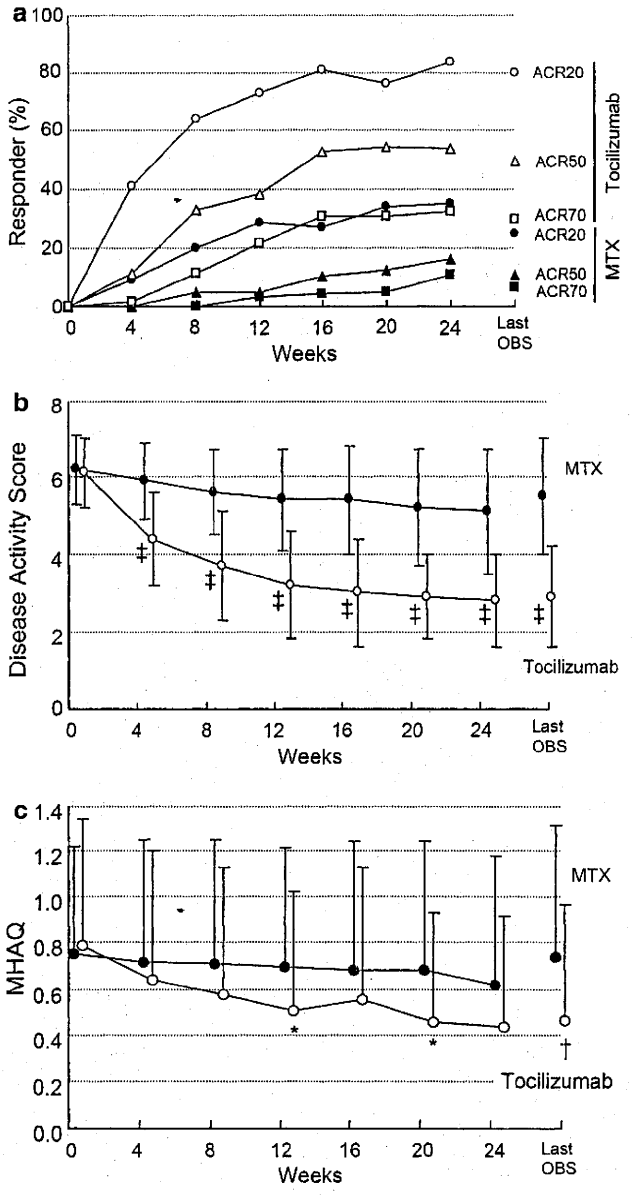


**Fig. 2** Percentage of responders according to the American College of Rheumatology (ACR) improvement criteria and the Disease Activity Score in 28 joints (DAS28) as well as mean change in Modified Health Assessment Questionnaire (MHAQ) scores. Percentage of responders according to the ACR improvement criteria (**a**) and the DAS28 (**b**) according to the ITT analysis over 24 weeks. Mean change in MHAQ scores from baseline to week 24 (**c**). Last *OBS* = last observation. $*P < 0.05$; $\dagger P < 0.01$; $\ddagger P < 0.001$ versus MTX by paired *t*-test

DAS28 than the control group did (Fig. 2b). At the last observation, the incidence of "Good" was 3.2 and 65.5%, and that of "Good or moderate" was 39.7 and 96.6% in the control group and the tocilizumab group, respectively. Similarly, remission was observed in 1.6% of the control group and 43.1% of the tocilizumab group at the last observation, and tocilizumab treatment achieved

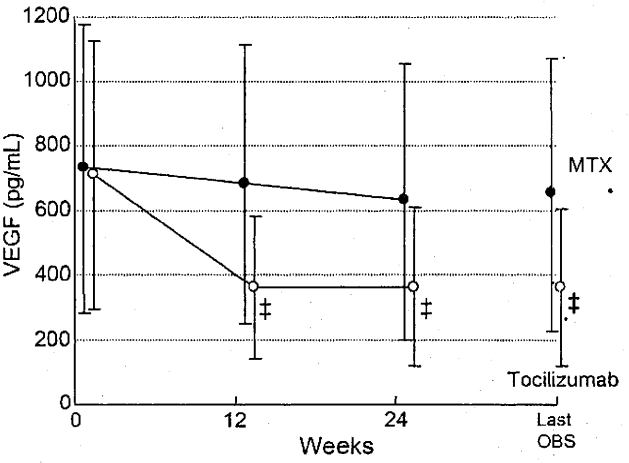


**Fig. 3** Change from baseline in serum levels of VEGF. Values are the mean for each group at each time point. VEGF was measured at three points (0, 12 and 24 weeks) over the study period. *Last OBS* = last observation. $\ddagger P < 0.001$ versus MTX by paired *t*-test

significantly higher remission rates than MTX treatment ($P < 0.001$).

Tocilizumab treatment also significantly improved MHAQ scores compared to MTX treatment (Fig. 2c). A decrease of ≥0.22 units in MHAQ scores represents significant clinical improvement and the minimum clinical important difference [12]. Such improvement was seen in 34% in the control group and 67% in the tocilizumab group at the last observation, demonstrating significant improvement in the tocilizumab group compared to the control group ($P < 0.001$).

The mean serum VEGF levels showed a marked decrease in the tocilizumab group (Fig. 3). Mean changes from baseline were −74.0 pg/ml in the control group and −346.9 pg/ml in the tocilizumab group at week 24 ($P < 0.001$).

## Safety

A total of 104 adverse events occurred in 46 of 64 patients (71.9%) in the control group and 211 adverse events occurred in 56 of 61 patients (91.8%) in the tocilizumab group. Most of the adverse events were mild or moderate. Table 2 shows frequent adverse events observed in at least 5% of the patients. Nasopharyngitis was the most common adverse event in the both groups (the control group 10.9%, the tocilizumab group 18.0%).

Serious adverse events were reported in 4.7% (3 of 64 patients) and 6.6% (4 of 61 patients) in the control group and tocilizumab group, respectively. In the control group, these consisted of one event each of pneumonia, spinal compression fracture and femoral neck fracture, among which a causal relationship with the study drug could not

**Table 2** Adverse events observed in at least 5% of patients

| | Control group ($n = 64$) | Tocilizumab group ($n = 61$) |
|---|---|---|
| Nasopharyngitis | 7 (10.9) | 11 (18.0) |
| Stomatitis | 0 | 7 (11.5) |
| Hyperlipidemia | 1 (1.6) | 4 (6.6) |
| Headache | 2 (3.1) | 4 (6.6) |
| Rash | 2 (3.1) | 4 (6.6) |
| Diarrhea | 1 (1.6) | 4 (6.6) |
| Upper respiratory tract inflammation | 4 (6.3) | 3 (4.9) |

Values are the number (%) of patients

*Tocilizumab* humanized anti-interleukin-6 receptor antibody

be ruled out for pneumonia. In the tocilizumab group, the serious adverse events consisted of one event each of pneumonia, infectious arthritis, colonic polyp and headache, among which a causal relationship with the investigational product could not be ruled out for pneumonia and infectious arthritis. All serious adverse events improved with appropriate treatments.

Tuberculosis was not observed in this study. No tuberculosis screening or prophylactic use of any anti-tuberculosis drugs was done for this study.

Drug-related infusion reactions were reported for eight events in seven patients (11.5%) of the tocilizumab group: two with pruritus, and one each with headache, flushing, rash, arthralgia, abnormal feeling and increased blood pressure. The severity of arthralgia was moderate while all other infusion reactions were mild, and all patients continued the study.

### Laboratory findings

Laboratory test abnormalities were reported in 23 and 56% of patients in the control and the tocilizumab groups, respectively. In the tocilizumab group, lipid metabolism-related changes such as an increase in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDLC) were common. Increases in TC, TG, and LDLC under non-fasting were reported in 36, 20, and 28% of the patients, respectively. Most of them were grade 1 according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC). Values increased until week 4 and thereafter remained constant. High-density lipoprotein cholesterol (HDLC) values were also raised in the tocilizumab group. Therefore, the atherogenic index, calculated by (TC-HDLC)/HDLC, did not change at all throughout the study period of 24 weeks.

Increases in AST, ALT, and total bilirubin were reported in 8, 11, and 0% of the patients in the control group, and 3, 5, and 2% in the tocilizumab group, respectively. These values did not continue to be increased, but became stable at week 16 in the tocilizumab group (Fig. 4). All the patients with these abnormalities in the tocilizumab group were NCI-CTC grade 1 except for 1 patient with grade 2 increase in total bilirubin. There was no patient who withdrew from this study for the reason of liver functional abnormality.

Anti-tocilizumab antibodies were not detected in this study.

## Discussion

This study was a multi-center, randomized, blinded, double-dummy trial of tocilizumab in active RA patients who had an inadequate response to low dose MTX treatment in Japan. The results of this study confirmed the excellent efficacy of tocilizumab monotherapy for signs and symptoms as shown in previous studies [7, 8]. Since MTX is an anchor drug in RA treatment, it is noteworthy that tocilizumab treatment is a very efficacious treatment for the patients with an inadequate response to MTX. In addition, switching MTX therapy to tocilizumab monotherapy was safe and effective.

The dose of MTX prior to enrollment was 10–25 mg/week in CHARISMA study, which was conducted in Europe [13], while all patients in this study were treated with MTX 8 mg/week. The dosage 8 mg of MTX/week in this trial is the maximum dosage approved in Japan. The Japanese government recommends 6–8 mg/week of MTX based on the evidence from the Japanese clinical trials of MTX for RA [14, 15]. The MTX dosage used in Japan is lower than that used in Western populations treated in the EU or US. The average body weight of the patients in this study was 54 kg, and much lower than those of EU and US patients. Additionally, all patients were given folic acid in the CHARISMA study, in contrast to only 51% of the patients in this study. Considering these factors, the differences in the clinical efficacy of MTX between the CHARISMA study and this study might not be so large as expected when looking at the difference in the MTX dose.

