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Activated mast cells synthesize and release soluble ST2-a decoy receptor for IL-33

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Abstract

Interleukin 33 (IL-33) released from damaged cells plays a central role in allergic inflammation by acting through its membrane-bound receptor, ST2 receptor (ST2L). IL-33 activity can be neutralized by the soluble spliced variant of ST2 (sST2) which has been associated with allergic inflammation but its source is not well defined. We investigated whether mast cells (MCs) are a significant source of sST2 following activation through FcεRI or ST2. We find that antigen and IL-33 induce substantial production and release of sST2 from human and mouse MCs in culture and do so synergistically when added together or in combination with stem cell factor. Moreover, increases in circulating sST2 during anaphylaxis in mice were dependent on the presence of MCs. Human MCs activated via FcεRI failed to generate IL-33 and IL-33 produced by mouse bone marrow-derived MCs was retained within the cells. Therefore, FcεRI-mediated sST2 production is independent of MC-derived IL-33 acting in an autocrine manner. These results are consistent with the conclusion that both mouse and human MCs when activated are a significant inducible source of sST2 but not IL-33 and thus have the ability to modulate the biologic impact of IL-33 produced locally by other cell types during allergic inflammation.

Keywords

Human Mast Cells; sST2; IL-33; Antigen; Stem Cell Factor

Introduction

Interleukin 33 (IL-33), one of the IL-1/IL-18 family of cytokines, plays a central role in type 2 immune responses and has been implicated in the pathogenesis of asthma [1–3], allergic rhinitis [4], allergic conjunctivitis [5], atopic dermatitis [6, 7] and several autoimmune diseases (reviewed in [8–11]). IL-33 is produced constitutively in stromal cells such as

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endothelial cells, epithelial cells, and fibroblasts where it is retained within the nucleus and can exert transcriptional repressor activity [12–14] but most likely acts as an “alarmin” when released from these cells after cell damage or injury (reviewed in [8, 9, 15]). Full length IL-33, which does not require proteolytic cleavage for activity, stimulates production of Th2 cytokines in Th2 cells [1, 16], mast cells [17, 18], basophils [1, 2], eosinophils [5, 19], and other lymphocyte subsets [20, 21] in addition to promoting differentiation, survival, and chemotaxis of Th2 cells and mast cells [16–18, 22, 23]. IL-33 also potentiates production of Th2 cytokines by antigen (Ag) and other stimulants in mast cells and basophils [24, 25]. In addition, IL-33 is cleaved by mast cell tryptase and chymase to liberate more potent fragments that can activate group-2 innate lymphoid cells and thereby amplify innate immune responses and allergic inflammation [26].

IL-33 acts specifically through the receptor, ST2 (also referred to as ST2L), which is primarily but not exclusively located in hematopoietic cells and is dependent on the co-receptor, IL-1 receptor accessory protein (IL-1RAcP), and the adaptor protein MyD88 for signaling [8, 27]. A soluble spliced variant of ST2 (sST2) also exists that lacks the ST2L cytosolic and transmembrane domains. Both ST2L and sST2 are transcribed from a dual promoter system to drive differential mRNA transcription of the *ST2* gene [28, 29]. sST2 is reported to act as a binding decoy for IL-33 and thus modulates IL-33 activity during inflammatory responses. It has been found to block IL-33 signaling in allergic airway inflammation in mice [30] while sST2-Fc fusion protein significantly attenuates collagen-induced arthritis [31]. Also, *ST2* transgenic mice, which have high tissue levels of sST2, no longer exhibit IL-33-induced Th2 immune responses [32]. sST2 is elevated along with IL-33 in several inflammatory diseases. These include atopic individuals with allergic symptoms or exacerbation of asthma [33, 34], atopic dermatitis [35], rheumatoid arthritis [36, 37], ulcerative colitis as well as Crohn’s disease [38], and systemic lupus erythematosus [39]. Although sST2 was found to be produced predominantly in lung, lymph nodes, thymus, spleen, and surprisingly ovaries following Ag challenge in a mouse asthma model [30], the specific cellular source(s) in allergic disease remains unknown.

