

Basophils: emerging roles in the pathogenesis of allergic disease

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Summary: After approximately 130 years since their discovery as rare granulocytes that circulate in blood, basophils are just now gaining respect as significant contributors in the pathogenesis underlying allergic inflammation and disease. While long known for secreting preformed and newly synthesized mediators and for selectively infiltrating tissue during immunoglobulin E (IgE)-mediated inflammation, their role has largely been viewed as redundant to that of tissue mast cells in functioning as effector cells. This line of thought has persisted even though it has been known in humans for approximately 20 years that basophils additionally produce relatively large quantities of cytokines, e.g. interleukin-4 (IL-4)/IL-13, that are central for the manifestations of allergic disease. Studies using novel IL-4 reporter mice have significantly added to the *in vivo* importance of basophils as IL-4 producing cells, with recent findings indicating that these cells also function as antigen-presenting cells essential in initiating T-helper 2 responses. If confirmed and translated to humans, these provocative findings will give new meaning to the role basophils have in allergic disease, and in immunology overall.

Keywords: basophil, cytokine, asthma, Fc receptors, allergy, inflammation

Introduction

The distinctive appearance that basophils have when stained with basic stains enabled the immunologist Paul Ehrlich to first describe these granulocytes over 130 years ago (Fig. 1). However, their low frequency in blood (<1% – the lowest of the major leukocytes) would greatly hinder, for nearly a 100 more years, any attempts to perform detailed studies regarding their biology. Only as recently as the 1950s, with the onset of density centrifugation, did clues begin to suggest basophils as a source of histamine, with this mediator tracking with suspensions enriched for these cells. It would take another decade, including the discovery of immunoglobulin E (IgE), before it was acknowledged that basophils bound this immunoglobulin with high affinity and released histamine upon cross-linking it using anti-IgE antibodies (1). Since this straightforward *in vitro* reaction was possible using mixed leukocytes readily assessable from blood, the belief soon arose that basophils might serve as surrogates for studying mast cells, which were not readily assessable from tissue. Even

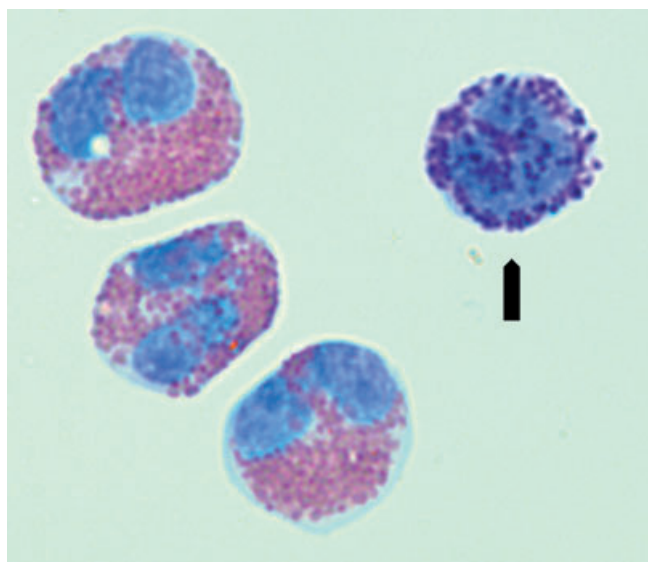


Fig. 1. Human basophil (arrow) stained with Wright's stain and among three eosinophils for comparison. Original magnification = $\times 1000$.

though comparative studies during the next 25 years would eventually reverse this concept, misunderstandings persist to this day, primarily among investigators outside of the field who generally think of basophils and mast cells being one in the same. As it stands, basophils are likely more developmentally related to eosinophils, despite sharing morphologic, phenotypic, and functional features with mast cells.

Only during the last 20 years, with the development of methods for isolating basophils and of novel tools and reagents, has there been a greater understanding of the biology these immune cells mediate. Perhaps most significant during these two decades is evidence that these cells secrete a third class of mediator – cytokines – in addition to vasoactive amines and leukotrienes. In fact, their capacity to secrete large quantities of interleukin-4 (IL-4) and IL-13 has implied a role

in driving and/or inducing the T-helper 2 (Th2) responses that are hallmark in allergic disease. Not only has this concept been recently validated *in vivo* using mouse models, there is also new provocative evidence that basophils possess antigen-presenting cell (APC)-like activity that is critical for the induction and/or amplification of Th2-type inflammation. Indeed, there has been an upsurge in the number of manuscripts describing new models, which have added a great deal of insight into the mechanistic roles that basophils mediate in disease and immunity. However, translating these concepts to human allergic disease, using what have been predominately parasitic-driven Th2 models in mice, seems more problematic in light of the biology long recognized for human basophils. Thus, while it seems certain that the murine studies will provide clues into the processes that basophils mediate in humans, until such concepts are actually translated, they remain only representative. This is particularly relevant in light of the estimated 65 million years of evolution separating the two species. Therefore, this review focuses on the advances made in understanding the role of basophils in human allergic disease but does so by drawing attention to relevant findings in the mouse, with the overall goal of determining those areas in common yet exposing inconsistencies that require further study.

Basophil development

Basophils arise from progenitors in the bone marrow and are released into circulation as mature granulocytes with a life span estimated in days (Fig. 2). For comparison, mast cells also arise from bone marrow precursors but are released into circulation as intermediates that only mature once they migrate and localize in tissue, where they are then thought to survive for months. Despite early beliefs, blood basophils do not give

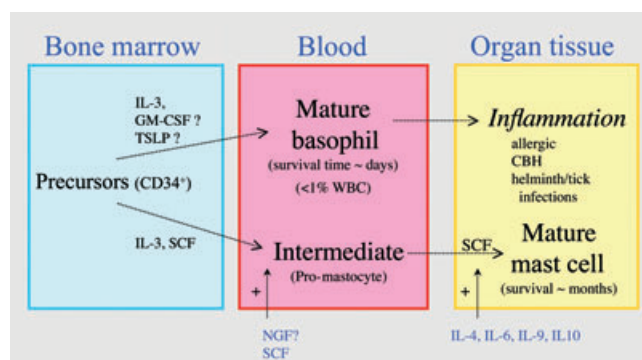


Fig. 2. Compartmental stages of basophil development. A diagram emphasizing the development of basophils from precursor cells in the bone marrow, their release into circulation as mature cells, and their recruitment into tissue during specific inflammatory conditions. This is contrasted this with that of mast cells.

rise to mast cells. Although, recent work in the mouse indicates that both cell types have the potential to evolve from a common progenitor cell (2).

Both *in vivo* and *in vitro* studies point to IL-3 playing an important role in the development of basophils, even though it is likely not the only growth factor for these cells. For example, early studies showed that non-human primates, when injected with recombinant IL-3, would develop a significant basophilia, although other granulocytes (e.g. eosinophils) also increased in these animals (3). *In vitro* evidence soon followed indicating that immature-like basophils could be grown from human cord blood precursors after culturing approximately 3 weeks in medium containing rIL-3. In fact, refinements in this approach are currently taking advantage of using purified CD34⁺ precursors, which allow for a greater expansion of basophils suitable for a variety of functional assays (4). Similar *in vitro* approaches are also being done to grow mouse basophils from bone marrow precursors. Interestingly, basophils predominate in these cultures after only 10–14 days with IL-3. However, these cultures consist of essentially all mast cells (and are devoid of basophils) after 3–4 weeks, implying that the former cell type can develop in IL-3 alone. Naturally, stem cell factor (SCF) facilitates mast cell development in these cultures, but its addition is not necessarily required. In contrast, the growth of human mast cells *in vitro* requires the addition of SCF, which is needed throughout the 8–12 weeks necessary for these cells to mature.

