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IL-1 Receptor Accessory Protein and ST2 Comprise the IL-33 Receptor Complex

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IL-33 (IL-1F11) is a recently described member of the IL-1 family of cytokines that stimulates the generation of cells, cytokines, and Igs characteristic of a type 2 immune response. IL-33 mediates signal transduction through ST2, a receptor expressed on Th2 and mast cells. In this study, we demonstrate that IL-33 and ST2 form a complex with IL-1R accessory protein (IL-1RAcP), a signaling receptor subunit that is also a member of the IL-1R complex. Additionally, IL-1RAcP is required for IL-33-induced in vivo effects, and IL-33-mediated signal transduction can be inhibited by dominant-negative IL-1RAcP. The implications of this shared usage of IL-1RAcP by IL-1(α and β) and IL-33 are discussed. ***The Journal of Immunology,*** **2007, 179: 2551–2555.**

Interleukin-33 is the 11th described member of the IL-1 family of cytokines. IL-1 family members share a common β-trefoil structure and are highly proinflammatory. Several naturally occurring antagonists and decoy receptors exist to regulate the potent inflammatory effects exhibited by these cytokines. The best characterized members of this family, IL-1 (α and β) and IL-18, are expressed as prodomain containing polypeptide precursors which are proteolytically cleaved to generate the active form of the cytokine. These cytokines require the usage of two receptor subunits: a primary ligand-binding receptor chain and a second receptor chain which does not bind to the ligand by itself, but is required to mediate signal transduction (reviewed in Ref. 1).

IL-33 was identified computationally based on a sequence profile generated by compiling β-trefoil structures of IL-1 family members and fibroblast growth factor. Like IL-1 and IL-18, IL-33 can be proteolytically cleaved in vitro by caspase-1 to generate a mature form of the protein. When administered i.p. to mice, IL-33 has broad proinflammatory effects, inducing eosinophilia, splenomegaly, goblet cell hyperplasia and mucous production at mucosal surfaces, and increased serum levels of IL-5 and IgE (2). Based on these in vivo effects, IL-33 is likely to be involved in Th2-mediated immune responses, including asthma, allergy, or parasitic helminth infections. ST2, which is expressed on Th2 and mast cells and is highly homologous to the ligand-binding subunits of the IL-1 and IL-18 receptor complexes, was shown to be one component of the IL-33R complex. On cells that express ST2, the presence of IL-33 leads to activation of a signaling pathway involving MyD88 and NF-κB (2).

To understand the precise role of IL-33 and ST2 in the immune response, it is necessary to identify the second component of the IL-33R complex. The most prominent members of the IL-1 family, IL-1 and IL-18, do not share usage of receptor subunits. However, three more recently discovered IL-1 family members (IL-1F6, IL-1F8, and IL-1F9) have been shown to use the second component of the IL-1R complex, the IL-1R accessory protein (IL-1RAcP)[2] (3). IL-1RAcP is necessary for IL-1α and IL-1β-mediated signal transduction, but is unable to bind to these cytokines by itself (4–8). We asked whether IL-1RAcP is also involved in IL-33 signaling. We show that IL-1RAcP$^{-/-}$ mice and polarized Th2 cells from IL-1RAcP$^{-/-}$ mice do not respond to IL-33 administration. Additionally, we can detect a ST2/IL-33/IL-1RAcP complex by ELISA. Finally, we show that dominant-negative (dn) IL-1RAcP can inhibit IL-33-mediated signaling. These results clearly show that IL-1RAcP is a member of the IL-33 signaling receptor complex.

Materials and Methods

Mice

IL-1RAcP$^{-/-}$ mice (on a mixed C57BL/6 and 129Sv background) were purchased from The Jackson Laboratory and bred at this institution. Wild-type (WT) B6.129SF2/J mice were purchased from The Jackson Laboratory. In some experiments, WT mice came from an internally produced mixed C57BL/6–129Sv colony. Mice were i.p. injected with 2 μg of recombinant human or murine IL-33 (produced as described previously; Ref. 2) or saline daily for 6 days and sacrificed on day 7. All animal studies were reviewed and approved by the DNAX Animal Care and Use Committee.

