

Commensal bacteria–derived signals regulate basophil hematopoiesis and allergic inflammation

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Commensal bacteria that colonize mammalian barrier surfaces are reported to influence T helper type 2 (T_H2) cytokine-dependent inflammation and susceptibility to allergic disease, although the mechanisms that underlie these observations are poorly understood. In this report, we find that deliberate alteration of commensal bacterial populations via oral antibiotic treatment resulted in elevated serum IgE concentrations, increased steady-state circulating basophil populations and exaggerated basophil-mediated T_H2 cell responses and allergic inflammation. Elevated serum IgE levels correlated with increased circulating basophil populations in mice and subjects with hyperimmunoglobulinemia E syndrome. Furthermore, B cell–intrinsic expression of myeloid differentiation factor 88 (MyD88) was required to limit serum IgE concentrations and circulating basophil populations in mice. Commensal-derived signals were found to influence basophil development by limiting proliferation of bone marrow–resident precursor populations. Collectively, these results identify a previously unrecognized pathway through which commensal-derived signals influence basophil hematopoiesis and susceptibility to T_H2 cytokine–dependent inflammation and allergic disease.

Allergic diseases have reached pandemic levels¹ and represent a major source of morbidity, mortality and healthcare cost². These chronic inflammatory diseases are characterized by interleukin-4 (IL-4), IL-5, IL-9 and IL-13 production by CD4⁺ T_H2 cells, IgE production and recruitment of effector cells to sites of tissue inflammation^{3,4}. It is thought that susceptibility to T_H2 cytokine–dependent allergic inflammation is influenced by both polymorphisms in mammalian genes⁵ and environmental factors including diet and exposure to pollutants or infectious agents^{6–8}. However, the specific genetic and environmental stimuli that influence allergy susceptibility and how these factors contribute to the development of allergic disease are ongoing fields of study.

The human intestine is colonized by 100 trillion microorganisms belonging to each of the three domains of life⁹. Of these, bacteria are the most abundant; the colon is home to trillions of commensal bacteria¹⁰ with a diversity of at least 1,000–15,000 species¹¹. Epidemiologic studies have identified associations between alterations in the composition of commensal bacterial communities and the development of allergic disease. For example, infants who develop allergies have altered commensal populations early in life¹², and children who have undergone treatment with broad-spectrum antibiotics are at an increased risk of developing allergic diseases^{13,14}. Studies in animal

model systems have further implicated commensal-derived signals in influencing the development of T_H2 cytokine–mediated allergic inflammation^{15–18}. However, the mechanisms through which the innate immune system recognizes commensal-derived signals and regulates T_H2 cytokine responses remain poorly characterized¹⁹.

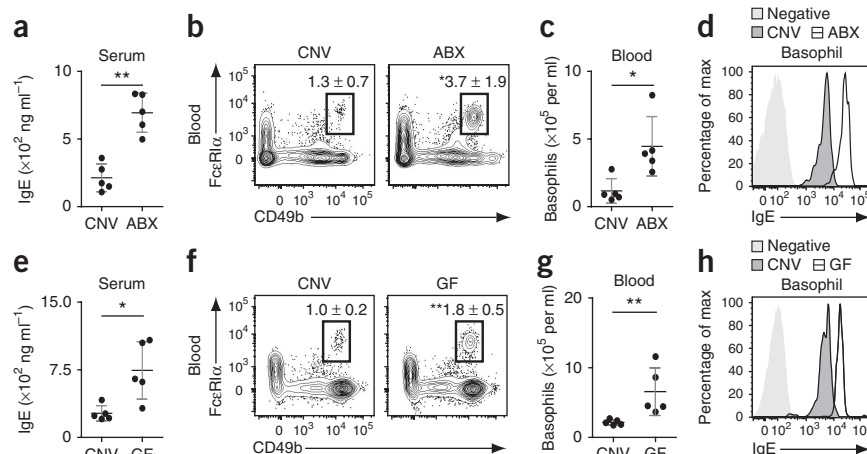
Here we used oral delivery of broad-spectrum antibiotics to determine the influence of commensal bacteria–derived signals on innate cell populations that contribute to the development of T_H2 cytokine–dependent allergic inflammation. Depletion or deletion of bacterial communities was associated with elevated serum IgE concentrations, increased circulating basophil populations and exaggerated T_H2 cell responses and allergic inflammation. Exaggerated T_H2 cell responses were reduced upon depletion of basophils, implicating this cell type in contributing to the exaggerated allergic inflammation observed in antibiotic-treated mice. We found IgE to be a crucial regulator of steady-state basophil responses in mice, and human subjects with hyperimmunoglobulinemia E syndrome had elevated frequencies of circulating basophils compared to controls. Additionally, B cell–intrinsic expression of myeloid differentiation factor 88 (MyD88) was required to limit serum IgE concentrations and circulating basophil populations in mice. Finally, commensal-derived signals influenced circulating basophil populations by regulating the proliferative

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Figure 1 Elevated steady-state serum IgE levels and circulating basophils in antibiotic-treated or germ-free mice. **(a)** Serum IgE from conventionally reared (CNV) or antibiotic-treated (ABX) mice, as measured by ELISA. **(b)** Flow cytometric analysis of blood basophils from CNV or ABX mice. Numbers adjacent to outlined areas indicate percentage cells in each gate. Gated on CD3-CD4-CD8-CD19-CD117⁺ cells. **(c)** Number of basophils per milliliter of blood from CNV or ABX mice. **(d)** Surface-bound IgE levels on blood basophils from CNV or ABX mice, as determined by flow cytometry. **(e)** Serum IgE from CNV or germ-free (GF) mice, as measured by ELISA. **(f)** Flow cytometric analysis of blood basophils from CNV or GF mice. **(g)** Number of basophils per milliliter of blood from CNV or GF mice. **(h)** Surface-bound IgE levels on blood basophils

from CNV or GF mice as determined by flow cytometry. Data representative of three or more independent experiments, results shown as mean $\pm$ s.d., significance determined by Mann-Whitney test (CNV, $n = 5$; ABX, $n = 5$; GF, $n = 5$; * $P \leq 0.05$; ** $P \leq 0.01$).



capacity of bone marrow-resident basophil progenitor populations. Together, these findings provide therapeutically relevant insights into the molecular and cellular mechanisms through which commensal bacteria-derived signals influence the development of T_H2 cytokine-dependent inflammation and susceptibility to allergic diseases.

RESULTS

Elevated serum IgE and basophils in antibiotic-treated mice

Combined oral antibiotic treatment with ampicillin, gentamicin, metronidazole, neomycin and vancomycin resulted in quantitative and qualitative alterations to commensal bacteria colonizing the mouse intestine including reductions in bacteria of the Firmicutes and Bacteroidetes phyla (Supplementary Fig. 1a,b) and significant increases in serum IgE levels^{20,21} (Fig. 1a). As IgE has been reported to influence granulocyte homeostasis²², we investigated whether antibiotic-induced elevations in IgE were associated with alterations in the frequency or number of circulating mast cells, eosinophils or basophils. Antibiotic treatment did not alter blood mast cell (Supplementary Fig. 2a,b) or eosinophil (Supplementary Fig. 2c,d) populations. However, frequencies and numbers of basophils (identified as non-B, non-T (NBNT) CD117⁺CD49b⁺FceRIα⁺) were significantly increased in the blood (Fig. 1b,c) and spleen (Supplementary Fig. 3a,b) of antibiotic-treated compared to conventionally reared mice. Basophils from antibiotic-treated mice showed increased amounts of surface-bound IgE compared to controls (Fig. 1d), whereas expression of other basophil-associated surface markers (CD69, CD123, CD200R, FcεRIα, FcγR, Gr1) was unaltered (Supplementary Fig. 3c).

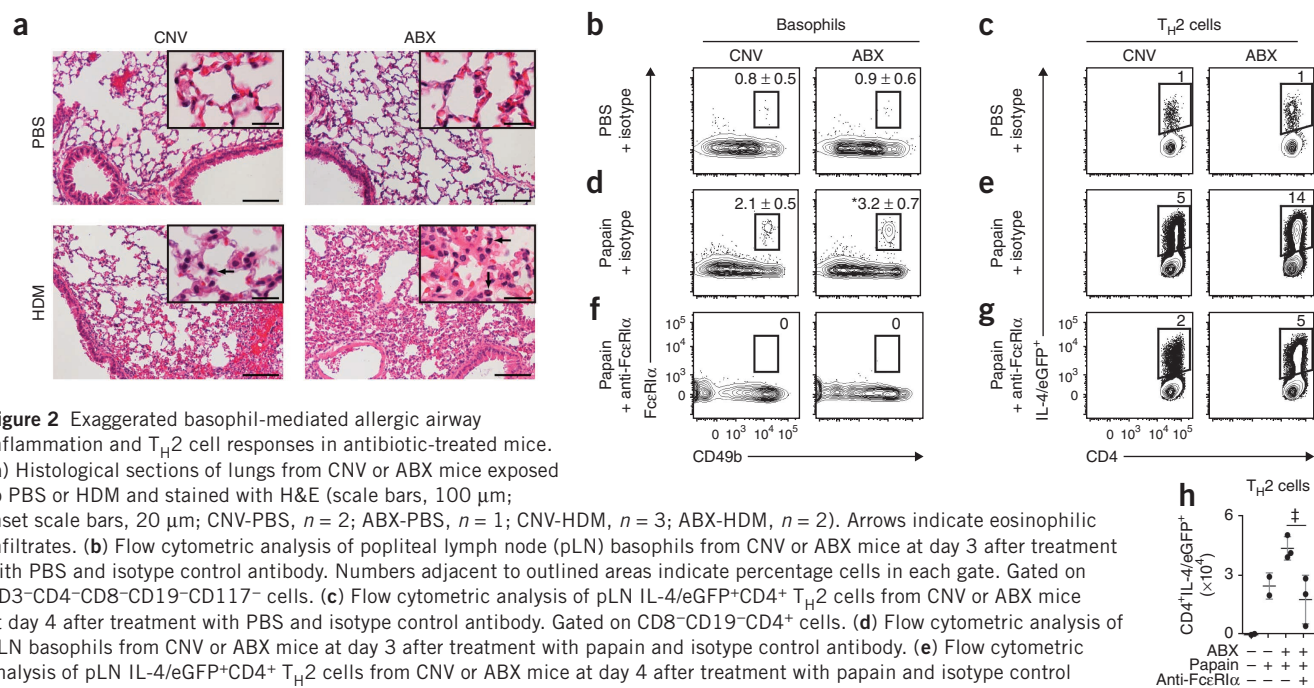
Antibiotic treatment does not eliminate all commensal bacteria²³. Therefore, to investigate whether steady-state levels of IgE or basophil populations were altered in the absence of all live microbial stimuli, we used germ-free mice²⁴. Consistent with the effects of antibiotic treatment, compared to conventionally reared controls, germ-free mice (that were not treated with antibiotics) showed higher serum IgE levels (Fig. 1e), higher frequencies and numbers of basophils in the blood (Fig. 1f,g) and spleen (Supplementary Fig. 3d,e) and higher basophil-surface-bound IgE levels (Fig. 1h). Consistent with commensal-derived signals being sufficient to limit serum IgE concentrations and circulating basophil populations, conventionalization of germ-free mice resulted in reductions in serum IgE concentrations (Supplementary Fig. 3f) and blood and spleen basophil populations

(Supplementary Fig. 3g,h). Collectively, these data indicate that commensal bacteria-derived signals limit serum IgE concentrations and circulating basophil populations in the steady state.

Commensals limit basophil-mediated allergic inflammation

Given the role for commensal-derived signals in limiting circulating numbers of basophils, we hypothesized that antibiotic-treated mice might show exaggerated inflammation in models of basophil-associated allergic disease. It has previously been shown that basophils contribute to the inflammation that results from inhalation of house dust mite allergen (HDM)²⁵. To test whether commensal-derived signals influenced the development of HDM-induced inflammation, we exposed conventionally reared or antibiotic-treated IL-4/eGFP reporter mice²⁶ (which express both IL-4 and eGFP under control of the IL-4 promoter) to HDM and examined innate cell responses, T_H2 cell responses and lung inflammation. Compared to conventionally reared mice, antibiotic-treated mice exposed to HDM showed higher frequencies of circulating blood basophils (Supplementary Fig. 4a) and IL-4/eGFP⁺ CD4⁺ T_H2 cells in the draining mediastinal lymph node (Supplementary Fig. 4b), increased lymph node cell-derived IL-4 and IL-13 secretion (Supplementary Fig. 4c) and a higher proportion of eosinophils in the lung and bronchoalveolar lavage (BAL) fluid (Supplementary Fig. 4d). Compared to conventionally reared controls, antibiotic-treated mice showed more HDM-elicited inflammation characterized by exaggerated alveolar infiltrates (Fig. 2a). Together, these findings indicate that commensal-derived signals limit allergic inflammation in the lung.

