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Emerging functions of basophils in protective and allergic immune responses

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Basophils that were long thought to have a redundant role in mast cells in the effector response to allergens and parasites are now being recognized to have important roles in the regulation of adaptive immune responses. Recent data have revealed their role in the initiation of the T helper cell 2 (Th2)-mediated immune response. Not only do basophils guide the Th1–Th2 balance by providing an early source of crucial Th2-skewing cytokines, interleukin (IL)-4 and thymic stromal lymphopoietin, but recent findings have also illustrated their capacity to function as antigen-presenting cells. Thus, basophils activate and instruct naive CD4 T cells, and guide their development into Th2 cells. Not only do basophils directly interact with T cells, but new insights have illustrated that they may also directly guide antibody responses in both the primary and memory responses. These and other studies have illustrated the emerging role of basophils in the regulation of type 2 immunity.

INTRODUCTION

Basophils were first described by Paul Ehrlich in 1879, who named them after their avid staining with basophilic dyes.[1] However, despite their early discovery, they remained an enigmatic cell, studied more as a surrogate for the tissue resident mast cell than as a unique contributor to the immune response. Thus, throughout much of the twentieth century, their function remained unstudied and underappreciated, with common theories identifying them as either circulating mast cells or mast cell precursors. This confusion was aided by the "absence" of basophils in the most common experimental animal model, the mouse. Not until the early 1980s, over 100 years after their discovery in humans, were basophils finally discovered in mice.[2] But basophil research remained fettered by their scarcity and despite the accumulation of *in vivo* and *in vitro* research identifying specific capacities of basophils, it was not until this decade that the necessary, non-redundant role of basophils has begun to be appreciated. Now a rapidly developing area of research, the field owes much to earlier work defining the immunophenotype of human and mouse basophils. However, it was not until the production of mouse models linking interleukin (IL)-4 production to reporter expression and the incidental observation that basophils constitutively expressed these IL-4 reporters that identification of basophils became easy and straightforward.[3,4] These incidental but essential observations paved the way for the recent renaissance in basophil research.

Basophils make up $<1\%$ of the peripheral blood leukocytes in mice and humans.[5] In both species, basophils can be identified by their high surface expression of FcεRI, immunoglobulin E (IgE), CD49b, and IL-3R.[5,6] They can also be functionally identified by their rapid production of cytokines, such as IL-4 and IL-13, and release of histamine and leukotriene C4 after activation of FceRI by IgE crosslinking.[5,7] Under homeostatic conditions, mature basophils can be found circulating in the peripheral blood as well as in highly vascularized organs such as the liver, spleen, and bone marrow. As mature cells, studies in mice indicate that they have an estimated lifespan of only 60 h and the basophilia observed in response to a type 2 immune response is thought to be secondary to increased hematopoetic production.[8]

Although this review will focus upon the role of basophils as initiators and regulators of the adaptive immune response, they are better known as effector cells of the type 2 immune response. As part of this role in the effector response, basophils avidly bind circulating IgE through the high-affinity IgE receptor, FcεRI. FcεRI is highly expressed in basophils and its expression level

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is directly correlated with and regulated by levels of circulating IgE.[9–11] High levels of IgE can thus be accommodated by mast cells and basophils and IgE of one specificity is not "diluted out" by the addition of IgE of another specificity. Classical activation of basophils and mast cells occurs when antigen binds and crosslinks FcεRI-bound IgE. This activation leads to a Syk-mediated signaling cascade and ultimately the production of cytokines and chemokines and the release of preformed factors through a process called degranulation.[12] FcεRI-activated basophils classically release high levels of IL-4 and IL-13, along with chemokines involved in mediating lymphocyte and eosinophil chemotaxis.[5] Degranulation of basophils and mast cells leads to the characteristic symptoms of the allergic response, from sneezing and rhinorrhea in the upper respiratory tract to angioedema and pruritis of the skin. These effects are because of, among others, release of vasoactive amines and eicosanoids such as histamine and leukotriene C4, respectively.[5] These effects are meant to target the evolutionarily relevant pathogens of type 2 immunity, multicellular helminth parasites, that cannot be targeted by the type 1 effector mechanisms of phagocytosis and cell-mediated cytotoxicity. Instead, the classical type 2 effector response mediated by basophils focuses on pathogen expulsion.

