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Experimental Models of Allergic Disease

Targeting the interleukin-7 receptor alpha by an anti-CD127 monoclonal antibody improves allergic airway inflammation in mice

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Abstract

Background: Interleukin-7 (IL-7) is the most important cytokine for T-cell homeostasis. IL-7 signals through the IL-7 receptor (IL-7R) which is composed of an alpha chain (IL-7R α), also called CD127 and a common gamma chain. T lymphocytes, especially T helper type 2, play a crucial role in the pathobiology of allergic asthma.

Objective: To study the effects of an anti-CD127 monoclonal antibody (mAb) in a murine model of allergic airway inflammation induced by house dust mite (HDM).

Methods: Allergic airway inflammation was induced in mice using a protocol comprising 4 weekly percutaneous sensitizations followed by 2 weekly intranasal challenges with total HDM extracts and treated by intraperitoneal injections of an anti-CD127 mAb. Because CD127 is shared by both IL-7R and the receptor for thymic stromal lymphopoietin (TSLP), a group of mice was also treated with an anti-IL-7 mAb to block only the IL-7 signalling pathway.

Results: Anti-CD127 mAb-treated mice showed significantly lower airway resistance in response to methacholine and improvement in lung histology compared with isotype mAb-treated animals. Anti-CD127 mAb treatment significantly decreased the mRNA expression of Th2 cytokines (IL-4, IL-5, and IL-13) and chemokines (CCL5/RANTES) in lung tissue, decreased the secretion of Th2 cytokines (IL-4, IL-5, and IL-13) and chemokines (CXCL1 and CCL11/eotaxin) in bronchoalveolar lavage fluid (BALF), decreased serum HDM-specific IgE, and reduced the number of total leucocytes and leucocyte subpopulations such as eosinophils, macrophages, lymphocytes, T lymphocytes, and ILC2 in BALF and lung tissue. Mice treated with anti-IL-7 mAb also showed less allergic airway inflammation as evidenced by significantly lower airway resistance and fewer leucocytes in BALF and lung tissue compared to mice treated with the corresponding isotype control mAb.

Conclusion and Clinical Relevance: Targeting the IL-7R α by an anti-CD127 mAb improves allergic airway inflammation in mice and presents as a potential therapeutic approach for allergic asthma.

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KEYWORDS

animal models, asthma, lymphocytes

1 | INTRODUCTION

Asthma affects more than 300 million people worldwide, among whom 5%-10% fail to respond to standard treatments combining inhaled long-acting beta-adrenergic agonists and inhaled corticosteroids and represent a burden for the healthcare system.¹ Biologically, asthma is a heterogeneous disorder with two main endotypes: the type 2/T helper 2 (Th2)-high asthma characterized by increased serum IgE, increased blood and sputum eosinophils, and increased secretion of Th2 cytokines and the type 2/Th2-low asthma, characterized by neutrophilic inflammation of the airway and absence of the Th2 cytokine signature.²

Monoclonal antibodies (mAbs) that inhibit the Th2 cytokine signalling pathways, especially the anti-interleukin-5 (IL-5)³ or anti-IL-5R α monoclonal antibodies (mAbs) and the anti-IL-4/IL-13R α mAb,⁴ have recently been approved for the treatment of asthma. Nevertheless, not all asthmatic patients respond to these drugs. Therefore, it is necessary to explore new therapeutic targets in asthma. Among the cytokines that affect lymphocyte functions, IL-7 is the most important cytokine for the lymphopoiesis and homeostasis of both naïve and memory T cells.^{5,6} The IL-7 receptor is composed of 2 chains, a common gamma chain (CD132) and an alpha chain (IL-7R α), also called CD127. IL-7R blockade by anti-CD127 mAbs has been shown to have therapeutic effects in various murine models of transplantation⁷ and autoimmune diseases such as type 1 diabetes,^{8,9} lupus,¹⁰ and colitis.¹¹ Given the importance of IL-7 for T-cell functions, IL-7R blockade is expected to reduce T-cell allergic immune responses. Moreover, type 2 innate lymphoid cells (ILC2), a special cell type whose roles in asthma are increasingly recognized,¹² also express CD127 and require IL-7 for survival.¹³ Finally, since IL-7R α is also a component of the receptor for thymic stromal lymphopoietin (TSLP), anti-CD127 mAbs can also block TSLP signalling,¹⁴ a central pathway in allergic asthma.¹⁵

For all those reasons, we aim to study the therapeutic effects of a blocking anti-CD127 mAb in a mouse model of allergic asthma induced by house dust mites (HDM). We found that anti-CD127 mAb treatment reduces airway hyperresponsiveness, reduces serum HDM-specific IgE levels, and decreases bronchopulmonary leucocyte infiltration and inflammatory cytokine expression, suggesting that targeting CD127 is a novel potential therapeutic approach for allergic asthma.

2 | MATERIALS AND METHODS

2.1 | Animal model and treatments

Female BALB/c mice aged 6 weeks were purchased from Charles River Laboratories France. All experimental procedures were authorized

by the Regional Ethical Committee for Animal Experimentation of Pays de la Loire conforming with the European Directive 2010/63/EU. Allergic airway inflammation was induced using total HDM extract (Stallergenes Greer, Antony, France) as previously described.¹⁶ The HDM extract contains both *Dermatophagoides farinae* (Der f) and *Dermatophagoides pteronyssinus* (Der p). Briefly, mice were sensitized on day (D) 0, 7, 14, and 21 by percutaneous (PC) application on to the ears with 500 μ g of HDM in 20 μ l of DMSO (Sigma), and then received intranasal (IN) challenges with 250 μ g of HDM diluted in PBS on D27 and D34. This protocol induces airway inflammation that contains both eosinophil and neutrophils.¹⁶ HDM-induced allergic mice were treated with intraperitoneal (IP) injections of either an anti-CD127 mAb (A7R34, in-house production) 400 μ g on D22, 24, 26, 28, 30, 32, and 34 or a rat IgG2a isotype mAb (LO-DNP-61, Université Catholique de Louvain, Brussel, Belgium) on the same schedule. A7R34 has been shown to be a blocking but not a lytic mAb.^{7,17} Non-allergic control mice received DMSO and PBS for PC sensitizations and IN challenges, respectively, and were treated with IP injections of PBS. In a group of HDM-induced allergic mice, A7R34 or LO-DNP-61 was given in a reduced dose schedule: 200 μ g IP on D28, 30, 32, and 34. Another group of HDM-induced allergic mice was treated with a blocking anti-IL-7 mAb (M25)¹⁸ or a mouse IgG2b isotype mAb (MPC-11) (both from BioXcell) at 500 μ g IP on D28, 30, 32, and 34. All analyses were performed on D35.

