

IL-13 receptor $\alpha 2$ contributes to development of experimental allergic asthma

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Background: IL-13 receptor $\alpha 2$ (IL-13R $\alpha 2$) binds IL-13 with high affinity and modulates IL-13 responses. There are soluble and membrane forms of IL-13R $\alpha 2$ generated by alternative splicing in mice, but human subjects express only the membrane form of IL-13R $\alpha 2$ (memIL-13R $\alpha 2$).

Objective: We determined the role of memIL-13R $\alpha 2$ in the development of allergic inflammation in mouse models of asthma.

Methods: IL-13R $\alpha 2$ -deficient and memIL-13R $\alpha 2$ lung epithelium-specific transgenic mice were challenged with house dust mite (HDM). Airway hyperresponsiveness (AHR) and inflammation were assessed based on the airway pressure-time index, bronchoalveolar lavage (BAL) cell counts, and lung histology. Mucus production was determined by means of periodic acid-Schiff 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. The expression of cytokines and chemokines was determined by using RT-quantitative PCR.

Results: In IL-13R $\alpha 2$ -deficient mice AHR and airway inflammation were attenuated compared with levels seen in

wild-type mice after HDM challenge. Lung epithelial overexpression of memIL-13R $\alpha 2$ in the IL-13R $\alpha 2$ -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-13R $\alpha 2$ -deficient mice, whereas lung epithelial overexpression of memIL-13R $\alpha 2$ increased mucus production. Lung epithelial overexpression of memIL-13R $\alpha 2$ had no effect on levels of the soluble form of IL-13R $\alpha 2$ in serum or BAL fluid and did not affect IL-13-dependent signal transducer and activator of transcription 6 activation in the lungs.

Conclusion: These data collectively support a distinct role for memIL-13R $\alpha 2$ in the lung and suggest that memIL-13R $\alpha 2$ might contribute to allergic inflammation. (J Allergy Clin Immunol 2013;■■■■:■■■-■■■.)

Key words: IL-13, IL-13 receptor, lung, airway hyperresponsiveness, airway inflammation

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

The precise role of IL-13R $\alpha 2$ in IL-13 signaling and responses is unclear. Characterization of IL-13R $\alpha 2$ -deficient mice revealed that these mice have increased IgE levels, which is consistent with a role of IL-13R $\alpha 2$ as a decoy receptor.⁹ The decoy role for IL-13R $\alpha 2$ is further supported by the finding that a soluble form of IL-13R $\alpha 2$ (sIL-13R $\alpha 2$) exists *in vivo*, and administration of sIL-13R $\alpha 2$ 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-13R $\alpha 2$ downregulates 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-13R $\alpha 2$ -deficient mice compared with those seen in wild-type mice.¹⁴ IL-13 activity is enhanced in IL-13R $\alpha 2$ /IL-10 double-deficient mice compared with IL-10-deficient mice.¹⁵ These observations indicate

Abbreviations used

AHR:	Airway hyperresponsiveness
APTI:	Airway pressure-time index
BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
CLCA3:	Chloride channel calcium activated 3
EMSA:	Electrophoretic mobility shift assay
HDM:	House dust mite
hGH:	Human growth hormone
IL-4R α :	IL-4 receptor α
IL-13R α 1:	IL-13 receptor α 1
IL-13R α 2:	IL-13 receptor α 2
MCP:	Monocyte chemoattractant protein
memIL-13R α 2:	Membrane form of IL-13R α 2
PAS:	Periodic acid-Schiff
qPCR:	Quantitative PCR
rCC10:	Rat Clara cell 10 kDa
sIL-13R α 2:	Soluble form of IL-13R α 2
STAT6:	Signal transducer and activator of transcription 6

that IL-13R α 2 functions as a decoy receptor in IL-13–signaling pathways in mice.

Although IL-13R α 2 can act as a decoy receptor, it might contribute to IL-13 responses as well. Studies have shown that IL-13–induced TGF- β –dependent fibrosis is dependent on IL-13R α 2 through activation of the transcription factor activator protein 1.^{16,17} In human subjects studies have also supported a contributory signaling role for IL-13R α 2.¹⁸

A potential reason for the confusing findings involves the data from our group and others, which clearly demonstrate that human and murine IL-13R α 2 biology are markedly different.^{19,20} There are distinct membrane and soluble isoforms of IL-13R α 2 generated by means of alternative splicing in mice.²¹ Mice have nanogram quantities of sIL-13R α 2 in their sera, even at baseline, whereas human subjects lack sIL-13R α 2.^{19,20} Thus conclusions from IL-13 studies done in mice might not be fully applicable to human subjects. Human subjects express only the membrane form of IL-13R α 2 (memIL-13R α 2), which might have unique roles independent of sIL-13R α 2. In current studies we used IL-13R α 2–deficient mice and memIL-13R α 2 lung transgenic mice to elucidate the biological functions of IL-13R α 2 in the development of allergic asthma. Our data demonstrate that memIL-13R α 2 contributes to allergen-induced AHR and airway inflammation.

METHODS

Mice

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

Generation of memIL-13R α 2 transgenic mice

The pBluescript II KS+/rat Clara cell 10 kDa (rCC10)/mIL-13R α 2/human growth hormone (hGH) transgenic construct was generated from mouse memIL-13R α 2 cDNA and hGH intronic and polyadenylation sequences by using the rCC10 promoter for lung epithelial cell–specific expression.²² The rCC10 promoter was kindly provided by Dr Jeffrey Whitsett (Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio).²³ FVB/N IL-13R α 2 transgenic mice were generated by using standard

microinjection procedures in the transgenic core facility at the Cincinnati Children's Hospital Medical Center. IL-13R α 2–deficient mice were kindly provided by Dr Michael Grusby (Harvard School of Public Health, Boston, Mass).⁹ The FVB/N IL-13R α 2 lung transgenic mice were crossed to BALB/c IL-13R α 2–deficient mice to generate memIL-13R α 2 transgenic/IL-13R α 2–deficient mice.

Genotyping and Northern blotting

The memIL-13R α 2 transgenic mice were genotyped by using both Southern blotting and PCR. Southern blotting was performed with an hGH gene probe with AatII-digested genomic tail DNA from the mice. The memIL-13R α 2 transgenic mice were genotyped with the forward primer 5'-AGAGGACACAGGTGCCTACAGTTC-3' (in the 3' end of the rCC10 promoter) and the reverse primer 5'-AGCCTGGATAAGGGAATGGTTG-3' (in the 5' end of hGH gene). Total RNA was isolated from mouse lung tissue by using Trizol reagent (Invitrogen, Carlsbad, Calif), and Northern blot analysis was performed with a 1-kb IL-13R α 2 cDNA probe. The IL-13R α 2–deficient mice were genotyped by means of PCR by using the IL-13R α 2 forward primer 5'-CTGGGGGTGTTTCATGTAGACTT-3', the IL-13R α 2 reverse primer 5'-CTTCAATAATCATGTGGGTAAAC-3', and the neomycin cassette reverse primer 5'-CTCGTCAAGGCGATAGAAGGCGAT-3'.

Immunization protocol

For an acute asthma model, on days 0 and 7, mice were sensitized intraperitoneally with 10 μ g of house dust mite (HDM; Greer Laboratories, Lenoir, NC) in 100 μ L of PBS or an equivalent amount of PBS alone. On days 14 and 21, mice were challenged intratracheally with 100 μ g of HDM in 50 μ L of PBS or PBS alone.²¹ On days 22 or 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 (25 mg/kg). Acetylcholine (50 μ g/kg) was injected into the inferior vena cava, and changes in airway pressure were recorded and expressed as the airway pressure-time index (APTI).^{24,25}

Bronchoalveolar lavage and lung histology

After AHR measurement, blood samples were collected, and bronchoalveolar lavage (BAL) was performed. Total cell numbers and cell differential percentages were determined, as previously described.^{25,26} After completion of BAL, lungs were removed, fixed with 10% neutral formalin, and embedded in paraffin, and 5- μ m lung sections were cut. Hematoxylin and eosin and periodic acid-Schiff (PAS) staining were performed, as previously described.²⁶ Collagen staining was done with Accustain Trichrome Stains (Masson) from Sigma (St Louis, Mo). Histologic analysis was performed with the Nikon Eclipse 90i Microscope System (Nikon, Tokyo, Japan).

