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**Method:**

**Generations of iIL-25Tg and *Il17rb*$^{-/-}$ Mice.** Murine *Il25* cDNA was inserted behind intestinal fatty acid–binding protein promoter (iFABP), and the expression plasmid was linearized by *EcoRI* digestion for the generation of iIL-25Tg mice[1]. For *Il17rb*$^{-/-}$ mice, a targeting vector was constructed to delete exons 5 to 11, resulting in the loss of the transmembrane and intracellular regions, as well as the alternative splicing isoforms of IL-17RB. Three independent iIL-25Tg founders and two *Il17rb*$^{-/-}$ founders were screened, selected, and backcrossed into the BALB/c background for more than ten generations. BALB/c and 4GET (*Il4*–IRES–eGFP; stock number 004190) mice and *Stat6*$^{-/-}$ (stock number 002828) mice were purchased from the National Cancer Institute (Bethesda, MD, USA) and Jackson Laboratory, respectively, and maintained in our animal facility under specific pathogen-free conditions. *Il17rb*$^{-/-}$ mice were crossed with 4GET mice to generate *Il17rb*$^{-/-}$/4GET mice. *Il13*$^{-/-}$ mice were a kind gift of Dr. Andrew McKenzie. All genetically modified mice used in this study were backcrossed onto BALB/c background for more than ten generations. All mice were used at 8-9 weeks of age, and all experiments employed age- and gender-matched controls to account for any variations in data sets compared across experiments. All animal experiments were approved by the animal care and use committee of Cincinnati Children's Hospital Medical Center and performed in accordance with institutional guidelines.

**LP mononuclear cell isolation.** Small intestines were isolated, cut, and washed before vigorous vortexing to remove epithelial and intraepithelial cells. After enzyme digestion with collagenase A (Roche) and DNase I (Roche) at 37$^{o}$C for 30 minutes and the removal of tissue debris, liberated cells were suspended in 44% Percoll and loaded on 67% Percoll for centrifugation. LP cells were collected from the interface for subsequent analysis.

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**Flow cytometry analysis and cell sorting.** LP cells from the small intestines were first stained with PE-conjugated anti-CD127 [A7R34], APC-conjugated anti–IL-17RB or anti-KLRG1 [2F1/KLRG1], V500-conjugated anti-CD4 [GK1.5], PE-Cy7–congjuated anti-CD3e [145-2C11], FITC-conjugated anti-KLRG1 or GFP (for phenotype, anti-CD44 [IM7], CD62L [MEL-14], CD69 [H1.2F3], Thy1.2 [30-H12], ICOS [C398.4A], and Sca-I [D7]), biotinylated anti–T1/ST-2 [DJ8] or anti-CD127, and APC-Cy7–conjugated anti–c-KIT [2B8] antibodies. Subsequently, cells were counterstained with PerCP-Cy5.5–conjuaged monoclonal antibodies against lineage (Lin) markers (CD11b [M1/70], CD11c [HL3], CD8 [53-6.7], B220 [RA3-6B2], Gr-1 [RB6-8C5], and CD335 [NKP46, 29A.4]) and with Brilliant Violet 421™labeled Streptavidin (Biolegend) before analyses with a FACSCanton II (BD Bioscience) or cell sorting with a FACSAria II (BD Bioscience). After lysis of red blood cells, peripheral blood cells were stained with Brilliant Violet 421™labeled Siglect-F, FITC-conjugated CCR3, APC-conjugated CD11b, and PerCP-Cy5.5–conjuaged monoclonal antibodies against CD11c, CD4, CD8, B220, and Gr-1 for the analysis of blood eosinophils. For intracellular cytokine analyses, $Lin^+$ LP cells from BALB/c mice were labeled with microbeads conjugated with monoclonal antibodies against CD11b, CD8α, and B220 and then removed with a MACS column (Miltenyi Biotech). After re-stimulation with PMA/ionomycin or IL-25 and treatment with Golgi blocker, enriched $Lin^-$ LP cells were stained with APC-conjugated anti–IL-17RB, APC-Cy7–conjugated anti-CD3e, V500-conjugated anti-CD4, PE-Cy7–conjugated anti-FcεRIα, and biotinylated anti–IL-7Rα antibodies. Subsequently, cells were counterstained with PerCP-Cy5.5–conjugated monoclonal antibodies against Lin markers as described above and with Brilliant Violet 42™–labeled Streptavidin. Stained cells were fixed and permeabilized for intracellular cytokine staining using FITC-conjugated anti–IFN-γ [XMG 1.2] and PE-conjugated anti–IL-13 [eBio13A].

