

Selective control of SIRP- α -positive airway dendritic cell trafficking through CD47 is critical for the development of T_H2-mediated allergic inflammation

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Background: Dendritic cells (DCs) are essential for the initiation and maintenance of T_H2 responses to inhaled antigen that lead to the establishment of allergic diseases. Two subpopulations of nonplasmacytoid DCs (ie, CD11b^{low}CD103⁺ and CD11b^{high}CD103⁻) are found in lung/airway tissues. Yet the identification and migratory properties of the DC subset that contributes to T_H2-mediated responses remain to be clarified. CD47, a signal regulatory protein (SIRP)- α partner, reportedly governed skin DC migration.

Objective: We here thought to investigate the role of CD47/SIRP- α interactions in airway DC trafficking and the development of allergic airway inflammation.

Methods: We characterized the DC influx into lungs and mediastinal lymph nodes in CD47^{-/-} and CD47^{+/+} BALB/c mice by using experimental models of allergic asthma. Mice were systemically (intraperitoneal ovalbumin/alum) or locally (intratracheal ovalbumin-loaded bone marrow-derived DCs) immunized and challenged by ovalbumin aerosol. We also evaluated the consequences of SIRP- α -Fc fusion molecule administration on the induction of airway disease in BALB/c mice. **Results:** SIRP- α selectively identified the CD11b^{high}CD103⁻ DC subset that predominantly accumulated in mediastinal lymph nodes during airway inflammation. However, CD103⁺SIRP- α ⁺ DC trafficking, T_H2 responses, and airway disease were impaired in CD47^{-/-} mice. Importantly, the adoptive transfer of CD103⁺SIRP- α ⁺CD47^{+/+} but not CD47^{-/-} DCs elicited a strong T_H2 response in CD47^{-/-} mice. Finally, the administration of SIRP- α -Fc molecule protected BALB/c mice from allergic airway inflammation.

Conclusion: Lung CD11b^{high}CD103⁻SIRP- α ⁺ DC migration is governed by self-CD47 expression, and manipulation of the CD47/SIRP- α pathway suppresses CD103⁺SIRP- α ⁺ DC-driven

pathogenic T_H2 responses and airway inflammation. (J Allergy Clin Immunol 2009;123:1000-1010.)

Key words: Dendritic cells, migration, CD103, allergic inflammation, T_H2, CD47, SIRP- α

Allergic asthma is characterized by elevated serum IgE and the production of proinflammatory type 2 cytokines both in the bronchial tissues and the draining lymph nodes (LNs).¹⁻⁵ Dendritic cells (DCs) play a central role in the induction and perpetuation of T_H2-driven allergic airway inflammation.⁶ Conditional gene depletion of airway CD11c⁺ DCs before aerosol challenge, or even in mice with established airway inflammation, abolishes asthma features.⁷ Compounds that include sphingosine-1-phosphate and prostacyclin analogs inhibit asthma development by directly manipulating DC function and migration.⁸⁻¹²

Different lung DC subsets have been identified with distinct anatomical localizations and functions.¹³ Plasmacytoid DCs (pDCs; CD11c^{low}CD11b⁺GR1⁺B220⁺120G8⁺) are found in the lung interstitium and conducting airways and dictate T_H cell fate to innocuous antigens by stimulating the generation of regulatory T cells.^{14,15} The adoptive transfer of pDCs protects them from developing asthma.¹⁶ The nonplasmacytoid DCs (mDCs; CD11c^{high}CD11b⁺) promote the differentiation of naive T cells and the activation of T_H2 effector cells.¹⁷ The mDCs are further subdivided into CD11b^{high}CD103⁻ and CD11b^{low}CD103⁺ DC subsets.¹⁸⁻²⁰ Although both are specialized in presenting innocuous antigen, which of these 2 DC subpopulations contributes to the induction and exacerbation of airway inflammatory responses remains to be clarified. CCR6 and CCR2 regulate DC precursor and monocyte transmigration into lungs early after an inflammatory insult,²¹ whereas airway DC trafficking to mediastinal lymph nodes (mLNs) is controlled by CCR7 expression at steady state.²²⁻²⁴ However, other chemokine receptors are likely involved in lung DC trafficking because appreciable T_H2 responses can still be mounted in *plt* mutant mice, a strain displaying a disruption of CCR7 ligands.^{25,26} Therefore, the identification of immunogenic airway DC subsets and the molecules that control lung egress is crucial to unravel novel therapeutic targets for T_H2-mediated disorders.

CD47 is a marker of self that is ubiquitously expressed on the surface of immune and nonimmune cells.²⁷ Through interactions with its 2 ligands coexpressed on DCs, signal regulatory protein (SIRP)- α and thrombospondin, CD47 is implicated in the negative regulation of the immune response.^{28,29} By contrast, CD47 expression on skin myeloid DCs positively governs *in vivo* DC

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Abbreviations used

BALF: Bronchoalveolar lavage fluid
 BMDC: Bone marrow–derived dendritic cell
 DC: Dendritic cell
 FITC: Fluorescein isothiocyanate
 Foxp3: Forkhead box protein 3
 LN: Lymph node
 MFI: Mean fluorescence intensity
 mLN: Mediastinal lymph node
 pDC: Plasmacytoid dendritic cell
 SIRP: Signal regulatory protein

migration toward draining LNs, whereas CD47^{-/-} DCs express normal levels of CCR7.^{30,32} By performing a series of complementary approaches *in vivo*, we here identify CD11b^{high}CD103⁻SIRP- α ⁺ DCs as the primary immunogenic DC subset that migrates out of tissues to promote T_H2 responses in mLNs and drive airway disease. By using CD47^{-/-} mice and BALB/c mice treated with SIRP- α -Fc fusion molecule, we provide evidence that CD47/SIRP- α interactions are implicated in the pathogenesis of DC-driven allergic airway inflammation and demonstrate that CD47/SIRP- α interactions may be targeted to alter the development of T_H2-mediated responses.

METHODS**Mice**

The CD47^{-/-} mice were backcrossed onto CD47^{+/+} BALB/c mice for 16 to 18 generations. Control BALB/c mice and BALB/c expressing DO11.10 (ovalbumin-specific) T cell receptor (TCR) were purchased from Charles River Laboratories. Female mice 6 to 8 weeks old were used in all experimental protocols as approved by the Canadian Council on Animal Care and maintained under specific pathogen-free conditions.

Mouse model of asthma by systemic intraperitoneal immunization

CD47^{+/+} and CD47^{-/-} mice were sensitized by intraperitoneal injection of 10 μ g ovalbumin (Grade V; Sigma, USA) adsorbed to 1 mg Imject Alum (Pierce, USA) on days 0 and 5. On days 12, 16, and 20, mice were challenged for 30 minutes with a 0.5% ovalbumin aerosol (Grade V; Sigma). In some experiments, CD47^{+/+} mice were treated intraperitoneally with 50 μ g control human CD47-Fc³¹ or 50 μ g murine SIRP- α -Fc (Novartis, Basel, Switzerland) on days 0 and 5. Bronchoalveolar lavage fluid (BALF) was collected 4 times with 0.5 mL physiologic saline, and lung and mLNs were isolated. "mLN cells (4 $\times$ 10⁶ cells/mL) were cultured with ovalbumin (100 μ g/mL) for 72 hours in culture medium (RPMI 1640; Wisent Inc, Canada), supplemented with 10% FBS, 500 U/mL penicillin, 500 μ g/mL streptomycin, 10 mmol/L HEPES buffer and 1 mmol/L 2-mercaptoethanol)."

