

# Increased Levels of Interleukin 33 in Sera and Synovial Fluid from Patients with Active Rheumatoid Arthritis

YASUSHI MATSUYAMA, HITOAKI OKAZAKI, HIROYUKI TAMEMOTO, HIROTAKA KIMURA, YASUYUKI KAMATA, KATSUYA NAGATANI, TAKAO NAGASHIMA, MORISADA HAYAKAWA, MASAHIRO IWAMOTO, TAKU YOSHIO, SHIN-ICHI TOMINAGA, and SEIJI MINOTA

**ABSTRACT. Objective.** To determine levels of interleukin 33 (IL-33) in serum and synovial fluid (SF) and their clinical associations in patients with rheumatoid arthritis (RA). To evaluate the ability of activated peripheral blood mononuclear cells (PBMC) and fibroblast-like synoviocytes (FLS) from RA patients to release IL-33.

**Methods.** Sera were obtained from 59 patients with RA, 10 patients with infectious diseases, and 42 healthy volunteers. SF samples were obtained from 15 patients with RA and 13 with osteoarthritis. IL-33 levels were measured using a sandwich ELISA after removal of rheumatoid factor with protein A-Sepharose beads. FLS were stimulated with IL-1 $\beta$  and tumor necrosis factor, and treated with or without chemical damage. PBMC were stimulated with anti-CD3/CD28 antibodies. The levels of IL-33 were measured in the culture supernatants and cell lysates by ELISA or immunoblotting.

**Results.** Serum IL-33 levels were significantly higher in RA patients, especially in the high disease activity group compared to the moderate or low activity group. IL-33 levels in SF were elevated in all 15 RA patients measured. IL-33 levels were higher in SF samples than in sera in 7 RA patients measured simultaneously. The 30-kDa IL-33 precursor was detected in the culture supernatants of damaged FLS but was not detected in those of activated PBMC and non-damaged FLS.

**Conclusion.** IL-33 levels were elevated in sera and SF samples from patients with RA, and correlated with disease activity. IL-33 was produced mainly in inflamed joints; IL-33/ST2L signaling might play an important role in joint inflammation of human RA. (First Release Nov 15 2009; J Rheumatol 2010;37:18–25; doi:10.3899/jrheum.090492)

## Key Indexing Terms:

RHEUMATOID ARTHRITIS

DISEASE ACTIVITY

INTERLEUKIN 33

Interleukin 33 (IL-33) was recently identified as a member of the IL-1 family and a ligand for the IL-1 family receptor ST2L (IL1RL1). IL-33 is synthesized as a 30-kDa precursor with 270 amino acids<sup>1</sup>. *In vitro* studies have shown that the synthetic precursor of IL-33 is cleaved by caspase-1 to become a mature form containing 112–270 residues and that this form acts as a cytokine by binding to ST2L, which is mainly expressed on type 2 helper T lymphocytes (Th2 cells)<sup>1,2</sup>, mast cells<sup>3,4</sup>, and eosinophils<sup>5</sup>. IL-33/ST2L signaling enhances the production of cytokines such as IL-5, IL-6,

and IL-13 via the activation of nuclear factor- $\kappa$ B and MAP kinases<sup>1,2,6</sup>. However, details regarding the *in vivo* process are not known. Recent studies with human samples show that caspase-1 does not cleave the IL-33 precursor at the proposed site Ser-111<sup>7-10</sup>, and 3 studies revealed that the IL-33 precursor itself can act as a cytokine by binding to ST2L<sup>7-9</sup>. One study showed that the IL-33 precursor is released from human umbilical vein endothelial cells by physical and chemical (detergent) cell destruction<sup>8</sup>. Two studies showed that caspase-3, which is associated with cell apoptosis, cleaves the IL-33 precursor and inactivates it<sup>8,9</sup>. We recently reported that calpain, which is a calcium-dependent protease, cleaved the IL-33 precursor at a different cleavage site from the proposed site Ser-111<sup>10</sup>. However, the bioactivity of calpain-cleaved IL-33 remains unknown.

In a murine model, IL-33 induces asthmatic symptoms<sup>2</sup> or enhances joint inflammation<sup>11,12</sup> by binding to ST2L and promoting inflammatory cytokine production. In particular, mouse IL-33 exacerbates antigen-induced arthritis by binding to ST2L on mast cells<sup>12</sup>. On the other hand, little is known about the function of IL-33 in the pathogenesis of human diseases. Immunohistochemistry and *in situ* hybridization have identified IL-33 inside the synovial cells of rheumatoid arthritis (RA)<sup>13</sup>, and increased production of

From the Division of Rheumatology and Clinical Immunology and the Department of Biochemistry, Jichi Medical University, Tochigi, Japan.

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Y. Matsuyama, MD; H. Okazaki, MD, PhD, Professor; H. Kimura, MD, PhD; Y. Kamata, MD; K. Nagatani, MD, PhD; T. Nagashima, MD, PhD; M. Iwamoto, MD, PhD, Associate Professor; T. Yoshio, MD, PhD, Associate Professor; S. Minota, MD, PhD, Professor, Division of Rheumatology and Clinical Immunology; H. Tamemoto, MD, PhD, Associate Professor; M. Hayakawa, PhD; S. Tominaga, MD, PhD, Professor, Department of Biochemistry, Jichi Medical University.

