

Expression of IL-4 receptor α on smooth muscle cells is not necessary for development of experimental allergic asthma

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Background: Airflow in the lungs of patients with allergic asthma is impaired by excessive mucus production and airway smooth muscle contractions. Elevated levels of the cytokines IL-4 and IL-13 are associated with this pathology. *In vitro* studies have suggested that IL-4 receptor α (IL-4R α) signaling on smooth muscle cells is critical for airway inflammation and airway hyperresponsiveness.

Objective: To define the contribution of IL-4 and IL-13 to the onset of asthmatic pathology, the role of their key receptor IL-4R α in smooth muscle cells was examined *in vivo*.

Methods: By using transgenic smooth muscle myosin heavy chain^{cre}IL-4R α ^{-lox} mice deficient in IL-4R α in smooth muscle cells, *in vivo* effects of impaired IL-4R α signaling in smooth muscle cells on the outcome of asthmatic disease were investigated for the first time. Allergic asthma was introduced in mice by repeated sensitization with ovalbumin/aluminum hydroxide on days 0, 7, and 14, followed by intranasal allergen challenge on days 21 to 23. Mice were investigated for the presence of airway hyperresponsiveness, airway inflammation, allergen-specific antibody production, T_H2-type cytokine responses, and lung pathology.

Results: Airway hyperresponsiveness, airway inflammation, mucus production, T_H2 cytokine production, and specific antibody responses were unaffected in smooth muscle myosin heavy chain^{cre}IL-4R α ^{-lox} mice compared with control animals. **Conclusion:** The impairment of IL-4R α on smooth muscle cells had no effect on major etiologic markers of allergic asthma. These findings suggest that IL-4R α responsiveness in airway smooth muscle cells during the early phase of allergic asthma is

not, as suggested, necessary for the outcome of the disease. (J Allergy Clin Immunol 2010;■■■■:■■■-■■■.)

Key words: Smooth muscle cell, allergy, asthma, cytokine receptors, IL-4, IL-13, gene-deficient mice

Allergic asthma is characterized by airway obstruction, inflammation, and airway hyperresponsiveness (AHR). Patients with allergic asthma experience impaired respiratory air flow, which is caused by a combination of increased airway mucus secretion and airway smooth muscle cell (ASMC) contraction in response to allergens.

Immunologically, it is established that T_H2-type cytokines, particularly IL-4 and IL-13, play an important role in asthma pathogenesis. Indeed, overexpression of these cytokines results in the development of asthmatic symptoms in murine models.¹⁻⁴ Although IL-4 plays an important role in establishing T_H2 responses,^{5,6} IL-13 is the cytokine critical for driving actual asthmatic pathology. In the absence of IL-13, mucus production, AHR, and airway inflammation are inhibited. Conversely, administration of recombinant IL-13 will induce these pathological features in nonallergic mice.^{7,8} Such IL-13–driven effects occur irrespective of IL-4; nevertheless, induction of a strong T_H2 response and immunoglobulin class-switching to IgE in mice requires IL-4. These IL-4 and IL-13–mediated effects require signaling through heterodimeric receptors containing IL-4 receptor α (IL-4R α). Mice deficient in IL-4R α show protection from experimentally induced allergic asthma,⁹ and IL-4R α antagonists abrogate the development of allergic airway symptoms.¹⁰

It can be appreciated from this that an understanding of the role of IL-4R α in specific cell types would enhance the understanding and treatment of asthma. Key cells involved in the onset and pathogenesis of asthma include T cells, B cells, epithelial cells, and smooth muscle cells. To date, the cell-specific role of IL-4R α in allergy has only been described *in vivo* for epithelial cells. Here, disrupted epithelial IL-4R α signaling results in protection from airway mucus production, whereas AHR was not affected.¹¹

In addition to immune and epithelial cells, it is apparent that ASMCs may play an important role in the onset of asthma. Several *in vitro* studies have shown that IL-4 and IL-13 induce hypercontractility in smooth muscle cells in a signal transducer and activator of transcription 6 (STAT6)–dependent manner.¹²⁻¹⁴ This IL-4 and IL-13–dependent hypercontractility response of ASMCs to carbachol was attenuated in STAT6^{-/-} mice. Use of anti-IL-4R α antibodies also inhibited the contractile responsiveness of IgE-sensitized ASMCs to acetylcholine, whereas pretreatment with recombinant IL-13 enhanced contractile ability.¹⁵ In addition to effects on ASMC hypercontractility, human ASMCs have been shown to

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Abbreviations used

AHR:	Airway hyperreactivity
ASMC:	Airway smooth muscle cell
BAL:	Bronchoalveolar lavage
EPO:	Eosinophil peroxidase
IL-4R α :	IL-4 receptor α
mMCP-1:	Mouse mast cell protease 1
OVA:	Ovalbumin
PAS:	Periodic acid-Schiff
Penh:	Enhanced pause
SM-MHC:	Smooth muscle myosin heavy chain
STAT6:	Signal transducer and activator of transcription 6

release allergy-associated chemokines, such as eotaxin,^{16,21} in response to IL-4 and/or IL-13 *in vitro*. This IL-4/IL-13–eotaxin expression in ASMCs can be inhibited by anti-IL-4R α antibodies¹⁸ and antisense oligonucleotides to STAT6.²²

Because of this substantial body of *in vitro* data, it was suggested that ASMC responsiveness to IL-4 and IL-13 contributes significantly to asthmatic pathology. To investigate the *in vivo* role of IL-4R α signaling in smooth muscle cells, we used smooth muscle myosin heavy chain [SM-MHC]^{cre}IL-4R α ^{-lox} mice a mouse model with smooth muscle cell-specific disruption of the *Il4ra* gene. These mice express Cre recombinase under control of the smooth muscle cell-specific myosin heavy chain promoter. As a consequence of this loxP flanked exons of the *il4ra* gene are excised in smooth muscle cells only. Recently characterized by our laboratory, SM-MHC^{cre}IL-4R α ^{-lox} mice showed delayed mucus production, T_H2 cytokine production, and worm expulsion in a model of nematode infection.¹³ This was the first *in vivo* demonstration of the influence of IL-4R α expression in smooth muscle cells on T_H2-type immune responses of the host.

