

Basophils: A Nonredundant Contributor to Host Immunity

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The role of basophils, the rarest of blood granulocytes, in host immunity has been a mystery. Long considered the poor relative of mast cells, basophils have received much recent attention because of the availability of new reagents and models that reveal unique properties of these cells. Basophils are known to have distinct roles in allergic hypersensitivity reactions and in the immune response to intestinal helminthes. In this review, we highlight these advances and summarize our current understanding of the repertoire of functions attributed to these cells. Despite these recent insights, we are likely only beginning to gain a full understanding of how and where these cells lend effector functions to vertebrate immunity. Advances are likely to come only with the development of specific reagents that enable the finer study of basophil lineage and function. Although many fundamental aspects of basophil biology remain unanswered, the prospects remain bright for unmasking new contributions by these unusual cells.

Introduction

Basophils make up less than 1% of circulating blood leukocytes and are present in all vertebrates. Recognized by Paul Ehrlich by their cytoplasmic granules that stain with basophilic dyes, basophils are typically grouped with mast cells based on their appearance in tissues during allergic and antihelminth immune responses and on their expression of high-affinity IgE receptor (FcεRI) that renders both of these cell types responsive to activation by crosslinking IgE bound to the surface. Progress in basophil research was slowed for many years by the inability to follow these cells by other than morphologic criteria. Advances largely over the past 5 years, however, have enabled a more comprehensive look at the role of basophils in immunity and suggest that these cells may provide unique functions unmet by other hematopoietic cells, particularly during Th2-associated allergic and antihelminth responses. Here, we review new findings of basophil function that support a closer look at this infrequently examined cell lineage.

Basophil Lineage

The appreciation of a role for basophils in immunity was hampered greatly by their obscurity. Until relatively recently, their existence in mice was actively questioned or these cells were assumed to be an evolutionary relic of the mast cell lineage. The initial descriptions of cells in the mouse that possessed the morphological characteristics, including electron microscopic features, of basophils appeared in the literature only in the early 1980s (Dvorak et al., 1982; Galli et al., 1982; Nagao et al., 1981; Urbina et al., 1981). Since then, a number of flow cytometry-based purification schemes and microarray analyses have established criteria for the recognition of basophils in the mouse, a process that was aided greatly by the establishment of various interleukin-4 (IL-4) reporter mouse strains (see below), which, serendipitously at the time, labeled basophils (Min et al., 2004; Voehringer et al., 2004b). Use of these various markers has

enabled studies seeking to determine the lineage of these cells during hematopoietic differentiation.

All cells of the immune system develop from rare bone-marrow-resident hematopoietic stem cells (HSC). Common lymphoid and myeloid progenitors (CLP and CMP, respectively) derived from the HSC represent the earliest progenitors committed to populating the cellular subsets within their respective lineages. Whereas CLPs exclusively give rise to T, B, and NK cells, CMPs are the antecedents of erythrocytes, monocytes, and granulocytes. Eosinophils, basophils, and mast cells derive from the granulocyte-monocyte progenitor (GMP), a direct descendent of the CMP (Iwasaki and Akashi, 2007). Recent studies have begun to identify the lineage-restricted progenitors and molecular determinants required for terminal differentiation of basophils from the GMP.

Basophil (BaP) and mast cell progenitors (MCP) are derived from the GMP and are defined by expression of FcεRI. Eosinophil progenitors (EoP), as tracked by a reporter for GATA-1, a key hematopoietic lineage determinant, also arise from GMPs as shown by expansion in bone marrow after intestinal helminth infection. EoPs are marked by increased GATA-1 and IL-5Rα expression, consistent with the requisite role for IL-5 in reactive eosinophilia (Iwasaki et al., 2005). A common basophil-mast cell progenitor (BMCP) was isolated from the spleen of mice (Arinobu et al., 2005; Gurish and Boyce, 2006). The splenic BMCP exhibited high expression of the intestinal homing β7-integrin, gave rise to BaP and MCP in culture, and was capable of reconstituting intestinal β7^{hi} MCPs when transferred to mast cell-deficient mice (Arinobu et al., 2005). In the bone marrow, BaPs were identified within a β7^{lo} GMP population (Gurish and Boyce, 2006) and MCPs by expression of the T1(ST2) receptor (Chen et al., 2005); however, the precise relationship between bone-marrow-resident progenitors and the splenic BMCP remains unclear.

The developmental decisions associated with lineage commitment reflect a series of patterned transcription factor profiles that

result ultimately in a defined, lineage-restricted gene profile. One study suggests that a complex interplay between the transcription factors GATA-2 and CEBP/ α determines the terminal cell fate decisions that define eosinophils, basophils, and mast cells. Prior work showed that CEBP/ α was highly expressed in purified bone marrow BaPs as compared to splenic BMCPs, yet was undetectable in intestinal or cultured MCPs (Arinobu et al., 2005). Further, ectopic expression of CEBP/ α reinforced basophil differentiation from cultured BMCPs, whereas conditional deletion of CEBP/ α resulted in populations of pure mast cells. Thus, CEBP/ α appears to be a critical determinant whose presence or absence specifies terminal basophil and mast cell differentiation, respectively.

