Human Basophils and Cytokines/Chemokines

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ABSTRACT
Basophils comprise the smallest population in human peripheral blood leukocytes. The role of basophils in the pathogenesis of allergic diseases has long been obscure, although their accumulation and activation in tissues have suggested their potential importance. Recent advances in the field of basophil biology have indicated that cytokines and chemokines are the primary regulators of basophil functions. In addition, various functions of these cells seem differently modulated. The evidence strongly supports the notion that basophils exposed to these substances and allergens will behave as unique effector cells that presumably play proinflammatory roles in type I allergic reactions.

KEY WORDS
allergy, basophils, cell activation, chemokines, cytokines, human

INTRODUCTION
Basophils are the least abundant cell type among human peripheral blood leukocytes. They have a high affinity receptor for IgE (FcεRI) on their surface, and cross-linking of IgE molecules by specific antigens or anti-IgE antibodies, or ligation of FcεRI molecules by anti-FcεRI antibodies, leads to the liberation of preformed mediators such as vasoactive amines. Basophil activation also results in de novo synthesis of lipid mediators such as LTC4, in addition to cytokines including IL-4 and IL-13. Through release of these mediators and cytokines, basophils are thought to be active participants in the pathogenesis of IgE-mediated allergic inflammation.¹ ²

Several lines of evidence have suggested that basophils represent important effector cells in the pathogenesis of allergic late-phase reactions. Local accumulation of these cells is observed at sites of nasal and cutaneous late-phase reactions,³ ⁴ and it is reported that late-phase nasal secretions and bronchoalveolar lavage fluid contain a cell population which is morphologically identified as basophils.⁵ ⁶ In addition, analysis of the local chemical mediator profile in nasal secretions has indicated that basophils may be a significant source of the mediators in late-phase reactions.⁴ ⁷ Recently, Karasuyama et al. elegantly showed that basophils are a critically important player in the pathogenesis of IgE-mediated very-late-phase allergic responses and IgG-mediated anaphylactic reactions in mice⁸-¹⁰ (also see the article by Karasuyama appearing in this issue).

Although the precise mechanisms of basophil accumulation and activation in local tissues of antigen-induced allergic inflammation and chronic allergic diseases such as asthma remain unclear, it is thought that there must be an active pathway that attracts and stimulates basophils, since those cells usually reside in circulating blood. Basophils are motile cells, and they seem to have a sophisticated sensor system relative to their surrounding environment.¹¹,¹² In a series of analyses of basophils’ biological functions, we have identified certain cytokines and chemokines as potentially important regulators that act on them. Interestingly, these biologically potent regulators modulate various arrays of basophil functions. In this article, we discuss how each function of basophils is regulated by cytokines and chemokines. And, in the last section, we describe our recent findings that a new cytokine of the IL-1 family, IL-33, is able to regulate diverse basophil functions.
Activating IL-3 receptor α (IL-3Rα) on basophils results in marked enhancement of histamine release initiated by anti-IgE, formylmethionyl-leucyl-phenylalanine (FMLP), calcium ionophore A23187 and phorbol ester. IL-3 also renders basophils susceptible to stimulation with secretory IgA, complement C3a or platelet-activating factor (PAF), which alone are unable to transduce signals sufficient for basophil degranulation.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-5 also enhance histamine releasability from basophils. Based on inspection of the ED50 values, IL-3 exerted the most potent priming effects (ED50: 0.3 and 1 pM for anti-IgE- and FMLP-induced degranulation, respectively), while IL-5 and GM-CSF were ~30-fold less active compared with IL-3. The difference in basophil sensitivity to the cytokines may reflect the varied receptor levels. However, the maximal responses at the optimal doses of IL-5 and GM-CSF were equivalent to those induced by IL-3.

