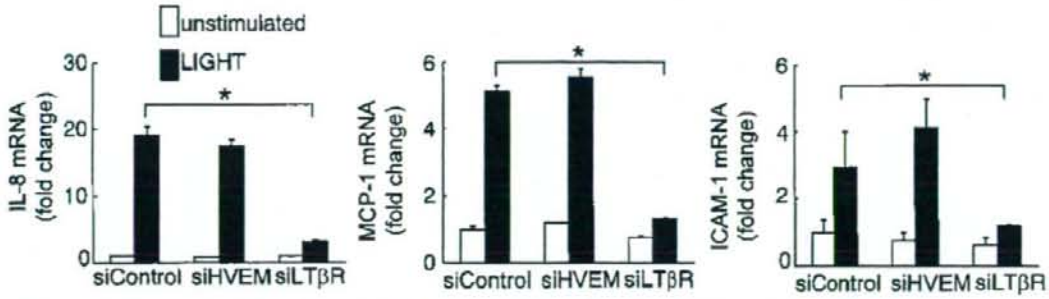

Figure 5. LIGHT-induced expression of IL-8, MCP-1, and ICAM-1 in RA-FLS via LTβR. RA-FLS were transfected with control, HVEM, or LTβR siRNA using Lipofectamine 2000. After 96 h incubation, cells were stimulated with 10 ng/ml LIGHT for an additional 3 h. Levels of IL-8, MCP-1, and ICAM-1 mRNA were analyzed by real-time quantitative PCR. Values are shown as means ± SD per fold change compared with controls. All analyses were carried out on 4 RA-FLS lines. *$p < 0.05$.

target cells of LIGHT. Indeed, we first showed that LIGHT had a stronger RA-FLS growth-promoting activity than PDGF, in lower concentrations. The proliferation of RA-FLS is one of the most critical pathological changes in RA. Thus, our findings suggest that increased expression of LIGHT might lead to the synovial hyperplasia of RA. Anticytokine therapies targeting TNF-α, IL-1β, and IL-6 have been used to treat patients with RA, and it has been demonstrated that such treatments may suppress the accompanying bone destruction as well as the synovitis[28,29]. In addition, recent studies have indicated that LIGHT reduces Fas-mediated apoptosis in FLS[30], that LIGHT may function as a mediator of bone resorption through the induction of osteoclastogenesis[31], and that LTβR-Ig protein blocks the induction of experimental arthritis in mice[18]. Thus, a neutralizing antibody against LIGHT could be a useful tool for inhibition of synovial hyperplasia and bone destruction in RA.

The enhanced effects of LIGHT on RA-FLS proliferation were significantly inhibited by LTβR siRNA, but not by HVEM siRNA, suggesting that LTβR, rather than HVEM, is involved in the LIGHT-induced proliferation of RA-FLS. The exact mechanism by which LIGHT influences RA-FLS proliferation through LTβR is unknown. A potential mechanism underlying RA-FLS proliferation induced by LIGHT may involve cell-cycle regulators, including cyclin-dependent kinases (CDK). The mammal cell cycle is controlled by holoenzymes composed of a catalytic CDK and regulatory cyclin. The expression level of p21 was reduced in RA synovial linings and FLS compared with the level in patients with OA[32]. Overexpression of p21 or p16 by adenoviral-mediated delivery suppresses FLS growth *in vitro*[33,34]. Further, LIGHT induces cell proliferation, downregulates the CDK inhibitors p21, p27 and p53, and inversely upregulates cyclin D and Rb hyperphosphorylation in vascular smooth muscle cells[13]. Thus, it is possible that LIGHT promotes FLS proliferation by shortening the cell cycle of FLS in RA. Wang, *et al* reported that LTβR-null mice show reduced BrdU incorporation in dendritic cells[35]. This supports our claim that LTβR signaling is involved in the proliferation of RA-FLS.

We observed that LIGHT also induces the production of inflammatory cytokines and chemokines and expression of adhesion molecules on RA-FLS. Inflammatory cytokines and chemokines induce the migration of cells and release of mediators of inflammation and angiogenesis, and could be involved in the pathogenesis of RA[1,2,36]. The increased expression of ICAM-1 and VCAM-1 adhesion molecules on activated endothelial cells enhances the recruitment of monocytes, lymphocytes, and neutrophils, leading to inflammation. These findings indicate that LIGHT might play an important role in inflammation in the synovial lining layer, as well as in its hyperplasia. A recent study revealed that LIGHT upregulates the expression of ICAM-1, VCAM-1, and IL-6 in RA-FLS via NF-κB activation[30,37]. Although these reports are consistent with our present results, it has not been clear which of 2 receptors is involved in the induction of these genes in FLS. Our knockdown analysis using siRNA revealed that LIGHT induces proliferation and gene expression by signaling via LTβR, but not HVEM. Braun, *et al* have shown that LTβR is expressed on RA-FLS, and that LTa1β2, a ligand for LTβR, induces expression of inflammatory cytokines, chemokines, and ICAM-1[38]. This supports our claim that LTβR signaling is involved in the activation of RA-FLS. The NF-κB transcription factor is certainly involved in cytokine- and chemokine-driven responses and is a point of convergence for several upstream proinflammatory pathways[23]. Indeed, NF-κB activation appears to be an important factor in RA, as the expression of NF-κB is enhanced in lining cells[39,40] and in the cartilage-pannus junction in the RA synovium[41]. In our study, treatment with PDTC blocked LIGHT-induced IL-8, MCP-1, and ICAM-1 expression, suggesting that the effects of LIGHT are mediated through NF-κB. The involvement of NF-κB in LIGHT-induced proinflammatory responses was further confirmed

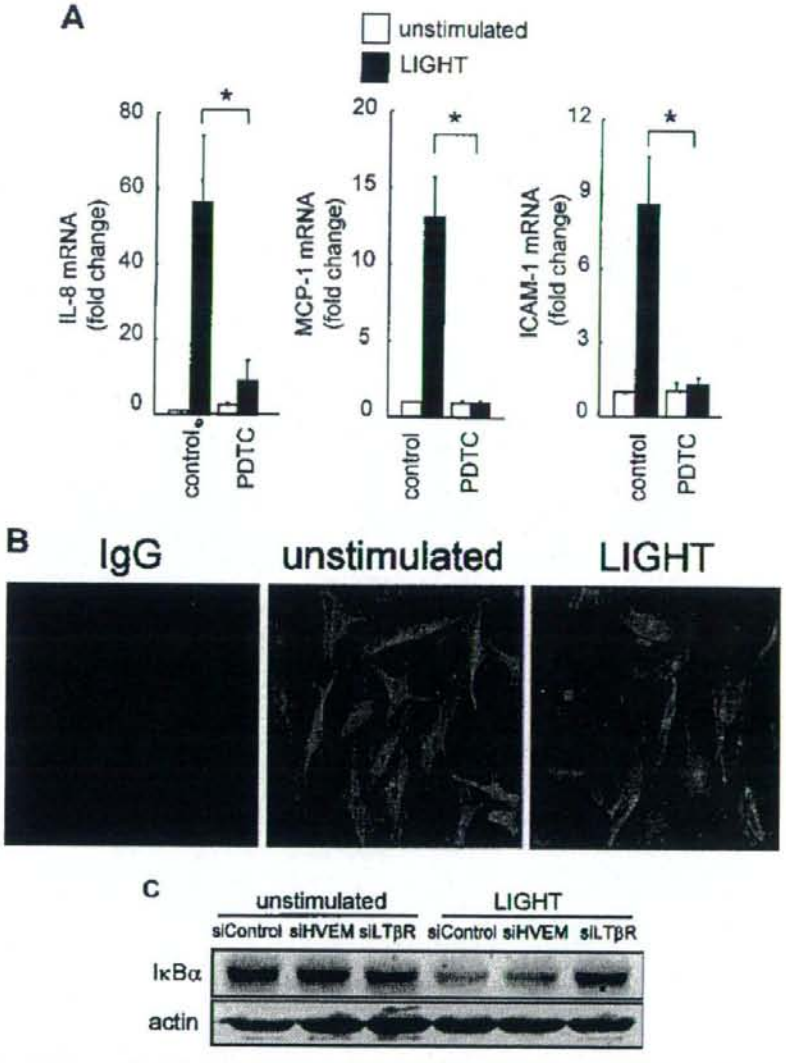

Figure 6. LIGHT-induced expression of IL-8, MCP-1, and ICAM-1 through NF-κB-mediated pathways. A. FLS were stimulated with 10 ng/ml LIGHT for 3 h with or without preincubation for 30 min with 30 μM PDTC. Levels of IL-8, MCP-1, and ICAM-1 mRNA were analyzed by real-time quantitative PCR. Values are shown as means ± SD per fold change compared with control. All analyses were carried out on 4 RA-FLS lines. *$p < 0.05$. B. Immunofluorescence staining for NF-κB p65 in RA-FLS. Control in which primary antibodies were replaced with control IgG (left panel); unstimulated RA-FLS (middle); and RA-FLS stimulated with 10 ng/ml LIGHT for 30 min (right). Results are representative of 2 experiments using 2 FLS lines. C. 96 h after siRNA transfection, cells were stimulated with 10 ng/ml LIGHT for 40 min. IκBa degradation was analyzed by immunoblotting. Results are representative of 2 experiments using 2 RA-FLS lines.

