

Abbreviations used

AAMs: Alternatively activated macrophages
 AHR: Airway hyperreactivity
 FIZZ-1: Found in inflammatory zone 1 (RELM- α)
 HDM: House dust mite
 IL-4R α : IL-4 receptor α
 iNOS: Inducible nitric oxide synthase
 OVA: Ovalbumin

suppressive properties in diseases such as schistosomiasis,⁷ filarial infection,²² arthritis,²³ and experimental autoimmune encephalomyelitis,²⁴ and can downregulate T-cell proliferation.^{11,22,25-27} Therefore, we aimed to test the hypothesis that AAMs contribute to allergic airway disease by using LysM^{cre}IL-4R α ^{-flox} mice, which have abrogated IL-4R α signaling on macrophages and neutrophils and as a result lack IL-4R α -dependent AAMs.⁷ Our results demonstrate no reduction in allergic airway disease in the absence of IL-4R α -dependent AAMs, suggesting that AAMs may not be as important in allergic airway disease as is commonly suggested.

METHODS**Mice**

Eight- to 10-week-old LysM^{cre}IL-4R α ^{-flox}, IL-4R α ^{-flox}, and IL-4R α ^{-/-} mice²⁸ on a BALB/c background were housed in independently ventilated cages under specific pathogen-free conditions in the University of Cape Town Animal Facility. Animal procedures were approved by the University of Cape Town Animal Ethics Committee.

Models of allergic airway disease**Acute ovalbumin-induced allergic airway disease.**

Mice were sensitized and challenged as previously described²⁹ and AHR was measured on day 24. After the procedure, mice were euthanized and tissue samples were taken for analysis.

Chronic ovalbumin-induced allergic airway disease.

Mice were sensitized subcutaneously with 20 μ g ovalbumin (OVA) adsorbed to 0.65% alum on days 0, 7, 14, and 21. On days 27 and 29 and then twice weekly for 4 weeks, mice were administered intranasal challenges with 20 μ g OVA in 50 μ L PBS under anesthesia.³⁰ AHR was measured on day 60.

House dust mite-induced allergic airway disease.

Mice were anaesthetized and sensitized intranasally with 100 μ g house dust mite (HDM) extract (Greer Laboratories, Lenoir, NC) on day 0 and 10 μ g HDM on days 7 to 11, as previously described.³¹ AHR was measured on day 14.

AHR

Airway resistance and elastance of the whole respiratory system (airways, lung chest wall) after challenge with increasing doses of acetyl- β -methylcholine (methacholine, Sigma-Aldrich, Aston Manor, South Africa) were determined by forced oscillation measurements as previously described²⁹ with a Flexivent system (SCIREQ, Montreal, Canada) by using the single-compartment ("snapshot") perturbation. Differences in the dose-response curves were analyzed by repeated-measures ANOVA. Only mice with acceptable measurements for all doses (coefficient of determination >0.95) were included in the analysis. After the procedure, mice were killed and tissue samples were taken for analysis.

Flow cytometry

Bronchoalveolar lavage (BAL) cells were obtained as previously described.²⁹ Single-cell suspensions were prepared from lungs after digestion for

1 hour at 37°C in Iscove's Modified Dulbecco's Medium (IMDM) (Invitrogen, Carlsbad, Calif) containing 13 μ g/mL DNase I (Roche, Randburg, South Africa) and 50 U/mL collagenase IV (Sigma-Aldrich). For intracellular cytokine staining, lung cells were incubated at 37°C for 4 hours with phorbol myristate acetate (Sigma-Aldrich) (50 ng/mL), ionomycin (Sigma-Aldrich) (250 ng/mL), and monensin (Sigma-Aldrich) (200 μ M in IMDM/10% FCS). See additional Methods information in this article's Online Repository at www.jacionline.org.

Sorting of alveolar macrophages

Alveolar macrophages were sorted as CD11c⁺SiglecF⁺ cells from the pooled lungs of OVA-sensitized and -challenged mice ($n = 5$). Cells were sorted on a FACS Vantage flow cytometer (BD Immunocytometry Systems, San Jose, Calif). Purity was determined by flow cytometry and checked by cyto-spin and staining with the Rapidiff Stain set (Clinical Diagnostics CC, Southdale, South Africa) and was at least 99%.

NO and arginase assays

Alveolar macrophages sorted from the lungs of OVA-sensitized and -challenged IL-4R α ^{-flox} and LysM^{cre}IL-4R α ^{-flox} mice ($n = 5$, pooled) were plated in triplicates in 96-well plates at 2×10^5 cells per well and 2×10^4 cells per well for NO and arginase assays, respectively, in the presence or absence of 100 ng/mL LPS (Sigma-Aldrich). NO was quantified in supernatants by the Griess reaction.³² The arginase assay was carried out as previously described.³²

Quantitative RT-PCR

Total RNA was extracted from sorted alveolar macrophages by using Tri reagent (Applied Biosystems, Carlsbad, Calif) according to the manufacturer's protocol and purified with RNeasy Microprep kit (Qiagen Hilden, Germany). cDNA was synthesized with Transcriptor First Strand cDNA synthesis kit (Roche), and real-time PCR was performed by using Lightcycler FastStart DNA Master^{PLUS} SYBR Green I reaction mix (Roche) on a Lightcycler 480 II (Roche). Primers for inducible nitric oxide synthase (iNOS), arginase, Ym-1, and FIZZ-1 have been previously described.³³ Values were normalized according to the expression of the housekeeping gene ribosomal *RPS12* or *RPL32*.

Histology and immunohistochemistry

Lungs were fixed in 4% formaldehyde/PBS and embedded in paraffin. Tissue sections were stained with periodic acid-Schiff for mucus and Sirius red for collagen. Image analysis was performed on NIS Elements (Nikon Instruments, Tokyo, Japan). Lung sections from individual mice were assessed, and data from 3 experiments were pooled ($n = 4$ -6 per experiment).

ELISAs

Antibody ELISAs were carried out as previously described,³⁴ using 1 mg/mL OVA to coat for specific IgE and 5 μ g/mL OVA to coat for specific IgGs.

Statistical analysis

P values were calculated in GraphPad Prism 4 (GraphPad Software, Inc, San Diego, Calif) by using ANOVA with Bonferroni's posttest or repeated-measures ANOVA, and results are presented as mean $\pm$ SE of the mean. Differences were considered significant if *P* was <.05.

