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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.

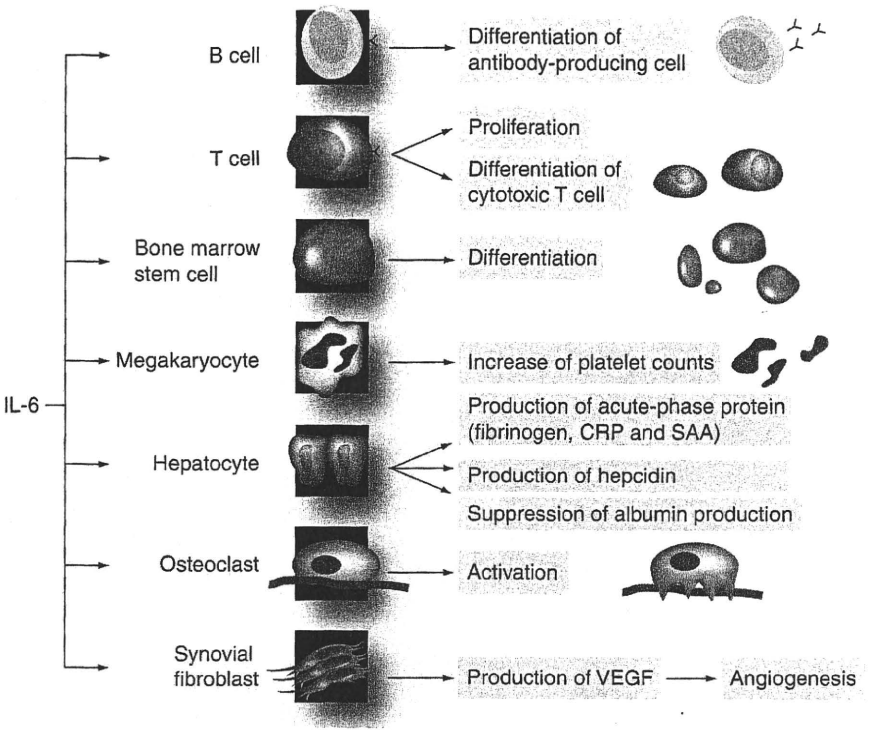

Figure 1. Biological role of IL-6 in the pathological condition of rheumatoid arthritis.
CRP: C-reactive protein; SAA: Serum amyloid A.

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].

