Published in final edited form as:

Ann N Y Acad Sci. 2011 January ; 1217: 166–177. doi:10.1111/j.1749-6632.2010.05918.x.

New insights into basophil biology: initiators, regulators and effectors of type 2 inflammation

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Abstract

Recent studies indicate that basophils perform essential functions in multiple models of Th2 cytokine-dependent immunity and inflammation. In addition to their role as late phase effector cells, basophil populations can express MHC class II and co-stimulatory molecules, migrate into draining lymph nodes, present antigen to naive CD4⁺ T cells and promote Th2 cell differentiation. In this context, basophils have been shown to contribute to the induction and propagation of Th2 cytokine responses following exposure to some helminth parasites or allergens. In this review, we discuss recent studies that provide new insights into basophil development, regulation and effector function. In addition, we discuss the ability of basophils to act both independently and cooperatively with dendritic cells to support Th2 cytokine-mediated inflammation.

Keywords

basophile; Th2 cell; helminth; cytokine; immunity; inflammation

Introduction

Basophils are the least abundant granulocyte population accounting for less than 1% of leukocytes in the blood and spleen. Although originally discovered in 1879 by Paul Erlich, who identified them by treating blood samples with aniline dyes, basophil effector functions were not appreciated for almost a century. In 1972 basophils were demonstrated to bind immunoglobulin (Ig)E and release histamine, yet they were considered to be a redundant mast cell-like population that lacked unique effector functions^{1, 2}. However, subsequent studies directly comparing mast cells and basophils illustrated that these cell populations differ in their differentiation pathways, ultrastructural phenotype, surface marker expression and release of inflammatory mediators^{3–5}. Despite these advances, the effector functions of basophils could not be properly interrogated due to the inability to isolate or deplete basophils *in vivo* and the lack of a relevant animal model.

In 1981, a “persisting cell” or P cell was identified and ultimately became the first reported identification of a basophil-like population in mice. The P cell contained histamine and was found to be unique from mast cell populations⁶. However, the first cell population termed

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“mouse basophils” was not officially identified until 1982, when Dvorak and colleagues characterized a granular cell population in the bone marrow (BM) of mice that resembled the ultrastructural characteristics of human and other mammalian basophils⁷. Significant advances in understanding basophil biology in murine models was aided by the development of two interleukin (IL)-4/eGFP reporter mice and the discovery that basophils acquire constitutive IL-4 mRNA expression during their development^{8–10}. These advances allowed for a comprehensive analysis of surface marker expression on basophils by flow cytometry and eventually provided the basis for isolation and depletion strategies. Murine basophils were found to have a surface phenotype consistent with that of human basophils (FcεRI⁺, CD49b⁺, CD69⁺, Thy-1.2⁺, CD123⁺, CD200R⁺, CD117⁺, CD19⁺, CD14⁺, CD122⁺, CD11c⁺, Gr-1⁺, NK1.1⁺, B220⁺, CD3⁺, γδTCR⁺, αβTCR⁺)^{2, 8, 9, 11, 12}. Methods of depleting basophils were also established by targeting the high-affinity IgE receptor or the membrane glycoprotein CD200R^{13–16}. The ability to identify and deplete basophils in mice allowed for a series of studies that significantly advanced the understanding of basophil functions. More specifically, these studies identified a fundamental role for basophils in the induction and maintenance of Th2 cytokine-dependent immunity and inflammation and have promoted a renewed interest in the factors that regulate basophil development, activation and function. The purpose of this review is to provide a comprehensive overview of findings that describe the pathways that regulate the development, phenotype, activation and functions of both murine and human basophils. We will first review the established role of basophils as mediators of allergic disease in humans. Next, studies that have identified non-redundant functions of basophils as both initiators and propagators of Th2 cytokine-mediated immunity and inflammation will be highlighted. Lastly, recent studies that implicate basophil populations as antigen presenting cells (APCs) that may work independently of, or cooperatively with, dendritic cells (DCs) and other professional APC populations to promote Th2 cell development will be discussed.