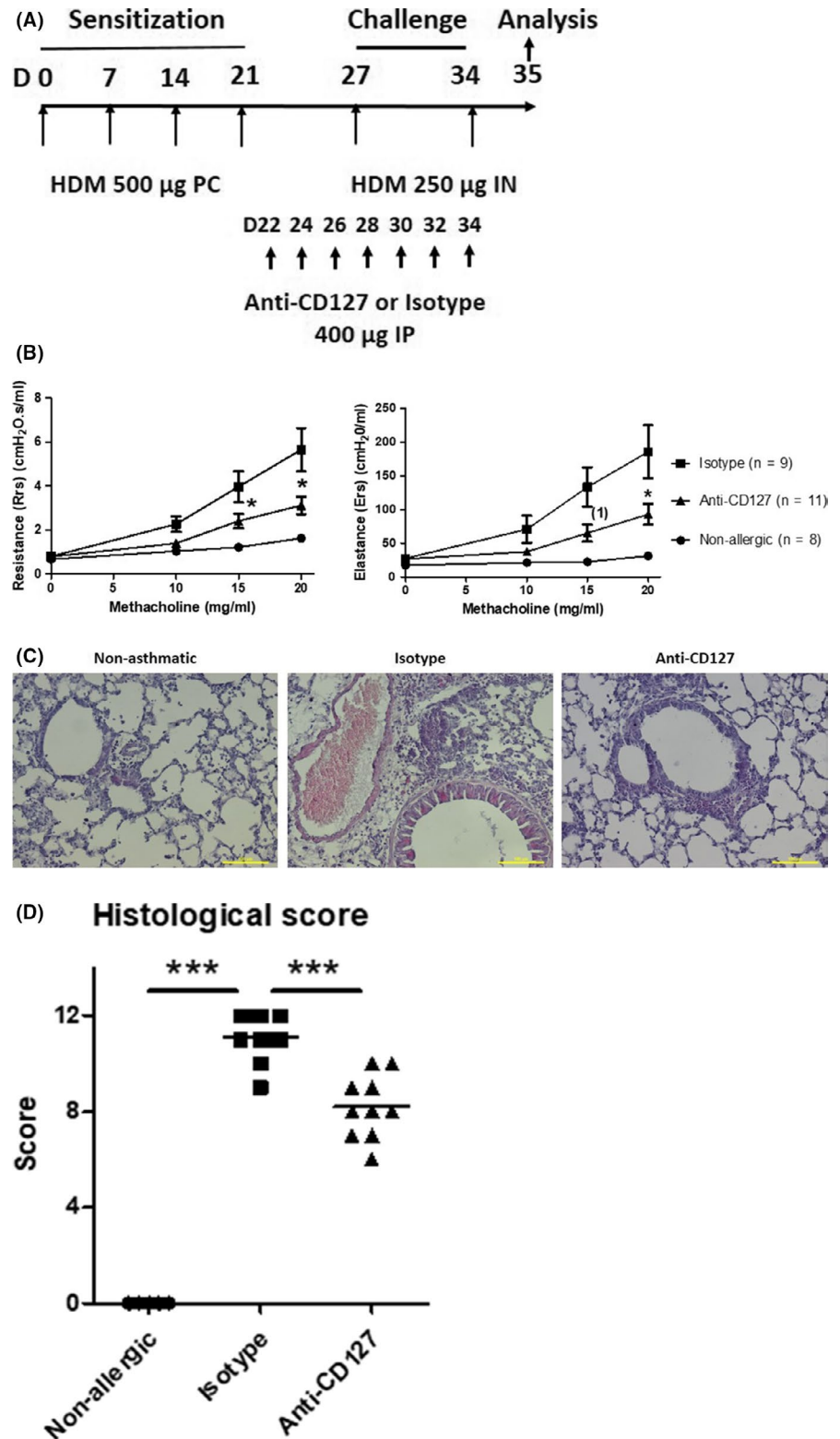
2.2 | Measurement of airway responsiveness

Dynamic lung resistances were measured by the FlexiVent system (SCIREQ, Emka) according to the manufacturer's instructions and as previously described.¹⁹ Briefly, mice were anaesthetized with ketamine and xylazine, connected to the FlexiVent machine via an endotracheal cannula, paralysed with rocuronium bromide, and mechanically ventilated at a respiratory rate of 150 breaths/min and a tidal volume of 10 mL/kg against a positive end expiratory pressure of 3 cmH₂O. Respiratory mechanics were assessed using a 1.2-second, 2.5-Hz single frequency forced oscillation manoeuvre. Increasing doses of methacholine (0, 5, 10, 15, and 20 mg/ml) were nebulized via the endotracheal cannula to induce bronchoconstriction. Total respiratory system resistance (Rrs) and elastance (Ers) were calculated in the flexiware software (SCIREQ) by linear regression using the single compartment model.^{20,21}

2.3 | Histology

Mouse lungs were fixed in 4% formaldehyde, paraffin-embedded, sectioned at 6 μ m thickness, and then stained with haematoxylin-eosin and periodic acid-Schiff (PAS). Histological findings were

FIGURE 1 Anti-CD127 mAb treatment reduces airway hyperresponsiveness and improves lung histology in a murine model of allergic asthma induced by house dust mite (HDM). (A) Schema showing the allergic airway inflammation induction protocol in mice and the treatment schedule. (B) FlexiVent measurement of airway resistance (Rrs) and elastance (Ers) in response to metacholine challenges. Data points and error bars indicate mean $\pm$ SEM, * $P < .05$, (1) $.05 < P < .06$. Representative microphotographs (C) and semi-quantitative histological scores (D) of lung sections from non-allergic control and HDM-induced allergic mice treated by anti-CD127 mAb or isotype control mAb (haematoxylin & eosin stain, original magnification $\times 200$). *** $P < .001$. Each data point represents one mouse



evaluated based on bronchial morphology and inflammation as previously described.²² A semi-quantitative score was given for each histological sample including bronchial epithelial cell hyperplasia and dystrophy, (0 to 4) cellular infiltration (0 to 4), and formation of nodules (0 to 4).

2.4 | Quantitative PCR

Total RNA extraction from mouse lung tissue was performed using TRIzol Reagent (Thermo Fisher Scientific). cDNA was synthesized using MMLV reverse transcriptase. Taqman gene expression assays

(Thermo Fisher Scientific) were performed according to the manufacturer instruction for the following genes: IL-4, IL-5, IL-13, IL-7, IL-17, IL33, TSLP, IFN γ , TNF, CCL11/eotaxin, and CCL5/RANTES using the ViiA 7 Real-Time PCR System (Thermo Fischer Scientific) (see Table S1 for the list of Taqman probes). Differential gene expression was calculated by the ddCT method.