Address correspondence to Dr. H. Okazaki, Division of Rheumatology and Clinical Immunology, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi, 329-0498, Japan. E-mail: hitoaki@jichi.ac.jp

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messenger RNA and the IL-33 precursor have been found in the synovial cells of RA after stimulation with IL-1 $\beta$  and/or tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )<sup>11,12</sup>. However, it is not known whether IL-33 is secreted in sera or synovial fluid (SF) in human RA. Therefore, we analyzed levels of IL-33 in sera and SF from RA patients using a sandwich enzyme-linked immunosorbent assay (ELISA) after removal of rheumatoid factor (RF) with protein A-Sepharose beads. The ability of activated peripheral blood mononuclear cells (PBMC) and fibroblast-like synoviocytes (FLS) from RA patients to release IL-33 was also evaluated using ELISA and immunoblotting.

## MATERIALS AND METHODS

**Sera and synovial fluid.** Sera and SF were obtained from patients referred to the Division of Rheumatology and Clinical Immunology, Jichi Medical University, from 2005 to 2009. Patients with infectious diseases and healthy volunteers were used as controls. RA and osteoarthritis (OA) patients were diagnosed according to the American College of Rheumatology (ACR) criteria for RA<sup>14</sup> and OA<sup>15</sup>. To eliminate the influence of RF on IL-33 levels in ELISA, all sera and SF samples were pre-cleared by protein A-Sepharose beads [10% (v/v) pelleted beads; GE Healthcare, UK] overnight at 4°C as described<sup>16,17</sup>. The titers of RF in pre-cleared serum samples were evaluated with the RA particle agglutination (RAPA) test (SERODIA<sup>®</sup>RA; Fujirebio Inc., Tokyo, Japan). RF-negative specimens were defined as those not agglutinating with the sensitized particles (1:40 final dilution). In this study, RF-negative samples were included in the analysis only after the pre-clearing treatment.

Informed consent was obtained from all participating subjects. This study was reviewed and approved by our institutional review board for human studies.

**PBMC and FLS from RA patients.** PBMC were isolated by subjecting heparinized venous blood from 3 patients with active RA to Ficoll density-gradient centrifugation. Primary FLS from RA patients were purchased from Cell Applications, Inc. (San Diego, CA, USA). FLS from passages 3–4 were used for the experiments.

**Antibodies and reagents.** Two different goat anti-human IL-33 polyclonal antibodies, horseradish peroxidase (HRP)-conjugated streptavidin, recombinant human TNF- $\alpha$ , recombinant human IL-33, and the TMB (3, 3', 5, 5'-tetramethylbenzidine) peroxidase substrate system were purchased from R&D systems (Minneapolis, MN, USA). RPMI-1640, Dulbecco's modified Eagle's medium (DMEM), and bovine serum albumin (BSA) were purchased from Sigma Aldrich (St. Louis, MO, USA). Anti-human CD3 antibody and anti-human CD28 antibody were purchased from BD Biosciences (San Jose, CA, USA). Recombinant human IL-1 $\beta$ , caspase-3, fetal bovine serum (FBS), and Nonidet<sup>®</sup> P-40 (NP-40) were purchased from Peprotec Inc. (Rocky Hill, NJ, USA), Calbiochem (San Diego, CA, USA), Nichirei Co. (Tokyo, Japan), and Nacalai Tesque, Inc. (Tokyo, Japan), respectively.

**ELISA for IL-33 in sera and SF samples.** To establish an appropriate protocol for serum and SF analysis, several commercial antibodies and solution diluents were tested. We found that 2 different kinds of goat anti-human IL-33 polyclonal antibodies (AF3625 and BAF3625) and 100% heat-inactivated FBS were applicable as the coating antibody, the detecting antibody, and the diluents, respectively (data not shown). Recombinant human IL-33 protein was used as a standard.

The ELISA protocol in this study was as follows. A 96-well polystyrene plate (Maxisorp, Nalge Nunc International K.K., Tokyo, Japan) was coated with 100  $\mu$ l goat anti-human IL-33 polyclonal antibody (AF3625) diluted to 0.8  $\mu$ g/ml with phosphate-buffered saline (PBS), and allowed to stand at 4°C overnight. After washing, 300  $\mu$ l of blocking solution consisting of 1% (w/v) BSA in PBS was added to each well, and the plate was incubated at

room temperature for 1 h. After washing 3 times, 100  $\mu$ l of pre-cleared samples and standards diluted with 100% FBS were added. After 2 h incubation at room temperature, the plate was washed 3 times, and 100  $\mu$ l of 0.2  $\mu$ g/ml biotinylated goat anti-human IL-33 antibody (BAF3625) was added to each well. After 1 h, the plate was washed 3 times; 100  $\mu$ l of HRP-labeled streptavidin diluted 1:200 was added; and the mixture was allowed to react for 20 min. After washing 3 times, the color was developed with 100  $\mu$ l of TMB peroxidase substrate system for 20 min. In order to stop the TMB reaction, 50  $\mu$ l of 2 N sulfuric acid was added to each well. The absorbance at 450 nm was measured using a microplate reader with a wavelength correction set at 540 nm to subtract the background. Each sample was measured in duplicate.