Basophils in human disease

Basophils, like mast cells, are capable of producing abundant quantities of secreted mediators that contribute to immediate hypersensitivity reactions and basophil responses in human disease have primarily been associated with allergic disorders^{2, 17}. A number of recent studies have revealed previously unrecognized functions of basophils and identified mediators that support the population expansion, survival and functional responses of these rare circulating leukocytes. As such, a new appreciation for the potential contribution of basophils to host protective and aberrant inflammatory responses in human disease has come to light. Although to date no basophil-specific therapeutics have been tested in humans and their explicit contributions to human disease are yet to be determined, basophils are associated with several human diseases.

For example, basophils are elevated in the airways of asthmatics¹⁸, increase with exacerbations¹⁹ and elevated basophil numbers are associated with fatal asthma²⁰. Additionally, airway allergen challenge of asthmatic patients results in the accumulation of higher numbers of basophils relative to mast cells²¹. Peripheral blood basophil counts are also reported to be increased in patients diagnosed with asthma and are associated with asthma symptoms including airway hyper-responsiveness and decreased lung function²². One likely contribution of basophils to the pathogenesis of asthma is through their production of factors capable of influencing both early and late stage asthmatic responses. Along with mast cells, activated basophils are capable of rapid secretion of histamine, leukotriene C₄ (LTC₄) and platelet activating factor (PAF), all major mediators of acute bronchoconstriction^{3, 23}. Additionally, basophils secrete an expansive array of other inflammatory mediators including cytokines and chemokines that further sustain and orchestrate heterogeneous asthmatic responses²⁴.

Although not normally present in tissues of healthy subjects, basophils are detected in and associated with a variety of skin disorders suggesting a role in the etiology of these diseases. Basophils are found in skin biopsies of atopic and allergic contact dermatitis patients and similar to airway disease, are increased following allergen challenge^{25–27}. Systemic sclerosis patients demonstrate reduced basophil responses to anti-IgE and are refractory to the priming effects of IL-3 on IgE-mediated histamine release compared to controls²⁸. Basophils are also implicated in chronic urticaria (hives), a common skin disease with autoimmune components involving skin mast cells, blood basophils and recruitment of basophils to skin lesional sites²⁹.

While basophils are most commonly associated with allergic diseases, basophils were recently reported to play a role in systemic lupus erythematosus (SLE), an autoimmune disease characterized by autoantibody production. Individuals with SLE were found to have self-reactive IgE and circulating basophils in an activated state expressing CD62L and HLA-DR, which were associated with active disease compared to healthy controls. Basophils were also found in the spleen and lymph nodes of SLE patients examined in the study whereas control subjects without SLE lacked basophils in these secondary lymphoid organs³⁰.

The potential involvement of basophils in diseases with autoimmune components such as SLE broadens the perception of basophils as exclusively effector cells of allergic inflammation to cells that may play a central role in a variety of human disease conditions. However, technical limitations have prevented researchers from definitively demonstrating a role for basophils in these processes. The therapeutic blockade of IgE interactions with FcεRI by omalizumab, a monoclonal antibody specific for IgE, has been useful in distinguishing the contributions of mast cells versus basophils in human allergic disease and is to date the most basophil-specific therapy tested in humans^{31–32}. As no specific genetic deficiency has been identified that leads to a selective loss of basophils in humans³³, development and testing of basophil targeted therapeutics will be necessary to determine their functional role in the variety of human diseases they are associated with.

Despite the challenges and limitations of working with patient-derived samples, recent data identifying unique effector functions of murine basophils have significantly expanded our understanding of the factors that regulate basophil development and function and further implicate them as contributors to human disease.

Basophil development

Basophils develop from hematopoietic stem cells in the BM, however, the characterization of the molecular events and signaling pathways that determine the lineage commitment of basophils remains incomplete. In this section, we will discuss the progenitor cell populations and molecular events that contribute to basophil development.

Basophils are reported to develop from a common granulocyte-monocyte precursor in the BM that has the capacity to develop into an eosinophil precursor (EoP), a basophil-mast cell precursor (BMCP), a mast cell precursor (MCP) and a basophil precursor (BaP) *in vitro*³⁴. MCPs and BaPs have been reported to reside in the BM where they give rise to mature mast cells and basophils respectively³⁵. In contrast, BMCP are thought to migrate to the spleen where they also have the capacity to develop into mature basophil populations³⁵.

