Functional heterogeneity in the basophil cell lineage

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Abstract

CD4^{+} T-helper type 2 (Th2) cells, characterized by their expression of interleukin (IL)-4, IL-5, IL-9 and IL-13, are required for immunity to helminth parasites and promote the pathological inflammation associated with asthma and allergic diseases. Recent reports from a number of laboratories have indicated that basophils can influence the induction and/or effector stages of Th2 cytokine-mediated inflammation. However, the impact of basophils appears to depend on the anatomical location and nature of the infectious or inflammatory stimulus. This review will highlight the factors that regulate basophil development and activation and will describe known basophil effector functions. Further, we will discuss the recent identification of phenotypic and functional heterogeneity within murine and human basophil populations and discuss how these findings may explain the context-dependent influence of basophils on either the propagation, regulation or effector phases of Th2 cytokine-associated inflammation.

Introduction

Basophils are the least abundant granulocyte population, accounting for less than 1% of leukocytes in the blood and spleen. Although originally described by Paul Erlich in 1879, their effector functions were not appreciated until 1972 when basophils were shown to bind immunoglobulin (Ig)E and release histamine[1, 2]. Despite these findings, basophils were thought to be a redundant cell population with effector functions similar to those of mast cells. However, subsequent studies directly comparing mast cells and basophils illustrated that these cell populations differ in their differentiation from progenitor cells, life span, anatomical location, surface marker expression and release of effector molecules[3–5]. Although these data demonstrated that basophils represent a unique cell population, the lack of an animal model prevented further interrogation of their functional properties *in vivo*. In 1981, a histamine containing basophil-like population termed a "persisting cell" or P cell was identified in mice[6]. However, the first cell population recognized as mouse basophils was not reported until 1982, when Dvorak et. al. characterized a granular cell population in murine bone marrow with ultrastructural characteristics similar to other mammalian basophil populations[7]. Advances in basophil biology were further aided by the development of interleukin (IL)-4/eGFP reporter mice and the determination that basophils acquire constitutive IL-4 mRNA expression during their development and can be easily identified by flow cytometric analysis[8–10]. These technical advances facilitated the identification of murine basophils as expressing surface markers consistent with that of human basophils

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(FcεRI$^+$, CD49b$^+$, CD69$^+$, Thy-1.2$^+$, CD123$^+$, CD200R$^+$, CD117$^-$, CD19$^-$, CD14$^-$, CD122$^-$, CD11c$^-$, Gr-1$^-$, NK1.1$^-$, B220$^-$, CD3$^-$, αβTCR$^-$, γδTCR$^-$)[2, 8, 9, 11, 12].

Methods of depleting murine basophils, including delivery of monoclonal antibodies targeting the high-affinity IgE receptor or the membrane glycoprotein CD200R3, lineage restricted expression of the diphtheria toxin receptor (DTR) or lineage restricted expression of Cre recombinase have also been developed[13–22]. The ability to deplete basophils has allowed for a series of *in vivo* studies that significantly advanced the understanding of basophil functions. Specifically, these studies identified a previously unrecognized role for basophils in contributing to optimal Th2 cytokine responses and prompted a renewed interest in the factors that regulate basophil development, activation and function.

This review will provide an overview of the molecules and pathways that regulate the development, activation and functions of murine and human basophil populations. In addition, we will highlight the recent discovery of phenotypic and functional heterogeneity in basophil populations and discuss how these findings may explain some of the paradoxical reports regarding the influence of basophils on the development to Th2 cytokine-mediated immunity or inflammation following exposure to either helminth parasites or allergens.

Basophil development

Like other myeloid lineages, basophils are thought to develop from hematopoietic stem cells in the bone marrow. However, many of the cytokines and growth factors that regulate basophil lineage commitment remain unknown. This section will discuss known progenitor cell populations and the molecular mechanisms that regulate basophil development. In addition, this section will highlight the recent discovery of basophil precursors in the periphery and the previously unrecognized ability of thymic stromal lymphopoietin (TSLP) to promote the development of a distinct basophil population from bone marrow-resident progenitor cells[23].

Basophils are reported to arise from a common granulocyte-monocyte precursor in the bone marrow that has the capacity to develop into eosinophils, basophil-mast cell precursors (BMCP), mast cell precursors (MCP) and basophil precursors (BaP)[24]. Bone marrow-resident MCPs and BaPs are reported to give rise to mast cells and basophils respectively, while BMCPs are reported to exit to the periphery but maintain the capacity to migrate back to the bone marrow where they can develop into basophils[25]. Unlike mast cells which mature in peripheral tissues, basophils are reported to enter the periphery with a fully mature phenotype. However, recent studies have also demonstrated that an IL-25-responsive multipotent progenitor cell population termed MPP^{type2} cells is present in the periphery, undergoes population expansion in response to heminth infection and has the capacity to mature into basophils[26]. The lineage commitment of basophil precursor populations is reported to be dependent on the expression of the transcription factors GATA-2 and the CCATT enhancer-binding protein C/EBPα[25]. In addition, studies have also demonstrated that mast cells, eosinophils and basophils initiate and maintain expression of IL-4 and IL-13 mRNA transcripts during their maturation[10]. Despite our understanding of these events, the cytokines and growth factors that initiate basophil development and whether plasticity exists between precursor populations remain poorly characterized.

