Basophil Functions During Type 2 Inflammation: Initiators, Regulators and Effectors

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Abstract: Recent studies have identified several previously unrecognized functions of basophils in multiple models of Th2 cytokine-dependent immunity and inflammation. In addition to their established role as effector cells in inflamed tissues, findings now indicate that basophils express MHC class II and co-stimulatory molecules, can migrate into draining lymph nodes, present antigen to naive $CD4^+$ T cells and promote Th2 cell differentiation. In this context, basophils have been shown to be critically important for the induction and propagation of Th2 cytokine responses following exposure to helminth parasites and allergens. This article reviews recent conceptual advances in our understanding of basophil biology in the context of allergy and helminth infection.

Key Words: Basophil, Th2 cells, allergic inflammation.

INTRODUCTION

Basophils are the least abundant granulocyte population, making up less than 1% of all circulating leukocytes. Originally identified by the German scientist Paul Ehrlich in 1879, more than a century passed before basophils were shown to have the ability to bind IgE and produce histamine [1]. Despite these findings, basophils were considered to be a redundant population of cells that represented circulating or immature mast cells [2]. This prevailing dogma persisted until recent technical advances allowed researchers to study *in vivo* basophil biology in greater depth.

Significant conceptual advances in the functional biology of basophils were driven by technological developments and coincided with the generation of interleukin-4 (IL-4)/eGFP reporter mouse models. These tools facilitated the discovery that eosinophils, mast cells and basophils acquired constitutive IL-4/eGFP expression during their development [3-5]. These studies also allowed researchers to establish the expression of surface molecules on resting and activated basophils, which allowed them to be distinguished from tissue-resident mast cells. Shortly after a specific surface phenotype was established, methods of depleting basophil populations by targeting the high-affinity IgE receptor or the membrane glycoprotein CD200R3 were developed [6-9]. The ability to identify and deplete basophils *in vivo* allowed for a series of studies that defined basophils as non-redundant contributors that play critical roles in the induction, regulation and propagation of Th2 cytokine-mediated immune responses.

The purpose of this article is to highlight recent conceptual advances in understanding the functions of basophils. We describe the mediators of basophil activation and effector functions in response to both endogenous and exogenous signals. Further, we focus on recent reports demonstrating that basophils function as antigen presenting cells (APCs) that provide a critical link between innate and adaptive forms of Th2 cytokine-mediated immunity and inflammation.

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BASOPHIL EFFECTOR FUNCTIONS

Although recent studies have fundamentally altered the way we think about basophil biology, historically basophils are best known as effector cells that release pre-formed mediators in response to activation via surface bound IgE [1]. Circulating basophils bind IgE through the high affinity IgE receptor FcεRI and degranulate upon FcεRI crosslinking [10, 11]. Basophils activated via surface bound IgE produce histamines, leukotrienes, cytokines and chemokines [2]. However, more recent studies have demonstrated that basophils can be activated by an array of stimuli in both IgE- dependent and -independent manners. Further, basophils are capable of secreting a variety of cytokines including IL-4, IL-6, IL-13, tumor necrosis factor (TNF)α and thymic stromal lymphopoietin (TSLP) [2]. The following section will summarize our current knowledge of the stimuli that activate basophils and the resulting effector molecules they secrete.

IgE-, IgG- and IgD-mediated Activation of Basophils

As discussed above, basophils produce pre-formed mediators such as histamines and leukotrienes in response to FcεRI crosslinking via surface bound IgE. Thus, similar to tissue resident mast cells, basophils are capable of immediately responding to antigens found in the blood [12]. The ability of basophils to rapidly produce pre-formed mediators upon antigen exposure has implicated them as contributors to systemic anaphylaxis in humans [12]. In addition to IgE-mediated anaphylaxis, basophils have also been shown to contribute to a novel form of IgE-mediated chronic allergic inflammation in mice [8].