2.5 | Flow cytometry

Leucocyte phenotyping was performed on bronchoalveolar lavage fluids (BALF) and on cells isolated from the lungs and tracheobronchial lymph nodes (LN) of mice using 70 μ m BD Falcon cell strainers (BD Biosciences). BALF were recovered following administration of 1 mL of PBS into the tracheal catheter, centrifuged, the supernatants were kept for cytokine measurement and the cell pellets were resuspended and counted before staining. Cells were stained using fluorescence-conjugated antibodies to the following cell markers: CD3, CD4, CD8, CD19, CD25, CD44, CD45, CD45R (B220), CD49b, CD62L, CD127, Ly6G (all from BD Biosciences), CCR3 (R&D Systems), F4/80, ST2 (IL-33R), and FOXP3 (eBioscience) (see Table S2 for the list of antibodies used). A lineage cocktail (BioLegend) containing antibodies to mouse CD3, Ly6G/Ly6C, CD11b, CD45R, and Ter-119 was also used to stain for ILC2, which was defined as lineage negative and ST2 positive cells. Data were acquired using a BD LSR II flow cytometer with BD FACSDiva software and then analysed using FlowJo software.

2.6 | Measurement of cytokines and chemokines in BALF

The following cytokines and chemokines were measured in mouse BALF by luminex multiplex technique using a Procartaplex kit (Thermo Fisher Scientific): IL-2, IL-4, IL-5, IL-7, IL-10, IL-13, IL-17A, CXCL1/KC, CCL5/RANTES, and CCL11/Eotaxin. The luminex multiplex assay was performed at the CIMNA platform (Center for Immuno Monitoring Nantes Atlantic, CHU de Nantes) according to the manufacturer instruction. The detection range of the Procartaplex assays is 5–2000 pg/mL (see Biomarker quantitation assay guide on thermofisher.com).

2.7 | Measurement of serum HDM-specific IgE

Serum HDM-specific IgE was measured by ELISA as previously described.¹⁶ Briefly, plates were coated overnight with total HDM extract (the same as used for sensitizations and challenges), washed,

incubated with mouse serum, washed, and revealed with HRP-conjugated anti-mouse IgE (AbD Serotec).

2.8 | Statistics

Data are presented as mean $\pm$ SEM. ANOVA with post hoc Dunn's test was used for multiple comparisons, and unpaired t test (Mann-Whitney) was used to compare between 2 groups. Data analysis was performed using the GraphPad Prism software. The threshold for statistical significance was set at $P < .05$.

3 | RESULTS

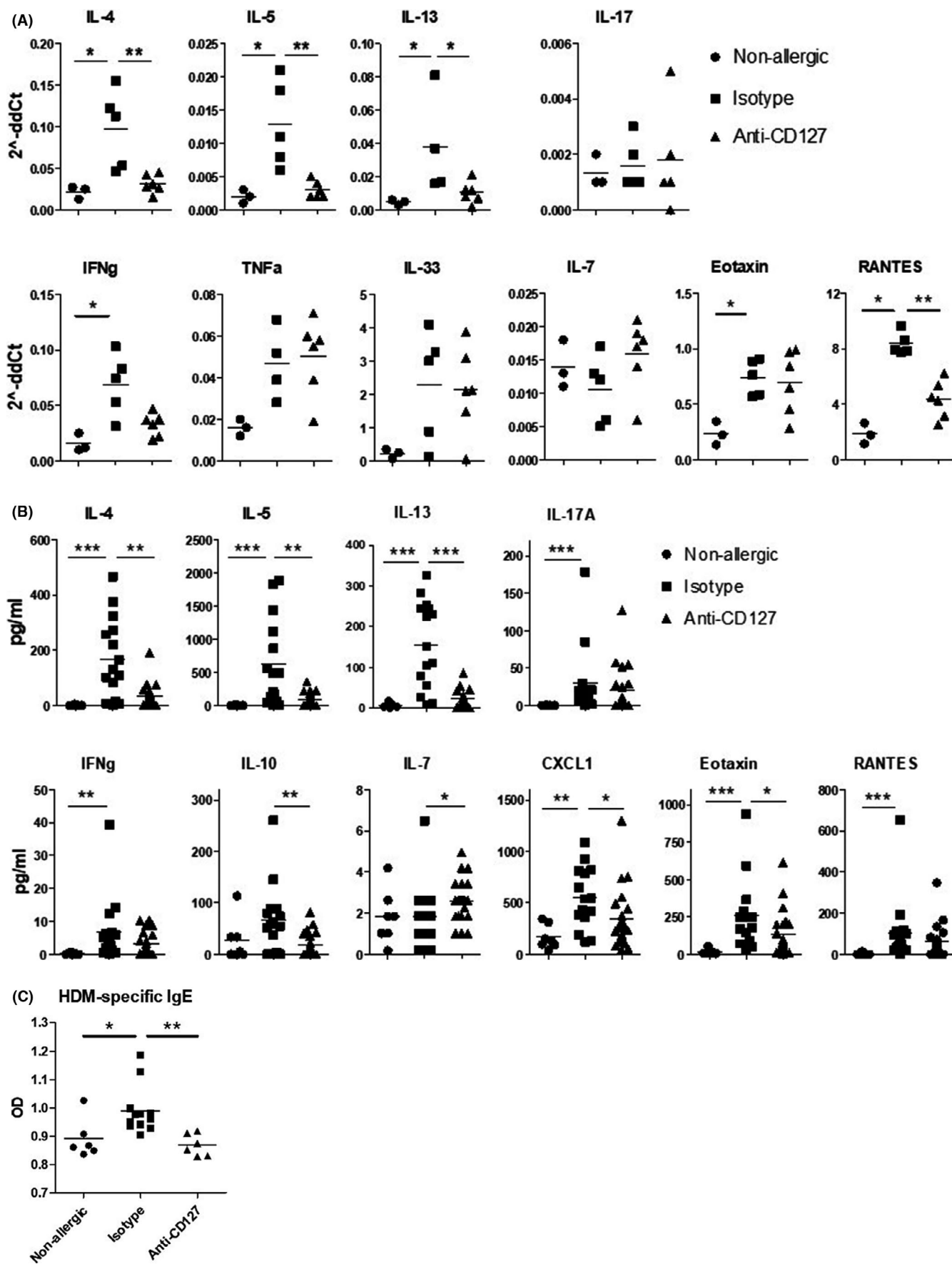
3.1 | Anti-CD127 mAb reduces airway hyperresponsiveness and improves lung histology

Mice underwent percutaneous sensitizations and intranasal challenges with HDM extract to induce allergic airway inflammation and were treated with an anti-CD127 mAb 400 μ g IP every other day for 7 injections (Figure 1A). This dosing schedule of anti-CD127 mAb was based on our previous experience in murine transplant models.⁷ In order to evaluate the effect of treatment on respiratory functions, we measured respiratory system resistance (Rrs) and elastance (Ers) of mice by FlexiVent. We found that HDM-induced allergic mice treated with anti-CD127 mAb as indicated in Figure 1A had significantly lower resistance and elastance in response to methacholine challenges at the dose of 15 and 20 mg/mL compared to allergic mice treated with isotype mAb (Figure 1B). Non-allergic control mice, as expected, showed negligible responses to methacholine challenges. Anti-CD127-treated allergic mice also showed improvement in lung histology with less bronchial epithelial cell hyperplasia and dysplasia and less inflammatory infiltration compared to allergic mice that received isotype mAb (Figure 1C and 1D).