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**Measuring cytokines and mediators.** Sorted ILC2s ($1x10^4$) and $CD4^+T_H2$ cells ($1x10^4$) from mice with active allergic diarrhea were cultured with IL-25 (50 ng/mL) in the presence or absence of IL-2 (50 ng/mL) and IL-7 (50 ng/mL) (R&D Systems) for 3 days. To examine IL-25 responsiveness, enriched LP cells ($3x10^5$ cells per well) from the small intestines of wild-type or wild-type mice reconstituted with wild-type BM, *Rora*$^{-/-}$ BM or *Il17rb*$^{-/-}$ BM with or without active allergic diarrhea were cultured with IL-25 (50 ng/mL) in 96-well plates for 3 days before the collection of supernatants. In some experiments, sorted ILC2s ($1x10^4$ cells per well) from wild-type mice that were injected daily with 0.4 μg recombinant mouse IL-25 (R&D Systems) for 4 days were cultured with or without *in vitro*–derived $CD4^+T_H2$ cells ($4x10^4$ cells per well) in the presence of IL-25 or IL-25 plus αIL-2 monoclonal Abs (clone: S4B6-1, BioXcell) for 3 days before the collection of supernatants. Secreted cytokines in supernatants were assessed by ELISA kits for IL-4, IFN-γ (BD Pharmingen), IL-5 (R&D Systems), and IL-13 (Antigenic American).

**RNA isolation and quantitative real-time PCR analysis.** RNA from sorted cell populations was isolated using an RNeasy Plus Mini kit (Qiagen) and served as templates to synthesize cDNA using iScript cDNA synthesis kit (Bio-rad). Quantitative real-time PCR analyses were performed with SYBR Green Chemistry (Applied Biosystems) in an ABI Prism 7900 detection system using previously described primer sets[2-6], or the following primer sets: *Gapdh* (F5'-TGCACCACCAACTGCTTAGC, R5'-GGCAT GGACTGTGGTCATGAG), *Il13* (F5'-TGACCAACATCTCCAATTGCA, R5'-TTGTTATAAAGTG GGCTACTTCGATTT), *Il25* (F5'-TGTACCAGGCTGTTGCATTC, R5'-CTCCACTTCAGCCACTCCTC), *Tarc* (F5'-CAGGAAGTTGGTGAGCTGGT, R5'-GGGTCTGCACAGATGAGCTT), *Cxcl1* (F5'-TAGGGTGAGGACATGTGTGG, R5'-AAATGTCCAAGGGAAGCGT), *Ccl11* (eotaxin 1, F5'-CCCAACTTCCTGCTGCTTTA, R5'-AGATCTCTTTGCCCAACCTG), and *Ccl24* (eotaxin 2*,* F5'-

CTCCTTCTCCTGGTAGCCTGCG, R5'-GTGATGAAGATGACCCCTGCCTT). Expression levels of target genes were normalized to endogenous *Gapdh* transcript levels, and relative quantification of samples was compared to the lowest expression levels as the baseline.

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**Reference:**


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## FIGURE LEGENDS

**FIG E1.** (A) Detection of and phenotypic analysis of $CD3^+CD4^+$ T cells in LP of small intestine of IL-4-eGFP (4GET) mice after six intragastric OVA challenges. (B) Measurements by ELISA of indicated cytokines secreted by sorted indicated T cell subsets ($100x10^3$ cells) after stimulation with CD3 and CD28 for 24hrs. (C) Expression of the indicated genes by intestinal ILC2, $CD4^+$ TH2 cells, eosinophils, and a panel of other indicated hematopoietic cell lineages from the spleens of mice with active allergic diarrhea, as well as by *in vitro*-generated bone marrow-derived mast cells (BMMCs), was analyzed by quantitative real-time PCR using primers referenced in the methods. Data are expressed as relative fold difference and represent one of three independent experiments. mDC, myeloid dendritic cells ($CD11c^{hi}CC11b^{hi}CD4^+$); pDC, plasmacytoid dendritic cells ($CD11c^{med}CD11b^-Gr\text{-}1^+B220^+$). Data represent one of three independent experiments. Error bars denote mean ± S.E.M. * $p<0.05$, **$p\leq 0.01$. ns, not significant.

