IL-28A(IFN-\(\lambda\)2) modulates lung DC function to promote Th1 immune skewing and suppress allergic airway disease

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Running title: IL-28 promotes Th1 skewing and suppresses asthma
SUPPLEMENTARY MATERIALS AND METHODS

Cytokines and antibodies
Purified recombinant anti-CD3 (BD PharMingen, Heidelberg, Germany) and anti-
CD28 (BD PharMingen, Heidelberg, Germany) antibodies were used for cell culture.
Recombinant IL-28 (IFN-λ) was purchased from Peprotech (Rocky Hill, NJ) or
eBioscience (San Diego, CA). LPS was from Sigma. Neutralizing anti-IL-12 p40
antibody (Clone C17.8) and its isotype control (Clone C1.18.4) were purchased from
Bio-X-Cell (West Lebanon, USA). For the analysis of murine primary cells from the
lung the following fluorochrome-labelled antibodies for FACS analysis and sorting
FITC-anti-mouse MHC class II (clone M5/114.15.2), PE-Cy5-anti-mouse CD11b
(clone M1/70), PE-Cy7-anti-mouse CD11c (clone HL3), PE-anti-mouse OX40L
(clone RM134L) and FITC-anti-mouse CD4 (clone L3T4) were purchased from
eBioscience (San Diego, CA).

Recombinant adenoviruses
Recombinant replication-deficient E1/E3-deleted adenovirus expressing IL-28A
(AdIL-28) and mock control adenovirus (Ad0) were constructed using the Gateway
system (Invitrogen, Carlsbad, CA). The IL-28A cDNA was kindly provided by T.
Murakami. Viruses were propagated and titrated with the Adeno-X-Titer kit
(Clontech, Mountain View, CA).

Allergen sensitization and challenge protocol and treatment regimens
Wild-type mice and IL-28Rα−/− deficient mice were on a C57BL/6J genetic
background and maintained as previously described (Hausding et al., 2004). IFNγ−/−
mice on a C57BL/6J background were purchased from the Jackson Laboratories. IL-28Rα−/− knockout mice were generated by homologous recombination technology and screened as described elsewhere (Ank et al., 2008). In some experiments, as indicated, BALB/cJ mice were also used. All mice were kept in a specific-pathogen free (SPF) facility according to FELASA recommendations. Unless indicated otherwise in Results, six to eight week old mice were sensitized with two intraperitoneal injections on days 0 and 7 of 7.5 μg OVA (Grade V; Sigma) complexed with aluminum hydroxide (alum) as adjuvant (Sigma, Deisenhofen, Germany). On days 14, 15, 16, mice received aerosol challenge with 5% w/v OVA in PBS (OVA/OVA) for 30 min per day. Control mice received intraperitoneal injections and challenges of PBS alone (PBS/PBS). In some cases, mice were treated with 1 μg/mouse recombinant IL-28A concurrently with OVA challenges or 5×10^8 i.u. of an adenovirus expressing IL-28A (AdIL-28) and its mock control (Ad0) one day before allergen challenge as indicated in the experimental protocol. For IL-12 p40 neutralization experiments, 300 μg/mouse of anti-IL-12p40 antibody or its isotype control were administered i.p. just before each treatment with recombinant IL-28. One day after the last challenge mice were examined for airway hyperresponsiveness, bronchoalveolar lavage performed and tissues (mediastinal lymph nodes, spleen and lung) collected for further analysis. All procedures had received prior approval from the Institutions and Regional Ethical Review Boards and were in accordance with the US National Institutes of Health Statement of Compliance (Assurance) with Standards for Humane Care and Use of Laboratory Animals (#A5736–01) and with the European Union Directive 86/609/EEC for animal research.
Airway hyperresponsiveness

Airway hyperresponsiveness was measured in anesthetized mechanically ventilated mice (flexiVent, SciReq, Montreal, Canada) 24 hours after the last aerosol exposure as previously described (Xirakia et al., 2010). Aerosolized metacholine was delivered in-line through the inhalation port for 10 sec. Lung resistance was determined as the highest of twelve measurements obtained for each concentration of metacholine and is reported as the percent increase from baseline. In BALB/c mice, airway hyperresponsiveness was measured invasively using a body plethysmograph (Buxco Electronics, Inc., Wilmington, NC) as previously reported (Hausding et al., 2007).