Mast cells respond to IL-33 by releasing inflammatory cytokines [17, 18] and express ST2L in both mouse [40, 41] and human [35] mast cells even before expression of the IgE receptor, FcεRI, during mast cell development [40]. This expression is regulated at the transcriptional level by GATA proteins in LAD2 human mast cells [42]. Given these considerations and the fact that mast cells have been implicated in the pathogenesis of many of the diseases noted above, we have now investigated whether human as well as mouse mast cells are a significant source of sST2, and if so, under what conditions. Data to be presented support the conclusion that both human and mouse mast cells produce substantial amounts of sST2 along with cytokines when activated via ST2, FcεRI, or the mast cell growth factor receptor KIT. However, mast cells do not appear to be a source of IL-33 following FcεRI engagement and, by implication, may not be a significant source of IL-33 in allergic disorders. Instead, the data are consistent with the conclusion that mast cells in the inflammatory environment modulate IL-33 activity by de novo synthesis of sST2 which may act to limit the later phases of the allergic inflammatory response along with other mast cell-derived anti-inflammatory mediators.

Results

Both human and mouse mast cells express sST2 as well as ST2L and release sST2 upon IL-33 stimulation

Primary human peripheral blood-derived mast cells (HuMCs), human LAD2 mast cells, and mouse BMMCs constitutively express mRNAs for sST2 and ST2L (Fig. 1A). The effect of IL-33 on sST2 expression was examined because of its increase in some allergic diseases. After stimulation with IL-33, expression of sST2 mRNA was increased in all three cell types (Fig. 1B–D) with variable changes in ST2L mRNA that did not reach statistical significance (Fig. 1E–G). The IL-33-induced increase in sST2 mRNA reached a maximum by 4 h and then declined to near basal levels by 8 h.

Soluble ST2 protein was released into the medium following stimulation with IL-33 in HuMCs, LAD2 cells, and mouse BMMCs, reaching concentrations of 1–2 ng/ml that are in the range or higher than levels found in tissue and serum from patients with elevated sST2 [33–39]. The time-course of sST2 release was similar in all three cell types (Fig. 2). Release was apparent by 2 h and continued progressively thereafter for up to at least 24 h (Fig. 2), after sST2 mRNA had returned to basal levels (Fig. 1B–D). In comparison, release of GM-CSF, a cytokine abundantly produced by mast cells, reached a plateau by 8 to 12 h after the addition of IL-33.

The release of sST2 was dependent on the concentration of IL-33 but specific mast cell types varied in their sensitivity to IL-33 (Fig. 3). Both HuMCs and LAD2 cells responded robustly to as little as 1 ng/ml human IL-33 to produce near equivalent molar concentrations of sST2 in the medium (Fig. 3A and D). Maximal release of sST2 occurred at 3 to 10 ng/ml IL-33, although at higher concentrations (30 and 100 ng/ml) this response diminished in HuMCs but not in LAD2 cells. BMMCs exhibited progressive increases in sST2 levels over the concentration range of 1 – 100 ng/ml IL-33 (Fig. 3G). All three cell types also released GM-CSF and MIP1 α in response to IL-33 over the same range of IL-33 concentrations (Fig. 3 B, C, E, F, H, and I).

Collectively, these data demonstrate that mast cells respond to IL-33 with release of sST2 as well as cytokines. Control experiments with IL-33 and culture media indicated that neither interfered with the assay of sST2 by ELISA (data not shown). Also, it is unlikely that the appearance of sST2 had substantial effect on the potency of IL-33 in this series of experiments because of the late appearance of sST2 in the medium in amounts that would neutralize 1 to 2 ng/ml IL-33. However, this might not be so in pathological conditions where, for example, levels of IL-33 in serum and synovial fluid generally range from 0.1 to 2 ng/ml in patients with rheumatoid arthritis [36, 37] or at levels that appear to be sufficient to stimulate sST2 production in human mast cells (as in Fig. 3A).