The current study investigated for the first time the *in vivo* effect of IL-4R α signaling in smooth muscle cells in a model of acute allergic asthma. The findings of several *in vitro* studies have suggested a role for IL-4/IL-13 responsiveness in smooth muscle cells on the outcome of allergic airway disease, but our results demonstrated that smooth muscle cell-specific disruption of the IL-4R α did not influence the acute phase of the allergic airway response caused by ovalbumin (OVA).

METHODS**Mice**

Generation and characterization of the SM-MHC^{cre}IL-4R α ^{-lox} (C.Cg-*Il4ra*^{tm1Fbb}/*Il4ra*^{tm2Fbb}Tg(Myh11-cre)5013Gko) mice was described previously.¹³ SM-MHC^{cre} mice on a BALB/c background²³ were crossed with IL-4R α ^{-/-} mice.²⁴ To generate SM-MHC^{cre}IL-4R α ^{-lox} mice, they were then crossed with IL-4R α ^{-lox} mice²⁵ because the hemizygous IL-4R α ^{-lox} genotype increases the probability of Cre-mediated *il4ra* gene disruption. All mice were genotyped by PCR before the experiments to confirm smooth muscle cell-specific disruption of the *il4ra* gene. IL-4R α ^{lox/lox} mice produce functional IL-4R α , and hemizygous IL-4R α ^{-lox} littermates were used as control groups. IL-4R α ^{-/-} (*Il4ra*^{tm1Fbb}/*Il4ra*^{tm1Fbb}) mice were used as global knockout controls for the IL-4R α . Mice were housed in the animal unit of the University of Cape Town under specific pathogen-free conditions by using individually ventilated cages. All work was approved by the University of Cape Town animal ethics committee.

Allergen sensitization and challenge of mice

Sensitization and challenge of mice was performed as described previously.¹¹ SM-MHC^{cre}IL-4R α ^{-lox}, IL-4R α ^{-lox}, and IL-4R α ^{-/-} mice were

sensitized with 50 μ g OVA (grade V; Sigma-Aldrich, Aston Manor, South Africa) in 200 μ L PBS/1.3% alum (Sigma-Aldrich) on days 0, 7, and 14. On days 21, 22, and 23, mice were anesthetized with ketamine (Anaket-V; Centaur Labs, Johannesburg, South Africa)/xylazine (Rompun; Bayer, Isando, South Africa) and challenged with 1 mg OVA in 50 μ L PBS by nasal administration. Control groups were treated identically except OVA was missing in the solutions. Mice were killed and studied on day 24.

Measurement of airway hyperresponsiveness

Two different methods for measurement of airway resistance were used in this study. First, measurements of airway resistance in response to intravenously administered acetylcholine were performed as described previously.¹¹ Anesthetized and paralyzed mice were mechanically ventilated, and lung resistance was measured at baseline and after increasing intravenous doses of acetylcholine. For the second approach, a flexiVent system (SCIREQ, Montreal, Canada) was used for airway resistance measurements in response to increasing doses of aerosolized methacholine (0, 5, 10, 20, 40 mg/mL). Mice were anesthetized with ketamine and xylazine, intubated with a 18G cannula, and mechanically ventilated at a frequency of 2.5 Hz. Mice were exposed for 10 seconds to methacholine aerosol, and 15 single-frequency forced oscillation maneuvers were performed to determine dynamic resistance, the maximum response for each concentration. In addition to the 2 invasive methods, enhanced pause (Penh) values in response to β -methacholine (Sigma-Aldrich) were assessed in conscious unrestrained mice by using a whole body plethysmograph (emka Technologies, Paris, France). This system measures pressure changes induced by the breathing of the mice and computes Penh values, a dimensionless parameter reflecting airway hyperresponsiveness.²⁶ Baseline readings were measured for 5 minutes before each exposure to increasing doses of aerosolized β -methacholine in PBS (0, 5, 10, 20 mg/mL). Results are computed as the average of first 5 minutes minus the average of baseline readings.

