

The influence of commensal bacteria-derived signals on basophil-associated allergic inflammation

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Commensal bacteria that colonize mammalian mucosal surfaces are reported to influence T helper type 2 (T_H2) cytokine-dependent inflammation and susceptibility to allergic disease. However, the mechanisms that underlie these observations are only beginning to be understood. We recently utilized studies of murine model systems and atopic patient populations to elucidate a mechanism by which commensal bacteria-derived signals limit serum immunoglobulin E levels, influence basophil development and steady-state circulating basophil populations and regulate basophil-associated T_H2 cell responses and allergic inflammation. In this addendum, we summarize the findings of our recent work and other developments in the field, discuss the broader implications of these findings and generate new hypotheses regarding our understanding of host-commensal relationships. These areas of investigation may be applicable to the development of new preventative or therapeutic approaches to reduce the burden of allergic disease.

Introduction

Allergic diseases have reached pandemic levels¹ and represent a significant source of morbidity, mortality and healthcare cost.² These chronic inflammatory diseases are characterized by interleukin (IL)-4, IL-5, IL-9 and IL-13 production by CD4⁺ T helper type 2 (T_H2) cells, immunoglobulin E (IgE) production by B cells and the 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 as well as environmental factors including host 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 research.

The human intestine is colonized by 100 trillion microorganisms belonging to each of the three domains of life.^{9,10} Of these, bacteria are the most abundant; the colon is home to a population of trillions of commensal bacteria¹¹ that is estimated to be composed of thousands of different species.¹² 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 display altered intestinal bacterial populations early in life,¹³ and children who have undergone treatment with broad-spectrum antibiotics are at an increased risk of developing allergic disease.^{14,15} Together, these and other observations provoke the hypothesis that commensal bacteria influence the severity of allergic disease. However, the molecular and cellular mechanisms by which commensal bacteria-derived signals regulate allergic inflammation are not well understood.^{16,17}

Studies of animal model systems have identified correlations between alterations

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Abbreviations: IL, Interleukin; T_H2 , T Helper Type 2; IgE, Immunoglobulin E; IL-3R, IL-3 receptor; DC, Dendritic cell; BaP, Basophil precursor; TSLP, Thymic stromal lymphopoietin

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in commensal bacteria-derived signals and exaggerated allergic inflammatory responses.¹⁸⁻²⁰ For example, work by Noverr et al. indicated that disruption of normal murine commensal bacterial populations via oral antibiotic treatment, followed by subsequent oral inoculation of the gastrointestinal tract with *Candida albicans*, results in exaggerated inflammatory responses in an IL-13-dependent model of allergic airway inflammation.^{21,22} Similarly, work by Bashir et al. indicated that oral antibiotic treatment potentiates the development of IgE-mediated allergy to food antigens in mice, a phenomenon that is mediated in part by TLR-dependent signaling.²³ More recent studies have focused on identifying the specific effects of antibiotic treatment on commensal bacterial populations^{24,25} and have correlated allergic propensity with alterations in specific innate or adaptive immune cell populations.^{18,26} For example, work by Olszak et al. indicated that germ-free mice display exaggerated lung inflammation in a model of asthma that is concurrent with increased pulmonary expression of the chemokine ligand CXCL16 and accumulation of invariant natural killer T cells in lung tissue.²⁷ Despite these advances, the specific cellular and molecular mechanisms responsible for recognition of commensal bacteria-derived signals and the initiation and propagation of subsequent Th2 cytokine-dependent inflammatory responses are poorly understood.