Anaphylaxis, the extreme and immunopathologic form of type 2 immunity, is the result of overwhelming release of vasoactive amines and eicosanoids, leading to potentially deadly vascular permeability. Both mast cells and basophils release such factors after IgE crosslinking; however, whether FcεRI-mediated basophil degranulation can lead to anaphylaxis remains unclear. Obata *et al.*[13] showed that depletion of basophils did not affect the development of anaphylaxis in mice "sensitized" through injection of antigen-specific IgE and then immunized intravenously with specific antigen. When the passively sensitized mice were instead challenged subcutaneously with antigen, they developed chronic skin inflammation that was dependent on basophils. Interestingly, the skin infiltrate was composed primarily of eosinophils and neutrophils, leading the researchers to speculate that basophils may exert an effect as "initiators" of the effector response by promoting cell entry to the site of inflammation.[13] However, basophils have been implicated in another model of anaphylaxis. Using a similar model of passive sensitization, mice were given either antigen-specific IgG1 followed by immunization with the corresponding antigen or preformed immune complexes that induced systemic anaphylaxis, as measured by reduction in body temperature. This response was wholly dependent on basophils, was lost when basophils were depleted, and was found to be specifically the result of platelet-activating factor release from basophils.[14] Interestingly, mast cells had no role in IgG-mediated anaphylaxis, setting up two non-overlapping routes to systemic anaphylaxis, in IgE-induced anaphylaxis mediated by mast cells, and in IgG-induced anaphylaxis mediated by basophils.[13,14]

MODES OF BASOPHIL ACTIVATION

Basophil activation can be roughly separated into antibody-mediated activation and direct activation. Although recent studies have increased awareness and interest in direct activation of basophils by pathogen-associated molecular patterns, parasite products, and allergens, most of what is known about basophil activation is based on activation by antibody crosslinking. Basophils bind IgE through FcεRI, IgG through FcγRIII and, at least in humans, IgD through an undescribed receptor.[5,15] Although basophils are not directly activated by antibody binding to its Fc receptor, they are quickly and robustly activated upon antibody crosslinking in the presence of its cognate antigen. In the case of IgE, crosslinking leads to the stereotypical basophil effector cell response characterized by degranulation and release of allergic effector molecules.[12] As discussed above, IgG1 crosslinking can also lead to a similar effector cell response that is capable of inducing systemic anaphylaxis.[14] New data also indicate that human basophils can bind IgD and are activated by IgD crosslinking to release antimicrobial peptides and proinflammatory stimuli.[15] Chen *et al.*[15] showed that IgD-activated basophils can inhibit the growth of both *Haemophilus influenzae* and *Moraxella catarrhalis*, exposing a novel role for basophils in the effector response to bacteria. Interestingly, IgD crosslinking also leads to cytokine release and upregulation of cell surface ligands involved in antibody production that will be discussed in detail later.

Human basophils express mRNAs for Toll-like receptor (TLR)2, TLR4, TLR9, and TLR10.[16,17] Although the full spectrum of TLR-mediated basophil activation is unknown, it has been shown that human basophils activated by peptidoglycan release IL-4 and moderate amounts of IL-13.[17,18] This IL-13 production is augmented in "allergic" individuals after peptidoglycan stimulation.[18] Unfortunately, little is known about the role of lipopolysaccharide (LPS) stimulation apart from the fact that human basophils must be pre-treated with interferon-γ to respond to LPS.[16] Little is known about murine basophils. Unpublished observations have indicated that they express TLR1, TLR2, TLR4, and TLR6, and produce IL-4, IL-6, and IL-13 after stimulation with peptidoglycan or LPS in the presence of IL-3.[19] However, under similar culture conditions, we found murine basophils to produce only IL-6 and tumor necrosis factor in response to LPS stimulation, but not IL-4 or IL-13.[20] Basophils clearly express and respond to TLR ligands, but future studies will need to clarify the full spectrum of TLR-induced responses in basophils and how it differs from TLR-induced responses in macrophage and dendritic cells.

There is a growing consensus that basophils can be directly activated by T helper cell 2 (Th2)-inducing agents in the absence of antigen-specific IgE. Some of these mechanisms use the presence of IgE, but in an antigen-independent manner. For example, the IPSE/α-1 secretory glycoprotein from Schistosome mansoni eggs has been shown to directly activate basophils from unsensitized humans and mice.[21,22] This occurs by IPSE/alpha-1 binding to FcεRI-bound IgE on the surface of basophils, leading to antigen-independent IgE crosslinking. The HIV viral envelope glycoprotein gp120 similarly exerts an effect, activating basophils by binding to, and thus crosslinking, the VH3 portion of IgE bound through the FcεRI.[23] The significance of this interaction is unknown, although it may be used to enhance

