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Basophils contribute to T_H2-IgE responses *in vivo* via IL-4 production and presentation of peptide–MHC class II complexes to $CD4^+$ T cells

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Basophils express major histocompatibility complex class II, CD80 and CD86 and produce interleukin 4 (IL-4) in various conditions. Here we show that when incubated with IL-3 and antigen or complexes of antigen and immunoglobulin E (IgE), basophils internalized, processed and presented antigen as complexes of peptide and major histocompatibility complex class II and produced IL-4. Intravenous administration of ovalbumin-pulsed basophils into naive mice 'preferentially' induced the development of naive ovalbumin-specific $CD4^+$ T cells into T helper type 2 (T_H2) cells. Mice immunized in this way, when challenged by intravenous administration of ovalbumin, promptly produced ovalbumin-specific IgG1 and IgE. Finally, intravenous administration of IgE complexes rapidly induced T_H2 cells only in the presence of endogenous basophils, which suggests that basophils are potent antigen-presenting cells that 'preferentially' augment T_H2-IgE responses by capturing IgE complex.

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Atopic people, after repeated exposure to a particular antigen, develop strong T helper type 2 (T_H2) responses and produce immunoglobulin E (IgE). IgE then sensitizes mast cells and basophils by binding to their FcεRI receptor (A000543)[1–3]. Subsequent exposure to the same antigen activates the mast cells and basophils to secrete the chemical mediators, cytokines and chemokines that result in the pathological reactions of immediate hypersensitivity. IgE is a unique antibody that upregulates expression of FcεRI on mast cells and basophils, thereby providing a mechanism for the amplification of IgE-mediated reactions[4,5]. Indeed, a strong positive correlation exists between FcεRI expression on basophils and IgE titers in human peripheral blood[6]. Furthermore, as with the inhalation of ragweed pollen, low antigen dose without adjuvant can induce IgE production, which suggests that there is an amplification loop for IgE production *in vivo*. Thus, once atopic people begin to produce IgE, they develop progressive allergic inflammation by increasing production of IgE and expression of FcεRI on effector cells.

Basophils and mast cells are important effector cells in IgE-mediated allergic inflammation[1–3]. Progenitors of mast cells in the bone marrow migrate to the peripheral tissues as immature cells and undergo differentiation *in situ*[1,7]. Thus, normally, mature mast cells are not found in the circulation. In contrast, basophils are rare circulating granulocytes that originate from progenitors in the bone marrow. Basophils constitute less than 1% of blood leukocytes and are normally not present in tissues. However, they may be recruited to some inflammatory sites where antigen is present and contribute to immediate hypersensitivity reactions[8–11]. Studies also suggest that basophils induce IgE-mediated chronic allergic inflammation and IgG1-mediated systemic anaphylactic shock[12–14]. Thus, basophils are primary effector cells in allergic disorders.

However, some lines of evidence have shown that these cells are important regulators of T_H2 responses *in vivo*, particularly in helminth-infected mice[15–20]. In general, the entry of an invading pathogen triggers recognition by dendritic cells (DCs) through Toll-like receptors (TLRs) and their subsequent maturation to express costimulatory molecules and produce interleukin 12 (IL-12) and IL-18, which favor T_H1 responses[21–24]. In contrast, infection with helminths strongly induces T_H2 cells and the proliferation of basophils in the spleens and livers of host mice[18], which suggests a contribution of basophils to the induction and/or augmentation of T_H2 responses. The development of naive $CD4^+$ T cells into T_H2 cells is dependent on IL-4 (A001262) in the milieu[25]. However, the nature of cells that produce 'early' IL-4, required for the development of naive $CD4^+$ T cells into T_H2 cells, remains unknown[26]. IL-18 with IL-3 or IL-33 with IL-3 strongly induces basophils but not mast cells to produce both IL-4 and IL-13 *in vitro*[27,28], which suggests basophils are involved in the induction of T_H2 cells by functioning as early IL-4-producing cells. Other published studies have also indicated that basophils are critically involved in T_H2 responses by their unique ability to produce early IL-4 and thymic stromal lymphopoietin in response to papain or

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bromelain[29]. Thus, here we studied the mechanism that accounts for the induction and progression of allergic response by positive feedback loops between IgE and basophils *in vivo*. We demonstrate the contribution of basophils to the T_H2-IgE response *in vitro* and *in vivo* through the production of IL-4 and presentation of complexes of peptide and major histocompatibility complex (MHC) class II to naive $CD4^+$ T cells, in contrast to the T_H1 cell–inducing action of DCs.

RESULTS

Basophils induce the development of T_H2 cells *in vitro*

We first examined the ability of splenic basophils from naive mice and mice infected with *Strongyloides venezuelensis*[30] to produce T_H2 cytokines and to induce the development of naive $CD4^+$ cells into T_H2 cells *in vitro*. We prepared non–T cell, non–B cell fractions from spleens of naive mice and infected mice and determined the proportion of $Fc\varepsilon RI^+$c-Kit$^-$ cells (basophils) in those fractions. Non–T cell, non–B cell fractions from spleens of naive mice contained 0.20% $Fc\varepsilon RI^+$c-Kit$^-$ cells, whereas those from *S. venezuelensis*–infected mice had a much greater proportion of these cells (5.84%; **Fig. 1a**), as reported for mice infected with *Nippostrrongylus brasiliensis*[18]. Furthermore, *S. venezuelensis*–infected mice had a greater proportion of $Fc\varepsilon RI^+$c-Kit$^+$ cells (mast cells) in those fractions (0.02% in naive mice compared with 0.39% in infected mice). We purified basophils from the spleens of naive mice and infected mice (**Fig. 1a**) and examined their production of cytokines and expression of MHC class II molecules. Splenic basophils from infected mice cultured for 24 h with IL-3 produced large amounts of IL-4, IL-6 and IL-13, whereas those from naive mice produced small amounts of these T_H2 cytokines, although both types of basophils produced similar amounts of IL-10 (**Fig. 1b**). However, the production of IL-17A, interferon-γ (IFN-γ) and tumor necrosis factor was low (**Fig. 1b**). As reported before[27], basophils from infected mice were able to produce IL-4 without IL-3 stimulation, whereas basophils from naive mice did not produce IL-4 in the absence of IL-3 *in vitro* (**Supplementary Table 1** online), which suggests that basophils in infected mice gain the ability to produce substantial IL-4 even in the absence of IL-3. Flow cytometry of basophils from naive mice and infected mice showed that they had abundant and comparable expression of MHC class II (**Fig. 1c**). Peripheral blood basophils from naive mice also expressed MHC class II molecules (**Fig. 1c**).

As splenic basophils from infected mice expressed MHC class II and had the potential to produce substantial IL-4, IL-6 and IL-13 in cultures containing IL-3, we next examined their ability to induce ovalbumin (OVA)-specific naive $CD4^+$ T cells to develop into T_H2 cells *in vitro* in the presence of OVA peptide (amino acids 323–339 (OVA(323–339)), IL-2 and IL-3 without IL-4 ('neutral' culture conditions). We simultaneously cultured naive $CD4^+$ T cells with conventional antigen-presenting cells (APCs; T cell–depleted splenic cell samples (ΔT-spleen cells)) in the presence of OVA(323–339) in neutral conditions (**Fig. 1d**). Splenic basophils from *S. venezuelensis*–infected mice showed a notable ability to induce naive $CD4^+$ T cells to develop into T_H2 cells (**Fig. 1d**). In contrast, as reported elsewhere[25], conventional APCs failed to induce T_H2 cells in these neutral conditions, although both types of APC strongly induced the development of T_H2 cells in T_H2 conditions (**Fig. 1d**). We found that like typical T_H2 cells that developed in T_H2 conditions