**Disease activity evaluations and other clinical characteristics.** Tender joint count (TJC) in 46 joints and swollen joint count (SJC) in 48 joints according to the ACR criteria<sup>18</sup> were scored by 8 rheumatologists who had no access to the laboratory data. Disease activity in RA was scored with the Disease Activity Score 28 based on C-reactive protein levels (DAS28-CRP)<sup>19</sup> by the same 8 rheumatologists. Patients with RA were classified into 3 groups according to their DAS28-CRP: the high-activity group was defined as DAS28-CRP  $\geq$  4.1; the moderate activity group as  $2.7 \leq$  DAS28-CRP  $<$  4.1; and the low-activity group as DAS28-CRP  $<$  2.7<sup>19</sup>. Serum TNF- $\alpha$ , IL-1 $\beta$ , IL-5, IL-6, and IL-13 levels were measured using an ELISA kit (R&D systems). Matrix metalloproteinase-3 (MMP-3) levels in sera were measured by latex turbidimetric immunoassay at SRL, Inc. (Tokyo, Japan).

**Measurement of IL-33 levels in culture supernatants of PBMC.** PBMC isolated from 3 RA patients were cultured in RPMI-1640 supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin, 4 mM glutamine, and 0.05 mM 2-mercaptoethanol. After overnight incubation on RepCell cultureware (Cellseed Inc., Tokyo, Japan), PBMC were seeded at  $1 \times 10^6$ /well in 6-well polystyrene plates (BD Biosciences) and coated with 20  $\mu$ g/ml anti-human CD3 antibody or PBS. Anti-CD28 antibody (5  $\mu$ g/ml) or PBS was also added to each well. The culture supernatants were collected at 0, 24, and 72 h after anti-CD3/28 antibody stimulation. IL-33 levels were measured using the ELISA we developed, and IL-1 $\beta$ , TNF- $\alpha$ , and interferon- $\gamma$  (IFN- $\gamma$ ) levels were measured using an ELISA kit (R&D Systems). Each sample was measured in duplicate.

**Measurement of IL-33 levels in culture supernatants of FLS.** The FLS were seeded in 10 ml DMEM with 10% heat-inactivated FBS, 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin, and 4 mM glutamine at  $1 \times 10^6$  cells on a 10 cm diameter dish. After 24 h incubation, the medium was changed to DMEM with 1% FBS and 2 mM glutamine. After incubation for 3 h, recombinant IL-1 $\beta$  and TNF- $\alpha$  or PBS as a control was added to the dishes. After 24 h incubation, 5 ml of the culture supernatant was collected and 0.2% (v/v) NP-40 was added to the dishes. After 5 min, the culture supernatants were collected and IL-33 levels were measured. Several samples were incubated with caspase-3 at 37°C for 3 h before the assay.

**Immunoblotting for IL-33 protein.** Cell lysates were obtained using lysis buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% (v/v) NP-40, 0.5% (w/v) deoxycholate, 0.1% (w/v) sodium dodecyl sulfate (SDS)]. The protein samples were separated by electrophoresis on SDS polyacrylamide gels (12.5%) and transferred to PVDF membranes (Millipore, Billerica, MA, USA). The PVDF membranes were probed with biotinylated goat anti-human IL-33 (BAF 3625). After washing, the membranes were incubated with horseradish peroxidase (HRP)-conjugated streptavidin (KPL Inc., Gaithersburg, MD, USA). Can Get Signal (Toyobo, Osaka, Japan) and Superblock (Thermo Fisher Scientific Inc., Waltham, MA, USA) were used as an antibody diluent and blocking agent, respectively. Proteins were visualized using ECL Western blotting detection reagents (GE Healthcare), and the membranes were exposed to x-ray films (Fuji Photo Film, Japan).

**Statistical analysis.** Continuous data from the patient samples were analyzed using the Kruskal-Wallis test and/or the Mann-Whitney U test. Continuous data from the culture supernatants were analyzed by ANOVA and/or the Student's t test. Categorical data were compared using the

chi-squared test or Fisher's exact probability test. Differences of  $p < 0.05$  were considered significant.

## RESULTS

**Evaluation of ELISA for IL-33.** The intraassay and interassay precision values were 6.5% and 5.2%, respectively. The linearity of the assay was preserved within the range of 1:2 to 1:8 sample dilutions with 100% FBS. The absorbance of IL-33 solution at 450 nm was not influenced after adding 2000 pg/ml recombinant ST2 protein, which is a soluble receptor for IL-33 (data not shown).