Analysis of airway inflammation

Lungs from sacrificed mice were flushed once with 1 mL PBS/1% FCS to obtain bronchoalveolar lavage (BAL) fluid, and total cell number was determined by using a hemocytometer. A maximum of 2×10^5 cells was centrifuged on a microscope slide and stained with Rapidiff Stain Set (Clinical Diagnostics CC, Johannesburg, South Africa). Differential cell counts were made at $\times 400$ magnification, and at least 100 cells were counted per slide. Lung tissue was homogenized in TRIS/Nonidet P-40 buffer and then centrifuged for 20 minutes at 20000g for mouse mast cell protease 1 (mMCP-1) ELISA and eosinophil peroxidase (EPO) assay. A colorimetric assay for EPO was used to quantify eosinophils in the lung tissue.²⁷ Fifty microliters of the substrate solution consisting of 16 mmol/L o-phenylenediamine (Sigma-Aldrich) in 100 mmol/L TRIS-HCl buffer pH 8.0 containing 0.1% Triton and 0.01% hydrogen peroxide was added to 50 μ L tissue sample (protein concentration 1 mg/mL) and peroxidase standard (Sigma-Aldrich) in 96-well plates. The reaction was stopped after 5 minutes with 50 μ L 2 mol/L H₂SO₄, and absorption was measured at 490 nm. Mouse mast cell protease concentrations were determined by ELISA (Moredun Scientific Ltd, Midlothian, United Kingdom) according to the manufacturer's protocol. Serum eotaxin levels were determined by ELISA according to the manufacturer's protocol (R&D Systems, Minneapolis, Minn).

Histology and immunohistochemistry

For histologic analysis of goblet cell hyperplasia, tissue samples were fixed in 4% phosphate-buffered formalin, embedded in paraffin and cut into sections of 5 to 7 μ m, and stained with periodic acid-Schiff (PAS) reagent. Mucin was stained with anti-Mucin 5AC (MUC5AC) antibody (clone 45M-1, 1:100 dilution; Thermo Scientific, Fremont, Calif) and a mouse-on-mouse staining kit (M.O.M.; Vector Laboratories, Burlingame, Calif).

Measurement of serum antibodies

Blood samples were collected in plasma separator tubes (BD Vacutainer Systems, Franklin Lakes, NJ) by tail vein bleeding and centrifuged for 15

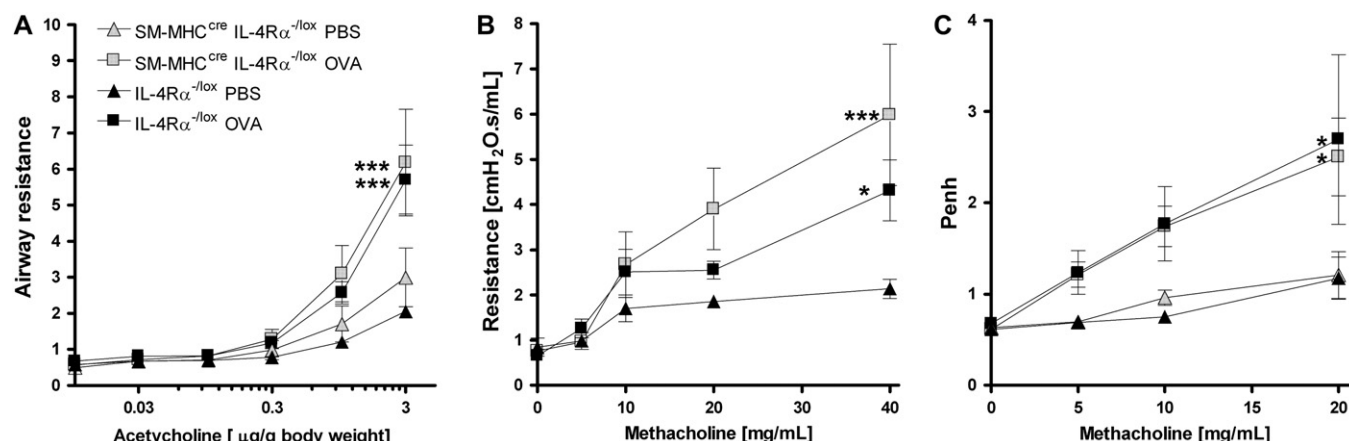


FIG 1. Airway resistance is not affected by loss of the IL-4Rα on smooth muscle cells. Airway resistance to increasing concentrations of intravenously administered acetylcholine (A), aerosolized methacholine (B), or Penh values in response to methacholine (C) were measured in IL-4Rα^{-/-} and SM-MHC^{cre} IL-4Rα^{-/-} mice after OVA or PBS sensitization and challenge. Values represent pooled data with SD from 2 experiments with 4 to 8 mice per group. **P* < .05; ****P* < .001 vs PBS control.

minutes at 6000g. OVA-specific antibody isotypes were measured in blood serum dilution series by endpoint titration ELISA. Plates were coated with 5 μg/mL (for IgG) or 1 mg/mL (for IgE) OVA. alkaline phosphatase-conjugated antimouse isotype specific antibodies (Southern Biotechnology, Birmingham, Ala) and 4-nitrophenyl phosphate (Sigma-Aldrich) were used for detection. Absorbance was measured at 405 nm with 492 nm as a reference wavelength.

Analysis of *in vitro* cytokine production

For analysis of *in vitro* cytokine production, splenocytes and lymphocytes were isolated from pooled spleens or mediastinal lymph nodes of OVA-treated and control mice. Cells were incubated for 120 hours at 2×10^6 cells/mL in 200 μL Iscove's Modified Dulbecco's Medium (Gibco, Paisley United Kingdom)/10% FCS (Delta Bioproducts, Kempton Park, South Africa) in 96-well plates. Cells were stimulated with OVA (100 μg/mL), and supernatants were collected after 120 hours. Concentrations of IL-4, IL-5, IFN-γ (BD Biosciences, Erembodegem, Belgium) and IL-13 (R&D Systems) were measured by using sandwich ELISA assays according to the manufacturer's protocol.

Statistics

Values are given as means ± SDs, and significant differences were determined by using ANOVA (GraphPad Prism 4 software, GraphPad Software Inc, San Diego, Calif). *P* values ≤ .05 were considered significant.

