

Basophil-derived IL-4 plays versatile roles in immunity

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Abstract Recent studies demonstrated that basophils play crucial and non-redundant roles in the immune system, in spite of the fact that they are the rarest granulocytes and represent less than 1 % of peripheral blood leukocytes. In response to various stimuli, basophils release effector molecules stored in their cytoplasmic granules, including chemical mediators and proteases, and also secrete cytokines and chemokines. In this review, we will focus on the physiological and pathological roles of basophil-derived IL-4. Basophils can readily produce large quantities of IL-4 and are therefore the important source of IL-4. Basophil-derived IL-4 has been shown to regulate other immune cells, including T cells, B cells, group 2 innate lymphoid cells, monocytes, and macrophages. It also acts on non-hematopoietic cells such as fibroblasts and endothelial cells. Those cells stimulated with basophil-derived IL-4 contribute to the positive or negative regulation of a variety of immune responses in health and disease, including protection against parasitic and bacterial infections, allergy, and autoimmune diseases. Thus, basophil-derived IL-4 plays versatile roles in immunity.

Keywords Basophil · IL-4 · Th2 response · Allergy · Parasitic infection

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Introduction

Basophils share common features with tissue-resident mast cells, including the cell surface expression of the high-affinity IgE receptor (FcεRI) and the ability to release chemical mediators such as histamine in response to IgE plus antigen stimulation [1]. Despite their phenotypic similarities with mast cells, the study on basophils remains behind because of their rarity and short life-span that make their functional analysis difficult. Therefore, the physiological significance of basophils had long been unknown, until the studies in the early 1990s have highlighted the importance of basophils by providing evidence that both human and murine basophils can rapidly secrete a large amount of IL-4 in response to FcεRI cross-linking [2, 3]. This finding puts basophils on the main stage of immunology, allowing researchers to appreciate previously neglected roles of basophils [4, 5]. In addition, the recent advantages of analytical tools for basophils, including genetically engineered mice in which only basophils are constitutively or inducibly ablated [6–9], have accelerated in vivo research on basophils. It is now clear that basophils function as an important source of IL-4 in vivo and that basophil-derived IL-4 has a significant effect on a variety of cells under both physiological and pathological conditions. In this review, we focus on versatile roles of basophil-derived IL-4 in the immune system.

Stimuli and signaling that induce IL-4 production by basophils

Basophils can release IL-4 within 5–10 min after stimulation even under pharmacological inhibition of transcriptional factors, indicating the existence of preformed IL-4 in steady-state basophils [10]. Indeed, basophils from IL-4/green fluorescent

protein (GFP) reporter mice, in which IL-4 expression in basophils can be visualized, show constitutive GFP expression [11]. However, basophil-derived IL-4 mostly results from de novo synthesis of protein after basophil activation.

Basophils release IL-4 in IgE-dependent and IgE-independent manners [2, 3, 12–14] in response to a variety of stimuli such as IgE plus antigens; cytokines; pathogen-derived molecules including Toll-like receptor (TLR) ligands; and allergenic proteases, suggesting the involvement of basophils in both acquired and innate immunities (Fig. 1). The IgE-dependent mechanisms of basophil activation have been extensively examined and characterized, in which the Fc receptor γ -chain (FcR γ), spleen tyrosine kinase (Syk), and nuclear factor of activated T cells (NFAT) play pivotal roles in Fc ϵ RI-mediated signal transduction [15, 16], leading to a robust IL-4 production of basophils in response to IgE plus antigens.