Stimulation of sST2 release by IL-33 related cytokines

There are indications that other members of the IL-1/IL33 family including IL-1 β [43, 44] and IL-18 [45, 46] regulate mast cell activities and were examined accordingly for their ability to stimulate production of sST2 and GM-CSF (Fig. 4). HuMCs responded to IL-1 β , albeit modestly when compared to IL-33, but not IL-18 (Fig. 4A and B) while BMMCs

responded only to IL-33 and not to murine IL-1 β or IL-18 (Fig. 4C and D). Stimulation with IL-1 β or IL-18 in combination with IL-33 did not further enhance release of sST2 and GM-CSF in either HuMCs or BMMCs. Therefore, the ability to stimulate sST2 production appeared to be restricted mainly to IL-33 among this family of cytokines in mast cell cultures.

Ag and stem cell factor (SCF) stimulate release of sST2 and augment responses to IL-33

Because IL-33 interacts synergistically with Ag in stimulating cytokine production in mast cell cultures [24, 25], as does KIT ligand SCF with Ag [47], we examined whether similar synergistic interactions occur in the production of sST2. As reported [41], Ag (DNP-HSA) itself stimulated a modest release of sST2 in BMMCs but this release was markedly enhanced on co-stimulation with SCF (Fig. 5A and legend). The response to the Ag/SCF combination was rapid when compared to IL-33 alone (compare Fig. 5B with Fig. 2E). The increase in sST2 in culture medium was apparent within 30 min and reached a maximum by 4 h to suggest that production had ceased by then. Consistent with this relatively transient response, significant increases in serum sST2 were also apparent 4 and 6 h after antigen challenge in a passive systemic anaphylaxis mouse model with sST2 levels returning to basal levels by 24 h (Fig. 5C). Mast cells appear to be the major, if not exclusive, cause of sST2 release as none was observed in the mast cell deficient *Kit^{W-sh/W-sh}* mice (Fig. 5C).

Ag also interacted synergistically with IL-33 as sST2 production was markedly augmented when BMMCs were co-stimulated with optimal concentrations of Ag (100 ng/ml) and IL-33 (10 ng/ml) (Fig. 5D). As an experimental control, MyD88-deficient BMMCs, which lack the ability to efficiently signal through ST2L [25], failed to produce sST2 (Fig. 5E) in response to IL-33 but still released sST2 to the same extent in response to Ag whether IL-33 was present or not. These results also indicated that Ag can act independently of and synergistically with IL-33.

We next examined the effect of SCF on IL-33 responses because of the report that SCF enhances IL-33-induced cytokine production through interaction of activated KIT with IL-1RAcP and ST2L [48]. SCF also augmented production of sST2 on costimulation of BMMCs with SCF and IL-33 (Fig. 5F). The synergistic interactions noted above were also evident in the production of sST2 mRNA (Fig. 5G). Ag and SCF were relatively weak stimulants by themselves but together production of sST2 mRNA was markedly enhanced and even more so in combination with IL-33. IL-3, the growth factor for BMMCs, neither stimulated nor enhanced IL-33-induced sST2 production in IL-3-deprived BMMCs (Supporting Information, Fig. 1A). The same was true for Ag stimulation (Supporting Information, Fig. 1B).

The pattern of responses of HuMCs was similar to that of BMMCs in that both streptavidin (used as a cross-linking agent with biotinylated-IgE primed HuMCs) and SCF, although relatively weak stimulants by themselves, enhanced sST2 production in response to IL-33 (Fig. 5H and I). Collectively, these studies indicated that Ag and SCF are relatively weak stimulants individually, but together they become potent stimulants of sST2 production especially in combination with IL-33. In addition, the production of sST2 is inducible and dependent on gene transcription.

