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ORIGINAL ARTICLE

Shunsuke Mori

A relationship between pharmacokinetics (PK) and the efficacy of infliximab for patients with rheumatoid arthritis: characterization of infliximab-resistant cases and PK-based modified therapy

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Abstract Infliximab, a chimeric anti-tumor necrosis factor α (TNF-α) monoclonal antibody, has been recognized as significantly improving the course of rheumatoid arthritis (RA); however, a subset of patients shows poor responses. To understand the mechanism underlying such unresponsiveness, I examined the clinical pharmacokinetics (PK) of infliximab, using time–serum concentration profiles obtained from 21 RA patients who had received infliximab therapy in combination with methotrexate (MTX). At week 14 of therapy, 15 cases achieved good or moderate responses in the European League Against Rheumatism (EULAR) criteria, and 3 cases resulted in nonresponders. The others discontinued therapy because of severe adverse effects or aggravation of disease activities. The means of distribution volume and elimination half-life ($t_{1/2}$) during the first 2 weeks were 0.05 l/kg and 9.5 days, respectively. Through 14 weeks, most good and moderate responders maintained serum concentrations of more than 1 μg/ml, even immediately before the next infusions. Only 3 cases among good or moderate responders showed undetectable levels of trough serum concentration at week 14. In contrast, the PK profiles of all nonresponders except one showed rapid clearance during therapy. These data support the idea that the rapid clearance of infliximab is the main cause of poor therapeutic responses. I also found that the $t_{1/2}$ during the first 2 weeks is inversely correlated to the disease activity scores for 28 joints at the start of treatment, suggesting that TNF-α levels may determine the disease activity of RA. For patients who showed a rapid clearance of infliximab, the increased use of prednisone or MTX was beneficial to achieve sufficient clinical responses. The addition of tacrolimus was effective to improve the clinical outcomes of nonresponders. Thus PK data apparently offer guidance when modified treatment for infliximab-resistant RA patients is being considered.

Key words Infliximab · Pharmacokinetics · Rheumatoid arthritis · Tumor necrosis factor α

S. Mori (✉)
Department of Rheumatology, Clinical Research Center for Rheumatic Disease, National Kumamoto Saishunsou Hospital, 2659 Suya, Kohshi 861-1196, Japan
Tel. +81-96-242-1000; Fax +81-96-242-2619
e-mail: moris@saisyunsou.hosp.go.jp

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that primarily targets synovial tissues. Over the course of the disease, these tissues proliferate uncontrollably, resulting in persistent synovitis, destruction of cartilage, and erosion of surrounding bone, ligaments, and tendons. Most patients have relentless progressive polyarthritis with marked functional impairment. Treatment in the past aimed at symptomatic relief of pain with nonsteroidal anti-inflammatory drugs. Further, clinical experience has delineated several agents to alter the course of RA. This class of agents, which are referred to as disease-modifying antirheumatic drugs (DMARDs), includes methotrexate (MTX), gold compound, D-penicillamine, and sulfasalazine. Although DMARDs were initiated to slow the progression of RA, conventional DMARD therapy was incapable of retarding irreversible joint destruction observed on radiographs. Recently, the prognosis of RA has dramatically improved because of the development of novel therapeutic strategies targeted at specific cytokines. Tumor necrosis factor α (TNF-α) is a proinflammatory cytokine that is believed to play a pivotal role in inflammatory responses in RA. In fact, high levels of TNF-α have been detected in both serum[1] and synovial fluids.[2] In a variety of clinical settings, the blocking of TNF-α activity with biological agents is demonstrated as an effective therapy for DMARD-refractory RA patients.[3–6] The use of anti-TNF-α agents often induces significant decreases in the radiographic progression of disease. However, we have learned by experience that all RA patients treated with anti-TNF-α agents do not show good therapeutic responses. Several groups reported that 20%–40% of individuals

trying these agents are nonresponders and most patients have only a partial response.[3–6] Despite the significant number of patients treated with these agents, why some patients show inadequate clinical responses to anti-TNF-α blocking therapy remains an unanswered question.

Infliximab, a chimeric IgG_1 anti-TNF-α monoclonal antibody composed of the human constant and mouse variable regions, has been approved in Japan for the treatment of RA in combination with MTX. It can bind to both the soluble and membrane forms of TNF-α, an event that reduces the amount of TNF-α available for binding to its physiological receptors. Etanercept is a dimeric fusion protein consisting of the extracellular ligand-binding portion of human p75 TNF receptor and the Fc portion of human IgG1. Despite their sharing the mechanism of action for TNF-α blocking, it has been broadly appreciated that for RA patients who have failed to respond to one anti-TNF-α agent, a trial of another such agent often causes favorable outcomes under certain situations.[7–9] What causes a different therapeutic efficacy of each anti-TNF-α agent for individual RA patients? Several biological differences exist between infliximab and etanercept. The former has an ability to induce antibody-dependent cellular cytotoxicity, thereby causing a cytolysis of cells bearing TNF-α on their surfaces,[10,11] but the latter does not lyse these cells.[10] Only etanercept binds to lymphotoxin (TNF-β).[12] An additional difference in biological activity may be related to the induction of apoptosis in synovial cells,[13] although controversial results have been reported. Whether these distinctions may be involved in different therapeutic responses observed in RA patients, however, is unknown. Recently it has been shown that the anti-TNF-α agents possess different pharmacokinetics (PK) profiles, which are derived from the inherent properties of molecules, such as absorption, distribution, and clearance, or induced by differences in dose amount, administration frequency, and route.[14] Nestorov stressed that PK is one of the first considerations whenever different clinical outcomes are observed between anti-TNF-α agents with common mechanisms of action.[14] Pharmacokinetics analysis is useful for the determination of effective and well-tolerated dosage regimens for individual patients. The anti-TNF-α agents are used to eliminate surplus TNF-α from blood circulation and inflammation sites; however, elevated TNF-α levels should not be decreased below physiologically normal values, since abnormally low levels of TNF-α increase the risk of opportunistic infections. Thus rheumatologists should maintain serum concentrations of anti-TNF-α agents within a particular range that is sufficient to obtain effective clinical responses but that does not lead to an occurrence of adverse effects.[15,16] Serum concentrations of agents at the end of a dosing period (trough serum concentrations) may be useful indicators to examine whether the exposure to the anti-TNF-α agents is kept above therapeutic limit levels during the course of therapy. Using data on trough levels of infliximab obtained from 428 subjects, St Clair et al. indicated that favorable therapeutic outcomes depend in part on sufficient exposure to infliximab and that higher trough levels of infliximab may be beneficial for the treatment of certain cases of RA.[17]