viral entry through IL-4-mediated upregulation of CXCR4 receptors on T cells.[23] In addition to glycoproteins, proteases derived from parasites and allergens have been shown to directly activate basophils.[20,24,25] The dust mite protease, Der p 1, as well as the proteolytically active fraction of the excretory–secretory products of *Necator americanus* induce IL-4, IL-5, and IL-13 production in the KU812 basophilic cell line.[25] The cysteine protease allergen papain also directly activates basophils to produce IL-2, IL-4, IL-6, and IL-13 in the absence of IgE.[20] In addition, papain stimulation induces upregulation of surface major histocompatibility complex (MHC) class II and costimulatory molecule expression, allowing basophils to function as antigen-presenting cells.[26] This will be discussed in further detail below, but it is important to note that these responses occur in the absence of degranulation.[20] Thus, unlike IgE crosslinking that promotes an effector phenotype, papain exposure (and likely exposure to other cysteine proteases) induces a distinct initiator phenotype in basophils. The target of papain remains unknown, but experiments showing that basophils can be directly activated by papain in serum-free conditions make it likely that the target molecule is a component of basophils, as opposed to a secreted factor.[20] Protease-activated receptors are reasonable candidates and murine basophils express protease-activated receptors1 and 3, although stimulation with its agonist peptides does not lead to basophil activation and these antagonists do not inhibit papain-mediated activation of basophils (Sokol and Medzhitov). Thus, the sensor of protease activity remains unknown. It is likely that the proteases and glycoproteins examined thus far only constitute a fraction of the possible direct activators of basophil function. For instance, it was recently shown that chitin can induce a type 2 immune response in naive mice.[27] Considering the significant toll of allergy and asthma, more research into the nature of activators of type 2 immunity is needed.

BASOPHILS AS INDUCERS OF Th2 DIFFERENTIATION

Because of the role of IgE crosslinking in basophil activation, there has long been a keen understanding of the importance of B cells in basophil function. A role for T cells directly affecting basophil function was only recently described. In a mouse model of *Nippostrongylus brasiliensis* infection, $CD4^+$ Th2 cells were shown to be necessary for recruitment of basophils to effector sites.[28] First described as degranulated eosinophils and then later identified as basophils, their recruitment to the effector sites was lost in T-cell-deficient mice.[28,29] When antigen-specific $CD4^+$ T cells were transferred into a T-cell-deficient host, basophil recruitment to sites of the effector response was restored.[28] These important insights illustrated that similar to type 1 response, T helper cells coordinate the effector response to type 2 pathogens. However, not only did $CD4^+$ T cells effect basophils and eosinophils, but IL-4 production from an undetermined innate immune cell was shown to be essential for the differentiation of functional Th2 cells.[29] This observation underlined two major questions in type 2 immunity of what innate immune cells instruct Th2 differentiation and what is the cellular source of IL-4.

Th1 differentiation from Th_0 cells requires both cell surface-associated signals and secreted cytokines, all delivered by dendritic cells, to both activate naive T cells and to induce their differentiation into Th1 cells. In the case of Th2 differentiation, however, the situation seems to be more complex, with multiple pathways contributing to Th2 differentiation. In contrast to IL-12 and other Th1-instructing cytokines, dendritic cells do not produce IL-4. Nevertheless, Lambrecht *et al.*[30] and others[31] have clearly shown in several models of Th2 differentiation that dendritic cells have an essential role in both inductive and effector phases. These dendritic cell-mediated Th2 responses may use multiple pathways: the expression of notch ligands on dendritic cells can directly instruct Th2 differentiation,[32] thymic stromal lymphopoietin produced by epithelial cells activates dendritic cells to induce Th2 differentiation,[33,34] and OX40 ligand-dependent induction of Th2 responses is also mediated by dendritic cells.[35] On the other hand, IL-4 production is also essential for Th2 differentiation,[36] although the relevant source of IL-4 has been unclear. One possible source of this IL-4 is from Th2-differentiating cells themselves.[37] However, Voehringer *et al.*[29] showed in an *in vivo* infection model using *N. brasiliensis* that when IL-4 secretion is limited to T cells that Th2 cells can differentiate in the lymph nodes, they do not become functional effector Th2 cells; that is, they do not migrate to the site of infection and are thus incapable of coordinating a functional effector response as measured by influx of effector cells and worm expulsion. Thus, IL-4 production from an innate immune cell is clearly important for Th2 differentiation, but the specific cellular source remained unclear.

