CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector

Immunity, Vol. 23, 479–490, November, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.immuni.2005.09.015

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

Jochen Schmitz,[1] Alexander Owyang,[1]
Elizabeth Oldham,[1] Yaoli Song,[1] Erin Murphy,[1]
Terril K. McClanahan,[1] Gerard Zurawski,[1]
Mehrdad Moshrefi,[1] Jinzhong Qin,[2] Xiaoxia Li,[2]
Daniel M. Gorman,[1] J. Fernando Bazan,[1,3,*]
and Robert A. Kastelein[1,*]
[1]Schering-Plough Biopharma
(formerly DNAX Research, Inc.)
901 California Avenue
Palo Alto, California 94304
[2]Department of Immunology
Cleveland Clinic Foundation
Cleveland, Ohio 44195

Summary

Cytokines of the interleukin-1 (IL-1) family, such as IL-1α/β and IL-18, have important functions in host defense, immune regulation, and inflammation. Insight into their biological functions has led to novel therapeutic approaches to treat human inflammatory diseases. Within the IL-1 family, IL-1α/β, IL-1Ra, and IL-18 have been matched to their respective receptor complexes and have been shown to have distinct biological functions. The most prominent orphan IL-1 receptor is ST2. This receptor has been described as a negative regulator of Toll-like receptor-IL-1 receptor signaling, but it also functions as an important effector molecule of T helper type 2 responses. We report a member of the IL-1 family, IL-33, which mediates its biological effects via IL-1 receptor ST2, activates NF-κB and MAP kinases, and drives production of T_H2-associated cytokines from in vitro polarized T_H2 cells. In vivo, IL-33 induces the expression of IL-4, IL-5, and IL-13 and leads to severe pathological changes in mucosal organs.

Introduction

IL-1 family members are known to alter the host response to an inflammatory, infectious, or immunological challenge (Dinarello, 1994, 2000). The four best known members of this family are IL-1α/β, IL-1Ra, and IL-18. IL-1α/β and IL-18 are highly inflammatory cytokines, and dysregulation of their expression can lead to severe pathobiological effects. Accordingly, the expression of these cytokines is highly regulated via both soluble receptors (e.g., type 2 IL-1 receptor) and natural antagonist proteins (e.g., IL-1Ra and IL-18 binding protein) as well as alternatively spliced forms of both ligands and receptors (Dinarello, 1997; Dunne and O'Neill, 2003). With the exception of IL-18, all IL-1 genes are clustered on human chromosome 2, including several new IL-1 family members called IL-1F5-F10 for which no clear function has yet emerged (Sims et al., 2001).

IL-1 cytokines exert their function through a family of receptors that belong to the Toll-like receptor-IL-1 receptor (TLR-IL-1R) superfamily, which is defined by the presence of an intracellular Toll-IL-1R (TIR) module. This superfamily can be divided into two main groups, the Toll-like receptors and receptors of the IL-1 family. The IL-1 receptor family currently has 10 members, and IL-1 ligands typically bind to a cellular receptor complex that consists of two members of this family. The receptor complex for IL-1α/β consists of IL-1R1 and IL-1RAcP, with IL1Ra acting as a natural antagonist of IL-1α/β by trapping IL-1R1 molecules. IL-18 mediates its function via IL-18Rα and IL-18Rβ, with IL-18 binding protein (IL-18bp) acting as a negative regulator (Dunne and O'Neill, 2003). The only other IL-1 receptor that has been matched to ligands is IL-1Rrp2 (Debets et al., 2001; Towne et al., 2004). The hallmark of IL-1 receptors signaling is the activation of the transcription factor NF-κB and the mitogen-activated protein (MAP) kinases p38, JNK, and ERK1/2 (Dunne and O'Neill, 2003).

Thus far, the IL-1 receptor family members ST2, SIGIRR, IL-1RAPL, and IL-1RAPL2 have eluded ligand identification. Of these, the best-known orphan IL-1 receptor is ST2 (also called DER4, Fit-1, or T1). Originally identified 16 years ago as a serum-inducible secreted protein in murine fibroblast (Bergers et al., 1994; Klemenz et al., 1989; Tominaga, 1989; Tominaga et al., 1991, 1992), ST2 in its transmembrane form is expressed primarily on mast cells and on T_H2 cells and is linked to important T_H2 effector functions (Coyle et al., 1999; Lohning et al., 1998, 1999; Moritz et al., 1998; Townsend et al., 2000; Xu et al., 1998). Treatment of mice either with an antagonistic antibody against ST2 or with an IgG-ST2 fusion protein leads to an enhancement of T helper type 1 (T_H1) responses and has an inhibitory effect on T_H2-associated allergic airway inflammation (Lohning et al., 1998; Xu et al., 1998). Although two putative ligands for ST2 have been reported, neither of these proteins trigger activation of NF-κB, nor are they related to IL-1 family cytokines (Gayle et al., 1996; Kumar et al., 1995). Recently, ST2 has been described as a negative regulator of TLR-IL-1R receptor signaling (Brint et al., 2004), in line with an earlier report that ST2 was unable to activate the classical IL-1-activated transcription factor NF-κB (Brint et al., 2002; Thomassen et al., 1999).

In this study, we present a member of the IL-1 family that binds to IL-1 receptor ST2. Like IL-1β and IL-18, IL-33 (also labeled IL-1F11, following IL-1 family nomenclature [Sims et al., 2001]) is produced in a precursor form and can be cleaved by caspase-1. The binding of IL-33 to cells that express ST2 results in the activation of NF-κB and MAP kinases. Administration of purified IL-33 either in vitro or in vivo leads to the production of T_H2-associated cytokines. Furthermore, in mice, IL-33 induces eosinophilia, splenomegaly, and increased levels of serum Ig—leading to profound histopathological changes in the lungs and gastrointestinal (GI) tract.

*Correspondence: jfbazan@gene.com (J.F.B.); rob.kastelein@dnax.org (R.A.K.)
[3]Present address: Department of Protein Engineering, Department of Molecular Biology, Department of Immunology, Genentech, Inc., Mail Stop 37, 1 DNA Way, S. San Francisco, California 94080.

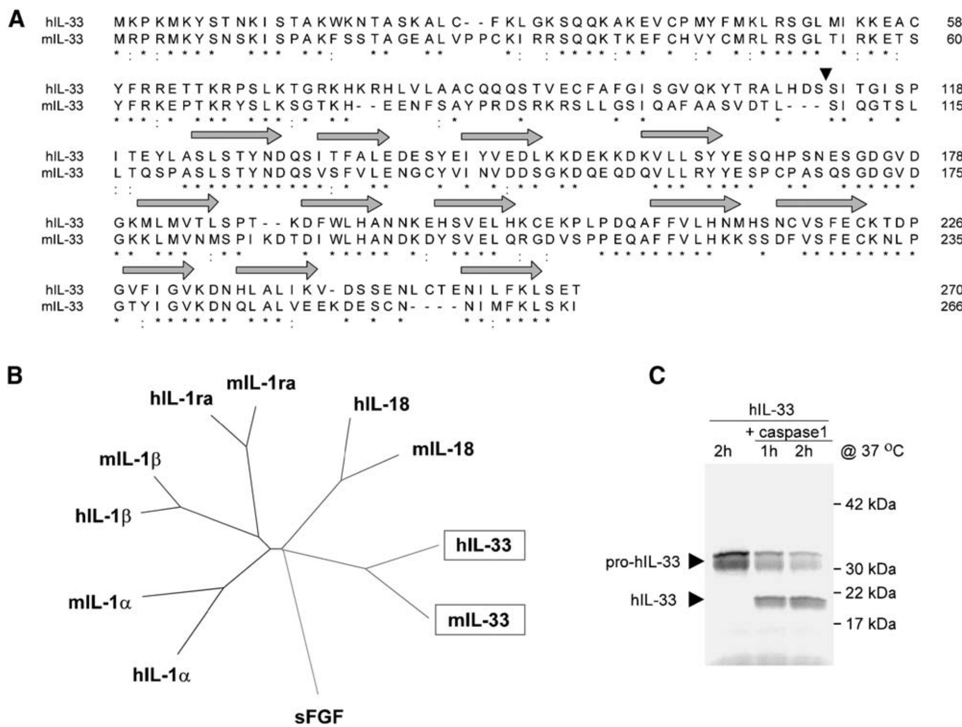

Figure 1. Alignment of Human and Mouse IL-33 Protein Sequences

(A) Sequence alignment of human and mouse IL-33 proteins. Gray arrows indicate the 12 predicted β strands of the IL-1 fold. The arrowhead indicates the start of the recombinant proteins.

(B) Phylogenetic tree for representative members of the human IL-1 family. The dendrogram was derived by the neighbor-joining method within ClustalX and is rooted by the FGF relatives of IL-1.

(C) In vitro translated ^{35}S-labeled full-length IL-33 is cleaved by caspase-1. Pro-hIL-33 and mature IL-33 are indicated.

Results

Identification of IL-33 as an IL-1 Family Member

We searched sequence databases with a computationally derived profile of members of the IL-1 cytokine family (see Experimental Procedures). This effort led to the identification of an IL-1 family member that we have named IL-33 (IL-1F11). The human gene is located on chromosome 9p24.1, while its mouse counterpart can be found on the syntenic chromosome 19qC1 region. The IL-33 cDNA sequences encode 270 and 266 amino acid polypeptides for human and mouse, respectively (Figure 1A), corresponding to full-length proteins with calculated masses of 30 and 29.9 kDa. IL-33 is expressed as a prodomain containing polypeptide. Incubation of in vitro translated IL-33 with caspase-1 led to the production of mature IL-33 with a mass of 18 kDa as determined by SDS-PAGE (Figure 1C). Human and mouse IL-33 are 55% identical at the amino acid level. The IL-1 family member most closely related to IL-33 is IL-18 (Figure 1B).

