Published in final edited form as:

Curr Opin Hematol. 2008 January ; 15(1): 59–63. doi:10.1097/MOH.0b013e3282f13ce8.

BASOPHILS AND TYPE 2 IMMUNITY

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Abstract

Purpose of review—Mechanisms involved in the development of *in-vivo* type 2 immunity are poorly defined. Basophils are potent IL-4-producing cells and may contribute to the process of polarizing immune responses.

Recent findings—Although basophils represent fewer than 0.5% of blood leukocytes, their frequency dramatically increases under certain circumstances, particularly Th2-related responses including parasitic infection and allergic inflammation. Recent studies proposed the hypothesis that basophils could contribute to the development of type 2 immunity by providing initial IL-4 important in T cell polarization and by recruiting other effector cells such as eosinophils or neutrophils. Multiple stimuli of IgE-dependent and IgE-independent pathways that lead to release of cytokines and mediators from activated basophils have been identified. In addition, progenitors that differentiate into mature basophils have recently been identified.

Summary—The current review revisits basophils with the goal of providing insights into understanding unappreciated roles of basophils *in vivo*.

Keywords

basophils; IL-4; parasites; Th2 type immunity

Introduction

Basophils are the least common leukocytes found in the circulation, representing less than 0.5% of total blood leukocytes and of nucleated bone marrow cells. The paucity of basophils has been the main obstacle in understanding their biological importance. While earlier *in-vitro* studies of human basophils have provided insights into understanding basophil activation, *in-vivo* basophil biology remains poorly defined. The recent demonstration of increases in basophil numbers in murine parasitic infection and in allergic inflammation models provide an important impetus to investigate basophil function in animal models. The close association of basophil responses with Th2 type immunity suggests that basophils may play a role in the induction of Th2 type immune responses or in Th2 effector immunity.

Basophil development

Basophils develop in the bone marrow, and enter the periphery as fully differentiated forms. Basophil progenitors have recently been identified as Lin[−]CD34⁺FcεRI^{hi}c-Kit[−] cells in the

bone marrow that arise from granulocyte-macrophage progenitors in the bone marrow and basophil-mast cell progenitors in the spleen [1]. The transcription factor, C/EBP α , plays a key role in the differentiation of the precursors into the basophil lineage [1]. Steady-state basophil levels are maintained under normal circumstances. Conditions such as parasitic infection or allergy disrupt this homeostasis, however, resulting in increases in numbers of peripheral basophils.

The nature of stimuli promoting basophil development during parasitic infections is unclear. The hematopoietic cytokine IL-3 has been suggested to play a key role in the differentiation of basophil precursors into mature basophils. Culture of bone marrow cells in IL-3 has been shown to induce basophil differentiation *in vitro* [2]. *In vivo*, we recently reported that IL-3 administration results in substantial increases in basophil development in mice [3]. As IL-3 is mainly produced upon T cell activation, it is possible that the initial burst of IL-3 produced by activated T cells enhances basophil development in the bone marrow. This hypothesis is further supported by the following observations. Mice deficient in IL-3 displayed defective basophil production following parasitic infection [4]. Basophil accumulation in the liver of *Nippostrongylus brasiliensis*-infected mice was partially diminished by anti-IL-3 antibody treatment [5]. T cell deficient mice fail to accumulate basophils in the periphery following *N. brasiliensis* infection. Adoptive transfer of CD4 T cells at the time of infection was sufficient to restore basophil development [5]. There is also evidence, however, suggesting that IL-3 is not the only factor responsible for the basophil development. First, steady-state basophil production is not dependent on IL-3; IL-3-deficient mice have normal levels of basophils in the circulation and in the bone marrow, although infection-induced increase in basophils frequency is severely impaired [4]. Second, IL-3 production is not exclusively seen in Th2 cells, although Th2 cells have been reported to make more IL-3 than Th1 cells [6]. Given that enhanced basophil production is typically observed during type 2 immune responses, it is likely that there may be an additional factor that initiates basophil production in the bone marrow.

Parasite-associated molecules such as proteases [7], glycoproteins [8], or structural components such as chitin [9] have been demonstrated to stimulate basophils to produce cytokines or to recruit basophils into inflammatory sites. Whether these parasite components have a direct impact on basophil development needs to be determined. Alternatively, T cell activity in the bone marrow has been suggested to play a critical role in maintaining normal hematopoiesis. Particularly, subsets of CD8 T cells were shown to be important for eosinophilopoiesis [10]. Likewise, it is possible that some CD4 T cells activated by infection may migrate into the bone marrow and enhance basophil production by producing IL-3. In support of this, it was recently demonstrated that activated bone marrow CD4 T cells maintain normal hematopoiesis [11].