Although the lineage commitment of BaPs and BMCPs to mature basophils is not fully understood, it appears that the expression of the transcription factor GATA-2 and the CCATT enhancer-binding protein alpha (C/EBPα) are critical³⁵. Granulocyte-monocyte precursors are known to constitutively express C/EBPα. Maintenance of C/EBPα

expression, coupled with upregulation of GATA-2 expression, promotes development of EoPs and ultimately eosinophils³⁵. In contrast, if C/EBP α expression is downregulated while GATA-2 expression is increased in granulocyte-monocyte precursors, these cells develop into BMCPs that have the capacity to mature into mast cells or basophils³⁵. It is thought that the expression of C/EBP α in BMCP populations is critical to their lineage commitment to either a mast cell or a basophil³⁶. It is reported that the continued downregulation of C/EBP α expression in BMCPs results in the development of MCPs that express GATA-2 and have the capacity to mature into mast cells³⁶. However, if BMCPs regain the expression of C/EBP α they are reported to develop into BaPs which possess the capacity to develop into mature basophils³⁶. In addition to the expression of transcription factors, it is also known that eosinophils, mast cells and basophils express IL-4 and IL-13 mRNA during their maturation. However the stage of development at which this occurs, and the stimuli and signaling pathways that regulate this have not been determined¹⁰.

Unlike mast cells which exit the BM as precursors and fully mature in peripheral tissues, basophils migrate from the BM to the periphery exhibiting a mature phenotype (Fig. 1; ③)³⁷. The life span of a mature basophil is estimated at 60–70 h, much shorter than that of mast cells, which has been estimated at weeks to months^{35, 38, 39}. Basophil populations are very small in number at baseline, but are known to expand in response to the production of growth factors such as IL-3, which is thought to be critical for basophil activation, population expansion and subsequent survival (Fig. 1; ①)^{40–43}.

Basophil activation and effector function

Basophils can be activated by a broad spectrum of signals including those mediated by cytokines, antibodies, proteases, toll-like receptor (TLR) ligands and complement factors (Fig. 1; ④). Once activated, basophils produce a variety of effector molecules including preformed mediators such as histamines, LTC₄ and antimicrobial peptides. Basophils are also reported to produce the cytokines IL-4, IL-5, IL-13 and TSLP, and various chemotactic factors (Fig. 1; ⑤, ⑥, ⑦)^{2, 17}. In the following sections we will review the stimuli known to activate basophils and discuss the mediators they produce.

Cytokine-mediated activation of basophils

IL-3 has been shown to expand mature basophils from BM precursor cells *in vitro* and administration of IL-3 *in vivo* induces the generation of basophils in mice^{42, 43}. Furthermore, IL-3–IL-3R signaling is reported to be critical for the population expansion of peripheral basophil populations in response to the helminth parasites *Nippostrongylus brasiliensis* and *Strongyloides venezuelensis*⁴⁰. Further, IL-3 can enhance the release of mediators and other effector functions of basophil populations. For example, IL-3 enhances the production of IL-4 and IL-13 from basophils in response to IgE-mediated activation^{44, 45}. Collectively, these data support the hypothesis that basophil responses are critically dependent on IL-3–IL-3R signaling. However, basophil development is normal in the absence of IL-3–IL-3R signaling, suggesting that other cytokines or growth factors may be capable of regulating basophil development and function (Fig. 1; ②)⁴².

In addition to IL-3, it is now appreciated that the IL-1 family members IL-18 and IL-33 can activate and enhance basophil functions (Fig. 1; ④)^{46, 47}. IL-18 is produced by macrophages, Kupffer cells and mast cells and is associated with allergic disease and immunity to helminth parasites^{24, 48–51}. Consistent with its role in Th2 cytokine-mediated immunity and inflammation, administration of IL-18 to mice enhanced basophil-specific IL-4 and histamine production⁵⁰. IL-18 signals through MyD88 to enhance the production of Th2 cytokines from murine BM-derived basophils⁴⁶. Although human basophils are known to express the IL-18 receptor, their activation by IL-18 has not been reported^{47, 52}.

IL-33 is expressed by dermal fibroblasts, airway epithelial cells and bronchial smooth muscle cells and is associated with IL-4, IL-13 and IgE production^{53, 54}. Similar to IL-18, IL-33 signals through MyD88 to enhance the production of IL-4 and IL-13 from murine BM-derived basophils⁴⁶. In addition, IL-33 can induce the production of IL-4, IL-5, IL-6 and IL-13 from human blood-derived basophils⁵⁵. Collectively, these data illustrate that IL-18 and IL-33 both directly activate and enhance basophil activation.

