

REVIEW ARTICLE

Newly appreciated roles for basophils in allergy and protective immunity

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Abstract

Basophils are evolutionarily conserved in many animal species, in spite of the fact that they account for <1% of peripheral blood leukocyte. This suggests that basophils have an indispensable and nonredundant role *in vivo*, even though they show some phenotypic similarity with tissue-resident mast cells. However, their functional significance remained uncertain long after Paul Ehrlich discovered them as blood-circulating cells with basophilic granules more than 130 years ago. The study of basophils has been far behind that of mast cells, owing to the rarity of basophils and the paucity of tools for their detection and functional analysis. Recent development of novel analytical tools, including basophil-depleting antibodies and genetically engineered mice deficient only in basophils, has greatly advanced basophil research and illuminated previously unrecognized roles of basophils. We now appreciate that basophils and mast cells play distinct roles in immune responses. Basophils have crucial roles in the development of acute and chronic allergic responses, the protective immunity against ecto- and endoparasites, and the regulation of acquired immunity, including the augmentation of humoral memory responses and the initiation of Th2 responses. Thus, basophils are no longer the neglected minority and are key players in the immune system.

Basophils are basophilic granulocytes and were first described at the end of the nineteenth century by Paul Ehrlich as blood-circulating cells with basophilic granules in their cytoplasm that stain in purple with basic dyes. They are very rare compared to neutrophils and represent <1% of peripheral blood leukocytes. In addition to basophilic granules in the cytoplasm, basophils share some important features with mast cells (1). Both basophils and mast cells express high-affinity IgE receptor FcεRI on their surface and release allergy-inducing chemical mediators such as histamine in response to various stimuli, including IgE/FcεRI-mediated one. Therefore, basophils have often erroneously been considered as minor relatives of mast cells (2). From the clinical point of view, basophils are much more accessible than tissue-resident mast cells. Accordingly, basophils are a practical and convenient surrogate for mast cells in *in vitro* allergy test,

in that basophils isolated from allergic patients are incubated with allergen candidates to determine which allergen indeed stimulates basophils (3, 4). Basophils were some times considered even as blood-circulating precursors of tissue-resident mast cells. Although we now appreciate that basophils and mast cells are distinct cell lineages, the developmental origin of basophils still remains poorly defined, compared to that of other hematopoietic cells. A recent study in mice demonstrated a close lineage relationship between basophils and mast cells, by identifying common progenitors that can differentiate into basophils and mast cells but not other lineages (5). Curiously, the same study also identified basophil-committed monopotent progenitors, probably derived from granulocyte/monocyte progenitors (5), while another study identified mast cell-committed progenitors that are directly derived from multipotent progenitors (6). Even more compli-

cated, human basophils seem to have a closer relationship with eosinophils rather than with mast cells (7), based on the result of *in vitro* colony assay, the presence of granulocytes with a basophil–eosinophil hybrid phenotype in some leukemic patients, and two case reports of patients who lacked both basophils and eosinophils.

Basophils and mast cells differ in several important aspects, in spite of the above-mentioned similarity. Developmentally, basophils originate and mature in the bone marrow, circulate as terminally differentiated cells in the peripheral blood, and do not usually infiltrate peripheral tissues unless inflammation takes place. In contrast, mast cells mature in peripheral tissues, after their bone marrow-derived precursors enter the circulation and migrate into peripheral tissues. Mature mast cells reside in peripheral tissues and do not usually circulate in the peripheral blood. Thus, the anatomical localization differs between basophils and mast cells. They also differ in the life span: basophils are short-lived (approximately 2.5 days in mice) (8) while mast cells survive for weeks and months. Basophils do not proliferate after their maturation, unlike mast cells. These apparent differences between basophils and mast cells, together with the evolutionary conservation of basophils in many animal species (9), suggest that basophils and mast cells may have distinct roles *in vivo*. However, the functional significance of basophils remained an enigma long after their discovery by Paul Ehrlich. At last, recent studies have uncovered previously unappreciated roles of basophils and placed the once-overlooked basophils on the main stage of immunology (10–19). This review focuses on emerging roles of basophils in physiological and pathological settings, based mainly on animal studies performed in the past 5 years.