Collection and analysis of the BALF

Twenty-four hours after the last aerosol challenge with either OVA or saline (PBS), BALF of the whole lung was performed with 0.5 ml saline for two times. BALF was collected and an aliquot was stained with trypan blue solution and cells counted for viability determination using a Neubauer chamber. Samples were centrifuged at 1500 rpm for 5 min, supernatants kept for ELISA and cell pellets subjected to cytopsin centrifugation at 500 rpm for 5 min. Cytospins were stained with May-Grumwald-Giemsa and analyzed by differential cell counting (Xirakia et al., 2010). At all cases, <5x10^3 eosinophils or neutrophils were detectable is BALF from PBS/PBS control mice. For the analysis of IL-28 levels, BALF was concentrated 10X using Amicon Ultra-15 centrifugal filter columns (Millipore, USA) with a molecular weight cutoff of 10 KDa according to the manufacturer's instructions.

Histological analysis

Lung tissue was fixed in 10% (vol/vol) neutral buffered formalin and embedded in
paraffin. Paraffin-embedded tissue slices were stained with hematoxylin/eosin (H&E) or periodic acid–Schiff’s (PAS) solution (Sigma, Deisenhofen, Germany). Histopathologic analysis of inflammatory cells in H&E stained lung sections from at least six mice was performed in a blinded fashion using a semi-quantitative scoring system as previously described (Xirakia et al., 2010). Both peribronchiolar and perivascular inflammation were scored giving a maximum score of 8 as follows: 0, normal; 1, few cells; 2, a ring of inflammatory cells one cell layer deep; 3, a ring of inflammatory cells two to four cells deep; and 4, a ring of inflammatory cells of more than four cells deep. Histological score for PBS/PBS control mouse lungs was always 0. Morphometric analysis of PAS stained sections was performed by quantifying PAS pixels per μm length distance of bronchial epithelium of central airways using the Image J software. At least six areas from similar sections per mouse and at least six mice were assessed blindly. PAS score for PBS/PBS control mouse lungs was constantly <2.

**Immunohistochemistry**

Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin and cut into 5 μm thick sections. Sections were mounted on adhesive-coated slides, deparaffinized, and rehydrated through xylene and ethanol. For antigen retrieval, tissues were incubated in sodium citrate buffer (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0) for 15 minutes at 80°C, rinsed with PBS and endogenous peroxidase activity blocked with 1.5% H2O2 in methanol for 30 minutes. Sections were blocked with 5% rabbit serum PBS for 30 min, washed with PBS and incubated with a goat anti-mouse IL-28Rα antibody (raised against amino acids 44-72, Abcam, Cambridge, UK) diluted 1:300 in 2% rabbit serum PBS overnight. Sections were then washed
with PBS, incubated with an HRP conjugated rabbit anti-goat antibody (Dako, Hamburg, Germany) diluted 1:200 in 2% rabbit serum PBS for 1h and color was developed with diaminobenzidine tetrahydrochloride as a substrate (DAB substrate kit; Vector Laboratories Inc., Burlingame, CA) according to the manufacturer’s instructions, and counterstained with Mayer’s hematoxylin.

**Primary T cell responses**

IL-28Rα−/− and wild-type mice were immunized with 7.5 µg OVA complexed in alum or emulsified in complete Freud’s adjuvant (CFA). Alternatively, wild-type mice received 5x10⁸ i.u. of AdIL-28, Ad0 or vehicle control (PBS) one day before immunization. Six days after immunization, spleen was collected, single cell suspensions prepared and cells cultured at 5x10⁵/well in 96-well plates in the presence or absence of various concentrations of ovalbumin or 10 µg/ml of OT-II or OT-I peptides. After 72h supernatants were collected and examined by ELISA for the presence of Th1, Th2 and Th17 cytokines. Background levels of cytokine production from PBS-immunized mice were always below 20 pg/ml for IL-4, IL-5, IL-10, IL-13, IL-17 or IFNγ.