Expression Profile of IL-33

Analysis of a panel of human and mouse cDNA libraries by real-time quantitative PCR showed that IL-33 mRNA is broadly expressed in many tissues but is more restricted at the level of cell types (Figures 2A and 2B). High levels of mouse IL-33 mRNA can be found in the stomach, lung, spinal cord, brain, and skin. Lower levels of mouse IL-33 mRNA were observed in lymph tissue, spleen, pancreas, kidney, and heart. Human smooth muscle cells (SMC) of various tissues as well as epithelial cells forming the bronchus or small airways showed a constitutive expression of IL-33 mRNA, whereas in primary lung or dermal fibroblasts and keratinocytes, IL-33 gene expression was induced after activation with TNF-α and IL-1β. Activated dendritic cells and macrophages are the only hematopotietic cells that show low quantities of human IL-33 mRNA. In contrast, mouse IL-33 mRNA was found in resting dendritic cells and activated macrophages.

IL-33 Forms a Complex with the Orphan IL-1 Receptor ST2

For both IL-1α/β and IL-18, one of the receptor chains functions as a primary binding component, while the second chain contributes little to binding but is necessary for signal transduction (Dunne and O'Neill, 2003). We tested ST2, the orphan IL-1 receptor most closely related to IL-1R1 and IL-18Rα, as the primary binding receptor for IL-33. *E. coli*-expressed and purified mature human IL-33 (residues 112–270) was biotinylated and precipitated via avidinbeads in the presence of ST2-Fc fusion protein. The precipitates were monitored for the presence of ST2-Fc by Western blot by use of antibodies directed against human ST2 (Figure 3A). ST2 was immunoprecipitated in the presence of IL-33 (Figure 3A, lane 3). Some background binding of the ST2-Fc occurred in the absence of IL-33 (Figure 3A, lane 2), but the corresponding ST2-Fc protein band was significantly more

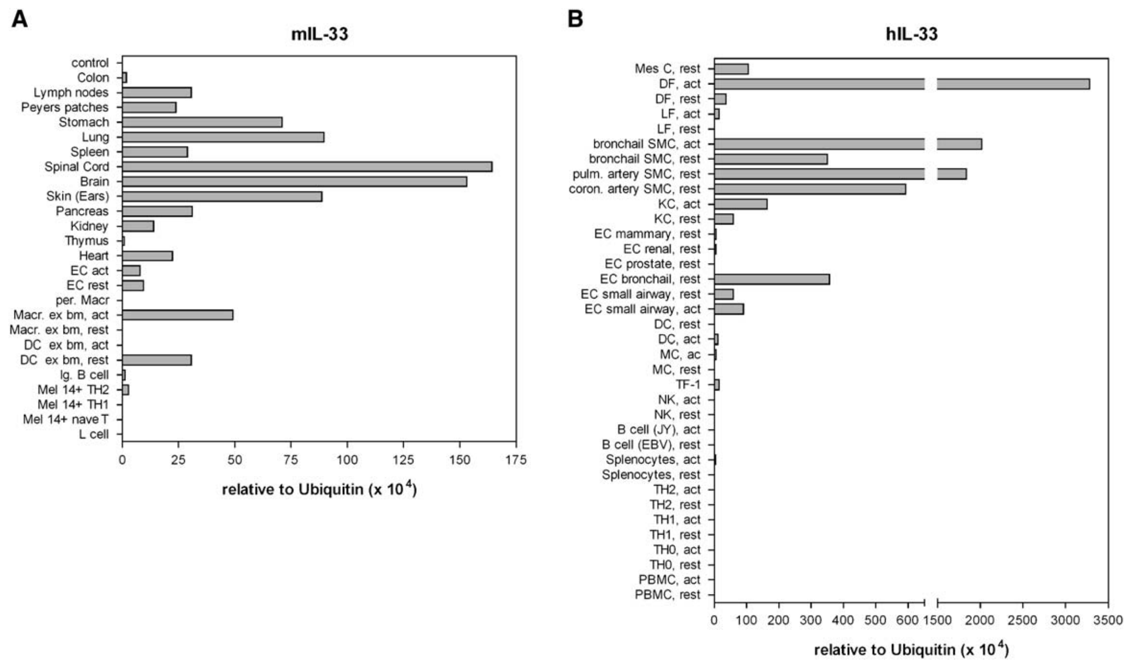

Figure 2. Distribution of IL-33 in Tissues and Cell Types

Real-time quantitative PCR was performed for IL-33 on various mouse (A) and human (B) cDNA libraries. Human cDNA libraries index (top to bottom): mesangial cells resting; dermal fibroblasts (DF) activated and resting; lung fibroblast (LF) activated and resting; bronchial smooth muscle cells (SMC) activated and rested; pulmonary artery SMC resting; coronary artery SMC resting; keratinocytes activated and resting; various resting epithelial cells (EC) and activated small airway EC; monocyte-derivate dendritic cells (DC) activated with LPS and resting; monocytes (MC) activated with LPS and resting; hematopoietic precursor TF1; natural killer cells activated and resting; B cell lines activated and resting; splenocytes activated and resting; T cell subsets activated and resting; peripheral blood mononuclear cells activated and resting. Mouse cDNA libraries index (top to bottom): various tissues; epithelial cells activated and resting; peritoneal macrophages; macrophages from bone marrow activated with LPS and resting; dendritic cells from bone marrow activated and resting; B cells; Mel14+ T cell subset; L-fibroblast.

prominent in the presence of IL-33 (Figure 3A, lane 3). Next we investigated pull-down of IL-33 via ST2. ST2 fusion protein was precipitated via Protein-G-Sepharose in the presence of biotinlyated IL-33. ST2 was capable of binding biotinylated IL-33 (Figure 3B, lane 2). No binding occurred when only Protein-G-Sepharose and biotinylated IL-33 were present (Figure 3B).

IL-33 Signals via ST2

The ability of ST2 to bind IL-33 suggested that this receptor is part of a functional IL-33 receptor complex. To test this hypothesis, we investigated whether IL-33 could initiate signaling via this receptor by using an NF-κB-dependent reporter assay. We transiently transfected HEK293FT cells either with a mock mammalian expression vector or with an expression vector encoding ST2 in conjunction with an NF-κB-driven GFP expression construct. Real-time PCR analysis of untransfected HEK293 cells showed no endogenous expression of ST2 (data not shown). After stimulation of the transiently transfected cells with mouse IL-33 for 16 hr, cells were analyzed for GFP expression by FACS analysis (Figure 3C). A small population of cells, transfected only with the GFP-reporter gene construct, expressed GFP, but this population did not increase after stimulation of these cells with IL-33. In contrast, expression of ST2 led to the induction of GFP upon stimulation with IL-33 (Figure 3C). To examine what proximal signaling components are utilized by IL-33-ST2-mediated signaling, cell extracts of 293 cells transfected with human ST2 untreated or stimulated with 10 ng/ml IL-33 were immunoprecipitated with anti-ST2 antibody, followed by Western analysis with anti-MyD88, IRAK4, IRAK, TRAF6, and ST2 antibodies. As shown in Figure 3D, MyD88, IRAK, IRAK4, and TRAF6 are all recruited to ST2 upon IL-33 stimulation. We also monitored these extracts for activation of downstream signaling pathways. As shown in Figure 3E (right panel), IL-33 stimulation of ST2-transfected 293 cells leads to phosphorylation of IκBα as well as the kinases Erk1/2, p38, and JNK with a peak of phosphorylation occurring at 15 min. This pattern of IL-33/ST2-induced phosphorylation is similar to the signaling events induced in 293 cells transfected with IL-1R1 and stimulated with IL-1β (Figure 3E, left panel).