Recent technological advances in basophil research

The study of basophils has long been hampered not only by their rarity but also by the lack of tools for their detection and functional analyses. During the past 5 years, a series of groundbreaking tools have been developed and brought new era of basophil research.

Novel tools for definitive identification of basophils

One can readily stain and identify human basophils in blood smear with basic dyes as Paul Ehrlich described. However, this is not true for histochemical identification of tissue-infiltrating basophils in formalin-fixed and paraffin-embedded tissue sections, in sharp contrast to relatively easy identification of tissue-resident mast cells. This difficulty in basophil detection has rendered the basophil research far behind the mast cell research. Although successful identification of tissue-infiltrating basophils was reported with use of electron microscopy and epon-embedded tissue sections (20), more practical and convenient methods for basophil identification have long been sought. A panel of monoclonal antibodies (mAbs) specific to human basophils has been established for the immunohistochemical detection of basophil infiltration into peripheral tissues, including BB1 (basogranulin-specific),

J175-7D4 (pro-major basic protein-specific), and 2D7 (21–23). We have recently established TUG 8, a mAb specific to mouse mast cell protease 8 (mMCP-8), which is suitable for immunohistochemical identification of mouse basophils (24). mMCP-8 is a granzyme B-like serine protease that is selectively expressed by basophils and stored in their secretory granules (24).

Recent studies have identified useful markers on the surface of human and mouse basophils, including CD49b (DX5), CD123 (IL-3 receptor α chain), CD200R3, CD203c, 2B4, CCR2, CCR3, and the intermediate level of CD45R expression (13, 25–30). These surface markers, in combination with other surface phenotypes such as Fc ϵ RI⁺ and c-Kit⁺ as well as their characteristic side/forward scatter profile distinct from that of other granulocytes, allow basophils to be readily identified and isolated by flow cytometry, in spite of their rarity. This has greatly advanced the characterization and functional analysis of basophils.

Generation of basophil-deficient animal models

The discovery of natural mutant mice deficient in mast cells, such as WBB6F1-Kit^{W/W-v} and C57BL/6-Kit^{W-sh/W-sh} mice, has greatly advanced our understanding of mast cell functions *in vivo* (31). In contrast, model animals deficient only in basophils have long been unavailable until recently. The development of basophil-depleting mAbs has partly overcome this obstacle (32–34). We found that *in vivo* administration of Ba103, a mAb specific to CD200R3 (29), leads to transient ablation of basophils (32), while others demonstrated that MAR-1, a mAb specific to Fc ϵ RI α , also displays basophil-depleting activity (33, 34). Although these mAbs react with both basophils and mast cells *ex vivo*, they preferentially deplete circulating basophils and show minimal effect on the number of tissue-resident mast cells, when administered *in vivo* (Fig. 1A). Taking advantage of these basophil-depleting mAbs, previously unappreciated roles for basophils have been illuminated, as discussed below. We recently found that anti-asialo GM1 antibody efficiently depletes basophils *in vivo* in addition to natural killer cells (35).

A potential problem with the Ba103- or MAR-1-mediated basophil depletion is the unwanted side-effects on mast cells. Indeed, intravenous injection of Ba103 or MAR-1 stimulates mast cells *in vivo* and induces systemic anaphylaxis (32, 36), and repeated intraperitoneal administration of MAR-1 leads to reduced number of mast cells in the peritoneal cavity (33). Accordingly, when mast cells substantially contribute to the immune response of interest, the antibody-mediated basophil depletion cannot provide clear evidence for the role of basophils in the response. The phenotype of antibody-treated mice may be ascribed to basophil depletion, to deleterious effects on mast cells, or to both. To solve this potential problem, we recently created *Mcpt8*^{DTR} mice in that only basophils express the human diphtheria toxin receptor, and therefore administration of diphtheria toxin induce selective and transient ablation of basophils, without any deleterious effect on other cell lineages including mast cells (37) (Fig. 1B). Others generated *Mcpt8Cre* transgenic mice that are