RESULTS

Allergen-induced AHR is not affected in SM-MHC^{cre}IL-4Rα^{-/-} mice

Airway reactivity was measured in sedated and paralyzed, mechanically ventilated SM-MHC^{cre}IL-4Rα^{-/-} and IL-4Rα^{-/-} mice after OVA or saline challenge. Airway resistance was increased in allergen-challenged IL-4Rα^{-/-} and SM-MHC^{cre}IL-4Rα^{-/-} mice, indicating the development of AHR. These increases in airway resistance were not observed in saline-challenged mice. Similar increases in airway reactivity were seen in SM-MHC^{cre}IL-4Rα^{-/-} and IL-4Rα^{-/-} mice with no statistically significant differences between the mouse strains (Fig 1, A). The development of airway hyperresponsiveness in both SM-MHC^{cre}IL-4Rα^{-/-} and IL-4Rα^{-/-} mice was confirmed by a second invasive method for determination of airway

resistance in response to increasing doses of methacholine aerosol. Airway resistance in both strains treated with OVA was significantly increased compared with PBS-treated IL-4Rα^{-/-} mice with no significant differences between SM-MHC^{cre}IL-4Rα^{-/-} and IL-4Rα^{-/-} mice (Fig 1, B). Similar results were obtained by measuring responses to methacholine by noninvasive whole-body plethysmography. Both OVA-treated IL-4Rα^{-/-} mice and SM-MHC^{cre}IL-4Rα^{-/-} mice responded to methacholine challenges with increased Penh values compared with PBS-treated control groups, and these increases were not significantly different between the mouse strains (Fig 1, C).

Allergic airway inflammation is not affected in SM-MHC^{cre}IL-4Rα^{-/-} mice

As a consequence of allergic sensitization and challenge inflammatory cells are recruited to the lungs with eosinophils being a characteristic cell type for allergic airway inflammation. The total number of cells in the BAL fluid of OVA-treated SM-MHC^{cre}IL-4Rα^{-/-} mice did not differ from the cell number recovered from IL-4Rα^{-/-} mice but was significantly higher than the cell number in the BAL fluid of IL-4Rα^{-/-} mice (Fig 2, A). Differential cell counts revealed that the main cell type in the BAL fluid in both OVA-treated IL-4Rα^{-/-} mice and SM-MHC^{cre}IL-4Rα^{-/-} mice was eosinophils, with no significant differences between the strains, whereas in IL-4Rα^{-/-} mice, it was neutrophils (Fig 2, B). To quantify the number of eosinophils in the lung tissue, the amount of EPO, which is stored in intracellular vesicles in eosinophils until activation, was measured in lung tissue lysate. There was no significant difference in the amount of EPO in the lung tissue between SM-MHC^{cre}IL-4Rα^{-/-} mice and IL-4Rα^{-/-} mice (Fig 2, C). In IL-4Rα^{-/-} mice, the concentration of EPO was significantly reduced compared with SM-MHC^{cre}IL-4Rα^{-/-} mice but was higher than in PBS-treated control mice. During allergic airway inflammation, the lung tissue and ASMCs are infiltrated by mast cells. The amount of mMCP-1, a marker for mast cell degranulation, was increased in the lung tissue after challenge with OVA, with no significant differences in all mouse strains (Fig 2, D). It has been shown that after *in vitro* stimulation with IL-13,

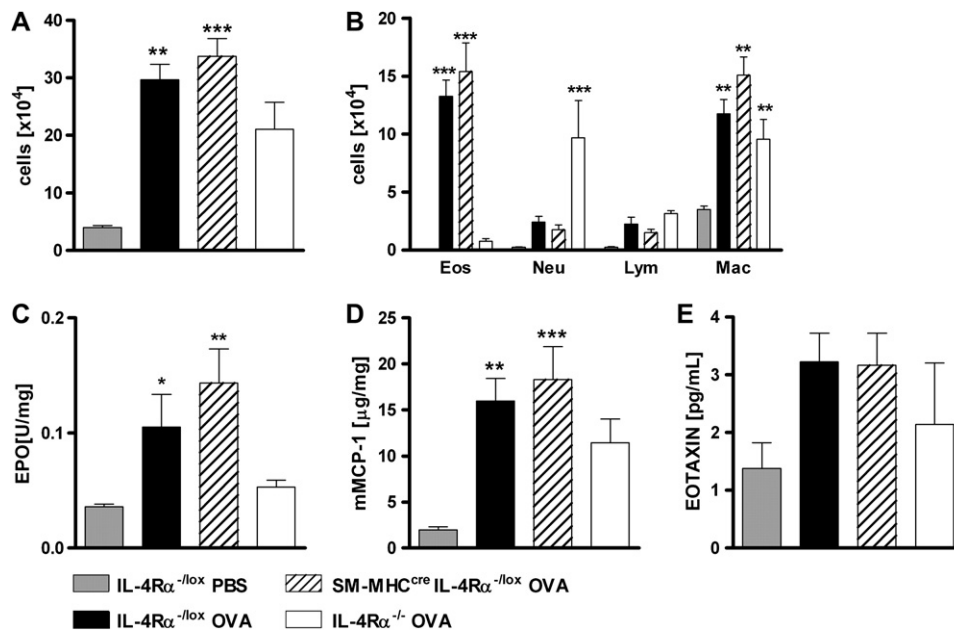


FIG 2. Markers of lung inflammation are not affected by loss of the IL-4R α on smooth muscle cells after sensitization and challenge with OVA. Total cell numbers (A) and numbers of eosinophils (*Eos*), neutrophils (*Neu*), lymphocytes (*Lym*), and macrophages (*Mac*) were counted in the BAL fluid. EPO (B) and mouse mast cell protease (mMCP-1; C) levels were measured in lung tissue lysate. Eotaxin concentration was measured in the blood serum by ELISA (D). Values represent pooled data with SD from 2 experiments with 4 to 6 mice per group. *P < .05; **P < .005; ***P < .001 vs PBS control.