Stimulation of sST2 production by Ag and SCF is not dependent on mast cell-derived IL-33

As BMMCs produce IL-33 in response to Ag [41, 49], the possibility exists that Ag acts, in addition, through release of IL-33 which in turn stimulates production of sST2. We confirmed that BMMCs produce intracellular IL-33 mRNA (Fig. 6A and Supporting Information Table 1) and protein (Fig. 6B upper blot, 6C and Supporting information Fig. 2A), especially on co-stimulation with Ag and SCF but weakly so by Ag and SCF individually (Fig. 6A). Nevertheless, and consistent with another study [49], IL-33 was retained within the cells and was not detectable in the medium by ELISA or Western blotting, even when ATP or thapsigargin were added 4 h later to promote IL-33 release as reported in other cell types [50, 51] (data not shown). In contrast to BMMCs, we were unable to detect intra- or extracellular IL-33 in stimulated HuMCs and LAD2 cells by Western blotting (Fig. 6B, lower two panels), flow cytometry (Supporting Information Fig. 2B and C) or ELISA (data not shown). The ability of BMMCs, but not human mast cells, to produce IL-33 was not due to conditioning of cells by IL-3. HuMCs and LAD2 cells grown in IL-3 for 1 week, still failed to produce IL-33 when stimulated by SA and SCF (Supporting Information Fig. 3). Therefore, mouse and human mast cells release sST2 in response to Ag/SCF regardless of whether they produce IL-33 or not and, as noted for MyD88-deficient cells (as in Fig. 5E), Ag can act independently of IL-33 by stimulating sST2 production. The corollary based on these data is that mast cells may not be a significant source of extracellular IL-33 in allergic reactions.

Discussion

We report here that the ST2 receptor is expressed in human mast cells and BMMCs and that all mast cell subtypes produce significant amounts of sST2 mRNA (Fig. 1) and protein upon stimulation with IL-33 (Fig. 2). Of the other members of the IL-1/IL-33 family tested, IL-1 β but not IL-18 minimally stimulated production of sST2 (Fig. 4) in HuMCs and had no effect on BMMCs. Our findings also indicate that production of sST2 is an implicit part of the response to mast cell activation by Ag, especially in the presence of IL-33 and SCF. Also, the coincident production of sST2 mRNA and protein (Fig. 5) suggest that production of sST2 is an inducible response resulting from alternative splicing rather than proteolytic cleavage of ST2L. Although the induction of sST2 production by Ag and SCF is relatively modest when compared to the effects of IL-33 alone, Ag interacts synergistically with IL-33 as does SCF with Ag to robustly enhance sST2 production in HuMCs and BMMCs (Fig. 5 and 6) as is true for generation of cytokines [25, 47]. As with cytokine production [25], IL-33-induced production of sST2 as well as its synergistic interaction with Ag is prevented in MyD88-deficient BMMCs while Ag itself remains fully capable of stimulating production of sST2 (Fig. 5C). Therefore, sST2 production can be induced through either the ST2L/MyD88- or Fc ϵ RI-mediated signaling pathways although both pathways interact synergistically to enhance production of sST2 (Fig. 5) as well as cytokines [25].

It is unlikely that stimulation of sST2 release by Ag is mediated through an autocrine/paracrine action of IL-33. Apart from the studies with MyD88-deficient BMMCs which indicated that Ag can act independently of IL-33, we were unable to detect production or release of IL-33 in Ag-stimulated HuMCs or LAD2 cells even though these cells produced

sST2. Although IL-33 was produced by BMMCs it appeared to be retained entirely within the cell as noted by others [41]. Rather than being actively secreted, IL-33 is generally considered to be one of a group of alarmins or danger signaling molecules that, when released from necrotic or damaged cells, they act on immune cells to initiate a type 2 inflammatory responses [15]. In this context, mast cells might be considered a target [17,18, 22, 24, 25] rather than a source of extracellular IL-33. Indeed, it is reported that necrotic cells specifically activate mouse mast cells via release of IL-33 in a ST2L/MyD88-dependent manner [52]. Mast cells so activated release leukotrienes and inflammatory cytokines without degranulation, consistent with the actions of IL-33 itself [25]. The subsequent production of sST2 by IL-33 (this paper), which can prevent the effects of IL-33 on mast cells [53], might then limit the duration of action of IL-33.

