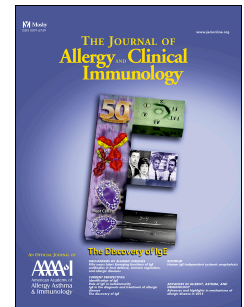


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Multifaceted roles of basophils in health and disease

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Multifaceted roles of basophils in health and disease

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Summary

Until recently, basophils had often been neglected in immunological studies because of their minority status among immune cells, or confused with tissue-resident mast cells due to some phenotypic similarities between them in spite of different anatomical localization. It is now appreciated that basophils and mast cells are distinct cell lineages, and that basophils play important and non-redundant roles distinct from those played by mast cells. On the one hand, basophils contribute beneficially to protective immunity, particularly against parasitic infections. On the other hand, basophils are involved in the development of various disorders, including allergy and autoimmune disease. Basophils interact with other immune cells and non-hematopoietic cells through cell to cell contact or basophil-derived factors such as cytokines and proteases, contributing to the regulation of immune and allergic responses. In this review article, we highlight recent advances in our understanding of basophil pathophysiology in humans and animal models by consolidating research findings reported during the past five years. Further studies on basophils and their products will help identify suitable targets for novel therapeutics in allergy and effective vaccines against parasitic infection.

Key words

Basophil, mast cell, development, allergy, anaphylaxis, inflammation, autoimmune disease, infection, antigen-presentation, IL-4, protease

Abbreviations used

APC: antigen-presenting cells

APRIL: a proliferation-induced ligand

BAFF: B cell activating factor

CIU: chronic idiopathic urticaria

CML: chronic myeloid leukemia

EoE: eosinophilic esophagitis

HGF: hepatocyte growth factor

IgE-CAI: IgE-mediated chronic allergic inflammation

ILC2: group 2 innate lymphoid cell

ICD: irritant contact dermatitis

IVIG: intravenous immunoglobulin

MHC-II: MHC class II

mMCP: mouse mast cell protease

Nb: *Nippostrongylus brasiliensis*

PAF: platelet-activating factor

SLE: systemic lupus erythematosus

TSLP: thymic stromal lymphopoietin

Key points

- Basophils and mast cells are distinct cell lineages, and their development is differentially regulated by distinct transcription factors.
- Basophils contribute to the development of allergic inflammation in the skin, respiratory and gastrointestinal tracts as well as systemic anaphylaxis.
- Basophils are involved in the pathogenesis of non-allergic inflammation, including autoimmune and inflammatory bowel disease.
- Basophil-like neoplastic cells contribute to CML progression.
- Basophils can both promote pathology in tissue allograft and limit pathology in graft-versus-host disease.
- Basophils play beneficial roles in protective immunity against parasitic infections.
- Basophils interact with other immune cells to positively or negatively regulate various immune responses.
- Further studies are needed to verify that the findings in mouse basophils are relevant to human basophils.
- Basophils and their products could be promising therapeutic targets for the treatment of immune disorders.

Introduction

Basophils are the least abundant granulocytes, representing less than 1% of peripheral blood leukocytes. The existence of basophils in the blood, as cells possessing basophilic granules, was first described by Paul Ehrlich in 1879, much earlier than the discovery of T and B cells. Nevertheless, the functional significance of basophils remained uncertain until recently. Difficulty in collecting sufficient number of basophils, absence of cell lines corresponding to primary basophils, and the paucity of analytical tools such as animals deficient for only basophils hampered the advance of basophil research, in contrast to extensive studies on mast cells. Basophils share some characteristics with tissue-resident mast cells, including the presence of basophilic granules in the cytoplasm, the surface expression of high-affinity of IgE receptor, and the release of chemical mediators such as histamine when activated. For these reasons, basophils had often been considered as minor and redundant relatives of mast cells. In a clinical setting, basophils are commonly used as a surrogate of less accessible mast cells for diagnosis of allergen sensitization in allergic patients.

