In situ hematopoiesis: a regulator of T_H2 cytokine-mediated immunity and inflammation at mucosal surfaces

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Hematopoiesis refers to the development of blood cells in the body through the differentiation of pluripotent stem cells. Although hematopoiesis is a multifocal process during embryonic development, under homeostatic conditions it occurs exclusively within the bone marrow. There, a limited number of hematopoietic stem cells differentiate into a rapidly proliferating population of lineage-restricted progenitors that serve to replenish circulating blood cells. However, emerging reports now suggest that under inflammatory conditions, alterations in hematopoiesis that occur outside of the bone marrow appear to constitute a conserved mechanism of innate immunity. Moreover, recent reports have identified previously unappreciated pathways that regulate the egress of hematopoietic progenitor cells from the bone marrow, alter their activation status, and skew their developmental potential. These studies suggest that progenitor cells contribute to inflammatory response by undergoing *in situ* hematopoiesis (ISH). In this review, we highlight the differences between homeostatic hematopoiesis, which occurs in the bone marrow, and ISH, which occurs at mucosal surfaces. Further, we highlight factors produced at local sites of inflammation that regulate hematopoietic progenitor cell responses and the development of T_H2 cytokine-mediated inflammation. Finally, we discuss the therapeutic potential of targeting ISH in preventing the development of inflammation at mucosal sites.

INTRODUCTION

Hematopoiesis occurs during fetal development and is necessary for the generation of all blood cells, including erythrocytes and cells of the lymphoid and myeloid lineage. During embryonic development, hematopoiesis is a multifocal process occurring first in the extraembryonic yolk sac, followed temporally at a series of intraembryonic sites, including the aorta–gonad–mesonephrous region, fetal liver, fetal spleen, and finally the neonatal bone marrow.[1,2] After its development, the bone marrow becomes the primary site of hematopoiesis in the body.[1,2] Under steady-state conditions in adults, the earliest multipotent hematopoietic progenitors reside in the subendosteal region of the bone marrow and in the trabecular bone. As these cells differentiate, they give rise to a rapidly proliferating population of "transient amplifying" cells that are increasingly committed to a given lineage. These lineage-restricted progenitors, in turn, replenish and maintain circulating blood cells.[1,2] Under conditions of stress, such as injury or infection, hematopoiesis can increase in capacity, to provide the blood cells necessary to promote inflammation, wound healing, and/or the elimination of pathogens.[3–7] Although coordinated alterations in hematopoiesis can be beneficial, dysregulated responses can result in the development of unchecked inflammation and thus contribute to various disease states, including colitis, asthma, and allergies.[6,8–10] Moreover, recent studies have demonstrated that the contributions of hematopoiesis to the development of inflammation are not limited to events occurring in the bone marrow, and that hematopoietic progenitor cells can enter the periphery and traffic directly to sites of inflammation.[11]

Hematopoietic progenitors express the stage-specific antigen, CD34, which is present at the highest levels on early hematopoietic lineage cells, and is progressively lost on terminally differentiated cells,[12] with the exception of some

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murine mast cells.[13] CD34 has been reported to facilitate the mobilization of progenitors from the bone marrow[14,15] and, together with other surface markers and additional determinants such as small size and low granularity, it enables hematopoietic progenitor cells to be accurately identified and enumerated in the bone marrow and peripheral tissues by flow cytometry.[16] These advances have allowed investigators to identify increased populations of hematopoietic progenitor cells at local sites of inflammation where, under control of local factors, they differentiate into effector cells via "on site" or "*in situ*" hematopoiesis (ISH). Collectively, these studies have demonstrated that hematopoietic stem/progenitor cells (HSPCs) are capable of sensing external cues that directly regulate their activation, migration, and developmental potential.[11,17] In addition, it has been shown that HSPCs are acutely tuned to negative feedback from cytokines produced by maturing progeny and produce their own factors in response to innate receptor activation, as a means of responding to systemic inflammation.[18] In this review we examine recent studies that identify previously unrecognized pathways through which ISH contributes to the development of T_H2 cytokine-mediated inflammation at mucosal surfaces. In addition, we highlight the emerging concept that ISH represents a conserved mechanism of the innate immune system capable of combating multiple infectious threats.

The hypothesis that HSPCs can operate as sentinels of the immune system was first supported by the identification of Toll-like receptors (TLRs) on the surface of progenitor cell populations and the demonstration that microbial-derived signals can influence the hematopoietic potential of HSPCs in a MyD88-dependent manner. This concept was further strengthened by subsequent studies demonstrating that small populations of TLR-expressing HSPCs exit the bone marrow and enter the periphery under steady-state conditions.[19–21] Moreover, these studies demonstrated that circulating HSPCs stop migrating and undergo ISH on encountering microbial-derived signals in peripheral tissues.[19] In this regard, it has long been known that virtually all well-characterized molecules that regulate HSPC trafficking to the appropriate bone marrow niche during transplantation (PSGL1/P-selectin, CXCL12/CXCR4, CD44/hyaluronic acid, $\alpha4\beta1$ integrin, and CD34 itself) are also used by inflammatory cells to migrate to peripheral tissue sites of inflammation.[22] These data led to the hypothesis that HSPCs recognize pathogen-derived signals via TLR signaling at the local site of inflammation, facilitating their differentiation in order to provide an immediate source of innate immune cells capable of containing an infectious threat.[19,20,23] Therefore, the ability of HSPCs to recognize viral and bacterial antigens in TLR- and/or MyD88-dependent manners allows them to contribute to the inflammatory responses necessary to eliminate these specific classes of pathogens. It is also well established that robust T_H2 cytokine responses necessary to initiate immunity to helminth parasites and promote the development of allergic inflammation can occur in the absence of TLR- and/or MyD88-dependent signaling.[24,25] Collectively, these studies suggest that the contributions that ISH may make to T_H2 cytokine-mediated inflammation operate via distinct and previously unappreciated pathways.