Granulocytes, including mast cells, eosinophils and basophils can be activated by an array of stimuli including antibodies, cytokines and proteases,^{28,29} have been shown to express MHC class II,³⁰⁻³² to be potent sources of IL-4 and, depending on the model, contribute to the development of optimal T_H2 cytokine responses.³³⁻³⁵ Given the proposed role for commensal bacteria in influencing allergy susceptibility, and the identification of granulocytes as innate cells that promote T_H2 cytokine-mediated inflammation, our recent publication examined whether commensal bacteria-derived signals influence granulocyte homeostasis or allergic inflammation.⁷⁷ We found that depletion or the absence of commensal bacteria, achieved via treatment with broad-spectrum oral antibiotics or utilization of a germ-free

mouse model, is associated with elevations in serum IgE levels, increased frequencies and numbers of circulating basophil populations and exaggerated T_H2 cytokine-dependent inflammation in a model of allergic airway 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 commensal-depleted hosts. Furthermore, IgE was found to be a critical regulator of steady-state basophil responses in antibiotic-treated or germ-free mice, and B cell-intrinsic expression of MyD88 was found to limit serum IgE levels and circulating basophil populations in vivo. Commensal-derived signals influenced circulating basophil populations by regulating the proliferative capacity of bone marrow-resident basophil progenitor populations in an IL-3 receptor (IL-3R)-dependent manner, providing one mechanism by which commensal bacteria limit basophil populations in the steady-state. Finally, subjects with hyperimmunoglobulinemia E syndrome were found to have elevated frequencies of circulating basophils compared with control subjects, an observation that is consistent with IgE influencing circulating basophil responses in human patients. Here, we discuss these and other relevant findings that suggest commensal bacteria-derived signals act via IgE and basophils to influence the development of T_H2 cytokine-dependent inflammation and allergic disease.

The Commensal Bacteria-IgE-Basophil Axis of Allergic Inflammation

Commensal-deficient mice display elevated serum IgE levels compared with commensal-sufficient mice.³⁶ Distinct from allergen-specific IgE that mediates anaphylactic reactions, IgE found in the serum of germ-free mice is composed of germ-line encoded proteins with natural specificities, implicating this molecule in functions other than antigen detection.³⁷ Consistent with this hypothesis, IgE has recently been shown to influence multiple aspects of granulocyte homeostasis in an antigen-independent manner including surface protein expression, cell survival

and cell adhesion.³⁸⁻⁴⁰ Building upon these findings, we hypothesized that antibiotic-induced elevations in serum IgE levels might be associated with quantitative or qualitative alterations in systemic granulocyte populations. Consistent with this hypothesis, increases in steady-state serum IgE levels that occurred upon antibiotic treatment of mice correlated with elevated blood basophil populations. A similar correlation was observed in germ-free mice⁴¹ indicating that the elevations in circulating basophils were the result of a reduction in commensal-derived signals. To determine whether the correlation between serum IgE levels and blood basophil populations represented a causal relationship, an IgE-deficient mouse strain (*Igh-7^{-/-}*) was utilized. Antibiotic treatment of IgE-deficient mice did not influence blood basophil populations, indicating that IgE contributes to the elevations in circulating basophils observed in antibiotic-treated mice. Additionally, injection of IgE-deficient mice with monoclonal IgE resulted in antigen-independent elevations in blood basophils. Together, these findings identified commensal bacteria and IgE as regulators of circulating basophil populations in mice.

In addition to their roles as late phase effector cells that infiltrate inflamed tissues, basophils are also recruited to draining lymph nodes early in the response to some infectious⁴² or allergic^{29,43} stimuli. While the development of T_H2 cell responses can occur independently of basophils in some models of allergy or helminth infection,^{28,44-46} basophils can promote optimal T_H2 cell responses in others.^{33-35,42,43,47,48-50} In some circumstances, basophils have been reported to cooperate with dendritic cells (DCs) to promote optimal T_H2 cell responses.^{42,43,49} Based on these findings, we hypothesized that elevated circulating basophil populations in antibiotic-treated mice might contribute to the well-established phenotype of exaggerated allergic inflammation in this model system.^{22,23} To test this hypothesis, we utilized two models in which basophils are thought to contribute to the development of optimal T_H2 cytokine-mediated allergic inflammation; the papain protease model of T_H2 cell induction and the house dust mite model of allergic airway

inflammation.^{28,29} Compared with conventionally-reared mice, antibiotic-treated mice displayed exaggerated T_H2 cell development and allergic airway inflammation using these models. Depletion of basophils, using a monoclonal antibody to the FcεRIα receptor,²⁹ resulted in reduced T_H2 cell responses upon allergen exposure. However, mast cells and some dendritic cells can express FcεRIα so caution must be exercised in interpreting findings using this approach. As an alternative strategy, we employed a diphtheria toxin-dependent basophil depletion mouse system.⁵¹ Efficient and selective depletion of basophils using this system also resulted in reduced T_H2 cell responses. Together, these results indicate that commensal bacteria-derived signals limit basophil-mediated T_H2 cell responses and allergic inflammation.