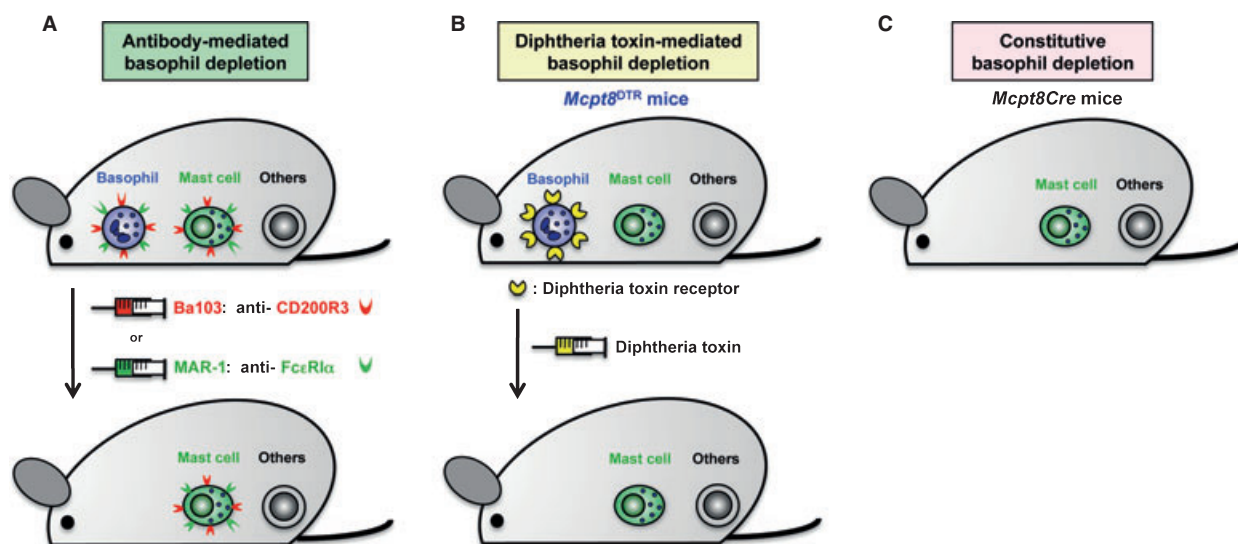


Figure 1 Generation of basophil-deficient mouse models. (A) Antibody-mediated basophil depletion. Treatment of mice with Ba103 (anti-CD200R3 mAb) or MAR-1 (anti-FcεRIα mAb) preferentially ablates basophils, even though both mAbs react with mast cells in addition to basophils. (B) Diphtheria toxin-mediated basophil depletion.

Mcpt8^{DTR} mice express human diphtheria toxin receptor only on basophils, and therefore diphtheria toxin administration induces selective and transient depletion of basophils. (C) Constitutive basophil depletion. *Mcpt8Cre* transgenic mice constitutively lack most basophils.

constitutively deficient for basophils (36) (Fig. 1C). The establishment of these engineered mice with inducible or constitutive ablation of basophils has truly enabled the functional characterization of basophils, including clear distinction between basophil and mast cell in their functional roles *in vivo*.

Role of basophils in allergy

While basophils circulate in the peripheral blood under homeostatic conditions, they are often recruited to the affected tissues in allergic disorders, including asthma, atopic dermatitis and rhinitis (13, 38). However, no appropriate animal model of allergy was available until recently to address the issue whether basophils indeed play a crucial role or just redundant with mast cells in allergic responses. In the 1970s, extensive studies were performed on a delayed-onset cutaneous hypersensitivity reaction that is associated with a massive dermal infiltration of basophils (comprising 50–90% of the infiltrates), and was therefore designated cutaneous basophil hypersensitivity (CBH) in guinea pigs or Jones–Mote hypersensitivity in humans (39). In contrast to the classical delayed-type hypersensitivity reaction, CBH skin reaction is characterized by erythema and thickening without apparent induration, peaks 18–24 h after the antigen challenge, and fades by 48 h. Unexpectedly, the treatment of guinea pigs with anti-basophil antiserum before the antigen challenge did not diminish the CBH reaction even though basophils were efficiently depleted from the peripheral blood and the skin lesion (40). This disappointingly argued against the importance of basophils as initiators or effectors in the CBH reaction, and the enthusiasm for CBH and basophils faded

out. As discussed below, recent studies have revisited the functional significance of basophils in allergic reactions and illuminated their crucial and nonredundant roles in mouse models of allergic disorders.