The first indication that growth factors other than IL-3 might regulate basophil development arose from studies showing that these cells persist in IL-3-deficient mice (5). While the exact cytokines mediating this activity remain to be determined, recent evidence has pointed to thymic stromal lymphopoietin (TSLP) having a role (6). Studies in IL-3-deficient mice do indicate, however, an important role for IL-3 in markedly increasing basophil numbers following helminth infection (5, 7).

The frequencies of circulating basophils in humans can vary significantly among individual subjects, but there is currently no solid evidence to support the notion that increased numbers correlate with allergic disease. Likewise, there is no evidence that IL-3 levels in plasma/serum are increased in allergic disease, let alone with increased numbers of basophils in general. Although, as discussed below, increased numbers of basophils are often seen in the inflamed tissue of individuals who suffer from allergic diseases.

Basophils (both human and mouse) retain very high levels of IL-3 receptor (CD123) throughout their life span. This expression most certainly accounts for the fact that IL-3

augments essentially every function mediated by this cell. Human basophils share this distinction of expressing high levels of CD123 with none other than plasmacytoid dendritic cells (pDCs). As discussed below, this peculiarity has contributed to the notion that these cells are intricately linked in ways yet to be fully explained, thus warranting studies that further probe this odd relationship.

Inflammatory mediators

There has been in recent years a greater understanding of the mediators stored in the granules of basophils. Chondroitin sulfate, rather than heparin sulfate, is the major glycosaminoglycan (GAG) responsible for the staining properties of basophils when treated with basic dyes. Naturally, histamine is the best-known prestored inflammatory mediator, averaging approximately 1 pg/cell – a finding that is remarkably consistent among individuals, including those who suffer from allergic disease. This level is in contrast to what is found in human mast cells, which typically average 3–5 pg/cell, but is considerably greater (approximately 5- to 10-fold) than what has been reported for mouse basophils. There is evidence that basophils contain active forms of α and β tryptase, despite the notion of this protease being a mast cell-specific marker (8). In fact, the actual levels comprise just 1% of those measured in mast cells, but there is at least one flow-based assay showing equal staining in basophils (9). Whether this has resulted in instances where basophils have been mistaken for mast cells is not known, but the possibility exists and should warrant caution in the future depending on the assays used in detecting tryptase. Basophils are also known to store serine proteases that are potentially released upon degranulation. In particular, cathepsin G was first described in basophils some 26 years ago (10). While its function in basophils remains unknown, it is recently reported to play a central role in processing antigen in DCs and other APCs (11). Thus, it is intriguing to think that it may mediate a similar function in basophils in light of the recent evidence in mice that these cells possess APC activity (see below). Granzyme B is yet another serine protease recently reportedly to be released as an inflammatory mediator from human basophils, although its function too remains obscure (12). Its expression in basophils is upregulated following IL-3 exposure, more so than with IgE-dependent activation. Therefore, its generation tracks more closely with IL-13 than any other mediator released from basophils. Finally, basogranulin is a preformed mediator specific only to basophils (13). It is identified by the BB1 antibody, and, like histamine, is released upon

degranulation (14). Its role in allergic inflammation remains unclear but is suggested by evidence that it is found in lavage fluid recovered from sites of allergic inflammation (15).

Activated basophils generate *de novo* two additional classes of mediators central to allergic disease, namely leukotrienes (LTs) and cytokines. LTC₄ is the predominate and likely only arachidonic acid metabolite generated in basophils, and it is rapidly released upon activation with a time course nearly transposable to that of histamine. While the levels of LTC₄ released by basophils are considerably less than those seen for histamine, this mediator is estimated to be some 6000-fold more potent at inducing bronchoconstriction. Unlike mast cells, basophils have thus far shown no capacity to synthesize prostaglandins and are therefore devoid of cyclooxygenase activity. With regards to cytokines, IL-4 and IL-13 are currently the two most relevant to allergic disease and are produced by human basophils at comparatively high levels compared to other immune cells. A more in depth description of these and the parameters regulating their secretion is provided below.

Phenotypic markers

FcεRI

The ever-expanding development of fluorescently labeled antibodies targeting cell surface proteins has made it possible to identify phenotypic markers expressed on most cell types, including basophils. Of course, no marker on basophils has received more attention than has the high affinity IgE receptor, FcεRI. Only basophils and mast cells express the full tetramer, which consists of one α-, one β-, and two γ-chain molecules that together form the αβγ₂ structure. Yet, it is now evident, at least in humans, that these two cell types are not alone in binding IgE with high affinity. A variant of FcεRI, αγ₂, which lacks the β-subunit, is expressed on a variety of other cell types, namely immature DC subtypes where it is thought to play a role in facilitating antigen/allergen-presentation (16). With the development of anti-IgE therapy using omalizumab (Xolair™), there has been a greater appreciation for the role IgE mediates in driving the expression of FcεRI and its αγ₂ variant on basophils, mast cells, and DCs. Mechanistic studies utilizing omalizumab as a 'tool' are also providing novel insight into how IgE/FcεRI expression regulates basophil sensitivity to allergen, why this treatment fails in some individuals, and is providing new insight into other functions mediated by this immunoglobulin (17–19). For a detailed review regarding how IgE receptor expression on basophils is regulated, the reader is referred elsewhere (20). Depending

on IgE serum levels, the number of FcεRI receptors found on a basophil typically ranges from a few thousand to as high as 1 million, with the median levels among non-atopic and atopic subjects being approximately 100 000 and approximately 250 000, respectively. However, the overall receptor number is not the most important factor regulating the sensitivity of a basophil to an IgE-dependent stimulus. Recent work by MacGlashan and colleagues (21) has demonstrated that such responses are very much determined by the intracellular levels of the tyrosine kinase syk with higher ratios accounting for greater responsiveness.

FcγR

There remains considerable debate regarding the role IgG mediates in basophil biology, and controversy as to exactly what Fcγ receptors are expressed by these cells. First, there is a general consensus that the low affinity IgG receptor, FcγRIIβ (CD32), is found on basophils. Moreover, there is evidence suggesting that co-crosslinking this receptor with FcεRI, via allergen binding specific IgG and IgE, effectively inhibits basophil mediator release normally resulting from FcεRI-dependent activation alone (22, 23). Efforts to capitalize on this mechanism for therapeutic purposes using Fcγ–Fcε fusion proteins are in place, with encouraging results reported in mice and non-human primate model of allergic inflammation (24–26). However, it remains unclear whether and how these fusion proteins might interact with high affinity Fcγ receptors associated with activation in humans. In particular, human mast cells express FcγRI and FcγRIIα but not FcγRIIβ, suggesting that the fusion proteins might instead activate these cells (27). In addition, at least one study has reported evidence that basophils from atopic dermatitis subjects express FcγRIIIβ (CD16b), an IgG receptor associated with activation (28). Yet, in spite of these most recent findings, there is no known function for this receptor on human basophils. Then again, it has been proposed that the IgE-independent anaphylaxis described in mouse models results from the release of platelet-activating factor (PAF) from basophils activated in an IgG-dependent fashion (29). The possibility of this occurring in humans becomes even more intriguing in light of recent evidence that PAF levels correlate with anaphylaxis severity and that the enzyme that metabolizes this mediator, PAF acetylhydrolase, inversely correlates (30). However, the idea that human basophils generate and secrete PAF is clearly lacking, with only one study thus far providing evidence (31). Nonetheless, the possibility that basophils express IgG receptors linked to activation requires further exploration.

