

Targeting SIRP- α protects from type 2-driven allergic airway inflammation

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The interplay between innate and adaptive immune responses is essential for the establishment of allergic diseases. CD47 and its receptor, signal regulatory protein α (SIRP- α), govern innate cell trafficking. We previously reported that administration of CD47^{+/+} but not CD47^{-/-} SIRP- α ⁺ BM-derived DC (BMDC) induced airway inflammation and Th2 responses in otherwise resistant CD47-deficient mice. We show here that early administration of a CD47-Fc fusion molecule suppressed the accumulation of SIRP- α ⁺ DC in mediastinal LN, the development of systemic and local Th2 responses as well as airway inflammation in sensitized and challenged BALB/c mice. Mechanistic studies highlighted that SIRP- α ligation by CD47-Fc on BMDC did not impair Ag uptake, Ag presentation and Ag-specific DO11.10 Tg Th2 priming and effector function *in vitro*, whereas *in vivo* administration of CD47-Fc or CD47-Fc-pretreated BMDC inhibited Tg T-cell proliferation, pinpointing that altered DC trafficking accounts for defective Th priming. We conclude that the CD47/SIRP- α axis may be harnessed *in vivo* to suppress airway SIRP- α ⁺ DC homing to mediastinal LN, Th2 responses and allergic airway inflammation.

Key words: Airway inflammation · CD47 · DC · Signal regulatory protein α · Th2



Supporting Information available online

Introduction

Allergic disorders result from aberrant Th2 responses to common environmental Ag [1]. Allergic asthma is characterized by increased IgE production and the accumulation of Th2 effector/memory cells in the bronchial tissues. Cytokine release by innate cells combined with the accumulation of Th2 cells in lung tissues are largely responsible for the massive local recruitment of eosinophils, basophils and other precursors, which ultimately promote airway hyper responsiveness and mucus secretion [2–5].

DC are critical players in the initiation and maintenance of airway inflammation [6]. Three lung DC subsets have been identified in the conductive airways and the lung parenchyma with distinct functions [7]. The non-plasmacytoid signal regulatory protein α (SIRP- α)⁺ DC (CD11c^{high}CD11b⁺CD103⁻SIRP- α ⁺) induce Th2 polarization as well as the activation of Th2 effector cells, while the CD103⁺ DC (CD11c^{high}CD11b^{low}CD103⁺SIRP- α ⁻) and the plasmacytoid DC (CD11c^{low}CD11b⁻Gr-1⁺B220⁺120G8⁺, pDC) may promote tolerance [8–11]. Hence, when pDC are depleted, OVA-sensitized mice develop a severe asthma [12].

Initial triggering of allergic asthma appears to occur at the bronchial epithelial surface. Exposure of epithelial barrier to allergens with endogenous endotoxin or protease activity triggers a TLR4-dependent program that results in the activation of DC,

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basophils and mast cells releasing IL-4 and IL-13, which promote Th2-mediated airway inflammation [13]. Agents that pharmacologically manipulate DC precursors recruitment from blood-stream to lung tissues as well as DC migration from airways to mediastinal LN (mLN) impair the induction as well as the perpetuation of Th2 responses and successfully alleviate airway inflammatory responses [14]. For instance, sphingosine 1-phosphate receptor antagonist FTY720 alters migratory behavior of airway DC, inhibits DC/T stable interactions with naïve T cells and effector Th2 cells and suppresses airway disease development [15]. Indeed, the number and activation status of lung DC during secondary challenge appears essential for controlling allergic inflammation. In experimental model of asthma, allergen challenge results in the accumulation of CD11b⁺ DC in the lungs and the mLN [16]. CCR2 and not CCR6 are implicated in the attraction of immature Th2-cell-inducing DC to the lungs while CCR7 is involved in their egress en route to regional LN [17–19].

CD47 is expressed on all hematopoietic cells and serves as a counter-receptor for SIRP- α , while SIRP- α expression is restricted to myeloid and neuronal cells [20]. We recently reported that airway SIRP- α ⁺ DC trafficking to mLN is critical for the development of Th2-mediated airway allergic inflammation, which are both impaired in CD47-deficient mice. CD47 expression on

murine CD11b⁺ SIRP- α ⁺ DC positively governs cell trafficking [8, 21] whereas SIRP- α ligation by a CD47-Fc fusion molecule inhibits human DC migration *in vitro* [22]. We demonstrate here that *in vivo* administration of a CD47-Fc fusion molecule inhibited local and systemic Th2 responses and protected BALB/c mice from airway inflammation. We explored the mode of action of the CD47-Fc protective effect and showed that SIRP- α targeting by CD47-Fc did not interfere with Th2 priming and re-stimulation *in vitro* but impaired SIRP- α ⁺ DC accumulation in mLN and compromised Ag-specific CD4⁺T-cell proliferation *in vivo*.

