

Supplemental Figures

Group 2 Innate Lymphoid Cells Promote Development of T follicular Helper (Tfh) Cells and Initiate Allergic Sensitization to Peanuts

Running Title: Role of ILC2s in Development of Peanut Allergy

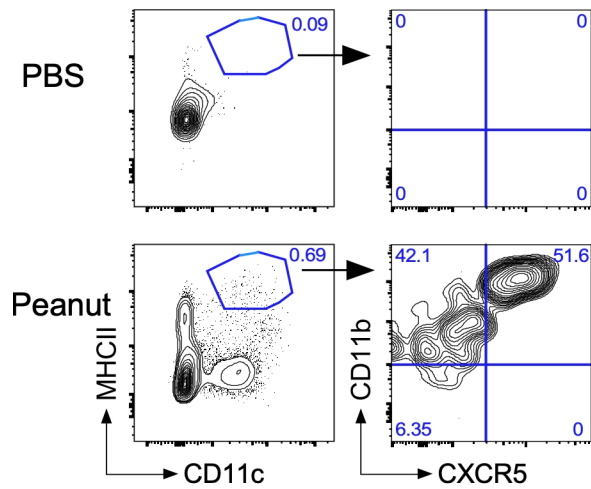
James W. Krempski, M.S.^{*}, Takao Kobayashi, Ph.D.[†], Koji Iijima, Ph.D.[†], Andrew N.
McKenzie, Ph.D.[‡], Hirohito Kita, M.D.^{*,†}

^{*}Department of Immunology, Mayo Clinic, Rochester, MN 55905

[†]Division of Allergic Diseases and Department of Medicine, Mayo Clinic, Scottsdale, AZ 85259

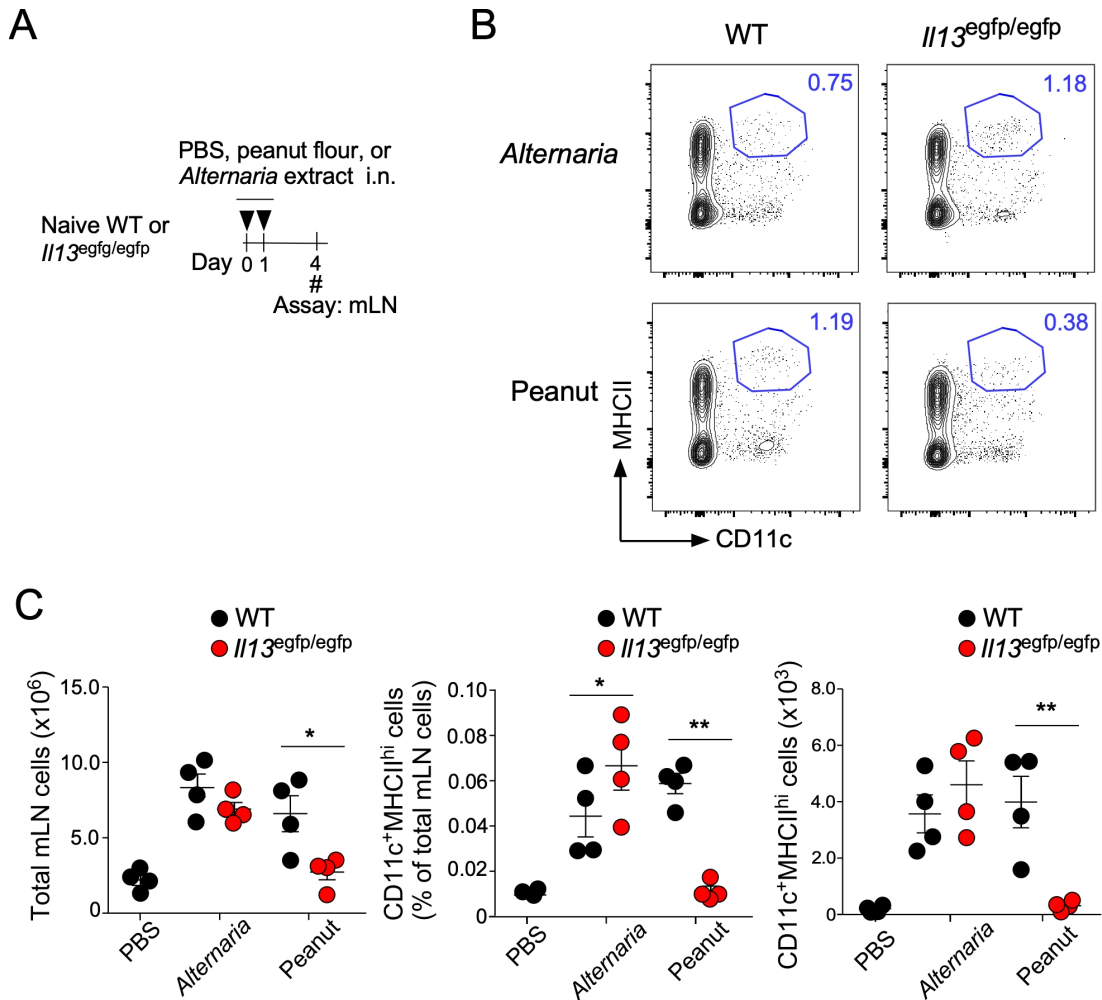
[‡]MRC Laboratory of Molecular Biology, Cambridge, CB2 0QH, UK