ASMCs release eotaxin, a chemoattractant for eosinophils. Levels of this chemokine were measured in the serum of OVA-sensitized and challenged SM-MHC^{cre}IL-4R α ^{-/-}, IL-4R α ^{-/-}, and IL-4R α ^{-/-} mice. Eotaxin concentrations were similar in both SM-MHC^{cre}IL-4R α ^{-/-} and IL-4R α ^{-/-} mice but significantly lower in IL-4R α ^{-/-} mice (Fig 2, E). These results show that SM-MHC^{cre}IL-4R α ^{-/-} mice are not protected from allergic airway inflammation, as is the case with IL-4R α ^{-/-} mice, which showed no signs of eosinophilic cell infiltration in the lungs.

Airway mucus production is not affected in SM-MHC^{cre}IL-4R α ^{-/-} mice

Hyperplasia of mucus-producing goblet cells in the lungs is a characteristic of allergic asthma. The predominant mucin glycoprotein secreted in the respiratory tract of healthy individuals and individuals with asthma is MUC5AC, and its expression in goblet cells is upregulated by IL-4R α signals in murine models of asthma.¹¹ Histologic examination of PAS and anti-MUC5AC-stained lungs of OVA-sensitized and challenged IL-4R α ^{-/-} mice (Fig 3, A) and SM-MHC^{cre}IL-4R α ^{-/-} mice (Fig 3, B) showed similar severity of goblet cell hyperplasia and mucin production, whereas IL-4R α ^{-/-} mice (Fig 3, C) and the alum-treated control group showed no signs of increased mucus production (Fig 3, D).

Antigen-specific T_H2-type cytokine and antibody responses in IL-4R α ^{-/-} SM-MHC^{cre}IL-4R α ^{-/-} mice

To determine whether T-cell-dependent immune responses were affected by disrupted smooth muscle cell IL-4R α , lymphocytes from lung draining mediastinal lymph nodes (Fig 4, A) and

splenocytes (Fig 4, B) were stimulated *in vitro* with OVA. Sensitized and challenged SM-MHC^{cre}IL-4R α ^{-/-} and IL-4R α ^{-/-} mice showed antigen-specific T_H2-type cytokine responses characterized by production of IL-4, IL-5, and IL-13 in lymphocytes, with production of IL-5 and IL-13 significantly higher in SM-MHC^{cre}IL-4R α ^{-/-} mice (Fig 4, A). Compared with cytokine production by lymphocytes, splenocytes produced similar levels of IL-4 but only low levels of IL-5 and IL-13 (Fig 4, B). IL-4R α ^{-/-} mice developed a T_H1-type cytokine response to OVA characterized by production of IFN- γ by lymphocytes but not splenocytes. Antigen-specific cytokine responses were not detected in PBS control groups. To investigate whether antibody responses to allergens are affected by IL-4R α signaling on smooth muscle cells, specific antibody levels in SM-MHC^{cre}IL-4R α ^{-/-} mice were compared with those of IL-4R α ^{-/-} and IL-4R α ^{-/-} mice after sensitization and challenge with OVA. Compared with alum-treated control groups, serum levels of OVA-specific IgE (Fig 5, A) and IgG₁ (Fig 5, B) were increased in IL-4R α ^{-/-} and in SM-MHC^{cre}IL-4R α ^{-/-} mice with no significant differences between the 2 mouse strains. As expected, IL-4R α ^{-/-} mice showed significantly increased levels of specific IgG_{2a} and IgG_{2b} (Fig 5, C and D) but impaired OVA-specific IgE and IgG₁ antibody titers.¹⁰

DISCUSSION

To investigate the contribution of smooth muscle cell IL-4 and IL-13 signaling in the onset of allergic asthma *in vivo*, SM-MHC^{cre}IL-4R α ^{-/-} mice were used, which have a cell-specific disruption of IL-4R α on smooth muscle cells.¹³ Comparison of these mice with IL-4R α ^{-/-} and IL-4R α ^{-/-} mice showed that impaired IL-4R α signaling in smooth muscle cells had no effect