**Evaluation of RF removal.** The titers of RAPA in 22 serum samples were evaluated before and after preclearing treatment with protein A-Sepharose. The titers of RAPA decreased in all samples after preclearing treatment and in 18 of 22 samples became completely negative (data not shown). Then, the titers of RAPA in a total of 156 precleared samples (128 serum samples and 28 SF samples) were measured. Two precleared serum samples from RA patients tested RF-positive at 640-fold. Among serum samples from other RA patients, 1 tested positive at 160-fold; 2 at 80-fold; and 8 at 40-fold. To avoid the influence of RF on ELISA, these samples were excluded in this study. Thirteen SF samples (6 RA, 7 OA) were agglutinated with nonsensitized particles due to nonspecific materials other than RF. These samples were included in this study. One hundred eleven other serum samples and 15 SF samples tested RF-negative, and the IL-33 levels ranged from undetectable levels to 10,110 pg/ml. We therefore concluded that RF had no influence on the measurement of IL-33 levels after they were precleared.

**Patient characteristics.** After exclusion of patients with RF-positive status despite preclearing treatment, 67 patients with RA [55 women; mean (SD) age 57.7 (13.9) yrs], 10 patients with infectious diseases [4 women; mean (SD) age 73.1 (19.9) yrs], 13 patients with OA [8 women; mean (SD) age 74.5 (11.2) yrs], and 42 healthy volunteers were enrolled in the analysis for IL-33 levels in sera and/or SF. The mean (SD) disease duration of RA was 9.3 (10.3) years. Of the 67 patients with RA, 63 patients received one or more antirheumatic drugs [48 methotrexate (mean [SD] dose 6.6 (1.8) mg/wk), 29 biologics (24 infliximab, 5 etanercept), 7 tacrolimus, 4 bucillamine, and 50 prednisolone (mean [SD] dose 5.3 [2.2] mg/day)]. Four patients (3 in sera and SF analysis, and 1 in SF analysis) did not receive any treatment for RA. All 15 RA patients and 13 OA patients subjected to SF analysis had joint symptoms and needed intraarticular steroid or hyaluronic acid injection. Of the 10 patients with infectious diseases, none had rheumatic diseases. The infectious diseases included 2 cases of pneumonia, 2 urinary tract infection, 2 biliary tract infection, 1 typhoid fever, 1 bacterial meningitis, 1 skin infection, and 1 pulmonary tuberculosis.

**IL-33 levels in sera.** Serum IL-33 levels were significantly higher in patients with RA than in those with infectious dis-

eases and healthy controls ( $p = 0.013$ ,  $p < 0.001$ , respectively; Figure 1).

**Disease activity and serum IL-33 levels.** In RA patients, serum IL-33 levels were significantly higher in the high disease activity group than in the moderate and low activity groups ( $p = 0.011$ ,  $p = 0.015$ , respectively; Figure 2).

**IL-33 levels in synovial fluid.** IL-33 levels in SF were elevated in all cases of RA, while they were undetected in 11 of 13 OA cases ( $p < 0.001$ ; Figure 3A). IL-33 levels were higher in SF samples than in sera in all 7 RA patients measured simultaneously ( $p = 0.009$ ; Figure 3B).

**IL-33 levels in the culture supernatants of FLS.** IL-33 levels in the culture supernatants were undetectable after stimulation of IL-1 $\beta$ /TNF- $\alpha$  unless detergent NP-40 was added. In contrast, IL-33 levels were significantly elevated when NP-40 was added to the above culture (Figure 4A). Immunoblotting with the same antibodies used in the ELISA revealed that the molecular weight of the detected protein was ~30 kDa, which is the same as that of the IL-33 precursor (Figure 4B). Moreover, the levels of IL-33 in ELISA decreased dose-dependently after caspase-3 treatment (Figure 5A). In immunoblotting, caspase-3-cleaved IL-33 was not detected, and the thickness of the 30 kDa precursor band decreased after treatment with caspase-3 (Figure 5B).

**IL-33 levels in the culture supernatant of PBMC.** Levels of IL-33 were undetectable in the culture supernatants of PBMC, although the levels of IL-1 $\beta$ , which is released after caspase-1 processing, were elevated. Levels of TNF- $\alpha$  and IFN- $\gamma$  were also elevated (Figure 6).