by the LIGHT-induced nuclear translocation of NF-κB p65. Moreover, LIGHT induced IκBa degradation in RA-FLS, an effect that was inhibited by LTβR siRNA, but not by HVEM siRNA. These findings are consistent with studies showing that LTβR ligation can lead to activation of NF-κB[24,42-45]. However, it is unknown why LIGHT prefers the LTβR signaling pathway in RA-FLS, even though HVEM is also expressed on these cells.

We have demonstrated that LIGHT is overexpressed in RA synovial tissues and SF. LIGHT induced increased production of inflammatory cytokines, chemokines, and adhesion molecules through NF-κB activation, as well as proliferation of RA-FLS. These findings indicate that LIGHT signaling via LTβR plays an important role in the pathogenesis of RA by affecting key processes such as the proliferation and activation of RA-FLS. Therefore, regulation of LIGHT-LTβR signaling may represent a new therapeutic target for the treatment of RA.

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（IV）RA 患者末梢血細胞遺伝子発現の網羅的解析プロフィールからの新規遺伝子選択研究

Tocilizumab

Norihiro Nishimoto and Toru Mima

CHAPTER 10J

Interleukin (IL)-6 was originally identified as B-cell stimulatory factor-2 (BSF-2), a T-cell–derived factor that induces B-cell differentiation.[1] After the cDNA for BSF-2 was found to be identical to that of interferon-β2, a hybridoma/plasmacytoma growth factor, a 26-kDa protein, and a hepatocyte stimulating factor, these entities were unified under the name IL-6.[2] IL-6 is produced not only by T cells but by B cells, monocytes, fibroblasts, keratinocytes, endothelial cells, and some tumor cells.[3] Although IL-6 plays important physiologic roles in the regulation of immune response, inflammatory reaction, and hematopoiesis, constitutive overproduction of IL-6 has been implicated in the development of immune-mediated and inflammatory diseases.[4]

In patients with rheumatoid arthritis (RA), IL-6 levels are elevated in both serum and synovial fluids.[5–8] IL-6 augments autoimmune responses through the activation of both B cells, resulting in hyper-γ-globulinemia and increased autoantibody titers, and autoreactive T cells. In animal models, it has been shown that in the presence of IL-6 transforming growth factor-β (TGF-β) induces differentiation of Th17 cells—which in turn produce IL-17, IL-6, and TNF.[9–13] In contrast, TGF-β in the absence of IL-6 induces $CD4^+CD25^+$Forkhead box P3 $(FOXP3)^+$ T regulatory cells—which inhibit autoimmune reactions. It appears that IL-6 may therefore be a key cytokine in the pathogenesis of effector Th17 cells and autoimmune responses, although further confirmatory studies will be needed in humans.

IL-6 acts synergistically with IL-1β and TNF to induce production of vascular endothelial growth factor (VEGF), which plays a role in stimulating angiogenesis in the hyperplastic synovial tissues of RA patients.[14] In the presence of soluble IL-6 receptors, IL-6 also induces osteoclast differentiation[15]—which mediates the joint destruction and osteoporosis associated with RA. As a proinflammatory mediator, IL-6 causes systemic symptoms of fever and fatigue,[16] as well as increased production of acute-phase proteins[17] such as C-reactive protein (CRP), fibrinogen, α1-antitrypsin, and serum amyloid A (SAA). Overexpression of IL-6, as a megakaryocyte-activating factor, induces thrombocytosis.[18] Finally, IL-6 induces production of the iron regulatory peptide hormone hepcidin.[19,20] By suppressing iron absorption and increasing hepatic iron storage, hepcidin promotes a hypoferremic anemia of chronic inflammation.

Recognition that IL-6 is involved in numerous inflammatory responses led to the idea that inhibition of IL-6 might be a plausible therapy for RA. Tocilizumab, a humanized antihuman IL-6 receptor (IL-6R) monoclonal antibody specifically targeting IL-6,[21] has been the subject of recent safety and efficacy trials in patients with RA.

Pharmacologic Features of Tocilizumab

Mode of Action

Tocilizumab was engineered by grafting complementarity-determining regions (CDRs) from mouse antihuman IL-6R antibody to human IgG1 to create a human IL-6R binding site on a human antibody.[21] This strategy proved effective for minimizing immunogenicity, as fewer neutralizing antibodies have been detected with repetitive tocilizumab administration than with treatments using mouse antibodies or mouse and human chimeric antibodies. This results in a prolonged half-life for tocilizumab.

IL-6 signal transduction is mediated by a ligand-binding IL-6R and a non ligand-binding but signal-transducing chain, gp130, on the surface of target cells. Soluble forms of IL-6R, found in blood and synovial fluids, are also capable of signal transduction through "trans-signaling"[22]—a signaling mechanism unique to the IL-6R system. Tocilizumab recognizes IL-6 binding sites on both the membranous and soluble forms of IL-6R, and competitively inhibits IL-6 binding to IL-6R (Figure 10J-1). Using the standard tocilizumab dosing regimen, neither antibody-dependent nor complement-dependent cellular cytotoxicity has been observed in cells that express IL-6R.

Pharmacokinetics

Serum tocilizumab concentrations showed nonlinear pharmacokinetics in the dose range of 2 to 8 mg/kg when intravenously administered by drop infusion for 2 hours.[23]. The half-life of tocilizumab (t1/2) was dose dependent, and approximated the half-life of human IgG1 (241.8 ± 71.4 hours) by the third dose of 8 mg/kg. The mean area under the curve (AUC) for serum tocilizumab concentration peaked at approximately 10.66 ± 4.07 mg*hour/ml.[23]

Interestingly, CRP and SAA levels were undetectable in RA patients with serum concentrations of free tocilizumab greater than 1 μg/ml, suggesting that IL-6 is essential for CRP and SAA production *in vivo*. In fact, CRP levels have been shown to function as a surrogate marker for the level of tocilizumab activity.[23]

Clinical Studies of Tocilizumab in RA Patients

Clinical studies of tocilizumab in patients with immune-inflammatory diseases were initially performed in the Osaka University Hospital of Japan. The pilot studies, which were performed in patients with refractory disease, demonstrated the therapeutic potential of tocilizumab.[14,24,25] Tocilizumab

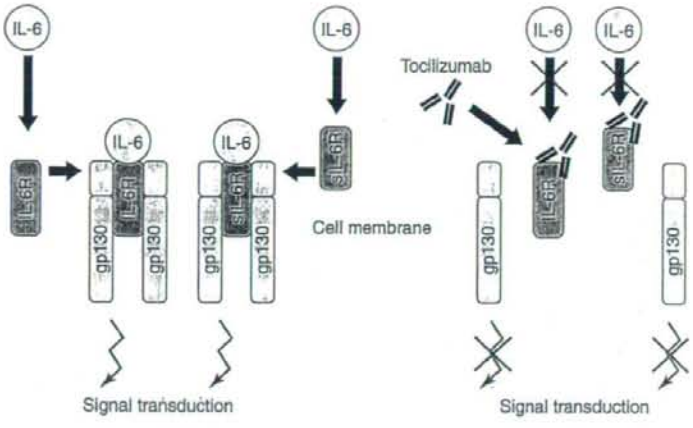

Figure 10J-1. Inhibitory action of tocilizumab in IL-6 signaling. IL-6 signal transduction is mediated by a ligand-binding IL-6R and a non–ligand-binding but signal-transducing chain, gp130, on the cell surface. Soluble IL-6R (sIL-6R) is also capable of signal transduction. Tocilizumab recognizes IL-6 binding sites on both the membranous IL-6R and sIL-6R and inhibits IL-6 signal transduction.

treatment dramatically improved both inflammatory symptoms and laboratory abnormalities, including serum levels of acute-phase proteins, albumin, hemoglobin, and VEGF. On the basis of these findings, clinical trials were designed and conducted.