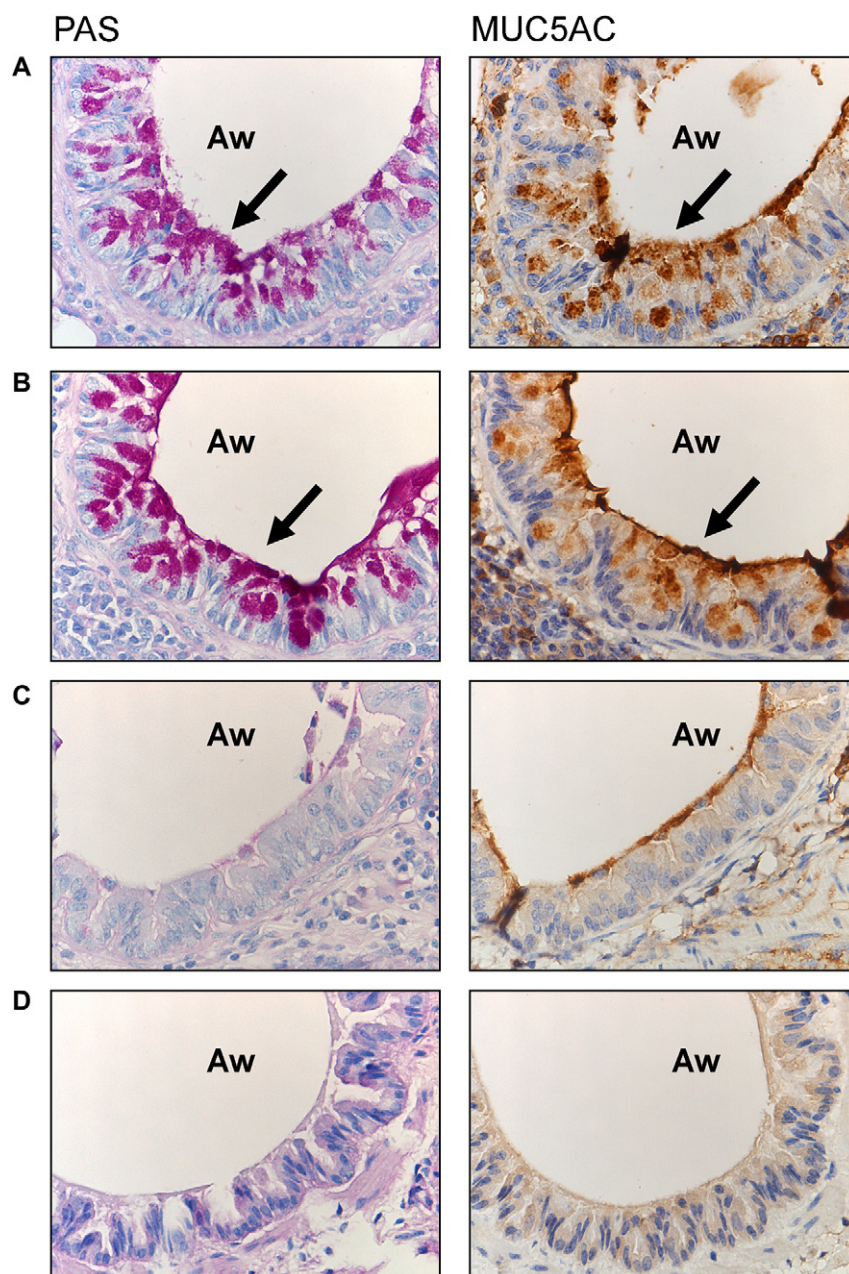


FIG 3. Goblet cell hyperplasia and mucin production after sensitization and challenge with OVA is not affected by loss of the IL-4R α on smooth muscle cells. Mucus-producing goblet cells were stained with PAS or anti-MUC5AC antibody in the airways of OVA-sensitized and challenged IL-4R $\alpha^{-/-}$ (A), SM-MHC^{cre} IL-4R $\alpha^{-/-}$ (B), and IL-4R $\alpha^{-/-}$ mice (C) and PBS-sensitized and challenged IL-4R $\alpha^{-/-}$ mice (D). Pictures were taken with a magnification of $\times 400$, and representative examples of 3 experiments are shown here. Arrows indicate mucus-producing goblet cells. Aw, Airways.

on the major etiologic markers of allergic asthma. In a recently published study, we demonstrated delayed mucus production, T_H2 cytokine production, and worm expulsion in SM-MHC^{cre}IL-4R $\alpha^{-/-}$ mice in a model of gut infection with the nematode *Nippostrongylus brasiliensis*.¹³ This disparity between our 2 studies might be explained by intrinsic differences between the 2 disease models and different roles for IL-4R α in smooth muscle cells of the gut and the lung in clearance of intestinal worm infections or induction of allergic airway disease. Airway hyperresponsiveness is a characteristic feature of allergic asthma.

Because the best method for the determination of AHR in mice is under debate,²⁸ 2 invasive and 1 noninvasive method were used in this study. The results obtained from each method were comparable, and both SM-MHC^{cre}IL-4R $\alpha^{-/-}$ and IL-4R $\alpha^{-/-}$ mice developed AHR after sensitization and challenge with OVA but not PBS. Furthermore, equivalent responses to acetylcholine and methacholine were seen in both groups. In agreement with our results, a study using a different mouse model with smooth muscle cell-specific IL-4R α disruption (SMP8^{cre} IL-4R $\alpha^{-/-}$ mice) found development of AHR in response to house dust mite