A similar analysis incorporating GATA-2 with CEBP/ α proposed a detailed model of lineage differentiation from the GMP stage. Whereas the combination of CEBP/ α and GATA-2 in GMPs promoted eosinophil development, GATA-2 expression in the absence of CEBP/ α supported the generation of BMCPs. Re-expression of CEBP/ α at the intermediate bipotent BMCP stage enforced basophil development over the mast cell lineage (Iwasaki et al., 2006). The strength of this model was apparent by the ability to redirect CLPs to derive each of these myeloid lineages by kinetically regulated ectopic expression of C/EBP α and GATA-2 (Iwasaki et al., 2006). All of these suggest a tight developmental relationship between basophils, mast cells, and eosinophils, perhaps not unexpected considering the functional interactions that occur between these three innate cell types in allergic and helminth immunity characterized by a predominance of Th2-associated responses. The precise systemic signals that guide the coordinated expansion of these cell types, however, remain incompletely defined, although the IL-3-IL-5-GM-CSF axis is at least partially involved (see below). Further evidence for their intertwined origins comes from the finding that the capacity to produce IL-4 is shared by each of these cell types, a process that is developmentally acquired (Gessner et al., 2005). The mechanisms that activate this effector program in innate cells remain incompletely explored but of much interest.

Despite these advances, full understanding of the basophil lineage and by what means these cells acquire their innate effector functions is still lacking. The elucidation of the requisite transcriptional programs that control the cell fate decisions involved will require further genetic and transcriptional dissection, but will be important in gaining an understanding of the primary evolutionary pressures that have sustained the basophil lineage.

Antibody-Mediated Basophil Activation Hypersensitivity and Anaphylaxis

Basophils and mast cells both express the high-affinity IgE receptor, Fc ϵ RI, a characteristic of the two lineages that is central to their function in allergic immunity. Crosslinking of Fc ϵ RI and IgE complexes with antigen triggers a rapid signal transduction cascade, resulting in the release of a large number of allergic mediators, including eicosanoids, vasoactive amines and peptides, and proteolytic enzymes, including granzyme B (Figure 1; Kawakami and Galli, 2002; Tschopp et al., 2006). Hypersensitivity reactions can be generally divided between immediate responses (which are typically IgE mediated and include syndromes such as allergy, asthma, and anaphylaxis)

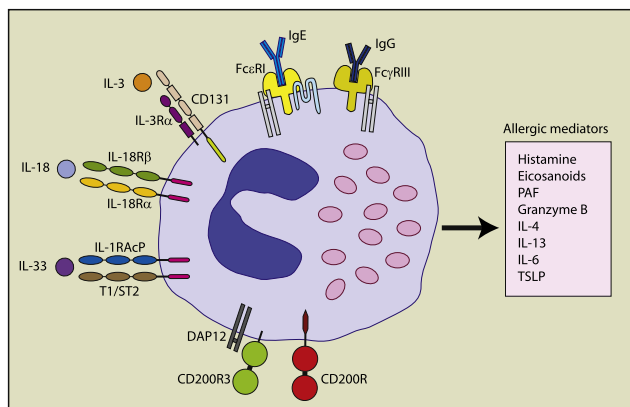


Figure 1. Extracellular Signals, Surface Receptors, and Mediators Central to Basophil Effector Function

Basophils respond to a number of extracellular stimuli, including immunoglobulins, cytokines, and ligands for the CD200-receptor family (left). Upon activation, basophils release effector molecules that contribute to Th2-associated immunity (right). IgE and IgG are bound to the surface of basophils through association with their respective Fc receptors and activate basophils after crosslinking by antigen. Basophils express receptors for the IL-1 cytokine family members IL-18 and IL-33, which induce basophil cytokine secretion. Two CD200-receptor homologs, CD200 and CD200R3, are expressed by basophils. CD200R3 ligation activates basophils through association with DAP12, whereas CD200R has been shown to inhibit IgE-mediated activation of basophils. Signaling through the IL-3 receptor, the cytokine IL-3 serves several roles in basophil function, including expansion, survival, and activation in the context of additional stimuli.

and delayed reactions (which are often CD4⁺ T cell dependent and include chronic conditions such as contact dermatitis).

Distinguishing the contributions between basophils and mast cells in IgE-mediated hypersensitivity reactions has centered on their disparate anatomical locations. Mast cells exit the bone marrow as immature precursors and terminally differentiate only after entering tissues, where they establish long-term residence. Mast cells have been long associated with local acute phase reactions that occur within minutes of antigen exposure. In contrast, basophils enter the circulation fully mature, but their recruitment to tissues is generally believed to be restricted to delayed late-phase allergic inflammation that occurs hours after antigen exposure (Galli et al., 2008). Indeed, in several model systems, optimal basophil accumulation in tissues was highly CD4⁺ T cell dependent (Min et al., 2004; Voehringer et al., 2004b).