**FIG E2** (A) Phenotype of $Lin^-$IL-17RB$^+$c-KIT$^-$ and $Lin^-$IL-17RB$^-$c-KIT$^+$ in lamina propria (LP) of the small intestine from mice with experimental food allergy was analyzed using flow cytometry. (B) Detection and frequency of ILC2s in mesenteric lymph node of BALB/c mice injected with recombinant IL-25 (0.5 mg) for 4 consecutive days. Filled histograms represent the staining of indicated markers on indicated cell subsets (A); open histograms represent isotype control. Data represent one of three independent experiments (n=4 mice per group). Error bars denote mean ± S.E.M. **$p\leq 0.01$.

**FIG E3** Origin of intestinal ILC2s and $CD4^+T_H2$ cells in irradiated recipient mice after reconstitution in a murine model of food allergy. (A) Diagram of adoptive transfer protocol in the mouse model of food allergy. (B, C) Detection (B) and frequency (C) of donor-derived ILC2s ($Lin^-$IL-17RB$^-$c

-Kit$^{-}$Thy1.1$^{lo/hi}$Thy1.2$^{-}$) and recipient-derived CD4$^{+}$Thy1.1$^{-}$Thy1.2$^{+}$ T cells in the small intestine of irradiated recipient Thy1.2$^{+}$ wild type (WT) or *STAT6*$^{-/-}$ BALB/c mice reconstituted with bone marrow progenitors from WT Thy1.1$^{+}$ BALB/c mice after six intragastric OVA challenges. Data represent one of three independent experiments (n=4 mice per group). LP, laminar propria. ns, not significant.

**FIG E4**. (A) Measurement of indicated cytokines produced by laminar propria cells of naïve or mice developed experimental food allergy after culture with medium only or $OVA_{323-339}$ peptides (10μM) for 3 days by ELISA. (B) Measurement of IL-5 and IL-13 production by ILC2s, CD4$^{+}$$T_H2$ cells, or ILC2s co-cultured with CD4$^{+}$$T_H2$ cells after stimulation with $OVA_{323-339}$ peptides, IL-25, or both for 3 days by ELISA. Data is the representative of two independent experiments. Error bars denote mean ± S.D. **p ≤ 0.01; ***p ≤ 0.001. ND, not detected; ns, not significant.

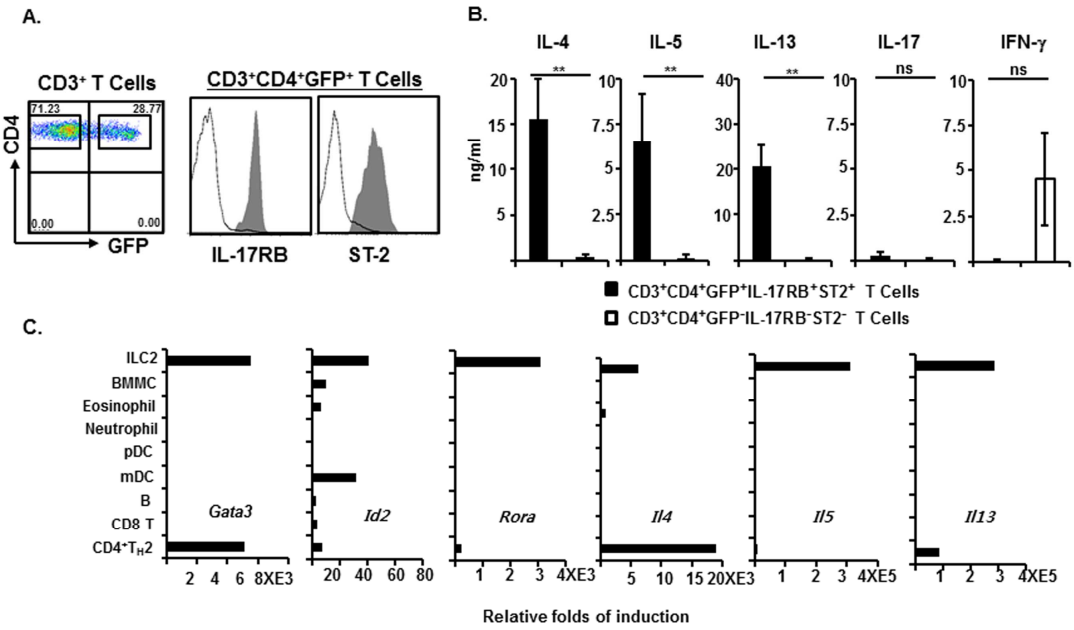
A.
CD3+ T Cells
CD3+CD4+GFP+ T Cells
CD4
GFP
71.23
28.77
0.00
0.00
IL-17RB
ST-2
B.
IL-4
IL-5
IL-13
IL-17
IFN-γ
ng/ml
**
**
**
ns
ns
CD3+CD4+GFP+IL-17RB+ST2+ T Cells
CD3+CD4+GFP-IL-17RB-ST2- T Cells
C.
ILC2
BMMC
Eosinophil
Neutrophil
pDC
mDC
B
CD8 T
CD4+TH2
Gata3
Id2
Rora
Il4
Il5
Il13
2 4 6 8XE3
20 40 60 80
1 2 3 4XE3
5 10 15 20XE3
1 2 3 4XE5
1 2 3 4XE5
Relative folds of induction