The following sections will examine emerging studies demonstrating that in addition to combating bacterial and viral infections, ISH uniquely contributes to the development of protective immunity to helminth parasites and the initiation and progression of allergic inflammation at mucosal surfaces. First, we highlight the recently identified role for epithelial cell-derived cytokine alarmins in promoting T_H2 cytokine-mediated inflammation via their effects on ISH. Next, we discuss the known roles of ISH on the development of protective immunity to gastrointestinal helminth infections as well as the propagation of allergic inflammation. Finally, we discuss the need to better understand the factors that regulate ISH and highlight the potential targeting of this pathway as a therapeutic strategy to regulate the development of inflammation at mucosal barriers.

EPITHELIAL CELL-DERIVED CYTOKINES AND ISH

Aside from classical T_H2 cytokines, the predominantly epithelial cell-derived cytokines thymic stromal lymphopoietin (TSLP), interleukin (IL)-25, and IL-33 are secreted following epithelial stimulation due to tissue damage, microbial-derived signals, or allergen exposure. Although their unique and redundant contributions remain to be fully defined, these epithelial-derived factors have the capacity to initiate T_H2 cytokine-mediated inflammation at mucosal sites via multiple pathways.[26–28] A major recent discovery is that TSLP, IL-25, and IL-33 promote T_H2 cytokine-mediated inflammation via their effects on unique lineage negative (Lin^-) cell populations. For example, TSLP, IL-25, and IL-33 regulate the population expansion and activation of type 2 innate lymphoid cells (ILC2), which produce T_H2-associated cytokines and contribute to the development of protective immunity to helminth parasites and the initiation of allergic inflammation.[29–31] In addition to their effects on ILC2s,[32,33] recent studies have also shown that TSLP, IL-25, and IL-33 have effects on the accumulation, activation, and differentiation of HSPCs in peripheral tissues.[4,5,26,34,35] Collectively, these studies provide direct support for the process of ISH as a significant contributor to the development of inflammation at mucosal surfaces. Further, these studies promote the idea that epithelial-derived cytokines produced at barrier surfaces are critical links between local inflammation, ISH, and innate immune responses that promote T_H2 cytokine-mediated inflammation. The following sections highlight the known influences of TSLP, IL-25, and IL-33 on murine and human HSPCs.

Thymic stromal lymphopoietin

TSLP–TSLP receptor (TSLPR) interactions are critical to the development of T_H2 cytokine-mediated inflammation in murine models of helminth infection and multiple models of allergic disease.[27] Although TSLP can influence the activation of multiple terminally differentiated immune cells,[27] early studies investigating its biological activity reported that

overexpression in mice also results in increased populations of HSPCs in peripheral tissues.[36] Consistent with these data, more recent murine studies reported that TSLPR is expressed on HSPCs, and that TSLP can directly promote basophil development from bone marrow-resident, lineage-committed basophil progenitor cells.[5,37] Further, it was also demonstrated that TSLP expression regulates the presence and developmental potential of HSPCs in the periphery of mice, following helminth infection or the induction of atopic dermatitis-like inflammation.[37] Collectively, these murine studies suggest that TSLP promotes inflammation, in part, through its hematopoietic effects.

Consistent with murine studies, tissue and systemic TSLP expression is closely associated with human allergic disease states, including atopic dermatitis, asthma, and eosinophilic esophagitis.[27] In addition, it has been reported that human $CD34^+$ progenitor cells express the TSLPR,[34,38] and that TSLP signaling cooperates with other hematopoietic cytokines in the differentiation of human $CD34^+$ cells.[38] Further, TSLP can directly activate human $CD34^+$ progenitor cells isolated from peripheral tissues.[34,38] These studies suggest that the TSLP-ISH pathway operates to promote type 2 inflammatory responses in humans. The influence of TSLP on $CD34^+$ progenitor cells with subsequent development of mucosal inflammation, will be further discussed below.

Interleukin-25

IL-25 is an IL-17 family member (IL-17E) that is produced by epithelial cells at mucosal surfaces in response to helminth parasites and exposure to allergens.[39,40] In addition, IL-25 has been shown to promote the development of protective immunity to gastrointestinal helminths, eosinophil responses, and the development of inflammation in murine models of allergic disease.[4,41] Further, IL-25 is expressed at higher levels in the serum and sputum of patients suffering from asthma and in the skin of patients suffering from atopic dermatitis.[42] Similar to TSLP, IL-25 acts on multiple, terminally differentiated immune cells known to express its receptor (IL-17Rb), including $CD4^+$ T_H2 T cells, eosinophils, and ILC2s; in addition, IL-25 promotes the expansion of HSPCs in the peripheral tissues of mice.[4,26,41] The unique ability of IL-25-induced HSPCs to contribute to T_H2 cytokine-mediated inflammation will be highlighted below.