Phase I/II Studies in the United Kingdom and Japan

The phase I/II study in the United Kingdom was a double-blind placebo-controlled trial testing the pharmacokinetics, safety, and efficacy of single-dose intravenous administration of tocilizumab at the following doses: 0.1, 1, 5, and 10 mg/kg.[26] The treatment was well tolerated, and normalization of CRP was achieved in the 5- and 10-mg/kg groups. In the 5-mg/kg group, 55.6% of the patients met the American College of Rheumatology (ACR) response criteria ACR 20, compared to 0% of the patients in the placebo group.

A Japanese phase I/II study then tested the pharmacokinetics, safety, and efficacy of repeated intravenous tocilizumab doses at 2, 4, and 8 mg/kg every 2 weeks in an open-label trial.[23] At 6 months, 86% and 33% of the patients had met ACR 20 and ACR 50 criteria, respectively. Treatments were again well tolerated.

Phase II Studies for RA

In 2001, the safety and efficacy of tocilizumab monotherapy for RA was evaluated in a multicenter double-blind randomized placebo-controlled phase II trial in Japan. In this study, 164 patients with refractory RA were randomized to receive intravenous treatment with placebo, tocilizumab treatment at 4 mg/kg, or tocilizumab treatment at 8 mg/kg. Treatment was administered at three sessions spaced 4 weeks apart, and the clinical responses were evaluated at 12 weeks using the ACR criteria.[27] Tocilizumab treatment significantly improved all measures of disease activity, and a dose-response relationship was observed across the 4- and 8-mg/kg groups. Patients had a mean disease duration of 8 years, and had used an average of four to five antirheumatic drugs before beginning tocilizumab treatment. Despite their advanced disease, 78% of the patients in the 8-mg/kg group met ACR 20 criteria—compared to 57% in the 4-mg/kg group ($p = 0.02$) and only 11% in the placebo group ($p < 0.001$). ACR 50 and ACR 70 responses in the 8-mg/kg group were 40% and 16%, respectively, and both were superior to those in the placebo group ($p < 0.001$, $p = 0.002$). The efficacy of tocilizumab monotherapy was also confirmed by the disease activity score in 28 joints (DAS28): 91% of patients in the 8-mg/kg group reported "good or moderate" joint scores, as opposed to 19% in the placebo group ($p < 0.001$). Complete normalization of CRP was observed in 76% of the patients in the 8-mg groups, but only 1.9% of patients in the placebo group. Significant improvement was also noted in lab values for hemoglobin, platelet count, fibrinogen, SAA, RF, albumin, bone formation markers, and bone resorption markers.

Safety data did not show a dose-dependent relationship. The overall incidence for adverse events was 56%, 59%, and 51% in the placebo, 4-, and 8-mg/kg groups, respectively. Serious adverse events were reported in three patients receiving tocilizumab (2.8%) and two patients receiving placebo (3.7%). One patient died from hemophagocytosis syndrome associated with reactivation of chronic active Epstein-Barr virus (EBV) infection after receiving a single dose of 8 mg of tocilizumab. This patient had increased serum EBV DNA levels before study enrollment, and it was later determined that she had developed Hodgkin's disease prior to tocilizumab treatment.[28] Although the mechanism of EBV reactivation is currently unclear, a careful pretreatment examination is necessary to assess whether the patient has a concurrent infectious disease. Other serious adverse events included allergic pneumonitis and super-infection of a burn, both of which were adequately treated with no long-term sequelae.

Abnormalities in laboratory values were also reported, notably including increased total cholesterol (TC) in 44% of patients receiving tocilizumab. High-density lipoprotein (HDL) cholesterol also increased, however, leaving the atherogenic index (total cholesterol – HDL cholesterol/HDL cholesterol) unchanged throughout the study period. The TC values tended to stabilize in the upper normal range, and no cardiovascular complications were reported. Similar increases in TC concentrations were also reported with TNF inhibitor treatments,[29] suggesting that the dyslipidemia may be secondary to the improvement in disease activity. Mild to moderate increases in liver function tests were observed in 14 of 109 patients (12.8%) receiving tocilizumab. They were all transient and normalized with the repeated administration of tocilizumab. There were no increases in antinuclear antibodies or anti-DNA antibodies, as is occasionally reported with TNF inhibitor treatments. These results indicate that treatment with tocilizumab was generally well tolerated.

A European phase II trial later tested the safety and efficacy of tocilizumab in combination with methotrexate (MTX) among patients with inadequate responses to MTX monotherapy.[30] This multi-arm trial randomized 359 patients into seven parallel arms. Patients received MTX (10–25 mg/week) with tocilizumab placebo; tocilizumab at 2, 4, or 8 mg/kg with MTX; or tocilizumab at 2, 4, or 8 mg/kg with MTX placebo. Tocilizumab or tocilizumab placebo was infused every 4 weeks for a total of 16 weeks.

ACR 20 criteria were met by 61% and 63% of patients, respectively, receiving 4 mg/kg and 8 mg/kg of tocilizumab as monotherapy, and by 63% and 74% of patients, respectively, receiving 4 mg/kg and 8 mg/kg of tocilizumab plus MTX. In contrast, only 41% of patients receiving placebo plus MTX satisfied the ACR 20 criteria. However, no statistical difference was observed in the ACR 20 response rate between the groups receiving monotherapy with 8 mg/kg tocilizumab and those receiving combination therapy with 8 mg/kg tocilizumab and MTX. ACR 50 and ACR 70 responses, as well as the European League Against Rheumatism (EULAR) remission rates, were significantly higher in patients receiving a combination therapy than in patients receiving MTX alone. EULAR remission was achieved in 34% of patients receiving tocilizumab 8 mg/kg plus MTX, compared to 17% in the group receiving 8 mg/kg tocilizumab monotherapy and 8% in the group receiving only MTX. The clinical benefits of tocilizumab, however, appeared to be similar with and without concomitant MTX therapy—although concomitant use of MTX increases the efficacy of TNF inhibitors.

In this study, tocilizumab was also well tolerated—with approximately 50% of patients experiencing adverse events, the majority of which were mild or moderate. No clear dose-dependent pattern was observed. Thirty-five serious adverse events were reported, with higher rates in the 2-mg/kg tocilizumab monotherapy group. Serious anaphylactic reactions were observed only in the groups receiving monotherapy with either 2 or 4 mg/kg of tocilizumab, and may be related to the development of anti-tocilizumab antibodies. Two cases of sepsis developed in patients receiving combination therapy with 8 mg/kg tocilizumab and MTX. Clinically significant laboratory abnormalities included increased transaminase levels, which seemed to be exacerbated by MTX. It is possible that tocilizumab treatment diminishes the protective effect of IL-6 on hepatocytes against MTX-induced toxicity. As in earlier trials, increased TC and decreased neutrophil counts were noted.

Phase III Studies for RA

The Japanese phase III trials consisted of two studies: The SATORI study evaluated the safety and efficacy of tocilizumab monotherapy for RA patients with inadequate responses to MTX treatment,[31] and the SAMURAI trial investigated the efficacy of tocilizumab monotherapy in slowing joint damage.[32]

In the SATORI study, 127 RA patients with inadequate responses to MTX (8 mg/week) were randomized to receive tocilizumab 8 mg/kg every 4 weeks in addition to a MTX placebo (tocilizumab group) or tocilizumab placebo in addition to MTX 8 mg/week (MTX group) for 24 weeks. ACR 20 improvement rates at 24 weeks served as the primary endpoint, with additional outcome measures including ACR 50 and ACR 70 improvement rates, DAS28, EULAR response, and ACR-N AUC.[31] At 24 weeks, ACR 20 response rates were significantly higher in the tocilizumab group than in the MTX group (80.3% vs. 25.0%, $p < 0.001$). Patients in the tocilizumab group also reported significantly higher ACR 50 and ACR 70 rates than patients treated by MTX [49.2% vs. 10.9% ($p < 0.001$) and 29.5% vs. 6.3% ($p < 0.001$), respectively]. Similarly, tocilizumab therapy was associated with improvements in both the EULAR response rates and the ACR-N AUC. Tocilizumab was very well tolerated, and withdrawals due to adverse events (tocilizumab, $n = 2$; MTX, $n = 3$) as well as the occurrence of serious adverse events (tocilizumab, $n = 4$; MTX, $n = 3$) were similar between tocilizumab and MTX groups. The frequency of liver function test abnormalities was higher in the MTX group (mean alanine transaminase [ALT] increase of 10.9%), although most cases were grade 1.

