The Journal of Immunology

Cutting Edge: The ST2 Ligand IL-33 Potently Activates and Drives Maturation of Human Mast Cells

Zoulfia Allakhverdi, Dirk E. Smith, Michael R. Comeau and Guy Delespesse

J Immunol 2007; 179:2051-2054; ;
doi: 10.4049/jimmunol.179.4.2051
http://www.jimmunol.org/content/179/4/2051

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

THE JOURNAL OF IMMUNOLOGY

CUTTING EDGE

Cutting Edge: The ST2 Ligand IL-33 Potently Activates and Drives Maturation of Human Mast Cells[1]

Zoulfia Allakhverdi, Dirk E. Smith,† Michael R. Comeau,† and Guy Delespesse[2]**

IL-33, the natural ligand of the IL-1 receptor family member ST2L, is known to enhance experimental allergic-type inflammatory responses by costimulating the production of cytokines from activated Th2 lymphocytes. Although ST2L has long been known to be expressed by mast cells, its role in their biology has not been explored. In this study we report that IL-33 directly stimulates primary human mast cells (MCs) to produce several proinflammatory cytokines and chemokines and also exerts a permissive effect on the MCs response to thymic stromal lymphopoietin, a recently described potent MCs activator. IL-33 also acts both alone and in concert with thymic stromal lymphopoietin to accelerate the in vitro maturation of CD34$^+$ MC precursors and induce the secretion of Th2 cytokines and Th2-attracting chemokines. Taken together, these results suggest that IL-33 may play an important role in mast cell-mediated inflammation and further emphasize the role of innate immunity in allergic diseases. The Journal of Immunology,* 2007, 179: 2051–2054.

The recently discovered cytokine IL-33 is the natural ligand of ST2L, a long known orphan member of the IL-1 receptor superfamily (1). The gene encoding the IL-33 receptor also encodes a soluble molecule (sST2)[3] by alternative splicing as well as a variant form expressed in humans, ST2v, the functional significance of which is unclear (2, 3). In addition to being selectively expressed on Th2 but not Th1 lymphocytes (4), ST2L is also expressed on mature mouse mast cells (MCs) and on their very early precursors at the initial stage of MC lineage commitment (5, 6). T cell-associated ST2L augments Th2 immune responses in vivo and in vitro, whereas macrophage-associated ST2L as well as sST2 have been reported to display anti-inflammatory activity (7). Disruption of ST2L activity through the use an ST2 Ab or soluble ST2 protein markedly attenuates Th2-dominated immune responses and disease activity without affecting the development of naive T cells into Th2 effectors (8, 9). In line with these earlier observations on ST2L, IL-33 augments the in vitro production of IL-5 and IL-13 by activated mouse Th2 cells. In vivo, treatment of mice with IL-33 selectively induces the expression of high levels of IL-4, IL-5, and IL-13 in various tissues, together with eosinophilia and elevated serum levels of IgE and IgA. IL-33-treated mice display severe inflammatory lesions of the lungs and the digestive tract with eosinophilic infiltrates, epithelial cell hyperplasia, and increased production of mucus (1). A role for ST2/IL-33 in human allergic diseases is supported by the recent finding that a single nucleotide mutation in the ST2 promoter results in enhanced ST2 expression and is associated with an increased risk of developing atopic dermatitis (10). The cellular origin of IL-33 and the mechanisms regulating its secretion remain to be determined; however, human IL-33 mRNA is constitutively expressed in airway epithelial cells, bronchial smooth muscles, and he smooth muscles of the coronary and pulmonary arteries (1). Like other members of the IL-1 superfamily such as IL-1β, IL1-α, and IL-18, IL-33 is produced as a precursor protein that is cleaved by caspase-1 into mature IL-33 (1). Recent data further indicate that IL-33 is a dual function protein that may play the role of either a proinflammatory cytokine or an intracellular NF with transcriptional regulatory properties (11). Although IL-33 has been demonstrated to act on T cells in vitro, the biological effects of IL-33 on MCs has not been described, although this cytokine was reported to induce the activation of NF-κB and MAPKs in mouse MCs (1). Our data provides the first evidence that IL-33 alone or together with thymic stromal lymphopoietin TSLP may play a significant role in MC-mediated inflammation by directly acting upon mature as well as precursor human MCs.