Although basophils are not essential players in the immediate- or late phase- responses that result after multivalent antigens are administered to mice via a subcutaneous injection into the ear, they are required for the chronic inflammation that follows [8]. More specifically, when basophils,

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which represented only 1-2% of the cellular infiltrate in the ear, were depleted, there was a dramatic reduction in the number of infiltrating eosinophils and neutrophils and a marked reduction in ear thickness [8]. These data suggest that lesion-resident basophils may either directly produce chemokines that recruit inflammatory granulocytes, or produce mediators that indirectly induce the production of chemokines from tissue resident cells. Further, these studies demonstrate the potency of small numbers of basophils and illustrate their ability to significantly influence inflammatory responses.

In addition to IgE-mediated activation, basophils can also be activated by binding IgG or IgD antibodies [7, 13]. For example, basophils produce platelet activating factor (PAF) during an IgG1-mediated alternative pathway of anaphylaxis [7]. Specifically, when C57BL/6 mice and mast cell deficient $Kit^{W\text{-}sh/W\text{-}sh}$ mice were sensitized with Penicillin V (Pen V) and subsequently challenged with Pen V-bovine serum albumin, both wild-type and mast cell-deficient mice developed systemic anaphylaxis. Depleting basophils prior to secondary challenge proved to be protective, implicating basophils as essential mediators of IgG1-mediated anaphylaxis in this model [7]. Further, basophils were also shown to be the dominant cell type that captured Pen V in an IgG1-dependent manner [7].

Human basophils are also capable of binding and being activated by IgD, a class of antibody expressed by mature B cells before class switching has occurred [13]. Although the functions of IgD remain unclear, it is highly expressed in the upper respiratory tract of humans and can bind the bacteria *Haemophilus influenzae* and *Moraxella catarrhalis* [13]. IgD-activated basophils produce a unique array of molecules including IL-4, B cell-activating factor, and a broad spectrum of antimicrobial peptides, but do not release histamine. Further, supernatants from IgD-activated basophils promoted B cell class switching to IgA and IgD and were able to prevent the replication of both *H. influenzae* and *M. catarrhalis* [13]. Collectively, these data suggest that IgD and basophils may be critical in providing protection against pathogens found in the human respiratory tract.

Cytokine Activation of Basophils: the Role of IL-3, IL-18 and IL-33

Although baseline levels of basophils are relatively low, basophil populations significantly expand following exposure to *Nippostrongylus brasiliensis* and *Strongyloides venezuelensis*, two models of murine hookworm infection [14]. The expansion of basophil populations in response to both *N. brasiliensis* and *S. venezuelensis* is IL-3-dependent, indicating that IL-3 is an important basophil growth factor [14]. The ability of IL-3 to expand basophil populations was further demonstrated by studies showing that IL-3 promotes the development of basophils *in vivo* and the differentiation of basophils from bone marrow (BM) precursors *in vitro* [15, 16]. Further, IL-3 can augment the basophil-specific secretion of IL-4 and IL-13 after IgE-mediated activation and is also necessary for the migration of basophils to the draining lymph nodes (LNs) following *N. brasiliensis* infection [17-19]. Collectively, these data have prompted the hypothesis that the development and activation of basophils is highly dependent on IL-3-IL-3R signaling. However, IL-3 is not necessary for the emergence of baseline numbers of basophils *in vivo*, suggesting that other cytokines or bioactive molecules may be capable of regulating basophil development [14, 15]. For example, a recent study suggests that basophil development may arise in the periphery from an IL-25-dependent multipotent cell population termed MPP^{type2} cells, although the role of IL-3 in this response has not been examined [20]. Further, the cytokines IL-18 and IL-33 have been shown to be capable of activating basophils, although their influence on basophil development has not been examined in detail [15, 21, 22].

IL-18 and IL-33 are members of the IL-1 cytokine family and contribute to Th2 cytokine production and Th2 cytokine-mediated inflammation [23-27]. Consistent with their connection to Th2 cytokine-mediated immunity and inflammation, IL-18 and IL-33 are both capable of directly activating or augmenting the activation of basophils. For example, murine BM-derived basophils produce more IL-4 and IL-13 when activated with IL-3 and IL-18 than when they are activated with IL-3 alone [25]. IL-18 is also capable of enhancing IL-4, histamine production and basophil survival *in vivo* [21, 25]. Similar to IL-18, IL-33 is also capable of enhancing IL-3-induced secretion of IL-4 and IL-13 from basophils [21]. Further, IL-33 alone can activate human basophils to produce IL-4, IL-5, IL-6 and IL-13 [28]. Collectively, these data demonstrate that cytokines other than IL-3 may be capable of regulating basophil development and activation.