IL-33 Induces NF-κB Phosphorylation and Activates MAP Kinases in Mast Cells

We next investigated whether IL-33 is able to induce signaling in cells that naturally express ST2. We tested mouse mast cells (WTMC) for the cell-surface expression of ST2. Expression of ST2 was readily detected by FACS analysis using a ST2 antibody (Figure 4A, left panel). Binding of the ST2 antibody could be abrogated with IL-33, and we could demonstrate binding of biotinylated IL-33 to these cells (Figure 4A, right panel). To analyze the signaling pathways induced by IL-33, we stimulated this mast cell line with IL-33 and monitored the phosphorylation of NF-κB and various MAP kinases (Figures 4B and 4C). Phosphorylation of NF-κB was

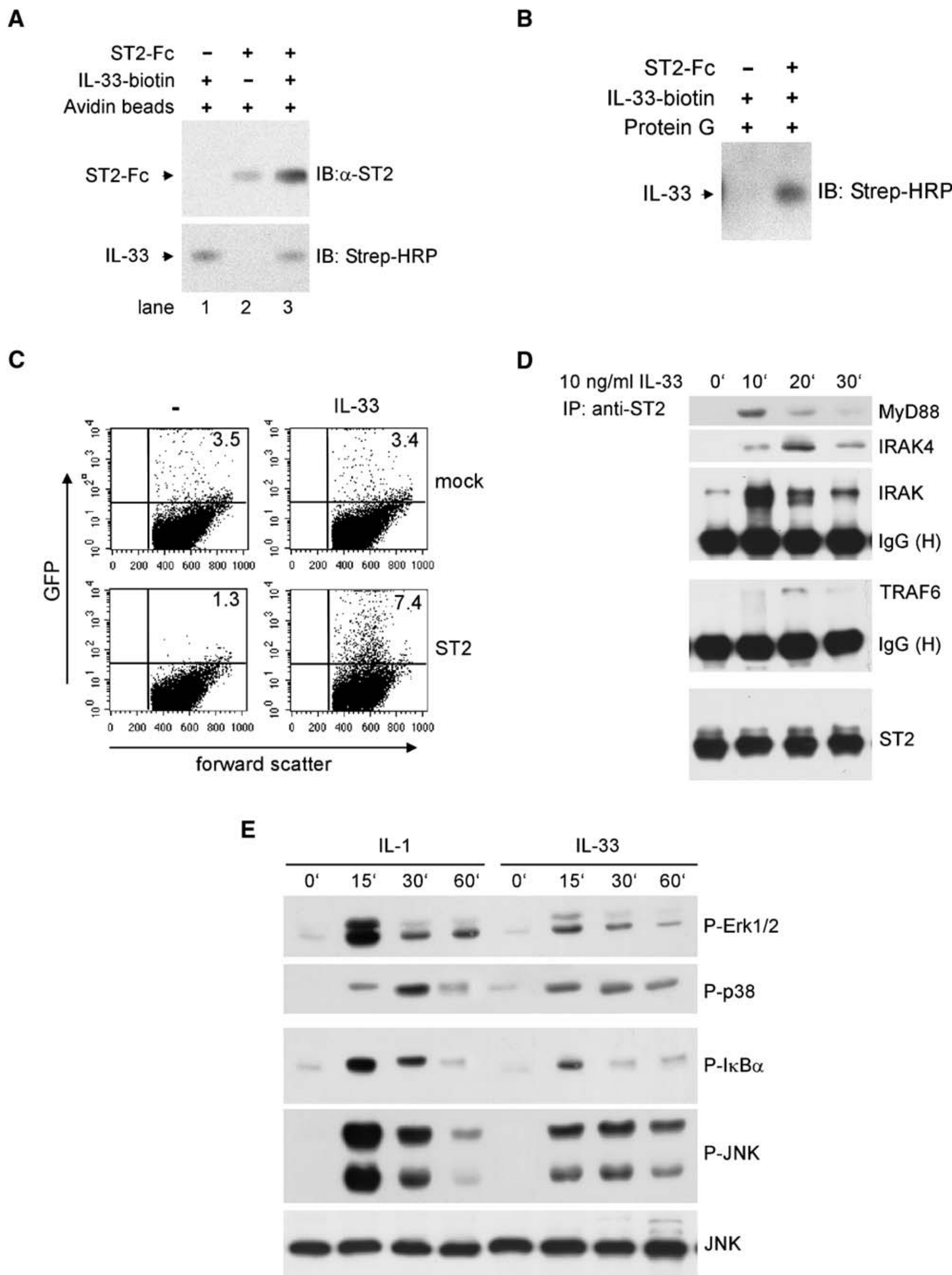

Figure 3. IL-33 Receptor Matching and ST2-Receptor Complex

(A) Pull-down of recombinant extracelluar ST2 Fc-fusion protein via biotinylated IL-33. Biotinylated IL-33 was precipitated via Avidinbeads in the presence or absence of ST2-Fc as indicated. The precipitated proteins were separated by SDS-PAGE, electroblotted, and visualized by Western blot/ECL reaction with anti-ST2.

(B) Pull-down of biotinylated IL-33 with ST2-Fc fusion protein. ST2-Fc fusion protein was incubated with IL-33-biotin and precipitated with Protein G-Sepharose. The precipitates were separated by SDS-PAGE, electroblotted, and monitored for the presence of biotinylated IL-33.

(C) HEK293FT cells (4×10^6) were transfected with 1.3 μg of pNF-κB-GFP reporter gene plasmid and with either 1.3 μg of pME18S-mST2 plasmid or with empty control vector. 24 hr after transfection, cells were split. 24 hr later, cells were left untreated or were stimulated for 16 hr with IL-33 (50 ng/ml). GFP expression was monitored by FACS analysis.

(D) Cell extracts prepared from 293/ST2 cells untreated or stimulated with 10 ng/ml IL-33 were immunoprecipitated with anti-ST2 antibody, followed by Western analysis with anti-MyD88, IRAK4, IRAK, TRAF6, or ST2 antibodies.

(E) Cell extracts prepared from 293 cells transfected with IL-1RI (left) or ST2 (right), untreated or stimulated with 10 ng/ml IL-1β or 10 ng/ml IL-33, respectively, were blotted with anti-phospho-Erk1/2, anti-phospho-p38, anti-phospho-IκBα, or anti-phospho-JNK antibodies. Loading of equal amounts of proteins was determined by restaining of the blots with anti-JNK antibody.

observed in the total cell lysate after IL-33 stimulation with a peak of phosphorylation occurring at 15 min and decreased levels at 30 min. NF-κB phosphorylation could be significantly blocked by preincubation of the cells with a neutralizing anti-ST2 antibody prior to stimulation with IL-33 (Figure 4B). A control antibody was not

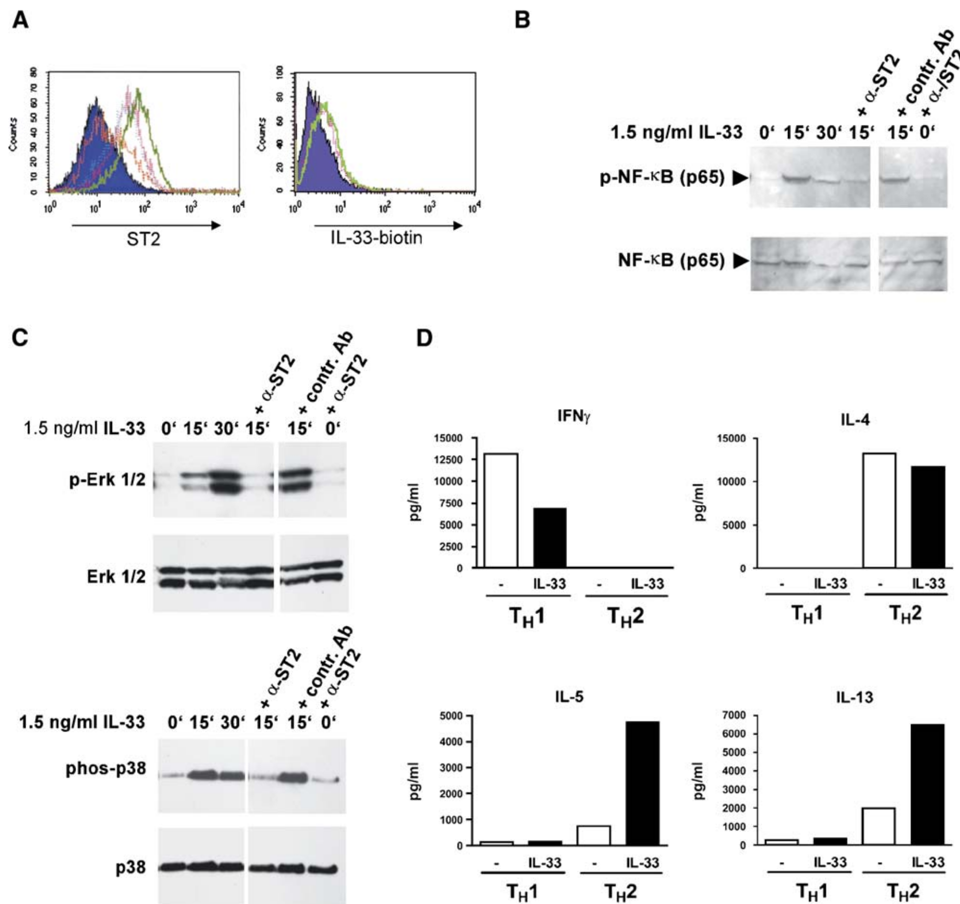

Figure 4. IL-33-Induced NF-κB Phosphorylation and MAPK Activation and T_H2 Cytokine Production

(A) Left: WTMC cells were incubated with either 1.5 μg of isotype control antibody (purple), 1.5 μg anti-ST2 antibody (green), 1.5 μg anti-ST2 antibody and 0.8 μg of rhIL-33 (magenta), 1.5 μg anti-ST2 antibody and 2 μg of hIL-33 (blue), or 1.5 μg anti-ST2 antibody and 4 μg of hIL-33 (orange). Cells were washed and incubated with an anti-rat IgG-PE antibody. Cells were analyzed by FACS. Right: WTMC cells were either untreated (purple) or incubated with 0.4 μg of biotinylated hIL-33 (green) or incubated with a combination of 0.4 μg of biotinylated and 4 μg of hIL-33 (magenta). Cells were washed, incubated with streptavidin-PE, and analyzed by FACS.