Materials and Methods

MC cultures

Human peripheral blood- or cord blood-derived CD34$^+$ progenitor cells were isolated and cultured in stem cell factor-supplemented medium as reported (12). After 10–12 wk of culture, >98% of the cells were stained for c-Kit (BD), FcεRI (eBioscience), and tryptase (Chemicon). MCs were cultured at 1×10^5 cells/ml in 0.2 ml in 96-well flat-bottom plates for 24 h in the presence of exogenous cytokine (+/−)-neutralizing Abs as indicated. Abs and recombinant

*Laboratory on Allergy, Centre Hospitalier de l'Université de Montréal Research Center, Notre-Dame Hospital, Montreal, Quebec, Canada and †Inflammation Research, Amgen, Seattle, WA 98101

Received for publication May 22,2007. Accepted for publication June 15, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by a grant from Amgen.

[2] Address correspondence and reprint requests to Dr. Guy Delespesse, Centre Hospitalier de l'Université de Montréal Research Center, Notre-Dame Hospital, Laboratory on Allergy, 1560 Sherbrooke East Street, Pavillon Mailloux, M4211-K, Montreal, Quebec, Canada H2L-4M1. E-mail address: guy.delespesse@sympatico.ca

[3] Abbreviations used in this paper: SST2, soluble ST2; LTC_4, 5(*S*)-hydroxy-6(*R*)-S-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; MC, mast cell; TSLP, thymic stromal lymphopoietin.

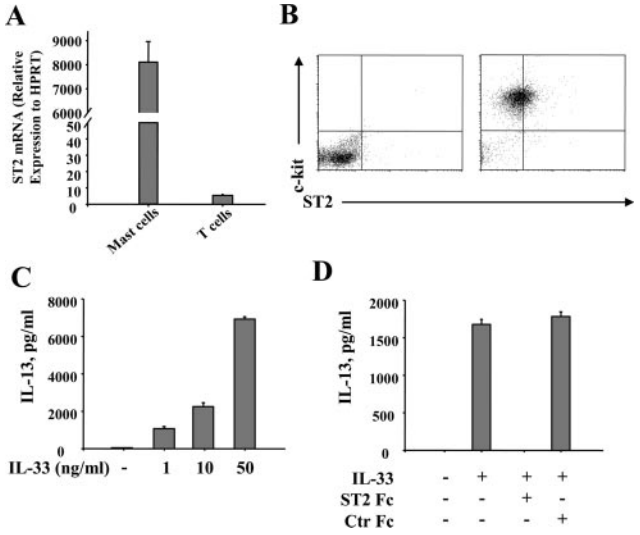

FIGURE 1. Human MCs express a functional receptor for IL-33. *A* and *B*, Membrane ST2L expression was determined at mRNA (mean ± SEM of eight experiments on different MC lines) and protein levels (one representative experiment of five) on MCs and peripheral blood T cells. *C*, MCs were stimulated with varying concentrations of IL-33, and IL-13 production was measured in the supernatants after 24 h of culture. One representative experiment of three is shown; mean ± SD of triplicates. *D*, MCs were stimulated in the presence of IL-33 (10 ng/ml) and neutralizing ST2 Fc or control Fc protein (each at 10 μg/ml). One representative experiment of three is shown; mean ± SD of triplicates. Ctr, Control.

cytokines used included anti-ST2-PE (MBL), anti-MC tryptase (Chemicon), rIL-33 (Amgen), and recombinant TSLP (Amgen).