The life span of a mature basophil is relatively short and is estimated to be on the order of 60–70 hours[25, 27, 28]. Thus, it is thought that basophil differentiation from precursor populations is continually occurring in order to maintain basophil populations in the periphery. Although present in very small numbers at baseline levels, basophil populations can significantly expand in response to certain factors, such as IL-3 (IL-3)[29–32]. IL-3 promotes the differentiation of basophils from bone marrow cells (Fig. 1A), induces the

generation of basophils in mice, and IL-3 signals are necessary for infectioninduced basophilia following *Nippostrongylus brasiliensis* and *Strongyloides venezuelensis* infections[29, 31, 32]. In addition, IL-3 can augment many aspects of basophil function including the release of effector molecules after IgE-dependent stimulation (Fig. 1B)[33, 34]. Collectively, these studies supported the hypothesis that basophil responses were critically dependent on IL-3 signaling.

In recent studies IL-3-IL-3R-independent basophil development and peripheral basophil responses were observed *in vivo*, and the epithelial cell-derived cytokine TSLP was critical in promoting these responses[23]. Previous studies have shown that murine TSLP is critical for the development of Th2 cytokine-mediated immunity and inflammation[35, 36, 37]. Consistent with these findings, studies in patients identified that gain-of-function mutations in the gene encoding TSLP and elevated TSLP expression are associated with the allergic diseases asthma, atopic dermatitis and eosinophilic esophagitis[38–41]. In *in vitro* studies, TSLP promoted the selective population expansion of murine basophils from bone marrow-resident progenitor cells (Fig. 1C). In addition, overexpression of TSLP resulted in the population expansion of basophils *in vivo*[23]. Critically, TSLP was capable of promoting murine basophil responses in the absence of IL-3-IL-3R signaling, demonstrating a previously unrecognized pathway of basophil development and activation[23]. The phenotypic and functional heterogeneity that distinguish TSLP-elicited versus IL-3-elicited basophils will be discussed in greater detail below.

Basophil activation and effector functions: the traditional view

Basophils can be activated by an array of stimuli including those mediated by antibodies, cytokines, proteases, TLR ligands and complement factors. Activated basophils are known to produce cytokines (IL-4, IL-13, IL-6, and TNFα), effector molecules (histamine, leukotriene C4 (LTC4) and antimicrobial peptides) and chemotactic factors[2, 42, 43]. The following sections will highlight current knowledge of the mediators of basophil activation and the effector functions they promote.

Some populations of basophils rapidly produce preformed effector molecules such as histamines, LTC4 and cytokines in response to crosslinking of FcεRI via surface bound IgE[2]. The rapid release of effector molecules in response to antibody-mediated crosslinking allows basophils to contribute to the development of systemic anaphylaxis. Several studies of human basophils suggest they degranulate via surface bound IgE stimulation and contribute to anaphylaxis when exposed to blood-borne antigens[44]. Although studies in mouse models have failed to identify a similar role for basophils during IgE-mediated anaphylaxis, reports have demonstrated that basophils produce platelet-activating factor and contribute to anaphylaxis mediated by IgG1[45]. Collectively, these studies indicate that some basophils are capable of contributing to anaphylaxis in both mice and humans.

Basophils can also contribute to a type of chronic allergic inflammation in mice[46]. Following subcutaneous injection of multivalent antigens into the ear, basophils were required for the development of IgE-mediated chronic allergic inflammation (IgE-CAI) in the skin. IgE-CAI was found to be mast cell- and T cell-independent, but was dependent on an FcεRI+, CD49b+ basophil population[46]. Although basophils comprised only a small percentage of the cellular infiltrate at the site of the chronic lesion in the skin, their depletion led to a dramatic reduction in inflammation. Specifically, basophil depletion resulted in a decrease in the number of eosinophils and neutrophils present at the site of the lesion and a marked reduction in ear thickness. The loss of infiltrating eosinophils and neutrophils suggests that basophils produce cytokines and/or other factors that directly result in cell recruitment, or indirectly induce the production of chemokines from tissue-resident cells[46].

Taken together, these results illustrate the potent inflammatory effects of small numbers of basophils and indicate a novel, non-redundant role for basophils in the initiation and maintenance of chronic IgE-mediated inflammatory responses in mice.

In addition to IgG1- and IgE-mediated activation, human basophils have been reported to selectively bind IgD, a class of antibody produced early in B cell development[43]. Although the biological function of IgD remains unclear, this study demonstrates that IgD is highly expressed in the human upper respiratory tract and can bind the respiratory bacteria *Haemophilus influenzae* and *Moraxella catarrhalis*. IgD-activated basophils exhibited distinct effector functions from IgE-activated basophils and expressed a broad spectrum of antimicrobial peptides, IL-4 and B cell activating factor[43]. Furthermore, IgD-activated basophils induced IgM, IgD, and IgA class switching from B cells, and supernatants from IgD-activated B cells were capable of preventing the replication of *M. catarhalis* and *H. influenzae*. Collectively, these data illustrate that IgD-activated basophils are functionally distinct from IgE-activated basophils and may play a protective role against respiratory pathogens[43].

