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Review

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Basophil modulation by cytokine instruction

David Voehringer

Department of Infection Biology, Universitätsklinikum Erlangen and Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

Basophils have recently been recognized as critical effector cells in allergic reactions and protective immunity against helminths. Precise characterization of basophil biology could help to develop specific therapies that interfere with differentiation, tissue recruitment, or induction of effector functions and thereby ameliorate allergic disorders. The development, homeostasis, and effector functions of basophils are tightly regulated by extrinsic signals and in particular by cytokines. IL-3, GM-CSF, and thymic stromal lymphopoietin activate the STAT5 pathway that promotes proliferation, activation, and cytokine secretion but also induces a negative feedback loop via Pim-1 and SOCS proteins. Basophils further express receptors for IL-18 and IL-33, which are associated with the signaling adaptor MyD88 and activate the NF-κB and MAP kinase pathways. This review focuses on positive and negative regulation of basophils by these cytokines.

Keywords: Basophilia · IL-3 · IL-18 · IL-33 · TSLP

Introduction

Basophils are a subset of granulocytes and constitute less than 1% of circulating leukocytes in the peripheral blood. However, they increase in number and are recruited to sites of inflammation especially during immune responses against allergens or parasites. Basophils are closely related to mast cells as both cell types express the high-affinity receptor for IgE and a similar set of effector molecules, including histamine and Th2-associated cytokines. However, in contrast to mast cells, mature basophils lack the receptor tyrosine kinase c-Kit, express distinct serine proteases, can rapidly be mobilized from precursor cells during inflammatory conditions, and have a much shorter lifespan. Basophil in vivo functions could be elucidated by selective depletion of basophils with antibodies or use of genetically basophil-depleted mouse strains. These studies resulted in controversial findings, which are not subject of this mini-review and are discussed elsewhere [1–5]. Basophils express a variety of cytokine receptors and can therefore respond to numerous extrinsic signals that regulate their development, homeostasis, and effector function. Recent studies revealed novel insights in the regulation of basophil biology by cytokines of the hematopoietin family or the IL-1 family as outlined below.

Cytokine families and their receptors

Cytokines are small soluble proteins that regulate the generation, survival, and function of cells by binding to cell surface receptors. They can be grouped into different families based on structural features. Type I (or hematopoietic) cytokines adopt a four-helix bundle structure. Their heteromeric receptors are composed of ligand-specific chains that pair with signal-transducing chains used by a number of different cytokines. The common gamma chain (γ_c) is used by members of the IL-2 family (IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, and IL-21). The gp130 chain is used by IL-6 family members and the common beta chain (β_c) is used by IL-3, IL-5, and GM-CSF. In contrast to these structurally related type I cytokines, type II cytokines are more diverse and include members of the IL-10 family and interferons [6]. Type I and type II cytokines mediate their signals mainly by activation of Janus kinases (JAKs), which subsequently phosphorylate tyrosine residues in cytokine receptor chains and in signal transducer and activator of transcription (STAT) proteins. Phosphorylated STAT proteins form dimers and translocate to the nucleus where they serve as transcription factors [7]. In contrast to these JAK-STAT-coupled receptors, receptors for

Correspondence: Prof. David Voehringer
e-mail: david.voehringer@uk-erlangen.de

members of the IL-1 and IL-17 family belong to the STIR domain superfamily that consists of receptors that contain either the TIR (Toll/IL-1R) domain or the SEFIR (similar expression of fibroblast growth factor genes/IL-17R) domain. TIR and SEFIR domains serve as docking sites for downstream adaptors that mediate signals that lead to activation of NF-κB and MAP kinase pathways [8].

