



Supplementary Material for

Communication

## Th17-Dependent Nasal Hyperresponsiveness Is Mitigated by Steroid Treatment

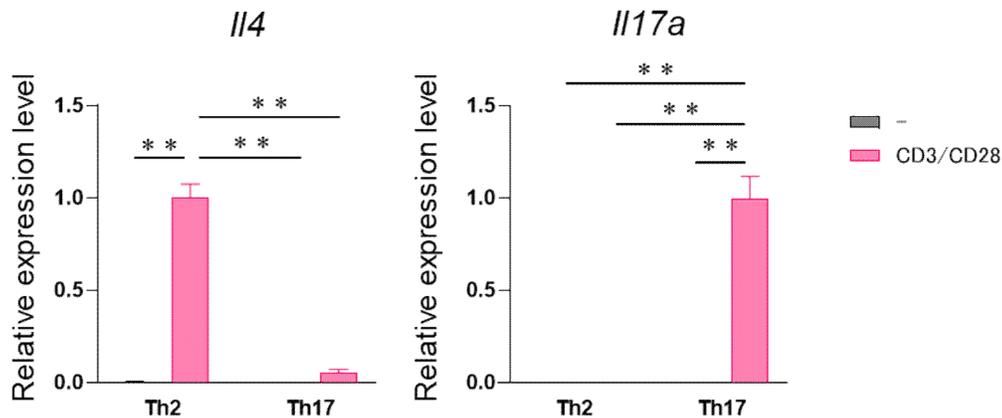
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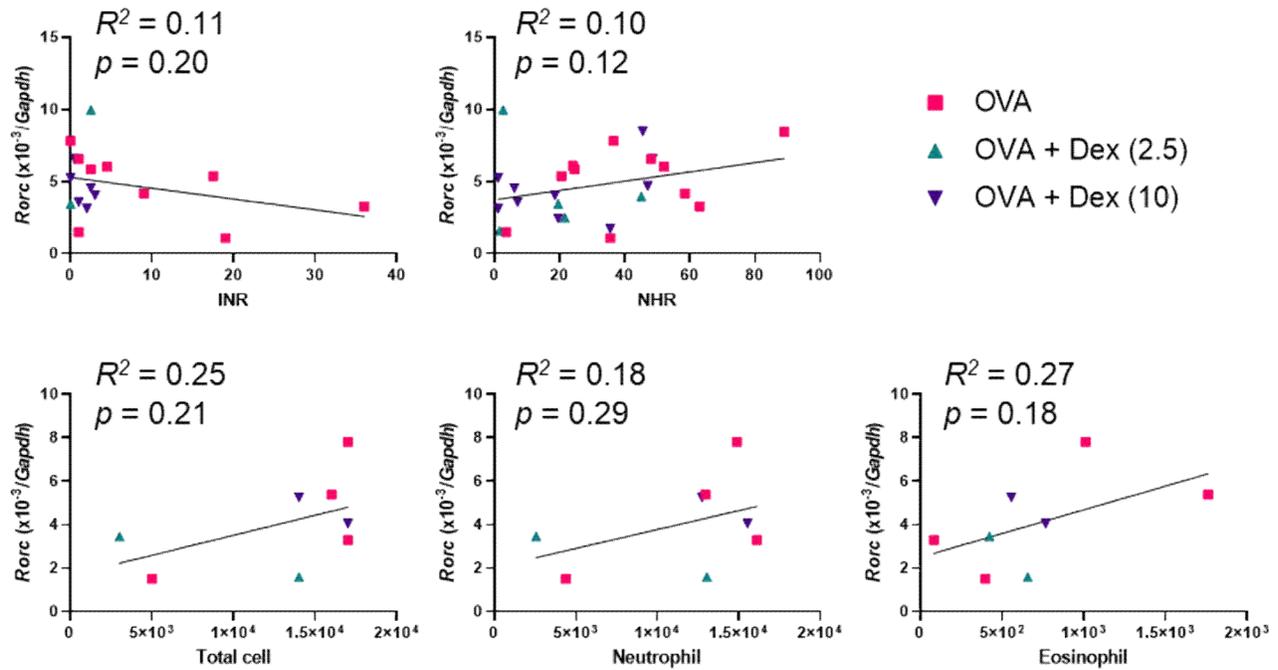
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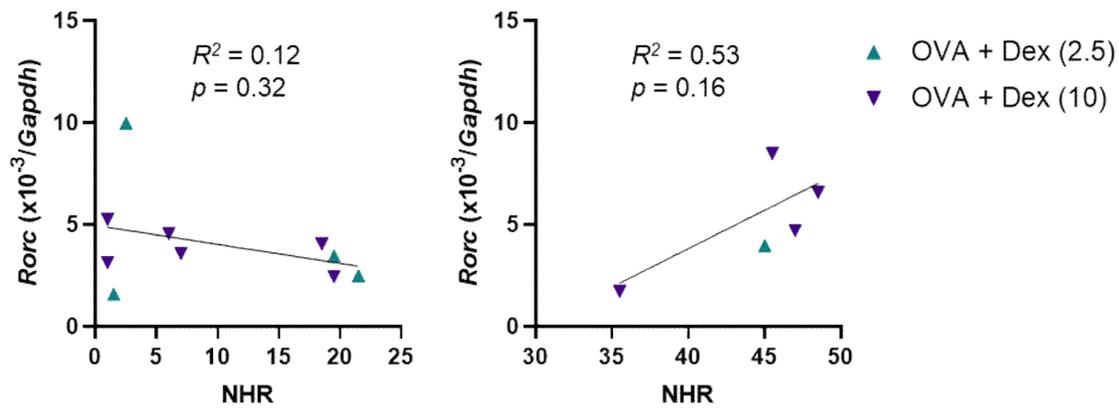
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**Figure S1.** Expression of *Il4* and *Il17a* mRNA in *in vitro*-differentiated Th2 and Th17 cells. The relative expression levels were normalized to *Gapdh* expression as an endogenous reference. Data are displayed as the mean  $\pm$  standard error of the mean ( $n = 4$ ). The mean value of Th2 or Th17 activated with anti-CD3/CD28 was set as 1.0 for *Il4* or *Il17a*, respectively. Statistical analyses were conducted by one-way analysis of variance and additional Dunnett's test.  $**p < 0.01$  (Dunnett's test).