Maini et al. demonstrated in the CHARISMA study, that adding MTX to tocilizumab increased the efficacy in terms of ACR50 and ACR70 response rates, although there was no difference in ACR20 response rates between the tocilizumab 8 mg/kg monotherapy and the tocilizumab 8 mg/kg plus MTX. Thus, the combination with MTX may be a therapeutic option, if the toxicity is not increased. In this study, however, even monotherapy with tocilizumab 8 mg/kg induced DAS28 remission at 6 months in about 40% of patients. Furthermore, since anti-tocilizumab antibodies are not detected without use of MTX, the combination with MTX is not required to suppress the emergence of anti-

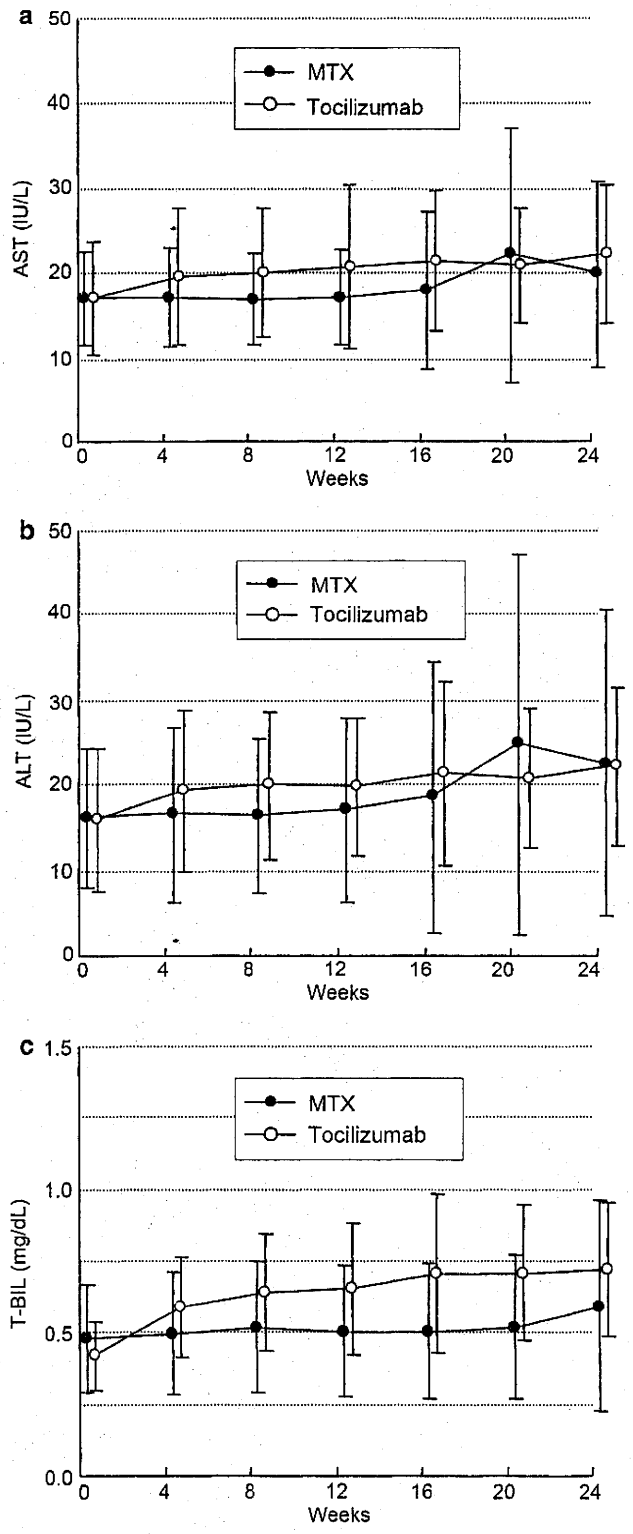


**Fig. 4** Change from baseline in serum levels of aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin (T-BIL). Values are the mean for each group at each time point

tocilizumab antibodies as long as 8 mg/kg of tocilizumab is used. Therefore, tocilizumab will be useful for the patients who do not tolerate MTX.

This double-blind study of tocilizumab also confirms the previous finding that IL-6 receptor inhibition improves serum VEGF levels of RA patients [16]. Serum VEGF levels markedly decreased during tocilizumab therapy compared to the treatment with MTX. VEGF is produced by macrophages, fibroblasts surrounding microvessels, vascular smooth muscle cells, synovial lining cells in synovium [17], neutrophils in synovial fluid [18], and peripheral blood mononuclear cells [19] from patients with RA. VEGF is a potent angiogenic factor and thought to be responsible for the angiogenesis necessary to oxygenate the hypertrophic synovial tissues of the affected joints of RA patients [20, 21]. VEGF also induces vascular permeability and mediates inflammation [22–24]. Therefore, the decrease in VEGF may be an important part of the mechanism how IL-6 receptor inhibition with tocilizumab exerts its therapeutic efficacy in RA. Since serum VEGF levels correlate with disease activity scores and radiographic progression in RA patients [16, 25], the dramatic decrease in VEGF also underlines the efficacy of tocilizumab. The normalization of VEGF by blockade of IL-6 function alone indicates that IL-6 is essential for the VEGF production in this disease.

Tocilizumab monotherapy was generally well tolerated. There was no specific type of infection related to tocilizumab therapy. There is no indication for an increased risk of reactivation of latent tuberculosis, which is often a problem in anti-TNF therapy [26]. In this study patients did not receive prophylactic medication nor were they screened for latent or active tuberculosis at the time of screening.

The increase in TC observed is in concordance with observations in previous studies of tocilizumab [7, 8]. This may be secondary to the improvement in inflammation. Furthermore, there was no cardiovascular adverse event related to the increase in TC. However, further investigation will be required to evaluate whether or not tocilizumab might increase the risk for developing ischemic heart diseases.

The mean value elevations of liver functional tests (AST, ALT and total bilirubin) were seen in the tocilizumab group as well as in the control group, but they were within normal range. Liver functional tests abnormalities were more frequently observed in the control group than in the tocilizumab group. Moreover, most of them were grade 1 according to the NCI-CTC. These abnormalities were clinically not significant and no hepatitis was observed. Therefore, tocilizumab monotherapy appears to be as tolerable as MTX in terms of liver function.

## Conclusion

This study demonstrates that tocilizumab monotherapy in patients with active RA who had an inadequate response to

low dose MTX treatment in Japan has an excellent efficacy with a positive benefit-risk ratio.

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**Conflict of interest statement** NN has served as a consultant to and received honoraria from Chugai Pharmaceutical, the manufacturer of tocilizumab. NN also works as a scientific advisory board of Hoffmann-La Roche. TK holds a patent for tocilizumab. The other authors have no competing interests.

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# Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti–IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease

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**Interleukin-6 (IL-6) plays pathologic roles in immune-inflammatory diseases such as rheumatoid arthritis (RA) and Castleman disease. By inhibiting IL-6 receptors (IL-6Rs), tocilizumab (a humanized anti–IL-6R antibody) ameliorates the symptoms of these diseases and normalizes acute-phase proteins, including C-reactive protein (CRP). We found that tocilizumab treatment increased serum levels of IL-6 and soluble IL-6R (sIL-6R). To investigate the pathologic significance of these increases, we analyzed the kinetics of serum IL-6 and sIL-6R and the proportion of sIL-6R saturated with tocilizumab after tocilizumab administration in patients with RA and Castleman disease and then compared the results with the CRP values. Serum IL-6 and sIL-6R markedly increased after tocilizumab administration in both RA and Castleman disease. As long as free tocilizumab was detectable, sIL-6R was saturated with tocilizumab and IL-6 signaling was completely inhibited. We concluded that it is likely that sIL-6R increased because its elimination half-life was prolonged by the formation of tocilizumab/sIL-6R immune complex, and that free serum IL-6 increased because IL-6R–mediated consumption of IL-6 was inhibited by the unavailability of tocilizumab-free IL-6R. We also concluded that the increased level of free IL-6 during tocilizumab treatment closely reflects the actual endogenous IL-6 production and true disease activity. (Blood. 2008;112:3959-3964)**

## Introduction

Interleukin-6 (IL-6) is a multifunction cytokine that has a wide range of biological activities in various target cells and regulates immune responses, acute phase reactions, hematopoiesis, and bone metabolism.[1] IL-6 signaling is mediated by a unique IL-6 receptor system consisting of 2 functional membrane proteins: an 80-kDa ligand-binding chain (known as IL-6 receptor [IL-6R], IL-6R α-chain, or CD126)[2] and a 130-kDa non–ligand-binding signal-transducing chain (known as glycoprotein 130 [gp130], IL-6R β-chain, or CD130).[3] In cells with sufficient membrane-bound IL-6R, IL-6 binds to these receptors, the IL-6/IL-6R complex induces homodimerization of the gp130 molecule, and a high-affinity functional receptor complex of IL-6, IL-6R, and gp130 is formed.[4] In cells that do not express sufficient cell-surface IL-6R, IL-6 signal transduction starts with the binding of IL-6 to the free soluble form of IL-6R (sIL-6R), which lacks the membrane and intracytoplasmic portion of the 80-kDa membrane-bound IL-6R molecule.[3,4] Thus, either membrane-bound or soluble IL-6R can mediate IL-6 signal into cells, as long as they express gp130. Considerable amounts of sIL-6R are observed in serum and body fluids,[5,6] and sIL-6R may play physiologic roles as well as its pathologic role in immune-inflammatory and malignant diseases.[7]

Because IL-6 plays important physiologic roles, deregulated overproduction of IL-6 causes various pathologic conditions, including autoimmune, inflammatory, and lymphoproliferative disorders. It has been shown that IL-6 is involved in immune-inflammatory diseases such as rheumatoid arthritis (RA), Castleman disease, juvenile idiopathic arthritis (JIA), and Crohn disease.[8-11] Elevated serum IL-6 has been observed in patients with these diseases and the IL-6 levels correlate with disease activity,[5,10,12-14] although there are differences in IL-6 levels among the diseases.

Tocilizumab is a humanized anti-human IL-6R antibody engineered by grafting the complementarily determining regions of a mouse anti–human IL-6R antibody into human IgG1κ to create a human antibody with a human IL-6R binding site.[15] Tocilizumab binds to the IL-6 binding site of human IL-6R and competitively inhibits IL-6 signaling. A series of clinical studies have shown that inhibition of IL-6 signaling by tocilizumab is therapeutically effective in RA, JIA, Castleman disease, and Crohn disease.[16-20] In all of these diseases, tocilizumab ameliorates inflammatory manifestations and normalizes acute phase protein levels, including C-reactive protein (CRP), thus confirming the observation that IL-6 is essential for the production of CRP.

It was noticed that both serum IL-6 and serum sIL-6R increased in patients after administration of tocilizumab while the disease symptoms continued to be ameliorated, but the mechanisms of these increases and the pathologic significances of the increased levels remained obscure.

The objective of this study, therefore, was to elucidate the mechanisms and the pathologic significances of the increases in serum IL-6 and serum sIL-6R that occur when IL-6 signaling is inhibited by tocilizumab. To achieve this, we analyzed serum and blood samples collected in the previous clinical studies, and considered the results in combination with laboratory data already obtained in those studies.

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## Methods

### Participants and tocilizumab treatment methods

The serum and blood samples and laboratory data analyzed in this study were from the following people: (1) 20 healthy adult volunteers who received tocilizumab in a phase 1 study (N.N. and T.K., unpublished data, November 1998); (2) 15 patients with RA who were treated with tocilizumab in a phase 1/2 study in RA;[16] (3) 18 patients with RA who were treated with tocilizumab in a clinical pharmacology study in RA (K.T., T. Tsuru, M. Suzaki, T. Amamoto, H. Nakashima, S. Higuchi, T.K., and N.N., unpublished data, August 2008); and (4) 28 patients with Castleman disease who were treated with tocilizumab in a phase 2 study in Castleman disease.[19] All of these clinical studies were approved by the Japanese Ministry of Health, Labor and Welfare and also by the independent ethics committees of the respective medical institutions. Written informed consent was obtained from the patients before enrollment in accordance with the Declaration of Helsinki.