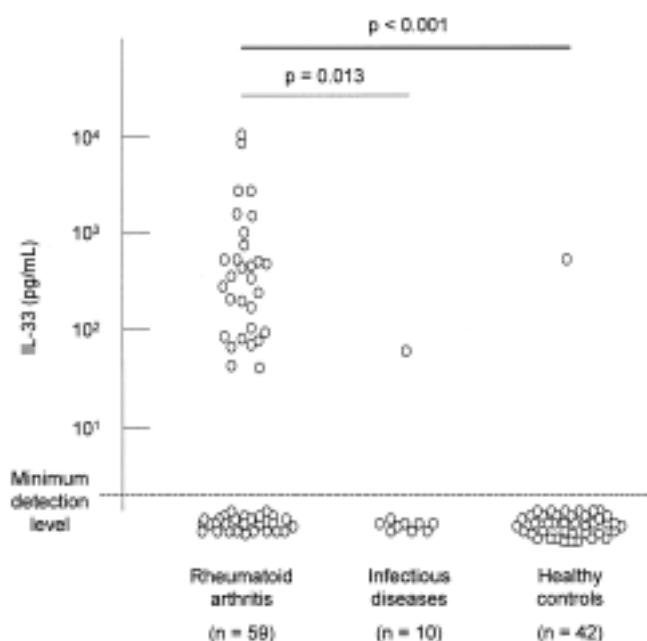


Figure 1. Interleukin 33 (IL-33) levels in sera from patients with rheumatoid arthritis, infectious diseases, and healthy volunteers. Symbols represent individual samples.

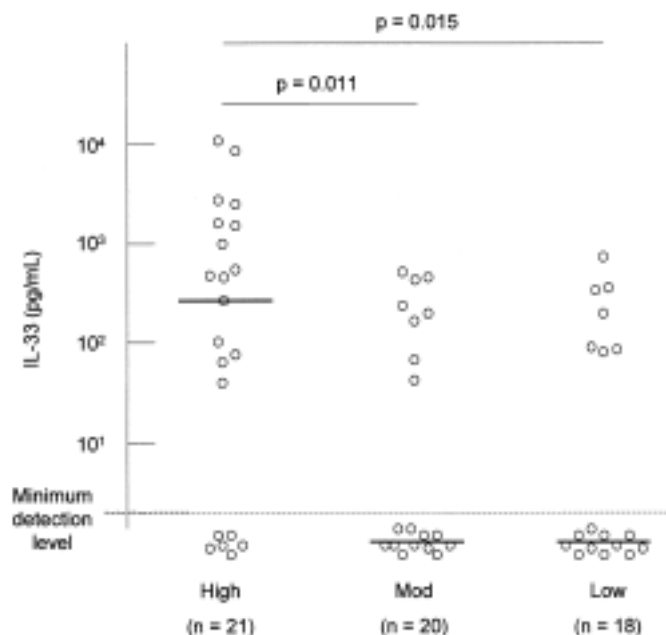


Figure 2. Interleukin 33 (IL-33) levels in sera from RA patients. RA patients were categorized into 3 groups according to the Disease Activity Score based on C-reactive protein levels. High: high activity group; Mod: moderate activity group; Low: low activity group. Symbols represent individual samples; horizontal bars represent median IL-33 levels.

Comparison between patients with detectable and undetectable levels of IL-33 in sera. TJC, SJC, and DAS28-CRP were significantly higher in patients with detectable levels of IL-33 (IL-33+ group) in sera than in those with undetectable levels (IL-33- group). Serum IL-5 levels were sig-

nificantly higher in the IL-33+ group. However, the number and the percentage of eosinophils in the peripheral blood cells did not differ between the 2 groups. No difference was observed between the 2 groups in serum levels of MMP-3, IL-6, IL-13, IL-1 $\beta$ , and TNF- $\alpha$ . Moreover, the number of patients treated with prednisolone, methotrexate, or anti-TNF- $\alpha$  agents and the doses of these drugs did not differ between the 2 groups (Table 1).

## DISCUSSION

To our knowledge, this is the first report to identify increased IL-33 levels in sera and SF samples from patients with RA. Interestingly, the IL-33 levels correlated with the disease activity estimated by DAS28-CRP. Moreover, IL-33 levels in SF were elevated in all 15 RA patients evaluated. The IL-33 precursor was detected in the culture supernatants of FLS stimulated with IL-1 $\beta$  and TNF- $\alpha$  only after detergent damage. These findings support results of recent studies indicating that IL-33 expression in human RA synovial cells depends on proinflammatory cytokines and that IL-33/ST2L signaling plays an important role in the pathogenesis of autoimmune arthritis<sup>11,12</sup>.

In our study, serum IL-33 levels were elevated in 30 of 59 RA patients. We separated RA patients into IL-33+ and IL-33- groups to analyze their clinical characteristics. The results showed that TJC, SJC, and DAS28-CRP were higher in the IL-33+ group than in the IL-33- group, while serum CRP, IL-1 $\beta$ , IL-6, and TNF- $\alpha$  levels did not differ between the 2 groups. The treatment modalities between the 2 groups were also similar. These results indicated that IL-33 was closely associated with joint inflammation rather