Insulin-like growth factor (IGF-I) enhanced histamine release initiated by anti-IgE, calcium ionophore A23187 and phorbol ester. Stem cell factor (SCF) also weakly enhanced degranulation triggered by some, but not all, secretagogues. Interestingly, IGF-I and SCF co-operatively enhanced surface expression of an activation marker, CD69, on basophils. IL-1, interferon (IFN)-γ, and nerve growth factor (NGF), but not IL-4, tumor necrosis factor (TNF)-α, or transforming growth factor (TGF)-β, can also enhance basophil degranulation.

Several studies, including ours, have shown that prolonged incubation with IL-3 profoundly affects basophil releasability. For example, basophils from 10 to 20% of donors completely fail to release histamine in response to anti-IgE, and such donors are called non-releasers. Long-term culturing (>3 d) with IL-3 results in these basophils becoming able to release histamine in response to anti-IgE or specific antigen. Detailed intracellular signal analysis indicated that a defect in the tyrosine kinase Syk accounts for the lack of basophil response to IgE crosslinking stimulus in those donors, and that the defect is corrected after >3 days of culture with IL-3.

IL-3 is also known to enhance the secretion of de novo synthesized mediators such as LTC4. At picomolar concentrations, short-term preincubation with IL-3 primes basophils for enhanced LTC4 secretion after challenge with anti-IgE or FMLP. Basophils are reported to be an important source of Th2 cytokines, including IL-4 and IL-13; although short-term pretreatment with IL-3 (30 min) does not affect IL-4 release from basophils, long-term incubation with IL-3 (18 h) results in a marked increase in IL-4 secretion.

In addition to their chemotactic activities on leukocytes, chemokines are able to directly activate basophils. As demonstrated by us and others, MCP-1/ CCL2 was the most potent secretagogue among chemokines. MCP-1 induced approximately 20%
of histamine release from freshly isolated basophils, which was apparently amplified by priming with IL-3. IL-8/CXCL8 elicited weak degranulation from basophils, which was also enhanced by IL-3 pretreatment. On the other hand, freshly isolated basophils did not degranulate significantly in response to SDF-1/CXCL12, MIP-1α/CCL3 or MIP-1β/CCL4 in our study.

We recently reported that basophils, retrieved by bronchoalveolar lavage from patients with asthma, showed elevated levels of surface CD69 compared to the patients’ peripheral blood basophils, and that strong induction of surface CD69 expression occurs in vitro in the presence of relatively high doses of IL-3. This was in clear contrast to the finding that other surface molecules on basophils such as CD44 or CD54 failed to demonstrate differences between healthy and asthmatic subjects. Thus, CD69 was thought to be a useful marker for basophil activation, with potential clinical relevance, although we failed to observe any functional significance for basophil CD69. We previously reported that expression of CD69 by basophils was preferentially upregulated by IL-3. CD69 analysis using basophils cultured for 24 h with various concentrations of cytokines showed that expression of CD69 required considerably large amounts of IL-3 (ED50: 50 pM), whereas even as large as nanomolar orders of either IL-5 or GM-CSF induced only marginal levels of CD69 expression.

Interestingly, very low doses of antigen or anti-FcεRI chain mAb (CRA-1) (1 ng/ml) clearly enhanced basophil CD69 expression in the presence of IL-3; such concentrations of antigen or CRA-1 mAb correspond to subthreshold doses for triggering degranulation of basophils.

RT-PCR analysis demonstrated that basophils possessed transcripts for several Toll-like receptors (TLR), such as TLR4, TLR2, TLR9 and TLR10. Various TLR ligands were tested on freshly isolated basophils, but no functional changes were detected. After incubation of basophils with IFN-γ, a TLR4 ligand lipopolysaccharide slightly upregulated CD11b expression on basophils.