Interleukin-33

IL-33 is an IL-1 family cytokine member expressed at barrier surfaces by multiple cell types, including epithelial cells and mast cells, in response to injury and/or infection.[43,44] The IL-33 receptor (ST2) is expressed on multiple, terminally differentiated cells, including $CD4^+$ T_H2 T cells, eosinophils, mast cells, basophils, and ILC2s.[26,43,45,46] Moreover, IL-33 has been shown to contribute to T_H2 cytokine-mediated inflammation following helminth infection or the induction of allergic responses via its effects on multiple immune cell populations.[47] In addition to terminally differentiated immune cells, ST2 is also expressed on murine HSPCs, and IL-33 indirectly promotes basophil differentiation from bone marrow-resident progenitors by inducing granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-3 production.[48,49] Further, IL-33 is reported to promote TSLP production by epithelial cells and ST2 is highly expressed on TSLP-elicited granulocyte/monocyte progenitor (GMP)-like cells in the peripheral tissues of mice.[5,50] These studies suggest that TSLP and IL-33 may cooperate to influence ISH in the context of T_H2 cytokine-mediated inflammation.

Consistent with reports in murine studies, human $CD34^+$ HSPCs also express the IL-33R (T1/ST2) and IL-33 signaling can directly influence immune cell maturation.[51] Moreover, IL-33 cooperates with TSLP to directly activate human $CD34^+$ HSPCs.[34] Although the exact role of IL-33 in hematopoietic differentiation remains to be fully elucidated, these studies support a model in which IL-33 acts in concert with other epithelial cell-derived cytokines to influence the hematopoietic program.

ISH AND IMMUNITY TO HELMINTH PARASITES

Gastrointestinal helminth infections represent a significant public health concern with over two billion people infected worldwide.[52,53] Protective immunity to helminth parasites is dependent on the generation of $CD4^+$ T_H2 cells and the production of the T_H2 cytokines, IL-4, IL-5, and IL-13. The production of T_H2 cytokines leads to smooth muscle contractility, goblet cell formation, and intestinal mucus production necessary for worm expulsion.[52,53] As described above, an emerging body of literature has demonstrated that the epithelial cell-derived cytokines TSLP, IL-25, and IL-33 are produced at barrier surfaces in response to helminth infections, where they activate terminally differentiated cells and are critical to the development of intestinal inflammation and worm expulsion.[52] In addition, emerging studies suggest that the effects of TSLP, IL-25, and IL-33 on HSPCs directly influence protective immunity to helminths by promoting ISH at local sites of inflammation (**Figure 1**).

For example, recent studies demonstrate that TSLP expression promotes the accumulation of Lin^-, $CD34^+$, and c-Kit^+ GMP-like cells in the periphery following *Trichinella spiralis* infection.[5] TSLP-elicited GMP-like cells were shown to have altered transcriptional profiles and a significantly greater capacity to develop into basophil and mast cell populations, compared with bone marrow-resident GMPs.[5] Associated with their increased capacity to develop into basophils and mast cells, adoptive transfer of TSLP-elicited GMP-like cells promoted T_H2 cytokine production in gut-associated lymphoid tissues, increased intestinal mucin responses, and significantly reduced *Trichuris muris* worm burdens in normally susceptible TSLPR-deficient mice.[5] Further, when directly compared with bone marrow-resident GMPs, TSLP-elicited GMP-like cells selectively promoted T_H2 cytokine responses *in vivo*.[5] The skewed developmental potential and enhanced ability of TSLP-elicited GMP-like cells to promote T_H2 cytokine-mediated inflammation is likely a result of their altered transcriptional profiles. However, the exact pathways and transcription factors

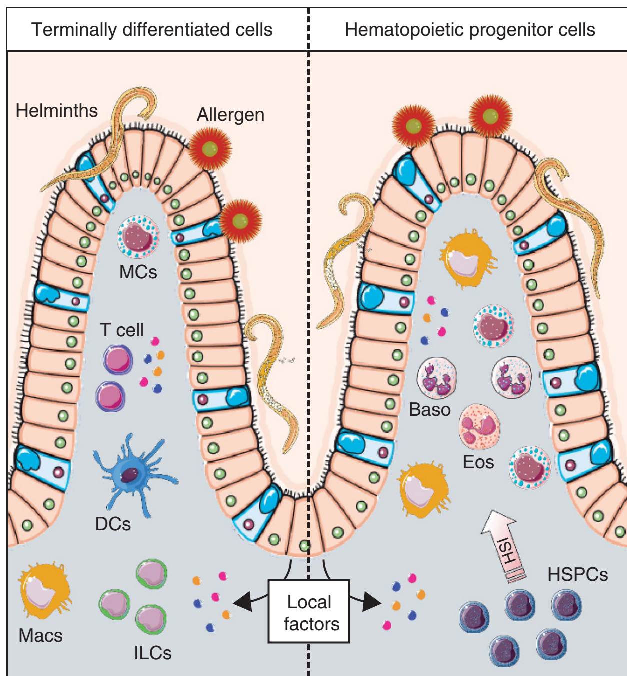

Figure 1 It is well established that local factors such as epithelial cell-derived cytokines (thymic stromal lymphopoietin (TSLP), interleukin (IL)-25, and IL-33) that are produced at the site of inflammation activate multiple terminally differentiated effector cells (T cells, mast cells (MCs), dendritic cells (DCs), macrophages (Macs), and innate lymphoid cells (ILCs)) that contribute to the development of protective immunity to helminth parasites, and the inflammation associated with allergies and asthma. In addition to terminally differentiated cells, emerging studies have demonstrated that hematopoietic stem/progenitor cells (HSPCs) also traffic to affected tissues where, under the influence of inflammatory factors produced at the local site of inflammation, they differentiate into various effector cell populations (Macs, basophils, MCs, and eosinophils) that contribute to T_H2 cytokine-mediated immunity and inflammation.

that regulate HSPC differentiation and whether TSLP acts directly or indirectly on HSPCs remains unknown.