In addition to antibody-mediated activation, some basophils can be activated by the cytokines IL-18 and IL-33[47, 48]. IL-18 is produced by innate immune cells such as macrophages and Kupffer cells and is known to play a role in allergic disease and immunity to helminth parasites[49–52]. 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]. Consistent with their ability to enhance Th2 cytokine-associated immune responses, IL-18 and IL-33 are also capable of activating basophil populations. IL-18 induces IL-4 production from bone marrow-derived basophils *in vitro*, and administration of IL-18 *in vivo* enhanced the production of IL-4 and histamine in mice[51]. Interestingly, although human blood basophils express the IL-18R at high levels, a role for IL-18 in the activation of human basophils has yet to be reported[47, 55]. Similar to IL-18, IL-33 induces IL-4 production from murine bone marrow-derived basophils, and IL-33 stimulation induces the production of IL-4, IL-5, IL-6 and IL-13 from human blood-derived basophils[56]. Furthermore, recent studies demonstrate that IL-33-responsive basophils produce IL-4 and are protective in a model of murine arthritis[57]. Collectively, these data demonstrate that the IL-1 family cytokines IL-18 and IL-33 are capable of inducing and/or enhancing human and murine basophil responses.

Emerging functions of basophils

Historically, basophils have been thought of as late phase effector cells that migrate into inflamed tissues after Th2 cytokine-dependent inflammation is established. As such, basophil population expansion is associated with chronic allergic inflammation and helminth infections. In the context of ongoing inflammatory responses, basophils are known to incorporate surface bound IgE via FcεRI and degranulate in response to FcεRI crosslinking[2]. In addition to IgE-mediated late phase effector functions, some basophil populations were reported to contribute to the induction and propagation of Th2 cytokine-mediated immunity and inflammation in the context of either allergen exposure or helminth infection[13, 14, 17, 19, 20, 23]. Subsequent reports in other model systems demonstrated that although basophils contribute, they are not necessary for the development of optimal Th2 cytokine-mediated immunity and inflammation, indicating that the requirement for basophils may be stimulus- or tissue location-dependent. [Hammad, #47][Phythian-Adams, #54]. Collectively, these reports have provoked many questions and generated some controversies regarding the ability of basophils to function as initiators of Th2 cell responses. The following sections will review recent reports on murine models of infection or allergic inflammation and highlight the recent discovery of functional heterogeneity between IL-3-

elicited versus TSLP-elicited basophil populations. We will discuss how basophil heterogeneity may inform the interpretation of these conflicting studies. Lastly, we will introduce a model illustrating how basophil heterogeneity within the basophil lineage may explain the differential requirement for basophils in the induction of Th2 cytokine responses in the context of some murine models of helminth infections or allergic disease but not others.

Influence of basophils on Th2 cell responses

It is well established that basophils are potent producers of IL-4 and as such may contribute to the induction and propagation of CD4^{+} Th2 cell responses. Consistent with this hypothesis, *in vitro* studies demonstrated that IL-4-producing basophils isolated from the spleen, liver or bone marrow are capable of initiating Th2 cell responses in the presence of DCs and antigen[58, 59]. In addition, IL-3-elicited bone marrow-derived basophils are also able to induce Th2 cell differentiation in the presence of DCs and antigen[59]. These data provided the proof of principle that IL-4-producing basophils can promote Th2 cell differentiation in the context of DCs and antigen. *In vivo* studies have further confirmed these data and demonstrated that CD4^{+} T cells stimulated in the presence of basophil populations preferentially adopt a Th2 phenotype. Collectively, these studies implicated basophils as a critical source of innate IL-4 required for the development of optimal Th2 cytokine-mediated immunity and inflammation.

In vivo basophil populations are primarily found in the blood and spleen, but are recruited to the tissues during inflammatory responses initiated by exposure to allergens or helminth parasites[23]. 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, house dust mite antigen (HDM), *Schistosoma mansoni* eggs or *N. brasiliensis* infection[13, 14, 17, 60]. Consistent with these data, MHC class II[+] human basophils are also found in the LNs of patients suffering from systemic lupus erythematosus[61]. These data further suggested that IL-4-producing basophils might be capable of interacting with T cells and influencing their activation and/or differentiation. The ability of basophils to interact with T cells in the LN was recently confirmed using two-photon imaging techniques. Specifically, Basoph8 mice, which express yellow fluorescent protein under the basophil-specific gene *Mcpt8*, were used to demonstrated that basophils make transient interactions with CD4^{+} T cells in the LN post-*S. mansoni* egg challenge[62]. However, unlike DC-T cell interactions, basophil-T cell interactions were of short duration and did not appear to form stable conjugates[62]. Similar results were also observed in the LN post-papain immunization. In addition, these studies also indicated that basophils failed to produce IL-4 in the secondary lymphoid tissues, but were capable of producing IL-4 in the lung tissue post- *N. brasiliensis* infection in a CD4^{+} T cell-dependent, IL-3-dependent manner[62]. Collectively, these data demonstrate that basophils are capable of interacting with CD4^{+} T cells in secondary lymphoid tissues, but that these interactions differ from those made by DC populations.