Transgenic expression of IgE specific for 2,4,6-trinitrophenol (TNP) or intravenous administration of TNP-specific IgE in mice followed by a single dose of TNP antigen has been used to study the multiple stages of an IgE-Fc ϵ RI-dependent cutaneous allergic reaction (Sato et al., 2003). Ear swelling occurs within minutes of TNP exposure, termed the immediate phase, and this is followed by a milder reaction with swelling at 6–10 hr, termed the late phase. Finally, infiltration of inflammatory cells into the site days after antigen exposure results in further swelling and inflammation, termed the chronic phase. Although the immediate phase response was mast cell dependent, the chronic phase response was intact in mast cell-deficient, Kit^{W/W^v}, and combined T cell- and NK cell-deficient, Rag2^{-/-} × Il2rg^{-/-} mice. Basophil-enriched, DX5⁺ bone marrow cells were sufficient to restore chronic phase inflammation in otherwise

resistant *Fcgr^{-/-}* mice (Mukai et al., 2005). Karasuyama and colleagues generated a basophil-specific antibody, Ba103, by immunizing rats with basophil-enriched bone marrow from helminth-infected mice. A single injection of Ba103 depleted blood basophils for 10 days while having no effect on the numbers of peritoneal mast cells. Ba103-mediated basophil depletion suppressed the IgE-mediated chronic phase of inflammation in both wild-type and mast cell-deficient mice, but did not affect models of mast cell-mediated (type I) or T cell-dependent (type IV) hypersensitivity reactions (Obata et al., 2007). This study further suggested that basophils, which accounted for only 1%–2% of the skin-infiltrating cells, could orchestrate the recruitment and activation of inflammatory eosinophils and neutrophils, although a role for basophils as effector cells within the affected tissues was not expressly ruled out. Cytokine production by basophils, particularly IL-4 (see below), remains a likely mechanism through known effects in upregulating vascular adhesion molecules, such as VCAM-1, for leukocytes (Falcone et al., 2000).

Anaphylaxis has traditionally been ascribed to antigen and IgE-mediated crosslinking of FcεRI receptors on the surface of mast cells, and this remains accepted as the classical pathway. However, anaphylaxis also occurs in mast cell-, FcεRI-, and IgE-deficient mice. This alternative mechanism of anaphylaxis requires IgG, FcγRIII, macrophages, and platelet-activating factor (PAF) (Finkelman, 2007). As studied via two different antigen models of this pathway, depletion of basophils in both wild-type and mast cell-deficient mice abrogated IgG-mediated anaphylaxis, while having no effect on mast cell-dependent IgE-mediated anaphylaxis (Tsujiura et al., 2008). Further, basophils proved to be a potent source of PAF (Figure 1), and interestingly, macrophages, though critical in previous reports, were dispensable for the effects according to these models.

Immune Memory

Immunological memory is a hallmark of adaptive immunity. Memory responses are by nature considerably more accelerated and robust than are primary immune reactions after the initial exposure to antigen, resulting in part from contributions from long-lived antibody-secreting plasma cells and high-affinity antibody, memory B cells, and the expansion and maintenance of memory T cells (Kaeche et al., 2002). In an effort to address the relative impact of innate immune cells during secondary antigen challenge, two studies implicated basophils as an unsuspected component of immunological memory.

Immunization with goat anti-mouse IgD (GAMD) induced a robust Th2-dominated response characterized by elevated serum IgE. Upon secondary challenge, serum IL-4 peaked rapidly, within hours, to concentrations nearly 100-fold greater than those observed during the primary response (Khodoun et al., 2004). IL-4 reporter mice were used to show that the secondary response was dominated by greatly increased numbers of IL-4-competent eosinophils, Th2 cells, and basophils. Systematic investigation of the candidate populations by various methods demonstrated that basophils, through an IgE- and FcεRI-dependent mechanism, accounted for the initial IL-4 produced during the secondary response. Similar findings occurred in mast cell-deficient mice. The sustained production of IL-4, however, lasting for several days, required memory CD4⁺ T cells.

Antigen-specific B cells can be identified by their capacity to bind antigen by high-affinity surface immunoglobulin. The

precise identity of an “antigen-capturing” population devoid of the B cell lineage markers B220 and CD19 had been controversial (Bell and Gray, 2003; Driver et al., 2001), but a recent report presented evidence that basophils serve in this capacity by binding antigen to surface IgE-FcεRI (Mack et al., 2005). Indeed, immunization with a model antigen, allophycocyanin (APC), led to rapid, uniform uptake of APC on the surface of basophils upon secondary challenge. In vitro, these APC-labeled basophils secreted IL-4 and IL-6 when stimulated through the FcεRI or FcγRIII receptors (Figure 1). Further, mice depleted of basophils prior to antigen challenge had lower antibody titers. Protein vaccination failed to protect basophil-depleted mice from sepsis after *S. pneumoniae* infection, although survival was not substantially affected in the absence of basophils. Basophil cytokine secretion in response to antigen also improved CD4⁺ T cell-mediated B cell activation and proliferation in vitro (Denzel et al., 2008), perhaps providing some mechanistic insight to this process.

