memIL-13Rα2 transgene or in response to HDM. As shown in Fig. E5A-E5C of Online Repository Materials, expression of IL-13Rα1 and IL-4Rα were largely unaffected by the presence of memIL-13Rα2-hGH transgene and by treatment with HDM in this mouse model of asthma. As shown in Fig. E5D of Online Repository Materials, memIL-13Rα2 transgenic mice had much higher ratio of IL-13Rα2 to IL-13Rα1 compared to the nontransgenic mice, suggesting that the expression level of memIL-13Rα2 may determine the impact on IL-13 responses during allergic inflammation.

IL-13-induced STAT6 activation was unaffected in lungs from memIL-13Rα2 transgenic mice

In order to elucidate potential mechanisms underlying enhanced HDM-induced AHR and airway inflammation in memIL-13Rα2 transgenic mice, we examined STAT6 activation in the lungs of the mice in response to IL-13 treatment. The mice were given 1 μg of IL-13 daily from day 1 to day 10. On day 15, the mice were given a single dose of 1 μg of IL-13 and the STAT6 activation was determined by EMSA. No statistical difference in STAT6 activation in lungs from IL-13-treated non-transgenic mice and IL-13-treated memIL-13Rα2 transgenic mice was observed under this condition (Fig. E6 of Online Repository Materials).

Discussion

Mouse IL-13Rα2 exists in soluble and membrane forms (sIL-13Rα2 and memIL-13Rα2), encoded by distinct transcripts generated by alternative splicing [21]. In contrast, sIL-13Rα2 is undetectable in human plasma and has a limited role in humans [1920]. Herein, we examined the role of IL-13Rα2 in allergen-induced asthma using IL-13Rα2-deficient mice and memIL-13Rα2 lung transgenic mice. HDM-induced AHR and airway inflammation were attenuated in IL-13Rα2-deficient mice and overexpression of memIL-13Rα2 in the lungs of these mice reconstituted the phenotype. Thus, memIL-13Rα2 has a contributory role in allergen-induced AHR and airway inflammation. Our data showed that the expression level of IL-13Rα2 affected mucus production in the airways as the the expression of CLCA3 was decreased in lungs of IL-13Rα2-deficient mice. We did not observe significant change in expression of several cytokines and chemokines in lung in wild type, IL-13Rα2-deficient and memIL-13Rα2-transgenic mice in our models of asthma. It is likely that the change in expression of these genes precedes the effect on AHR and airway inflammation.

Previous studies done in mice have been complicated by the presence of both sIL-13Rα2 and memIL-13Rα2, making it impossible to assess the role of each form independently. sIL-13Rα2 has been shown to act as a decoy receptor in studies of mouse models of asthma following administration of sIL-13Rα2. The effect of endogenous sIL-13Rα2 on allergen-induced airway inflammation has not been studied. In our mouse models of asthma, AHR and airway inflammation are attenuated in IL-13Rα2-deficient mice, indicating that under these conditions, deficiency of memIL-13Rα2 plays a major role compared to deficiency of sIL-13Rα2. Mice have large quantities of sIL-13Rα2 in their serum even at baseline, while humans lack sIL-13Rα2 [19, 20]. Soluble IL-13Rα2 has been measured at nanogram levels in mouse serum, up to 10 times higher molar concentrations than IL-13 [9, 28]. Thus, the amount of sIL-13Rα2 is high enough to inhibit IL-13 signaling in serum and suggests that sIL-13Rα2 is a major modifier of IL-13 activity and allergic inflammation in mice *in vivo*. Thus, conclusions from IL-13 studies done in mice may not be fully applicable to humans that have no sIL-13Rα2.

IL-13-induced TGFβ-dependent fibrosis has been shown to be promoted by IL-13Rα2, suggesting that IL-13Rα2 may have some signaling roles [16]. One report showed that in intestinal epithelial cells from ulcerative colitis or colorectal cancer patients, IL-13Rα2 can inhibit STAT6 signaling or initiate MAPK signaling depending on relative expression of IL-13Rα2 and the local concentration of IL-13 [19].