RESULTS**Characterization of macrophages from LysM^{cre}IL-4R α ^{-flox} mice**

The LysM^{cre}IL-4R α ^{-flox} mouse strain was previously characterized by our laboratory and found to have impaired expression

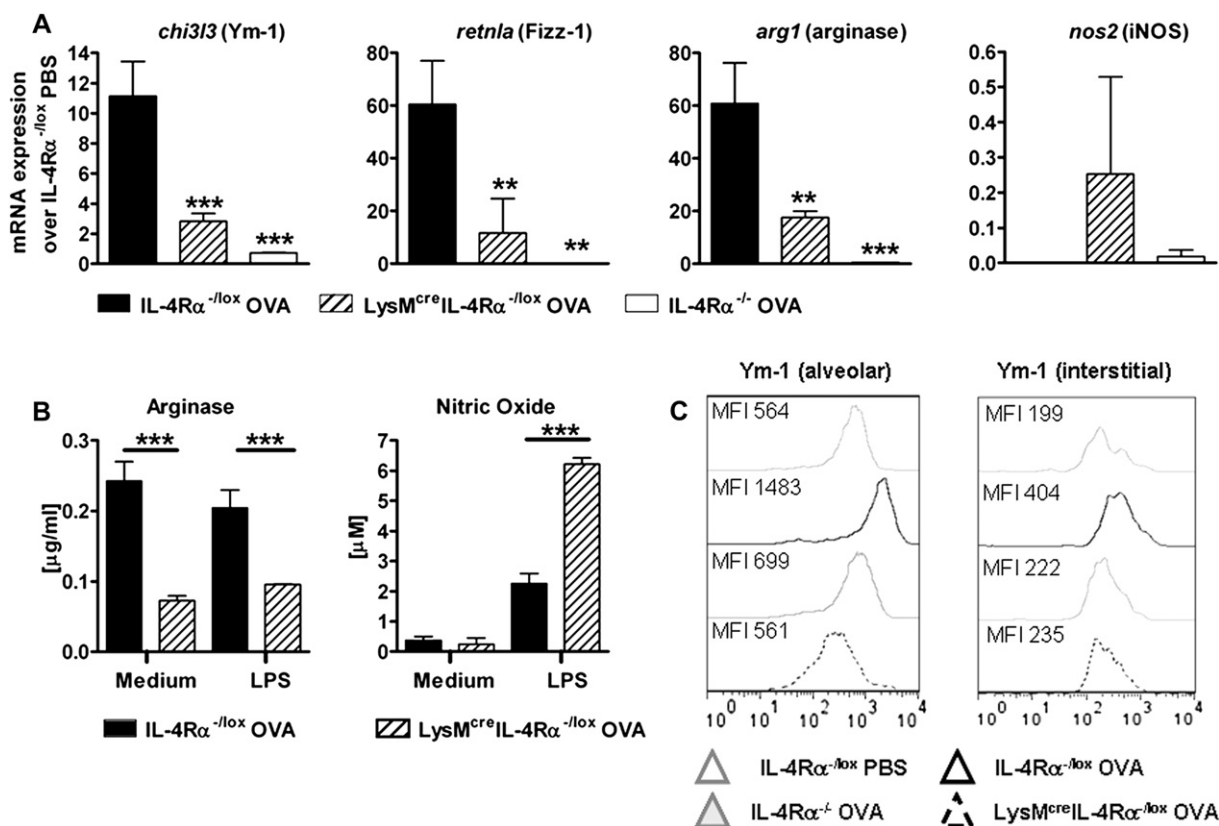


FIG 1. A, mRNA expression in alveolar macrophages (chronic OVA model). Expression was normalized by using the housekeeping gene *RPS12* and is shown as fold difference to the PBS group. Data are representative of 2 experiments with triplicate pooled samples. B, Arginase activity and NO production by alveolar macrophages. C, Ym-1 expression on alveolar and interstitial macrophages. ****P* < .05; *****P* < .001, compared with littermate controls. MFI, Mean fluorescence intensity.

of IL-4Rα on spleen macrophages and neutrophils but not dendritic cells or lymphocytes.⁷ Flow cytometry was used to further characterize IL-4Rα expression in the lungs of OVA-challenged mice (chronic model). The results showed that IL-4Rα expression is effectively abrogated on alveolar macrophages, interstitial macrophages, inflammatory macrophages, monocytes, and neutrophils, but not on dendritic cells, in LysM^{cre}IL-4Rα^{-/-} mice (see Fig E1 in this article's Online Repository at www.jacionline.org).

Alveolar macrophages from OVA-challenged mice (chronic model) were sorted, and mRNA expression of markers for alternative activation (Ym-1, FIZZ-1, and Arg-1) and classical activation (iNOS) were quantified (Fig 1, A). Macrophages from IL-4Rα^{-/-} mice (hemizygous littermate controls) had increased expression of Ym-1, FIZZ-1, and arginase compared with PBS controls, suggesting alternative activation of macrophages. The expression of arginase, Ym-1, and FIZZ-1 was significantly decreased in LysM^{cre}IL-4Rα^{-/-} macrophages compared to IL-4Rα^{-/-} controls, while iNOS expression was increased, demonstrating a shift toward classical macrophage activation in the absence of IL-4Rα signaling. Reduced arginase activity and increased iNOS activity in alveolar macrophages of LysM^{cre}IL-4Rα^{-/-} mice was confirmed by *ex vivo* assays (Fig 1, B), and reduced expression of Ym-1 in alveolar and interstitial macrophages was confirmed by flow cytometry (Fig 1, C). An overall reduction in Ym-1 was also observed in the lungs of

LysM^{cre}IL-4Rα^{-/-} mice in the acute model by immunohistological staining (see Fig E2 in this article's Online Repository at www.jacionline.org). Interestingly, the majority of Ym-1-positive cells were large and located in the airway spaces, indicating that these cells were primarily alveolar macrophages and not interstitial macrophages, which are located in the interstitium. Together, these results show impaired IL-4Rα-dependent AAMs in OVA-challenged mice.

Airway resistance and elastance are maintained in LysM^{cre}IL-4Rα^{-/-} mice

The airway responses of LysM^{cre}IL-4Rα^{-/-} mice and littermate controls were examined in OVA-induced allergic airway disease. Airway resistance and elastance were measured in response to increasing doses of methacholine after both short-term (acute) (Fig 2, A and B) and long-term (chronic) (Fig 2, C and D) exposure to OVA. In both models, LysM^{cre}IL-4Rα^{-/-} and IL-4Rα^{-/-} mice developed AHR, with no significant differences between LysM^{cre}IL-4Rα^{-/-} mice and IL-4Rα^{-/-} littermate controls (Fig 2; see Table E1 in this article's Online Repository at www.jacionline.org). A trend toward a slight, but not significant, increase in both airway resistance and elastance was observed in LysM^{cre}IL-4Rα^{-/-} mice. Global IL-4Rα^{-/-} mice did not show a significant increase in AHR compared with PBS controls, demonstrating protection. Whereas airway