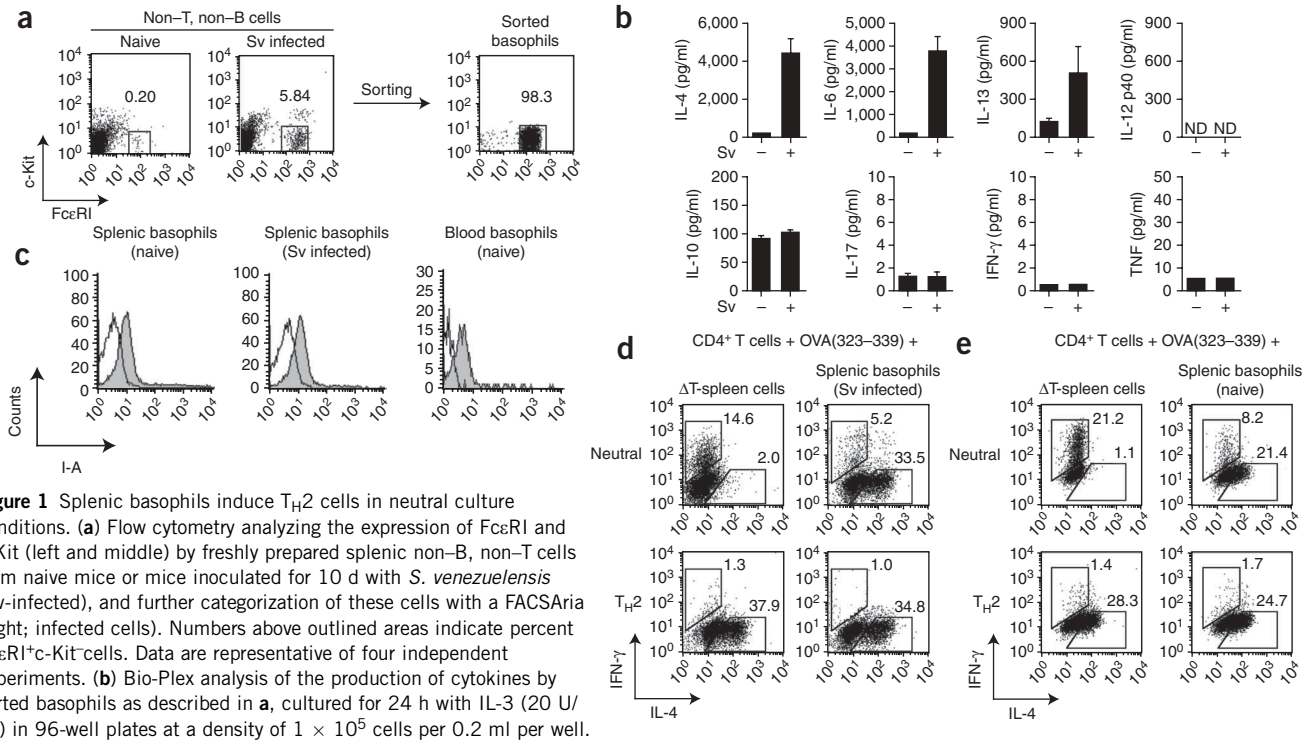

Figure 1 Splenic basophils induce T_H2 cells in neutral culture conditions. (**a**) Flow cytometry analyzing the expression of FcεRI and c-Kit (left and middle) by freshly prepared splenic non–B, non–T cells from naive mice or mice inoculated for 10 d with *S. venezuelensis* (Sv-infected), and further categorization of these cells with a FACSAria (right; infected cells). Numbers above outlined areas indicate percent $Fc\varepsilon RI^+$c-Kit$^-$cells. Data are representative of four independent experiments. (**b**) Bio-Plex analysis of the production of cytokines by sorted basophils as described in **a**, cultured for 24 h with IL-3 (20 U/ml) in 96-well plates at a density of 1×10^5 cells per 0.2 ml per well. ND, not detected. Data are representative of two independent experiments (mean and s.e.m. of three mice). (**c**) Flow cytometry of sorted splenic basophils from naive mice (left) or *S. venezuelensis*–infected mice (middle), stained for MHC class II molecules (I-A), and of peripheral blood mononuclear cells from naive mice, stained for MHC class II molecules and gated on $Fc\varepsilon RI^+DX5^+B220^-CD3^-$ cells (right). Filled histograms, markers; lines, unstained cells. Data are representative of two independent experiments with five mice. (**d**,**e**) Flow cytometry analyzing cytosolic IL-4 and IFN-γ in naive splenic DO11.10 $CD4^+CD62L^+$ T cells (1×10^5 cells per ml) stimulated for 7 d in 48-well plates with IL-2 (100 pM), IL-3 (20 U/ml) and OVA(323–339) (1 μM) in the presence of irradiated BALB/c ΔT-spleen cells or irradiated splenic basophils (5×10^5 cells per ml each) from *S. venezuelensis*–infected mice (**d**) or naive mice (**e**), with (T_H2) or without (Neutral) IL-4 (1,000 U/ml), then washed and recultured for 4 h with the phorbol ester PMA (50 ng/ml) plus ionomycin (0.5 μg/ml). Numbers adjacent to outlined areas indicate percent IL-4$^+$ or IFN-γ$^+$ cells gated on $CD4^+$ T cells. Data are representative of three independent experiments.

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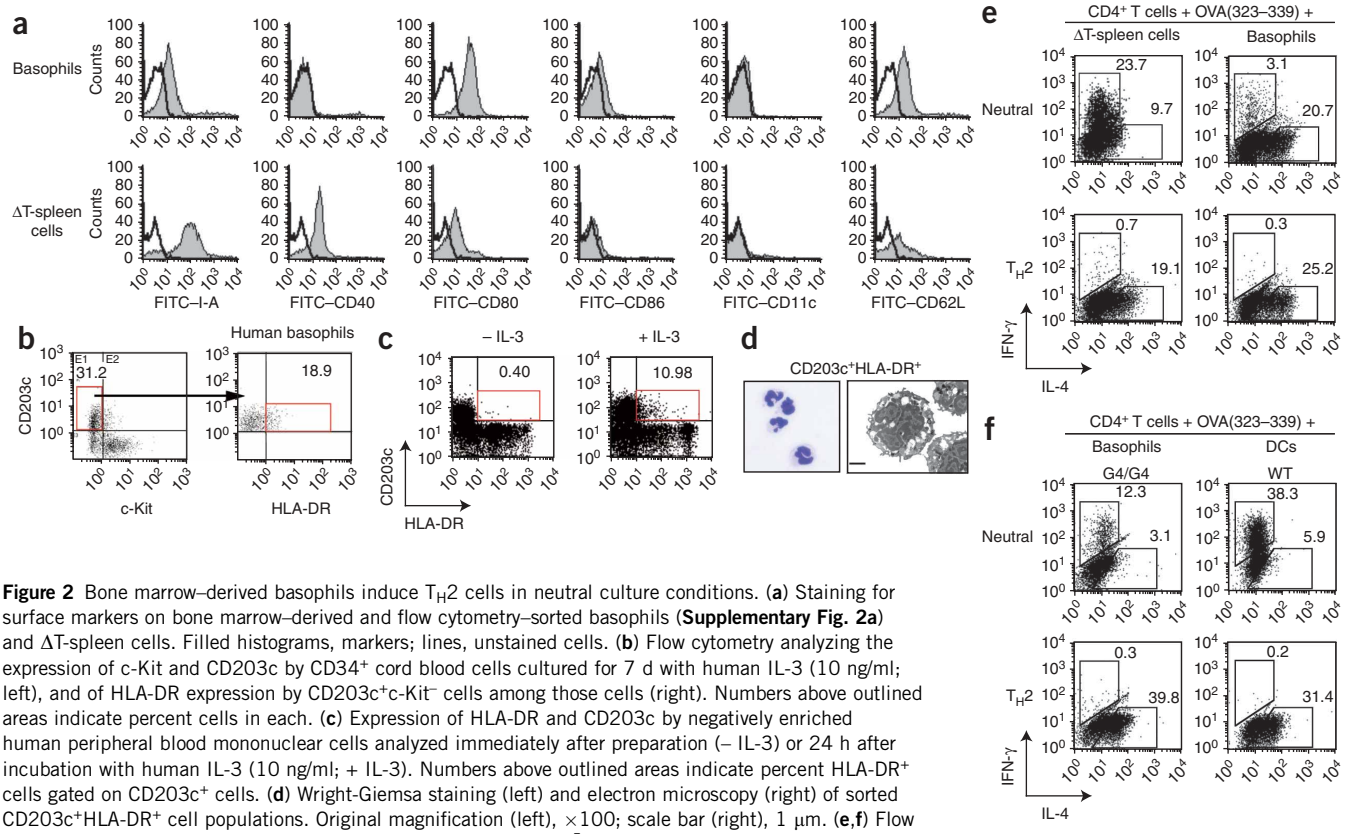

Figure 2 Bone marrow–derived basophils induce T_H2 cells in neutral culture conditions. (**a**) Staining for surface markers on bone marrow–derived and flow cytometry–sorted basophils (**Supplementary Fig. 2a**) and ΔT-spleen cells. Filled histograms, markers; lines, unstained cells. (**b**) Flow cytometry analyzing the expression of c-Kit and CD203c by $CD34^+$ cord blood cells cultured for 7 d with human IL-3 (10 ng/ml; left), and of HLA-DR expression by $CD203c^+$c-Kit^- cells among those cells (right). Numbers above outlined areas indicate percent cells in each. (**c**) Expression of HLA-DR and CD203c by negatively enriched human peripheral blood mononuclear cells analyzed immediately after preparation (– IL-3) or 24 h after incubation with human IL-3 (10 ng/ml; + IL-3). Numbers above outlined areas indicate percent HLA-DR^+ cells gated on $CD203c^+$ cells. (**d**) Wright-Giemsa staining (left) and electron microscopy (right) of sorted $CD203c^+$HLA-DR^+ cell populations. Original magnification (left), ×100; scale bar (right), 1 μm. (**e**,**f**) Flow cytometry of naive splenic DO11.10 $CD4^+CD62L^+$ T cells (1×10^5 cells per ml) stimulated and analyzed as described in **Figure 1d**, with irradiated ΔT-spleen cells, purified bone marrow–derived wild-type (WT) or IL-4-deficient (G4/G4) basophils or wild-type splenic DCs as APCs. Data are representative of three or four independent experiments.

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(**Fig. 1d**), T_H2 cells that developed after culture of naive $CD4^+$ T cells together with basophils in neutral culture conditions (**Fig. 1d**) produced IL-4, IL-5, IL-6, IL-10 and IL-13 (**Supplementary Fig. 1a** online), which suggested that these were true T_H2-polarized cells.

Next we assessed whether basophils from naive mice were also able to induce the development of T_H2 cells *in vitro*. We found that those splenic basophils also had a potent T_H2 cell–inducing function (**Fig. 1e**). As expected, some of the T_H2 cells induced in neutral conditions (**Fig. 1d**) expressed T_H2 cell marker IL-33Rα[31] (**Supplementary Fig. 1b**) and increased their production of T_H2 cytokines other than IL-4 when challenged with antigen plus IL-33 *in vitro* (**Supplementary Fig. 1c**).

Bone marrow basophils induce T_H2 cells *in vitro*

We next examined the ability of highly purified bone marrow basophils (**Supplementary Fig. 2a** online), devoid of other potential APCs, to induce T_H2 cell development *in vitro*. We first examined their expression of MHC class II molecules and the costimulatory molecules CD80 and CD86 (**Fig. 2a**). We simultaneously examined the expression of these molecules by conventional APCs. Bone marrow–derived basophils and conventional APCs expressed MHC class II, CD80 and CD86 but not CD11c. Basophils also expressed the lymph node–homing molecule CD62L, which suggested their potential to enter into lymphoid tissues[32]. As reported before[33], a fraction of human immature basophils ($CD203c^+$c-Kit^-) derived from cord blood expressed HLA-DR (18.9%; **Fig. 2b**). Although immature basophils decrease their expression of HLA-DR after maturation[33], we found mature peripheral blood basophils re-expressed HLA-DR after being cultured for 24 h with IL-3 (**Fig. 2c**,**d** and **Supplementary Fig. 2b**). In contrast, mouse peripheral basophils that expressed MHC class II failed to increase this expression in IL-3-containing medium (**Supplementary Fig. 2c**).