The SAMURAI study was designed to assess the change in total Sharp score (TSS), a quantitative radiographic evaluation of bone erosion and joint space narrowing in hand and foot joints of RA patients. This x-ray reader-blinded open-label randomized 1-year controlled trial enrolled RA patients who had been diagnosed less than 5 years prior to the study start date.[32] A total of 306 patients with active RA were randomly allocated to receive tocilizumab 8 mg/kg every 4 weeks or conventional disease-modifying antirheumatic drugs (DMARDs) for 52 weeks. Patients in the tocilizumab group showed significantly less radiographic progression, as measured by the change in TSS, than those receiving DMARDs (2.3 ± 5.6 versus 6.1 ± 11.4, $p = 0.001$). Tocilizumab was also superior to DMARDs in preventing both erosion and joint space narrowing ($p < 0.001$ and $p = 0.018$, respectively). The overall incidence of adverse events (including laboratory abnormalities) was 89% and 82% in the tocilizumab and DMARDs groups, respectively (serious adverse events: 18% and 13%, respectively; serious infections: 7.6% and 4.1%, respectively).

The results of two tocilizumab global phase III studies, OPTION and TOWARD, were reported in 2007.[33,34] In the OPTION study, 623 patients with moderate to severe RA received maintenance pre-study doses of MTX therapy in addition to placebo, 4 mg/kg of tocilizumab, or 8 mg/kg of tocilizumab. A significantly higher proportion of tocilizumab-treated patients achieved the primary endpoint of meeting ACR 20 criteria at 24 weeks (59% in the tocilizumab 8-mg/kg group; 48% in the tocilizumab 4-mg/kg group; 27% in the placebo group; $p < 0.0001$). ACR 50 endpoints were achieved in 44%, 32%, and 11% (respectively)—and ACR 70 endpoints were achieved in 22%, 12%, and 2% of patients in the tocilizumab 8-mg/kg group, the tocilizumab 4-mg/kg, and the placebo group, respectively.[33]

Safety profiles revealed a similar frequency of adverse events for tocilizumab and placebo groups, although serious infections were observed more frequently in the tocilizumab group (2.9% in the tocilizumab 8-mg/kg group; 1.4% in the tocilizumab 4-mg/kg group; 1% in the placebo group). Tocilizumab treatment significantly improved quality of life in patients with RA, as assessed by the health assessment questionnaire (HAQ), functional assessment of chronic illness therapy-fatigue scale (FACIT-fatigue), and short form-36 health survey (SF-36).[35]

Similarly, the TOWARD study evaluated the efficacy and safety of tocilizumab in a larger population of 1,216 RA patients with inadequate responses to a range of DMARDs.[34] Patients continued maintenance DMARD treatment while receiving intravenous tocilizumab 8 mg/kg or placebo every 4 weeks for 24 weeks. ACR 20, ACR 50, and ACR 70 responses were significantly higher in the tocilizumab group than in the placebo group (61% vs. 25%, 38% vs. 9%, and 21% vs. 3%, respectively; $p < 0.0001$). This study also

confirmed that tocilizumab treatment improved physical function, fatigue, and physical and mental health scores as assessed by HAQ, the FACIT-fatigue scale, and SF-36.[36]

Tocilizumab Safety Concerns

Because IL-6 plays a crucial role in immunity regulation, IL-6 inhibition may result in increased susceptibility to infectious diseases. In addition, suppression of inflammatory symptoms and laboratory value abnormalities may delay detection of infections. Clinical trials have demonstrated that the incidence of infections is similar to that of control groups, and similar to that reported for TNF inhibitors. It is noteworthy that tuberculosis was observed in only 2 of more than 4000 patients treated with tocilizumab, without pretrial screening or prophylactic use of anti-tuberculosis drugs. In comparison, tuberculosis frequently emerges during treatment with TNF inhibitors. This may be explained by the fact that TNF plays an important role in the formation of granulomas, whereas IL-6 is largely uninvolved in this immune response.

Several reports have claimed that TNF blockade may increase the risk of malignancy in RA patients, and it is possible that suppression of IL-6 action may also be carcinogenic. To investigate the risk of malignancy in RA patients treated with tocilizumab, outcomes from patients treated with long-term tocilizumab were compared to those of a Japanese cohort of RA patients (IORRA cohort) and a Japanese population database.[37] The incidence of malignancy, adjusted for age and gender, was calculated by direct and indirect methods.

In the tocilizumab cohort (mean age 51.5, female 79.8%, mean disease duration 6.21 years), 11 malignancies (male 3, female 8) were identified among 618 patients (1,459 person-years). In the IORRA cohort (mean age 55.9, female 81.8%, mean disease duration 8.73 years), 173 malignancies (male 56, female 115) were identified among 7,656 patients (25.567 person-years). The crude incidence of malignancies was 753.7 (male 1171.3, female 664.8) per 100,000 in the tocilizumab cohort and 676.7 (male 1331.5, female 542.2) per 100,000 in the IORRA cohort. Compared to the IORRA cohort, the standardized incidence ratio (SIR) of malignancies in the tocilizumab cohort was overall 1.33 (95% CI: 0.74–2.40). The SIR for males in this study was 1.18 (95% CI: 0.38–3.66), and that for females was 1.39 (95% CI: 0.70–2.79). Compared to the Japanese population database, the SIR was overall 1.66 (95% CI: 0.92–3.00)—and for males was 1.58 (95% CI: 0.51–4.88) and for females 1.70 (95% CI: 0.85–3.39). The incidence of malignancy in the tocilizumab cohort, therefore, did not exceed those of the Japanese RA cohort nor the Japanese population database.

Conclusion

Clinical studies have demonstrated that IL-6R inhibition by tocilizumab is a promising therapeutic approach for RA. The mechanisms by which IL-6 inhibition mediates improvements in RA, however, are not yet fully understood. Because regulation of inflammatory cytokines, including IL-6, is involved in the pathogenesis of RA future research will be needed to identify compounds that may act synergistically with tocilizumab. The long-term safety of tocilizumab treatment—particularly involving infection, malignancy, and cardiovascular risk—will also require investigation.

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EXPERT REVIEWS

Tocilizumab for the treatment of rheumatoid arthritis

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Tocilizumab is a humanized anti-human IL-6 receptor antibody that specifically inhibits the biological activity of IL-6 by competitively inhibiting the binding of IL-6 to the IL-6 receptor. Clinical trials have shown that tocilizumab 8 mg/kg administered by monthly infusion not only improves clinical signs and symptoms of refractory rheumatoid arthritis but also suppresses radiographic progression. In regard to safety, the most common adverse event was nonsevere infection, such as nasopharyngitis, although the incident rate of adverse events was slightly higher than that of disease-modifying antirheumatic drug treatment. Studies have shown that there is no specific infection or prolongation of infection related to tocilizumab treatment. Tocilizumab is a promising therapeutic agent with statisfactory efficacy and safety for rheumatoid arthritis.

KEYWORDS: anticytokine therapy • biologics • IL-6 • rheumatoid arthritis • tocilizumab

Rheumatoid arthritis (RA) is one of the most common systemic autoimmune diseases with a prevalence of 0.5–1.1% in the total population [1]. RA is characterized clinically by chronic synovitis and joint destruction in multiple joints. Joint destruction impairs functional ability and consequently may reduce the quality of life of patients. For preventing joint damage, the recommended management strategy for RA involves early diagnosis, tight control of inflammation and introduction into remission. In order to achieve this, the mechanisms of new therapeutic drugs for RA need to be based on the pathology of the disease and can thereby lead to highly effective results.

Proinflammatory cytokines, such as TNF, IL-1 and IL-6 are involved in the pathogenesis of RA, although the etiology is still obscure [2–4]. IL-6 levels are elevated in the synovial fluid of patients with RA and have been shown to correlate with disease activity [5–7]. The sources of IL-6 in the synovial fluid are T cells and macrophages infiltrating in synovial tissues, as well as synovial cells themselves [8,9]. Serum levels of IL-6 were also elevated and strongly correlated with erythrocyte sedimentation rate (ESR) and rheumatoid factor [10,11].