ELISA

IL-13R α 2 from bronchoalveolar lavage fluid (BALF), sera, and lung lysates was determined by using ELISA, as previously described.²¹ The IL-13 binding assay for IL-13R α 2 was performed, as previously described.²⁵ To prepare lung lysate, the lung tissue was minced in lysis buffer (10 mmol/L HEPES [pH 7.9], 10 mmol/L KCl, 0.1 mmol/L EDTA, 1.5 mmol/L MgCl₂, 0.2% Nonidet P-40, 1.0 mmol/L dithiothreitol, and 0.5 mmol/L phenylmethylsulfonyl fluoride), incubated on ice for 5 minutes, and centrifuged at 10,000g for 5 minutes, and the supernatant was collected as lung lysate. The protein concentration of lung lysate was determined by using the Protein Assay Kit (Bio-Rad Laboratories, Hercules, Calif). For free and IL-13–bound IL-13R α 2 assay, standards and samples were preincubated with 10 ng/mL mouse IL-13 (Pepro-Tech, Rocky Hill, NJ) at 37°C for 1 hour. Anti-mouse IL-13R α 2 polyclonal antibody (1 μ g/mL) and biotinylated anti-mouse IL-13 polyclonal antibody (0.5 μ g/mL) were used for capture and detection, respectively. Serum total and HDM-specific IgE ELISAs were performed, as previously described.¹⁴ For Muc5ac ELISA, goat anti-Muc5ac (K20) polyclonal antibody

TABLE I. Primers for qPCR

Primer	Sequence
mHPRT forward	TGCCGAGGATTGGAAAAAG
mHPRT reverse	CCCCCCTTGGACACACAG
mIL-13 α 2 forward	GGACTCATCAGACTATAAAGATT
mIL-13 α 2 reverse	GTGTGCTCCATTTCATTCTA
mIL-13 α 1 forward	TAATACTCAAACCGACCGACATAAT
mIL-13 α 1 reverse	CTTCACTCCAATCACTCCAC
mIL-4 α forward	TCTGCATCCCGTTGTTTTCG
mIL-4 α reverse	GCACCTGTGCATCCTGAATG
hGH forward	AACAGGGAGGAAACACAAC
hGH reverse	CAGCCACGACTGGATGA
mIL-13 forward	GCTTGCCTTGGTGGTCTCGCC
mIL-13 reverse	GGGTACACAGAACCCGCCA
mIL-4 forward	CTGTAGGGCTTCCAAGGTGCTTCG
mIL-4 reverse	CCATTTGCATGATGATGCTCTTTAGGC
mIFN- γ forward	CAGCAACAGCAAGGCGAAAAAGG
mIFN- γ reverse	TTCCGCTTCCTGAGGCTGGAT
mEotaxin forward	GAATCACCAACAACAGATGCAC
mEotaxin reverse	ATCCTGGACCCACTTCTCTT
mMDC forward	CTTCTTGCTGTGGCAATTC
mMDC reverse	TGATGGCAGAGGGTGAC
mTARC forward	AGTTGGTGAGCTGGTATAAG
mTARC reverse	TGTTGAAACCATGGACAGCA
mMuc5ac forward	ACTGTTACTATGCGATGTGTAGCCA
mMuc5ac reverse	GAGGAAACACATTGCACCGA

HPRT, Hypoxanthine-guanine phosphoribosyltransferase; *m*, murine; MDC, macrophage-derived chemokine; TARC, thymus and activation-regulated chemokine.

(Santa Cruz Biotechnology, Santa Cruz, Calif) and biotinylated wheat germ agglutinin (Sigma) were used for capture and detection.

RT-PCR

Total RNA from mouse lung was digested with RNase-free DNase and purified by using the RNeasy MinElute Kit (Qiagen, Hilden, Germany). Reverse transcription was done with the Superscript II First-strand cDNA Synthesis Kit (Invitrogen) by using random hexamers and purified RNA. Quantitative PCR (qPCR) was performed with the LightCycler 480 SYBR Green I Master (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions, and the values were normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT).¹⁹ The primers used are listed in Table I.

Western blot analysis

Western blot analysis was performed on lung homogenates with the following antibodies: chloride channel calcium activated 3 (CLCA3; Abcam, Cambridge, United Kingdom) and β -actin (Sigma). Horseradish peroxidase-conjugated secondary antibodies were goat anti-rabbit and goat anti-mouse (Santa Cruz Biotechnology). Data were imaged with an LAS4000 imaging system (Fujifilm) and quantitated with Quantity One Software (Bio-Rad Laboratories).

Electrophoretic mobility shift assay

FVB/N nontransgenic and memIL-13 α 2 transgenic mice were given 1 μ g of IL-13 (PeproTech) in 50 μ L of PBS intratracheally for 10 consecutive days. On day 15, the mice were given 50 μ L of PBS or 1 μ g of IL-13 in 50 μ L of PBS intratracheally, and lungs were harvested at 1, 2, 4, 8, 16, and 24 hours after treatment. Nuclear extract was isolated from lung tissue, and an electrophoretic mobility shift assay (EMSA) was performed, as previously described.¹¹ Five micrograms of nuclear extract was used for EMSA with ³²P-labeled signal transducer and activator of transcription 6 (STAT6) probe (Santa Cruz Biotechnology). The acquired digital images of EMSA were quantified by using Quantity One (Bio-Rad Laboratories).

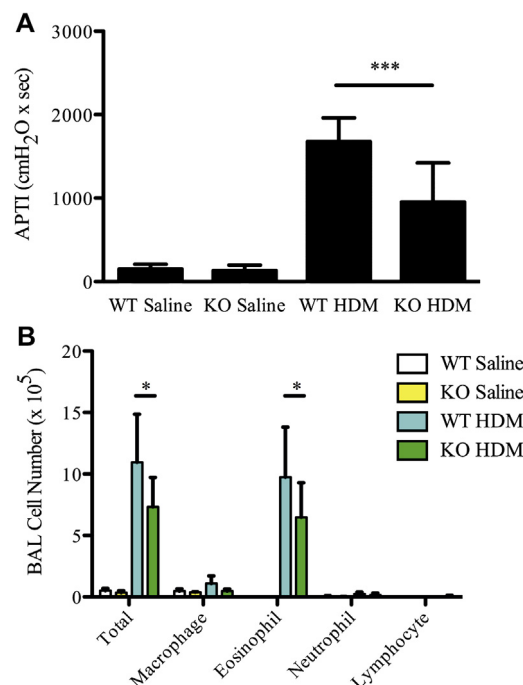


FIG 1. HDM-induced AHR and airway inflammation in wild-type and IL-13 α 2-deficient mice. Data are shown as means $\pm$ SDs. **A**, APTI of PBS- or HDM-treated wild-type and IL-13 α 2-deficient mice ($n = 8-10$). **B**, Total cells and eosinophils in BAL samples of PBS- or HDM-treated wild-type and IL-13 α 2-deficient mice ($n = 8-10$). KO, IL-13 α 2 deficient; WT, wild type. * $P < .05$ and *** $P < .001$.

Statistical analysis

All values are expressed as means $\pm$ SDs. Data were analyzed with the 2-tailed nonparametric Mann-Whitney test or 1-way ANOVA with the Newman-Keuls post test using Prism 5.0c for Mac OS X from GraphPad Software (San Diego, Calif). A P value of less than .05 was considered statistically significant.