**ADHESION**

Several lines of evidence indicate that IL-3 is involved in the regulation of basophil adherence to vascular endothelial cells. As reported by Bochner et al., IL-3 stimulates in vitro adherence of basophils to vascular endothelial cells. And their enhanced adherence was thought to be mediated by upregulation of surface β2 integrin (CD11b/CD18) expression. We found that not only IL-3 but also other hemopoietic growth factors, i.e., IL-5 and GM-CSF, up-regulated CD11b expression on basophils. The receptors for these cytokines are known to share a common β subunit as a signal-transducing apparatus, but the potency of the three cytokines in enhancing CD11b expression was not equal. IL-3 was the strongest inducer of surface CD11b, and half-maximal induction was obtained at as low as 6 pM of IL-3. IL-5 and GM-CSF were less potent compared to IL-3, and ~10-fold higher doses of these two cytokines were necessary for significant induction of CD11b expression on basophils. In a recent study analyzing basophil rolling and adhesion under physiological shear flow conditions, P-selectin and β1 integrins (CD49d and CD49e/CD29) on basophils were involved in both rolling and adhesion on IL-3-treated endothelial cells.

**MIGRATION**

In previous studies using a modified Boyden chamber and polycarbonate membrane with 5-μm pores, serially diluted hemopoietic growth factors were tested for basophil migratory activity. We found that GM-CSF, IL-3 and IL-5 had marked effects on basophil locomotion: the maximal numbers of migrated basophils increased to more than 10 times the background levels. GM-CSF was slightly more potent than IL-3 and IL-5. Checkerboard analyses indicated that these cytokines had mainly chemokinetic rather than chemotactic effects.

We next examined the expression profile of a panel of chemokine receptors in basophils, and found that basophils expressed transcripts of CCR1, CCR2, CCR3, CCR5, CXCR1, CXCR2 and CXCR4. Among 16 chemokines tested, eotaxin/CCL11 induced the most potent basophil migration. In addition, migration of basophils toward eotaxin was enhanced by very weak FcεRI-crosslinking stimulus, CRA-1 mAb at 1 ng/ml. SDF-1 also induced a strong, migratory response comparable to that induced by eotaxin in 24-h cultured basophils, reflecting CXCR4 expression induced during culture. SDF-1 did not elicit any calcium influx in freshly isolated basophils but caused strong influx in 24-h cultured basophils. Similar to as we already demonstrated for eosinophils, expression of CXCR4 on basophils was also regulated by several cytokines: it was markedly suppressed by IL-3 and slightly suppressed by GM-CSF, IL-5 and IL-4. However, IFN-γ, TGF-β and TNF-α each had no effect on basophil CXCR4 expression. CXCR4 expression on basophils was highly sensitive to IL-3, a femtomolar order of which was sufficient for inhibition of the expression.

**TRANSENDOTHELIAL MIGRATION (TEM)**

We have established a basophil TEM assay using human basophils and Transwell systems (Costar, Cambridge, MA, USA) with cultivated human umbilical vein endothelial cells (HUVEC) on the surface. HUVEC were activated by stimulation with IL-1β for 4 h before performing assays.

Figure 2 depicts the effects of various chemokines

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on TEM of Percoll-separated human basophils. Highly purified basophil preparations after Percoll and MACS separation gave essentially the same results. TEM was determined after 3 h of incubation using IL-1β-activated HUVEC. Among the various chemokines tested, CCR3 ligands, eotaxin/CCL11 and RANTES/CCL5 induced strong basophil TEM. Eotaxin binds specifically and exclusively to CCR3, whereas RANTES binds to both CCR1 and CCR3, although with higher affinity for CCR1. To determine the chemokine receptors responsible for RANTES-induced basophil TEM, we next performed blocking experiments using receptor-specific mAbs. As expected, eotaxin-induced TEM was almost completely inhibited by anti-CCR3 mAb. Although RANTES-induced TEM was also markedly blocked by anti-CCR3 mAb, treatment with anti-CCR1 mAb exerted virtually no effect, suggesting that CCR3 is the responsible receptor mediating the transmigratory response for both eotaxin and RANTES. This is also interesting in that it is the reverse of the relative affinities of RANTES for these two receptors, indicating that the affinity and TEM inducing activity of RANTES are not equivalent. Moreover, there was yet another chemokine receptor which induced basophil TEM: SDF-1, a specific ligand for CXCR4. Although freshly isolated basophils failed to exhibit TEM in response to SDF-1, strong TEM was observed with 24-h cultured basophils. In parallel to our previous findings regarding basophil chemotaxis, these results show that both CCR3 and CXCR4 are involved in basophil TEM. We found that β2 integrin is the key adhesion molecule that mainly accounts for basophil TEM. β1 integrin is also slightly involved, since TEM of fresh basophils toward eotaxin or IL-3 was significantly suppressed by treatment of basophils with anti-CD18 mAb, and weakly suppressed by anti-CD29 mAb.

