Mini Review

IL-33-induced activation of human basophils and eosinophils via ST2

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Objective: A cytokine of the IL-1 family, IL-33, has recently been recognized as one of the key cytokines enhancing Th2-balanced immune regulation through its receptor, ST2. However, the action of IL-33 on allergic effector granulocytes has remained unclear. We thus tested whether IL-33 acts directly on, and affects the functions of, human basophils and eosinophils.
Methods: Basophils and eosinophils were prepared from normal human peripheral blood. Cells were treated with IL-33, and their adhesion, migration, surface CD11b expression, mediator release and survival were assessed *in vitro*. Expression of ST2 was analyzed at both the mRNA and protein levels.
Results: Analysis by real-time PCR and flow cytometry demonstrated that both basophils and eosinophils expressed mRNA and protein for ST2. Expressions of IL-4 and IL-13 mRNA in basophils were upregulated by IL-33. IL-33 at 1-100 ng/ml potently enhanced the adhesiveness and CD11b expression of basophils and eosinophils. Although IL-33 failed to directly induce degranulation or migration of basophils, it exerted priming effects by enhancing basophil migration toward eotaxin and degranulation in response to IgE-crosslinking stimulus. IL-33 prolonged survival of eosinophils, but not basophils, via suppression of their apoptosis. In addition, blocking of ST2 with neutralizing antibody inhibited IL-33-induced upregulation of basophil and eosinophil functions.
Conclusion: IL-33 is a potent regulator of various functions of basophils and eosinophils. IL-33 may be a key cytokine in the pathogenesis of Th2-dominant allergic diseases, at least partly by acting on effector cells, including basophils and eosinophils.

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Introduction

Basophils and eosinophils are both recognized as important effector cells in allergic diseases such as bronchial asthma and allergic rhinitis[1,2]. Although the contents and biochemical features of the cytoplasmic granules in both types of cells are mostly different, recent research has indicated that the cells share many characteristics in terms of the regulatory systems for their maturation and biological functions.

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 histamine[1]. Basophil activation also results in *de novo* synthesis of lipid mediators, including leukotriene (LT) C4, in addition to cytokines 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[3]. In mouse models, Karasuyama et al. recently showed that basophils are a critically important player in the pathogenesis of IgE-mediated, very-late-phase allergic responses[4].

Eosinophils are recognized as very important effector cells. They constitute the major line of effector cells in the allergic inflammation observed locally in bronchial asthma and atopic dermatitis. Eosinophils store and release a wide array of preformed mediators such as major basic protein, and they also secrete newly synthesized mediators, including LTC_4 and platelet-activating factor. These mediators show diverse actions, can induce smooth muscle contraction and increased vascular permeability and also affect the activation of other inflammatory cells[5].

IL-33 is a recently identified cytokine of the IL-1 family[6]. The receptor of IL-33, ST2, is reported to be expressed on mast cells and Th2 cells, but not on Th1 cells[7]. The actions of this cytokine are thought to be intimately involved in Th2-dominant situations. For example, IL-33 enhances production of Th2-associated cytokines by *in vitro* polarized Th2 cells[6]. IL-33 also prolongs the survival of human umbilical cord blood-derived mast cells and promotes their adhesion to fibronectin and production of IL-8 and IL-13[8]. IL-33 is increasingly recognized as an important cytokine that enhances Th2-balanced immune regulation, but its action on allergic effector cells was only recently reported. In this review, we summarize and discuss the evidence that IL-33 can affect the adhesiveness, CD11b expression and other functions of basophils and eosinophils.

ST2 expression analysis

First, we assessed the expression of mRNA for the IL-33 receptor, ST2, in peripheral blood granulocytes[9]. Both basophils and eosinophils clearly expressed ST2 mRNA. The ST2 mRNA expression level by basophils was significantly higher than the levels expressed by eosinophils and neutrophils: judging from the copy number ratio versus β-actin, the expression level of ST2 mRNA in basophils was 2-4-fold higher than that in eosinophils and more than 10-fold higher than in neutrophils[10,11]. Flow cytometry hardly detected expression of ST2 on the surface of freshly isolated basophils and eosinophils, but intracellular staining showed the presence of ST2 in the cytoplasm of both cells.