Sample preparation and analysis

The percentage of eosinophils was determined by counting ≥300 leukocytes from smears of tail blood. Serum cytokines were quantified by multiplex immunoassay using the 22-Plex Mouse Cytokine kit (Linco Research). Serum IgE was quantified with a mouse IgE ELISA set (BD Biosciences). Gene expression was analyzed by RT-PCR of RNA prepared from snap-frozen tissue as previously described (2). The ST2 primer used detects message for both the soluble and membrane-bound forms of this receptor. For histology, formalin-fixed, paraffin-embedded tissue was sectioned and stained via periodic-acid Schiff.

Th2 polarization

CD4$^+$ cells were isolated from WT and IL-1RAcP$^{-/-}$ spleen and peripheral lymph nodes using the MACS CD4$^+$ T cell isolation kit (Miltenyi

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[2] Abbreviations used in this paper: IL-1RAcP, IL-1R accessory protein; dn, dominant negative; WT, wild type; TIR, Toll/IL-1R.

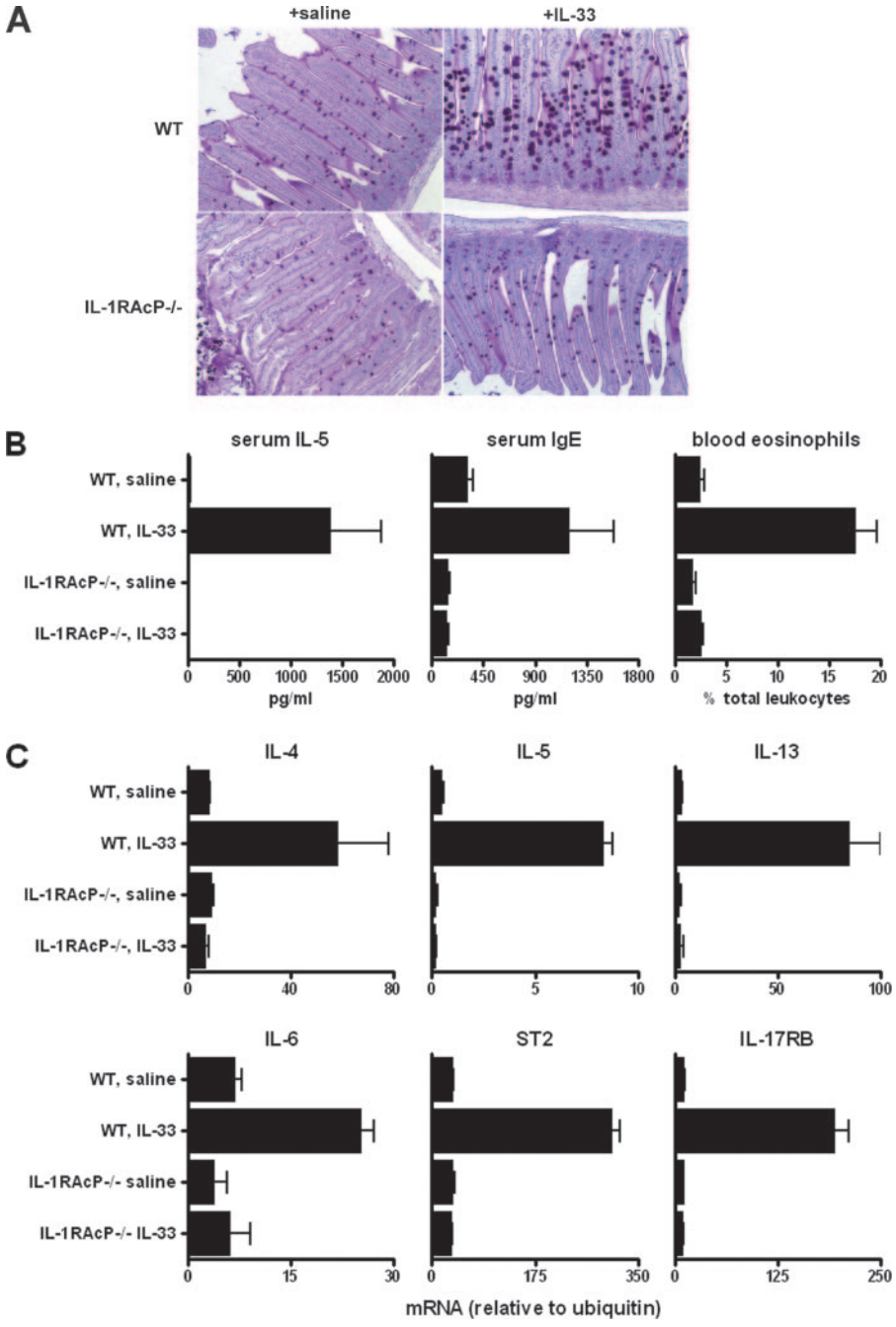

FIGURE 1. IL-1RAcP$^{-/-}$ mice do not respond to IL-33 administration. WT and IL-1RAcP$^{-/-}$ mice were given six daily injections of 2 μg of IL-33 and sacrificed on day 7. *A*, Periodic-acid Schiff stain of the small intestine showing the small intestinal villi with prominent goblet cell hyperplasia in the IL-33-treated WT animals. *B*, Serum IL-5 and IgE, and percent of total blood leukocytes that are eosinophils. *C*, Gene expression in the lung. A similar pattern was seen in the spleen. These results are representative of three experiments. Shown are the results with human IL-33. Murine IL-33 administration yielded similar results. Mean $\pm$ SEM, n = 3–5 mice/group.

Biotec), and cultured for 5 days on anti-CD3-coated plates in the presence of 5 ng/ml IL-2, 10 ng/ml IL-4, 10 μg/ml anti-IFN-γ, and 1 μg/ml anti-CD28. Cells were washed and cultured for 3 days in medium containing IL-2, washed again, and incubated with 5 ng/ml IL-2 $\pm$ 10 μg/ml anti-ST2 (MD Biosciences) and 50 ng/ml IL-33. Supernatants were harvested after 20 h and cytokines were measured by multiplex immunoassay.