The production of sST2 adds to the list of known inhibitory molecules released by activated mast cells and supports the perception that mast cells play a protective as well as inflammatory role in immune reactions [54]. In addition to sST2, the IL-1 receptor antagonist (IL-1Ra) is another cytokine antagonist that is released from activated mast cells in human lung [55] and synovial tissue [56] and may counteract elevated IL-1 β in these tissues in allergic asthma and rheumatoid arthritis. Protective mast cell-derived cytokines include IL-2, IL-6, and IL-10 which limit chronic allergic dermatitis [57], onset of innate lung inflammation [58], and contact hypersensitivity [59] respectively in animal models. In addition, release of mast cell proteases can have protective as well as inflammatory functions [60] and heparin, which is released exclusively from mast cells, binds to anti-thrombin to prevent clot formation.

In summary, our studies indicate that in acute allergic reactions mast cells are unlikely to be a significant source of IL-33 but appear to be essential for the generation of sST2 which would minimize the actions of ambient IL-33. However, sST2 release is relatively transient during Ag stimulation in vitro and in vivo as compared to IL-33. During more chronic inflammatory conditions associated with elevated IL-33 levels, IL-33 would stimulate progressively increasing amounts of sST2 limiting activity of IL-33 at later times. IL-33 itself desensitizes mast cells to Ag after prolonged IL-33 exposure [61]. These interactions could conceivably contribute towards suppression of mast cell and IL-33 activities as part of a resolution process that is dysfunctional in atopic diseases including atopic asthma [62], dermatitis [35], and chronic rhinosinusitis (reviewed in [63]) that are associated with elevated sST2 production and missense variants of ST2 [64].

Materials and Methods

Reagents

Reagents were obtained as follows: mouse SCF, IL-3, and IL-33 as well as human IL-33 from Peprotech Inc. (Rocky Hill, NJ); ELISA Duo Set kits for all cytokines and sST2, human SCF and IL-6, mouse IL-33, anti-human IL-33, anti-mouse IL-33, goat IgG control and Phycoerythrin (PE)-anti-goat antibody from R&D Systems (Minneapolis, MN); mouse anti-dinitrophenylated (DNP) IgE and DNP-human serum albumin (DNP-HSA) from Sigma-Aldrich (St. Louis, MO); human myeloma IgE (EMD-Millipore, MA) biotinylated in-house [65]; anti-goat IgG 800 CW and anti-mouse IgG 680 RD conjugates from LICOR

Biosciences, (Lincoln, NE); TaqMan gene expression assays from Life Technologies (Carlsbad, CA); Fluorescein isothiocyanate (FITC)-anti-FcεRI from eBioscience Inc. (San Diego, CA); Allophycocyanin (APC)-anti-Kit and rat anti-mouse CD16/CD32 from BD Biosciences (San Jose, CA); Human truestain FcX from Biolegend (San Diego, CA); Human umbilical vein cells (Huvec) from Lonza (Walkersville, MD). Mouse IL-33 biological effects varied in potency between different lots of IL-33 purchased from the two vendors mentioned above. Thus each lot of IL-33 purchased was tested for potency against IL-33 of known potency and only lots with maximum and equivalent potency were used for experiments. Mouse IL-33 from R & D systems was used for experiments in Fig. 2, Fig. 5–G and Supporting Information Fig 1. IL-33 from Peprotech was used in all other experiments.