Effects of IL-33 on basophil functions

We next examined the effects of IL-33 on IL-4 and IL-13 synthesis by human basophils. Based on real-time PCR analysis, IL-4 mRNA levels were significantly enhanced by treatment of basophils with 100 ng/ml of IL-33. IL-13 mRNA was also significantly upregulated by IL-33. ELISA assay found that the supernatants of IL-33-stimulated basophils contained significantly greater amounts of IL-4 after 24 h compared to those of non-stimulated cells[10].

Next, basophil adhesion was analyzed. In the presence of IL-33 at 100 ng/ml, a significantly increased number of basophils adhered to the plates compared to the untreated control level of adhesion. The adhesion-inducing effect of IL-33 was stronger than that of 300 pM IL-3. In addition, IL-33 at 10-100 ng/ml significantly induced adhesion of human basophils to fibronectin-, ICAM-1- and VCAM-1-coated microplates. Surface CD11b expression by basophils was upregulated by IL-33, and this enhancement was slightly weaker than that by 300 pM IL-3. This effect of IL-33 was dose-dependent, and the EC50 of IL-33 in terms of enhancement of basophil CD11b expression was approximately 1 ng/ml, which corresponds to 33 pM on a molar basis.

IL-33 enhanced basophil migration toward eotaxin when IL-33 was added to the upper chemotaxis chamber[12]. When neutralizing antibody for ST2 was added to the upper chamber, the effect of IL-33 on basophil migration toward eotaxin diminished, suggesting that IL-33 regulates basophil locomotion via the ST2 receptor.

We next tested the effect of IL-33 on basophil degranulation. Importantly, pretreatment with IL-33 at 100 ng/ml for 15 min significantly enhanced degranulation of basophils stimulated with anti-IgE antibody. However, freshly isolated basophils did not

degranulate in response to IL-33. Lastly, we analyzed the effect of IL-33 on the viability of highly purified basophils. Although IL-33 enhances the survival of eosinophils, as mentioned later, this cytokine induced no change in the number of viable or apoptotic basophils compared to cells cultured in medium alone. We further assessed whether IL-33 affects the viability of IL-3-cultured basophils, but it did not show any effect.

Effects of IL-33 on eosinophil functions

In vivo administration of exogenous IL-33 in murine models is known to induce local accumulation of eosinophils[6,13]. We thus performed a migration assay on eosinophils with IL-33. However, IL-33 added to the lower chamber failed to attract eosinophils. In addition, IL-33 added to the upper chamber did not enhance eosinophil migration toward eotaxin. Next, eosinophil adhesion was quantified by measuring EPO released from lysed adherent cells after 45-min incubation. Eosinophil adhesion to albumin-, fibronectin-, ICAM-1- and VCAM-1-coated wells was significantly upregulated by IL-33. This effect was apparent at a concentration of 1 ng/ml and increased up to 100 ng/ml. Surprisingly, the effect of IL-33 at 100 ng/ml on eosinophil adhesion was significantly greater than that of IL-5 at 300 pM[11]. The adhesion of eosinophils to albumin-, fibronectin- and ICAM-1-coated wells in the presence of IL-33 was almost completely blocked by anti-CD18 neutralizing antibody, indicating that mainly $\beta 2$ integrin on IL-33-treated eosinophils is involved in the adhesion process to albumin, fibronectin and ICAM-1. On the other hand, adhesion to VCAM-1-coated wells was strongly diminished by the combination of anti-CD18 plus anti-CD29 antibodies, suggesting that eosinophil $\beta 1$ integrin is also involved in adhesion to VCAM-1.

IL-33 at 1 ～ 100 ng/ml significantly upregulated the expression of CD11b on eosinophils dose-dependently, and this effect of IL-33 at 100 ng/ml was stronger than that of an eosinophil-active cytokine, IL-5, at 300 pM. On the other hand, other IL-1 family cytokines, such as IL-1β and IL-18, did not show any effect on eosinophil CD11b expression. When eosinophils were pretreated with anti-ST2 neutralizing antibody, the effect of IL-33 on CD11b expression was diminished, indicating that the effect of IL-33 on eosinophil CD11b expression was mediated by the ST2 receptor.