Cytokine/growth factor receptors

Flow cytometric studies have long shown that human basophils express a variety of other cytokine receptors, although at levels far less than what is seen for CD123. This includes receptors for IL-2, IL-4, IL-5, nerve growth factor (NGF), and granulocyte-monocyte colony-stimulating factor (GM-CSF). However, functional responses are reported for only a few. In particular, NGF, a cytokine that is increased in allergic inflammation and disease (32, 33), binds to Trk A receptors (34) and, as noted below, mediates a variety of functional responses. This cytokine as well as IL-5 and GM-CSF are known to induce signaling events in basophils that resemble those induced by IL-3 but are far less robust in doing so. For example, all three cytokines (like IL-3) acutely activate the extracellular signal-regulated kinase pathway, the signaling elements that precede this as well as cytosolic phospholipase A2 phosphorylation, all of which is consistent with their redundant ability to acutely prime basophils much like IL-3 (35). Naturally, IL-5 and GM-CSF are also reported to be increased in allergic disease and could conceivably contribute to basophil hyper-responsiveness (or priming).

There is growing evidence to support the belief that epithelial-derived cytokines activate basophils, particularly those known to act on DCs to initiate Th2 development (e.g. TSLP and IL-33). Marked differences are currently reported for those affecting human versus mouse basophils. Again, in mice, TSLP seemingly acts like IL-3 by promoting basophil maturation from precursors and possibly activating mature basophils for IL-4 secretion (6). In contrast, there is currently no evidence that TSLP activates human basophils. Instead, several groups have shown that IL-33 markedly increases IgE-dependent and -independent mediator release and cytokine secretion from basophils (36–38). Our own data are consistent with these findings, but indicate that IL-33 is particularly proficient at inducing IL-13 secretion and does so only when in the context of IL-3 exposure (JTS, unpublished observations). In fact, it has been shown that IL-3 induces the expression of ST2 – the receptor for IL-33 (38). Thus, whether basophils from allergic/asthmatic subjects directly respond to IL-33 as a result of *in vivo* exposure to IL-3 (or any other basophil-priming cytokine) is not yet reported but seems highly probable in light of their overall increased responsiveness in these subjects.

There is a long history regarding histamine-releasing factors (HRFs) that activate basophils (reviewed in 39). Many of these, in fact, turn out to be chemokines whose levels are elevated in allergic disease and under certain instances will induce histamine from basophils. Among those best known

are several from the CCL family (e.g. MCPs, RANTES, and eotaxin), but stromal cell-derived factor-1 (SDF1) (CXCL12) and IL-8 (CXCL8) are also reported to possess this activity. It is important to stress, however, that the capacity of these to act as so-called HRFs is very much subject dependent in that basophils from most subjects are unresponsive in this manner. This is particularly true for the so-called human recombinant HRF (HrHRF), a 23 kDa protein that directly induces histamine release from basophils of selected allergic subjects, particularly those deficient in SHIP (40). Interestingly, HrHRF will prime basophils (much like IL-3), and from most subjects, for greater responsiveness to IgE-dependent stimuli. It is thought to do so by binding a yet unknown receptor whose signaling is distinct from FcεRI (40, 41).

In contrast to cytokines or factors that either directly activate or prime basophils for mediator release and/or cytokine secretion, less is known regarding those factors that function in suppressing these cells. To date, type I interferons (IFNs) are the only cytokines/factors reported to possess inhibitory activity against basophil function and do so at relatively low concentrations (42). Specifically, both IFN- α/β have been shown to suppress IL-3-dependent responses, including basophil survival and induction of IL-13 secretion, and apparently do so by acting through IFN α R2. These innate immune-associated cytokines, however, do not inhibit IgE-dependent responses but only that which is augmented by IL-3 priming (42).

It is often implied that IL-10 inhibits basophil responses, but this concept thus far has proved incorrect and has likely stemmed from reports that have instead used culture-derived mast cells (43). For example, IL-10 is reported to suppress IgE-dependent mediator release from mouse and human mast cells derived in culture from progenitors by affecting their maturation (44, 45). However, there are actually no reports of this happening with blood basophils (human or mouse), let alone these cells even expressing IL-10 receptors. Moreover, we have, on several occasions, been unable to show any inhibitory effect of IL-10 on basophils (JTS, unpublished observations). In contrast, we have recently shown that this immunomodulatory cytokine potently suppresses FcεRI-dependent responses in human DCs, thus verifying its activity (46). Whether other immunomodulatory cytokines (e.g. IL-10-like or IL-17 family members) affect basophil function is not currently known.

Chemotaxis/transendothelial migration

Like any blood leukocyte, basophils have the potential to leave the circulation under homeostatic conditions and to migrate

into inflamed tissue sites. As expected, this is most prominent during allergic inflammation, where basophils (along with eosinophils, Th2, etc.) selectively infiltrate lesion sites, often during the late phase response (LPR) that arises several hours after an acute reaction to allergen (see below). *In vitro* studies utilizing human basophils have provided a great deal of insight into the chemoattractants and adhesion molecules likely involved in this trafficking. Foremost, PGD₂ is now thought to play an important role as an initial chemoattractant released by mast cells during acute responses. It does so by acting through the chemoattractant-homologous receptor expressed on Th2 cells (CrTH2) – a receptor (also known as DP2) that is selectively expressed on basophils, eosinophils, and, as its name implies, Th2 cells (47). PGD₂ and other agonists have been shown to induce calcium influx and enhance mediator release (48). As might be expected, there is an ongoing effort to develop antagonists targeting CrTh2 for therapeutic intervention in a variety of allergic diseases (49).

Many CCR3-binding chemokines, while better known as eosinophil attractants, are also thought to play a significant role in basophil trafficking. These include several monocyte chemotactic proteins: MCP-1 (CCL2), MCP-3 (CCL7), and MCP-4 (CCL13), eotaxin-1 (CCL11) and eotaxin-2 (CCL24), RANTES (CCL5), and monocyte inhibitory protein-1 α (MIP-1 α) (CCL3). All of these likely play an overlapping role recruiting basophils to allergic lesion sites and, in some instance, may also play a role in activating basophils for mediator release and cytokine secretion. The notion that basophils can be recruited to non-allergic lesion sites is supported by evidence that chemokines not associated with allergy also play a role in basophil trafficking, including SDF-1 and IL-8.

As with all leukocytes, basophil transendothelial migration into tissue is mediated by specific adhesion molecules expressed by the basophil interacting with endothelium proteins. Again, *in vitro* studies indicate this process being initiated by basophil-bound L-selectin (CD62L) loosely attaching to mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on endothelium to initiate 'rolling'. Firm adhesion is then instructed through β 1- and β 2-integrins, with intracellular adhesion molecule-1 (ICAM-1) on the basophil the primary molecule mediating this interaction. Finally, irreversible transmigration (or diapedesis) is likely achieved with basophil-associated VLA-4 interacting with VCAM-1 expressed on endothelial cells. These adhesion molecule interactions working in concert with the chemoattractants noted above account for the selective migration of basophils (and eosinophils) into allergic lesion sites.