In summary, the antigen-capturing capacity of the immunoglobulin receptors on basophils has been expanded to include roles in chronic IgE-mediated skin reactions, in acute IgG-mediated anaphylaxis, and in antigen capture that can facilitate memory B cell responses. The mechanisms by which basophils enter systemic and lymphoid tissues in order to interact with the various target cells important to these responses, however, remain important areas for further definition.

Basophils as a Source of IL-4

In a seminal study, Paul and colleagues at the NIH described a splenic, non-B, non-T cell source of IL-4 that was apparent after crosslinking of the high-affinity FcεRI receptor on cells (Ben-Sasson et al., 1990). Further, T cell- and B cell-independent IL-4 secretion could be enhanced by IL-3 and the capacity to produce IL-4 increased substantially when assayed after the induction of Th2-associated allergic inflammation, such as in models of *Nippostrongylus brasiliensis* infection or immunization with goat anti-mouse IgD antibody (Conrad et al., 1990; Le Gros et al., 1990; Seder et al., 1991b). Purification of the responsible FcεR⁺ innate cells from the spleen and bone marrow yielded a population greatly enriched for basophils, as based on staining with alcian blue, histamine content, and their characteristic electron microscopic appearance (Seder et al., 1991a). Likewise, considerable data have confirmed the capacity of human basophils to secrete large amounts of IL-4 and IL-13 after FcεR1 crosslinking (Falcone et al., 2000).

Despite these early observations, the function of basophils in vivo remains poorly defined. The recent development of sensitive transgenic IL-4 reporter mice has forwarded the field considerably. While highlighting the potential importance of basophils as an innate, IL-4-competent population, cells from these mice provided a surrogate marker that has greatly facilitated the tracking, isolation, and description of the basophil lineage. Detailed analysis, combining both flow cytometry profiling and gene array technology, has resulted in a much more definitive characterization of basophils. As a result, murine basophils can now be reliably identified based on a distinct combination of features, including expression of CD49b (DX5), FcεRI, and a characteristic forward-side scatter profile that is slightly more granular than lymphocytes. Basophils can be distinguished from mast cells by the differential expression of c-kit, which is expressed

at low and high amounts, respectively, on the two cell types. Basophils are constitutively fluorescent in lines of IL-4 reporter mice with GFP to mark expression from the endogenous IL-4 gene by various means (Min et al., 2004; Voehringer et al., 2004b).

In summary, the discovery that basophils are a potent source of IL-4 induced in response to allergic and parasitic helminth infections could be explained, more than 10 years later, by the finding that basophils acquire the capacity to make IL-4 after stimulation during their development in the bone marrow. The precise mechanism by which this lineage achieves activation of the Th2-associated cytokine locus remains undefined, as does the functional role of IL-4 generated by basophils during systemic responses to allergens or helminthes.

Initiating Th2 Differentiation

For more than two decades CD4⁺ T helper cells have been categorized into specialized subsets, defined by their distinct cytokine secretion profiles (Mosmann et al., 1986). Among the number of factors associated with CD4⁺ T helper cell differentiation, the cytokines present during T cell receptor engagement and expression of the corresponding cytokine receptors have proven key determinants of T helper cell fate (Murphy et al., 2000). It has been long established that IL-4, the hallmark cytokine produced by Th2 cells, selectively drives the Th2 developmental program while inhibiting the opposing Th1 pathway, a process defined in cell-culture systems. Considerable in vitro data substantiate the importance of IL-4 and the signaling components, IL-4R α and STAT6, in this process, although the initial source of IL-4 and/or additional cues that influence Th2 polarization in vivo have remained elusive.

Recent work has suggested that IL-4 derived from basophils may play a role in the initiation of Th2 differentiation under certain circumstances. Basophils isolated from spleen, liver, or bone marrow supported Th2 development of naive TCR transgenic CD4⁺ T cells cultured in the presence of antigen and dendritic cells in an IL-4-dependent manner in vitro. Of interest, basophils purified from IL-3-cultured bone marrow progenitors were capable of promoting Th2 polarization, but mast cells sorted from the same IL-3-induced cultures were not. Exogenous IL-3, administered continuously through an implanted miniosmotic pump, promoted the expansion in vivo of DX5⁺, Fc ϵ RI⁺ basophils in *Rag2*^{-/-} mice. After adoptive transfer of TCR transgenic T cells, challenge with cognate peptide induced greater amounts of IL-4 in such basophil-expanded mice (Oh et al., 2007).