Intriguingly, basophils are evolutionally conserved in many animal species, suggesting crucial and beneficial roles of basophils *in vivo*. Moreover, basophils circulate in the peripheral blood and are rarely present in peripheral tissues unless inflammation occurs, in contrast to mast cells. The lifespan of basophils is much shorter than that of mast cells, and therefore newly-generated basophils are constantly supplied from the bone marrow to the peripheral blood. These differences imply that basophils and mast cells have distinct functions *in vivo*.

Recent development of analytical tools such as basophil-deficient mice has enabled us to examine *in vivo* roles of basophils in a variety of immune responses in mouse models, including allergic inflammation, autoimmune diseases, protective immunity against pathogenic infections, and regulation of innate and acquired immunity. In this article, we

review basophil-related papers published during the past five years, and illustrate pivotal and non-redundant roles of basophils in health and disease.

Ontogeny of basophils

Basophils were sometimes regarded erroneously as blood-circulating mast cell precursors that could migrate to peripheral tissues and mature into tissue-resident mast cells. It is now widely accepted that basophils and mast cells are distinct cell lineages differentiated from hematopoietic stem cells in the bone marrow. However, the developmental relationship between basophils, mast cells and other hematopoietic cells remains to be fully elucidated¹. Several studies suggest that human basophils develop from common basophil-eosinophil progenitors^{2,3} (Fig.1). Hybrid-type cells possessing both basophil and eosinophil phenotypes have been detected in the bone marrow, cord blood, and peripheral blood of patients with myeloid leukemia. Further studies are needed to clarify whether these hybrid cells are indeed the common precursors of basophils and eosinophils or intermediates of the normal eosinophilopoiesis process. The existence of bi-potential human basophil-mast cell progenitors has not yet been conclusive either. Comprehensive analysis of mast cell transcriptome demonstrated that mast cells have no close relative in the hematopoietic network and that the similarity between mast cells and basophils is fairly limited in both humans and mice^{4,5}.

In mice, multiple differential pathways that give rise to basophils might exist¹ (Fig.1). A recent study identified a novel population of common basophil-mast cell progenitors in the mouse bone marrow⁶. A series of transcription factors are involved in the differentiation and commitment of basophils and mast cells. The *Cebpa* gene is highly expressed in basophils but not mast cells whereas the *Mitf* gene is highly expressed in mast cells but not in basophils. C/EBP α and MITF appear to determine basophil and mast cell fates

respectively, by silencing each other's transcription in a directly antagonistic fashion⁶. Murine basophils can be generated *in vitro* by culturing bone marrow cells in the presence of cytokine IL-3 or thymic stromal lymphopoietin (TSLP). IL-3-elicited basophils and TSLP-elicited basophils differ in terms of gene expression, phenotype and function (Fig. 1), suggesting heterogeneity among the basophil population⁷. Notably, basophils can be detected in mice deficient for both IL-3 and TSLP signaling, indicating that neither of them is essential for basophil development. Thus, the molecular regulation of basophil differentiation remains enigmatic even in mice.

The role of basophils in allergic disorders (illustrated in Fig. 2 and 3)

Allergy in the skin

In the 1970s, basophils attracted much attention due to the observation of substantial cutaneous infiltration of basophils at the site of Jones-Mote hypersensitivity in humans and cutaneous basophil hypersensitivity in guinea pigs^{8,9}. However, the pathological role of basophils in these delayed-onset reactions remained uncertain. Recent studies revisited the basophil infiltration in the skin, demonstrating that basophils accumulate in the skin lesions of various skin disorders, including atopic dermatitis, urticaria, prurigo, bullous pemphigoid, Stevens-Johnson syndrome, eosinophilic pustular folliculitis, and Henoch-Schönlein purpura¹⁰.

Basophils infiltrate the skin lesions of atopic dermatitis, particularly patch tested sites^{10,11}. In mice, topical treatment with the vitamin D analogue MC903 induces the production of TSLP in the skin, leading to the development of atopic dermatitis-like skin inflammation with basophil accumulation^{7,12}. Basophil depletion ameliorated skin inflammation^{7,11,13,14}, implying the important role of basophils in the pathogenesis of atopic

dermatitis. In this model, basophil-derived IL-4 appears to play a key role in promotion of Th2 responses.