CD63/CD203c/CD69: markers associated with activation

There are several molecules whose expression on human basophils is either induced or enhanced following activation. In particular, CD203c is a marker restricted to basophils, mast cells, and their precursors, yet its exact function on these cells remains unknown. It belongs to the family of ecto-nucleotide pyrophosphatases/phosphodiesterases (E-NPP3) that catalyze the hydrolysis of oligonucleotides, nucleoside phosphates, and NAD. It is expressed both intracellularly and on the cell surface of resting basophils, and its expression is increased upon activation. Likewise, CD63 is a LAMP-like granule-associated protein that is induced on the surface of basophils during activation as a result of granule membranes fusing with the plasma membrane. Its association with granules containing histamine has provided the rationale for it being an indirect marker for the release of this mediator. However, unlike CD203c, CD63 is found on other leukocytes including platelets, which raises the risk of its expression not necessarily being specific to basophils. This can be an issue when whole blood is employed in flow-based assays, with the risk of CD63⁺ platelets non-specifically adhering to basophils. Also, many such assays (some now commercially available) typically test just one concentration of allergen, despite extreme variability in responses among subjects. Other assays include the addition of IL-3 to augment allergen-dependent responses, yet IL-3 itself will induce all three markers. Such issues are potentially problematic, since CD203c and CD63 are both widely used as surrogates for histamine release and are being routinely employed in various clinical trials investigating the efficacy of immunomodulation therapies. Many of these concerns, in fact, have been addressed in more detail elsewhere (50, 51). Finally, CD69 has also been used as a marker of basophil activation, even though its expression (like CD63) is not specific to these cells. *In vitro* studies also indicate that CD69 is perhaps a better indicator of IL-3 exposure rather than a marker of IgE-dependent activation (52).

Innate immune-associated

Like most granulocytes and other cells of myeloid origin, basophils express a variety of molecules associated with innate immunity. In particular, it is long known that basophils release histamine in response to complement factors (e.g. C5a) and to bacterial components (i.e. fMLP). The receptors mediating these responses – CD88 for C5a and a 7-membrane transverse receptor for fMLP, work through signal transduction pathways very different than those for Fc ϵ RI. Obviously, this implies that basophil mediator release plays a role in immune responses not

necessarily linked to Type-I hypersensitivity reactions, yet the exact nature of such responses remains poorly understood.

Further evidence that basophils are responsive to microorganisms comes from data showing that they express specific Toll-like receptors (TLRs). In fact, several laboratories have identified TLR2 and TLR4 on basophils, which are the two TLRs most commonly associated with recognizing bacterial components (53, 54). In our hands, however, basophils are only responsive to ligands known to interact with TLR2. Both peptidoglycan (PGN) and the synthetic lipopeptide Pam₃Cys were shown to induce IL-4 and IL-13 from basophils as well as augment IgE-mediated responses. As expected, TLR1 and TLR6, which independently form dimers with TLR2, are also found on basophils and are needed in modulating TLR2 activity. Basophils actually express relatively high levels of TLR4 (both mRNA and protein) yet typically lack CD14 expression – an important adapter molecule needed in the initial binding of ligands (e.g. LPS) signaling through this TLR (54). Interestingly, TLR7 and TLR9 are two intracellular receptors more common to pDCs but are molecules that we, and others, have also seen expressed in basophils (55). With these receptors playing a critical role in recognizing viral nucleic acids, it may be reasoned that basophils too play a role in viral immunity. If so, then the exact nature of this interplay requires confirmation and further elucidation.

Immunoglobulin-like receptors (LIRs) are found on most types of blood leukocytes, but the natural ligands that bind them are poorly understood. This is particularly evident for basophils, which reportedly express LIR3 and LIR7 (56). Cross-linking LIR7 molecules on basophils using specific monoclonal antibodies mediates activation for histamine release and cytokine secretion. In contrast, doing the same for LIR3 molecules renders inhibitory signals for the release of these mediators, including in response to IgE-dependent stimulation. Determining exactly what is the ligand(s) for LIR3 and the inhibitory signal(s) it mediates could have therapeutic potential for Type I hypersensitivity.

Basophil cytokine secretion

IL-4

Since the early 1990s, when studies began reporting that human basophils produce IL-4, two observations were made that indicated the secretion of this cytokine differed markedly from the release of preformed mediators (57). Foremost, the time course for IL-4 is significantly slower than the rates seen for both histamine and LTC₄, implying a *de novo* synthesis of this cytokine. Secondly, the concentrations of stimulus opti-

mal for cytokine secretion are on the order of 10-fold less than those required for mediator release. At the time, it was well known that histamine is stored preformed within the cytoplasmic granules of basophils and is released within minutes after activation. In fact, half-maximal responses occur between 5 and 10 min and are essentially over in 20 min. Likewise, the release of LTC₄ is comparatively rapid and is also measured in minutes despite its formation being dependent first on the metabolism of arachidonic acid released from phospholipid stores. In contrast, secreted levels of IL-4 are first detected at approximately 60 min, are half-maximal at approximately 120 min, and essentially peak after 240 min.

There is to this day some controversy regarding whether preformed levels of IL-4 are present prior to activation. With regard to our experience, we have long acknowledged that basophils constitutively express mRNA; however, we see little evidence, if any, for preformed IL-4 protein (58). For example, IL-4 levels after 4 h in medium alone are generally <10 pg/10⁶ cells, compared to the 100–500 pg/10⁶ levels often seen after this time when cells are activated with anti-IgE antibody or by allergen. Our early studies found no detectable IL-4 protein in supernatants from pure basophil suspensions having undergone sonication (58). In addition, we typically never detect IL-4 (or IL-13) after stimulating with fMLP or C5a, even though these stimuli are quite capable of inducing degranulation resulting in histamine release and theoretically any preformed cytokine as well (57–59). Finally, super-optimal concentrations of allergen (or anti-IgE antibody), which induce maximal histamine release, are actually inhibitory towards cytokine secretion, thus implying that IL-4 (and IL-13) secretion can completely dissociate from degranulation (Fig. 3).

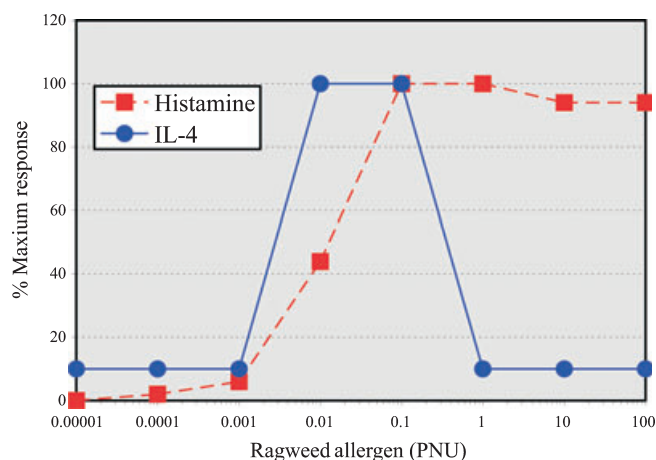


Fig. 3. Basophil mediator release vs. cytokine secretion. Allergen concentrations inducing histamine release and IL-4 secretion from human basophils, emphasizing that it is possible for these mediators to dissociate from one another. PNU, protein nitrogen units.

In spite of the above observations, it is important to stress that simply using stimuli that cross-link IgE/FcεRI does not necessarily result in basophil IL-4 secretion, let alone mediator release. For instance, little to no cytokine is secreted from basophils obtained from the 5–10% of subjects bearing the so-called 'non-releaser' phenotype, which results mostly from a deficiency in syk kinase but also in other signaling molecules (60, 61). In addition, there is a wide range of basophil IL-4 responses possible among subjects (just as there is for mediator release), with the ratio of syk/FcεRI playing an important role (21). And it is equally important to note that allergen, as expected, does not typically induce IL-4 secretion (let alone mediator release) from basophils not expressing specific IgE. In light of these observations, it is accurate to conclude that human basophils produce IL-4 best when activated through FcεRI.

