An IL-1 Cytokine Member, IL-33, Induces Human Basophil Activation via Its ST2 Receptor

Maho Suzukawa, Motoyasu Iikura, Rikiya Koketsu, Hiroyuki Nagase, Chise Tamura, Akiko Komiya, Susumu Nakae, Kouji Matsushima, Ken Ohta, Kazuhiko Yamamoto and Masao Yamaguchi

*J Immunol* 2008; 181:5981-5989; doi: 10.4049/jimmunol.181.9.5981
http://www.jimmunol.org/content/181/9/5981

**References** This article cites 42 articles, 22 of which you can access for free at: http://www.jimmunol.org/content/181/9/5981.full#ref-list-1

**Subscription** Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
An IL-1 Cytokine Member, IL-33, Induces Human Basophil Activation via Its ST2 Receptor

Maho Suzukawa,2*† Motoyasu Iikura,*‡ Rikiya Koketsu,* Hiroyuki Nagase,† Chise Tamura,* Akiko Komiya,* Susumu Nakae,§ Kouji Matsushima,¶ Ken Ohta,† Kazuhiko Yamamoto,* and Masao Yamaguchi*

Basophils are thought to play pivotal roles in allergic inflammation through rapid release of chemical mediators in addition to sustained production of Th2 cytokines, including IL-4. A newly identified cytokine, IL-33, has been recognized as one of the key cytokines enhancing Th2-balanced immune regulation through its receptor, ST2. The present study was conducted to elucidate whether IL-33 acts directly on, and affects the functions of, human basophils. Real-time PCR analysis showed that basophils express transcripts for ST2. The expression levels were significantly higher compared with eosinophils and neutrophils, and treatment with IL-33 significantly up-regulated basophil ST2 mRNA expression. Expressions of IL-4 and IL-13 mRNA were also up-regulated by IL-33, and there was also enhanced secretion of IL-4 protein. IL-33 increased the surface levels of basophil CD11b expression and enhanced basophil adhesiveness. Although IL-33 failed to directly induce degranulation or attract basophils, it exerted priming effects on basophils. It enhanced degranulation in response to IgE-crosslinking stimulus and also enhanced basophil migration toward eotaxin without changing surface CCR3. Also, IL-33 synergistically enhanced IL-4 production and CD11b expression by IL-3-stimulated basophils. Neutralization using Ab specific for ST2 significantly diminished the enhancing effects of IL-33 on both basophil CD11b expression and migration toward eotaxin, indicating that IL-33 signals via ST2 expressed on basophils. This study revealed that IL-33 potently regulates migration and activation of human basophils. IL-33 may be a key cytokine in the pathogenesis of Th2-dominant inflammation by acting not only on lymphocytes but also on effector cells such as basophils. The Journal of Immunology, 2008, 181: 5981–5989.

S
ince first having been described by Paul Ehrlich (1), basophils have been increasingly recognized as one of the important effector cell types in allergic inflammation although they constitute only <1% of circulating leukocytes. A hallmark aspect of basophils is the abundant expression of a high-affinity receptor for IgE, FcεRI, on their surface. When surface-bound IgE is cross-linked by specific Ags, basophils rapidly release potent vasoactive mediators such as histamine that are stored in their cytoplasmic granules. In addition, basophils synthesize cytokines such as IL-4 and IL-13 as well as lipid mediators such as leukotriene (LT) C4. Through the release of these proinflammatory mediators, basophils are thought to play pivotal roles in allergic inflammation.
in vitro polarized Th2 cells. In vivo, treatment of mice with IL-33 induces expression of IL-4, IL-5, and IL-13, resulting in severe pathological changes in mucosal organs such as infiltration by inflammatory cells (18). In addition, Ikikura et al. (24) very 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. IL-33 is now recognized as a potentially important cytokine that enhances Th2-balanced immune regulation.

