Supplemental Data

IL-17A inhibits airway reactivity induced by RSV infection during allergic airway inflammation

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Methods:

Mice. Pathogen-free eight- to ten-week-old female BALB/c mice were purchased from Charles River Laboratories (Wilmington, MA). IL-17A knockout (KO) mice on a BALB/c background were provided by Jay Kolls (University of Pittsburgh).

Allergic sensitization/challenge and RSV infection of mice. Mice were categorized into 4 groups: mock, OVA, RSV, and OVA/RSV. The protocol for OVA sensitization/challenge and RSV infection is previously described\(^1\) and also in the methods section of the text.

Flow cytometry and intracellular staining. Mononuclear cells were isolated from lungs of mice and restimulated for 6 hours with PMA (50µg/ml) and ionomycin (1mg/ml) (both from Sigma-Aldrich) in the presence of 0.07% golgi-stop (BD Bioscience). One million cells/mouse were FcR Ab 2.4G2 blocked (BD Biosciences) followed by staining with the following antibodies: anti-CD4, anti-CD3, anti-CD8, anti-CD11c, anti-Gr-1 and/or anti-γδ TCR (all from BD Biosciences). Cells were permeabized with cytofix/cytoperm (eBiosciences), washed thoroughly, and stained intracellularly with anti-IL-17A antibody (BD Biosciences). Cells were analyzed using a LSR II flow cytometer (BD Biosciences), and data were analyzed using Flow Jo 7.2 software (Tree Star, INC, Ashland, OR).

Airway reactivity measurements. Mice were anesthetized with i.p. injections of pentobarbital sodium (85 mg/kg) and a tracheostomy tube was placed. The internal jugular vein was cannulated and a microsyringe was attached to intravenous tubing for methacholine
administration. The mice were then placed in a plethysmography chamber and mechanically ventilated. Lung resistance was measured following administration of intravenous acetyl-ß-methacholine (0-3700µg/kg body weight) (Sigma-Aldrich) as previously described.\(^1,2\)

**Statistical analyses.** Data is presented as mean ± SEM with data shown from 1 experiment. Each experiment was repeated three times to ensure the reproducibility of the data. In Supplemental Figure 1, data were analyzed by ANOVA followed by the Tukey posthoc test. In Supplemental Figure 2 data were analyzed by repeated measures ANOVA with Bonferroni posthoc test. Values were considered significant when p< 0.05.
Supplemental Figure Legends:

Supplemental Figure 1: IL-17 expression occurs in CD3+CD4+ T cells with RSV infection during ongoing allergic airway inflammation. WT mice were OVA sensitized and challenged followed by RSV infection (OVA/RSV group) as described in Figure 1. Lungs were harvested and mononuclear cells were surface stained for anti-CD3, anti-CD4, anti-CD8, anti-γδ TCR, anti-CD11c, and anti-Gr-1. IL-17A expression was determined by intracellularly staining with anti-IL-17A antibody. (A-B) Representative dot plots gated on live/CD3+ cells. (C-E) Representative dot plots gated on live cells. (F) Total number of IL-17 cells. n=5 mice, ANOVA, *p<0.05 compared to CD3+CD8+, γδ TCR+, Gr-1+, and CD11c+ cells.
Supplemental Figure 2: IL-17A negatively regulates AR in RSV mice. WT and IL-17A KO mice were either mock or RSV infected and on day 14, AR was measured by detecting changes in airway resistance in response to increasing concentrations of methacholine. Scale on y-axis is the same as Figure 5. n=5-17 mice, ANOVA, *p<0.05 compared to WT mock mice, † p<0.05 compared to WT RSV mice.
REFERENCES