(B and C) WTMC cells were preincubated in the presence or absence of either anti-mST2 mAb or an isotype control mAb at a concentration of 1 μg/ml. Cells were stimulated with 1.5 ng/ml IL-33 for various times or were left unstimulated as indicated. Cell lysates were separated by SDS-PAGE and electroblotted. The presence of phosphorylated proteins was monitored by Western blot/ECl reaction using phospho-specific antibodies. Loading of equal amounts of proteins was determined by restaining of the blots with antibodies directed against NF-κB, Erk1/2, or p38.

(D) T_H1- and T_H2-polarized cells were restimulated with anti-CD3/28 in the presence or absence of IL-33 (20 ng/ml). After 48 hr, the supernatant were monitored for IFNγ, IL-4, IL-5, and IL-13.

capable of reducing phosphorylation (Figure 4B), and neither was the anti-ST2 antibody on its own able to induce activation of NF-κB. In addition to NF-κB, IL-33 also induced activation of Erk1/2 and p38 (Figure 4C). Phosphorylation of these kinases could also be inhibited by anti-ST2 antibody. Activation of JNK kinases was also observed (data not shown).

IL-33 Stimulates Cytokine Expression of In Vitro T_H2-Polarized Naive T Cells

ST2 is required for development of effective T_H2 responses (Trajkovic et al., 2004). To test whether IL-33 could stimulate T_H2 cells to produce T_H2 cytokines, we isolated naive T cells from C57BL/6 spleens and polarized these cells into T_H1 with IL-12 or T_H2 cells with IL-4 (Boonstra et al., 2001). After three rounds of polarization, cells were restimulated with anti-CD3 and anti-CD28 with or without IL-33 (20 ng/ml), and supernatants were tested for the expression of IFNγ, IL-4, IL-5, and IL-13. T_H2 cells but not T_H1 cells responded to IL-33 stimulation with increased production of IL-5 and IL-13 (Figure 4D). The production of IL-4 was already high and not further increased with the addition of IL-33. As expected, T_H1 cells produced high levels of IFNγ. Interestingly, this level was reduced when the cells were incubated with IL-33.

IL-33 Protein Treatment of Mice Induces Splenomegaly, Eosinophilia, and Serum IgE

To understand the in vivo biological function of IL-33, we injected wt mice intraperitoneally (i.p.) with IL-33 protein and monitored the mice for changes in hematopoietic parameters. Mice treated daily with 0.4 or 4 μg of IL-33 for 7 days developed splenomegaly with significantly higher numbers of eosinophils, mononuclear cells, and plasma cells, but not neutrophils (Figures 5A and 5B). The increased number of splenic eosinophils and mononuclear cells correlated with an increase in blood

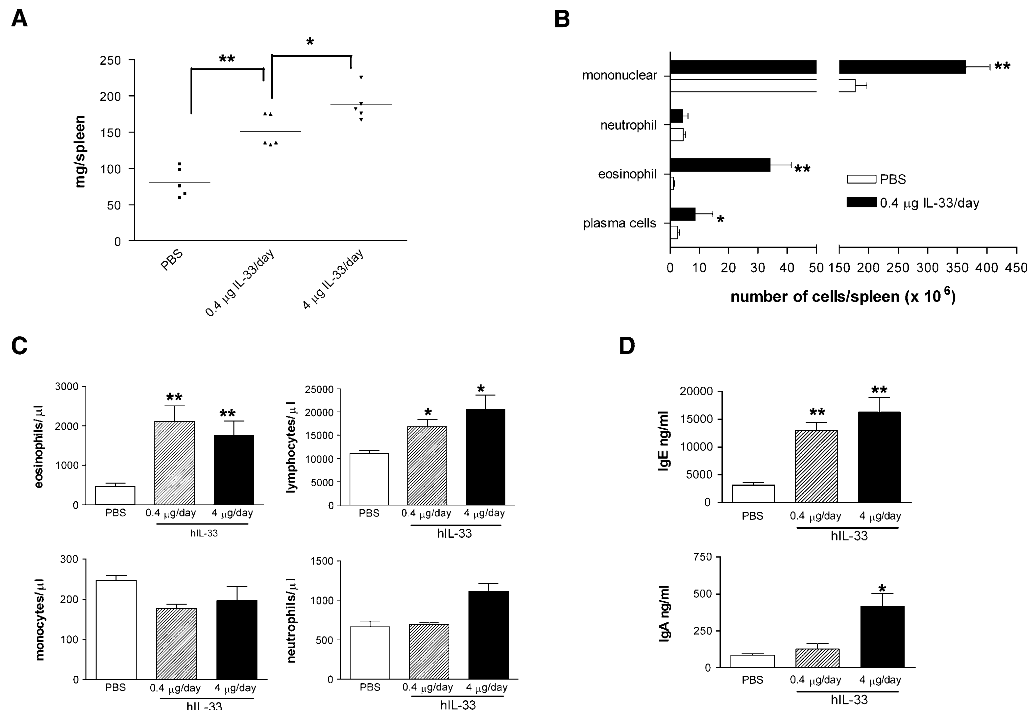

Figure 5. In Vivo Treatment with IL-33 Leads to Splenomegaly, Eosinophilia, and Increased Levels of Serum IgA and IgE

(A and B) C57BL/6 mice (n = 4–5) were injected i.p. daily with either 0.4 μg or 4 μg IL-33 or with PBS for 7 days. Spleens were harvested, weighed, and made into single-cell suspension to determine total cellularity. Relative populations were determined microscopically with Haema3-stained cytospins (*p < 0.05; **p < 0.01).

(C) C57BL/6 mice (n = 5) were treated for 7 days with either 0.4 μg or 4 μg IL-33 protein or with phosphate-buffered saline. At day 8, blood was taken for total white blood cell and differential blood cell count (*p < 0.05; **p < 0.01).

(D) Serum Ig isotype levels were determined by sandwich ELISA. Results shown are representative of two independent experiments (*p < 0.05).

eosinophils and lymphocytes (Figure 5C). No significant changes were observed in the numbers of blood neutrophils and monocytes. Evaluation of circulating Ig levels showed that IL-33-treated mice had significantly higher serum levels of IgE and IgA (Figure 5D).

IL-33 Induces Gene Expression of T_H2-Associated Cytokines

The increased eosinophil levels induced by IL-33 could be associated with an increase in IL-5 expression, while IgE and IgA synthesis could be associated with IL-4 and IL-13 expression (Bost et al., 1996; Chomarat and Banchereau, 1998; Roboz and Rafii, 1999). Therefore, we tested whether IL-33 could induce expression of T_H2-associated cytokines in vivo. Total tissue mRNA was isolated from mice treated with either 0.4 or 4 μg IL-33 per day and tested for the presence of IL-4, IL-5, and IL-13 mRNA by quantitative PCR analysis. IL-33 treatment strongly induced gene expression of IL-13 in all tissues tested, including thymus, spleen, liver, small intestine, and lung (Figure 6A and data not shown). mRNA expression of both IL-4 and IL-5 was higher in thymus, spleen, liver, and lung of IL-33-treated mice. There was no change in the gene expression of IL-1α, IL-2, TNFα, IL-10, IL-12, or IFNγ (data not shown). The serum of IL-33-treated mice showed highly increased levels of IL-5 and IL-13 (Figure 6B). Serum levels of IL-2, IL-4, IL-6, IL-10, IL-12, TNFα, IFNγ, or MCP-1 were either not detectable or did not change in comparison to PBS-treated animals (data not shown).

IL-33 Induces Pathological Changes in the Lung and the Digestive Tract

We also observed gross anatomical changes in IL-33-treated mice, including enlargement of spleen and stomach and mucus- and bile-filled duodenum. Microscopic examination showed striking histological changes in mucosal tissues, including the lung, esophagus, and small intestine. In the lung, vascular changes were observed in medium and small muscular arteries compared to untreated lung (Figures 7A–7D). Changes included minimal to moderate medial hypertrophy and the presence of myeloid cells within the vascular lumen and infiltrates of eosinophils and/or mononuclear cells beneath the endothelium, within the vessel wall, and adjacent to the vessels. Changes in the airways were primarily restricted to the bronchi and larger bronchioles (Figures 7E and 7F). The epithelial lining of the airways was hypertrophied and appeared to contain large amounts of mucus. Periodic acid-Schiff (PAS) and alcian blue staining highlighted the presence of mucus within the epithelial lining. In some mice, mucus filled the entire airway lumina. In the digestive tract, epithelial hyperplasia was evident in the esophagus (data not shown). Inflammatory infiltrates of eosinophils, neutrophils, and mononuclear cells were seen in the epithelium and lamina propria of the esophagus. Epithelial cells often contained prominent esoninophilic cytoplasmic inclusions (data not shown). In the small and large intestines, the goblet cells were hypertrophied and hyperplastic (Figures 7G and 7H), and lumen of the intestines sometimes