Chronic idiopathic urticaria (CIU) is clinically diagnosed as long-lasting hives with unknown etiology. Several observations suggest a possible involvement of basophils in the pathogenesis of CIU. Basophil infiltration is detected in CIU skin lesions¹⁵, and blood basopenia is frequently observed in CIU patients, probably due to basophil migration into the skin. Anti-IgE monoclonal antibody omalizumab has been approved as a novel drug for CIU treatment. Omalizumab administration reduces free IgE levels in circulating blood and downregulates high affinity IgE receptor FcεRI on the surface of basophils and mast cells. In the phase 3 studies, many patients experienced a major clinical effect of omalizumab within 2 weeks^{16,17}. Of note, FcεRI expression on basophils was significantly reduced by day 8 whereas no apparent reduction was detected until day 85 in IgE-/FcεRI-positive cells in skin biopsy specimens¹⁸, suggesting the possible contribution of basophils and IgE/FcεRI to the development of CIU.

A mouse model of IgE-mediated chronic allergic inflammation (IgE-CAI) in the skin has been established¹⁹. A single intradermal administration of allergens into the ear skin of allergen-specific IgE-sensitized mice elicits three waves of ear swelling, namely mast cell-mediated, immediate- and late-phase reactions, followed by delayed-onset IgE-CAI reaction with massive infiltration of leukocytes including eosinophils, neutrophils and monocytes/macrophages. Basophils also accumulate in the skin lesion to a much lesser extent, representing only 1 or 2 % of infiltrates. Nevertheless, basophil depletion completely abolishes the IgE-CAI development^{20,21}, demonstrating the essential and non-redundant role of basophils in the development of IgE-CAI. Recent studies revealed that basophil-derived serine proteases, mouse mast cell protease (mMCP)-8 and mMCP-11, elicit allergic inflammation with microvascular hyperpermeability and leukocyte infiltration^{22,23}.

Intriguingly, basophils also contribute to prevent excess of allergic inflammation through their release of IL-4, which acts on inflammatory monocytes infiltrating the IgE-CAI skin lesion, leading to the generation of anti-inflammatory M2-type macrophages²⁴. CD203c (ATP-hydrolyzing ectoenzyme E-NPP3) expressed on basophils also dampens the IgE-CAI reaction through hydrolysis of basophil-derived ATP that activates basophils in an autocrine manner²⁵. Basophil-derived amphiregulin has been shown to contribute to UVB irradiation-induced suppression of cutaneous hypersensitivity²⁶, but its role in IgE-CAI remains to be investigated. IgE-CAI can be induced by a single administration of allergen to passively IgE-sensitized mice¹⁹, whereas repeated intradermal injection of allergen to IgE-transgenic mice causes persistent and pruritic papulonodular skin lesions mimicking prurigo²⁷. Basophil depletion ameliorates this reaction, suggesting the involvement of basophils in the pathogenesis of prurigo.

Allergy in the respiratory tract

Allergic rhinitis is a common allergic disease. Basophils have been detected in nasal washes or mucosa of patients with allergic rhinitis^{28,29}. In mouse models of allergic rhinitis, sequential involvement of mast cells and basophils has been reported: histamine release from FcεRI-activated mast cells in the nasal tissue, followed by histamine-mediated recruitment of histamine H4 receptor-expressing basophils and their activation, leading to the production of Th2 cytokines and other effector molecules³⁰. In another model using ragweed pollen as allergen, allergen-stimulated epithelial cells in the nasal mucosa produced IL-33 which acts on mast cells and basophils, resulting in the early- and late-phase responses through histamine release and production of chemoattractants for leukocytes such as eosinophils, respectively³¹. TSLP is also involved in activation of mast cells and basophils in this model³².