The designs of these studies were as follows: (1) phase 1 study—a randomized, single-blind, placebo-controlled, intersubject dose-escalation study in which healthy adult men received 0.15, 0.5, 1, or 2 mg/kg tocilizumab intravenously over 2 hours (n = 5/cohort), and safety, tolerability and pharmacokinetics were evaluated; (2) phase 1/2 study in RA[16]—an open-label trial in which 15 patients with active RA received 2, 4, or 8 mg/kg tocilizumab intravenously over 2 hours every 2 weeks for 6 weeks (n = 5/cohort), and safety, tolerability, pharmacokinetics, and efficacy at each dosage level were assessed on days 0, 14, 28, and 42. CRP and erythrocyte sedimentation rate (ESR) were measured as markers of IL-6R inhibition; (3) clinical pharmacology study in RA—an open-label single-dose study in which 18 patients with baseline CRP of at least 1.5 mg/dL received 8 mg/kg tocilizumab intravenously over 1 hour, and drug-disease interaction was assessed; (4) phase 2 study in Castleman disease[19]—an open-label study in which 28 patients with active Castleman disease received 8 mg/kg tocilizumab intravenously over 1 hour every 2 weeks for 16 weeks (8 times in total), and improvement in disease activity was assessed based on biochemical markers such as CRP, hemoglobin, and serum albumin, and on general fatigue (visual analog scale).

Tocilizumab is a humanized anti–human IL-6R monoclonal antibody of the IgG1κ subtype, and was supplied by Chugai Pharmaceutical (Tokyo, Japan).

### Determination of free tocilizumab in serum

Serum-free tocilizumab was determined by enzyme-linked immunosorbent assay (ELISA). In brief, 100 μL human sIL-6R (SR-344, 1 μg/mL; Chugai Pharmaceutical) was added to the wells of an immunoplate precoated with mouse anti–human IL-6R antibody (MT-18; Chugai Pharmaceutical), and the plate was incubated at room temperature for 2 hours. After washing, 100 μL of 1000-fold diluted serum specimen was applied to the plate, and the plate was incubated for another 2 hours to bind the free tocilizumab to the plate. After washing, the bound tocilizumab was measured using biotin-labeled goat anti–human IgG antibody, followed by development with avidin-labeled alkaline phosphatase and p-nitrophenyl phosphate substrate. The colorimetric reaction was quantified by measuring the absorbance at 405 nm (and at 490 nm as reference) using a microplate reader. The concentration of free tocilizumab in the specimen was calculated from a calibration curve prepared from the absorbance of calibration standard solutions. The lower detection limit for free serum tocilizumab was 1 μg/mL. All serum samples were stored below −20°C, and the assay was applied within 4 weeks after drawing.

### Determination of IL-6 in serum

Serum IL-6 was determined by chemiluminescent enzyme immunoassay (CLEIA)[21] using a Human IL-6 CLEIA Fujirebio (Fujirebio, Tokyo, Japan), which detects IL-6 whether it is free or bound to sIL-6R. In brief, a mixture of 20 μL of serum sample and the alkaline phosphatase–conjugated mouse anti–human IL-6 monoclonal antibody included in the kit was incubated at 37°C for 10 minutes and then added to particles covalently linked to another murine anti–human IL-6 monoclonal antibody that recognizes an epitope different from that recognized by the original antibody. After incubation for 10 minutes at 37°C, the particles were separated magnetically and washed in buffer. Subsequently, an enhanced luminol/peroxide substrate solution containing 3-(2′-spiroadamantane)-4-methoxy-4-(3′-phosphoryloxy)phenyl-1,2-dioxetane disodium salt was added at 37°C, and after 5 minutes the chemiluminescence was measured using a photon counter (Lumipulse 1200′ Fujirebio). The lower detection limit for serum IL-6 was 0.1 pg/mL. All serum samples were stored below −20°C, and the assay was applied within 4 weeks after drawing.

### Determination of sIL-6R in serum

Serum sIL-6R was determined by ELISA using a Quantikine Human IL-6sR kit (R&D Systems, Minneapolis, MN). We preliminarily examined and found that this assay kit could detect 3 forms of sIL-6R: sIL-6R free from IL-6 or tocilizumab, sIL-6R in a complex of IL-6/sIL-6R, and sIL-6R in an immune-complex of tocilizumab/sIL-6R (data not shown). In brief, sIL-6R in the sample was bound to a murine anti–IL-6R monoclonal antibody–coated microtiter plate and incubated with a horseradish peroxidase (HRP)–conjugated polyclonal anti–IL-6R antibody at room temperature for 2 hours. To determine the quantity of peroxidase bound, the plate was incubated with tetramethylbenzidine at room temperature for 20 minutes. The colorimetric reaction was quantified by measuring the absorbance at 450 nm (and at 540 nm as reference) using a microplate reader and the concentration of sIL-6R in the sample was calculated as described in "Determination of free tocilizumab in serum." All serum samples were stored below −20°C, and applied the assay within 4 weeks after drawing.

### Determination of the proportions of tocilizumab-bound and tocilizumab-free sIL-6R in serum

Tocilizumab-bound sIL-6R and tocilizumab-unbound sIL-6R in serum samples were separated by gel filtration chromatography in a Superdex 200HR 10/30 column (300 × 10 mm internal diameter [ID]; GE Healthcare Bio-Science AB, Uppsala, Sweden) at 4°C, and the amount of sIL-6R in each fraction was determined. The eluent was phosphate-buffered saline with 0.05% Tween 20, and the fraction volume was 0.5 mL.

### Determination of IL-6 mRNA expression

In the clinical pharmacology study in RA (study 3), peripheral blood for measurement of IL-6 mRNA was drawn from 18 patients at 4 time points: 7 days and immediately before, and 14 and 35 days after administration of 8 mg/kg tocilizumab.

Total RNA was extracted from the blood samples using a PAXgene Blood RNA Kit (QIAGEN, Valencia, CA) and quantified spectrophotometrically by measuring absorbance at 260 nm. The purity and integrity of the RNA extracted from each PAXgene Blood RNA tube were estimated by the ratio of 28S to 18S ribosomal RNA using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA samples that showed severe degradation were excluded from further analysis. Expression of IL-6 mRNA was measured using a DNA microarray (Human oligo chip; Hitachi Software Engineering, Tokyo, Japan). After amino allylRNA (aRNA) was synthesized from 1 μg total RNA using the Amino Allyl Message Amo aRNA kit (Ambion, Austin, TX), the Cy3 and Cy5 dyes were then used to label RNA sequences. Human peripheral blood leukocyte total RNA purchased from BD Biosciences Clontech (San Jose, CA) was used as a universal control (reference RNA) and labeled with Cy3 in all the microarray experiments so that the ratio (Cy5/Cy3) means the relative expression level to the reference in the time course experiment series. The ratio (relative expression level) was plotted against the sampling time. All RNA samples were stored below −80°C until applied the assay. The DNA microarray dataset is available at the Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo) under the accession number GSE12653.

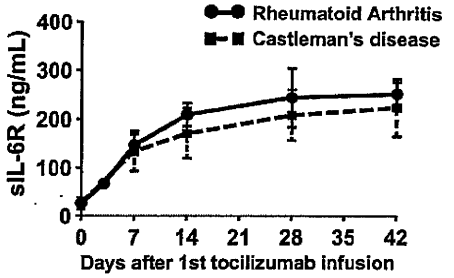


**Figure 1. Serum sIL-6R after tocilizumab infusion.** Tocilizumab was administered to patients with RA (●; n = 5) or Castleman disease (■; n = 28) on days 0, 14, and 28 at a dose of 8 mg/kg. Points and error bars show geometric means plus or minus SDs.

### Determination of CRP

CRP was determined in all clinical studies in which the serum was collected. CRP levels were measured at each clinical study site.

## Results

### Elevation of serum sIL-6R after administration of tocilizumab

When patients with RA or Castleman disease were treated with 8 mg/kg tocilizumab once every 2 weeks, serum sIL-6R increased markedly in patients with a detectable serum tocilizumab concentration (1 μg/mL or more) and reached a steady state at day 42 of treatment (RA: 27.7 ± 4.4 ng/mL at baseline, 251.4 ± 24.7 ng/mL at day 42; Castleman disease: 26.4 ± 11.6 ng/mL at baseline, 224.2 ± 58.3 ng/mL at day 42; Figure 1). There was no significant difference between patients with RA and patients with Castleman disease with respect to either serum sIL-6R at baseline or serum sIL-6R at day 42. In the healthy individuals, increase in serum sIL-6R was transient because of single administration of tocilizumab (Figure 2A).

### Relationship between free tocilizumab and the proportions of tocilizumab-free and tocilizumab-bound sIL-6R in serum

Total serum sIL-6R peaked 14 days after a single administration of 2 mg/kg tocilizumab to healthy individuals in the phase 1 study. Serum sIL-6R was tocilizumab-free before administration of tocilizumab, but after administration of tocilizumab, more than 95% of the sIL-6R molecules were bound in a sIL-6R/tocilizumab immune complex as long as the free tocilizumab concentration remained detectable in serum (at least 1 μg/mL). Representative data are shown in the Figure 2A. Similar data were obtained in the sIL-6R, which increased after repetitive administration of tocilizumab to patients with RA in the phase 1/2 study (Figure 2B). When free tocilizumab concentration fell below the detection limit in serum, the level of sIL-6R/tocilizumab complex decreased, and the proportion of free sIL-6R increased (Figure 2A,B).