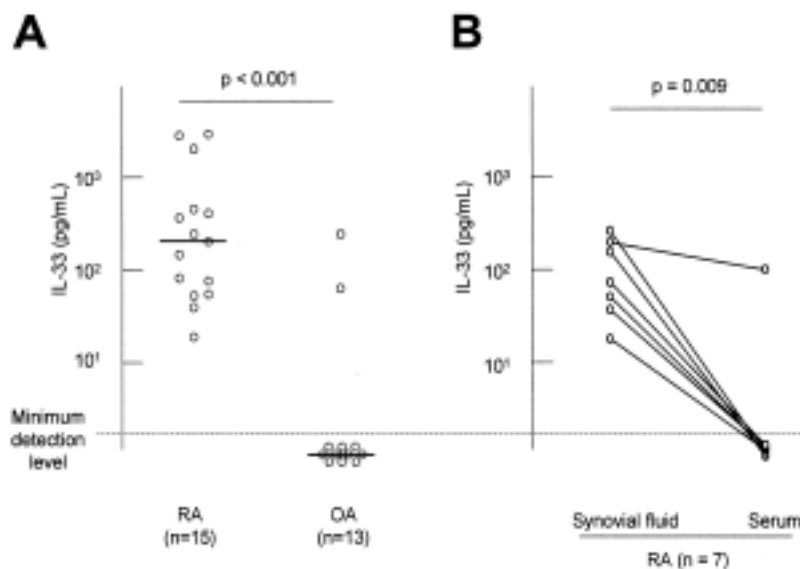
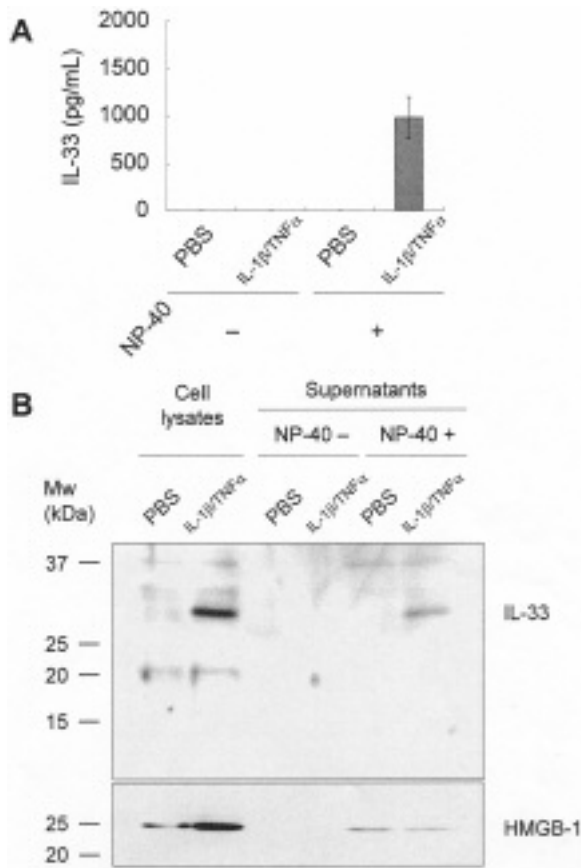


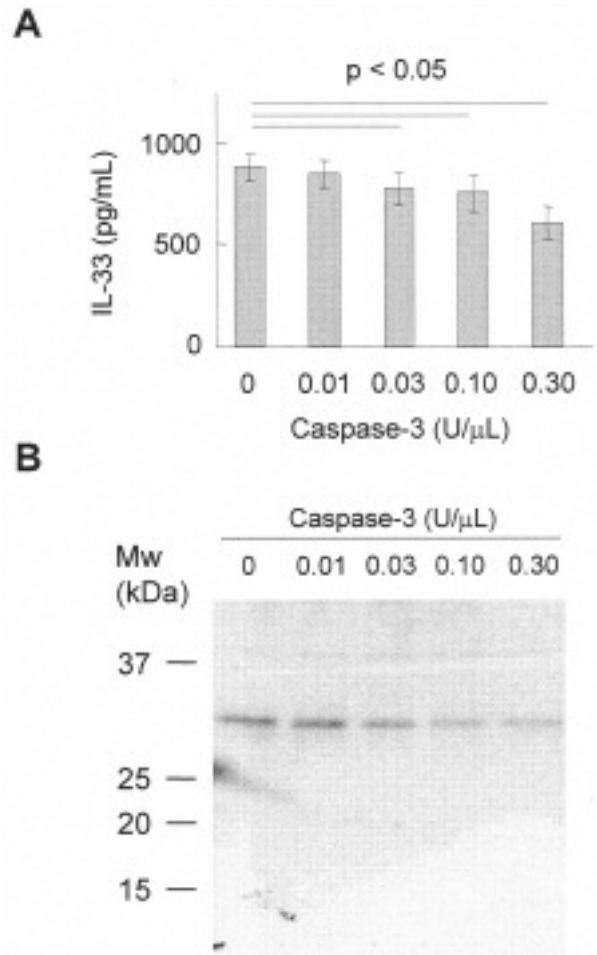
Figure 3. A. Interleukin 33 (IL-33) levels in synovial fluid (SF) samples from patients with rheumatoid arthritis (RA) and osteoarthritis (OA). Symbols represent individual samples and bars represent median IL-33 levels. B. IL-33 levels in sera and SF samples in 7 RA patients evaluated simultaneously.