Spontaneous expansion of basophils was observed in mice deficient in the transcription factor interferon regulatory factor-2 (IRF-2) (Hida et al., 2005). CD4⁺ T cells isolated from *Irf2*^{-/-} mice exhibited enhanced IL-4 production after stimulation in vitro and elevated surface expression of a component of the IL-33 receptor, T1(ST2), consistent with a Th2-biased phenotype that had been acquired in vivo. Likewise, IL-4 secretion from naive TCR transgenic CD4⁺ T cells was substantially higher when stimulated in the presence of T cell-depleted splenocytes from *Irf2*^{-/-} mice as compared to wild-type splenocytes. Whereas basophil depletion, with antibodies against IgE and Fc ϵ RI, or IL-3 blockade inhibited the Th2 polarizing capacity of IRF-2-deficient splenocytes, basophil enrichment by positive selection of DX5-positive cells enhanced Th2 differentiation in an IL-4-dependent manner.

Despite these findings, several observations have questioned the in vivo relevance of IL-4 and STAT6 in Th2 development. Th2 cells and associated effector functions have been observed in STAT6-deficient mice (Mohrs et al., 2001; Shinkai et al., 2002; van Panhuys et al., 2008; Voehringer et al., 2004b). Immunization with allergy-provoking proteases, such as papain and bromelain, resulted in the acquisition of IL-4 competence by TCR transgenic CD4⁺ T cells, as assessed by the 4get IL-4 reporter strain (Sokol et al., 2008). Interestingly, basophils were observed in the draining lymph node 1 day prior to activation of CD4⁺ T cells in response to protease immunization. Papain stimulated IL-4 secretion by cultured basophils and also induced the expression of several allergy-associated genes, including thymic stromal lymphopoietin (TSLP) (Liu et al., 2007). Depletion of basophils by anti-Fc ϵ RI (MAR-1) treatment or antibody blockade of TSLP resulted in a diminished Th2 response to papain. This study highlights the possibility that basophil-derived TSLP, and potentially additional factors other than IL-4, may contribute to Th2 differentiation in vivo, at least to certain types of allergens.

Helminthes

Helminth infections represent a considerable burden on global health, with an estimated 2–3 billion people affected worldwide (Chan, 1997). The stereotyped host response against helminthes is associated with elevated serum IgE, mucus epithelial cell hyperplasia, and tissue inflammation dominated by Th2 cells, eosinophils, and basophils. The factors that drive basophil mobilization and tissue recruitment during infection and even their precise role in antihelminth immunity, however, have been elusive (Falcone et al., 2001).

N. brasiliensis parasites, a natural rat pathogen that has been adapted for mice, enter the bloodstream after subcutaneous inoculation and migrate to the lung within hours. In the lung, the third-stage larvae molt, traverse the alveoli (inducing a profound Th2-associated allergic reaction), are swallowed, and enter the small intestine, where they mature to adult egg-laying worms (Finkelman et al., 1997). Analysis of IL-4 reporter strains after infection with *Nippostrongylus* revealed that basophils are mobilized from the bone marrow into the peripheral blood and are recruited systemically to affected tissues, like the lung, but also to organs unaffected by the parasites, like the liver and spleen (Min et al., 2004; Voehringer et al., 2004b). Indeed, systemic basophilia was also observed after infection with *Heligmosomoides polygyrus*, a helminth that remains in the bowel throughout its life span (Mohrs et al., 2005). Intranasal administration of chitin, an abundant natural polymer found in helminthes, insects, and fungi, was capable of inducing rapid eosinophil and basophil recruitment to the lung of mice by a process dependent on LTB₄ elaborated by alternatively activated macrophages (Reese et al., 2007). To what extent chitin or proteases mediate basophil recruitment in response to allergens or organisms like fungi and nematodes, however, awaits further investigation.

The advent of various IL-4 reporter mice allowed substantial advances to be made in understanding the signals required for basophil entry into tissues in response to allergens or helminthes. Intriguingly, basophil recruitment was highly CD4⁺ T cell dependent but did not require IL-4 or STAT6 signaling (Min et al., 2004; Voehringer et al., 2004b). This is in contrast to eosinophils, which rely on a STAT6-dependent signal in tissues

to generate chemokines like eotaxins required for tissue entry. Unexpectedly, IL-4- and IL-13-deficient CD4⁺ T cells, which are unable to secrete these canonical Th2-associated cytokines, were nonetheless sufficient to restore tissue basophil numbers in T cell-deficient mice after infection with helminthes (Voehringer et al., 2004b). Further, according to the *Nippostrongylus* model, depletion of basophils by targeting Thy1 antigens was sufficient to impede normal worm clearance from the bowel in the setting of IL-4- and IL-13-deficient T cells, implicating basophils in primary intestinal immunity (Ohnmacht and Voehringer, 2008). Future studies will clearly be focused on not only elucidating the factors needed for basophil recruitment to affected tissues, but also in defining the contributions of Th2 effector cells independent of IL-4 and IL-13 that seem to be necessary for basophil activation, whether directly or indirectly.

