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 (TH2) 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 TH2 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 TH2 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+ TH2 cells, IgE production and recruitment of effector cells to sites of tissue inflammation3,4. It is thought that susceptibility to TH2 cytokine–dependent allergic inflammation is influenced by both polymorphisms in mammalian genes5 and environmental factors including diet and exposure to pollutants or infectious agents6–8. 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 diseases13,14. Studies in animal model systems have further implicated commensal-derived signals in influencing the development of TH2 cytokine–mediated allergic inflammation15–18. However, the mechanisms through which the innate immune system recognizes commensal-derived signals and regulates TH2 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 TH2 cytokine–dependent allergic inflammation. Depletion or deletion of bacterial communities was associated with elevated serum IgE concentrations, increased circulating basophil populations and exaggerated TH2 cell responses and allergic inflammation. Exaggerated TH2 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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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) and significant increases in serum IgE levels20–21 (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*FcεRε+) 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, FcεRε, 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 influence 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 allergic28,29 stimuli, where they may cooperate with dendritic cells (DCs) to promote optimal T H2 cell responses27,29–31. To test whether antibiotic-treated mice showed 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. Antibiotic treatment did not alter the frequency or number 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 influence 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.

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of a FcRRIα-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 IL-2 cells, compared to conventionally reared mice. DT treatment of conventionally reared or antibiotic-treated BaS-TRECK mice, but not littermate controls, resulted in increased circulating basophil frequencies and numbers (Supplementary Fig. 5g) and reductions in the frequency (Supplementary Fig. 5h) and number (Supplementary Fig. 5i) of T IL-2 cells. Together with the results of FcRRIα-specific antibody–mediated basophil depletion, DT receptor–mediated deletion indicates that basophils contribute to the exaggerated allergen-induced T IL-2 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

To further examine the contributions of basophils to the exaggerated T IL-2 cell responses observed in antibiotic-treated mice, we adopted a loss-of-function approach complementary to FcRRIα-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 IL-2 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 IL-2 cells. Together with the results of FcRRIα-specific antibody–mediated basophil depletion, DT receptor–mediated deletion indicates that basophils contribute to the exaggerated allergen-induced T IL-2 cell responses observed in antibiotic-treated mice.
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−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 as measured by ELISA (ND, not detected). (f) Flow cytometric analysis of blood basophils from CNV or ABX WT or IgH-7−/− mice as measured by ELISA (ND, not detected). (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 as measured by ELISA (IgG, n = 4; IgE, n = 4). Data representative of three or more independent experiments, results shown as mean ± s.d., significance determined by Mann-Whitney test unless otherwise indicated (* P ≤ 0.05).
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. To test whether IgE-specific antibody treatment influences serum IgE concentrations or circulating basophil populations, we treated conventionally reared or germ-free mice with control or IgE-specific antibody (anti-IgE (+)). Numbers adjacent to outlined areas indicate percentage cells in each gate (CNV-control, n = 10; GF-control, n = 10). 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). 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 PRRs. Compared to littermate controls, conventionally reared Myd88−/− mice had higher serum IgE concentrations (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 signals. 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 basophil 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 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 basophils (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. 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...
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 influence 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 populations. 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 from germ-free mice (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.

**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) signaling, 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−CD34+ cells (CNV, experiments, results shown as mean ± 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−CD19−CD34+ cells (CNV, n = 5; GF, n = 5).

(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 ≤ 0.01, * P ≤ 0.05). (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 ≤ 0.01, * P ≤ 0.05).

(c) 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 ≤ 0.01, * P ≤ 0.05).

(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−CD19−CD34+ cells (CNV, n = 2; ABX, n = 2; GF, n = 2).

(f) Flow cytometric analysis of blood basophils from CNV or ABX Csf2rb2−/− mice. Gated on CD3−CD4−CD19−CD117− cells (CNV, n = 3; ABX, n = 3).

(d) 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; GF-control versus GF–anti-IgE, * P ≤ 0.05, GF-control versus GF–anti-IgE, t 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; GF-control versus GF–anti-IgE, * 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).

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 IgE-mediated signal influences bone marrow BaP populations in part by regulating IgE-mediated expression of CD123.

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-3Rβ3 and IL-3Rε receptor subunits (Csf2rb2−/−/Csf2rb2−/−). Antibiotic treatment of Csf2rb2−/−/Csf2rb2−/− 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.