Basophils in delayed-onset allergy

It is well known that IgE and mast cells crucially contribute to immediate-type allergic reactions. We recently demonstrated that IgE is also involved in a delayed-onset allergic inflammation in the skin, where basophils but not mast cells play an essential role in the development of inflammation, providing the first clear evidence that basophils and mast cells play distinct roles *in vivo* (32, 41). In antigen-specific IgE transgenic mice or in normal mice that have been passively sensitized with antigen-specific IgE, a single subcutaneous administration of antigens in the ear skin elicits three consecutive waves of ear swelling (41, 42) (Fig. 2A). The first two waves, the early-phase ear swelling within 1 h after the antigen challenge and the late-phase ear swelling 6–10 h later, occur in a mast cell-dependent manner. The third wave is delayed-onset (starting on day 2 with a peak on day 4 post-challenge) and more intense ear swelling with massive infiltration of eosinophils in the skin lesions (Fig. 2B) as well as hyperplastic epidermis with hyperkeratosis. Therefore, we designated this delayed-onset cutaneous response IgE-mediated chronic allergic inflammation (IgE-CAI) (41).

Notably, IgE-CAI can be elicited even in mast cell- or T cell-deficient mice. Adoptive transfer of cells isolated from wild-type mice into FcεRI-deficient mice, that cannot mount IgE-CAI, identified FcεRI⁺ basophils to be responsible for the development of IgE-CAI (41). The essential role of

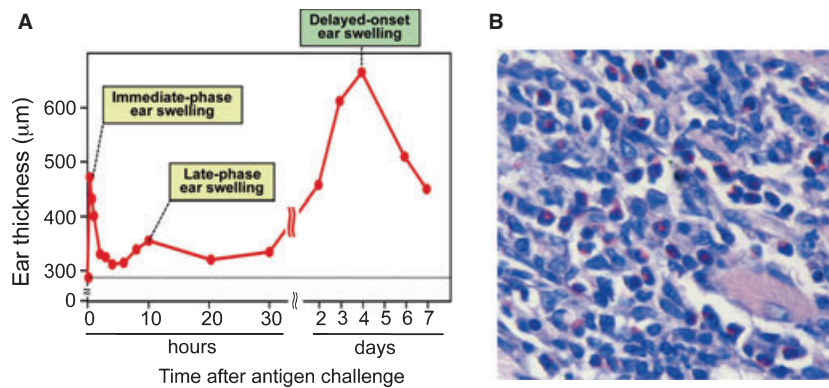


Figure 2 IgE-mediated chronic allergic inflammation (IgE-CAI). (A) Three consecutive waves of ear swellings are elicited when IgE transgenic mice or IgE-sensitized mice are challenged with subcutaneous injection of the corresponding antigen in the ear skin. The first (immediate-phase) and second (late-phase) ear swellings are

immediate-type allergic reactions and occur in a mast cell-dependent manner. In contrast, the third one (IgE-CAI) is delayed-onset and dependent on basophils. (B) In a Giemsa-stained specimen of the IgE-CAI skin lesion, massive infiltration of cells, including eosinophils, is observed.

basophils in IgE-CAI was further highlighted by the observation that IgE-CAI is not elicited in the absence of basophils, such as in Ba103-treated mice (32), *Mcpt8Cre* mice (36), and diphtheria toxin-treated *Mcpt8^{DTR}* mice (M. Egawa, K. Mukai, T. Wada, and H. Karasuyama, unpublished observation). Intriguingly, basophils represent only approximately 2% of the infiltrates in the IgE-CAI skin lesions (not like in the CBH reaction observed in guinea pigs), while eosinophils and neutrophils are abundant (41). The treatment of mice with Ba103 during the progress of dermatitis showed a therapeutic effect on the ongoing inflammation and lead to the reduction in ear swelling and in the number of eosinophils and neutrophils infiltrating the skin lesions, concomitantly with elimination of basophils from the site of inflammation (32). This strongly

suggests that basophils may function as initiators as well as, or rather than, effectors of the allergic inflammation and contribute to the recruitment of other proinflammatory cells such as eosinophils and neutrophils (Fig. 3). Although it remains to be determined whether basophils play the similar role in human allergic responses, it would be reasonable to consider the possible contribution of basophils to the pathogenesis of chronic allergic inflammation in humans even when the frequency of basophils is low in affected tissues.