Antigen presenting cell capacity of basophils

Consistent with the ability of MCH class II[+], IL-4/eGFP[+] basophils to migrate into peripheral LNs, three independent laboratories 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 IgE-allergen complexes, express MHC class II and costimulatory molecules, migrate to draining LNs and promote Th2 cell differentiation *in vitro* and *in vivo*[13, 20, 63]. For example, antigenpulsed basophils were sufficient to promote papain-specific Th2 cell differentiation after adoptive transfer into an MHC class II deficient host[20]. 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 antigens[13]. Further demonstrating a role for some basophil populations in the induction of Th2 cytokine responses, basophil depletion impaired protective immunity to *Trichuris* and eliminated Th2 cell development post-papain challenge [Sokol, 2009 #44][Perrigoue, 2009 #40]. Collectively, these studies demonstrated that in the context of some allergens and helminth infections, basophils function as inducers of Th2 cytokine-mediated inflammation[13].

It is well established that DCs can directly promote Th2 cell responses. For example, in a murine model of asthma using intratracheal instillation of ovalbumin (OVA), pulsed DCs were sufficient to sensitize mice to airway hyper-responsiveness, and depletion of DCs prior to OVA challenge resulted in decreased Th2 cytokine production and reduced lung pathology[64]. DCs were also reported to play a critical role in the induction of Th2 cell responses during *S. mansoni* infection. In addition, work from our own lab demonstrated that DCs are sufficient to promote Th2 cytokine responses and protective immunity to *Trichuris* in the absence of IFNγ[13]. Further strengthening these previous reports, a series of additional studies were published directly addressing the relative contributions of DCs and basophil populations in induction of Th2 cytokine responses[21]. These studies demonstrated that FcεRI^{+}, CD11c^{+}, MHC class II^{+} inflammatory DCs were both necessary and sufficient for the induction of Th2 cytokine responses in an HDM model of airway inflammation; however, depletion of basophils in this model still resulted in a significant reduction in IL-4-expressing T cells suggesting that basophils also contribute to optimal Th2 cell responses in vivo[19]. In addition, *in vivo* depletion of CD11c^{+} DCs following *S. mansoni* infection severely disrupted Th2 cytokine responses, while depletion of basophils had no effect on Th2 cell development in response to subcutaneous challenge with *S. mansoni*-eggs[21]. A recent study also showed that Th2 cell development in response to immunization with the cysteine protease papain is dependent on cooperative responses between DCs and basophil populations[22]. Specifically, 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 events that mediated the recruitment of basophil populations to the LN, and these basophils worked in concert with DCs to promote optimal Th2 cytokine responses[22]. Thus, these reports indicate that there are likely to be multiple pathways leading to Th2 cell differentiation, some of which may be DC-dependent, some of which may be basophil-dependent and some of which may require cooperation between DC and basophil populations.

Functional heterogeneity in basophil populations

Despite the likely existence of multiple pathways that promote the development of optimal of Th2 cell responses, conflicting reports describing the differential requirement for basophils in contributing to the induction of Th2 cytokine responses have been attributed to the limitations associated with the methods of basophil depletion employed. Multiple methods of basophil depletion have been developed including administration of mAb targeting the high affinity IgE receptor (FcεRI) or the membrane glycoprotein CD200R, basophil-specific expression of the DTR or lineage restricted expression of Cre recombinase (which was demonstrated to be toxic to basophils)[13–22]. As is commonly found with many cellular depletion strategies, different methods of basophil depletion have limitations. For example, treatment of mice with anti- FcεRI is capable of depleting basophil, mast cell and inflammatory dendritic cell populations, while lineage restriction of Cre-recombinase to basophils results in an incomplete loss of basophil populations[17, 19]. These limitations, coupled with the use of multiple model systems, has created some controversies regarding the functions of basophils as initiators or regulators of Th2 cytokines responses. The recent discovery of phenotypic and functional heterogeneity in basophil populations provides an

additional explanation for the differential requirements for basophils in different disease models. The following section will highlight functional heterogeneity between IL-3-elicited versus TSLP-elicited basophil populations and discuss how these data could influence the interpretations of conflicting reports on basophil function.

Functional heterogeneity in basophil populations

As mentioned above, a previously unrecognized pathway of IL-3-independent, TSLP-dependent basophil development and activation was recently described. TSLP-elicited basophils were demonstrated to be phenotypically and functionally distinct from IL-3-elicited basophils[23]. Further, genome-wide transcriptional profiling illustrated that TSLP-elicited basophils expressed genes associated with biological functions of linoleic acid metabolism, cell adhesion and cell communication, while IL-3-elicited basophils expressed genes consistent with the biological functions of dendritic cell (DC) and macrophage maturation. Interestingly, increased linoleic acid metabolism has previously been associated with atopic disorders of the skin[65]. Further, TSLP-elicited basophils expressed higher levels of CD123, IL-18Ra and T1/ST2 compared to IL-3-elicited basophils, while IL-3-elicited basophils were larger and more granular that TSLP-elicited basophils[23]. Consistent with phenotypic data, TSLP-elicited basophils demonstrated an enhanced ability to produce IL-4, IL-6 and other cytokines and chemokines compared to IL-3-elicited basophils (Fig. 2A). In contrast, IL-3-elicited basophils exhibited a significantly enhanced ability to degranulate in response to FcεRI crosslinking via surface bound IgE compared to TSLP-elicited basophils (Fig. 2B)[23]. These data demonstrate that heterogeneity exists in basophil populations and suggests that TSLP-elicited basophils may produce more cytokines and chemokines following stimulation, while IL-3-elicited basophils exhibit more classical basophil functions such as degranulation and histamine production in response to FceRI crosslinking[23].