The involvement of IL-3 was also assessed in that study. When no chemokines were added to the lower wells, a significant increase in basophil TEM was observed when the cell suspension in the upper wells contained as low as 3 pM of IL-3. Furthermore, IL-3 showed an additive effect on eotaxin-directed TEM: the number of basophils that transmigrated toward eotaxin was significantly increased in the presence of IL-3. However, the magnitude of IL-3’s effect on basophil TEM was modest compared to the strong effect of eotaxin.

**TRANS-BASEMENT MEMBRANE MIGRATION**

Basophil trans-basement membrane migration assay was performed using an experimental model, Matrigel (BD Biosciences, Bedford, MA, USA), a gel containing basement membrane components, i.e., laminin, type IV collagen, heparan sulfate, proteoglycan and entactin. Various chemokines were placed in the lower chamber. After 18 h of incubation, approximately 10 to 16% of the basophils had transmigrated spontaneously to the lower chamber. However, no apparent induction of transmigration was observed with any of the tested chemokines (“Nil” columns in Fig. 3A), showing a clear contrast to the findings for basophil TEM, in which cells actively transmigrated towards certain chemokines. We next included the potent basophil-active cytokine, IL-3, at 300 pM in the upper chamber. As shown in Figure 3A (“IL-3” columns), IL-3 induced statistically significant basophil trans-basement membrane migration even in the absence of chemoattractants in the lower chambers. Moreover, among the chemokines tested, IL-8 and RANTES added to the lower chambers at 50 nM induced a significant increase in migration compared to the spontaneous migration of IL-3-treated basophils. Other chemokines, including MCP-1 and eotaxin, exhibited weak basophil attracting potency, but it did not reach statistical significance. Another well-
known chemoattractant for basophils, complement C5a, failed to show significant transmigration-inducing activity. On the other hand, among the lipid mediators tested, 5-oxo-ETE and PAF at 1 μM significantly induced basophil transmigration, but only when IL-3 was included in the upper chamber (data not shown). Thus, the repertoire of attracting substances involved in this basophil transmigration system was unique compared to that in TEM or simple migration. Blocking experiments clearly demon-
strated that both CCR1 and CCR3 were involved in the RANTES-directed trans-basement membrane migration of basophils, and that CXCR1, but not CXCR2, was involved in the case of IL-8 (Fig. 3B).51 These results showed that not only CCR3 but also two other chemokine receptors, CCR1 and CXCR1, are involved in this transmigration across Matrigel. Anti-CD18 mAb but not anti-CD29 significantly inhibited IL-3 plus RANTES-induced transmigration, indicating that β2 integrin, but not β1 integrin, plays a central role in trans-Matrigel migration of basophils.

Importantly, IL-3 was essential for chemoattractant-induced transmigration, since, in the absence of IL-3, none of the tested chemokines upregulated basophil transmigration above the control level. In addition, an elastase inhibitor that blocks certain matrix metalloproteinases (MMPs) obviously attenuated transmigration of basophils. The results of quantitative real-time PCR and immunohistochemical studies suggested that MMP-9 is synthesized, stored in the cytoplasm, expressed on the cell surface and released by basophils, and flow cytometric analysis indicated that IL-3 upregulated surface MMP-9 levels on basophils.51 Presumably the effects of IL-3 on the expression of both β2 integrin and MMP-9 are importantly involved in the transmigration: cell-surface MMP-9 and/or MMP-9 that has been released will cooperatively help basophils expressing enhanced levels of β2 integrin cross the basement membrane.