IL-6 was originally identified as a B-cell stimulatory factor (BSF)-2 which is produced by T cells and induces B cells to differentiate into antibody-forming cells [12,13]. BSF-2, was later found to be identical to IFN-β2, hepatocyte-stimulating factor and hybridoma/plasmacytoma growth factor [14]. These names were unified as IL-6. IL-6 is also produced by B cells, macrophages, neutrophils, bone marrow stromal cells, synovial fibroblasts, fibroblasts, endothelial cells and keratinocytes [15,16]. IL-6 induces T-cell proliferation and terminal differentiation to cytotoxic T cells in cooperation with IL-2 [17,18]. IL-6 induces fever and the production of acute phase proteins, such as C-reactive protein (CRP), serum amyloid A (SAA), and fibrinogen, and complement by hepatocytes, while IL-6 suppresses albumin production [19,20]. IL-6 also induces hepcidin, which regulates the iron metabolism and is the causative molecule of anemia in chronic inflammatory diseases [21]. IL-6 induces osteoclast differentiation and maturation in the presence of soluble IL-6 receptor and contributes to bone absorption [22].

VEGF plays an important role in angiogenesis in RA [23]. Serum VEGF levels in RA patients correlate with disease activity and radiologic progression [24]. The IL-6 blockade suppresses VEGF production of synovial fibroblasts and reduces serum levels in RA patients [25]. Therefore, IL-6 is responsible for angiogenesis in RA through inducing VEGF production.

Production of IL-6 is elevated in murine arthritis models, such as collagen-induced arthritis (CIA) and adjuvant-induced arthritis [26].

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IL-6 is a key cytokine in the development of arthritis in CIA [27,28] and anti-murine IL-6 receptor (IL-6R) antibody ameliorates the arthritis [29]. In the SKG mouse, which has a mutation of the gene encoding an SH2 domain of ζ-associated protein 70 (ZAP-70) and spontaneously develops T-cell-mediated autoimmune arthritis, genetic deficiency of IL-6 suppresses the development of arthritis [30]. These suggest that IL-6 plays an important role in the development of arthritis in murine models.

Recently, Th 17 cells, which produce IL-17 and proinflammatory cytokines, have been shown to contribute to the pathogenesis in murine models of RA and multiple sclerosis [31]. Th17 cells are developed from naive T cells (Th0) in the presence of TGF-β1 together with IL-6, while Treg cells develop from Th0 cells in the presence of TGF-β1 alone [31]. Therefore, IL-6 is one of the key cytokines for determining T-cell development, especially in Th17 and Treg cells. The inhibition of IL-6 signal may influence the balance of Th17 and Treg cells *in vivo*, especially under chronic inflammatory conditions. Under IL-6 deficiency, the number of Th17 cells is decreased and arthritis is ameliorated in the SKG mouse [32]. Moreover, mice with a point mutation of tyrosine 759 in gp130 as a signal-transducing chain of IL-6 receptor complex, which induces over-signal of IL-6 in mice, develop autoimmune arthritis caused by insufficient clonal selection of T cells in the thymus and periphery [33]. Therefore, IL-6 may modify T-cell clonal selection and differentiation.

All of these evidences indicate that overproduction of IL-6 may contribute to the pathological condition of RA (FIGURE 1) and that inhibition of the IL-6 signal could be effective for the treatment of RA.

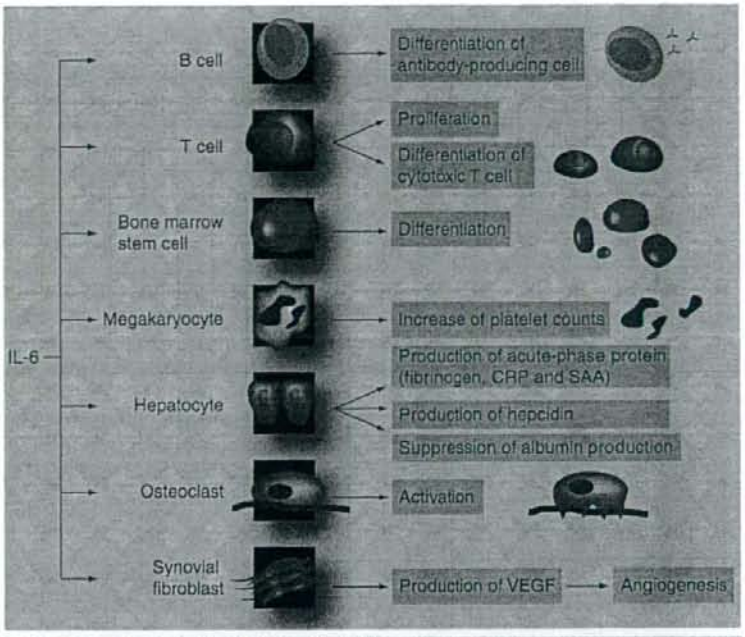

Figure 1. Biological role of IL-6 in the pathological condition of rheumatoid arthritis.
CRP: C-reactive protein; SAA: Serum amyloid A.

Overview of the market

At present, there is no monotherapy or combination therapy which is fully effective in all patients with RA. The American College of Rheumatology (ACR) response rates achieved by disease-modifying antirheumatic drug (DMARD) therapies, including combination therapies, is 36–71% of patients [34]. TNF-blocking therapy, especially in combination with methotrexate (MTX), is more successful but 20–30% of the patients have insufficient response [35–37].

Since the goals of RA treatment are to introduce the remission of symptoms, to inhibit joint destruction and to improve the functional ability of the patients as soon as possible, ineffective treatments should be changed to effective ones. For this aim, it is necessary to have a wide range of drugs with different modes of actions. Tocilizumab is a new drug targeting for IL-6, the different molecule from TNF, as well as abatacept targeting for T cells and rituximab targeting for B cells [38–40].

Toclizumab monotherapy has been shown to be effective in the treatment of RA and may be an option for patients that have side effects with combination biologics and DMARDs.

In addition, tocilizumab is effective treatment for patients with systemic-onset juvenile idiopathic arthritis (JIA), Crohn's disease and Castleman's disease, which are also caused by overproduction of IL-6 [41].

Introduction to tocilizumab

The first human study of the IL-6 signal blockade in RA was performed in five patients with refractory RA using a murine neutralizing monoclonal anti-human IL-6 antibody (B-E8) [42]. The patients received B-E8 10 mg by daily infusion for 10 days. The treatment was shown to improve clinical symptoms and reduce CRP levels [42]. Clinical improvement lasted for a mean of 2 months but the disease flared in all the patients. Thus, repeat treatment is necessary for controlling the activity of RA.

Tocilizumab is a humanized anti-human IL-6R antibody [43]. To enable the antibody to be repeatedly dosed to humans, murine anti-human IL-6R antibody was humanized by grafting the complementarity determining region onto human IgG1 by recombinant DNA technology, in order to reduce the antigenicity of the murine antibody [44]. By reducing the antigenicity in humans neutralizing antibodies appeared less frequently even with repeated dosing, and consequently the half-life of tocilizumab was prolonged.

Since tocilizumab does not cross-react with rodent IL-6R, the anti-arthritis effect of tocilizumab was examined using CIA in cynomolgus monkey [45] and severe combined immunodeficiency (SCID) mice grafted with human synovial tissue [46]. Treatment with tocilizumab inhibited the elevation of CRP, fibrinogen levels and ESR and suppressed joint destruction in the cynomolgus monkey with CIA. In SCID mice grafted with human tissue, the number of inflammatory cells, matrix metalloproteinase (MMP)-positive cells and osteoclasts identified as tartrate-resistant acid phosphatase-positive cells decreased when treated with tocilizumab. These findings suggested that tocilizumab would be effective for treatment of human RA.

Pharmacodynamics

IL-6 binds to membrane-expressed IL-6R, which does not have a signal-transducing domain. The soluble form of IL-6R (sIL-6R) exists in the serum and synovial fluid. Also IL-6 can bind to sIL-6R. Both types of IL-6R can form an IL-6–IL-6R complex. The complex associates with gp130, which is an actual signal-transducing chain, and induces its dimerization [47–49]. Dimerized gp130 activates the JAK–STAT cascade and nuclear factor IL-6 (Figure 2A, 2B). Tocilizumab binds to both soluble and membrane IL-6R and by competitively inhibiting the binding of IL-6 to IL-6Rs blocks the IL-6 biological activity (Figure 2C). The dissociated constant (Kd value) of tocilizumab determined by scatchard analysis using [^{125}I]-labeled tocilizumab was 2.54 ± 0.12 nmol/l (mean ± standard error [SE]) [50].