IL-3-dependent models of basophil activation

Many of the studies investigating the role of basophils as inducers of Th2 cytokine responses have employed the helminth infection models *N. brasiliensis* or *S. mansoni*[17, 18, 21, 62]. Critically, *Nippostrogylus* and *Schistosoma* infections are known to initiate Th2 cytokine-mediated immunity and inflammation independently of TSLP-TSLPR signaling[66, 67]. This suggests that the basophil populations elicited by *N. brasiliensis* and *S. mansoni* may be IL-3-dependent and exhibit more classical IgE-mediated effector functions. This hypothesis is supported by previous studies demonstrating that *N. brasiliensis*-induced basophilia and basophil recruitment to secondary lymphoid tissues are CD4+ T cell- and IL-3-dependent events[29, 60]. Further, the ability of basophils to produce IL-4 in the lung following *N. brasiliensis* infection is also dependent on CD4+ T cells and IL-3[62].

In the case of *S. mansoni*, the directly dependence of infection-induced basophilia on CD4+ T cells and IL-3 has not been investigated. However, it has been reported that the production of IL-4 from an FcεRI+, non B, non T (NBNT) cell population in the spleen of infected mice is dependent on CD4+ T cells and IL-3[68]. Further, studies from our own lab demonstrated that the recruitment of basophils to the draining LN post- *S. mansoni* egg challenge is dependent on CD4+ T cells and IL-3-IL-3R signaling (Siracusa, Pearce and Artis, unpublished). Collectively, these data suggest that *S. mansoni* also promotes IL-3-elicited basophil populations.

The demonstration that IL-3-elicited basophils appear to specialize in IgE-mediated effector functions suggests that *N. brasiliensis*- and *S. mansoni* induced-basophils may exhibit the same functions at TSLP-elicited basophils. Thus, it is likely that *N. brasiliensis*- and *S. mansoni*-induced basophils function more as late phase effector cells that migrate to tissues

after Th2 cytokine responses have been initiated. This hypothesis is supported by the recent demonstration that basophils migrate to the lungs and produce IL-4 after CD4^{+} T cell activation has occurred[17, 21, 62]. In addition, depletion of basophils in the context of *N. brasiliensis* infection or *S. mansoni* egg challenge did not prevent the induction of Th2 cytokines responses[21]. Further, it has also been reported that basophil populations initiated in response to a primary *N. brasiliensis* infection are capable of providing protective immunity to a secondary challenge in an IgE-dependent manner[18]. These data further suggest that IL-3-elicited basophils are late phase effector cells that mediate their *in vivo* effector functions via antigen-specific IgE (Fig. 3A).

TSLP-dependent models of basophil activation

In contrast to *N. brasiliensis* and *S. mansoni* infections, Th2 cytokine responses and protective immunity to *Trichuris muris* is dependent on TSLP-TSLPR signaling[36]. Consistent with these data, *Trichuris*-induced basophilia was shown to be dependent on TSLP-TSLPR interactions. Further, depletion of basophils via anti-FcεRI treatment resulted in a loss of Th2 cytokine production and protective immunity while TSLPR[+] basophils could restore immunity in TSLPR^{−/−} mice, suggesting *Trichuris*-induced TSLP-dependent basophils play a critical role in the induction of optimal Th2 cytokine responses and protective immunity in the context of *Trichuris* infection[13].

The importance of TSLP-elicited basophils in the induction of Th2-cytokine responses has also been demonstrated in a model of TSLP-TSLPR-dependent atopic dermatitis. Topical treatment with the vitamin D analogue MC903 resulted in the robust accumulation of basophils in the skin and the induction of significant atopic dermatitis-like pathology. MC903-induced basophilia was shown to be IL-3-IL-3R-independent, but was dependent on TSLP, as treatment with neutralizing anti-TSLP antibody prevented skin basophilia[23]. Further, blocking TSLP-TSLPR interactions resulted in reduced expression of Th2 cytokines and less cutaneous inflammation[69]. Critically, Th2 cytokine responses were also reduced when basophils were depleted via lineage-specific expression of DTR[23]. Together, these data are consistent with a role for TSLP-elicited basophils as potent producers of IL-4 and suggest that unlike IL-3-elicited basophils, TSLP-elicited basophils may function as initiators of Th2 cytokine responses (Fig. 3B).

Summary and future challenges

Recent studies regarding the relative contributions of DCs and basophils to the induction and propagation of CD4^{+} Th2 cells have resulted in conflicting reports and controversy. Many of the varied reports on the ability of basophils to contribute to Th2 cytokine mediated immunity and inflammation have been attributed to different experimental designs and basophil depletion strategies. However, the recent identification of phenotypic and functional heterogeneity in basophil populations provokes a fundamental reassessment of basophil effector functions and how they may vary depending on the infectious agent or antigen being used, the route of exposure and the cytokine environment induced. Much like M1 and M2 macrophages, basophils may exhibit distinct functional qualities based on the cytokine milieu in which they develop or are activated, and these differences should be considered when evaluating the ability of basophils to contribute to the induction of optimal Th2 cytokine-mediated immunity and inflammation.