Preparation of bone marrow–derived dendritic cells and DC/T-cell cocultures

Bone marrow cell suspensions were obtained from femurs and tibias of CD47^{+/+} and CD47^{-/-} mice and cultured in culture medium supplemented with 5% FBS and 20 ng/mL GM-CSF (PeproTech, USA) for 13 days. The medium was refreshed every 3 days. Bone marrow–derived DCs (BMDCs) were pulsed with 100 μ g/mL LPS-low ovalbumin (Grade V; Sigma) on day 12 of the culture. For coculture experiments, 5 $\times$ 10⁴ CD47^{+/+} or CD47^{-/-} BMDCs were cultured with 1 $\times$ 10⁵ DO11.10 TCR transgenic CD47^{-/-}CD4⁺ T cells for 5 days with 1 μ g/mL ovalbumin peptide (residues 323–339 of chicken ovalbumin; Peptides International, USA)

under T_H2 polarizing conditions (IL-4, 20 ng/mL; and anti-IFN- γ , 1 μ g/mL). After washing, cells were restimulated with phorbol 12-myristate 13-acetate and ionomycin.

Mouse models of airway inflammation induced by the adoptive transfer of BMDCs

Mice sensitized intraperitoneally with ovalbumin/alum received intratracheal injections of 1 $\times$ 10⁶ ovalbumin-pulsed BMDCs. In a second model, mice were instilled with 1 $\times$ 10⁶ ovalbumin-pulsed BMDCs and challenged with ovalbumin aerosol.

Migration assays

CD47^{+/+} and CD47^{-/-} mice were instilled intratracheally with 0.1% fluorescein isothiocyanate (FITC)–conjugated carboxylate YG microspheres (0.5 μ m, 2.5% solid-latex; Polysciences Inc, USA). Twenty-four or 48 hours later, bronchoalveolar lavage was performed, and mLNs were collected and treated with Liberase (0.4 mg/mL; Roche, USA). For some experiments, CD47^{+/+} and CD47^{-/-} mice were passively transferred with 2 $\times$ 10⁶ carboxy-fluorescein succinimidyl ester (CFSE)-labeled transgenic CD4⁺ T cells 1 day before ovalbumin/alum intraperitoneal immunization in the presence or absence of SIRP- α -Fc or control Fc molecule. On day 2, LNs were analyzed for CFSE cell dilution (mean fluorescence intensity [MFI]). Anticlonotypic mAb KJ126 phycoerythrin (BD Biosciences) was used with anti-CD4 allophycocyanin (APC) (BD Biosciences) to retrace divided and undivided transgenic cells.

Airway histology

After BALF was performed, optimal cutting temperature compound was injected into the lungs through the lavage canula. Lungs were frozen in optimal cutting temperature compound, and 5 μ m sections were prepared for hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining and examined by light microscopy.

Determination of airway hyperresponsiveness

Mice were anesthetized with xylazine hydrochloride (NovaPharm, Canada) followed by sodium pentobarbital (CEVA Santé Animale, France). Airway hyperresponsiveness was measured by using a small animal mechanical ventilator (FlexiVent; Scireq, Canada). On initiating ventilation, mice were paralyzed (pancuronium bromide; Sabex, Canada) and exposed to aerosolized saline followed by increasing doses of aerosolized methacholine (Sigma).

Flow cytometry

Total BAL cells were stained for 30 minutes with anti-CCR3 phycoerythrin (R&D Systems, USA), anti-CD3 FITC (clone 145-2C11) and anti-B220 FITC (R&D Systems), as described by van Rijt et al.³³ To characterize the DC subsets, mLNs and lungs were treated with Liberase (0.4 mg/mL; Roche). Lung cell suspensions were treated with NH₄Cl for red blood cell lysis before staining with the following antibodies: anti-CD11c FITC, anti-CD11b FITC, anti-I-Ad/I-Ed PE, 120G8 FITC, CD103 phycoerythrin, anti-CD47 FITC, biotinylated anti-CD103- and biotinylated anti-SIRP- α mAbs followed by streptavidine (SA)-APC or peridinin chlorophyll protein (PerCP) (BD Biosciences, USA), anti-Ly6G and Ly6C (GR-1), anti-B220 FITC (R&D Systems), anti-CD11c APC (clone N418), and anti-CD11b phycoerythrin (Caltag, USA). In lungs, autofluorescent alveolar macrophages were excluded from the analysis gates by using an empty phycoerythrin–Texas Red or PerCp fluorescent channel. For regulatory T cells, cells were first stained with anti-CD4 APC and anti-CD25 FITC (BD Biosciences), fixed, permeabilized, and stained with anti-Forkhead box protein 3 (Foxp3) phycoerythrin (kit from eBiosciences). All the data were acquired on a FACSCalibur or CantoII Flow Cytometer by using Cellquest or DIVA software (BD Biosciences, USA).