RESULTS

HDM-induced AHR and airway inflammation were attenuated in IL-13 α 2-deficient mice

To assess the role of IL-13 α 2 in allergen-induced AHR and airway inflammation, we examined IL-13 α 2-deficient mice in an HDM-challenged model of asthma. BALB/c wild-type and IL-13 α 2-deficient mice were sensitized twice intraperitoneally with 10 μ g of HDM in PBS or PBS alone and then challenged twice intratracheally with 100 μ g of HDM in PBS or PBS alone. Airway responsiveness was assessed based on APTI 48 hours after the second challenge. HDM-treated IL-13 α 2-deficient mice showed decreased AHR, BAL total cell numbers, and eosinophil numbers compared with those seen in HDM-treated wild-type mice (Fig 1), suggesting that IL-13 α 2 contributes to allergen-induced AHR and airway inflammation in this model of allergic asthma.

AHR and airway inflammation were enhanced in HDM-treated memIL-13 α 2 lung transgenic mice

To elucidate the role of memIL-13 α 2 in allergen-induced AHR and airway inflammation, we generated memIL-13 α 2

lung epithelial cell-specific transgenic mice using rCC10 promoter followed by memIL-13R α 2 cDNA and hGH intronic and polyadenylation sequences (see Fig E1, A, in this article's Online Repository at www.jacionline.org). Several founder lines of FVB/N memIL-13R α 2 transgenic mice were obtained that had various transgene copy numbers and mRNA expression levels. One founder line selected for our studies displayed a high expression level with approximate 50 copy numbers of the IL-13R α 2 transgene, as estimated by using Southern blot analysis (see Fig E1, B). Northern blot analysis of total RNA isolated from mouse lung tissue with a mouse IL-13R α 2 cDNA probe confirmed lung expression of the memIL-13R α 2 transgene because the expression of endogenous IL-13R α 2 was not detected in nontransgenic mice (see Fig E1, C). RT-qPCR and ELISA showed higher mRNA and protein expression levels of IL-13R α 2 in lungs from transgenic mice (see Fig E1, D and E).

The memIL-13R α 2 transgenic and nontransgenic mice were sensitized and challenged with PBS or HDM, as described above. Airway responsiveness was assessed based on the APTI 24 hours after the second challenge. HDM-treated transgenic mice displayed increased AHR compared with HDM-treated nontransgenic mice (Fig 2, A). HDM-treated transgenic mice also showed increased BAL total cell and eosinophil numbers compared with those seen in the HDM-treated nontransgenic mice but showed no difference in macrophage, neutrophil, and lymphocyte numbers (Fig 2, B). No significant difference was observed in lung mucus production and collagen deposition between HDM-treated transgenic mice and HDM-treated nontransgenic mice assessed by using PAS staining and Masson Trichrome staining (data not shown). We determined the expression of several cytokines and chemokines (including IL-4, eotaxin-1, monocyte chemoattractant protein [MCP] 1, MCP-2, MCP-3, MCP-5, macrophage inflammatory proteins 1 β and 2, and thymus and activation-regulated chemokine) using RT-qPCR but did not observe a difference in expression of the tested genes (data not shown).

To further study the function of memIL-13R α 2, we crossed the memIL-13R α 2 lung transgenic mice with the IL-13R α 2-deficient mice to generate mice that expressed exclusively memIL-13R α 2 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 based on APTI. The IL-13R α 2-deficient mice displayed decreased AHR compared with the wild-type mice. Lung overexpression of memIL-13R α 2 in the IL-13R α 2-deficient mice restored allergen-induced AHR to levels observed in wild-type mice (Fig 2, C). Serum total IgE levels were increased in IL-13R α 2-deficient mice, but overexpression of memIL-13R α 2 had no effect on the total IgE levels before and after HDM treatment (Fig 2, D). IL-13R α 2 deficiency had no effect on serum HDM-specific IgE levels (see Fig E2 in this article's Online Repository at www.jacionline.org). Lung hematoxylin and eosin staining showed decreased cellular infiltration around the airways in HDM-treated IL-13R α 2-deficient mice, and overexpression of memIL-13R α 2 increased the cellular infiltration (Fig 3). PAS staining showed increased mucus production in the airways after HDM treatment (Fig 4, A). Quantification of mucus production by using Western blot analysis for CLCA3 production showed decreased expression of CLCA3 in the lungs of HDM-treated IL-13R α 2-deficient mice compared with HDM-treated wild-type mice. Overexpression of memIL-13R α 2 increased the expression of CLCA3 (Fig 4, B and C). RT-qPCR and ELISA showed decreased expression of Muc5ac in lungs of

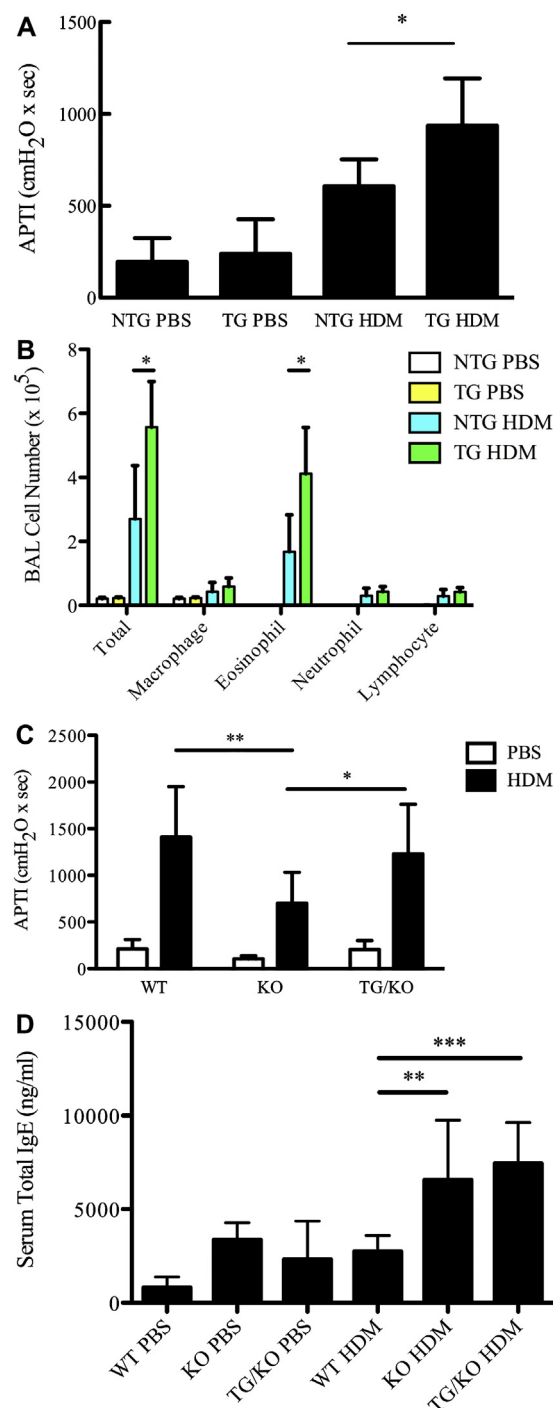


FIG 2. HDM-induced AHR and airway inflammation in nontransgenic and memIL-13R α 2 transgenic mice. Mice were sensitized and challenged with PBS or HDM. Data are shown as means $\pm$ SDs. **A**, APTI of PBS- or HDM-treated nontransgenic mice and memIL-13R α 2 transgenic mice ($n = 4-5$). **B**, Total cells and eosinophils in BAL samples from PBS- or HDM-treated nontransgenic mice and memIL-13R α 2 transgenic mice ($n = 4-5$). **C**, APTI of PBS- or HDM-treated wild-type, IL-13R α 2-deficient, and memIL-13R α 2 transgenic/IL-13R α 2-deficient mice ($n = 5-8$). **D**, Serum total IgE levels in PBS- or HDM-treated wild-type, IL-13R α 2-deficient, and memIL-13R α 2 transgenic/IL-13R α 2-deficient mice ($n = 5-8$). KO, IL-13R α 2-deficient; NTG, nontransgenic; TG, memIL-13R α 2 transgenic; TG/KO, memIL-13R α 2 transgenic/IL-13R α 2-deficient; WT, wild type. * $P < .05$, ** $P < .01$, and *** $P < .001$.