**FIG E1**

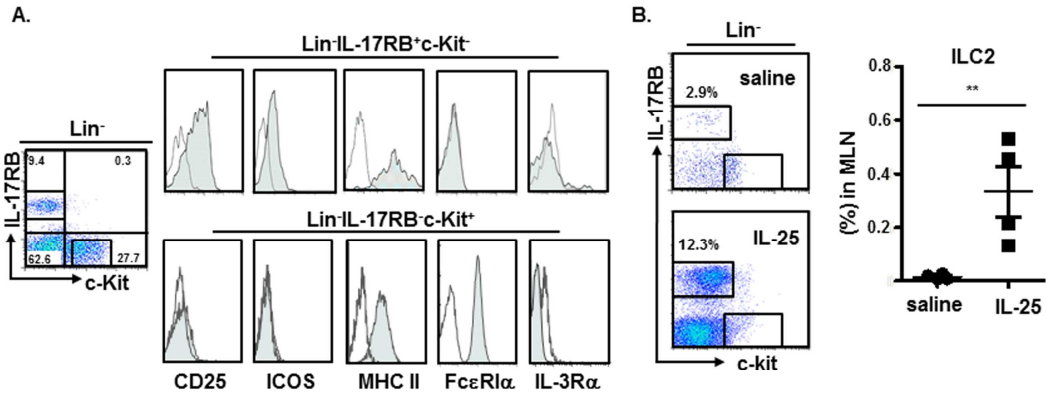
A.
Lin-IL-17RB+c-Kit-
Lin-
IL-17RB
9.4
0.3
62.6
27.7
c-Kit
Lin-IL-17RB-c-Kit+
CD25
ICOS
MHC II
FcεRIα
IL-3Rα
B.
Lin-
IL-17RB
2.9%
saline
12.3%
IL-25
c-kit
ILC2
**
(%) in MLN
0.8
0.6
0.4
0.2
saline
IL-25