Basophils are recruited to draining lymph nodes early in the response to infectious²⁷ or allergic^{28,29} stimuli, where they may cooperate with dendritic cells (DCs) to promote optimal T_H2 cell responses^{27,29–31}. To test whether antibiotic-treated mice show altered allergen-induced T_H2 cell responses, we exposed conventionally reared or antibiotic-treated IL-4/eGFP reporter mice to PBS or papain, a cysteine protease associated with occupational allergy in humans³² and T_H2 cell responses in mice²⁸. After PBS exposure, we observed similar frequencies of basophils (Fig. 2b) and IL-4/eGFP⁺CD4⁺ T_H2 cells (Fig. 2c) in the popliteal lymph nodes of conventionally reared or antibiotic-treated mice. However, after exposure to papain, antibiotic-treated mice showed higher frequencies of lymph node basophils (Fig. 2d) and IL-4/eGFP⁺CD4⁺ T_H2 cells (Fig. 2e) compared to conventionally reared controls. Depletion of basophils by administration



of a Fc ϵ R1 α -specific antibody²⁸ resulted in a lower frequency of lymph node basophils (Fig. 2f) and lower frequencies (Fig. 2g) and numbers (Fig. 2h) of papain-elicited IL-4/eGFP⁺CD4⁺ T_H2 cells, indicating that basophils contribute to the exaggerated T_H2 cell responses observed in antibiotic-treated mice.

DCs are crucial for the development of optimal T_H2 cell responses to some allergens and helminth parasites^{25,33}. To investigate the influence of commensal-derived signals on steady-state DC populations, we examined DCs in conventionally reared or antibiotic-treated mice. DC frequencies in the spleen were similar between conventionally reared or antibiotic-treated mice (data not shown), as was DC expression of major histocompatibility complex class I (MHC-I), MHC-II, CD40, CD80, CD86 and Fc ϵ R1 α (Supplementary Fig. 5a–c), indicating that commensal-derived signals do not substantially influence splenic DC populations under the conditions tested. Nonbasophil Fc ϵ R1 α ⁺ cells are important for the development of inflammatory responses to some allergens²⁵. Papain-elicited, nonbasophil Fc ϵ R1 α ⁺ cell frequencies in the lymph node were similar between conventionally reared and antibiotic-treated mice (Supplementary Fig. 5d–f), indicating that nonbasophil Fc ϵ R1 α ⁺ cell responses are not substantially influenced by commensal-derived signals in this setting. As Fc ϵ R1 α ⁺ cells are influenced by Fc ϵ R1 α -specific antibody treatment²⁵, we examined the effect of treatment with this antibody on nonbasophil Fc ϵ R1 α ⁺ cell populations. Compared to control treatment, Fc ϵ R1 α -specific antibody treatment did not substantially influence the frequency of papain-elicited, nonbasophil Fc ϵ R1 α ⁺ lymph node cells (Supplementary Fig. 5d–f). Together, these findings suggest that neither steady-state DC responses nor papain-elicited nonbasophil Fc ϵ R1 α ⁺ cell responses are dysregulated in antibiotic-treated mice under the conditions tested.

To further examine the contributions of basophils to the exaggerated T_H2 cell responses observed in antibiotic-treated mice, we adopted a loss-of-function approach complementary to Fc ϵ R1 α -specific antibody treatment by using a basophil-specific diphtheria toxin (DT)-dependent depletion mouse system (BaS-TRECK)³⁴. Antibiotic treatment resulted in increased frequencies of circulating basophils (Supplementary Fig. 5g), and exaggerated frequencies (Supplementary Fig. 5h) and numbers (Supplementary Fig. 5i) of papain-elicited lymph node T_H2 cells, compared to conventionally reared mice. DT treatment of conventionally reared or antibiotic-treated BaS-TRECK mice, but not littermate controls, resulted in efficient depletion of lymph node basophils (Supplementary Fig. 5g) and reductions in the frequency (Supplementary Fig. 5h) and number (Supplementary Fig. 5i) of T_H2 cells. Together with the results of Fc ϵ R1 α -specific antibody-mediated basophil depletion, DT receptor-mediated deletion indicates that basophils contribute to the exaggerated allergen-induced T_H2 cell responses observed in antibiotic-treated mice.

Commensals regulate basophils via an IgE-dependent pathway

We next sought to determine the mechanisms by which commensal-derived signals regulate steady-state circulating basophil populations. The epithelial cell-derived cytokine thymic stromal lymphopoietin (TSLP) was recently found to expand circulating basophil populations³⁴. To test whether TSLP is required for the elevated serum IgE concentrations or circulating basophil populations observed in antibiotic-treated mice, we used TSLP-deficient (*Tslp*^{−/−}) mice. As in wild-type (WT) mice, antibiotic treatment of *Tslp*^{−/−} mice resulted in elevated serum IgE concentrations (Supplementary Fig. 6a) and increased circulating basophil frequencies and numbers

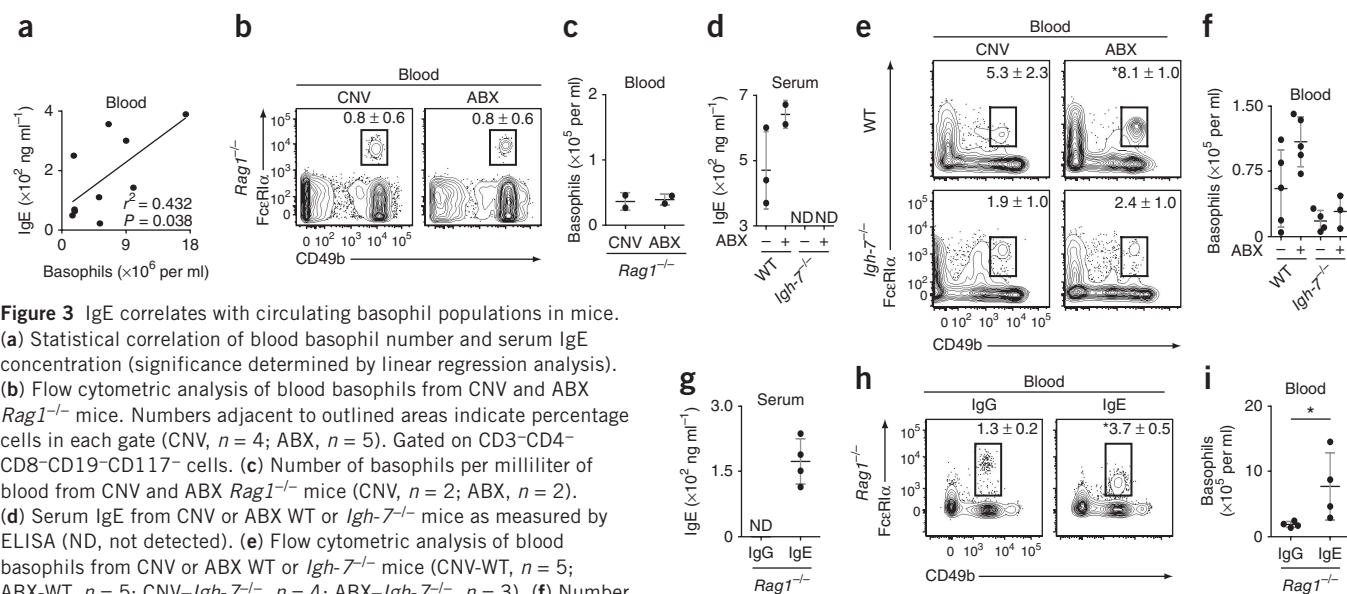


Figure 3 IgE correlates with circulating basophil populations in mice.

(a) Statistical correlation of blood basophil number and serum IgE concentration (significance determined by linear regression analysis).

(b) Flow cytometric analysis of blood basophils from CNV and ABX *Rag1*^{-/-} mice. Numbers adjacent to outlined areas indicate percentage cells in each gate (CNV, *n* = 4; ABX, *n* = 5). Gated on CD3⁺CD4⁺CD8⁻CD19⁻CD117⁻ cells.

(c) Number of basophils per milliliter of blood from CNV and ABX *Rag1*^{-/-} mice (CNV, *n* = 2; ABX, *n* = 2).

(d) Serum IgE from CNV or ABX WT or *Igh-7*^{-/-} mice as measured by ELISA (ND, not detected).

(e) Flow cytometric analysis of blood basophils from CNV or ABX WT or *Igh-7*^{-/-} mice (CNV-WT, *n* = 5; ABX-WT, *n* = 5; CNV-*Igh-7*^{-/-}, *n* = 4; ABX-*Igh-7*^{-/-}, *n* = 3).

(f) Number of basophils per milliliter of blood from CNV or ABX WT or *Igh-7*^{-/-} mice (CNV-WT, *n* = 5; ABX-WT, *n* = 5; CNV-*Igh-7*^{-/-}, *n* = 4; ABX-*Igh-7*^{-/-}, *n* = 3).

(g) Serum IgE concentrations from IgG- or IgE-treated *Rag1*^{-/-} mice as measured by ELISA (IgG, *n* = 4; IgE, *n* = 4; ND, not detected).

(h) Flow cytometric analysis of blood basophils from IgG- or IgE-treated *Rag1*^{-/-} mice (IgG, *n* = 4; IgE, *n* = 4).

(i) Number of basophils per milliliter of blood from IgG- or IgE-treated *Rag1*^{-/-} mice (IgG, *n* = 4; IgE, *n* = 4).

Data representative of three or more independent experiments, results shown as mean $\pm$ s.d., significance determined by Mann-Whitney test unless otherwise indicated (**P* $\leq$ 0.05).

(Supplementary Fig. 6b,c), indicating that TSLP is not necessary for these responses following antibiotic-mediated alterations in commensal bacteria in this setting.

As IgE regulates mast cell homeostasis²² and has been hypothesized to have similar roles in basophils³⁵, we next examined the relationship between elevated IgE concentrations and increased circulating basophil populations in antibiotic-treated mice. Serum IgE concentrations significantly correlated with circulating basophil numbers in conventionally reared and antibiotic-treated mice (Fig. 3a), suggesting that IgE may influence steady-state basophil homeostasis. To test whether the increase in circulating basophils observed in antibiotic-treated mice was dependent on lymphocytes, we used recombination activating gene 1-deficient (*Rag1*^{-/-}) mice³⁶. Consistent with a role for IgE in regulating basophil homeostasis, blood basophil frequencies and numbers were similar between conventionally reared and antibiotic-treated (Fig. 3b,c) or germ-free *Rag1*^{-/-} mice (Supplementary Fig. 7a,b). As IL-4 is necessary for IgE production³⁷, we used IL-4-deficient (*Il4*^{-/-}) mice to test the role of IL-4 in mediating the elevated serum IgE concentrations and basophil responses observed in antibiotic-treated mice. Whereas antibiotic treatment of WT mice resulted in increased serum IgE concentrations (Supplementary Fig. 7c) and frequencies of blood basophils (Supplementary Fig. 7d), IgE was not detected in *Il4*^{-/-} mice (Supplementary Fig. 7c), and antibiotic treatment of *Il4*^{-/-} mice did not substantially influence the frequency or number of blood basophils (Supplementary Fig. 7d,e). Together, these results suggest that IL-4-induced IgE production contributes to the increased circulating basophil responses observed in antibiotic-treated mice.

To directly test the role of IgE in mediating commensal bacteria-dependent regulation of steady-state circulating basophil populations, we used IgE-deficient (*Igh-7*^{-/-}) mice. Whereas antibiotic treatment of WT mice increased serum IgE concentrations (Fig. 3d) and the frequency and number of blood basophils (Fig. 3e,f), IgE was not

detected in *Igh-7*^{-/-} mice (Fig. 3d), and blood basophil populations were similar between conventionally reared and antibiotic-treated *Igh-7*^{-/-} mice (Fig. 3e,f). Together, these results indicate that IgE contributes to the exaggerated steady-state circulating basophil responses observed in antibiotic-treated mice. To test whether IgE is sufficient to promote the population expansion of circulating basophils, we treated *Rag1*^{-/-} mice with IgE and examined blood basophil populations. Compared to transfer of IgG, transfer of IgE into *Rag1*^{-/-} mice resulted in recovery of serum IgE concentrations (Fig. 3g) and significantly increased frequencies and numbers of blood basophils (Fig. 3h,i). Collectively, these gain- and loss-of-function approaches identify IgE as a regulator of peripheral basophil responses in mice.