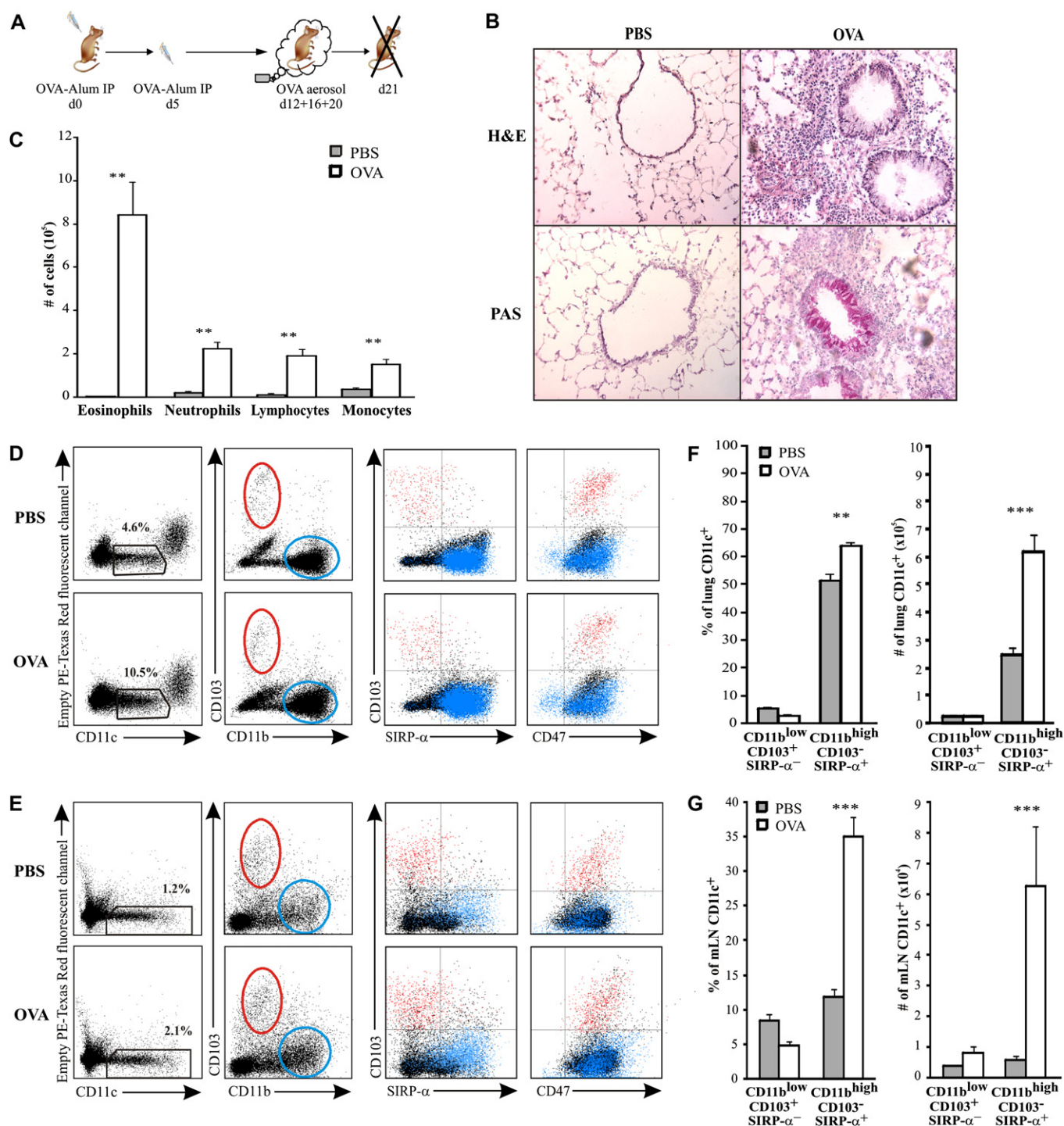


FIG 1. CD11b^{high}CD103⁺ DCs preferentially expressed SIRP-α and are recruited during allergic airway inflammation. **A**, Immunization procedure. **B**, Lung sections stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). **C**, BALF cell numbers. CD103 and CD11b expression gated on CD11c⁺ DCs in lungs (**D**) and mLNs (**E**). SIRP-α and CD47 expression gated on CD11c⁺CD11b^{low}CD103⁺ (red) and CD11c⁺CD11b^{high}CD103⁺ (blue) cells. Percentage and absolute numbers of lung (**F**) and mLN (**G**) DC subsets, means $\pm$ SEMs (5–10 mice/group). IP, Intraperitoneal; OVA, ovalbumin; PE, phycoerythrin. *** $P < .001$; ** $P < .01$.

Cytokine and immunoglobulin measurements

IL-4, IL-5, IL-10, IFN-γ (BD Biosciences), and IL-13 (R&D Systems) were measured in mLN supernatants by ELISA. Total and ovalbumin-specific IgE and IgG₁ production were quantified in the serum by ELISA.

Proliferation

For the last 18 hours of culture, mLN cells were pulsed with 1 μ Ci 3[H] thymidine (Amersham Biosciences, UK) and harvested for liquid scintillation counting. Data are expressed as counts per minute.

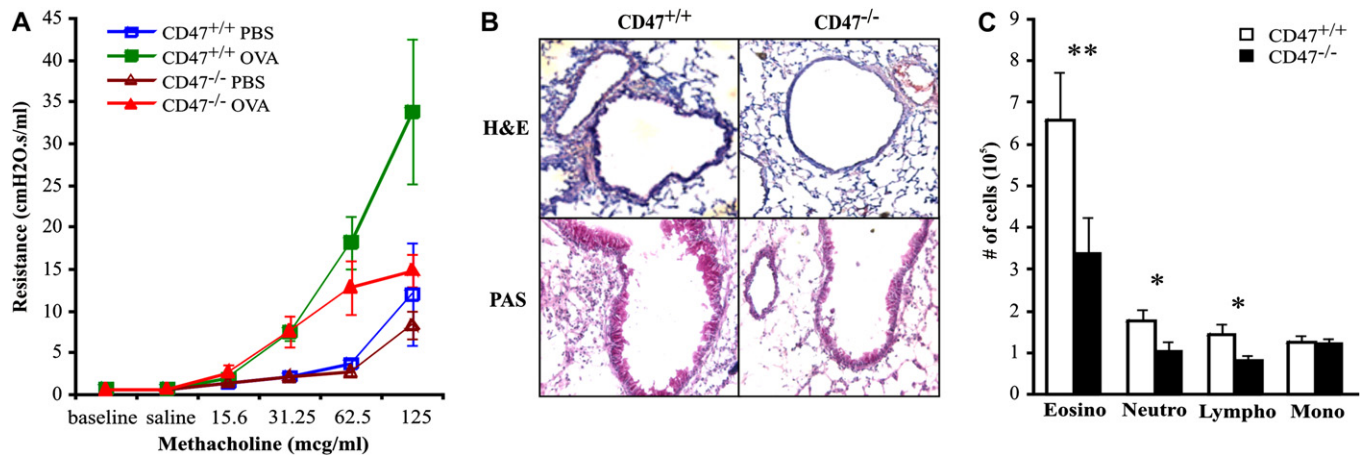


FIG 2. CD47 deficiency reduces the severity of experimental allergic asthma. **A**, Resistance to various doses of methacholine of CD47^{+/+} and CD47^{-/-} sensitized and challenged mice. **B**, Lung sections stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Data are representative of 5 individually analyzed lungs. **C**, BALF cell numbers. Means $\pm$ SEMs (5-10 mice/group). OVA, Ovalbumin; Eosino, Eosinophils; Neutro, neutrophils; Lympho, lymphocytes; Mono, monocytes. ** $P < .01$; * $P < .05$.