We compared the ability of conventional APCs and basophils to induce T_H2 cell development *in vitro* in neutral and T_H2 conditions (**Fig. 2e**). In the absence of any other APC, bone marrow–derived basophils were able to induce naive $CD4^+$ T cells to develop into T_H2 cells in neutral culture conditions as described above (20.7%), whereas conventional APCs induced T_H2 cells only in T_H2 conditions (**Fig. 2e**). Additional IL-4 stimulation (T_H2 conditions) resulted in an only modestly enhanced capacity of basophils to induce T_H2 cell development (25.2%; **Fig. 2e**), which suggests that bone marrow basophils produce sufficient IL-4 for maximum development of T_H2 cells. Indeed, basophils from IL-4-deficient G4/G4 mice[34] could not induce the development of T_H2 cells (3.1%) in neutral conditions (**Fig. 2f**). However, such IL-4-deficeint basophils did induce T_H2 cells in T_H2 conditions (39.8%), which allowed us to conclude that endogenous IL-4 from basophils was essential for the development of naive $CD4^+$ T cells into T_H2 cells. As reported elsewhere[25], splenic DCs induced T_H2 cells only in T_H2 cell–inducing conditions (**Fig. 2f**).

Basophils pulsed with antigen-IgE complexes are potent APCs

It was important to demonstrate the ability of basophils to take up and process OVA protein into OVA(323–339). We used 2,4-dinitrophenyl (DNP)-conjugated OVA (DNP-OVA) instead of OVA protein in this experiment and subsequent experiments, as DNP-OVA can also yield OVA(323–339) after processing. We were able to induce OVA-specific

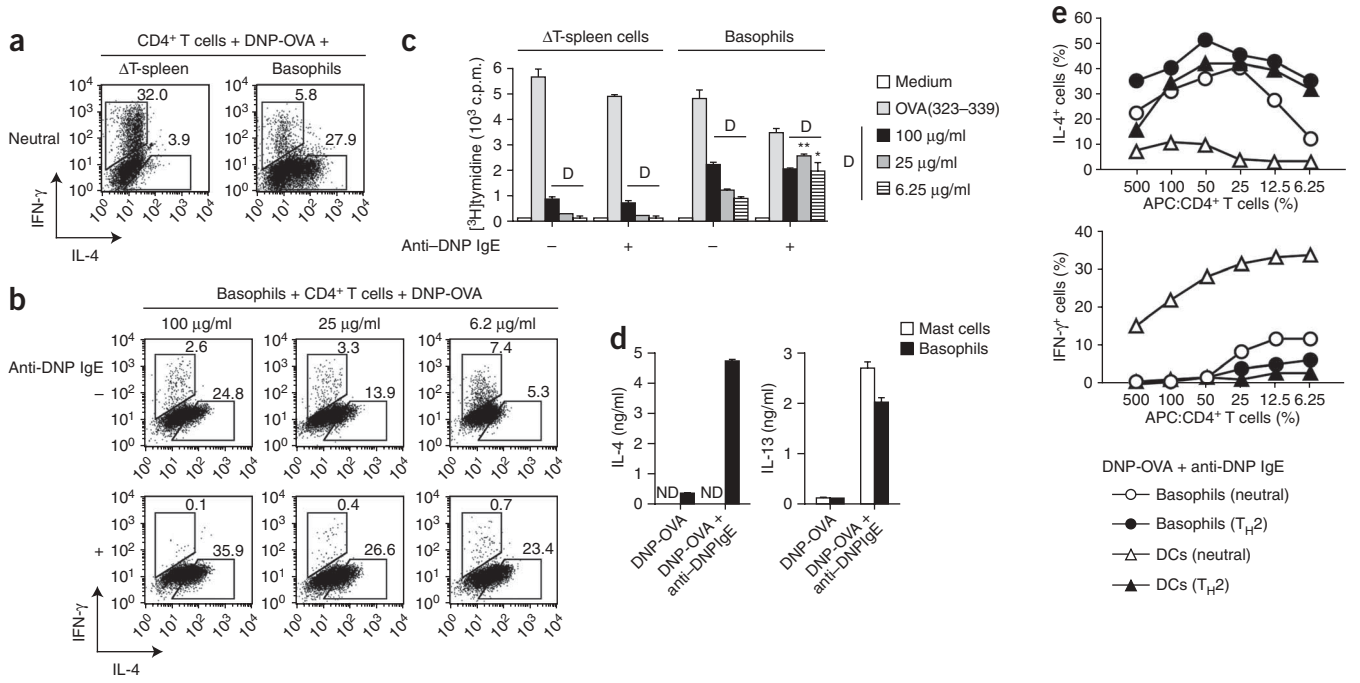

Figure 3 IgE complex enhances uptake of OVA by basophils. (**a**,**b**) Flow cytometry (as described in **Fig. 1d**) of naive splenic DO11.10 CD4+CD62L+ T cells (1×10^5 cells per ml) stimulated for 7 d with IL-2, IL-3 and DNP-OVA (100 μg/ml; **a**), or DNP-OVA (6.25–100 μg/ml) with or without IgE anti-DNP (10 μg/ml; **b**) in the presence of irradiated ΔT-spleen cells or bone marrow–derived basophils (5×10^5 cells per ml each). (**c**) Proliferative responses of naive splenic DO11.10 CD4+CD62L+ T cells (5×10^4 cells per 0.2 ml per well) cultured for 4 d in 96-well plates with OVA(323–339) (1 μM) or DNP-OVA (D; 6.25–100 μg/ml; in key) with or without IgE anti-DNP (10 μg/ml). *, $P < 0.05$ and **, $P < 0.005$, versus cells without anti–DNP IgE (Student's *t*-test). (**d**) Cytokine production by bone marrow–derived basophils or mast cells (5×10^5 cells per ml each) stimulated for 16 h in 48-well plates with IL-3 and DNP-OVA (100 μg/ml) with or without IgE anti-DNP (10 μg/ml). (**e**) Flow cytometry (as described in **Fig. 1d**) of naive splenic DO11.10 CD4+CD62L+ T cells (1×10^5 cells per ml) stimulated for 7 d with IL-2, IL-3, DNP-OVA (100 μg /ml) and IgE anti-DNP (10 μg /ml) in the presence of various numbers (6.25×10^3 to 5×10^5 cells per ml) of irradiated splenic DCs or bone marrow basophils with IL-4 (T_H2) or without IL-4 (Neutral). Data are representative of four (**a**–**d**) or two (**e**) independent experiments (mean and s.e.m. in **c**,**d**).

T_H2 cells by culturing naive CD4+ T cells with basophils in the presence of IL-2, IL-3 and DNP-OVA (100 μg/ml) without IL-4 (**Fig. 3a**). Again, conventional APCs failed to induce the development of T_H2 cell in these neutral culture conditions (**Fig. 3a**). Thus, basophils are able to process DNP-OVA into OVA(323–339) and to display peptide fragment in association with MHC class II and to produce IL-4.

It was also important to demonstrate the unique potential of basophils to increase their capacity to act as APCs when pulsed with antigen in the presence of antigen-specific IgE. Thus, we pulsed basophils with various doses of DNP-OVA in the presence or absence of monoclonal antibody to DNP (IgE anti-DNP; **Fig. 3b**). Basophils pulsed with a low dose (6.2 μg/ml) of DNP-OVA modestly induced T_H2 cells (5.3%), whereas pulsation with a higher dose (100 μg/ml) of DNP-OVA resulted in a higher proportion of 24.8%. The addition of IgE anti-DNP resulted in much higher proportions, particularly at lower concentrations of DNP-OVA (no IgE anti-DNP, 5.3%, 13.9% and 24.8%, versus with IgE anti-DNP, 23.4% 26.6% and 35.9%, for 6.2 μg/ml, 25 μg/ml and 100 μg/ml of DNP-OVA, respectively; **Fig. 3b**). Thus, the enhancing effect of IgE anti-DNP on basophil-induced T_H2 cell development was most apparent when basophils were pulsed with low concentrations of DNP-OVA.

We next examined whether IgE anti-DNP could enhance the capacity of conventional APCs to function as APCs. Thus, we cultured OVA(323–339)-specific T cells with conventional APCs or basophils in the presence of OVA(323–339) or DNP-OVA with or without IgE anti-DNP. Basophils pulsed with DNP-OVA in the presence of IgE anti-DNP had a significantly greater capacity to induce the proliferation of OVA–specific T cells (**Fig. 3c**). In contrast, conventional APCs pulsed with DNP-OVA in the presence of IgE anti-DNP did not have a greater capacity to induce T cell proliferation (**Fig. 3c**). These results suggest that basophils, taking advantage of their expression of FcεRI, might efficiently take up low doses of antigen in an IgE-dependent way.