Cell cultures

Primary human mast cell cultures were derived from CD 34⁺ peripheral blood progenitors obtained from healthy volunteers following informed consent under a protocol (NCT00001756) approved by the National Institutes of Health Internal Review Board. Cells were cultured as previously described [66, 67] and used between 7–9 weeks of culture when >95% cells were identified as mast cells (see Supporting Information Fig. 4A). LAD2 cells were cultured in StemPro-34 supplemented with L-Glutamine (2mM), penicillin (100U/ml), streptomycin (100 µg/ml) and recombinant human SCF (100 ng/ml) [68]. Mouse bone marrow-derived mast cells (BMMCs) from wild type (WT) and MyD88^{–/–} mice (C57BL/6 background, Jackson Laboratory) were cultured from femoral bone marrow cells as previously described [69] to achieve more than 95% purity (see Supporting Information Fig. 4B) and used between 4–6 weeks of culture.

Cell activation

For measurement of Ag dependent effects, HuMCs and LAD2 cells were sensitized overnight with biotinylated human myeloma IgE (100 ng/ml). Next day, cells were rinsed twice with cytokine-free growth medium and maintained in this medium for 3 h prior to activation with streptavidin in the presence or absence of SCF (100 ng/ml). Mouse BMMCs were sensitized overnight in cytokine free medium containing 100ng/ml monoclonal mouse anti-DNP IgE. After rinsing twice and resuspending in cytokine-free medium, cells were activated with DNP-HSA with or without mSCF (100ng/ml). Where indicated, cells were also stimulated with various concentrations of IL-33 (0–100 ng/ml).

Immunoblotting

Cell lysates were prepared from 1×10^6 cells as previously described [70]. 20 µl of each cell lysate was loaded on to 4–12% polyacrylamide gels, proteins separated by electrophoresis and transferred to nitrocellulose membranes. Membranes were blocked for 60 min using Odyssey blocking buffer (LICOR Biosciences) and incubated overnight with the primary antibodies. After 60 min incubation with the IR-dye (680RD or 800CW)-labeled secondary antibodies (1:20,000) immunoreactive proteins were visualized using an Odyssey imager (LI-COR Biosciences).

Passive Systemic anaphylaxis

Wild type and *Kit^{W-sh/W-sh}* C57Bl/6 mice were obtained from the Jackson Laboratory and used in accordance with National Institutes of Health (NIH) guidelines and an animal study protocol (LAD2E) approved by the National Institute of Allergy and Infectious Diseases Institutional animal Care Use Committee. Mice were sensitized overnight with 3 µg of anti DNP-IgE and challenged with 250 µg of antigen (DNP-HSA) in PBS. Anaphylactic responses were verified by measuring core body temperature and blood was collected by cardiac puncture after euthanasia by CO₂ inhalation at 0, 4, 6 and 24 hours after challenge.

Release of cytokines and sST2

Release of cytokines and sST2 from mast cells into culture medium was measured using Duo Set ELISA kits (R & D Systems) according to manufacturer's instructions.

Quantitative Real Time PCR

Total RNA from 1×10^6 cells were extracted using RNeasy plus mini kit (Qiagen, Valencia, CA) and cDNA prepared using random hexamers and Superscript III reverse transcriptase (Life Technologies) according to manufacturer's instructions. A 1 µg aliquot of RNA from each sample was used for cDNA synthesis. qRT-PCR analysis was performed using ABI 7500 system and TaqMan gene expression assays (Life Technologies).

The ST2 primers for the reverse transcriptase experiment for Figure 1A were designed to recognize sequences unique for each mRNA namely, human sST2-forward primer 5'-GAAAAACGCAACCTAACT-3', reverse primer 5'-TCAGAAACACTCCTTACTTG-3' and human ST2L- forward primer 5'-AGGCTTTTCTCTGTTTCCAGTAATCGG-3', reverse primer 5'-GGCCTCAATCCAGAACATTTTATAGGATGATAAC-3'; mouse sST2-forward primer 5'-CGTGGGTCGTCTGCAGAAAT-3', reverse primer 5'-GCTCTCTGAGGTAGGGTCCA-3' and mouse ST2L- forward primer 5'-TGAGGTTGCTCTGTTCTGGAG-3', reverse primer 5'-TTTCAAGAACGTCGGGCAGA-3'. For the remaining experiments, the relative levels of ST2 transcripts were determined by use of TaqMan assay kits as follows: human sST2 (Hs01073297_m1), human ST2L (Hs00249389_m1), mouse sST2 (Mm04176272_s1), mouse ST2L (Mm01233982_m1) and normalized against GAPDH as the endogenous control gene.