I present here 21 patients with active RA who have received infliximab therapy in our hospital. Based on the EULAR (European League Against Rheumatism) improvement criteria,[18] the patients were classified into the following three groups at week 14 of therapy: 7 cases of good responders, 8 moderate responders, and 3 nonresponders. The other three cases discontinued therapy because of severe adverse effects or aggravation of disease activity after the first infusion of infliximab. The purpose of this study is to explore the cause of clinical unresponsiveness to infliximab therapy, especially by considering PK profiles in individual patients for each infusion of infliximab. I also examine a distribution of trough serum concentrations of infliximab at week 14 in each of the clinical response groups. I then present modified therapy for poor responders and nonresponders, and their clinical outcomes are reported. The PK data provide helpful information for the selection of optimal treatment for infliximab-resistant RA patients.

Patients and methods

Patients and the assessment of RA

The eligibility for infliximab treatment was determined according to guidelines of the Japan College of Rheumatology.[19] Patients were eligible if they fulfilled the 1987 American College of Rheumatology (ACR) criteria for RA diagnosis[20] and had active RA defined as the presence of at least 6 tender joints and at least 6 swollen joints, plus an erythrocyte sedimentation rate (ESR) of at least 28 mm/h or a serum C-reactive protein (CRP) level of at least 2.0 mg/dl. All patients were enrolled in this study after their informed consents were received. The Institutional Review Board approved the study.

Study protocol and evaluation

The patients were scheduled to receive an intravenous infusion of infliximab (3 mg/kg or 200 mg) at weeks 0, 2, and 6, followed by maintenance therapy every 8 weeks. During this therapy, MTX (8 mg/week) was given concomitantly. For patients receiving 5 mg/day prednisone at the start of infliximab therapy, the same dose was continued throughout the therapy, unless alternations were mentioned. Clinical responses were evaluated immediately before each infusion according to the ACR improvement criteria[21] and the EULAR response criteria.[18] Disease activity was measured with the Disease Activity Score for 28 joints (DAS28 score), which was calculated by using a swollen joint count (28 joints assessed), a tender joint count (28 joints assessed), ESR levels, and patient assessment of pain (scale of 0 to 100).

Infliximab serum assay and human antichimeric antibody (HACA) detection

Infliximab serum concentration was measured based on previous reported methods.[22] Blood samples were collected

immediately prior to each infusion and 1 h after their completion. During the period without infusion, blood samples were collected every 2 weeks. The serum levels of infliximab were measured by an enzyme-linked immunosorbent assay using a monoclonal antibody specific for the TNF-α binding site. The captured infliximab was detected by a biotinylated monoclonal antibody specific for an epitope in its variable region. The lowest level that could be reliably detected was 0.1 μg/ml. Human antichimeric antibody (HACA) was measured by a modified method of the enzyme-linked immunoassay originally reported by LoBuglio et al.[23]

Statistical analysis

A correlation between the DAS28 score and elimination half-life was calculated with KaleidaGraph 4.0J software (Synergy Software, Reading, PA, USA).

Results

Baseline characteristics of RA patients and an evaluation of therapeutic responses to infliximab

The clinical characteristic of 21 subjects at baseline are shown in Table 1. The median disease duration of these patients was 3 years (range 0.4–40), and 4 cases were early RA with a disease duration of less than 1 year. The positive rates of IgM rheumatoid factor (RF) and anticyclic citrullinated peptide antibody (anti-CCP Ab) were 100% and 95%, respectively. The median values of serum CRP and ESR at baseline were 2.02 mg/dl and 62 mm/h, respectively. The median DAS28 score was 6.4 (range 5.1–8.7). The median counts of tender joints and swollen joints were 12 (range 5–26) and 10 (range 3–26), respectively. All subjects were diagnosed as active RA. Fourteen patients were being treated with 5 mg/day of prednisolone (PSL) at the start of infliximab therapy.