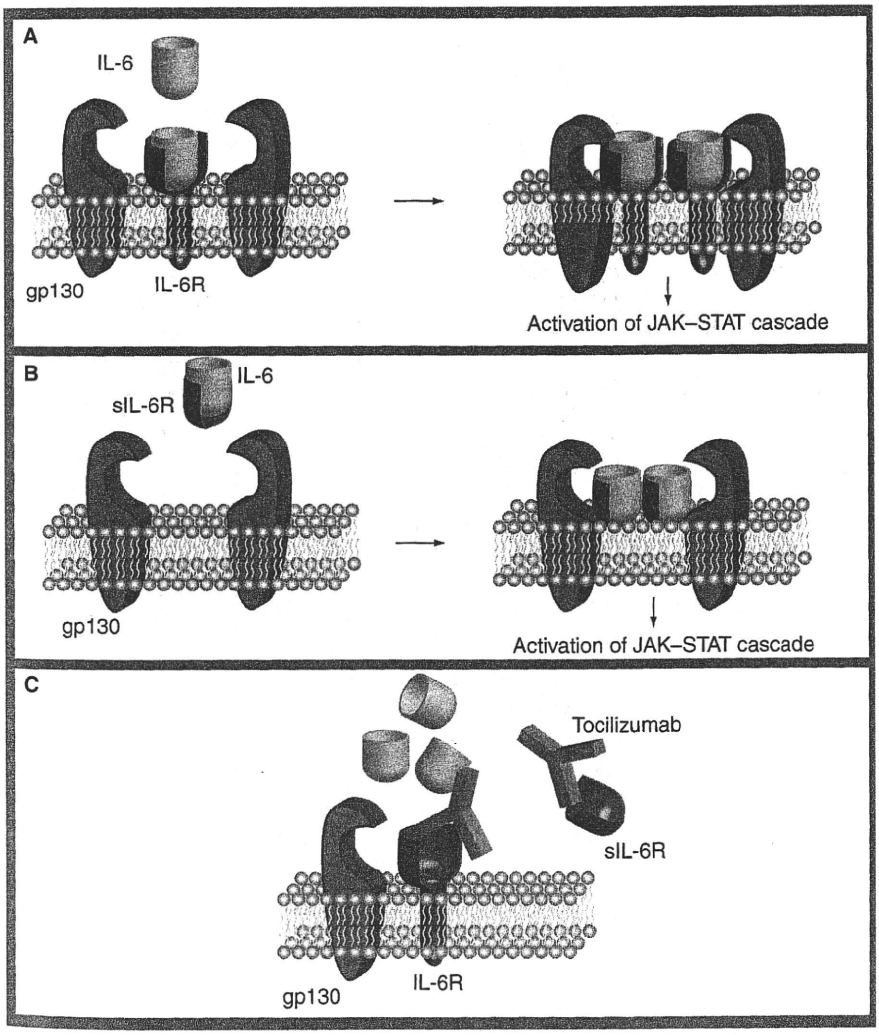

Figure 2. IL-6 signaling system and blockade of the signal by tocilizumab. (A) IL-6 signal transduction through the membrane IL-6R. **(B)** IL-6 signal transduction through the sIL-6R. **(C)** IL-6 signal blocked by tocilizumab.
IL-6R: IL-6 receptor; sIL-6R: Soluble IL-6 receptor.

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

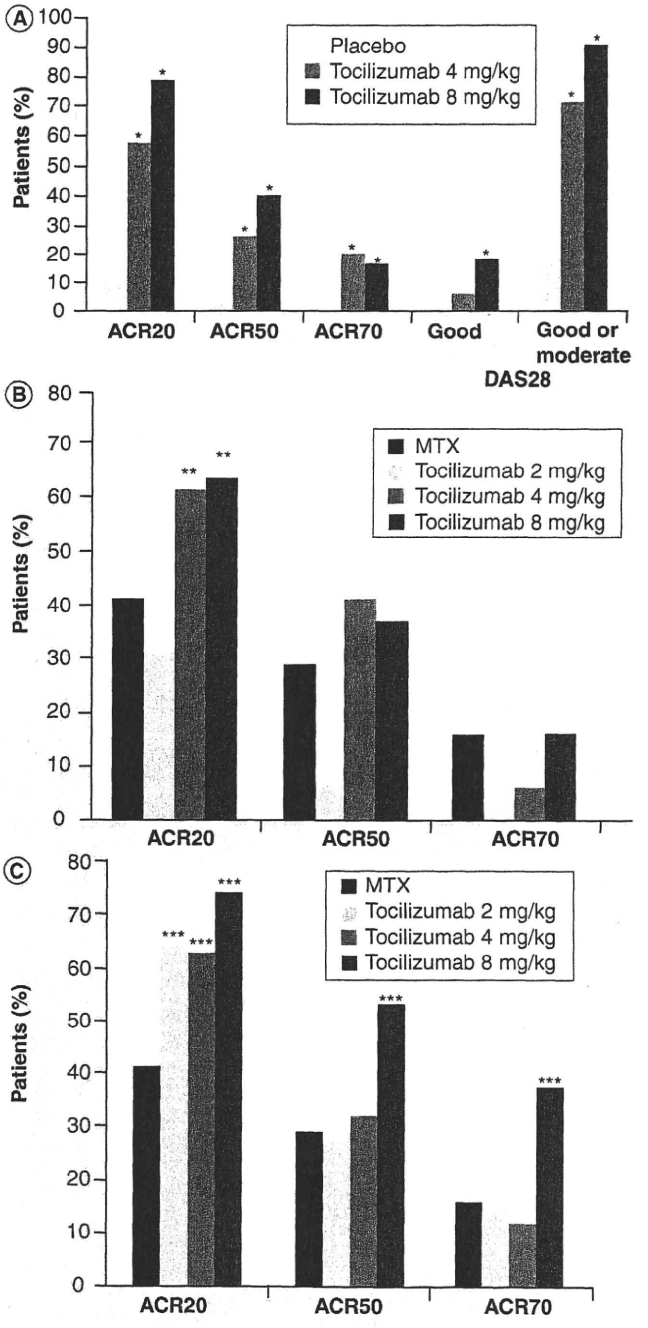

Figure 3. ACR response rate in Phase II clinical trial in both Japan and Europe. (A) Tocilizumab monotherapy in Japan. **(B)** Tocilizumab monotherapy in the CHARISMA study. **(C)** Tocilizumab combination therapy with MTX in the CHARISMA study.
*$p < 0.05$; tocilizumab-monotherapy group versus placebo 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.