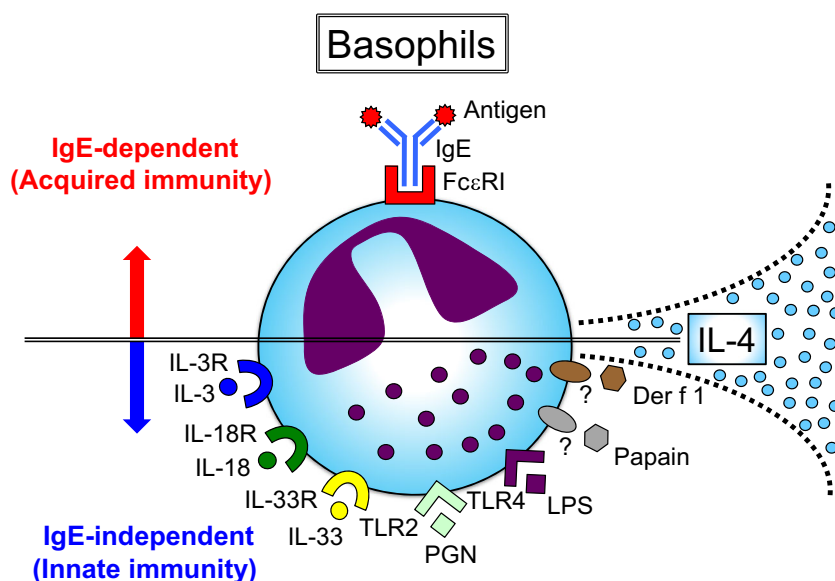
On the other hand, IgE-independent mechanisms underlying basophil IL-4 production remain incompletely defined. IL-3 (an important cytokine for basophil expansion [17]) can directly induce basophil IL-4 production and also enhance IL-4 production elicited by other types of stimuli [18, 19]. Intriguingly, it is reported that IL-3-induced IL-4 production of basophils requires FcR γ that is constitutively associated with the common β -chain of IL-3 receptor on basophils [20]. IL-18 and IL-33, which belong to the IL-1 family cytokines and have recently been shown to contribute to Th2 responses [21–24], can also induce basophil IL-4 production via a MyD88- and p38 α -dependent pathway in an Fc ϵ RI-independent manner [25, 26]. Interestingly, basophils appear to show functional heterogeneity depending on cytokine milieu. Thymic stromal lymphopoietin (TSLP), as well as IL-3, has recently been identified as a key regulator of basophil expansion [27]. TSLP-elicited basophils exhibit higher expression of receptors for IL-18 and IL-33, along with lower

responsiveness to IgE plus antigens, thereby producing more IL-4 in response to these cytokines than do IL-3-elicited conventional basophils, suggesting that the former may preferentially play roles in innate immunity rather than IgE-mediated acquired immunity [27, 28].

As basophils are thought to be involved in innate immunity, the roles of TLRs and their ligands in basophil activation have been investigated. Murine bone marrow-derived basophils (BMBAs) express TLR1, TLR2, TLR4, and TLR6, so that they can produce IL-4 in response to bacterial components including peptidoglycan (PGN) or lipopolysaccharide (LPS) in the presence of IL-3, even without Fc ϵ RI cross-linking [19, 22]. Human basophils are found to express high levels of TLR2 and TLR4 [29] and can secrete IL-4 in response to PGN but not to LPS via activation of nuclear factor- κ B (NF- κ B) [30]. The absence of CD14, a coreceptor for detection of LPS by TLR4, may account for the lack of LPS responsiveness in human basophils [29]. Notably, the TLR-mediated signaling has more impact on IL-4 production of basophils than that of mast cells [22]. These results suggest that basophils and basophil-derived IL-4 may play an important role in initiation of allergic diseases triggered by bacterial infections.

Basophils can also produce IL-4 in response to allergenic proteases such as papain and house dust mite proteases Der f 1 and Der p 1 [14, 31]. Interestingly, Rosenstein et al. have recently demonstrated that papain-induced IL-4 production is dependent on FcR γ , even though its immunoreceptor tyrosine-based activation motif (ITAM) and canonical signaling molecule Syk are not phosphorylated by papain stimulation [32]. More recently, it has been reported that FcR γ is indispensable for basophil IL-4 production not only by papain but also by other innate stimuli including Der f 1, IL-18, IL-33, and LPS, suggesting that FcR γ broadly controls basophil

Fig. 1 Stimuli and their sensors for basophil IL-4 production. Basophils produce a large amount of IL-4 in response to a variety of stimuli, including IgE plus antigens, cytokines, TLR ligands, papain, and house dust mite protease Der f 1. Basophils release IL-4 in both IgE-dependent and IgE-independent manners, suggesting that basophil-derived IL-4 plays roles in both acquired and innate immunities, respectively



IL-4 production even in the case of IgE-independent fashion [19].