Basophil infiltration was reported in the lung of asthmatic patients³³⁻³⁷. In a mouse

model of asthma-like lung inflammation, intranasal administration of papain induced eosinophil influx into bronchoalveolar lavage fluid, mucin production, and airway hyperresponsiveness. Papain acts on lung epithelial cells to release IL-33 that in turn activates basophils and group 2 innate lymphoid cells (ILC2s). IL-33-stimulated basophils secrete IL-4 that activates ILC2s to produce large amounts of IL-5, IL-13 and CCL11, leading to eosinophilia in the lung³⁸.

Allergy in the gastrointestinal tract

Patients with food allergy show symptoms ranging from itching, hives and diarrhea to acute anaphylaxis after exposure to a given food. It is generally thought that food allergen-mediated cross-linking of allergen-specific IgE on mast cells plays an important role in the pathogenesis of food allergy. However, a recent study of peanut allergy treated with omalizumab demonstrated that early clinical response to the therapy correlated with basophil suppression rather than mast cell suppression³⁹ as reported in CIU patients treated with omalizumab¹⁸. This suggests the possible contribution of basophils to the pathogenesis of peanut allergy. In a mouse model of peanut allergy, ablation of either basophils or mast cells resulted in ameliorated anaphylaxis⁴⁰, indicating the importance of both basophils and mast cells in this model of peanut allergy.

Atopic dermatitis is a risk factor for the development of food allergy. Epicutaneous sensitization with food allergens and subsequent oral intake of them may elicit food allergy. In mouse models of food allergy with cutaneous allergen sensitization, expansion of TSLP-elicited basophils in the skin lesion promotes Th2 cytokine responses, increase in serum levels of antigen-specific IgE, and accumulation of mast cells in the intestine, leading to the development of intestinal food allergy⁴¹⁻⁴³. Basophil depletion reduces the susceptibility to intestinal food allergy, demonstrating the pivotal role of basophils in this setting.

TSLP-elicited basophils have also been demonstrated to contribute to the pathogenesis of eosinophilic esophagitis (EoE), a food allergy-associated inflammatory disease characterized by esophageal eosinophilia⁴⁴. Gain-of-function polymorphisms in the *TSLP* gene are strongly associated with the development of EoE. Esophageal biopsies revealed elevated *TSLP* expression and accumulation of basophils in patients with active EoE. Mice develop EoE-like inflammation in the esophagus by means of epicutaneous allergen sensitization followed by repeated intragastric challenges with allergens⁴⁴. Importantly, TSLP neutralization or basophil depletion ameliorated EoE-like disease, suggesting that the TSLP-basophil axis could be therapeutically targeted to treat EoE. In another mouse model of EoE, blocking the IL-33 receptor or basophil depletion ameliorated EoE, suggesting the contribution of the IL-33-basophil axis to the EoE pathogenesis⁴⁵.

Systemic anaphylaxis

Anaphylaxis is an acute-onset and life-threatening systemic allergic reaction. The classical pathway of systemic anaphylaxis involves IgE, mast cells, and histamine. Histamine released from mast cells stimulated with IgE and allergen induces anaphylactic manifestations such as hypotension and dyspnea. This pathway may not account for all anaphylactic responses⁴⁶. Indeed, IgG but not IgE antibodies specific to allergens were detected in some individuals who showed systemic anaphylaxis in response to medicines. Moreover, levels of platelet-activating factor (PAF) in serum are positively correlated with the severity of anaphylaxis⁴⁷. Analysis of blood from patients with acute anaphylaxis to bee venom or peanut demonstrated a significant decrease in circulating basophil counts and FcεRI expression as well as an increase in basophil chemotactic CCL2, suggesting the possible involvement of basophils in a certain type of anaphylaxis⁴⁸.

In mice, IgG- and PAF-mediated systemic anaphylaxis can be elicited in a manner

independent of IgE and mast cells. Allergen-IgG immune complex-stimulated basophils, macrophages or neutrophils, dependent on the experimental conditions, are the major producers of PAF⁴⁹⁻⁵². A recent study suggested that IgG subclass may determine which type of leukocyte predominantly contributes to IgG-mediated systemic anaphylaxis⁵³.