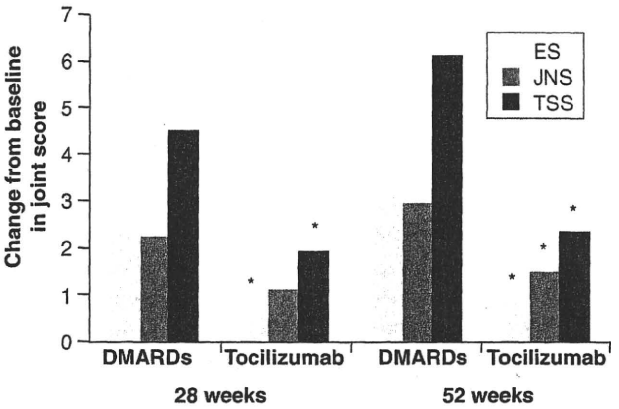

Figure 4. Tocilizumab monotherapy inhibiting the progression of joints damage in patients with rheumatoid arthritis in the SAMURAI trial.

*$p < 0.05$; 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 signaling.
- 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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Humanized Antihuman IL-6 Receptor Antibody, Tocilizumab

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Abstract Interleukin-6 (IL-6) is a pleiotropic cytokine that regulates immune responses and inflammatory reactions. Overproduction of IL-6 has been shown to play a role in inflammatory autoimmune diseases such as rheumatoid arthritis (RA), and juvenile idiopathic arthritis (JIA) and, therefore, an agent blocking IL-6 actions can be a therapy of these diseases. IL-6 belongs to a cytokine family, which shares the cytokine receptor subunit glycoprotein (gp) 130. This family also includes IL-11, oncostatin-M, and leukemia inhibitory factor (LIF). In the IL-6 receptor (IL-6R) system, both a membrane-bound IL-6R and a soluble form of IL-6R are able to mediate IL-6 signals into the cells through the interaction of gp130. Tocilizumab is a humanized antihuman IL-6 receptor antibody designed using genetic engineering technology. Tocilizumab recognizes both the membrane-bound and the soluble form IL-6R and specifically blocks IL-6 actions. Tocilizumab is expected to ameliorate the autoimmune inflammatory diseases with IL-6 overproduction and has been clinically developed as a therapeutic agent for RA, systemic-onset and articular types of JIA, Crohn's disease, etc. Tocilizumab has been shown to be effective not only

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for improving signs and symptoms but also for preventing joint destruction of RA. Immunopharmacology and clinical benefit of tocilizumab in RA is addressed.

1 Introduction

Interleukin-6 (IL-6) is a multifunctional cytokine that regulates immune responses and inflammatory reactions, and is likely to mediate the autoimmune, inflammatory, and joint destruction aspects of rheumatoid arthritis (RA) (Nishimoto 2006). Thus, agents that block the actions of IL-6 are potential therapeutic options for RA treatment. Tocilizumab is a humanized antihuman IL-6 receptor antibody designed using genetic engineering technology (Sato et al. 1993). Since it specifically blocks the actions of IL-6, it is effective in treating conditions resulting from excessive IL-6 production. Moreover, it was approved in April 2005 as the world's first drug for Castleman's disease (Nishimoto et al. 2005), an atypical lymphoproliferative disorder (trade name: ACTEMRA® 200 for intravenous infusion). Tocilizumab has also been developed as a treatment for RA, juvenile idiopathic arthritis (Yokota et al. 2005), and Crohn's disease (Ito et al. 2004). This review describes the immunopharmacology and clinical utility of tocilizumab mainly in RA.

2 Structure of Tocilizumab

Tocilizumab is a genetically-engineered monoclonal antibody, humanized from a mouse antihuman IL-6 receptor antibody using the CDR grafting method (Sato