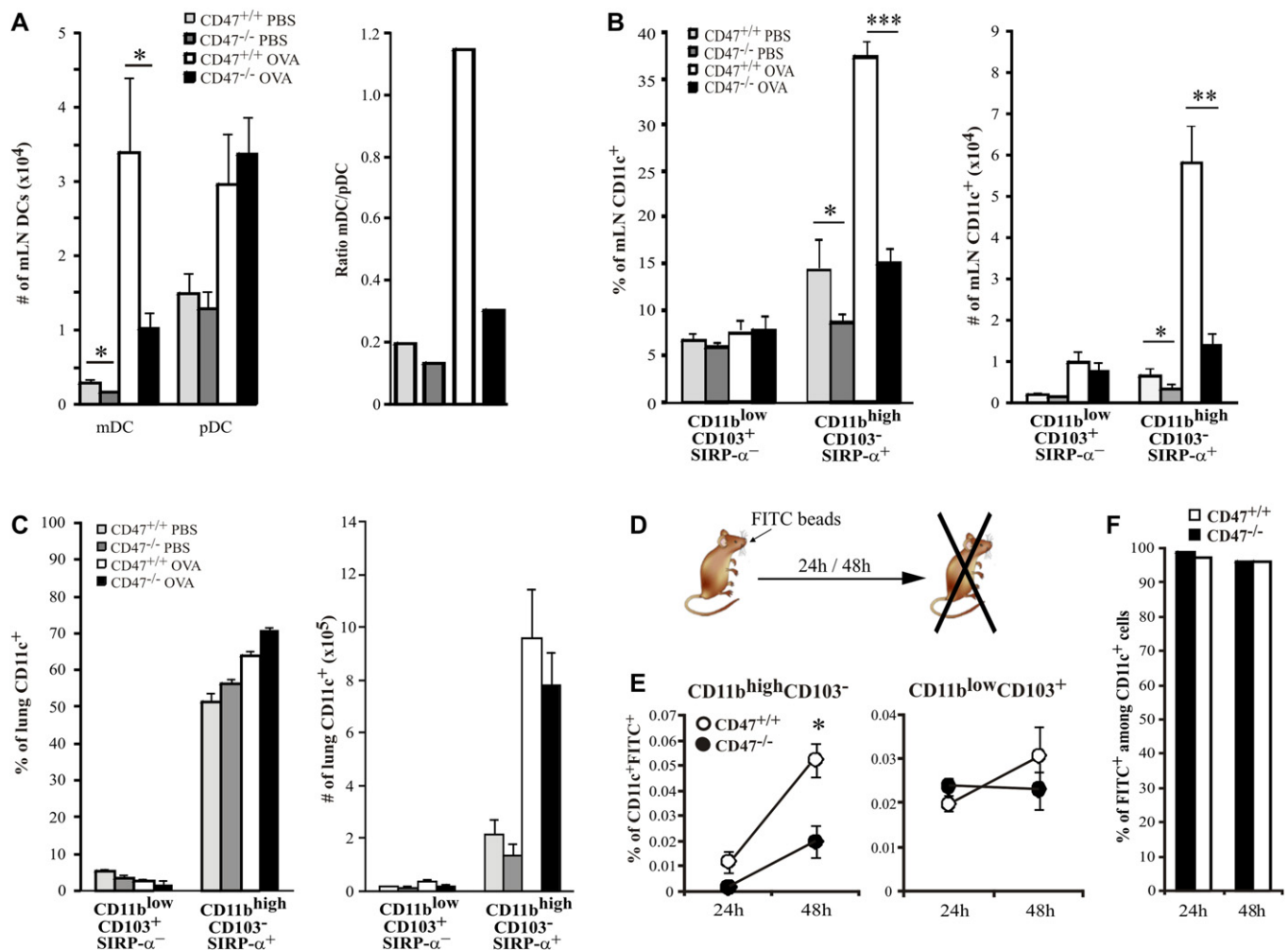


FIG 3. CD47 selectively controls airway CD11b^{high}CD103⁻SIRP-α⁺ DC migration at steady state and during allergic airway disease. **A**, CD11c^{high}/I-A⁺ (mDCs) and CD11c^{low}/B220⁺120G8⁺ (pDCs; left) and mDC/pDC ratio (right). Percentage and absolute numbers of DCs subsets (gated on CD11c⁺) in mLN (B) and lung (C) (8-10 mice/group). **D**, Naive CD47^{+/+} and CD47^{-/-} mice injected intratracheally with FITC-conjugated microspheres and CD11c⁺FITC⁺ cells in mLN (E) and BALF (F). Means $\pm$ SEMs (3 mice/group). OVA, Ovalbumin. *** $P < .001$; ** $P < .01$; * $P < .05$.

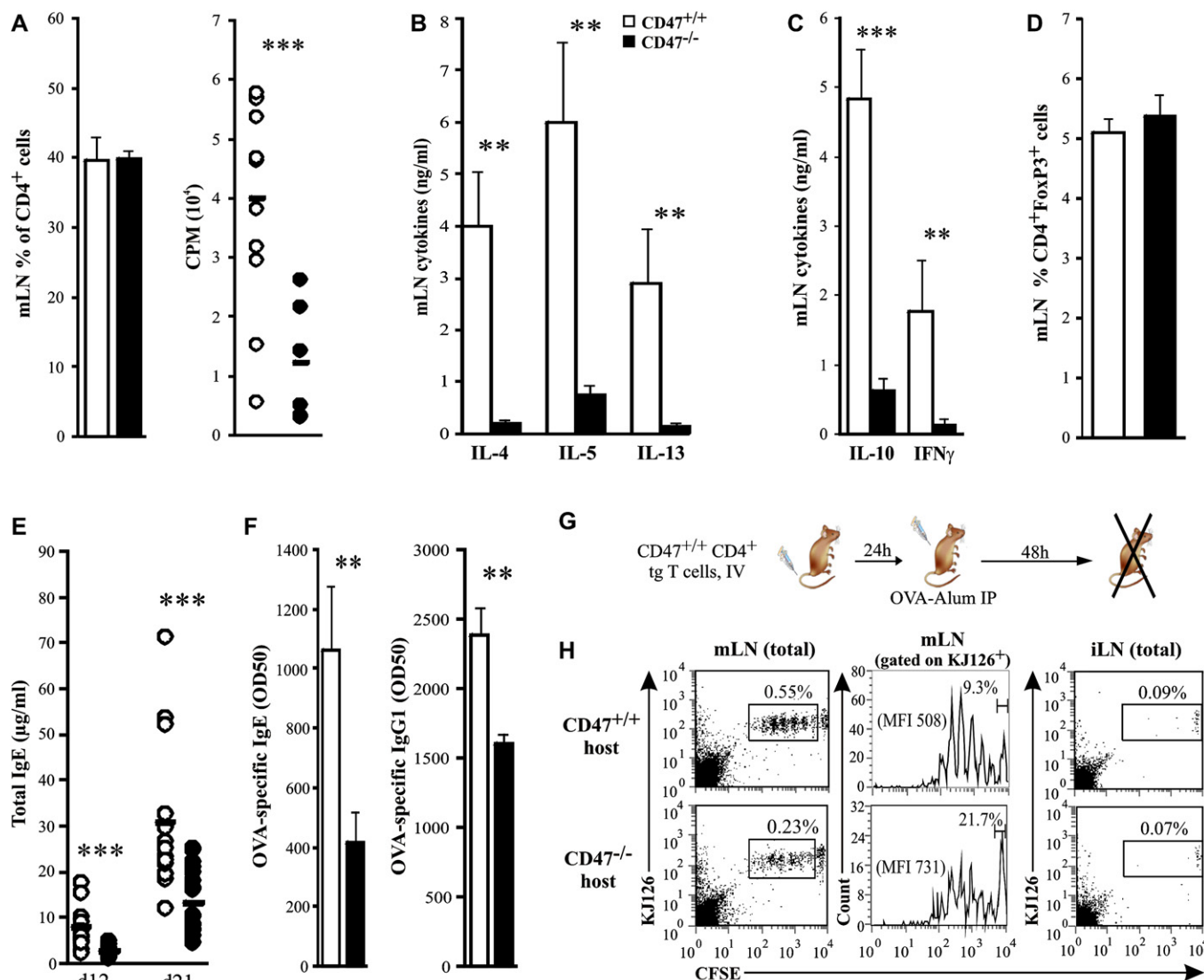


FIG 4. CD47 regulates airway SIRP- α ⁺ DC-driven T_H2 responses. **A**, left Percentage of CD4⁺ T cells in mLN. **A**, right Proliferation and (**B** and **C**) cytokine release after ovalbumin (OVA) restimulation *in vitro*. **D**, CD4⁺FoxP3⁺ T cells. Total (**E**) and OVA-specific (**F**) immunoglobulin in sera (5-20 mice/group). **G** and **H**, After adoptive transfer and immunization, CFSE-labeled transgenic cells were retraced in mLNs (divided [left], MFI and undivided cells [middle]) and iLNs (mean of 5-7 mice/group). CPM, Counts per minute; IP, intraperitoneal; IV, intravenous; iLN, Inguinal lymph nodes. ****P* < .001; ***P* < .01.

Statistical analysis

Statistical analyses were performed by using the unpaired Student *t* test and the nonparametric Mann-Whitney *U* test. Data represent mean $\pm$ standard errors of the mean (SEM) or when mentioned, standard deviation (SD). (***)*P* < .001; (**)*P* < .01; (**P* < .05).