The steady-state changes in basophil populations observed in antibiotic-treated or germ-free mice were primarily quantitative. Other than an increase in surface bound IgE, significant alterations in surface marker phenotype were not observed in our experimental systems when comparing basophils from commensal-sufficient or commensal-deficient hosts. However, these findings do not exclude a role for commensal-derived signals in regulating qualitative measures of basophil function such as inflammatory cytokine production. One mechanism by which commensal-derived signals could influence qualitative measures of basophil function is by altering expression of thymic stromal lymphopoietin (TSLP). TSLP, a cytokine derived from epithelial cells as well as other immune cell populations, has been found to be regulated by IgE and is elevated in the intestines of germ-free mice.^{52,53} Compared with the basophil growth factor IL-3, TSLP can elicit a functionally distinct population of basophils from bone marrow progenitor populations.⁵¹ While TSLP was not critical for the quantitative expansion of steady-state circulating basophil populations in our experimental systems, commensal bacteria-derived signals may influence TSLP-TSLP receptor interactions and basophil populations in ways not yet appreciated. As such, further analysis of the effects of selective manipulation of commensal bacteria on TSLP

expression and TSLP-dependent basophil responses is warranted.

As discussed above, basophils cooperate with DCs to promote optimal T_H2 cell responses in some cases.^{42,43,49} Non-basophil antigen presenting cell populations were not significantly influenced by commensal bacteria-derived signals in our experimental systems. However, while basophils were shown to contribute to the development of exaggerated allergen-induced T_H2 cell responses using two methods of basophil depletion, T_H2 cell frequencies were slightly higher in antibiotic-treated mice depleted of basophils compared with conventionally-reared mice depleted of basophils. This observation suggests that other immune cell types or molecules contribute to the exaggerated T_H2 cell responses observed in antibiotic-treated or germ-free mice. Further examination of DCs, regulatory T cells and other immune cell populations known to influence T_H2 cytokine-mediated inflammatory responses should therefore be undertaken. When undertaking such studies, it is important to note that DCs, basophils, eosinophils and mast cells can share expression of certain cell surface markers such as FcεR1 and CD123.⁵⁴⁻⁵⁸ As such, gating strategies based on multiple cell surface markers, utilization of cell sorting and subsequent cytological and transcription analyses as well as cell-specific depletion methods must be utilized to ensure that resulting phenotypes are accurately attributed to the cell population in question.

Finally, while we did not observe significant differences between the germ-free or antibiotic-treated models in our study, these model systems are not identical.¹⁰ One of the best characterized differences between these two models is the significantly reduced lymphoid compartment of germ-free compared with antibiotic-treated mice.⁵⁹⁻⁶¹ Due to such limitations, it is essential that observations made in germ-free mice be confirmed in an antibiotic treatment system. However, there are also considerable benefits to germ-free systems such as the ability to perform monoclonization studies with defined microbial species. Gnotobiotic studies, in which the intestine is selectively colonized with a single bacterial species or a

cocktail of known bacteria, have led to the identification of specific commensal populations with distinct immunomodulatory functions. For example, *Clostridium* species have been shown to induce intestinal regulatory T cell populations and promote resistance to colitis⁶² while segmented filamentous bacteria have been shown to induce T_H17 cell populations in the small intestine and mediate protective responses to the intestinal pathogen *Citrobacter rodentium*.⁶³ Because of their various strengths and weaknesses, complementary use of both the germ-free and the antibiotic-treated model systems will be essential to furthering our understanding of mammalian host-commensal mutualistic relationships.