Serum IgE levels correlate with blood basophils in humans

As IgE was found to regulate circulating basophil populations in mice, we hypothesized that IgE would have a similar role in humans. Subjects with loss-of-function polymorphisms in the dedicator of cytokinesis 8 gene (*DOCK8*⁻) have an autosomal recessive form of hyperimmunoglobulinemia E syndrome^{38,39} characterized by recurrent infections, increased susceptibility to atopic eczema and average serum IgE concentrations 10 times higher than those found in control subjects^{38,39}. Given our identification of a role for IgE in regulating basophil responses in mice, we hypothesized that elevated serum IgE levels in *DOCK8*⁻ subjects may be associated with increased circulating basophil populations. As previously reported, *DOCK8*⁻ subjects showed elevated serum IgE concentrations compared to control subjects^{38,39} (Fig. 4a). In control subjects, human blood basophils (identified as TCR β ⁺CD11c⁻CD19⁻CD117⁻CD123⁺Fc ϵ RI α ⁺) comprised approximately 1% of the NBNT cell compartment (Fig. 4b). In contrast, in four of five *DOCK8*⁻ subjects examined, basophils comprised approximately 4% of the NBNT compartment (Fig. 4c,d). Further, basophils isolated from *DOCK8*⁻ subjects showed higher surface-bound IgE levels compared to those of control subjects (Fig. 4e). These data are consistent with our

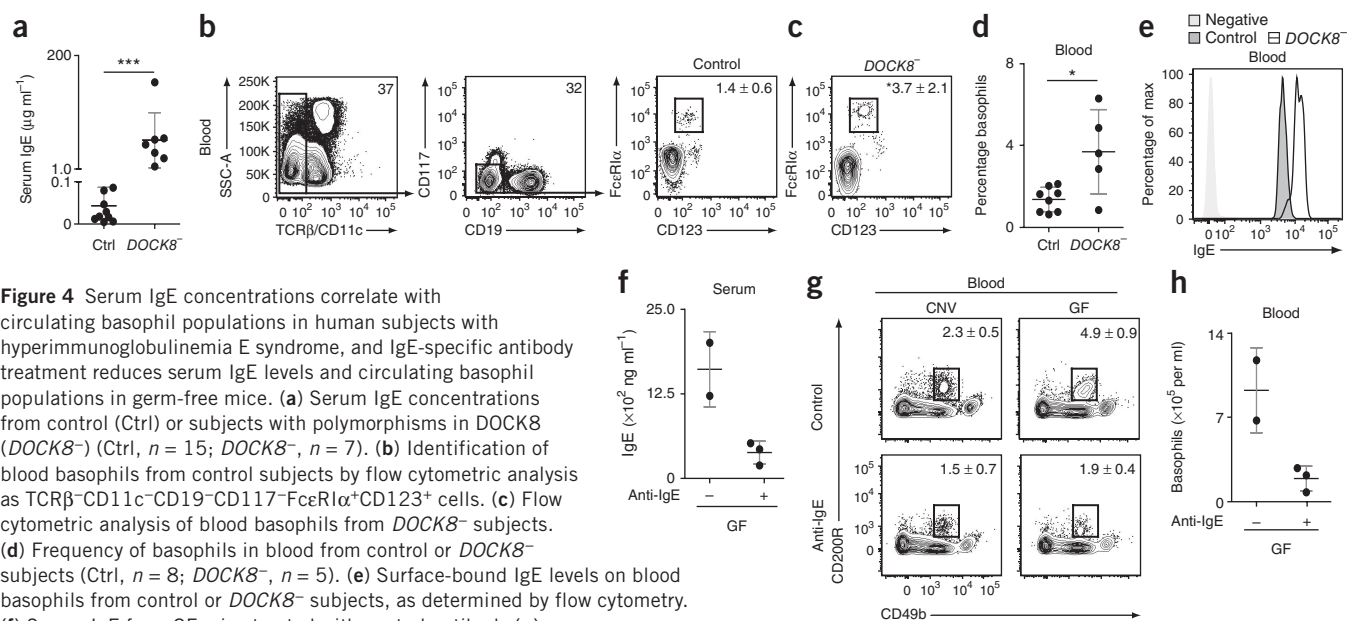


Figure 4 Serum IgE concentrations correlate with circulating basophil populations in human subjects with hyperimmunoglobulinemia E syndrome, and IgE-specific antibody treatment reduces serum IgE levels and circulating basophil populations in germ-free mice. (a) Serum IgE concentrations from control (Ctrl) or subjects with polymorphisms in *DOCK8* (*DOCK8*^{-/-}) (Ctrl, *n* = 15; *DOCK8*^{-/-}, *n* = 7). (b) Identification of blood basophils from control subjects by flow cytometric analysis as TCRβ⁺CD11c⁻CD19⁻CD117⁻FcεRIα⁺CD123⁺ cells. (c) Flow cytometric analysis of blood basophils from *DOCK8*^{-/-} subjects. (d) Frequency of basophils in blood from control or *DOCK8*^{-/-} subjects (Ctrl, *n* = 8; *DOCK8*^{-/-}, *n* = 5). (e) Surface-bound IgE levels on blood basophils from control or *DOCK8*^{-/-} subjects, as determined by flow cytometry. (f) Serum IgE from GF mice treated with control antibody (-) or IgE-specific antibody (anti-IgE; +) as measured by ELISA. (g) Flow cytometric analysis of blood basophils from CNV or GF mice treated with control or anti-IgE. Numbers adjacent to outlined areas indicate percentage cells in each gate (CNV-control, *n* = 7; GF-control, *n* = 7; CNV-αIgE, *n* = 9; GF-αIgE, *n* = 10 across four independent experiments). Gated on CD3⁺CD4⁺CD8⁻CD19⁻CD117⁻ cells. (h) Number of basophils per milliliter of blood from GF mice treated with control or anti-IgE. Data representative of three or more independent experiments, results shown as mean ± s.d., significance determined by Mann-Whitney test (***P* ≤ 0.01; ****P* ≤ 0.001).

findings in mouse models and suggest that IgE may influence peripheral basophil homeostasis and the development of basophil-mediated allergic inflammation in humans.

Given the potential role for IgE in regulating circulating basophil populations in mice and in human subjects, we hypothesized that therapeutic depletion of serum IgE may reduce circulating basophil populations. Omalizumab (an IgE-specific antibody) is a monoclonal, humanized mouse antibody to human IgE and a US Food and Drug Administration–approved anti-allergy drug^{40,41}. To test whether IgE-specific antibody treatment influenced serum IgE concentrations or circulating basophil populations, we treated conventionally reared or germ-free mice with control or IgE-specific antibody and examined serum IgE levels and blood basophil populations. As IgE-specific antibody treatment decreases FcεRIα expression on basophils⁴², we identified basophils as NBNT CD117⁺CD49b⁺CD200R⁺ cells⁴³. IgE-specific antibody treatment of germ-free mice resulted in reductions in serum IgE levels (Fig. 4f) and reduced frequencies and numbers of blood basophils (Fig. 4g,h). Together, these results identify IgE-specific antibody treatment as a potential therapeutic intervention to limit IgE-mediated increases in circulating basophil populations.

MyD88-dependent signaling in B cells limits IgE and basophils

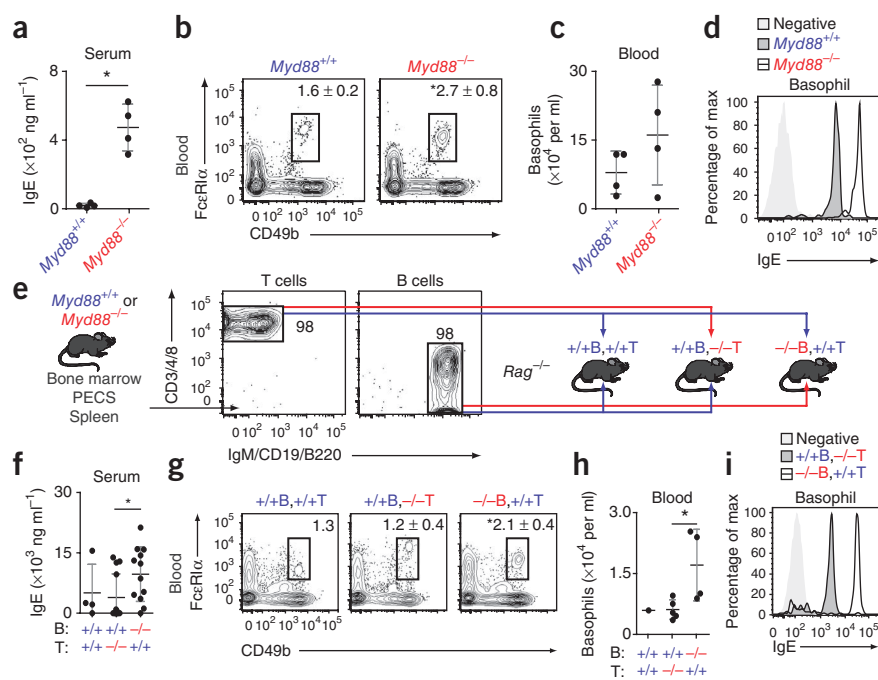
It has been hypothesized that commensal bacteria–derived signals influence allergic responses by signaling through pattern recognition receptors (PRRs)^{15,17}. To determine the molecular mechanisms through which commensal-derived signals influence serum IgE concentrations and circulating basophil responses, we used mice deficient in MyD88 (*Myd88*^{-/-}), a crucial adaptor molecule that regulates signaling through multiple PRRs⁴⁴. Compared to littermate controls, conventionally reared *Myd88*^{-/-} mice had higher serum IgE concentrations⁴⁴ (Fig. 5a), higher frequencies and numbers of blood basophils (Fig. 5b,c) and higher basophil-surface-bound IgE levels (Fig. 5d), indicating that MyD88 signaling pathways limit these responses.

Nucleotide-binding oligomerization domain-containing protein 1 (NOD1) is a MyD88-independent intracellular PRR that mediates innate and adaptive immunity by recognizing commensal bacteria–derived signals⁴⁵. To test whether NOD1 influences steady-state circulating basophil populations, we examined serum IgE concentrations and circulating basophil populations in control and NOD1-deficient (*Nod1*^{-/-}) mice. Neither serum IgE concentrations (Supplementary Fig. 8a) nor blood basophils populations were altered in *Nod1*^{-/-} compared to control mice (Supplementary Fig. 8b,c), indicating that NOD1-dependent signaling does not substantially influence steady-state serum IgE concentrations or circulating basophil responses in this setting. To examine whether MyD88-dependent bacterial-derived signals were sufficient to limit serum IgE concentrations or circulating basophil populations, we treated conventionally reared or antibiotic-treated mice with the commensal-bacterial DNA motif CpG^{15,46} and examined serum IgE concentrations and circulating basophil populations. CpG treatment of antibiotic-treated mice resulted in reductions in serum IgE concentrations (Supplementary Fig. 8d) and reduced frequencies and numbers of blood basophil populations (Supplementary Fig. 8e,f). Together, these findings identify MyD88-dependent signaling pathways as important regulators of steady-state serum IgE levels and circulating basophil populations in mice.

MyD88-dependent signaling in B cells can inhibit IgE class switching *in vitro*⁴⁷. To test whether B cell–intrinsic expression of MyD88 influences serum IgE concentrations or basophil homeostasis *in vivo*, we generated chimeras by sorting and adoptively transferring B or T cells from *Myd88*^{+/+} or *Myd88*^{-/-} mice into *Rag1*^{-/-} recipients (Fig. 5e). Compared to controls, mice that received *Myd88*^{-/-} B cells with *Myd88*^{+/+} T cells showed higher serum IgE concentrations (Fig. 5f), higher frequencies (Fig. 5g) and numbers (Fig. 5h) of blood basophils and higher basophil-surface-bound IgE levels (Fig. 5i). Together, these results indicate that B cell–intrinsic

Figure 5 Elevated serum IgE concentrations and circulating basophil populations in mice lacking B cell–intrinsic MyD88 signaling.