Corresponding author: Hirohito Kita, M.D., Division of Allergic Diseases and Department of
Medicine, Mayo Clinic Arizona, 13400 E Shea Blvd, Scottsdale, AZ 85259, USA, Telephone:
480-301-9616, Fax: 480-301-7017, E-mail: kita.hirohito@mayo.edu



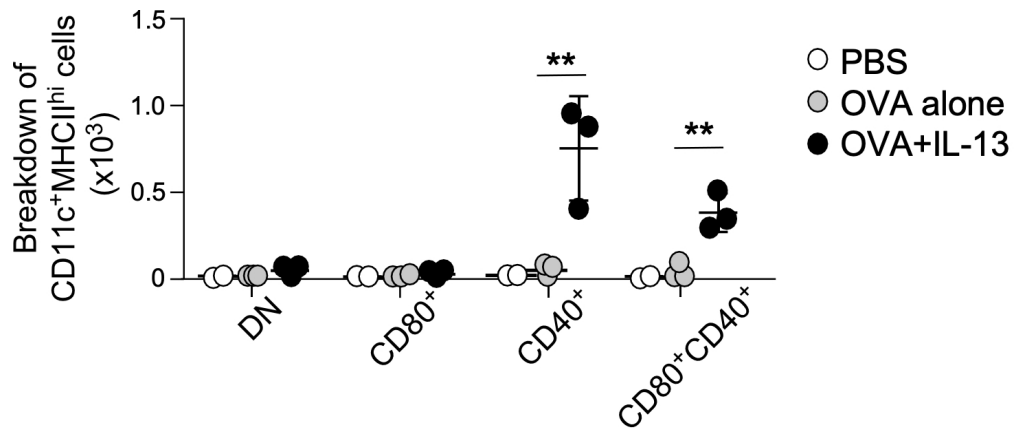
Supplemental Figure 1

Supplemental Figure 1. The CD11c⁺MHCII^{hi} DC population expresses CD11b and CXCR5. WT mice were exposed to peanut flour or PBS on days 0 and 1 as described in Fig 3A, and mediastinal lymph nodes were collected on day 4. Sample flow plots for CD11c⁺MHCII^{hi} population and CD11b⁺CXCR5⁺ population are shown. The cells were gated on the CD45⁺, singlet, living, high FSC-A/SSC-A population.