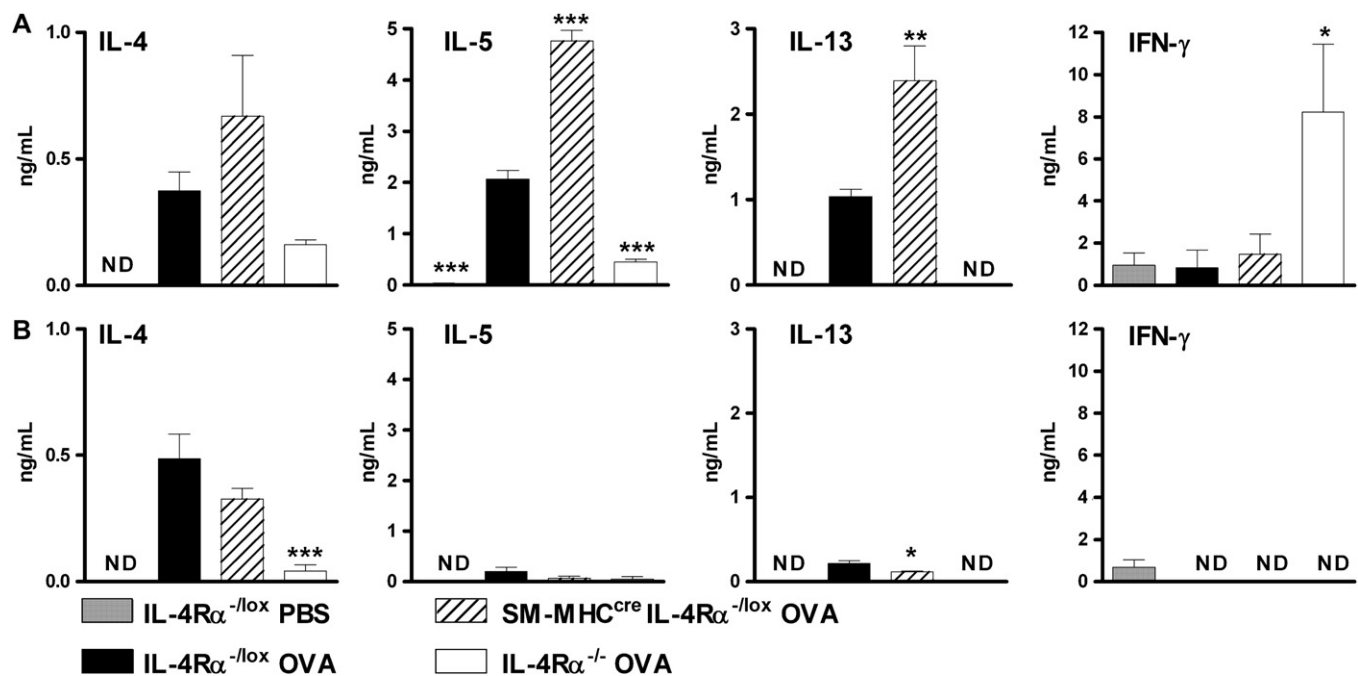


FIG 4. Sensitization and challenge with OVA induces a T_H2 -type immune response in IL-4Rα^{-/-lox} and SM-MHC^{cre}IL-4Rα^{-/-lox} mice. Lymphocytes (**A**) from mediastinal lymph nodes and splenocytes (**B**) were restimulated *in vitro* with OVA (100 μg/mL), and cytokine concentrations were measured in the supernatant by ELISA. ND, Not detected. * $P < .05$; ** $P < .005$; *** $P < .001$ vs IL-4Rα^{-/-lox} OVA.

allergens or recombinant IL-13 in the absence of smooth muscle IL-4Rα (C. Perkins, F. Finkelman, personal communication, 2010). These results indicate that IL-4Rα expression on smooth muscle cell is not required for the development of AHR in mice. This does not exclude the possibility that IL-4Rα signaling on smooth muscle cells contributes to the onset of AHR in wild-type animals, because the airway response observed in our model could have been induced by other cell types, like epithelial cells. It has been demonstrated that IL-13 signals on airway epithelial cells alone are sufficient to induce AHR in mice²⁹ despite the fact that disruption of IL-4Rα on epithelial cells did not affect development of AHR.¹¹

Allergic asthma is characterized by an inflammatory response in the lungs. Comparable levels of airway inflammation were observed in SM-MHC^{cre}IL-4Rα^{-/-lox} and IL-4Rα^{-/-lox} mice after treatment with allergen. No difference in concentration of eotaxin in blood serum or was seen between SM-MHC^{cre}IL-4Rα^{-/-lox} and IL-4Rα^{-/-lox} mice. As expected, IL-4Rα^{-/-} mice failed to generate a hyperplastic goblet cell mucus response; however, both SM-MHC^{cre}IL-4Rα^{-/-lox} and IL-4Rα^{-/-lox} mice showed hyperplastic goblet cell-driven mucus responses. Finally, lymphocytes from SM-MHC^{cre}IL-4Rα^{-/-lox} mice produced similar amounts of IL-4 and increased amounts of IL-5 and IL-13 in after *in vitro* stimulation with OVA compared with IL-4Rα^{-/-lox} mice. The differences were observed after antigen-specific stimulation but not after stimulation with anti-CD3 antibodies (data not shown), and increased *in vitro* IL-5 and IL-13 production did not result in different outcomes of AHR, mucus production, BAL eosinophils, and T_H2 -type antibody response typical of allergic reactions.

Major symptoms of allergic asthma are mediated by IL-4 and IL-13, and a recent study demonstrated that IL-4Rα signaling in epithelial cells is required for enhanced mucus production in response to allergens. Using mice with disrupted expression of IL-

4Rα on Clara cells (goblet cell precursors), it was possible to demonstrate a cell-specific requirement for IL-4Rα for allergen-induced mucus production in a nonimmune cell type.¹¹ Although epithelial cell IL-4Rα signaling was required for mucus production, it was not required for development of AHR, suggesting that IL-4Rα signals on other cell types must contribute to induction of AHR.