(a) Serum IgE from CNV *Myd88*^{+/+} or *Myd88*^{-/-} mice as measured by ELISA (*Myd88*^{+/+}, *n* = 4; *Myd88*^{-/-}, *n* = 4). (b) Flow cytometric analysis of blood basophils from CNV *Myd88*^{+/+} or *Myd88*^{-/-} mice. Numbers adjacent to outlined areas indicate percentage cells in each gate (*Myd88*^{+/+}, *n* = 4; *Myd88*^{-/-}, *n* = 4). Gated on CD3⁺CD4⁺CD8⁺CD19⁺CD117⁺ cells. (c) Number of basophils per ml of blood from CNV *Myd88*^{+/+} or *Myd88*^{-/-} mice (*Myd88*^{+/+}, *n* = 4; *Myd88*^{-/-}, *n* = 4). (d) Surface-bound IgE levels on blood basophils from CNV *Myd88*^{+/+} or *Myd88*^{-/-} mice, as determined by flow cytometry. (e) Experimental diagram of sort and transfer of *Myd88*^{+/+} or *Myd88*^{-/-} B or T cells into *Rag1*^{-/-} recipients. (f) Serum IgE from *Rag1*^{-/-} mice reconstituted with *Myd88*^{+/+} or *Myd88*^{-/-} B or T cells as measured by ELISA. (g) Flow cytometric analysis of blood basophils from *Rag1*^{-/-} mice reconstituted with *Myd88*^{+/+} or *Myd88*^{-/-} B or T cells. (h) Number of basophils per milliliter of blood from *Rag1*^{-/-} mice reconstituted with *Myd88*^{+/+} or *Myd88*^{-/-} B or T cells (+/+B +/+T, *n* = 4; +/+B -/-T, *n* = 12; -/-B +/+T, *n* = 12 across three independent experiments). (i) Surface-bound IgE levels on blood basophils from *Rag1*^{-/-} mice reconstituted with *Myd88*^{+/+} or *Myd88*^{-/-} B or T cells, as determined by flow cytometry. Data representative of three or more independent experiments, results shown as mean ± s.d., significance determined by Mann-Whitney test (**P* ≤ 0.05).



MyD88 expression limits steady-state serum IgE concentrations and circulating basophil populations *in vivo*.

Commensals limit bone marrow–resident basophil precursors

We hypothesized that commensal-derived signals may regulate circulating basophil populations by influencing basophil survival. To test this, we sort-purified basophils from conventionally reared or antibiotic-treated mice and cultured them in the presence of IL-3. Basophils isolated from conventionally reared mice and antibiotic-treated mice showed similar survival (Supplementary Fig. 9a), suggesting that commensal-derived signals do not readily influence basophil survival in this setting. As IgE was found to regulate commensal-dependent alterations in circulating basophil populations, we sought to investigate whether IgE influenced basophil survival in this assay. Basophils isolated from control and *Igh*-7^{-/-} mice showed comparable survival rates (Supplementary Fig. 9b), suggesting that IgE does not readily influence basophil survival in this setting.

We next hypothesized that commensal-derived signals may influence basophil development from the bone marrow rather than influencing survival. To test whether proliferation of basophils from bone marrow cells is influenced by commensal-derived signals, we CFSE-labeled bone marrow from conventionally reared or antibiotic-treated mice, cultured it in the presence of IL-3 and examined mature basophil populations⁴⁸. Compared to basophils derived from bone marrow of conventionally reared mice, a higher proportion of basophils derived from bone marrow of germ-free mice had diluted CFSE fluorescence and expressed the cell proliferation marker Ki67 (Fig. 6a), indicating that they originated from precursors that had undergone more rounds of cell division. Consistent with this, cultures of bone marrow cells from germ-free (Fig. 6b) or antibiotic-treated (Supplementary Fig. 9c) mice showed a larger population expansion

of basophils compared to equal numbers of bone marrow cells isolated from conventionally reared mice. Together, these results indicate that commensal-derived signals limit the proliferative capacity of bone marrow–resident basophil precursor populations.

Bone marrow–resident basophil precursors (BaPs) are characterized by expression of FcεRIα and the hematopoietic progenitor adhesion molecule CD34 (ref. 49). To determine whether BaPs are influenced by alterations in commensal-derived signals, we isolated bone marrow from conventionally reared and germ-free mice and examined BaP populations. Frequencies of BaPs (identified as NBNT, CD117⁺FcεRIα⁺CD34⁺) were significantly higher in the bone marrow of germ-free compared to conventionally reared mice (Fig. 6c,d). Having identified a role for IgE in mediating the influence of commensal-derived signals on circulating basophil populations, we hypothesized that IgE might mediate commensal-dependent effects on bone marrow BaP populations. To test this, we isolated bone marrow from conventionally reared and antibiotic-treated *Igh*-7^{-/-} mice and examined BaP populations. Frequencies of BaPs were similar between conventionally reared mice and antibiotic-treated *Igh*-7^{-/-} mice (Supplementary Fig. 9d), suggesting that IgE contributes to the increased frequency of bone marrow BaP cells observed following antibiotic-treatment.

Commensals limit IL-3 responsiveness in BaP cells

As the development of basophils from bone marrow–resident precursors is regulated in part by IL-3–IL-3 receptor (IL-3R) signaling⁵⁰, we examined whether commensal-derived signals influence BaP expression of the IL-3R subunit CD123. Compared to conventionally reared controls, BaPs in the bone marrow of antibiotic-treated or germ-free mice showed greater expression of CD123 (Fig. 6e). Given the higher expression of CD123 on BaPs from antibiotic-treated mice, we hypothesized that BaPs from antibiotic-treated mice may

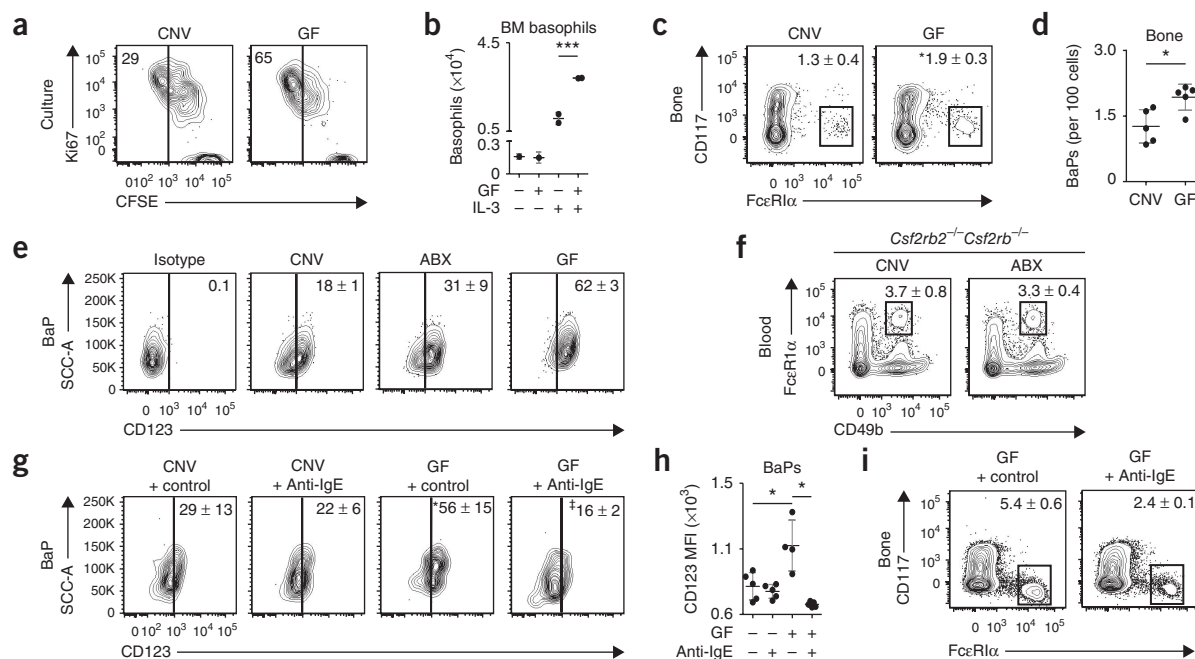


Figure 6 Dysregulated basophil development in germ-free or antibiotic-treated mice. **(a)** Bone marrow of CNV or GF mice was CFSE labeled and cultured in the presence of IL-3, and basophil populations were examined by flow cytometry. Numbers adjacent to outlined areas indicate percentage cells in each gate. Gated on CD3⁺CD4⁺CD8⁺CD19⁺CD117⁺CD49b⁺FcεR1α⁺ cells. **(b)** Bone marrow of CNV (–) or GF (+) mice was cultured in the absence (–) or presence (+) of IL-3, and basophil numbers were determined (means of two experiments ± s.d.; CNV-medium, *n* = 5; GF-medium, *n* = 3; CNV-IL-3, *n* = 10; GF-IL-3, *n* = 8; significance determined by two-way ANOVA; ***, *P* ≤ 0.001). **(c)** Flow cytometric analysis of BaPs in bone marrow of CNV or GF mice. Gated on CD3⁺CD4⁺CD8⁺CD19⁺CD34⁺ cells (CNV, *n* = 5; GF, *n* = 5). **(d)** Number of BaPs in bone marrow of CNV or GF mice (CNV, *n* = 5; GF, *n* = 5). **(e)** Frequency of CD123⁺ BaPs in bone marrow of CNV, ABX or GF mice, as determined by flow cytometry. Gated on CD3⁺CD4⁺CD8⁺CD19⁺CD117⁺CD34⁺FcεR1α⁺ cells (CNV, *n* = 2; ABX, *n* = 2; GF, *n* = 2). **(f)** Flow cytometric analysis of blood basophils from CNV or ABX *Csf2rb2*^{−/−}*Csf2rb*^{−/−} mice. Gated on CD3⁺CD4⁺CD8⁺CD19⁺CD117⁺ cells (CNV, *n* = 3; ABX, *n* = 3). **(g)** Frequency of CD123⁺ BaPs in bone marrow of CNV or GF mice treated with control or IgE-specific antibody (anti-IgE) (CNV-control, *n* = 5; CNV-anti-IgE, *n* = 5; GF-control, *n* = 4; GF-anti-IgE, *n* = 5; CNV-control versus GF-control, **P* ≤ 0.05; GF-control versus GF-anti-IgE, †*P* ≤ 0.05). **(h)** Mean fluorescence intensity of CD123 on BaPs in bone marrow of CNV or GF mice treated with control or anti-IgE (CNV-control, *n* = 5; CNV-anti-IgE, *n* = 5; GF-control, *n* = 4; GF-anti-IgE, *n* = 5; **P* ≤ 0.05). **(i)** Flow cytometric analysis of BaPs in bone marrow of GF mice treated with control or anti-IgE (GF-control, *n* = 2; GF-anti-IgE, *n* = 3). Data representative of two or more independent experiments, results shown as mean ± s.d., significance determined by Mann-Whitney test unless otherwise indicated (**P* ≤ 0.05).

be more responsive to IL-3 compared to BaPs from conventionally reared mice. To test this, we sort-purified BaPs from conventionally reared or antibiotic-treated mice and cultured them in equal numbers in the presence or absence of IL-3 (**Supplementary Fig. 9e**). In the presence of IL-3, BaPs from both conventionally reared and antibiotic-treated mice developed into mature basophil populations, as indicated by low expression of CD34 (**Supplementary Fig. 9f**), expression of CD49b and FcεR1α (**Supplementary Fig. 9g**) and mature cellular morphology (**Supplementary Fig. 9h,i**). However, culture of BaPs isolated from antibiotic-treated mice resulted in the development of a higher number of mature basophils compared to culture of an equal number of BaPs isolated from conventionally reared mice (**Supplementary Fig. 9j**). Together, these results indicate that commensal-derived signals limit BaP populations in the bone marrow, limit surface expression of CD123 on BaPs and regulate responsiveness of BaPs to IL-3.