IgE-, IgG1- and IgD-mediated activation of basophils

Like mast cells, which also express the high-affinity IgE receptor FcεRI, basophils release inflammatory mediators such as histamine and LTC₄ in response to IgE-mediated activation (Fig. 1; ④)². Basophils are known to contribute to allergic responses by inducing smooth muscle contraction once they migrate to the area of inflammation during the late phase response. In addition to their well-established role as late phase effector cells, the ability of basophils to release preformed mediators in response to surface bound IgE-mediated activation suggests that they may contribute to systemic anaphylaxis. Several studies in patients indicate that basophils contribute to anaphylaxis when exposed to blood borne antigens⁵⁶. Although similar studies have been done in mouse models, basophils have not been shown to contribute to IgE-mediated anaphylaxis. However, studies in C57BL/6 mice have shown that basophils are activated and produce PAF in an alternative pathway of IgG1-mediated anaphylaxis¹⁴. C57BL/6 mice and C57BL/6-Kit^{W-sh/W-sh} mast cell-deficient mice sensitized with Penicillin V-bovine serum albumin and subsequently challenged with Penicillin V-bovine serum albumin develop IgG1-, FcγRII/III-dependent systemic anaphylaxis¹⁴. Critically, depletion of basophils prior to a secondary challenge with Penicillin V-bovine rescues both mouse models from anaphylaxis¹⁴.

In addition to their role inducing immediate anaphylaxis, basophils have also been shown to contribute to a novel type of chronic IgE-mediated allergic inflammation in mice¹⁵. Although basophils are not necessary for the immediate or late-phase inflammation that is induced after multivalent antigens are administered subcutaneously into the ear, they are required for the IgE-mediated chronic inflammation that follows¹⁵. IgE-mediated chronic inflammation was found to occur independently of T cell and mast cell populations, but was dependent on CD49b⁺, FcεRI⁺ basophils. Notably, basophils only comprised 1–2% of the cellular infiltrate in the lesion, but their depletion resulted in a dramatic reduction in inflammation marked by reduced neutrophil and eosinophil infiltrates¹⁵.

In addition to the well-characterized ability of basophils to be activated by antigen-IgE complexes, basophils have also been shown to be activated by the IgD antibody class (Fig. 1; ④)⁵⁷. IgD is a class of antibody that is known to be produced during early phases of B cell development, but its biological functions remain poorly defined. Although its functions remain in question, IgD is highly expressed in the human upper respiratory tract and is known to bind the bacteria *Haemophilus influenzae* and *Moraxella catarrhalis*⁵⁷. Further, IgD can activate basophils to produce a broad spectrum of antimicrobial peptides. Interestingly, the IgD-mediated activation of basophils had no effect on histamine release, suggesting that this pathway of activation differs significantly from that of IgE-mediated activation⁵⁷. In addition to antimicrobial peptides, IgD-mediated activation also results in the increased production of IL-4 and B cell activating factor. Furthermore IgD-activated basophils induced IgM, IgD, and IgA class switching from B cells and supernatants from IgD-activated basophils were capable of inhibiting the replication of *H. influenzae* and *M. catarrhalis*⁵⁷. Collectively these data demonstrate that basophils can activate B cell populations and possess antimicrobial functions. In addition, the presence of IgD and basophils in the upper respiratory tract of humans suggests that IgD-activated basophils may provide critical protection against upper respiratory tract infections.

Direct activation of basophils by proteases and glycoproteins

Although basophil activation is thought to be largely antibody- and cytokine-mediated, allergens and parasite antigens are also capable of activating basophils directly. Specifically, the house dust mite antigen Derp1, an active protease, can induce the production of IL-4, IL-5 and IL-13 from human KU812 basophils in the absence of antigen-specific IgE⁵⁸. In addition, proteases secreted by the hookworm parasite *Necator americanus* directly induced the production of Th2 cytokines from KU812 basophils in the absence of IgE⁵⁸. It was also demonstrated that treating Derp1 and *N. americanus* antigens with protease inhibitors eliminated the ability of those antigens to activate basophils, suggesting that basophils may express mechanisms that are capable of sensing protease activity⁵⁸. Protease-activated receptors (PARs) are a potential family of receptors that are known to sense proteolytic activity and activate innate and adaptive immune cells⁵⁹. However, there is no evidence indicating that either human or murine basophils express known PAR family members. These data suggest that basophils may express other PAR-like receptors, or sense proteolytic activity through other molecular mechanisms⁶⁰.