SURVIVAL

It was already reported that IL-3 maintained the viability of purified basophils during culture.52 In the absence of cytokines, basophils rapidly died, and survived in vitro for only 3 days. Addition of IL-3 resulted in marked enhancement of basophil survival, such that nearly half of the cells remained viable for 14 days, as assessed by trypan blue stain. Several years after that initial study, the anti-apoptotic potencies of cytokines were analyzed by flow cytometry.20,53 As judged by their plateau levels, IL-5 and GM-CSF were equally effective with IL-3 in protecting basophils from apoptosis. The rank order of potency as assessed by the ED₅₀ values was IL-3 > IL-5 = GM-CSF, with IL-3 being ~10-fold more potent than the others. It should be noted that even a very low concentration of IL-3 was sufficient for survival enhancement (ED₅₀: 20 fm). IFN-γ is also reported to enhance basophil survival, but IL-4 or TNF-α is not.11 On the other hand, SCF, in concert with IL-3, was shown to prolong survival of basophils.23

A glucocorticoid, dexamethasone, is known to induce apoptosis in basophils. To determine whether IL-3 can overcome the apoptosis-inducing effect of dexamethasone, basophils were incubated with serially diluted IL-3 in the presence and absence of dexamethasone (100 nM).53 The enhanced life span induced by low concentrations of IL-3 (300 fm) was clearly shortened by dexamethasone, with statistical significance. On the other hand, dexamethasone exerted no significant effect on the rate of basophil apoptosis in the presence of higher concentrations of IL-3 (3–300 μM). Similar results were observed for the relationships between the effects of IL-3 and basophil-apoptotic substances other than glucocorticoids.54

EFFECT OF A NEW CYTOKINE, IL-33 ON ADHERENCE AND ACTIVATION OF BASOPHILS

IL-33 is a recently identified cytokine that belongs to the IL-1 family.55 This cytokine binds to the ST2 receptor (also called DER4, Fit-1 or T1), which has high homology to IL-1 receptor.55 ST2 receptor is reported to be expressed on mast cells56 and Th2 cells,57 but not on Th1 cells. ST2 has been considered to mediate the biological action of its ligand, IL-33, which can cause Th2-biased allergic inflammation. Accumulating evidence suggests that IL-33 can exert significant biological effects both in vivo and ex vivo.55,57–59 For example, IL-33 enhances production of Th2-associated cytokines by in vitro polarized Th2 cells. In addition, in mast cells, we recently demonstrated that IL-33 enhanced the survival of human umbilical cord blood-derived mast cells and promoted their adhesion to fibronectin as well as their production of IL-8 and IL-13.60 IL-33 is now recognized as a potentially important cytokine that enhances Th2-balanced immune regulation, but the action of IL-33 on allergic effector cells had not been known until recently.

In our very recent study analyzing the effects of IL-33 on human basophils, we demonstrated that basophils express the transcript and protein for ST2, a receptor for IL-33. IL-33 affected several arrays of basophil functions: this cytokine upregulated CD11b expression, induced eosinophil migration and degranulation, and enhanced eosinophil chemotaxis, induced Th2 cytokine IL-4 secretion and augmented the IgE-mediated histamine release reaction.61 Importantly, basophil adhesion was potently enhanced by IL-33, and this action of IL-33 was stronger than that of IL-3, a well-known basophil-active cytokine. Neutralization experiments demonstrated that ST2 mediates IL-33’s effects on basophils. In other studies analyzing the actions of IL-33, we found that this cytokine also activates human eosinophils.62 However, the precise effects of IL-33 on eosinophils differ somewhat from those on basophils: IL-33 failed to enhance migration and degranulation of eosinophils but suppressed eosinophil apoptosis,61 whereas basophil apoptosis was not affected by IL-33. The different spectra of IL-33’s effects on basophils and eosinophils may in part account for the different behaviors and fates of these effector cells in the controlling mechanisms of allergic inflammation.