**Figure 4.** A. Interleukin 33 (IL-33) levels in the culture supernatants of fibroblast-like synoviocytes (FLS) from RA patients. FLS were cultured with IL-1 $\beta$  and tumor necrosis factor (TNF- $\alpha$ ), or phosphate-buffered saline (PBS). After 24 h incubation, FLS were reacted with NP-40+ or not (NP-40-). Values are means and SD of at least 3 independent experiments using 2 FLS lines derived from different donors. B. Immunoblotting for IL-33 and high mobility group box 1 (HMGB-1) in cell lysates or supernatants of FLS. The figure shows representative results of 3 independent experiments.

than reflecting systemic inflammation in RA. On the other hand, IL-33 levels in SF were elevated in all 15 RA patients measured. IL-33 levels were higher in SF than in sera from 7 RA patients measured simultaneously. In 6 of the 7 patients, IL-33 levels of sera were undetectable while those of SF were elevated. Additionally, IL-33 was elevated in the culture supernatants of FLS but undetectable in the culture supernatants of activated PBMC. Thus, IL-33 proteins released into circulation may be mainly produced in inflamed joints.

Recent studies have shown that the IL-33 precursor does not require protease processing to act as a cytokine<sup>7-10</sup>. We therefore evaluated the molecular weight of IL-33 detected by ELISA. In our study, the 30-kDa protein was detected only in the supernatant of FLS stimulated with IL-1 $\beta$  and TNF- $\alpha$  and treated with detergent NP-40. A recent report found that the IL-33 precursor was released from endothe-



**Figure 5.** A. Interleukin 33 (IL-33) levels in the culture supernatants of fibroblast-like synoviocytes (FLS) after reaction with caspase-3. Values are the means and SD of at least 3 independent experiments using 2 FLS lines derived from different donors. B. Immunoblotting for IL-33 in the supernatants of FLS, as representative results of 3 independent experiments.

lial cells only after they were damaged with detergents or physical stress, and it acted as a cytokine by binding to ST2L8. Thus, we believe that IL-33 is released from damaged RA synovial cells as a 30-kDa precursor *in vivo* and functions as a cytokine, in a way similar to high mobility group box 1, which exists in the nucleus of synovial cells and is released into SF after synovial cell damage<sup>20</sup>. We also attempted to detect the molecular weight of IL-33 in sera and SF samples by immunoblotting. Immunoprecipitation with paired anti-human IL-33 antibodies revealed the 30-kDa protein in the serum sample from the RA patient in our study whose serum IL-33 level was the highest, at 10,110 pg/ml. However, probably due to the poor sensitivity of immunoprecipitation, we could not detect any notable bands in other samples, of which serum IL-33 levels were less than 3000 pg/ml (data not shown).

Recent studies have also shown that caspase-3, which is

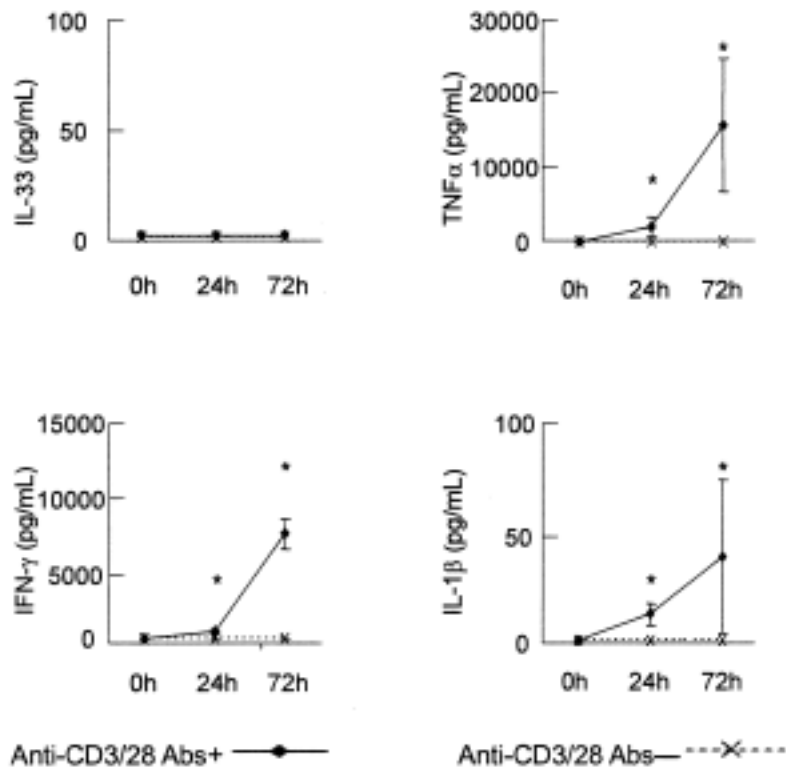


Figure 6. Interleukin 33 (IL-33), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ ), and IL-1 $\beta$  in the culture supernatants of peripheral blood mononuclear cells (PBMC) from RA patients. PBMC were stimulated with anti-CD3/CD28 antibodies (anti-CD3/28 Abs+) or vehicles (anti-CD3/28 Abs-). Values are means and SD of samples from 3 patients. \* $p < 0.005$  vs vehicle.

active in apoptotic cells, inactivates the 30-kDa precursor of IL-33 by cleaving 178 aspartic acid from 179 glycine<sup>8,9</sup>. In our study, the thickness of the 30-kDa precursor band in immunoblotting decreased, and cleaved IL-33 bands were not detected after treatment with caspase-3. The levels of IL-33 in ELISA decreased dose-dependently after caspase-3 treatment. These results indicated that the FLS-derived IL-33 precursor was processed by caspase-3, and the antibodies used in ELISA and immunoblotting did not react with cleaved and inactivated IL-33.