Receptor-ligand complex ELISA

A total of 3 μg/ml murine ST2-Fc or IL-1R1-Fc fusion proteins (R&D Systems) in PBS were coated overnight onto MaxiSorp plates (Nunc). After washing, plates were incubated with 3 μg/ml recombinant his6-tagged murine IL-1RAcP and increasing concentrations of murine IL-33 or IL-1β in PBS/1% BSA/0.05% Tween 20 for 1.5 h. Plates were washed and incubated with anti-his6-peroxidase (Roche Diagnostics) for 1 h, washed again, developed with tetramethylbenzidine peroxidase substrate (KPL), stopped with H_2PO_4, and read on a plate reader at 450–570 nm.

Dominant-negative experiment

HEK293FT cells (Invitrogen Life Technologies), which endogenously express human IL-1R1 and IL-1RAcP, were transfected with 3 μg of a NF-κB-driven reporter gene construct (pNF-κB-hrGFP; Stratagene), increasing amounts of cMyc-tagged murine IL-1RAcP dn construct (with a stop codon inserted before the Toll/IL-1R (TIR) domain), and 1 μg of a plasmid encoding murine ST2 (for cells stimulated with IL-33). Twenty-four hours posttransfection, cells were stimulated with 20 ng/ml murine IL-1α (R&D Systems) or murine IL-33 for 24 h and then analyzed for GFP expression by FACS.

Results

Administration of IL-33 to mice has potent inflammatory effects, including massive blood eosinophilia, increased IL-5 and IgE serum levels, and goblet cell hyperplasia at mucosal surfaces (2). To investigate the possibility that IL-1RAcP is important for these effects, we administered IL-33 or saline to WT and IL-1RAcP$^{-/-}$ mice. The results are quite clear. In all readouts examined, both human and murine IL-33 induce robust responses in WT mice, but there is a complete absence of response in the IL-1RAcP$^{-/-}$ mice (Fig. 1). WT mice receiving IL-33 have enlarged spleens and