The levels of sIL-13Rα2 was similar in memIL-13Rα2 transgenic and nontransgenic mice, indicating sIL-13Rα2 did not play a role in the enhanced HDM-induced AHR and airway inflammation in memIL-13Rα2 transgenic mice. We observed that in the absence of the endogenous IL-13Rα2 gene, lung overexpression of memIL-13Rα2 did not yield appreciable sIL-13Rα2 in the BALF. These data support that sIL-13Rα2 detected in memIL-13Rα2 transgenic and nontransgenic mice is coming predominantly from the soluble form transcript that is absent in memIL-13Rα2 transgenic/IL-13Rα2-deficient mice. There was no appreciable cleavage of memIL-13Rα2 in this model.

Recent evidence reveals that the relative expression levels of IL-4Rα, IL-13Rα1, and γc are critical for determining the IL-4 and IL-13 sensitivities of various cell types [29], however, the effect of IL-13Rα2 expression level remains unclear. IL-13 has been postulated to be an “effector” cytokine while IL-4 is an “immunoregulatory” cytokine, which is at least in part due to the distribution of IL-4 and IL-13 receptors on relevant immunoregulatory and effector cells [29]. Our data showed that the transgenic mice expressed high levels of memIL-13Rα2 while IL-4Rα and IL-13Rα1 expression did not change, suggesting that the

effect of overexpression of memIL-13Rα2 on allergen-induced AHR and airway inflammation is due to enhanced signaling through memIL-13Rα2 rather than IL-4Rα and IL-13Rα1.

In summary, our data demonstrate that memIL-13Rα2 can contribute to allergen-induced AHR and airway inflammation. IL-13 is an attractive target for therapeutic intervention in allergic inflammation and asthma. Anti-IL-13 monoclonal antibody is being evaluated in a clinical trial to treat asthma (ClinicalTrials.gov Identifier: NCT01402986). Delineation of the roles of membrane versus soluble IL-13Rα2 will enable the design of the most effective targeted therapies. The development of humanized mouse models that express exclusively membrane IL-13Rα2 would be most optimal for study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AHR	airway hyperresponsiveness
APTI	airway pressure time index
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
CC10	Clara cell 10-kDa
GH	growth hormone
HDM	house dust mite
IL-13Rα1	IL-13 receptor alpha 1
IL-13Rα2	IL-13 receptor alpha 2
IL-4Rα	IL-4 receptor alpha
memIL-13Rα2	membrane form of IL-13Rα2
qPCR	quantitative PCR
sIL-13Rα2	soluble form of IL-13Rα2

Key Messages

- memIL-13Rα2 has a distinct role in lung and contributes to the development of allergic inflammation.
- Models that better reflect human IL-13Rα2 biology are needed since the absence of sIL-13Rα2 in human plasma supports the importance of memIL-13Rα2 in human immunity.
- These data may enable novel asthma therapeutics targeting airway epithelial cells.