FIG E2

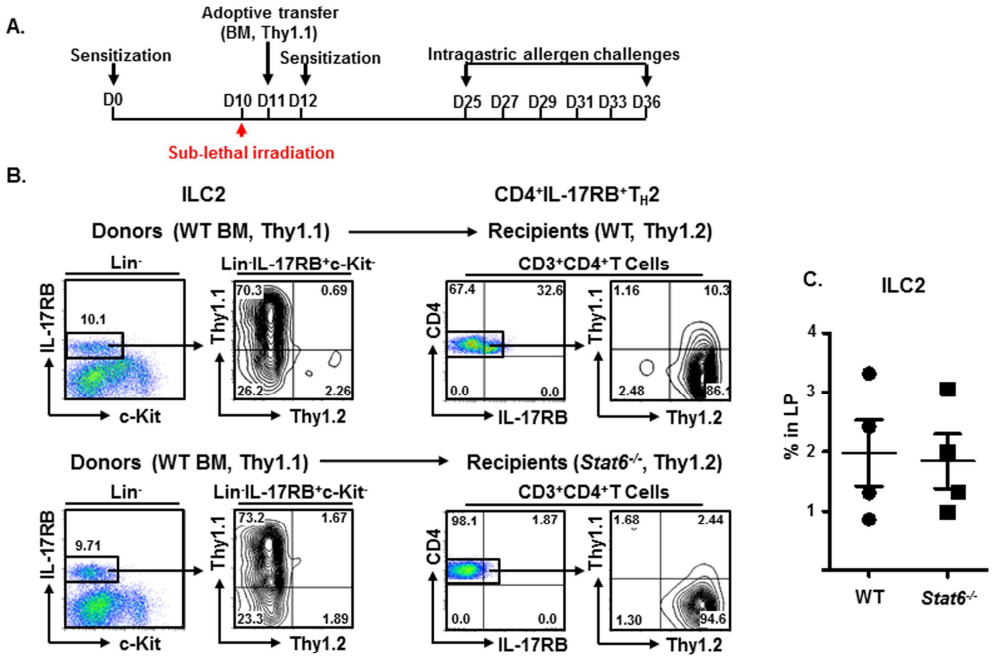
A.
Adoptive transfer
(BM, Thy1.1)
Sensitization
Sensitization
Intragastric allergen challenges
D0
D10 D11 D12
D25 D27 D29 D31 D33 D36
Sub-lethal irradiation
B.
ILC2
CD4+IL-17RB+TH2
Donors (WT BM, Thy1.1)
Recipients (WT, Thy1.2)
Lin-
Lin-IL-17RB+c-Kit-
CD3+CD4+T Cells
IL-17RB
c-Kit
Thy1.1
Thy1.2
CD4
10.1
70.3
0.69
26.2
2.26
67.4
32.6
0.0
0.0
1.16
10.3
2.48
Donors (WT BM, Thy1.1)
Recipients (Stat6-/-, Thy1.2)
9.71
73.2
1.67
23.3
1.89
98.1
1.87
1.68
2.44
1.30
94.6
C.
ILC2
% in LP
WT
Stat6-/-

FIG E3

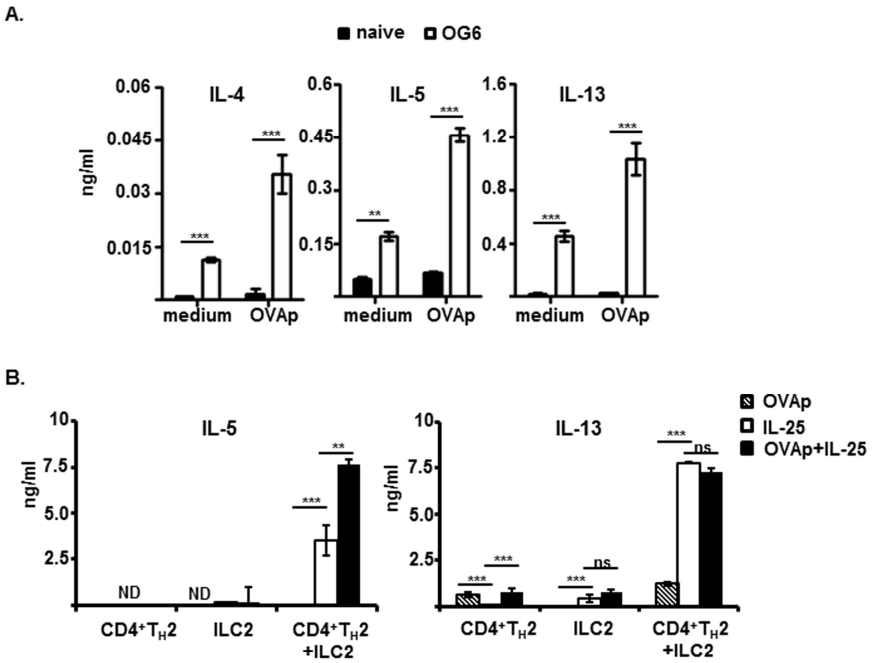
A.
naive
OG6
IL-4
IL-5
IL-13
ng/ml
0.06
0.045
0.03
0.015
0.6
0.45
0.3
0.15
1.6
1.2
0.8
0.4
***
**
medium
OVAp
B.
OVAp
IL-25
OVAp+IL-25
10
7.5
5.0
2.5
ND
ns
CD4+TH2
ILC2
CD4+TH2 +ILC2

FIG E4