3.2 | Anti-CD127 mAb decreases Th2 cytokine and chemokine expression in lung and BALF and decreases allergen-specific IgE response

We next compared the expression of inflammatory cytokines and chemokines in lung tissue between anti-CD127 mAb-treated and isotype mAb-treated allergic mice using quantitative PCR (Figure 2A). We found that anti-CD127 mAb treatment significantly reduced the mRNA expression of the Th2 cytokines IL-4, IL-5, IL-13, and the chemokine CCL5/RANTES. On the other hand, the expression of Th1 (IFN γ and TNF α), Th17 (IL-17), as well as other cytokines and chemokines such as IL-33, IL-7, CCL11/eotaxin was not statistically different. To confirm the effects

FIGURE 2 Anti-CD127 mAb treatment reduces bronchopulmonary inflammatory cytokine and chemokine expression and decreases allergen-specific humoral immune response in allergic mice. Allergic airway inflammation was induced and treated according to the schema shown in Figure 1A. A, Cytokine and chemokine gene expression in lung tissue as determined by quantitative PCR. B, Measurement of cytokine and chemokine concentrations in BALF by multiplex Luminex. C, Serum HDM-specific IgE measured by ELISA. * $P < .05$, ** $P < .01$, *** $P < .001$. Each data point represents one mouse



of treatment on the expression of inflammatory cytokines at the protein level, we measured the concentration of 10 cytokines and chemokines in bronchoalveolar fluids (BALF) of mice using a Luminex multiplex assay (Figure 2B). Interestingly, we found again that anti-CD127 mAb treatment significantly reduced the Th2 cytokines IL-4, IL-5, and IL-13 but not the Th1 cytokine IFN γ and the Th17 cytokine IL-17A. IL-10, CXCL1, and eotaxin were also reduced by treatment, whereas IL-7 was increased. In order to evaluate the effect of treatment on allergic humoral immune response, we measured allergen-specific IgE in mouse sera by ELISA and found that serum HDM-specific IgE levels were significantly reduced in anti-CD127-treated allergic mice (Figure 2C).

3.3 | Anti-CD127 mAb reduces airway cellular infiltration

Next, we performed leucocyte phenotyping by flow cytometry of cells isolated from BALF and lungs of mice to investigate the effects of treatment on bronchopulmonary cellular infiltration in HDM-induced allergic mice. The gating strategies to define leucocyte subpopulations were shown in Figure S1. We found that compared with isotype control, anti-CD127 mAb significantly reduced the absolute numbers of total leucocytes, macrophages, eosinophils, ILC2, lymphocytes, and T cells in BALF (Figure 3A). Similar results were obtained in lungs (Figure 3B), with, in addition, a marginally significant reduction in neutrophils ($P = .052$). We also carried out lymphocyte phenotyping of tracheobronchial LN and found that anti-CD127 mAb treatment significantly decreased the absolute numbers of total lymphocytes and lymphocyte subsets including T cells, CD4+ and CD8+ T cells, CD4+ memory T cells (defined as CD44hiCD62Llo), NK cells, and B cells

(Figure 3C). The absolute numbers of CD4+ FoxP3+ Tregs in lungs and in tracheobronchial LN were also significantly reduced by anti-CD127 mAb although the relative frequencies CD4+ FoxP3+ Tregs as percentages of CD4+ T cells were unchanged (Figure 3D). Finally, the absolute numbers of total lymphocytes and lymphocyte subsets in the spleen were also reduced in anti-CD127 mAb-treated compared with isotype mAb-treated mice (Figure S2A).

3.4 | Reduced dose of anti-CD127 mAb maintains the therapeutic effects without systemic lymphocyte reduction

Since the reduction in the number of lymphocytes and lymphocyte subset in lymphoid organs may not be desirable, we investigated whether a reduced dosing schedule of anti-CD127 mAb can reproduce the therapeutic effects while avoiding lymphocyte reduction in lymphoid organs. HDM-induced allergic mice were administered IP with 200 μ g of anti-CD127 mAb or isotype mAb for a total of 4 injections (Figure 4A). Respiratory function measurements by FlexiVent show that allergic mice treated with reduced dose of anti-CD127 mAb have significantly lower resistance (Rrs) and elastance (Ers) in response to methacholine challenge compared with isotype mAb-treated mice (Figure 4B). Leucocyte phenotyping by flow cytometry of BALF showed a significant reduction in the absolute numbers of total leucocytes, macrophages, eosinophils, ILC2, lymphocytes, and T cells in anti-CD127 mAb-treated compared with isotype mAb-treated allergic mice (Figure 4C). Concordantly, in lung, anti-CD127 mAb treatment reduced the absolute numbers of all cell types studied (Figure 4D). These data