Basophils are capable of quick production and secretion of IL-4, making them a possible candidate for the innate source of IL-4 necessary for Th2 differentiation, at least in some settings. An important clue to their role was provided by a study analyzing $IRF2^{-/-}$ mice. These mice have increased numbers of basophils and Th2 cells under steady-state conditions.[38] Interestingly, Hida *et al.*[38] showed that if the number of basophils was decreased by crossing these mice to cKit-mutant mice, the spontaneous Th2 polarization was lost. Basophilia and steady-state Th2 polarization has also been described in Lyn-deficient mice.[39] In this model, Th2 polarization was shown to be dependent not only on IL-4, but also on IgE despite the antigen-independent response.[39] However, was this spontaneous Th2 polarization due to specific effects by basophils or just increased environmental IL-4? Evidence for the specific role of basophils in instructing Th2 differentiation was provided in the model system of papain-induced Th2 differentiation, in which subcutaneous papain immunization (without any additional adjuvants) leads to Th2 differentiation in the draining lymph node at 4 days after immunization. Unexpectedly, basophils entered the draining lymph node immediately before Th2 differentiation, at 3 days after immunization. Although they were largely undetectable by 4 days after immunization, their transient presence in the T-cell zone of the draining lymph node was necessary for Th2 differentiation. Depletion of basophils, but not mast cells, using the MAR-1 antibody to FcεRI led to the loss of Th2 differentiation. Thus, basophils were required for Th2 differentiation.

Because basophils produced IL-4 after papain stimulation, this observation added to the mounting evidence that basophils were not just type 2 effector cells, but were also cells essential for the initiation of Th2-mediated immunity. Interestingly, basophils not only produced IL-4, but also thymic stromal lymphopoietin, which was shown through antibody neutralization to have a partial role in Th2 differentiation in response to papain immunization. Basophil migration into the draining lymph node was also induced by another plant-derived protease allergen, bromelain, as well as by the dust mite protease Der p 1 (Sokol and Medzhitov) and *Schistosoma mansoni* soluble egg antigen. Thus, basophil induction of Th2 differentiation is a common feature shared by allergens and parasite products.

The influence of basophils on T-cell differentiation is not limited to CD4+ T cells. As was recently shown by Kim *et al.*,[40] basophils may have a role in CD8 T-cell differentiation. Using an *in vitro* system, they illustrated that basophils can produce soluble factors that lead to preferential differentiation of CD8+ T cells into IL-10-producing cells. As provision of exogenous IL-4 and IL-6 to cultures induces the differentiation of IL-10-producing CD8+ T cells, basophil production of these cytokines was implicated as instructors of this CD8+ T-cell differentiation.[40] However, the physiological significance of both this pathway and IL-10-producing CD8+ T cells remain to be determined *in vivo*.

Basophils and basophil-derived cytokines are necessary for Th2 differentiation in response to immunization with protease allergens, but how is cytokine production controlled in basophils? As previously discussed, many parasite products and allergens can directly activate basophils in an IgE-independent manner. However, one common element between these studies is the use of IL-3 in the culture medium. IL-3 is an important promoter of basophil development *in vitro* and *in vivo*. Murine basophil development can be promoted *in vitro* by culturing bone marrow progenitors in the presence of IL-3.[41] *In vivo*, basophilia can be induced by IL-3 treatment of mice and parasite-induced basophilia is due to increased endogenous production of IL-3.[42,43] Interestingly, IL-3 does not seem to be necessary for basophil differentiation under homeostatic conditions, as IL-3-deficient mice have basophils but are not able to mount basophilia in response to parasite infection.[43] However, the effects of IL-3 are not simply limited to basophil development. IL-3 stimulation can directly induce moderate IL-4 production in resting basophils.[6] In addition, IL-3 has been described to have an important priming role in antibody-dependent basophil activation and cytokine production.[44,45] Whereas IL-4 production can be detected after IgE crosslinking or protease activation of basophils, IL-3 pretreatment significantly increases IL-4 production.[6] Interestingly, this IL-3-mediated priming effect seems to exert an effect through a different receptor complex than that inducing IL-3-mediated basophil development.[6] This may be particularly relevant for basophil-T cell crosstalk *in vivo* as activated T cells produce IL-3.[43] When starved of IL-3, basophils will produce small amounts of IL-4 directly in response to subsequent IL-3 stimulation. This depends on the presence of the Fc receptor common g-chain (FcRγ), as FcRγ knockout mice are unable to produce IL-4 in response to IL-3 stimulation.[6] This was shown to be due to interactions between the FcRg chain and the IL-3 receptor complex; IL-3R signaling through FcRg induces IL-4 production.[6] Interestingly, basophils can be derived from bone marrow precursors of FcRγ knockout mice. Thus, the common β-chain, which has long been thought to be the only signaling component of the IL-3R, is responsible for other consequences of IL-3 signaling, such as basophil differentiation from bone marrow precursors. How it is possible to select between the different IL-3-mediated signaling pathways remains unclear, as does the role of IL-3 in basophil-mediated Th2 differentiation *in vivo*.

