Pulmonary Epithelial Cell-Derived Cytokine TGF-β1 Is a Critical Cofactor for Enhanced Innate Lymphoid Cell Function

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Figure S1, related to Figure 1. Schematic of the generation of epithelial TGF-β knockout mice and the subsequent expression of Cre and TGF-β1 in the lung. (A) Double transgenic mice CCSP-rtTA/tetO-Cre are crossed with mice expressing homozygous Tgfb1 genes with LoxP sites (Floxed) and treated with doxycycline (DOX) at 6 weeks of age. Upon DOX administration the Tet-on system is activated and cells expressing club cell secretory protein (CCSP) transcribe Cre recombinase which binds LoxP sites and excises exon 6 of the Tgfb1 gene leading to the transcription of truncated and non-functional TGF-β. Littermate control mice lack one transgenic allele and are without a functional Tet-on system so therefore after DOX treatment continue to express TGF-β. (B) Immunohistochemistry images showing Cre expression 48 hr post DOX (or mock) treatment of transgenic mice and littermate controls. (C) Immunohistochemistry images showing TGF-β expression 72 hr post Dox treatment of transgenic mice and littermate controls. (D) mRNA levels of Tgfb1 in sorted club cells (CCSP+CD45neg) and CCSPnegCD45+ cells in control and Ccsp-creTgfb1-/- mice. n=3 groups of >5 mice per group. Mann–Whitney *P<0.05. (E-H) Cell counts in the (E) blood, (F) spleen, (G) bone marrow and (H) mediastenial lymph nodes in Ccsp-creTgfb1-/- mice (DOX) and Tgfb1+/- (mock) treated mice 7, 21 and 42 days after treatment. Box and whisker plots depict the median and IQR and minimum and maximum values. Bar charts are expressed as mean +/- SEM. n=4-5 mice per group (Mann–Whitney).
Figure S2, related to Figure 1. Doxycycline administration alone does not result in an altered allergic lung phenotype (A) Tgfb1, Tgfb2, and Tgfb3 mRNA levels in whole lung tissue of Ccsp-cre Tgfb1\(-/-\) mice (DOX) and Tgfb1\(+/+\) (mock) treated mice after HDM administration. Representative data is shown from 2 independent experiments with a total of n=8-12 mice per group (Mann–Whitney). (B-C) Airway hyperreactivity after intranasal house dust mite (HDM) or PBS administration as measured by (B) resistance and (C) compliance to an ascending methacholine concentration challenge in littermate control mice lacking either CCSP\(_{rtTA}\) or tetO-Cre unable (upon DOX administration) to ablate TGF-\(\beta\) expression in their epithelium (baseline; BL). (D-F) Cell counts in the (D) BAL and (E) lung tissue after Dox treatment and HDM exposure and (F) Numbers of macrophages (MAC), eosinophils (EOS) and neutrophils (NEU) in the airways of littermate control mice lacking either CCSP\(_{rtTA}\) or tetO-Cre. (G) Airway hyperreactivity measured by airway compliance to ascending methacholine concentration in Ccsp-cre Tgfb1\(-/-\) mice (DOX) and Tgfb1\(+/+\) (mock) treated mice (baseline; BL) (H) Histological scoring of lung inflammation after HDM (or PBS) treatment. (I) Eosinophils, macrophages and neutrophils in the lung tissue. Box and whisker plots depict the median and IQR and minimum and maximum values. Line graphs and bar graphs are expressed as mean +/- SEM. n=4-6 mice per group (Mann–Whitney).
Figure S3, related to Figures 2 and 3. Phenotype of *Ccsp-cre Tgfb1*−/− mice treated with HDM. (A-C) Levels of (A) KC, (B) MIP-2 and (C) MCP-1 in the lung tissue. (D) IgA levels in the BAL. (E) Flow cytometry plots show Th2 (CD3+CD4+IL-13+) cell gating strategy example shown is HDM treated mouse lung. (F-G) Frequencies of pulmonary T cells (F) IL-4+ T cells in the lung and (G) Th17 cells and (H) Th1 cells in the BAL. (I-J) Levels of (I) IL-17A and (J) IFN-γ levels in the lung. Representative data is shown from 2 independent experiments, with a total of n=8-12 mice per group, Mann–Whitney *P<0.05. Box and whisker plots depict the median and IQR and minimum and maximum values.