As reported before[35], basophils had a much greater capacity to produce IL-4 and IL-13 after being pulsed with DNP-OVA in the presence of IgE anti-DNP (**Fig. 3d**). In contrast, mast cells pulsed with DNP-OVA–IgE anti-DNP immune complexes produced only IL-13, not IL-4 (**Fig. 3d**). We prepared basophils and mast cells from bone marrow cells cultured for 14 d with IL-3 (**Supplementary Fig. 2a**). We stimulated those cells with mixture of DNP-OVA and IgE anti-DNP. Mast cells prepared from bone marrow cells cultured for 4–6 weeks in medium conditioned by mouse leukemic WEHI-3 cells (containing IL-3) are reported to produce IL-4 after sequential IgE anti-DNP sensitization and subsequent DNP-protein challenge[36]. In addition to that unique function, we found that only basophils, when stimulated with IL-3 plus IL-18, IL-33, peptidoglycan or lipopolysaccharide, produced both IL-4 and IL-13 (**Supplementary Fig. 3a**,**b** online). Thus, basophils became producers of large amounts of IL-4 in *S. venezuelensis*–infected mice (**Fig. 1b**) or *in vitro* when stimulated with DNP-OVA–anti-DNP IgE in the presence of IL-3. Next we compared the APC activity of basophils and sorted CD11c+ splenic DCs[37] by changing the ratio of APCs to naive CD4+ T cells. Again, only basophils 'preferentially' induced the development of T_H2 cells in

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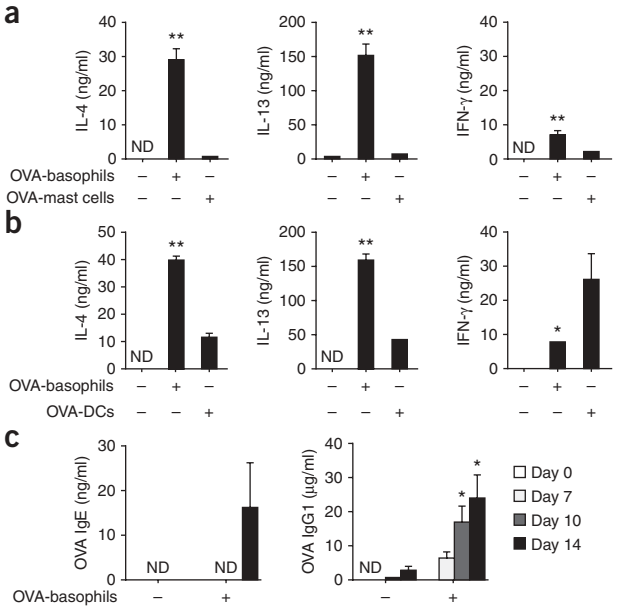

Figure 4 Intravenous administration of OVA-pulsed basophils induces a T_H2 response. (**a**,**b**) Enzyme-linked immunosorbent assay (ELISA) of IL-4, IL-13 and IFN-γ in supernatants of cells prepared as follows: purified bone marrow–derived basophils, mast cells (**a**) or splenic DCs (**b**) stimulated with DNP-OVA plus IgE anti-DNP as described in **Figure 3d** were adoptively transferred into BALB/c mice through the tail vein (2.5×10^5 cells of each per mouse); 4 d later, mice were intravenously challenged with OVA protein (100 μg per mouse) and, 2 d after this challenge, splenic $CD4^+$ T cells from each mouse were restimulated for 5 d in 96-well plates with OVA protein (100 μg/ml) in the presence of irradiated ΔT-spleen cells (1×10^5 cells per 0.2 ml per well for all cells). OVA- (left margin), processed OVA. (**c**) ELISA of OVA-specific IgE and IgG1 in serum from mice treated as follows: after priming of basophils as described in **a**, basophils (5×10^5 cells per mouse) were adoptively transferred into BALB/c mice through the tail vein; 1 week later (day 0), all mice were intravenously challenged with OVA protein (100 μg per mouse) and serum was then collected on days 0–14 (key). *, $P < 0.05$ and **, $P < 0.0001$, OVA-pulsed basophils versus cells from mice given mast cells and injected with OVA protein (**a**), given DCs and injected with OVA protein (**b**) or injected with OVA protein alone (**c**; Student's *t* test). Data are representative of two independent experiments (mean and s.e.m. of five mice).

neutral conditions. Furthermore, basophils had this T_H2-inducing capacity even when the ratio of basophils to $CD4^+$ T cells was decreased to 1:8. As expected, in T_H2 conditions, both types of APCs showed similar APC function. Furthermore, we were able to decrease the ratio of APC to $CD4^+$ T cells to 1:16 without substantially diminishing T_H2 cell development (**Fig. 3e**). In contrast, DCs 'preferentially' induced IFN-γ-producing cells in neutral conditions. Thus, basophils incubated with DNP-OVA and IgE anti-DNP showed very potent OVA-specific T_H2 cell–inducing activity *in vitro*.

OVA-pulsed basophils induce T_H2 cells *in vivo*

We compared the activity of OVA-pulsed basophils and mast cells to induce T_H2 cells *in vivo*. We pulsed basophils and mast cells with OVA by culturing cells with complexes of DNP-OVA and IgE anti-DNP, then transferred these basophils or mast cells into normal mice through the tail vein. Then, 4 d later, we challenged mice intravenously with intact OVA protein in PBS, and 2 d after this challenge, we prepared splenic $CD4^+$ T cells and stimulated them with OVA–pulsed APCs (ΔT-spleen cells) and measured IL-4, IL-13 and IFN-γ in the culture supernatants. We found that only OVA-pulsed basophils promptly and strongly induced T_H2 cells and modestly induced T_H1 cells in the spleen (**Fig. 4a**). We also examined the effect of OVA-pulsed DCs on T_H1 and T_H2 response *in vivo*. Intravenous administration of OVA-pulsed DCs into naive mice dominantly induced T_H1 cells, whereas intravenous administration of OVA-pulsed basophils again induced T_H2 cells strongly and T_H1 cells moderately (**Fig. 4b**), which suggests the importance of basophils in inducing T_H2 responses *in vivo*.

We next examined the ability of mice immunized with OVA-pulsed basophils to produce OVA-specific IgE and IgG1 after OVA challenge. Intravenous administration of OVA solution in naive mice did not induce IgE response but induced a very modest IgG1 response (**Fig. 4c**). In contrast, mice primed with OVA-pulsed basophils produced both IgE and IgG1 in response to OVA (**Fig. 4c**). Indeed, these mice developed $CD4^+CD62L^{lo}IL\text{-}33R\alpha^+$ T_H2 cells in their spleens (**Supplementary Fig. 4a**,**b** online) that help OVA-activated B cells produce IgE and IgG1 *in vivo*.

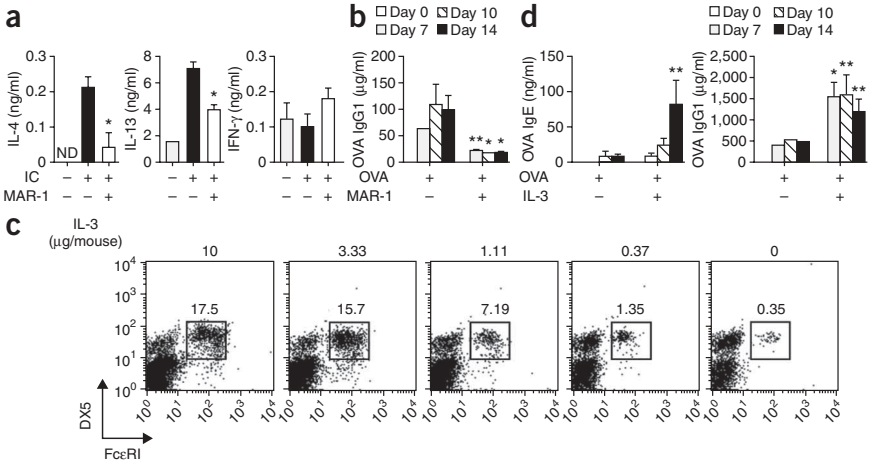

Figure 5 Intravenous administration of antigen-IgE complex induces T_H2 responses. (**a**,**b**) Analysis of cytokines (**a**) and antibodies (**b**) in BALB/c mice pretreated with PBS or anti-FcεRIa (MAR-1) and then injected intravenously with a mixture of DNP-OVA (10 μg) and IgE anti-DNP (20 μg; immune complex (IC)). (**a**) ELISA of IL-4, IL-13 and IFN-γ in supernatants of splenic $CD4^+$ T cells obtained 4 d after injection of the immune complex and restimulated as described in **Figure 4a**. (**b**) OVA-specific IgE and IgG1 antibodies in serum of mice intravenously challenged with OVA protein 4 d after injection of the immune complex, analyzed as described in **Figure 4c**. *, $P < 0.05$ and **, $P < 0.005$, compared with mice given immune complex without MAR-1 pretreatment (Student's *t*-test). (**c**) Frequency of basophils ($FcεRI^+DX5^+$ cells) in BALB/c mice injected with IL-3 (0–10 μg per mouse (above plots) for 2 weeks) via osmotic pump. Numbers above outlined areas indicate percent $FcεRI^+DX5^+$ cells gated on splenic non–B, non–T cells. (**d**) OVA-specific IgE and IgG1 in serum of BALB/c mice pretreated with PBS or IL-3 and then injected intravenously with immune complexes as described in **a**,**b**, then intravenously challenged 4 d later with OVA protein, assessed as described in **Figure 4c**. *, $P < 0.01$ and **, $P < 0.05$, compared with mice without IL-3 pretreatment (Student's *t*-test). Data are representative of two independent experiments (mean and s.e.m. of five mice; **a**,**b**,**d**) or two experiments with five mice (**c**).

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Antigen-IgE induces T_H2 cells in a basophil-dependent way

Finally, to demonstrate the contribution of basophils to the development and upregulation of T_H2-IgE response *in vivo*, we intravenously injected complexes of DNP-OVA and IgE anti-DNP into naive mice or mice depleted of basophils by treatment with anti-FcεRIα (MAR-1). As reported by others[38], daily injection of MAR-1 for 3 d almost completely depleted the spleen and liver of basophils (**Supplementary Fig. 5a**,**b** online). At day 4 after intravenous injection of DNP-OVA (10 μg) and IgE anti-DNP (20 μg), we prepared splenic $CD4^+$ T cells, stimulated them with OVA-pulsed APCs and measured IL-4, IL-13 and IFN-γ in the culture supernatants. We found that IgE immune complexes induced T_H2 cells in the spleens of naive mice (**Fig. 5a**). Depletion of basophils (**Supplementary Fig. 5c**,**d**) resulted in significantly diminished T_H2 cell development and T_H2-dependent IgG1 responses (**Fig. 5a**,**b**). These results suggest that basophils efficiently take up DNP-OVA–IgE anti-DNP immune complexes and induce OVA-specific T_H2 cells, which in turn stimulate OVA-stimulated B cells to produce IgG1.