BASOPHILS AS ANTIGEN-PRESENTING CELLS

After papain immunization, dendritic cells migrate to the draining lymph node with similar kinetics as is observed after immunization with the Th1-polarizing LPS. Thus, dendritic cells were suspected to exert an effect as antigen-presenting cells after papain immunization and basophils were thought to exert an effect primarily as essential cytokine providing accessory cells. However, an earlier clue that basophils could be providing more than simply soluble cytokines was provided by Min *et al.*[42] They clearly showed that co-culturing basophils with dendritic cells and T cells in the presence of antigen led to Th2 differentiation *in vitro*. In accordance with later *in vivo* studies, they found that IL-4 from basophils was the major factor leading to Th2 skewing. Interestingly, though, they also showed that at least part of the *in vitro* skewing ability of basophils was due to direct cell–cell interaction with T cells.[42] However, the identity and the primary target of these cell surface-associated signals remained unknown. At least part of this story was answered recently with the discovery of antigen-presenting function of basophils.

In a surprising insight into basophil biology, basophils were recently discovered to exert an effect as antigen-presenting cells in three separate models of a type 2 immune response: protease allergen mediated, IgE mediated, and the response to infection with a helminth parasite (**Figure 1**). Thus, although all studies share the same conclusion, they show that basophils exert an effect as antigen-presenting cells during the initial response to protease allergens and helminth parasites as well as during re-exposure to antigens (i.e., in the presence of antigen-specific IgE). Although not previously appreciated, murine and human basophils express MHC class II on their surface.[26,46,47] Under homeostatic conditions, peripheral blood basophils homogenously express surface MHC class II, which is retained and slightly increased on basophils in the lymph node after papain immunization. Perhaps because of their earlier stage of development, bone marrow-derived basophils heterogeneously express MHC class II, but this expression is increased after papain treatment.[26] MHC class II expression was accompanied by expression of costimulatory molecules, although there are conflicting data as to the panel of expression. Yoshimoto *et al.*[47] reported that bone marrow-derived basophils expressed CD80 and low levels of CD86, but not CD40. Our data while analyzing costimulatory molecule expression on peripheral blood basophils and lymph node basophils after papain immunization identified

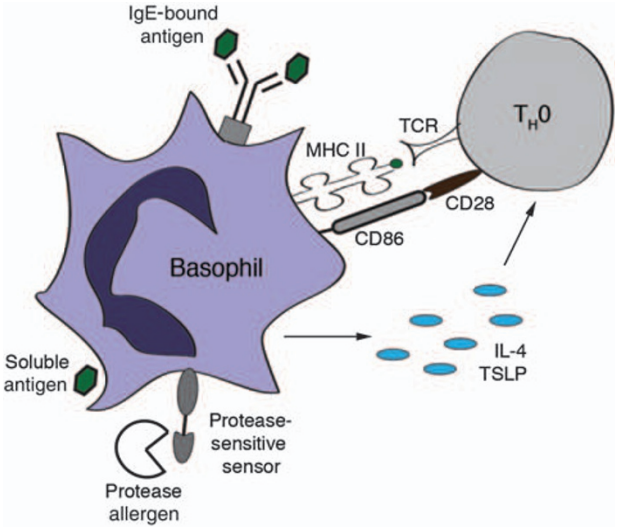

Figure 1 Basophils can be activated by immunoglobulin E (IgE) crosslinking or by cysteine protease allergens. Antigens, which are taken up through FceRI-mediated uptake or through endocytosis of soluble antigens, are processed and presented in major histocompatibility complex (MHC) class II. Basophils can then activate naive CD4 T cells through antigen presentation and expression of costimulatory molecules such as CD86. Activated basophils also produce cytokines including interleukin-4 (IL-4) and thymic stromal lymphopoietin (TSLP) that promote Th2 differentiation.

expression of CD40 and CD86, but not CD80. Expression of CD40 and CD86 was detectable in peripheral blood basophils and increased in lymph node basophils after papain immunization.[26] Such differences are likely due to differences in maturation status between bone marrow-derived and endogenous basophils; immature bone marrow resident basophils express lower levels of CD40 and CD86 than mature basophils.[26]