Basophils in immediate-type allergy

Anaphylaxis is an acute-onset, systemic, and potentially fatal allergic reaction, typically associated with IgE, mast

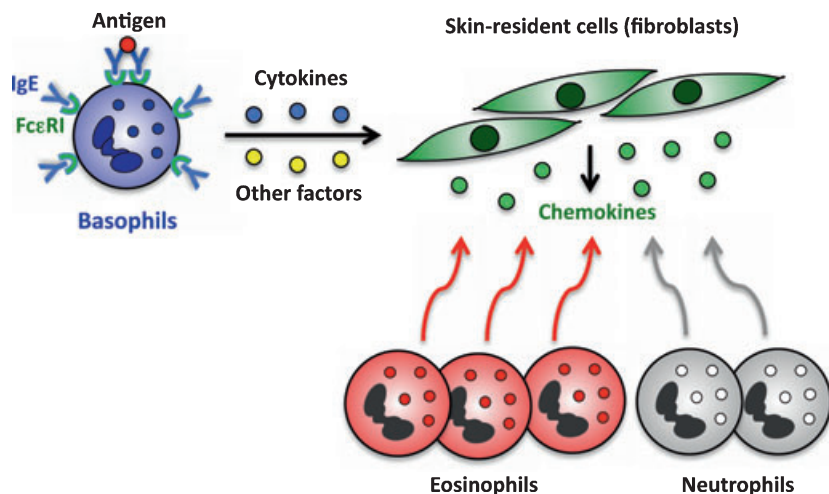


Figure 3 A crucial role for basophils in development of IgE-mediated chronic allergic inflammation (a proposed model). When IgE-armed basophils in the circulation migrate into the skin tissue and encounter the corresponding antigens, they are activated to secrete soluble factors, including cytokines, which in turn stimulate

skin-resident cells such as fibroblasts. The skin-resident cells stimulated with basophil-derived factors produce large amounts of cytokines to attract proinflammatory cells such as eosinophils and neutrophils, leading to chronic allergic inflammation.

cells, and histamine (43). Because basophils can release histamine and leukotriene C₄ in response to FcεRI-mediated stimulation (3), basophils have been considered likely contributors to IgE-mediated systemic anaphylaxis. However, given the fact that the absolute number of basophils in the body is far less than that of mast cells, it remains uncertain to what extent basophils contribute to systemic anaphylaxis. In this regard, we recently demonstrated that basophils, unlike mast cells, have no significant contribution to IgE-mediated systemic anaphylaxis in a mouse model (44). Instead, basophils are involved in IgG-mediated systemic anaphylaxis under our experimental conditions (44). Previous studies reported that the classical pathway of anaphylaxis, in which IgE, mast cells, and histamine are involved, cannot explain every case of anaphylaxis (45). Mice deficient in mast cells, IgE, or FcεRI still develop systemic anaphylaxis, while FcγR-deficient mice fail to do so (46–49). This suggests that the alternative pathway of anaphylaxis exists, in which nonmast cells and IgG (instead of IgE) are involved (45). Indeed, passive sensitization with antigen-specific IgG confers on mast cell-deficient mice the ability to develop systemic anaphylaxis in response to the antigen challenge (44, 48, 49). We found that basophils efficiently captured antigen/IgG1-immune complexes on their surface immediately after the intravenous injection of antigens in mice that had been passively sensitized with penicillin V-specific IgG1 mAb (44). Ba103-mediated basophil depletion suppressed IgG1-mediated systemic anaphylaxis, demonstrating basophils contribute to IgG1-mediated systemic anaphylaxis (44). While the treatment of mice with anti-histamine before the antigen challenge showed little or no inhibitory effect on IgG1-mediated anaphylaxis, the treatment with a platelet-activating factor (PAF) antagonist almost completely abolished it, indicating that PAF rather than histamine is the major chemical mediator of the anaphylaxis (44). PAF is far more potent than histamine in induction of anaphylaxis. Thus, immune complex-activated basophils can induce severe systemic anaphylaxis through PAF release, in spite of the fact that the absolute number of basophils is far less than that of mast cells.