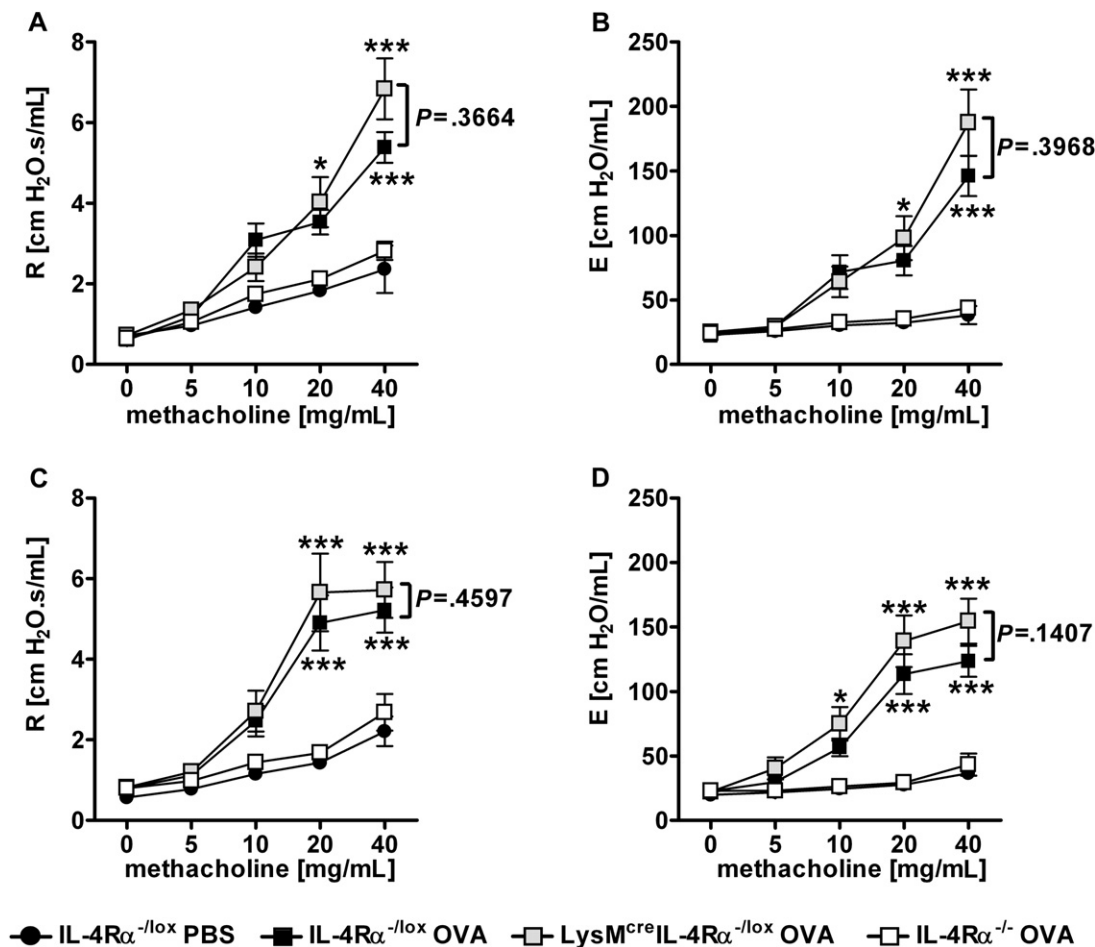


FIG 2. Resistance (A) and elastance (B) in acute OVA-induced allergic airway disease. N = 8 to 11 mice, pooled data from 2 experiments. Resistance (C) and elastance (D) in chronic OVA-induced allergic airway disease. N = 12 to 16 mice, pooled data from 3 experiments. Significant differences to IL-4Rα^{-/-} PBS: **P* < .05, ****P* < .001. *P* values: Comparison of dose-response curves to inhaled methacholine.

resistance assesses constriction of the airways, an increase in airway elastance is a measure of rigidity of the whole respiratory system and may indicate similar airway remodeling in LysM^{cre}IL-4Rα^{-/-} mice and littermate controls. These results provide strong evidence that the absence of IL-4Rα-dependent AAMs does not protect against AHR.

Airway inflammation and collagen deposition are maintained in the absence of IL-4Rα-dependent AAMs

In both the acute model (see Fig E3 in this article's Online Repository at www.jacionline.org) and the chronic model (Fig 3), IL-4Rα^{-/-} mice had increased mucus hypersecretion and airway inflammation compared with PBS controls, with infiltrating cells observed around airways and blood vessels (Fig 3, A, and Fig E3, A). Cellular infiltration was also observed in LysM^{cre}IL-4Rα^{-/-} and IL-4Rα^{-/-} mice, with no significant differences in total lung cell counts between the OVA-treated mouse strains (Fig 3, C, and Fig E3, B). Mucus hypersecretion (quantified by image analysis) was similar in IL-4Rα^{-/-} and LysM^{cre}IL-4Rα^{-/-} mice but abrogated in the global IL-4Rα^{-/-} strain (Fig 3, D, and Fig E3, C).

It was suggested that AAMs may play an important role in collagen deposition through increased production of prolines as a result of arginase activity.^{15,16} By using the chronic model,³⁰ we were able to assess the effect of AAMs on collagen deposition. Increased collagen deposition (shown by Sirius red staining and quantified by image analysis on NIS Elements software) was found around airways and blood vessels in OVA-challenged mice (Fig 3, B), with similar levels of deposition in IL-4Rα^{-/-} and LysM^{cre}IL-4Rα^{-/-} mice (Fig 3, E). Analysis of the cellular composition of BAL fluid found that IL-4Rα^{-/-} and LysM^{cre}IL-4Rα^{-/-} mice both had increased percentages of eosinophils compared with PBS controls in both the acute and chronic models (Fig 4, A and B). Numbers of eosinophils in the lung tissue were also increased in both IL-4Rα^{-/-} and LysM^{cre}IL-4Rα^{-/-} mice (Fig 4, C). There were also no significant differences in numbers of neutrophils, CD4⁺ T cells, CD8⁺ T cells, B cells, dendritic cells, and alveolar macrophages between IL-4Rα^{-/-} and LysM^{cre}IL-4Rα^{-/-} mice (see Fig E4 in this article's Online Repository at www.jacionline.org). Together, these data illustrate that IL-4Rα-dependent AAMs are not necessary for the development of OVA-induced allergic airway inflammation and do not appear to regulate collagen deposition.