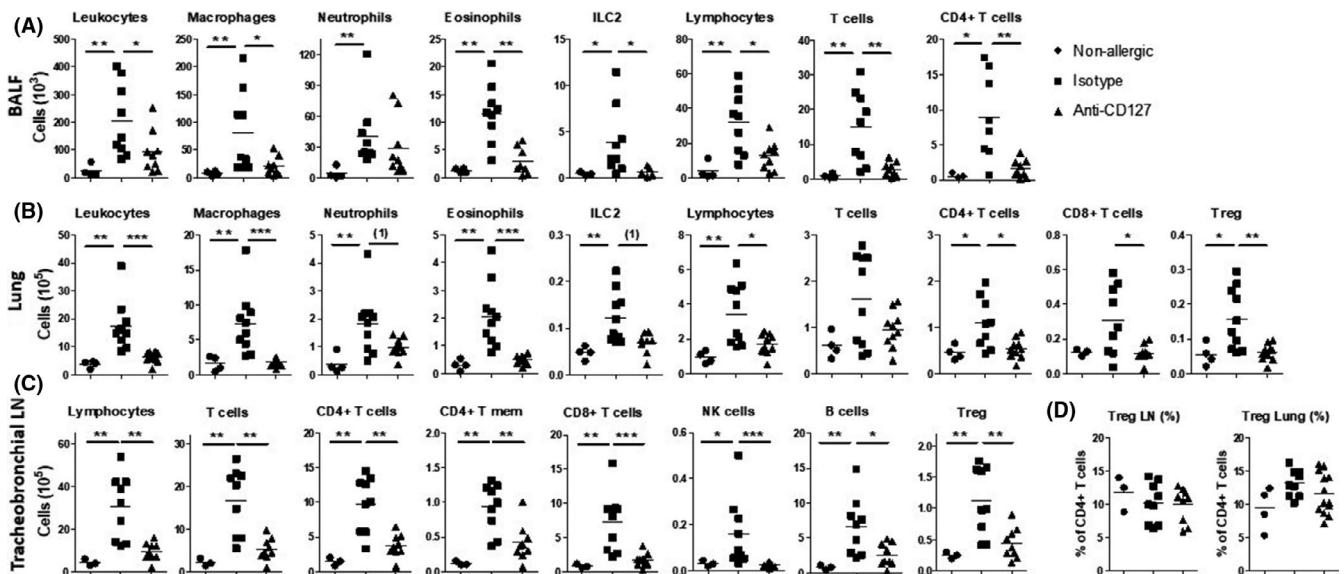


FIGURE 3 Leucocyte phenotyping by flow cytometry. Allergic airway inflammation was induced and treated according to the schema shown in Figure 1A. Anti-CD127 mAb-treated allergic mice have reduced total leucocyte and leucocyte subset numbers in BALF (A), lungs (B), and tracheobronchial lymph nodes (LN) (C) compared to isotype mAb-treated allergic mice (see also Figure S2A for the spleen). D, The relative frequencies of CD4 + FoxP3+ Treg are not affected by anti-CD127 mAb treatment. * $P < .05$, ** $P < .01$, *** $P < .001$, (1) $0.05 < P < .06$. Each data point represents one mouse

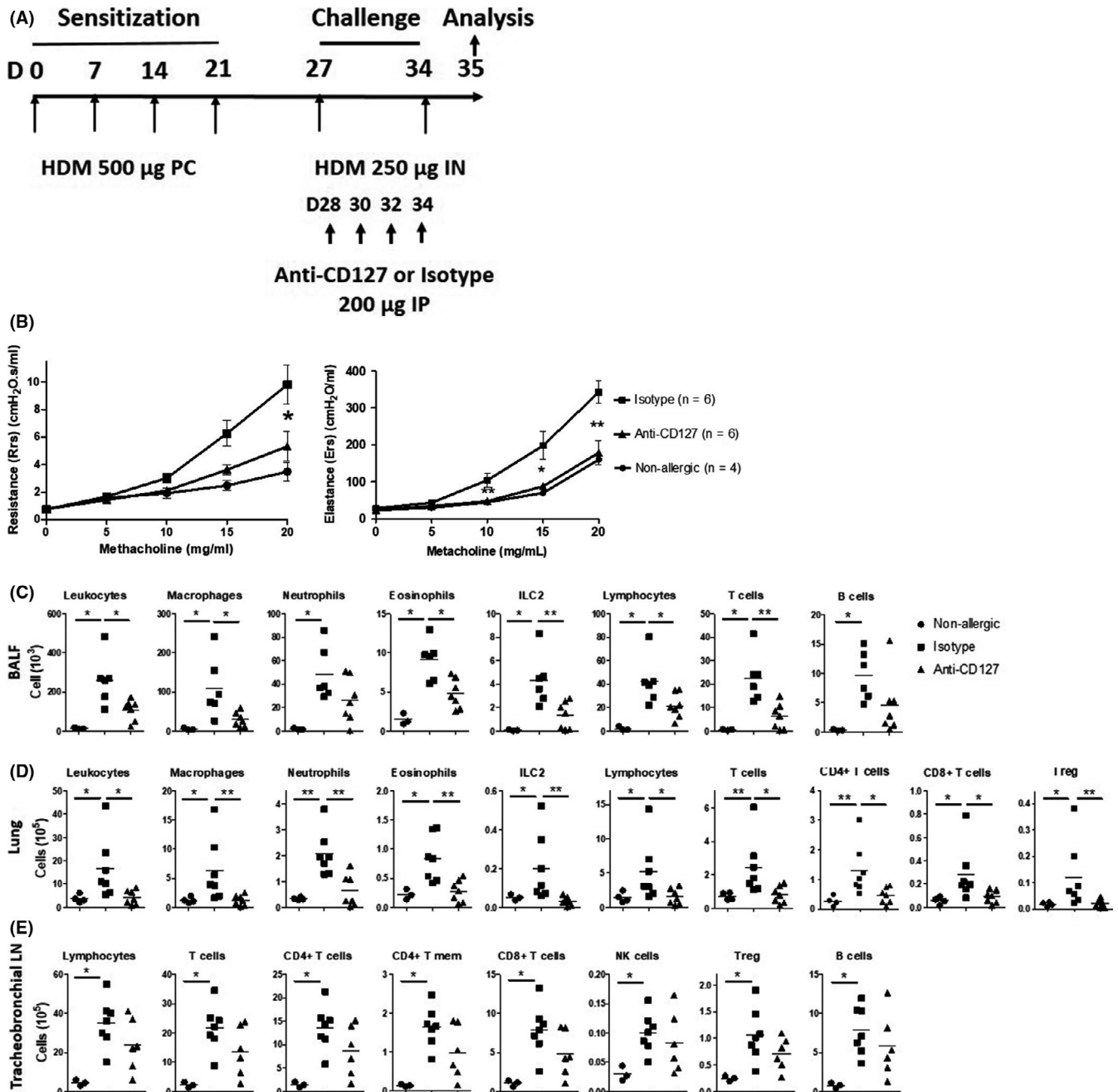


FIGURE 4 Reduced dose of anti-CD127 mAb maintains the therapeutic effects without lymphocyte reduction in lymphoid organs. A, Schema showing the allergic airway inflammation induction protocol in mice and the treatment schedule. Indeed, the induction protocol was the same as shown in Figure 1A, but mice were treated with lower dose and fewer injections of anti-CD127 mAb. B, FlexiVent measurement of airway resistance (Rrs) and elastance (Ers) in response to metacholine challenges. Data points and error bars indicate mean $\pm$ SEM. C-E, This reduced dosing regimen of anti-CD127 mAb decreases the absolute numbers of leukocytes and leukocyte subsets in BALF (C) and lungs (D) but not in tracheobronchial LN (E) (see also Figure S2B for the spleen). Each data point represents one mouse. * $P < .05$, (1) $0.05 < P < .06$

confirm that the reduced dosing schedule is also effective in inhibiting allergic airway inflammation. On the other hand, anti-CD127 mAb given at this dosage schedule did not lead to significant reduction of total lymphocyte and lymphocyte subset numbers in the tracheobronchial LN (Figure 4E) or in the spleen (Figure S2B), indicating that the reduction in lymphocyte numbers in lymphoid organs is not a prerequisite for the therapeutic efficacy of anti-CD127 mAb in our allergic asthma model.