Similar to TSLP, IL-25 has been shown to promote the expansion of a Lin^- c-Kit^+ multipotent progenitor cell population, termed MPP^{type2} cells, following infection with the helminth parasite, *Nippostrongylus brasiliensis*.[4] MPP^{type2} cells are induced in the gut-associated lymphoid tissues following *N. brasiliensis* infection and have distinct surface phenotypes and gene expression profiles compared with ILC2s.[4,41] Moreover, although IL-25-induced ILC2s are terminally differentiated cells, MPP^{type2} cells possessed the capacity to differentiate into basophils, mast cells, and macrophages, and promote T_H2 cytokine responses both *in vitro* and *in vivo*.[4] Further, IL-25 treatment of ILC2-depleted mice results in the induction of MPP^{type2} cells, increased T_H2-associated cytokine production, and goblet cell responses in the lung and gut.[41] Finally, the adoptive transfer of MPP^{type2} cells is sufficient to promote T_H2 cytokine production in the gut-associated lymphoid tissues, as well as intestinal mucin responses and the clearance of *T. muris* in normally susceptible IL-25-deficient mice.[4]

In addition to MPP^{type2} cells, IL-25 also promotes the accumulation of lineage-committed mast cell progenitors in the lamina propria and gut-associated lymphoid tissues following infection with *N. brasiliensis*, *T. muris* or *Heligmosomodies polygyrus bakeri*.[4,54] Lin^- $CD34^+$ mast cell progenitors actively express IL-4 transcripts and produce robust amounts of the proinflammatory cytokines, IL-5, IL-6, and IL-9, when isolated and cultured in the presence of IL-33 (refs 4, 54). These data further suggest that epithelial cell-derived cytokines produced at local sites of inflammation may cooperate to influence hematopoiesis and demonstrate that the IL-25-ISH pathway contributes to the development of T_H2 cytokine-mediated inflammation and protective immunity to helminth parasites.

Similar to TSLP and IL-25, IL-33 is highly produced at barrier surfaces in response to helminth parasites.[43] Although recent studies have defined an important role of IL-33 in promoting T_H2 cytokine-mediated inflammation and protective immunity to helminths via its potent effects on ILC2s,[33] the contributions of IL-33 to helminth-induced ISH remain poorly defined. Despite the fact that few studies have investigated the effects of IL-33 on the hematopoietic potential of progenitor cells following intestinal helminth infection, as mentioned above, it is likely that IL-33 acts either independently or cooperatively with other cytokines to influence ISH and the development of helminth-induced inflammation.

ISH AND ALLERGIC INFLAMMATION

Similar to pathways identified in the context of helminth infections, a growing body of evidence suggests that HSPCs contribute to allergic inflammation not only by giving rise to mature effector cells in the bone marrow, but also through their recruitment via circulation into peripheral tissues. These mobilized HSPCs traffic to sites of allergic inflammation where they undergo ISH and differentiate into effector cells under the control of locally produced inflammatory cytokines and growth factors[55] (**Figure 1**). The first studies to provide indirect evidence that progenitor cells may indeed traffic from the bone marrow to inflamed tissues were performed in subjects with allergic rhinitis.[56–58] A series of studies reported a significant elevation of eosinophil–basophil (Eo/B) progenitors before seasonal allergen exposure, followed by rapid reductions in the number of circulating progenitors in subjects during the season as they developed symptoms of rhinitis (**Figure 2**). This, in turn, was followed by a rebound back to basal (high circulating) levels at the end of the season (**Figure 2**). It was later demonstrated in an *ex vivo* explant model that allergen- and/or IL-5-induced *in situ* differentiation of $CD34^+$ progenitors was accompanied by decreased numbers of $CD34^+$/IL-5 receptor subunit α (IL-5Rα) $mRNA^+$ cells and increased mature eosinophils in the nasal mucosa of allergic subjects.[59] Sergejeva *et al.*[60] extended these studies further by demonstrating that the numbers of $CD34^+$ progenitors in the nasal mucosa of subjects with allergic rhinitis were increased during the allergen/pollen season. Collectively, these studies support a model in which $CD34^+$ Eo/B progenitors traffic to the nasal mucosa, undergo ISH, and contribute to the development of allergic rhinitis in the context of seasonal allergies (**Figure 2**).

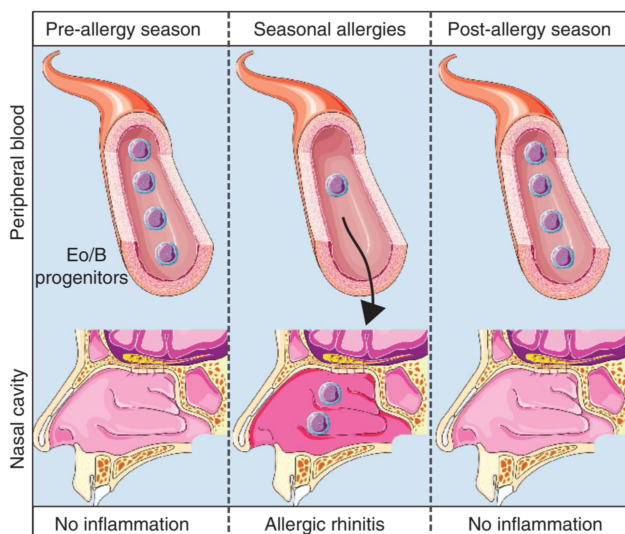

Figure 2 Multiple studies now support a model in which patients that suffer from season allergies present with elevated eosinophil–basophil (Eo/B) progenitor cells in their peripheral blood before the onset of allergic inflammation. Following the induction of seasonal allergies, circulating levels of Eo/B progenitors are thought to leave the periphery and traffic to the nasal mucosa where they undergo *in situ* hematopoiesis (ISH) and contribute to the development of allergic rhinitis. As the seasonal exposure to allergens subsides and allergic rhinitis is resolved, Eo/B progenitors are no longer called to the local site of inflammation and return to the periphery where they await the signals necessary to traffic to allergen-exposed tissues.