Direct Activation of Basophils by Antigens

Although it is well documented that basophils can be activated through antibody- and cytokine-mediated pathways, recent data demonstrated that basophils can be directly activated by antigens. In particular, the house dust mite protease Der p 1 and protease antigens secreted by the hookworm parasite *Necator americanus* induce the production of IL-4, IL-5 and IL-13 from a human basophil cell line [29]. In addition, papain, a cystein protease allergen, induces the production of IL-4 and IL-6 from murine BM-derived basophils and also induces the migration of basophils to the draining LN when administered subcutaneously [30]. Critically, the activation of basophils by proteases can occur in the absence of antigen-specific IgE, but requires the protease to be functionally active, suggesting that basophils express receptors that are capable of sensing protease activity [30]. Protease activated receptors (PARs) are a family of receptors that recognize protease activity and have been shown to be expressed on other innate and adaptive immune cells, however there is no evidence to date that human or murine basophils express PARs [31]. Collectively these studies suggest that basophils may express PAR-like receptors and/or possess other innate mechanisms by which they recognize protease antigens.

Monitoring Basophil Activation

As mentioned above, basophils can be activated by a wide range of endogenous and exogenous signals. The following section will summarize the surface marker phenotypes that are known to be associated with basophil activation.

Activated basophils accumulate in peripheral tissues during late-phase and chronic allergic responses as well as in response to parasitic infections [3, 4, 8]. The ability to identify activated basophils in various tissue microenvironments by flow cytometry has proven to be an invaluable tool. Several surface markers have now been shown to be upregulated by human and murine basophils in response to various stimuli. Analysis of human basophils identified that the expression of CD63, a member of the transmembrane 4 superfamily, is elevated on basophils that are activated in response to allergens including pollens and venom [32, 33]. CD63 is anchored in the basophil granule membrane and upon activation and degranulation gains access to the cell surface [34, 35]. Although CD63 is upregulated on basophils after activation, its level of expression can vary and it was not significantly upregulated on basophils isolated from individuals with drug allergies [36]. In addition, the high expression of CD63 on activated platelets that are capable of adhering to basophils, further complicates its use as an activation marker during flow cytometry studies [37]. Therefore, although useful, CD63 is not considered the most sensitive or accurate marker of basophil activation [36].

Another human basophil marker, the type II-transmembrane protein CD203c has been shown to be upregulated in response to FcεRI crosslinking [38]. CD203c shows higher expression levels after basophil activation than CD63 and is upregulated rapidly after allergen challenge. Thus, CD203c is considered a reliable marker that is differentially expressed on resting and activated basophils [38, 39]. Whether co-expression of CD63 and CD203c can be used in combination to provide a more comprehensive panel of activation markers remains to be tested [36].

Perhaps the most promising activation marker on human basophils is the disulfide-linked homodimer CD69. Human basophils exhibit elevated expression of CD69 in response to IL-3 and to a lesser extent in response to antigen stimulation [39-41]. Thus, the induction of CD69 in response to cytokines and antigens suggests it may be a reliable activation marker. Furthermore, CD69 appears to be an activation marker expressed on murine basophils. For example, CD69 expression is elevated on activated basophils in the lung after infection with *N. brasiliensis* [4]. Although CD69 shows promise as a murine basophil activation marker, further studies are necessary to determine its reliability. Few studies have focused on identifying activation markers for murine basophils. In fact, CD200R, an inhibitory receptor that belongs to the immunoglobulin superfamily, is the only recognized activation marker for murine basophils. CD200R is transiently upregulated in response to both IgE-dependent and –independent stimuli [42]. However, the transient nature of CD200R upregulation may make it difficult to use when tracking basophil activation *in vivo* during allergic inflammation and in response to helminth infections.