Assessment of mediator, β-hexosaminidase, cytokine, and chemokine release

MCs were incubated for 90 min with IL-33 (10 ng/ml) or PMA/ionomycin as a positive control, and ELISA for 5(*S*)-hydroxy-6(*R*)-S-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (LTC_4) and PGD_2 was performed (Cayman Chemical) according to manufacturer's instructions. β-Hexosaminidase release was analyzed as previously described (13). IL-4, IL-5, IL-6, IL-10, IL-13, GM-CSF, CCL1, CCL2, CCL17, CCL22, and CXCL8 were examined via commercial kits in supernatants harvested after 24 h of MC activation or 1 wk of stimulation of $CD34^+$ MC progenitors. All assays were conducted in triplicate.

Quantitative real-time PCR

RNA was isolated using the RNeasy mini kit (Qiagen). cDNA synthesis was performed using the ABI first-strand cDNA synthesis kit (Applied Biosystems). Quantitative real-time PCR was performed via TaqMan using ABI gene expression assays. Hypoxanthine phosphoribosyltransferase was used as a control for cDNA input.

Results and Discussion

We first sought to demonstrate that primary human MCs express the functional IL-33 receptor. As illustrated in Fig. 1, *A* and *B*, $CD34^+$ progenitor-derived MCs express membrane ST2L mRNA and protein as revealed by quantitative real-time PCR and flow cytometry. The ST2 receptor is functional in