The initial immune response against the trematode *Schistosoma mansoni* is Th1 associated in nature. After 5–6 weeks, adult females produce eggs that cross the vascular endothelium and lodge in organs, such as the liver and small intestine. Once deposited in tissues, the eggs elicit a robust Th2-dominated granulomatous response, which is dependent on IL-4 and IL-13 and which is associated with high IgE titers and eosinophilia (Pearce and MacDonald, 2002). Indeed, immunization with *S. mansoni* eggs or with soluble egg antigens (SEA) elicits a robust Th2-associated response (Vella and Pearce, 1992). Early work demonstrated substantial IL-4 production after *S. mansoni* infection by an innate FcεRI-positive population that responded to IL-3 and which, when isolated, included basophils and mast cells (Jankovic et al., 1997; Williams et al., 1993). SEA also induced IL-4 secretion by human basophils in vitro (Falcone et al., 1996). Interestingly, basophils from both sensitized and nonsensitized donors secreted IL-4 in response to SEA in an IgE-dependent manner. The IL-4-inducing component within the SEA preparations was identified as IPSE-α-1, a glycoprotein located in the subshell region of *S. mansoni* eggs, where it can be secreted (Haisch et al., 2001; Schramm et al., 2003). Intriguingly, IgE antibodies to IPSE-α-1 are present in serum from nonsensitized individuals. The nonspecific nature of the IgE antibody response was also demonstrated in mice, where animals infected with *H. polygyrus* to raise basophil numbers and serum IgE could be induced to release IL-4 from basophils in vitro and in vivo via SEA (Schramm et al., 2007). Consistent with the human basophil data, the response to SEA was dependent on IPSE-α-1 in the preparation and on basophil-bound IgE.

In summary, recent work has suggested that basophils may play a role in modulating Th2 cell differentiation in response to certain protease allergens and likely contribute to the host response to intestinal and migratory helminthes. Still left undetermined is whether either of these contributions is dependent on IL-4 (or the related cytokines expressed from the same locus) secreted by activated basophils or on some other functions attributed to these cells.

Non-Antibody-Mediated Mechanisms for Basophil Mobilization, Activation, and Effector Function

IL-3

Interleukin-3, particularly in combination with c-kit ligand or stem cell factor (SCF), supports the development of mast cells from bone marrow-derived progenitor cells (Kawakami and Galli,

2002). Several lines of evidence support a role for IL-3 as a basophil growth factor as well. When cultured in media enriched with IL-3 alone, bone marrow cells support the expansion of both mast cells and basophils in vitro (Dvorak et al., 1994). Over time, the combination of SCF with IL-3 inhibits basophil growth and selects exclusively for mast cells. However, even in IL-3, basophils do not survive beyond 2 weeks and cultures result in highly purified mast cells after about 4 weeks. Despite this, mice deficient in IL-3 had normal numbers of basophils and mast cells under resting conditions (Lantz et al., 1998). Thus, additional factors are likely working in concert with IL-3 to promote basophil differentiation in vivo.

Although unaffected at baseline, basophil and mast cell expansion was compromised in IL-3-deficient mice after infection with either *Strongyloides venezuelensis* or *N. brasiliensis* (Lantz et al., 1998, 2008; Shen et al., 2008). Basophil expansion in response to helminth infection depended on CD4⁺ T cells (Min et al., 2004; Voehringer et al., 2004b). Adoptive transfer of IL-3-deficient CD4⁺ T cells failed to restore basophilia entirely in *Rag2*^{−/−} mice after *N. brasiliensis* infection (Shen et al., 2008), thus implicating IL-3 from activated CD4⁺ T cells as necessary for optimal basophil mobilization and/or tissue survival after infection.

Mechanistically, in vitro data suggest that IL-3 protects basophils from apoptosis. Constitutive expression of the prosurvival factor bcl-2 was elevated in human basophils, thus perhaps prolonging basophil survival in vitro as compared to other short-lived granulocytes, like neutrophils or eosinophils, although the lifespan of the latter can be prolonged markedly with IL-5 (Rothenberg and Hogan, 2006). Without growth factors, basophils underwent spontaneous caspase-mediated apoptosis. IL-3, unlike several other cytokines, including IL-5 and GM-CSF, enhanced basophil survival by blocking caspase-3 cleavage of bcl-2 while also inducing expression of the serine-threonine kinase Pim1 (Didichenko et al., 2008). Ectopic expression of Pim1 blocked apoptosis in a kinase-dependent manner. Further, Pim1 protected basophils independently and in conjunction with the previously described PI3K pathway (Didichenko et al., 2008; Zheng et al., 2002). Activated CD4⁺ T cells enhanced basophil survival in vitro by an IL-3-dependent process. However, basophil apoptosis and proliferation in vivo were comparable in the presence of either wild-type or *Il3*^{−/−} CD4⁺ T cells after helminth infection (Shen et al., 2008). CD4⁺ T cell-derived IL-3 may be more critical for helminth-induced basophil development and expansion than for prolonging survival of basophils in peripheral tissues.

In short, IL-3 has been implicated in basophil survival and some of the mechanistic pathways have been defined. Complete understanding of the signals that mobilize, recruit, and sustain basophils in immunologically involved tissues, however, will be important areas for further study.