### Relationship between free tocilizumab and CRP level in serum

CRP is a representative acute-phase reactant, and change in CRP correlates with severity of inflammation. It has been shown that inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor induce the production of CRP by hepatocytes, and that IL-6 is essential for the production of CRP.[22]

Tocilizumab normalized the CRP level in patients with RA in the phase 1/2 study[16] as long as the free tocilizumab, which is capable of binding IL-6R and of inhibiting IL-6 actions, remained

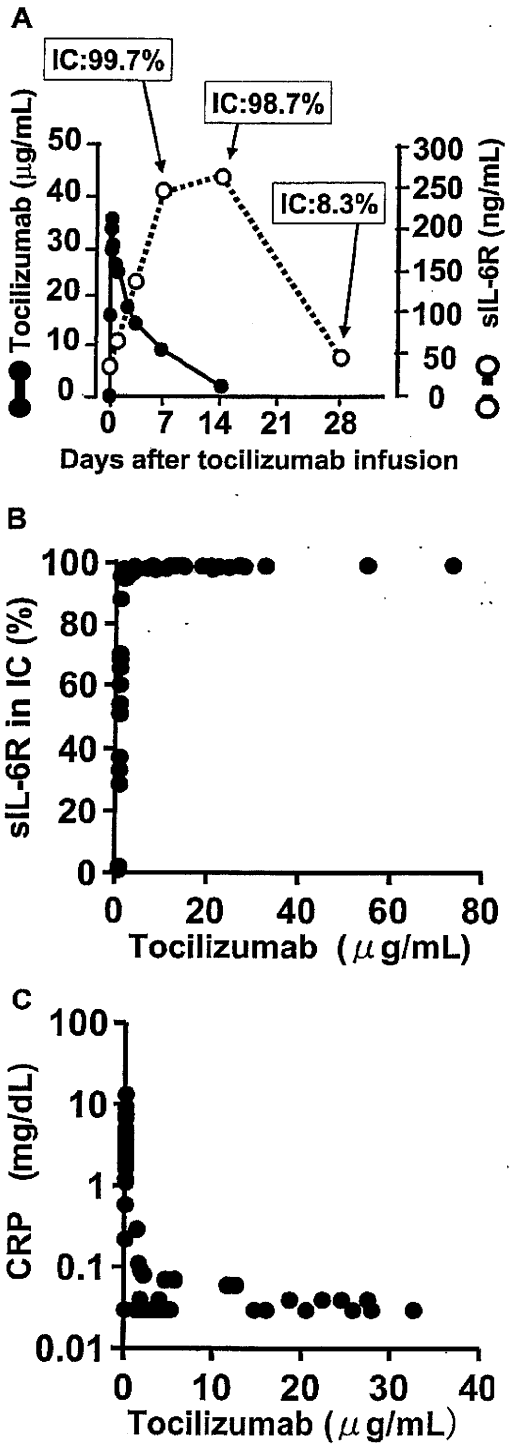


**Figure 2. Relationships between free tocilizumab, sIL-6R, percentage of sIL-6R bound to tocilizumab, and CRP in serum.** (A) Relationship between serum free tocilizumab (●), serum sIL-6R (○), and the percentage of sIL-6R bound to tocilizumab in an immune complex (IC). Serum sIL-6R (○) includes all sIL-6R: free, bound to tocilizumab, and bound to IL-6. Increased sIL-6R after tocilizumab infusion formed an immune complex with tocilizumab. Almost all sIL-6R was bound to tocilizumab while serum-free tocilizumab was detectable (1 μg/mL or more). This figure shows a representative data from the phase 1 study in healthy individuals. (B) Relationship between serum tocilizumab and percentage of sIL-6R bound to tocilizumab (phase 1/2 study in RA). More than 95% of sIL-6R was bound to tocilizumab while serum-free tocilizumab remained 1 μg/mL or more. (C) Relationship between serum tocilizumab and CRP. Serum CRP was normalized as long as the free tocilizumab concentration remained 1 μg/mL or more (phase 1/2 study in RA). The sensitivity of CRP assay in the present study was 0.03 mg/dL, and the normal range was less than 0.2 mg/dL.

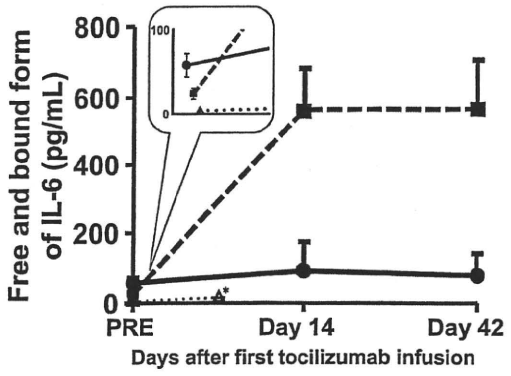


**Figure 3. Change in serum IL-6 after administration of tocilizumab.** Serum IL-6 increased after tocilizumab infusion and reached steady state at different levels in patients with RA (●; n = 5), patients with Castleman disease (■; n = 28), and healthy volunteers (△; n = 4). Points and error bars show geometric means plus or minus SEs. *Samples from healthy volunteers were drawn on day 7.

above 1 μg/mL in serum (Figure 2C). This shows that tocilizumab effectively inhibits IL-6 signaling when it is detectable in serum.

### Serum IL-6 after administration of tocilizumab

Serum IL-6 concentrations after a single dose of tocilizumab in healthy adult volunteers after the start of dosing (once every 2 weeks) in patients with RA, and in patients with Castleman disease were compared. At baseline, serum IL-6 was significantly higher in RA and Castleman disease than the normal range (< 4 pg/mL). Note that serum IL-6 was significantly higher in RA than in Castleman disease (58.4 ± 13.8 pg/mL vs 24.5 ± 6.5 pg/mL [geometric mean ± SE]; $P < .05$), yet serum CRP was significantly higher in Castleman disease than in RA (8.7 ± 5.0 mg/dL vs 5.4 ± 1.7 mg/dL; $P < .05$) and serum IgG was also higher in Castleman disease than in RA (5220 ± 1957 mg/dL vs 1516 ± 409 mg/dL; $P < .05$).

In healthy volunteers, serum IL-6 showed a significant increase at day 7 after tocilizumab administration (3.0 ± 0.6 pg/mL at baseline, 9.3 ± 1.0 pg/mL at day 7). In patients with RA, serum IL-6 showed a greater increase at day 14 and had not changed at day 42 (58.4 ± 13.8 pg/mL at baseline, 92.8 ± 82.4 pg/mL at day 14, 89.7 ± 63.7 pg/mL at day 42; n = 5). In patients with Castleman disease, serum IL-6 showed an even more significant increase at day 14, even though it was significantly lower in Castleman disease than in RA at baseline, and had not changed at day 42 (24.5 ± 6.5 pg/mL at baseline, 564.8 ± 127.6 pg/mL at day 14, 567.0 ± 141.0 pg/mL at day 42; n = 28; RA vs Castleman disease: $P < .001$ at day 14 and at day 42; Figure 3). These data clearly indicate that the degree of increase in serum IL-6 after IL-6R inhibition is not the same in RA and Castleman disease.

Furthermore, there observed much stronger correlation between the CRP at baseline and the serum IL-6 increasing after IL-6R blockade than that between the CRP at baseline and the serum IL-6 before IL-6R blockade in patients with RA and in Castleman disease (Figure 4).

### IL-6 mRNA expression in peripheral blood cells before and after administration of tocilizumab

In order to know whether the IL-6R inhibition augmented the IL-6 production through the elimination of possible negative feedback by IL-6 on IL-6 production, changes in IL-6 mRNA expression after tocilizumab administration in peripheral blood cells were examined. However, there was no significant difference in IL-6 mRNA expression at these 4 sampling time points, so administration of tocilizumab did not increase the production of IL-6 (Figure 5).

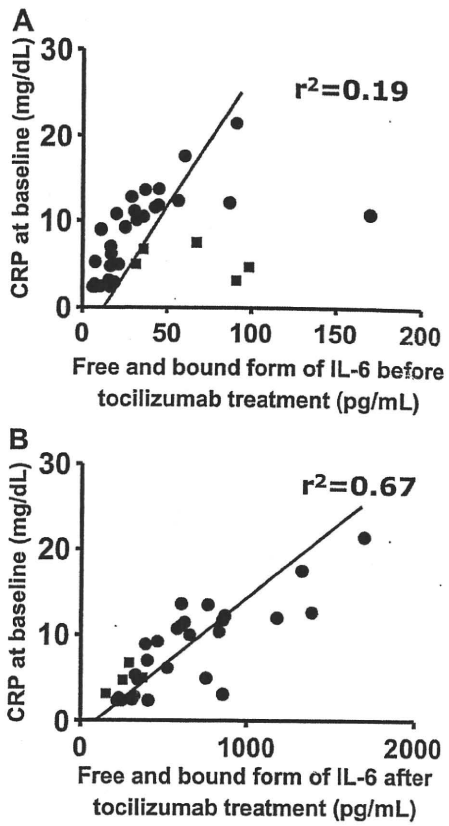


**Figure 4. Correlation between serum CRP at baseline and serum IL-6 at baseline or after tocilizumab administration.** ■ and ● show patients with RA and patients with Castleman disease, respectively. The x-axis shows (A) serum IL-6 at baseline ($r^2 = .19$) and (B) serum IL-6 after tocilizumab infusion ($r^2 = .67$).

## Discussion

This study demonstrates that IL-6R inhibition with tocilizumab results in an increase in the levels of serum IL-6 and serum sIL-6R. The normalization of serum CRP, however, shows that IL-6 signaling was inhibited as long as free tocilizumab was detectable in serum. This matches the finding that a very high percentage of

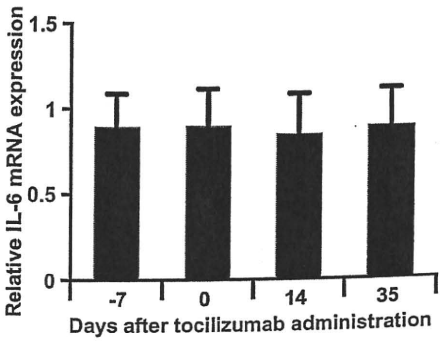


**Figure 5. Relative expression of IL-6 mRNA.** The relative expression levels of IL-6 mRNA compared with the reference calculated by the logarithm base 2 of ratio (Cy5/Cy3) at each time point were plotted. Expression of IL-6 mRNA in peripheral blood cells from 18 patients with RA was measured using a DNA microarray before and after administration of tocilizumab. Bars and error bars show means plus SEs.

serum sIL-6R (> 95%) was bound to tocilizumab while free tocilizumab was detectable in serum. Since CRP is mainly produced by hepatocytes, which express cell-surface IL-6R, membrane-bound IL-6R would be also fully occupied by tocilizumab. CRP is therefore a useful surrogate marker for tocilizumab levels that are high enough to inhibit the effects of IL-6 in patients.

Since immune complex formation of antigen and antibody prolongs the elimination half-life of antigen in serum,[23] the increase in serum sIL-6R seen after administration of tocilizumab is probably due to the formation of a sIL-6R/tocilizumab immune complex. This hypothesis is supported by the decrease in the C3, C4, and CH50 levels after tocilizumab administration that we observed in another study (data not shown) because complement factors are consumed during the elimination process of immune complexes.[23]

It is noteworthy that there was no difference between RA and Castleman disease with respect to serum sIL-6R either at baseline or after tocilizumab administration. This suggests that there is no difference in sIL-6R production between these 2 diseases.

For serum IL-6, on the other hand, the level after tocilizumab administration differed greatly between the diseases as well as between individual patients (Figures 3,4). This suggested to us that the degree of increase in serum IL-6 after inhibition of IL-6R by tocilizumab may reflect different levels of endogenous IL-6 production in these diseases and in individual patients.

The serum IL-6 level depends on the balance between IL-6 production and elimination, so tocilizumab could potentially have caused serum IL-6 to increase either by stimulating production or by inhibiting elimination. One possibility is that tocilizumab might stimulate the production of IL-6 if its blockade of IL-6 signaling inhibits a negative feedback effect of IL-6 on IL-6 production. This seems unlikely, however, because serum IL-6 did not continue to increase but remained steady between day 14 and day 42, and because there was no significant increase in IL-6 mRNA expression in peripheral blood cells after administration of tocilizumab. The relevance of the latter observation may be limited, however, by the fact that we did not examine IL-6 mRNA expression in cells of the affected joints of patients with RA or the affected lymph nodes of patients with Castleman disease, which are important sources of IL-6 in these diseases.