Table 1. Baseline characteristics of subjects (n = 21)

Age, years, median (range)	50 (33–75)
Sex n (%)	
Female	18 (86)
Stage, n (%)	
I	3 (14)
II	7 (33)
III	9 (43)
IV	2 (10)
Disease duration (years), median (range)	3 (0.4–40)
Swollen joint numbers (28), median (range)	10 (3–26)
Tender joint numbers (28), median (range)	12 (5–26)
Subjects with positive RF at baseline (%)	100
Subjects with positive anti-CCP Ab at baseline (%)	95
Serum CRP (mg/dl), median (range)	2.02 (0.19–14)
ESR (mm/h), median (range)	62 (31–108)
DAS28, median (range)	6.4 (5.1–8.7)
Patients taking PSL (5 mg daily) at baseline, n (%)	14 (67)

RF, IgM rheumatoid factor; anti-CCP Ab, anti-cyclic citrullinated peptide antibody; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; DAS28, Disease Activity Score; PSL, prednisolone

The patients initially received an intravenous infusion of infliximab (3 mg/kg or 200 mg) at weeks 0, 2, and 6 and subsequently every 8 weeks for maintenance therapy. MTX (8 mg/ml) was given concomitantly. For the 14 patients receiving PSL (5 mg/day), the same dose was maintained unless alternations were mentioned. Of 21 patients who were being treated with infliximab, 18 completed a total of 3 infusions, but 2 cases were withdrawn because of adverse effects (one a result of *Pneumocystis jiroveci* pneumonia,[15] the other a result of a severe hypersensitivity reaction). One patient discontinued treatment after the first infusion because of an aggravation of disease activity. Of 18 individuals who had completed the infusion three times, the number of patients who had achieved the ACR20, ACR50, and ACR70 response criteria at week 14 were 15, 14, and 6, respectively. Three cases failed to accomplish the ACR20 response criteria (nonresponders). Similar results were obtained with the EULAR criteria (7 good responders, 8 moderate responders, and 3 nonresponders).

Pharmacokinetics of infliximab

A model of concentration–time profiles for infliximab and the mean value of PK properties was obtained (Fig. 1A). The average volume of distribution (Vd) per body weight (kg) at steady state was 0.050 l/kg (n = 15), suggesting that the distribution of infliximab shows an intravascular pattern. There were no differences in Vd/kg between one group receiving 3 mg/kg of infliximab and another group receiving 200 mg. The mean values of the elimination half-life ($t_{1/2}$) of infliximab was 9.5 (n = 8), 8.2 (n = 5), and 9.5 (n = 9) days during weeks 0 to 2, 2 to 6, and 6 to 14, respectively. These data were similar to previous findings that the average $t_{1/2}$ of infliximab in RA patients ranges from 8.5 to 9.8 days. Figure 1B shows two cases of concentration–time profiles based on serum concentrations of infliximab obtained during the maintenance therapy. The elimination rate was apparently higher during the first 2 weeks after the infusion, compared with the following periods, thereby indicating that infliximab is eliminated from plasma in a nonlinear fashion. It is reported that HACA formation may possibly alter the pharmacokinetics of infliximab; however, HACA was not detected in patients with rapid clearance when their lowest serum concentrations of infliximab were below a detectable limit of assays.

Relationship between clinical improvement and trough serum concentrations of infliximab

The serum concentration of infliximab measured 1 h after each infusion approximates the maximum serum concentration (Cmax). In RA patients receiving 3 mg/kg of intravenous infusion, the mean Cmax for the first infusion was 57 μg/ml (n = 5). The serum concentration of infliximab measured immediately prior to the next infusions represents the lowest serum concentration (a trough concentration). Figure 2 shows the trough serum concentrations at week 14 for each of three groups defined by the EULAR

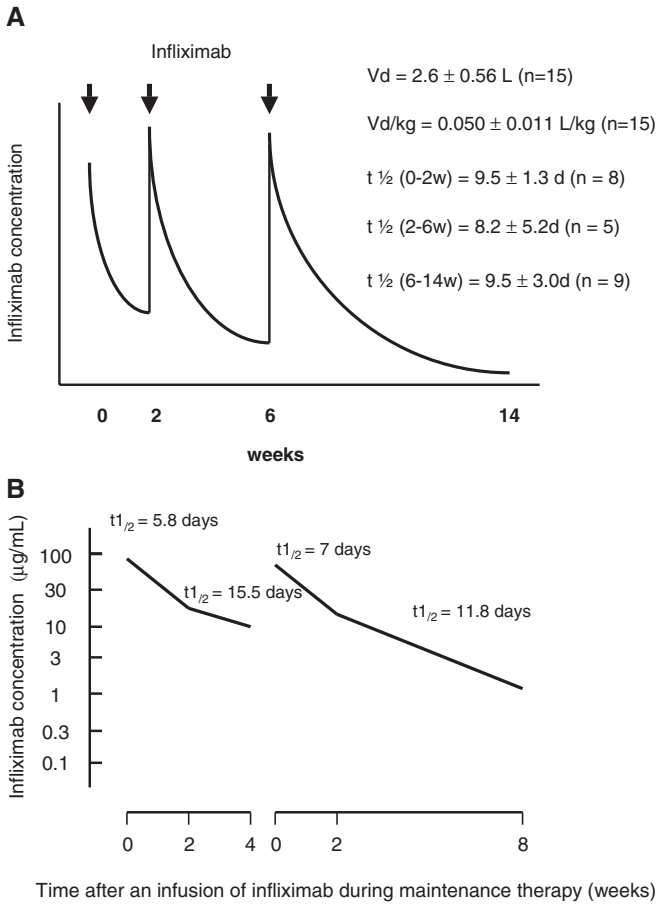

Fig. 1. **A** Pharmacokinetics modeling of infliximab in rheumatoid arthritis (RA) patients. Based on the serum concentrations of infliximab observed with RA patients, a schematic pattern of elimination is shown. Blood samples were collected immediately before and 1 h after each infusion of infliximab. *Vd*, the volume of distribution; *Vd/kg*, Vd per body weight (kg); $t_{1/2}$, elimination half-life. **B** Elimination half-life of infliximab during maintenance therapy. Blood samples were collected 1 h and 2, 4, and 8 weeks after the infliximab infusion during maintenance therapy. $t_{1/2}$, elimination half-life