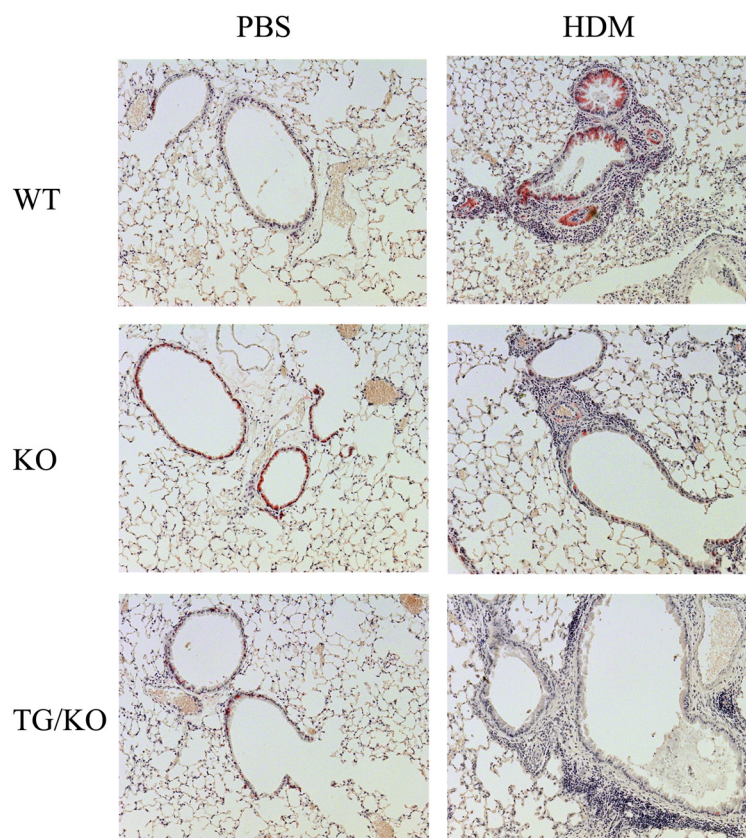


FIG 3. Hematoxylin and eosin staining of lung sections from PBS- or HDM-treated wild-type, IL-13R α 2-deficient, and memIL-13R α 2 transgenic/IL-13R α 2-deficient mice. KO, IL-13R α 2-deficient; TG/KO, memIL-13R α 2 transgenic/IL-13R α 2-deficient; WT, wild type.

HDM-treated IL-13R α 2-deficient mice compared with HDM-treated wild-type mice, and overexpression of memIL-13R α 2 increased expression of Muc5ac (Fig 4, D and E). We determined the expression of several cytokines and chemokines in the lungs. As shown in Fig 5, there was no difference in expression of the T_H2 cytokines IL-13 and IL-4. The expression of IFN- γ and eotaxin was increased in HDM-treated TG/KO mice. No difference in the expression of macrophage-derived chemokine and thymus and activation-regulated chemokine was observed among the HDM-treated groups. Thus memIL-13R α 2 contributes to the development of AHR in allergen-exposed mice, suggesting a distinct role of memIL-13R α 2 in the development of AHR.

sIL-13R α 2 levels were unchanged in memIL-13R α 2 lung transgenic mice

Because sIL-13R α 2 inhibits IL-13 responses and can be generated from cleavage of memIL-13R α 2, we determined the potential effect of memIL-13R α 2 overexpression on the levels of sIL-13R α 2. As shown in Fig E3 in this article's Online Repository at www.jacionline.org, sIL-13R α 2 was absent in the serum from memIL-13R α 2 lung transgenic/IL-13R α 2-deficient mice, and a significantly increased level of sIL-13R α 2 in BALF was only observed in HDM-treated wild-type mice, suggesting that sIL-13R α 2 in BALF was derived exclusively from the sIL-13R α 2 transcript and overexpression of memIL-13R α 2 did not alter the level of sIL-13R α 2 in mouse lung and serum. Total sIL-13R α 2 and IL-13-bound sIL-13R α 2 levels in serum and

BALF from nontransgenic mice and memIL-13R α 2 transgenic mice were similar at baseline and after HDM challenge (see Fig E4 in this article's Online Repository at www.jacionline.org). These data suggest that overexpression of memIL-13R α 2 had no effect on sIL-13R α 2 levels.

Expression of IL-13R α 1 and IL-4R α was unaffected in memIL-13R α 2 transgenic mice

Because IL-13 responses are also affected by IL-4R α and IL-13R α 1, we next determined whether alterations in expression of IL-13R α 1 or IL-4R α were occurring secondary to overexpression of the memIL-13R α 2 transgene or in response to HDM. As shown in Fig E5, A-C, in this article's Online Repository at www.jacionline.org, expression of IL-13R α 1 and IL-4R α was largely unaffected by the presence of the memIL-13R α 2-hGH transgene and by treatment with HDM in this mouse model of asthma. As shown in Fig E5, D, memIL-13R α 2 transgenic mice had a much higher ratio of IL-13R α 2 to IL-13R α 1 compared with nontransgenic mice, suggesting that the expression level of memIL-13R α 2 might determine the effect on IL-13 responses during allergic inflammation.

IL-13-induced STAT6 activation was unaffected in lungs from memIL-13R α 2 transgenic mice

To elucidate potential mechanisms underlying enhanced HDM-induced AHR and airway inflammation in memIL-13R α 2