However, to date, there have been no reports regarding possible direct effects of IL-33 on allergic effector cells such as basophils. We thus conducted analyses of IL-33-induced human basophil activation in vitro. In this report, we show that IL-33 potently activates various arrays of basophil functions via ST2 by enhancing cytokine production in vitro polarized Th2 cells. In vivo, treatment of mice with IL-33 induces expression of IL-4, IL-5, and IL-13, resulting in severe pathological changes in mucosal organs such as infiltration by inflammatory cells (18). In addition, Ikikura et al. (24) very 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. IL-33 is now recognized as a potentially important cytokine that enhances Th2-balanced immune regulation.

Materials and Methods

Reagents

The following reagents were purchased as indicated: human recombinant IL-33 (Adipogen); human IL-18 (MBL); human recombinant IL-1β (WAKO); human eotaxin/CCL11, human recombinant VCAM-1 and ICAM-1 (R&D Systems); Percoll (Pharmacia Fine Chemicals); PBS and RPMI 1640 (Life Technologies); and PIPES and fibronectin (0.1% solutions) (Sigma-Aldrich). Human IL-3 was donated by the Kirin Brewery.

The following Abs were purchased as indicated: mouse anti-IL-4 mAb (IgG1, clone 8D4 – 8) and biotin-conjugated rat anti-IL-4 mAb (IgG1, clone MP4 –25D2) (eBioscience); FITC-conjugated goat anti-human IgE Ab (Biosource International); mouse neutralizing anti-ST2 mAb (IgG1, clone 97203) (R&D Systems); rabbit anti-IL-33 pAb (Adipogen); mouse anti-ST2 mAb (IgG1, clone HB12) (MBL); control mouse IgG1 (MOPC21) and mouse IgG2a (UPC10) (Sigma-Aldrich); mouse IgG2b mAb (MOPC 195) (Cappel); and FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch). Anti-CCR3 mAb (IgG1, clone 444) was donated by Dr. H. Kawasaki (Institute of Medical Science, University of Tokyo, Tokyo, Japan). Anti-human FcεRI α-chain mAb (CRA-1) was provided by Dr. C. Ra (Nihon University, Tokyo, Japan).

Cell preparation

Leukocytes were isolated from venous blood obtained from consenting volunteers with no history of atopic diseases. Basophils were semipurified by density centrifugation using Percoll solutions of two different densities (1.080 and 1.070 g/ml) (17). The purity of these Percoll-separated basophil preparations was usually 5–15%, and the yield was ~2.4 × 10⁴ basophils/ml of peripheral blood. For some experiments, Percoll-separated basophils were further purified by negative selection with MACS beads (Basophil Isolation Kit, Miltenyi Biotec) according to the manufacturer’s instructions (purity: 97–100%).

Eosinophils were purified by density gradient centrifugation followed by negative selection using anti-CD16-bound beads as previously described (purity: 97–100%).

Human neutrophils were separated by density gradient centrifugation followed by positive selection using anti-CD14-bound micromagnetic beads (Miltenyi Biotec) (purity: 97–99%).

Real-time quantitative PCR analysis

Real-time quantitative PCR analysis was performed as previously described (17). In brief, total RNA was extracted from highly purified cell preparations from separate donors using RNeasy Mini Kit (Qiagen). For some experiments, MACS-separated basophils were treated with and without IL-33 at 100 ng/ml in RPMI 1640 medium with 0.3% human serum albumin (HSA) for 4 h before RNA extraction. Real-time PCR was performed using a 7500 Real Time PCR System (PE Applied Biosystems). The primers and the probes for ST2 and IL-4 and IL-13 were designed by PE Applied Biosystems. Standard curves were constructed with serial dilutions of specific PCR products, which were obtained by amplifying peripheral leukocyte cDNA as previously described (17).
Flow cytometric analysis of surface-expressed molecules

Highly purified basophils were used for flow cytometric analysis of ST2 expression. Basophils were incubated for 30 min at 4°C with 10 μg/ml of either anti-ST2 mAb (MBL) or control Ab and then stained with PE-conjugated goat anti-mouse IgG at 10 μg/ml for 60 min at 4°C. For intracellular staining, the cells were fixed with PBS containing 4% PFA at 4°C for 30 min followed by permeabilization in PBS containing 0.1% Tween 20 at 4°C for 30 min. The cells were then stained and analyzed by flow cytometry.