**Secondary T cell responses**

Mediastinal lymph nodes were removed, single cell suspensions prepared and cells cultured at 5x10⁵/well in 96-well plates in the presence or absence of 100 µg/ml ovalbumin. After 48h, supernatants were collected and examined by ELISA for the presence of Th1, Th2 and Th17 cytokines. Background levels of cytokine production from PBS/PBS mice were always below 20 pg/ml for IL-4, IL-5, IL-10, IL-13, IL-17 or IFNγ.
**Lung CD11c⁺ and CD4⁺ T cell isolation and culture**

Murine CD11c⁺ cells were isolated by using immunomagnetic separation (Miltenyi Biotech, Germany) and FACS sorting twenty four hours after the last allergen challenge as previously described (Hausding et al., 2007). Purified lung CD11c⁺ cells (>90% by FACS) were cultured overnight in the presence or absence of LPS (1 µg/ml; Invivogen, San Diego, CA) in RPMI medium containing 5% fetal calf serum and OVA (500 µg/ml; Calbiochem, San Diego, CA). In addition, lung CD4⁺ T cells were purified by using immunomagnetic separation, as previously described (Doganci et al., 2005). Lung CD4⁺ T cells were cultured in the presence or absence of immobilized anti-CD3 (5 µg/ml) and anti-CD28 (1 µg/ml) antibodies overnight. Th1, Th2 and Treg cells were generated after *in vitro* culture of spleen CD4⁺ T cells for 2 weeks with anti-CD3 (1 µg/ml) and anti-CD28 (1 µg/ml) stimulation in the presence of IL-12 (10 ng/ml) plus anti-IL-4 Ab (1 µg/ml) for Th1, IL-4 (10 ng/ml) plus anti-IL-12 Ab (1 µg/ml) for Th2 and IL-2 (100 U/mL) plus TGFβ (1 ng/ml) for Treg polarization.

**FACS analysis**

Whole lung digests stained with antibodies against CD11c, MHC II, CD11b and OX40L and examined using an FC500 flow cytometer (Beckman Coulter, USA). Beads-purified or FACS-sorted CD11c⁺ cells or CD4⁺ T cells were routinely analyzed for purity using FACSCalibur and the Cell Quest Pro version 4.02 software (BD PharMingen, Heidelberg, Germany). For FACS sorting of CD11c⁺ and CD11c⁺MHCI⁺CD11b⁺ cells, a BD FACS Aria sorter was employed. All antibodies used for FACS staining are listed above.
ELISA

Mouse IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17, IFN-\(\gamma\), and serum IgE were detected by using OptEIA\textsuperscript{TM} sandwich ELISA kits from eBioscience (California, USA) or BD PharMingen (Heidelberg, Germany). Mouse IL-28 cytokines were detected by using a Duo set\textsuperscript{TM} sandwich ELISA kit from R&D Systems picking up both IL-28A and IL-28B (Wiesbaden, Germany).

RNA isolation and real time–PCR

Total lung tissue and purified lung cells were homogenized without chilling and RNA was then extracted by using the RNA Micro Kit (Qiagen, Hilden, Germany). RNA (1\(\mu\)g) was reverse transcribed using the first strand cDNA synthesis kit for RT-PCR (MBI Fermentas, St. Leon-Rot, Germany). The resulting template-cDNA was amplified by quantitative real-time PCR by using specific primers for the IL-28 receptor \(\alpha\)-chain (QuantiTect RT–PCR Kit, Qiagen, Hilden, Germany) in 20 \(\mu\)l reactions in an iCycler (BioRad, München, Germany). For real-time qRT–PCR (30 min 50°C, 15 min 95°C and 40 cycles of 15 s 94°C, 60 s 60°C) a cyber green technique for DNA labeling was used. For primers detecting T-bet was used a forward primer with the sequence 5’-CCT GGA CCC AAC TGT CAA CT-3’ and a reverse primer with the sequence 5’-AAC TG T GTT CCC GAG GTG TC-3’. For the housekeeping gene hypoxanthine phosphoribosyltransferase 1 (HPRT) following sequences for the primers were used: 5’-GCC CCA AAA TGG TTA AGG TT-3’ for forward and 5’-TTG CGC TCA TCT TAG GCT TT-3’ as reverse primer.
Generation of BMDCs and functional analysis

Bone marrow DCs (BMDCs) were generated as previously described (Xirakia et al., 2010). They were plated at $10^6$ cells/ml, pulsed overnight with $10 \mu g/ml$ OVA, treated for 3h with 100 ng/ml IL-28A (eBioscience) and administered to mice. Seven days later, mice were challenged with inhaled OVA, mediastinal lymph nodes collected and assessed for cytokine production by ELISA.