Effects of basophil IL-4 on T cells

It is widely accepted that IL-4 has a principal role in promoting differentiation of naïve CD4⁺ T cells to Th2 cells that in turn produce more IL-4, leading to allergic responses and protection against parasites [33, 34]. Although the initial source of IL-4 remains unclear, basophils are proposed as providers of initial IL-4 that primarily induces Th2 differentiation [35, 36]. Indeed, basophils can rapidly produce larger amounts of IL-4 with lower threshold for activation than do T cells [37]. Coculture of naïve CD4⁺ T cells with wild-type (WT) but not *I14*^{-/-} basophils can promote a robust Th2 differentiation in the presence of conventional antigen-presenting cells (APCs) and antigens, suggesting the importance of basophil-derived IL-4 in promoting Th2 differentiation in vitro [38, 39]. The in vivo relevance of these findings is supported by analysis of mice with genetically or artificially induced basophilia in which Th2 differentiation is markedly accelerated [38–40]. Importantly, Sokol et al. have shown that subcutaneous injection of papain, a potent inducer of Th2 response, elicits a transient basophil migration into the draining lymph nodes where basophils produce IL-4 and encounter CD4 T cells [14]. Indeed, antibody-mediated depletion of basophils leads to a lack of basophil migration into the lymph nodes, resulting in a failure of Th2 differentiation [14]. These results suggest that basophil-derived IL-4 critically contributes to papain-induced Th2 differentiation in vivo, presumably in the lymph nodes.

In 2009, it has been reported by three independent groups that basophils act as not only providers of IL-4 but also critical APCs for driving a robust Th2 differentiation [41–43]. They have demonstrated that basophils express MHC class II and costimulatory molecules, such as CD80, CD86, and CD40, and can present antigens to naïve CD4⁺ T cells with providing IL-4, leading to Th2 differentiation even in the absence of dendritic cells (DCs). This is an exciting discovery as it is generally assumed that basophils and DCs have distinct roles in Th2 differentiation, a source of IL-4 and antigen presentation [44, 45], respectively. However, the full extent of capacity of basophils for antigen presentation and Th2 differentiation remains uncertain because conflicting results have appeared, probably depending on different experimental settings [7, 8, 46–55]. To address this issue, further investigation with more sophisticated tools, such as basophil-specific MHC class II-deficient mice, will be necessary.

Basophil-derived IL-4 also regulates other T cell subsets. Basophils appear to limit disease activity of experimental murine colitis, in which basophil-derived IL-4 suppresses harmful Th1 responses such as IFN- γ , IL-2, and TNF- α production of CD4⁺ T cells [56]. Unlike CD4⁺ T cells, naïve CD8⁺ T cells

stimulated in the presence of basophils can produce IL-10, which requires basophil-derived IL-4 [57].

Effects of basophil IL-4 on B cells

It is well known that antigen-specific antibodies, including IgE, are produced by B cells in response to the first antigen exposure during the primary responses and that antigen-specific IgE-armed basophils produce robust IL-4 in response to corresponding antigens in the secondary memory responses [58]. However, physiological relevance of basophil-derived IL-4 in the memory responses had largely been unknown. Recent studies have revealed that basophil-derived IL-4 plays roles in protective immunity [59, 60] and in autoimmunity [61] through enhanced production of pathogen-specific and self-reactive antibodies, respectively. Thus, basophils have the potential to activate B cells through production of IL-4 that amplifies humoral memory responses.

Denzel and colleagues have demonstrated that basophils act together with CD4⁺ T cells to enhance B cell proliferation and immunoglobulin production by providing IL-4 and IL-6 in vitro [59]. Notably, they have also delineated the physiological significance of basophils in humoral memory responses in vivo through analysis of murine vaccination-infection model. When mice vaccinated with pneumococcal surface protein A (PspA) are subjected to sepsis induced by *Streptococcus pneumoniae*, depletion of basophils before second vaccination results in increased mortality with much lower titers of plasma PspA-specific IgG1 and IgG2a [59]. Another group has reported that circulating IgD constitutively binds to human basophils through an unknown calcium-fluxing receptor [60]. IgD cross-linking on basophils triggers sustained intracellular calcium flux that elicits basophils' production of IL-4, IL-13, B cell-activating factor (BAFF), and a proliferation-inducing ligand (APRIL), leading to IgM secretion as well as IgG and IgA class switching in B cells. Since IgD⁺IgM⁻ B cells derived from the upper respiratory mucosa are specialized to produce IgD that recognizes respiratory bacteria and their components, IgD-armed basophils may be involved in amplification of humoral memory responses for protection against bacterial infections in humans [60]. Although the full extent of its capacity remains to be determined, these results suggest that basophil-derived IL-4 may play roles in enhancement of production of pathogen-specific antibodies in B cells and thus contribute to humoral protective immunity against pathogens.