Results

CD47-Fc protects BALB/c mice from Th2-mediated airway inflammation

We previously demonstrated that CD47-deficient mice are protected from allergic airway inflammation [8]. Here we evaluated the impact of CD47-Fc fusion protein on the development of allergic airway inflammation in BALB/c mice. Mice administered with CD47-Fc but not control human CD47-Fc (ctrl-Fc) on days 0 and 5 of OVA immunization showed decreased

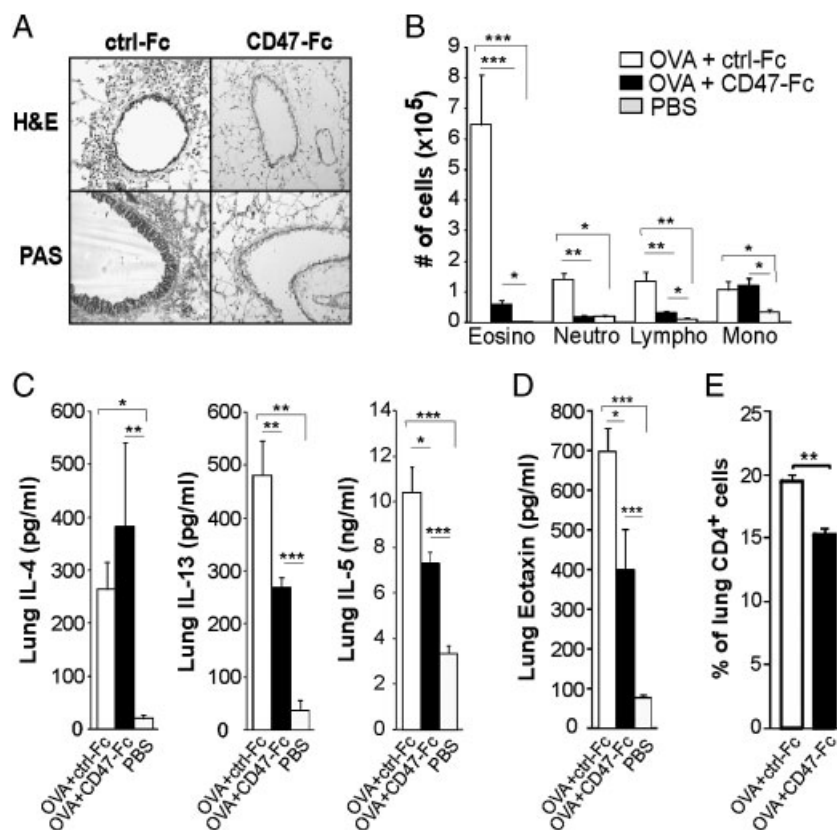


Figure 1. CD47-Fc delivered at priming inhibits airway inflammation. BALB/c mice were injected i.p. on days 0 and 5 with PBS (Control, grey bars) or OVA/Alum in the presence of CD47-Fc (black bars) or ctrl-Fc (open bars). Mice were OVA-aerosol challenged on day 12, 16, 20 and sacrificed at day 21. (A) Lung sections stained with H&E and PAS (magnification 1 ×). Data are one representative of three to five mice per group. (B) Differential BALF cell numbers. (C) IL-4, IL-5, IL-13 and (D) eotaxin release in lung explants. (E) Proportion of CD4⁺ T cells in lung tissues. Data show mean ± SEM ($n = 4–8$ mice per group) of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (U-test).

mucus secretion by goblet cells (periodic acid–Schiff (PAS) staining) and very few inflammatory cells infiltrating lung tissues (H&E) when examined 24 h after the last OVA aerosol challenge (day 21) (Fig. 1A). CD47-Fc treatment reduced the total cell counts in the BALF as well as the numbers of eosinophils, neutrophils and lymphocytes analyzed by flow cytometry (Fig. 1B and Supporting Information Fig. 1). We next examined cytokine and chemokine expression in the culture supernatants of lung explants of CD47-Fc-treated mice and found that IL-5, IL-13 and eotaxin release was significantly decreased, whereas IL-4 expression remained unchanged (Fig. 1C and D). We also found a reduction in the proportions of CD4⁺ T cells that infiltrated the lung tissues in CD47-Fc-treated mice (Fig. 1E).

The reduction in airway inflammation occurred together with an inhibition of systemic and local Th2 responses (Fig. 2). CD47-Fc administration resulted in a drop in serum OVA-specific IgE (Fig. 2A) and inhibited IL-4 and IL-5 release by OVA-re-stimulated mLN cells (Fig. 2B). The production of IL-13 as well as the proportion of CD4⁺ T cells secreting IL-13 was significantly suppressed in CD47-Fc-treated mice (Fig. 2B and C), whereas the compound did not promote Th1 (IFN- γ) (Fig. 2D) and Tr1 (IL-10) responses (data not shown). The frequency of Foxp3⁺ regulatory T cells was comparable in untreated and CD47-Fc-treated mice (Fig. 2E). These data indicate that the early *in vivo* administration of CD47-Fc prevents the late Th2-associated airway inflammation in sensitized and challenged BALB/c mice without deviating the immune response towards Th1 or inducing regulatory T cells.