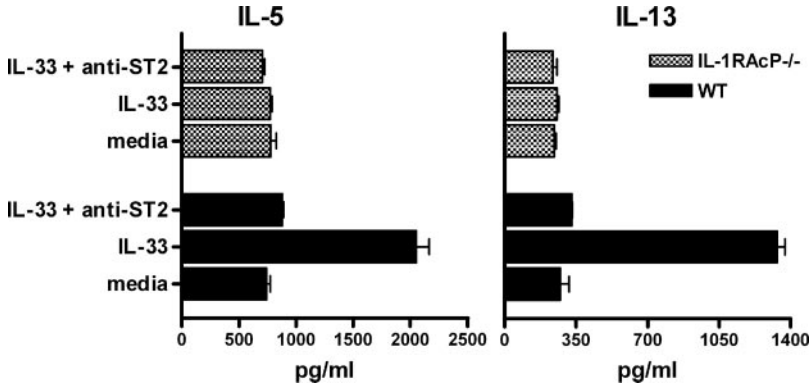

FIGURE 2. IL-1RAcP is required for IL-33 effects on Th2 cells. Cytokine production after 24 h of stimulation of resting polarized $CD4^+$ Th2 cells from WT or IL-$1RAcP^{-/-}$ in the presence or absence of anti-ST2 Ab. Mean ± SEM of duplicate wells.

lymph nodes, while IL-$1RAcP^{-/-}$ mice treated with IL-33 look identical with mice receiving saline (data not shown). Further analysis revealed that WT mice treated with IL-33 have increased levels of IL-5 and IgE in the serum, blood eosinophilia, increased message for IL-4, IL-5, IL-13, IL-6, ST2, and IL-17RB (IL-25R) in the lung and spleen, and goblet cell hyperplasia in the small intestine and upper airways of the lung (Fig. 1, and data not shown). These results confirm that IL-33 is a potent activator of the cells and cytokines involved in type 2 immune responses. Interestingly, IL-33 can stimulate the expression of its own receptor, ST2, as well as the receptor for the Th2 cytokine IL-25. In contrast, IL-$1RAcP^{-/-}$ mice treated with IL-33 exhibit none of these responses. These results strongly suggest that IL-1RAcP is a member of the IL-33R complex.

ST2, the ligand binding chain of the IL-33R complex, is expressed on mast cells and Th2 cells. IL-33 stimulates both of these cell populations to produce cytokines (Ref. 2 and data not shown). Interestingly, ST2 is not required for initial Th2 polarization, nor does it appear to be necessary to generate a Th2 immune response, although blockade or absence of ST2 can attenuate Th2 responses in some disease models (reviewed in Ref. 9). We tested the influence of IL-1RAcP on Th2 polarization and cytokine production. $CD4^+$ T cells from WT and IL-$1RAcP^{-/-}$ mice were polarized in Th2 conditions and, after a brief rest, were stimulated with IL-33 in the presence or absence of anti-ST2 Ab. Resting Th2 cells from both WT and IL-$1RAcP^{-/-}$ mice make substantial amounts of IL-5 and IL-13, but only cells from WT mice respond to IL-33 treatment with enhanced production of these cytokines. IL-33 effects could be blocked if WT cells were incubated with anti-ST2 Ab (Fig. 2). IL-33 had no effect on IL-4 production by either cell genotype (data not shown). It is interesting to note that Th2 cells respond to IL-33 in the absence of TCR stimulation. This suggests that once a Th2 response is initiated, IL-33 can promote Ag-independent enhancement of the response.

Although IL-1RAcP is required to mediate the effects of IL-33 in vivo and on Th2 cells, the possibility exists that this is due to an indirect rather than a direct interaction of IL-33 and IL-1RAcP. Therefore, we wished to detect formation of the ligand-receptor complex in vitro. In the IL-1R complex, IL-1RAcP cannot bind IL-1 directly, rather it is hypothesized to interact with the complex of IL-1 and its primary receptor, IL-1R1 (4, 7, 10). We were also unable to see a direct interaction of IL-33 with IL-1RAcP (data not shown). In contrast, the interaction between ST2 and IL-33 can be measured. The affinity of the IL-33/ST2 interaction was investigated by equilibrium binding using Biacore with immobilized ST2-Ig fusion protein; we measured the apparent K_D to be $8 \pm 2 \times 10^{-9}$ M (data not shown). A specific ST2/IL-33/IL-1RAcP complex was measured by ELISA. We titrated IL-33 or IL-1β into wells containing plate-bound ST2 and soluble his6-tagged IL-1RAcP (Fig. 3). We detected IL-33R complex formation with an anti-his6 Ab as the concentration of IL-33, but not IL-1β, increases. Conversely, if the plates are coated with IL-1R1 instead of ST2, we detect IL-1R complex formation with increasing amounts of IL-1β but not IL-33. This result demonstrates that ST2, IL-33, and IL-1RAcP can form a ligand/receptor complex.