Basophil-derived IL-4 also has the potential to amplify autoantibody production in B cells. It has been reported that *Lyn*^{-/-} mice exhibit a significant increase of basophil proliferation and IL-4 production because of the transcriptional factor GATA-3 upregulation [40]. Interestingly, basophils contribute to the production of autoantibodies including self-reactive IgE from B cells by providing IL-4, which causes lupus-like

nephritis in aged *Lyn*^{-/-} mice [61]. The self-reactive IgE in turn leads to basophil activation with IL-4 production, accelerating B cell autoantibody production and disease progression. Notably, either the depletion of basophils or the absence of IL-4 attenuates autoantibody production and thus protects mice from severe lupus nephritis [61], implicating the contribution of basophil-derived IL-4 to the pathogenesis of humoral autoimmunity.

Effects of basophil IL-4 on group 2 innate lymphoid cells

Innate lymphoid cells (ILCs) are the most recently identified cell population in the innate immune system [62]. Among them, group 2 ILCs (ILC2s) have been shown to play crucial roles in allergic inflammation and protective immunity against parasites through a robust production of Th2 cytokine in response to epithelial cell-derived factors such as IL-25, IL-33, and TSLP [62, 63]. Although ILC2s are a primary source of innate IL-5 and IL-13, there is little evidence that they produce IL-4 in mice [64], suggesting their unique role is distinct from that of IL-4-producing basophils in Th2 responses. In addition, recent studies have revealed that ILC2s express IL-4R α and can proliferate in response to IL-4 [65, 66]. Collectively, these results suggest that basophil-derived IL-4 acts on ILC2s and that basophils and ILC2s have cooperative roles by providing different types of Th2 cytokines in controlling Th2 responses.

Motomura et al. have demonstrated that basophil-derived IL-4 contributes to the activation of ILC2s in cysteine protease-induced allergic asthma (Fig. 2) [65]. In mice administered intranasally with papain, basophils promote a robust production of IL-5 and IL-13 from ILC2s by providing IL-4 in collaboration with epithelial cell-derived IL-33, resulting in the exacerbation of lung eosinophilia and goblet cell hyperplasia [65]. Another group has also reported the importance of basophil-derived IL-4 in promoting ILC2 activation during allergic skin inflammation [66]. They have shown that basophils and ILC2s accumulate in close proximity in the skin lesions from both atopic dermatitis (AD) patients and AD-

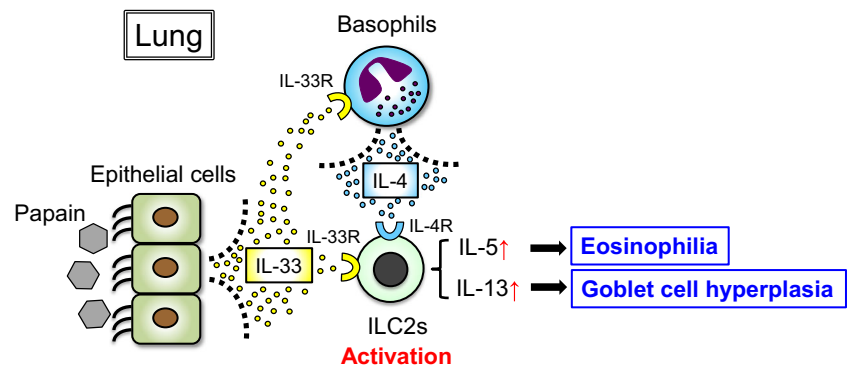
like mice induced by topical application of a vitamin D analog MC903. Analysis of mice with diphtheria toxin-based genetic depletion of basophils has revealed that basophils are required for substantial accumulation of ILC2s in the skin lesions. Notably, supernatants from in vitro activated WT basophils promote the proliferation of ILC2s while those from *Il4*^{-/-} basophils fail, suggesting that basophil-derived IL-4 is crucial for ILC2 proliferation [66].