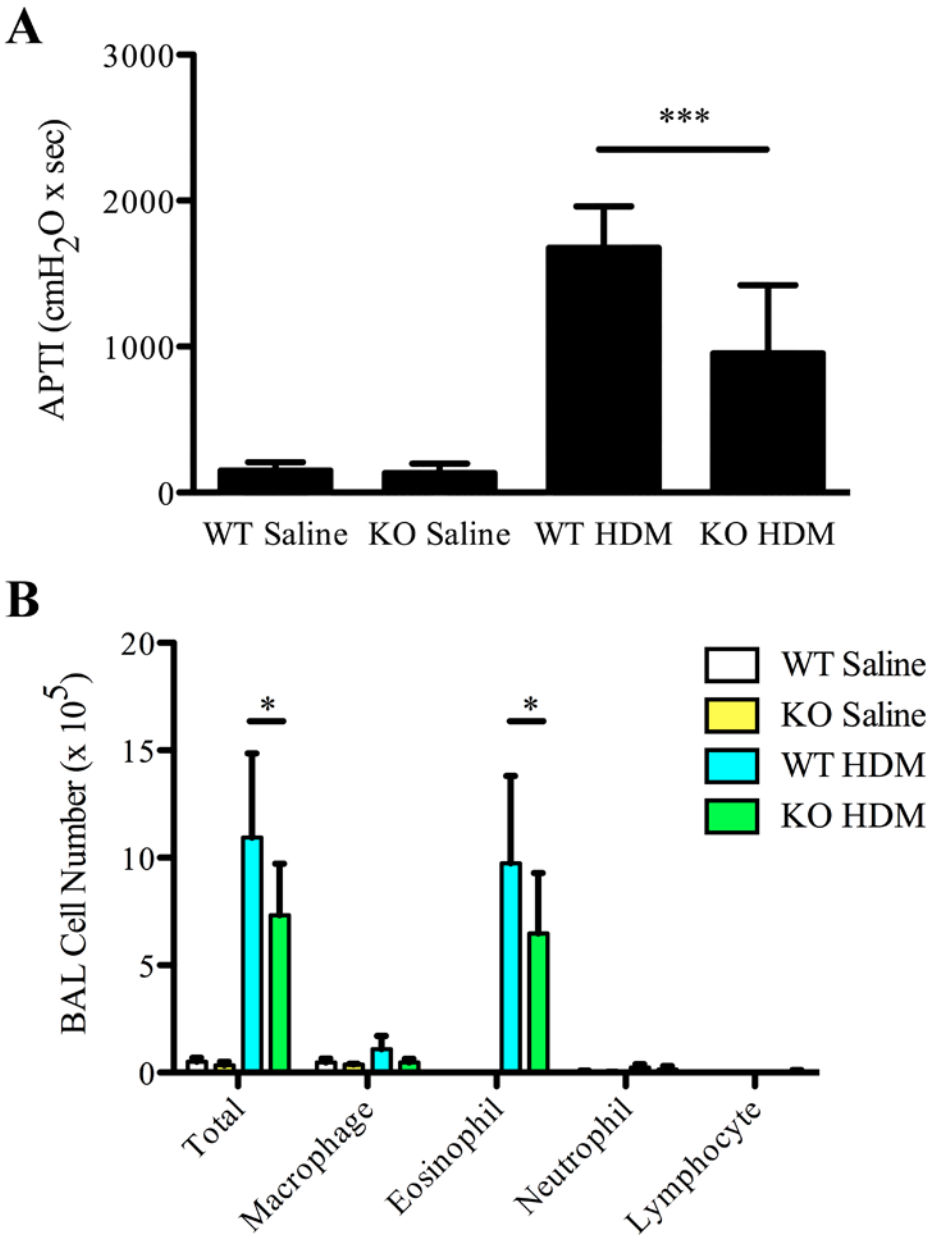

Figure 1. HDM-induced AHR and airway inflammation in wild type and IL-13Rα2-deficient mice

The data are shown as mean±SD. *, p<0.05; **, p<0.01; ***, p<0.001. (A) APTI of PBS or HDM-treated wild type and IL-13Rα2-deficient mice (n=8-10). (B) Total