3.5 | Anti-IL-7 mAb treatment produces similar therapeutic effects

Because the anti-CD127 mAb used in our study has also been shown to partially block TSLP signalling,¹⁴ a well-known pathway involved in allergic asthma, it is important to demonstrate that blockade of IL-7/IL-7R signalling alone can produce therapeutic effects. To this end, we treated HDM-induced allergic mice with a blocking anti-IL-7

mAb¹⁸ at 500 µg IP for 4 injections as indicated in Figure 5A. We found that anti-IL-7 mAb-treated allergic mice had significantly lower resistance (Rrs) and elastance (Ers) in response to methacholine challenge compared with animals treated by the respective isotype control (Figure 5B). Lymphocyte phenotyping of BALF, lung, and tracheobronchial LN by flow cytometry revealed significant or marginally significant ($0.05 < P < .06$) reduction of several immune cell types including total leucocytes, macrophages, neutrophils, eosinophils, ILC2, lymphocytes, T cells, and B cells (Figure 5C-E). Those results show that blockade of IL-7/IL-7R signalling alone also inhibits allergic airway inflammation in our model.

4 | DISCUSSION

In this study, using a mouse model of HDM-induced allergic airway inflammation, we showed that treatment with anti-CD127 mAb reduced airway hyperresponsiveness and decreased bronchopulmonary cellular infiltration of almost all immune cell types studied including lymphocytes, T cells, ILC2s, eosinophils, macrophages, and neutrophils. IL-7/IL-7R signalling is present in several immune cell types involved in the pathogenesis of asthma,^{6,23,24} explaining the effects of IL-7R blockade on these cells. Firstly, since IL-7 is critical for T-cell homeostasis,⁶ the reduction in bronchopulmonary T-cell infiltration by IL-7R blockade is expected. Moreover, IL-7 signalling has been shown to be necessary for the survival of allergen-specific CD4 T cells in a murine model of allergic asthma induced by ovalbumin.²⁵ Secondly, IL-7 is also required for the development of ILC2s as their number is reduced in IL-7-deficient mice.²³ The reduction of ILC2 in BALF and lung of anti-CD127 mAb-treated asthmatic mice suggests that anti-CD127 mAb could be a novel treatment to target ILC2. Thirdly, eosinophils have also been shown to express functional IL-7R and respond to IL-7 stimulation *in vitro*²⁴; however, it is unclear whether IL-7R blockade can directly affect their survival or functions *in vivo*. Interestingly, in patients with allergic asthma, IL-7 concentrations were shown to increase in BALF after allergen challenge and correlate with BALF eosinophils.²⁴ Finally, since the expression of CD127 is lower in CD4⁺ FoxP3⁺ Treg, it might be expected that anti-CD127 mAb should relatively spare Treg. However, IL-7 signalling has also been shown to be necessary for Treg survival,²⁶ explaining the reduction in the absolute numbers of Treg in anti-CD127 mAb-treated mice. Although the roles of Treg in asthma have been documented,²⁷ the reduction of Treg number by anti-CD127 mAb did not prevent its therapeutic effects in this allergic asthma model.

Since anti-CD127 mAb reduced Th2 but not Th1 or Th17 cytokine expression in lung tissue and BALF of HDM-induced allergic mice, this treatment might be more appropriate for type-2-high asthma. Interestingly, a recent report showed that IL-7R blockade reduced the production of the Th17 cytokine IL-22 in a murine model of fungal asthma.²⁸ Besides Th2 cytokines, anti-CD127 mAb also decreased the expression of some chemokines such as CXCL1 and eotaxin in BALF and CCL5/RANTES in lung tissue, which might explain

the overall reduction in bronchoalveolar leucocyte infiltration, including cells that do not express CD127 such as macrophages and neutrophils. Interestingly, IL-7 concentration was increased in the BALF of anti-CD127 mAb-treated mice, probably as a compensatory mechanism in response to IL-7R blockade.

Because IL-7R α is shared by both IL-7R and TSLPR, anti-127 mAbs may also block TSLP signalling, a key player in allergic immune responses. Both anti-TSLP and anti-TSLPR mAbs have been shown to reduce airway inflammation and Th2 cytokine expression in murine models of allergic asthma.²⁹⁻³¹ Moreover, human anti-TSLP mAb has been shown to lower asthma exacerbation rates in a recent phase 2 clinical trial.³² Therefore, it is important to show that the therapeutic effects of anti-CD127 mAb in our model are not just simply due to TSLPR blockade. To this end, we treated HDM-induced allergic mice with a blocking anti-IL-7 mAb and obtained a reduction in airway hyperresponsiveness and cellular infiltration, suggesting that blockade of the IL-7/IL-7R pathway itself contributes to the therapeutic effects of anti-CD127 mAb. However, it can be remarked that anti-IL-7 mAb appears less effective than anti-CD127 mAb in reducing bronchopulmonary cellular infiltration (Figure 3A and 3B), suggesting that the dual blocking activity of the anti-CD127 mAb confers an advantage. Therefore, anti-CD127 mAb might be a better choice than anti-IL-7 mAb for further development as a drug candidate in allergic asthma.

Based on our previous experience in murine allograft model,⁷ we initially used a relatively large dose of anti-CD127 mAb which was associated with lymphocyte reduction in the tracheobronchial LN and in the spleen, although the former effect can be attributed at least in part to the improvement of allergic airway inflammation. By reducing the dose of anti-CD127 mAb, we were able to reproduce the therapeutic effects without causing a reduction of lymphocyte numbers in these lymphoid organs. Therefore, the beneficial effects of anti-127 mAb in our murine model of allergic airway inflammation are not dependent on a systemic reduction of lymphocyte number, suggesting that targeting the IL-7R α by an anti-CD127 mAb could be a clinically relevant therapeutic approach for allergic asthma.

In this study, we did not treat non-allergic control mice with anti-CD127 mAb to confirm that there were no unwanted side effects in normal mice. However, it is likely that these control groups could be omitted because knowledge on anti-CD127 mAbs has been extended beyond rodent models. Indeed, therapeutic human anti-CD127 mAbs have been tested in nonhuman primate as well as in human phase 1 trials. We recently reported that anti-human CD127 mAb administered to nonhuman primates did not reduce lymphocyte and lymphocyte subset numbers despite full receptor occupancy,^{33,34} a result confirmed by another research group.³⁵ In human, single-dose administration of anti-CD127 mAb to healthy individuals did not affect lymphocyte numbers,³⁶ whereas repeated administration of anti-CD127 mAb in type 1 diabetic patients at dosages of 1-3 mg/kg for 12 weeks selectively inhibits memory T cells while preserving naïve T cells and Tregs, an effect which may serve to eliminate pathologic T cells in autoimmune diseases.³⁷ Importantly, treatment with anti-CD127 mAbs