RESULTS

CD11b^{high}CD103⁻ DCs that express SIRP- α are mobilized during airway inflammation

The identification and migratory properties of the lung DC subset that contributes to T_H2 responses remain unclear. Because CD47 expression on SIRP- α ⁺ skin DCs but not on lymphatic vessels positively controls their trafficking to LNs,³¹ we first evaluated the level of SIRP- α and CD47 expression on the 2 major airway DC subsets. To this end, we used an experimental

model of chronic allergic asthma induced by ovalbumin (Fig 1, A), which is characterized by lung cellular infiltration, mucus [F1-4/C] production, and eosinophil accumulation in the BALF (Fig 1, B and C). We here showed for the first time that CD11b^{high}CD103⁻ but not CD11b^{low}CD103⁺ DCs were identified by SIRP- α expression in lungs as well as in the mLNs of naive and sensitized mice (Fig 1, D and E). In fact, the great majority (greater than 98%) of the CD11b^{high}CD103⁻ DC subpopulation expressed SIRP- α . Interestingly, CD103⁺SIRP- α ⁻ and CD103⁻SIRP- α ⁺ DCs displayed similar amounts of CD47. We next demonstrated that the characteristic influx of CD11c⁺ DCs into the lungs and mLNs in sensitized mice was specifically attributed to an increase in the percentage and absolute numbers of the CD11b^{high}CD103⁻SIRP- α ⁺ DC subset (Fig 1, F and G). In contrast, the frequency and numbers of CD11b^{low}CD103⁺

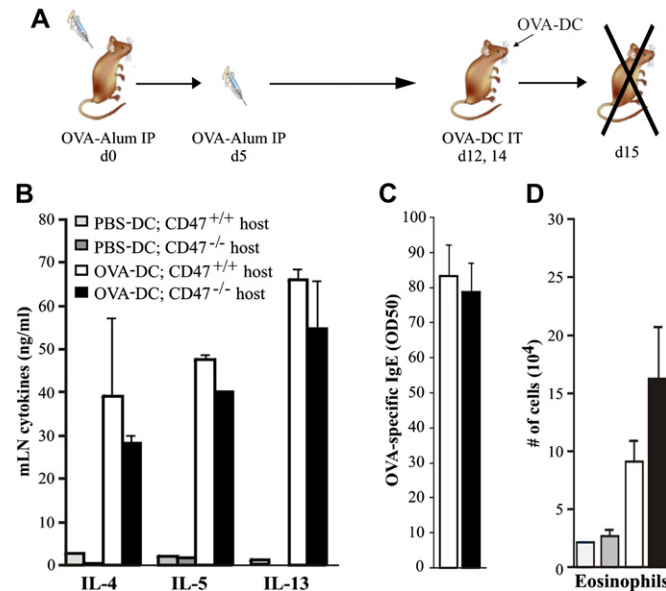


FIG 5. Local delivery of SIRP- α^+ CD47 $^{+/+}$ DCs elicits a strong T_H2 response in CD47 $^{-/-}$ mice. **A**, Sensitized mice were instilled intratracheally (IT) with ovalbumin (OVA)-pulsed or PBS-pulsed BMDCs. **B**, IL-4, IL-5, and IL-13 production were measured in the supernatant of mLN cell cultures. Serum levels of OVA-specific IgE (**C**) and number of eosinophils in the BALF (**D**). Means $\pm$ SEMs (4–7 mice/group). IP, Intraperitoneal.

SIRP- α^- DCs were not augmented on development of airway inflammation. On the basis of these findings, we hypothesize that CD103 $^+$ SIRP- α^+ DCs are implicated in the development of T_H2 -mediated airway allergic inflammation and that their recruitment to mLNs is regulated by CD47.

CD47 governs airway CD11b ^{high} CD103 $^+$ SIRP- α^+ DC migration during experimental asthma

Therefore, to gain mechanistic insight into the role of CD47/SIRP- α interactions in allergic airway inflammation, we induced experimental asthma in CD47 $^{-/-}$ mice and examined the distribution of CD103 $^+$ SIRP- α^+ DCs in their lungs and mLNs. We observed a decrease in bronchial airway hyperresponsiveness [F2-4/C] (Fig 2, A) that occurred together with a significant reduction in pulmonary cellular inflammation, mucus secretion (Fig 2, B) and the total number of inflammatory cells in the BALF (Fig 2, C). Among CD11c $^+$ MHC class II $^+$ cells, mDCs were recruited in lower numbers in CD47 $^{-/-}$ than CD47 $^{+/+}$ mLN, whereas pDC accumulation was comparable in both strains of mice (Fig 3, A, left). Accordingly, the mDC/pDC cell ratio was strongly enhanced in the mLN of sensitized CD47 $^{+/+}$ mice compared with CD47 $^{-/-}$ mice (Fig 3, A, right). Among the mDC subsets examined, we found a drastic reduction in the percentage and absolute numbers of CD103 $^+$ SIRP- α^+ DCs in CD47 $^{-/-}$ mice (Fig 3, B). The trafficking of the CD103 $^+$ SIRP- α^+ DC subset to mLN appeared to be independent of CD47. The impaired homing to mLN of CD103 $^+$ SIRP- α^+ DCs was unlikely to be attributed to altered DC precursor recruitment from the bloodstream into the lungs, because DC subsets accumulated in similar proportions and numbers in the inflamed lungs of both CD47 $^{-/-}$ and CD47 $^{+/+}$ mice (Fig 3, C).

CD47 is required for DC migration from lung to mLN at steady state

Because we observed a significant reduction in the frequency and absolute numbers of CD103 $^+$ SIRP- α^+ DCs in the mLN

of sham-treated CD47 $^{-/-}$ mice (Fig 3, B), we evaluated DC trafficking over time in draining mLN of mice that were instilled intratracheally with FITC-conjugated microspheres (Fig 3, D). We observed a reduction in the percentage of CD11c $^+$ CD11b ^{high} CD103 $^+$ FITC $^+$ cells that accumulated in the mLN of CD47 $^{-/-}$ mice compared with CD47 $^{+/+}$ mice, demonstrating an altered migration of CD47 $^{-/-}$ CD11b ^{high} CD103 $^+$ DCs carrying antigen at steady state (Fig 3, E). BALF isolated from the 2 strains of mice contained a similar proportion of DCs that had phagocytosed fluorescent beads, suggesting that the absence of CD47 did not interfere with antigen uptake by interstitial DCs recovered in the lavage (Fig 3, F). Taken together, these results have provided the first evidence that CD47 promotes CD11b ^{high} CD103 $^+$ SIRP- α^+ DC homing to mLN in naive and sensitized mice that developed airway disease.