Macrophages reportedly play a critical role in another model of IgG- and PAF-mediated systemic anaphylaxis, in that mice are actively immunized with goat anti-mouse IgD antiserum and then challenged with goat IgG (46). Recent study demonstrated that neutrophils play a predominant role in a distinct model of IgG-mediated, active systemic anaphylaxis, in which IgG2, FcγRIV, and PAF are involved (50). In this model, neither macrophages nor basophils play the major role. Basophil-deficient *Mcp18Cre* mice were shown to develop active anaphylaxis as well as passive IgG1-mediated anaphylaxis (36). It should be noted that the experimental condition employed varies from study to study, including the mouse strains and immunization protocols as well as types and doses of antigen and antibody. Thus, the mechanism underlying the IgG-mediated systemic anaphylaxis appears multifaceted, and not as simple as IgE-mediated one. Basophils, neutrophils and macrophages among others have potential to induce IgG-mediated systemic anaphylaxis

through PAF release, and their relative contribution may depend on experimental settings.

It was thought that much higher amounts of antigen and antibody are needed to elicit IgG-mediated systemic anaphylaxis than IgE-mediated one, suggesting that IgG-mediated anaphylaxis may not occur in real life. We recently demonstrated that IgG-mediated anaphylaxis can be induced even under conditions of limited amounts of antibody and antigen in a mouse model using a cocktail of IgG1 mAbs (51). Moreover, a relatively small dose of antigen induces severe anaphylaxis through the combination of the IgE- and IgG-mediated pathways (51). Thus, IgG-mediated systemic anaphylaxis, particularly in conjunction with IgE-mediated one, may not rare in real life, in contrast to previous thought. In this regard, it is notable that treatment with the combination of anti-histamine and PAF antagonist efficiently suppressed systemic anaphylaxis in a mouse model of peanut allergy (52). Several case reports indicated human anaphylaxis that occurred in the apparent absence of detectable antigen-specific IgE or in the absence of a sign of mast cell activation (increase in serum tryptase levels) (53, 54). Serum PAF levels are well correlated with the severity of anaphylaxis in humans (55). These observations suggest that the alternative pathway of anaphylaxis may be operative in humans as well.

Role of basophils in protective immunity

The evolutionary conservation of basophils in many animal species (9) strongly suggests that a primary role of basophils is not an initiator or effector of allergic reactions and should be host-beneficial. Basophilia, increased number of basophils, is frequently observed in association with parasitic infections, suggesting that basophils contribute to protective immunity against parasites (56). Recent studies have clearly demonstrated that basophils indeed play crucial roles in protection from infections with ecto- and endo-parasites (19).

Ticks are blood-feeding ectoparasites and can transmit a variety of microorganisms that can cause serious infectious diseases in humans and animals (57). Lyme disease is a common tick-borne disease, and its incidence has increased in the United States these days. Many animal species develop resistance to tick feeding once they experienced tick bites (58), leading to reduced number and body weight of engorged ticks in subsequent infestations. This acquired tick resistance greatly contributes to reducing pathogen transmission from infected ticks to host animals. Elucidation of cellular and molecular mechanisms underlying the acquired tick resistance would help develop efficient vaccination against tick-feeding and tick-borne diseases. However, previous studies reported conflicting results regarding cells responsible for manifestation of tick resistance: basophils play an important role in guinea pigs (59) while mast cells instead of basophils do so in mice (60). This may suggest that basophils and mast cells have redundant roles in tick resistance, and their relative contribution varies in different species of host animals and ticks.