Antigen Presenting Cell Functions of Basophils: a New Link Between Innate and Adaptive Immunity

It has long been appreciated that basophils are potent producers of the Th2 cytokines IL-4 and IL-13 and that they accumulate at sites of inflammation after exposure to allergens or helminth parasites. However, recent evidence now suggests that basophils serve as liaisons between innate and the adaptive immune system. It is now known that IL-4 producing basophils express major histocompatibility complex (MHC) class II, co-stimulatory molecules and migrate to draining LNs where they come in contact with naïve $CD4^+$ T cell populations [30, 43-45]. Further, basophils are capable of capturing antigen in an IgE-dependent manner and can directly induce $CD4^+$ T cell proliferation and Th2 cytokine production [45]. The following section will summarize these findings and highlight the role of basophils as a link between innate and adaptive forms of Th2 cytokine-dependent immunity and inflammation.

Basophil-derived IL-4 promotes Th2 Cell Responses

The ability of DCs to promote Th1 cell differentiation during viral, bacterial or protozoan infections via their activation through pattern recognition receptors (PRRs), up regulation of co-stimulatory molecules and production of IL-12 and other proinflammatory cytokines is well-documented [46-48]. However, the inability of DCs to produce IL-4 has provoked questions regarding how DC populations are sufficient to induce and sustain $CD4^+$ Th2 cell differentiation [47-49]. These questions formed the basis for a hypothesis that an innate cell population capable of producing IL-4 may be required to initiate and/or maintain optimal Th2 cell differentiation [49]. Several innate cell populations can produce IL-4, including NKT cells, eosinophils, mast cells and basophils, and thus are capable of providing a link between innate and adaptive Th2 cytokine responses [49].

There is strong evidence from previous studies that directly implicates basophils as a likely candidate cell population capable of initiating Th2 cell development. For example, basophils isolated from the spleen, liver and BM are capable of promoting IL-4-dependent Th2 cell development when added to cultures containing naïve T cells, DCs and antigen [50, 51]. Further, interferon regulatory factor (IRF) $2^{-/-}$ mice, which exhibit substantially increased basophil populations in the periphery, were shown to have enhanced steady state levels of $CD4^+$ Th2 cells [50]. *In vitro* studies demonstrated that basophils were the dominant population of IL-4 producing cells in the spleen of $IRF2^{-/-}$ mice capable of initiating Th2 cytokine production from $CD4^+$ T cells [50]. Collectively, these studies illustrated the potential of basophils to promote Th2 cytokine-dependent immune responses.

Basophil Migration Into Lymph Nodes

Recent studies demonstrated that IL-4/eGFP$^+$, MHC class II^+ basophils migrate to the draining LNs following exposure to papain, *Schistosoma mansoni* eggs and *N. brasiliensis* infection [19, 30, 44]. Although basophils are only transiently present in the LNs, these data suggest that basophils are capable of directly interacting with LN-resident $CD4^+$ T cells, B cells and DC populations post-challenge. Critically, it was also shown that depleting basophil populations prior to papain challenge prevented the accumulation of IL-4/eGFP expressing $CD4^+$ T cells, suggesting an essential role for basophils in the induction of papain-induced Th2 cell differentiation [30].

Although the mechanisms by which basophils enter LNs and their importance in inducing $CD4^+$ T cell differentiation remain unknown, a recent study suggests that IL-3 may be a critical regulator of basophil LN recruitment. Infection with

N. brasiliensis induced the accumulation of basophils to the mediastinal LNs on days 4 and 10 post-infection [19]. Interestingly, basophils failed to accumulate in the LNs in the absence of IL-3-IL-3R signaling. In addition, the loss of basophils in the LNs of IL-3$^{-/-}$ mice did not prevent the induction of Th2 cell differentiation, suggesting that in the specific case of *N. brasiliensis*, basophils are not essential for the induction of Th2 cytokine-mediated immunity [19]. The basophil-independent nature of Th2 cell differentiation in this model is perhaps not surprising considering that multiple redundant mechanisms for Th2 cell development are initiated post- *N. brasiliensis* infection. For example, it has been shown that Th2 cell development post-*N. brasiliensis* infection can also occur in the absence of STAT6 or the combined absence of IL-5, IL-9 and IL-13 signaling [52, 53]. These findings also highlight that the potential contribution of basophils to local IL-4 production or antigen presentation may depend on the nature of the stimulus, the strength of signal and the route of antigen or allergen exposure.