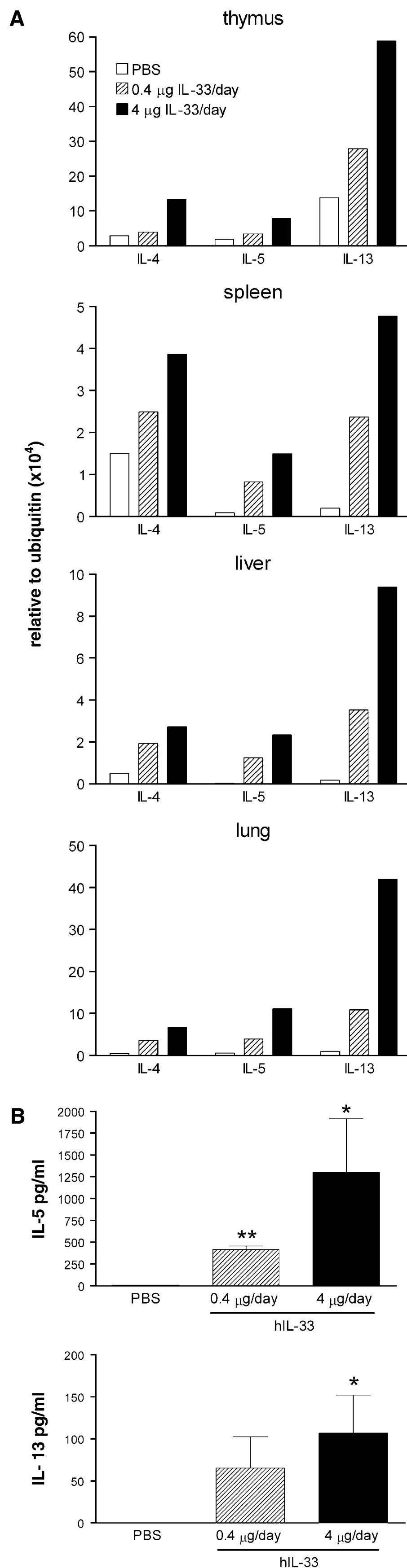

Figure 6. IL-33 Leads to Induction of IL-4, IL-5, and IL-13 Genes and Protein Expression

(A) C57BL/6 mice (pool of 3 mice) were treated daily with either 0.4 μg or 4 μg of IL-33 protein or PBS for 7 days, and then organs were harvested. Total RNA was isolated from individual tissues, and IL-4, IL-5, and IL-13 mRNAs were quantified by real-time PCR as described in Experimental Procedures.

(B) C57BL/6 mice (n = 3–5 per group) were treated daily with either 0.4 μg or 4 μg of IL-33 protein or PBS for 7 days and blood was collected. IL-5 and IL-13 serum concentrations were measured either by cytometric bead array (IL-5) or by sandwich ELISA (IL-13). The results shown are typical of three independent experiments (*$p < 0.05$; **$p < 0.01$).

contained increased amounts of mucus. The microscopic investigation of the enlarged spleen showed extramedullary hematopoesis attended by the distortion of red and white pulp (Figures 7I and 7J).

Discussion

We describe an IL-1 family member called IL-33 that is most closely related in structure to IL-18 and IL-1β. Like these two "classical" IL-1 family members, IL-33 is expressed as a prodomain-containing protein, which is processed for optimal biological activity. IL-1β and IL-18 have profound biological activities as regulators of immune and inflammatory responses (Dinarello, 1997, 2000), and indeed, we find that IL-33 also displays strong immunomodulatory functions. However, whereas IL-1β and IL-18 promote proinflammatory and T_H1-associated responses (Dinarello, 1994; Robinson et al., 1997), IL-33 leads to the production of T_H2-associated cytokines and increased serum immunoglobulin levels. The existence of an IL-1 family member with the biological profile as described here for IL-33 has long been anticipated, based on the presence of the IL-1R family member ST2 as a selective marker of T_H2 cells and its role in regulation of effective T_H2 responses (Coyle et al., 1999; Lohning et al., 1998; Xu et al., 1998). However, how ST2 contributes to T_H2 responses has remained unclear. Two previously identified putative ST2 ligands do not affect T_H2 functions and are not related to IL-1 cytokines, and their physiological relevance remains uncertain (Gayle et al., 1996; Kumar et al., 1995). The close structural relationship between IL-33, IL-18, and IL-1β and the equally close relationship between receptor family members IL-1R1, IL-18Rα, and ST2 prompted us to rapidly identify ST2 as a subunit of the IL-33 receptor. The T_H2-associated biological activities that we report are in agreement with IL-33 being the true ligand for ST2. We have started a search for the second chain of the IL-33 receptor complex. Possible candidates are the orphan IL-1 receptor SIGIRR or IL-1RAcP. Both receptors are widely expressed and present on cells we have used in the experiments described here (Towne et al., 2004). Experiments are currently underway in our laboratories.

IL-1 and FGF family members display 12 core β strands that make up the characteristic β-trefoil fold (Priestle et al., 1988). The β strands identified by structural alignment of mature IL-33 with members of the IL-1 family are shown in Figure 1A. The phylogenetic tree of the IL-1 family shows the outlier relationship of (mature) IL-33 to the rest, though IL-18 appears as the

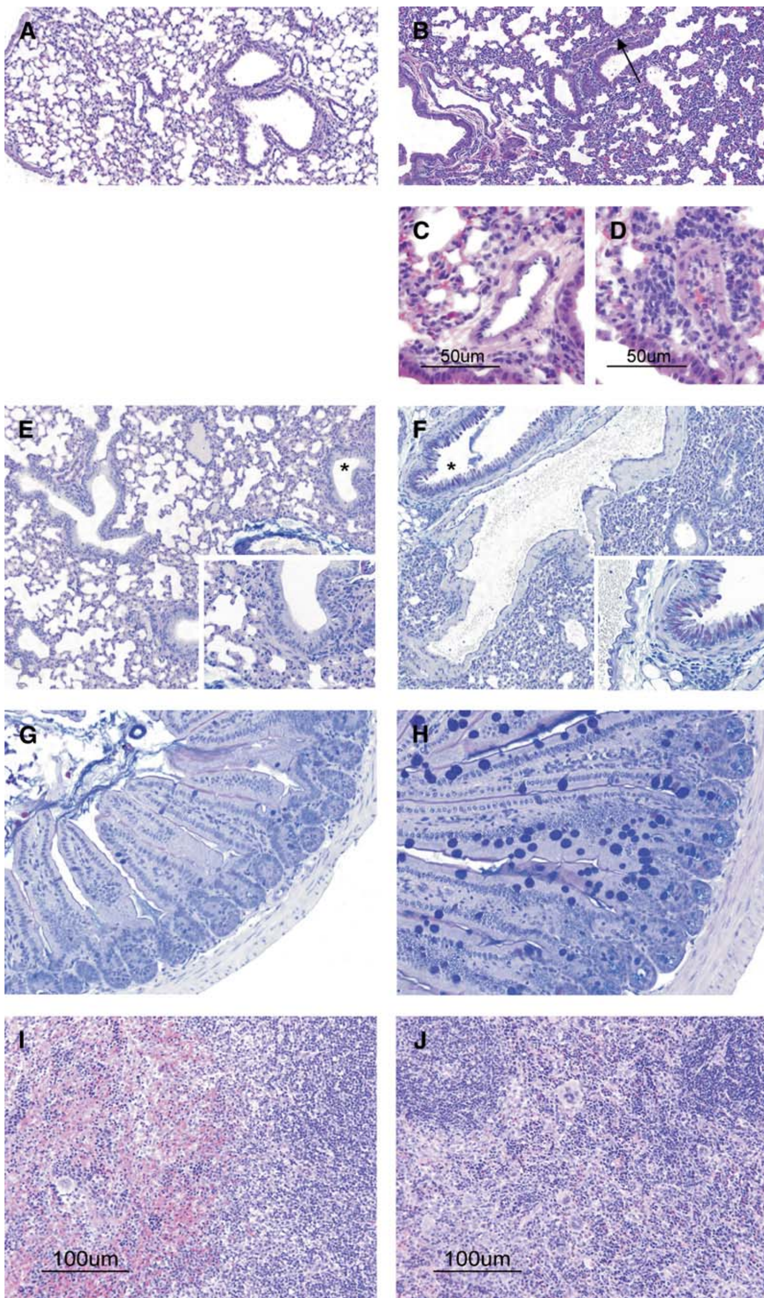

Figure 7. Microscopic Changes in Mice Receiving 0.4 μg of IL-33 Protein for 7 Days

(A–D) Pulmonary changes in mice receiving IL-33 protein (hematoxylin and eosin [H&E]).
(A) Section of normal lung from PBS-treated mice (PAS-AB).
(B) Vascular changes are localized to medium and small muscular arteries. The larger muscular artery had no changes, while the smaller branch (arrow) has mild medial hypertrophy and infiltrates of inflammatory cells (80×).
(C) Higher magnification of a control muscular artery.
(D) Mononuclear and a few myeloid cells almost occluded the arterial lumen as well as focal periateriolar mononuclear and myeloid cell infiltrates.
(E and F) Epithelial changes in the lungs. Proximal airway (*) (periodic acid-Schiff and alcian blue [PAS-AB] stain, ×40 [inset ×120]).
(E) Lung from mouse that received PBS. Inset higher magnification of bronchiole.
(F) Lung from mouse that received IL-33 protein. Intense PAS-AB-positive staining in the proximal airway epithelium. Inset: higher magnification of bronchiole.
(G and H) Jejunal changes (PAS-AB, ×80).
(G) Jejunum from mouse receiving PBS.
(H) Jejunum from mouse receiving IL-33 protein. Hypertrophy and hyperplasia of goblet cells.
(I and J) Spleen changes (H&E, ×120).
(I) Spleen from mouse receiving PBS.
(J) Spleen from mouse receiving IL-33 protein. Loss of the organizational structure of white and red pulp; extramedullary hematopoiesis.

closest homolog (Figure 1B). Of the now 11 members of the IL-1 family, only IL-33 and IL-18 are not linked to the IL-1 gene cluster on human chromosome 2. Like IL-18 and IL-1β, IL-33 is produced with a long prodomain that can be cleaved, at least in vitro, by caspase-1. We do not yet know if caspase-1 is also responsible for processing of pro-IL-33 in vivo. Efforts to identify a cellular source to isolate and characterize natural IL-33 protein are in progress.