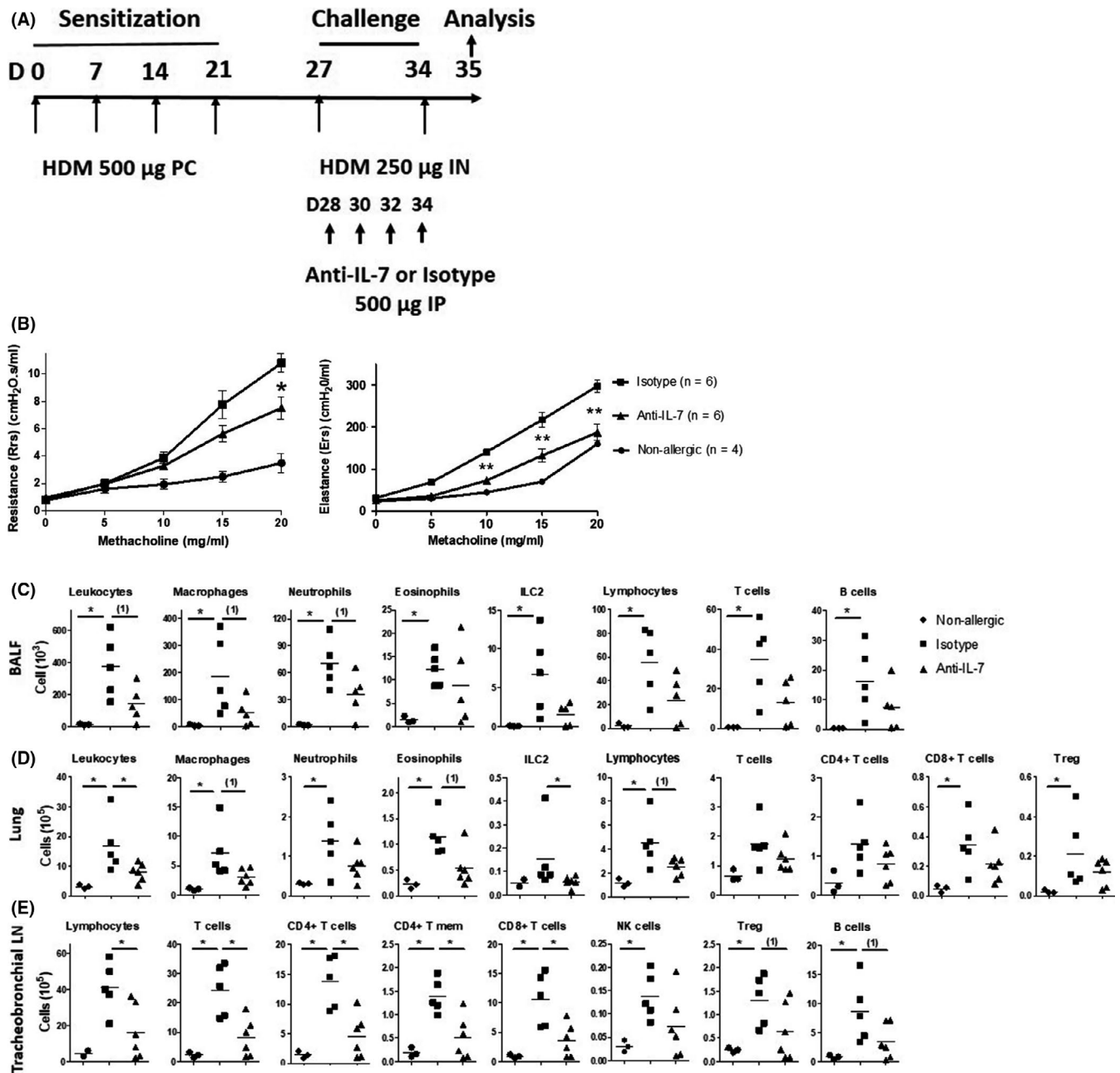


FIGURE 5 Anti-IL-7 mAb produces similar therapeutic effects. A, Allergic airway inflammation was induced by HDM and treated with an anti-IL-7 mAb as shown in the schema. B, FlexiVent measurement of airway resistance (Rrs) and elastance (Ers) in response to metacholine challenges. Data points and error bars indicate mean $\pm$ SEM. C-E, Treatment of allergic mice with anti-IL-7 mAb leads to a reduction in the absolute numbers of leukocytes and leukocyte subsets in BALF (C), lungs (D), and tracheobronchial LN (E). Each data point represents one mouse. * $P < .05$, (1) $0.05 < P < .06$

in human was well-tolerated, no significant adverse effects were seen in more than 40 individuals participating in these two phase 1 trials.^{36,37}

Taking advantage of the availability of human anti-CD127 mAbs, further studies using humanized mouse³⁸ or nonhuman primate models³⁹ of allergic asthma should be performed to confirm the therapeutic potentials of anti-CD127 mAbs in allergic asthma. With regard to safety issues, however, as with any novel treatment targeting a cytokine signalling pathway, the effects of the long-term use of human anti-CD127 mAbs on the immune

system are still unknown. We expect that ongoing clinical trials using anti-CD127 mAbs in autoimmune diseases will provide us with knowledge on their long-term safety profiles, helping us to weigh the risks and benefits of this potential therapeutic approach in allergic asthma.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

HLM, GB, AM, and SB designed the study and wrote the manuscript. HLM, TVHN, KH, and MAC carried out experiments.

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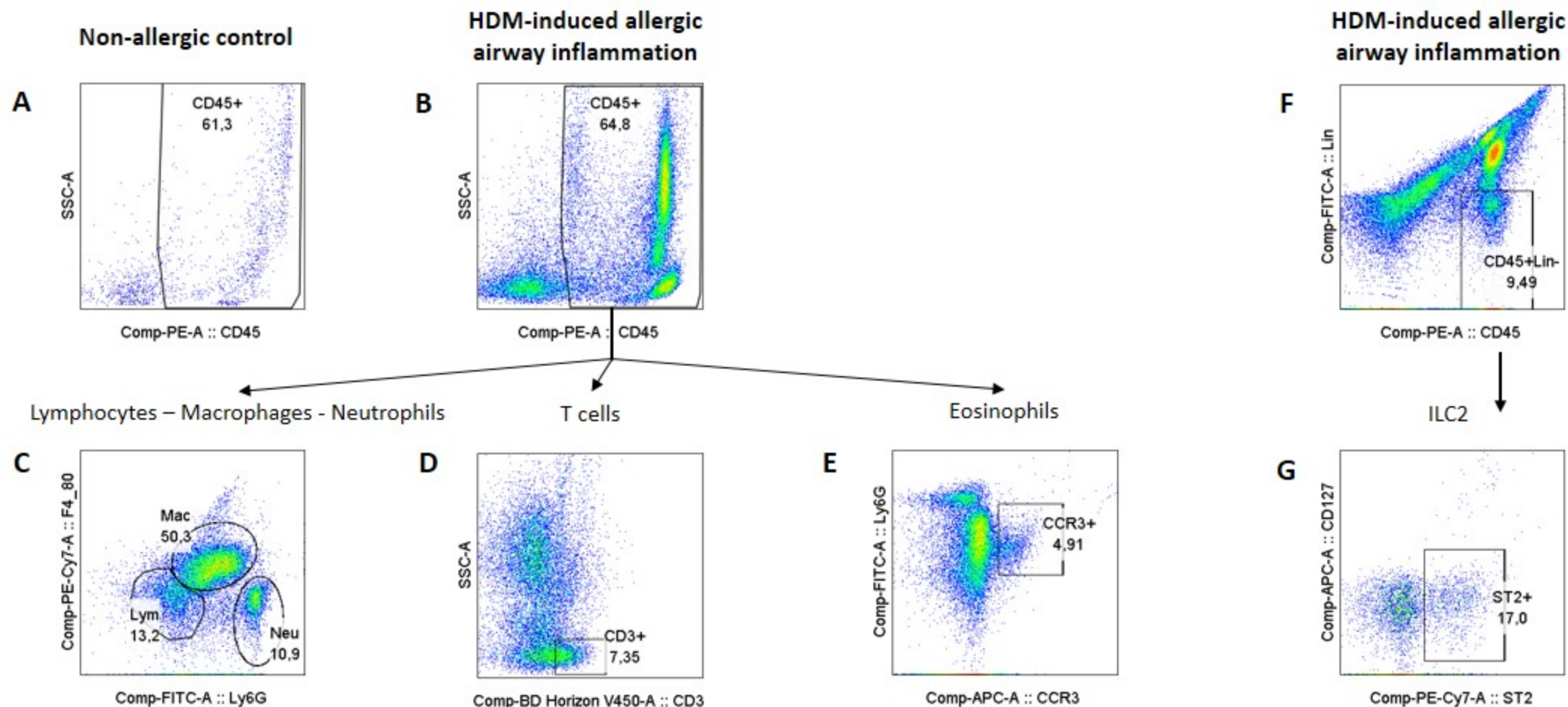
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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