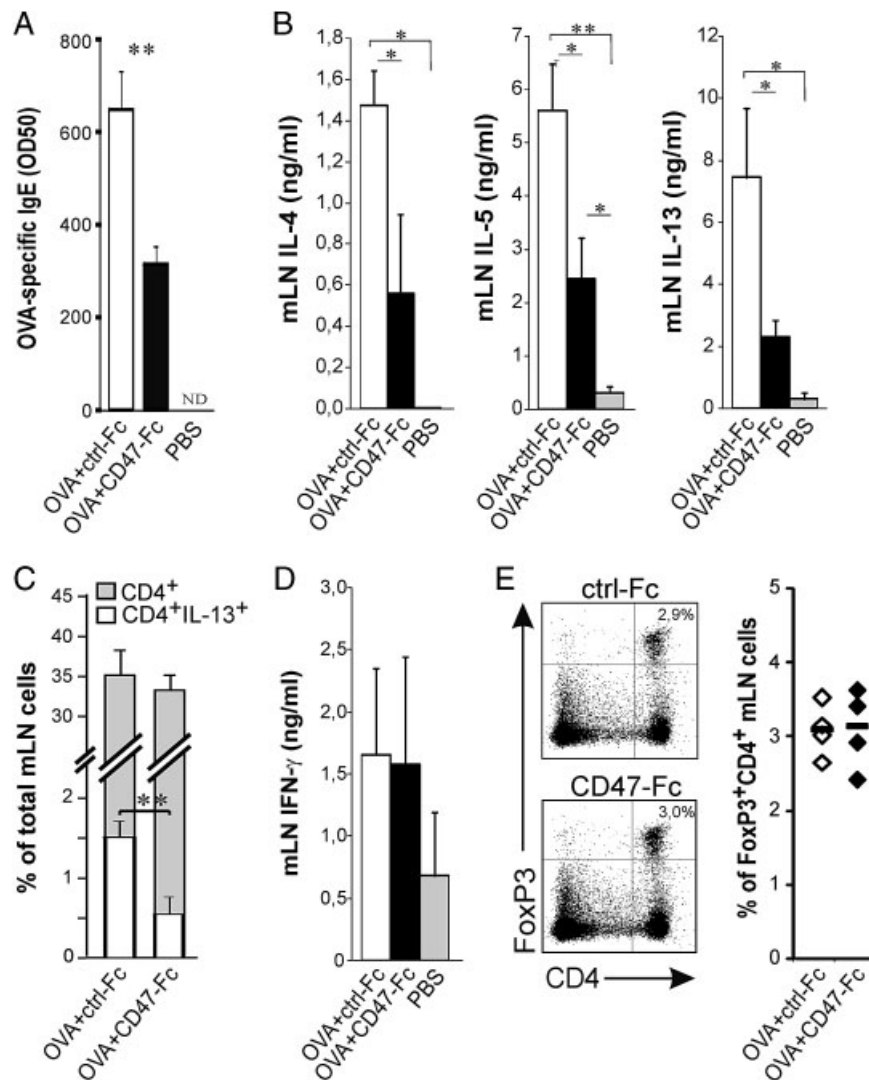


Figure 2. CD47-Fc delivered at priming suppresses late Th2 responses *in vivo*. BALB/c mice were injected i.p. on days 0 and 5 with PBS or OVA/Alum in the presence of CD47-Fc or ctrl-Fc and OVA-aerosol challenged as in Fig. 1. (A) Serum level of OVA-specific IgE at day 21. (B) IL-4, IL-5 and IL-13 release in the supernatants of OVA-stimulated mLN cells. (C) Proportions of CD4⁺ T cells and IL-13-producing CD4⁺ T cells in mLN. (D) IFN- γ release in the supernatants of OVA-stimulated mLN cells. (E) Proportion of Foxp3⁺CD4⁺ T cells in total mLN. Data show mean $\pm$ SEM ($n = 4$ –8 mice per group) of three independent experiments. ND; non detectable. * $p < 0.05$, ** $p < 0.01$ (U-test)

Th2-cell priming and effector functions are not impaired by CD47-Fc *in vitro*

We first explored *in vitro* the potential mechanisms behind the CD47-Fc inhibitory effect of the Th2-responses seen in sensitized and challenged BALB/c mice. DC drive Th2-cell differentiation and amplify Th2 responses. We thus postulated that CD47-Fc suppressed DC/naïve CD4⁺ T-cell priming and/or DC stimulation of a new round of division of circulating memory CD4⁺ T cells. To test this hypothesis, OVA-loaded SIRP- α^+ BM-derived DC (BMDC) were co-cultured with purified naïve CD4⁺ Tg T cells under Th2 conditions in the presence of CD47-Fc or ctrl-Fc. BMDC were more than 98% CD11c⁺CD11b⁺CD103[−] SIRP- α^+ [8]. We found that CD47-Fc added to the primary culture did not prevent Ag-specific T-cell proliferation as assessed by CFSE cell dilution (Fig. 3A) and Th2 (IL-4 and IL-13) cell differentiation (Fig. 3B). Polarized Th2 Tg T cells were next co-cultured with OVA-BMDC in the presence of CD47-Fc or ctrl-Fc. CD47-Fc added during the secondary culture did not modify Th2 cytokine expression (Fig. 3C). These data suggest that the inhibition of Th2 responses induced by CD47-Fc *in vivo* is likely not attributed to the ability of CD47-Fc to alter DC/T interactions, Th2 polarization and Th2-cell effector function.

CD47-Fc treatment alters SIRP- α^+ DC migration *in vivo*

Migration of airway SIRP- α^+ DC to the mLN is essential for the priming of naïve T cells and the initiation of Th2 responses [8]. We next examined how the early CD47-Fc administration affected the late accumulation of CD11c^{high}CD11b⁺ MHC class II^{high} DC (mDC) and pDC in the mLN of sensitized and challenged mice. We found a reduction in the mDC/pDC ratio (Fig. 4A), which correlated with a selective decrease in the accumulation of

SIRP- α^+ DC in the mLN of CD47-Fc-treated mice (Fig. 4B). CD47-Fc treatment did not modify the proportion of pDC (data not shown). To support the hypothesis that altered DC trafficking to mLN is mechanistically linked to a reduced Th cell priming, BALB/c mice were passively transferred with CFSE-labeled CD4⁺ Tg T cells 1 day prior OVA-alum i.p. immunization together with CD47-Fc. CFSE cell dilution was examined in mLN after 2 days (Fig. 4C). At this early time point, no T-cell proliferation was observed in non-draining LN (*i.e.* inguinal LN) [23]. The administration of CD47-Fc resulted in a decrease in the proportion of CFSE-labeled Tg T cells among CD4⁺ T cells without reducing the frequency of total CD4⁺ T cells in the mLN of OVA-immunized mice.