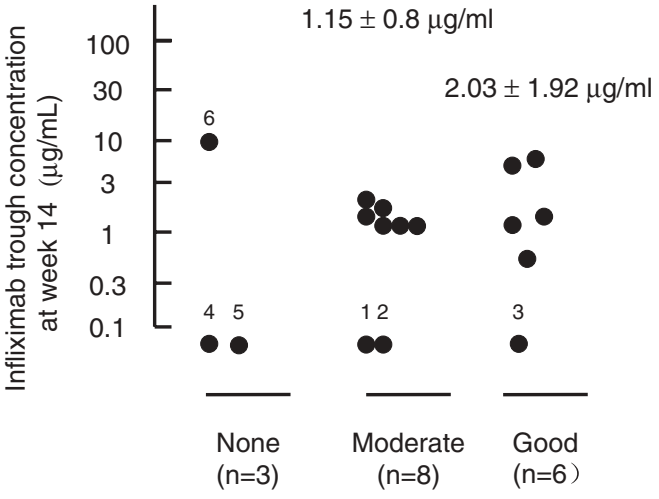

Fig. 2. Relationship between trough serum concentrations of infliximab and clinical responses defined by European League Against Rheumatism (EULAR) criteria. Blood samples were collected immediately prior to a fourth infusion of infliximab. The trough serum concentrations are presented for each of three clinical response groups defined by EULAR criteria. The average values of trough serum concentrations for each group are shown in the figure. The *number above the circle* corresponds to the number of cases shown in the text. The value of case 1 was measured at week 10. The data for one patient in the good response group were not available

response criteria. In the good and moderate responder groups, a greater proportion of patients had trough concentrations of more than 1 μg/ml. The average of trough concentrations among the good and moderate responders was 2.03 and 1.15 μg/ml, respectively. Of 11 cases with trough concentrations of more than 1 μg/ml, 10 patients showed good or moderate responses at week 14. Two cases in 3 nonresponders showed undetectable levels of a trough serum concentration of infliximab (less than 0.1 μg/ml) at week 14. Thus it seems reasonable to assume that differences in clinical responses are derived from variable rates of infliximab clearance from the plasma of patients. Rapid clearance appears to be one of the main causes of unresponsiveness. Three patients are noted who achieved good or moderate responses at week 14 despite a failure to maintain sufficient levels of trough serum concentration of infliximab. The details of serum concentration–time profiles and clinical courses of these cases are presented in the latter half of this paper.

Relationship between initial DAS28 scores and the elimination half-life of infliximab

Tumor necrosis factor α is believed to play a key role in inflammatory responses in RA, and its elevations are detected in both serum and synovial fluids of patients. I questioned whether the serum TNF-α level might determine the disease activity of RA. To explore this problem, I examined a relationship between DAS28 scores at baseline and the elimination half-life ($t_{1/2}$) values of infliximab during the first 2 weeks of therapy. If there are many TNF-α molecules in RA patients, the elimination of infliximab is accelerated by forming TNF-α–infliximab immune complexes. Figure 3 shows the presence of an inverse correlation of the initial DAS28 score to the $t_{1/2}$ value. Using these data, I formulated the following equation: $t_{1/2}$ (days) = 17.394 – 1.5936 × initial DAS28. Thus the elimination rate of infliximab from the body is more rapid in RA patients with higher disease activity at baseline. Since the presence of high levels of TNF-α increases the disappearance of infliximab from the body, the inverse correlation between DAS28 scores and $t_{1/2}$ of infliximab strongly suggests that the disease activity of RA may be affected by the elevation of serum TNF-α.

Serum concentration–time profiles and clinical courses of cases with good or moderate responses despite undetectable trough serum concentrations of infliximab

As mentioned above, three patients who had not maintained therapeutically sufficient levels of serum concentra-

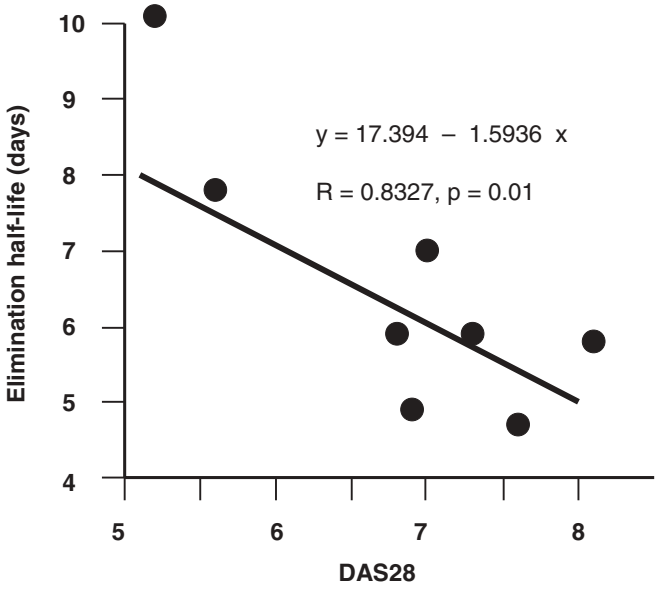

Fig. 3. Inverse correlation between Disease Activity Score (*DAS28*) and elimination half-life. The elimination half-life was measured for 2 weeks after the first infusion. The DAS28 score was measured at the start of infliximab therapy. A correlation between the DAS28 score and the elimination half-life was calculated with KaleidaGraph 4.0J software (Synergy Software, Reading, PA, USA). The *R* value was 0.8327 and the *P* value was 0.01