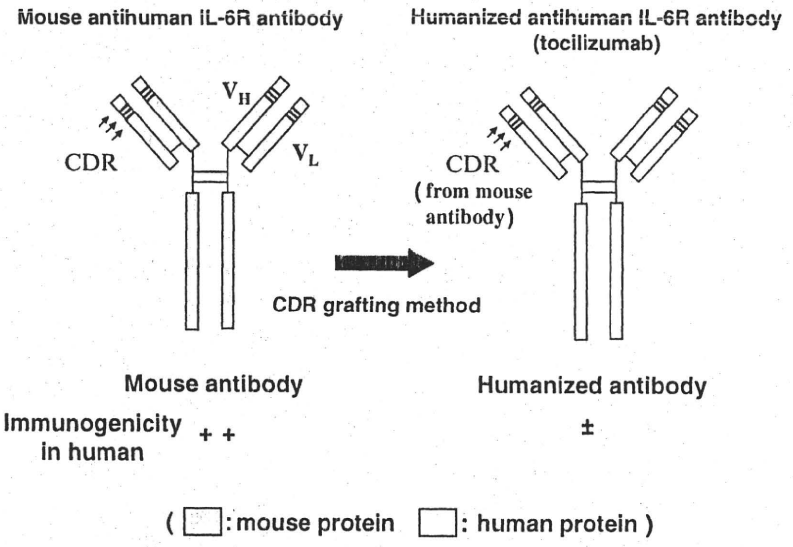

Fig. 1 Humanized antihuman IL-6 receptor (tocilizumab). Antigenicity in humans is reduced by humanizing a mouse antihuman IL-6 receptor with the CDR grafting method (Sato et al. 1993)

et al. 1993) (Fig. 1). It was initially called MRA for myeloma receptor antibody, because of potential applications in multiple myeloma treatments (Sato et al. 1993; Nishimoto et al. 1994), but was then renamed tocilizumab. Humanization of tocilizumab has resulted in decreased antigenicity in the human body. Therefore, the drug's half-life is prolonged and repetitive treatment with tocilizumab rarely causes production of neutralizing antibodies compared with mouse antibodies or mouse and human chimeric antibodies.

3 Immunopharmacological Characteristics of Tocilizumab

3.1 Mechanism of Action

Tocilizumab recognizes the IL-6 binding site of the human IL-6 receptor (IL-6R) and inhibits IL-6 signaling through competitive blockade of IL-6 binding (Fig. 2). Despite being an IgG1 antibody, a regular dose of tocilizumab in humans causes no antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity in cells that express IL-6R.

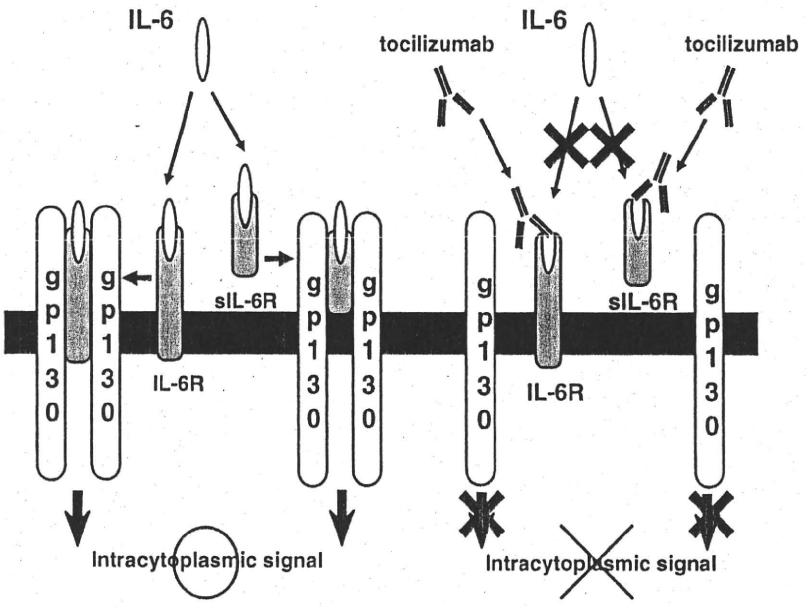

Fig. 2 IL-6 receptor system and mechanism for tocilizumab inhibition of IL-6 signaling. IL-6 triggers dimerization of signal-transducing gp130 molecules on the cell membrane when binding with membrane IL-6 receptors (IL-6R) or soluble receptors (sIL-6R) in body fluids, thus transmitting signals into the cells. Tocilizumab recognizes both IL-6R on the cell membrane and sIL-6R, and blocks IL-6 signaling

Soluble IL-6 receptors (sIL-6R), on the other hand, are found in body fluids such as blood and synovial fluid. Unlike tumor necrosis factor (TNF), whose signals are blocked by soluble TNF receptors, IL-6 signals are transmitted into the cell by sIL-6R. This mechanism is called trans-signaling (Scheller et al. 2006) and occurs when IL-6 binds sIL-6R, assembles with gp130 molecules on the cell membrane, and triggers formation of the high affinity IL-6R complex. Tocilizumab recognizes both IL-6R on the cell membrane and sIL-6R, and inhibits IL-6 signaling by preventing ligand-receptor binding (Mihara et al. 2005).