CpG and MyD88 as Regulators of Circulating Basophil Populations

To further elucidate the molecular and cellular mechanisms by which commensal bacteria-derived signals influence serum IgE levels and circulating basophil populations, we next sought to identify specific commensal bacteria-derived signals and immune cell types that were mediators of the commensal bacteria-IgE-basophil axis. Unmethylated CpG motifs in bacterial DNA are recognized by Toll-like receptor 9 and inhibit IgE class switching by B cells in vitro in a MyD88-dependent manner.⁶⁴ Additionally, administration of CpG has been shown to reduce the severity of food allergy in a TLR4-deficient mouse model.²³ Consistent with CpG regulating IgE levels and circulating basophils in vivo, treatment of germ-free mice with CpG motifs resulted in reductions in both serum IgE levels and blood basophil populations. We next sought to expand upon these observations by examining the role of MyD88-dependent signaling in B cells on serum IgE levels and circulating basophils in vivo. To do so, we adoptively transferred MyD88-sufficient or deficient B cells into B cell-deficient mice. We found that mice reconstituted with MyD88-deficient B cells displayed elevated serum IgE levels and blood basophil populations compared to controls. Together, these findings indicate that B cell-intrinsic MyD88-dependent signaling limits serum IgE levels and circulating

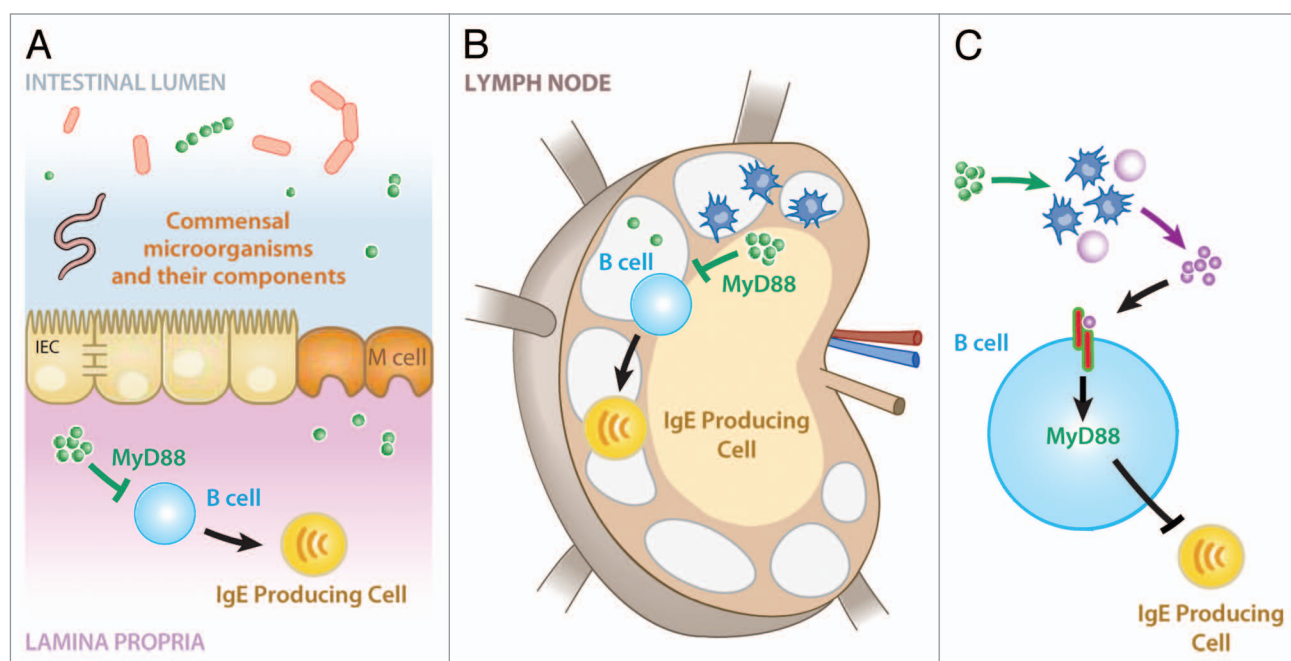


Figure 1. Potential mechanisms by which commensal-derived signals may act in a B cell-intrinsic, MyD88-dependent manner to influence IgE production. (A) Commensal-derived signals (green spheres) may act in a MyD88-dependent manner on B cells in the lamina propria or other intestinal-associated tissues to limit production of IgE. (B) Alternatively, commensal bacteria or their signals may be transported to mesenteric lymph nodes where they could act in a MyD88-dependent manner on B cells to limit production of IgE. (C) Finally, commensal-derived signals may act via one or more intermediate cell types or signaling molecules to influence B cell production of IgE in a MyD88-dependent manner. Figure adapted from reference 10.