CD47 promotes CD103 $^+$ SIRP- α^+ DC-mediated T_H2 -mediated responses

Because the DCs are essential to the development of T_H2 responses *in vivo*, we next examined the ovalbumin-specific T-cell responses in mLN of CD47 $^{+/+}$ and CD47 $^{-/-}$ sensitized mice. Although the proportion of CD4 $^+$ T cells (Fig 4, A, left) and different [F4-4/C] CD4 $^+$ T-cell subsets (data not shown) were comparable in the 2 groups of mice, the proliferation of cells isolated from CD47 $^{-/-}$ mLN was significantly reduced after *ex vivo* restimulation with ovalbumin (Fig 4, A, right). Accordingly, the production of T_H2 cytokines was strongly suppressed in CD47 $^{-/-}$ mice (Fig 4, B). The inhibition of the T_H2 response in CD47 $^{-/-}$ mice was not linked to an altered proportion of naturally occurring CD4 $^+$ Foxp3 $^+$ cells, or to an immune deviation toward T_H1 or adaptive regulatory T cells (Tr1), because IFN- γ and IL-10 secretion was inhibited (Fig 4, C and D). Total IgE and ovalbumin-specific IgE and IgG $_1$ antibody responses were significantly diminished in CD47 $^{-/-}$ mice, an indication of their compromised capacity to mount a systemic T_H2 response (Fig 4, E and F). The decreased IgE levels readily seen at day 12 after sensitization immediately

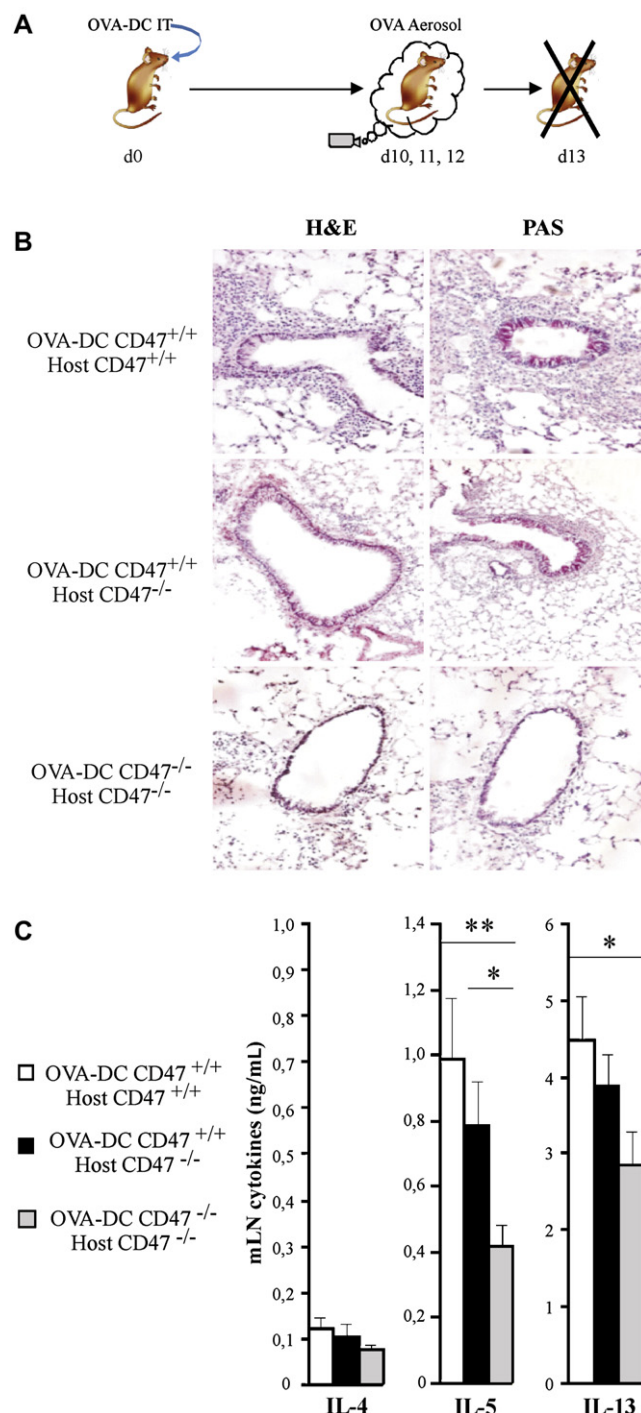


FIG 6. CD47 expression on SIRP- α^+ DCs is required to promote T_H2 -mediated airway inflammation. **A**, Ovalbumin (OVA)-pulsed BMDCs sensitized mice received 3 OVA-aerosol challenges. **B**, Lung sections stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). One representative of 5 individually analyzed lungs. **C**, IL-4, IL-5, and IL-13 levels in the supernatants of mLN cell cultures after *in vitro* restimulation with OVA. Means $\pm$ SEMs (8 mice/group). IT, Intratracheal. ** $P < .01$; * $P < .05$.

before ovalbumin challenge (Fig 4, E, day 12 (d12)) pinpointed an early defect in the recruitment of DC precursors from the periphery to the mLNs of CD47^{-/-} mice, resulting in impaired T_H priming. To validate this point, CD47^{+/+} and CD47^{-/-} mice were passively transferred with CFSE-labeled CD47^{+/+} CD4⁺

transgenic T cells 1 day before intraperitoneal ovalbumin/alum immunization (Fig 4, G). At day 2, no T-cell proliferation was observed in other lymph nodes (ie, inguinal LNs) because the intraperitoneal delivery of ovalbumin/alum primes adaptive T_H2 responses directly in the mLNs, bypassing the spleen.³⁴ The proportion of divided CD47^{+/+} transgenic T cells was significantly reduced in CD47^{-/-} compared with CD47^{+/+} mLNs (Fig 4, H; 0.55% vs 0.23%; $P < .05$). Accordingly, the frequency of undivided cells and the MFI of CFSE KJ126⁺ cells were significantly increased in CD47^{-/-} mice (Fig 4, H; $P < .01$). These data suggest that impaired local and systemic T_H2 responses seen in CD47^{-/-} mice are linked to a defective CD103⁺SIRP- α^+ DC homing to mLNs.

***In vivo* antigen delivery by local transfer of CD11b^{high}CD103⁺SIRP- α^+ DCs restores CD47^{-/-} T_H cell function**

To establish direct causative links between defective DC trafficking and impaired T_H2 responses, we next show that local delivery of antigen by 2 intratracheal injections of ovalbumin/LPS^{low}-pulsed BMDCs, in the absence of aerosol challenge, was sufficient to restore a T_H2 response in ovalbumin-sensitized CD47^{-/-} mice (Fig 5, A). Notably, the BMDCs are CD11b⁺ CD103⁺SIRP- α^+ (see this article's Fig E1, A, in the Online Repository at www.jacionline.org), supporting the concept that BMDCs may function like immunogenic CD11b^{high}CD103⁺SIRP- α^+ lung DCs.¹⁶ The amounts of IL-4, IL-5, and IL-13 elicited by BMDCs in CD47^{-/-} and CD47^{+/+} mice were 8-fold to 20-fold higher than those induced in intraperitoneal ovalbumin-sensitized and aerosol-challenged mice (Fig 5, B). Accordingly, the ovalbumin-specific IgE response and eosinophil numbers in the BALF were comparable in the 2 hosts (Fig 5, C and D). We conclude that CD47^{-/-} T cells were capable of mounting a robust T_H2 response *in vivo* and that airway CD47⁺CD103⁺SIRP- α^+ DC recruitment to mLNs was sufficient to drive a T_H2 response in otherwise protected immunized CD47^{-/-} mice.

CD47 expression is required on CD103⁺SIRP- α^+ DCs to promote T_H2 -mediated airway inflammation

We further analyzed the relative contribution of CD47 expression on BMDCs to their trafficking into mLNs and ability to directly prime or reactivate effector T_H2 cells. To this end, naive CD47^{-/-} mice were sensitized with a single intratracheal administration of CD47^{+/+} and CD47^{-/-} OVA-loaded BMDCs and challenged by OVA aerosol (Fig 6, A). Both phenotype and T_H2 polarizing capacity were found to be independent of CD47 expression on the BMDCs ($<MMC = 1 >$ Fig E1). We found that CD47^{-/-} mice instilled with CD47^{-/-}SIRP- α^+ BMDCs displayed no cellular infiltration or mucus production in lungs (Fig 6, B) and lower IL-5 and IL-13 secretion *in vivo* when compared to hosts immunized with CD47^{+/+} BMDCs (Fig 6, C). Taken collectively, CD47 expressed by CD103⁺SIRP- α^+ DCs themselves governs their trafficking to mLNs to promote the development and maintenance of T_H2 -driven allergic airway inflammation.