Whereas IL-4 secretion by human basophils appears most dependent on IgE/FcεRI-dependent stimulation, recent studies in mouse models indicate that less stringent requirements are needed. For example, mouse basophils (defined as FcεRI⁺ CD49b⁺c-kit⁻ cells) have been shown to secrete IL-4 in response to certain serine proteases (e.g. papain) and seemingly do so independent of IgE (62). The significance of these findings relate to the fact that many allergens (e.g. Der p 1 of dust mite) and helminth components are in fact serine proteases, which theoretically could initiate Th2 responses in the absence of IgE by activating basophils to produce IL-4. While the *in vivo* portion of these murine studies cannot exclude this response arising from unknown indirect effects, the *in vitro* experiments showing that papain also induces IL-4 from culture-derived basophils do indeed support a direct mechanism – perhaps mediated through PAR receptors. However, it is worth commenting that our own investigations have been unsuccessful in showing that papain (or thrombin) induces IL-4 from human basophils nor have we been able to identify PAR receptors on these cells (JTS, unpublished observations). Thus, these are inconsistencies that require further investigation, given the ramifications from the mouse studies that basophils initiate, let alone amplify, Th2 responses.

IL-13

There has long been evidence that IL-13 secretion is not limited to IgE-dependent stimulation to the extent observed for IL-4 (63, 64). As noted above, basophils treated with IL-3 alone produce relatively large quantities of IL-13, which often exceed those levels seen following IgE-dependent activation. Importantly, this response is not simply seen using cells only

from atopic individuals but occurs using basophils from most subjects including those non-atopic. Although, greater levels of IL-13 are generally produced when using basophils isolated from allergic subjects (65). The same is true when activating with NGF, although as expected, this stimulus induces a less robust response. Again, this NGF response is mediated through TrkA receptors expressed on the surface of basophils, suggesting some similarity with CD123 signaling for inducing IL-13. In contrast, IL-5 and GM-CSF do not typically induce basophils to produce IL-13 (or IL-4), which is somewhat surprising given that they share with IL-3 a common receptor beta subunit, induce similar intracellular signals in basophils, and generally prime for mediator release (35).

Another significant difference regarding basophil IL-13 production relates to its time course. Early studies indicated that its secretion is ongoing and peaks after approximately 24 h as opposed to the transient production of IL-4, which is essentially over after 4–6 h (63, 64, 66). This is certainly true for IL-3-dependent secretion but is also evident when IL-13 is produced in response to FcεRI-dependent activation. It was initially thought that the relatively early secretion of IL-4 might promote the subsequent production of IL-13 and/or that IL-13 might suppress IL-4. However, neither of these hypotheses has proven valid, despite several experimental attempts to show IL-4/IL-13-dependent effects on basophils. However, it has recently been determined that basophils themselves transiently produce IL-3 following IgE-dependent activation, which, through autocrine activity, functions in prolonging IL-13 secretion (67). Thus, the ongoing production of IL-13 observed when basophils are stimulated with anti-IgE antibody or allergen (relative to transient IL-4 secretion) is partially dependent on the indirect effects of autocrine IL-3. As discussed below, this finding is thought to also have relevance to the primed phenotype seen among basophils from allergic subjects.

The concept that at least two distinct pathways for IL-13 generation exist in human basophils is further supported by pharmacologic studies. In particular, we had shown early on that cytokines produced in response to IgE/FcεRI-dependent activation (e.g. IL-4 and IL-13) are markedly inhibited by FK506 and cyclosporine A (CsA) (64). This observation in and of itself suggests a calcium-dependent calcineurin pathway in regulating this IgE/FcεRI-dependent response. It also accounts for why the generation of these cytokines are very much dependent on extracellular calcium and are produced at very high levels when activated by calcium ionophores. In contrast, neither FK506 nor CsA were found to have any effect on the IL-13 secreted in response to IL-3 stimulation (64).

More recently, we have demonstrated a similar finding using a specific inhibitor for Bruton's tyrosine kinase (Btk), PCI-32765. This compound also markedly inhibits IgE-dependent responses in basophils, including histamine release and cytokine secretion in the low nanomolar range (68). However, like FK506, it too does not prevent IL-13 secreted in response to IL-3. Collectively, these data point to the notion that multiple pathways exist in basophils for the generation of IL-13, and that simply targeting IgE/Fc ϵ RI-dependent mechanisms for clinical intervention may not completely prevent its production.

It is clear that many of the same upstream signals necessary for IgE-dependent mediator release are also needed for cytokine secretion. For example, syk phosphorylation is as critical for cytokine secretion as it is for mediator release, with specific inhibitors of this kinase blocking both responses. Even the selective inhibition of more distal events associated with IgE/Fc ϵ RI-mediated activation, such as Btk activation, is incapable of fully dissociating cytokine secretion from mediator release. Whereas the mobilization of cytosolic calcium is critical for both responses, it is the prolonged changes, such as those occurring with ionophores and sub-optimal IgE/Fc ϵ RI cross-linking, that appear optimal for cytokine production. Taking into consideration these parameters and the added evidence that FK506 and CsA are potent inhibitors of calcineurin phosphatase activity, we proceeded to investigate whether the nuclear factor of activated T cell (NFAT) family of transcription factors might be regulating basophil cytokine responses. NFAT2/c1 had been shown in earlier studies to be an important cofactor for IL-4 production, in mouse bone marrow-derived mast cells (BMMCs) (69), but that its expression in these cells first required cellular activation. Interestingly, we found that human basophils differ from most leukocytes in that they constitutively express NFAT2/c1 (rather than NFAT1/c) – a finding that may account for their ability to rapidly produce IL-4. Indeed, we found further evidence of NFAT2 in the cytosol of activated basophils and its ability to bind IL-4 promoter sequences (70).

It seems probable that other signaling events and transcription factors play a role in basophil IL-4/IL-13 expression but are yet to be identified in human cells. In particular, GATA-3 is recently described to play an important role in IL-4 expression in murine basophils, just as it is in rodent Th2 cells and mast cells (71). At least one early study reported evidence for GATA-3 in human leukemic basophils (72). Our own studies, however, have been unsuccessful in identifying this transcription factor (mRNA or protein) in mature human blood basophils (JTS, unpublished observations).

As noted above, human basophils constitutively express IL-4 mRNA, which is another feature consistent with their rapid ability to generate this cytokine (58, 73). Taking into account this observation and that transcripts for IL-4 contain AU-rich elements in the 3'-untranslated region (UTR), it seems feasible that expression of this cytokine, in part, is post-transcriptionally regulated in basophils by miRNAs and/or RNA-binding proteins that affect its half-life. Should this hypothesis prove correct, then identifying and characterizing the miRNAs and RNA-binding proteins potentially involved, and how they themselves are regulated, could provide much needed information on how basophils regulate IL-4 expression as well as IL-13.

Other cytokines

While quite proficient in producing IL-4 and IL-13, human basophils have thus far not demonstrated a great capacity to produce other pro-inflammatory cytokines, at least to the extent of what is reported for mouse basophils. Nonetheless, there are several additional cytokines, growth factors, and chemokines produced *in vitro* by human basophils when stimulated through Fc ϵ RI cross-linking. As already noted, basophils rapidly generate IL-3 with a time course (approximately 4 h) nearly identical to that seen for IL-4. Our studies show that this IL-3 has the capacity to prime basophils for other cell functions by acting in an autocrine fashion (67). In fact, we hypothesize that the ability to do this accounts for the increased baseline expression of activation-linked markers seen on basophils from individuals suffering from a variety of allergic conditions (52, 74, 75). As noted above, this also includes an increased capacity to produce IL-13. In addition, basophils produce VEGF/VEGF receptors (76) and amphiregulin (77), both of which implicate these cells as having a role in the tissue remodeling often associated with allergic disease. A variety of chemokines made by basophils, including RANTES, eotaxin, MIP-1 α , IL-8, and MIP-5, further imply a role in regulating the trafficking of other cell types, particularly eosinophils.

