Basophils have emerged as a key player in immunity

Short title: Emerging roles of basophils

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Abstract

Basophils had long been neglected in immunological studies, because of their paucity and phenotypic similarity with tissue-resident mast cells. However, recent development of analytical tools has cast new light on this neglected minority, and revealed previously-unappreciated roles of basophils, distinct from those of mast cells, in various immune responses. Primary function of basophils appears to be the protection against infections with parasites, including ticks and helminths. This is why basophils are evolutionally conserved well in many animal species, albeit a small number. Nevertheless, basophils sometimes exert host-deleterious functions in immunological disorders such as allergy. Here we summarize recent advance in our understanding of basophil ontogeny and their *in vivo* roles under physiological and pathological conditions.

Introduction

Basophils are the rarest granulocytes (less than 1% of peripheral blood leukocytes), and named after basophilic granules in the cytoplasm, as documented first by Paul Ehrlich more than 130 years ago. Since then, the study of basophils had long been hindered by their rarity and short life span, the absence of long-lived cell lines, and the paucity of tools for their detection and functional analysis. Based on their rarity and phenotypic similarity with mast cells, including the presence of basophilic granules in the cytoplasm and the surface expression of high-affinity of IgE receptor, basophils had often erroneously been considered as minor and redundant relatives or blood-circulating precursors of tissue-resident mast cells. This view was held for many years, until the early 1990s when the long-neglected minority gained new respect by the discovery that basophils are an important cellular source of a Th2 cytokine IL-4, suggesting a potential role of basophils in Th2-type immune responses such as allergy and anti-parasitic protective immunity [1]. Indeed, recent development of novel

analytical tools, in particular, genetically engineered mice in which only basophils are inducibly or constitutively deficient [2-5], has enabled the functional characterization of basophils *in vivo*, and revealed that basophils play pivotal and non-redundant roles in a variety of immune responses, as outlined in this review.

Ontogeny of basophils

The life span of circulating basophils is very short (~2.5 days in mice), and therefore they are continuously replenished from progenitors in the bone marrow. The origin and developmental pathway of basophils in the bone marrow long remained enigmatic until recently. Accumulating evidence indicates a close lineage relationship of basophils with mast cells in mice [6,7]. The ordered expression of two transcription factors, GATA-2 and C/EBP α , regulates the lineage commitment of basophils [8]. C/EBP α and MITF silence each other's transcription in a directly antagonistic fashion, and specify basophil and mast cell fate, respectively [9*]. Ikaros limits basophil development by suppressing C/EBP α expression [10]. Interestingly, mice deficient for P1-Runx1 show severe basophilopenia with no apparent anomaly of mast cells, indicating a crucial role of P1-Runx1 in the differentiation of basophils but not mast cells [11]. Besides GATA-2, GATA-1 also plays an important role in the generation and function of basophils [12*]. Accordingly, Δ dblGATA mice carrying the mutated *Gata1* promoter show numerical and functional aberrancy in basophils in addition to the **known** deficiency of eosinophils. It is notable that a transforming *MYB-GATA1* fusion gene was identified in acute basophilic leukemia of humans [13].

In humans, in contrast to mice, basophils seem to have a closer lineage relationship with eosinophils, as demonstrated, for example, by the presence of immature basophils with a basophil-eosinophil hybrid phenotype [14]. Recent establishment of mice reconstituted with human myeloid cells [15] may help study the differentiation pathway of human basophils.

Intriguingly, basophil hematopoiesis appears to be influenced by serum IgE levels [16*], and elevated IgE concentration correlates with increased numbers of circulating basophils in both mice and humans [16*,17]. It is suggested that IgE binding to its high affinity receptor on basophil precursors in the bone marrow upregulates their IL-3R α (CD123) expression, leading to increased responsiveness to IL-3 and hence their expansion [16*].

Do basophils function as antigen-presenting cells (APCs)?

This has remained a matter of debate since 2009 when three groups independently reported the exciting discovery that basophils, rather than dendritic cells, are the critical APCs for driving Th2 cell differentiation in mice [18-20]. Basophils express surface molecules necessary for APC function, including MHC class II and co-stimulatory molecules such as CD80, CD86 and CD40, and can process and present antigens and provide IL-4 to naïve CD4 T cells, leading to their differentiation into the Th2 cell type. However, the full extent of the role of basophils in antigen presentation and Th2 differentiation remains uncertain, and conflicting conclusions were drawn, depending on different experimental settings [3,4,21-29]. Of note, Otsuka et al. recently reported that basophils are responsible for Th2 skewing to haptens and peptide antigens, but not protein antigens *in vivo*, while basophil can promote Th2 skewing for protein antigens in the presence of dendritic cells [30*]. So far no evidence for APC activity of human basophils has been provided [31-35].