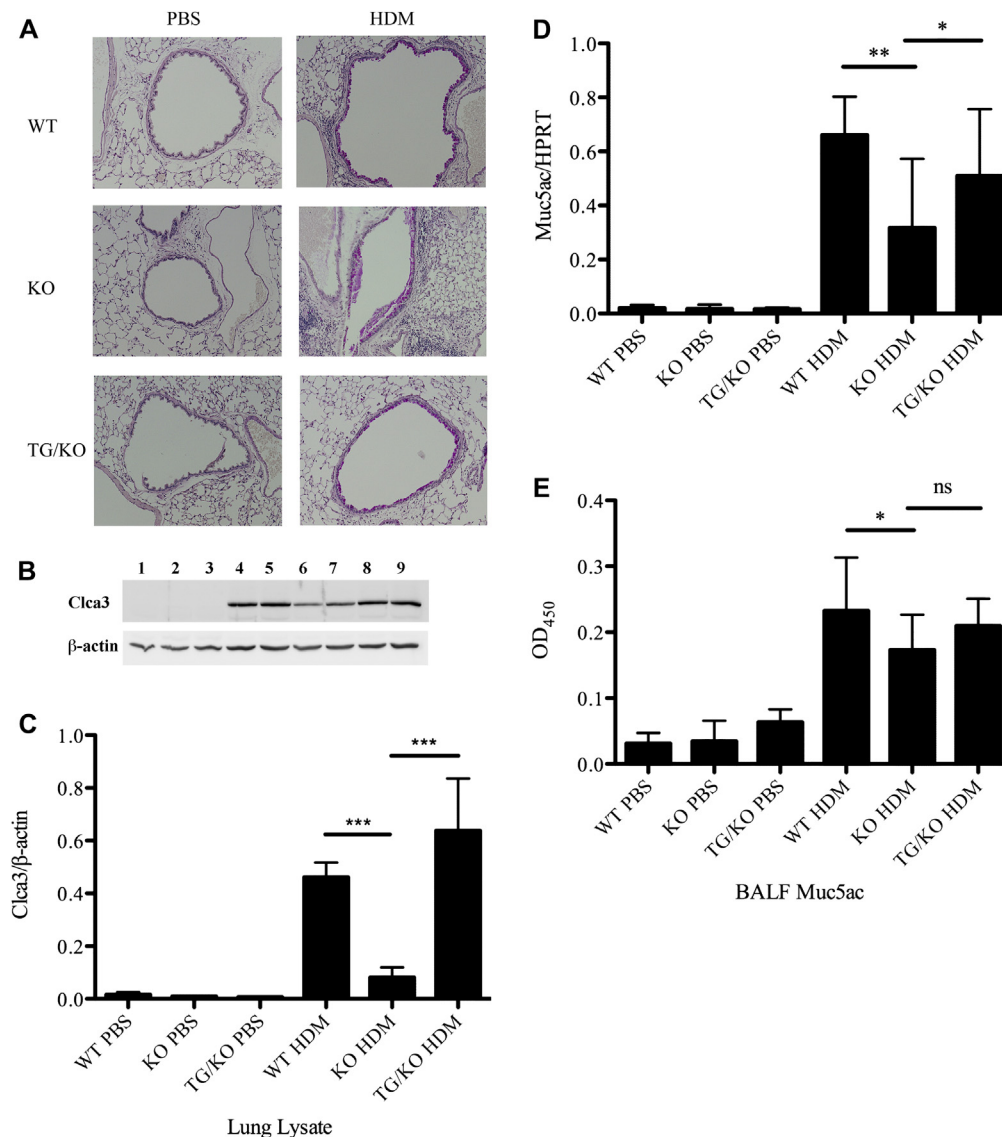


FIG 4. Lung mucus production in HDM-treated wild-type, IL-13R α 2-deficient, and memIL-13R α 2 transgenic/IL-13R α 2-deficient mice. **A**, PAS staining of lung sections. **B** and **C**, Western blot analysis and quantification of CLCA3 expression in lung homogenates ($n = 3$). Lane 1, WT PBS; lane 2, KO PBS; lane 3, TG/KO PBS; lanes 4 and 5, WT HDM; lanes 6 and 7, KO HDM; lanes 8 and 9, TG/KO HDM. **D**, mRNA expression of Muc5ac in lungs ($n = 9-10$). **E**, Muc5ac levels in BALF ($n = 9-10$). KO, IL-13R α 2-deficient; ns, not significant; TG/KO, memIL-13R α 2 transgenic/IL-13R α 2-deficient; WT, wild type. Data are shown as means $\pm$ SDs. * $P < .05$, ** $P < .01$, and *** $P < .001$.

transgenic mice, we examined STAT6 activation in the lungs of the mice in response to IL-13 treatment. The mice were given 1 μ g of IL-13 daily from days 1 to 10. On day 15, the mice were given a single dose of 1 μ g of IL-13, and STAT6 activation was determined by using EMSA. No statistical difference in STAT6 activation in lungs from IL-13-treated nontransgenic mice and IL-13-treated memIL-13R α 2 transgenic mice was observed under this condition (see Fig E6 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Mouse IL-13R α 2 exists in soluble and membrane forms (sIL-13R α 2 and memIL-13R α 2) encoded by distinct transcripts

generated by means of alternative splicing.²¹ In contrast, sIL-13R α 2 is undetectable in human plasma and has a limited role in human subjects.^{19,20} Herein we examined the role of IL-13R α 2 in mice with allergen-induced asthma by using IL-13R α 2-deficient mice and memIL-13R α 2 lung transgenic mice. HDM-induced AHR and airway inflammation were attenuated in IL-13R α 2-deficient mice, and overexpression of memIL-13R α 2 in the lungs of these mice reconstituted the phenotype. Thus memIL-13R α 2 has a contributory role in allergen-induced AHR and airway inflammation. Our data showed that the expression level of IL-13R α 2 affected mucus production in the airways because the expression of CLCA3 was decreased in lungs of IL-13R α 2-deficient mice. We did not observe a significant change in expression of several cytokines and chemokines in

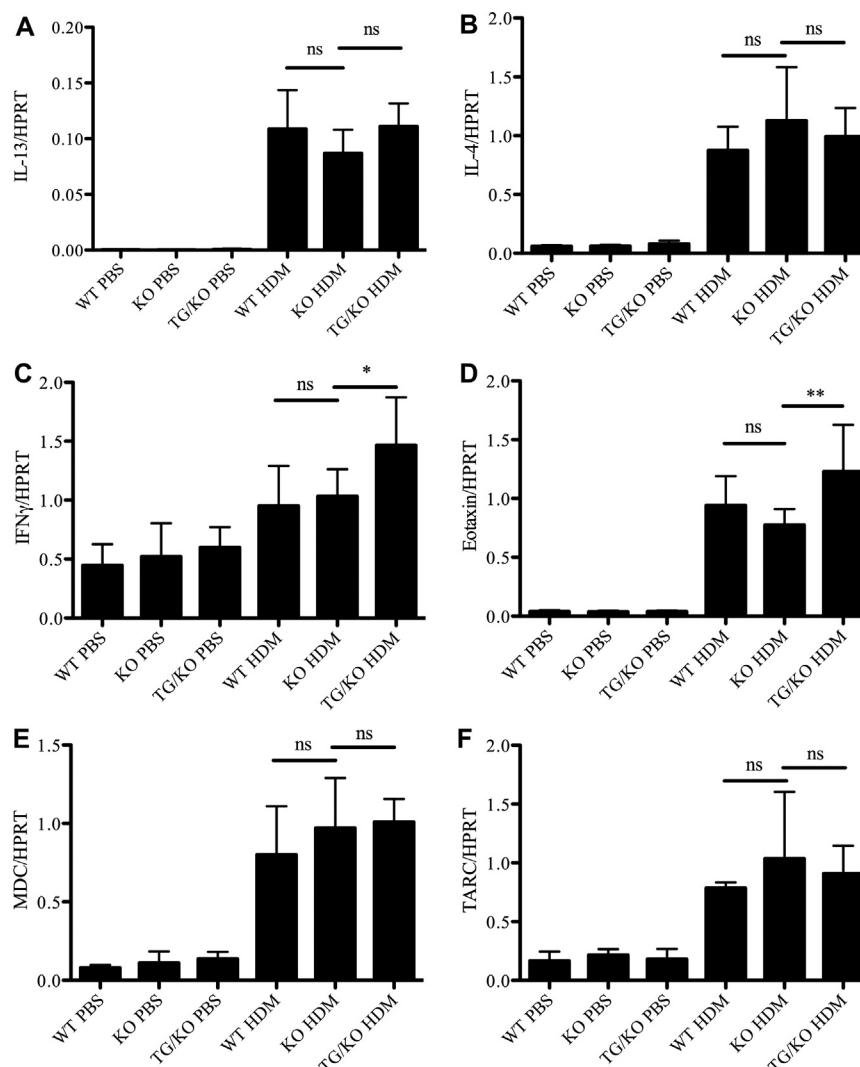


FIG 5. Lung mRNA expression of cytokines and chemokines in production in HDM-treated wild-type, IL-13R α 2-deficient, and memIL-13R α 2 transgenic/IL-13R α 2-deficient mice. **A**, IL-13. **B**, IL-4. **C**, IFN- γ . **D**, Eotaxin. **E**, Macrophage-derived chemokine (MDC). **F**, Thymus and activation-regulated chemokine (TARC). Data are shown as means $\pm$ SDs (n = 4-6). KO, IL-13R α 2-deficient; ns, not significant; TG/KO, memIL-13R α 2 transgenic/IL-13R α 2-deficient; WT, wild type. * P < .05 and ** P < .01.

the lung in wild-type, IL-13R α 2-deficient, and memIL-13R α 2-transgenic mice in our asthma models. It is likely that the change in expression of these genes precedes the effect on AHR and airway inflammation.

Previous studies performed in mice have been complicated by the presence of both sIL-13R α 2 and memIL-13R α 2, making it impossible to assess the role of each form independently. sIL-13R α 2 has been shown to act as a decoy receptor in studies of mouse models of asthma after administration of sIL-13R α 2. The effect of endogenous sIL-13R α 2 on allergen-induced airway inflammation has not been studied. In our mouse models of asthma, AHR and airway inflammation are attenuated in IL-13R α 2-deficient mice, indicating that under these conditions, deficiency of memIL-13R α 2 plays a major role compared with deficiency of sIL-13R α 2. Mice have large quantities of sIL-13R α 2 in their sera, even at baseline, whereas human subjects lack sIL-13R α 2.^{19,20} sIL-13R α 2 has been measured at nanogram levels in mouse serum, with up to 10 times higher molar

concentrations than IL-13.^{9,27,28} Thus the amount of sIL-13R α 2 is high enough to inhibit IL-13 signaling in serum and suggests that sIL-13R α 2 is a major modifier of IL-13 activity and allergic inflammation in mice *in vivo*. Thus conclusions from IL-13 studies done in mice might not be fully applicable to human subjects who have no sIL-13R α 2.