Additional studies have expanded on these earlier findings and now suggest that ISH functions as a conserved source of innate effector immune cell populations that contribute to multiple allergic disease states. For example, increased numbers of CD34$^+$ progenitors are found at multiple sites of allergic inflammation, including the nasal mucosa of subjects with nasal polyposis[61] and the bronchial mucosa of patients with asthma.[62] Moreover, CD34$^+$ progenitor cell numbers correlate with the level of airflow obstruction in asthmatics.[62] Finally, enhanced numbers of CD34$^+$ progenitor cells, as well as both mature and immature eosinophils, are found at higher levels in the peripheral blood[8,63] and bone marrow[9] of atopic individuals, compared with non-atopics.

In addition to the increased presence of CD34$^+$ HSPCs, a rise in bone marrow and circulating, committed Eo/B progenitors (defined as CD34$^+$/IL-5Rα^+ cells[9,64]) in response to airway inhaled allergen exposure in atopic asthmatic subjects[9,10,65,66] provides further evidence for local airway mucosal (*in situ*) control of hematopoietic differentiation. Likewise, in a proof-of-concept study for the development of anti-IL-5 therapies (see below) in human subjects, systemically administered IL-5 stimulated the appearance of increased numbers of circulating CD34$^+$/IL-5Rα^+ cells.[67] In addition, several groups have reported the increased expression of IL-5Rα on CD34$^+$ progenitor cells in the bone marrow of atopic asthmatics.[68] This supports the concept that HSPCs in atopic subjects are primed to respond to IL-5 (ref. 8), likely as a result of increased levels of bone marrow CD34$^+$/IL-5Rα^+ cells, a feature of atopic disease.[69] Along these lines, compared with asthmatics who do not develop eosinophilia, there is a higher proportion of CD34$^+$/IL-5Rα^+ cells in the bone marrow after allergen challenge in mild asthmatic subjects who develop airway eosinophilia and increased clinical symptoms to methacholine challenge.[9,10] Taken together, these data support a model in which eosinophil-lineage skewing of CD34$^+$ HSPCs occurs following allergen stimulation and this then contributes to the subsequent development of blood and tissue eosinophilia in multiple disease states.[9]

Consistent with data presented in the studies cited above, ovalbumin-sensitized mice exhibit similar increases in HSPCs that coincide with increased airway eosinophilia.[70] Furthermore, *in vivo* experiments demonstrate increased IL-5Rα[9] and CCR3 (ref. 71) expression on human bone marrow-derived CD34$^+$ cells following allergen challenge, events which may subsequently facilitate the mobilization of these cells from the bone marrow to sites of allergic inflammation,[71] and their differentiation *in situ*, leading to the development of local eosinophilic–basophilic inflammation. These studies suggest that HSPCs in the peripheral blood and bone marrow are sensitive to allergic stimuli in the airways and respond with enhanced systemic and local eosinophilo- and basophilopoiesis. This is supported by the close relationships that exist among increased production of Eo/B lineage-committed progenitors within the bone marrow, development of blood and tissue eosinophilia,[9] and maintenance of allergic tissue inflammatory responses.[8,10]

Although the exact mechanisms that regulate control of HSPC responses and ISH in the context of allergic inflammation in humans remain unknown, recent studies suggest that in addition to the effects of IL-5, the epithelial cell-derived cytokines TSLP and IL-33 may, in part, regulate these pathways, as has been shown in mice (see above). For example, HSPCs express the TSLPR and TSLP signaling upregulates IL-3Rα on peripheral blood CD34$^+$ cells, rendering them more responsive to the effects of IL-3, with concomitant functional Eo/B colony forming unit differentiation.[38] Furthermore, TSLP stimulation of, and signaling in, human CD34$^+$ HSPCs results in the rapid release of high levels of cytokines and chemokines, further contributing to specific lineage commitment and differentiation.[34,38] Related to this, TSLP induces IL-5$^+$/IL-13$^+$ CD34$^+$ cells to appear in sputum after airway allergen challenge of atopic asthmatics.[34] The enhanced production of T_H2-associated cytokines suggests TSLP-activated HSPCs may function as inflammatory effector cells even before undergoing further differentiation; such modes of action and cytokine production could help to explain the induction of eosinophilic inflammation in the absence of T_H2 cell activation in eosinophilic asthma.[72,73] However, as will be discussed further below, additional studies are required to better differentiate the relative contributions of, and relationships between, IL-5- and IL-13-producing CD34$^+$ progenitor cells and ILC2 populations.

In addition to TSLP, human CD34$^+$ HSPCs also express a functional receptor for IL-33 which, on ligation with IL-33,

accelerates maturation of tryptase-containing cells (mast cells).[51] Moreover, IL-33 alone or together with TSLP is a potent activator of $CD34^+$ progenitors, resulting in the secretion of several cytokines (IL-5, GM-CSF, and TNFα) and chemokines (CCL1).[51] Collectively, these studies suggest that local inflammatory factors may promote the release of effector cytokines and chemokines that directly influence HSPC populations and promote ISH in the context of allergic inflammation.