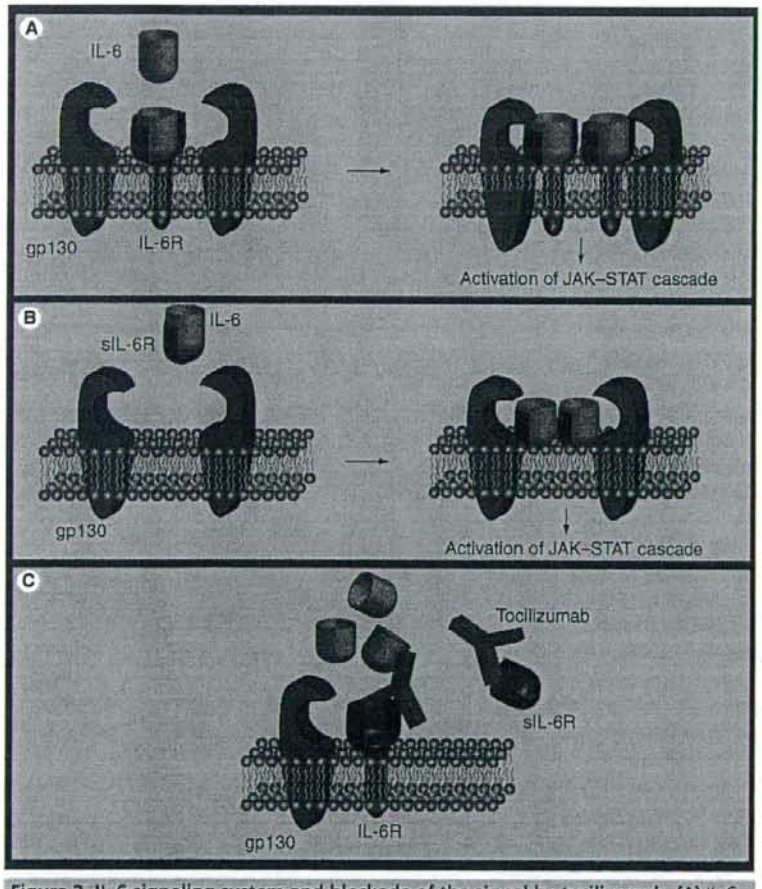

Figure 2. IL-6 signaling system and blockade of the signal by tocilizumab. (A) IL-6 signal transduction through the membrane IL-6R. **(B)** IL-6 signal transduction through the sIL-6R. **(C)** IL-6 signal blocked by tocilizumab.
IL-6R: IL-6 receptor; sIL-6R: Soluble IL-6 receptor.

Pharmacokinetics & metabolism

In an open label dose-escalation study patients with RA received tocilizumab 2, 4 or 8 mg/kg bodyweight intravenously once every 2 weeks [51]. Only in the 8-mg/kg group could serum tocilizumab concentration always be detected in all the patients. The AUC value increased as the tocilizumab dose increased and tocilizumab was repeatedly administered. The half-life also increased and reached 241.8 ± 71.4 h following the third infusion in the 8-mg/kg group [52].

CRP levels were completely normalized in patients whose serum tocilizumab levels could be maintained, while CRP levels were not normalized in patients whose serum tocilizumab levels could not be maintained. Therefore, the action of IL-6 can be blocked if free tocilizumab levels are detectable in the serum, and CRP can be a surrogate maker for tocilizumab concentration sufficient enough to block this action [52].

Clinical efficacy

Phase I/II studies

In an open label, Phase I/II clinical study, 15 patients with RA were treated with tocilizumab 2, 4 or 8 mg/kg bodyweight once every 2 weeks for 24 weeks [51]. Inflammatory indicators, such as CRP, ESR and SAA, were completely normalized in 12 out of 15 patients at 6 weeks, although there were no statistically significant differences in ACR clinical response rate among the three dose groups. At 24 weeks, 13 out of 15 patients achieved ACR20 and five achieved ACR50.

In a randomized, double-blind, placebo-controlled, dose-escalation clinical study in the UK, 45 patients with active RA received a single infusion of tocilizumab 0.1, 1, 5 or 10 mg/kg bodyweight or placebo [53]. In the 5 and 10 mg/kg groups, CRP and ESR were normalized at 2 weeks after the administration of tocilizumab. In the 5 mg/kg group, 55.6% of patients achieved ACR20 at 2 weeks while no patients in the placebo group achieved ACR20, although there was no statistically significant efficacy in the other three groups [53]. The results of these Phase I/II studies encouraged us the move to Phase II trials.

Phase II studies

In a multicenter, double-blind, placebo-controlled Phase II study in Japan, 164 patients with refractory RA were randomized to receive tocilizumab 4 or 8 mg/kg or placebo every 4 weeks for a total of 3 months [54]. At the last observation, 78% of patients in the 8-mg/kg group, 57% in the 4-mg/kg group and 11% in the placebo group achieved ACR20 (FIGURE 3A). The proportions of patients achieving ACR50 response were 40% for the 8-mg/kg group, 26% for the 4-mg/kg group and 1.9% for the placebo group. ACR70 response rates were 16% for the 8-mg/kg group and 20% for the 4-mg/kg group. No patients achieved ACR70 in the placebo group. Therefore, tocilizumab treatment reduced disease activity.

In patients treated with tocilizumab, laboratory findings, such as hemoglobin levels, platelet counts, SAA, MMP-3 and rheumatoid factor values, were improved. As for markers related to bone metabolism, serum levels of osteocalcin and C-terminal propeptide of type I procollagen (markers of bone formation) increased during tocilizumab treatment, while urinary pyridinoline and deoxypyridinoline (markers of bone absorption) decreased. These findings indicated that tocilizumab improved bone metabolism associated with RA.

In a double-blind, randomized controlled Phase II study in Europe (the Chugai Humanized Anti-Human Recombinant IL-6 Monoclonal Antibody [CHARISMA] study), 359 RA patients with an inadequate response to MTX at more than 10 mg/week were randomized to receive 2, 4, 8 mg/kg bodyweight of tocilizumab monotherapy or combination with previous MTX dose, or MTX monotherapy [55]. At 16 weeks, 61% of patients in the 4- and 63% in the 8-mg/kg tocilizumab-monotherapy group achieved ACR20 compared with 41% of patients in the MTX-monotherapy group (FIGURE 3B). ACR20

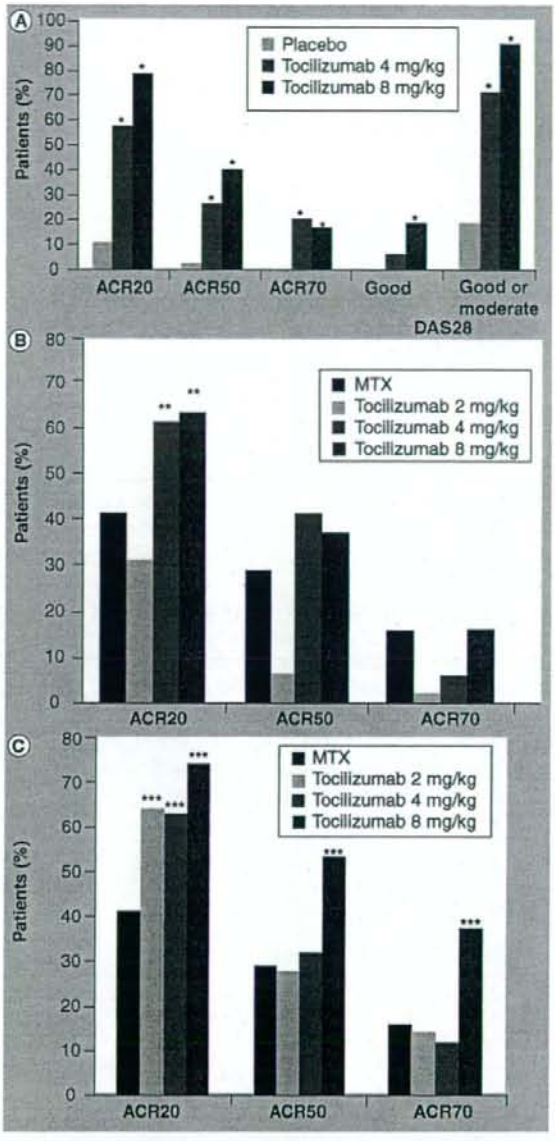

Figure 3. ACR response rate in Phase II clinical trial in both Japan and Europe. (A) Tocilizumab monotherapy in Japan. **(B)** Tocilizumab monotherapy in the CHARISMA study. **(C)** Tocilizumab combination therapy with MTX in the CHARISMA study.
*$p < 0.05$; tocilizumab-monotherapy group versus placebo group.
**$p < 0.05$; tocilizumab-monotherapy group versus MTX monotherapy group.
***$p < 0.05$; tocilizumab combination therapy with MTX versus MTX-monotherapy group.
ACR: The American College of Rheumatology; DAS: Disease activity score; MTX: Methotrexate.