We next examined whether CD47-Fc delivers a signal to SIRP- α^+ DC to alter their functional properties, which include Th-cell stimulatory capacity *in vitro* and *in vivo*. To this end, BMDC were loaded with OVA protein in the presence of CD47-Fc or Ctrl-Fc and then co-cultured with naïve CD4⁺ Tg T cells or established Th2 cells (Fig 5A and B). Alternatively, OVA-loaded BMDC were administered i.p., in the absence of adjuvant, to BALB/c mice passively transferred with CFSE-labeled CD4⁺ Tg T cells and cell division was examined 2 days later (Fig 5C, andD). Ligation of SIRP- α on BMDC did not preclude *in vitro* Ag uptake and presentation, Th2 priming and re-stimulation since CD47-Fc and Ctrl-Fc-pretreated OVA-BMDC induced similar Tg T-cell proliferation (Fig. 5A) and Th2 cytokine expression in primary and secondary cultures (Fig. 5B). In contrast, we found a four- to fivefold reduction in the frequency and absolute numbers of proliferating Tg T cells in the mLN of mice that were injected with CD47-Fc-pre-treated OVA-BMDC when compared with mice administered with Ctrl-Fc-pre-treated OVA-BMDC (Fig 5C and D). The mLN cellularity was not significantly altered. Of note, CD47-Fc pretreatment did not affect BMDC cellular viability. CD47-Fc and Ctrl-Fc-pre-treated OVA-BMDC expressed

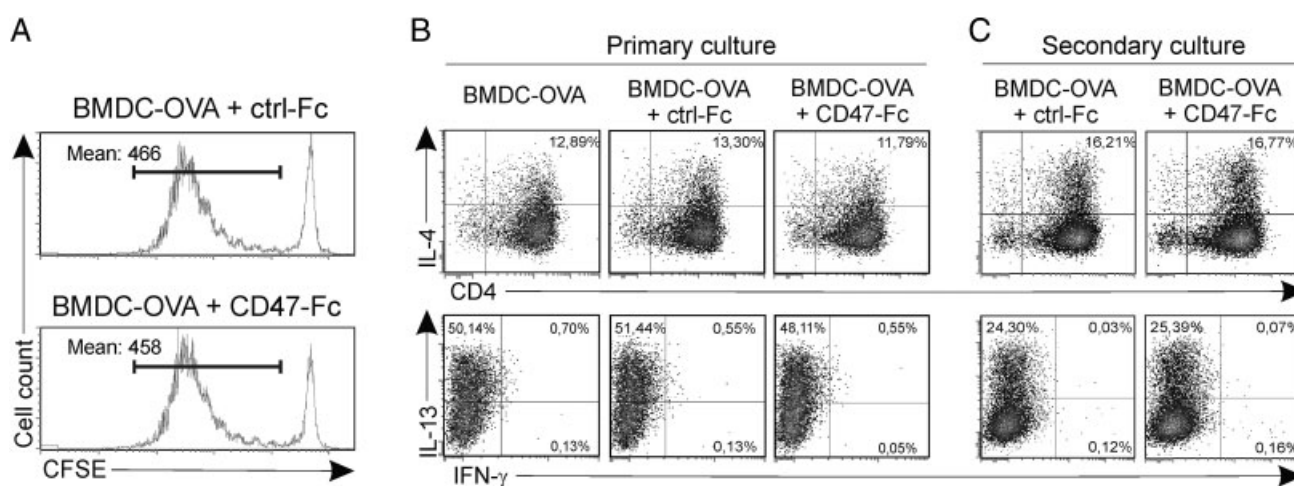


Figure 3. CD47-Fc does not impair Th2 priming and effector function *in vitro*. (A and B) OVA-loaded BMDC were co-cultured with purified naïve CD4⁺ KJ126⁺ T cells under Th2 polarizing conditions for 5 days in the absence or presence of CD47-Fc or ctrl-Fc (primary culture). (A) T-cell proliferation measured by CFSE dilution. (B) IL-4, IL-13 and IFN- γ expression gated on CD4⁺ T cells after re-stimulation with PMA/ionomycin. (C) OVA-loaded BMDC were co-cultured with Th2 polarized cells for 5 days in the absence or presence of CD47-Fc or ctrl-Fc (secondary culture). IL-4, IL-13 and IFN- γ expression is after gating on CD4⁺ T cells. Dot plots are one representative of three independent experiments.