basophil populations in vivo and that CpG is sufficient to limit serum IgE levels and circulating basophil populations in the absence of other commensal bacteria-derived signals.

While extending the molecular and cellular understanding of how commensal bacteria influence serum IgE levels in the steady-state, the observation that B cell-intrinsic MyD88-dependent signaling limits serum IgE levels and circulating basophils in vivo raises further questions. The simplest explanation for our observation is direct detection of commensal bacteria-derived signals by B cells resulting in reduced IgE class switching and production. However, this raises the question as to where in the body B cells interact with commensal-derived signals. One possibility is that B cells circulate through intestinal-associated compartments such as the lamina propria where they are exposed to commensal bacteria-derived signals (Fig. 1A). Alternatively, B cells may encounter commensal bacteria-derived signals in mesenteric lymph nodes⁶⁵ or commensal bacteria-derived signals may be present systemically at high enough levels to impart effects on systemic B cell populations

(Fig. 1B). Finally, commensal-derived signals may act on one or more intermediary cell populations to influence production of a signaling molecule that is detected by B cells in a MyD88-dependent manner (Fig. 1C). Elucidating these mechanisms may lead to the development of specific therapeutics designed to manipulate production of IgE by B cells.

Regulation of Basophil Development by Commensal Bacteria and IgE

We next sought to determine the mechanism by which commensal-derived signals and IgE regulate circulating basophil populations. Initially, based on previous studies of other granulocyte populations, we hypothesized that commensal bacteria-derived signals would act via IgE to influence basophil survival.³⁸⁻⁴⁰ However, our initial studies suggested that basophil survival is not significantly dysregulated in antibiotic-treated or germ-free mice. These findings provoked the hypothesis that commensal bacteria-derived signals may influence basophil development. Consistent with this hypothesis, we found that bone

marrow cells from antibiotic-treated or germ-free mice exhibited increased potential for basophil development compared with an equal number of bone marrow cells from conventionally-reared mice. These findings indicate that bone marrow from antibiotic-treated or germ-free mice has an increased hematopoietic potential for the development of mature basophils.

To further examine the mechanisms by which commensal bacteria influence basophil development, we first examined bone marrow-resident basophil precursor (BaP) populations. We found that BaPs from antibiotic-treated or germ-free mice displayed elevated surface expression of IL-3R compared with BaPs from conventionally-reared mice. To determine whether the increased IL-3R expression on BaPs from antibiotic-treated mice conferred increased cell responsiveness to IL-3, we sort-purified BaPs from conventionally-reared or antibiotic-treated mice and cultured them in the presence of IL-3. Cultures of BaPs from antibiotic-treated mice displayed increased basophil development in the presence of IL-3 compared with cultures of BaPs from conventionally-reared mice, indicating that BaPs from