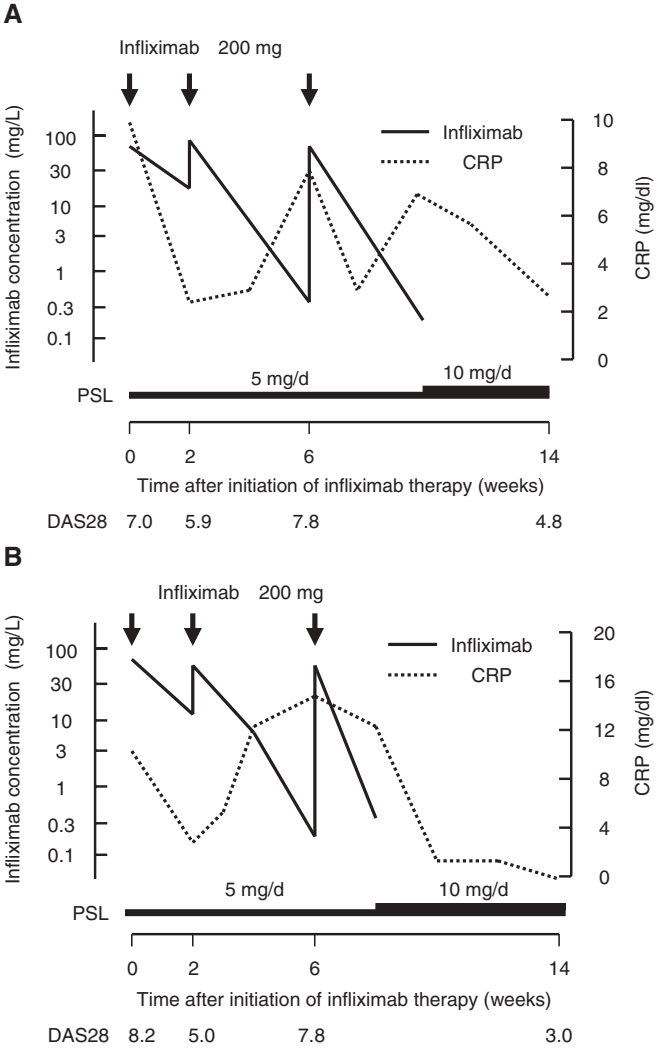

Fig. 4A,B. Pharmacokinetics profile and clinical response of moderate responders who showed undetectable levels of trough serum concentration at week 14. Case 1 (**A**) and case 3 (**B**) received an intravenous infusion of infliximab (200 mg) at weeks 0, 2 and 6. Methotrexate (8 mg/week) was given concomitantly. Blood samples were collected immediately prior to each infusion and 1 h after their completion. During the period without infusion, the blood samples were collected every 2 weeks. The DAS28 score was evaluated immediately before each infusion. *CRP*, C-reactive protein; *PSL*, prednisolone

tions of infliximab succeeded in achieving good or moderate responses defined by EULAR criteria at week 14 of therapy. Case 1, a 50-year-old woman with seropositive RA for 10 years, was treated with 200 mg of infliximab in combination with 8 mg/week of MTX (Fig. 4A). Prednisolone was maintained at the baseline dosage (5 mg/day) during therapy. At week 2, the DAS28 score was decreased from 7.0 to 5.9, and the CRP value was also dramatically improved; however, her disease activity flared at week 6. Despite a third infusion, the CRP value was increased to a high level at week 10, and the trough serum concentration was decreased to 0.2 µg/ml. The dose of PSL was increased from 5 to 10 mg/day at week 10. At week 14, the patient achieved a moderate response. In this case, the clearance rate of infliximab was slow after the first infusion but became much faster after the second and third infusions. The elimination half-life during weeks 2–6 and weeks 6–14 was 3.6 days and 3.4 days, respectively. In case 2, a 74-year-old woman with seropositive RA for more than 25 years showed very high levels of rheumatoid activity (DAS28 score, 8.7; CRP value, 14 mg/dl). The disease activity was gradually improved at every infusion of infliximab (200 mg), and at week 14 she achieved a moderate response in EULAR criteria. At this time the DAS28 score and the CRP value were 4.5 and 3.4 mg/dl, respectively, but the trough serum concentration was undetectable. By an increase of the MTX dose to 10 mg/week, from 8, at week 14, the DAS28 scores had decreased to 3.2 at week 54. After 70 weeks of therapy, the patient reached clinical remission and CRP value was maintained below 0.1 mg/dl. The trough serum concentration remained at levels below the detectable lower limit. In case 3, a 69-year-old woman with seropositive RA for 10 years responded well to the first infusion of infliximab: DAS28 score and CRP value were markedly decreased at week 2 (Fig. 4B). Prednisolone was continued at the baseline dose (5 mg/day). In spite of a second infusion, these data were aggravated, and the serum concentration of infliximab was rapidly decreased. After a third infusion, infliximab was more rapidly eliminated from serum, and the CRP value was not improved at week 8. At this time the dosage of PSL was increased to 10 mg/day, from 5. At week 14, this patient succeeded in achieving a good response defined by EULAR criteria, although trough serum concentrations were below the detectable lower limit. DAS28 score was 3.0, and CRP was undetectable at week 14. Similar to case 1, the elimina-