To confirm the contribution of basophils to the initiation and amplification of T_H2-IgE responses, we injected IL-3 into naive mice using an osmotic pump (10 μg IL-3 per 100 μl PBS) to increase the number of basophils and then examined their responsiveness to the treatment with antigen-IgE complex. IL-3-treated mice markedly increased the number of basophils in their spleens (**Fig. 5c**) and other organs, somewhat resembling atopic people, who also increase the number of basophils in inflammation sites[8–11]. We found IL-3-treated mice died of systemic anaphylactic shock when challenged with a high dose of IgE complex (for example, 100 μg DNP-OVA and 200 μg IgE anti-DNP). Therefore, we intravenously injected low doses of IgE complex (5 μg DNP-OVA and 10 μg IgE anti-DNP). In contrast to mice that received no pretreatment with IL-3, IL-3-treated mice significantly increased their production of OVA-specific IgG1 and IgE in response to OVA challenge (**Fig. 5d**), which suggests that the number of basophils might determine the responsiveness to IgE complex that 'preferentially' induces T_H2 cells. These results collectively indicated that basophils are responsible for inducing OVA-specific T_H2 cells by taking up DNP-OVA–IgE anti-DNP complexes, presenting OVA peptide with MHC class II and producing abundant IL-4.

DISCUSSION

Basophils can induce T_H2 cells *in vitro* and *in vivo* by producing early IL-4 (refs. 18,20,29,39). Other studies have shown that basophils that transmigrate to draining lymph nodes after papain stimulation are stimulated to produce IL-4 and/or thymic stromal lymphopoietin, which promote T_H2 differentiation *in vivo*[29]. However, it has remained uncertain whether basophil-derived IL-4 is indeed involved in the development of T_H2 cells in response to stimuli other than protease allergens.

Here we have demonstrated that protein antigen without enzymatic activity induced antigen-specific T_H2 cells *in vitro* and *in vivo* in a basophil- and IL-4-dependent way, which suggests involvement of basophil-derived IL-4 in the development of T_H2 cells. Another important issue that needs to be addressed is the mechanism by which basophils induce T_H2 cells. We could propose at least two mechanisms for basophil-mediated promotion of T_H2 responses. One is that basophils produce IL-4 and/or thymic stromal lymphopoietin and simply transfer foreign protein to DCs, which do the actual antigen presentation. The other is that basophils are APCs that also produce IL-4. We have demonstrated that basophils induce T_H2 cells in the absence of 'professional' APCs *in vitro*. Furthermore, we have shown that basophils are potent APCs that directly and 'preferentially' induce T_H2 cells *in vivo*.

We first demonstrated that basophils derived from mice inoculated with *S. venezuelensis* produced substantial amounts of IL-4, IL-6 and IL-13 in IL-3-containing medium. Then we demonstrated that they expressed MHC class II and strongly induced the development of naive $CD4^+$ T cells into T_H2 cells in neutral conditions. In contrast, basophils from naive mice produced relatively small amounts of IL-4, IL-6 and IL-13 in IL-3-containing medium.

We initially regarded only basophils from infected mice as potent APCs. Then we recognized that basophils from naive mice and infected mice expressed almost identical amounts of MHC class II, which suggested they had a potent APC function. Indeed, splenic basophils in naive mice were also immunologically competent APCs. Both types of basophils produced substantial amounts of IL-10, which suggests the possibility that this IL-10 might enhance the ability of IL-4 from basophils to induce the development of T_H2 cells *in vitro*. Bone marrow basophils also recapitulated well the APC function of splenic basophils. In particular, they were able to efficiently take up a low dose of antigen-IgE complex, present antigen–MHC class II and produce IL-4, which suggests that they are also very potent T_H2 cell–inducing APCs.

We further demonstrated that intravenous administration of OVA-pulsed basophils, which we prepared by culturing basophils with complexes of DNP-OVA and IgE anti-DNP, strongly induced OVA-specific T_H2 cells in the spleens of naive mice. In contrast, OVA-pulsed mast cells failed to do so. These results indicated their difference in inducing antigen-specific T_H2 cells *in vivo*. Basophils have been shown to have a very short half-life after adoptive transfer[40]. Because we cultured basophils with immune complexes of DNP-OVA and IgE anti-DNP, we suspect such crosslinking might induce signals that sustain their survival *in vivo*.

Finally, we demonstrated a single intravenous administration of a low dose of DNP-OVA–IgE anti-DNP complex into naive mice rapidly and 'preferentially' induced OVA-specific T_H2 cells in an endogenous basophil–dependent way. Such sensitized mice then promptly produced antigen-specific IgG1 in response to intravenous administration of antigen solution. As expected, IL-3 treatment prepared mice highly susceptible to the T_H2 cell–inducing action of IgE complex by increasing the number of basophils.

Although basophil MHC class II expression was less than that on conventional APCs, basophils showed more potent T_H2 cell–inducing activity than did conventional APCs in both neutral and T_H2 conditions. We found that basophils had a greater APC activity when pulsed with DNP-OVA in the presence of IgE anti-DNP. We also found that basophils still had the notable T_H2 cell–inducing ability in neutral conditions even when the ratio of APCs to $CD4^+$ T cells was low (1:8). In contrast, OVA-pulsed DCs failed to induce T_H2 cells at any APC/$CD4^+$ T cell ratio in neutral conditions, although they induced IFN-γ-producing cells. Thus, basophils are very potent T_H2 cell–inducing cells *in vitro*.

Our study has indicated that endogenous basophils are important for promotion of the T_H2-IgE response *in vivo*. We demonstrated that intravenous administration of immune complexes of DNP-OVA and IgE anti-DNP 'preferentially' induced OVA-specific T_H2 cells in an endogenous basophil–dependent way. Studies have shown that basophils can also capture antigen by binding to surface antigen-specific IgE-FcεRI (refs. 38,41). Activated basophils then produce IL-4 and IL-6 and possibly express CD40L[42], the ligand for the costimulatory molecule CD40, which in combination induce B cells to proliferate and to produce IgE. Thus, basophils promote both T_H2 and IgE responses *in vivo*.

Atopic people are characterized by having more basophils in sites of allergic inflammation[8–11]. Once atopic people start to produce antigen-specific IgE, they can steadily increase the amount of complexes of

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antigen and antigen-specific IgE, which allows basophils to augment their uptake of IgE complex. Although mature human basophils lack HLA-DR, we have shown here that some can re-express HLA-DR when stimulated with IL-3. Thus, it is plausible that mature basophils in the allergic inflammation site, which might be characterized by abundant production of IL-3 and other factors, do express HLA-DR. Basophils, then, could become potent APCs and induce progressive allergic inflammation in these people.

Here we have demonstrated that basophils are important in the amplification of the T_H2-IgE response. Indeed, depletion of basophils by a specific antibody inhibited IgE complex–induced T_H2-IgE responses. Published work has suggested that anti-IgE therapy is effective for T_H2-IgE–mediated diseases[43–45]. The rationale for such therapy is that it is believed to interfere with IgE-mediated activation of mast cells and basophils. On the basis of our results here, we can add another rationale: inhibition of the generation of antigen-pulsed basophils. Thus, basophils might represent an important therapeutic target cell.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureimmunology/.

Accession codes. UCSD-Nature Signaling Gateway (http://www.signaling-gateway.org): A000543 and A001262.

Note: Supplementary information is available on the Nature Immunology website.

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AUTHOR CONTRIBUTIONS

K.N. and T.Y. envisaged the possible APC function of basophils; T.Y. and K.N. designed the experiments; T.Y. did the main part of this study and analyzed the data; K.Y., M.N. and Y.I. helped with some experimental procedures; H.T. and Y.F. analyzed human cells; and T.Y. prepared the draft of manuscript and K.N. completed it.

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ONLINE METHODS

Mice. BALB/c mice were from Jackson Laboratory. Mice transgenic for αβ TCR recognizing OVA(323–339) (DO11.10) and BALB/c G4-homozygous (IL-4-deficient) mice[34] were bred in specific pathogen–free conditions at the animal facilities of Hyogo College of Medicine. All animal experiments were done in accordance with guidelines of the Institutional Animal Care Committee of Hyogo College of Medicine.

Antibodies and reagents. Anti–mouse IL-4 (11B11)[25] was purified in our laboratory in the Department of Immunology and Medical Zoology, Hyogo College of Medicine. Phycoerythrin (PE)–anti–mouse CD4 (GK1.5), fluorescein isothiocyanate (FITC)–anti–mouse CD62L (MEL-14), FITC–anti–mouse I-A^d (AMS-32.1), FITC–anti–mouse CD40 (HM40-3), FITC–anti–mouse CD80 (16-10A1), FITC–anti–mouse CD86 (GL1), FITC–anti–mouse CD11c (HL3), PE–anti–mouse c-Kit (2BB), FITC–anti–mouse c-Kit (2BB), FITC–anti–mouse CD49b (DX5), FITC–anti–human HLA-DR (TÜ36) and biotin–human CD203c (FR3–16A11) were from BD Biosciences. FITC–anti–mouse ST2 (DJ8), biotin–anti–mouse FcεRIα (MAR-1), streptavidin-PE and streptavidin-allophycocyanin were from eBioscience. The following PE-labeled monoclonal antibodies to human cell surface markers were from BD Biosciences: anti-CD3 (HIT3a), anti-CD7 (M-T701), anti-CD14 (M5E2), anti-CD15 (HI98), anti-CD16 (3G8), anti-CD19 (HIB19), anti-CD36 (CB38), anti-CD45RA (HI100) and anti-CD235a (GAR-2). Recombinant mouse IL-2, IL-3, IL-4 and human IL-3 were from R&D Systems. IL-18 was from MBL. Recombinant human IL-33 was purified in our laboratory in the Department of Immunology and Medical Zoology, Hyogo College of Medicine[28]. Monoclonal IgE anti-DNP (SPE-7), OVA (grade V), lipopolysaccharide from *Salmonella minnesota* Re-595 or *Escherichia coli* 055:B5, and peptidoglycan from *Staphylococcus aureus* were from Sigma. DNP-OVA was prepared according to a published method[46].