Owing to the increased surface-bound CD123 on BaPs of antibiotic-treated mice and the increased responsiveness of these BaPs to IL-3, we hypothesized that the elevations in circulating basophils observed upon antibiotic treatment of mice might be mediated by IL-3R-dependent signaling. To test this, we examined basophil responses in conventionally reared or antibiotic-treated mice deficient in both the IL-3Rβ13 and IL-3Rβc receptor subunits (*Csf2rb2*^{−/−}*Csf2rb*^{−/−}). Antibiotic treatment of *Csf2rb2*^{−/−}*Csf2rb*^{−/−} mice did

not substantially alter the frequency of blood basophil populations (**Fig. 6f**), suggesting that IL-3R signaling is necessary for antibiotic-induced elevations in circulating basophil populations. Therefore, the effects of commensal-derived signals on basophils are dependent in part on both IgE and IL-3R signaling.

IgE increases CD123 expression on BaPs of germ-free mice

On the basis of our findings that antibiotic-induced elevations in circulating basophils are dependent on IgE and IL-3R signaling and correlate with bone marrow BaP surface-expression of CD123, we hypothesized that IgE may regulate the increased CD123 surface expression on BaPs of germ-free mice. To test this hypothesis, we treated conventionally reared mice and germ-free mice with control or IgE-specific antibody and examined both surface expression of CD123 on BaPs and total bone marrow BaP frequencies. Compared to BaPs in the bone marrow of conventionally reared mice, BaPs in the bone marrow of germ-free mice had significantly elevated expression of CD123 (**Fig. 6g,h**). Treatment of germ-free mice with IgE-specific antibody, but not control antibody, significantly reduced surface expression of CD123 on germ-free bone marrow BaPs (**Fig. 6g,h**) and reduced frequencies of BaPs in the bone marrow of germ-free mice (**Fig. 6i**). Collectively, these results indicate that commensal-derived signals influence bone marrow BaP populations in part by regulating IgE-mediated expression of CD123.

DISCUSSION

In this study, we used oral delivery of broad-spectrum antibiotics to determine the influence of commensal bacteria-derived signals on innate immune cell populations known to contribute to the development of T_H2 cytokine-dependent allergic inflammation. Antibiotic treatment resulted in elevated serum IgE concentrations, increased circulating basophil populations and exaggerated basophil-associated T_H2 cell responses and allergic inflammation. We identified IgE as a crucial regulator of steady-state circulating basophil populations in mice. These findings are consistent with studies that indicate that IgE can influence mast cell survival and function and suggest a broader immunoregulatory role for IgE in settings of infection and allergy²². Additionally, we found serum IgE concentrations to correlate with high circulating basophil frequencies in subjects with hyperimmunoglobulinemia E syndrome, implicating IgE-mediated regulation of basophil populations in contributing to the increased susceptibility to allergies observed in these and other subjects with genetic or pathogenic elevations in circulating IgE concentrations^{38,39}.

Previous reports that used genomic deletions in *Myd88* identified MyD88-dependent signaling as a major regulator of T_H2 cytokine-associated inflammation^{44,51}. Here B cell-intrinsic expression of MyD88 was identified as one regulatory pathway that limits steady-state serum IgE concentrations and circulating basophil populations in mice. This finding is supported by *in vitro* studies that indicate that CpG can inhibit IgE class switching by B cells⁴⁷. Consistent with this, human subjects with deficiencies in the MyD88 or interleukin-1 receptor-associated kinase-4 signaling pathways show elevated serum IgE concentrations⁵². Given these associations, further examination of circulating basophil populations or susceptibility to allergic inflammation in these subject populations may be of value.

We found that commensal bacteria-derived signals regulate basophil development from bone marrow-resident precursor populations by influencing the responsiveness of these precursors to IL-3. These findings suggest that, in addition to regulating immune cells in the periphery, commensal-derived signals can alter mammalian hematopoietic programs in the bone marrow to promote or protect against the development of allergic responses. The identification of a role for commensal-derived signals in regulating hematopoiesis could have implications beyond allergy to other chronic inflammatory disease states that are associated with alterations in commensal bacteria, including cancer, infection and autoimmunity.

Although our focus here has been on the influence of commensal bacteria-derived signals on steady-state basophil populations and basophil-associated allergic inflammation, these findings do not exclude a potential role for DCs or regulatory T cells in influencing aspects of commensal regulation of allergic responses^{25,53}. Additionally, the findings described here do not exclude a synergistic role for other mediators of allergic inflammation such as TSLP, a cytokine that is regulated by IgE and elevated in the intestines of germ-free mice^{54,55}. Further analysis of the effects of selective manipulation of commensal bacteria on DCs and regulatory T cells (including inducible LAP⁺ cell populations⁵⁶) in animal models and human subjects is therefore warranted.

In summary, the data presented here indicate that commensal bacterial-derived signals limit steady-state serum IgE concentrations, circulating basophil populations, and basophil-associated allergic inflammation. We propose that IgE, in addition to influencing mature basophils in the periphery, binds bone marrow-resident basophil precursors, increasing their responsiveness to IL-3 and resulting in both the population expansion of mature circulating basophils

in the steady state and exaggerated allergen-induced inflammation (Supplementary Fig. 10). These mechanistic insights into the influence of the commensal bacteria-IgE-basophil axis on the development of allergic inflammation may have utility in the design of new strategies to prevent or treat allergic diseases in humans.

METHODS

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

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

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AUTHOR CONTRIBUTIONS

D.A.H., M.C.S., M.C.A., B.S.K., D.K. and D.A. designed and performed the research; D.F.L., E.D.R., J.S.O., M.K., T.K. and F.D.B. provided reagents; D.A.H., M.C.S., M.C.A., B.S.K. and D.A. analyzed the data; D.A.H., M.C.S. and D.A. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Mice. BALB/c, Swiss-Webster, C57BL/6, *Rag1*^{-/-}, BaS-TRECK, *Csf2rb2*^{-/-}, *Csf2rb*^{-/-}, *Igh*^{-/-}, IL-4/eGFP reporter, *Il4*^{-/-}, *Myd88*^{-/-}, *Nod1*^{-/-} and *Tslp*^{-/-} mice (8–24 weeks of age) were bred at the University of Pennsylvania, purchased from Jackson, Taconic or Charles River Laboratories, or provided by Amgen. Germ-free mice were provided by the University of Pennsylvania Gnotobiotic Mouse Facility. Conventionally reared mice were housed in specific pathogen-free conditions. Experiments were performed under Institutional Animal Care and Use Committee (IACUC)-approved protocols and in accordance with the guidelines of the IACUC of the University of Pennsylvania.

Reagents and treatments. We fed mice autoclaved water with or without ampicillin (0.5 mg ml⁻¹), gentamicin (0.5 mg ml⁻¹), metronidazole (0.5 mg ml⁻¹), neomycin (0.5 mg ml⁻¹) and vancomycin (0.25 mg ml⁻¹) continuously via water bottle for 4 weeks. In the event of poor mouse hydration, we supplemented control and antibiotic water with artificial sweetener. We conventionalized germ-free mice by housing them with conventionally reared mice for 4–8 weeks. We inoculated mice intranasally on day -17, day -14 and day -7 with 50 µl of PBS with or without 100 µg of *Dermatophagoides pteronyssinus* extract (Greer)²⁵ and killed them on day 0 for further analyses. We injected mice subcutaneously in contralateral footpads with 50 µl of PBS with or without 50 µg of papain (Calbiochem)²⁸ and killed them on day 3 or 4 after injection for basophil or T_H2 cell analyses. We treated mice by intraperitoneal injection (i.p.) with 10 µg of FcεRIα-specific (MAR1) antibody or isotype control (eBio299Arm) antibody (eBioscience) on day -3, day -2 and day -1 before killing them for further analyses. We treated littermate control or BaS-TRECK mice i.p. with 750 ng DT on day -2 and day 0 before killing them for further analyses. We treated *Rag1*^{-/-} mice i.p. with 50 µg of IgG or IgE (557079) antibody (BD Bioscience) daily for 7 d before killing them for further analyses. We treated mice i.p. with 200 µg of control IgG (I4131, Sigma) or omalizumab (IgE-specific) antibody (Genentech) daily for 7–9 days before killing them for further analyses. We treated mice i.p. weekly with 100 µl of PBS containing 100 µg of control phosphorothioate GpC (5'-ZOOZFEOZZOOZEFEOZZT-3') or phosphorothioate CpG (5'-ZOOZFEOZZOOZEFEOZZT-3') before killing them for further analyses.

Flow cytometry, cell sorting and adoptive transfer. We collected blood, spleen, bone marrow, lung, bronchoalveolar lavage and lymph node tissues, digested with collagenase and dispase (lung), homogenized, purified of red blood cells by histopaque (Sigma-Aldrich) or red blood cell lysis, and stained at a 1:200 dilution with mouse fluorochrome-conjugated monoclonal antibodies specific for CD3ε (145-2C11), CD4 (GK1.5), CD8 (53-6.7), CD11c (N418), CD19 (eBio103), CD34 (RAM34), CD40 (HM40-3), CD45R (RA3-6B2), CD49b (DX5), CD69 (H1.2F3), CD80 (16-10A1), CD86 (GL1), CD117 (28B), CD123 (5B11), CD200R (OX108), FcεRIα (MAR1), FcγR (2.4G2), Gr-1 (RB6-8L5), IgE (R35-72), IgM (II/41), IL-4 (11B11), IL-13 (eBio13A), Ki67 (SolA15), MHC-I (SF1-1.1.1), MHC-II (2G9) and Siglec F (E50-2440) (BD Bioscience, BioLegend, eBioscience, AbD Serotec).

We obtained samples from subjects with hyperimmunoglobulinemia E or control subjects with participant consent, assent, and/or parental consent under protocols approved by the Children's Hospital of Philadelphia and the Ludwig Maximilians University Institutional Review Boards. We isolated peripheral blood mononuclear cells by Ficoll (GE) gradient, stained at a 1:200 dilution with antibodies against human CD11c (3.9), CD19 (HIB19), CD117 (104D2), CD123 (6H6), FcεRIα (AER-37), IgE (Ige21) or TCRβ (IP26) (BD Bioscience, eBioscience) and fixed with 4% paraformaldehyde. We acquired or sorted cells with a FACSCanto II, LSR II or FACSaria with DiVa software (BD Bioscience) and analyzed with FlowJo software (version 8.7.1; Tree Star). We transferred purified B or T cells i.p. in equal ratios and allowed them to reconstitute for 8–10 weeks.

Culture, enzyme-linked immunosorbent assay and histology. We cultured single-cell lymph nodes suspensions for 2–4 d at 200–250,000 cells in 200 µl of complete medium with or without antibody to CD3 (17A2) and antibody to CD28 (37.51) at a final concentration of 0.5–1.0 µg ml⁻¹ (eBioscience), stimulated for 4 h with 50 ng ml⁻¹ phorbol 12-myristate 13-acetate, 750 ng ml⁻¹ ionomycin and 10 µg ml⁻¹ brefeldin A (Sigma-Aldrich) and treated them with Fix/Perm (eBioscience). We CFSE labeled and cultured bone marrow cells for 4–5 d at 2–2.5 × 10⁶ cells per ml with or without 10 ng ml⁻¹ of IL-3 (R&D Systems). We cultured sort-purified BaPs for 4–5 d at 50,000 cells per milliliter plus or minus 10 ng ml⁻¹ of IL-3 (R&D Systems). We assayed IgE, IL-4 and IL-13 levels by sandwich ELISA (BD Bioscience) or chart review. We fixed lung tissue sections in 4% paraformaldehyde, embedded in paraffin, cut 5-µm sections and stained with H&E.

16S rDNA sample acquisition and quantification of 16S rDNA. We collected stool samples and extracted DNA with the QIAamp DNA Stool Mini Kit (Qiagen) as described previously²³. We quantified 16S rDNA by real-time RT-PCR with degenerate bacterial 16S rDNA-specific primers (5'-AGAGTTTGATCCTGGCTCAG-3'; forward), (5'-CTGCTGCCTYCCGTA-3'; reverse), (5'-FAM-TA+ACA+CATG+CA+AGTC+GA-BHQ1-3'; probe; + precedes position of LNA base).