We have recently revisited this issue by using novel tools and illuminated the pivotal contribution of basophils to acquired tick resistance in mice (37). In accordance with the

earlier report (60), Giemsa staining of skin tissue sections failed to detect basophils infiltrating tick-feeding sites in mice, unlike in guinea pigs. However, immunohistochemical analysis with TUG8, a newly developed mAb specific to mMCP-8 (24), clearly demonstrated that basophils are recruited to and clustered around the tick mouthparts during the second, but not the first infestation with ticks (37), suggesting the possible involvement of basophils in acquired tick resistance. The selective basophil depletion before the second tick infestation, by means of diphtheria toxin treatment of *Mcp8^{DTR}* mice, completely abolished the acquired tick resistance in the second infestation (37). We also confirmed the previous observation that mast cell-deficient mice fail to acquire tick resistance in the second infestation, indicating the essential role of mast cells. Thus, both basophils and mast cells play pivotal roles in the manifestation of acquired tick resistance. In the first infestation, mice raise antibodies, particularly of IgE class, against tick saliva antigens. In the second infestation, IgE-armed basophils are recruited to the tick-feeding sites and activated upon encounter with tick antigens, leading to the release of a variety of mediators, including proteases, which interfere with tick feeding. Mast cells contribute to the tick resistance in an antibody-independent manner (37). It remains to be determined how mast cells cooperate with basophils to manifest tick resistance.

Basophils also contribute to protection against helminthic endoparasites. Helminths are the most common infectious agents of humans in developing countries. In the mouse model of hookworm infection, basophil depletion results in impaired worm expulsion in secondary but not primary infection (36, 61), indicating a crucial role of basophils in protective immunity during repeated infections, as observed in tick infestation. In *Trichuris muris* infection, basophils contribute to worm expulsion in primary infection (62). In chronic infection with filarial nematodes, basophils amplify Th2-type immune responses, including IgE production, but do not contribute to worm expulsion in primary infection (63). Thus, the extent of basophil's contribution to the protection from helminthic infections varies, depending on the kinds of helminths and types of infection.

Basophils are also involved in protective immunity to bacterial infections. In mice, basophils enhance humoral memory responses by activating B and T cells in the secondary immune response through their IL-4 and IL-6 production (33). Basophils also support the survival of plasma cells (64). Of note, basophil depletion before a second vaccination with pneumococcal antigens resulted in reduced production of antigen-specific IgGs and rendered mice more susceptible to *Streptococcus pneumoniae* infection (33). Human basophils also stimulate B cells to secrete IgM and to undergo class switching to IgG and IgA (65). Moreover, activated human basophils produce antimicrobial peptides such as β -defensin and cathelicidin, which inhibit bacterial replication (65). Notably, the basophil-mediated enhancement of B-cell activation and plasma cell survival is also attributed to the accelerated production of autoantibodies in a mouse model of systemic lupus erythematosus (66).

Role of basophils in regulation of Th2 cell differentiation

In addition to the role of basophils in the above-mentioned B-cell activation, recent studies have illuminated the role of basophils in initiating Th2 cell differentiation (34, 62, 67, 68) (Fig. 4). IL-4 has a crucial role in driving the differentiation of naïve T cells to Th2 cells, which produce Th2-type cytokines such as IL-4 and IL-13, and are involved in protective immunity against parasites and allergic responses (69). Studies in the early 1990s discovered that basophils from both humans and mice can rapidly secrete large quantities of IL-4 as well as IL-13 (3, 70, 71). The establishment of IL-4/GFP reporter mice revealed that basophils indeed produce IL-4 *in vivo* during parasitic infections (25, 26). Of note, basophils are the major source of IL-4 at the early phase of immune responses (72, 73). These findings suggest that basophils may be involved in providing initial IL-4 necessary for Th2 cell differentiation and mediating Th2 immune responses. However, it remained uncertain whether basophils and naïve CD4⁺ T cells interact with each other in the lymph node, in which Th2 differentiation takes place. A recent study demonstrated that basophils indeed migrate into the draining lymph node after subcutaneous injection of papain, a protease that induces a strong Th2 response (34). Papain directly stimulates basophils to secrete IL-4 in an IgE-independent manner (34). Importantly, the antibody-mediated basophil depletion abol-