Flow cytometry and cell purification. For the preparation of bone marrow–derived basophils, bone marrow cells were cultured for 14 d with IL-3 (10 U/ml) in RPMI-1640 medium supplemented with 10% (vol/vol) FBS, 50 μM 2-mercaptoethanol, 2 mM L-glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin (complete RPMI) and were washed twice. Cells were first treated for 30 min at 4 °C with anti–FcγRII/III (10 μg/ml), followed by treatment for 1 h at 4 °C with biotin–anti–mouse FcεRIα (5 μg/ml) in staining buffer (1% (vol/vol) FCS in PBS). After being washed twice, cells were stained for 30 min with streptavidin-allophycocyanin and PE–anti–mouse c-Kit. Samples were analyzed on a FACSCalibur (BD Biosciences) and were separated into $FcεRI^+c\text{-}Kit^-$ cells (basophils) or $FcεRI^+c\text{-}Kit^+$ cells (mast cells) with a FACSAria (BD Biosciences). The purity of each population was over 96%. The resultant populations were further stained with FITC-labeled antibodies for analysis of surface makers. For preparation of splenic basophils, spleen cell samples from BALB/c mice were first depleted of Thy-1.2^+ T cells and $B220^+$ cells with a MACS system (MiltenyiBiotec), then the residual cells were further stained and separated into $FcεRI^+c\text{-}Kit^-$ or $FcεRI^+c\text{-}Kit^+$ cells with a FACSAria. The purity of each population was over 96%. For the preparation of splenic $CD4^+CD62L^+$ resting T cells and for intracellular cytokine staining, published methods were followed[47].

Human peripheral blood from normal volunteers and umbilical cord blood obtained from normal full-term deliveries were obtained and processed after informed consent was given. The Institutional Review Board approved the experimental plan. Mononuclear cells were isolated from peripheral blood and cord blood by Ficoll density-gradient centrifugation. Peripheral blood mononuclear cell samples were further depleted of T cells, monocytes, eosinophils, natural killer cells, B cells, platelets, DCs and erythroid cells magnetically with a 'cocktail' of PE-labeled monoclonal antibodies to human CD3, CD7, CD14, CD15, CD16, CD19, CD36, CD45RA and CD235a and anti-PE MicroBeads (MiltenyiBiotec). Umbilical cord blood mononuclear cells were further enriched to $CD34^+$ cells with MicroBeads. These $CD34^+$ progenitor cells were plated at a density of 5×10^5 cells per ml in 12-well plates and were cultured for 7 d in StemPro-34 SFM (GIBCO) supplemented with 10% (vol/vol) FBS, 50 μM 2-mercaptoethanol, 0.5 mM L-glutamine, 50 U/ml of penicillin, 50 μg/ml of streptomycin and 10 ng/ml of human IL-3.

***In vitro* culture.** Naive splenic $CD4^+CD62L^+$ T cells (1×10^5 cells per ml) from DO11.10 mice were stimulated in 48-well plates with IL-2 (100 pM), IL-3 (20 U/ml) and OVA(323–339) (1 μM) or DNP-OVA (6.25–100 μg/ml) in the presence of conventional APCs (irradiated T cell–depleted BALB/c splenocyte samples), irradiated splenic $CD11c^+$DCs prepared as described[37] or irradiated purified basophils (5×10^5 cells per ml each). For the induction of T_H2 cells, IL-4 (1000 U/ml) was also added to the culture. On the third or fourth day of culture, cells were diluted 1:2 or I:3 in complete RPMI medium with IL-2 (100 pM) and their populations were expanded into 48-well plates. Then, 7 d after the initial stimulation, cells were collected and washed, then were recultured for 4 h with PMA (phorbol 12-myristate 13-acetate; 50 ng/ml) plus ionomycin (500 ng/ml) and were analyzed by flow cytometry for cytosolic IL-4 and IFN-γ. In some experiments, after initial priming, $CD4^+$ T cells (1×10^5 cells per 0.2 ml per well) were restimulated for 48 h in 96-well plates with IL-2 (100 pM) and OVA(323–339) (1 μM) in the presence of 1×10^5 irradiated conventional APCs. Supernatants were collected and cytokine production was assessed with ELISA kits (R&D Systems) or the Bio-Plex system (BioRad) as described before[28].

***In vivo* treatment of mice.** Bone marrow–derived and flow cytometry–sorted basophils, mast cells and splenic $CD11c^+$ DCs (5×10^5 cells per ml each) were cultured for 16 h in 48-well plates with IL-3 (20 U/ml), DNP-OVA (100 μg/ml) and IgE anti-DNP (10 μg/ml). After priming, basophils, mast cells and splenic DCs (2.5×10^5 cells per mouse) were transferred through the tail vein into BALB/c mice. At 4 d or 1 week after reconstitution, mice were intravenously challenged with OVA protein (100 μg) in PBS. In some experiments, BALB/c mice were injected intravenously with a mixture of DNP-OVA (5–100 μg per mouse) and IgE anti-DNP (10–200 μg per mouse). For *in vivo* depletion of basophils, a published method of was followed[38]. Mice were injected intraperitoneally twice daily for 3 d with 5 μg anti–mouse FcεRIα (MAR-1) or PBS. Mice were allowed to 'rest' for 2 d and then were injected with a mixture of DNP-OVA plus IgE anti-DNP, then these mice were injected twice daily for additional 3 d with MAR-1 or PBS. IL-3 was infused subcutaneously into mice via osmotic pumps (Durect) filled with IL-3 (100 μg) in 100 μl PBS in mice as described[48].

ELISA. OVA-specific serum IgE was measured with a Mouse OVA-IgE ELISA kit (Dainippon Sumitomo Pharma). OVA-specific serum IgG1 was measured with a Mouse OVA-IgG1 ELISA kit (AKRIE-04; Shibayagi).

Parasites. BALB/c mice were subcutaneously inoculated with 5,000 *S. venezuelensis* third-stage larvae to initiate complete infection as described[27,30].

Electron microscopy. Sorted human $CD203c^+HLA\text{-}DR^+$ cells were fixed with 2% (wt/vol) paraformaldehyde and 1.25% (wt/vol) glutaraldehyde, were post-fixed with 1% (wt/vol) OsO_4 and were embedded in Epon. Ultrathin sections were double-stained with uranyl acetate and lead citrate and were examined with a JEM 1220 transmission electron microscopy (Jeol).

Proliferation assay. Naive splenic $CD4^+CD62L^+$ T cells from DO11.10 mice (5×10^4 cells per 0.2 ml per well) were stimulated for 4 d in 96-well plates with IL-2 (100 pM), IL-3 (20 U/ml) and OVA(323–339) (1 μM) or DNP-OVA (6.25–100 μg/ml) with or without monoclonal anti-DNP IgE (10 μg/ml) in the presence of conventional APCs or purified basophils (2.5×10^5 cells per well each). DNA synthesis was assessed by measurement of the incorporation of 0.2 μCi [^{3}H]thymidine during the final 16 h.

Analysis of expression of TLR mRNA. Total RNA was extracted from sorted basophils and mast cells with TRIzol reagent, was treated with DNase I and was reverse-transcribed with SuperScript II Reverse Transcriptase and oligo$(dT)_{12–18}$ primer (Invitrogen). As a positive control for each TLR, RNA extracted from total spleen cells was used. For analysis of expression of TLR mRNA, mRNA was amplified by a modified standard RT-PCR amplification procedure. The specific TLR primer sequences and their annealing

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temperatures were according to a published report[49]. PCR conditions were as follows: cDNA was amplified by 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, followed by further extension at 72 °C for 10 min, then samples were stored at 4 °C until analysis. After amplification, PCR products were separated by electrophoresis through 1.7% agarose gels and were visualized by illumination with ultraviolet light.

Statistics. Statistical comparisons between two experimental groups were made with a paired Student's *t*-test using GraphPad Instat Software. *P* values of less than 0.05 were considered significant.

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Basophils contribute to T_H2-IgE responses *in vivo* by interleukin 4 production and presentation of peptide-MHC class II complexes to $CD4^+$ T cells