Another possible explanation for the increase in serum IL-6 after tocilizumab administration is that tocilizumab may inhibit the clearance of IL-6 from serum. There are 2 possible elimination pathways of IL-6 from serum: one is receptor-mediated clearance via the binding of IL-6 to IL-6R; the other is direct degradation of IL-6 protein. The main elimination pathway may be receptor-mediated clearance. If so, this would explain why free IL-6 accumulates in serum when IL-6R is occupied by tocilizumab. IL-6 levels reach a steady state when the IL-6 production rate matches the IL-6 degradation rate.

We would like to explain this mechanism of action with the help of the schematic bathtub model illustrated in Figure 6. In this model, endogenous IL-6 production is represented by the stream of water flowing from the faucet into the tub at a constant rate that depends on the level of true disease activity. Receptor-mediated IL-6 clearance is represented by water flowing out of the bathtub drain. Direct degradation of IL-6 is represented by minor water flowing out from the side small drain in this figure. When the bathtub drain is plugged, the water level will depend on the flow rate from the faucet. Likewise, when IL-6R is inhibited by tocilizumab, the serum IL-6 level will reflect the actual level of endogenous IL-6 production that correlates with the level of true

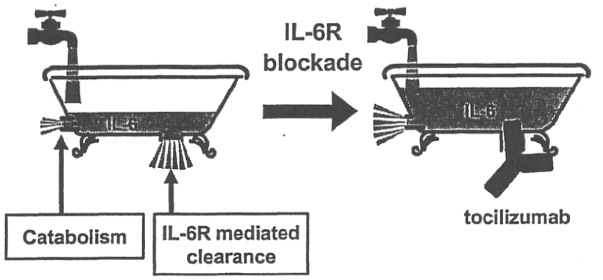


**Figure 6. Schematic model of the mechanism by which serum IL-6 is increased when IL-6 receptor is blocked by tocilizumab.** The bathtub model explains the elimination of IL-6 from serum before and after administration of tocilizumab. The rate of water flowing from the faucet into the tub (the IL-6 production rate) remains constant. Before tocilizumab administration, the rate of water flowing out of the bathtub (the elimination of receptor-bound IL-6 from serum and IL-6 catabolism) is also constant, so the water level (serum IL-6 level) remains constant. In the second diagram, the flow of IL-6 from the bathtub is greatly restricted by a "plug" (IL-6R–mediated elimination is inhibited by tocilizumab). The water level increases and then remains constant at a higher level (serum IL-6 increases to a new steady-state level when the IL-6 production rate matches the IL-6 degradation rate).

disease activity while inflammatory symptoms are ameliorated by the inhibition of IL-6 signaling through IL-6R.

In practice, the correlation between CRP (an indicator of resultant inflammation) at baseline and serum IL-6 level after administration of tocilizumab was much closer than that between CRP at baseline and serum IL-6 level before tocilizumab administration. Furthermore, serum IL-6 was much higher in patients with Castleman disease than in patients with RA after tocilizumab administration, even though serum IL-6 in patients with Castleman disease was lower than that in patients with RA at baseline. The difference in increased IL-6 level between RA and Castleman disease after tocilizumab treatment closely reflected the difference between RA and Castleman disease in baseline inflammatory activity and in laboratory abnormalities such as increased CRP and IgG values, whereas the IL-6 levels before tocilizumab treatment did not. The fact that IL-6 was lower in Castleman disease than in RA at baseline indicates that the elimination of serum IL-6 is much faster in Castleman disease than in RA without tocilizumab treatment, and the fact that this faster elimination in Castleman disease was greatly slowed by tocilizumab suggests that it is receptor-mediated elimination.

We conclude that the serum IL-6 level during inhibition of IL-6R by tocilizumab represents the actual endogenous production of IL-6 and the true disease activity of patients with different diseases much better than the serum IL-6 level before tocilizumab treatment. If the causal factors of IL-6 overproduction are neutralized by adequate therapy (the faucet of the bathtub is closed), the serum IL-6 level will decrease by natural protein degradation. Decrease in serum IL-6 during tocilizumab treatment may therefore indicate disease remission and may allow us to safely discontinue tocilizumab treatment without the risk of an acute flare. This idea remains to be confirmed in future studies.

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## Authorship

Contribution: N.N. wrote the manuscript; N.N. and K.T. planned the study and wrote the study protocol; N.N., T.M., and H.N. treated and documented the patients; K.T., N.T., and T.K. organized and monitored the study; and K.T. performed the statistical evaluation.

Conflict-of-interest disclosure: K.T., N.T., and T.K. are employees of Chugai Pharmaceutical, whose product (tocilizumab) was studied in the present work. N.N. and T.M. received a consulting fee as medical advisers from Chugai Pharmaceutical. N.N. is on the scientific advisory board of Hoffmann-La Roche. H.N. declares no competing financial interests.

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# Long-term safety and efficacy of tocilizumab, an anti-IL-6 receptor monoclonal antibody, in monotherapy, in patients with rheumatoid arthritis (the STREAM study): evidence of safety and efficacy in a 5-year extension study

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## ABSTRACT

**Objectives:** To evaluate the safety and efficacy of 5-year, long-term tocilizumab monotherapy for patients with rheumatoid arthritis.

**Methods:** In an open-label, long-term extension trial following an initial 3-month randomised phase II trial, 143 of the 163 patients who participated in the initial blinded study received tocilizumab monotherapy (8 mg/kg) every 4 weeks. Concomitant therapy with non-steroidal anti-inflammatory drugs and/or oral prednisolone (10 mg daily maximum) was permitted. All patients were evaluated with American College of Rheumatology (ACR) improvement criteria, disease activity score (DAS) in 28 joints, and the European League Against Rheumatism response, as well as for safety issues.

**Results:** 143 patients were enrolled in the open-label, long-term extension trial and 94 (66%) patients had completed 5 years as of March 2007. 32 patients (22%) withdrew from the study due to adverse events and one patient (0.7%) due to unsatisfactory response. 14 patients withdrew because of the patient's request or other reasons. The serious adverse event rate was 27.5 events per 100 patient-years, with 5.7 serious infections per 100 patient-years, based on a total tocilizumab exposure of 612 patient-years. Of the 88 patients receiving corticosteroids at baseline, 78 (88.6%) were able to decrease their corticosteroid dose and 28 (31.8%) discontinued corticosteroids. At 5 years, 79/94 (84.0%), 65/94 (69.1%) and 41/94 (43.6%) of the patients achieved ACR20, ACR50, and ACR70 improvement criteria, respectively. Remission defined as DAS28 less than 2.6 was achieved in 52/94 (55.3%) of the patients.

**Conclusion:** In this 5-year extension study, tocilizumab demonstrated sustained long-term efficacy and a generally good safety profile.

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by persistent synovitis and progressive joint damage.[1] Although the causes of RA are not fully understood, constitutive overproduction of IL-6, a multifunctional cytokine that regulates the immune response, inflammatory reaction and bone metabolism, is thought to play a major pathological role in RA.[2]

Tocilizumab is a humanised anti-human IL-6 receptor monoclonal antibody,[2] which has been demonstrated to improve the signs and symptoms of RA[3–9] and prevent radiographic progression[10] in previous clinical trials. Those controlled trials provided evidence for a rapid reduction in disease activity in response to tocilizumab in patients with active RA as measured by American College of Rheumatology (ACR) responses, disease activity scores (DAS) and a modified health assessment questionnaire (MHAQ).[5–9] The efficacy was dose related and 8 mg/kg tocilizumab provided a marked clinical benefit. The success in the treatment of patients with RA using tocilizumab confirmed that IL-6 plays an important pathological role in RA, and further studies were therefore required to determine the long-term safety and efficacy of tocilizumab treatment. We report here the safety and efficacy of tocilizumab in a 5-year long-term extension study.

## METHODS

### Patients

This study was registered with http://www.clinicaltrials.gov (NCT00144651). The study protocol was approved by the Ministry of Health, Labor and Welfare of Japan and by the ethical committee of each institute, and patients gave their written informed consent.

The eligibility criteria and the study design of the initial 12-week, randomised, double-blind, placebo controlled study have been reported previously.[5] Briefly, eligible patients were 20 years of age or older and fulfilled the 1987 criteria for RA of the American Rheumatism Association[11] with a disease history of longer than 6 months. All subjects had been insufficient responders to treatment with at least one disease-modifying anti-rheumatic drug (DMARD) or immunosuppressant. Patients had active disease at the time of enrollment into the initial controlled trial, as defined by the presence of six or more swollen joints, six or more tender joints and one of the following two criteria: a Westergren erythrocyte sedimentation rate (ESR) of at least 30 mm/h or a C-reactive protein (CRP) level of more than 1.0 mg/dl. Patients receiving prednisolone (10 mg daily maximum) and/or non-steroidal anti-inflammatory drugs (NSAID) were eligible if the dose had not increased during the washout period of 1 month. Doses of both medications remained stable during the blinded study period of 12 weeks. Patients who had received tocilizumab or placebo twice or more were given the opportunity to receive tocilizumab in this open-label extension trial.

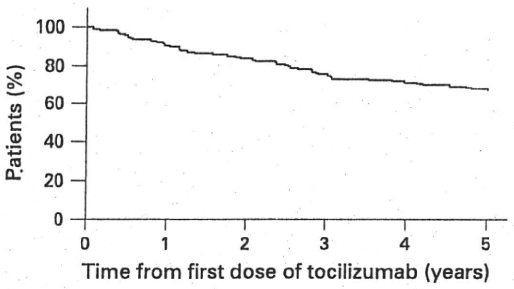


**Figure 1** Kaplan–Meier estimate of the probability of the patients remaining on study. Treatment time was calculated beginning with the first infusion of tocilizumab at any dose, excluding the time receiving placebo.

In the extension study, the use of prednisolone (10 mg daily maximum) and one NSAID was permitted. Sexually active premenopausal women were required to have a negative urine pregnancy test at entry and to use effective contraception during the study period.

### Treatment

Patients were randomly assigned to receive either placebo, or 4 or 8 mg/kg body weight of tocilizumab every 4 weeks in the initial blinded 12-week trial. In the first 12 weeks of the open-label extension study, patients received 8 mg/kg tocilizumab every 4 weeks and thereafter dose reduction and treatment interval changes (minimum 2 weeks) were allowed.