The role of basophils in non-allergic and non-infectious disorders (illustrated in Fig. 3)

Autoimmune diseases

Autoreactive IgE specific to dsDNA can be detected in sera of systemic lupus erythematosus (SLE) patients, whose IgE titers correlate positively with SLE disease activity^{54,55}. Basophils in SLE patients display an activated phenotype such as upregulated CD203c expression (Table 1). Mice deficient for Lyn, a Src family kinase, develop an SLE-like disease including nephritis⁵⁶. Aged Lyn-deficient mice produce autoantibodies, including IgE isotype, against nuclear antigens, which form immune complexes that can deposit and accumulate in the kidney, leading to kidney dysfunction (lupus nephritis). Activation of basophils by autoreactive IgE causes their homing to lymph nodes, promoting Th2 cell differentiation through IL-4 production and enhancing the production of self-reactive antibodies, leading to lupus-like nephritis (Table 1 and Fig. 3). Basophil depletion ameliorates the disease, illustrating the important role of basophils in this SLE model⁵⁶. In spontaneous lupus-prone MRL-*lpr/lpr* mice, basophil depletion lowers serum levels of autoantibodies and IL-17 and improves renal function⁵⁷ (Table 1), further supporting this hypothesis.

Intravenous immunoglobulin (IVIG) therapy is used in the treatment of autoimmune and inflammatory diseases, but the mechanism underlying anti-inflammatory action of IVIG in autoimmune disorders remains incompletely understood. In a mouse model of rheumatoid arthritis, IVIG administration results in the production of IL-33 which in turn induces basophil expansion, and basophil-derived IL-4 promotes increased expression of the inhibitory Fc

receptor FcγRIIB on effector macrophages, resulting in suppression of arthritis⁵⁸. However, a subsequent study showed no apparent contribution of basophils to the therapeutic effect of IVIG in a similar model⁵⁹, and therefore the involvement of basophils in the IVIG-mediated suppression remains to be further investigated.

Inflammatory bowel disease

Basophilia is observed in the blood of patients with inflammatory bowel disease including Crohn's disease and ulcerative colitis⁶⁰. Basophils accumulate in the inflamed colon of patients with these diseases^{60,61}. Basophils appear to augment IL-17 and IFN-γ production by memory CD4⁺ T cells and therefore exacerbate inflammation. The augmentation is partially mediated by basophil-derived histamine⁶¹ (Fig. 3). In mice, basophils also contribute to Th17 cell differentiation through IL-6 production, and basophil depletion ameliorates Th17-dependent experimental autoimmune encephalomyelitis⁶² (Table 1 and Fig. 3). In contrast, in the colitis model using adoptive transfer of naive CD4⁺ T cells into lymphopenic mice, basophils were shown to ameliorate inflammation through production of IL-4 and IL-6 that downregulate the expression of IFN-γ, IL-2 and TNF in T cells⁶³.

Irritant contact dermatitis

Irritant contact dermatitis (ICD) is a non-allergic skin inflammation that occurs after irritant chemical exposure, and the infiltration of basophils and eosinophils is detected in the skin lesions of human and murine ICD⁶⁴. In a mouse model, basophil depletion suppressed cutaneous swelling and eosinophil infiltration, suggesting the important role of basophils in the development of ICD. Co-culture experiments *in vitro* revealed that activated basophils can attract eosinophils directly via CCL5 production and indirectly through the promotion of CCL11 production by fibroblasts. Thus, basophils appear to contribute to the pathogenesis of

ICD in cooperation with eosinophils and fibroblasts⁶⁴.

Hyper-IgD syndrome and IgG4-related disease

Hyper-IgD syndrome, an autoinflammatory disorder associated with hyper-IgD production, is characterized by periodic fever, systemic antibiotic-resistant inflammation, and increased circulating IgM⁺IgD⁺ B cells. Patients show fewer circulating but more mucosal IgD-armed basophils. Intriguingly, cross-linking of IgD but not IgE on the surface of basophils promotes the release of proinflammatory cytokines such as IL-1 β and TNF that are involved in autoinflammation⁶⁵, suggesting a contribution of basophils in the pathogenesis of hyper-IgD syndrome (Table 1 and Fig. 3).