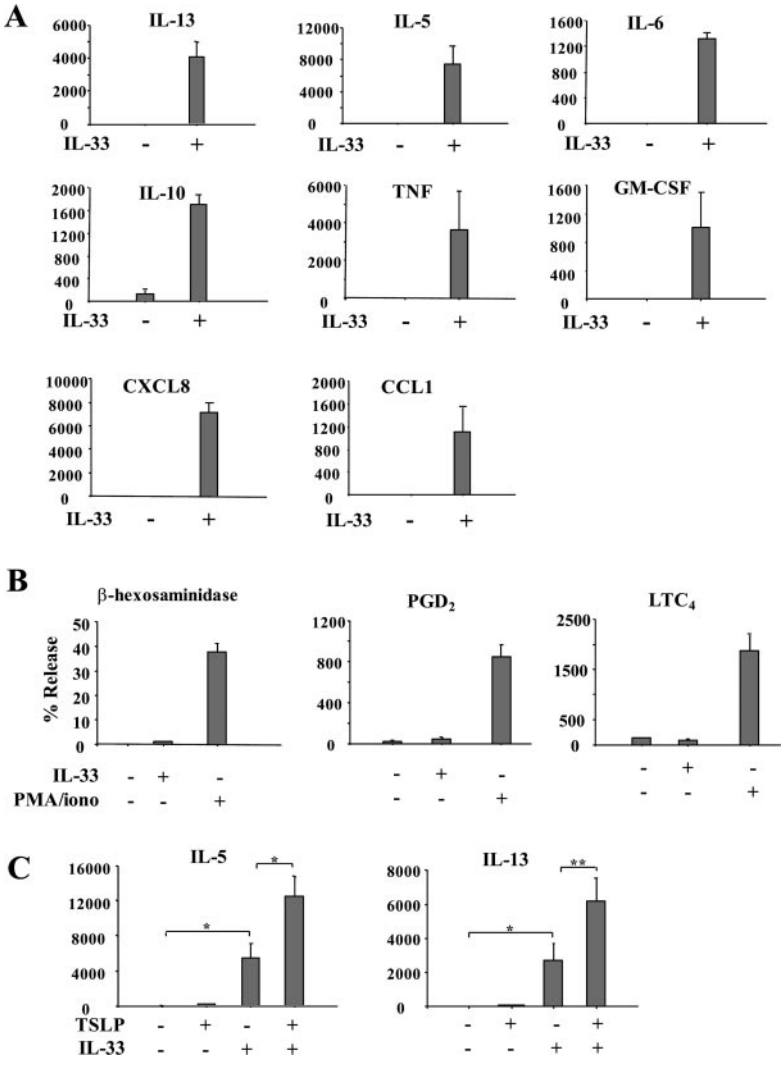

FIGURE 2. Effect of IL-33 on mature MCs. *A*, Cytokines and chemokines secretion (pg/ml) by MCs (10^5 cells/ml) stimulated for 24 h with IL-33 (10 ng/ml). Mean ± SEM of 5–14 experiments is shown. *B*, MCs were stimulated by IL-33 (10 ng/ml) or PMA/ionomycin (as a positive control); β-hexosaminidase release was measured after 30 min and LTC_4 or PGD_2 (pg/ml) after 90 min of stimulation. *C*, MC activation and release of IL-5 or IL-13 (pg/ml) in the presence or absence of IL-33 and/or TSLP. $^*p < 0.05$; $^{**}p < 0.01$. Mean ± SEM of eight experiments is shown.

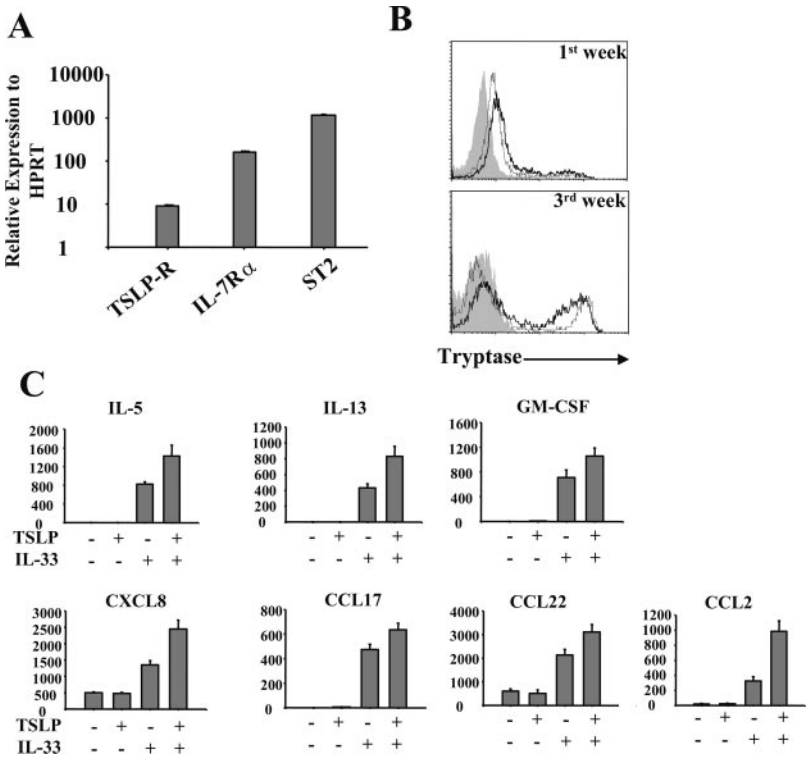

FIGURE 3. Effect of IL-33 on $CD34^+$ MCs precursors. *A*, mRNA for membrane ST2L, TSLP-R, and IL-7Rα-chains of the TSLP receptor were determined on 3-day-old $CD34^+$ MC precursors by quantitative real-time PCR. *B*, Intracytoplasmic tryptase staining of 1- and 3-wk-old $CD34^+$ MCs precursors cultured in the presence of SCF (100 ng/ml) with or without IL-33. Isotype control mAb is depicted as a gray histogram and unstimulated and IL-33-stimulated cultures are represented as thin and bold lines, respectively. One representative experiment of three is shown. *C*, Cytokines and chemokines secretion by $CD34^+$ MCs precursors stimulated for 1 wk with or without IL-33 and/or TSLP (10 ng/ml each). Results are expressed in pg/ml; mean ± SEM of four experiments.