### Efficacy assessments

Disease activity was assessed at baseline and at every visit during the initial blinded trial and the first 12 weeks of the extension study, and thereafter every 3 months. All patients were evaluated with ACR improvement criteria, DAS28 and the European League Against Rheumatism response. The DAS28 was calculated using the ESR. Clinical assessments included the following: complete counts of swollen and tender joints (49 joints evaluated; cervical spine and hips evaluated only for tenderness); physician's and patient's global assessment of disease status, on a visual-analogue scale from 0 (asymptomatic) to 100 (severe symptoms); patient's assessment of pain on a scale from 0 (no pain) to 100 (severe pain); functional disability measured with a MHAQ; ESR and CRP levels.[12] Treatment time was calculated beginning with the first infusion of tocilizumab, excluding the time receiving placebo.

### Safety assessments

Safety was assessed for all patients who received at least one dose of tocilizumab in the extension study. Serious adverse events (SAE) were defined as events that were fatal or life-threatening, leading to permanent or significant disability or incapacity, a congenital anomaly or birth defect, or requiring prolonged inpatient hospitalisation. Adverse events were classified using the Medical Dictionary for Drug Regulatory Affairs (MedRA version 8.0).

### Statistical analysis

Patients who had remained in the study and had completed visit reports were analysed. No imputation was used for missing data. A paired t test was employed to detect statistically significant differences in disease activity and functional outcomes from baseline. Statistical analyses were performed with SAS version 8.2 TS2M0. The continuation rate, defined as the cumulative percentage of patients still receiving medication, was analysed using the Kaplan–Meier method. Analysis of adverse events was performed with the person-year method.

**Table 1** Demographics and baseline clinical characteristics of patients with RA who received tocilizumab at any time during the blinded period or open-label extension of the tocilizumab study

| | Tocilizumab (n = 143) |
|---|---|
| Demographics | |
| Age, years (SD) | 54.3 (11.1) |
| No of men/no of women | 34/109 |
| Clinical characteristics | |
| RA duration, years (SD) | 9.9 (8.4) |
| No of failed DMARD, mean (range) | 4.5 (1–11) |
| Functional class,* I/II/III/IV | 10/93/40/0 |
| RA stage,* I/II/III/IV | 3/34/56/50 |
| Tender joint count, 0–49 scale (SD) | 20.3 (10.3) |
| Swollen joint count, 0–46 scale (SD) | 14.5 (8.7) |
| ESR, mm/h (SD) | 68.7 (29.9) |
| CRP, mg/dl (SD) | 4.7 (3.3) |
| DAS28 (SD) | 6.7 (1.0) |

Values are mean (SD) unless stated otherwise. The data were calculated from the baseline of the double-blind trial (4 mg/kg group, 8 mg/kg group) and from the extension trial (placebo group). *Rheumatoid arthritis (RA) functional status determined by American College of Rheumatology criteria. RA stage determined by Steinbrocker's criteria. CRP, C-reactive protein; DAS28, disease activity score in 28 joints; DMARD, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate.

## RESULTS

### Characteristics of the patients

A total of 143 patients was enrolled in the open-label, long-term extension trial; 108 patients (76%) had completed 3 years and 94 patients (66%) had completed 5 years, as of March 2007 (fig 1). The median duration of treatment with tocilizumab was 66.7 months (range 0.95–73.2).

Thirty-two patients (22%) withdrew due to adverse events. Only one patient (0.7%) withdrew due to unsatisfactory response. Other reasons for withdrawals were as follows: eight for patient's personal requests; one for the emergence of anti-tocilizumab antibodies and five for other reasons.

The baseline demographic and clinical data are summarised in table 1. The patients' mean age was 54 years and the mean disease duration was 9.9 years. Patients had very active disease at baseline, in terms of the increased number of tender and swollen joint counts and elevated ESR of 68.7 mm/h and CRP levels of 4.7 mg/dl. Furthermore, the baseline DAS28 was 6.7.

### Safety

A total of 148 SAE was reported in 77 patients (53.8%) for an overall rate of 27.5 events per 100 patient-years. Table 2 shows SAE (occurring in at least 1% of patients). Joint surgery related to RA was the most common SAE and occurred in 20 patients (14.0%). In addition, a variety of musculoskeletal disorders was reported as SAE, which were classified as not related to tocilizumab.

Serious infections were reported in 25 patients (17.5%) at a rate of 5.7 events per 100 patient-years. The most frequently reported infections were as follows: pneumonia (nine patients, 1.5 events per 100 patient-years); herpes zoster (seven patients, 1.1 events per 100 patient-years); acute bronchitis (five patients,

**Table 2** Serious adverse events observed in at least 1% of patients

| SAE | No (%) |
|---|---|
| Any SAE | 77 (53.8) |
| Joint surgery | 20 (14.0) |
| Pneumonia | 9 (6.3) |
| Herpes zoster | 7 (4.9) |
| Tendon rupture | 5 (3.5) |
| Humerus fracture | 4 (2.8) |
| Spinal osteoarthritis | 3 (2.1) |
| Femoral neck fracture | 3 (2.1) |
| Joint dislocation | 2 (1.4) |
| Back pain | 2 (1.4) |
| Lumbar spinal stenosis | 2 (1.4) |
| Bronchitis acute | 2 (1.4) |
| Pyelonephritis | 2 (1.4) |
| Brain stem infarction | 2 (1.4) |
| Cataract | 2 (1.4) |
| Pneumothorax | 2 (1.4) |
| Liver function abnormality | 2 (1.4) |

SAE, serious adverse event.

0.8 events per 100 patient-years) and pyelonephritis (three patients, 0.5 events per 100 patient-years).

Four malignancies were reported in four patients (2.8%; 0.7 events per 100 patient-years). The types of malignancies were bladder cancer, breast cancer, large intestine carcinoma and intraductal papilloma.

Temporary prolongation of treatment intervals with tocilizumab was observed throughout the study. Although 163 events of prolonged intervals of 8 weeks or more occurred, the majority of the prolongation of intervals was due to transition from the randomised study to the extension study (median interval of the transition was 10.1 weeks). No particular adverse events were reported when tocilizumab was re-administered except for one patient with a severe infusion reaction. The patient had received 4 mg/kg tocilizumab in the initial 3-month trial, and IgE anti-tocilizumab antibodies appeared at the second infusion of the extension trial. Two more patients were positive for anti-tocilizumab antibodies, when tocilizumab was not detectable in their blood. No adverse event was reported related to the anti-tocilizumab antibodies.

Mean non-fasting total blood cholesterol increased after treatment initiation and stabilised (mean values 185 mg/dl at baseline; 220 mg/dl at 12 months; 214 mg/dl at 60 months; fig 2A). A total of 112 patients experienced total cholesterol abnormalities at at least one point and 15 patients had abnormal values at baseline. Thirty-nine patients (34.8%) were treated with statins, including two patients who had started statin treatment before the trial. There were no cardiovascular SAE related to tocilizumab except for ischaemic heart disease reported in one patient whose total blood cholesterol increased from 168 mg/dl at baseline to 227 mg/dl without statin treatment. The patient also had the risk factor of diabetes mellitus.

Mean neutrophil counts decreased but remained within the normal range (fig 2B). Grade 2 neutropenia was observed in 17 patients and grade 3 in nine patients. All the events were transient, and no patients experienced febrile neutropenia or withdrew as a result of neutropenia.

Mean aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increased slightly, but remained roughly within the normal ranges (fig 2C). Grade 2 or higher increases in AST and ALT occurred in nine (6.3%) and 14 (9.8%) of 143 patients,

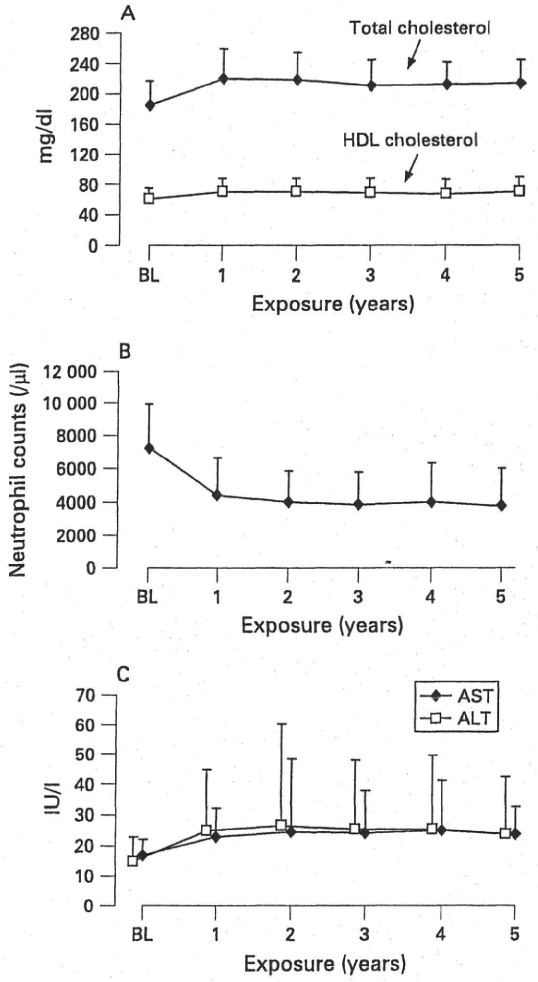


**Figure 2** Change in serum total cholesterol, high-density lipoprotein (HDL) cholesterol, neutrophil counts, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Values are means. Bars indicate SD. BL, baseline.

respectively, during the study, but most were transient and resolved without any particular treatment. No serious liver disorders, such as fulminant hepatitis, were seen during this study.

## Efficacy

The response rate according to the ACR improvement criteria increased during the initial year and remained constant throughout the study period (fig 3A). At 5 years, 79 (84.0%), 65 (69.1%) and 41 (43.6%) of 94 patients met ACR20, ACR50 and ACR70, respectively. These response rates analysed with the last observation carried forward were 77.3%, 58.9% and 37.6%, respectively.

Tocilizumab treatment significantly improved all measures, including tender joint counts, swollen joint counts (fig 3B), CRP levels (fig 3C), MHAQ score (fig 3D) and DAS28 score (fig 3E), and the efficacy was sustained throughout the 5-year treatment. The percentage of patients who achieved clinical remission defined as DAS28 less than 2.6[13 14] was 55.3% (52/94) at 5 years. Most patients exhibited anaemia at baseline and the mean haemoglobin level was 11.3 mg/dl (SD 1.4). Tocilizumab treatment significantly improved anaemia in these patients, and the mean haemoglobin level was increased to 13.2 mg/dl (SD 1.5) at year 5 (fig 3F).