Interruption of CD47/SIRP- α protects BALB/c mice from T_H2 -driven airway inflammation

Because CD47 on CD103⁺SIRP- α^+ airway DCs and not on lymphatic cells appears as a critical molecule in the initiation

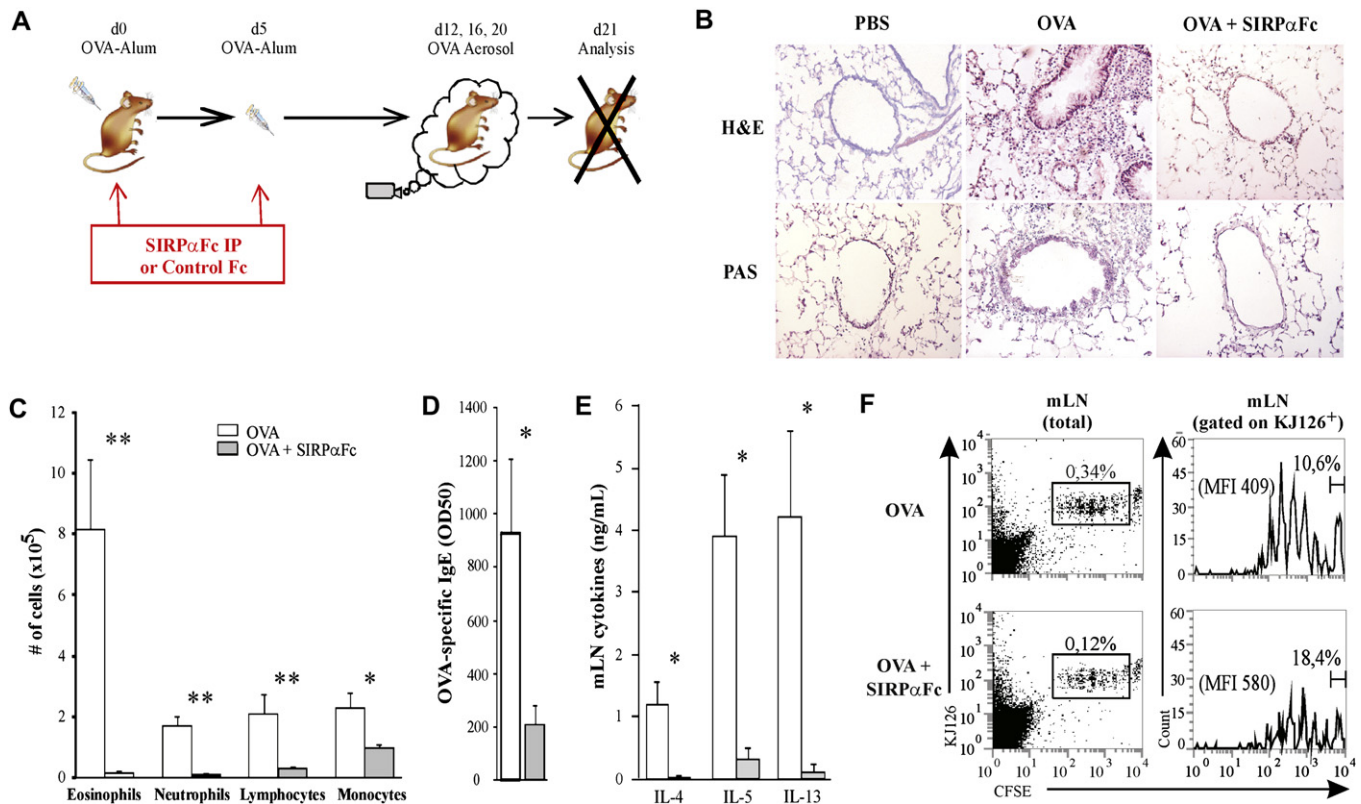


FIG 7. Targeting CD47 protects BALB/c mice from T_H2 -mediated airway allergic inflammation. **A**, Immunization and treatment procedure. **B**, Lung sections stained with hematoxylin (H&E) and periodic acid-Schiff (PAS). **C**, BALF cell numbers. Ovalbumin (OVA)-specific IgE in sera (**D**) and T_H2 cytokine release in mLN culture (4-8 mice/group) (**E**). **F**, CFSE-labeled transgenic cells retraced in mLNs after adoptive transfer and immunization with or without SIRP- α -Fc treatment (mean of 5-6 mice/group). IP, Intraperitoneal. ** $P < .01$; * $P < .05$.

and perpetuation of T_H2 immunity, it may be harnessed therapeutically to reduce lung inflammation and ameliorate airway disease. BALB/c mice administered SIRP- α -Fc on days 0 and 5 of ovalbumin immunization (Fig 7, A) had very few or no inflammatory cell infiltration of the lung tissues after ovalbumin aerosol challenge (Fig 7, B). A strong reduction of inflammatory cells in the BALF (Fig 7, C) occurred together with a drop in serum ovalbumin-specific IgE (Fig 7, D) and a drastic inhibition in T_H2 cytokine production in the mLNs (Fig 7, E). The protection from airway inflammation was not correlated with increases in IL-10 or IFN- γ release, which in fact were also suppressed in treated mice (data not shown). Moreover, the administration of SIRP- α -Fc led to significant changes in the proportion of divided and undivided CFSE-labeled transgenic T cells in the mLNs of ovalbumin-immunized mice (Fig 7, F; $P < .01$ and $P < .05$, respectively), underscoring the impaired DC-driven T_H responses seen in sensitized and challenged CD47 $^{-/-}$ mice (Fig 4, H). These data demonstrate that CD47/SIRP- α interruption at primary antigen sensitization largely prevents T_H2 responses in mLNs as well as IgE-dependent airway inflammation.

DISCUSSION

Our present findings provide evidence that CD47 and SIRP- α are critically involved in the development of T_H2 -mediated chronic allergic inflammation. We show that SIRP- α is

preferentially expressed on the lung CD11b^{high}CD103⁻ DC subset, whose accumulation in the draining mLNs is governed by CD47. We further demonstrate that CD103⁻SIRP- α ⁺ DCs promote pathogenic T_H2 responses and airway disease. First, CD47 deficiency significantly inhibits CD103⁻SIRP- α ⁺ DC-driven T_H2 responses and attenuates airway disease. Second, the local delivery of CD103⁻SIRP- α ⁺CD47 $^{+/+}$ DCs elicits strong T_H2 responses and lung cellular infiltration in CD47-deficient mice, establishing causative links among CD103⁻SIRP- α ⁺ DCs, perpetuation of T_H2 responses, and allergic disease. Finally, disruption of CD47 and SIRP- α interactions by SIRP- α -Fc administration at primary antigen sensitization protects BALB/c mice from T_H2 -mediated airway inflammation.