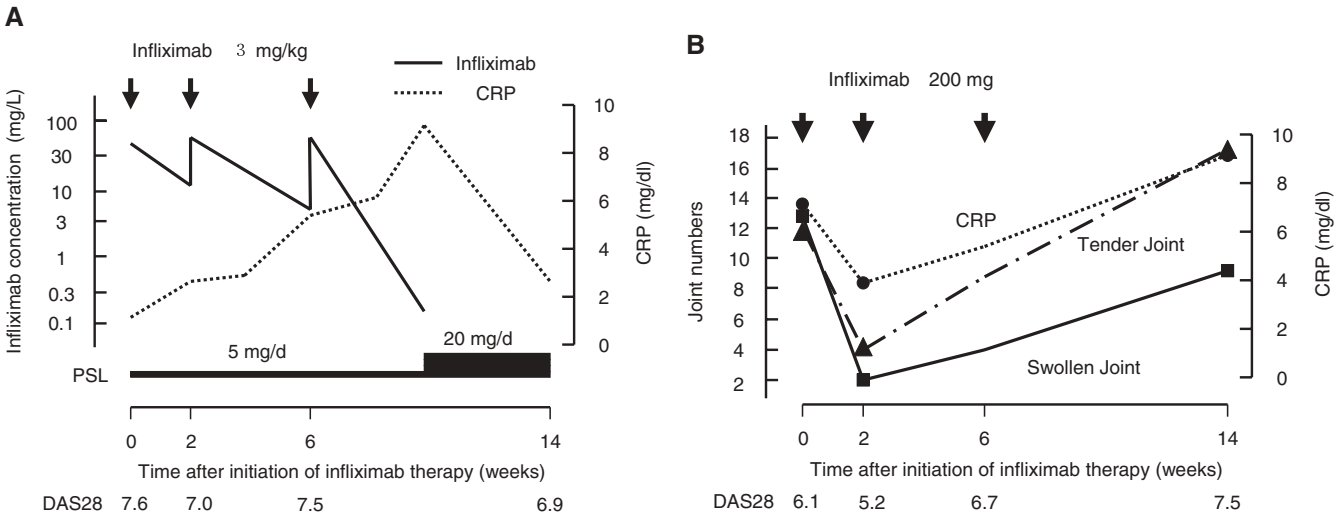

Fig. 5A,B. Pharmacokinetics profile and clinical responses of nonresponders. Case 5 (**A**) and case 6 (**B**) received an intravenous infusion of infliximab (A, 3 mg/kg; B, 200 mg) at weeks 0, 2, and 6. Methotrexate (8 mg/week) was given concomitantly. Blood samples were collected immediately prior to each infusion and 1 h after their completion. During the period without infusion, they were collected every 2 weeks. The DAS28 scores and counts of swollen joints and tender joints were evaluated immediately before each infusion. *CRP*, C-reactive protein; *PSL*, prednisolone

tion of infliximab became much faster after the second and third infusions, compared with the first 2 weeks.

Serum concentration–time profiles and clinical courses of three nonresponders to infliximab

In this study, three patients failed to accomplish a good or moderate improvement of EULAR criteria by week 14. Among these nonresponders, two showed undetectable levels of trough serum concentrations of infliximab, but one did maintain sufficient serum levels throughout the course of therapy. Case 4 was a 38-year-old woman with a 6-year history of seropositive RA. Her DAS28 score and CRP value at baseline were 7.8 and 4.3 mg/dl, respectively. She was treated with 200 mg of infliximab in combination with 8 mg/week of MTX. An administration of 5 mg/day PSL was continued. At week 2 these data were dramatically improved (DAS28 score, 2.8; CRP value, 0.05 mg/dl). Unfortunately, the rheumatic activity aggravated after a second infusion. At week 14, the DAS28 score and CRP value were increased to 7.8 and 2.4 mg/dl, respectively, and the trough serum concentration was below the detectable lower limit. It was decided to increase the dose of MTX to 12 mg/week, from 8, and the infliximab therapy was further continued. At week 54, she achieved an ACR50 response, although the trough serum concentrations were still undetectable. In case 5, a 74-year-old woman with seropositive RA for 6 years was resistant to infliximab therapy (Fig. 5A). Her DAS28 score remained high. The CRP value went from bad to worse during the first 6 weeks, although sufficient levels of infliximab were maintained. After a third infusion, the clearance rate became faster compared with the previous two infusions. The serum concentration was decreased to an undetectable level at week 10. The dose of PSL was increased to 20 mg/day at week 10; however, the DAS28 score hardly improved and the CRP value was still more than 2 mg/dl at week 14. It was decided to discontinue the infliximab therapy and start a new treatment of 1.5 mg/day of tacrolimus and 8 mg/week of MTX. An administration of 20 mg/day of PSL was continued during the new treatment. After 2 weeks the disease activity began to decrease and the dose of PSL was then decreased to 10 mg/day. Three months later it reached a low range (DAS28 score, 3.2; CRP value, 0.12 mg/dl). Case 6 was a 47-year-old woman who had early RA with 6 months of disease duration. Her disease was progressive, and bone erosion was observed on plain X-ray films of the foot. Methotrexate monotherapy for 2 months induced marked improvement, but the disease activity flared again. Throughout the course of infliximab therapy (200 mg per infusion), DAS28 remained at high levels (Fig. 5B). The sufficient trough serum concentration was maintained at week 14 (9.1 μg/ml). It was decided to switch the anti-TNF-α agent from infliximab to etanercept (25 mg, twice/week by subcutaneous injection). After 6 months, clinical responses were still poor, and a decrease of disease activity was also insufficient. An addition of tacrolimus (3 mg/day) was therefore decided upon. Six months later, she achieved a good response in EULAR criteria, and the CRP value was decreased to 0.18 mg/dl. She reached clinical remission.