There are several points to be considered in evaluating the APC activity of basophils, including the purity of isolated basophils, the cytokine milieu surrounding basophils, off-target effects of basophil-depleting reagents, and possible difference between peripheral blood basophils and those accumulating in lymph nodes and affected tissues. Establishment of more sophisticated tools such as basophil-restricted MHC class II-deficient

mice would help to solve this controversial issue.

Versatile roles of basophil-derived IL-4

Basophils from both humans and mice rapidly secrete large quantities of IL-4 in response to various stimuli. Recent studies have identified versatile roles of basophil-derived IL-4 in immune responses, in addition to its role in Th2 cell differentiation as described above (Figure 1). Basophil-derived IL-4 stimulates B cells to enhance antibody production in protective immunity [36,37] and in autoimmunity [38]. In papain-induced eosinophilic lung inflammation, activated basophils secrete IL-4 that acts on natural helper cells, a member of group 2 innate lymphoid cells, to enhance their production of chemokine CCL11 (eotaxin) and cytokines IL-5, IL-9 and IL-13, leading to eosinophilic inflammation [39**]. Basophil-derived IL-4 can act on fibroblasts *in vitro* to promote CCL11 expression, and basophils appear to contribute to eosinophil recruitment in a mouse model of irritant contact dermatitis [40].

Intriguingly, basophil-derived IL-4 also plays a regulatory role in allergic inflammation. In basophil-elicited, IgE-mediated chronic allergic inflammation [41], skin-infiltrating basophils secrete IL-4, which in turn act on inflammatory monocytes recruited to the skin, leading to the generation of M2-type macrophages that dampen allergic inflammation [42**]. Basophil-derived IL-4 has also been shown to act on macrophages to promote increased expression of the inhibitory Fc receptor FcγRIIB on the surface, leading to the suppression of inflammation in an arthritis model of mice when treated with high dose intravenous immunoglobulin [43], even though a later study contradicted the basophil involvement in the suppression [44]. Basophils appear to limit disease activity in a mouse model of colitis through production of IL-4 that downregulates Th1 cytokine expression in T cells [45].

Distinct roles of TSLP-elicited basophils

Recent studies suggest that basophils may be a heterogeneous population of cells, and their development can be influenced by distinct cytokines, including IL-3 and thymic stromal lymphopoietin (TSLP), although basophils can be generated at a steady-state level even in the absence of both cytokines [46]. TSLP-elicited basophils exhibit a distinct pattern of gene expression, compared with classical IL-3-elicited basophils. The former displays higher expression of receptors for IL-18 and IL-33 to respond more robustly to these cytokines and produce more IL-4 and IL-6 than the latter while the latter shows higher responsiveness to IgE-antigen stimulation [46,47]. The unique association of the IL-3 receptor with FcR γ , a component of the IgE receptor Fc ϵ RI, in basophils may be related with the IgE responsiveness of IL-3-elicited basophils [48]. Collectively, TSLP- and IL-3-elicited basophils appear to have tendency to contribute to IgE-independent (innate) and IgE-dependent (acquired) immune responses, respectively (Figure 2). Indeed, a recent study has demonstrated in mice that TSLP-elicited basophils contribute to the pathogenesis of eosinophilic esophagitis, a food allergy-associated inflammatory disease, in an IgE-independent manner [49**]. Importantly, elevated TSLP expression and exaggerated basophil response were observed in esophageal biopsy samples from eosinophilic esophagitis patients, and a gain-of-function of TSLP polymorphism was associated with increased basophil responses in patients. TSLP-elicited basophils are also attributed to the pathogenesis of atopic dermatitis [46,50] and cutaneously-sensitized food allergy [51,52], as well as the promotion of Th2 cytokine responses to helminth infection [53]. Thus, the TSLP-basophil pathway could be a promising target for controlling Th2-type immunity and inflammation.

Basophils in allergy

Anaphylaxis is an acute-onset, life-threatening allergic reaction. Besides the classic mode of anaphylaxis in that IgE, mast cells and histamine play crucial roles, IgG- and platelet-activating factor (PAF)-mediated anaphylaxis has been demonstrated in mouse models. Mast cells are dispensable for the latter type of anaphylaxis, and three distinct cell lineages (basophils, macrophages, and neutrophils) have been reported as PAF-producing effector cells **under different experimental conditions** [54-56]. A series of recent studies tried to elucidate which cell type is **indeed** crucial for IgG- **and PAF**-mediated systemic anaphylaxis, and **concluded that either** basophils [57], macrophages [58] or all three cell types [59] **are** the major players, confirming that their relative contribution depends on experimental settings. Interestingly, intravenous injection of anti-asialo GM1 antibody activates basophils but not mast cells to produce PAF, and results in anaphylaxis-like shock in mice [60], illustrating the ability of basophils to elicit PAF-mediated anaphylaxis. Both basophils and mast cells contribute to peanut-induced active systemic anaphylaxis in mice [61], most likely in IgG- and IgE-dependent manner, respectively. While previous studies reported the dispensable role for basophils in IgE-mediated anaphylaxis [5,54], a recent study using IgE knock-in (**in place of IgG1**) mice suggested some contribution of basophils to **IgE-mediated anaphylaxis in an active sensitization model** [62], **even though the involvement of IgG subclasses other than IgG1** [59] **cannot be formally excluded in this model of anaphylaxis.**