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 CD123 (Supplementary Fig. 9f).

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 IgE-mediated signal influences 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 Th2 cytokine-dependent allergic inflammation. Antibiotic treatment resulted in elevated serum IgE concentrations, increased circulating basophil populations and exaggerated basophil-associated Th2 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 allergy. 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 pathologic elevations in circulating IgE concentrations.

Previous reports that used genetic deletions in Myd88 identified MyD88-dependent signaling as a major regulator of Th2 cytokine-associated inflammation. Here B cell–intrinsic expression of IgE can inhibit IgE class switching by B cells. Consistent with this, human subjects with deficiencies in the MyD88 or interleukin-1 receptor-associated kinase 4 signaling pathways showed elevated serum IgE concentrations. 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. 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. Further analysis of the effects of selective manipulation of commensal bacteria on DCs and regulatory T cells (including inducible LAP+ cell populations) 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/.

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AUTHOR CONTRIBUTIONS

D.A.H., M.C.S. and D.A. designed and performed the research; D.E.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−/−, Ilg-7−/−, IL-4/eGFP reporter, Il4−/−, Myd88−/−, Nod1−/− and Tlrp−/− mice (8–24 weeks of age) were bred at 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) and killed them on day 0 for further analyses. We injected mice subcutaneously in contralateral legpads with 50 µl of PBS with or without 50 µg of papain (Calbiochem) and killed them on day 3 or 4 after injection for basophil or Tg_{α2} cell analyses. We treated mice by intraperitoneal injection (i.p.) with 10 µg of FcRα-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 (eBioscience) on day −3, day −2 and day −1 before killing them for further analyses. We quantified 16S rDNA by real-time RT-PCR with degenerate bacterial 16S rDNA-specific primers (5′-AGAGTTTGATCCTGGCTCAG-3′; probe; + precedes position of LNA base).

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 (lungen), homogenized, purified of red blood cells by histopaque (Sigma-Aldrich) or red blood cell lysis, and stained at a 1:200 dilution with antibodies against human CD11c (3.9), CD19 (HB19), CD117 (1H2.3F3), CD204 (145-2C11), CD40 (HT4), CD45R (RA3-6B2), CD49b (DX5), CD69 (H1.2F3), CD80 (1B10), CD86 (GL1), CD117 (2B8), CD123 (SB11), CD200R (OX108), FcRγII (MAR1), FcγR (2.4G2), Gr-1 (RB6-8L5), IgE (R35-72), IgM (II/41), IL-4 (11B11), IL-13 (eBio13A), Kβ6 (SoLA15), MHC-I (SF1-1.1), MHC-II (2G9) and Siglec F (ES0-2440) (BD Bioscience, BioLegend, eBioscience, AbD Serotec).

We obtained samples from subjects with heperimmunoglobulinemia 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 (HB19), CD117 (14D2), CD123 (6H6), FcεRα (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 node 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 brefeldia 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 × 10^6 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 collected sorted and untreated control peritoneal lavages and stained with FixDect (eBioscience). We pooled and stained fetal liver mononuclear cells by Ficoll (GE) gradient, stained at a 1:200 dilution with antibodies against human CD11c (3.9), CD19 (HB19), CD117 (1H2.3F3), CD204 (1B10), CD86 (GL1), CD117 (2B8), CD123 (SB11), CD200R (OX108), FcγRII (MAR1), FcγR (2.4G2), Gr-1 (RB6-8L5), IgE (R35-72), IgM (II/41), IL-4 (11B11), IL-13 (eBio13A), Kβ6 (SoLA15), MHC-I (SF1-1.1), MHC-II (2G9) and Siglec F (ES0-2440) (BD Bioscience, BioLegend, eBioscience, AbD Serotec).

We carried out RT-PCR with degenerate bacterial 16S rDNA-specific primers (5′-AGAGTTTGATCCTGGCTCAG-3′; forward), (5′-CTGCTGCTCYCGTA-3′; reverse), (5′-FAM-TA+ACA+CATG+CA+AGTC+GA-BHQ1-3′; probe; + precedes position of LNA base).

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′-CTGCTGCTCYCGTA-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.e. for individual mice. We determined significance by nonparametric two-tailed Mann-Whitney test, unweighted means analysis two-way ANOVA, or linear regression analysis.