cells and eosinophils in BAL samples of PBS or HDM-treated wild type and IL-13Rα2-deficient mice (n=8-10). WT, wild type; KO, IL-13Rα2-deficient.

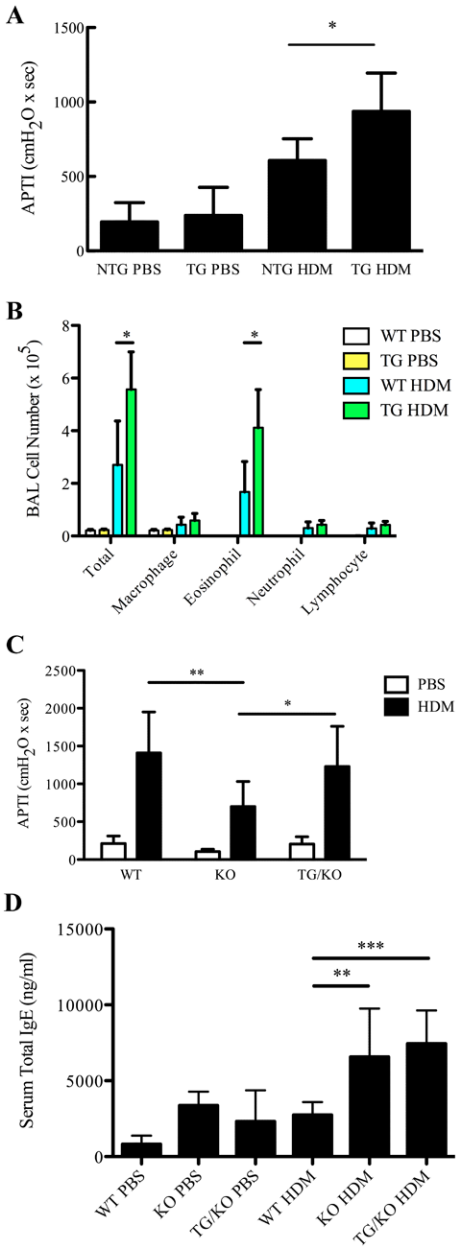

Figure 2. HDM-induced AHR and airway inflammation in nontransgenic and memIL-13Rα2 transgenic mice