IL-13-induced TGF- β -dependent fibrosis has been shown to be promoted by IL-13R α 2, suggesting that IL-13R α 2 might have some signaling roles.¹⁶ One report showed that in intestinal epithelial cells from patients with ulcerative colitis or colorectal cancer, IL-13R α 2 can inhibit STAT6 signaling or initiate mitogen-activated protein kinase signaling depending on the relative expression of IL-13R α 2 and the local concentration of IL-13.¹⁹

sIL-13R α 2 levels were similar in memIL-13R α 2 transgenic and nontransgenic mice, indicating sIL-13R α 2 did not play a role in the enhanced HDM-induced AHR and airway inflammation seen in memIL-13R α 2 transgenic mice. We observed that in the

absence of the endogenous IL-13R α 2 gene, lung overexpression of memIL-13R α 2 did not yield appreciable sIL-13R α 2 in BALF. These data support that sIL-13R α 2 detected in memIL-13R α 2 transgenic and nontransgenic mice is coming predominantly from the soluble form of the transcript that is absent in memIL-13R α 2 transgenic/IL-13R α 2-deficient mice. There was no appreciable cleavage of memIL-13R α 2 in this model.

Recent evidence reveals that the relative expression levels of IL-4R α , IL-13R α 1, and γ c are critical for determining the IL-4 and IL-13 sensitivities of various cell types²⁹; however, the effect of the IL-13R α 2 expression level remains unclear. IL-13 has been postulated to be an “effector” cytokine, whereas 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-13R α 2, whereas IL-4R α and IL-13R α 1 expression did not change, suggesting that the effect of overexpression of memIL-13R α 2 on allergen-induced AHR and airway inflammation is due to enhanced signaling through memIL-13R α 2 rather than IL-4R α and IL-13R α 1.

In summary, our data demonstrate that memIL-13R α 2 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 mAb is being evaluated in a clinical trial to treat asthma (ClinicalTrials.gov Identifier no. NCT01402986). Delineation of the roles of memIL-13R α 2 versus sIL-13R α 2 will enable the design of the most effective targeted therapies. The development of humanized mouse models that express exclusively memIL-13R α 2 would be most optimal for study.

Key messages

- memIL-13R α 2 has a distinct role in the lung and contributes to the development of allergic inflammation.
- Models that better reflect human IL-13R α 2 biology are needed because the absence of sIL-13R α 2 in human plasma supports the importance of memIL-13R α 2 in human immunity.
- These data might enable novel asthma therapeutics targeting airway epithelial cells.

REFERENCES

1. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261-3.
2. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-61.
3. Aman MJ, Tayebi N, Obiri NI, Puri RK, Modi WS, Leonard WJ. cDNA cloning and characterization of the human interleukin 13 receptor alpha chain. *J Biol Chem* 1996;271:29265-70.
4. Hilton DJ, Zhang JG, Metcalf D, Alexander WS, Nicola NA, Willson TA. Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor. *Proc Natl Acad Sci U S A* 1996;93:497-501.
5. Caput D, Laurent P, Kaghad M, Lelias JM, Lefort S, Vita N, et al. Cloning and characterization of a specific interleukin (IL)-13 binding protein structurally related to the IL-5 receptor alpha chain. *J Biol Chem* 1996;271:16921-6.
6. Zhang JG, Hilton DJ, Willson TA, McFarlane C, Roberts BA, Moritz RL, et al. Identification, purification, and characterization of a soluble interleukin (IL)-13-binding protein. Evidence that it is distinct from the cloned IL-13 receptor and IL-4 receptor alpha-chains. *J Biol Chem* 1997;272:9474-80.
7. Miloux B, Laurent P, Bonnin O, Lupker J, Caput D, Vita N, et al. Cloning of the human IL-13R alpha1 chain and reconstitution with the IL4R alpha of a functional IL-4/IL-13 receptor complex. *FEBS Lett* 1997;401:163-6.
8. Donaldson DD, Whitters MJ, Fitz LJ, Neben TY, Finnerty H, Henderson SL, et al. The murine IL-13 receptor alpha 2: molecular cloning, characterization, and comparison with murine IL-13 receptor alpha 1. *J Immunol* 1998;161:2317-24.
9. Wood N, Whitters MJ, Jacobson BA, Witek J, Sypek JP, Kasaian M, et al. Enhanced interleukin (IL)-13 responses in mice lacking IL-13 receptor alpha 2. *J Exp Med* 2003;197:703-9.
10. Kawakami K, Taguchi J, Murata T, Puri RK. The interleukin-13 receptor alpha2 chain: an essential component for binding and internalization but not for interleukin-13-induced signal transduction through the STAT6 pathway. *Blood* 2001;97:2673-9.
11. Daines MO, Tabata Y, Walker BA, Chen W, Warriar MR, Basu S, et al. Level of expression of IL-13R alpha 2 impacts receptor distribution and IL-13 signaling. *J Immunol* 2006;176:7495-501.
12. Mentink-Kane MM, Cheever AW, Thompson RW, Hari DM, Kabatereine NB, Vennervald BJ, et al. IL-13 receptor alpha 2 down-modulates granulomatous inflammation and prolongs host survival in schistosomiasis. *Proc Natl Acad Sci U S A* 2004;101:586-90.
13. Morimoto M, Zhao A, Sun R, Stiltz J, Madden KB, Mentink-Kane M, et al. IL-13 receptor alpha2 regulates the immune and functional response to *Nippostrongylus brasiliensis* infection. *J Immunol* 2009;183:1934-9.
14. Sivaprasad U, Warriar MR, Gibson AM, Chen W, Tabata Y, Bass SA, et al. IL-13-Ralpha2 has a protective role in a mouse model of cutaneous inflammation. *J Immunol* 2010;185:6802-8.
15. Wilson MS, Ramalingam TR, Rivollier A, Shenderov K, Mentink-Kane MM, Madala SK, et al. Colitis and intestinal inflammation in IL10-/- mice results from IL-13Ralpha2-mediated attenuation of IL-13 activity. *Gastroenterology* 2011;140:254-64.
16. Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A. IL-13 signaling through the IL-13Ralpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. *Nat Med* 2006;12:99-106.
17. Fichtner-Feigl S, Young CA, Kitani A, Geissler EK, Schlitt HJ, Strober W. IL-13 signaling via IL-13R alpha2 induces major downstream fibrogenic factors mediating fibrosis in chronic TNBS colitis. *Gastroenterology* 2008;135:2003-13, e1-7.
18. Mandal D, Levine AD. Elevated IL-13Ralpha2 in intestinal epithelial cells from ulcerative colitis or colorectal cancer initiates MAPK pathway. *Inflamm Bowel Dis* 2010;16:753-64.
19. Chen W, Sivaprasad U, Tabata Y, Gibson AM, Stier MT, Finkelman FD, et al. IL-13Ralpha2 membrane and soluble isoforms differ in humans and mice. *J Immunol* 2009;183:7870-6.
20. O'Toole M, Legault H, Ramsey R, Wynn TA, Kasaian MT. A novel and sensitive ELISA reveals that the soluble form of IL-13R-alpha2 is not expressed in plasma of healthy or asthmatic subjects. *Clin Exp Allergy* 2008;38:594-601.
21. Tabata Y, Chen W, Warriar MR, Gibson AM, Daines MO, Hershey GK. Allergy-driven alternative splicing of IL-13 receptor alpha2 yields distinct membrane and soluble forms. *J Immunol* 2006;177:7905-12.
22. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999;103:779-88.
23. Stripp BR, Sawaya PL, Luse DS, Wikenheiser KA, Wert SE, Huffman JA, et al. cis-acting elements that confer lung epithelial cell expression of the CC10 gene. *J Biol Chem* 1992;267:14703-12.
24. Walters DM, Breyse PN, Schofield B, Wills-Karp M. Complement factor 3 mediates particulate matter-induced airway hyperresponsiveness. *Am J Respir Cell Mol Biol* 2002;27:413-8.
25. Chen W, Tabata Y, Gibson AM, Daines MO, Warriar MR, Wills-Karp M, et al. Matrix metalloproteinase 8 contributes to solubilization of IL-13 receptor alpha2 in vivo. *J Allergy Clin Immunol* 2008;122:625-32.
26. Chen W, Erickson MB, Levin LS, Khurana Hershey GK. Functional effect of the R110Q IL13 genetic variant alone and in combination with IL4RA genetic variants. *J Allergy Clin Immunol* 2004;114:553-60.
27. Khodoun M, Lewis CC, Yang JQ, Orekov T, Potter C, Wynn T, et al. Differences in expression, affinity, and function of soluble (s)IL-4Ralpha and sIL-13Ralpha2 suggest opposite effects on allergic responses. *J Immunol* 2007;179:6429-38.
28. Morimoto M, Morimoto M, Zhao A, Madden KB, Dawson H, Finkelman FD, et al. Functional importance of regional differences in localized gene expression of receptors for IL-13 in murine gut. *J Immunol* 2006;176:491-5.
29. Junttila IS, Mizukami K, Dickensheets H, Meier-Schellersheim M, Yamane H, Donnelly RP, et al. Tuning sensitivity to IL-4 and IL-13: differential expression of IL-4Ralpha, IL-13Ralpha1, and gamma c regulates relative cytokine sensitivity. *J Exp Med* 2008;205:2595-608.