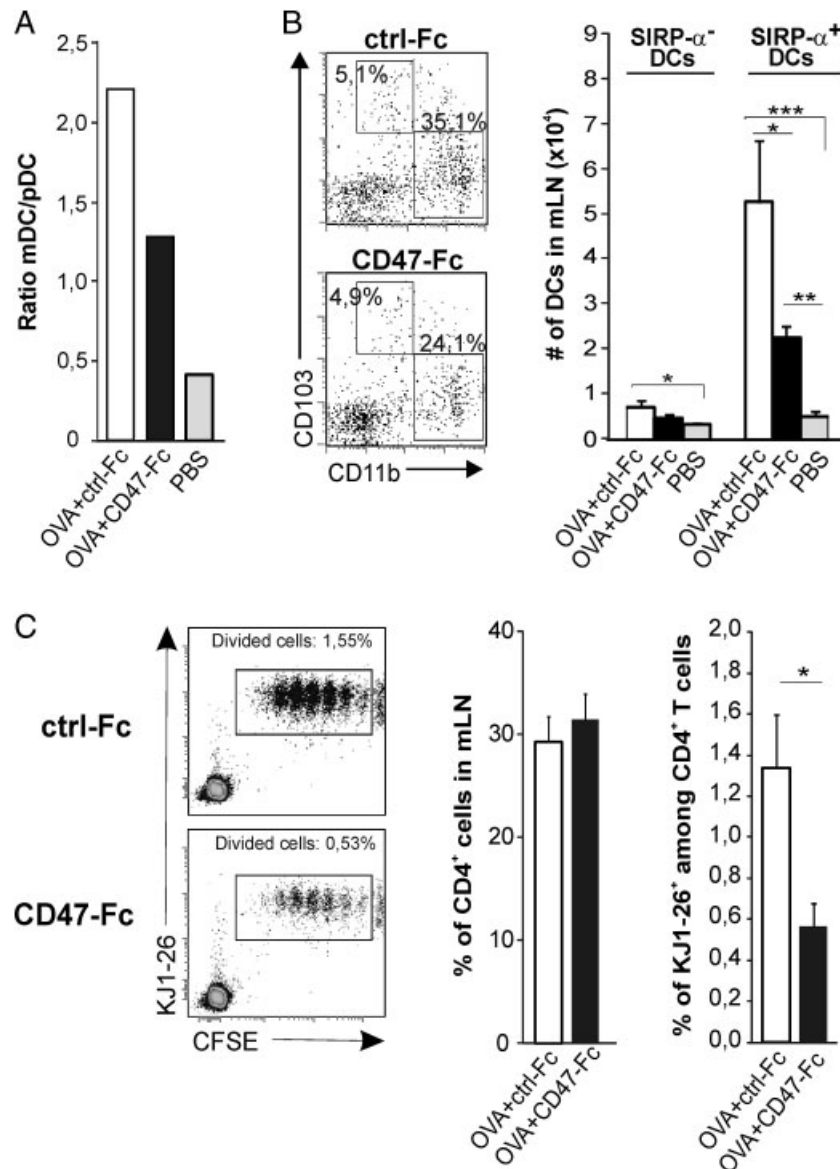


Figure 4. CD47-Fc alters SIRP α ⁺ DC migration to mLN and Th cell priming *in vivo*. (A and B) BALB/c mice were injected i.p. on days 0 and 5 with PBS (grey bars) or OVA/Alum in the presence of CD47-Fc (black bars) or ctrl-Fc (open bars) and OVA-aerosol challenged as in Fig. 1. MLN were examined for (A) the proportion of CD11c^{high}I-A⁺ (mDC) and CD11c^{low}B220⁺120G8⁺ (pDC), expressed as mDC/pDC ratio. (B) Frequency and absolute numbers of DC subpopulations, gated on CD11c⁺ cells. Mean $\pm$ SEM ($n = 4$ –6 mice per group) of two independent experiments. (C) BALB/c mice were passively transferred with CFSE-labeled CD4⁺Tg T cells a day prior i.p. OVA-alum immunization in the presence of CD47-Fc or ctrl-Fc. CFSE cell dilution was examined in mLN after 2 days (left panel). Frequency of CD4⁺ T cells and KJ126⁺ T cells among CD4⁺ T cells (middle and right panels). Mean $\pm$ SEM ($n = 9$ –11 mice per group) of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (B; U-test, C; t-test).

similar quantities of CD86, MHC Class II and CCR7 (data not shown). These data indicate that SIRP- α ligation on BMDC impairs, by a yet unknown mechanism, SIRP- α ⁺ DC trafficking and thus Th priming in the mLN.

Taken all, these data suggest that the altered homing of SIRP- α ⁺ DC to mLN accounts for the defective Th2-cell priming, the altered recruitment of innate and effector T cells to lung tissues, which both contribute to the decreased local and systemic Th2 responses seen in CD47-Fc-treated mice.

Discussion

Our current findings provide evidence that CD47 and SIRP- α are critically involved in the induction of type 2-mediated chronic allergic inflammation and that the CD47/SIRP- α axis can be harnessed by CD47-Fc to suppress Th2 responses. We demonstrate that the early CD47-Fc administration suppresses the late local and systemic Th2 responses and protects mice from developing airway inflammation. The following observations