Mice were sensitized and challenged with PBS or HDM. The data are shown as mean±SD. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. (A) APTI of PBS or HDM-treated nontransgenic mice and memIL-13Rα2 transgenic mice (n=4-5). (B) Total cells and eosinophils in BAL samples from PBS or HDM-treated nontransgenic mice and memIL-13Rα2 transgenic mice (n=4-5). (C) APTI of PBS or HDM-treated wild type, IL-13Rα2-deficient and memIL-13Rα2 transgenic/IL-13Rα2-deficient mice (n=5-8). (D) Serum total IgE levels in PBS or HDM-treated wild type, IL-13Rα2-deficient and memIL-13Rα2 transgenic/IL-13Rα2-deficient mice (n=5-8). NTG, nontransgenic; TG, memIL-13Rα2 transgenic; WT, wild type; KO, IL-13Rα2-deficient; TG/KO, memIL-13Rα2 transgenic/ IL-13Rα2-deficient.

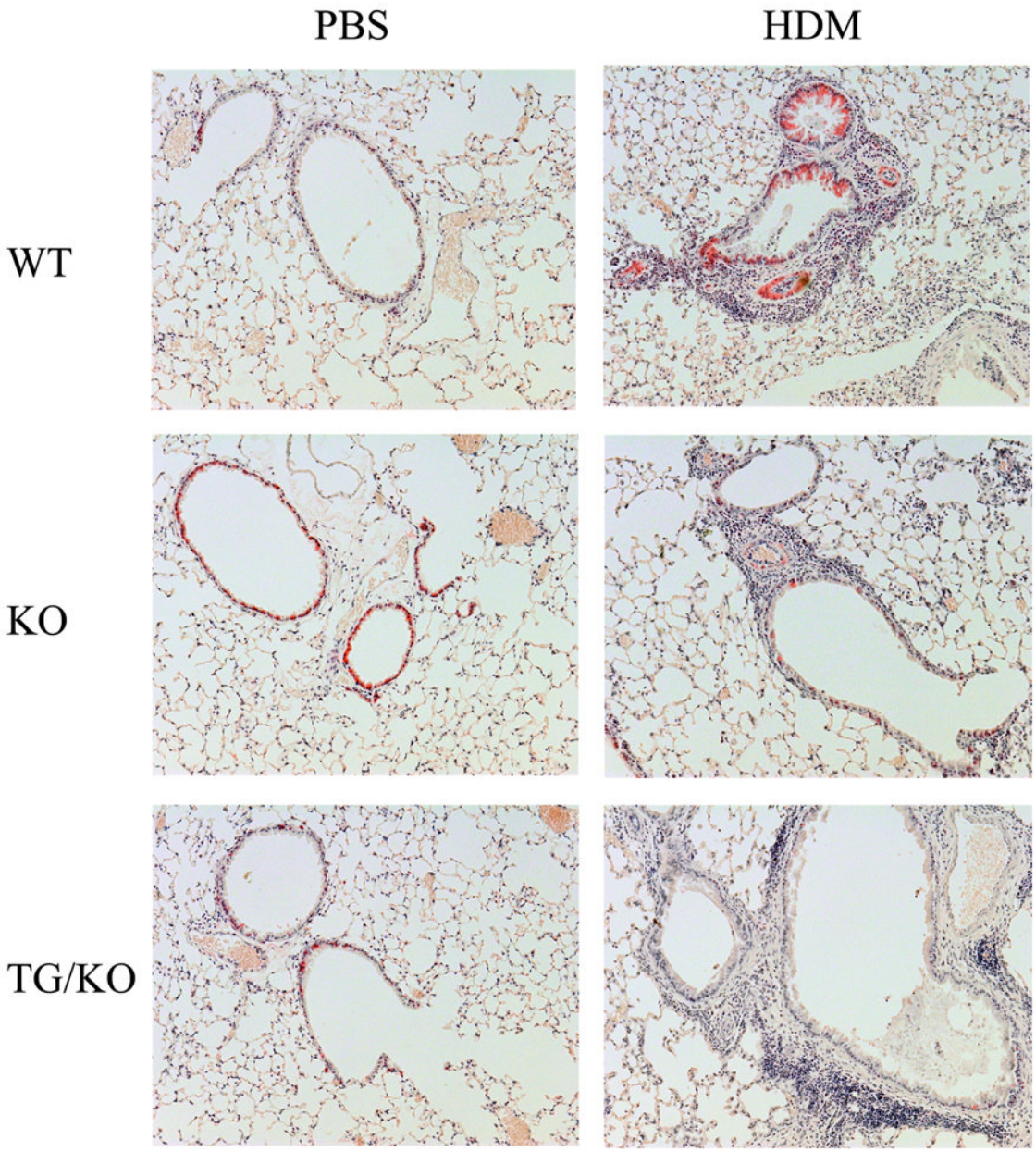

Figure 3. H&E staining of lung sections from PBS or HDM-treated wild type, IL-13Rα2-deficient and memIL-13Rα2 transgenic/IL-13Rα2-deficient mice

NTG, nontransgenic; TG, memIL-13Rα2 transgenic; WT, wild type; KO, IL-13Rα2-deficient; TG/KO, memIL-13Rα2 transgenic/ IL-13Rα2-deficient.