Basophil expression of MHC class II and costimulatory molecules was correlated with function. All three groups illustrated that basophils mixed with OVA peptide can activate naive Th0 cells and induce Th2 differentiation in the absence of any other cells or stimuli *in vitro*. Basophils were necessary antigen-presenting cells *in vitro*, as even dendritic cells sorted from the draining lymph node of papain-immunized mice were incapable of inducing Th2 differentiation in the absence of basophils.[26] Basophils were capable of forming immunological synapses with T cells *in vitro* after only 60 min of co-culture.[26] These *in vitro* observations were illustrative of their *in vivo* role. Basophils were shown to be essential antigen-presenting cells *in vivo* after infection with Trichuris muris or papain exposure. Th2 differentiation was lost in both models when MHC class II expression was restricted to dendritic cells.[26,46] Thus, dendritic cells are neither necessary nor sufficient for initiation of the Th2-mediated response. However, specific identification of basophils as being functional antigen-presenting cells *in vivo* required transfer of basophils, a difficult procedure because of the fragility and short life span of basophils. Yoshimoto *et al.*[47] accomplished this by transferring antigen-loaded basophils into naive mice and then challenging them 4 days later to boost the response. To analyze specifically the primary response induced by the transferred basophils, we injected antigen-loaded basophils from Bcl2 transgenic mice and examined Th2 differentiation in the absence of additional immunizations.[26] Both techniques illustrated that antigen-loaded basophils were capable of initiating Th2 differentiation in recipient mice.[26,47] One caveat to the transfer experiment was that host cells may be capturing antigen on basophils and exerting an effect as antigen-presenting cells as opposed to the transferred basophils. This was ruled out by experiments transferring antigen-loaded basophils into MHC class II-deficient mice. In these experiments, the transferred basophils were the only cells with MHC class II, and thus the only cells capable of antigen presentation. Th2 differentiation was completely intact using this transfer model, illustrating that basophils are relevant antigen-presenting cells *in vivo*.[26]

Basophils clearly have an important role in initiating Th2-mediated immunity to certain stimuli through their actions as both antigen-presenting cells and cytokine-providing cells. Although it is certainly not clear that they can supplant the role of the dendritic cell or other antigen-presenting cells, their role and actions in type 2 immunity seem to closely mimic that of the dendritic cells in type 1 immunity. From their antigen-presentation ability to their cytokine production to their migration into the draining lymph node, the two antigen-presenting cells share many of their basic roles in common. However, dendritic cells and basophils have many qualitative and quantitative differences: dendritic cells express higher levels of MHC and costimulatory molecules, unlike basophils, and they can migrate to the lymph nodes from the peripheral tissues, and basophils are very short-lived cells compared with dendritic cells. Interestingly, these differences may be due to the fact that basophils and dendritic cells target different types of antigens during the primary immune response. Immature dendritic cells are well known to avidly and efficiently phagocytose antigens. Basophils, however, do not efficiently phagocytose particulate antigens.[26] The source of antigen for basophils consist of soluble antigens; basophils are capable of endocytosing fluorescently labeled ovalbumin with an efficiency equal to, if not surpassing, that of dendritic cells.[26] This may reflect the fact that although many type 2 pathogens are multicellular helminth parasites that are too large to be phagocytosed, these parasites are constantly shedding soluble antigens from their surface. The preferential uptake of soluble antigens by basophils may represent a specific strategy to target type 2 pathogens. Having said that, Fc-mediated uptake of antigens is also an important strategy used by basophils, one that would allow for uptake of both soluble and particulate antigens. Yoshimoto *et al.*[47] clearly showed that antigens targeted for uptake through FcεRI can be presented through MHC class II. Although basophils may target soluble antigens during the primary response, they are not similarly restricted during the secondary response. How the preferential uptake of antigens based on their solubility affects the polarization of the immune response remains to be understood.

After immunization with protease allergens, parasite excretory–secretory products, or helminth parasites themselves, basophils do transiently migrate into the draining lymph node.[20,46,48] Basophils apparently leave the bloodstream and enter the lymph nodes through the high endothelial venules, as

basophil entry into the draining lymph node is CD62 L dependent.[20] This brings up the important questions of where blood resident basophils interact with their antigens and what leads to basophil migration specifically to the draining lymph node. As previously mentioned, *in vitro* evidence has shown that in the absence of antigen-specific IgE, basophils seem to be specialized in uptake of soluble, but not particulate antigens. However, do they directly encounter that antigen in the bloodstream or lymph node, or are they reliant on an additional cell to bring that antigen from the periphery to the lymph node? Evidence that basophils directly interact with soluble antigen without assistance of another cell was provided by experiments in which the injection site was removed within hours of immunization. By removing the injection site of mice immunized in the pinna with papain, cell-mediated traffic to the draining lymph node was abolished, whereas passive movement of soluble antigens through the afferent lymphatics was retained. Basophil migration and Th2 differentiation remained even when the injection site was removed, indicating that basophils do not require other antigen-capturing cells to transfer antigen from the periphery to the basophil.[26] Of course, these observations do not rule out the existence of another antigen-capturing cell in the lymph node or elsewhere that captures and retains the antigen for basophils. And it remains unknown whether basophils first encounter antigen in the draining lymph node or the bloodstream. Regardless of where basophils encounter antigen, they are likely reliant on additional signals to promote entry into the specific draining lymph node. What cells provide these signals and what these signals are remain unknown.