Signal transduction by IL-1RAcP in the IL-1 signaling cascade is mediated by its cytoplasmic TIR domain and involves recruitment of the adaptor MyD88 and activation of NF-κB (reviewed in Ref. 1). IL-33 uses the same signaling components as IL-1, including IL-1R-associated kinase, IL-1R-associated kinase 4, MyD88, and TNFR-associated factor 6, leading to the activation of NF-κB and MAPKs (2). MyD88 is required for IL-33 signaling, as $MyD88^{-/-}$ mice do not respond to IL-33 administration (data not shown). To investigate the contribution of IL-1RAcP to IL-33 signal transduction, we used a dn form of IL-1RAcP that contains a stop codon before the TIR domain. Titration of dn IL-1RAcP into 293FT cells that were transfected with mouse ST2 and a NFκB-GFP reporter gene construct was able to decrease the reporter gene signal in response to IL-33. A similar effect with dnIL-1RAcP was seen when these cells were stimulated with IL-1α as a positive control (Fig. 4). Thus, IL-1RAcP is likely to be involved in IL-33-mediated signal transduction. The different relative levels of GFP^+ cells measured after stimulation with IL-1α vs IL-33 may relate to different affinities of the two cytokines for their specific primary receptor components and/or relate to different primary receptor numbers for IL-33 and IL-1α expressed on the cell surface (Table I).

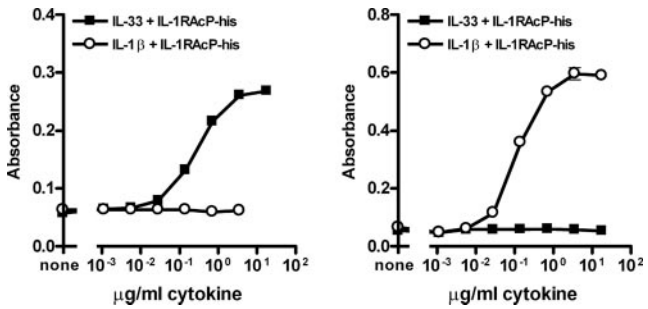

FIGURE 3. Detection of the ST2/IL-33/IL-1RAcP complex. Murine IL-1β or IL-33 was titrated into plates containing soluble his6-tagged murine IL-1RAcP and plate-bound ST2-Fc (*A*) or IL-1R1-Fc (*B*). Peroxidase-conjugated anti-his6 was used as a detection Ab. Data are representative of two experiments. Mean ± SEM of duplicate wells.

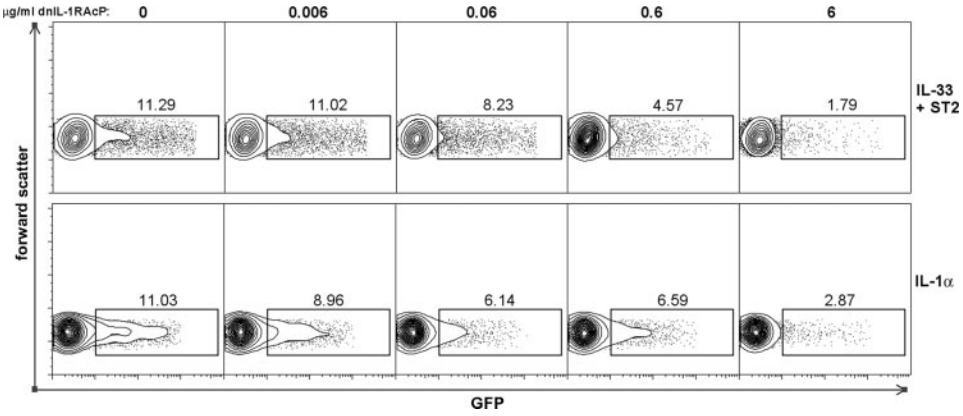

FIGURE 4. dn IL-1RAcP inhibits IL-33 signaling. NFκB-GFP reporter gene expression in IL-33 or IL-1α-treated 293FT cells that had been transiently transfected with murine ST2 (IL-33-treated cells only) and increasing concentrations of murine dnIL-1RAcP. A representative of two experiments is shown.