CYTOKINES AND GROWTH FACTORS THAT INFLUENCE ISH

In addition to the pathways described above, the differentiation of mature hematopoietic cells from HSPCs is under the permissive control of a range of hematopoietic cytokines and growth factors. Under inflammatory conditions, many of these factors are highly produced at local sites of inflammation where $CD34^+$ cells are enriched.

Currently, many hematopoietic cytokines and growth factors have been identified that regulate hematopoiesis by binding specific receptors on the cell surface.[74] Although stem cell factor and IL-6 have been shown to exert effects very early in the hematopoietic lineage hierarchy,[75] other cytokines act at later stages in the development of myeloid progenitors. The differentiation of committed progenitors towards an Eo/B lineage is regulated by a triad of cytokines found systemically and in tissue, and predominantly produced by T_H2 cells and mast cells, with some epithelial contribution: IL-3, IL-5, and GM-CSF.[76,77] The gene loci for encoding these cytokines are closely linked on human chromosome 5, likely reflecting a recent triplication of a common ancestral gene that then acquired more specialized functions.[78] This is further supported by the finding that these cytokines are both pleiotropic and redundant, acting in an autocrine and paracrine manner—signaling through a common receptor β-chain[79] and highly specific α-chain, which regulate cell proliferation, specific lineage commitment, and differentiation and survival,[80,81] through the activation of signal transduction pathways and lineage-specific transcription factors.[76,77]

It has been shown that up to 50% of $CD34^+$ cells from asthmatic subjects express intracellular IL-5 protein and exhibit high expression of IL-5 mRNA.[67] Further, progeny of Eo/B progenitors in colonies express GM-CSF protein and mRNA,[82] and there is an autocrine GM-CSF-dependent signaling mechanism for Eo/B differentiation mediated through bacterial lipopolysaccharide signaling.[83] In addition, as $CD34^+$ progenitors undergo ISH in the nasal mucosa of allergic subjects,[59] IL-5, along with IL-9 signaling, can upregulate the surface expression of IL-5Rα during terminal eosinophil differentiation.[84,85] These studies suggest that HSPCs may aid in their own differentiation through autocrine production of growth factors, such as IL-5 and GM-CSF, at local sites of inflammation.

In addition to cytokines, HSPCs also express receptors that bind chemokines, including eotaxin, macrophage inflammatory protein-1α and stromal cell-derived factor (SDF-1/CXCL12), which have been implicated in the regulation of progenitor proliferation and movement.[86,87] Given that hematopoietic cells develop under the influence of the tissue milieu, the rich combination of cytokines and chemokines present in the bone marrow sinusoids, circulation, or mucosal and other tissues probably have a critical role in guiding and modulating hematopoiesis. Changes in the composition of the local milieu, such as increased expression of T_H2 cytokines (particularly IL-5) in individuals with atopy, may promote differentiation of lineage-committed Eo/B progenitors[88] and help to explain increased eosinophils and basophils at sites of inflammation in atopic individuals.

DISTINGUISHING HEMATOPOIETIC PROGENITOR CELLS FROM ILCS

The recent identification of Lin^- ILC populations and their shared characteristics with many hematopoietic progenitor cells have made it difficult to reliably identify and distinguish these distinct cell populations from one another. For example, both HSPCs and ILCs accumulate in inflamed tissues, respond to epithelial cell-derived cytokines and contribute to the development of T_H2 cytokine-mediated inflammation. Further, the reported ability of $CD34^+$ progenitor cells to produce IL-5 and IL-13 makes it difficult to functionally distinguish HSPCs from ILCs. There is a need to further investigate their distinct contributions to the development of inflammation at mucosal sites.

As mentioned above, TSLP is known to have multiple cellular targets and has been demonstrated to be important in the generation of T_H2 cytokine-mediated inflammation by promoting production of IL-5 and IL-13 (refs 89, 90). For example, TSLP is reported to promote the production of IL-5 and IL-13 from $CD34^+$ progenitor cells and nascent mast cells;[34] as already noted, TSLP induces double-positive $IL\text{-}5^+/IL\text{-}13^+$ $CD34^+$ cells to appear in sputum after airway allergen challenge of atopic asthmatics.[34] In addition, nasal polyp epithelial-derived TSLP has also been shown to act on ILC2s[73] and, in combination with IL-33, stimulates the production of T_H2 cytokines, especially IL-5 and IL-13. Given the fact that TSLPR is expressed on HSPCs and ILCs, along with the reported capacity of both HSPCs and ILCs to promote expression of IL-5 and IL-13 (refs 29, 31, 45, 91), it is difficult to determine whether these studies have identified distinct cell populations, or whether CD34-expressing ILCs or ILC progenitor cell populations are separately and distinctly recruited to inflamed tissues.

Despite the difficulty in identifying and distinguishing the contributions of Lin^- HSPC and ILC populations, recent studies by Saenz *et al.*[41] demonstrated that IL-25 promotes inflammation in the lung and gut via its combined effects on ISH and ILC2 populations. For example, IL-25 treatment was capable of promoting ISH and T_H2 cytokine responses in mice lacking T cell and ILC populations, suggesting that ISH is sufficient to promote T_H2 cytokine-mediated inflammation.[41] These data support a model though which ISH operates as a major contributor of mucosal inflammatory responses and cooperates with ILC responses to promote optimal T_H2 cytokine production (**Figure 3**). In support of this model,