The finding that loss of IL-4Rα on smooth muscle cells had no effect on any of the major etiologic markers of allergic asthma is surprising. A large body of *in vitro* data indicated a contribution of smooth muscle cell IL-4Rα in allergic asthma. Several studies showed enhanced responses of cultured murine smooth muscle cells to contractile agonists after treatment with IL-4 and IL-13.^{12,14,30} In human smooth muscle cells, IL-13 increased intracellular Ca²⁺ responses induced by contractile agonists¹⁴ and decreased responsiveness to bronchodilators.³¹ On the basis of these results, it was believed that narrowing of the airways in allergic asthma was influenced by direct effects of IL-4 and IL-13 on ASMCs. However, it was shown here that AHR in this model was independent of IL-4Rα signals in ASMCs, indicating that enhanced responses to contractile agonists after treatment with IL-4 or IL-13 observed *in vitro* are not necessary for AHR development *in vivo*. Furthermore, it was shown that IL-4Rα signaling on smooth muscle cells is not essential for inflammatory responses and eotaxin release during acute-phase allergic asthma. Because lung endothelial cells express eotaxin in response to IL-13³² in a STAT6-dependent manner,³³ these cells may be responsible for the IL-4Rα-dependent increases in serum eotaxin observed here contributing to the airway inflammation. On the other hand, we have previously shown that direct effects of IL-13 on airway epithelial cells did not induce airway inflammation *in vivo*.²⁹

Using the SM-MHC^{cre}IL-4Rα^{-/-lox} mice enabled us, for the first time, to test *in vivo* the assumptions about the role of IL-4Rα on

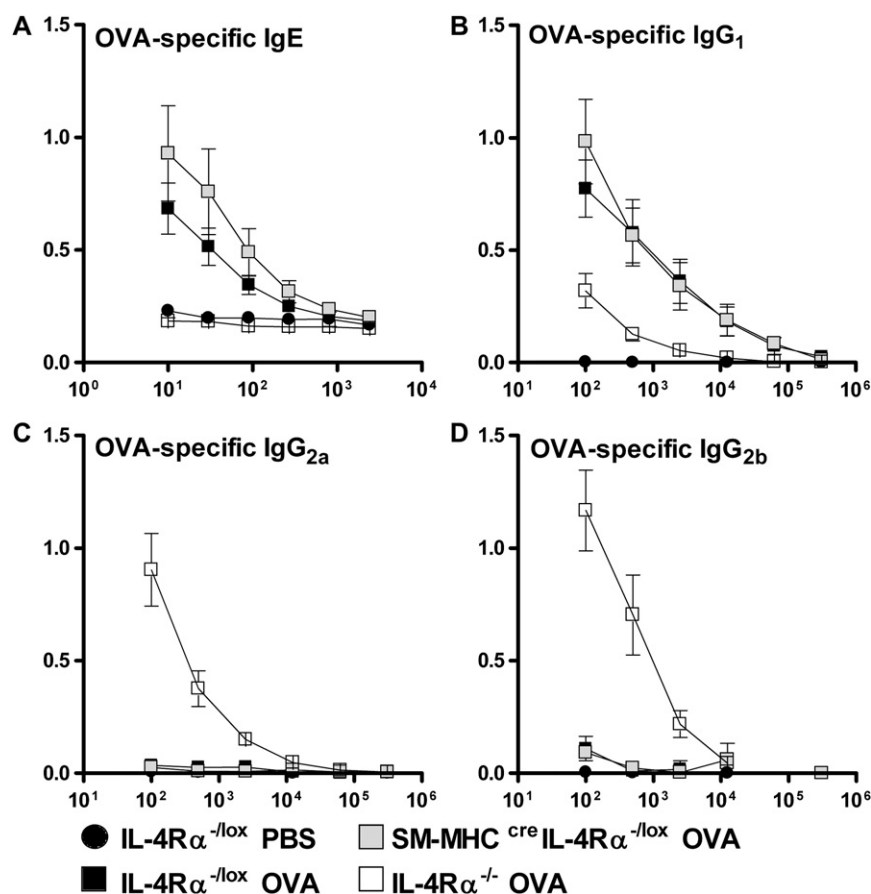


FIG 5. The allergen-specific antibody response of SM-MHC^{cre}IL-4Rα^{-/-lox} mice resembles the antibody response of IL-4Rα^{-/-lox} mice. OVA-specific antibodies were measured by endpoint titration ELISA. Tested subclasses were IgE (A), IgG₁ (B), IgG_{2a} (C), and IgG_{2b} (D). Data represent pooled values with SD from 2 experiments with 4 to 6 mice per group.

ASMCs derived from *in vitro* studies. No evidence was found to support an important role for smooth muscle cell IL-4Rα in allergic asthma. The presence of IL-4Rα on ASMCs in allergic asthma was not, as previously suggested, critical for the disease outcome, highlighting the importance of *in vivo* studies of complex diseases such as allergic asthma that are characterized by complicated interactions of different cell populations. However, despite not being necessary for the development of allergic airway disease in this model, our results do not rule out a contribution of IL-4Rα on ASMCs to allergic asthma. ASMCs do have the ability to produce a wide range of cytokines and chemokines on stimulation,³⁴ which are likely to play an important immunoregulatory role in allergic asthma independent of direct responsiveness to IL-4Rα. Such candidates are likely to define the role of ASMCs in allergic asthma.

The results presented here were derived from a mouse model of allergic asthma, and the situation might be similar in the human disease. As such, the data presented here could have important consequences for the development of new treatments of allergic asthma, and therapies targeting IL-4Rα in the lung might have no direct effect on ASMCs.

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Clinical implications: Therapies targeting the IL-4Rα might have no direct effect on smooth muscle cells in an allergic asthma response.

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