Activation of Basophils by IL-1 Family Members

The IL-1 cytokine family consists of several proinflammatory mediators, several of which, including IL-1β, IL-18, and IL-33, require caspase-1-mediated cleavage of the procytokine by the activated inflammasome to produce the mature cytokine (Arend et al., 2008). Although IL-18 was first cloned as IFN-γ-inducing factor and was initially defined as an amplifier of IFN-γ expression by Th1, CD8, and NK cells, IL-18 also enhanced Th2-type responses, depending on the context of the stimulus (Nakanishi

et al., 2001). Evidence now suggests that IL-18 activates basophil cytokine secretion. Basophils derived from IL-3-conditioned bone marrow cultures and from tissues of *N. brasiliensis*-infected mice express the IL-18R α chain (Voehringer et al., 2004b; Yoshimoto et al., 1999). Cultured basophils (Fc ϵ RI⁺, ckit⁺) produced substantially higher amounts of IL-4 and IL-13 in response to IL-18 and IL-3 as compared to mast cells (Fc ϵ RI⁺, ckit⁺) from the same cultures, despite comparable IL-18R α expression by both cell types. IL-18 administered during helminth infection enhanced Fc ϵ RI-mediated cytokine secretion by splenic non-B, non-T cells and induced IL-4 and histamine release independent of Fc ϵ RI ligation when given together with IL-3 (Yoshimoto et al., 1999). Exogenous IL-18 and IL-3 also elicited basophil cytokine secretion in mice previously infected with *H. polygyrus* to mobilize the basophil pool (Figure 1; Mohrs et al., 2005).

IL-33 was identified with an IL-1 sequence homology-based screen and shown to be the natural ligand of the T1(ST2) receptor expressed on differentiated Th2 cells and mast cells (Schmitz et al., 2005). Consistent with the expression profile of T1(ST2), IL-33 augmented cytokine production by polarized Th2 cells, analogous to IL-18-mediated enhancement of IFN- γ secretion by Th1 cells; IL-33 also stimulated histamine and cytokine release from mast cells (Allakhverdi et al., 2007; Ho et al., 2007; Schmitz et al., 2005). T1(ST2) expression was also demonstrated in mouse basophils via microarray analysis and in human basophils via polymerase chain reaction (Figure 1; Smithgall et al., 2008; Voehringer et al., 2004b). Further, both IL-3-conditioned bone marrow-derived basophils and freshly isolated human basophils secreted Th2-associated cytokines, including IL-4 and IL-13, after incubation with IL-33 (Smithgall et al., 2008; Suzukawa et al., 2008). Additionally, IL-33 enhanced human basophil adhesion, chemotaxis, and IgE-mediated degranulation (Suzukawa et al., 2008). However, basophil depletion was not sufficient to abrogate the IL-13-dependent goblet cell hyperplasia observed in *Rag2*^{-/-} mice after intranasal administration of recombinant IL-33 (Kondo et al., 2008), consistent with direct effects of IL-13 on epithelial cells in the airway. Additional studies are needed to address the importance of the IL-33 and T1(ST2) pathways on basophil function in vivo.

In summary, further study is needed to test the role of the caspase-1-dependent IL-1 family members in basophil activation in vivo. The recent associations of the inflammasome, the multi-molecular complex required for caspase-1 activation, in alum-mediated adjuvanticity suggests that a closer look at the role of basophils and basophil activation in contributing to the activity of certain adjuvants may be a productive line of inquiry.

CD200 Receptors

The CD200R family of membrane-bound glycoprotein receptors, expressed predominantly on myeloid cells, including mast cells and basophils, has been implicated in both positive and negative immune regulation (Barclay et al., 2002; Minas and Liversidge, 2006). Ligation of CD200R (Figure 1) inhibited Fc ϵ RI- and IgE-mediated activation of basophils and mast cells (Cherwinski et al., 2005; Shiratori et al., 2005) through tyrosine phosphorylation of the cytoplasmic tail of CD200R and recruitment of the inhibitory adaptor proteins Dok1 and Dok2 (Zhang et al., 2004; Zhang and Phillips, 2006). Microarray analysis revealed selective expression of the CD200R-like receptor CD200R3 on basophils as compared to eosinophils from helminth-infected mice (Voeh-

ringer et al., 2004a). CD200R3 paired with the ITAM-containing signaling adaptors DAP12 and DAP10. An effort to generate basophil-specific antibodies produced two antibodies that recognize CD200R3, Ba103 and Ba91, which both stained basophils and mast cells (Kojima et al., 2007). Analysis of DAP12-deficient mice confirmed a requisite association between CD200R3 and DAP12 (Figure 1). Further, ligation of CD200R3 by Ba103 or Ba91 induced degranulation and cytokine secretion independent of IgE. Taken together, the balance between activating and inhibitory signals generated by members of the CD200R family may prove to be important in modulating basophil function in tissues, analogous to the regulation of NK cell activity.