IL-33 shows a different expression pattern in comparison to IL-1β and IL-18. Although hematopoietic cells seem to be the main source of IL-1β and IL-18, we can detect only low levels of human IL-33 mRNA in this cell type. Despite its relative scarcity in hematopoietic cells, IL-33 mRNA is broadly expressed in various organs. Expression within these organs is restricted to a few cell types, such as epithelial cells from the bronchus or the small airways, fibroblasts, and smooth muscle cells. Some expression of mouse IL-33 mRNA was seen in dendritic cells, activated macrophages, and in T_H2 cells, but in general levels are low.

IL-33 plays an important role in T_H2-associated immunology. We show that in vitro polarized T_H2 cells upon restimulation respond to IL-33 by significantly increasing secretion of IL-5 and IL-13. In these highly polarized cells, we did not observe a further increase in the already high production of IL-4, although in vivo administration of IL-33 clearly induced IL-4 mRNA in multiple tissues. These findings point to an important role for IL-33, and its receptor ST2, in the induction of T_H2 effector functions. While these results confirm an effector function for ST2, there has not been agreement on the question

of whether ST2 plays a role in T_H2 development (Hoshino et al., 1999; Townsend et al., 2000). In initial experiments to address this issue, we do not see, at least under in vitro culture conditions, a role for IL-33 in driving T_H2 cell development (J.S., unpublished results). Interestingly, IL-18 plays a similar role in T_H1 cells: while IL-18 has important T_H1 effector functions and strongly synergizes with IL-12 to produce IFNγ, it does not play a role in the development of T_H1 cells (Robinson et al., 1997). When administered in vivo, IL-33 has no effects on mRNA levels of IL-1β, IL-2, IL-10, IL-12p40, TNFα, INFγ, and G-CSF (data not shown), but we detect significant increases in gene expression of prominent T_H2-associated cytokines IL-4, IL-5, and IL-13. In addition, we can detect IL-5 and IL-13 in the serum of mice as early as 3 days after administration of IL-33. Furthermore, treatment of mice with IL-33 leads to blood eosinophilia, splenomegaly, and increased IgE and IgA isotypes. The increased serum IgE is likely related to the increased production of IL-4 (Chomarat and Banchereau, 1998; Corry, 1999; Pene et al., 1988). Exposure to IL-33 causes striking histological changes in the lungs and GI tract, including eosinophilic and mononuclear infiltrates, increased mucus production, and epithelial cell hyperplasia and hypertrophy. Since IL-5 is critical for the differentiation and the release of eosinophils from the bone marrow (Karlen et al., 1998; Roboz and Rafii, 1999), the observed blood eosinophilia in IL-33-treated mice is likely directly related to the elevated serum level of IL-5. The histological changes seen in the lungs and digestive tracts in IL-33-treated animals can be expected to be linked to enhanced IL-13 protein expression as the primary mediator of these changes, since IL-13 has been shown to be necessary for the increased mucus production and airway hyperresponsiveness (Corry, 1999). We confirmed this by treating IL-13 KO mice with IL-33 for 7 days. No epithelial changes were observed in the lungs of these IL-33-treated IL-13 KO mice (see Figure S1 in the Supplemental Data available with this article online).

We demonstrate that IL-33 signal transduction depends on expression of ST2. The result of IL-33-induced signal transduction is the recruitment of IRAK, IRAK4, MyD88, and TRAF6 to ST2, ultimately leading to the activation of NF-κB and MAP kinases. This is in contrast to studies that showed no NF-κB activation downstream of this receptor (Brint et al., 2002; Thomassen et al., 1999). However, in the absence of a true ligand, these studies invariably rely on the use of chimeric receptors composed of extracellular domains of (for example) IL-1R1 and IL-1RAcP, combined with the intracellular domains of ST2. Triggering of such chimeric receptors with IL-1α/β does not always lead to productive complexes capable of initiating signal transduction and NF-κB activation. On the basis of its inability to activate NF-κB, ST2 has been described together with SIGIRR as the only two members of the TIR family that are incapable of inducing inflammatory responses (Brint et al., 2002; Thomassen et al., 1999). Instead, both receptors have been identified as negative regulators of TLR-IL-1 receptor signaling (Brint et al., 2004; Wald et al., 2003). The mechanism by which these receptors regulate TLR-IL-1R-driven inflammatory responses appears to be by sequestration of "downstream" shared adaptor proteins. Both ST2-deficient and SIGIRR-deficient mice accordingly show enhanced inflammatory responses to either LPS or IL-1 injections with enhanced serum levels of inflammatory cytokines such as IL-12 and IL-6. For ST2, the data we present here point to an alternative explanation of these findings. On cells that express ST2 such as mast cells and T_H2 cells, IL-33 administration leads to the activation of a pathway that involves NF-κB. This activation ultimately results in the production of the T_H2-associated cytokines IL-4, IL-5, and IL-13 and their downstream biological responses. These T_H2 cytokine-dependent responses can potentially balance ongoing inflammatory T_H1 responses.

Because of their profound biological functions and the severe pathophysiological consequences of dysregulated expression, all IL-1 family members are tightly regulated, either at the ligand or receptor level or both (Dinarello, 1997, 2000; Dunne and O'Neill, 2003). The well-known soluble form of ST2 could play an important role in regulating the biological activity of IL-33. The importance of soluble ST2 was highlighted in several recent reports that described greatly increased serum levels of this protein in a number of human diseases, including asthma and allergic airway inflammation, acute myocardial infarction, and sepsis (Brunner et al., 2004; Oshikawa et al., 2001; Shimpo et al., 2004; Tajima et al., 2003; Weinberg et al., 2003), and several studies have demonstrated beneficial effects of soluble ST2 treatment in animal models of disease (Coyle et al., 1999; Leung et al., 2004; Sweet et al., 2001; Xu et al., 1998). It is, however, not yet clear how soluble ST2 functions in these diseases. Identification of IL-33 as the ligand for ST2 will allow us to understand the potentially competing roles of the membrane and soluble forms of ST2. Identification of IL-33 and its receptor ST2 as an IL-1-related cytokine-receptor system, capable of activating NF-κB and MAP kinases, and leading to the induction of T_H2 cytokines, opens new opportunities to investigate and ultimately understand the intricacies of the human immune system.

Experimental Procedures

Identification of Human and Mouse IL-33

A structural superposition of available IL-1 and FGF structures (Holm and Sander, 1998) served as the basis for a greater alignment of diverse IL-1 family sequences and was used to create computational tools (position-specific scoring matrices, HMMs, and profiles) to search sequence databases for distant IL-1- and FGF-like proteins. The full-length sequence of dog DVS-27 (Onda et al., 1999) (accession # BAA75891) was identified by this effort from GenBank, and its IL-1 family assignment was secured by Superfamily (Gough and Chothia, 2002) and 3D-PSSM (Kelley et al., 2000) fold recognition methods that matched it to IL-1/FGF β-trefoil fold templates. The orthologous human and mouse sequences were deduced by sequencing ESTs (AL545605 and BE915331, respectively); the corresponding mouse gene was located with the UCSC Genome Browser (Karolchik et al., 2003), and the predicted genomic sequence was used to isolate a full-length cDNA by use of PCR from a mouse lung Marathon-Ready library. Both sequences are deposited at GenBank (accession numbers AY905581 and AY905582, respectively).

RNA Extraction and Real-Time Quantitative PCR for Gene Expression

For TAQMAN analysis, total RNA was isolated using RNA STAT-60 (Tel-Test, Friendswood, TX). After isopropanol precipitation, total RNA was reextracted with phenol:chloroform:isoamyl alcohol

(25:24:1) (Sigma Chemicals) via phase-lock light tubes (Eppendorf). Total RNA (5 μg) was subjected to treatment with DNase (Roche Molecular Biochemicals). Total RNA was reverse-transcribed using Superscript II (GIBCO-BRL). Primers were designed using Primer Express (PE Biosystems) or obtained commercially from Applied Biosystems. Real-time PCR on 10 ng of cDNA was performed as described before (Fort et al., 2001).

In Vitro Translation of IL-33 and Digestion with Caspase-1

The cDNA of full-length IL-33 was subcloned in the pSPORT6 vector (Invitrogen) containing the SP6 promoter. In vitro translation was performed by the TNT Coupled Reticulocyte Lysate System (Promega) with [^{35}S]methionine (Amersham Bioscience). In vitro translated IL-33 was either untreated or treated with 1.5 units of caspase-1 (GTS Inc.) for 1 or 2 hr at 37°C. Untreated IL-33 was incubated for 2 hr at 37°C. Samples were separated by SDS-PAGE. Gels were fixed, dried, and autoradiographed.