These findings suggest that IL-33 may be a potent and unique regulator acting not only on lymphocytes
Fig. 4 Cytokines and chemokines affect human basophils. Representative cytokines and chemokines that either enhance or directly trigger various functions of these cells are described. TEM: transendothelial migration. TBMM: trans-basement membrane migration.

or mast cells, but also on effector cells such as basophils and eosinophils.\textsuperscript{61-64} Further studies assessing the precise roles of IL-33 in various allergic diseases will be important.

**CONCLUSION**

Basophils constitute the smallest subpopulation of leukocytes, but they seem to be powerful cells capable of secreting various bioactive mediators in tissues and in the blood, where they encounter allergens and/or their affecting cytokines/chemokines. In human peripheral blood, basophil density is strictly regulated: the percentage of basophils among total peripheral leukocytes usually does not exceed 2%. After undergoing maturation steps in the bone marrow, basophils enter the blood stream. After a rather short (hours to days) stay in the circulating blood, basophils are thought to be trapped in tissues with elevated levels of attracting molecules (Fig. 4). Although several features, such as metachromatic stain, histamine content and surface expression of FceRI, indicated great similarities between basophils and mast cells, these two cell types are not very close in origin. Mast cells reside in the bone marrow only during the initial steps of their differentiation; mast cell progenitors enter the blood and then move into tissues where they proliferate and mature.\textsuperscript{10} Now we believe that eosinophils show a closer association with basophils; the mechanisms regulating differentiation/maturation of these cells are quite similar.\textsuperscript{11} We thus think that the detailed analyses of the biological functions of mature basophils and eosinophils focusing on cytokines and chemokines, as presented in this manuscript, are important. Interestingly, these substances discussed in this manuscript are also known to be important regulators of mature eosinophil functions,\textsuperscript{11,65} and the repertoires of cytokines/chemokines acting on basophil and eosinophil functions are mostly overlapping. For example, a CCR3 ligand, eotaxin, is a very potent chemoattractant for both basophils and eosinophils freshly isolated from peripheral blood.

However, in our studies, we have also found that the actions of cytokines and chemokines on basophils are not identical to those on eosinophils. Of course, substances that either modify or directly induce basophil degranulation cannot be theoretically extrapolated to eosinophils, since eosinophils do not manifest rapid degranulation. It has long been known that, among the three hemopoietic growth factors, IL-3, IL-5 and GM-CSF, IL-3 most potently activates various types of basophil functions, whereas IL-5 efficiently activates eosinophils. Their differences have so far been explained on the basis of the levels of their receptor expression in these cells. IL-3 receptor α chain (IL-3Rα) mRNA is abundantly expressed in basophils, but in eosinophils IL-5Rα mRNA is dominant among the α chains of the three cytokines (Fig. 1).\textsuperscript{20} Thus, the receptor expression levels may be an important factor that determines the sensitivity and magnitude of the cells’ responses to each cytokine/chemokine. The expression levels of cytokine/chemokine receptors are not always stable; transient or inducible expression of receptors will significantly
modify the actions of basophils at inflammatory sites. For example, basophil CXCR4 expression is strongly induced during culture for a few hours or one day, and basophils acquire responsiveness to the ligand, SDF-1, during culture.\textsuperscript{35} Interestingly, basophil CXCR4 expression is almost completely suppressed by IL-3, and cells exposed continuously to IL-3 presumably retain relatively low sensitivity to SDF-1. In addition, IL-33 enhances basophil expression of the IL-3 receptor, ST2, and these cells, having been once exposed to IL-3, may maintain their response to this cytokine.\textsuperscript{91}

Recent progress, especially regarding the \textit{in vivo} roles of basophils, has greatly increased the attention paid to this small leukocyte subpopulation. Moreover, in this context, clarification of basophil-unique mechanisms will provide us with important insights into disease pathogenesis. Such information will, in turn, contribute to our understanding of the pathogenesis of various allergic diseases, including asthma and anaphylaxis, in which basophils (and eosinophils) play significant roles. Future studies analyzing these issues can also be expected to provide us with important clues that will be useful for establishing novel therapeutic strategies.

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