Figure S4, related to Figure 3. Innate lymphoid cell identification and gating. (A) Flow cytometry gating strategy for IL-13+ LinnegICOS+CD45+ ILCs where lineage cocktail contains CD3, CD45R, CD11b, TER-119, Ly-6G (GR1). (B) Flow cytometry gating strategy for IL-13+, IL-4+ and IL-5+ LinnegICOS+CD45+ ILCs. (C) Flow cytometry plots depicting Linneg and Lin+ populations with additional lineage markers (CD5, CD4, NK1.1, CD8, CD11c, β-TCR, γδ-TCR. (D) Plots show expression of ST2, CD127 and Thy1 on IL-13+ LinnegICOS+CD45+ ILC population.
Figure S5, related to Figures 3 and 4. **Innate cells in the lung** (A) Flow cytometry plots depicting IL-5+ and IL-13+ ILCs from PBS treated control mice and HDM treated Ccsp-cre Tgfb1−/− mice and littermate controls. (B) IL-4+ ILC numbers in the lung. (C) IL-5+ ILC numbers in the lung. (D-E) GATA-3+ ILCs in the (D) lung and (E) BAL. (F) IL-33 levels in the lung. (G) NK cell (NKp46+ CD3neg) numbers in lung tissue of Ccsp-cre Tgfb1−/− mice (DOX) and Tgfb1+/+ (mock) treated mice. (H) Frequency of ILC3 defined as IL-17A+ lineagenegCD45+ICOS+ in lung. (I) Histogram showing T1/ST2 expression on club cells (CCSP+CD45neg) recovered from the lung of naïve mice. (J) Levels of bioactive TGF-β in the BAL of control and Ccsp-cre Tgfb1−/− mice. Representative data is shown from 2 independent experiments, with a total of n=8-12 mice per group Mann–Whitney *P<0.05 and **P<0.01 Box and whisker plots depict the median and IQR and minimum and maximum values.
Figure S6, related to Figures 4 and 7. Phenotype of *Ccsp-cre Tgfb1−/+* mice mice treated with rIL-33. (A) Eosinophils (EOS), macrophages (MAC) and neutrophils (NEU) in the lung tissue. (B) Levels of IL-5 and (C) eotaxin-2 in the lung. (D) Total cell counts and (E) eosinophil numbers in the BAL. (F) Th2 cells and (G) ILC2 numbers in the BAL. (H) IL-13 levels in the BAL. (I) Accumulated distance and (J) track velocity of ILCs from control (*Tgfb1+/+*) and *Ccsp-cre Tgfb1−/+* mice exposed to gradients of rTGF-β1 (5μg/ml) assessed using TAXIScan methodology. Representative data is shown from 2 independent experiments, with a total of n=8-12 mice per group. Box and whisker plots depict the median and IQR and minimum and maximum values. Bar graphs are expressed as mean +/- SEM.
A

Epithelial specific TGF-β1 Transgenic mice

Truncated non-functional TGF-β1 transcribed

Littermate control mice

TGF-β1 transcribed

B

Ccsp-cre Tgfb1−/− (DOX)

Tgfb1+/+ (MOCK)

Litter mate control Tgfb1+/+ (DOX)

Ccsp-cre Tgfb1−/− (DOX)

5X

Anti-CRE

40X

Ccsp-cre Tgfb1−/− (DOX)

Anti-CRE

Bronchiolar Epithelium

Parenchyma

C

Litter mate control Tgfb1+/+

Ccsp-cre Tgfb1−/−

20X

(DOX)

Anti-TGF-β

D

Tgfb1 mRNA expression

\[ \Delta \Delta C_{t} Tgfb1 \]

E

Cell counts Blood

F

Cell Counts Spleen

G

Cell Counts Bone Marrow

H

Cell Counts Mediastinal Lymph Nodes
IL-4+

T cells Lung

MOCK+PBS
MOCK+HDM
DOX+PBS
DOX+HDM

0
1.0 \times 10^4
2.0 \times 10^4
3.0 \times 10^4
4.0 \times 10^4

IL-4+ CD4+ T cells (cell/ml)

MIP-2 Lung

MOCK+PBS
MOCK+HDM
DOX+PBS
DOX+HDM

0
50
100
150
200
250

MIP-2 (pg/ml)

KC Lung

MOCK+PBS
MOCK+HDM
DOX+PBS
DOX+HDM

0
50
100
150
200
250

KC (pg/ml)

IFN- \gamma

Lung

MOCK+PBS
MOCK+HDM
DOX+PBS
DOX+HDM

0
10
20
30
40
50

IFN- \gamma (pg/ml)

IgA BAL

MOCK+PBS
MOCK+HDM
DOX+PBS
DOX+HDM

0
10
20
30
40

IgA (ng/ml)

MCP-1 Lung

MOCK+PBS
MOCK+HDM
DOX+PBS
DOX+HDM

0
100
200
300
400

MCP-1 (pg/ml)

Cytokine expression gating

E

T cell gating strategy and cytokine expression gating

F

IL-4+ T cells Lung

MOCK+PBS
MOCK+HDM
DOX+PBS
DOX+HDM

IL-4+ CD4+ T cells (cell/ml)

IL-17+ CD4+ T cells (cell/ml)

Th17 Cells BAL

MOCK+PBS
MOCK+HDM
DOX+PBS
DOX+HDM

IL-17A Lung

MOCK+PBS
MOCK+HDM
DOX+PBS
DOX+HDM

IL-17A (pg/ml)

Th1 Cells BAL

MOCK+PBS
MOCK+HDM
DOX+PBS
DOX+HDM

IFN- \gamma+ CD4+ T cells (cell/ml)

IFN- \gamma (pg/ml)

IFN- \gamma+ CD4+ T cells (cell/ml)

IFN- \gamma (pg/ml)