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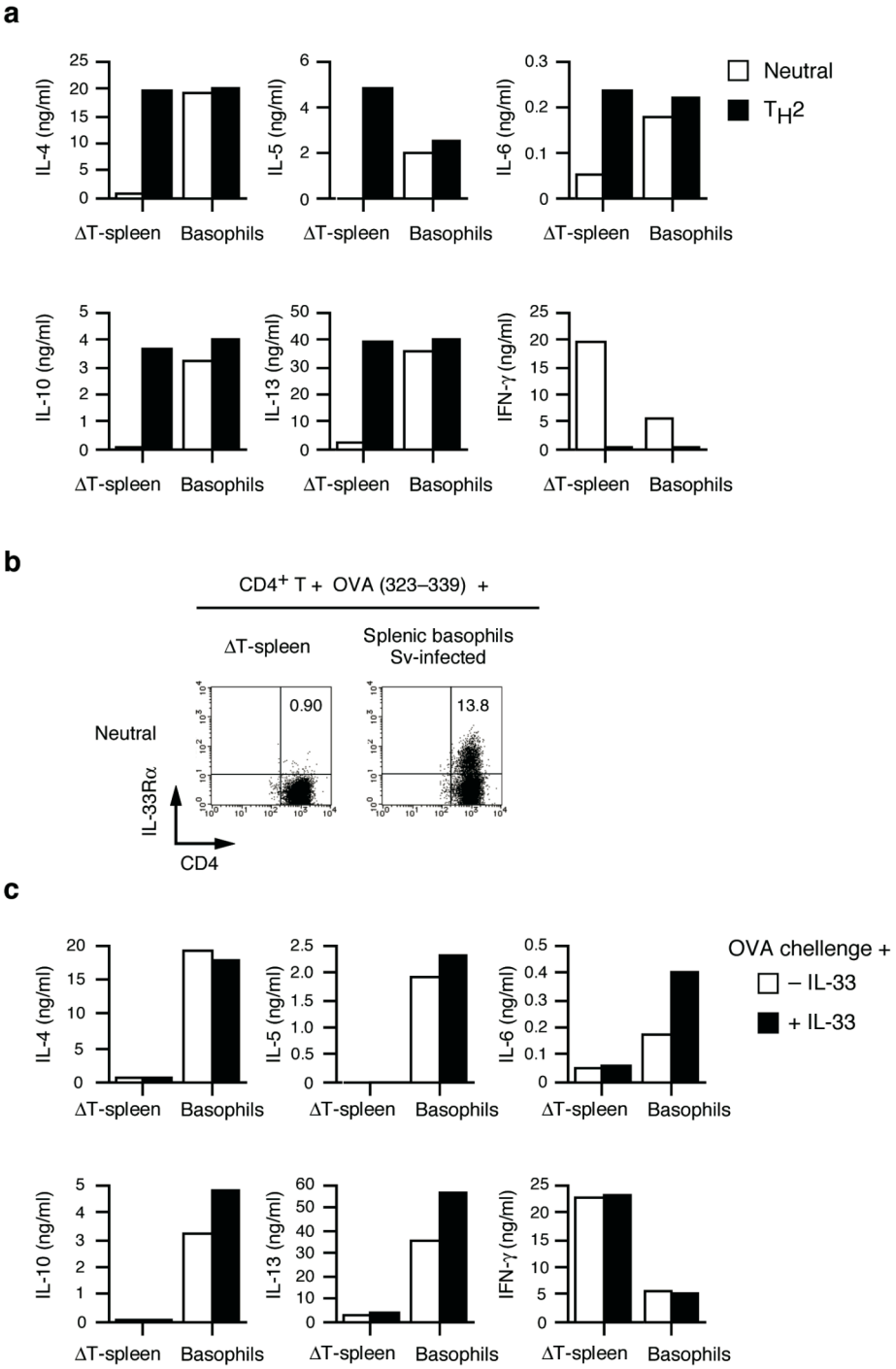
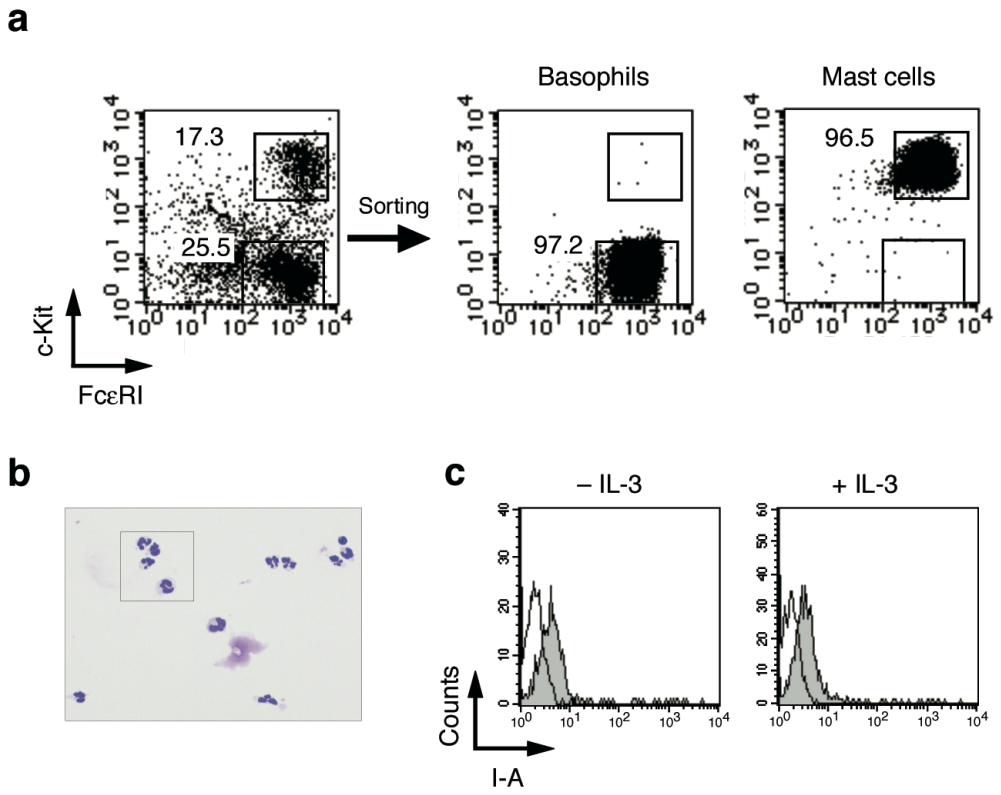
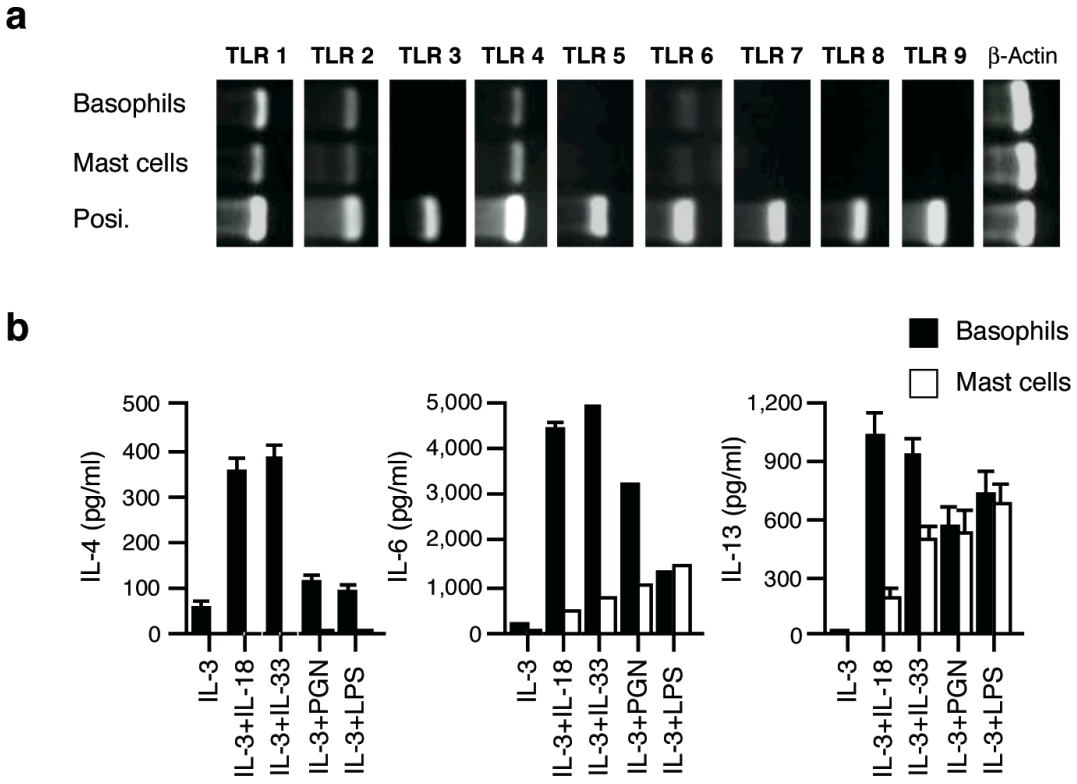

a
IL-4 (ng/ml)
IL-5 (ng/ml)
IL-6 (ng/ml)
Neutral
TH2
IL-10 (ng/ml)
IL-13 (ng/ml)
IFN-γ (ng/ml)
ΔT-spleen
Basophils
b
CD4+ T + OVA (323–339) +
ΔT-spleen
Splenic basophils
Sv-infected
Neutral
0.90
13.8
IL-33Rα
CD4
c
OVA chellenge +
– IL-33
+ IL-33

Supplementary Fig. 1. Cytokines production from basophils–driven T_H2 cells.
Naive splenic $CD4^+CD62L^+$ T cells ($1x10^5$/ml) from DO11.10 mice were stimulated with IL-2 (100 pM), IL-3 (20 U /ml) and $OVA_{323-339}$ (1 μM) in the presence of $5x10^5$/ml irradiated ΔT-spleen or irradiated splenic basophils from Sv–infected mice in the absence (Neutral) or presence (T_H2) of 1000 U/ml IL-4 in 48-well plates for 7 days. (**a**) After initial priming, $CD4^+$T cells ($1x10^5$/0.2 ml per well) were restimulated with IL-2 (100 pM) and $OVA_{323-339}$ (1 μM) in the presence of $1x10^5$ irradiated ΔT-spleen in 96–well plates for 48h. Supernatants were harvested and tested for cytokine production by the Bio-Plex system. (**b**) Surface expression of IL-33Rα chain on $CD4^+$ T cells, which were stimulated with ΔT-spleen or basophils from Sv–infected mice under neutral condition (Neutral) for 7 days by flow cytometry. The percentages shown represent the population of IL-33Rα chain $^+$ cells among $CD4^+$ cells. (**c**) After initial priming without IL-4 (Neutral), $CD4^+$T cells ($1x10^5$/0.2 ml per well) were restimulated with IL-2 (100 pM) and $OVA_{323-339}$ (1 μM) in the presence of $1x10^5$ irradiated ΔT-spleen in 96–well plates with or without IL-33 (100 ng/ml) for 48h. Supernatants were harvested and tested for cytokine production by the Bio-Plex system. Data are representative of three independent experiments.