Next, we analyzed the effect of IL-33 on the viability of highly purified eosinophils. IL-33 at 10-100 ng/ml significantly enhanced the survival of eosinophils dose-dependently, although the effect was weaker than that of IL-5 at 300 pM[14]. IL-33 at 100 ng/ml increased the number of live eosinophils by approximately 20% after 24 h. Apoptotic cells, i.e., cells positive for annexin V staining and negative for PI staining, were significantly decreased by addition of IL-33[11].

We lastly assessed whether IL-33 can regulate degranulation and lipid mediator synthesis in human eosinophils. Eosinophil degranulation was analyzed by measuring EDN[15], but we found no evidence of direct degranulation by IL-33. LTC_4 synthesis was analyzed by ELISA, but no apparent release of LTC_4 was induced by IL-33.

Conclusion

In this review, we have described that IL-33 can potently activate human basophil and eosinophil functions (Table 1)[10,11]. The effects of IL-33 were much greater than those of other IL-1 family cytokines, such as IL-1 or IL-18. IL-33's actions on basophils and eosinophils were mediated by the ST2 receptor.

Among the cell populations we examined in this study, eosinophils have long been regarded as central effector cells in allergic diseases such as asthma[16]. And recent findings, especially regarding the *in vivo* roles of basophils in experimental models, have greatly increased allergists' interest in this smallest of leukocyte subpopulations[4,17]. Although the functional regulation of these granulocytes has not yet been fully elucidated, extracellular bioactive proteins, especially cytokines, are thought to be important participants in the pathogenesis of allergic diseases[18-20]. In this review, we showed that IL-33 is a basophil- and eosinophil-active cytokine. Our results are in line with the findings reported from other laboratories[21-23]. Elucidation of the effects of various

Table 1 Effects of IL-33 on basophils and eosinophils

	Basophils	Eosinophils
Adhesion	+	+
Surface CD11b expression	+	+
Migration toward eotaxin	+	-
Survival	-	+
Direct degranulation	-	-
Enhancement of degranulation	+	-
Direct LTC_4 production	-	-
Enhancement of LTC_4 production	+	ND
Cytokine production (IL-4, IL-13)	+	ND

+: induction/enhancement; -: no effect; ND: not determined

cytokines on basophils and eosinophils will provide us with greater insight into the pathogenesis of allergic inflammation. Such information will contribute to our understanding of the mechanisms of allergic diseases, including asthma[24] and anaphylaxis, in which basophils and eosinophils play significant roles.

Recent progress has vastly expanded our knowledge regarding the biological importance of IL-33. Initially, this cytokine was reported to be closely related to Th2-dominant conditions: IL-33 acts on *in vitro* polarized Th2 cells and enhances the production of IL-5 and IL-13[6]. It was demonstrated that intraperitoneal administration of IL-33 causes peripheral blood eosinophilia and lymphopoiesis, in addition to elevated levels of serum IL-5, IL-13, IgE and IgA in mice[6]. We and others have shown that this cytokine is potentially important as an activator of various types of cells involved in the pathogenesis of allergic inflammation, such as mast cells[8], basophils[10,21,23], eosinophils[11,22,23], and NK and NKT cells[21], and the action of IL-33 is mediated by ST2. IL-33 could thus be regarded as both a director and a local mediator of Th2-biased allergic inflammation. Moreover, very recent evidence has shown that, *in vivo*, IL-33 is found predominantly in the nuclear compartment of keratinocytes and vascular endothelial cells. This implies that the mode of secretion of this cytokine might be closely related to cell death and/or tissue damage[25]. Further studies are necessary to complete our understanding of ST2-IL-33 biology. Through those studies, we hope to elucidate whether IL-33 and ST2 will be useful and appropriate for establishing novel therapeutic strategies for allergies and various other diseases.

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