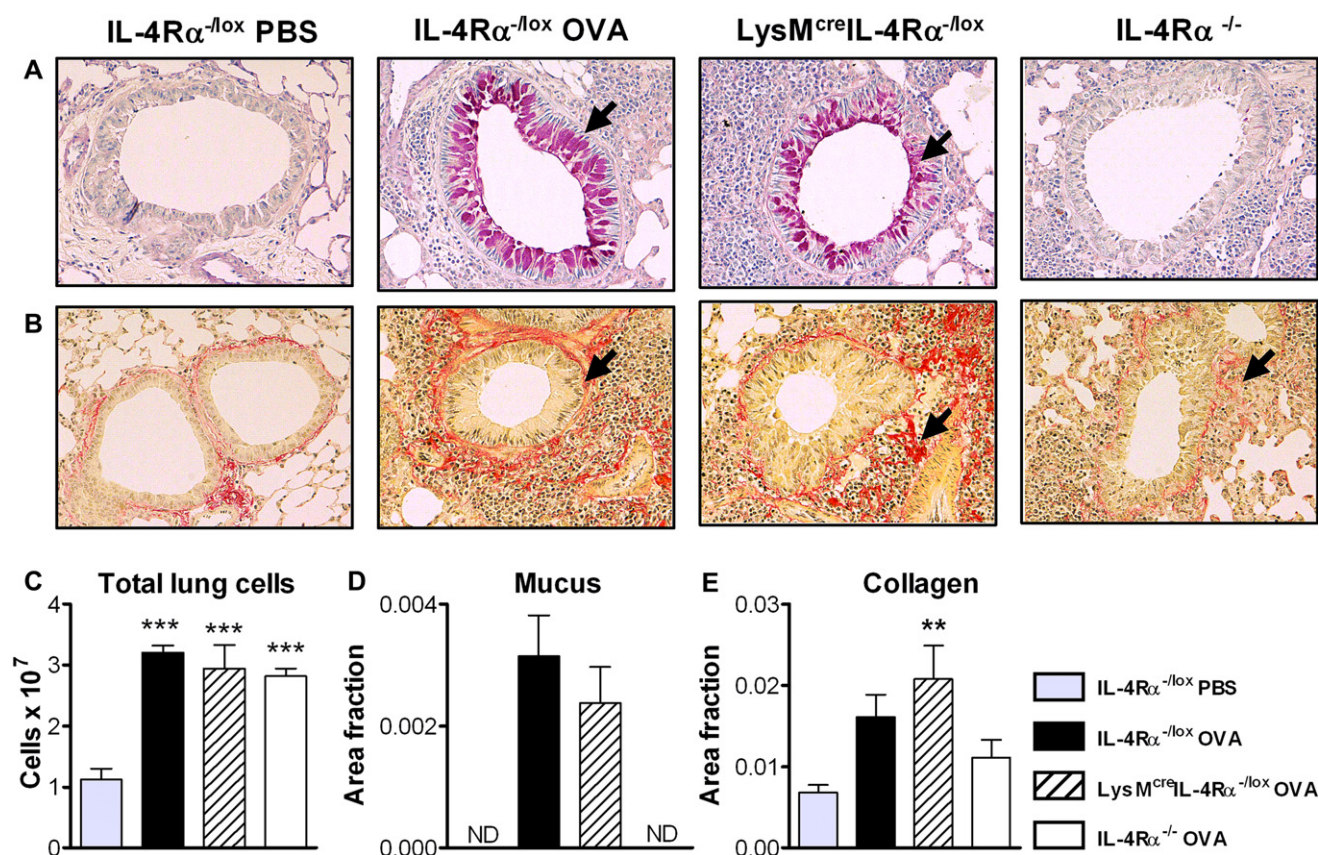


FIG 3. Lung pathology (chronic model). **A**, Mucus hypersecretion and inflammation around airways. Arrows indicate mucus hypersecretion. **B**, Collagen deposition around airways and blood vessels illustrated by Sirius red staining. Arrows indicate collagen deposition. **C**, Total lung cells. **D**, Mucus quantification. **E**, Collagen quantification. Representative of 3 experiments (n = 4-6 each). Significance versus PBS control: ** $P < .01$, *** $P < .001$.

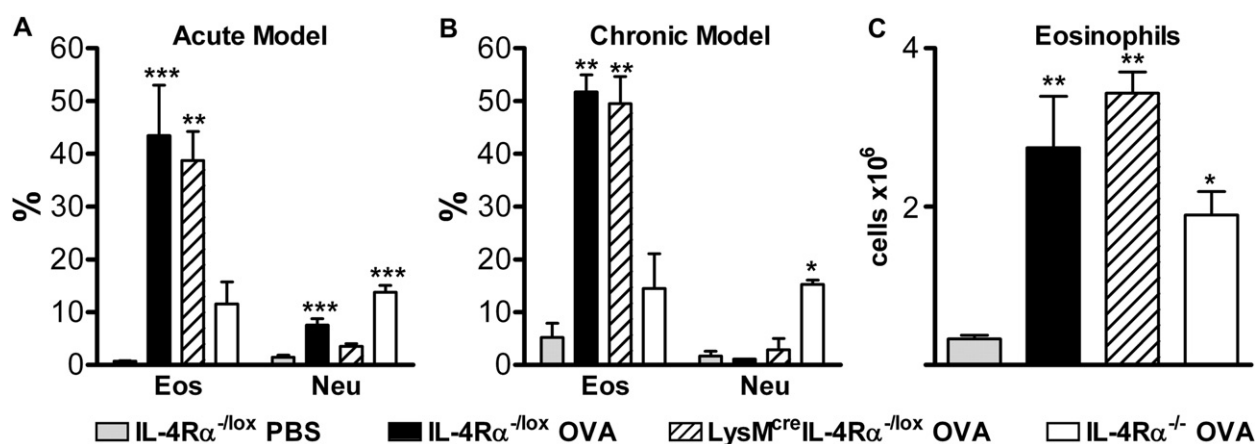


FIG 4. Eosinophils (*Eos*) and neutrophils (*Neu*) in BAL fluid (n = 5-6) after acute (**A**) and chronic (**B**) OVA-induced allergic airway disease. **C**, Numbers of eosinophils in the lungs (n = 5) after chronic OVA exposure. Significant differences to IL-4R $\alpha^{-/-}$ PBS are represented by stars (* $P < .05$, ** $P < .01$, *** $P < .001$).

Similar antibody and cytokine responses in the absence of IL-4R α -dependent AAMs

OVA-specific IgE and total IgE levels were similar in LysM^{cre}IL-4R $\alpha^{-/-}$ and IL-4R $\alpha^{-/-}$ mice in both the acute

model (data not shown) and the chronic model (Fig 5, A), as were OVA-specific IgG levels (see Fig E5 in this article's Online Repository at www.jacionline.org). Both LysM^{cre}IL-4R $\alpha^{-/-}$ and IL-4R $\alpha^{-/-}$ mice had similar increases in total and

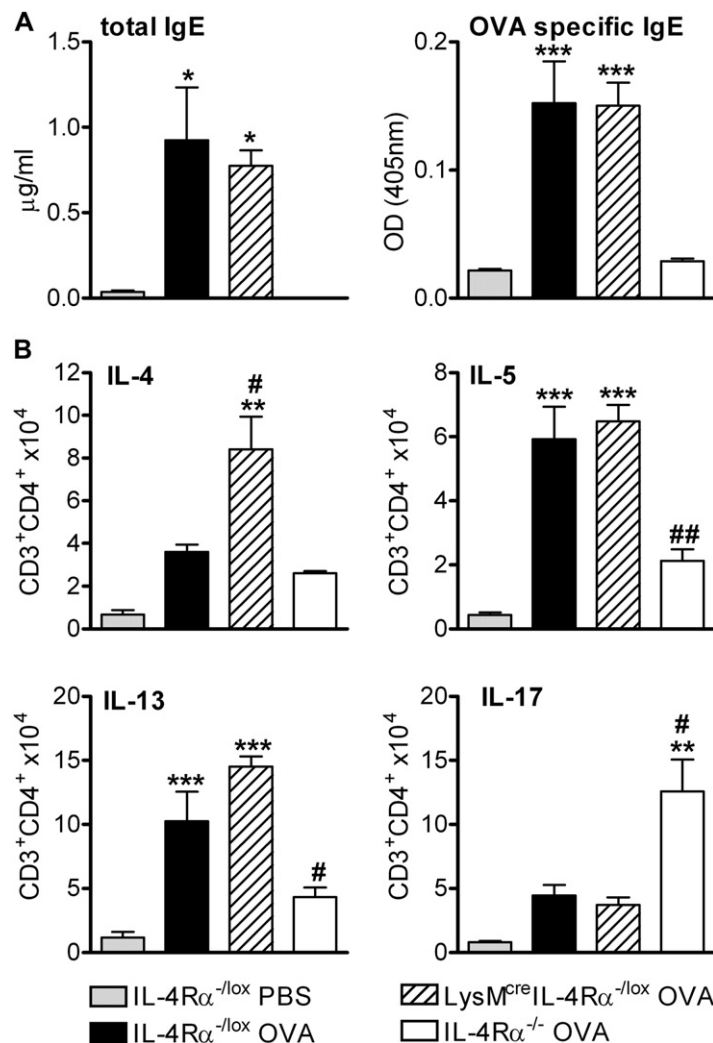


FIG 5. Antibody and cytokine production in chronic OVA-induced allergic airway disease. **A**, Total and OVA-specific IgE (n = 5). **B**, Number of cytokine-producing lung CD4⁺ T cells (n = 3-5). Significant differences to IL-4Rα^{-/-} PBS: * *P* < .05, ** *P* < .01, *** *P* < .001. Significant differences to IL-4Rα^{-/-} OVA: # *P* < .05, ## *P* < .01. Representative of 2 experiments.