Adoptive transfer experiments

Naïve mice were treated i.v. with AdIL-28, Ad0 or vehicle control (PBS). One day later, CD11c$^+$ DCs from the spleen were isolated using the MACS CD11c$^+$ positive selection kit (Miltenyi Biotec, Mönchengladbach, Germany). Purity assessed by FACS was routinely 85-90%. CD11c$^+$ DCs were pulsed with $10 \mu g/ml$ ovalbumin for 3h and then administered to mice one day before challenge for three consecutive days with 5% (w/v) OVA for 30 min per day.

ELISA

For cytokine measurements, ELISA kits from eBioscience (California, USA) or BD PharMingen (Heidelberg, Germany) were used.

Acknowledgments

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Figure S1. IL-28A treatment suppresses Th2 and Th17 responses in the lung of OVA-sensitized and challenged mice.

BALB/c mice were sensitized twice with OVA/alum and challenged for three consecutive days with inhaled OVA in the absence (OVA/OVA+PBS) or presence of recombinant IL-28A (OVA/OVA+IL-28A). Control mice were challenged with PBS (PBS/PBS).

A. CD4⁺ T cell responses in the lung of OVA-sensitized and challenged mice treated with IL-28A. Levels of IL-4 and IL-17 of anti-CD3/CD28 stimulated CD4⁺ T cells for 24h are shown. Results are expressed as mean values ± SEM of five mice per group.

B. Western blot analysis of GATA-3 expression in lung digests from IL-28A-treated OVA-sensitized and challenged mice. One representative of three independent experiments is shown. Bands correspond to individual mice.

P< 0.05*

Figure S2. Comparison of remodeling changes in the airways of OVA sensitized and challenged IL-28Rα⁺/⁺ and IL-28Rα⁻/⁻ mice

Wild-type (IL-28Rα⁺/⁺) and IL-28Rα⁻/⁻ C57BL/6 mice were sensitized twice with OVA/alum and challenged for three consecutive days with inhaled OVA (OVA/OVA). Control mice were challenged with PBS (PBS/PBS).

A. CD4⁺ T cell responses in the lung of OVA-sensitized and challenged IL28Rα⁺/⁺ and IL28Rα⁻/⁻ mice. CD4⁺ T cells isolated from lung digests were stimulated with anti-CD3/CD28 and supernatants examined by ELISA. Levels of IL-4, IL-5, IL-13...
and IL-17 expressed as mean values ± SEM of five mice per group of one representative experiment are shown.

**B.** Percentage of lung CD4+ T cells in total lung digests from naive (PBS/PBS) and OVA-challenged mice (OVA/OVA). Results are expressed as mean values ± SEM of 5 mice per group.

**C.** Western blot analysis of GATA-3 expression in lung digests from OVA-challenged IL28Rα+/+ and IL28Rα−/− mice. GATA-3 protein was significantly increased in OVA-treated IL-28Rα−/− mice as compared to IL28Rα+/+ controls. One representative of three independent experiments is shown. Bands correspond to individual mice.

**D.** Collagen deposition in the airways of OVA sensitized and challenged wild-type (IL-28Rα+/+) and IL-28Rα−/− mice was measured as previously described (Finotto et al., 2002). Lung sections were stained with Goldner staining and analyzed by using a Axioobserver. Results are expressed as mean collagen layer thickness ± SEM of four mice per group.

P< 0.05*; P< 0.001***; ns: non-significant

**Figure S3. IL-28Rα−/− mice exhibit augmented Th2 responses after primary immunization with OVA emulsified in complete Freud’s adjuvant**

**A.** IL-28Rα−/− mice develop augmented Th2 responses and reduced Th1 responses upon primary immunization with OVA/CFA. Levels of IL-5 and IFNγ in supernatants of OVA-stimulated splenocyte cultures expressed as mean values ± SEM of 5 mice per group are shown. One representative of two independent experiments.

**B.** IFNγ production from CD4+ and CD8+ T cells of OVA/CFA immunized IL28Rα+/+ and IL28Rα−/− mice. Levels of IFNγ in supernatants of OT-II and OT-I-
stimulated splenocyte cultures expressed as mean values ± SEM of 5 mice per group are shown. One representative of two independent experiments.