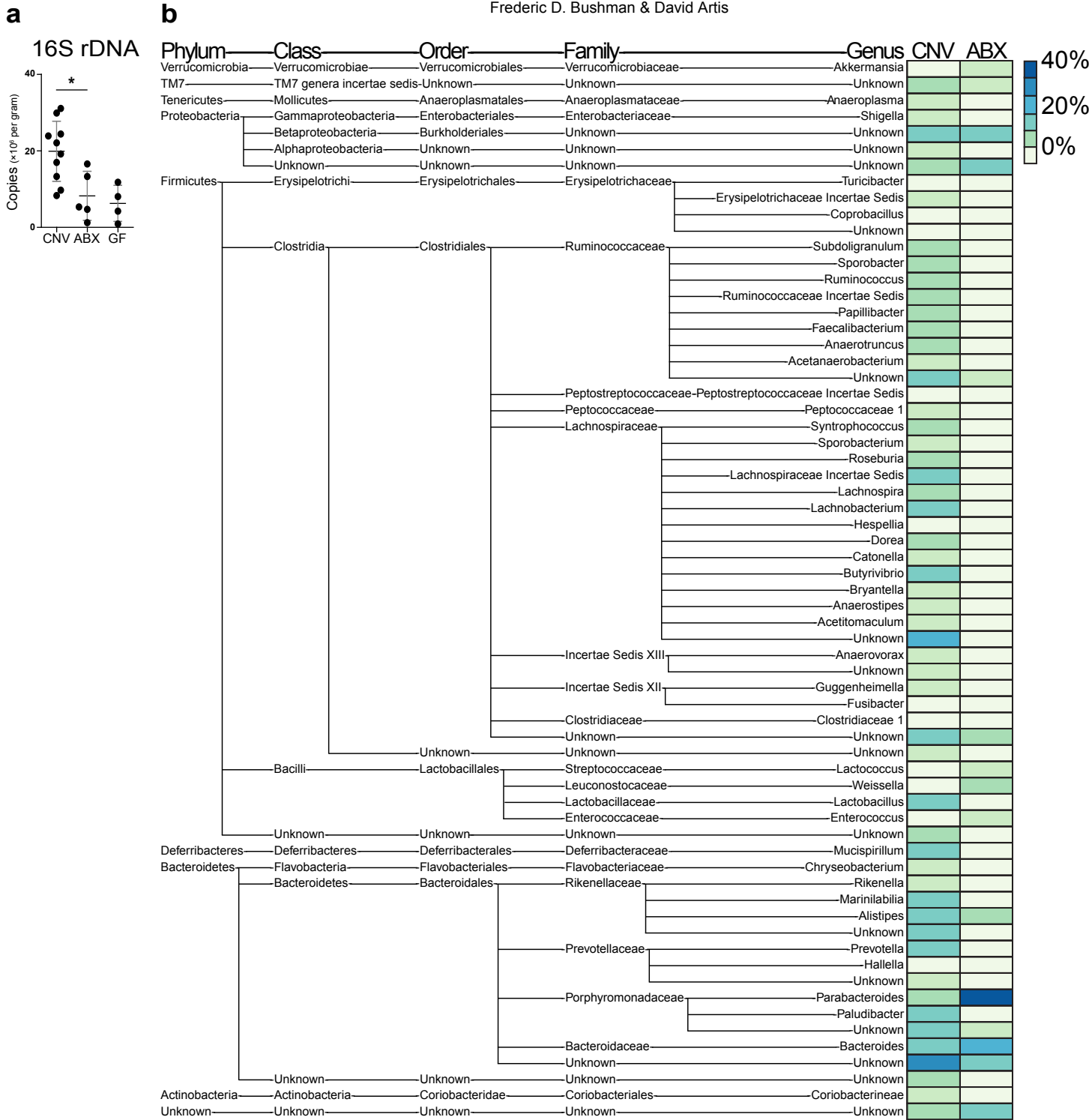
DNA manipulations, sequencing and bioinformatic analysis. We obtained 16S rRNA gene fragments as described previously²³. We carried out PCR reactions (50 µl) with the AmpliTaq System (Applied Biosystems). We gel-purified PCR products with the QIAquick Gel extraction kit (Qiagen). We pooled and pyrosequenced amplicons (100 ng). We assessed sequence quality and discarded samples with <100 sequences, and sequences were inserted into a 16S rRNA gene tree using parsimony insertion implemented in ARB software (The ARB Project). We obtained taxonomic assignments using RDP Classifier.

Statistical analyses. Results are shown as mean ± s.d. for individual mice. We determined significance by nonparametric two-tailed Mann-Whitney test, unweighted means analysis two-way ANOVA, or linear regression analysis.

Supplementary Figure 1

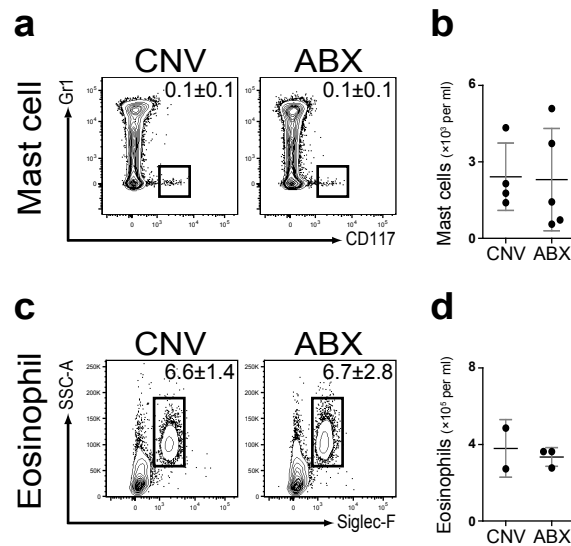
Commensal bacterial-derived signals regulate basophil hematopoiesis and allergic inflammation

David A. Hill, Mark C. Siracusa, Michael C. Abt, Brian S. Kim, Dmytro Kobuley, Masato Kubo, Taku Kambayashi, David F. LaRosa, Ellen D. Renner, Jordan S. Orange, Frederic D. Bushman & David Artis



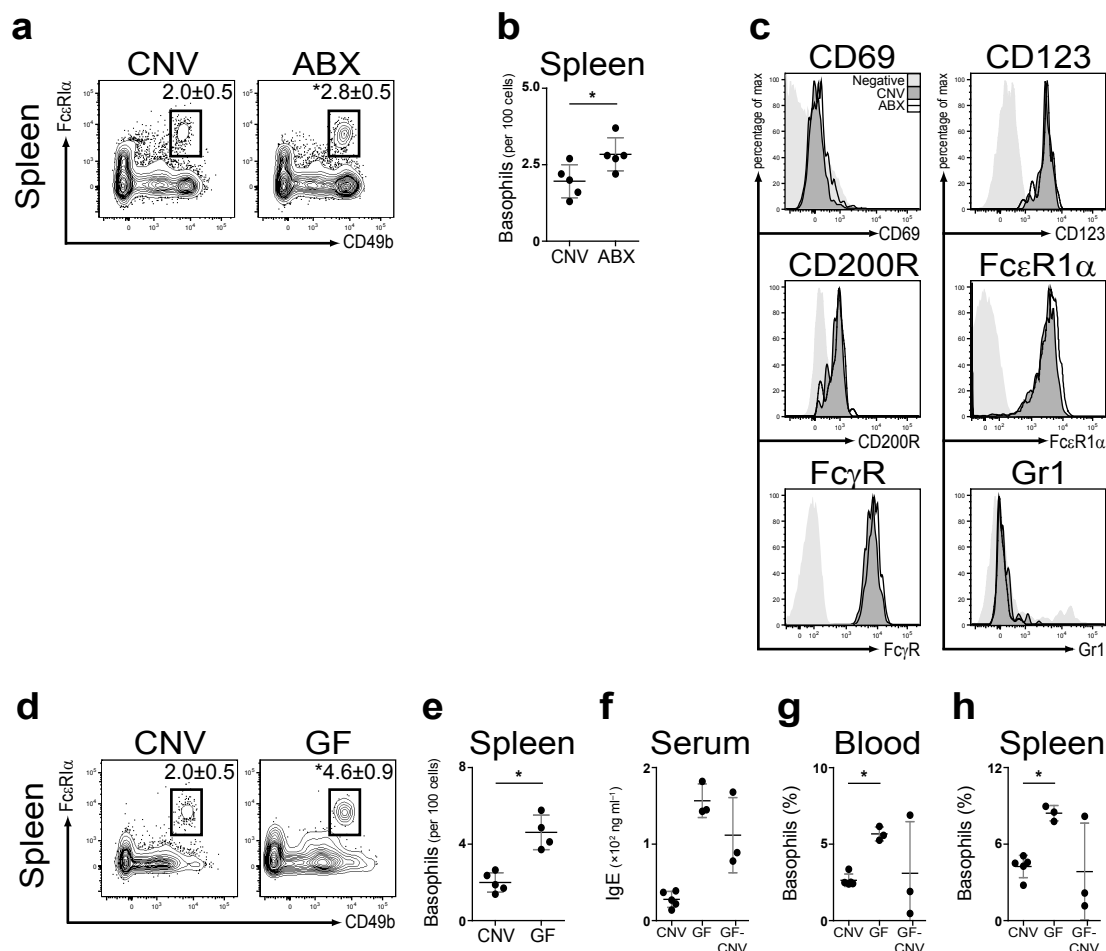
Supplementary Figure 1 Oral antibiotic treatment results in quantitative and qualitative alterations to commensal bacterial communities of the murine intestine. **(a)** Copies of bacterial 16S rDNA genes in stool from conventionally-reared (CNV), ABX-treated (ABX), or germ-free (GF) mice (mean $\pm$ s.d.; CNV, $n=10$; ABX, $n=5$; GF, $n=4$; significance determined by Mann-Whitney test (*, $P \leq 0.05$). **(b)** Genus-level phylogenetic classification of 16S rDNA segments in stool pellets collected from CNV or ABX mice. Colors represent the percent contribution of each rDNA segment to the total population of rDNA segments recovered per treatment group.

Supplementary Figure 2



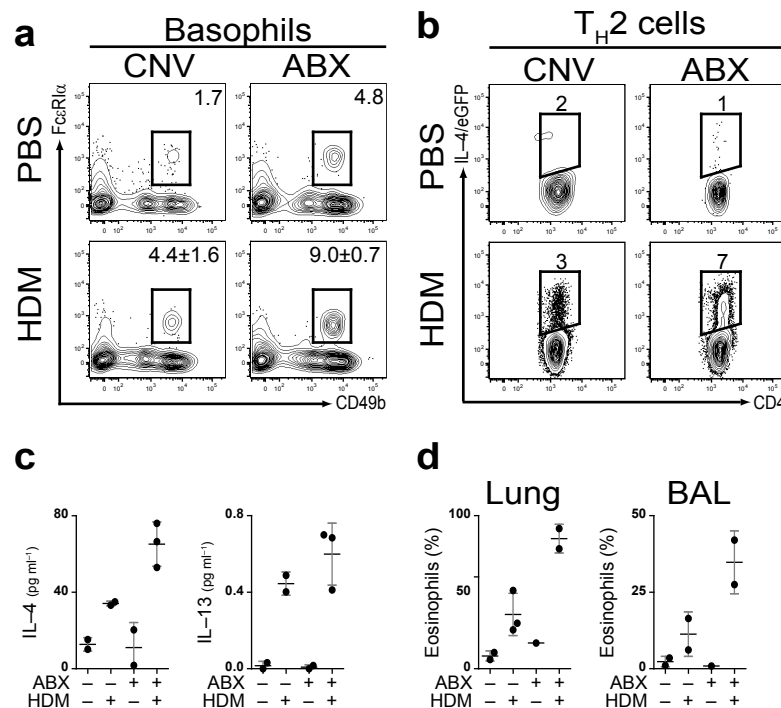
Supplementary Figure 2 Similar mast cell and eosinophil frequencies and numbers in blood of conventionally-reared or antibiotic-treated mice. **(a)** Flow cytometric analysis of blood mast cells from conventionally-reared (CNV) or antibiotic-treated (ABX) mice. Numbers adjacent to outlined areas indicate percent cells in each gate. Gated on CD3⁺, CD4⁺, CD8⁺, CD19⁺ cells. **(b)** Number of mast cells per ml of blood from CNV or ABX mice. **(c)** Flow cytometric analysis of blood eosinophils from CNV or ABX mice. **(d)** Number of eosinophils per ml of blood from CNV or ABX mice. Data representative of three or more independent experiments, results shown as mean ± S.D (CNV, n=2–4; ABX, n=3–5).