There is no to little evidence that human basophils secrete additional Th2-like cytokines other than IL-4 and IL-13, which again is markedly different from what is currently published for their murine counterpart. In fact, after numerous attempts during the past 20 years, our own laboratory has been unsuccessful in detecting several of the cytokines originally linked to Fc ϵ RI-dependent activation in rodent mast cell lines (78). These include IL-5, IL-6, and TNF- α , which we have never detected in culture supernatants using either enzyme-linked immunosorbent assay or multiplexing assays,

and after activating with a variety of stimuli (JTS, unpublished observations). Nonetheless, at least one study using human basophils has reported IL-5 and IL-6 among the cytokines detected with multiplexing (36).

One recent study has reported that human basophils (along with eosinophils) produce and secrete IL-25 (IL-17E) – a Th2-like cytokine critical for the expansion of CrTH2⁺ Th2 memory CD4⁺ cells (79). However, the basophils used in this study were defined as CrTH2⁺ CD4[−] cells that were co-isolated alongside CrTH2⁺ CD4⁺ cells during cell sorting. Thus, there remains doubt in whether these cells truly were basophils, since specific phenotypic markers (e.g. morphology, Alcian blue staining, histamine, IL-4, etc.) were not investigated. Our own experiments have been unable to confirm these findings using ultra-pure suspensions (>99% basophils). We have instead identified IL-17F mRNA in human basophils (80).

Basophils in human allergic disease

As noted above, the half-life of a circulating basophil is estimated at approximately 2 days, which, although not uncommon for a granulocyte, is far less compared to mast cells and most immune cells in general. Combine this with the fact that they represent <1% of circulating leukocytes, then it has been easy to conclude that basophils, at best, serve only a redundant role to that of mast cells and eosinophils. This has certainly been the dogma, even though basophils have long shown strong associations with Th2-associated disease (81), anti-helminth activity (82), and in specific delayed-type hypersensitivities in humans (83). However, there is increasing evidence that basophils do indeed play a non-redundant role in all three of these areas. Again, much of this newfound evidence has escalated with the development of IL-4 reporter mice (both the 4get and G4 strains), which have clearly implicated basophils as an important *in vivo* source of this cytokine (84–87). Yet, much of this evidence pertains to helminth infections and is beyond the scope of this review. Therefore, when appropriate, emphasis will be placed on those studies in mice that are most applicable to human allergic disease, yet the primary focus here is to discuss what we currently know regarding human basophils and allergic disease.

The late-phase response

It has been known in humans since the mid-1970s that basophils infiltrate tissue following acute allergic responses. This happens both in natural disease as well as in models where inflammation is provoked using experimental allergen challenge. In recent years, the development of monoclonal

antibodies that specifically stain basophils has greatly facilitated both areas of interest by allowing one to definitively identify basophils in a variety of tissues (13, 88–90). These reagents in fact have been instrumental in validating many findings that originated more than 30 years ago in studies that first characterized basophils (using less direct measures) in the so-called late phase response (LPR) (91).

In natural disease, increased numbers of basophils (but not mast cells) have been found in the lungs of individuals having died from asthma (92). Several studies also report increased numbers of basophils in the upper airways of allergic rhinitic subjects (93) and in the skin of individuals with atopic dermatitis and urticaria (89, 94). Yet, perhaps the most insight into the parameters associated with basophil infiltration into allergic lesion has come from work done with allergen challenge models investigating the LPR (95, 96). Thus, approximately 50% of subjects undergoing allergen challenge will have a LPR whereby basophils selectively infiltrate the lesion site approximately 4–20 h after the acute response (initiated by mast cell activation). This has been definitively shown in several models, including both the upper and lower airways as well as the skin (91, 97, 98). Naturally, the striking influx of eosinophils, neutrophils, and mononuclear cells in these responses has long overshadowed that of the basophil. Yet, basophil mediator release is thought to contribute to the re-emergence of symptoms that occur during this late response. Importantly, several studies investigating LPRs in the lung have shown that basophils are a significant source of IL-4 and IL-13 among the inflammatory cells recovered from the airways (99–101).

Basophil hyper-responsiveness in clinical disease: potential mechanisms

There are basophil phenotypic markers and functional characteristics that associate with allergic disease. Indeed, it was first suggested in studies conducted over 40 years ago that basophil histamine release responses observed *in vitro* were predictive of the symptoms one would experience during the ragweed season (102). As a result, there has been and continue to be efforts to determine the mechanisms underlying basophil hyper-responsiveness and whether such phenotypes are altered during therapies targeting immunomodulation, with the added goal of identifying biomarkers associated with clinical efficacy. The following section therefore focuses on a few examples.

It has been known since the mid-1970s that basophils from approximately 80% of food allergic children spontaneous release histamine *in vitro* – a phenotype not seen in children without food allergy (103, 104). Work conducted by

Sampson et al. (105) some 20 years later indirectly suggested a mechanism for this response that was partially dependent on IgE. Our own preliminary studies have shown that this activity is transferable to normal cells during *in vitro* passive sensitization using patient plasma. Yet, this response is blocked with the addition of omalizumab (Xolair™), which specifically inhibits IgE binding to FcεRIα (106). These results therefore support the concept that spontaneous histamine release (SHR) is indeed dependent on IgE, yet the exact mechanisms remain unclear. Previous studies have suggested a mechanism involving IgE/allergen immune complexes, but this has yet to be confirmed (107). Importantly, basophil SHR does not appear restricted to food allergy; the phenotype has also been seen using basophils from allergic asthmatics only less frequently.

There is data emerging in both food allergy and in asthma research that increased CD203c expression is also linked to the basophil SHR phenotype. Again, CD203c and CD63 are commonly referred to as activation-linked markers that increase on basophils following *in vitro* activation. However, it is recently reported that baseline expression of CD203c is increased on basophils from nut-allergic subjects compared to normal controls (108). Likewise, CD203c is increased on circulating basophils from asthmatic subjects recently suffering an exacerbation (109). In contrast, CD63 expression was not observed on basophils from either group of subjects. Recent *in vitro* studies now indicate that CD63 expression is associated more with anaphylactic-type degranulation, whereas CD203c is linked to so-called piecemeal degranulation (51, 110). If this is also an indication of what is happening *in vivo*, then it seems possible that these CD203c^{high} basophils are slowly releasing bioactive amines and/or cytokines from their granules in a manner similar to SHR. Interestingly, this baseline expression of CD203c significantly decreased in the nut-allergic subjects subsequently receiving omalizumab, suggesting that it too, like SHR, is dependent on IgE. Thus, it seems evident that basophils are activated and contributing to the pathogenesis of these diseases, but further investigation is needed to determine the role of IgE/allergen complexes and whether suppression of these responses associate with therapeutic efficacy.