P< 0.05*; ns: non-significant

Figure S4. IL-28A induces ISG15, OAS1 and T-bet expression in dendritic cells
A. IL-28A mediates signaling in bone marrow DCs from C57BL/6 mice. Real-time PCR results shown as fold induction ± SEM of ISG15 and OAS1 genes using the 2\(^{-\Delta\Delta Ct}\) method. A representative of two independent experiments is shown.
B. T-bet expression of lung CD11c\(^+\) cells from naïve C57BL/6 mice. Purified lung CD11c\(^+\) cells were cultured with LPS (1 µg/ml) in the presence or absence of IL-28A for 24h and T-bet mRNA levels assessed by real-time PCR. Results are expressed as mean fold induction ± SEM relative to T-bet levels in the absence of IL-28A using the 2\(^{-\Delta\Delta Ct}\) method.

P< 0.05*; P< 0.01**; P< 0.001***; ns: non-significant

Figure S5. IL-28A does not directly affect T cell differentiation or function
A. FACS analysis of spleen CD4+ T cells sorted at a purity of 99.9%.
B. Effect of IL-28A treatment on anti-CD3-stimulated CD4+ T cells. Spleen CD4+ T cells were stimulated with anti-CD3 for 48h in the presence or absence of IL-28A and supernatants assessed by ELISA. Levels of IFN-γ and IL-5 expressed as mean values ± SEM are shown. One representative of four independent experiments.
C. Effect of IL-28A treatment on IFN\(_\gamma\), IL-4 and IL-5 production of anti-CD3-stimulated Th0, Th1 and Th2 cells. CD4+ T cells were differentiated for 2 weeks with anti-CD3 (1 µg/ml) and anti-CD28 (1 µg/ml) stimulation in the presence of IL-12 (10 ng/ml) plus anti-IL-4 Ab (1 µg/ml) for Th1 polarization, IL-4 (10 ng/ml) plus anti-IL-
12 Ab (1 µg/ml) for Th2 polarization or the absence of additional factors for Th0 polarization. After resting for 3 days, Th0, Th1 and Th2 cells were stimulated for 24h with anti-CD3 Ab in the presence or absence of IL-28A. Results are expressed as mean values ± SEM. One representative of three independent experiments is shown.

D. Effect of IL-28A treatment during T helper cell differentiation on IFNγ and IL-4 production induced by anti-CD3 stimulation. CD4+ T cells were differentiated to Th0, Th1 or Th2 cells as described above in the presence or absence of IL-28A and then stimulated for 24h with anti-CD3 antibody. Results are expressed as mean values ± SEM. One representative of three independent experiments is shown.

ns: non-significant

Figure S6. IL-28A-treated bone marrow DCs promote Th1 polarization in vivo

A. FACS analysis of bone marrow DCs for MHC class II (I-A/I-E) and costimulatory molecule expression after IL-28A treatment. IL-28A treatment for 24h significantly down-regulated the expression of MHC class II and OX40L in these cells. Results are expressed as mean fluorescence intensity MFI ± SEM from 5 independent experiments.

B. Protocol for immunization of naïve mice with bone marrow DC (BMDC) pulsed with OVA.

C. IL-28A-treated BMDC pulsed with OVA promote Th1 over Th2 polarization in vivo. BMDC were cultured with OVA in the presence (DC/IL-28) or absence (DC/PBS) of IL-28A, or absence of OVA (DC/CTL) for 24h and used to immunize naïve C57BL/6 mice i.p. After three inhaled OVA challenges, mediastinal LNs were collected and T cell responses to OVA assessed by ELISA. Levels of IL-5, IL-10, IL-13, IL-17 and IFNγ expressed as mean ± SEM in supernatants of OVA-stimulated
mediastinal LN cultures are shown. One representative of two independent experiments is shown.

P< 0.05*; P< 0.01**; ns: non-significant

**Figure S7. Adoptive transfer of IL-28A-treated DCs before sensitization inhibits the development of allergic airway disease**

**A.** Experimental protocol used for adoptive transfer of CD11c⁺ DC from IL-28A-treated mice before allergen sensitization. C57BL/6 mice received OVA-pulsed spleen CD11c⁺ DCs from AdIL-28, Ad0 or vehicle control (PBS)-treated mice one day before OVA immunization.

**B.** BALF differential counts for eosinophils expressed as mean ± SEM of 11-14 mice per group pooled from two independent experiments are shown.

**C.** BALF differential counts for neutrophils expressed as mean ± SEM of 11-14 mice per group pooled from two independent experiments are shown.