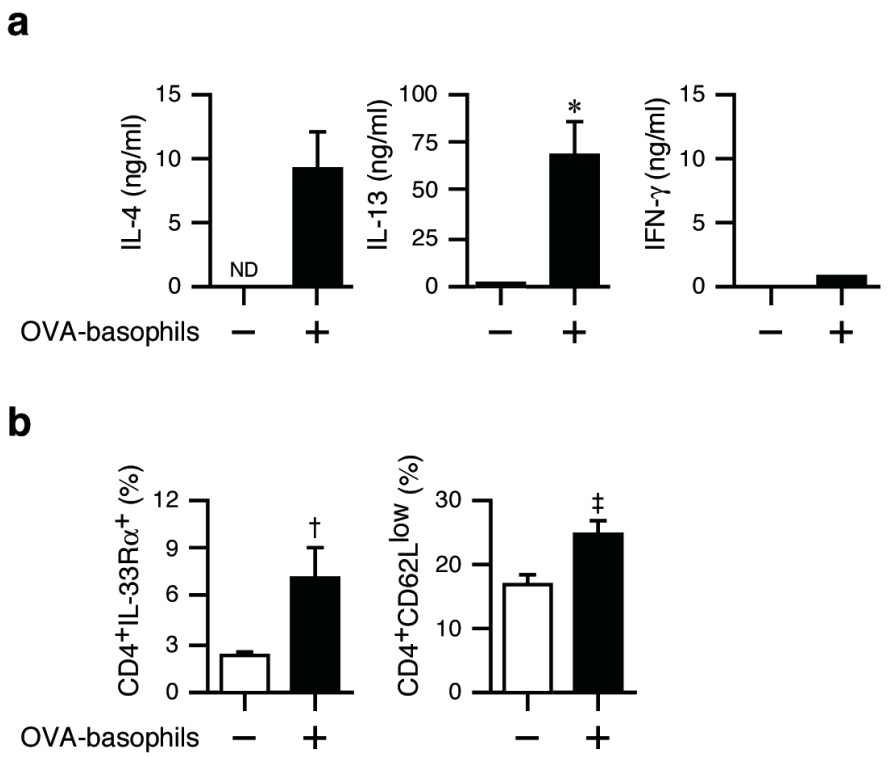


Supplementary Fig. 2. Mouse and human basophils.
(**a**) Bone marrow cells from BALB/c mice cultured with IL-3 (10 U/ml) for 14 days were analyzed for expression of FcεRI and *c-kit* by flow cytometry and then sorted into FcεRI^{+}/*c-kit*$^{-}$ (Basophils) or FcεRI^{+}/*c-kit*$^{+}$ (Mast cells) cell populations by FACS Aria. Percentages of cells in selected populations are indicated. (**b**) Negatively enriched human peripheral blood mononuclear cells stimulated with human IL-3 (10 ng/ml) for 24 h as described in **Figure 2 c**, were analyzed for the expression of HLA-DR and CD203c by flow cytometry and then sorted into CD203c^{+}/ HLA-DR^{+} cell populations by FACS Aria. Sorted CD203c^{+}/ HLA-DR^{+} cell populations were stained with Wright-Giemsa staining solution (20X). Boxed area is presented at higher magnification in **Figure 2 d**. (**c**) Mouse peripheral blood mononuclear cells cultured with or without IL-3 (20 U/ml) for 24 h *in vitro* were stained for MHC class II molecules gated on FcεRI^{+}/DX5^{+} /B220^{-}/ CD3^{-} cells. Shaded area, each marker staining; solid line, unstained.



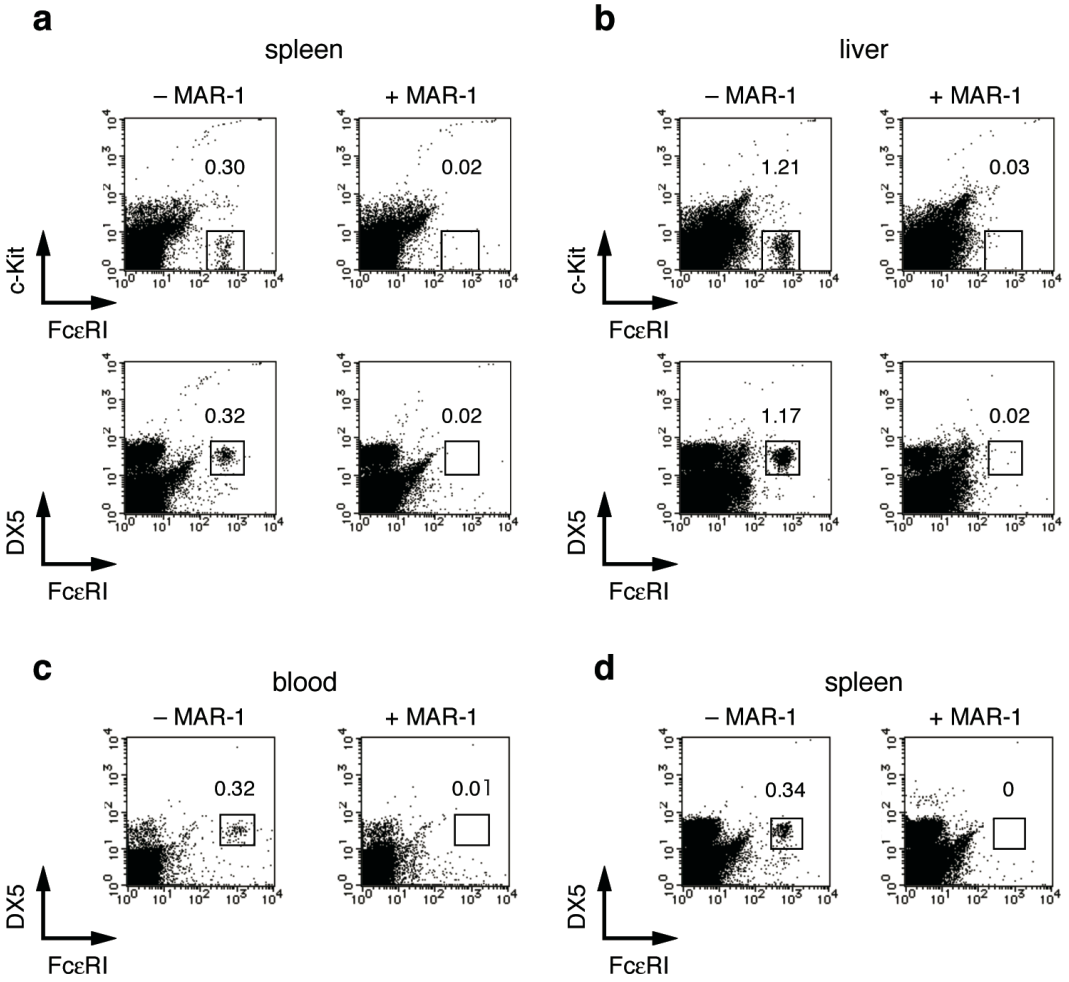
Supplementary Fig. 3. T_H2 cytokines production from bone marrow-derived basophils and mast cells.

(**a**) mRNAs extracted from bone marrow-derived and FACS sorted basophils or mast cells as described in **Supplementary Fig. 2a** were analyzed for TLR genes and β-actin gene expression by RT-PCR. As positive controls (Posi.) for TLRs mRNA, mRNAs extracted from murine splenic cells were used. Data are representative of three independent experiments. (**b**) Purified bone marrow-derived basophils or mast cells were restimulated with IL-3 (20 U/ml) plus IL-18 (50 ng/ml), IL-33 (100 ng/ml), LPS (1 μg/ml) or PGN (10 μg/ml) for 24 h. Supernatants were harvested and tested for production of IL-4, IL-6 and IL-13 by ELISA. Data are representative of five independent experiments (mean and s.e.m.).



Supplementary Fig. 4. Intravenous administration of OVA–pulsed basophils induces T_H2 response.

After priming of basophils as described in **Figure 4a**, basophils (5 x 10^5 per mouse) were transferred into BALB/c mice via tail vein. One week after adoptive transfer, all mice were iv challenged with OVA protein (100 μg per mouse). (**a**) Two weeks after challenge injection, splenic $CD4^+$ T cells (1x10^5/0.2ml per well) from each mouse were restimulated with OVA protein (100 μg/ml) in the presence of irradiated ΔT-spleen (1 x 10^5/0.2 ml per well) in 96-well plates for five days. Supernatants were harvested and tested for IL-4, IL-13 and IFN-γ production by ELISA. (**b**) Two weeks after challenge injection, splenic $CD4^+$ T cells from each mouse were analyzed by flow cytometry for the expression of IL-33Rα chain and CD62L, respectively. Data are representative of two independent experiments (**a**, **b**; mean and s.e.m. of five mice). ND; not detected. *P< 0.01, P< 0.05 and P< 0.005 by Student's t test, OVA-pulsed basophils as compared to mice injected with OVA protein alone.



Supplementary Fig. 5. Depletion of basophils.

(**a-d**) Flow cytometry and frequency of basophils (FcεRI$^+$/*c-kit*$^-$ or FcεRI$^+$/DX5$^+$ cells) in BALB/c mice injected ip twice daily for 3 days with 5 μg anti-mouse Fcε R1α [+ MAR-1] or PBS [–MAR-1]. Two days after the last injection, the numbers of basophils were quantified in the spleen (**a**) and liver (**b**). Mice were then iv injected with a mixture of DNP-OVA plus anti-DNP IgE as described in **Figure 5a**, followed by injection twice daily for additional 3 days with MAR-1 or PBS. After iv challenge with OVA at 4 days after iv IC injection, mice were additionally treated with MAR-1 or PBS continuous 5 days per week for 2 weeks. One week or two weeks after OVA challenge, the numbers of basophils were quantified in the blood (**c**) or spleen (**d**), respectively. The percentage shown represents the proportion of FcεRI$^+$/*c-kit*$^-$ or FcεRI$^+$/DX5$^+$ cells gated on splenic non-B, non-T cells (**a–d**).

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Supplement Table 1. Production of IL-4 by splenic basophils from naive and *S. venezuelensis*–infected mice. pg/ml

	Naive mice	Sv–infected mice
medium	< 5	432
IL-3	571	3295

Freshly prepared splenic basophils (1 X 10^5/ml/0.2 ml per well) from naive BALB/c mice or BALB/c mice inoculated with 5000 *S. venezuelensis* third-stage larvae (Sv–infected mice) for 10 days were stimulated with medium alone or IL-3 (20 U/ml) in 96–well plates for 24 h. Supernatants were analyzed for production of IL-4 by ELISA.

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