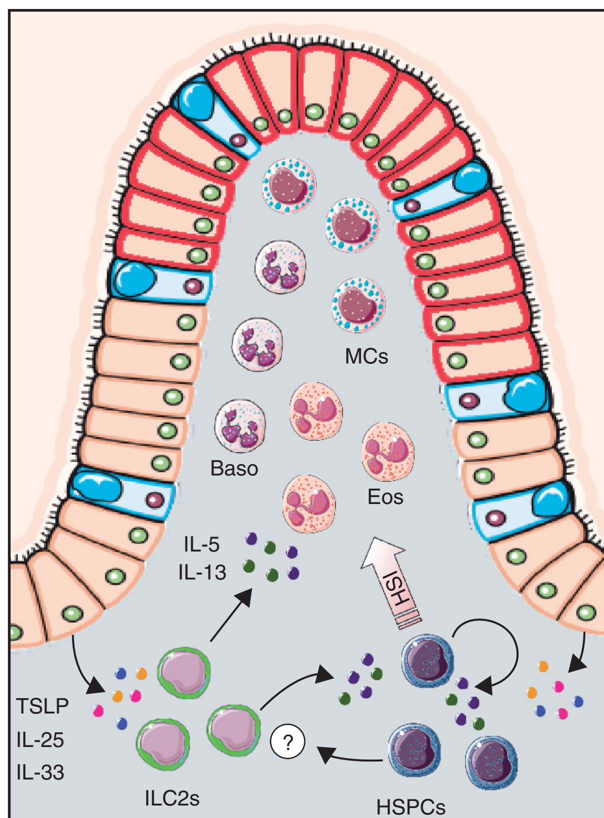

Figure 3 Thymic stromal lymphopoietin (TSLP), interleukin (IL)-25, and IL-33 are known to regulate the accumulation and activation of type 2 innate lymphoid cells (ILC2s) and hematopoietic stem/progenitor cells (HSPCs) in affected tissues. Both ILC2s and HSPCs are reported to produce IL-5 and IL-13 in response to TSLP and IL-33 signaling, respectively. In addition to the autocrine effects that HSPC-derived cytokines may have on *in situ* hematopoiesis (ISH), IL-5-, and IL-13-producing ILC2s are likely to directly influence HSPC differentiation and the activation of effector cells. Recent studies have demonstrated that basophil populations regulate ILC2 responses in inflamed skin and lung; however, whether HSPC-derived factors or effector cells influence ILC populations remain unknown.

recent studies have identified strong relationships between ILC2s and tissue-resident eosinophils, basophils, and mast cells.[92–95] Collectively, these studies suggest that coordinated interactions between HSPCs, ISH-derived effector cells, and ILCs may be occurring at the local site of inflammation.

THE THERAPEUTIC POTENTIAL OF TARGETING ISH

As described above, a growing body of evidence suggests that ISH significantly contributes to the development of T_H2 cytokine-mediated inflammation at mucosal surfaces. In addition, these studies strongly suggest that ISH-mediated pathways uniquely contribute to inflammatory responses and appear to be functionally distinct from central hematopoietic pathways occurring in the bone marrow. Therefore, the ISH pathway may represent a unique therapeutic target to treat the development and/or progression of T_H2 cytokine-mediated inflammation at mucosal sites. Previous efforts have been made to either target cytokines that initiate hematopoietic responses or to find therapeutics that could potentially interrupt the signals necessary for lineage commitment into effector cells known to contribute to inflammation at mucosal surfaces. Although it is difficult to determine the effects of these therapeutic pathways on central hematopoiesis versus ISH, the following sections will highlight therapeutic strategies known to alter the hematopoietic program in a manner that may prevent the development of inflammation at barrier surfaces.

Corticosteroids

Corticosteroids inhibit the development of airway eosinophilia and are considered the most effective current treatment for allergic asthma. Treatment with inhaled corticosteroids is sufficient to reduce baseline numbers of bone marrow Eo/B colony forming units.[96] In atopic asthmatics, the gradual withdrawal of inhaled corticosteroids results in a rise in circulating progenitors, which coincides with the development of clinical symptoms and reductions in airway peak flow (a measure of incipient airway obstruction).[97] Moreover, inhaled budesonide has been shown to have protective effects on allergen-mediated inflammation by inhibiting the number of allergen-induced circulating eosinophils and their progenitors grown in the presence of GM-CSF.[98] Similarly, Kim *et al.* reported that intranasal steroids were associated with an increase in $CD34^+/CD45^+$ progenitors and decrease in mature eosinophils in nasal polyp mucosa,[61] suggesting that corticosteroids block eosinophil differentiation, with a consequent accumulation of tissue (undifferentiated) $CD34^+$ progenitor cells. Together, these studies highlight and demonstrate the direct responsiveness of peripheral blood HSPCs to topical corticosteroids.

Anti-IL-5

Clinical investigational evidence supports systemic effects of IL-5 on lineage-committed HSPCs—as mentioned above, IL-5 given intravenously affects circulating Eo/B progenitors.[99] Aside from a possible role in the recruitment of mature eosinophils from the circulation to the airways,[99] IL-5 has been implicated in the local differentiation of progenitors in the tissue.[100] However, therapeutics targeting IL-5, a known maturation and differentiation factor for eosinophils,[101] have demonstrated inconsistent outcomes in abolishing eosinophilia. In an initial randomized, double-blinded trial, Leckie *et al.*[102] reported the inability of the monoclonal antibody to IL-5, to reduce the magnitude of late asthmatic response following allergen challenge; however, subsequent studies have shown benefits of IL-5 blockade in populations.[103,104] A clinical study using mepolizumab, an anti-IL-5 monoclonal antibody, showed inhibition of bone marrow eosinophil maturation and decreased Eo/B committed progenitors in the bronchial mucosa of atopic patients,[100] suggesting that local tissue eosinophilopoiesis may be, at least in part, IL-5 dependent. These studies confirm that the bone marrow and the lung in asthmatics with eosinophilia are both affected by anti-IL-5 treatment. Further, mepolizumab has been shown to lower eosinophil levels in patients with hypereosinophilic syndrome.[105] However, therapeutically

targeting IL-5 alone will likely not achieve complete regulation of eosinophilopoiesis in subjects with eosinophilic disorders, due to the functional redundancy among the hematopoietic cytokines, IL-3, IL-5 and GM-CSF, such that even in the absence of one of these cytokines, adequate production of some populations of HSPC is ensured.[106] Indeed, in IL-5-deficient mice responses to GM-CSF and IL-3 are normal.[107] These results highlight the multifaceted nature of allergic inflammation, suggesting that combined therapies may achieve higher clinical efficacy.