response rates in patients receiving combination therapies with tocilizumab 2, 4 or 8 mg/kg and MTX were 64, 63 and 74%, respectively (Figure 3C). In ACR50 and ACR70 response rates, only combination therapy with 8 mg/kg of tocilizumab and MTX was significantly superior to that of MTX monotherapy. The disease activity score in 28 joints (DAS28) remission rate (defined as DAS28 < 2.6) was achieved in 34% of patients in the 8 mg/kg of tocilizumab plus MTX group, 17% in the tocilizumab 8 mg/kg-monotherapy group and 8% in MTX-monotherapy group.

These data indicate that blocking the IL-6 signal with tocilizumab was highly efficacious in the treatment of patients with RA, and that use in combination with MTX may increase the efficacy.

Phase III studies

In an x-ray, reader-blinded, randomized controlled trial of tocilizumab in Japan (Study of Active Controlled Monotherapy for Rheumatoid Arthritis, an IL-6 inhibitor [SAMURAI] trial), 306 patients with active RA of less than 5 years' disease duration were assigned to receive tocilizumab 8 mg/kg every 4 weeks or conventional DMARDs for 52 weeks [56]. Radiographs of hands and feet were scored using the van der Heijde's modified Sharp score at baseline, week 28 and week 52 independently by two readers blinded for order of films, treatments and responses (Figure 4). At week 28, the tocilizumab group showed significantly less radiographic change in total modified Sharp score (TSS) (mean 1.9) and erosion score (ES) (mean 0.8) than the DMARDs group (mean TSS value 4.5; mean ES value 2.4). At week 52, the tocilizumab group showed less radiographic change in TSS (mean 2.3), ES (mean 0.9) and joints space narrowing score (JNS) (mean 1.5) than the DMARDs group (mean TSS value, 6.1; mean ES value 3.2; mean JNS value 2.9). A total of 52% of patients in the tocilizumab group had no radiographic progression compared with 39% of patients in the DMARDs group. These results suggested that tocilizumab monotherapy inhibited the progression of structural joints damage in patients with RA.

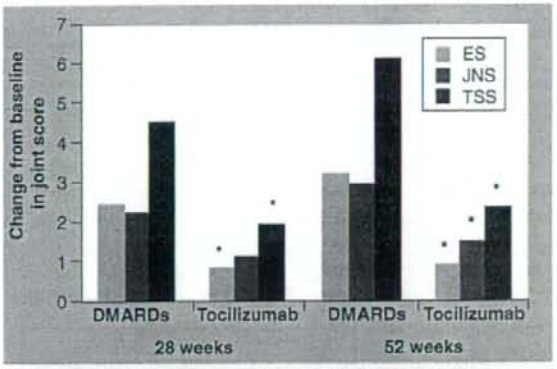

Figure 4. Tocilizumab monotherapy inhibiting the progression of joints damage in patients with rheumatoid arthritis in the SAMURAI trial.
*p < 0.05; tocilizumab monotherapy group versus DMARDs group.
p-values were analyzed with a rank-transformed analysis of covariance (ANCOVA) on the change scores that included factors for baseline score and baseline disease duration.
DMARD: Disease-modifying antirheumatic drugs; ES: Erosion score; JNS: Joints space narrowing score; TSS: Total Sharp score.

Safety & tolerability

No serious adverse reactions related to tocilizumab were observed in a Japanese Phase I/II dose-finding study [51] and a dose-escalation study conducted in the UK [53]. In Phase II clinical trials in Japan, the overall incidence rate for adverse events was similar among the placebo, 4- and 8-mg/kg groups (56, 59 and 51%, respectively). There was no dose dependency. In the SAMURAI trial, the rate of patients with adverse events was 89% in the tocilizumab group and 82% in the DMARDs group [56]. Most adverse events were mild or moderate in both groups.

Infection

In a Phase II clinical trial in Japan, two serious treatment-emergent infections in the tocilizumab groups were reported. One patient in the 8-mg/kg group died from hemophagocytosis syndrome associated with reactivation of Epstein–Barr virus (EBV) at 61 days following the first infusion of tocilizumab [57]. This patient had repeated episodes of lymphadenopathy developing during MTX treatment and disappearing after discontinuation of MTX. Retrospectively, an increase of EBV DNA was found in the patient's plasma before entering the clinical trial. By autopsy, Hodgkin's lymphoma cells were found to infiltrate the enlarged lymph nodes and EBV-encoded small RNA-1 was also identified. Pre-existing Hodgkin's disease might have worsened the clinical course. One patient in the 4-mg/kg group was hospitalized because of infection secondary to a burn on the leg. The patient was cured with adequate treatment.

In the CHARISMA study, two patients in the 8-mg/kg tocilizumab plus MTX group had a severe sepsis and they both improved by adequate treatment.

In the SAMURAI trial, 12 serious infections, including three pneumonia and two upper respiratory tract infections, in the tocilizumab group and eight serious infections, including three gastroenteritis and two pneumonia, in the DMARDs group were reported. All of the infection events were improved by appropriate treatment and no prolongation of the infection was observed with tocilizumab treatment. There was no specific infection related to tocilizumab treatment and no tuberculosis was reported for a 1-year period in the SAMURAI trial.

Hypersensitivity & abnormal laboratory findings

Drug-related infusion reaction were reported in five out of 102 patients treated in the CHARISMA study and 11 out of 157 patients in the SAMURAI trial. All the infusion reactions were

mild. Anti-tocilizumab antibodies were detected in four patients in the SAMURAI trial. One patient showed a skin eruption while the other three were asymptomatic. There was no increase in antinuclear antibodies (ANAs) or anti-DNA antibodies.

An increase in total cholesterol levels was frequently reported in the tocilizumab treatment groups in all the clinical trials. However, the total cholesterol levels did not continue to increase and became stable around the upper normal range in most cases. Since high-density lipoprotein also increased, the atherogenic index did not change. No cardiovascular complications were observed within the relatively short term of the clinical trials. Transient neutropenia and transient increase in liver-function tests were observed, but they returned to normal with repeated treatment with tocilizumab.

These clinical trials for patients with RA have demonstrated that tocilizumab monotherapy is generally well tolerated and safe.

Regulatory affairs

Tocilizumab was approved for the use in patients with Castleman's disease in Japan in 2005 and is under review for RA and JIA currently in Japan.

Conclusion

Tocilizumab is effective for patients with refractory RA in monotherapy as well as in combination with MTX. Tocilizumab significantly suppresses radiographic progression and improves the joint function in patients with RA. In addition, tocilizumab shows an excellent safety profile.

Expert commentary

The symptoms related to infection, such as fever and general fatigue, are also suppressed during tocilizumab treatment. Thus, it is sometimes difficult for patients to notice the occurrence of an infection and, consequently, the diagnosis of infections might be delayed. In addition, serum CRP levels are usually normalized by tocilizumab therapy so that their diagnostic value in detecting infections is limited. Therefore, infections should be carefully monitored and diagnosed. Even a mild symptom (e.g., slight cough, sputa) can be a sign for a severe infection. Increase in white blood cell counts and radiographic analysis are useful to detect infections.

Amyloid A (AA) amyloidosis is one of the severe complications of inflammatory rheumatic diseases, such as RA and JIA, especially in Eastern Asia. It is important for treating AA amyloidosis to suppress SAA production less than 10 μg/ml [58]. Since IL-6 is a key cytokine for SAA production [59], tocilizumab may be effective for treating human AA amyloidosis caused by IL-6 overproduction. Indeed, tocilizumab improved not only arthritis in an active JIA patient with AA amyloidosis but also eliminated the clinical symptoms related to AA amyloidosis [60]. Future studies are needed to confirm that tocilizumab may be an efficacious agent against AA amyloidosis in RA.