**D.** Effector T cell responses in the lung-draining mediastinal LNs of OVA sensitized and challenged mice after adoptive transfer of CD11c⁺ DCs before OVA sensitization. Levels of IL-5, IL-13, IL-10 and IFN-γ are expressed as mean values ± SEM in supernatants of OVA-stimulated mediastinal LN cultures of 11-14 mice per group from two independent experiments.

P< 0.05*; P< 0.01**; P< 0.001***; ns: non-significant

**Figure S8. IL-28A treatment inhibits the production of IL-25 and TSLP during the development of allergic airway disease**

C57BL/6 mice were subjected to vehicle (PBS), mock (Ad0) or IL-28A expressing adenovirus (AdIL-28) treatment in the lung and then challenged with aerosolized
OVA (OVA/OVA) or PBS (PBS/PBS). 24h after the last challenge, BALF was collected and IL-13, IL-25, TSLP and IL-33 levels assessed. Results are expressed as mean values ± SEM of 7-9 mice per group. A representative of two independent experiments is shown.

P<0.01**; P<0.001***; ns: non-significant
A. 

<table>
<thead>
<tr>
<th>Condition</th>
<th>IL-4 (ng/ml)</th>
<th>IL-17A (ng/ml)</th>
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<tr>
<td>OVA/OVA</td>
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<td>PBS</td>
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<td>PBS/PBS</td>
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B. 

- 53 kDa GATA-3
- 43 kDa β-actin

Fig. S1
A.

IL-28Rα+/+ IL-28Rα-/-
IL-4 (pg/ml)

IL-28Rα+/+ IL-28Rα-/-
IL-5 (pg/ml)

IL-28Rα+/+ IL-28Rα-/-
IL-13 (ng/ml)

IL-28Rα+/+ IL-28Rα-/-
IL-17A (pg/ml)

B.

Lung CD4+ T cells

IL-28Rα+/+ IL-28Rα-/-
PBS/PBS OVA/OVA

C.

OVA/OVA
GATA-3 β-actin

IL-28Rα+/+ IL-28Rα-/-

D.

IL-28Rα+/+ IL-28Rα-/-

Collagen layer (micrometers)

IL-28Rα+/+ IL-28Rα-/-

Fig. S2
Fig. S3
Fig. S4

A. ISG15 and OAS1 fold induction over time with IL-28 treatment.

B. T-bet mRNA expression with PBS and IL-28A treatments.
A. Pre-sorting

CD4
CD11c

Post-sorting

CD4
CD11c

B. IFN-γ (pg/ml)

No stimulus | Anti-CD3

IL-5 (pg/ml)

No stimulus | Anti-CD3

C. IFN-γ (pg/ml)

No stimulus | Anti-CD3

IL-4 (pg/ml)

No stimulus | Anti-CD3

D. IFN-γ (pg/ml)

No stimulus | Anti-CD3

IL-4 (pg/ml)

No stimulus | Anti-CD3

Fig. S5
A. 

![Graphs showing MFI for LC, CD80, CD86, I-A/I-E, OX40L, and CD40 under different conditions.](image)

B. 

BM-DC treated with IL-28A (100 ng/ml) 

Time (d) 

0 7 8 9 10 

Challenge Ova aerosol 

LN collection, assays

C. 

![Graphs showing cytokine levels (IL-5, IL-10, IL-13, IL-17, IFNγ) under different conditions.](image)

Fig. S6
A. CD11c+ DC from PBS, Ad0, AdIL-28 treated mice

Time (d)
-1 0 7 8 9 10

Sensitization Ova-alum i.p.
Challenge Ova aerosol
BAL, Tissue collection, assays

B.

Eosinophils in BAL x10^5/ml

PBS/Ad0 PBS/AdIL-28 PBS/Ad0 PBS/AdIL-28
PBS/PBS OVA/OVA

C.

Neutrophils in BAL x10^4/ml

PBS/Ad0 PBS/AdIL-28 PBS/Ad0 PBS/AdIL-28
PBS/PBS OVA/OVA

D.

IL-5 (pg/ml)

PBS Ad0 AdIL-28
0 100 200 300

IL-10 (pg/ml)

PBS Ad0 AdIL-28
0 400 800 1200

IL-13 (pg/ml)

PBS Ad0 AdIL-28
0 500 1000 1500

IFN-γ (pg/ml)

PBS Ad0 AdIL-28
0 500 1000 1500

Fig. S7
**Fig. S8**