In addition to proteases, it has also been demonstrated that a class of “super antigens” can activate basophils independently of antigen specific antibodies⁶¹. For example, the gp120 glycoprotein of the human immunodeficiency virus has been shown to interact with the VH3 region of IgE in a manner that induces IL-4 and IL-13 production from human basophils⁶². Furthermore, murine basophils are activated by the schistosome-derived glycoprotein IPSE/alpha-1, which induces the production of IL-4 from basophils in a nonspecific manner⁶⁴.

Activation of basophils through pattern recognition receptors

Several studies have reported the expression of TLRs on human basophil populations including TLR1, TLR2, TLR4, TLR6 and TLR9^{65–67}. These data suggest that basophils may be capable of recognizing pathogen-associated molecular patterns (PAMPs) expressed by various pathogens. However, only TLR2 ligands have been shown to activate basophils by inducing the production of IL-4 and IL-13⁶⁵. In addition to human basophils, it has also been reported that murine basophils express TLR1, TLR2, TLR4 and TLR6⁶⁸. Despite these reports, the ability of human and murine basophils to respond directly to TLR ligands requires further analysis.

In addition to TLRs, human basophils have also been shown to express the complement receptors CR1, CR3, CR4 and CD88. Furthermore, it has been shown that human basophils produce histamine in response to the complement component C5a in an IgE-independent manner⁶⁹. These data further illustrate innate mechanism of basophil activation.

Th2 cytokine-dependent immunity and inflammation

Basophils have historically been thought of as late phase effector cells that migrate to the site of inflammation after a Th2 cytokine response is established. However, recent data have demonstrated that in addition to effector functions, some basophil populations play a central role in the induction and propagation of Th2 cytokine-mediated immunity and inflammation. The following section will highlight recent studies that have demonstrated the ability of MHC class II⁺ basophils to migrate into secondary lymphoid tissues and promote CD4⁺ T cell differentiation and proliferation (Fig. 1; ⑧).

Basophil population expansion and activation are hallmarks of Th2 cytokine responses initiated during allergic responses and helminth infections. Basophils are known to be a potent source of IL-4 production in the context of these inflammatory responses that is likely

to promote CD4⁺Th2 cell-mediated inflammation⁷¹. Earlier studies demonstrated that basophils isolated from the spleen, liver or BM are capable of initiating Th2 cell development in the presence of antigens and DCs^{72, 73}. IL-4-deficient basophils failed to promote Th2 cell development *in vitro*, suggesting that IL-4 is the critical Th2 inducing factor produced by basophils⁷³. In addition to primary basophils, IL-3-elicited basophils from BM progenitor cells were also able to induce Th2 cell differentiation in the presence of DCs and antigen⁷³. Collectively, these data provided evidence to support the principle that basophils can produce IL-4 and promote Th2 cell differentiation when DCs and cognate antigen are present.

In vivo studies further confirmed the ability of basophils to promote Th2 cell development. For example, CD4⁺ T cells stimulated in the presence of enhanced basophil populations *in vivo* preferentially differentiated into Th2 cells. Further, mice deficient in interferon regulatory factor 2 (IRF2^{-/-}), which have significantly increased basophil populations, demonstrated enhanced IL-4 production by CD4⁺ T cells upon *in vivo* stimulation⁷². Collectively, these studies implicated basophils as potential liaisons between innate IL-4 production and the development of adaptive forms of Th2 cytokine-mediated immunity and inflammation.