Recombinant Protein Production

The cDNA for human IL-33 was subcloned into the expression vector pET3a, starting with amino acid 112 of the full-length protein. DL21(D3) (Novagen) was transformed and expression was induced with IPTG (Fisher Scientific) at OD_{600} of 0.6. 2 hr after induction, bacteria were harvested and the pellet was resuspended in ice-cold 50 mM Tris (pH 8.0) with 1 mM EDTA (Buffer A) in the presence of Halt-Protease inhibitor (Pierce). Bacteria were lysed by sonication. The lysate was centrifugated (20 min, 10,000 rpm). The supernatant was passed over HiTrap Q column (Amersham Bioscience). The bound protein was eluted with a NaCl gradient. IL-33-containing peak fraction was further purified via gel filtration over a Superdex200 HR 10/30 column (Amersham Bioscience). Peak fractions of >95% pure IL-33 were quantified by SDS-PAGE and Coomassie blue staining with lysozyme as a standard. Endotoxin levels (0.023 eu/μg protein) were determined (BioWhittaker Limulus Amebocyte Lysate QCL-1000 pyrogen testing).

Pull-Down Assay

IL-33 was biotinylated with EZ-Link Sulfo-NHS-Biotin (Pierce). Pull-down of 2 μg biotinylated IL-33 was performed in 500 μl RIPA-Lysis buffer (Upstate) with 50 μl of a 50% slurry of agarose bound Avidin D (Vector Laboratories). 5 μg of extracellular ST2 -Fc (R&D Systems) was used. After incubation overnight at 4°C, precipitates were washed 3× with 500 μl RIPA-Lysis buffer. Proteins were separated by SDS-PAGE, electroblotted, and visualized by Western blot/ECL with anti-ST2 (R&D Systems). Pull-down of biotinylated IL-33 with ST2-Fc was performed as described above, only Protein G-Sepharose (Amersham) was used instead of Avidin D. IL-33 was visualized via a Streptavidin-HRP conjugate (Pierce) and ECL reaction.

Flow Cytometry

Cells were collected, washed, and incubated with either anti-ST2 (MD Bioscience) or isotype control antibody (rat IgG1; BD PharMingen) and subsequently stained with an R-Phycoerythrin-conjugated anti-rat IgG antibody (Jackson ImmunoResearch). Or cells were incubated with biotinylated human IL-33 and the bound IL-33 was stained with R-Phycoerythrin-conjugated Strepdavidin (Pierce). Cells were analyzed on a FACSCalibur flow cytometer (Becton Dickinson).

Mice and In Vivo Treatment

C57BL/6, BALB/c mice were obtained from Jackson Laboratory and held under pathogen-free conditions at the DNAX Animal Care Facility. Mice were given i.p. phosphate-buffered saline, 0.4 or 4 μg IL-33 protein daily up to 7 days. All animal experiments described were approved by the institutional animal care and use committee of DNAX and are in compliance with the guidelines of the American Association for Accreditation of Laboratory Animal Care.

Blood and Spleen Cell Analyses

Blood was collected via cardiac puncture and transferred to either heparin collection tubes for blood analysis or serum tubes to obtain serum. Blood was run on a blood analyzer (Advia 120, Bayer). Mouse spleens were made into single-cell suspension, counted by hemocytometer, and an aliquot was used for a cytospin. Both splenic cytospin and blood smears were stained with Haema3 (Fisher Scientific) and counted to determine the relative percentage of mononuclear cells, neutrophils, and eosinophils. Absolute numbers of each cell type were calculated for individual mice.

Histologic Analyses

Microscopic examination of mouse tissues was performed on formalin-fixed tissue sections stained with either hematoxylin/eosin (H&E) or periodic acid-Schiff (PAS) and alcain blue.

Phosphorylation of NF-κB, IκB-α, and MAP Kinases

WTMC mast cells (Wright et al., 2003) were lysed in RIPA lysis Buffer (Upstate) containing Complete Mini protease inhibitor cocktail (Roche) and 10 mM Na_3VO_4. Proteins were separated by SDS-PAGE, transferred to Immobilon-P membranes (Millipore), and immunoblotted with antibodies to phosphorylated p65 NF-κB, p65 NF-κB, phosphorylated IκB-α, phosphorylated p44/42 MAP kinases, p44/42 MAP kinases, phosphorylated p38 MAP kinase, and p38 MAP kinase (all antibodies from Cell Signaling Technology).

Determination of Serum Ig Isotypes and Cytokine Levels

Determination of Serum Ig was described previously (Fort et al., 2001). Cytokine levels were determined by using Mouse Inflammation and T_H1/T_H2 Cytometric Bead Array Kits (BD Biosciences). IL-13 was detected by enzyme-linked immunoabsorbent assay (ELISA) with paired antibodies (R&D Systems). IFNγ, IL-4, IL-5, and IL-13 were detected using a Beadlyte Kit and Reagent (Upstate) and the luminex100 detection system (Luminex).

T_H1 and T_H2 Polarization of Naive Mouse Cells

Naive T cells were isolated from C57BL/6 spleens by use of the $CD4^+CD62L^+$ T Cell Isolation Kit and an AutoMACS separator (Miltenyi Biotec). Polarization of naive T cell into T_H1 or T_H2 cells has been described previously (Boonstra et al., 2001). After three rounds of polarization, 4×10^5 cells were restimulated with plate bound anti-CD3 and 1 μg/ml anti-CD28 with or without IL-33 (20 ng/ml). After 48 hr, supernatants were collected and monitored for cytokines.

Transient Transfection and Reporter Gene Assays

HEK293FT cells were seeded before transfection with an NF-κB-driven GFP reporter gene construct (pNF-κB-hrGFP; Stratagene) and with a plasmid encoding ST2 as indicated with Fugene-6 (Roche). Cells were split 24 hr after transfection. After 24 hr, cells were either left untreated or stimulated with mouse IL-33 at the concentration of 50 ng/ml. 16 hr after stimulation, cells were analyzed for GFP expression by FACS.

Immunoprecipitations

HEK293 cells were transiently transfected with human ST2 expression vector using the calcium-phosphate method. 36 hr after transfection, cells untreated or treated with IL-33 (10 ng/ml) were lysed (1.0% Nonidet P-40, 20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1.0 mM EDTA, 10 mM β-glycerophosphate, 10 mM NaF, 1 mM sodium orthovanadate, 2 mM EGTA, 20 μM aprotinin, 1 mM phenylmethylsulfonyl fluoride). Cell extracts were incubated with 1 μg of antibody or preimmune serum for 2 hr, followed by a 2 hr incubation with 20 μl of protein A-Sepharose beads. After incubation, the beads were washed four times with lysis buffer, separated by SDS-PAGE, transferred to Immobilon-P membranes (Millipore), and analyzed by immunoblotting. Antibodies ST2 (R&D Systems), MyD88 (Stressgen), TRAF6 (Santa Cruz Biotechnologies), IRAK (Santa Cruz Biotechnologies), and IRAK4 (Upstate) were used.

Supplemental Data

Supplemental Data include one figure and can be found with this article online at http://www.immunity.com/cgi/content/full/23/5/479/DC1/.

Acknowledgments

The authors thank Daniel J. Cua for his critical reading of the manuscript and the DNAX Animal Care Facility. DNAX is supported by Schering-Plough. The authors declare that they have no competing

financial interests. Competing financial interests: Two of this paper's authors (J.S. and R.A.K.) are listed as inventors of patents involving IL-33.

Received: February 2, 2005
Revised: September 2, 2005
Accepted: September 14, 2005
Published: November 15, 2005

References

Bergers, G., Reikerstorfer, A., Braselmann, S., Graninger, P., and Busslinger, M. (1994). Alternative promoter usage of the Fos-responsive gene Fit-1 generates mRNA isoforms coding for either secreted or membrane-bound proteins related to the IL-1 receptor. EMBO J. *13*, 1176–1188.

Boonstra, A., Barrat, F.J., Crain, C., Heath, V.L., Savelkoul, H.F., and O'Garra, A. (2001). 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J. Immunol. *167*, 4974–4980.

Bost, K.L., Holton, R.H., Cain, T.K., and Clements, J.D. (1996). In vivo treatment with anti-interleukin-13 antibodies significantly reduces the humoral immune response against an oral immunogen in mice. Immunology *87*, 633–641.

Brint, E.K., Fitzgerald, K.A., Smith, P., Coyle, A.J., Gutierrez-Ramos, J.C., Fallon, P.G., and O'Neill, L.A. (2002). Characterization of signaling pathways activated by the interleukin 1 (IL-1) receptor homologue T1/ST2. A role for Jun N-terminal kinase in IL-4 induction. J. Biol. Chem. *277*, 49205–49211.

Brint, E.K., Xu, D., Liu, H., Dunne, A., McKenzie, A.N., O'Neill, L.A., and Liew, F.Y. (2004). ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance. Nat. Immunol. *5*, 373–379.

Brunner, M., Krenn, C., Roth, G., Moser, B., Dworschak, M., Jensen-Jarolim, E., Spittler, A., Sautner, T., Bonaros, N., Wolner, E., et al. (2004). Increased levels of soluble ST2 protein and IgG1 production in patients with sepsis and trauma. Intensive Care Med. *30*, 1468–1473.

Chomarat, P., and Banchereau, J. (1998). Interleukin-4 and interleukin-13: their similarities and discrepancies. Int. Rev. Immunol. *17*, 1–52.

Corry, D.B. (1999). IL-13 in allergy: home at last. Curr. Opin. Immunol. *11*, 610–614.

Coyle, A.J., Lloyd, C., Tian, J., Nguyen, T., Erikkson, C., Wang, L., Ottoson, P., Persson, P., Delaney, T., Lehar, S., 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.