However, much remains to be clarified regarding the differentiation, regulation, and effector functions of heterogeneous basophil populations. For instance, future studies will be required to determine whether IL-3-elicited versus TSLP-elicited basophils represent two distinct cell lineages that arise from distinct precursors or whether they represent different activation states of the same cell (Fig. 1A,C). Future studies will also be needed to delineate

whether IL-3-elicited versus TSLP-elicited basophils develop in the bone marrow, or if these populations differentiate in the periphery in response to ongoing inflammation. In addition, it remains unclear whether IL-3-elicited versus TSLP-elicited basophils function in different contexts or tissues (Fig. 3A,B). Some of the data reviewed here suggest that TSLP-elicited basophils may function during the development of allergic responses at epithelial surfaces, while IL-3-elicited basophils may function as late phase effectors in the tissues in the context of helminth infection (Fig. 3A,B). Finally, further functional analysis will be required to determine if other factors, particularly epithelial-derived cytokines such as IL-25 and IL-33 can also shape the differentiation and function of basophil populations. A recent report described an IL 33-responsive IL-4 producing basophil population that was critical for the suppression of experimentally-induced arthritis[57], further suggesting that epithelial cell-derived cytokines may regulate basophil activation. Further exploration of the effects of these cytokines on basophil populations will be integral in gaining a more complete understanding of the regulation of basophil differentiation and effector function, which in turn could inform efforts to develop treatment strategies to target allergic diseases and helminth infections.

Summary

Recent studies regarding the relative contributions of DCs and basophils to the induction and propagation of CD4^{+} Th2 cells have resulted in conflicting reports and controversy. Many of the varied reports on the ability of basophils to contribute to Th2 cytokine mediated immunity and inflammation have been attributed to different experimental designs and basophil depletion strategies. However, the recent identification of phenotypic and functional heterogeneity in basophil populations provokes a fundamental reassessment of basophil effector functions and how they may vary depending on the infectious agent or antigen being used, the route of exposure and the cytokine environment induced. Much like M1 and M2 macrophages, basophils may exhibit distinct functional qualities based on the cytokine milieu in which they develop or are activated, and these differences should be considered when evaluating the ability of basophils to contribute to the induction of optimal Th2 cytokine-mediated immunity and inflammation.