Conclusions

We have attempted to summarize recent work that implicates basophils as more than bystander cells in allergic and antihelminth immunity. Increasingly, nonredundant roles for these cells are being recognized, as in IgG-mediated anaphylactic-type responses, host recognition of certain classes of injurious proteases, and mucosal reactions to helminthes. The presence of these cells in tissues of patients with fatal asthma (Gibbs, 2005; Kepley et al., 2001; Koshino et al., 1993), as well as experiments in model systems (Min et al., 2004; Mukai et al., 2005; Voehringer et al., 2004b, 2006), suggests that basophils may also contribute to this widely prevalent human affliction in a similarly nonredundant way. Hopefully, these efforts mark the beginning of studies that increasingly define the lineage and effector potential of this largely overlooked granulocyte population.

Despite these findings, we still do not have a complete understanding of how these cells expand in bone marrow in response to intestinal or allergic challenges at mucosal surfaces, an understanding that would bring the possibility of manipulating these responses in positive or negative ways. Nor do we comprehend how helper T cells regulate the entrance and/or survival of mobilized basophils into systemic tissues. The mechanisms by which tissue basophils become activated to secrete cytokines and vasoactive mediators have likewise been incompletely defined. Although immunoglobulin crosslinking of Fc γ R and Fc ϵ R on the basophil surface are undoubtedly important in enabling these cells to achieve antigen specificity, it is becoming clear that basophils can be activated to secrete cytokines, such as IL-4, IL-5, and IL-13, by mechanisms independent of antibody. The regulation of tissue signals, such as TSLP, caspase-1-dependent IL-1 family members, and CD200R ligands, will be important areas for further investigation. The activation of cytokine secretion by activated basophils in affected tissues itself remains unproven (Figure 2).

How will the field move forward? To date, methods to track or eliminate basophils have been relatively indirect, because surface markers are not absolutely specific for the lineage and can be modulated unpredictably under various conditions, thus obscuring the identification of these cells. Although investigators have tried to take into account contributions by other cells, the use of various lineage-deficient mice always raises issues of unintended compensatory effects. The field needs a specific lineage marker for basophils, such that cell fate decisions, lifespan analysis, and trafficking patterns can be reliably quantitated. Various genetic markers have been noted in transcriptional analysis of basophils (Liu et al., 2006; Nakajima et al.,

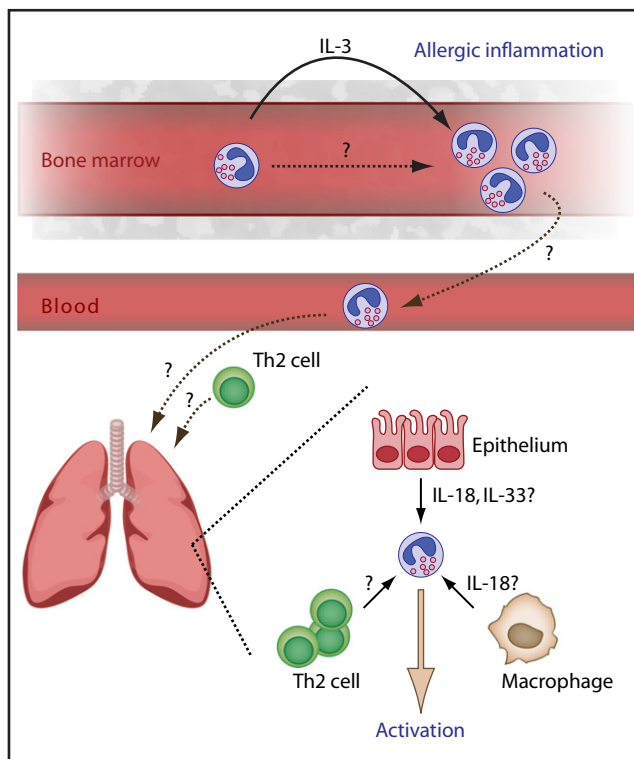


Figure 2. Fundamental Questions Surrounding Basophils in Allergic Immunity

Despite recent advances in our understanding of basophils, key questions remain. Although IL-3 is required for basophil expansion during allergic inflammation, the factors responsible for basophil development and maintenance under steady-state conditions are unknown. Further, the mechanisms by which basophils traffic from the bone marrow and from the circulation into peripheral tissues have not been identified. Although Stat6-dependent chemokines are required for the migration of Th2 cells (and eosinophils, not shown) into involved tissues (such as the lung and intestines), the mechanisms by which basophils enter tissues such as the lung, intestines, and liver (not shown) are Stat6 independent and remain undetermined. Finally, although the role of antigen-mediated immunoglobulin crosslinking in basophil activation has been well documented, the role for additional signals that activate basophils in tissues, such as cytokines, and the interplay of basophils with other cell types associated with allergic immunity, including the epithelium and Th2 cells, are poorly defined.

2004; Voehringer et al., 2004b), increasing the likelihood that lineage-specific marking of these cells will be possible.

What are the implications? If basophils truly perform nonredundant roles in allergic and helminth immunity, then mechanisms that target these cells may offer new strategies for disease control that have not been envisioned. For fields like asthma and atopic diseases, such strategies could bring welcome relief to huge numbers of patients.

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