There is a further case that had once discontinued infliximab therapy because of an aggravation of disease activity, but finally achieved a good response defined by the EULAR criteria. Case 7, a 74-year-old woman with a history of seropositive RA, was resistant to MTX monotherapy. Etanercept therapy had also failed. Two weeks after the start of infliximab therapy (200 mg per infusion), DAS28 score and CRP value were increased to 8.0, from 7.5, and to 7.4 mg/dl, from 6.1, respectively. At this time, the inflix-

imab therapy was discontinued. Several weeks later information was obtained that the elimination half-life of infliximab was 4.7 days for the first 2 weeks. Because the failure of therapy was due to rapid clearance, the infliximab therapy is restarted in combination with 15 mg/day of PSL. The patient achieved a moderate response in EULAR criteria at week 6; however, at week 14, clinical response became poor. One mg/day of tacrolimus was added to the therapy and the dose of PSL was decreased to 5 mg/day. One month later, the patient achieved a good response.

Discussion

Infliximab is generally recognized to provide impressive functional and radiological improvements for patients with active RA; however, some patients do not respond to it at all, and many patients show partial clinical responses.[3–6] In the present study, I have found that of 18 patients who had completed a total of three infusions of infliximab, 7 achieved good responses in the EULAR criteria and 8 showed moderate responses, but 3 cases became nonresponders at week 14. Understanding the event of insufficient clinical responses is useful to explore optimal strategies for infliximab-resistant RA patients. By examining a relationship between clinical efficacy and the PK of infliximab, I indicated that maintaining trough serum concentration above the therapeutic limit level is beneficial to achieve satisfactory clinical improvement (Fig. 2). In the good and moderate responder groups, most patients maintained trough concentrations of more than 1 μg/ml. The averages of the good and moderate responders were 2.03 and 1.15 μg/ml, respectively. Previous data showed that an inhibition of TNF-α is achieved in vitro within a range of 0.2–10 μg/ml of concentrations.[24] The clinical responses of RA patients are known to decline rapidly after serum infliximab levels drop under 1 μg/ml.[17] Using data obtained from a large-scale trial, St Clair et al. showed that the trough serum concentrations of infliximab are related to the clinical improvement of RA patients.[17] These reports are in good agreement with my findings. I showed here that an elimination of infliximab from the body is more rapid in RA patients with higher disease activity at baseline (Fig. 3). Because the formation of infliximab-TNF-α immune complexes increases the elimination of infliximab through the mononuclear cell phagocyte system, the differences in TNF-α levels of individual RA patients may influence the clearance speed of infliximab. Therefore for RA patients with higher disease activity, we need to pay special attention to trough serum concentrations of infliximab. It was reported that approximately 26% of subjects receiving an infusion of 3 mg/kg infliximab have undetectable levels of serum concentrations at the end of the 8-week dosing period.[17] I also found that 24% of the subjects failed to maintain serum levels above the quantification limit at week 14 (8 weeks after the third infusion) (Fig. 2). These findings suggest that 3 mg/kg of infliximab dose may be subtherapeutic at the end of the 8-week dosing period. Increasing the doses and/or shortening the administration periods may benefit patients with a rapid clearance of infliximab. However, van Vollenhoven et al. reported that an increased infliximab dose may have less clinical benefit than is expected in the clinical practice.[25] St. Clair et al. suggested that to maintain sufficient levels of trough serum concentrations of infliximab, frequent administrations are more effective than dosage increases.[17] Further, we must remember the adverse effects that may occur when the infliximab dose is raised.