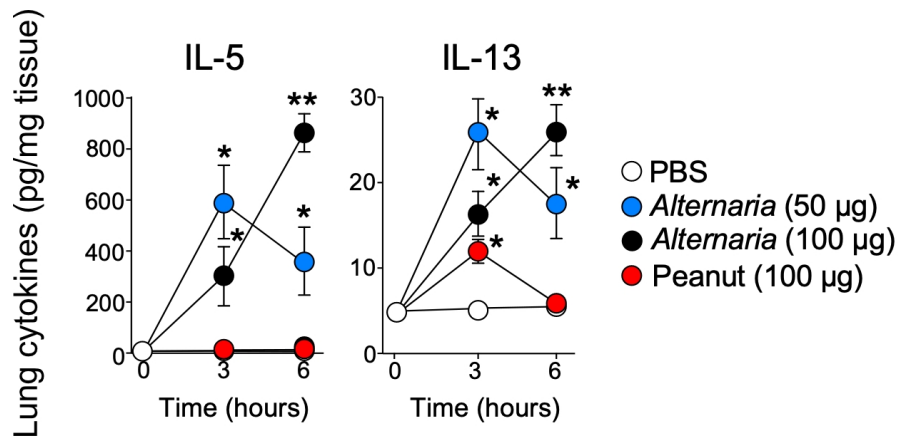
Supplemental Figure 2

Supplemental Figure 2. IL-13 deficiency does not affect the number of CD11c^{hi}MHCII^{hi} DCs in mediastinal lymph nodes (mLNs) after *Alternaria* extract exposure. **A.** WT and IL-13-deficient (i.e. *Il13*^{egfp/egfp}) BALB/c mice were exposed i.n. to peanut flour, *Alternaria* extract, or PBS on days 0 and 1. On day 4, mLN were harvested and analyzed by flow cytometry. **B.** Representative gating strategy for each experimental group, preceded by gating on the CD45⁺, singlet, living, high FSC-A/SSC-A cell population. **C.** Data represent total numbers of mLN cells and frequency and cell counts for the CD11c⁺MHCII^{hi} population. * $P < 0.05$ and ** $P < 0.01$ between the groups indicated by horizontal lines. Each circle represents individual mouse.



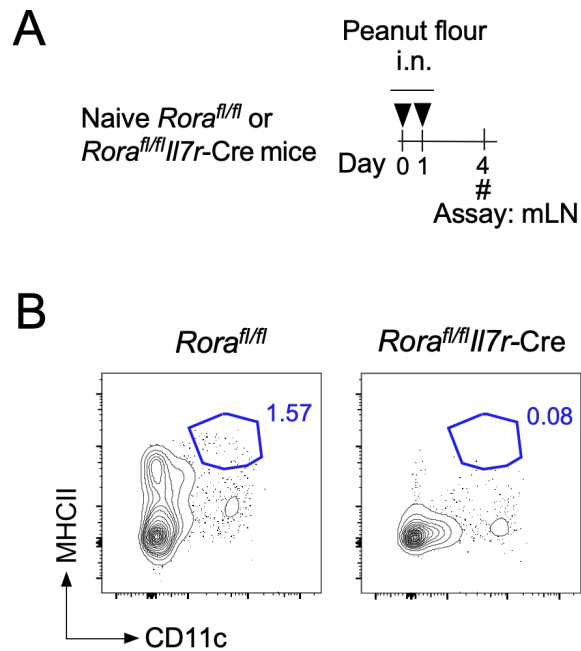
Supplemental Figure 3

Supplemental Figure 3. IL-13 in the presence of OVA antigen increases CD40⁺ and CD80⁺CD40⁺ CD11c⁺MHCII^{hi} DCs in mLNs. WT BALB/c mice were exposed i.n. to IL-13+OVA, OVA alone, or PBS for on days 0 and 1. On day 4, mLNs were harvested and analyzed by flow cytometry. Data represent cell counts (mean ± SEM) for the double-negative (DN), CD80⁺ or CD40⁺ single-positive, and CD80⁺CD40⁺ double-positive populations in CD11c⁺MHCII^{hi} DCs. ***P*<0.01 between the groups indicated by horizontal lines. Each circle represents individual mouse.



Supplemental Figure 4

Supplemental Figure 4. *Alternaria* extract induces robust type 2 cytokine production after airway exposure. **A.** WT BALB/c mice were exposed once to peanut flour (100 µg/dose) or *Alternaria* extract (50 µg/dose or 100 µg/dose) or PBS. Lungs were harvested 3 or 6 hours later, and IL-5 and IL-13 levels from lung lysate were assessed by ELISA. Data represent mean ± SEM from an experiment with 3–5 mice/group. * $P < 0.05$ and ** $P < 0.01$ compared to the mice exposed to PBS.



Supplemental Figure 5

Supplemental Figure 5. Decreased CD11c⁺MHCII^{hi} DC population in mLNs in *Rora^{fl/fl}Il7r-Cre* mice. **A.** *Rora^{fl/fl}Il7r-Cre* mice or *Rora^{fl/fl}* control mice were exposed to peanut flour on days 0 and 1 as described in Fig 3A, and mediastinal lymph nodes were collected on day 4. **B.** Sample flow plots for CD11c⁺MHCII^{hi} population are shown. The cells were gated on the CD45⁺, singlet, living, high FSC-A/SSC-A population.