Cytokines in basophil development and homeostasis

Murine basophils have a lifespan of about 60 h under steady-state conditions [9]. They develop from a common granulocyte macrophage precursor (GMP) population. Yet, the lineage relationship of basophils to other hematopoietic cells is not fully understood. Recent work showed that basophil development from GMPs is tightly regulated by timed expression of the transcription factors c/EBPα and GATA-2 [10]. c/EBPα is constitutively expressed in GMPs. Downregulation of c/EBPα and simultaneous upregulation of GATA-2 in GMPs in the murine spleen generates a population of bi-potent basophil/mast cell precursors (BMCPs). Re-expression of c/EBPα in BMCPs results in the development of basophils while cells that fail to re-express c/EBPα develop into mast cells [11]. The extrinsic signals that regulate timed expression of c/EBPα and GATA-2 remain to be determined. Of note, IL-3, IL-5, GM-CSF, and thymic stromal lymphopoietin (TSLP) are not required for the development of basophils during steady-state conditions since basophils are not reduced in mice that lack the receptors for these cytokines [12]. However, STAT5 appears important for basophil development as basophils could not develop from a population of STAT5-deficient fetal liver cells after transfer into sublethally irradiated recipient mice [13]. STAT5 is also required for mast cell development, suggesting that development of a common BMCP is regulated by this transcription factor. STAT5 is associated with many cytokine receptors, including those for IL-2, IL-3, IL-4, IL-5, IL-7, IL-9, IL-15, TSLP, GM-CSF, erythropoietin, thrombopoietin, growth hormone, prolactin, leptin, and SCF. It remains to be determined which extrinsic signal(s) directly stimulates the development of basophils from precursor cells under steady-state conditions.

Cytokine-induced expansion of the basophil population

IL-3-mediated basophilia

The increase of basophils during infection of mice with the helminth *Nippostrongylus brasiliensis* is caused by a combination of enhanced production of new basophils in the bone marrow and prolonged survival of mature basophils [9]. Both mechanisms are regulated by extrinsic signals including cytokines of the hematopoietin family.

IL-3 has been shown to induce basophilia and mastocytosis during infection of mice with helminth parasites [14]. Further studies demonstrated that T cells are a major source of IL-3 for induction of basophilia during helminth infection [15]. However, IL-3 can also be produced by other cell types, including basophils themselves, suggesting an autocrine enhancement of basophil expansion. IL-3 does not selectively expand basophils but also supports the growth of other cell types including mast cells, dendritic cells, eosinophils, and monocytes. The IL-3 receptor is constituted of the IL-3R alpha chain (IL-3Rα) and the covalently linked common beta chain (β_c) (Fig. 1). The β_c is a type I transmembrane protein with four extracellular fibronectin domains. Mice that lack the β_c have fewer eosinophils and impaired functionality of alveolar macrophages [16]. The IL-3Rα/β_c complex forms a high-affinity receptor for IL-3 ($K_D = 0.1$ nM), although IL-3 can also bind with 100- to 1000-fold lower affinity to IL-3Rα alone [17]. Despite the lack of an intrinsic signaling module, IL-3Rα is required for JAK2 phosphorylation by the β_c and downstream activation of STAT5 [18]. The detailed structure of the ternary IL-3/IL-3Rα/β_c complex has not yet been resolved. However, one may infer it from the GM-CSF/GM-CSFRα/β_c complex that forms a 2:2:2 hexamer that assembles into a signaling-competent dodecameric or even higher ordered complex (Fig. 1) [19].

A second IL-3 receptor exists in mice but not in humans. This receptor comprises an IL-3-specific beta chain ($\beta_{IL\text{-}3}$) that associates with IL-3Rα to form a high-affinity receptor [20]. Deletion of $\beta_{IL\text{-}3}$ results in no obvious defect [16]. IL-3Rα is already expressed on the cell surface of basophil-precursor cells in the fetal liver of mice at embryonic day 16.5 while other surface markers on mature basophils such as FcεRI, CD49b, or 2B4 are poorly expressed on these cells [9]. Direct evidence that IL-3 causes basophilia by increasing the de novo production of basophils comes from in vitro studies [12, 13]. An increase in total basophil numbers was observed when purified basophil-precursors from the bone marrow of mice were cultured in the presence of IL-3 [12, 13]. Interestingly, IL-3-treated bone marrow cultures from β_c-deficient mice were not impaired in basophil production [21]. In addition, it has been demonstrated that IL-3 increases the survival of basophils by induction of NF-κB and upregulation of the serine/threonine kinase Pim-1in human basophils [22, 23]. The enhanced de novo production in combination with reduced apoptosis of mature basophils leads to a rapid increase of basophil numbers in response to IL-3. With regard to the other two β_c-associated cytokines it was demonstrated that GM-CSF but not IL-5 could further enhance murine basophil expansion in the presence of low amounts of IL-3 [21]. In vivo administration of IL-33 leads to indirect basophil expansion by the induction of GM-CSF and IL-3 in bone marrow cells, including basophils themselves [21]. In the human system, it could be shown that IL-5 and GM-CSF enhance IL-4, IL-13, and leukotriene C4 release from basophils in response to the anaphylatoxin C5a [24].