Supplementary Figure 3



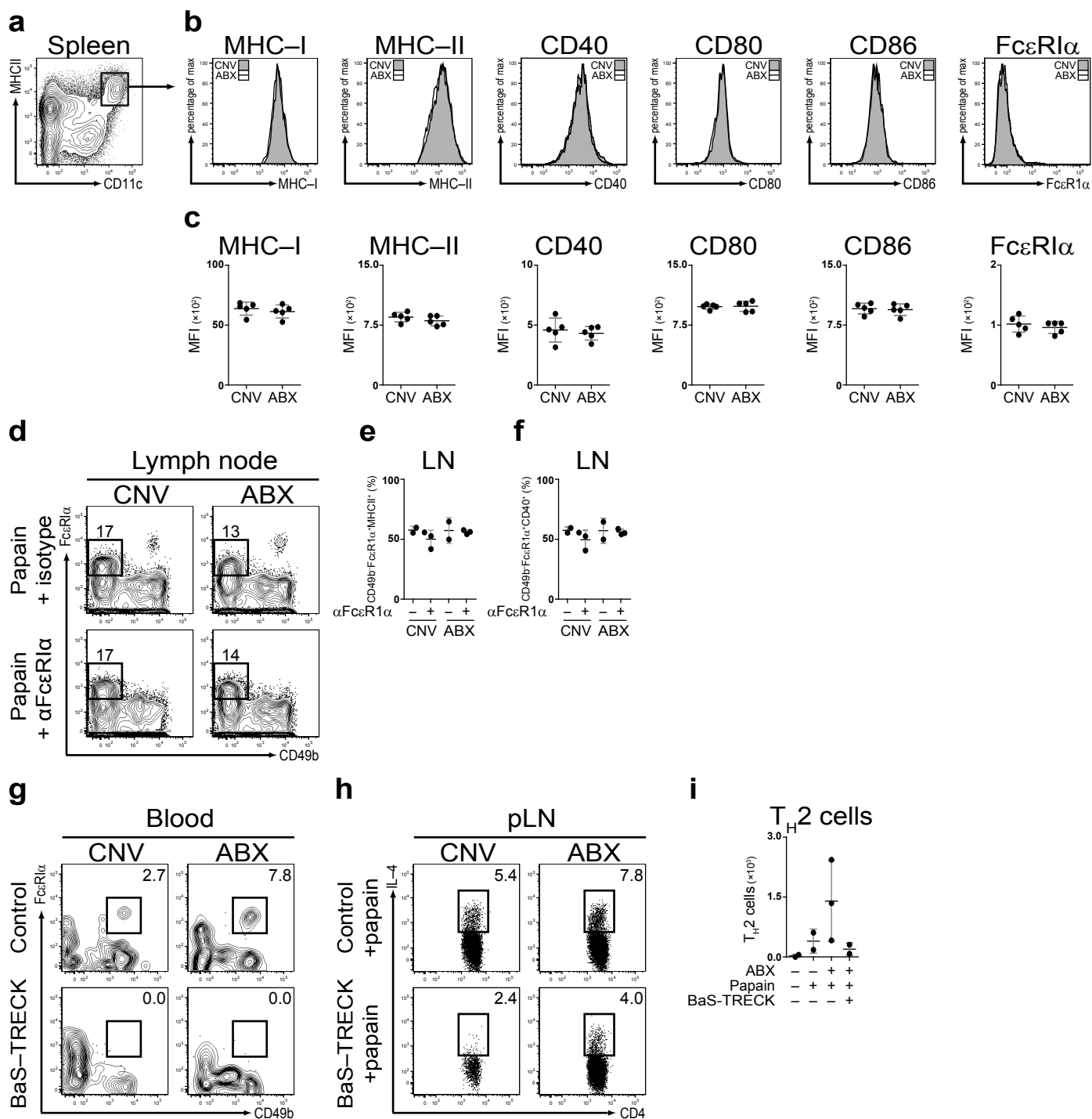
Supplementary Figure 3 Examination of steady-state basophil populations in antibiotic-treated, germ-free or conventionalized mice. **(a)** Flow cytometric analysis of spleen basophils from conventionally-reared (CNV) or antibiotic-treated (ABX) mice (CNV, *n*=5; ABX, *n*=5). Numbers adjacent to outlined areas indicate percent cells in each gate. Gated on CD3⁻, CD4⁻, CD8⁻, CD19⁻, CD117⁻ cells. **(b)** Number of spleen basophils from CNV or ABX mice (CNV, *n*=5; ABX, *n*=5). **(c)** Mean fluorescence intensity of surface CD69, CD123, CD200R, FcεR1α, FcγR and Gr1 on blood basophils from CNV-reared or ABX-treated mice as determined by flow cytometry. Gated on CD3⁻, CD4⁻, CD8⁻, CD19⁻, CD117⁻, CD49b⁺, FcεR1α⁺ cells. **(d)** Flow cytometric analysis of spleen basophils from CNV or germ-free (GF) mice (CNV, *n*=5; GF, *n*=4). **(e)** Number of spleen basophils from CNV or GF mice (CNV, *n*=5; GF, *n*=4). **(f)** Serum IgE from CNV, GF or conventionalized germ-free (GF-CNV) mice as measured by ELISA (CNV, *n*=5; GF, *n*=3; CNV-GF, *n*=3). **(g)** Frequency of blood basophils from CNV, GF or GF-CNV mice (CNV, *n*=5; GF, *n*=3; CNV-GF, *n*=3). **(h)** Frequency of spleen basophils from CNV, GF or GF-CNV mice (CNV, *n*=5; GF, *n*=3; CNV-GF, *n*=3). Data representative of three or more independent experiments, results shown as mean ± s.d., significance determined by Mann-Whitney test (*, *P* ≤ 0.05).

Supplementary Figure 4



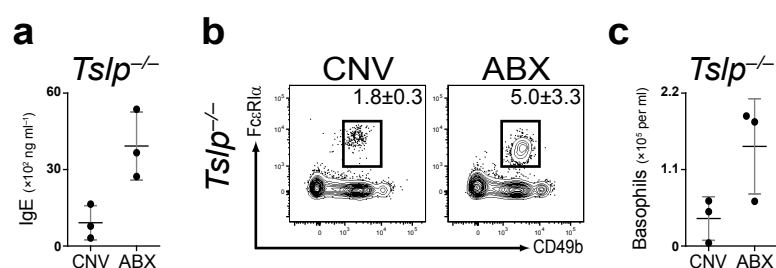
Supplementary Figure 4 Exaggerated house dust mite–elicited inflammation and T_H2 cell responses in antibiotic–treated mice. **(a)** Flow cytometric analysis of blood basophils from control (PBS) or house dust mite (HDM)–exposed, CNV or ABX mice. Numbers adjacent to outlined areas indicate percent cells in each gate. Gated on CD3[−], CD4[−], CD8[−], CD19[−], CD117[−] cells. **(b)** Flow cytometric analysis of mediastinal lymph nodes (MLN) IL-4/eGFP⁺CD4⁺ T_H2 cells from CNV or ABX mice exposed to PBS or HDM. Gated on CD8[−], CD19[−], CD4⁺ cells. **(c)** IL-4 or IL-13 levels in culture supernatants of MLN cells from CNV or ABX mice exposed to PBS or HDM as measured by ELISA. **(d)** Frequencies of eosinophils in lung digestions or bronchoalveolar lavage (BAL) samples from CNV or ABX mice exposed to PBS or HDM as measured by flow cytometry. Data representative of two or more independent experiments, results shown as mean ± S.D (CNV, n=2–3; ABX, n=2–3).

Supplementary Figure 5



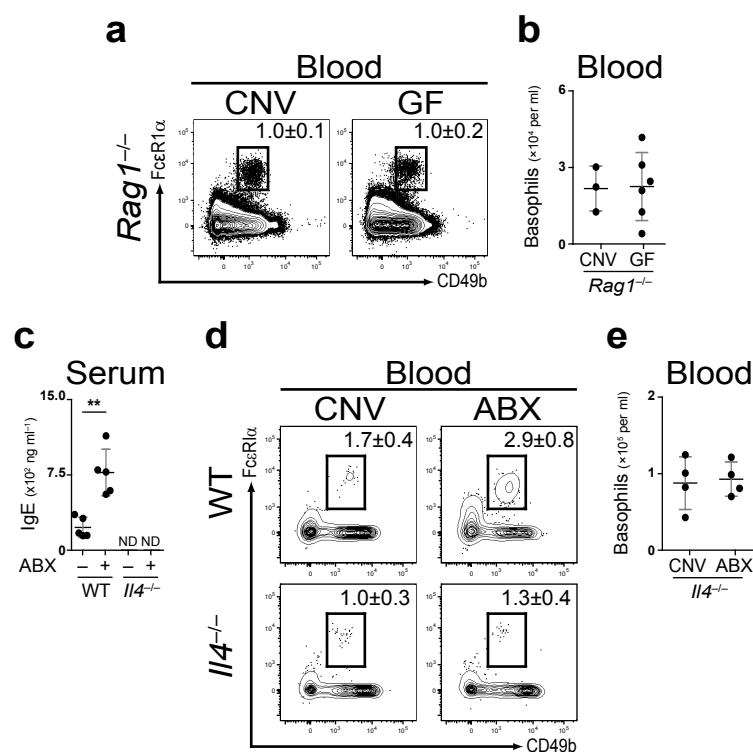
Supplementary Figure 5 Similar dendritic cell phenotype in conventionally-reared or antibiotic-treated mice. **(a)** Gating of splenic dendritic cells (DCs). **(b)** Mean fluorescence intensity (MFI) of surface MHC-I, MHC-II, CD40, CD80, CD86 and FcεRIα on splenic DCs from conventionally-reared (CNV) or antibiotic-treated (ABX) mice as determined by flow cytometry. Gated on CD3⁻, CD4⁻, CD8⁻, CD19⁻, MHC-II⁺, CD11c⁺ cells. **(c)** MFI of MHC-I, MHC-II, CD40, CD80, CD86 and FcεRIα on splenic DCs from CNV or ABX mice (CNV, n=5; ABX, n=5). **(d)** Flow cytometric analysis of popliteal lymph node (pLN) CD49b⁻ FcεRIα⁺ cells from CNV or ABX mice treated with papain and isotype or αFcεRIα antibody. Numbers adjacent to outlined areas indicate percent cells in each gate (CNV-ISO, n=2; CNV-αFcεRIα, n=3; ABX-ISO, n=2; ABX-αFcεRIα, n=3). Gated on CD3⁻, CD4⁻, CD8⁻, CD19⁻, CD117⁻ cells. **(e)** Frequencies of pLN CD49b⁻ FcεRIα⁺ MHC-II⁺ cells from CNV or ABX mice treated with papain and isotype or αFcεRIα antibody (CNV-ISO, n=2; CNV-αFcεRIα, n=3; ABX-ISO, n=2; ABX-αFcεRIα, n=3). **(f)** Frequencies of pLN CD49b⁻ FcεRIα⁺ CD40⁺ cells from CNV or ABX mice treated with papain and isotype or αFcεRIα antibody (CNV-ISO, n=2; CNV-αFcεRIα, n=3; ABX-ISO, n=2; ABX-αFcεRIα, n=3). **(g)** Flow cytometric analysis of pLN basophils from CNV or ABX, control or BaS-TRECK mice treated with diphtheria toxin (DT). Gated on CD3⁻, CD4⁻, CD8⁻, CD19⁻, CD117⁻ cells (Control-CNV, n=4; Control-ABX, n=3; BaS-TRECK-CNV, n=2; BaS-TRECK-ABX, n=3). **(h)** Flow cytometric analysis of pLN CD4⁺ TH2 cells from control or BaS-TRECK mice treated with PBS or papain and DT. Gated on CD8⁻, CD19⁻, CD4⁺ cells (Control-CNV, n=2; Control-ABX, n=1; BaS-TRECK-CNV, n=3; BaS-TRECK-ABX, n=2). **(i)** Number of day 4 pLN IL-4⁺ or IL-5⁺ CD4⁺ TH2 cells from CNV (-) or ABX (+) control (-) or BaS-TRECK (+) mice treated with PBS (-) or papain (+) and DT (control-PBS, n=2; control-papain, n=3; BaS-TRECK-PBS, n=1; BaS-TRECK-papain, n=3). Data representative of two or more independent experiments, results shown as mean ± s.d.