**Figure S2.** Relationship between nasal *Rorc* expression and inflammatory responses. The correlation of *Rorc* expression in the nasal-associated lymphoid tissue with allergen-induced immediate nasal response (INR), nasal hyperresponsiveness (NHR), and the number of total cell, neutrophils, and eosinophils in the nasal lavage fluids obtained in Th17 cell-transferred and ovalbumin (OVA)-challenged mice was evaluated. The Pearson correlation coefficient ( $R^2$ ) and  $p$ -value were also shown. Dex, dexamethasone (2.5 or 10 mg/kg).



**Figure S3.** Relationship between nasal *Rorc* expression and nasal hyperresponsiveness (NHR). The correlation between *Rorc* expression in the nasal-associated lymphoid tissue and NHR was separately evaluated for Dex-responder (left panel) and -non-responder (right panel) groups. The Pearson correlation coefficient ( $R^2$ ) and  $p$ -value were also shown for individual groups. OVA, ovalbumin; Dex, dexamethasone (2.5 or 10 mg/kg).

### ***In vitro* development of allergen-reactive Th2 and Th17 cells**

Allergen-reactive Th2 and Th17 cells were developed as previously described [4,8]. In brief, ovalbumin (OVA)-reactive CD4<sup>+</sup> T cells were prepared from spleen cells of BALB/c background transgenic mice, DO11.10/RAG2<sup>-/-</sup>, by magnetic cell sorting with an EasySep Mouse CD4<sup>+</sup> T Cell Isolation Kit (Veritas, Santa Clara, CA, USA). The cells were co-cultured in the presence of X-ray-irradiated spleen cells in AIM-V medium (Thermo Fisher Scientific, Waltham, MA, USA) with 10% fetal calf serum. At the beginning of culture, we added 0.3 μM OVA323-339 synthetic peptide (Scrum Inc., Tokyo, Japan), 20 U/mL IL-2 (PeproTech, Rocky Hill, NJ, USA), and 10 μg/mL anti-IFN-γ (R4-6A2, eBioscience, San Diego, CA, USA). We further added 10 U/mL recombinant IL-4 (PeproTech) for Th2 development, and 10 ng/mL human IL-1β (PeproTech), 20 ng/mL IL-6 (PeproTech), 10 ng/mL IL-23 (R & D Systems, Minneapolis, MN, USA), 1 ng/mL human TGF-β (Bio-Legend, San Diego, CA, USA), 10 ng/mL TNF-α (PeproTech), and 10 μg/mL anti-IL-4 (Abcam, Cambridge, UK) for Th17 development. Cells were collected following seven-day culture, activated for 6 h by Dynabeads coated with anti-CD3/CD28 (Thermo Fisher Scientific) in AIM-V with 10% fetal calf serum, and processed for the gene expression assessment.

### **Gene expression assessment**

Following the extraction of total RNA from the Th cells, reverse transcription was performed using random primers with SuperScript VILO cDNA Synthesis Kit (Thermo Fisher Scientific), and then quantitative RT-PCR for *Ii4* (Mm00439618\_m1) and *Ii17a* (Mm00439618\_m1) was carried out using Taqman gene expression probes (Thermo Fisher Scientific) on the ABI StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific). The relative transcript levels were normalized to *Gapdh* (4351309) expression as an endogenous reference using the ΔΔCt method.