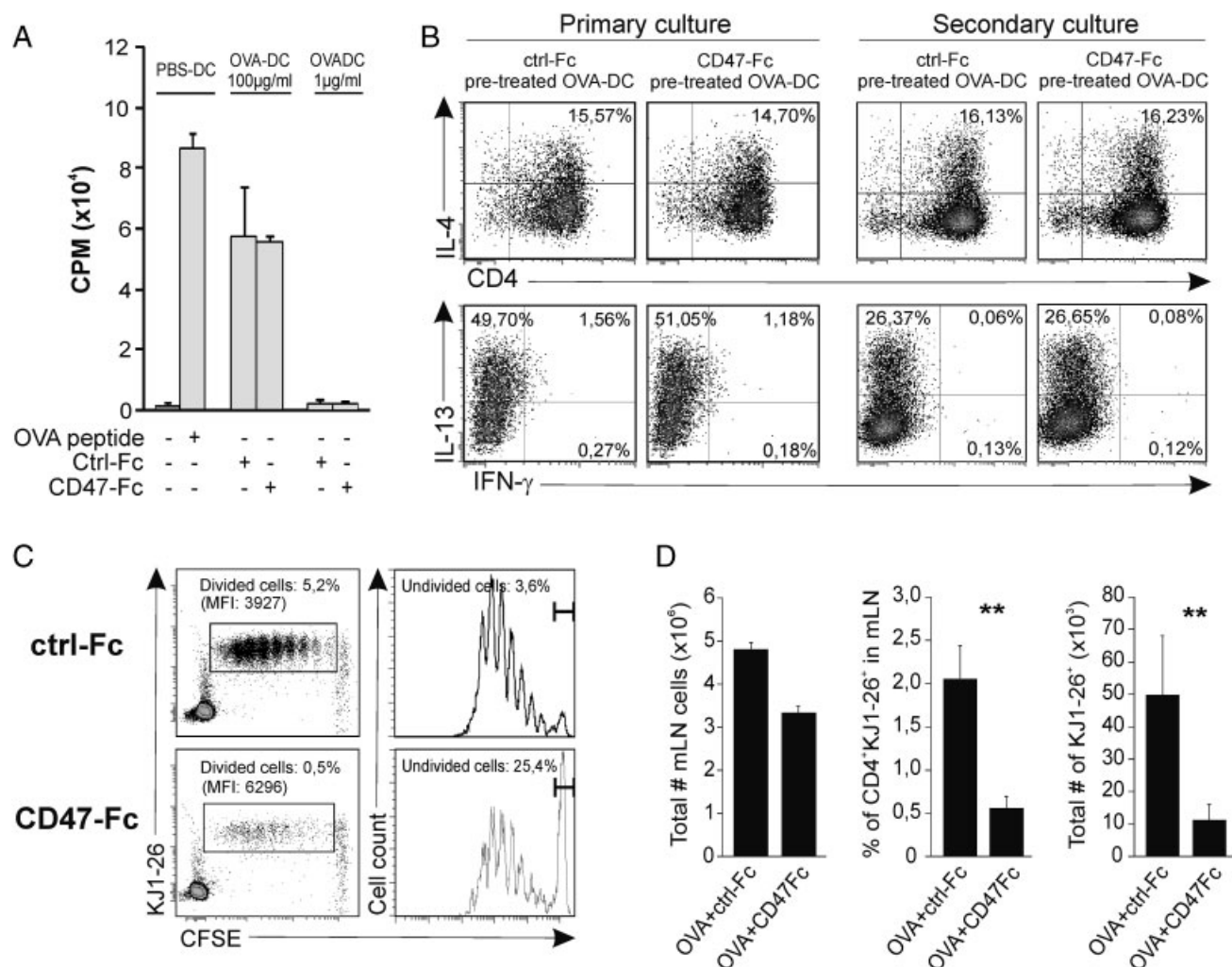


Figure 5. SIRP- α ligation on BMDC *in vitro* impairs Th cell priming *in vivo*. (A and B) BMDC were loaded with 100 or 1 μ g/mL OVA and pre-treated with CD47-Fc or ctrl-Fc and co-cultured for 5 days with either purified naive CD4⁺KJ126⁺ T cells under Th2 polarizing conditions (primary culture) or Th2 polarized cells (secondary culture). (A) Proliferation measured by thymidine incorporation (CPM) at day 4. PBS-loaded BMDC alone were used as negative control and as positive control in the presence of OVA-peptide. Mean $\pm$ SD of one representative out of two independent experiments (B) IL-4, IL-13 and IFN- γ expression gated on CD4⁺ T cells after PMA/iono re-stimulation. Dot plots are one representative of three independent experiments. (C and D) BALB/c mice were passively transferred with CFSE-labeled CD4⁺ Tg T cells a day prior i.p. injection of CD47-Fc or Ctrl-Fc-pre-treated OVA-loaded BMDC. (C) CFSE cell dilution was examined in mLN after 2 days. (D) LN cellularity, frequency and absolute numbers of CD4⁺ KJ126⁺ T cells. Mean $\pm$ SEM ($n = 6$ –9 mice per group) of three independent experiments. ** $p < 0.01$ (t-test).

pinpoint towards an impairment of SIRP- α ⁺ DC recruitment to mLN as one mechanism implicated in the *in vivo* inhibition of Th priming and suppression of the Th2 responses seen in CD47-Fc treated mice. First, the reduction in Th2 cytokine expression is linked to a selective decrease in the accumulation of SIRP- α ⁺ DC in the mLN in sensitized and challenged CD47-Fc-treated mice. Second, *in vivo* Ag-specific Tg T-cell proliferation is reduced in mice that have been injected i.p. with OVA-Alum together with CD47-Fc as well as in mice injected i.p. with CD47-Fc-pretreated BMDC. In contrast, CD47-Fc did not impair *in vitro* functional BMDC properties that include Ag presentation, DC/T interactions, Th2 polarization and Th2 effector function.

The current findings dovetail the concept that enhanced DC migration regulates LN vascular growth and Ag-specific T-cell responses [24–26]. Hence, a twofold decrease in the number of

activated BMDC that were injected subcutaneously and migrated to the LN results in up to fourfold decrease in the number of activated T-cell blasts recovered from the draining LN [25]. Therefore, a twofold reduction in SIRP- α ⁺ DC numbers seen in the mLN of CD47-Fc-treated mice (Fig. 4B) may account for the defect in Th priming (Fig. 4C) and Th2 responses (Fig. 2).