Supplementary Figure 6



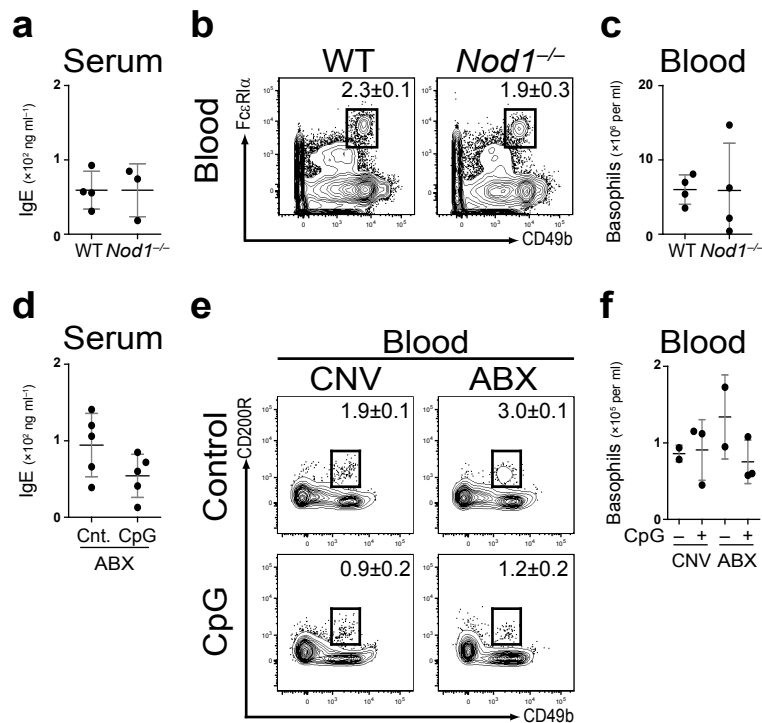
Supplementary Figure 6 Similar serum IgE levels and blood basophil frequencies and numbers in conventionally-reared or antibiotic-treated TSLP-deficient mice. **(a)** Serum IgE from conventionally-reared (CNV) or antibiotic-treated (ABX) TSLP-deficient (*Ts/p^{-/-}*) mice as measured by ELISA. **(b)** Flow cytometric analysis of blood basophils from CNV or ABX *Ts/p^{-/-}* mice. Numbers adjacent to outlined areas indicate percent cells in each gate. Gated on CD3⁻, CD4⁻, CD8⁻, CD19⁻, CD117⁻ cells. **(c)** Number of basophils per ml of blood from CNV or ABX *Ts/p^{-/-}* mice. Data representative of two or more independent experiments, results shown as mean ± S.D (CNV, n=3; ABX, n=3).

Supplementary Figure 7



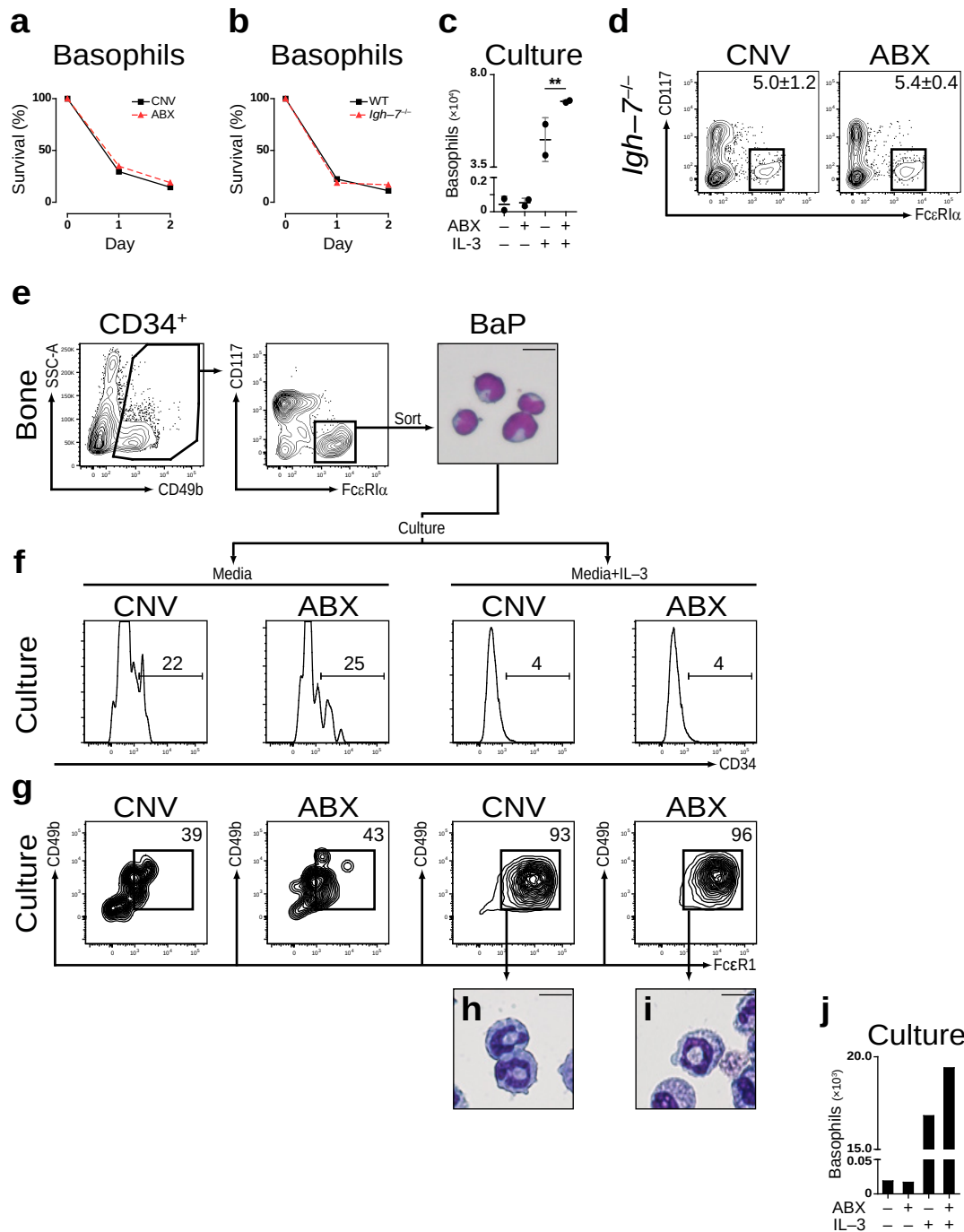
Supplementary Figure 7 Similar blood basophil frequencies in conventionally-reared or antibiotic-treated *Rag1* or *Il4*-deficient mice. **(a)** Flow cytometric analysis of blood basophils from conventionally-reared (CNV) or germ-free (GF) *Rag1*^{-/-} mice. Numbers adjacent to outlined areas indicate percent cells in each gate (CNV, n=3; GF, n=6). Gated on CD3⁻, CD4⁻, CD8⁻, CD19⁻, CD117⁻ cells. **(b)** Number of basophils per ml of blood from CNV or GF *Rag1*^{-/-} mice (CNV, n=3; GF, n=6). **(c)** Serum IgE from CNV or ABX wild-type (WT) or *Il4*^{-/-} mice as measured by ELISA (ND, not detected). **(d)** Flow cytometric analysis of blood basophils from CNV or ABX, WT or *Il4*^{-/-} mice (CNV-WT, n=5; ABX-WT, n=5; CNV-*Il4*^{-/-}, n=4-5; ABX-*Il4*^{-/-}, n=3-4). **(e)** Number of basophils per ml of blood from CNV or ABX *Il4*^{-/-} mice (CNV-WT, n=5; ABX-WT, n=5; CNV-*Il4*^{-/-}, n=4-5; ABX-*Il4*^{-/-}, n=3-4). Data representative of three or more independent experiments, results shown as mean ± s.d.

Supplementary Figure 8



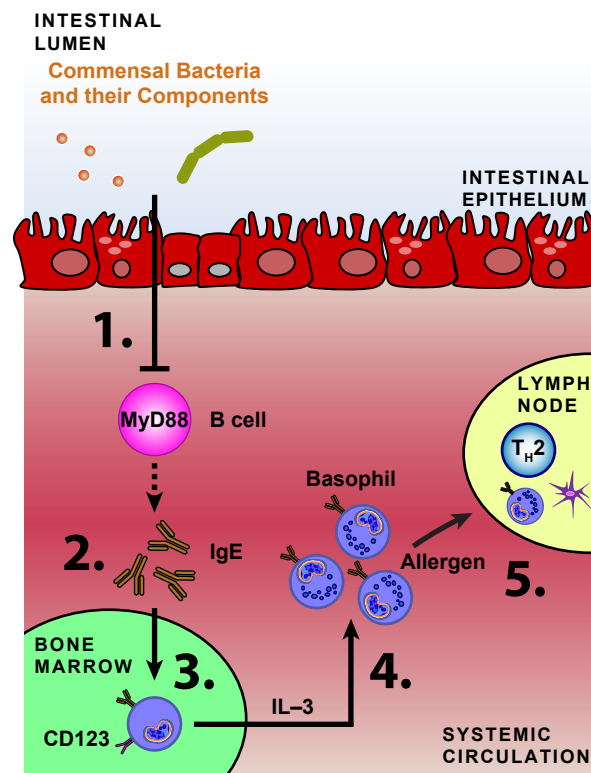
Supplementary Figure 8 Commensal regulation of steady-state basophils is independent of *Nod1* and CpG is sufficient to reduce serum IgE levels and blood basophils. **(a)** Serum IgE levels from wild-type (WT) or *Nod1* $^{-/-}$ mice as measured by ELISA. **(b)** Flow cytometric analysis of blood basophils from WT or *Nod1* $^{-/-}$ mice. Numbers adjacent to outlined areas indicate percent cells in each gate (WT, $n=4$; *Nod1* $^{-/-}$, $n=3$). Gated on CD3 $^{-}$, CD4 $^{-}$, CD8 $^{-}$, CD19 $^{-}$, CD117 $^{-}$ cells. **(c)** Number of basophils per ml of blood from WT or *Nod1* $^{-/-}$ mice (WT, $n=4$; *Nod1* $^{-/-}$, $n=4$). **(d)** Serum IgE from antibiotic-treated (ABX) mice treated with control or CpG as measured by ELISA. **(e)** Flow cytometric analysis of blood basophils from CNV or ABX mice treated with control or CpG (CNV–Control, $n=2$; CNV–CpG, $n=2$; ABX–Control, $n=5$; ABX–CpG, $n=5$). **(f)** Number of basophils per ml of blood from CNV or ABX mice treated with control or CpG (CNV–Control, $n=2$; CNV–CpG, $n=2$; ABX–Control, $n=2$; ABX–CpG, $n=3$). Data representative of two or more independent experiments, results shown as mean $\pm$ s.d.

Supplementary Figure 9



Supplementary Figure 9 Basophil survival and development in conventionally-reared or antibiotic-treated mice. **(a)** Basophils were sort-purified from conventionally-reared (CNV) or antibiotic-treated (ABX) mice and cultured in the presence of IL-3. **(b)** Basophils were sort-purified from wild-type (WT) or *Igh-7^{-/-}* mice and cultured in the presence of IL-3. **(c)** Bone marrow of CNV or ABX mice was cultured in the presence or absence of IL-3, and basophil numbers were determined (means of two experiments $\pm$ s.d.; CNV-Media, n=5; ABX-Media, n=3; CNV-IL3, n=10; ABX-IL3, n=8; significance determined by 2 way ANOVA; **, $P \leq 0.01$). **(d)** Flow cytometric analysis of bone BaPs from CNV or ABX *Igh-7^{-/-}* mice. Gated on CD3⁻, CD4⁻, CD8⁻, CD19⁻, CD34⁺ cells (CNV, n=4; ABX, n=4). **(e)** BaPs were sort purified from CNV or ABX mice and cultured in the presence or absence of IL-3 and **(f)** CD34 expression **(g)** CD49b and FcεR1α expression, **(h,i)** cellular morphology and **(j)** number of basophils was determined. Data representative of two or more independent experiments, results shown as mean $\pm$ s.d.

Supplementary Figure 10



Supplementary Figure 10 Model of commensal regulation of basophil development, circulating basophil populations, and basophil-associated T_H2 cell responses. Commensal bacterial-derived signals act via B cell-intrinsic, MyD88-dependent signaling pathways to limit production of IgE (1). In the absence of commensal-derived signals, serum IgE levels increase (2). Binding of IgE to bone marrow-resident basophil precursors directly or indirectly increases precursor expression of CD123 and responsiveness to IL-3, and results in maturation and exit of precursors from the bone marrow and expansion of the steady-state population of circulating basophils (4). In the event of allergen exposure, basophils are recruited to draining lymph nodes where they contribute to the generation of T_H2 cell responses (5).