BASOPHILS IN THE B CELL ANTIBODY RESPONSE

Although the role of basophils in T-cell activation and differentiation has only recently been appreciated, their role in B-cell antibody production has a longer history. In experiments using the human basophilic cell line, KU812, activated basophils promoted IgE production in human B cells *in vitro*.[49,50] This was dependent upon both IL-4 production and by CD40 L expression by KU812 cells.[49,50] In addition to the KU812 cell line, human basophils express CD40 L and are sufficient to induce IgE production from B cells *in vitro*.[49–51] Murine basophils also express low levels of CD40 L, which increase after activation.[15] A recent study showed that in addition to IgE-inducing cytokines such as IL-4, activated basophils produced B-cell activating factor and membrane-bound proliferation-inducing ligand, known to be involved in the T-independent Ig production. Interestingly, these investigators showed that although IgE crosslinking induced production of these B-cell activating factors, IgD crosslinking was even more efficient at this. Human basophils bind soluble IgD on their surface and crosslinking of IgD led to a specific activation profile characterized by production of cytokines, antimicrobial peptides, and B-cell stimulating factors, but not histamine production or release. This release pattern of inflammatory stimuli was hypothesized by the researchers to be behind the chronic inflammation in various syndromes characterized by elevated IgD levels. This remains to be determined precisely, but similar to the case of protease allergens, IgD crosslinking seems to activate a specific initiator phenotype in basophils as opposed to the stereotypical effector phenotype. Considering the similar phenotype induced between IgD crosslinking and stimulation with protease allergens, it is tempting to speculate that direct induction of IgE by basophils could be a common pathway for IgE production.

BASOPHILS IN THE MEMORY RESPONSE

Freely circulating IgE has a short half-life in the mouse, but this is overcome by binding to basophils and mast cells through FcεRI. By doing this, basophils and mast cells extend the effective life span of IgE and contribute to immune memory. This may be essential for IgE-mediated memory because as as opposed to other isotypes, long-lived IgE plasma cells have yet to be discovered. Although basophils are a rare cell type, they are coated with antigen-specific IgE and through this IgE they efficiently capture encountered antigen. Basophils have been identified as the predominant antigen-capturing cell in the bone marrow, spleen, and peripheral blood. The bound antigen crosslinks the surface-bound IgE and ultimately results in FcεRI-mediated basophil activation and production of cytokines, including IL-4 and IL-6. Both of these cytokines are involved in Ig production by B cells and a recent study addressed whether this could lead to basophil-mediated promotion of the memory response.

Denzel *et al.*[52] first immunized mice with fluorescent allophycocyanin (APC) mixed with heat-killed *Bordetella pertussis*. After 6 weeks, injected APC still efficiently bound to basophils and these basophils produced IL-4 and IL-6 in response. Depletion of basophils led to decreased production of antigen-specific IgG1 and IgG2a, which correlated with decreased antigen-specific B cells, after restimulation with APC.[52] In the presence of activated T cells, basophils directly promoted IgG1 production by B cells *in vitro*. These effects were mainly due to IL-6 production by activated basophils, but also IL-4 and CD40 L expression by basophils and their effects on both T cells and B cells.[52] These *in vitro* effects were echoed by an *in vivo* basophil transfer model. Likely to circumvent the difficulty of transferring purified basophil populations, investigators transferred a basophil-containing mixed population of total splenocytes and bone morrow cells depleted of CD138 + cells. Transfer of APC-specific IgE basophils (along with many other cell types) into naive mice led to increased APC-specific IgG1 production 7 days after restimulation.[52] However, the interpretation of results from basophils transfers in this study is complicated by the observation that basophil-depleted transfers also led to a reduced, but significant APC-specific IgG1 memory response.[52] Thus, although basophil transfer was most efficient at transferring humoral memory, this ability is not singular to basophils. In addition, investigators showed that the amplification of the humoral memory response by basophils is dependent on FcRγ, the common signaling component of both FcεRI and FcγRI/III.[52] Cytokine production is retained in the absence of FcεRI, indicating to investigators that signaling through both FcεRI and FcγRI/III led to the IL-4 and IL-6 production by basophils. Although this is likely part of the story, recent observations that FcRγ is a constituent of the IL-3R complex obscure the exact mechanisms behind basophil promotion of humoral memory.[6]

Table 1 Phenotypes of human vs. mouse basophils

	Human basophils	Mouse basophils
Nuclei	Lobulated	Lobulated, polymorphic
Granules	Abundant, large, intensely basophilic	Sparse, uneven size, basophilic
Histamine content	1 pg per cell	0.1 pg per cell
Frequency	0.5–1%	0.3–1%
Comparison with bone marrow-derived basophils	Decreased granules	Similar to *in vivo*
Commonly used cell surface antigens		
FcεRI	+ +	+ +
CD11b	+	+/−
CD11c	+	−
CD49b	+ +	+ +
CD69	+/+ + +[a]	+/+ + +[a]
CD117 (c-kit)	−	−
CD123 (IL-3R)	+ +	+
Cytokine production		
IL-3	+	+
IL-4	+ + +	+ + +
IL-6	+/−[b]	+ +
IL-13	+ +	+

[a]Marked upregulation on activation.
[b]Based only on transcript detection.