Basophil activation

Basophils and mast cells express the high affinity surface IgE receptor, Fc ϵ RI. Crosslinkage of the receptor, either physiologically through antigen cross-linkage of IgE bound to Fc ϵ RI, or artificially, through use of anti-Fc ϵ RI or anti-IgE antibodies, delivers an activation signal, rapidly releasing intracellular mediators, mainly histamine and leukotrienes and causing the production and secretion of cytokines. IL-4 production by basophils can be detected as early as 10 min after stimulation in the presence of transcription inhibitors, implying that some IL-4 production by basophils comes from preformed stores [12]. In agreement with this, basophils from reporter mice in which enhanced green fluorescent protein (GFP) is inserted after an internal reentry sequence down-stream of the *IL4* gene constitutively express GFP [13]. Much of the IL-4 produced by stimulated basophils, however, depends upon new transcription and translation.

Several proteins associated with parasites are capable of stimulating basophils. Proteases from helminths as well as dust mites have been shown to stimulate basophils to produce IL-4 [7]. Recently, it was reported that the secretory glycoprotein IPSE/α-1 from *Schistosoma mansoni* eggs induces basophil IL-4 production. Interestingly, IPSE mediated basophil IL-4 production requires the presence of IgE, yet in an antigen nonspecific manner [8]. Similarly, HIV glycoprotein gp120 was shown to bind to the VH3 domain of surface IgE, leading to cytokine production from basophils [14].

IgE-independent pathways that lead to basophil activation have also been described. IL-3, which is involved in basophil development, can also induce basophils to produce IL-4 and mediators without IgE and can enhance the production in response to FcR cross-linkage [15,16]. As basophils may be thought of as members of the innate immune system, the roles of Toll-like receptors (TLRs) in their activation have been examined. Yamaguchi and colleagues [17] recently reported the pattern of a panel of TLRs in human basophils. They constitutively express TLR2, TLR4, TLR9, and TLR10 mRNAs. The expression of TLR2 and TLR4 was particularly prominent. Despite the high degree of expression of TLR4 mRNA in basophils, the cells do not respond to lipopolysaccharides (LPS), the TLR4 ligand. Their failure to respond to LPS may be attributed to the absence of CD14 on human basophils, which is critical for LPS responsiveness [18].

Basophils displayed NF-κB nuclear localization in response to the TLR2 ligand peptidoglycan [19]. Basophils stimulated with TLR2 ligand produced both IL-4 and IL-13 [18,19]. The pattern of TLR expression on murine basophils has also been reported. Bone marrow derived murine basophils express TLR1, TLR2, TLR4 and TLR6 [20]. Stimulation of basophils with IL-3 plus peptidoglycan or with IL-3 plus LPS induced production of IL-4 and IL-13 in the absence of FcεRI cross-linkage [20]. These results imply that basophils may be activated to produce IL-4 or IL-13 during bacterial infection, which may then trigger the development of allergic inflammation. Along the same lines, bacterial products from *Helicobacter pylori* activate basophils through N-formyl-peptide receptor-like (FPRL) 1 and 2 expressed on basophils [21]. IL-18 used together with IL-3 also stimulates basophils to produce IL-4 and IL-13 [2]. Consistent with this observation, basophils isolated from MyD88-deficient mice fail to produce IL-4 in response to IL-18 or TLR ligands (our unpublished results). Whether activation of basophils through TLR/IL-18-mediated pathway plays a role *in vivo* requires further investigation but it is striking that treating mice with IL-2 plus IL-18 causes striking elevation of serum IL-4, IL-13 and IgE concentrations [22]. Whether basophils play a role in this process has not yet been resolved.

Basophils, potential inducers of type 2 immunity

Upon activation, basophils rapidly produce IL-4; the total amount of IL-4 produced by activated basophils is probably greater than that by T cells, although highly differentiated Th2 cells may produce more IL-4 than basophils on a per cell basis. [23]. Due to their rapid and robust IL-4 production, it has been proposed that basophils be considered 'innate type 2 cells', whose activation may lead to the development of adaptive type 2 immunity by providing the initial IL-4 used by CD4 T cells in their differentiation to the Th2 phenotype [24,*25]. Consistent with this possibility, basophils from healthy individuals produce IL-4 upon stimulation of *Schistosoma* egg antigens. This supports the hypothesis that IL-4 produced by activated basophils induces parasite-specific naive T cells to become Th2 type effector T cells [26]. Basophils also may play a role in immunoglobulin class switching to IgE *in vitro* [27]. Upon FcεRI stimulation, basophils induced IgE synthesis by cocultured B cells. IL-4 neutralization as well as blockade of CD40L/CD40 interaction abolished IgE synthesis [28,29]. Whether basophils exert roles in Th2 differentiation and immunoglobulin class switching *in vivo*, however, has not formally been tested.

We recently demonstrated that naive CD4 T cells stimulated *in vitro* in the presence of basophils preferentially differentiate into IL-4-producing Th2 type effector CD4 T cells in the absence of exogenous IL-4 [3]. Basophils are the source of the IL-4 required for this differentiation as IL-4-deficient basophils failed to aid in Th2 differentiation [3]. We also demonstrated that CD4 T cells stimulated *in vivo* in a basophil-enriched environment differentiated into IL-4-producing Th2 CD4 T cells, strongly suggesting that basophils could induce Th2 type immunity *in vivo*. In agreement with this, it was recently reported that mice deficient in IRF-2 spontaneously develop Th2 type immunity [30]. Interestingly, increased expansion of peripheral basophils was found in these mice, further supporting the hypothesis that the basophil population size may determine Th1/Th2 balance *in vivo* [30].