CD11b expression experiments were performed using Percoll-separated basophils as previously described (25). Following stimulation in PIPES buffer containing 25 mM PIPES, 119 mM NaCl, 5 mM KCl, 2 mM Ca²⁺, 0.5 mM Mg²⁺, and 0.03% HSA, basophils were incubated with 10 μg/ml of either PE-conjugated anti-CD11b mAb or PE-conjugated control mouse IgG1 at 4°C and then stained with FITC-conjugated anti-human IgE Ab at 10 μg/ml. Cells showing strong positive staining for IgE were considered to be basophils and were further analyzed for their PE fluorescence. The median values of fluorescence intensity for the basophils were converted to the numbers of molecules of equivalent soluble fluorochrome units (MESF), as previously described (25). Surface receptor levels were semi-quantified using the following formula: \[ \text{MESF} = (\text{MESF of cells stained with anti-CD11b mAb}) - (\text{MESF of cells stained with control IgG}) \]

MACS-separated basophils were used to analyze surface CCR3 expression as previously described (13, 25). In brief, cells were incubated for 30 min with 10 μg/ml of either anti-CCR3 mAb or control mouse IgG1, and then stained with FITC-conjugated goat anti-mouse IgG Ab before flow cytometric analysis.

FcɛRI expression was analyzed using MACS-separated basophils. Cells were incubated with 5 μg/ml of CRA-1 mAb or control mouse IgG2b and stained with FITC-conjugated goat anti-mouse IgG Ab.

Chemotaxis assay

Basophil chemotaxis experiments were performed using Percoll-separated or MACS-separated basophils and Chemotaxicell (Kurabo) as previously described (25). After incubation for 2.5 h at 37°C, cells which had migrated into the lower chamber were collected, stained with FITC-conjugated goat anti-human IgE, and counted by flow cytometry. Experiments were performed in duplicate or triplicate. Migration was expressed as a percentage of the inoculated cells after subtracting the spontaneous migration.

Degranulation assay

Basophil degranulation was assessed using Percoll-separated basophils, as previously described (25). The released histamine was measured using an automated fluorometric technique. Experiments were performed in duplicate. Histamine release was expressed as a percentage of the total cellular histamine after subtracting the spontaneous release (consistently <5%).

Adhesion assay

Culture plates (96-well; IWAKI) were coated overnight at 4°C with 100 μl of BSA (20 mg/ml), fibronectin (20 μg/ml), ICAM (100 ng/ml), or VCAM (100 ng/ml) dissolved in PBS. The coated wells were washed twice with blocking buffer (2% BSA in PBS) and incubated with 100 μl of this buffer.
FIGURE 3. IL-33 enhances adhesiveness of human basophils. Percoll-separated basophils were preincubated with and without IL-33 (1, 10, 100 ng/ml), IL-1β (100 ng/ml), IL-18 (100 ng/ml), or IL-3 (300 pM) for 45 min and then dispensed into BSA- (A), fibronectin- (B), ICAM-1- (C) or VCAM-1- (D) coated plates. Percentages of adherent cells were calculated based on the histamine content of the cellular histamine content. Error bars represent the SEM (n = 3). *, p < 0.05; **, p < 0.01 vs medium alone (nil).

for 1 h at 37°C. The wells were ready for use after washing twice with RPMI 1640 medium containing 0.3% HSA.

Approximately 3 × 10^5 Percoll-separated basophils were added to each well with and without cytokines in RPMI 1640 medium containing 0.3% HSA, and the plates were incubated at 37°C in 5% CO₂ for 45 min. After incubation, the wells were gently washed twice with RPMI 1640 medium to remove nonadherent cells. Then 2% perchloric acid was added to each well, and the plates were held overnight at 4°C. Basophil adherence was quantified by measuring basophil-derived histamine. Adherent basophils were expressed as a percentage of the total histamine content of the total basophils placed in each well.