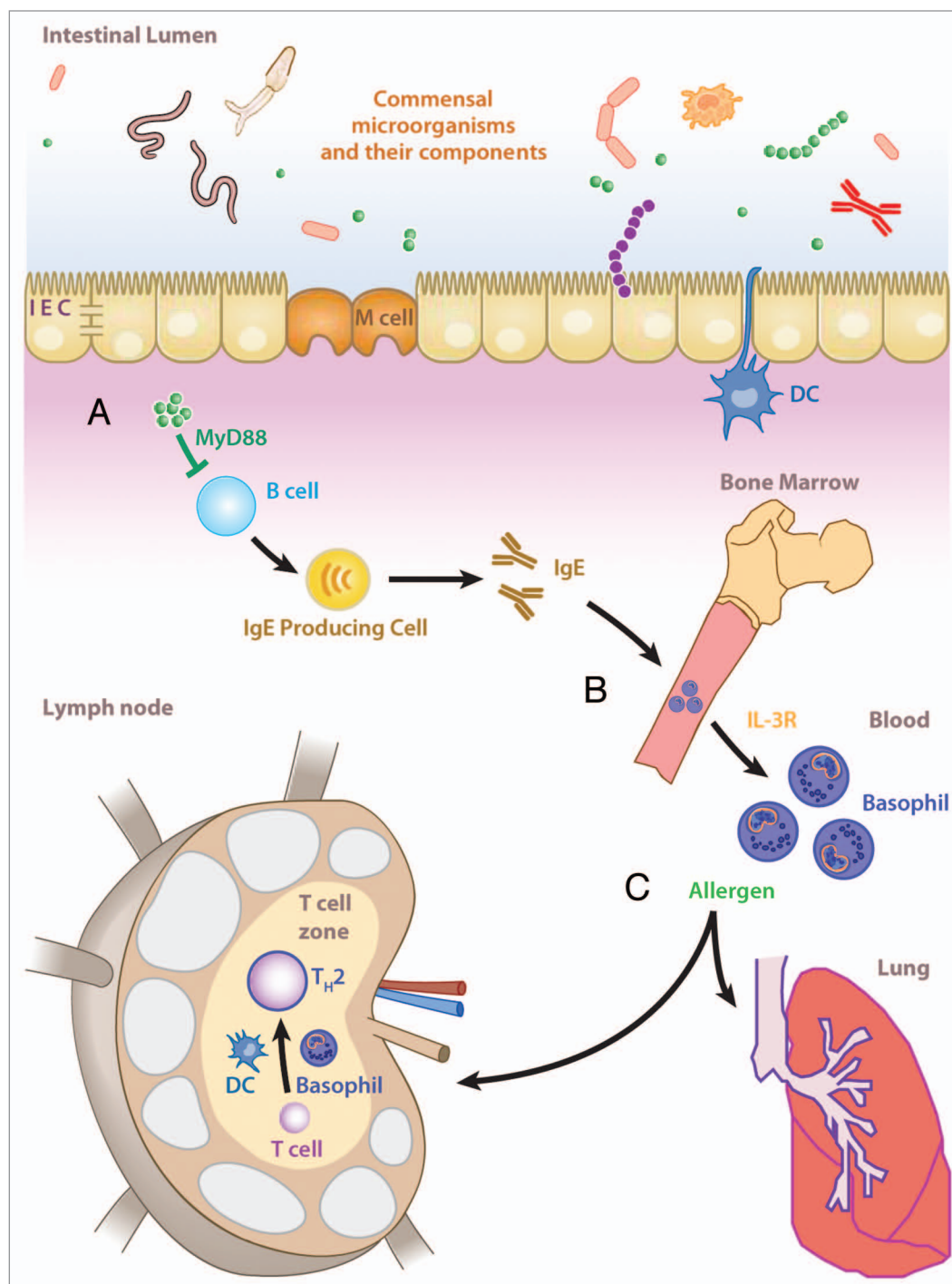


Figure 2. The commensal bacteria-IgE-basophil axis of allergic inflammation. (A) Commensal bacteria-derived signals (green spheres) act via B cell-intrinsic, MyD88-dependent signaling pathways to limit circulating levels of IgE. (B) IgE acts on bone marrow-resident basophil precursor populations to increase surface expression of the IL-3 receptor and development of mature circulating basophils. (C) Upon allergen exposure, basophils are recruited to draining lymph nodes where they potentiate the development of T_H2 cell responses and T_H2 cytokine-dependent allergic inflammation. Figure adapted from reference 10.

antibiotic-treated mice are more responsive to IL-3 stimulation. Finally, to determine whether IgE was required for commensal bacterial effects on BaP populations, we examined the influence of IgE depletion

on both bone marrow BaP IL-3R expression and total bone marrow BaP populations. Consistent with IgE mediating the alterations to BaP populations observed in germ-free mice, BaP expression of IL-3R,

as well as total BaP frequencies, were reduced in germ-free mice upon depletion of IgE. Together, these results indicate that commensal bacteria-derived signals limit basophil development from the bone

marrow by reducing circulating IgE and expression of IL-3R on BaPs.