The decrease in the accumulation of SIRP- α ⁺ CD103⁺ but not SIRP- α ⁺ CD103⁺ DC in the mLN of CD47-Fc-treated mice extends on previous observations that the CD47/SIRP- α axis controls the mobilization of SIRP- α ⁺ myeloid cells, which is defective in CD47-deficient mice. CD47-mediated regulation of innate cell trafficking includes neutrophils, epidermal DC, dermal DC, marginal zone splenic DC, airway and intestinal pathogenic CD103⁺ SIRP- α ⁺ DC [8, 21, 27–29]. Nonetheless, the precise mechanisms whereby CD47/SIRP- α interactions regulate the

function of innate SIRP- α ⁺ cells and/or transendothelial trafficking remain to be further investigated. Since SIRP- α is expressed on myeloid DC and endothelial cells, a signal delivered to SIRP- α as well as CD47/SIRP- α blockade represent two non-mutually exclusive pathways to interfere with the development and maintenance of airway inflammation.

The combined results of our *in vitro* and *in vivo* studies concurred with the hypothesis that CD47-Fc diminishes airway DC trafficking without altering Ag-presenting function, which results in impaired Th2 priming. Recent reports demonstrated that basophils are early producers of IL-4 that migrate to mLN whereby they may trump DC in their Ag-presenting function and ability to initiate naïve T-cell differentiation towards Th2 [30]. However, a cooperation between DC and basophils appears to be required to drive Th2 responses [31]. In our experimental model, we failed to detect basophils in mLN, either at an early time point after immunization (day 3, i.p. OVA/Alum) or in mice with established disease (day 21, immunized and challenged) in both untreated and CD47-Fc-treated mice.

Th2 cytokines (IL-4 and IL-13) produced by effector T cells are required and intimately linked to the induction of IgE and IgG1 antibody responses *in vivo* [32], while they are dispensable to the initiation of airway hyperreactivity and the recruitment of the first wave of innate cells to the lungs. This may underscore the decrease in systemic (OVA-specific IgE) and local Th2 responses seen in the mLN of CD47-Fc-treated and protected BALB/c mice. We also found that CD47-Fc treatment reduced IL-13 and eotaxin release in lungs, eosinophils and lymphocyte cell numbers in BALF and thus airway inflammation. In fact, IL-13/IL-13R α 1 interactions lead to eotaxin generation, increased airway resistance and mucus production, the key pathogenic components associated with asthma severity [33]. Furthermore, immediately after allergen challenge, eotaxin recruits eosinophils that attract Th2 effectors and promote their lung accumulation [34].

Collectively, we demonstrate that the manipulation of the CD47/SIRP- α pathway by CD47-Fc suppresses Th2 responses and airway inflammation at least by impairing the migration of pathogenic SIRP- α ⁺DC to the mLN. We propose that targeting the CD47/SIRP- α axis may offer previously unknown therapeutic perspectives for allergic asthma.

Materials and methods

Mice

Female BALB/c and DO11.10 TCR Tg mice (6–8 wk old) were purchased from Charles River and Jackson Laboratory, respectively, and maintained under specific pathogen-free conditions. All experiments were approved by the Ethics committee of CRCHUM and met the standards required by the Canadian Council on Animal Care.

A mouse model of asthma by systemic i.p. immunization

BALB/c mice were sensitized on days 0 and 5 by i.p. injection of 10 μ g OVA adsorbed to 1 mg Imject Alum (Pierce) in the absence or presence of 100 μ g of murine CD47-Fc (a fusion protein constructed with murine CD47 extracellular domain plus a mutated human IgG1-Fc with low FcR binding activity) or Ctrl-Fc molecules [21]. On days 12, 16 and 20, mice were challenged for 30 min with a 0.5% OVA aerosol (Sigma, Grade V). Mice were sacrificed 24 h after the last challenge.

Serum Ig measurement

Serum was isolated from whole blood on day 21 and OVA-specific IgE levels were determined by ELISA technique.

Airway histology

After BAL was performed, 1 mL Optimal Cutting Temperature (OCT) compound was injected into the lungs through the lavage canula. Lungs were frozen in OCT compound and 5 μ m sections were prepared for H&E or PAS staining.

Preparation of BMDC and DC/T-cell cocultures

As previously reported [8], SIRP- α ⁺BMDC were obtained from femur and tibia BM cells suspension and cultured for 13 days in culture medium supplemented with 5% fetal bovine serum and 20 ng/mL GM-CSF (PeproTech, USA). The medium was refreshed every 3 days and cells were pulsed on day 12 with 100 or 1 μ g/mL OVA (Grade V; Sigma). PBS-loaded-BMDC were used as negative control. OVA-loaded BMDC were co-cultured, in presence or absence of 10 μ g/mL of CD47-Fc or ctrl-Fc, either with purified naïve CD4⁺ Tg T cells (EasySep (StemCell)) under Th2 polarizing conditions (IL-4 (40 ng/mL) and anti-IFN- γ (10 μ g/mL)) (primary culture) or with Th2-polarized cells (secondary culture). As a positive control, PBS-loaded BMDC were cocultured with T cells in the presence of OVA peptide (0.4 μ g/mL, Peptides International). After 5 days, T cells were re-stimulated with phorbol 12-myristate 13-acetate (5 ng/mL) and ionomycin (500 ng/mL) for 5 h and stained for intracytoplasmic cytokine expression. For some experiments, BMDC were pre-treated with 10 μ g/mL of CD47-Fc or ctrl-Fc during the last 24 h of culture with OVA protein and co-cultured with naïve CD4⁺ Tg T cells or Th2 polarized cells. All cocultures were performed in 96-well plates in RPMI1640 (Wisent) supplemented with 10% fetal bovine serum, 500 U/mL Penicillin, 500 μ g/mL Streptomycin, 10 mM HEPES buffer and 1 mM 2-ME.