3.2 Pharmacokinetics

The pharmacokinetics of tocilizumab were examined in detail by conducting a phase I study of healthy adults and a phase I/II study of RA patients (Nishimoto et al. 2003). The phase I/II study was conducted with 15 RA patients who had previously had an insufficient response to one or more doses of disease modifying antirheumatic drugs or immunosuppressants, or had side effects that led to discontinued treatment. Patients underwent repetitive treatment with 2, 4, or 8 mg kg^{-1} body weight of tocilizumab at 2-week intervals for a total of three times, and the pharmacokinetics were examined. Figure 3A shows changes in blood-level tocilizumab during repetitive treatment, indicating nonlinear pharmacokinetics in the 2–8 mg kg^{-1} dose range. The half-life of tocilizumab (t1/2) was dose-dependent and prolonged as dosage increased from 2 to 8 mg kg^{-1}, as well as when the number of doses increased through repetitive treatment (Fig. 3B). In addition, the half-life at the third 8 mg kg^{-1} dose was about 240 h, close to the half-life of human immunoglobin (Ig) G1. Blood-level area under the curve (AUC) of tocilizumab also increased an average of 19.9 mg hr ml^{-1} (Nishimoto et al. 2003).

3.3 Pharmacological Characteristics

Blood-level tocilizumab in the second posttreatment week was absent in most patients who received 2 mg kg^{-1} tocilizumab at 2-week intervals, while it tended to accumulate in most patients who received 4 or 8 mg kg^{-1}. These patients were completely negative for CRP and serum amyloid A (Fig. 4A). On the other hand, those who showed no blood-level tocilizumab were positive for CRP and serum amyloid A (Nishimoto et al. 2003).

CRP and serum amyloid A are acute-phase proteins produced by the liver as a result of IL-6, IL-1, and TNF stimulation. Infliximab and etanercept, TNF inhibitors, also reduce CRP, but only a limited number of patients became completely negative for CRP (Charles et al. 1999). Production of CRP and serum amyloid A clearly requires IL-6, as IL-6 inhibition resulted in negative values for these markers. Therefore, it is important to maintain blood-level tocilizumab to inhibit the actions of IL-6.

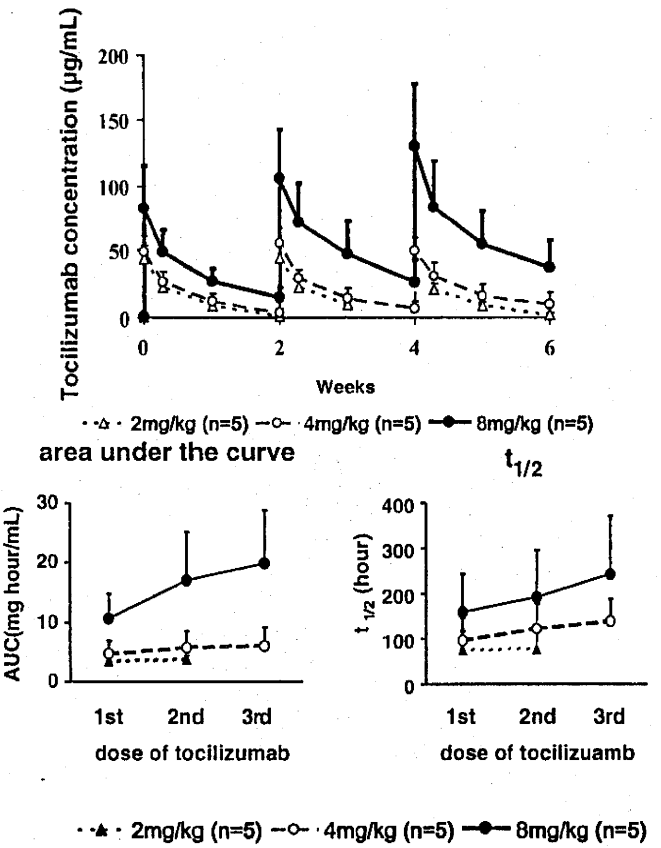

Fig. 3 Pharmacokinetics of tocilizumab. (**A**) Changes in blood-level tocilizumab. (**B**) Blood-level area under the curve (AUC) and blood half-life (t1/2) (adapted from Nishimoto et al. 2003) Repetitive treatment increases both AUC and t1/2

CRP may serve as an alternate indicator for sufficient blood levels of tocilizumab (Nishimoto et al. 2003).