Migration of basophils into peripheral lymph nodes

Basophils are predominantly found in the blood and spleen, but are recruited to the site of inflammation after exposure to stimuli such as allergens or helminth parasites⁷⁴. Although previously reported to be excluded from LNs, recent studies demonstrated that IL-4/eGFP⁺, MHC class II⁺ murine basophils migrate to the draining LNs following exposure to papain, *Schistosoma mansoni* eggs or *N. brasiliensis* infection^{75–78}. MHC class II⁺ human basophils are also found in the LNs of patients suffering from SLE³⁰. Although basophils are thought to be transiently present in the LNs, these data suggest that basophils are capable of directly interacting with LN-resident CD4⁺ T cells, B cells and APC populations. Critically, it was also shown that depleting basophil populations prior to papain challenge in mice prevented the accumulation of IL-4/eGFP expressing CD4⁺ T cells, suggesting an essential role for basophils in the induction of papain-induced Th2 cell differentiation⁷⁵.

Although the mechanisms by which basophils enter LNs and their importance in inducing CD4⁺ T cell differentiation remain unknown, a recent study suggests that IL-3 may be a critical regulator of basophil LN recruitment subsequent to *N. brasiliensis* infection. *N. brasiliensis* induced the recruitment of basophils to the mediastinal lymph nodes between days 4 and 10 post-infection^{77, 78}. Critically, basophils failed to accumulate in the LNs in the absence of IL-3-IL-3R signaling, however, the loss of basophils in the LNs of IL-3^{-/-} mice did not prevent the induction of Th2 cell differentiation, suggesting that in the specific case of *N. brasiliensis*, basophils are not essential for the induction of Th2 cytokine-mediated immunity⁷⁷. Further, although basophils are critical for secondary immunity to *N. brasiliensis*, the induction of Th2 cytokine-mediated inflammation during primary infection was not diminished in mice that lack, or were depleted of basophil populations^{78, 79} (refer to Fig. 2). The basophil-independent nature of Th2 cell responses in this model is perhaps not surprising considering that multiple redundant mechanisms for Th2 cell development are initiated subsequent to *N. brasiliensis* infection. Previous studies have demonstrated that Th2 cell development post-*N. brasiliensis* infection can also occur in the absence of STAT6 or the combined absence of IL-5, IL-9 and IL-13 signaling^{80, 81}. These studies provoke fundamental questions regarding the relative contributions of basophils to local IL-4 production or antigen presentation and suggest that the functions of basophils may vary depending on the nature of the antigen, the strength of signal and the location of challenge.

Basophils as APCs

Several studies demonstrated that DCs can directly promote Th2 cell development and contribute to Th2 cytokine-dependent immunity and inflammation. For example, intratracheal instillation of ovalbumin (OVA)-pulsed DCs was sufficient to sensitize mice to airway hyper-responsiveness in a murine model of asthma and depletion of DCs during airway challenge with OVA resulted in a decreased Th2 cytokine-mediated inflammation in the lungs.⁸² In a model of HDM-induced allergic airway inflammation, FcεRI⁺, CD11c⁺, MHC class II⁺ inflammatory DCs were both necessary and sufficient for the induction of Th2 cytokine-mediated airway inflammation⁸³. In addition, *in vivo* depletion of CD11c⁺ dendritic cells following *S. mansoni* infection, a helminth infection known to modify DCs towards a phenotype that initiates Th2 cell differentiation^{84, 85}, disrupted Th2 cytokine responses⁸⁶ (refer to Fig. 2). Further, work from our own lab also demonstrated that DCs are sufficient to promote Th2 cytokine responses and protective immunity to *Trichuris* in the absence of IFN-γ⁷⁶.

However, a number of recent studies indicate that depending on the stimulus and/or model system employed, DCs may not be essential for the development of Th2 cytokine-mediated immunity and inflammation. For example, in mice in which MHC class II expression is restricted to CD11c⁺ DC populations (MHC II^{CD11c}) Th2 cell development and Th2 cytokine-dependent immune responses are significantly impaired following exposure to helminth infection or allergens^{76, 87}. These studies showed that MHC II^{CD11c} mice failed to develop Th2 cytokine responses after infection with *Trichuris*, immunization with papain or allergic airway sensitization with ovalbumin^{76, 87, 88}. Further, employing the CD11c-DTR model to selectively deplete DC populations throughout the duration of helminth infection, or after immunization with papain, failed to abolish Th2 cell differentiation^{76, 88}, supporting the concept that DC-independent Th2 cell responses can also develop *in vivo*. Collectively these data indicate that although CD11c⁺ DCs may contribute to the development of Th2 cytokine responses, in some *in vivo* systems they may not be necessary or sufficient for the development of optimal Th2 cytokine responses (refer to Fig. 2). Instead, these reports suggest that alternative APC populations may be critical for Th2 cytokine-mediated immunity and inflammation.