Chronic idiopathic urticaria is defined as long-lasting itchy hives with no apparent triggers, and its pathogenesis remains unclear. A recent clinical trial has cast new light on our understanding of underlying mechanism. Anti-IgE antibody (Omalizumab) significantly reduced clinical symptoms and signs of patients who were resistant to H1-antihistamine therapy [63*]. **The Omalizumab administration shows a therapeutic effect** within a week. **Of note**, down-regulation of FcεRI on blood basophils is observed within 2 weeks after Omalizumab administration while that on mast cells typically occurs after 8 weeks. **Taken**

together with the observation that basophils infiltrated affected skin [64,65], IgE and basophils may contribute to the pathogenesis of chronic idiopathic urticaria.

Basophils in protective immunity against parasitic infections

Basophils are evolutionarily conserved in many animal species, albeit a minor population among leukocytes, suggesting a beneficial rather than deleterious role of basophils. Indeed, basophils have been demonstrated to play a crucial role in acquired protective immunity to infestation with blood-sucking ticks [2]. IgE-armed basophils are recruited to and accumulated in tick feeding sites of the skin during the second but not first infestation, and selective basophil ablation leads to the loss of resistance against tick feeding observed in the second infestation. By contrast, the role of basophils in acquired protective immunity against helminthic infections remained controversial [3,4]. A recent study with a mouse model of hookworm infections has revealed a novel mode of anti-helminth immunity in that skin-infiltrating basophils provide acquired protection against intestinal helminths by means of restraining skin-invading larvae from migration toward the lung and intestine, rather than expelling adult worms from the intestine [66**]. IgE-armed basophils recruited to the skin are activated in response to helminth antigens, and produce IL-4 that in turn promotes the generation of M2-type macrophages, leading to the larval trapping in the skin through ariginase-1 production. Thus, basophil- and IgE-mediated acquired immunity in the skin appears to be important for the resistance to reinfection of both ecto- and endo-parasites. Moreover, basophils have been shown to help establish protective immunity induced by irradiated larval vaccination for filariasis [67], and to contribute to granuloma formation induced by *Schistosoma mansoni* eggs [68].

Concluding remarks

Recent technological advances in basophil research have illustrated that basophils have pivotal roles *in vivo*, distinct from those of mast cells, in spite of their small number. Accumulating evidence suggests that the primary function of basophils is protection against parasitic infections while they often contribute to the development and exacerbation of allergic disorders under parasite-free conditions. Although functional study on human basophils is limited compared to murine one, recent analyses of human samples have extended the notion that basophils are recruited to affected tissues and may contribute to allergic responses or protective immunity against parasitic infections in humans [49,65,69,70]. Therefore, further elucidation of the molecular mechanisms underlying basophil-mediated allergic inflammation and protective immunity will help the establishment of a new strategy for allergy treatment and the development of efficient vaccination against parasites, respectively.

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Figure legends

Figure 1. Versatile roles of basophil-derived IL-4 in immune responses

Activated basophils secrete large quantities of IL-4 that shows pleiotropic functions in immune responses through action on a variety of cell types, including lymphocytes (T and B cells), innate lymphoid cells (ILC2), myeloid cells (monocytes and macrophages), and non-immune cells (fibroblasts).

Figure 2. Functional heterogeneity between TSLP- and IL-3-elicited basophils

Basophils appear to display functional heterogeneity, depending on the cytokine milieu during their development in the bone marrow. Epithelial cell-derived TSLP and T cell-derived IL-3 can independently promote the generation and expansion of the basophil cell lineage. TSLP-elicited basophils are less granular and produce higher levels of IL-4 and IL-6 in response to the IL-18 or IL-33 stimulation, compared to IL-3-elicited basophils. IL-3-elicited basophils are more granular and highly responsive to IgE-antigen complexes, compared to TSLP-elicited basophils. Thus, TSLP- and IL-3-elicited basophils trend to contribute to innate and acquired immune responses, respectively.

Figure 1

Figure 2