OVA-specific IgE concentrations compared with PBS controls and IL-4Rα^{-/-} mice, and higher IgG₁ but lower IgG_{2a} and IgG_{2b} titers than IL-4Rα^{-/-} mice, indicative of a predominant T_H2-type response. There were significantly increased numbers of IL-4-producing CD4⁺ T cells in the lungs of LysM^{cre}IL-4Rα^{-/-} mice compared with littermate controls, while numbers of cells producing IL-5, IL-13, and IL-17 cells were not significantly different (Fig 5, B). Therefore, the absence of IL-4Rα-dependent AAMs did not reduce T_H2 responses or affect T_H17 responses in allergic airway disease.

LysM^{cre}IL-4Rα^{-/-} mice are not protected against HDM-induced allergic airway disease

To confirm our findings with a clinically relevant allergen, we tested LysM^{cre}IL-4Rα^{-/-} mice in a model of HDM-induced allergic airway disease³¹ (see Fig E6 in this article's Online Repository at www.jacionline.org). In this model, LysM^{cre}IL-4Rα^{-/-} mice had no significant differences in eosinophil numbers in BAL, total IgE concentration, airway inflammation, mucus

hypersecretion, and AHR compared with littermate controls (Fig E6, A-D), demonstrating that IL-4Rα-dependent AAMs do not play a role in HDM-induced allergic airway disease. Numbers of T_H2 cytokine-producing lung CD4⁺ T cells (Fig E6, E) and cytokine production by mediastinal lymph node cells restimulated *ex vivo* with HDM extract (data not shown) were similar between IL-4Rα^{-/-} and LysM^{cre}IL-4Rα^{-/-} mice, while IL-4Rα^{-/-} mice produced both less T_H2 cytokines and less IFN-γ, suggesting impaired antigen-specific responses in the absence of IL-4/IL-13 signaling. Global IL-4Rα-deficient mice had decreased AHR and mucus hypersecretion, and percentages of BAL eosinophils were decreased while neutrophils were increased (Fig E6, A-D).

DISCUSSION

Although markers of AAMs are upregulated in both murine allergic airway disease and human asthma, to date no studies have shown clear evidence that these macrophages play a causative role in the disease. By using LysM^{cre}IL-4Rα^{-/-} mice, which

have impaired expression of IL-4R α on macrophages and neutrophils and therefore lack IL-4/IL-13–induced activation of macrophages through IL-4 receptor types 1 and 2, we aimed to determine the role of IL-4R α –dependent AAMs in murine allergic airway disease. Our results in 3 experimental mouse models demonstrate that IL-4R α –dependent AAMs are not necessary in the pathogenesis of allergic airway disease in mice.

Similar to previous studies,^{15,17,18,35} markers of AAMs such as arginase, Ym-1, and FIZZ-1 were upregulated in littermate control IL-4R α ^{−/lox} mice during OVA-induced allergic airway disease. LysM^{cre}IL-4R α ^{−/lox} mice had impaired expression of these markers on alveolar macrophages, although they still exhibited a slight increase in comparison to the PBS controls. IL-4R α –independent expression of AAM markers has been previously observed.^{33,36} Despite impaired AAM activation, LysM^{cre}IL-4R α ^{−/lox} mice had similar AHR, airway inflammation, mucus hypersecretion, and collagen deposition to littermate controls. Importantly, this indicates that IL-4R α –dependent AAMs do not play an important role in the regulation of collagen deposition as suggested on the basis of their expression of arginases.^{15,16} A previous study by our laboratory also showed normal collagen deposition in liver granulomas after *Schistosoma mansoni* infection in LysM^{cre}IL-4R α ^{−/lox} mice.⁷ Furthermore, mice with arginase-1–deficient macrophages had increased, rather than reduced, collagen deposition in the liver in a chronic schistosomiasis model,³⁷ suggesting that particular molecules expressed by AAMs may suppress fibrosis rather than promote it. Our results also showed that AAMs are not important for eosinophil infiltration, as was previously suggested because of their expression of eotaxin and Ym-1, both of which can act as chemoattractants for eosinophils.¹⁹ Therefore, it appears that the association between AAMs and allergic airway disease does not translate into a significant pathological role. On the contrary, we found a slight though nonsignificant increase in AHR in LysM^{cre}IL-4R α ^{−/lox} mice in OVA-induced allergic airway disease, associated with a trend toward slightly increased numbers of IL-4– and IL-13–producing CD4⁺ T cells in the lungs. There is evidence to suggest that macrophages can prevent an immunological overreaction in the lungs.^{38–42} Depletion of alveolar macrophages exacerbates AHR, eosinophilic inflammation, and levels of T_H2 cytokines,⁴³ while interstitial macrophages have been shown to have suppressive properties in allergic airway disease through the production of IL-10.¹⁰ It appears that interstitial macrophages are important for preventing the initial triggering of a response to innocuous allergens, while alveolar macrophages play a role in suppressing effector responses.¹⁰

Many studies show the downmodulation of inflammation by AAMs in various disease models.^{7,22,44} Furthermore, alveolar macrophages from asthmatic patients have different properties than do those isolated from healthy controls and release less IL-10 and more inflammatory cytokines such as TNF- α , IL-1- β , GM-CSF, MIP-1 α , IL-6, and IL-8,^{42,45} markers typically associated with classical activation of macrophages. Studies also demonstrate that AAMs suppress T-cell proliferation,^{11,22,25–27,37,46} and molecules associated with AAMs such as FIZZ-1 have been shown to negatively regulate helminth-induced T_H2 responses and associated inflammation.^{27,47} Studies examining the role of individual molecules associated with AAMs will therefore be valuable in clarifying whether individual markers of alternative activation associated with asthma are beneficial or detrimental to airway pathology. Interestingly, recent

studies indicate that neither FIZZ-1 nor arginase plays any significant pathological role in allergic airway disease in mice, which correlates with our findings.⁴⁸ It will, however, be interesting to determine how alternative activation influences the phenotype of distinct macrophage populations, such as alveolar macrophages, interstitial macrophages, and inflammatory macrophages.