Cell culture and assay of basophil-secreted products

For ELISA assay, 5 × 10^5 cells/ml of highly purified basophils were cultured at 37°C with cytokines in RPMI 1640 medium containing 0.3% HSA, and the supernatant was collected after centrifugation. Cell lysates were obtained by addition of 0.5% Nonidet P-40 (Sigma-Aldrich) to the cell pellets. Samples were stored at −80°C until assay.

Immunoreactive IL-4 was quantified using a modification of a double-ligand immunoassay. In brief, samples and standards were incubated at 4°C overnight in flat-bottom 96-well microtiter plates (Maxisorp; Nunc) pre-coated with a mouse anti-IL-4 mAb (eBioscience). After washing, biotin-conjugated rat anti-IL-4 mAb (eBioscience) was added to the plates and reacted for 3 h. The plates were then washed, followed by addition of HRP-conjugated streptavidin (Amershams Biosciences) and incubation for an additional 2 h. The plates were developed with a 3,3′,5,5′-tetramethylbenzidine microwell peroxidase substrate system (Kirkegaard & Perry Laboratories), and the reactions were stopped with 2 N H₂SO₄. Absorbance was measured at 450 nm, and a standard curve was generated for each assay. The ELISA method detected IL-4 concentrations of >0.69 pg/ml.

Immunoreactive LTC4 was measured using an ELISA kit for LTC4 (Cayman Chemicals; detection range: 10–1000 pg/ml) by following the manufacturer’s instructions.

Survival assay

Highly purified basophils were cultured in RPMI 1640 medium supplemented with 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma-Aldrich). Measurement of apoptotic and live cells was performed using an MEBCYTO apoptosis kit (MBL) and flow cytometer as previously described (26). In brief, early apoptotic cells were identified by their ability to bind annexin V and exclude propidium iodide (PI). Cells stained with PI were considered to be necrotic cells. Cells not stained by either annexin V or PI were judged to be alive.

Statistics

All data are expressed as the mean ± SEM. Differences between values were analyzed by the one-way ANOVA test. When this test indicated a significant difference, Fisher’s protected least significant difference test was used to compare individual groups.

Results

Human basophils express ST2

In the first series of experiments, we studied the expression of mRNA for the IL-33 receptor, ST2, in basophils and other granulocytes. Cells were purified to apparent homogeneity (>98.5%), and the expression levels of transcripts for ST2 were quantified by real-time PCR (Fig. 1A). Basophils 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 vs β-actin, the expression level of ST2 mRNA by basophils was 2–4-fold higher than by eosinophils and >10-fold higher than by neutrophils. It has been reported that human mast cells are clearly positive for ST2, and they possess abundant ST2 mRNA (19, 24). We very recently analyzed the ST2 mRNA levels using cultured human mast cells (the detailed methods are described in our previous manuscript; Ref. 27), and the data are shown in Fig. 1A. The levels of ST2 mRNA in basophils were significantly lower than those in human mast cells. Next, we examined the effect of IL-33 on ST2 expression, since certain cytokines have previously been reported to regulate the expression of their respective receptors (28). Interestingly, treatment with IL-33 at 100 ng/ml for 4 h significantly up-regulated the ST2 mRNA expression by basophils (p < 0.05) (Fig. 1B).

Next, using highly purified basophils, surface and intracellular ST2 levels were analyzed by flow cytometry. Expression of ST2 on the surface of freshly isolated basophils was hardly detectable, as shown in Fig. 1C. However, following incubation with IL-33 at 300 pM for 18 h, ST2 protein was clearly detectable on the basophils’ surface (Fig. 1D), a finding that is consistent with a recent
In addition, intracellular staining clearly showed the presence of ST2 in fresh basophils (Fig. 1E).