IgG4-related disease is characterized by elevated serum IgG4 levels and infiltration of IgG4-expressing plasma cells into multiple organs, leading to pancreatitis, cholangitis, sialoadenitis, nephritis, retroperitoneal fibrosis and inflammatory pseudotumors. In coculture of basophils and B cells *in vitro*, basophils activated via Toll-like receptors induce IgG4 production by B cells in association with enhanced production of B cell activating factor (BAFF) and IL-13⁶⁶, suggesting a possible involvement of basophils in the pathogenesis of IgG4-related disease.

Malignancy

Basophilia is a frequently observed hematological abnormality in chronic myeloid leukemia (CML), particularly in the accelerated phase. The transcription factor IKAROS is absent or reduced in bone marrow blasts from most CML patients with advanced myeloid disease⁶⁷. Forced expression of dominant-negative isoform of IKAROS in chronic-phase CML patients' CD34⁺ cells resulted in disrupted IKAROS activity and enhanced ability to differentiate into basophils⁶⁷, suggesting that a loss of IKAROS contributes to the myeloid

disease progression with basophilia. It has been reported that CML basophils but not other cell types abundantly express hepatocyte growth factor, which promotes CML cell expansion in an autocrine fashion⁶⁸ (Fig. 3). A recent study using a mouse model of CML demonstrated that basophil-like leukemia cells crucially contribute to CML development by providing the chemokine CCL3⁶⁹ (Fig. 3). A CML-like disease can be induced in mice by means of direct transplantation of *BCR-ABL* gene-transduced hematopoietic stem/progenitor cells into the bone marrow cavity⁷⁰. In this model, CML development induces a marked accumulation of basophil-like *BCR-ABL*⁺ leukemia cells that produce CCL3 in the bone marrow. Basophil-derived CCL3 negatively regulates the proliferation of normal hematopoietic stem/progenitor cells and supports the predominant expansion of leukemia initiating cells⁶⁹. Indeed, basophil depletion prevents the development of CML.

The role of basophils in tumor immunity remains poorly understood. In a mouse model of melanoma, tumor-infiltrating basophils produce chemokines CCL3 and CCL4 that in turn promote the recruitment of CD8⁺ T cells, leading to tumor rejection⁷¹.

Organ transplantation

Basophil infiltration and accumulation were observed in human skin allografts in the days immediately preceding graft rejection⁷². Basophils accounted for up to 5% of infiltrating cells and underwent a form of piecemeal degranulation, suggesting a possible contribution of basophils to graft rejection. Indeed, in a mouse model of pancreatic islet transplantation, basophil depletion enhanced and prolonged the survival of transplanted islet in allogeneic recipient mice⁷³. Basophils have also been shown to contribute to the development of allograft fibrosis in a mouse model of heart transplantation, in which basophil-derived IL-4 plays a crucial role in activation of fibroblasts and development of fibrotic organ remodeling, leading to chronic allograft rejection⁷⁴. Another study

demonstrated a beneficial role of murine basophils in the induction phase of an acute graft-versus-host disease, in that basophils limit the expansion of allogeneic CD4⁺ T cells through IL-4 production⁷⁵.

The beneficial role of basophils in protective immunity against pathogens

Parasitic infection

Basophil infiltration was observed in the skin lesion of scabies and tick bites¹⁰. A patient lacking basophils and eosinophils was reported to suffer from widespread scabies⁷⁶. These observations suggest the role of basophils in protection against ectoparasitic infection. In a mouse model of tick infestation, once host mice have experienced tick infestation, they produce IgE specific to tick saliva antigens and show resistance against tick feeding in subsequent infestations. IgE-armed basophils infiltrate tick feeding sites in the skin and surround tick mouthparts during the second but not first infestation. The use of genetically-engineered mice generated for selective and conditional basophil ablation clearly demonstrated that basophil depletion before the second infestation abolished the tick resistance⁷⁷. This finding, together with the pioneering study in guinea pigs using anti-basophil serum⁷⁸, illustrates the pivotal role of basophils in acquired immunity to tick infestation (illustrated in Fig. 3).