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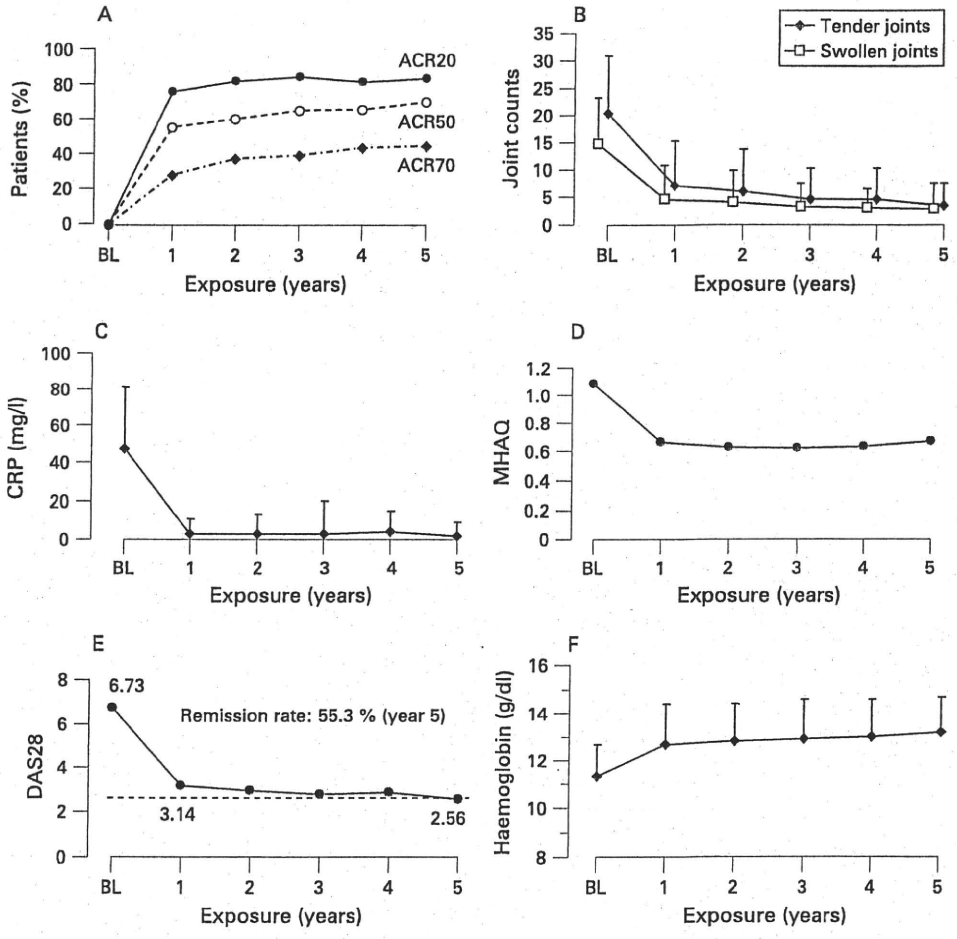


**Figure 3** Percentage of responders according to the American College of Rheumatology improvement criteria and the disease activity score in 28 joints (DAS28) as well as the mean change in modified health assessment questionnaire (MHAQ) scores, number of tender joints, number of swollen joints, C-reactive protein (CRP) and haemoglobin. BL, baseline.

Eighty-eight of the 94 patients who received tocilizumab for more than 5 years had received corticosteroids when they began the initial study. After 5 years of tocilizumab treatment, 78 of 88 (88.6%) had been able to decrease their corticosteroid dose and 28 of 88 (31.8%) had discontinued corticosteroids. The mean dose of corticosteroids for these patients decreased from 6.9 mg/day (median 7.5 mg/day) to 2.4 mg/day (median 2.0 mg/day) at 5 years.

## DISCUSSION

The STREAM study is the first study demonstrating the long-term safety and efficacy of tocilizumab monotherapy. This open-label extension trial of tocilizumab demonstrated a sustained good efficacy and a generally good safety profile over 5 years. The high retention rate at 5 years indeed indicates the favourable efficacy and safety profile. In particular, only one of 143 patients withdrew as a result of an unsatisfactory response, indicating that no general loss of response occurred during long-term treatment.

ACR responses and improvements in DAS28 scores and individual components of the ACR core set were all sustained during the long-term treatment with tocilizumab monotherapy. At 5 years, approximately half of patients had achieved ACR70 and more than half of patients had achieved clinical remission defined as a DAS28 of less than 2.6, although this study was open labelled.

Tocilizumab monotherapy markedly improved inflammation markers such as CRP and ESR and improvements were sustained throughout the study. Haemoglobin levels were also improved. It is well documented that hepcidin plays a key role in anaemia of chronic inflammatory diseases. IL-6 induces the secretion of hepcidin, an iron regulatory peptide hormone that is produced in the liver and that negatively regulates the absorption of intestinal iron and iron recycling by macrophages.[15] This increase in haemoglobin levels is expected to contribute to the improvement in patients' quality of life.

A steroid-sparing effect was another benefit of tocilizumab therapy for RA patients. As the use of corticosteroids is often associated with adverse events such as infection or steroid-induced osteoporosis, this also contributes to the improvement in patients' quality of life from the safety point of view.

A major objective of this study was to evaluate long-term safety. Long-term treatment with tocilizumab was well tolerated. Most of the adverse events were mild and acceptable compared with the benefit provided. The rate of serious infections of 5.7/100 patient-years after 612 patient-years of treatment was comparable to that reported with tumour necrosis factor (TNF) antagonists.[16 17] There was no systemic opportunistic infection or tuberculosis in this study. At least two patients with a history of tuberculosis were treated with tocilizumab because this study did not exclude patients who had a history of tuberculosis. Neither had any recurrence nor exacerbation of tuberculosis without the prophylactic use of antituberculosis drugs. However, two cases of tuberculosis were reported in another study (two cases in 1891 patient-years in Japan),[18] and we should therefore follow patients carefully during tocilizumab treatment.

Four malignancies were reported in four patients. Yamanaka *et al*[19] reported a comparison of the incidence of malignancies in the following three populations: (1) tocilizumab cohort: all clinical trials (including this trial) of tocilizumab in active RA patients; (2) IORRA cohort: an observational cohort of RA patients in the Institute of Rheumatology, Tokyo Women's Medical University and (3) a Japanese population database: cancer incidence in Japan

by the research group for population-based cancer registration in Japan supported by the Japanese Ministry of Health, Labour and Welfare. The incidence of malignancies in the patients receiving tocilizumab was almost equivalent to that in the observational cohort of RA patients or the Japanese population data. Further study will be required to evaluate whether tocilizumab treatment might influence the incidence of malignancies using a much larger population of RA patients treated with tocilizumab.

Throughout long-term treatment, a serious infusion reaction was observed in only one patient who received 4 mg/kg tocilizumab in the initial double-blind trial and developed IgE anti-tocilizumab antibodies. Maini *et al*[6] reported that anaphylaxis and anaphylactoid reactions occurred only at low doses of tocilizumab in the absence of methotrexate. Therefore, initial treatment with a relatively low dose (4 mg/kg) of tocilizumab without methotrexate may induce anti-tocilizumab antibodies.

Increases in total cholesterol, high-density lipoprotein cholesterol and triglycerides were observed in the initial controlled study. In this extension study, however, they did not continue increasing. Furthermore, the atherogenic index, calculated by (total cholesterol–high-density lipoprotein cholesterol)/high-density lipoprotein cholesterol, was stable throughout the 5-year treatment. Therefore, an increase in total cholesterol does not always mean an increased risk of cardiovascular disease. As IL-6 is thought to play a causative role in atherosclerosis, IL-6 blockade may decrease the incidence of cardiovascular events, as observed with anti-TNF therapy.[20] Further investigation will be required to evaluate whether tocilizumab might increase the risk of developing ischaemic heart disease. At present, we should introduce treatment according to the guideline for cholesterol management.

Neutropenia was also reported, as seen in previous studies,[4–7 9] but the incidence was less frequent than that observed in combination with methotrexate therapy.[6 7 9] This may be an advantage of tocilizumab monotherapy.

Although it has been established that TNF inhibitors should be given with methotrexate for maximal efficacy,[21 22] this study indicated that tocilizumab monotherapy offered a good safety profile and sustained efficacy throughout long-term treatment. Therefore, tocilizumab has considerable clinical benefit for patients who do not tolerate methotrexate. Short-term safety and efficacy studies of tocilizumab in combination with methotrexate or DMARD have been reported,[6–9] but further studies are required to determine long-term safety and efficacy.

In conclusion, this study clearly demonstrates excellent long-term efficacy and generally good safety of tocilizumab monotherapy in active RA patients.

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# Interleukin 11 and paired immunoglobulin-like type 2 receptor α expression correlates with the number of joints with active arthritis in systemic juvenile idiopathic arthritis

Systemic juvenile idiopathic arthritis (sJIA) is characterised by systemic inflammatory symptoms such as spiking fever, skin rash, pericarditis and hepatosplenomegaly, along with arthritis.[1] We reported the abnormal expression of genes involved in cytokine networks and mitochondrial function in patients with sJIA identified by DNA microarray.[2] The current study was performed to extend these results.

To identify genes that correlate with arthritis severity or systemic inflammation in patients with sJIA, we further analysed the 3491 genes identified by prior microarray analysis to be abnormally expressed in the peripheral blood of 51 patients with sJIA.[2] They all fulfilled International League of Associations for Rheumatology (ILAR) criteria.[3] Of these genes, 2267 were annotated in the Expression Analysis Systematic Explorer system (EASE) V. 2.0.[4] The statistical correlation between the expression level of each gene, the number of joints with active arthritis (median: 4, range: 0–39) and the serum level of C-reactive protein (CRP) (median: 4.3 mg/dl, range: 1.6–19 mg/dl), as a marker of the systemic inflammation, was analysed using the Pearson product-moment correlation coefficient.

Genes with expression levels showing a correlation index more than 0.4 or less than –0.4 with the number of joints with active arthritis and CRP levels are shown in tables 1 and 2. A total of 10 genes, including interleukin 11 (IL11) and paired immunoglobulin-like type 2 receptor α (PILRA), correlated with the number of joints possessing active arthritis. The expression levels of IL11 were upregulated in patients with sJIA compared to healthy children and positively correlated with the number of joints with active arthritis ($r = 0.48$). Because IL11 reportedly induces osteoclast formation by a receptor activator of nuclear factor κB ligand (RANKL)-independent mechanism, it may be involved in joint destruction.[5] PILRA expression was down-regulated in patients with sJIA and negatively correlated with the number of joints with active arthritis ($r = -0.43$). A signal through PILRA containing immunoreceptor tyrosine-based inhibitory motifs may contribute to counterbalancing the immunoreceptor tyrosine-based activation motifs signal in osteoclast differentiation cooperated with RANKL.[6–8] Therefore, down-regulation of PILRA may also be involved in joint destruction. Additionally, eight more molecules were shown to statistically correlate with the number of joints with active arthritis, but the roles of these molecules in arthritis are still unclear.

**Table 1** Correlation of gene expression with the number of joints with active arthritis

| Gene name | Index of correlation |
|---|---|
| Positive correlation: | |
| Interleukin 11 | 0.48 |
| Plant homeodomain (PHD) finger protein 21A | 0.40 |
| Negative correlation: | |
| Ras-related GTP binding B | −0.51 |
| Transient receptor potential cation channel, subfamily M, member 3 | −0.45 |
| Reticulon 3 | −0.45 |
| Paired immunoglobulin-like type 2 receptor α | −0.43 |
| Positive cofactor 2 (PC2; multiprotein complex) glutamine/Q-rich-associated protein | −0.42 |
| Su(var)3-9, enhancer-of-zeste, trithorax (SET) domain containing 5 | −0.42 |
| Glucosidase I | −0.41 |
| Ubinuclein 1 | −0.41 |

Genes identified correlated with the number of joints with active arthritis. Index of correlation was examined using the Pearson product-moment correlation coefficient test. The genes with expression levels showing a correlation index of more than 0.4 or less than −0.4 with the number of joints with active arthritis are shown.