Regardless, Denzel *et al.* clearly showed that basophils have a role in promoting the humoral memory response. Whether this is due to direct effects on B cells *in vivo* or basophil-mediated activation of Th2 differentiation and subsequent Th2-mediated antibody production remains unclear.

CONTROVERSIES AND QUESTIONS

Both human and mouse basophils share in common specific ultra-structural components: the presence of electron-dense granules and segmental nuclei.[2] However, despite the similarities between mouse and human basophils, there are specific and notable differences in the cells, particularly in their appearance by light microscopy after staining with hematologic dyes (**Table 1**). Using light microscopy, human basophils are easily identified by a preponderance of large granules that stain darkly with basophilic dyes, but murine basophils largely lack such granules. Instead, murine basophils are characterized by a multilobed nucleus and a lightly basophilic cytoplasm with few granules of uneven size.[5] Such differences in cell structure have prompted some investigators to posit that murine basophils may instead be immature basophils or an entirely different cell type altogether.[53] However despite histological differences, murine basophils and human basophils seem to have analogous functional roles. After FcεRI crosslinking, both cells behave in a functionally similar way, releasing preformed leukotriene C4 and histamine (although at a lesser quantity in mouse basophils) and producing type 2-associated cytokines such as IL-4 and IL-13.[5] Indeed, their antigen-presenting function and their role in B-cell activation and antibody production have been at least partially recapitulated using human basophils.[47,50,51] Given their similar role in allergic inflammation and helminth parasite infection, it is likely that human and mouse basophils do constitute analogous cells despite their histological differences.

One of the most controversial issues emerging from the discovery that basophils are necessary and sufficient antigen-presenting cells in at least some murine type 2 immune responses concerns the role of the dendritic cell. Recent studies showing basophils as the necessary antigen-presenting cells in initial immune responses against protease allergens and some helminth parasites as well as in the memory response, may suggest that dendritic cells do not have a role in initiating Th2-mediated immunity. Could dendritic cells be specialized inducers of Th1 and Th17 differentiation whereas basophils are specialized inducers of Th2 differentiation? The reality of the situation is likely more complicated than this. Dendritic cells have been clearly shown to be able to induce Th2 differentiation *in vitro* and *in vivo* through pathways involving Notch ligands and OX40.[32,54] Although dendritic cells are unnecessary for Th2 differentiation in response to papain immunization, they do migrate to the draining lymph node after papain injections.[20] This migration is in response to the protease activity of papain and not due to contamination by TLR agonists, as it is retained in TLR4-deficient animals.[20] However, although dendritic cells respond to active papain *in vivo*, they do not respond *in vitro*.[20] This dichotomy suggests that they are responding to secondary stimuli produced by an undetermined cell type, perhaps the same cell type that ultimately induces basophil migration to the draining lymph node. Whatever is the case, activated dendritic cells appear in the draining lymph node after papain immunization, suggesting that they are not superfluous to the type 2 immune response even in the case of protease allergens. It has long been assumed that the type 2 immune response is the default response because of studies showing increased Th2 differentiation when dendritic cell activation is inhibited.[55] Could dendritic cells have a role in inhibiting basophil-mediated Th2 differentiation and thus type 2-mediated inflammation? Basophils do not enter the draining lymph node after immunization with TLR agonists, but they do respond to TLR ligands. This and other puzzling observations underscore how little is still known about basophil biology and their role in adaptive immunity.

Although many questions remain concerning the role of basophils in both initiating and instructing the type 2 immune response, it is clear this previously underappreciated cell type has an important role in innate control of adaptive immunity. Recent research has shown that basophils not only directly promote the initiation of Th2-mediated immunity and Ig production, but they also function as antigen presenting-cells in type 2 immunity, at least in some cases. After years of being commonly held to be redundant to the mast cell, they have finally come into their own as critical regulators of type 2 immunity. Although this

newfound appreciation of their role as type 2 activators makes it vital to further characterize their mechanism of function and its regulation in future studies.

DISCLOSURE

The authors declared no conflict of interest.

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