In conclusion, we find no evidence to suggest that IL-4R α –activated AAMs are detrimental in allergic airway disease in mice, despite the importance of global IL-4R α expression, suggesting that effector functions of other cell types are more important in the development of AHR and pathology. The presence of AAMs in allergic airway disease may be merely associative—a consequence of elevated T_H2 responses.

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Key messages

- Mice lacking IL-4R α –induced alternative activation of macrophages were not protected against allergic airway disease.
- Alternatively activated macrophages are not necessary for allergic airway disease and may be only associative.

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METHODS

Flow cytometry

BAL cells were obtained as previously described.²⁶ Single-cell suspensions were prepared from lungs after digestion for 1 hour at 37°C in IMDM (Invitrogen, Carlsbad, Calif) containing 13 µg/mL DNase I (Roche, Randburg, South Africa) and 50 U/mL collagenase IV (Sigma-Aldrich, Aston Manor, South Africa). The following antibodies were used for flow cytometry: SiglecF-PE (clone E50-2440), CD11c-APC (clone HL3), CD11b-PE (clone M1/70), Ly6C-biotin (clone AL-21), F480 APC (clone BM-8), CD4-PerCP (clone L3T4), CD8-PE (clone Ly2), CD124-PE (clone mIL4R-M1), rat antimouse IgG_{2a}-PE (clone R35-95), CD19-biotin (clone 1D3), CD124-biotin (clone mIL-4R-M1) and rat-anti-mouse IgG_{2a} biotin (clone R35-95) with streptavidin-APC (all BD Bioscience, Erembodegem, Belgium), and CD fluorescein isothiocyanate (clone 145-2C11), GR-1-fluorescein isothiocyanate (clone RB68C5), and MHCII-biotin (clone M5/114) with PerCP streptavidin (BD Bioscience). For intracellular cytokine staining, lung cells were incubated at 37°C for 4 hours with phorbol myristate acetate (Sigma-Aldrich)

(50 ng/mL), ionomycin (Sigma-Aldrich) (250 ng/mL), and monensin (Sigma-Aldrich) (200 µM) in IMDM/10% FCS. Cells were stained with CD3-fluorescein isothiocyanate (clone 145-2C11) and CD4-PerCP (clone L3T4) (BD Bioscience), and intracellular cytokines were stained with anti-IL-4 (clone 11B11), anti-IL-5 (clone TRFK5), anti-IFN-γ (clone XMG 1.2), anti-IL-17 (clone TC11-18H10) (BD Bioscience), anti-IL-13 (clone eBio 13A) (eBioscience, San Diego, Calif), and rat anti-mouse IgG₁ isotype control (clone A85-1) (BD Bioscience) (all PE-labeled). Cells were acquired on a FACSCalibur machine (BD Immunocytometry Systems, San Jose, Calif), and data were analyzed by using Flowjo software (Treestar, Ashland, Ore).

Ym-1 immunohistochemistry

Formalin fixed sections were stained with biotinylated antimouse Ym-1 antibody (R&D Systems, Minneapolis, Minn) and peroxidase-labeled streptavidin. Ym-1-positive cells in the alveolar spaces were quantified in 10 fields of view per lung section for each mouse at ×400 magnification.

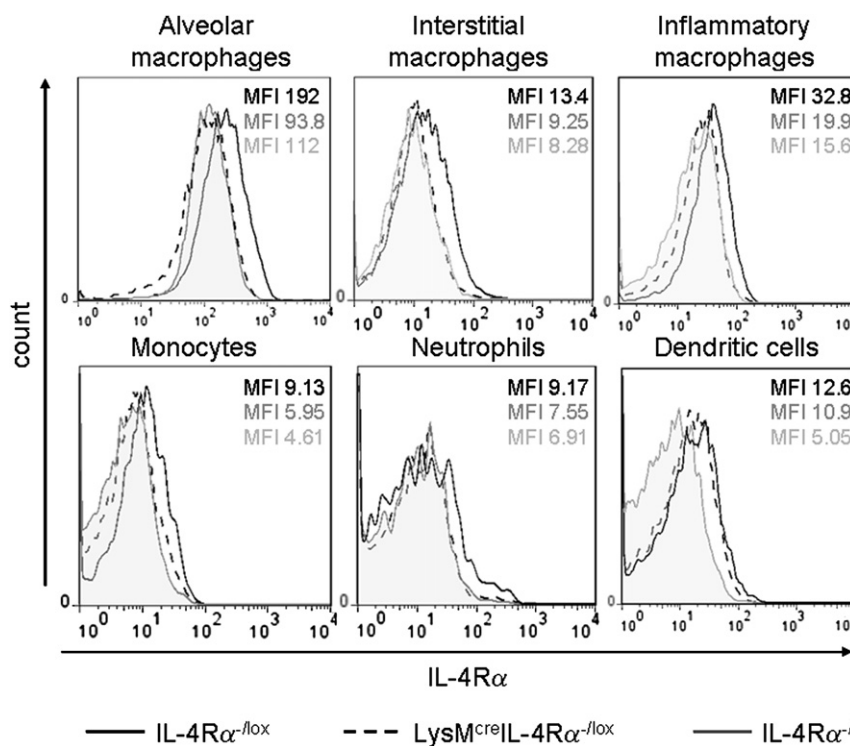


FIG E1. Impairment of IL-4R α expression on macrophages of OVA-challenged lungs of LysM^{cre}IL-4R α ^{-flox} mice (chronic model). Similar results were observed in the acute model. Representative of 3 individual mice for each strain. IL-4R α expression was evaluated by flow cytometry on alveolar macrophages (CD11c⁺SiglecF⁺), interstitial macrophages (F480⁺CD11c⁻SiglecF⁻), inflammatory macrophages (CD11c⁺MHCII⁺CD11c⁻), monocytes (Lyc6C⁺CD11b⁺CD11c⁻), neutrophils (GR-1^{high}CD11c⁻), and dendritic cells (CD11c⁺MHCII⁺SiglecF⁻). *MFI*, Mean fluorescence intensity.