Antisense therapy

The use of antisense oligonucleotides in treating allergic asthma has been reported by Gauvreau *et al.*[108,109] In these studies, TPI ASM8, a combination of two antisense oligonucleotides, was used to block translation of the IL-3/IL-5/GM-CSF receptor βc and the translation of CCR3. TPI ASM8 significantly inhibited the accumulation of mature eosinophils and $CD34^{+}$ IL-5Rα^{+} cells in the sputum following allergen challenge, in addition to inhibiting the late asthmatic response. The results of these studies suggest that TPI ASM8 exerts its effects by inhibiting multiple signals (that is, IL-3, IL-5, GM-CSF, eotaxin-1, and eotaxin-2) shown to be crucial for eosinophil hematopoiesis, migration, activation, and survival.

Anti-TSLP

A more promising therapeutic target that may interrupt ISH directly, and thus be effective in the treatment of diseases such as allergic asthma, is TSLP. In a recent clinical trial,[110] intravenous administration of AMG 157, a human anti-TSLP monoclonal antibody that inhibits its interaction with the TSLPR, was demonstrated to reduce both the early and late asthmatic response, following an allergen challenge in mild atopic asthmatics. This was accompanied by a significant decrease in baseline blood eosinophil counts, suggesting that eosinophil levels are controlled by epithelial-derived TSLP and/or TSLP-responsive factors. Hui *et al.*[38] recently demonstrated the ability of TSLP to mediate Eo/B differentiation from human peripheral blood hematopoietic progenitor cells *ex vivo*, which was dependent on TSLP–TSLPR interactions. These data provide a potential mechanism by which AMG 157 may operate to decrease eosinophils and basophils at sites of inflammation. However, it is as yet unclear whether the inhibition of the allergen-induced responses was causally related to the decreased numbers of eosinophils in the blood and sputum;[110] further mechanistic studies of AMG 157 are warranted.

To date, of all the available asthma treatments, inhaled glucocorticoids are the gold standard, as they are the only agents known to attenuate baseline levels of blood eosinophil counts and exhaled nitric oxide, while all other available treatments can only attenuate components of allergen-induced airway responses.[111] Therefore, although the full clinical value of anti-TSLP therapy cannot be ascertained yet, the capacity of AMG 157 to attenuate baseline indices of inflammation appears most promising. Collectively, these findings highlight a possible direct role for TSLP in the development and persistence of allergic asthmatic airway inflammation by initiating hematopoietic responses in the mucosa and/or promoting ISH through provision of signals necessary for lineage commitment of inflammatory effector cells at mucosal surfaces. Further clinical studies will be needed to carefully evaluate the potential clinical benefits and/or side effects of anti-TSLP therapy, given that the biological effects of TSLP are strongly dependent on the local tissue microenvironment: while in the skin and airway TSLP induces T_H2 type inflammation, in the intestinal mucosa it promotes tolerance.[112]

Anti-CD34

Targeting CD34 itself could offer a therapeutic strategy to dampen allergic inflammation. As noted above, CD34 has been shown to play a key role in the trafficking of HSPCs to the appropriate sites of hematopoiesis in transplantation assays.[113–115] Although targeted deletion of the *Cd34* gene in mice by two groups yields only very subtle phenotypes at steady state,[116,117] it has also been shown that these mice are refractory to a variety of mucosal inflammatory diseases including allergic asthma,[116,118] hypersensitivity pneumonitis,[119] colitis,[120] salmonella infection,[121] and intestinal polyposis.[122,123] This appears to be through a general role for CD34 in blocking adhesion and enhancing mobility, and also through a highly selective role in enhancing chemotaxis to a subset of chemokines.[114,118,119] Intriguingly, in at least some cases, sensitivity to inflammatory disease is restored by transgenic expression of human CD34. Thus, targeting the CD34-dependent migratory capacity of progenitors could prove therapeutic. The lack of an overt phenotype in CD34-deficient mice suggests that this strategy should have few adverse effects at steady state.

SUMMARY

A growing body of evidence suggests that ISH operates as a conserved mechanism of the innate immune system that significantly contributes to the development of immunity and inflammation at mucosal sites. In addition, these studies strongly suggest that the ISH pathway represents a viable therapeutic target to treat multiple inflammatory disease states. Despite these advances, the specific pathways that regulate peripheral HSPC trafficking and differentiation into effector cells at the local site of inflammation remain unknown. Future studies are needed to better determine the molecular events that promote ISH and skew HSPC potential, in order to aid the development of new therapeutic targets to treat ISH-driven inflammatory disorders. In addition, developing a better understanding of how ISH differs from central hematopoiesis may inform the administration and dosing strategies of known therapeutics in a manner that can differentially target these specific pathways to better regulate inflammatory responses at mucosal sites.

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DISCLOSURE
The authors declare no conflict of interest.

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