The finding that commensal bacteria-derived signals influence development of an immune cell progenitor population in the bone marrow has broad implications for understanding both normal mammalian physiology and the pathophysiology of multiple inflammatory diseases. By influencing bone marrow hematopoietic programs, relatively small alterations in commensal populations could dramatically influence the propensity of their host to develop protective or pathogenic T_H2 cell responses to helminth parasites, environmental allergens or other stimuli that provoke this module of the immune response. Recently, several innate progenitor cell populations have been identified that can give rise to multiple mature immune cell populations depending on immunologic stimuli to which they are exposed.^{66,67} Commensal bacteria-derived signals may influence these or other hematopoietic progenitor populations to tip the immunologic balance toward or away from the development of adaptive immune responses. Finally, given that alterations in commensal bacteria have been shown to influence T_H17 and Treg responses,^{62,63,68,69} commensal regulation of hematopoietic programs may have implications beyond T_H2 cell-mediated immunity and allergic inflammation to other inflammatory and autoimmune disease states.

Relevance to Atopic Patient Populations

Given the considerable public health and economic burden of allergic diseases, the need for the development of new preventative and therapeutic strategies has never been greater.^{1,2} As such, we sought to correlate our findings in animal models with observations in human subjects with atopic disease. Subjects with loss-of-function polymorphisms in the dedicator of cytokinesis 8 gene exhibit an autosomal recessive form of hyperimmunoglobulin IgE syndrome^{70,71} which is characterized by recurrent infections, increased susceptibility to atopic eczema and average serum IgE levels 10 times higher than those

found in control subjects.⁷² Consistent with our observations in mice, we found that subjects with hyperimmunoglobulin E syndrome display elevations in circulating basophil populations compared with controls suggesting that IgE-mediated regulation of basophil homeostasis may be relevant to the pathogenesis of this human disease. Additionally, Herbst et al. recently reported a role for isotype-switched antibodies in promoting basophil expansion and effector function following animal models of helminth infection.⁷³ Further investigation is needed to determine whether IgE-mediated regulation of basophil homeostasis contributes to the inflammation observed in patients with hyperimmunoglobulin E syndrome or other conditions in which serum IgE levels are elevated such as atopy or infection.

Omalizumab is a monoclonal, humanized mouse anti-human IgE-specific antibody and FDA approved anti-allergy drug.^{74,75} We hypothesized that omalizumab treatment could be a therapeutic option to influence antigen-independent, IgE-mediated regulation of systemic basophil populations. To test whether omalizumab treatment influenced circulating basophils, conventionally-reared or germ-free mice were treated with omalizumab or control antibody and serum IgE levels and blood basophils were examined. Omalizumab treatment of germ-free mice resulted in reductions in serum IgE levels and circulating basophil populations suggesting a potential role for this drug in therapeutically manipulating IgE-dependent basophil responses. Analysis of the effects of omalizumab on human basophil populations has also been undertaken. Depletion of IgE in human subjects results in downregulation of basophil surface FcεR1α receptor expression and impaired FcεR1α-mediated basophil activation indicating that omalizumab therapy influences some qualitative aspects of basophil physiology.⁷⁶ Despite these advances, further investigation is necessary to determine whether omalizumab treatment influences basophil progenitor populations, circulating basophil numbers, T_H2 cell responses or basophil recruitment to sites of tissue inflammation

in human subjects. An appropriately conducted clinical study would be required to test these hypotheses in humans. With further investigation, omalizumab therapy may be found to be an important intervention to influence antigen-independent effects of IgE on granulocyte populations.

Conclusion

Here we discuss our recent report of a cellular and molecular mechanism by which commensal bacteria-derived signals influence basophil development and basophil-associated allergic inflammation (Fig. 2). We also propose some hypotheses to be tested in future studies. Further investigation of the commensal bacteria-IgE-basophil axis may allow for the design of new strategies to prevent or treat allergic disease in atopic patients.

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