Proliferation measurement

To assess T-cell proliferation 1 μ Ci 3[H] thymidine (Amersham Biosciences, UK) was added during the last 16 h of a 4-day culture. Triplicate culture cells were harvested into glass fiber filters and the radioactivity was counted using liquid scintillation. Data are expressed as count *per* minute. In some experiments, CFSE-labeled naïve CD4⁺Tg T cells were used for co-cultures and cell proliferation was detected by flow cytometry by monitoring CFSE dilution.

Adoptive transfer experiments

BALB/c mice were passively transferred with 2×10^6 CFSE-labeled-CD4⁺ Tg T cells 1 day prior to immunization. Mice were immunized i.p. either with OVA/Alum together with CD47-Fc or ctrl-Fc or with 1×10^6 OVA-loaded BMDC pre-treated with CD47-Fc or ctrl-Fc. Two days after immunization, mLN and inguinal LN were collected and analyzed for CFSE cell dilution. Cells were stained with anti-clonotypic KJ126mAb (BD Biosciences) in combination with anti-CD4 (BD Biosciences) to retrace CFSE-labeled CD4⁺ KJ126⁺ Tg T cells. Data were acquired with a FACSAriaII (BD Biosciences).

Flow cytometry

Total BAL cells were stained with anti-CCR3, anti-B220 (R&D systems) and anti-CD3 (clone 145-2C11) and analyzed by flow cytometry according to van Rijt *et al.* [35] method, as shown in Supporting Information Fig. 1. For DC analysis, mLN were treated with Liberase at 0.4 mg/mL (Roche) before staining. Autofluorescent alveolar macrophages were excluded from the analysis gates using an empty fluorescent channel. The following mAb were used for cell phenotype analysis: Anti-CD4, anti-CD11b, anti-CD103, anti-SIRP- α , anti-I-Ad/I-Ed, anti-DO11.10 TCR, 120G8 (BD Biosciences), anti-CD11c and anti-B220 (R&D systems). Gating strategy to discriminate DC subpopulations was defined as described in [8]. For intracellular staining, cells were fixed, permeabilized and stained with anti-IL-4, anti-IL-13 anti-IFN- γ and anti-Foxp3 (eBiosciences) after surface staining with anti-CD4. All the data were acquired on an FACSAria II (BD Biosciences).

Cytokine and chemokine measurement

Lungs were rinsed in PBS supplemented with antibiotics, cut into small pieces and cultured in flat-bottom 24-well plates for 24 h in 1 mL RPMI1640 (Wisent) supplemented with 10% fetal bovine serum, 500 U/mL Penicillin, 500 μ g/mL Streptomycin, 10 mM HEPES buffer and 1 mM 2-ME. MLN cells (4×10^6 cells/mL) were cultured in flat-bottom 96-well plates and re-stimulated with OVA (100 μ g/mL) for 72 h. IL-4, IL-5, IFN- γ (BD Biosciences), IL-13

and CCL11/eotaxin (R&D Systems) were measured by ELISA techniques in culture supernatants of lung explants and OVA-stimulated mLN.

Statistical analysis

Statistical analyses were performed using unpaired Student's *t*-test and the non-parametric Mann–Whitney *U*-test. ****p*<0.001, ***p*<0.01, **p*<0.05.

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Conflict of interest: The authors declare no financial or commercial conflict of interest.

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Abbreviations: BMDC: BM-derived DC · ctrl-Fc: control human CD47-Fc · i.t.: intratracheal(ly) · mLN: mediastinal LN · PAS: periodic acid-Schiff · pDC: plasmacytoid DC · SIRP- α : signal regulatory protein α

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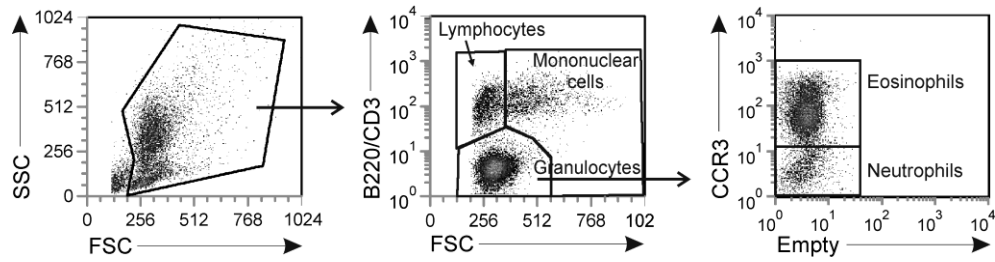
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Targeting SIRP- α protects from type 2-driven allergic airway inflammation

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Supplementary Figure 1. Differential cell count in BALF using flow cytometry.

BALF cells were stained with anti-CCR3, anti-B220 and anti-CD3 and analyzed by flow cytometry. After gating on live cells (left panel), granulocytes, lymphocytes and other mononuclear cells were detected using FSC and B220/CD3 staining (middle panel). After gating on granulocytes, eosinophils were distinguished from neutrophils by CCR3 expression (right panel). Shown is one representative of 3 independent experiments.