Debets, R., Timans, J.C., Homey, B., Zurawski, S., Sana, T.R., Lo, S., Wagner, J., Edwards, G., Clifford, T., Menon, S., et al. (2001). Two novel IL-1 family members, IL-1 delta and IL-1 epsilon, function as an antagonist and agonist of NF-kappa B activation through the orphan IL-1 receptor-related protein 2. J. Immunol. *167*, 1440–1446.

Dinarello, C.A. (1994). The biological properties of interleukin-1. Eur. Cytokine Netw. *5*, 517–531.

Dinarello, C.A. (1997). Interleukin-1. Cytokine Growth Factor Rev. *8*, 253–265.

Dinarello, C.A. (2000). Interleukin-18, a proinflammatory cytokine. Eur. Cytokine Netw. *11*, 483–486.

Dunne, A., and O'Neill, L.A. (2003). The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense. Sci. STKE *2003*, re3.

Fort, M.M., Cheung, J., Yen, D., Li, J., Zurawski, S.M., Lo, S., Menon, S., Clifford, T., Hunte, B., Lesley, R., et al. (2001). IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity *15*, 985–995.

Gayle, M.A., Slack, J.L., Bonnert, T.P., Renshaw, B.R., Sonoda, G., Taguchi, T., Testa, J.R., Dower, S.K., and Sims, J.E. (1996). Cloning of a putative ligand for the T1/ST2 receptor. J. Biol. Chem. *271*, 5784–5789.

Gough, J., and Chothia, C. (2002). SUPERFAMILY: HMMs representing all proteins of known structure. SCOP sequence searches, alignments and genome assignments. Nucleic Acids Res. *30*, 268–272.

Holm, L., and Sander, C. (1998). Touring protein fold space with Dali/FSSP. Nucleic Acids Res. *26*, 316–319.

Hoshino, K., Kashiwamura, S., Kuribayashi, K., Kodama, T., Tsujimura, T., Nakanishi, K., Matsuyama, T., Takeda, K., and Akira, S. (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.

Karlen, S., De Boer, M.L., Lipscombe, R.J., Lutz, W., Mordvinov, V.A., and Sanderson, C.J. (1998). Biological and molecular characteristics of interleukin-5 and its receptor. Int. Rev. Immunol. *16*, 227–247.

Karolchik, D., Baertsch, R., Diekhans, M., Furey, T.S., Hinrichs, A., Lu, Y.T., Roskin, K.M., Schwartz, M., Sugnet, C.W., Thomas, D.J., et al. (2003). The UCSC genome browser database. Nucleic Acids Res. *31*, 51–54.

Kelley, L.A., MacCallum, R.M., and Sternberg, M.J. (2000). Enhanced genome annotation using structural profiles in the program 3D-PSSM. J. Mol. Biol. *299*, 499–520.

Klemenz, R., Hoffmann, S., and Werenskiold, A.K. (1989). Serum- and oncoprotein-mediated induction of a gene with sequence similarity to the gene encoding carcinoembryonic antigen. Proc. Natl. Acad. Sci. USA *86*, 5708–5712.

Kumar, S., Minnich, M.D., and Young, P.R. (1995). ST2/T1 protein functionally binds to two secreted proteins from Balb/c 3T3 and human umbilical vein endothelial cells but does not bind interleukin 1. J. Biol. Chem. *270*, 27905–27913.

Leung, B.P., Xu, D., Culshaw, S., McInnes, I.B., and Liew, F.Y. (2004). A novel therapy of murine collagen-induced arthritis with soluble T1/ST2. J. Immunol. *173*, 145–150.

Lohning, M., Stroehmann, A., Coyle, A.J., Grogan, J.L., Lin, S., Gutierrez-Ramos, J.C., Levinson, D., Radbruch, A., and Kamradt, T. (1998). T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin *4*, interleukin *5*, and interleukin *10*, and important for Th2 effector function. Proc. Natl. Acad. Sci. USA *95*, 6930–6935.

Lohning, M., Grogan, J.L., Coyle, A.J., Yazdanbakhsh, M., Meisel, C., Gutierrez-Ramos, J.C., Radbruch, A., and Kamradt, T. (1999). T1/ST2 expression is enhanced on CD4+ T cells from schistosome egg-induced granulomas: analysis of Th cell cytokine coexpression ex vivo. J. Immunol. *162*, 3882–3889.

Moritz, D.R., Rodewald, H.R., Gheyselinck, J., and Klemenz, R. (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.

Onda, H., Kasuya, H., Takakura, K., Hori, T., Imaizumi, T., Takeuchi, T., Inoue, I., and Takeda, J. (1999). Identification of genes differentially expressed in canine vasospastic cerebral arteries after subarachnoid hemorrhage. J. Cereb. Blood Flow Metab. *19*, 1279–1288.

Oshikawa, K., Kuroiwa, K., Tago, K., Iwahana, H., Yanagisawa, K., Ohno, S., Tominaga, S.I., and Sugiyama, Y. (2001). Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. Am. J. Respir. Crit. Care Med. *164*, 277–281.

Pene, J., Rousset, F., Briere, F., Chretien, I., Paliard, X., Banchereau, J., Spits, H., and De Vries, J.E. (1988). IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-gamma. J. Immunol. *141*, 1218–1224.

Priestle, J.P., Schar, H.P., and Grutter, M.G. (1988). Crystal structure of the cytokine interleukin-1 beta. EMBO J. *7*, 339–343.

Robinson, D., Shibuya, K., Mui, A., Zonin, F., Murphy, E., Sana, T., Hartley, S.B., Menon, S., Kastelein, R., Bazan, F., and O'Garra, A. (1997). IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. Immunity *7*, 571–581.

Roboz, G.J., and Rafii, S. (1999). Interleukin-5 and the regulation of eosinophil production. Curr. Opin. Hematol. *6*, 164–168.

Shimpo, M., Morrow, D.A., Weinberg, E.O., Sabatine, M.S., Murphy, S.A., Antman, E.M., and Lee, R.T. (2004). Serum levels of the

interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction. Circulation *109*, 2186–2190.

Sims, J.E., Nicklin, M.J., Bazan, J.F., Barton, J.L., Busfield, S.J., Ford, J.E., Kastelein, R.A., Kumar, S., Lin, H., Mulero, J.J., et al. (2001). A new nomenclature for IL-1-family genes. Trends Immunol. *22*, 536–537.

Sweet, M.J., Leung, B.P., Kang, D., Sogaard, M., Schulz, K., Trajkovic, V., Campbell, C.C., Xu, D., and Liew, F.Y. (2001). A novel pathway regulating lipopolysaccharide-induced shock by ST2/T1 via inhibition of Toll-like receptor 4 expression. J. Immunol. *166*, 6633–6639.

Tajima, S., Oshikawa, K., Tominaga, S., and Sugiyama, Y. (2003). The increase in serum soluble ST2 protein upon acute exacerbation of idiopathic pulmonary fibrosis. Chest *124*, 1206–1214.

Thomassen, E., Renshaw, B.R., and Sims, J.E. (1999). Identification and characterization of SIGIRR, a molecule representing a novel subtype of the IL-1R superfamily. Cytokine *11*, 389–399.

Tominaga, S. (1989). A putative protein of a growth specific cDNA from BALB/c-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. FEBS Lett. *258*, 301–304.

Tominaga, S., Jenkins, N.A., Gilbert, D.J., Copeland, N.G., and Tetsuka, T. (1991). Molecular cloning of the murine ST2 gene. Characterization and chromosomal mapping. Biochim. Biophys. Acta *1090*, 1–8.

Tominaga, S., Yokota, T., Yanagisawa, K., Tsukamoto, T., Takagi, T., and Tetsuka, T. (1992). Nucleotide sequence of a complementary DNA for human ST2. Biochim. Biophys. Acta *1171*, 215–218.

Towne, J.E., Garka, K.E., Renshaw, B.R., Virca, G.D., and Sims, J.E. (2004). Interleukin (IL)-1F6, IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL-1RAcP to activate the pathway leading to NF-kappaB and MAPKs. J. Biol. Chem. *279*, 13677–13688.

Townsend, M.J., Fallon, P.G., Matthews, D.J., Jolin, H.E., and McKenzie, A.N. (2000). T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. J. Exp. Med. *191*, 1069–1076.

Trajkovic, V., Sweet, M.J., and Xu, D. (2004). T1/ST2-an IL-1 receptor-like modulator of immune responses. Cytokine Growth Factor Rev. *15*, 87–95.

Wald, D., Qin, J., Zhao, Z., Qian, Y., Naramura, M., Tian, L., Towne, J., Sims, J.E., Stark, G.R., and Li, X. (2003). SIGIRR, a negative regulator of Toll-like receptor-interleukin 1 receptor signaling. Nat. Immunol. *4*, 920–927.

Weinberg, E.O., Shimpo, M., Hurwitz, S., Tominaga, S., Rouleau, J.L., and Lee, R.T. (2003). Identification of serum soluble ST2 receptor as a novel heart failure biomarker. Circulation *107*, 721–726.

Wright, G.J., Cherwinski, H., Foster-Cuevas, M., Brooke, G., Puklavec, M.J., Bigler, M., Song, Y., Jenmalm, M., Gorman, D., McClanahan, T., et al. (2003). Characterization of the CD200 receptor family in mice and humans and their interactions with CD200. J. Immunol. *171*, 3034–3046.

Xu, D., Chan, W.L., Leung, B.P., Huang, F., Wheeler, R., Piedrafita, D., Robinson, J.H., and Liew, F.Y. (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.