It has been suggested that basophils may play an important role in HIV infection [*25]. Elevated serum IgE levels are often found in HIV-infected patients, which is believed due to a shift from Th1 to Th2 cytokine production [31]. Although the mechanisms responsible for the immune deviation during HIV infection remain to be determined, a possible contribution of basophils to this process was initially proposed based on the fact that HIV gp120 stimulates basophils to produce IL-4, a key inducer of Th2 type immunity [32,33]. Importantly, gp120s derived from divergent HIV-isolates induce basophil IL-4 production. In addition, IL-4 upregulates basophil CXCR4 expression, a primary coreceptor of HIV-1. It has been suggested that basophils together with mast cells, might be novel targets of HIV infection. Indeed, basophils isolated from allergic patients have been demonstrated to be susceptible to HIV-1 *in vitro* [34]. Taken together, basophil IL-4 production in response to HIV antigens and the subsequent immune deviation toward Th2 differentiation may be a significant issue in HIV infection.

Basophils also appear to play important roles in recruiting other effector cells to sites of inflammation. An earlier study [35] reported that depleting basophils reduced eosinophil recruitment to sites of inflammation in *Amblyomma americanum* infection. The role of immune regulators was further supported by an elegant study by Karasuyama and colleagues. They have previously reported an IgE-mediated chronic allergic dermatitis characterized by massive infiltration of eosinophils and basophils [*36]. Selective depletion of basophils, which only represent approximately 2% of the infiltrating cells, dramatically reduced the numbers of infiltrating eosinophils, and neutrophils in the lesion. These data strongly suggest that basophils may initiate the development of IgE-mediated chronic allergic inflammation by recruiting other effector cells.

Basophil recruitment

Basophils leave the circulation and migrate to inflammatory sites during allergic inflammation and infection. Like any other leukocytes, transmigration of basophils involves multistep processes including adhesion to the vascular endothelium, transendothelial migration, and locomotion to inflammatory sites [*25]. Measuring human basophil migration using HUVEC cell lines has revealed that P and E-selectin as well as $\beta 2$ integrin (CD11b/CD18)/ICAM-1 play a critical role in basophil migration [37]. Stimulation of basophils with cytokines such as IL-1 β or IL-3 significantly enhanced migration capacity, in part by upregulating $\beta 2$ integrin expression [38]. Basophils express multiple chemokine receptors including CCR1, CCR2, CCR3, CXCR1, CXCR3, and CXCR4. Eotaxin (CCL11), RANTES (CCL5), and SDF-1 (CXCL12) strongly stimulate basophil transmigration *in vitro* [*25,39]. Nonetheless, whether these adhesion molecules and chemokines are important during *in vivo* immune responses remains to be determined. In addition to integrins and chemokines, basophils may utilize matrix metalloproteinase-9 in the pathogenesis in allergic diseases [40].

The analysis of basophil accumulation patterns during parasitic infection raises interesting points. Following *N. brasiliensis* infection, we found the liver to be the major site in which substantial basophil accumulation occurs. Increased numbers of basophils were also found in the blood, the spleen, and the lung. Basophil accumulation in the draining lymph nodes was marginal and transient (our unpublished observation). The mechanisms underlying selective basophil accumulation in the course of *N. brasiliensis* infection are unclear. If basophils play a key role in the development of parasite specific Th2 type immunity, one would look to basophil accumulation in the draining lymph node as a crucial step through which parasite-specific naive T cells are activated by antigen bearing dendritic cells in the presence of basophil derived IL-4, which might then promote Th2 differentiation. Whether basophils do participate in this process, however, and what controls such basophil accumulation in the draining lymph nodes needs further investigation. Recently, Medzhitov and colleagues [3] reported transient basophil accumulation in draining lymph nodes at about day 3 after immunization and have shown that blocking this by treatment with anti-FcεRI can strikingly diminish priming for Th2 accumulation (personal communication). In addition, it is unclear why basophils accumulate in the liver. Basophil liver accumulation also occurs in response to IL-3 administration, where infection does not play a role in the process.

Since depletion of basophils in chronic allergic dermatitis completely abolishes the lesion by preventing recruitment of other effector cells (see above), preventing basophil recruitment into the inflammatory lesion might prove an effective therapeutic strategy to treat allergic disorders.

Conclusion

Although basophils have been recognized for more than 120 years [25], they are one of the least studied cell types, which should be attributed to the lack of animal models in which these cells can be analyzed. Evidence emerging from recent studies strongly suggests that basophils may play important roles in the development of type 2 immune responses as well as in allergic inflammation, but this still remains a possibility and not a certainty. Further investigations should focus on understanding molecular mechanisms of basophil development; defining *in-vivo* mechanisms of immunoregulatory functions; and establishing animal models deficient in basophils.

Understanding *in-vivo* basophil biology may allow us to develop therapeutic strategies not only in Th2 type disorders involving basophils but also in Th1/Th17 mediated autoimmunity such as experimental allergic encephalomyelitis.

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