In general, helminth infection induces strong Th2 immune responses. Infection with the nematode *Nippostrongylus brasiliensis* (Nb) in mice is widely used as a model of human hook worm infection. Infectious larvae first penetrate the skin, migrate into the lung through blood vessels, and finally reach the intestine. As in the case of tick infestation, host animals acquire resistance against Nb infection after the first infection. Basophils have been shown to contribute to this acquired immunity^{21,79}. During the second Nb infection, many larvae are trapped in the skin, reducing the spread of infection to the lung and intestine. IgE-armed

basophils accumulate in the skin lesion where larvae are trapped, and basophil depletion abolishes the larval trapping in the skin⁷⁹. Basophil-derived IL-4 acts on skin-infiltrating inflammatory monocytes to promote their differentiation into M2 macrophages which produce arginase 1, leading to the larval trapping⁷⁹ (illustrated in Fig. 3). In mice re-infected with another helminth, *Heligmosomoides polygyrus*, IgE-armed basophils are recruited to the small intestine and secrete IL-4/IL-13, leading to worm expulsion from the intestine⁸⁰. Basophils also contribute to the protection against the filarial nematode, *Litomosoides sigmodontis*, when mice were immunized with irradiated larvae⁸¹. Thus, basophils play a critical role in the acquired immunity against helminth infections. Basophils also show some contribution in mice to the protective immunity in primary infection with *Strongyloides ratti* or *Strongyloides venezuelensis*^{82,83}. In mice infected with other helminths such as *Trichuris muris* or *Trichinella spiralis*, TSLP-elicited basophils play an important role in Th2 responses to primary infection^{7,84}.

Bacterial infection

B cells in the human upper respiratory mucosa generate local and circulating plasmablasts producing IgD reactive to respiratory bacteria such as *Moraxella catarrhalis* and *Haemophilus influenzae*⁶⁵. Cross-linking of IgD bound to basophils activates basophils to secrete antimicrobial factors such as β -defensin and cathelicidin as well as B cell-activating factors including IL-4, IL-13, BAFF and a proliferation-induced ligand (APRIL) that further promote the production of antibodies against bacteria. The role of basophils in enhancement of humoral memory response to bacterial infections has also been shown in mice^{85,86}. Moreover, when activated, both human and mouse basophils can form extracellular DNA traps, containing mitochondrial DNA and granular proteins, that can kill bacteria⁸⁷.

Basophils appear to contribute to the resolution phase of *Listeria monocytogenes*

infection in mice⁸⁸. Upon infection, basophils are recruited to the liver, and produce IL-4 that in turn promotes M2 polarization of recruited monocytes, therefore damping bacteria-induced acute inflammation and limiting liver injury.

Viral infection

Basophils have been shown to contribute to HIV-1 dissemination throughout the body⁸⁹. Human basophils can capture HIV-1 and mediate viral trans-infection of CD4⁺ T cells through HIV-1 attachment factors including C-type lectins. The role of basophils in protective immunity against viral infections remains to be clarified.

A controversial issue: Do basophils function as antigen-presenting cells?

It is well appreciated that basophils in both humans and mice readily produce large quantities of IL-4 upon activation with a variety of stimuli. In mice, it has been shown under certain experimental conditions that basophils migrate to lymph nodes and secrete IL-4, therefore promoting the differentiation of naive CD4⁺ T cells toward Th2 cells⁹⁰. Moreover, three groups independently reported that murine basophils express MHC class II (MHC-II) and costimulatory molecules such as CD80, CD86 and CD40 that are necessary for antigen presentation to naive T cells⁹¹⁻⁹³. Thus, basophils can function dually as antigen-presenting cells (APCs) as well as IL-4 secreting cells, driving Th2 cell differentiation even in the absence of classical APCs such as dendritic cells. This novel finding cast new light on the mechanism underlying Th2 differentiation while other studies demonstrated the critical role of dendritic cells but not basophils in Th2 differentiation^{21,94,95}. Therefore, the functional significance of basophils as APCs remains controversial⁹⁶. Of note, a recent study in mice reported an unexpected mechanism of MHC-II acquisition by basophils¹⁴. Basophils produce few or no MHC-II by themselves but they can capture peptide-MHC-II complexes from