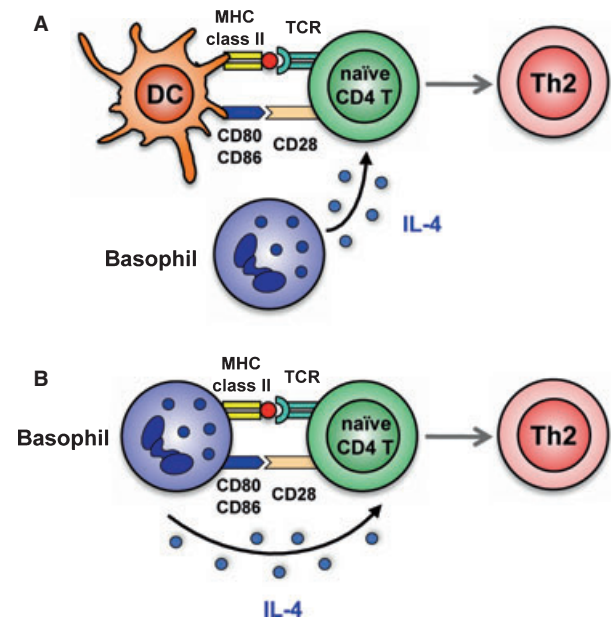


Figure 4 Roles for basophils in initiating Th2 cell differentiation. (A) Basophils function as IL-4 providers. Basophils are transiently recruited to the draining lymph node and provide IL-4 to naïve CD4⁺ T cells to promote their differentiation to Th2 cells, while dendritic cells (DCs) function as antigen-presenting cell (APCs). (B) Basophils express MHC class II and co-stimulatory molecules and can function as both APCs and IL-4 providers to promote Th2 cell differentiation even in the absence of DCs.

ished Th2 cell differentiation in the lymph nodes (34), demonstrating that basophils critically contribute to papain-induced Th2 cell differentiation. Papain administration also induces migration of dermal dendritic cells (DCs) to the draining node. Therefore, it was proposed that DCs process and present antigens to naïve CD4⁺ T cells, while basophils provide IL-4 necessary for Th2 differentiation (34) (Fig. 4A).

The role of basophils in mediating Th2 cell differentiation was further expanded by three independent studies showing that basophils can function as antigen-presenting cells (APCs) (62, 67, 68). Basophils express MHC class II (albeit to a lesser extent than do DCs and B cells) and costimulatory molecules such as CD80 and CD86. Therefore, basophils alone can induce Th2 cell differentiation through their functions as both APCs and IL-4 providers (Fig. 4B). Indeed, under experimental conditions employed, DC depletion had little or no impact on Th2 cell differentiation whereas basophil depletion abolished or diminished Th2 cell differentiation. The dual function of basophils in Th2 cell differentiation appears to be analogous to that of DCs in Th1 cell differentiation, in that DCs function as both APCs and IL-12 providers. This new paradigm that basophils are the genuine APCs for induction of Th2 responses got much attention and has brought basophils under the spotlight, but also met with severe criticism. The crucial role of DCs was demonstrated in papain-induced Th2 cell differentiation (36, 74) as well as in the induction of Th2 responses in asthma models (75, 76). Furthermore, Th2 responses to parasitic infections were not impaired by basophil depletion (36, 37, 73, 77). Thus, the relative contribution of basophils and DCs to Th2 cell differentiation appears to vary, depending on the model of Th2 responses employed (17, 69). It is notable that basophils can also act as APCs for CD8⁺ T-cell differentiation (78), although the *in vivo* relevance of this finding remains to be clarified.

Concluding remarks

Basophils were often considered to be minor and redundant 'circulating mast cells' because of their phenotypic similarity

with mast cells, and hence their functional significance remained questioned for a long time. As discussed in this review article, taking advantage of newly developed analytical tools, recent studies have illuminated previously unappreciated roles for basophils, that are distinct from those played by mast cells. Thus, a neglected minority gains new respect. Basophils play important and nonredundant roles in the regulation of acquired immunity, and the protective immunity against parasitic infections. Basophils are also critically involved in the development and deterioration of immune disorders such as allergy and autoimmunity in mouse models. Antibody-mediated basophil depletion showed therapeutic effects on these diseases (32, 44, 66). Treatment of mice with cyclosporine A or steroids almost completely suppressed basophil-mediated IgE-CAI even in the absence of T cells, suggesting that these immunosuppressants act on basophils (42). Considering the fact that the absolute number of basophils is much less than that of other effector cells, basophils and their products could be promising therapeutic targets for such immune disorders, even though further studies are essential to establish that the findings in mouse models are relevant to human diseases.

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Conflict of interest

The authors have no conflicting financial interests.

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