Effects of basophil IL-4 on macrophages

Macrophages have a phenotypic heterogeneity and exhibit distinct features for activation under different conditions of immunological status [67]. Alternatively activated macrophages (AAMs), also called M2-type macrophages, are elicited by canonical Th2 cytokines IL-4 and IL-13 and are involved in Th2 responses during parasitic infection and allergic inflammation [68]. Recent studies have expanded their roles beyond conventional type 2 immunity to include maintenance of physiological homeostasis [69–71] and tissue repair [72, 73]. Although various cell types, including T cells, natural killer T (NKT) cells, mast cells, eosinophils, and basophils, show the potential to release IL-4 that may impact on macrophages [68], we and other groups have recently highlighted the importance of basophil-derived IL-4 in alternative activation of macrophages (Fig. 3) [74–76].

Helminth infections provoke highly polarized Th2 responses, characterized by elevated levels of serum IgE, eosinophilia, mucus overproduction, and smooth muscle and fibroblast alternations along with AAM generation. Th2 cytokines IL-4 and IL-13 play a pivotal role at both the initiation and execution phases of the protective immunity against helminths [77]. Although the roles of both basophils and AAMs in Th2 responses during helminth infection remained controversial [7, 8, 68], our recent report has proposed an important role of basophil-derived IL-4 in AAM generation and protection against the intestinal helminth *Nippostrongylus brasiliensis* (Fig. 3) [75]. During the second infestation with *N. brasiliensis*, IgE-armed basophils infiltrate into the larva-

Fig. 2 Basophil-derived IL-4 provokes ILC2 activation. Basophils activate ILC2s by providing IL-4 in collaboration with IL-33 derived from papain-stimulated airway epithelial cells in the lung. Activated ILC2s produce IL-5 and IL-13 that trigger lung eosinophilia and goblet cell hyperplasia, respectively



infected skin and are activated in response to helminth antigens. Skin-infiltrating basophils produce IL-4 that subsequently promotes the generation of AAMs, leading to the larval trapping of the skin through arginase-1 production [75].

Th2 responses are also closely associated with allergic inflammation, in which basophil-derived IL-4 is implicated in AAM generation (Fig. 3). We have previously established a mouse model of basophil-dependent chronic skin allergic inflammation (designated IgE-CAI) [78], in which mice passively sensitized with antigen-specific IgE are challenged intradermally with corresponding antigens. The IgE-armed basophils produce IL-4 in response to antigens and initiate allergic inflammation in the skin lesions. Basophil-derived IL-4 acts on inflammatory monocytes recruited to the skin lesions and promotes their differentiation to AAMs, which in turn dampen inflammation [74]. Thus, basophil-derived IL-4 contributes to the termination of allergic skin inflammation.

Recent report has delineated a role of basophil-derived IL-4 in AAM generation to maintain liver homeostasis (Fig. 3) [76]. Bleriot and colleagues have demonstrated that, upon *Listeria monocytogenes* infection, bacteria-induced Kupffer cell death triggers IL-33 release from hepatocytes that induce IL-4 production by infiltrating basophils in the liver. Basophil-derived IL-4 elicits differentiation of recruited inflammatory monocytes into AAMs that in turn replace dead Kupffer cells, restoring liver homeostasis [76].

Basophil-derived IL-4 has been shown to promote increased expression of the inhibitory receptor Fc γ RIIB on

macrophages that may in part account for the immunosuppressive effect of high-dose intravenous immunoglobulin (IVIG) [79]. Sialylated IgG, an effector component of IVIG, binds to DC-SIGN⁺ myeloid cells, resulting in the production of IL-33, which induces IL-4 production of basophils. Intriguingly, basophil-derived IL-4 promotes upregulation of Fc γ RIIB on the cell surface of macrophages, leading to immunosuppression in an arthritis model of mice when treated with IVIG [79]. Although a conflicting result has appeared recently [80], this observation suggests that basophils can regulate inflammation by providing IL-4 through enhanced expression of Fc γ RIIB on macrophages.