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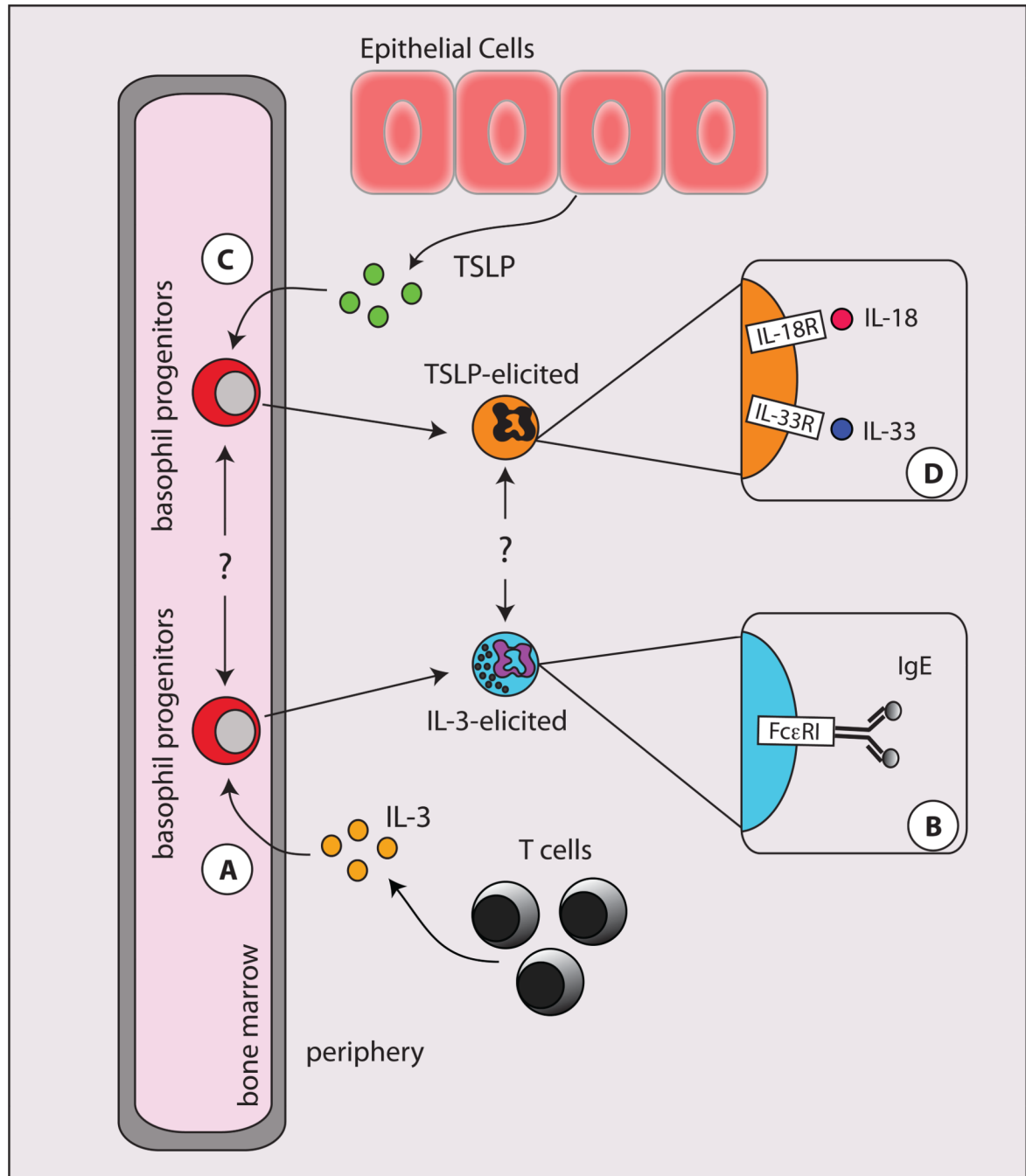
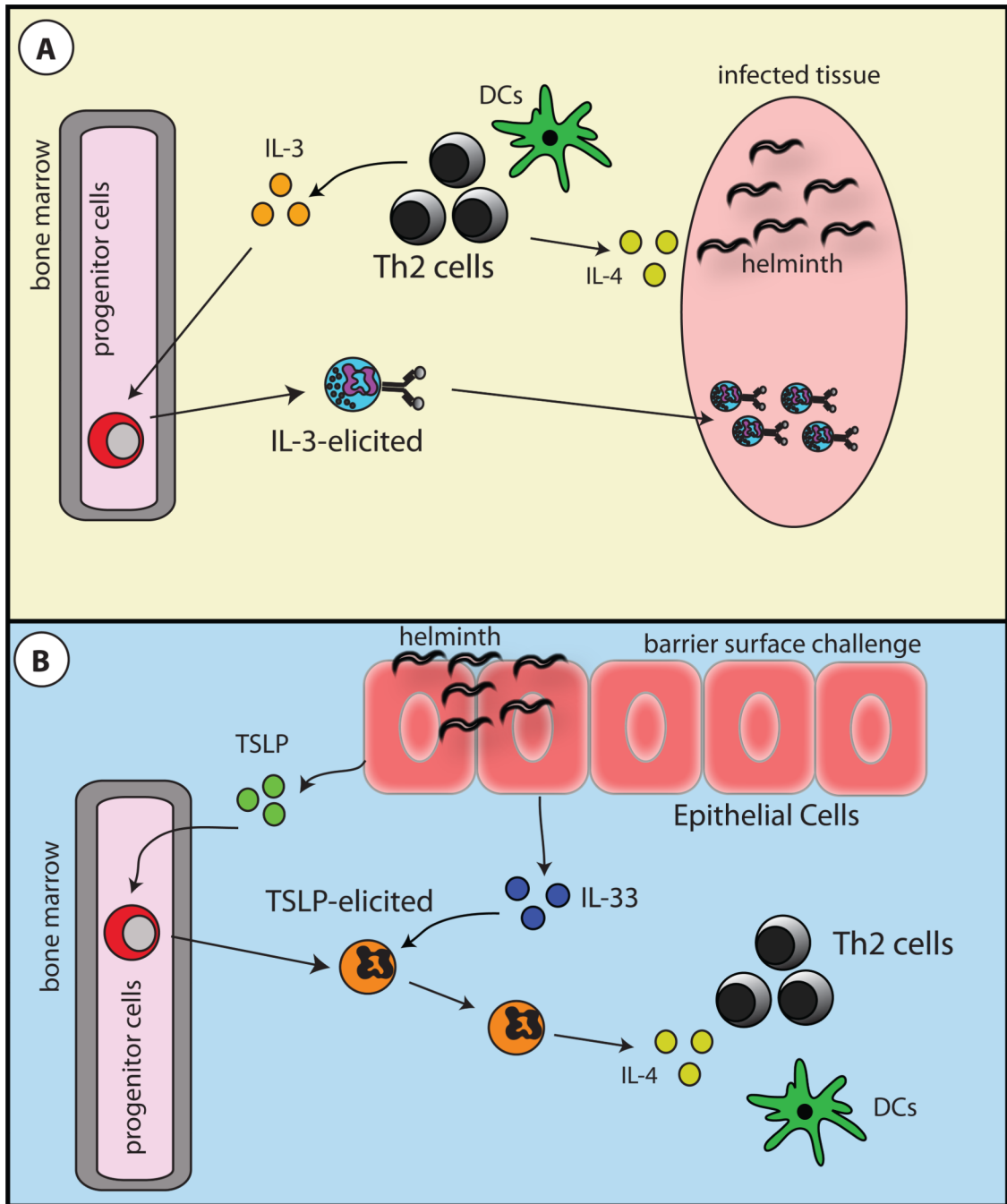**Figure 1. Developmental and functional heterogeneity in basophils populations**
A, In the context of an ongoing inflammatory response, CD4^{+} Th2 cells produce IL-3. IL-3 promotes the generation of mature basophils from bone marrow-resident precursor cells. **B**, Mature IL-3-elicited basophils enter the periphery and acquire surface bound IgE via FcεRI and are potent producers of histamines and leukotrienes. **C**, In the context of inflammation at barrier surfaces, epithelial cells produce TSLP. TSLP promotes the generation of mature basophils from bone marrow-resident precursor cells. **D**, Mature TSLP-elicited basophils express elevated levels of IL-18Rα and IL-33R and are potent producers of IL-4 in response to IL-18 or IL-33 stimulation.
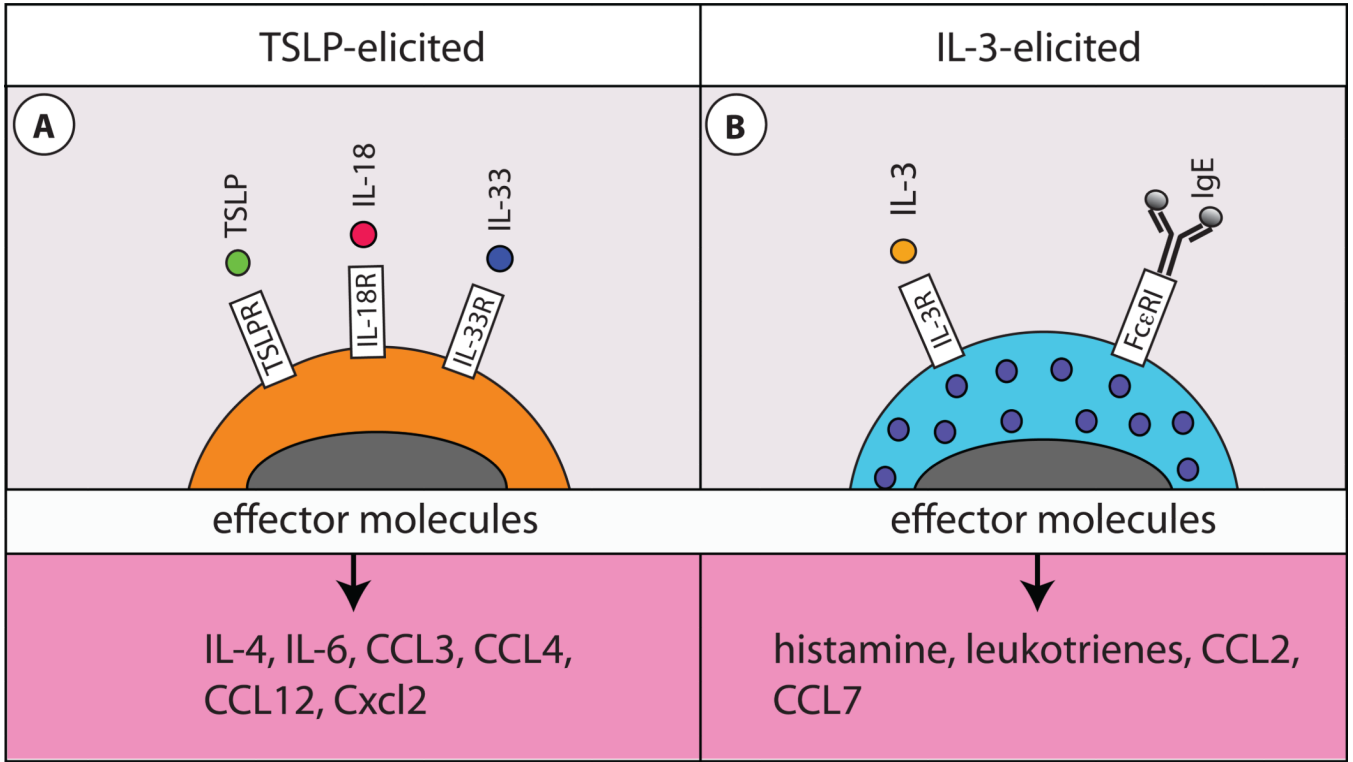
Figure 2. Effector mechanism of IL-3-elicited and TSLP-elicited basophils
A, TSLP-elicited basophils are smaller and less granular than IL-3-elicited basophils and express elevated levels of IL-18Rα and IL-33R on their surface. TSLP-elicited basophils produce exaggerated amounts of IL-4, IL-6, CCL3, CCL4, CCL12 and Cxcl2 compared to IL-3-elicited basophils. **B**, IL-3-elicited basophils are larger and more granular than TSLP-elicited basophils and are potent producer of histamines, leukotrienes, CCL2 and CCL7 in response to IgE-mediated FcεRI crosslinking.

Figure 3. Basophil functions during Th2 cytokine-mediated immunity and inflammation
A, In the context of an ongoing inflammatory response such as that initiated by tissue dwelling helminth infections, activated $CD4^+$ Th2 cells produce IL-3. IL-3 promotes the development of classical basophil populations from bone marrow-resident precursor cells. Mature IL-3-elicited basophils enter the periphery and function as late phase effector cells that contribute to chronic inflammation. **B**, In the context of an infection or insult at a barrier surface, the epithelial cell-derived cytokines TSLP and IL-33 are produced. TSLP promotes the development of basophils from bone marrow-resident precursor cells. Mature TSLP-elicited basophils enter the periphery where they are actvated by IL-33. TSLP-elicited,

IL-33-activated basophils are potent sources of IL-4 and along with DC populations, contribute the induction of CD4$^+$ Th2 cell responses.