As mentioned above, increased IL-13 secretion is yet another phenotype seen among basophils isolated from allergic subjects compared to normal controls. For example, we first reported a decade ago that the mean levels of IL-13 secreted by basophils prepared from allergic individuals are on average approximately 2.5-fold greater than those from normal controls, when activated with either IL-3 or NGF. Exactly why this hyper-responsiveness is observed is not fully understood, but a series of subsequent results have linked it to

in vivo allergen exposure. Thus, in our first study, we showed that blood basophils from allergic subjects having undergone repeated nasal allergen challenge spontaneously secrete more IL-13 compared to prechallenge responses (111). This was observed within 24 h following the third consecutive day challenge, but was no longer evident 7 days later. These results have recently been substantiated using a segmental allergen challenge (SAC) model in the lung. In this instance, circulating basophils obtained following a single challenge secreted significantly greater levels of IL-13 when stimulated with IL-3, compared to those prepared pre-SAC (101). Collectively, these results imply that allergen exposure, even when localized, can have a systemic effect on basophil function. Certainly, additional studies are needed to determine the exact mechanisms responsible. Nonetheless, it seems one hypothesis relates to the fact that basophils secrete IL-3, especially following low-level FcεRI-dependent activation, which, through autocrine activity, has the ability to prime for increased IL-13 production. Although IL-3 is generally thought to prime for FcεRI-dependent responses, we have confirmed *in vitro* that the opposite is also possible (JTS, unpublished observations). Thus, basophils encountering allergen in circulation, even at levels that do not induce mediator release, can become hypersensitive to non-IgE dependent stimuli. Whether autocrine-acting IL-3 accounts for this basophil priming (including CD203c expression and SHR) is not yet known but is one hypothesis being pursued.

Once again, Type I IFNs (IFN-α/β) are the only cytokines known to inhibit the IL-3 priming of basophils, and immature pDCs are a major source of these when stimulated with innate immune stimuli (42). As mentioned above, several of the DC subtypes within in the human system, including pDCs, express the αγ₂ variant of FcεRI. We and others (112, 113) have shown that the secretion of IFN-α/β by pDCs is impaired when these cells are activated with stimuli targeting IgE/FcεRI. Thus, it is logical to hypothesize that the primed basophil phenotype that is more evident among allergic individuals than controls may also arise, in part, from impaired IFN-α/β production by IgE-bearing pDCs having encountered allergen. Indeed, several groups have shown evidence that IFN-α secretion by pDCs is impaired in allergic rhinitic and asthmatic subjects and that this deficiency correlates with overall IgE levels (113–115). Whether neutralizing IgE levels using anti-IgE therapy (i.e. omalizumab) would improve these innate immune responses in pDCs is currently not known but seems probable. Interestingly, preliminary results using standard immunotherapy have shown significant increases in IFN-α production by PBMCs (and pDCs) from

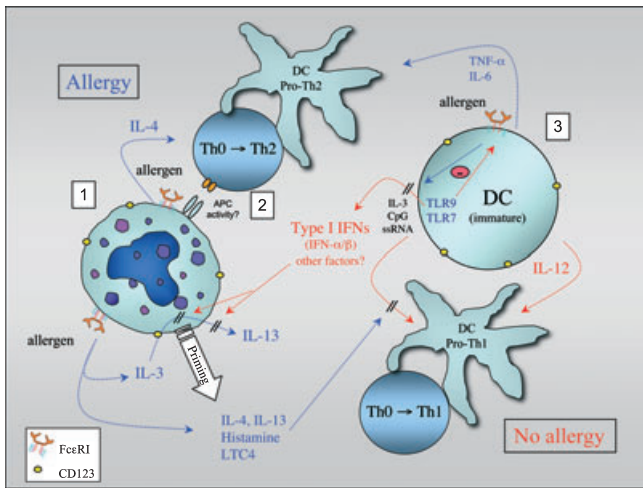


Fig. 4. An oversimplified conceptual representation of how networks between human basophils and DCs might promote (or suppress) Th2 development associated with allergic inflammation and disease. (1) Low-level binding of allergen to specific IgE on basophils triggers cytokine secretion (e.g. IL-3, IL-4 and IL-13) all of which favor Th2 polarization. IL-3 also works in an autocrine fashion to 'prime' basophils for greater responsiveness and directly induces IL-13 secretion. Histamine and LTC4 are released as allergen concentrations increase, with histamine negatively affecting DC function in supporting Th1 development. (2) Basophils possessing APC capabilities can also promote Th2 responses by directly activating naïve T cells and/or memory cell responses. (3) Immature DCs are inherently pro-Th1, and when exposed to specific innate immune stimuli (CpG, ssRNA) and/or cytokines (IL-3), produce pro-Th1 cytokines (e.g. Type I IFNs) that counter-regulate basophil priming. However, these same pro-Th1 responses are suppressed by allergen binding to FcεRI (the $\alpha\gamma_2$ variant) expressed on the DC. In this instance, DC maturation is altered, making these cells less responsive to the same innate immune stimuli that cause them to secrete IFNs that counter-regulate basophil priming. At the same time, the pro-Th2 potential of the DC is also facilitated by allergen binding to IgE/FcεRI (19, 43, 67, 85, 101, and 112–114).

subjects who reached maintenance injection doses of allergen, as assessed by TLR9-dependent activation (116). These increases in IFN- α were also consistent with increases in IgG4, even though the two did not significantly correlate with one another. Thus, it is intriguing to think that increases in pDC-derived IFN- α are playing a role in clinical efficacy by suppressing basophil priming. While this thought may currently reflect an oversimplification, the data do suggest counter-regulatory functions between the two cell types – a working hypothesis that requires further investigation (Fig. 4).

Future directions: resolving mouse versus human inconsistencies

APC activity

The newfound interest in basophils has occurred with several developments arising from work done in mice. First, the IL-4 reporter mice (both the 4get and G4 strains) have conclusively

showed that basophils (defined as FcεRI⁺CD49b⁺c-kit⁺ cells) do, indeed, constitute an important source of IL-4 *in vivo* (117–119). Basophils are additionally identified and tracked in these mice as green fluorescence protein (GFP) positive cells, as a result of inserting this probe as a bi-cistronic construct (i.e. 4get strain) or by using it to replace the entire IL-4 gene (G4 strain). Both models have added significantly to our understanding of basophils as IL-4-producing cells. Most significantly, the expression of IL-4 in these mouse basophils was shown to occur independently of STAT6. Basophils expressing IL-4 were also seen in rag deficient mice, implying that the phenotype is independent of B/T lymphocytes. Importantly, T cells do appear to play a critical role in the mobilization of basophils into the tissues of these animals, particularly in the mouse models involving helminth infection – a process that is partly dependent on T-cell-derived IL-3 (119). Overall, these data have suggested that basophils may very well be the initial source of IL-4 needed in driving Th2-like inflammation.

Second, the notion that basophils play an important role in the induction of Th2 responses gained additional momentum with the work of Sokol *et al.* (62), which showed evidence that a serine protease (i.e. papain) directly activates basophils for the production of IL-4. This finding provided commonality as to why Th2-like responses prevail in both allergic disease and helminth infection, since many known allergens and worm antigens are in fact serine proteases. Exactly how papain activates basophils remains unclear, but serine proteases are known to mediate activity through PAR receptors. This study additionally showed for the first time that basophils could be found in lymph nodes following injection with papain and migrated to this tissue at a time preceding lymphocyte infiltration.