that IL-33 dose-dependently stimulates MC production of IL-13, and this response is IL-33 specific because it is suppressed by recombinant sST2-Fc (Fig. 1, *C* and *D*). It is unlikely that the MC response to IL-33 is inhibited by endogenously released sST2, which is known to contain the ST2L exodomain (2, 3). Indeed, sST2 is not detectable in the culture supernatants of MCs stimulated with IL-33 or IL-1/TNF or by FcεR1 cross-linkage (< 20 pg/ml), whereas low levels (100–200 pg/ml) are released in response to PMA stimulation (data not shown). In addition to IL-13, IL-33-stimulated MCs release IL-5, IL-6, IL-10, TNF, GM-CSF, CXCL8, and CCL1 (Fig. 2*A*) but produce very little or no IL-1β (<100pg/ml in only two of eight cultures, data not shown). Finally, IL-33 does not trigger MC degranulation or the production of PGD_2 and LTC_4 (Fig. 2*B*). These effects of IL-33 are similar to those recently reported for TSLP with the noticeable exception that TSLP requires the presence of IL-1 to exert its activity (12). Given that IL-33 is an IL-1-like cytokine, we next examined the possible cooperation between IL-33 and TSLP. We found that similar to IL-1β, IL-33 confers a permissive effect on MC response to TSLP (Fig. 2*C*). ST2L is expressed at several stages during development of the MC lineage, including on the earliest detectable committed precursors in the mouse (5). The population of human $CD34^+$ progenitor cells is heterogeneous and may differentiate into several lineages including MCs, dendritic cells (14), and polynuclear leukocytes such as eosinophils/basophils (15, 16). We first show that ST2L mRNA is expressed in the population of $CD34^+$ precursor cells isolated from human umbilical cord blood, which also expresses mRNA encoding the two chains of the TSLP receptor (Fig. 3*A*). The addition of IL-33 to cultures of $CD34^+$ progenitors accelerates their maturation into tryptase-containing cells (Fig. 3*B*). The culture supernatants of these IL-33-stimulated progenitor cells contain high levels of proinflammatory cytokines and chemokines, including IL-5, IL-13, GM-CSF, CXCL8, CCL17, CCL22, and CCL2. TSLP significantly enhances the stimulatory activity of IL-33 on $CD34^+$ cells (Fig. 3*C*). It is of note that unlike mature MCs, $CD34^+$ precursors do not release TNF or IL-10. The data indicate that IL-33 alone or together with TSLP is a potent activator of $CD34^+$ precursor cells.

Although the role of IL-33/ST2L-mediated T cell stimulation has been well documented in Th2-mediated immune responses and diseases, this study is the first to document the ability of IL-33 to directly stimulate MCs and to exert a permissive effect, similar to that of IL-1β, on the response of these cells to TSLP. The two cytokines directly stimulate mature MCs and their locally recruited precursors to produce high levels of cytokines and chemokines that are reportedly sufficient to induce and maintain an allergic phenotype by a direct effect on tissue-resident cells and the local recruitment and activation of inflammatory cells (17).

The clinical relevance of these observations is underlined by the pivotal role of MCs in the exacerbation, maintenance, and initiation of allergic diseases (18). Given that like TSLP IL-33 is presumably produced by cells other than lymphocytes and that MCs are integral members of the innate immune system, our data suggest that allergic inflammation may be induced or aggravated by the innate immune system. It will be important to determine which natural triggers induce the expression, processing, and release of IL-33 in local MC-containing tissues.

Acknowledgments

We thank the group of nurses and obstetricians at St-Luc Hospital (Montreal, Quebec, Canada) for providing us with umbilical cord blood.
D.E.S and M.R.C. are Amgen shareholders.

Disclosures

The authors have no financial conflict of interest.