IL-33 induces cytokine production by human basophils

Basophils are one of the major sources of Th2 cytokines such as IL-4 (15, 30) and IL-13 (31–33). We next examined the effect of IL-33 on cytokine synthesis by human basophils. Using cDNA from IL-33-stimulated basophils, real-time PCR was performed to detect mRNA for IL-4 and IL-13. As a result, transcripts for IL-4 were significantly enhanced by treatment with 100 ng/ml of IL-33 (Fig. 2A). IL-13 mRNA was also significantly up-regulated by IL-33 (Fig. 2B). ELISA found that the supernatants of IL-33-stimulated basophils contained significantly greater amounts of IL-4 after 24 h (Fig. 2C). Another cytokine of the IL-1 family, IL-1β,
also significantly induced IL-4 secretion by basophils. As shown in Fig. 2D, time-course analyses showed a gradual increase of IL-4 in the supernatants of IL-33-stimulated basophils, reaching a plateau at 24 h. Furthermore, we assessed whether IL-33 affects IL-4 secretion from basophils stimulated by IL-3 or IgE-crosslinkage. As shown in Fig. 2E, IL-33 enhanced IL-4 secretion from basophils stimulated with IL-3. IL-4 production from basophils stimulated with CRA-1 mAb was also potently augmented by IL-33. We also tested for basophil LTC4 synthesis in response to IL-33, but only weak LTC4 secretion, below significant levels, was observed in IL-33-treated basophils (data not shown).

**IL-33 enhances adhesiveness of human basophils**

Next, we analyzed the adhesiveness of human basophils using Percoll-separated cell preparations. Plastic plates were coated with BSA and tested for basophil adhesion. As shown in Fig. 3A, in the presence of IL-33 at 100 ng/ml, a significantly increased number of basophils adhered to the plates compared with the baseline level of adhesion. Unexpectedly, the adhesion-inducing effect of IL-33 was much stronger than that of 300 pM IL-3. Similarly, IL-33 at 10–100 ng/ml significantly induced adhesion of human basophils to fibronectin-, ICAM-1- and VCAM-1-coated microplates, and, again, those effects were more potent than those of 300 pM IL-3 (Fig. 3, B–D). On the other hand, IL-1β and IL-18 failed to affect basophil adhesion to plates coated with fibronectin, ICAM-1 or VCAM-1.

**IL-33 up-regulates CD11b expression on human basophils**

Percoll-separated basophils were used to study the effect of IL-33 on basophil CD11b expression (Fig. 4, A and B). Consistent with previous reports (14), CD11b expression was markedly up-regulated by IL-3 at 300 pM (74 ± 8.7% above baseline, p < 0.01). IL-33 also significantly enhanced surface CD11b expression by basophils, although 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. The effect reached a plateau at 10–100 ng/ml of IL-33 (Fig. 4C). In the next experiments, we compared the effects of IL-33 incubation for 30 min and 18 h. The longer, 18-h incubation was not as effective at enhancing CD11b expression as the shorter, 30-min incubation (Fig. 4C). We next tested whether IL-33 affects the level of basophil CD11b expression induced by other well-known stimulants such as IL-3 and FceRI-crosslinkage. As shown in Fig. 4D, IL-33 synergistically augmented surface CD11b expression on IL-3-treated basophils. IL-33 also showed slight enhancement of CD11b levels on anti-FceRI mAb-treated basophils, but this effect was small and seemingly additive rather than synergistic. Neutralizing Ab for ST2 diminished the enhancement of basophil CD11b expression by IL-33, as shown in Fig. 4E. Pretreatment of basophils with anti-ST2 Ab at 10 μg/ml significantly suppressed the effect of 1 ng/ml IL-33 on CD11b expression, indicating that IL-33 regulates basophil CD11b expression by signaling through its receptor, ST2. However, IL-33 at 10 ng/ml or more seemed to be too high for anti-ST2 Ab to efficiently block the IL-33-induced up-regulation of CD11b (Fig. 4E).

**IL-33 enhances basophil migration toward eotaxin**

In vivo local administration of IL-33 was reported to attract inflammatory cells to inflammatory sites (18). Therefore, we investigated whether IL-33 regulates human basophil migration. IL-33 was added to the lower chamber of Chemotaxicell at 10–100 ng/ml, but no induction of basophil migration was observed (Fig. 5A). However, when added to the upper chamber with the cells, IL-33
IL-33 enhances degranulation of human basophils

Next, using Percoll-separated basophils, we studied the effect of IL-33 on basophil degranulation. As shown in Fig. 6A, freshly isolated basophils did not degranulate in response to IL-33. We next tested IL-33 for basophil priming. Importantly, pretreatment with IL-33 at 100 ng/ml for 15 min significantly enhanced degranulation of basophils stimulated with anti-IgE Ab. We confirmed that the expression level of surface FceRI remained the same even after IL-33 pretreatment of basophils, as shown in Fig. 6B.