Basophilia induced by TSLP

Injection of recombinant TSLP into mice leads to increased frequencies of basophils in the blood [25]. TSLP belongs to the hematopoietin family of cytokines and is structurally related to

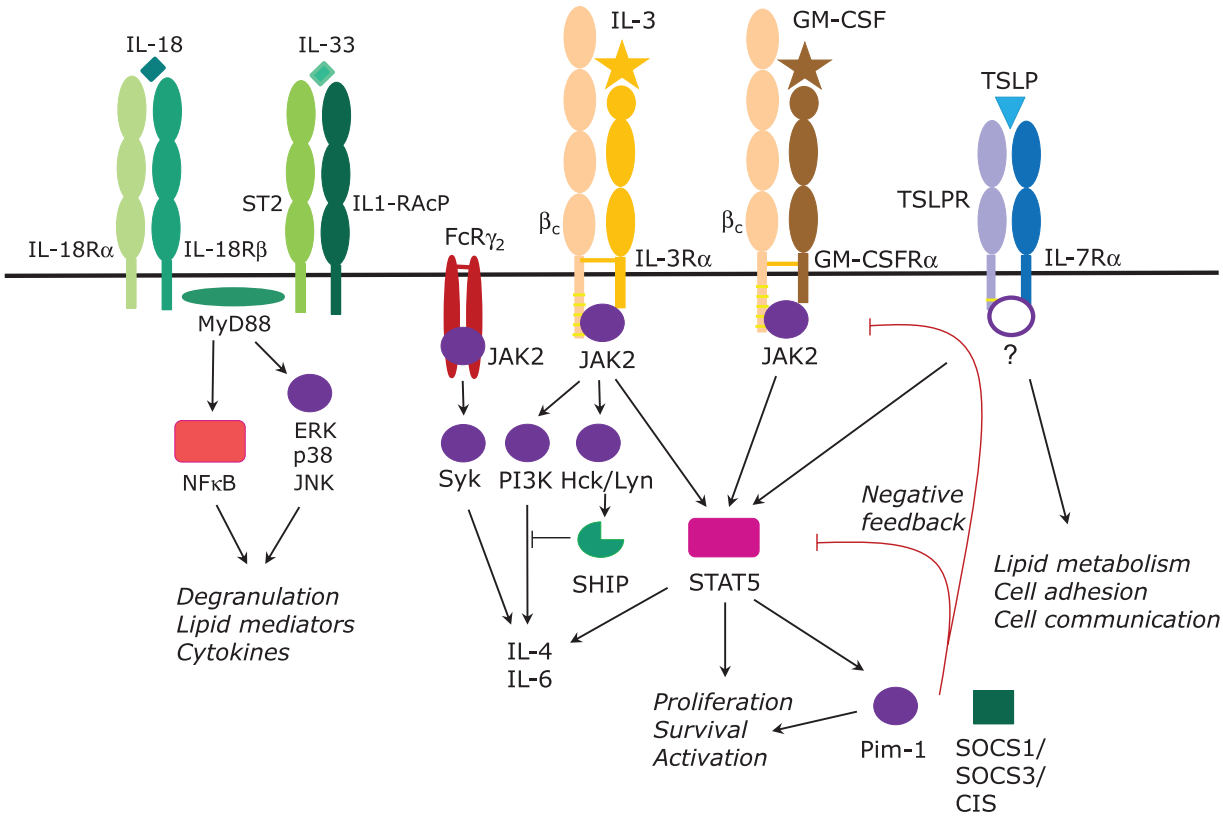

Figure 1. Schematic illustration of signaling cascades in basophils induced in response to IL-3, GM-CSF, TSLP, IL-18, or IL-33. IL-3 and GM-CSF induce cytokine secretion, activation, proliferation, and survival of basophils by JAK2-mediated phosphorylation of STAT5. TSLP can also activate STAT5 but probably independently of JAK2. In addition, TSLP induces genes that are involved in lipid metabolism, cell adhesion, and cell communication. A negative feedback loop of the JAK2-STAT5 pathway by Pim-1 and SOCS1/SOCS3/CIS proteins dampens this pathway. IL-3-induced release of IL-4 further requires the FcRγ2-mediated activation of Syk. This pathway is negatively regulated by Hck/Lyn-mediated phosphorylation of the phosphatase SHIP. The receptors for IL18 and IL-33 transmit their signals via MyD88 and activation of the NFκB or ERK/p38/JNK pathways, which ultimately lead to degranulation and secretion of lipid mediators and cytokines.

IL-7 [26]. The high-affinity receptor for TSLP is a heterodimer formed by the IL-7 receptor alpha chain (IL-7Rα) and the TSLP receptor (TSLPR), which is closely related to the γ_c chain [27] (Fig. 1). However, in contrast to the γ_c chain, the cytoplasmic tail of TSLPR lacks binding motifs for JAKs and contains only one tyrosine residue. TSLPR alone can bind TSLP with low affinity. Downstream signaling from the TSLPR/IL-7Rα complex leads to phosphorylation of STAT5 by a yet to be identified kinase. Although it seems likely that TSLP-induced basophilia is mediated by direct binding of TSLP to basophils, it is also possible that TSLP triggers other cells to release factors that promote basophil development and proliferation or enhance survival. It was demonstrated that 1 μg/mL TSLP promotes the survival of purified murine bone marrow derived basophil precursor (BaP) cells although the total number of cells did not increase [12]. In contrast, BaP cultured in the presence of 0.01 μg/mL IL-3 gave rise to about fivefold more basophils. This experiment suggests that TSLP may cause basophilia in vivo by reducing the rate of apoptosis in mature basophils rather than by increasing the de novo production of basophils in the bone marrow. Overexpression of TSLP in mice under control of a ubiquitous promoter resulted in severe reduction of B- and T-cell precursors and expansion of myeloid precursors indicating that lymphoid and myeloid precursors respond differently to TSLP [28]. Increased serum levels of IL-5 were observed in TSLP transgenic mice suggesting that myeloid expansion may be caused by indirect upregulation of IL-5 [28]. To further determine the relative contribution of TSLP for expansion of basophils in response to parasites or allergens, it would be informative to study such responses in mice that lack TSLPR only on basophils.

Negative regulation of basophil expansion

In contrast to IL-3, GM-CSF and TSLP, which promote the expansion of basophils, other cytokines have been reported to induce basophil apoptosis. IL-18 can indirectly induce Fas-mediated apoptosis of immature murine basophils in the presence of IL-12. It has been shown that IL-18 plus IL-12 cause increased production of IFN-γ and TNF-α from NK cells, which then sensitizes basophils for Fas-mediated apoptosis [29].

Furthermore, it has been shown that the transcription factor IRF-2 limits the expansion of murine basophils in response to IL-3

although the molecular details of this effect remain unresolved [30].

Overexpression of bcl-2 blocks the apoptosis of neutrophils [31]. A similar antiapoptotic effect of bcl-2 has been proposed for basophils. Basophils from H2K-bcl-2 transgenic mice were reported to survive better in adoptive transfer experiments as compared to basophils from normal mice [32]. However, other pathways may also regulate apoptosis in basophils. For example, conditional deletion of the antiapoptotic protein Mcl-1in murine mast cells and basophils leads to a complete deletion of mast cells and a 58–78% reduction of basophils [33].