Effects of basophil IL-4 on non-hematopoietic cells

Beyond the hematopoietic lineages, recent studies have illuminated the roles of basophil-derived IL-4 in the regulation of other cell types including fibroblasts [81] and endothelial cells [82]. Basophils promote eosinophil migration especially in the presence of fibroblasts. In coculture with fibroblasts, basophils produce IL-4 and TNF- α , which elicit CCL11 expression in fibroblasts, leading to the enhanced eosinophil migration in vitro [81]. In allergic skin lesions, IgE/allergen-stimulated basophils alter their migratory kinetics during the transmigration through blood vessels and stay in the endothelium [82]. Sustained interaction of basophils with endothelium promotes delivery of basophil-derived IL-4 to the endothelium and subsequent induction of endothelial vascular cell

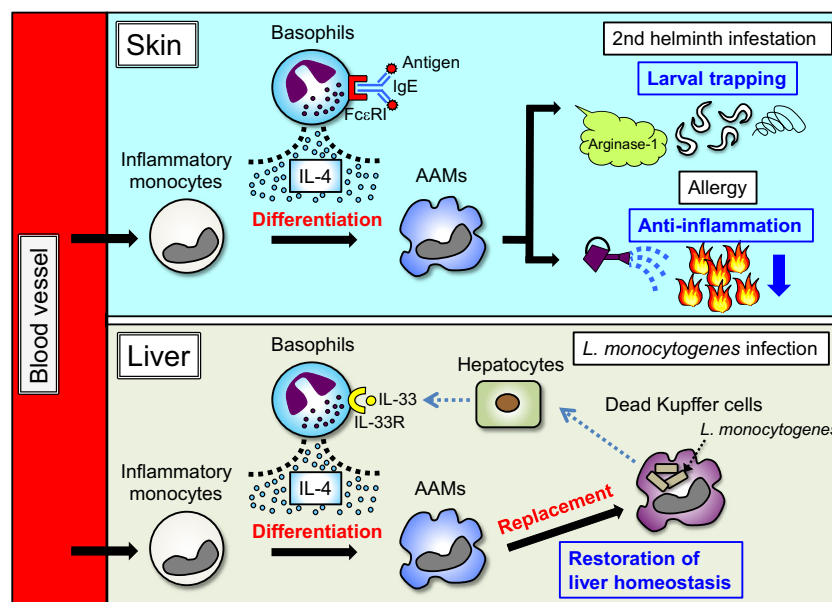


Fig. 3 Basophil-derived IL-4 contributes to AAM generation from inflammatory monocytes. Basophil-derived IL-4 acts on inflammatory monocytes recruited into the skin, leading to the generation of AAMs. The AAMs contribute to larval trapping through arginase-1 production during the second helminth infestation or to attenuation of allergic inflammation (upper panel). During *L. monocytogenes* infection,

bacteria-induced Kupffer cell death stimulates hepatocytes that release IL-33, triggering basophil IL-4 production in the liver. Basophil-derived IL-4 subsequently induces the generation of monocyte-derived AAMs that replace Kupffer cells ablated by bacterial infection, restoring liver homeostasis (lower panel)

adhesion molecule-1 (VCAM-1), leading to eosinophil accumulation in the skin.

Concluding remarks

Basophil research has been strongly accelerated over the past few years by generation of sophisticated analytical tools such as constitutive or inducible basophil-ablated mice. In addition to initiation of Th2 differentiation, recent advances have revealed the crucial roles of basophil-derived IL-4 in the activation of B cells, ILC2s, macrophages, and non-hematopoietic cells. Such advances have led to greater appreciation for functions of basophil-derived IL-4 beyond the conventional Th2 responses, including humoral memory responses and maintenance of physiological homeostasis. Thus, basophils now come into the forefront of immunological field. Given that the absolute number of basophils is relatively small, it is possible that basophils and their products including IL-4 become promising targets to treat immunological disorders, although we need to define the clinical relevance of findings in mice to human diseases.

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