Finally, we analyzed the effect of IL-33 on the viability of highly purified basophils. Although IL-33 is known to enhance the survival of eosinophils (27), this cytokine induced no change in the number of viable or apoptotic basophils compared with basophils cultured in medium alone (data not shown). We next assessed whether IL-33 affects the viability of IL-3-cultured basophils, but it did not show any effect (data not shown).

Discussion

In this study, we demonstrated that human basophils express transcripts and protein for ST2, a receptor for IL-33, and neutralization studies showed that basophil ST2 is functional. IL-33 affected several arrays of basophil functions: this cytokine up-regulated CD11b expression on the cell surface of basophils, enhanced eotaxin-directed chemotaxis, induced Th2 cytokine IL-4 secretion, and augmented the IgE-mediated histamine release reaction. This is the first study to identify the roles of IL-33 and its ST2 receptor in the functional regulation of basophils. Importantly, basophil adhesion was potently enhanced by IL-33, and this action of IL-33 was greater than that of IL-3, a well-known basophil-active cytokine.

The IL-1 cytokine family is known to regulate various inflammatory reactions; among its members, IL-1β and IL-18 are especially potent proinflammatory substances. However, our knowledge regarding the effects of these cytokines on basophil functions is limited. To date, IL-18 has been demonstrated to induce cytokine production by basophils (34, 35), and IL-1α and IL-1β have been demonstrated to potentiate IgE-mediated histamine release from human basophils (36, 37).

IL-33 is a new member of the IL-1 family of cytokines. Schmitz et al. demonstrated that IL-33 has biological activities such as driving Th2-polarized cells to produce Th2 cytokines such as IL-5 and IL-13. In addition, in vivo studies revealed that administration of IL-33 induces histological changes in the mucosa, including eosinophil infiltration, increased mucus production, and epithelial cell hyperplasia and hypertrophy (18). Thus, locally produced IL-33 may act as a potent inducer of Th2-dominant inflammation. IL-33 is produced by various cells, including epithelial cells and smooth muscle cells (38). Greater knowledge regarding the biological effects of IL-33 on basophils might shed light on the interplay between tissue structural cells and inflammatory granulocytes. In our present study, IL-33 potently enhanced basophil adhesiveness and surface CD11b expression, and these actions of IL-33 were by far the strongest among the tested IL-1 family members. Furthermore, the finding that IL-33 induced IL-4 secretion by basophils implies that this IL-1 family member may strengthen local Th2 dominance through effects not only on Th2 lymphocytes and mast cells but also on basophils, since IL-4 can exert multiple effects causing exacerbation of inflammation (39–41). In addition, our study indicates that transcripts for another Th2 cytokine, IL-13, are also increased by IL-33 in basophils. We further found that IL-4 secretion by basophils stimulated with IL-3 or FceRI crosslinkage was potently enhanced by IL-33. IL-33-stimulated basophils may thus be an important cellular source of Th2 cytokines in the pathogenesis of Th2-biased allergic inflammation.

IL-33 was recently identified as a biologically active ligand for ST2 (18), a Th2-associated receptor expressed on Th2 cells and mast cells. Before the ligand was identified, ST2 had been shown to function as an important effector molecule for Th2 responses in experimental models (20, 23). In addition, in the clinical setting, elevated ST2 protein expression was reported in the sera of patients suffering asthmatic exacerbation (42). Thus, ST2 is believed
IL-33 INDUCES HUMAN BASOPHIL ACTIVATION

References

Acknowledgments
We thank Dr. Koichi Hirai for helpful discussion, and Yasuko Asada for excellent secretarial work. We also thank Drs. H. Kawasaki and C. Ra for providing mAbs for this work.

Disclosures
The authors have no financial conflict of interest.