Three independent laboratories recently identified a previously unappreciated function for basophils as APCs in the context of helminth infections or exposure to allergens. In these studies, basophils were shown to endocytose soluble antigens and IgE-allergen complexes, express MHC class II and costimulatory molecules, migrate to draining LNs and promote Th2 cell differentiation *in vitro* and *in vivo*^{76, 88, 89}. For example, antigen-pulsed basophils were sufficient to promote papain-specific Th2 cell differentiation after adoptive transfer into an MHC class II-deficient host⁸⁸. Consistent with these findings, adoptively transferred IL-4/eGFP, MHC class II expressing basophils were capable of augmenting Th2 cell differentiation in response to *S. mansoni* egg antigens⁷⁶. In addition, depletion of basophils impaired protective immunity to *Trichuris* and eliminated Th2 cell development post-papain challenge, indicating an essential role for basophils in these systems of Th2 cytokine-mediated immunity and inflammation⁷⁶. Collectively, these data suggest that basophils are not only capable of producing soluble factors such as IL-4, IL-13 and TSLP, but also support the initiation and propagation of antigen-specific Th2 cell responses in an MHC class II-dependent manner.

Taken together, multiple studies demonstrated the importance of basophils and DCs in the induction of Th2 cytokine-mediated responses. The apparent identification of DC-dependent and DC-independent pathways of Th2 cell differentiation suggests that there are multiple pathways by which Th2 cell responses develop *in vivo*. For example, in some models of

helminth infection or allergic inflammation, Th2 cell differentiation may involve priming of naïve T cells by DCs coupled with the addition of IL-4 or other soluble factors by basophils. However, other models of antigen presentation by IL-4 and MHC class II-expressing basophils may be sufficient to promote Th2 cell differentiation. Alternatively, basophils and DCs may present antigen to naïve T cells cooperatively to initiate the propagation of optimal Th2 cell responses. A recent study suggests that Th2 cell development in response to immunization with the cysteine protease papain is dependent on cooperative responses between DC and basophil populations⁹⁰. Immunization with papain and OVA resulted in the release of reactive oxygen species (ROS) by DC populations. These studies reported that the release of ROS by DC populations initiated the production of oxidized lipids, triggered the production of TSLP by epithelial cells, which suppressed the production of IL-12 by DCs⁹⁰. This cascade of events induced production of CCL7 by DCs, which mediated the recruitment of basophil populations to the LN⁹⁰. This report suggests that DCs and basophils work in concert to initiate Th2 cytokine-mediated responses. The cooperative effects of DCs and basophils were also demonstrated in a model of HDM-induced airway inflammation⁸³.

In summary, these studies indicate that basophil-dependent, DC-dependent, and DC/basophil-codependent models of Th2 cytokine-mediated inflammation exist *in vivo* (summarized in Fig. 2). It is likely that the nature of these responses will depend on the characteristics of the antigen being studied, the duration and site of antigen exposure and whether the differentiation of Th1, Th17 or T_{reg} cells is occurring simultaneously.

Summary

Recent studies have significantly advanced our understanding of the factors that regulate basophil development, regulation and function. It is now appreciated that basophils develop from precursor cells in the BM that expand in number in response to growth factors such as IL-3 (Fig. 1; ①). Although IL-3 is a critical cytokine, it is clear that basophil development also occurs in the absence of IL-3-IL-3R signaling, suggesting that unknown factors and pathways may regulate basophil development and/or function *in vivo* (Fig. 1; ②). Upon their development, mature basophils exit the BM and enter the periphery (Fig. 1; ③). Once in the periphery, basophils can be activated by an array of signals including those mediated by cytokines (IL-3, IL-33 and IL-18), antibodies (IgG, IgD and IgE) and non specifically by allergens such as papain (Fig. 1; ④). Once activated, basophils are known to produce histamines (Fig. 1; ⑤), cytokines (Fig. 1; ⑥) and chemokines (Fig. 1; ⑦). Activated basophils are also capable of migrating to the LNs, where they produce TSLP, reduce IL-12 production from DCs, present antigen via MHC class II, and provide IL-4 that promotes the differentiation of naïve T cells (Fig. 1; ⑧). Basophils also function as late phase effector cells that migrate to inflamed tissues during an ongoing inflammatory response (Fig. 1; ⑨). Collectively, these data illustrate that basophils contribute to multiple components of the innate and adaptive immune response and suggest that further delineating the factors that regulate basophil development and function may lead to new therapeutic intervention strategies for helminth infection, allergic inflammation and some forms of autoimmunity.