References

1. Schmitz, J., A. Owyang, E. Oldham, Y. Song, E. Murphy, T. K. McClanahan, G. Zurawski, M. Moshrefi, J. Qin, X. Li, et al. 2005. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 23: 479–490.
2. Gachter, T., A. K. Werenskiold, and R. Klemenz. 1996. Transcription of the interleukin-1 receptor-related T1 gene is initiated at different promoters in mast cells and fibroblasts. *J. Biol. Chem.* 271: 124–129.
3. Tominaga, S., K. Kuroiwa, K. Tago, H. Iwahana, K. Yanagisawa, and N. Komatsu. 1999. Presence and expression of a novel variant form of ST2 gene product in human leukemic cell line UT-7/GM. *Biochem. Biophys. Res. Commun.* 264: 14–18.
4. Xu, D., W. L. Chan, B. P. Leung, F. Huang, R. Wheeler, D. Piedrafita, J. H. Robinson, and F. Y. Liew. 1998. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *J. Exp. Med.* 187: 787–794.
5. Chen, C. C., M. A. Grimbaldeston, M. Tsai, I. L. Weissman, and S. J. Galli. 2005. Identification of mast cell progenitors in adult mice. *Proc. Natl. Acad. Sci. USA* 102: 11408–11413.
6. Moritz, D. R., H. R. Rodewald, J. Gheyselinck, and R. Klemenz. 1998. The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. *J. Immunol.* 161: 4866–4874.
7. Brint, E. K., D. Xu, H. Liu, A. Dunne, A. N. McKenzie, L. A. O'Neill, and F. Y. Liew. 2004. ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance. *Nat. Immunol.* 5: 373–379.
8. Coyle, A. J., C. Lloyd, J. Tian, T. Nguyen, C. Erikkson, L. Wang, P. Ottoson, P. Persson, T. Delaney, S. Lehar, S. Lin, et al. 1999. Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. *J. Exp. Med.* 190: 895–902.
9. Hoshino, K., S. Kashiwamura, K. Kuribayashi, T. Kodama, T. Tsujimura, K. Nakanishi, T. Matsuyama, K. Takeda, and S. Akira. 1999. The absence of interleukin 1 receptor-related T1/ST2 does not affect T helper cell type 2 development and its effector function. *J. Exp. Med.* 190: 1541–1548.
10. Shimizu, M., A. Matsuda, K. Yanagisawa, T. Hirota, M. Akahoshi, N. Inomata, K. Ebe, K. Tanaka, H. Sugiura, K. Nakashima, et al. 2005. Functional SNPs in the distal promoter of the ST2 gene are associated with atopic dermatitis. *Hum. Mol. Genet.* 14: 2919–2927.
11. Carriere, V., L. Roussel, N. Ortega, D. A. Lacorre, L. Americh, L. Aguilar, G. Bouche, and J. P. Girard. 2007. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proc. Natl. Acad. Sci. USA* 104: 282–287.
12. Allakhverdi, Z., M. R. Comeau, H. K. Jessup, B. R. Yoon, A. Brewer, S. Chartier, N. Paquette, S. F. Ziegler, M. Sarfati, and G. Delespesse. 2007. Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *J. Exp. Med.* 204: 253–258.
13. Kulka, M., L. Alexopoulou, R. A. Flavell, and D. D. Metcalfe. 2004. Activation of mast cells by double-stranded RNA: evidence for activation through Toll-like receptor 3. *J. Allergy Clin. Immunol.* 114: 174–182.
14. Caux, C., C. Dezutter-Dambuyant, D. Schmitt, and J. Banchereau. 1992. GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells. *Nature* 360: 258–261.
15. Denburg, J. A., S. Telizyn, H. Messner, B. Lim, N. Jamal, S. J. Ackerman, G. J. Gleich, and J. Bienenstock. 1985. Heterogeneity of human peripheral blood eosinophil-type colonies: evidence for a common basophil-eosinophil progenitor. *Blood* 66: 312–318.
16. Robinson, D. S., R. Damia, K. Zeibecoglou, S. Molet, J. North, T. Yamada, A. B. Kay, and Q. Hamid. 1999. $CD34^+$/interleukin-5Rα messenger RNA^+ cells in the bronchial mucosa in asthma: potential airway eosinophil progenitors. *Am. J. Respir. Cell Mol. Biol.* 20: 9–13.
17. Wills-Karp, M. 2004. Interleukin-13 in asthma pathogenesis. *Immunol. Rev.* 202: 175–190.
18. Bradding, P., A. F. Walls, and S. T. Holgate. 2006. The role of the mast cell in the pathophysiology of asthma. *J. Allergy Clin. Immunol.* 117: 1277–1284.