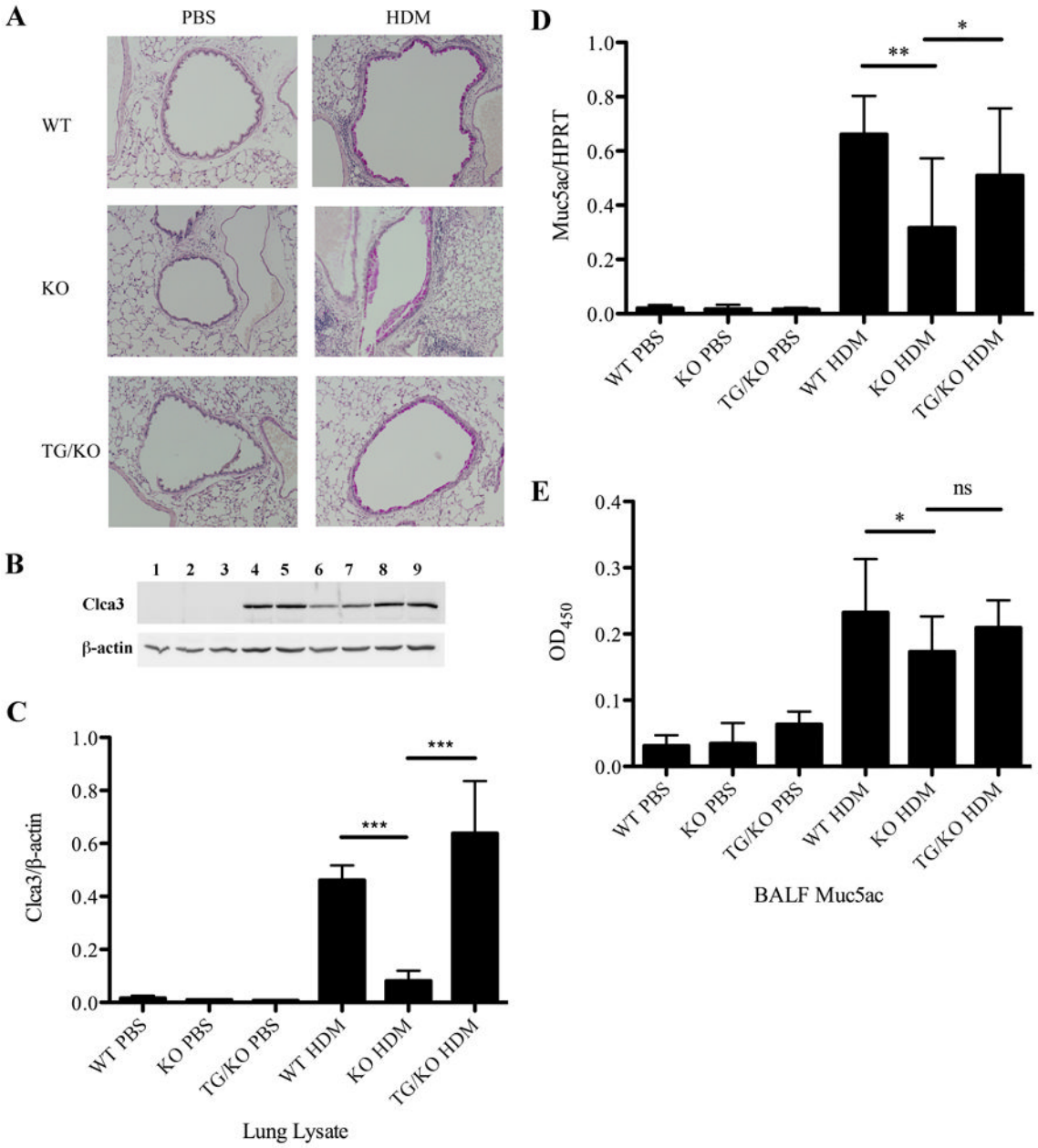

Figure 4. Lung mucus production in HDM-treated wild type, IL-13Rα2-deficient and memIL-13Rα2 transgenic/IL-13Rα2-deficient mice

(A) PAS staining of lung sections. (B-C) Western blot analysis and quantification of CLCA3 expression in lung homogenates (n=3). Lane 1: WT PBS; Lane 2: KO PBS, Lane 3: TG/KO PBS; Lane 4-5: WT HDM; Lane 6-7: KO HDM; Lane 8-9: TG/KO HDM. (D) mRNA expression of Muc5ac in lungs (n=9-10). (E) Muc5ac levels in BALF (n=9-10). TG, memIL-13Rα2 transgenic; WT, wild type; KO, IL-13Rα2-deficient; TG/KO, memIL-13Rα2 transgenic/ IL-13Rα2-deficient. Data are shown as mean±SD. ns, not significant; *, $p<0.05$; **, $p<0.01$.