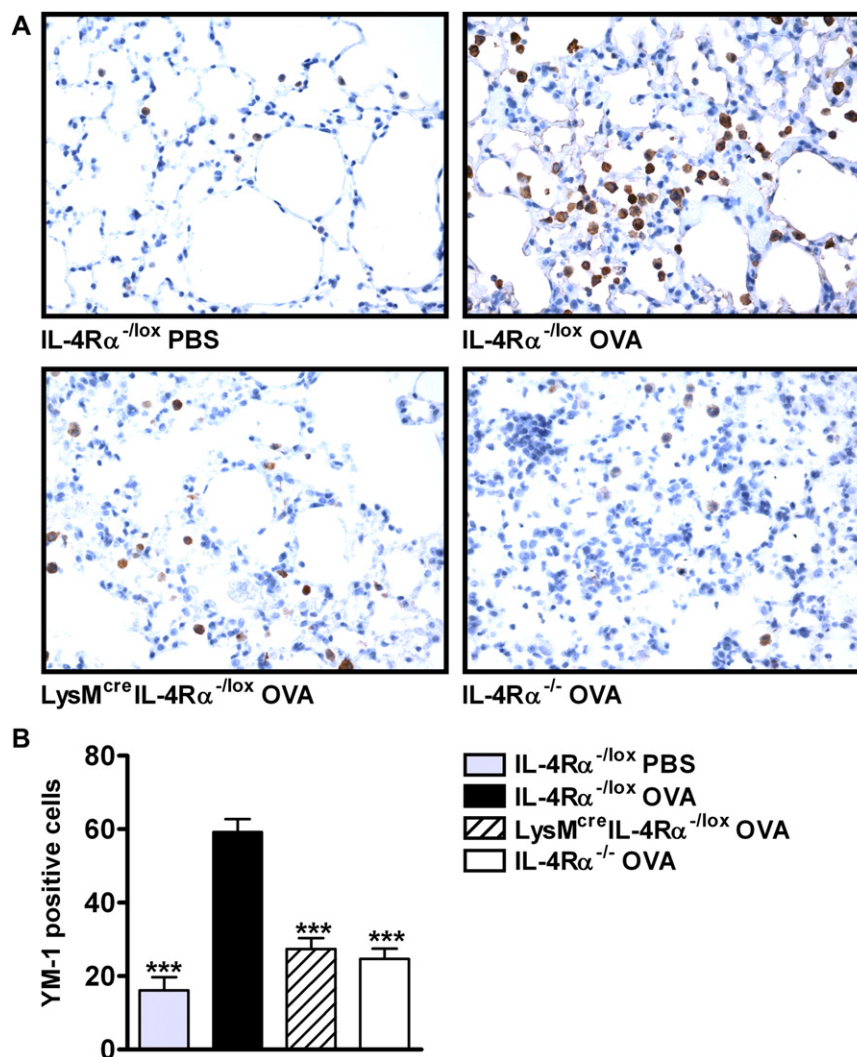


FIG E2. A, Immunohistochemical staining of Ym-1-positive cells in the lung. B, Quantification of Ym-1-positive cells in the alveolar spaces. Ym-1-positive cells in the alveolar spaces were quantified in 10 fields of view per lung section for each mouse at $\times 400$ magnification ($n = 6$ per group). Significant difference to IL-4R α ^{-lox} OVA group: *** $P < .001$.

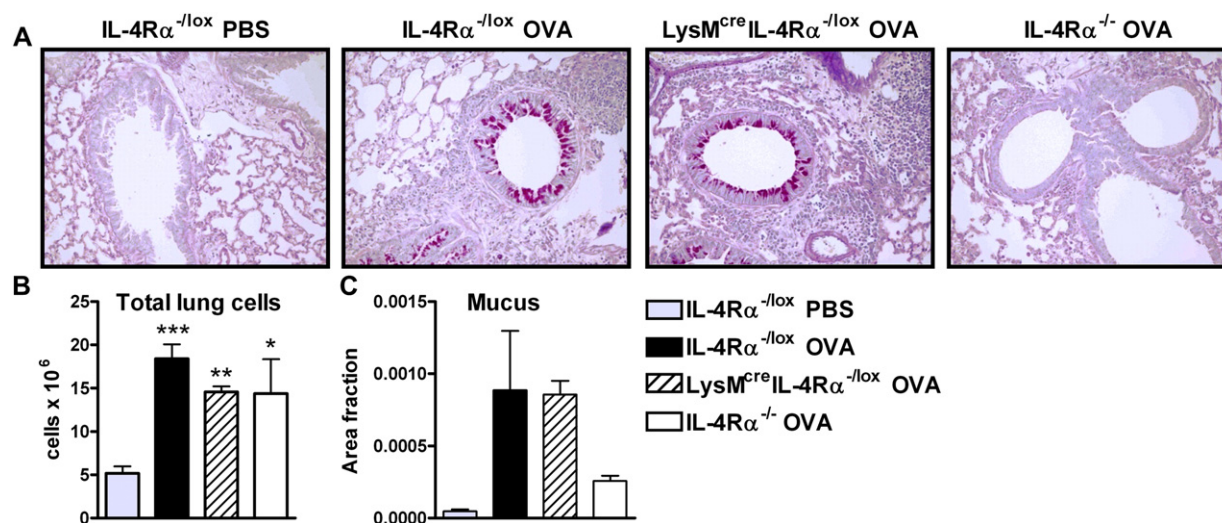


FIG E3. Lung pathology in the acute OVA model. **A**, Lung sections were stained with periodic acid-Schiff for mucus. **B**, Lungs were digested and total lung cells were counted ($n = 4-6$). **C**, Mucus was quantified in whole lungs by using NIS Elements. One lung section was quantified per individual mouse. Representative of 2 experiments pooled ($n = 7-9$). * $P < .05$, ** $P < .01$, *** $P < .001$.

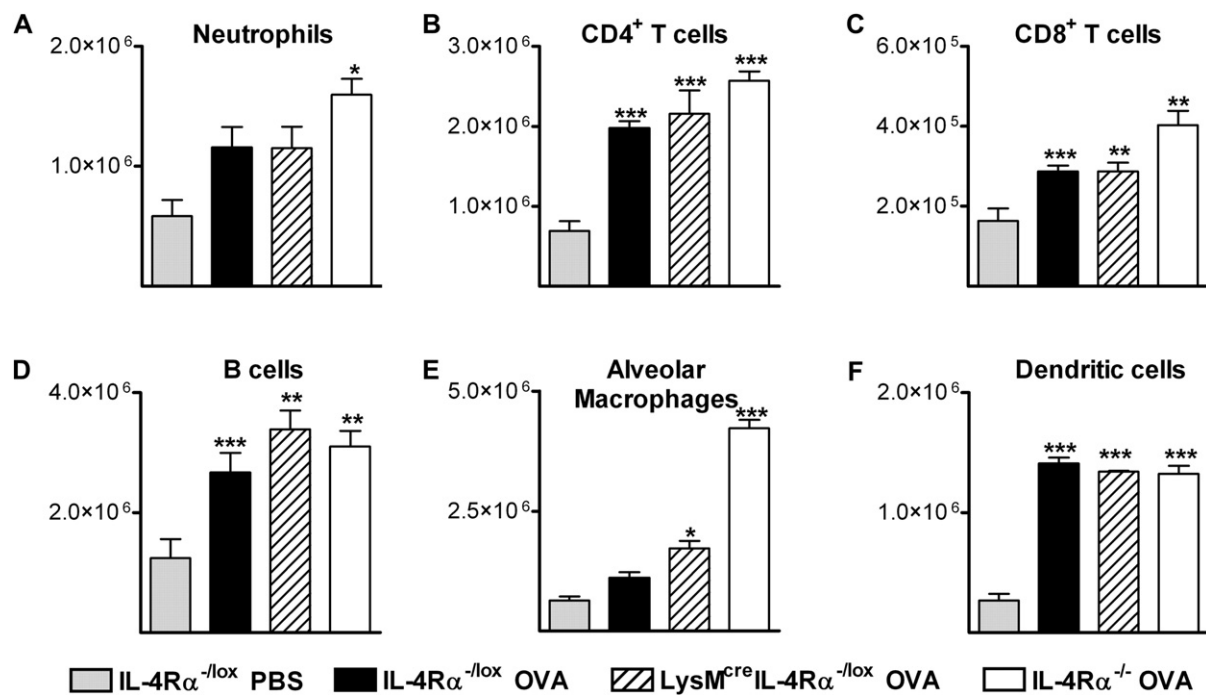


FIG E4. Immune cell populations in the lungs ($n = 5$) after chronic OVA exposure. **A**, Neutrophils (GR-1^{high} CD11c⁻). **B**, T helper cells (CD3⁺ CD4⁺). **C**, Cytotoxic T cells (CD3⁺ CD8⁺). **D**, B cells (CD19⁺ CD3⁻). **E**, Alveolar macrophages (CD11c⁺ SiglecF⁺ GR-1^{int}). **F**, Dendritic cells (CD11c⁺ SiglecF⁻). Significant differences to IL-4Rα^{-/-} PBS are represented by stars (* $P < .05$, ** $P < .01$, *** $P < .001$).