Acknowledgments

We thank members of the Artis laboratory for helpful discussions and Steven Saenz, Will Bailis, Mario Noti and Gregory Sonnenberg for critical reading of the manuscript. Research in the Artis laboratory is supported by the NIH (AI61570, AI74878, AI087990 and AI083480, F32-AI085828, F31-GM082187, T32-AI060516, T32-AI007532, T32-CA09140, T32-AI055438, T32-AI05528, and S10RR024525), the Burroughs Wellcome Fund (Investigator in Pathogenesis of Infectious Disease Award), the Crohn's and Colitis Foundation of America, and pilot grants from the University of Pennsylvania (VCID, PGFI, and URI). MC is an employee of Amgen, Inc.

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Figure 1. Basophil development, activation and function

Basophils develop from precursor cells in the BM that expand in number in response to growth factors including IL-3 (① and ②). Once mature, basophils exit the BM and enter the periphery (③). Mature basophils can be activated by an array of signals including those mediated by cytokines (IL-3, IL-33 and IL-18), antibodies (IgG, IgD and IgE) and antigens (④). Although the tissue-restricted signals that regulate basophil effector function are poorly characterized, activated basophils are known to produce histamines (⑤), cytokines (⑥) and chemokines (⑦). Some basophil populations migrate to draining LNs (⑧) while others accumulate in inflamed tissues during an ongoing inflammatory response (⑨).

Challenge/Infection	Basophil population	Th2 cytokine response	Indicators	Indicators of DC manipulation	Indicators of Th2 cytokine response	Indicators
Papain	MAR-1	Reduced Th2	NT	NT	NT	NT
Papain	Mcpt8Cre	Normal Th2	NT	NT	NT	NT
Papain + OVA	MAR-1	Reduced Th2	NT	CD11c-DTR	Reduced Th2	NT
Papain + OVA	Mcpt8Cre	Normal Th2	NT	CD11c-DTR	Reduced Th2	NT
HDM	MAR-1	Reduced Th2	NT	CD11c-DTR	Reduced Th2	NT
HDM	Ba103	Reduced Th2	NT	CD11c-DTR	Reduced Th2	NT
Nippo 1°	MAR-1	Reduced Th2	NT	NT	NT	NT
Nippo 1°	Mcpt8Cre	Normal Th2	NT	NT	NT	NT
Nippo 2°	MAR-1	Reduced Th2	NT	NT	NT	NT
Nippo 2°	Mcpt8Cre	Normal Th2	NT	NT	NT	NT
Schisto	MAR-1	Reduced Th2	NT	CD11c-DTR	Normal Th2	NT
Schisto	NT	NT	NT	CD11c-DTR	Reduced Th2	NT
Schisto egg	MAR-1	Normal Th2	NT	CD11c-DTR	Reduced Th2	NT

Figure 2. Basophil- and dendritic cell-specific contributions to Th2 cytokine responses

Summary of experiments in which mice were challenged with papain, papain + ovalbumin (OVA), house dust mite (HDM), primary infection with *Nippostrongylus brasiliensis* (Nippo 1°), secondary infection with *N. brasiliensis* (Nippo 2°), *Trichuris muris*, *Schistosoma mansoni* (Schisto), or *S. mansoni* eggs (Schisto egg). Basophil populations were modified by treatment with anti-FcεR1MAR-1), expression of Cre recombinase under the mast cell protease 8 (*Mcpt8Cre*), or treatment with anti-CD200R3 (Ba103). Dendritic cell populations were modified by delivery of diphtheria toxin to mice that express the diphtheria toxin receptor under the CD11c promoter (CD11c-DTR), DC ablated mice (DeltaDC), or treatment with MAR-1. Th2 cytokine responses were evaluated post-basophil or DC manipulation. Experiments that did not test (NT) the contributions of basophils or DCs on Th2 cytokine response are indicated.