APC Functions of Basophils

In a papain allergen model, an OVA/Alum allergic inflammation model and following *Trichuris* infection, it has been demonstrated that restricting MHC class II expression to DC populations is insufficient to induce optimal Th2 cell differentiation *in vivo* [43-54]. Further, depleting basophil populations prior to papain challenge and *Trichuris* infection significantly reduced the magnitude of Th2 cytokine responses, suggesting that basophils could be critical participants in the induction of optimal Th2 cytokine responses *in vivo* [43, 44]. These observations were further strengthened by gain of function studies illustrating that the transfer of antigen-loaded MHC class II^{+} basophils into CIITA$^{-/-}$ mice, which have deficiencies in MHC class II expression, was sufficient to induce papain-specific Th2 cell differentiation [43]. In addition, the adoptive transfer of IL-4/eGFP^{+} MHC class II^{+} basophils was capable of augmenting *S. mansoni* egg-induced Th2 cell differentiation *in vivo* [44]. The function of basophils as APCs was further demonstrated by *in vitro* studies demonstrating that basophils are capable of taking up antigens in an FcεRI-dependent manner and could present them via MHC class II [45]. Further, three independent studies demonstrated that basophils can induce OVA-specific CD4^{+} T cell proliferation and Th2 cytokine production in the absence of other APC populations *in vitro* [43-45].