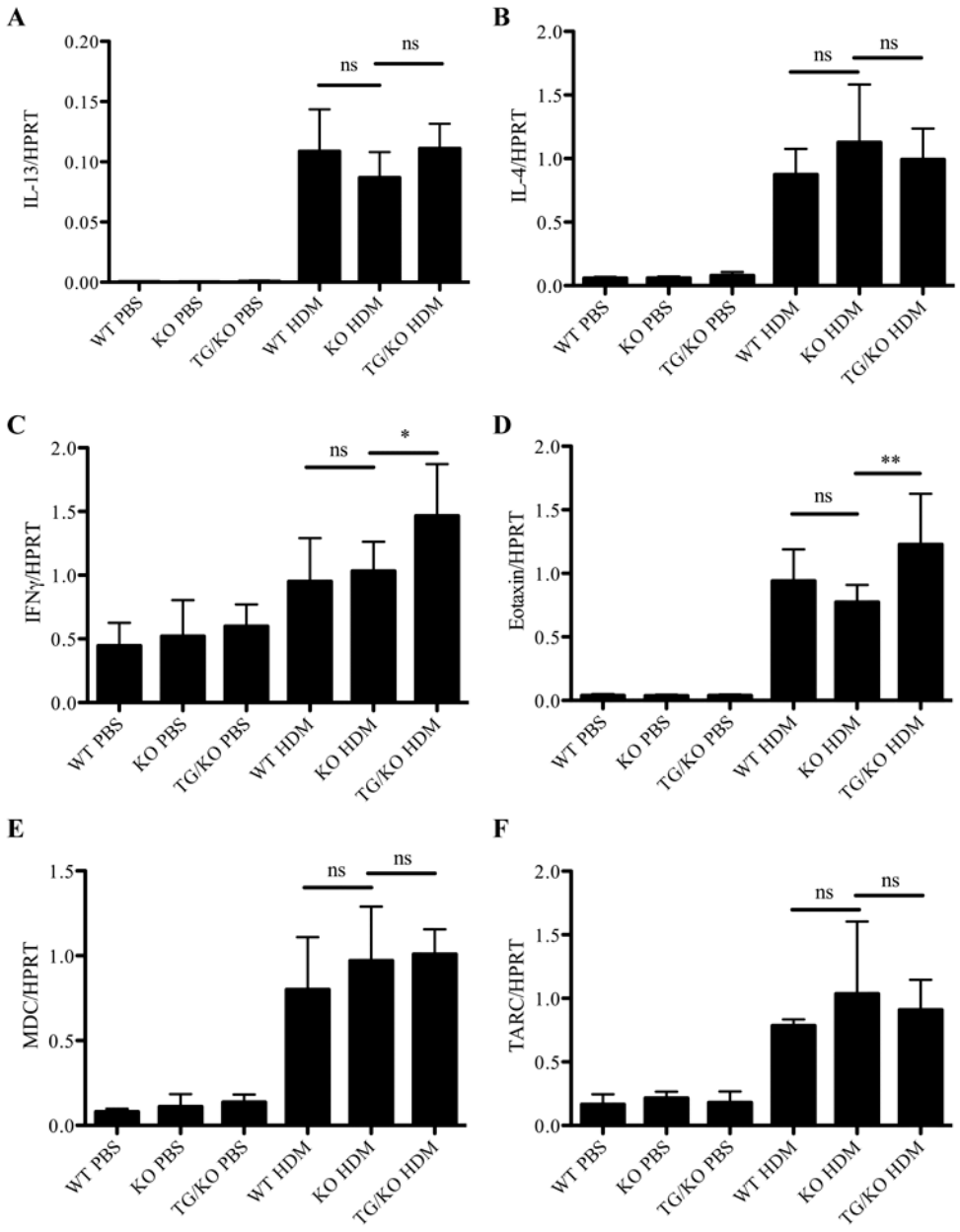

Figure 5. Lung mRNA expression of cytokines and chemokines in production in HDM-treated wild type, IL-13Rα2-deficient and memIL-13Rα2 transgenic/IL-13Rα2-deficient mice

(A) IL-13. (B) IL-4. (C) IFNγ. (D) Eotaxin. (E) MDC. (F) TARC. Data are shown as mean ±SD (n=4-6). ns, not significant; *, p<0.05; **, p<0.01; ***, p<0.001.

Table 1

Primers for qPCR

Primer	Sequence
mHPRT Forward	TGCCGAGGATTTGGAAAAAG
mHPRT Reverse	CCCCCCCTTGAGCACACAG
mIL-13Rα2 Forward	GGACTCATCAGACTATAAAGATT
mIL-13Rα2 Reverse	GTGTGCTCCATTTCATTCTA
mIL-13Rα1 Forward	TAATACTCAAACCGACCGACATAAT
mIL-13Rα1 Reverse	CTTCACTCCAATCACTCCAC
mIL-4Rα Forward	TCTGCATCCCGTTGTTTTGC
mIL-4Rα Reverse	GCACCTGTGCATCCTGAATG
hGH Forward	AAC AGG GAG GAA ACA CAA C
hGH Reverse	CAG CCA CGA CTG GAT GA
mIL-13 Forward	GCTTGCCTTGGTGGTCTCGCC
mIL-13 Reverse	GGGCTACACAGAACCCGCCA
mIL-4 Forward	CTGTAGGGCTTCCAAGGTGCTTCG
mIL-4 Reverse	CCATTTGCATGATGATGCTCTTTAGGC
mIFNγ Forward	CAGCAACAGCAAGGCGAAAAAGG
mIFNγ Reverse	TTTCCGCTTCCTGAGGCTGGAT
mEotaxin Forward	GAATCACCAACAACAGATGCAC
mEotaxin Reverse	ATCCTGGACCCACTTCTTCTT
mMDC Forward	CTTCTTGCTGTGGCAATTC
mMDC Reverse	TGATGGCAGAGGGTGAC
mTARC Forward	AGTTGGTGAGCTGGTATAAG
mTARC Reverse	TGTTGAAACCATGGACAGCA
mMuc5ac Forward	ACTGTTACTATGCGATGTGTAGCCA
mMuc5ac Reverse	GAGGAAACACATTGCACCGA