Dendritic cells have a pathophysiological role, beyond the sensitization phase, in the perpetuation of T_H2 -mediated airway inflammatory responses by restimulating resting central memory T cells and promoting the differentiation of a new wave of naive T cells into effector T cells.^{35,36} We here propose that a divergence in tasks is operated by the CD103⁺SIRP- α ⁻ and CD103⁻SIRP- α ⁺ DC subpopulations. These 2 mDC subsets originate from distinct precursors and appear to maintain a stable phenotype with no conversion of CD103⁻ into CD103⁺ DCs and vice versa.³⁷ When the airways of sensitized mice are exposed to airborne allergens in the presence of Toll-like receptor agonists (ie, ovalbumin contaminated by a low dose of LPS), CD103⁻ DCs are the major

chemokine-producing cells recruited from the bloodstream into lung tissues.²¹ Although both DC precursors are recruited to CD47^{-/-} inflamed lung tissues, our results point toward selective impeded egress of airway CD103⁺SIRP- α ⁺ DCs, which, combined with impaired recruitment of inflammatory CD47^{-/-} DCs directly from the bloodstream, results in their decreased accumulation in the mLN. In fact, CD47 expressed on the DCs positively regulated CD103⁺SIRP- α ⁺ DC trafficking to mLN, although it did not significantly alter the migration of CD103⁺ DCs and pDCs under steady-state or inflammatory conditions. Through the expression of tight junction proteins, CD103⁺ DCs are equipped to extend their pseudopods into the bronchiolar luminal space to capture antigen,²⁰ and it was recently established that lung CD11b^{low}CD103⁺ DCs display tolerogenic properties.³⁸

We here show, for the first time, that airway CD11b^{high}CD103⁻ DCs are positively identified by SIRP- α while expressing similar quantities of CD47 as CD103⁺ DCs. Notably, SIRP- α is expressed on CD8⁺ splenic, dermal, and thymic DCs.^{32,39} CD47 expression controls the mobilization of SIRP- α ⁺ cells, including neutrophils, epidermal DCs, dermal DCs and marginal zone splenic DCs.^{31,32,40} Of interest, SIRP- α is found on human alveolar macrophages, mast cells, and basophils.⁴¹ How CD47 expression on the SIRP- α ⁺ DCs precisely controls the *in vivo* transendothelial migration and whether it regulates other innate SIRP- α ⁺ cell trafficking and function remain to be clarified.

T_H2 cytokines produced by effector T cells (ie, IL-4 and IL-13), although dispensable to the initiation of airway hyperreactivity and recruitment of the first wave of innate cells to the lungs, are required and intimately linked to the induction of IgE and IgG₁ antibody responses *in vivo*.⁴² The primary and secondary antigen-specific type 2 humoral and cellular immune responses were significantly impaired in CD47-deficient mice and SIRP- α -Fc-treated mice. The altered T_H2 response does not reflect an intrinsic CD4⁺ T-cell defect because local delivery of ovalbumin-loaded SIRP- α ⁺CD47^{+/+} DCs elicit the release of large quantities of T_H2 cytokines by CD47^{-/-} T cells *in vivo*. Also, the reduced ovalbumin-specific IgE response could not be attributed to a B-cell intrinsic defect because *in vitro* IL-4-activated purified B cells isolated from CD47^{+/+} or CD47^{-/-} mice produced comparable amounts of IgG₁ and IgE.²⁹ Although the total IgG response to corpuscular T-cell-independent antigens was reduced in the C57BL/6 CD47^{-/-} mice,³² intraperitoneal or subcutaneous immunization with ovalbumin in complete Freund adjuvant (CFA) induced type 1-biased cellular responses as well as enhanced antigen-specific IgG₂ antibody production in BALB/c CD47^{-/-} mice.²⁹ However, antigen-specific T-cell IFN- γ production was reduced in the mLN of ovalbumin/alum-immunized and challenged SIRP- α -Fc-treated or mutant mice. Considering that acute T_H1 responses may aggravate T_H2-dependent allergic pulmonary disease *in vivo*,⁴³ the combined decreased T_H2 and T_H1 responses along with the unaltered proportion of Foxp3⁺ regulatory T cells conferred CD47^{-/-} or treated BALB/c mice a protection from airway inflammation.

Collectively, we here demonstrate for the first time that the manipulation of CD47/SIRP- α pathway inhibits CD103⁺SIRP- α ⁺ DC-driven pathogenic T_H2 responses and airway inflammation.

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Key messages

- CD11b^{high}CD103⁻ DCs express SIRP- α and are critically implicated in the development of T_H2-mediated airway allergic inflammation.
- CD47 expression on immunogenic SIRP- α ⁺ DCs regulates their trafficking.
- Blocking CD47/SIRP- α interaction prevents the development of airway allergic inflammation.

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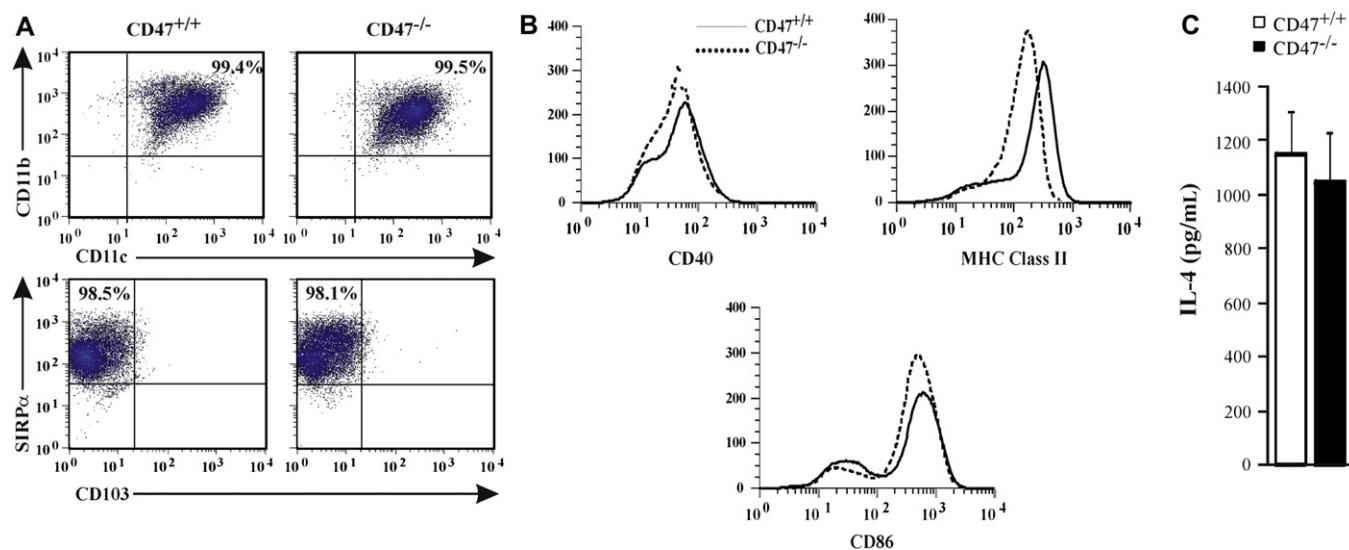


FIG E1. Phenotype and functional properties of BMDCs. **A and B**, Phenotype of ovalbumin (OVA)-pulsed CD47^{+/+} or CD47^{-/-} BMDCs. Shown is 1 representative experiment of 3. **C**, CD47^{+/+} or CD47^{-/-} BMDCs were cultured with transgenic CD47^{-/-}CD4⁺ T cells in the presence of OVA peptide under T_H2-polarizing conditions (IL-4 and anti-IFN- γ), and IL-4 secretion was measured in the supernatant. Means $\pm$ SDs of 3 independent experiments.