Cytokine modulation of basophil recruitment and effector functions

Regulation of cytokine-induced basophil recruitment

IL-3 has been shown to be required for recruitment of basophils into lymph nodes after infection of mice with the helminth *N. brasiliensis* [34]. In contrast, basophil recruitment to the skin in response to the vitamin D3 analog MC903 occurred independently of $\beta_c/\beta_{cIL\text{-}3}$ and was blocked by anti-TSLP antibodies [12]. These findings illustrate that selective tissue recruitment of basophils can be initiated by different cytokines. Most likely tissue-specific recruitment is caused by indirect effects of IL-3 and TSLP resulting from differential expression of chemokine receptors, selectins or integrins, on basophils. IL-33 has been shown to directly act on human basophils where it induced expression of CD11b and increased their responsiveness to eotaxin suggesting that homing into inflamed tissue may in part be regulated by IL-33 [35].

Regulation of basophil effector functions by cytokines

Basophils are a major source of IL-4. IL-4 is constitutively expressed at low levels and independently of STAT6. Instead, the transcription factor c/EBPα, which is also a critical component of the developmental program of the basophil lineage, was recently found to regulate IL-4 expression in basophils [36]. The secretion of IL-4 from basophils can be induced by IL-3. Interestingly, this stimulatory activity of IL-3 depends on association of the Fc receptor common gamma chain (FcRγ) with the β_c (Fig. 1) [37]. IL-3 also enhances degranulation by facilitating FcεRI-mediated basophil activation. This phenomenon might be explained by the fact that STAT5 is involved in signal transduction from the IL-3 receptor and FcεRI [38]. Although TSLP can activate STAT5, it probably triggers other signaling cascades that induce a distinct set of effector molecules involved in cell adhesion, cell communication, and lipid metabolism [12].

Besides IL-3 and TSLP, basophils can be activated by two members of the IL-1 family, namely IL-18 and IL-33. IL-18 is secreted from inflammatory monocytes and DCs in response to signals that activate inflammasomes. The core structure of inflammasomes consists of an NLR (nod-like receptor) family member, which can either directly of indirectly (via binding of ASC) recruit and process pro-caspase-1 to caspase-1 [39]. Caspase-1 processes pro-IL-18 to IL-18, which is then secreted. IL-18 is mainly known for its induction of IFN-γ secretion from Th1 cells and NK cells in combination with IL-12 or IL-15 [40]. However, IL-18 effector functions are not restricted to Th1-associated immune responses. It was shown that IL-18 can promote Th2 responses in combination with IL-2 [41]. Furthermore, IL-18 stimulates the histamine release, IL-4 production, and leukotriene synthesis from murine basophils and the human basophilic cell line KU812 in the presence of IL-3 or FcεRI cross-linking [42, 43]. However, additional factors might be required for activation of human basophils since freshly isolated human basophils did not respond to IL-18 [44]. The IL-18 receptor is formed by the IL-18Rα and IL-18Rβ chains (Fig. 1). IL-18R is highly expressed in basophils and IL-3 does not further enhance its expression level [44, 45].

IL-33 is rapidly released from fibroblasts, epithelial cells, and endothelial cells in response to injury or infection and serves as "alarmin" to initiate the immune response [46]. This cytokine plays a prominent role in Th2-associated immunity such as allergic inflammation and helminth infections. IL-33 has been shown to induce expression of IL-4, IL-5, IL-6, IL-8, IL-9, IL-13, and GM-CSF in human basophils [47, 48]. IL-33 also promotes upregulation of the leptin receptor on basophils. Leptin, a member of the IL-6 family, can enhance survival, induce migration, degranulation, and cytokine release in human basophils [49]. The activating receptor for IL-33 (IL-33R1) is composed of two subunits, ST2 and the IL-1 receptor associated protein (IL-1RAcP) (Fig. 1). ST2 was first described to be expressed by Th2 cells but not by Th1 cells and served as marker to distinguish both T-cell subsets [50]. ST2 further serves as activating receptor on other cells that are associated with type 2 immunity including mast cells, basophils, eosinophils, and innate type 2 lymphocytes (also named nuocytes). Surprisingly, ST2 is also expressed on activated CD8 T cells and appears to be required for efficient CTL responses against replicating viruses [51]. The expression level of ST2 on human basophils can be upregulated by IL-3 [44]. IL-3 also induced a soluble form of ST2 (sST2), which localized to basophil granules but the function of this soluble receptor in basophils remains unclear [44]. sST2 might be released from basophils by degranulation and serve as decoy receptor to block IL-33 signaling [52]. The IL-33R1 and IL-18 receptor transduce signals via recruitment of MyD88 and activation of NF-κB, ERK, p38, and JNK pathways [53]. These signaling cascades ultimately result in degranulation, cytokine production, and generation of lipid mediators.