RA is a chronic inflammatory joint disorder involving activation of immune cells, mainly monocytes, Th1, and Th17 cells<sup>21</sup>. However, recent studies have shown that mast cells, which express ST2L and produce inflammatory cytokines through IL-33/ST2L signaling<sup>3,4</sup>, are abundantly expressed in human synovial tissue and play an important role in the pathogenesis of RA<sup>12,22,23</sup>. Our study revealed that IL-33 levels in SF samples were significantly elevated in RA compared to OA, and IL-33 levels in SF samples were higher than those in sera. Therefore IL-33 might act as a cytokine mainly in the inflamed joints by binding to ST2L expressed on synovial mast cells. One possible explanation is that the IL-33 precursor is produced in RA synovial fibroblasts by IL-1 $\beta$  and/or TNF- $\alpha$  signaling, and severe

inflammation induces cell destruction and releases IL-33 in a precursor form. The released IL-33 then binds to the ST2L expressed on the synovial mast cells, producing inflammatory cytokines and exacerbating the inflammatory process in the affected joints.

Our study revealed the existence of IL-33 in sera and SF samples of patients with RA, and may support the importance of IL-33/ST2L signaling in the synovial joints of human RA.

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Table 1. Comparison between patients with detectable and undetectable levels of interleukin 33 in sera, by ELISA. Values are median unless otherwise stated.

	IL-33+, n = 30	IL-33 -, n = 29	p
Age, yrs	56.5 (30–74)	59 (23–92)	0.306
Sex, % female	80.0	82.8	0.785
Disease duration, yrs	6.5 (1–65)	6 (0–27)	0.494
Stage, no. of patients			0.173
I	5	8	
II	6	10	
III	11	4	
IV	8	7	
Class, no. of patients			0.196
1	3	7	
2	16	13	
3	7	9	
4	4	0	
Tender joint count, 0–46 joints	7 (0–36)	3 (0–19)	0.013
Swollen joint count, 0–48 joints	9.5 (0–34)	3 (0–9)	0.001
CRP, mg/dl	1.08 (0.00–15.6)	0.82 (0.00–27.4)	0.264
DAS28-CRP	4.2 (1.5–7.3)	3.3 (1.0–6.6)	0.017
MMP-3, ng/ml	173 (13–663)	119 (10–792)	0.682
IL-5, pg/ml	6.2 (0.0–142.7)	0.1 (0.0–56.1)	0.005
IL-6, pg/ml	25.2 (0.0–836.6)	1.3 (0.0–687.5)	0.123
IL-13, pg/ml	0.0 (0.0–109.1)	0.0 (0.0–6.3)	0.605
TNF- $\alpha$ , pg/ml	4.4 (0.0–332.8)	7.2 (0.0–726.2)	0.520
IL-1 $\beta$ , pg/ml	4.9 (0.0–156.2)	3.0 (0.0–32.0)	0.180
Eosinophil, %*	0.6 (0.0–8.0)	0.8 (0.0–4.7)	0.478
Eosinophil, / $\mu$ l*	23 (0–483)	47 (0–371)	0.713
Treatment			
PSL			
No. patients	24	20	0.330
Mg/day	5 (1.5–10)	5 (2–10)	0.135
MTX			
No. patients	23	20	0.506
Mg/week	8 (2–10)	6 (4–8)	0.249
Biologics			
No. patients			
Infliximab	10	13	0.365
Etanercept	1	3	0.293
Other DMARD,	TAC 5, SASP 3,	TAC 1, CyA 1,	
No. of patients	BUC 3, GST 1	BUC 1, ACT 1	

\* Eosinophil (%) and eosinophil (/ $\mu$ l) were analyzed in the 28 and 24 patients in the IL-33+ and IL-33- groups whose samples were available, respectively. IL: interleukin; IL-33+: patients with detectable levels of IL-33; IL-33-: patients with undetectable levels of IL-33; CRP: C reactive protein; DAS28-CRP: Disease Activity Score 28 based on C-reactive protein; MMP-3: matrix metalloproteinase 3; TNF- $\alpha$ : tumor necrosis factor- $\alpha$ ; PSL: prednisolone; MTX: methotrexate; TAC: tacrolimus; SASP: salazosulfapyridine; CyA: cyclosporin A; BUC: bucillamine; GST: gold sodium thiomalate; ACT: actarit; eosinophil (%): percentage of eosinophils in total white blood cells; eosinophil (/ $\mu$ l): cell numbers of eosinophils in peripheral blood.

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