Flow Cytometry

Prior to surface staining, FcγRs were blocked for 10 min at room temperature (RT) with either 2.4G2 or Human TruStain FcX (for human cells) or rat anti-mouse CD16/CD32 (for BMMCs) in all samples. For FcεRI and KIT surface expression, cells were washed with PBS and stained with FITC- anti- FcεRI and APC-anti-KIT antibodies for 30 min at 4°C and washed with PBS containing 0.5% w/v BSA. For IL-33 expression, surface stained cells were fixed with BD FACS Lysis buffer for 15 min at RT, washed with PBS containing 0.5% w/v BSA, and permeabilized (with PBS, 0.5% w/v BSA, 0.1% w/v saponin) for 30 min at 4°C. Cells were then washed and stained with goat anti IL-33 (2.5 µg/10⁶ cells) for 30 min

at RT, followed by PE-anti-goat antibody (5 µl/100 µl) for 30 min at RT. After washing with PBS containing 0.5% BSA w/v, the stained cells were analyzed by flow cytometry.

Statistical analysis

Means and SEM were computed from average values obtained from experiments conducted on cells derived from at least three donors or animals. Unpaired Student's *t*-test was used to determine statistically significant differences between groups and are indicated as follows; * $p < 0.05$. ** $p < 0.01$, *** $p < 0.001$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper

BMMC	mouse bone marrow derived mast cells
DNP-HSA	DNP-human serum albumin
HuMCs	primary human peripheral blood-derived mast cells
IL-1RAcP	IL-1R accessory protein
sST2	soluble ST2
ST2L	full length ST2 also called interleukin-1 receptor-like 1

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Figure 1.

Expression of ST2L and sST2 mRNAs in human and murine mast cells. (A) (left) Representative gel image obtained by RT-PCR of ST2L and sST2 mRNA extracted from non-stimulated HuMCs, LAD2 cells, and mouse BMMCs. Total RNA was extracted from 2×10^6 cells for preparation of cDNA and amplification using specific primers described in the Materials and Methods section. Data shown are representative of 3 independent cultures of each cell type. (right) GAPDH was amplified as a loading control (B–G) Changes in levels of sST2 and ST2L mRNA in cells after stimulation with 10 ng/ml IL-33 for the indicated times as determined by quantitative real time PCR. GAPDH was used as the internal control. Values are mean $\pm$ SEM of normalized values (0 h equals 1.0) from three independent cultures of each cell type. Significant differences are indicated thus; * $p < 0.05$. ** $p < 0.01$, *** $p < 0.001$, Student's t-test, between control (0 h) and stimulated levels.

Figure 2.

Time-course of release of sST2 and GM-CSF from human and murine mast cells in response to IL-33. HuMCs (A and B), LAD2 cells (C and D), and BMMCs (E and F) were stimulated with 10 ng/ml IL-33 for the times indicated. Levels of secreted sST2 and GM-CSF were determined by ELISA. Values are mean $\pm$ SEM values from experiments with three independent cultures of cells. Significant differences are indicated thus; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Student's t-test, between control (0 h) and stimulated levels.

Figure 3.

Release of sST2, GMCSF, and MIP1-α in response to various concentrations of IL-33.

HuMCs (A–C), LAD2 cells (D–F), and BMMCs (G–I) were stimulated with the indicated concentrations of IL-33 for 24 h. Levels of secreted sST2, GMCSF and MIP1-α were determined by ELISA. Values are mean ± SEM of values from three independent cultures of cells. Significant differences are indicated thus; * $p < 0.05$. ** $p < 0.01$, *** $p < 0.001$, Student's t-test, between 0 ng/ml and other concentrations.