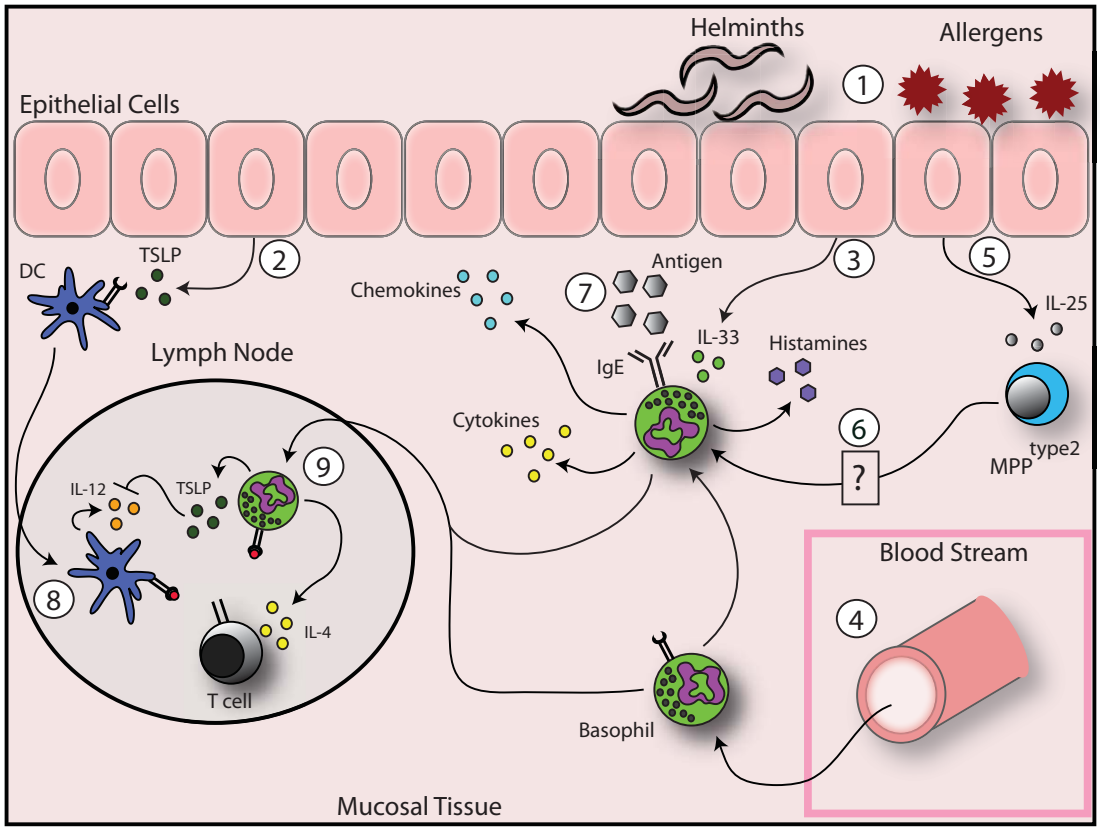

Fig. (1). Basophil activation, migration and effector function. Following exposure to helminth parasites or allergens at barrier surfaces❶, the epithelial derived cytokines TSLP, IL-33 and IL-25 are produced. TSLP can act directly on tissue-resident DC populations to prevent their upregulation of costimulatory molecules and IL-12/23p40 production, creating an environment permissive for Th2 cell differentiation ❷. IL-33 can directly activate basophil populations ❸ that have been recruited to the site of challenge from the blood stream ❹, while IL-25 has been shown to elicit a multipotent progenitor cell population termed MPP^{type2}❺. MPP^{type2} cells have the capacity to differentiate into basophils and may contribute to the expansion of basophil populations at the site of challenge ❻. Basophils can also be activated by antigen via surface bound IgE ❼ and are capable of producing effector molecules including histamines, cytokines and chemokines. MHC class II expressing DCs ❽and basophils ❾ can migrate to the draining lymph node where they come in contact with naive CD4^{+} T cell populations. Once in the LN, basophil-derived TSLP and IL-4 can limit the production of IL-12/23p40 by DC populations and can promote the differentiation of CD4^{+} T cells. Antigen presentation by both DCs and basophils may act cooperatively to expand Th2 cell populations.

Collectively, these studies demonstrated that basophils express MHC class II and co-stimulatory molecules, endocytose antigen and can promote $CD4^+$ T cell proliferation and Th2 cell differentiation both *in vitro* and *in vivo*. These data also support the hypothesis that basophils provide a link between innate and adaptive forms of Th2 cytokine responses and significantly advance our understanding of how Th2 cytokine-dependent immunity and inflammation are initiated. These data suggest that some basophil populations may share functional characteristics with DCs, expressing MHC class II and costimulatory molecules, endocytosing soluble antigens, migrating to draining LNs and producing cytokines that can promote the initiation and propagation of Th2 cells (Fig. **1**. ❹,❼,❾).

The ability of basophils to function as APCs in the absence of DCs does not preclude the possibility that DCs and basophils may function in concert to initiate Th2 cell development and propagation. There are several potential pathways through which basophils and DCs may act cooperatively to induce Th2 cytokines responses. Basophils may act as IL-4 producing accessory cells that promote the differentiation of T cells previously activated by DCs. Alternatively, basophils and DCs may present antigens to T cells in parallel (Fig. **1**❽,❾). In this scenario, basophils could augment antigen presentation, increase T cell proliferation and provide the IL-4 necessary for optimal Th2 cell differentiation. In the context of this model, basophils may further enhance Th2 cell development by producing TSLP that inhibits the ability of DCs to produce IL-12/23p40 and upregulate costimulatory molecules (Fig. **1**❾). Further studies will be required to directly assess the relative contributions of basophils and DCs in promoting Th2 cytokine responses in models of allergy and helminth infection. Understanding the mechanisms through which basophils cooperatively interact with DCs and other professional antigen presenting cells may provide new targets to limit or promote the development of Th2 cell-dependent immune responses.

SUMMARY AND FUTURE DIRECTIONS

Although relatively ignored for almost a century, recent studies have fundamentally altered our understanding of the functional biology of basophils. It is now appreciated that basophils can be activated by an array of signals including those mediated by cytokines, antibodies and directly by antigens themselves. Once activated, MHC class II^+ basophils can migrate into LNs, present antigen to naïve $CD4^+$ T cells and induce Th2 cell differentiation. The ability of basophils to function as APCs and their emerging central role in initiating and regulating Th2 cytokine responses suggest that further exploring the factors that regulate basophil development and function may lead to therapeutic strategies for the treatment of Th2-cytokine induced inflammation.

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