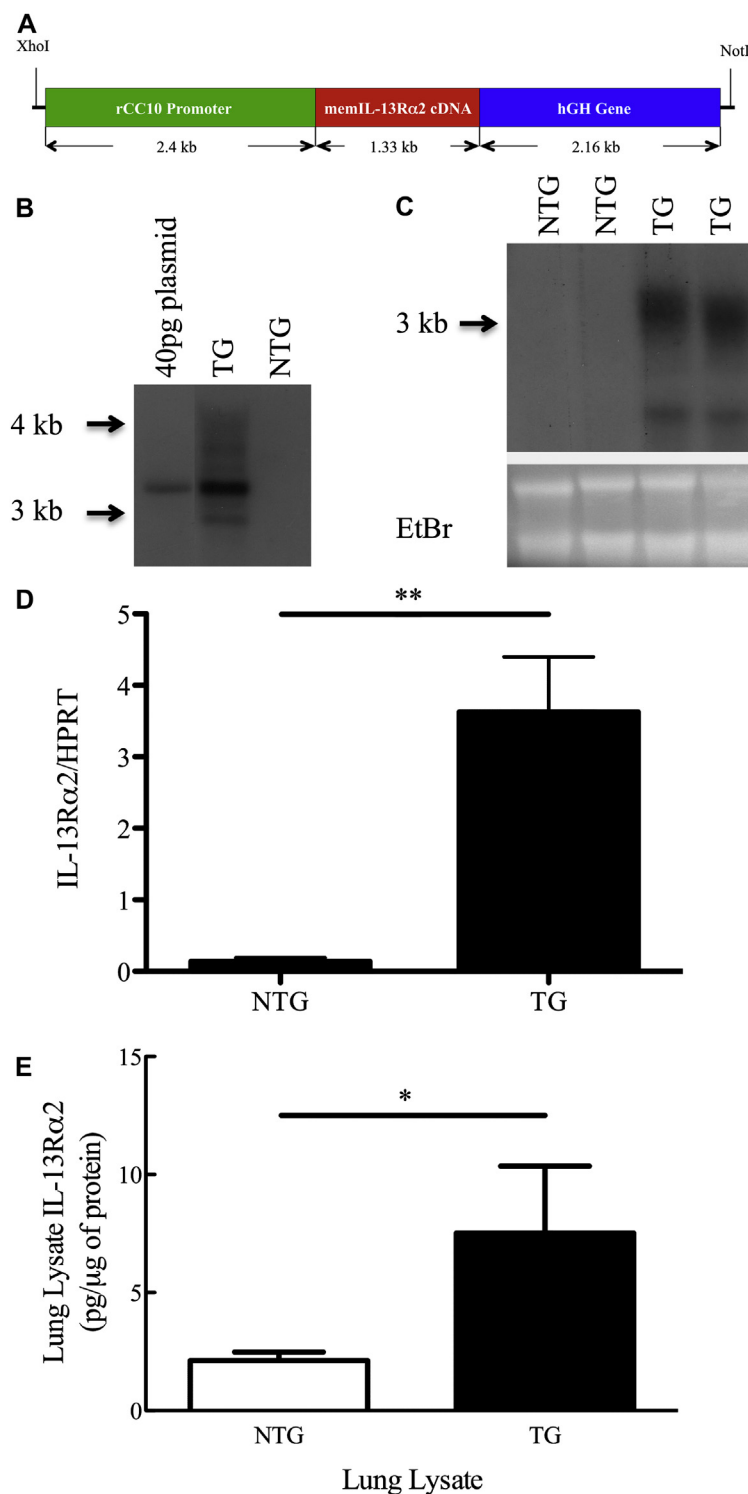


FIG E1. Characterization of memIL-13Rα2 lung transgenic mice. **A**, Schematic diagram of the construct for memIL-13Rα2 lung transgenic mice. **B**, Southern blot analysis of the memIL-13Rα2-hGH transgene in transgenic and nontransgenic mice. The control plasmid was used to estimate transgene copy numbers. **C**, Northern blot analysis of memIL-13Rα2 expression in the lung. **D**, RT-qPCR: lung expression of IL-13Rα2. Data are shown as means $\pm$ SDs ($n = 3$). **E**, ELISA for IL-13Rα2 in lung lysate from memIL-13Rα2 transgenic and nontransgenic mice. Data are shown as means $\pm$ SDs ($n = 5$). * $P < .05$ and ** $P < .01$. NTG, Nontransgenic; TG, memIL-13Rα2 transgenic.

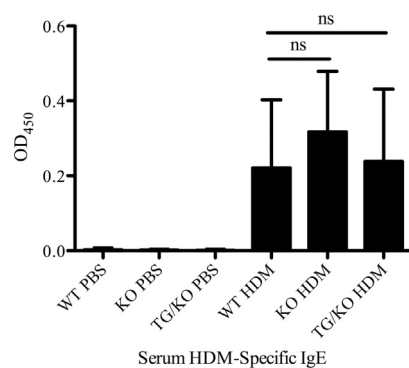


FIG E2. Serum HDM-specific IgE in PBS- or HDM-treated memIL-13R α 2 transgenic/IL-13R α 2-deficient mice. Serum HDM-specific IgE levels were determined by using ELISA. *KO*, IL-13R α 2-deficient; *ns*, not significant; *TG/KO*, memIL-13R α 2 transgenic/IL-13R α 2-deficient; *WT*, wild type. Data are shown as means $\pm$ SDs ($n = 9-10$).

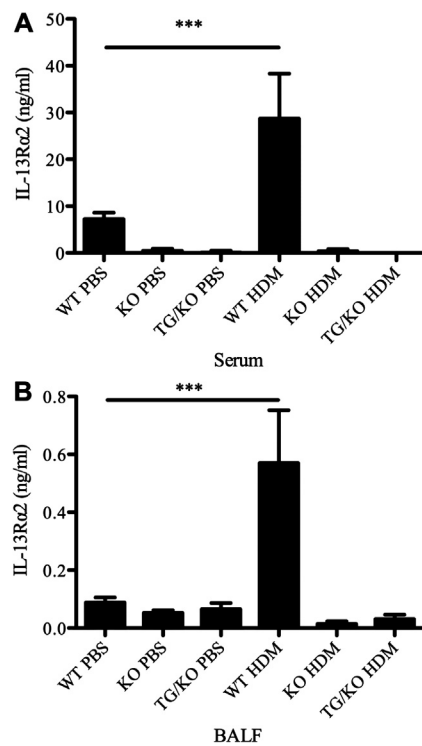


FIG E3. Levels of sIL-13Rα2 in PBS- or HDM-treated memIL-13Rα2 transgenic/IL-13Rα2-deficient mice. **A**, Serum. **B**, BALF. Data are shown as means $\pm$ SDs ($n = 4-8$). *KO*, IL-13Rα2-deficient; *TG/KO*, memIL-13Rα2 transgenic/IL-13Rα2-deficient; *WT*, wild type. *** $P < .001$.