4 Clinical Utility of Tocilizumab

Choy et al. have conducted a British phase I study with 45 patients treated with a single dose of 0.1, 1, 5, or 10 mg kg^{-1} tocilizumab, or placebo (Choy et al. 2002). This study evaluated tocilizumab safety and RA disease activity in the second posttreatment week using the American College of Rheumatology (ACR) improvement criteria. The ACR20 rate was 56% for the 5 mg kg^{-1} tocilizumab group and 0% for placebo group, indicating a significant difference. However, no significant

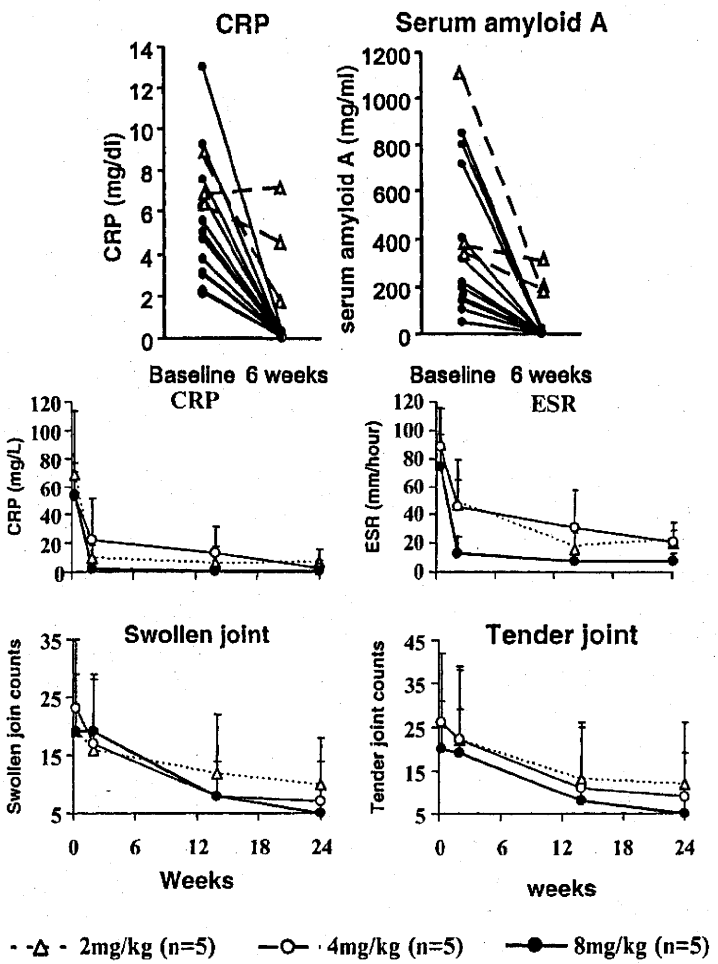

Fig. 4 Clinical effects of tocilizumab. (A) Quantitative changes in acute-phase proteins in the blood: acute-phase proteins, CRP, and serum amyloid A were normalized in patients with measurable blood-level tocilizumab (*filled circle*). Normalization of CRP and serum amyloid A was not observed in patients who did not maintain blood-level tocilizumab (*open triangle*). (B) Improved clinical findings: treatment with tocilizumab quantitatively improved swollen and painful joints, as well as CRP values and erythrocyte sedimentation rates (ESR) (adapted from Nishimoto et al. 2003)

difference in ACR20 rate was observed between other tocilizumab groups and the placebo group. The results of this study also confirmed an adequate level of safety with no serious side effects.

In the Japanese 24-week phase I/II study, patients that did not show serious side effects, had improved CRP or erythrocyte sedimentation rate (ESR) in the second week following the third dose, were further treated with tocilizumab if the

patients were willing to continue the tocilizumab therapy, and also if the principal investigator physician decided that the patients required to receive the treatment. Of all the patients, the ACR20 response rate at the sixth week was 60% and 86% at the sixth month (Nishimoto et al. 2003). In addition, the ACR50 rate was 13% at the sixth week and 33% at the sixth month. The findings of this open study suggested that tocilizumab has large treatment effects on RA disease activity (Fig. 4B), which lead to a phase II study.

A randomized, multicenter, double-blind, placebo-controlled trial of tocilizumab was conducted in a Japanese late phase II study, with 164 patients who previously had an insufficient response to one or more doses of antirheumatic or immunosuppressant drugs. Patients underwent intravenous therapy with either 4 or 8 mg kg^{-1} tocilizumab, or placebo given at 4-week intervals for a total of three times. RA disease activity was evaluated in the fourth posttreatment week (Nishimoto et al. 2004). The ACR20 response rate of 8 mg kg^{-1} tocilizumab was 78%, while that of placebo was only 11%, thus confirming clinical utility of tocilizumab in a double-blind study (Fig. 5).