Recent studies showed that there are two types of "lack of efficacy" in the failure of infliximab therapy. One is the absence of any clinical responses (primary lack of efficacy), and the other is the disappearance of an initial favorable response during therapy (secondary lack of efficacy). The detailed mechanisms of losing efficacy, predictive factors, and therapeutic strategies to avoid the lack of efficacy are poorly understood. In this study I have noted some individuals who initially had responded to infliximab therapy and subsequently showed a disappearance of therapeutic effects (secondary lack of efficacy). Cases 1 and 3 showed a recurrence of disease activity during the course of therapy. In these cases the clearance rate of infliximab was slow after the first infusion, but it became much faster after the second and third infusions. Thus a shortening of elimination rate is apparently related to the secondary lack of efficacy to infliximab. Recently, the development of HACA was reported for some of the RA patients.[22] Maini et al. reported that 53% of RA patients who received multiple infusions of infliximab (1 mg/kg) formed HACA, and this percentage dropped to 15% with the concomitant use of low-dose MTX. They also showed that the group with a higher incidence of HACA more rapidly cleared infliximab from the serum.[22] Although in patients without MTX treatment serum concentrations declined to 0.1 μg/ml during the dose interval of 4 weeks, concentrations in patients treated with MTX declined to only 2 μg/ml.[22] However, it has remained unclear whether the HACA formation has clinical significance. Wagner et al. showed that there were no differences in ACR20 response rates between HACA-positive and HACA-negative patients, although the clearance of infliximab in the HACA-positive group was significantly faster than in the HACA-negative group.[26] Of note, HACA was not detected in patients who had showed a rapid clearance of infliximab in this study, thereby suggesting that the rapid clearance observed here is not involved in HACA development. The occurrence of another mechanism for tolerance to infliximab should be considered. One possible explanation for rapid clearance is a revival of TNF-α-producing cells and a subsequent overproduction of TNF-α during infliximab therapy. These events may induce a rapid consumption of infliximab from the body. Moreover, I reported that the increase of MTX was effective for an improvement of clinical outcomes during the prolonged infliximab therapy with a dose interval of 8 weeks; however, trough serum concentrations of infliximab were still undetected (cases 2 and 4). Although the mechanisms of clinical improvement by increasing MTX are not yet clear, clinical practice guidelines in regard to MTX therapy for RA are proposed as follows:[27] the initial dosage should not be less than 10 mg/

week, and when a patient shows an inadequate response, the dosage should be increased at intervals of 6 weeks, up to 20 mg/week. Increasing the dose of MTX may be one choice for patients who show high disease activity or a rapid clearance of infliximab.

In the present study, I showed that the increased use of PSL is effective to achieve clinical improvements for 2 patients (cases 1 and 3) who are characterized by a secondary lack of efficacy resulting from the occurrence of rapid clearance after a second infusion. Infliximab has the potential to induce apoptosis in monocytes expressing membrane-bound TNF-α in a caspase-dependent pathway.[13] Since PSL is known to downregulate various cytokines in both membrane and soluble forms, it may help to avoid an excessive consumption of infliximab at local levels in the event of apoptosis by reducing the number of membrane forms of TNF-α on the cells. Case 4 also showed a secondary lack of efficacy by the occurrence of rapid clearance. I did not notice that a clearance rate of infliximab had changed to an even faster rate during the course of therapy. If the dose of PSL had been increased, this case might have belonged to a good or moderate responder at week 14, as mentioned with cases 1 and 3. On the contrary, an increase of PSL had little effect on the clinical outcome of the nonresponder who had a primary lack of efficacy (case 5). Case 7, the primary lack of efficacy with rapid clearance in the first trial of infliximab therapy, responded to an increase of PSL for the first 6 weeks in the second trial, but at week 14 she was identified as a nonresponder. Thus increasing the dose of PSL may be a useful way mainly for the RA case that is considered the secondary lack of efficacy by the rapid clearance of infliximab.

Tacrolimus is a newly approved immunosuppressant for RA, which has been reported to down-regulate the synthesis of inflammatory cytokines such as TNF-α and interferon-γ by inhibiting the ability of calcineurin to initiate gene transcription in a T-cell-specific fashion.[28] Because T-cell activation plays a major role in the pathogenesis of RA, the T-cell-suppressing property of tacrolimus is potentially beneficial for the RA treatment.[28] I stated here that the use of tacrolimus succeeded in the inducement of good clinical responses for three RA patients who were characterized by the primary lack of efficacy, regardless of the presence or absence of rapid clearance of infliximab (cases 5, 6, and 7). Tacrolimus was introduced with its single use (case 5) or in combination with the anti-TNF agents (cases 6 and 7). Methotrexate was continued for all three patients. Increased amounts of PSL were continued for case 5 during this therapy. These patients achieved similar favorable clinical response by the tacrolimus plus MTX therapy, alone or in combination with anti-TNF agents or PSL, although the possibility is undeniable that an increase of PSL may have contributed to the achievement of a good response by case 5. Besides targeting specific cytokine such as TNF-α, it may be required to focus on events upstream in the inflammatory cascade such as T-cell activation. Therefore tacrolimus may be an alternative for the treatment of infliximab-refractory cases. Cases 5 and 6 were considered to have a primary lack of efficacy without rapid clearance, at least during the first 6 weeks. In these cases, TNF-α may not be a trigger factor of inflammation in synovial tissues. It is necessary that infliximab therapy for patients with a primary lack of efficacy without rapid clearance be discontinued because of increased risks of adverse effects and costs associated with therapy.

A characterization of "failure of infliximab therapy" by using PK data provides important information to determine an optimal treatment for individual patients with infliximab-refractory RA. The rapid clearance appears to be the main cause of unresponsiveness to infliximab. Maintaining the trough serum concentrations above therapeutic limit levels is beneficial to favorable clinical outcomes. Patients showing higher disease activity are inclined to have a more rapid elimination of infliximab. For those with a secondary lack of efficacy by rapid clearance, the increased use of PSL or MTX induced good clinical outcomes. Tacrolimus benefited the nonresponders who showed a primary lack of efficacy. The effects of switching to etanercept were partial for infliximab-resistant RA patients. I hope that further prospective research to evaluate the efficacy of additional and/or modified treatment for infliximab-resistant RA patients with different types of PK is carried out.

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