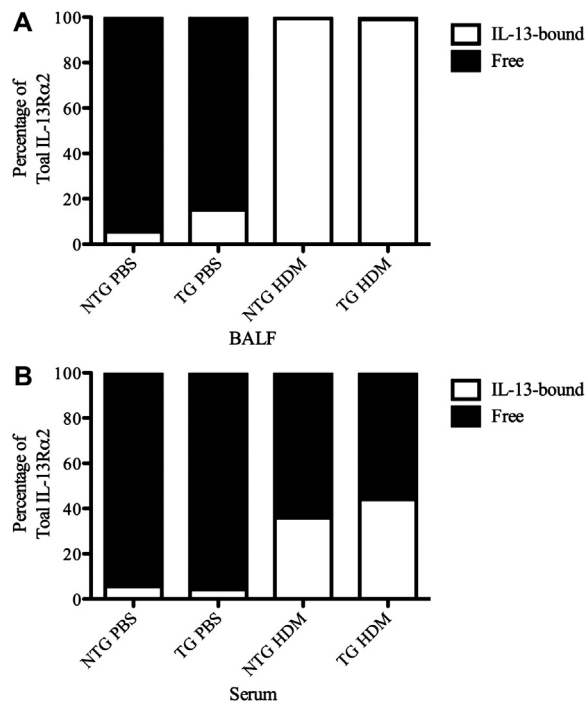


FIG E4. Percentage of IL-13-bound and free IL-13R α 2 in BALF and serum of PBS- or HDM-treated nontransgenic and memIL-13R α 2 transgenic mice ($n = 5$). **A**, BALF. **B**, Serum. *NTG*, Nontransgenic; *TG*, memIL-13R α 2 transgenic.

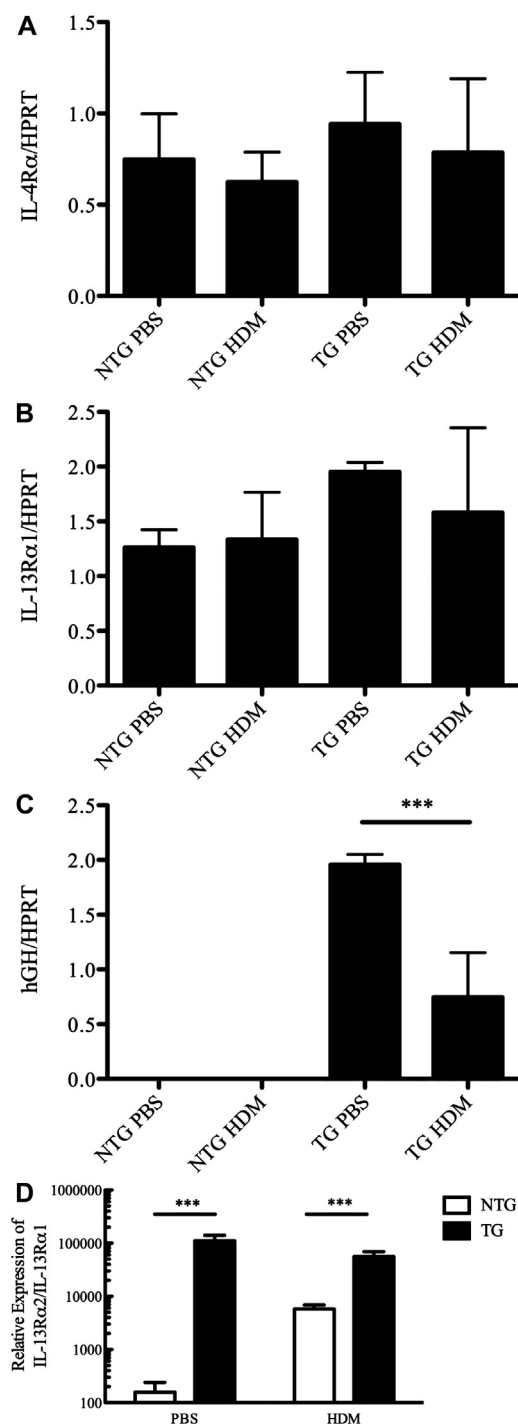


FIG E5. mRNA expression of IL-13Rα1 and IL-4Rα in lungs of PBS- or HDM-treated IL-13Rα2 transgenic and nontransgenic mice. **A-C**, Expression of IL-13Rα1, IL-4Rα, and the hGH transgene in lungs of PBS- or HDM-treated memIL-13Rα2 transgenic and nontransgenic mice. **D**, Relative expression of IL-13Rα2 to IL-13Rα1. Data are shown as means $\pm$ SDs ($n = 3-5$). *** $P < .001$.

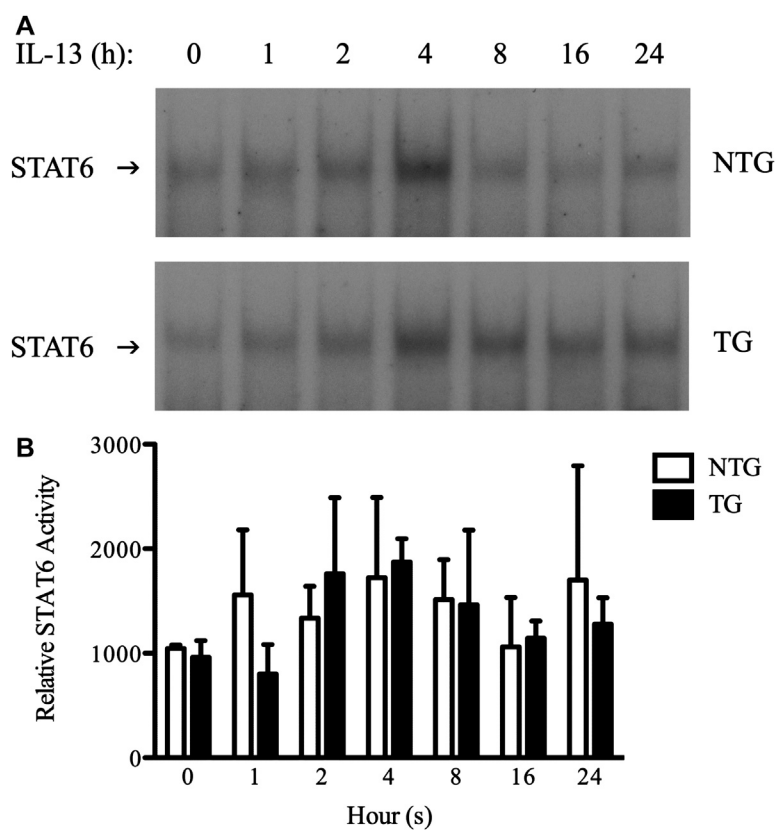


FIG E6. IL-13-induced STAT6 activation in lungs from nontransgenic and memIL-13R α 2 transgenic mice. **A**, Nuclear extracts from lungs of IL-13-treated nontransgenic and memIL-13R α 2 transgenic mice at different time points (1, 2, 4, 8, 16, and 24 hours) were used to assess STAT6 DNA-binding activity by using EMSA. **B**, Quantification of STAT6 activity. Data are shown as means $\pm$ SDs ($n = 3$). NTG, Nontransgenic; TG, memIL-13R α 2 transgenic.