Evaluation of drug safety showed no significant difference in adverse event rates between 4 and 8 mg kg^{-1} tocilizumab, and placebo groups. Laboratory findings, however, indicated a dose-dependent increase of total cholesterol values in 44% of patients treated with tocilizumab. This increase stabilized at around 240 mg dl^{-1}, and HDL cholesterol also increased in a similar way. Long-term safety evaluation is necessary to know whether or not the increased total cholesterol value indicates a higher risk of cardiovascular diseases. It has been reported that TNF inhibition also

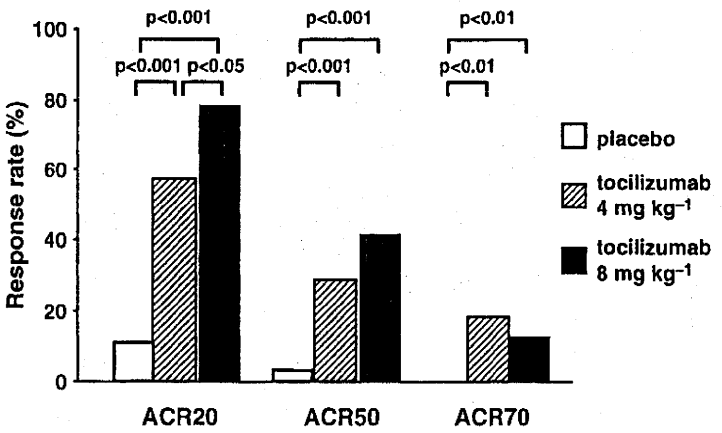

Fig. 5 Disease activity evaluation by ACR improvement criteria. Treatment with tocilizumab significantly improved RA disease activity when compared with the placebo treatment (adapted from Nishimoto et al. 2004). ACR improvement criteria: ACR20 is defined as improvement by 20% or more in tender and swollen joint counts and 20% improvement in 3 of the 5 remaining ACR core set measures: patient and physician global assessments, pain, disability, and an acute-phase reactant (CRP or ESR). Likewise, ACR50 is achieved with improvement by 50% or more and ACR70 with improvement by 70% or more

resulted in an increase in total cholesterol, suggesting that this effect may be due to a decrease in RA disease activity (Seriolo et al. 2006).

Tocilizumab monotherapy, with no combinatory use of methotrexate, resulted in an insignificant number of patients developing antinuclear antibodies or anti-DNA antibodies, which are frequently observed with anti-TNF antibody treatment. Moreover, the anti-tocilizumab antibody was observed only in 2% of the patients treated with tocilizumab, reconfirming the advantages of humanized antibodies. As IL-6 is a cytokine that induces antibody production, IL-6 inhibition may reduce the production of neutralizing antibodies. In terms of treatment strategy, it is highly advantageous that methotrexate is not required.

Serious adverse events have been observed in patients with infectious diseases treated with 8 mg kg^{-1} tocilizumab. A patient with Epstein-Barr virus (EBV) reactivation died about 2 months later from subsequent hemophagocytic syndrome. This patient manifested conditions similar to those of chronic active EBV infection, with fluctuation in abnormal liver function tests and CRP increase, which inversely correlated with the number of peripheral leukocytes. Plasma EBV DNA was detected prior to study participation, but later findings indicated that this patient also had Hodgkin's disease prior to the study (Ogawa et al. 2006). Subsequent studies examined more than 200 patients treated with tocilizumab, of which none had plasma EBV DNA. While the EBV reactivation mechanism is currently unclear, a careful pretreatment examination is necessary in tocilizumab therapy to determine whether or not the patient has an infectious disease.

The above studies indicate that tocilizumab is effective in reducing RA disease activity, even as a monotherapy, and has a tolerability within the permissible range. An 8 mg kg^{-1} dose at 4-week intervals appears to be the optimum regimen.

Maini et al. have conducted a European phase II study on the combinatory treatment of tocilizumab with methotrexate (Maini et al. 2006). This study yielded results that confirm the effectiveness of tocilizumab as a monotherapy. No difference was observed in the ACR20 rate between monotherapy with 8 mg kg^{-1} tocilizumab and combinatory treatment with 8 mg kg^{-1} tocilizumab and methotrexate. However, a synergistic effect was observed in combinatory treatment with 4 mg kg^{-1} or a lower dose of tocilizumab and methotrexate.

A Japanese phase III trial was conducted to examine the preventive effects of tocilizumab on progressive joint destruction. This study evaluated the 1-year change in the van der Heijde's modified Sharp score (i.e., a quantitative radiographic evaluation of bone erosion and joint space narrowing in hand and foot joints of RA patients). The results indicate that tocilizumab is also effective in preventing progressive joint destruction (Nishimoto et al. 2005).