Discussion

In the present manuscript, we demonstrate that IL-33 biology is mediated through a heteromeric receptor complex consisting of ST2 and IL-1RAcP. Our findings add yet another IL-1-related cytokine to the list of ligands that are functionally dependent on IL-1RAcP. Receptor promiscuity is a common theme in hemopoietic cytokine biology. IL-1α and IL-1β both use IL-1R1 and IL-1RAcP, and recently, IL-1F6, IL-1F8, and IL-1F9 were shown to also use IL-1RAcP (3). In addition to using the same coreceptor, these IL-1 family members are located in a cluster of genes on human chromosome 2. In contrast, the IL-1 family member IL-18 is located on a separate chromosome and uses two unique receptor subunits. Because IL-33 also resides on a separate chromosome from other IL-1 family members, it was unclear whether its receptors would be shared or unique. ST2 was identified as the primary ligand-binding receptor in the initial description of IL-33 (2), and these results show that IL-33, like IL-1 (α and β), IL-1F6, IL-1F8, and IL-1F9, uses IL-1RAcP as the second component of its signaling receptor complex.

The shared usage of IL-1RAcP by IL-33 and other IL-1 family members suggests that these cytokines may have overlapping activities. Alternatively, these cytokines could potentially compete for IL-1RAcP and its associated signaling components. Soluble ST2 has been shown to attenuate disease severity in collagen-induced arthritis, a model of autoimmune inflammation that is dependent on IL-1 (11, 12). In light of our findings, the mechanism for this effect may involve the inhibition of IL-1 signaling via the interaction of soluble ST2 and IL-33 with IL-1RAcP. However, IL-1RAcP is broadly expressed (13), and may not be a limiting factor in IL-1 vs IL-33 responses. It is more likely that expression of the primary ligand-binding chains is the limiting responsive factor. Indeed, ST2 is a selective marker for Th2 cells, while IL-1R1 is likely to be expressed on the recently described inflammatory Th17 cell subset (Ref. 14 and K. Bak-Jensen, unpublished observations). Based on the differential expression of these receptors on T cell subsets, it will be interesting to see whether IL-1RAcP is involved in effector T cell lineage commitment or persistence.

The identification of the complete IL-33 signaling receptor complex now paves the way for identifying the physiological role of IL-33. Based on our initial findings and the expression pattern of ST2, it is likely that IL-33 is involved during the effector phase of type-2 immune responses involving Th2 cells and/or mast cells. Interestingly, increased levels of soluble ST2, a likely natural antagonist of IL-33, have been associated with numerous human diseases including acute myocardial infarction, asthma with acute exacerbation, eosinophilic pneumonia, sepsis and trauma, and exacerbated idiopathic pulmonary fibrosis, pointing toward a possible role for IL-33 in these indications (15–20). Additionally, the cDNA sequence for what is now known as IL-33 was originally found as a gene that is highly up-regulated in cerebral arteries after cranial hemorrhage (21). This, coupled with the fact that IL-33 can be cleaved in vitro by caspase-1, an apoptotic protease, suggests IL-33 could be expressed during tissue injury. Further investigations on the regulation of IL-33 expression, activation, and secretion at the cellular level may provide insight into where and how IL-33 is expressed during disease.

Table I. *Titration of dn-mIL-1RAcP*

Amount dn-mIL-1RAcP Expression Vector (μg)	Percentage of GFP$^+$ Cells after Stimulation with IL-33	
	Experiment 1	Experiment 2
0	11	12
0.006	11	12
0.06	8	9
0.6	5	5
6	2	2

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Disclosures

The authors have no financial conflict of interest.

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