**Table 2** Correlation of gene expression with the serum levels of C-reactive protein (CRP)

| Gene name | Index of correlation |
|---|---|
| Positive correlation: | |
| H2A histone family, member X | 0.42 |
| Methyltransferase like 9 | 0.41 |
| Complement component 1, q subcomponent binding protein | 0.40 |
| Negative correlation: | |
| Mucin 5B, oligomeric mucus/gel-forming | −0.47 |
| Coiled-coil domain containing 113 | −0.46 |
| Plexin B2 | −0.44 |
| Brain-specific angiogenesis inhibitor 2 | −0.43 |
| DEAH (Asp-Glu-Ala-Asp/His) box polypeptide 57 | −0.43 |
| Cat eye syndrome chromosome region, candidate 2 | −0.41 |
| Zinc finger, DHHC-type containing 1 | −0.40 |

Genes identified correlated with the serum levels of CRP. Index of correlation was examined using the Pearson product-moment correlation coefficient test. The genes with expression levels showing a correlation index of more than 0.4 or less than −0.4 with CRP levels are shown.

Similarly, 10 genes correlated with CRP levels: 3 positively and 7 negatively. The former includes complement component 1q-binding protein (C1QBP), which is expressed on T cells, B cells and monocytes. A signal through C1QBP regulates the proliferation and differentiation of these cells, although the specific role of C1QBP in inflammatory responses is still to be elucidated. Since C1QBP is upregulated by inflammatory cytokines, upregulation of C1QBP may reflect the result of systemic inflammation.[9 10] Other molecules, such as mucin 5B and brain-specific angiogenesis inhibitor 2, have known functions, but others do not. Regardless, the pathological role of these molecules in systemic inflammation is not clear.

Our data indicate that IL11, PILRA and the expression of eight other molecules correlates with the number of joints with active arthritis in patients with sJIA. Further study will be required to understand their exact pathological roles in sJIA.

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# Clinical value of blocking IL-6 receptor

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**Purpose of review**

Interleukin-6 (IL-6) is a multifunctional cytokine that regulates inflammatory response and immune reaction. Overproduction of IL-6 is pathologically involved in inflammatory autoimmune diseases such as rheumatoid arthritis (RA) and juvenile idiopathic arthritis, and therefore, blocking IL-6 activity is one of therapeutic options for these diseases. Tocilizumab is a humanized anti-IL-6 receptor (IL-6R) antibody and inhibits IL-6 activity. There is now accumulating evidence that tocilizumab is therapeutically effective for patients with RA and other inflammatory autoimmune diseases. This article reviews the clinical value of blocking IL-6R.

**Recent findings**

Tocilizumab, as monotherapy and in combination with methotrexate, has been shown to be effective for RA patients with insufficient efficacy to methotrexate or other disease-modifying antirheumatic drugs. These findings of tocilizumab have been expanded to patients refractory to tumor necrosis factor inhibitors. Tocilizumab also retards the progression of structural joint damage. Furthermore, a 5-year long-term safety and efficacy has been shown. Tocilizumab is also a promising therapeutic option for other rheumatic diseases such as systemic-onset juvenile idiopathic arthritis, adult-onset Still's disease, and Takayasu arteritis.

**Summary**

Blocking IL-6R with tocilizumab represents a promising new treatment for RA and other inflammatory diseases. Large registry data will warrant the safety profile of tocilizumab.

**Keywords**

interleukin-6, joint destruction, juvenile idiopathic arthritis, rheumatoid arthritis, tocilizumab

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## Introduction

In the last decade, biological agents represented by tumor necrosis factor (TNF) inhibitors have been introduced into the therapy for patients with rheumatoid arthritis (RA). These powerful agents have sifted the therapeutic paradigm of RA, and clinical remission has become a realistic goal of RA therapy [1]. However, the powerful TNF inhibitors are not always effective for every patient. In addition, the majority of responders experience only a partial clinical improvement in their disease. Therefore, we still need another therapeutic option having high efficacy and safety. Interleukin-6 (IL-6) is another target molecule the biological activities of which should be inhibited for the treatment of RA.

IL-6 is a multifunctional cytokine with various biological activities such as induction of inflammatory response, regulation of immune reaction, and hematopoiesis [2]. IL-6 induces the proliferation and differentiation of T cells as well as the terminal differentiation of B cells, including autoantibody-producing cells. Thus, overproduction of IL-6 augments the autoimmune reaction. Recently, the pathogenic significance of T helper (Th) cells that produce IL-17 (and the related cytokine, IL-17F), IL-6, and TNF, but not IL-4 or interferon-γ, was focused on autoimmune diseases. A subset of $CD4^+$ T cells that produce IL-17 and are distinct from Th1 and Th2 are called Th17 cells [3–5]. They have been shown to have crucial roles in the induction of inflammation and autoimmune diseases [6]. Interestingly, transforming growth factor-β (TGFβ), in the presence of IL-6, was reported to induce the differentiation of pathogenic Th17 cells [7,8]. By contrast, TGFβ, in the absence of IL-6, induces naturally occurring $CD4^+CD25^+$ forkhead box P3 $(FOXP3)^+$ T regulatory (Treg) cells, which inhibit autoimmunity and protect against tissue injury [9,10]. Treg cells can also suppress Th1, Th2 as well as Th17 cells. In these reciprocal developmental pathways for the generation of pathogenic effector Th17 cells and Treg

cells, therefore, IL-6 is a key cytokine [8], although the pathological roles of Th17 cells in human diseases are still obscure.

IL-6 is responsible for both systemic and local inflammation. Systemically, IL-6 induces inflammatory symptoms such as fever, general fatigue, and anorexia as well as laboratory abnormalities, including increase in acute phase proteins such as C-reactive protein (CRP), serum amyloid A, and fibrinogen, decrease in serum albumin, and hypoferremic anemia through inducing the secretion of hepcidin, an iron regulatory peptide hormone [11]. In the affected joints, IL-6 causes angiogenesis through the induction of vascular endothelial growth factor (VEGF) production [12], which is necessary to oxygenate the hypertrophic synovial tissue in RA [13–15]. VEGF also increases vascular permeability and mediates inflammation. IL-6, in the presence of soluble IL-6 receptor (sIL-6R), induces the differentiation of osteoclast precursor cells to mature osteoclasts, which results in bone absorption and joint destruction of RA [16]. These evidences have encouraged us to develop an IL-6 inhibitor as a therapeutic agent.

Tocilizumab is a humanized antihuman IL-6R monoclonal antibody [17], which specifically blocks IL-6R and developed as a therapeutic agent for IL-6-related diseases. In April 2008, tocilizumab was approved for RA and juvenile idiopathic arthritis (JIA) in Japan, and a license application has been submitted in the USA and Europe.

## Blocking IL-6R with tocilizumab

IL-6R complex is composed of 80 kDa IL-6-binding receptor (IL-6R) and 130 kDa non-IL-6-binding but signal-transducing molecule called gp130 [18]. There are two forms of IL-6R, membrane IL-6R and sIL-6R which circulates in blood and synovial fluids. IL-6 can bind both forms of IL-6R, and IL-6/IL-6R complex is made. Then, the complex associates with gp130, which transduces IL-6 signal into the cells. Tocilizumab recognizes both forms of IL-6R and competitively inhibits IL-6 binding to IL-6R [19], and consequently inhibits the biological activity of IL-6.

When IL-6R is completely blocked with tocilizumab, an increase in the serum IL-6 levels is observed [20]. This is explained by the inhibition of IL-6R-mediated consumption of IL-6. Although serum IL-6 levels increase by tocilizumab treatment, the activity of IL-6 is completely inhibited as long as IL-6R is blocked by tocilizumab [20]. It is noteworthy that the IL-6 levels increasing after tocilizumab administration correlate well with the disease activities and reflect the amount of endogenously produced IL-6 [20].

## Clinical value of tocilizumab

The clinical value of tocilizumab has been evaluated in a series of clinical trials with various study designs all over the world. Overviews of clinical studies are shown in Fig. 1 and Tables 1 and 2. Phase I/II studies started in 1999 both in Japan and the UK. The Japanese phase II study showed that tocilizumab monotherapy (intravenous infusion of tocilizumab 8 mg/kg body weight every 4 weeks) significantly reduced disease activity of RA [21]. Similar efficacy was confirmed in the Chugai Humanized AntiRheumatic Interleukin Six Monoclonal Antibody (CHARISMA) phase II study in the UK [22], in which the safety and efficacy of tocilizumab was evaluated with or without methotrexate (MTX), an anchor drug for RA therapy. Both studies demonstrated that intravenous administration of tocilizumab (8 mg/kg) every 4 weeks is a recommended regimen.

Japanese phase III trials mainly consisted of two studies: Study of Active controlled TOcilizumab monotherapy for Rheumatoid arthritis patients with an Inadequate response to methotrexate (SATORI) [23•] and Study of Active controlled Monotherapy Used for Rheumatoid Arthritis, an IL-6 Inhibitor (SAMURAI) [24••]. The SATORI trial showed that tocilizumab monotherapy was an efficacious treatment for patients with an inadequate response to low-dose MTX (8 mg/week), and switching MTX therapy to tocilizumab monotherapy was well tolerated [23•]. Impairment of physical function was also improved. In addition, it was confirmed that blocking IL-6R by tocilizumab significantly decreased serum VEGF levels in association with improvement in disease activity. The tOcilizumab Pivotal Trial in methotrexate Inadequate respONders (OPTION) study is the first multinational study to assess the therapeutic efficacy of tocilizumab in RA patients with moderate-to-severe disease despite MTX treatment [25••]. Combination therapy of tocilizumab and stable prestudy dose of MTX was efficacious for RA patients with inadequate response to MTX [25••]. In this study, health-related quality of life was assessed using Medical Outcomes Study 36-Item Short-Form General Health Survey and Functional Assessment of Chronic Illness Therapy—Fatigue assessment. Tocilizumab improved health-related quality of life more than placebo. TOcilizumab in combination With traditional DMARD therapy (TOWARD), the second global trial, assessed the combination of tocilizumab with stable prestudy dose of traditional disease-modifying antirheumatic drugs (DMARDs) [26•]. At week 24, response rates of a 20% improvement in RA sign and symptom according to American College of Rheumatology (ACR) criteria (ACR20), ACR50, and ACR70 in the tocilizumab group were superior to those of the DMARD group, respectively. Thus, the addition of tocilizumab to the traditional DMARDs is effective.