Finally, three papers appearing simultaneously (6, 120, 121) have since presented the most striking evidence to date: that mouse basophils possess APC activity necessary for the induction of Th2 responses. Two of these studies demonstrated this in antigen-specific models (120, 121), whereas the third employed a helminth infection model (6). Surprisingly, all three studies presented evidence that basophils constitutively express MHC class II molecules and other accessory cell markers (e.g. CD80/86) required for this APC-like activity, and once again provided evidence that basophils infiltrate lymphoid tissue. As predicted, the IL-4 secreted by these basophils contributed to their pro-Th2 functions. Most significantly, the Th2 responses induced in each of the models were ablated by first depleting basophils using an anti-FcεRI antibody (MAR-1). Overall, all three studies reached the

conclusion that basophils are necessary for the induction of Th2 responses by not only secreting IL-4 but also by capturing, processing, and presenting antigen to naive T cells.

Despite the strong *in vivo* evidence for basophils acting as APCs required for Th2 responses, at least one study has challenged this concept. Using an inhaled dust mite allergen model, Hammad *et al.* (122) found evidence that basophils contribute but are not necessary or sufficient to induce Th2 responses. Instead, the authors concluded that so-called inflammatory DCs (iDCs) expressing high affinity IgE receptors (the $\alpha\gamma_2$ variant) were responsible for the Th2 immunity and stressed that previous studies targeting Fc ϵ RI positive basophils for depletion had inadvertently removed these Fc ϵ RI-positive DCs. Moreover, the response was additionally shown to be dependent on TLR4 – a concept consistent with previous studies indicating the importance of low-level endotoxin in inducing Th2 responses. Overall, these results are in of themselves quite remarkable, since transfection studies had previously shown that the $\alpha\gamma_2$ variant is not expressed in rodent cells but only in human cells. Indeed, the current study saw no evidence for $\alpha\gamma_2$ on conventional DCs, but only on iDCs, with additional *in vitro* data showing that its expression is partially regulated by IL-3 and/or GM-CSF. Importantly, there is one previous study reporting $\alpha\gamma_2$ expression on mouse DCs only after viral infection, further suggesting that this receptor may only be induced on mouse cells following specific inflammatory conditions (123).

Whether human basophils act as professional APCs has not been addressed, but there are several inconsistencies with the mouse model that currently make it difficult to accept. Foremost, and as noted above, flow-based analyses conducted during that past approximately 20 years have all shown results indicating that human basophils lack HLA-DR expression. Indeed, basophils are often distinguished from CD123⁺ pDCs as HLA-DR⁻ cells. Of course, these findings are the result of looking at resting cells, so it remains possible that basophils might acquire MHC class II expression following certain modes of activation. In support of this hypothesis, we have seen preliminary evidence for HLA-DR expression following IgE-dependent activation, although staining was far less (1–2 logs) than what is seen on resting blood DCs (JTS, unpublished observations). Moreover, IgE/Fc ϵ RI interactions have long been implicated in facilitating antigen presentation in many human APC types, with recent *in vivo* evidence to support this claim. Thus, it seems probable that other IgE-bearing cells, such as basophils (and mast cells), possess similar capabilities. Importantly, there is at least one report indicating that MHC class II expression on basophils is restricted to immature cells (124). In this study,

HLA-DR expression was identified on cultured-derived basophils, with levels peaking between 1 and 2 weeks. This expression, however, had waned by 3 weeks – a time when other markers indicated mature basophils. It is therefore possible that in humans, immature basophils possess APC activity and that this also accounts, in part, for some of the findings thus far observed in mice. Yet another explanation, as noted above, regards the issue of whether tissue- and/or lymphoid derived basophils acquire these molecules *in vivo*. In particular, many of the rodent studies thus far have investigated basophils in/from lymph nodes, spleen, and lung, which may differ considerably from those circulating in blood. As a result, it seems pertinent to investigate in humans whether basophils infiltrating allergic lesion sites might differ from blood basophils in expressing MHC class II and/or accessory cell molecules, let alone possess APC activity.

While there remains a great deal of uncertainty as to whether human basophils possess APC activity, there is indirect evidence that the mediators they secrete can influence T-cell and DC function in a way that is pro-Th2 in nature. Naturally, IL-4 is well known for its activity in promoting Th2 development. However, histamine is known to alter the cytokine profiles of maturing monocyte-derived DC resulting in a change from Th1 to Th2 in their T cell polarizing function (125). Likewise, histamine mediates similar activity in pDCs by inhibiting the production of IFN- α , a cytokine now recognized as much for its pro-Th1 activity as it is for mediating antiviral effects (126). Thus, these observations support the belief that basophils, rather than acting as professional APCs, may instead alter DC/T-cell maturation/function towards favoring Th2 responses (Fig. 4).

Cytokines

Mouse basophils are reported to make many more cytokines that have not yet been detected using human basophils (Table 1). Again, the current rationalization for this discrepancy is that the mouse studies have used tissue- and/or lymph node-derived cells rather than those isolated from blood, as is typically done when investigating human basophils. However, IL-3-dependent mouse bone-marrow-derived basophils were also shown to express IL-5, TNF- α , IL-6, and TSLP, thus implying that tissue-derived factors are not necessarily required for the production of these cytokines (62). Yet, there is some concern that several of the cytokines expressed by mouse basophils may actually be produced by contaminants, particularly DCs. For instance, we have shown that human DCs (both pDC and mDC) express the $\alpha\gamma_2$ variant of Fc ϵ RI,

Table 1. Primary cytokines secreted by human basophils in response to IgE-dependent and -independent activation

	IgE-dependent	IgE-independent (e.g. IL-3)	Method of detection
IL-3	+++	—	E
IL-4	+++	+	E, IC, MP
IL-5	—	—	E, MP
IL-6	—	—	E, MP
IL-8	+++	+	E, MP
IL-13	++	+++	E, IC, MP
TNF-α	—	—	E, MP
TSLP	—	—	E
VEGF	++	+	E, MP
MIP-1 α	++	+	E
RANTES	++	+	E, IC

Bold type denotes those cytokines additionally made by mouse basophils that are not produced by human basophils.

+, relative secretion response; E, ELISA; IC, intracellular staining; MP, multiplex.

and when stimulated in an IgE-dependent fashion, produced IL-6 and TNF- α at very high (ng/ml) levels (46, 112, 127). It is our experience that even a small 1% contamination by these cells could give the impression that basophils produce these cytokines when activated through Fc ϵ RI. Thus, the same issues stressed above regarding basophils being mistaken for DC-dependent APC activity may also apply here for cytokines. Nevertheless, these inconsistencies between the two models point to a need for translational studies, which are essential in moving the field forward not to mention towards understanding how basophils might be better targeted for therapeutic intervention.

Conclusion

Basophils can no longer be viewed as insignificant players in the allergic disease diathesis. These granulocytes produce and secrete three classes of mediators, including vasoactive amines (histamine), arachidonic acid metabolites (LTC₄), and proinflammatory cytokines (e.g. IL-4/IL-13), all of which are pivotal in promoting allergic inflammation and disease. In humans, these mediators are largely secreted as a result of allergen interacting with receptor-bound IgE. However, specific cytokines, IL-3 in particular, are critical modulators of this response and also have the capacity to function as complete stimuli for distinct effects. Recent studies show, in fact, that basophils themselves produce IL-3, and this ability to do so allows for autocrine priming, possibly resulting in the hyper-responsive phenotypes evident in allergic disease. Mounting evidence is also indicating that innate immune factors play a key role in modulating basophil responses, with some having augmentative effects on mediator release, while others suppress. Finally, new IL-4 reporter mice are providing mechanistic data that not only substantiate basophils as an important source of this cytokine *in vivo* but are further indicating that these cells migrate to lymphoid tissue to act as APCs in initiating Th2 responses. These provocative findings, while somewhat inconsistent with the biology known for human basophils, point to a greater need for translational studies, since confirmation will give new meaning to the role basophils have in human allergic disease.

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