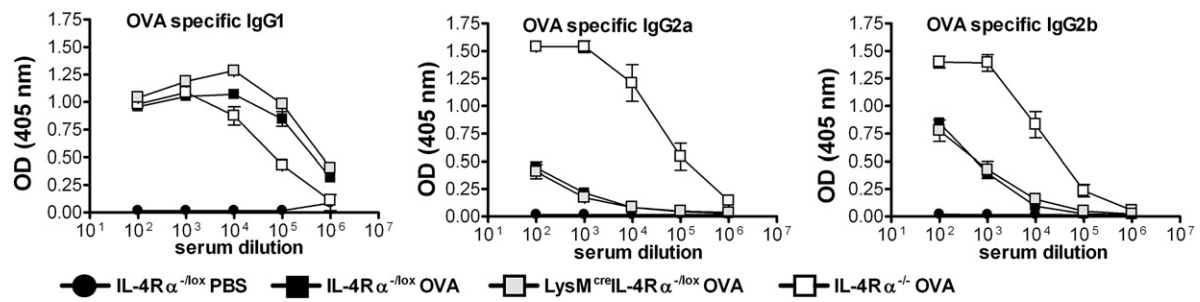


FIG E5. OVA-specific IgG antibodies in mice sensitized and challenged with OVA (chronic model).

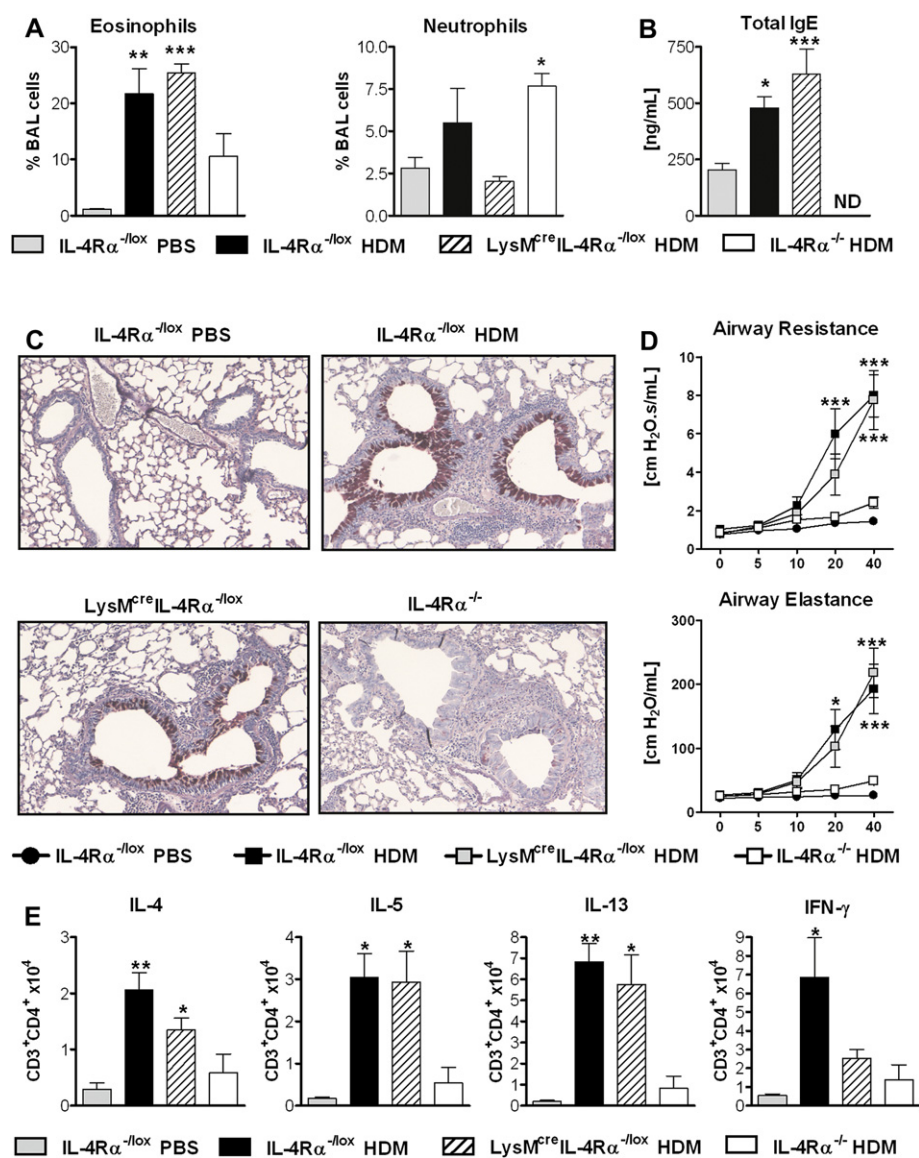


FIG E6. HDM-induced allergic airway disease. **A**, Eosinophils and neutrophils in BAL fluid ($n = 3-6$). **B**, Total IgE ($n = 4-6$). **C**, Airway mucus secretion and inflammation (periodic-acid-Schiff staining). **D**, AHR ($n = 9-11$, pooled data from 2 experiments). **E**, Number of cytokine-producing lung CD4⁺ T cells. Significant differences to IL-4R $\alpha^{-/-}$ PBS: * $P < .05$, ** $P < .01$, *** $P < .001$.

TABLE E1. Comparison of dose-response curves of airway resistance (R) and elastance (E) to inhaled methacholine by repeated-measures ANOVA*

	<i>P</i> value					
	Acute OVA-induced allergic airway disease		Chronic OVA-induced allergic airway disease		HDM-induced allergic airway disease	
	R	E	R	E	R	E
IL-4Rα ^{-/-lox} PBS vs IL-4Rα ^{-/-lox} OVA	<.001	<.001	<.001	<.001	<.001	<.001
IL-4Rα ^{-/-lox} PBS vs LysM ^{cre} IL-4Rα ^{-/-lox} OVA	<.001	<.001	<.001	<.001	.0010	.0005
IL-4Rα ^{-/-lox} PBS vs IL-4Rα ^{-/-} OVA	.2825	.4151	.1469	.331	.0153	.0037
IL-4Rα ^{-/-lox} OVA vs LysM ^{cre} IL-4Rα ^{-/-lox} OVA	.3664	.3968	.4597	.1407	.4031	.9290

*Acute OVA-induced allergic airway disease (n = 8-11), chronic OVA-induced allergic airway disease (n = 12-16), and HDM-induced allergic airway disease (n = 9-12).