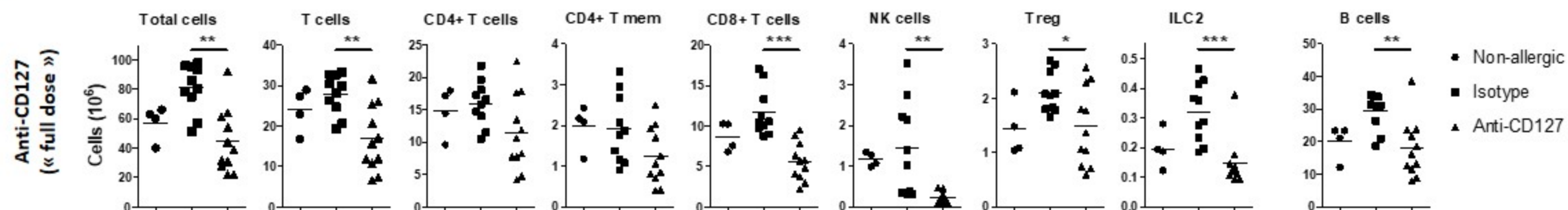
Figure S1



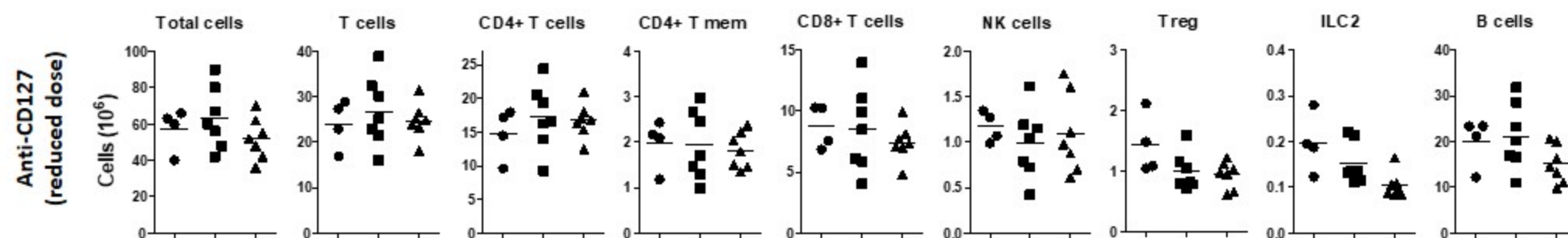
Gating strategy for bronchoalveolar fluid (BALF): BALF cellularity in non-allergic control (A) and HDM-induced allergic airway inflammation (B). Leukocytes (CD45+) were gated and further distinguished by their staining patterns for Ly6G, F4/80, CD3, CCR3, lineage cocktail (Lin), and ST2 (IL-33R). (C) Lymphocytes, macrophages, and neutrophils were identified as F4/80^{lo}Ly6G⁻, F4/80^{hi}Ly6G^{med}, and F4/80^{lo}Ly6G^{hi} cells, respectively. T cells (CD3+) (D) and eosinophils (CCR3+) (E) were shown. Type 2 innate lymphoid cells (ILC2) were defined as CD45⁺Lin⁻ST2⁺ (F and G).

Figure S2

A



B



Leukocyte phenotyping of the spleen of mice with HDM-induced airway inflammation treated with anti-CD127 mAb at 400 μ g IP every other day for 7 injections (« full dose ») (A) or 200 μ g IP every other day for 4 injections (reduced dose) (B). *p<0.05, **p<0.01, ***p<0.001. Each point represents one mouse.

Table S1: List of Taqman probes (Thermofisher) used for quantitative PCR

Gene	Reference
IL4	Mm00445259_m1
IL5	Mm00439646_m1
IL13	Mm00434204_m1
IL17a	Mm00439618_m1
IFNg	Mm01168134_m1
TNFa	Mm00443260_m1
IL33	Mm00505403_m1
IL7	Mm01295803_m1
CCL11	Mm00441238_m1
CCL5	Mm01302427_m1

Table S2: List of mAbs used for flow cytometry

Marker	Fluorochromes	Clone	Company
CD3e	V450	500A2	BD Biosciences
CD4	PE-Cy7	RM4-5	BD Biosciences
CD8a	PE or APC	53-6-7	BD Biosciences
CD19	APC	1D3	BD Biosciences
CD25	PerCP Cy5.5	PC61	BD Biosciences
CD44	APC	IM7	BD Biosciences
CD45	PE	30-F11	BD Biosciences
CD45R (B220)	PerCP Cy5.5	RA3-6B2	BD Biosciences
CD49b	FITC	DX5	BD Biosciences
CD62L	PerCP Cy5.5	MEL-14	BD Biosciences
CD127	APC	SB/199	BD Biosciences
CCR3	APC	FAB729A	R&D Systems
F4/80	PE-Cy7	BM-8	eBioscience
FoxP3	FITC	FJK-16s	eBioscience
ST2 (IL-33R)	PE-Cy7	RMST2-2	eBioscience
Ly6G	FITC	1A8	BD Biosciences
Lineage cocktail (CD3, Gr-1, CD11b, CD45R, Ter-119)	FITC	145-2C11, RB6-8C5, M1/70, RA3-6B2, Ter-119	Biolegend