dendritic cells through a mechanism called trogocytosis in a cell contact-dependent manner. MHC-II-dressed basophils can provide peptide-MHC-II complexes and IL-4 to naive CD4⁺ T cells that in turn differentiate to Th2 cells. This finding appears to reconcile some discrepancy observed in previous studies, and the APC activity of basophils may vary in different experimental settings, depending on the extent of the basophil-dendritic interaction and MHC-II trogocytosis.

Human basophils circulating in the peripheral blood express little or no HLA-DR at resting state, but they can be induced to express HLA-DR when activated *in vitro* with stimuli such as cytokines^{56,93,97,98}. Nevertheless, human basophils reportedly failed to induce antigen-specific T cell proliferation⁹⁸⁻¹⁰⁰. It remains to be investigated whether human basophils can acquire peptide-HLA-DR complexes from dendritic cells via trogocytosis and function as APCs.

Perspective

As reviewed in this article, our understanding of basophil function in health and disease has greatly advanced these days. We now appreciate that basophils are crucially involved in protective immunity against pathogens such as parasites, and in the development of immunological abnormalities, including allergy, asthma, autoimmune and inflammatory diseases. One thing we have to take into account when basophil functions are examined in mouse models is the possible “off-target effect” of basophil-depleting antibodies, anti-FcεRIα and anti-CD200R3, on mast cells expressing FcεRIα and CD200R3. This issue can be solved by using more recently-developed tools such as genetically-engineered mice deficient for only basophils^{21,77,101,102}. We also need to keep in mind that there are some phenotypic and functional differences between human and murine basophils, including the expression of protease-activated receptors and the responsiveness to proteases such as papain¹⁰³⁻¹⁰⁶.

Therefore, further extensive studies are necessary to verify that the findings in mouse models are indeed relevant to human physiology. Advances of basic and translational research on basophils would facilitate the development of novel strategy for treatment of immunological disorders and new generation of anti-parasite vaccines.

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765 Figure Legend:

766

767 Figure 1. Differentiation pathway of basophils (controversial)

768

769 Figure 2. Involvement of basophils in the pathogenesis of allergic disorders

770

771 Figure 3. Effector molecules involved in basophil-mediated immune responses

Table1. Roles of basophils in autoimmune and autoinflammatory diseases

Disease	Species	Model or Patients	Stimulants of basophils	Role of basophils	Ref
SLE	Mouse	<i>Lyn</i> ^{-/-}	Autoreactive-IgE + autoantigens	Promotion of autoantibody production	56
	Mouse	MRL <i>lpr/lpr</i>	Autoreactive-IgE + autoantigens	Promotion of IL-17 and autoantibody production	57
	Human	SLE patients	Autoreactive-IgE + autoantigens	Promotion of autoantibody production and Th17 differentiation	54-57
Autoimmune encephalomyelitis	Mouse	EAE model	??	Promotion of Th17 differentiation through IL-6 production	62
Hyper-IgD syndrome	Human	Patients with hyper-IgD syndrome	IgD	Release of pro-inflammatory cytokines upon stimulation with IgD crosslinking	65

Hematopoietic stem cell

HSC

SCRIPT

Common myeloid progenitor

CMP

Basophil / eosinophil
common precursor ?

Basophil / mast cell
common precursor

Eosinophil

Basophil
progenitor

Mast cell

IL-3R

TSLPR

IL-3

TSLP

IL-3-elicited basophil

TSLP-elicited basophil

Antigen

IgE FcεRI

IL-3R

IL-3

IL-3

IL-3R

IL-18R

IL-18

TSLPR

TSLP

IL-33R

IL-33

IgE-dependent degranulation

Release of histamine, leukotriene, protease etc.

Cytokine secretion

Release of IL-4, IL-6 etc.