Inhibition of basophil activity and development by cytokines

In contrast to its stimulatory activity, the IL-3 receptor may also inhibit the cytokine release of basophils. This function requires activation of the Src family kinases Hck and Lyn via JAK2 [54]. Hck/Lyn then phosphorylate SHIP, a phosphatase that counteracts the activity of PI3K by hydrolyzing PIP3 to PI-3,4-P2 resulting

in reduced IL-4 production from basophils [54] (Fig. 1). Therefore, IL-3 can either induce or inhibit IL-4 expression in basophils depending on the balance of the downstream signaling pathways from the IL-3 receptor. Lyn further seems to inhibit basophil development since Lyn-deficient mice have more basophils and a spontaneously Th2-skewed immune system [55]. However, this inhibitory effect of Lyn on basophil expansion occurs independently of SHIP [54].

A negative feedback loop of IL-3 signaling is mediated by *Pim-1*, a STAT5 target gene, which encodes an oncogenic serine/threonine kinase (Fig. 1). Pim-1 inhibits STAT5-dependent gene expression by interaction with suppressor of cytokine signaling (SOCS)1 and SOCS3 [56]. Another SOCS-family member, CIS (cytokine-inducible SH2-domain-containing protein) is also induced by STAT5 and represses STAT5 phosphorylation [57]. SOCS proteins inhibit signal transduction from various cytokine receptors. The SOCS proteins attach via their SH2-domains to phosphotyrosine sites on active JAKs or cytokine receptors and recruit the ubiquitin-transferase system via their SOCS box. SOCS proteins function as E3 ubiquitin ligases and cause degradation of proteins they bind to [58].

Although IL-18 and IL-33 are generally considered to have activating properties, it remains to be analyzed whether prolonged engagement of their receptors induces a negative feedback loop. Negative feedback regulation is probably an important pathway involved in the downregulation of basophil activity at later stages of an immune response.

Conclusions

The developmental program and the activity of basophils are tightly regulated by cytokines. IL-3 plays a major role in mobilizing basophils from precursor cells, recruiting basophils to lymph nodes, and enhancing their effector functions. TSLP has similar activities but appears less efficient and induces a different spectrum of gene expression. The IL-3 receptor can also mediate inhibitory functions by activation of SHIP, Pim-1, or SOCS proteins. At present, we know very little about the regulation of activating versus inhibiting signals that modulate basophil homeostasis and activity in vivo. With the development of new mouse models, we can now begin to fill these gaps of knowledge by selective deletion of genes in basophils or reconstitution of basophil-deficient mice with basophils from various knock-out mice. In addition, in vitro studies with human basophils are important to verify whether observations made in mouse models are translatable to the human immune system.

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Abbreviations: **BMCP**: basophil/mast cell precursor · β_c: beta chain · **TSLP**: thymic stromal lymphopoietin · **TSLPR**: TSLP receptor

Full correspondence: Prof. David Voehringer, Department of Infection Biology, Universitätsklinik Erlangen and Friedrich-Alexander-Universität, Wasserturmstrasse 3–5, D-91054 Erlangen, Germany
Fax: +49-(0)9131–85-32733
e-mail: david.voehringer@uk-erlangen.de

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