5 Conclusion

Ongoing clinical trials have shown the effectiveness of tocilizumab in treating RA, and the drug is currently undergoing the application process for approval in Japan. Although no direct comparison data with TNF inhibitors are available, it is notable

that tocilizumab is highly effective even in a monotherapy. Future studies include long-term safety evaluation and the examination of differential applications of IL-6 inhibition treatment and TNF or IL-1 inhibition treatments. These studies based on the biological functions of target molecules will elucidate how each treatment takes part in the strategy of RA treatment.

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Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial

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See Comment page 961

See Articles page 987

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Summary

Background Systemic-onset juvenile idiopathic arthritis does not always respond to available treatments, including antitumour necrosis factor agents. We investigated the efficacy and safety of tocilizumab, an anti-interleukin-6-receptor monoclonal antibody, in children with this disorder.

Methods 56 children (aged 2–19 years) with disease refractory to conventional treatment were given three doses of tocilizumab 8 mg/kg every 2 weeks during a 6-week open-label lead-in phase. Patients achieving an American College of Rheumatology Pediatric (ACR Pedi) 30 response and a C-reactive protein concentration (CRP) of less than 5 mg/L were randomly assigned to receive placebo or to continue tocilizumab treatment for 12 weeks or until withdrawal for rescue medication in a double-blind phase. The primary endpoint of the double-blind phase was an ACR Pedi 30 response and CRP concentration of less than 15 mg/L. Patients responding to tocilizumab and needing further treatment were enrolled in an open-label extension phase for at least 48 weeks. The analysis was done by intention to treat. This study is registered with ClinicalTrials.gov, numbers NCT00144599 (for the open-label lead-in and double-blind phases) and NCT00144612 (for the open-label extension phase).

Findings At the end of the open-label lead-in phase, ACR Pedi 30, 50, and 70 responses were achieved by 51 (91%), 48 (86%), and 38 (68%) patients, respectively. 43 patients continued to the double-blind phase and were included in the efficacy analysis. Four (17%) of 23 patients in the placebo group maintained an ACR Pedi 30 response and a CRP concentration of less than 15 mg/L compared with 16 (80%) of 20 in the tocilizumab group ($p<0·0001$). By week 48 of the open-label extension phase, ACR Pedi 30, 50, and 70 responses were achieved by 47 (98%), 45 (94%), and 43 (90%) of 48 patients, respectively. Serious adverse events were anaphylactoid reaction, gastrointestinal haemorrhage, bronchitis, and gastroenteritis.

Interpretation Tocilizumab is effective in children with systemic-onset juvenile idiopathic arthritis. It might therefore be a suitable treatment in the control of this disorder, which has so far been difficult to manage.

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Introduction

Systemic-onset juvenile idiopathic arthritis is a subtype of chronic childhood arthritis of unknown cause, manifested by spiking fever, erythematous skin rash, pericarditis, and hepatosplenomegaly.[1] Half of patients given non-steroidal anti-inflammatory drugs or corticosteroids continue to show progressive involvement of increasing number of joints and severe functional disability with striking growth impairment. Moreover, long-term use of systemic corticosteroids leads to various disorders, including iatrogenic Cushing's disease, growth suppression, bone fracture, or cataracts. Sometimes systemic-onset juvenile idiopathic arthritis progresses to macrophage-activation syndrome, in which the inflammation might be caused by cytokine storm;[2–4] therefore, effective and tolerable treatments are much needed.

One major development in rheumatology was the introduction of biological-response modifiers. Tumour necrosis factor α (TNFα) concentrations are increased in serum and synovial fluid of children with juvenile idiopathic arthritis, and concentrations are correlated with disease activity.[5] Etanercept has proven effective in the treatment of children with this type of arthritis, which is resistant to methotrexate.[6] To protect joints from inflammatory destruction, use of biological-response modifiers as early as possible is appropriate in patients with rheumatoid arthritis.[7] These findings also encourage the use of these drugs for treatment of juvenile idiopathic arthritis. However, patients with this type of arthritis have a higher rate of etanercept failure than those with other chronic arthritis subtypes,[8] indicating that TNFα is not the only cytokine implicated in the pathogenesis of the disease. Macrophage-activation syndrome has been reported during treatment with etanercept;[9] therefore inhibition of TNFα does not always prevent this potentially fatal complication.

Although serum concentrations of interleukin 1 are not increased in systemic-onset juvenile idiopathic arthritis, dysregulation of this cytokine might play a part in the pathogenesis.[10] Case reports and an early uncontrolled