Five-year view

Phase III clinical trials in Japan have already been completed. In Europe and worldwide, most of the Phase III clinical trials have been completed. Within a few years, tocilizumab will be available for routine clinical treatment of RA. Worldwide use of tocilizumab as the routine clinical treatment, it will be clear about the cost–effectiveness of treatment and whether it should be used before or after existing biologics, such as TNF inhibitors.

The broadening safety database will allow for the assessment of long-term risks, such as any specific infections related to tocilizumab and the development of malignancies, which are of a concern for some biologics. Tocilizumab could, theoretically, have a positive effect on some malignancies that are known to be stimulated by IL-6, such as multiple myeloma, malignant mesotheliomas, cervical cancer and renal carcinoma [61–64].

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Key issues

- IL-6 plays a key role in the pathogenesis of rheumatoid arthritis.
- Tocilizumab is a humanized anti-human IL-6 receptor (IL-6R) monoclonal antibody.
- Tocilizumab binds to both membrane-expressed and soluble IL-6R and inhibits the biological activity of IL-6.
- C-reactive protein is a surrogate marker for the maintenance of sufficiently high tocilizumab concentrations that ensures the blockade of IL-6 signalling.
- Tocilizumab monotherapy can improve the clinical signs and symptoms of RA.
- Tocilizumab suppresses radiographical progression in rheumatoid arthritis.
- There is no specific type of infection linked to tocilizumab therapy.

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- •• of considerable interest

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ORIGINAL ARTICLE

Study of active controlled tocilizumab monotherapy for rheumatoid arthritis patients with an inadequate response to methotrexate (SATORI): significant reduction in disease activity and serum vascular endothelial growth factor by IL-6 receptor inhibition therapy

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Abstract We investigated the clinical efficacy and safety of tocilizumab (a humanized anti-IL-6 receptor antibody) monotherapy in active rheumatoid arthritis (RA) patients with an inadequate response to low dose methotrexate (MTX). In a multicenter, double-blind, randomized, controlled trial, 125 patients were allocated to receive either tocilizumab 8 mg/kg every 4 weeks plus MTX placebo (tocilizumab group) or tocilizumab placebo plus MTX 8 mg/week (control group) for 24 weeks. The clinical responses were measured using the American College of Rheumatology (ACR) criteria and the Disease Activity Score in 28 joints. Serum vascular endothelial growth factor (VEGF) levels were also monitored. At week 24, 25.0% in the control group and 80.3% in the tocilizumab group achieved ACR20 response. The tocilizumab group showed superior ACR response criteria over control at all time points. Additionally, serum VEGF levels were significantly decreased by tocilizumab treatment. The overall incidences of adverse events (AEs) were 72 and 92% (serious AEs: 4.7 and 6.6%; serious infections: 1.6 and 3.3%) in the control and the tocilizumab groups, respectively. All serious adverse events improved by adequate treatment. Tocilizumab monotherapy was well tolerated and provided an excellent clinical benefit in active RA patients with an inadequate response to low dose MTX.

Keywords Clinical trial · Interleukin-6 · Rheumatoid arthritis · Tocilizumab · Vascular endothelial growth factor

Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by persistent synovitis and progressive destruction of cartilage and bone in multiple joints [1]. The affected joints exhibit hyperplasia of inflamed synovium infiltrated with a range of immunocompetent cells, which forms pannus tissue and invade cartilage and bone [2]. Angiogenesis is a characteristic histological feature of rheumatoid synovium for which vascular endothelial growth factor (VEGF) is responsible [3]. In addition, patients with RA show systemic inflammatory manifestations such as fever, fatigue, anemia, and laboratory findings, including elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), hyper-γ-globulinemia and emergence of various types of autoantibodies. These abnormalities can be explained, at least partly, by deregulated overproduction of interleukin (IL)-6, a pro-inflammatory cytokine, although the etiological causes are not fully understood.

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Recently biologics targeting T cells, B cells, as well as pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-1, have been successfully used in the treatment of RA. However, these therapies are not always effective. In addition, adverse reactions are also the problem for those treatments. Therefore, we need further new therapeutic agents that have a new mechanism of action and higher efficacy.

Tocilizumab is a humanized anti-human IL-6 receptor (IL-6R) monoclonal antibody that inhibits IL-6 binding to IL-6R [4]. This antibody was humanized by grafting the complementarity-determining regions from a murine anti-IL-6R antibody into human IgG1, thereby creating a functioning antigen-binding site in a reshaped human antibody and reducing the antigenicity of the antibody in human. We have demonstrated that treatment with tocilizumab improves the signs and symptoms and prevents joint damage of RA [5–8]. The objective of this clinical trial was to investigate the safety and efficacy of tocilizumab monotherapy in active RA patients with an inadequate response to MTX, an anchor drug for RA treatment, at a dose of 8 mg/week, which is approved for adult RA patients in Japan. In addition, tocilizumab effect on serum VEGF levels was also examined.

Patients and methods

Patients

Eligible patients were between 20 and 75 years old, fulfilled the American college of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria for the classification of RA [9], with disease duration of more than 6 months. All candidates were treated with MTX 8 mg/week for at least 8 weeks until enrolment. They all had ≥6 tender joints (of 49 evaluated), ≥6 swollen joints (of 46 evaluated), ESR of ≥30 mm/h or CRP of ≥10 mg/l at enrolment. An inadequate response to MTX was defined as the presence of active disease, as described above. Patients were not allowed to have received prior anti-TNF agents or leflunomide (within 12 weeks prior to the first dose), plasma exchange therapy or surgical treatments (within 4 weeks prior to the first dose), DMARDs other than MTX or immunosuppressants (within 2 weeks prior to the first dose). Oral corticosteroids (prednisolone, ≤10 mg/day) were allowed if the dosage had not been changed within 2 weeks. Eligible patients had white blood cell counts $\geq 3.5 \times 10^9$/l, lymphocyte counts $\geq 0.5 \times 10^9$/l and platelet count of at least the lower limit of normal as defined by the respective local laboratory used. Patients were excluded if they had functional class IV using Steinbrocker's criteria [10], aspartate transaminase (AST), alanine transaminase (ALT) and serum creatinine ≥1.5-fold the upper limit of normal, were HBs antigen and/or HCV antibody positive, had pulmonary fibrosis or active pulmonary disease, a history of serious adverse drug reaction to MTX, concomitant pleural effusion, ascites, varicella infection, or were excessive users of alcohol on a regular basis. Patients were also excluded if they had significant cardiac, blood, respiratory system, neurologic, endocrine, renal, hepatic, or gastrointestinal disease, or had an active infection requiring medication within 4 weeks before the first dose or medical history of a serious allergic reaction. Sexually active premenopausal women were required to have a negative urine pregnancy test at the entry to the study and to use effective contraception during the study period.

Study protocol

This study was conducted at 25 sites in Japan. The study protocol was approved by the Ministry of Health, Labor and Welfare of Japan, and by the local ethical committee. Patients gave their written informed consent. This trial was registered with http://www.clinicaltrials.gov (NCT00144521). The first patient was enrolled on January 27, 2004, and the last patient exited the study on February 15, 2005.

Patients were randomly assigned to receive either tocilizumab therapy or MTX therapy as a control: tocilizumab 8 mg/kg every 4 weeks plus MTX placebo (tocilizumab group) or tocilizumab placebo plus MTX 8 mg/week (control group) for 24 weeks. The randomization was done by registering the patients to the patient registration center utilizing a centralized allocation method. The dosage of tocilizumab used in this study was chosen according to a previous dose finding study [7]. The dose of MTX was the maximum dose allowed in Japan (see Discussion). Oral corticosteroids less than 10 mg prednisolone per day were allowed, and the dose could not be increased during the study. Intra-articular injections of corticosteroid (only one joint at one treatment) and hyaluronate preparations were allowed. Use of one nonsteroidal anti-inflammatory drug (NSAID), including switching to another NSAID, was allowed. DMARDs, intravenous or intramuscular corticosteroids, plasmapheresis and surgical treatment were not allowed. Patients who received three or more doses of tocilizumab or tocilizumab placebo were able to join an open-label extension study of tocilizumab.

The clinical responses were measured using the ACR criteria as well as the Disease Activity Score in 28 joints (DAS28) and the European League Against Rheumatism (EULAR) criteria based on DAS28. Remission was defined according to EULAR definition of a DAS28 <2.6 [11]. Serum VEGF levels were also monitored. Safety was