Figure 4.

Comparison of effectiveness of IL-33, IL-18, and IL-1 β in stimulating sST2 release. HuMCs (A and B) and BMMCs (C and D) were stimulated for 24 h with 10 ng/ml IL-33, 10 ng/ml IL-1 β or 20 ng/ml IL-18 alone or in the combinations indicated. Levels of secreted sST2 and GM-CSF were determined by ELISA. Values are mean $\pm$ SEM of values from three independent cultures of cells. Significant differences are shown thus; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Student's t-test, between control (c) and stimulated levels, and between indicated pairs.

Figure 5.

Ag, SCF, and IL-33 interact synergistically to enhance production of sST2. (A, B) Synergistic effects of SCF on sST2 release in BMMCs stimulated with different concentrations of (A) Ag for 6 h or (B) with 10 ng/ml Ag and SCF (100 ng/ml) for indicated times. Levels of secreted sST2 were determined by ELISA. The corresponding values for SCF alone in panel A were equivalent to or less than those for Ag alone (not shown). (C) Release of sST2 into circulation during IgE/Ag-induced passive systemic anaphylaxis. Mice were sensitized with 3 μ g of DNP-specific IgE and challenged 24 h later with 250 μ g DNP-HSA (Ag). Serum samples were obtained at the times indicated and sST2 was measured by ELISA. (D, E) Synergistic effects of IL-33 on Ag-induced sST2 release in WT BMMCs (D) or MyD88-deficient BMMCs (E) stimulated with Ag (100 ng/ml) and/or IL-33 (10 ng/ml) for 24 h. Levels of secreted sST2 were determined by ELISA. (F) Synergistic effects of SCF on IL-33-induced sST2 release in BMMCs stimulated with SCF (100 ng/ml) and/or IL-33 (10 ng/ml) for 24 h. Levels of secreted sST2 were determined by ELISA. (G) Synergistic effects of Ag and SCF on IL-33-induced sST2 gene expression in BMMCs. BMMCs were stimulated for 4 h with Ag (100 ng/ml), SCF (100 ng/ml) and IL-33 (10 ng/ml) individually or in combinations shown. Gene expression was measured by qRT-PCR. GAPDH was used as the internal control and data were normalized against the control (c). (H) sST2 released from HuMCs stimulated for 24 h with streptavidin (100 ng/ml) or IL-33 (10 ng/ml), individually or in the combinations shown. Levels of secreted sST2 were determined by ELISA. (I) sST2 released from HuMCs stimulated with SCF (100 ng/ml) or IL-33 (10 ng/ml) individually or in the combinations shown. Levels of secreted sST2 were determined by ELISA. All values are mean $\pm$ SEM from three independent experiments. Significant differences from 0 h or controls and between indicated pairs are shown thus; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Student's t-test between control and stimulated levels.

Figure 6.

Evidence that IL-33 is not released from stimulated mast cells. (A) Relative changes in IL-33 mRNA levels were measured by qRT-PCR after stimulation of BMMCs with 10 ng/ml Ag and 100 ng/ml SCF individually or in combinations shown. GAPDH was used as the internal control (0 min equals 1.0). Values shown are mean $\pm$ SD from one of three independent experiments each exhibiting identical patterns although maximal responses varied (mean $\pm$ SEM 610 ± 360 , 2 h after stimulation with Ag and SCF). (B) Representative Western blots from one of three experiments for IL-33 protein in lysed BMMCs, HuMCs, and LAD2 cells stimulated for the times indicated with SCF (100ng/ml) and Ag (10ng/ml) or streptavidin (10 ng/ml). GAPDH levels are shown as loading control. (C) Densitometric measurements of BMMC Western blots shown in B (upper panel). Data are from three independent experiments with different batches of cells and values depict mean $\pm$ SEM of values normalized to GAPDH. Significant differences are indicated as follows: * $p < 0.05$. ** $p < 0.01$, *** $p < 0.001$, Student's t-test, between control and stimulated levels.