Soluble urokinase plasminogen activator receptor as a useful biomarker to predict the response to adalimumab in patients with rheumatoid arthritis in a Japanese population

T. Koga¹, A. Okada¹, S. Kawashiri¹, J. Kita¹, T. Suzuki¹, Y. Nakashima¹, M. Tamai¹, K. Sato², T. Origuchi³, N. Iwamoto¹, S. Yamasaki¹, H. Nakamura¹, K. Migita⁴, H. Ida⁵, Y. Ueki⁶, K. Eguchi¹, A. Kawakami¹

¹Unit of Translational Medicine, Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; ²Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; ³Nagasaki University School of Health Sciences, Nagasaki, Japan; ⁴NHO Nagasaki Medical Center, Omura, Japan; ⁵Division of Rheumatology Respirology, Neurology, and Rheumatology, Department of Medicine, Kurume University School of Medicine, Kurume, Japan; ⁶Center for Rheumatic Disease, Sasebo Chuo Hospital, Sasebo, Japan.

Abstract

Objective
To determine whether soluble urokinase plasminogen activator receptor is a useful biomarker to predict the response to adalimumab (ADA) in Japanese patients with rheumatoid arthritis.

Methods
Rheumatoid arthritis (RA) patients administrated ADA (n=51) were classified as good responders (n=18) or non-responders (n=9) according to the EULAR response criteria after 8 weeks of bi-weekly ADA administration. We examined the expression of cytokines and chemokines in these groups by antibody array methods. Positive results obtained by antibody array methods were further confirmed by ELISA.

Result
Antibody array has identified that the macrophage migration inhibitory factor (MIF), vascular endothelial growth factor (VEGF) and soluble urokinase plasminogen activator receptor (uPAR) decreased in the good responders to ADA whereas these changes were not observed in the non-responders. The decrement of serum uPAR was confirmed by ELISA in the good responders to ADA. Furthermore, serum uPAR at baseline was significantly high in non-responders compared with good responders.

Conclusion
An antibody array is convenient for screening the expression of proteins of interest. Examination of serum uPAR at baseline and thereafter may be useful as a predictive biomarker for primary failure toward ADA in patients with RA.

Key words
rheumatoid arthritis, adalimumab, urokinase plasminogen activator receptor, primary failure
Introduction
Adalimumab (ADA) is an effective treatment for rheumatoid arthritis (RA). However, a substantial number of patients with rheumatoid arthritis either do not respond or lose their initial response (1). Anti-adalimumab antibodies have been previously reported in 12% of patients receiving ADA monotherapy 40 mg every other week for 26 weeks (2). Previous reports showed that the presence of especially high concentrations of antibodies against ADA is associated with EULAR non-response (3). Taken together, the production of anti-adalimumab antibody is thought to be one of the predictive factors for secondary failure. However, the pathogenesis in primary failure of ADA has not yet been well investigated. At the current moment, the choice of biologics such as tumor necrosis factor (TNF) inhibitor or interleukin-6 receptor (IL-6R) inhibitor depends on the clinician’s experience or the injection route. The prevalence of non-response to biologics represents a critical problem in the treatment of RA. Consequently, it is quite warranted to predict a patient’s response to biologics before their use.

TNF-α induces the expression of a number of molecules; thus, the monitoring of dynamic alteration of candidate molecules is considered to be beneficial for prediction of the therapeutic efficacy of TNF inhibitors (4). Considering that angiogenesis in the synovial tissues with the augmentation of fibrinolysis is one of the characteristic of RA (5), we have tried to find fibrinolysis-associated molecules whose expression is regulated by TNF-α during ADA therapy. In the present study we focus on the urokinase-type plasminogen activator receptor (uPAR, CD87), a cell receptor that binds urokinase-type activators (uPA) with high affinity. uPA / uPAR systems are known to be important factors in the pathogenesis of tumours and certain non-viral inflammatory diseases (6-8): in addition, increased serum uPAR concentrations were found in patients with both RA and primary Sjögren’s syndrome, which is closely related to a poor prognosis (6). Our present study has identified that serum uPAR is a useful surrogate biomarker to identify primary failure of ADA therapy.

Patients and methods
Patients
Patients were recruited from the Unit of Translational Medicine, Department of Immunology and Rheumatology, Graduate School of Biomedical Sciences, Nagasaki University, Sasebo Chuo Hospital and NHO Nagasaki Medical Center. A written informed consent form approved by the above hospitals was obtained from each patient. All patients fulfilled the American College of Rheumatology 1987 revised criteria for RA and had active disease, indicated by a disease activity score in 28 joints (DAS28) of >3.2 despite earlier treatment with disease-modifying anti-rheumatic drugs (DMARDs), according to the Japanese consensus statement on the initiation and continuation of TNF blocking therapy in RA. Patients were treated with either adalimumab and concomitant DMARD or adalimumab alone. All patients used 40 mg adalimumab subcutaneously every other week.

Clinical response
Disease activity was assessed at baseline and after 4 and 8 weeks of treatment using the DAS28 score (9). Clinical response was assessed by the European League Against Rheumatism (EULAR) criteria and changes in the DAS28 score (delta DAS28) (10). Serum samples were collected just before injection with adalimumab at baseline and after 8 weeks.

Cytokine and chemokine assays in patients with RA
The levels of cytokines and chemokines in the serum of RA patients at baseline and after 8 weeks of treatment were measured using a RayBio Human Inflammation Antibody Array 7 (Ray Biotech, Inc., Norcross, GA, USA), according to the manufacturer’s instructions. This assay employs a qualitative western screening technique. The standard array matrix consisted of an 11×8 dot grid on a 20 mm×30 mm nitrocellulose membrane with 79 unique capture antibodies. The array kit included the biotinylated-antibodies solution and a chemiluminescent substrate. These 79 humoral factors were detected using the indicated antibod-
ies for 1 h, followed by HRP-labeled streptavidin incubation for 1 h. An enhanced chemiluminescence (ECL) system (Amersham, Amersham, UK) was used for detection. In addition, the protein concentration of soluble uPAR was confirmed by enzyme-linked immunosorbent assays (ELISA) (Quantikine, R&D Systems, USA).

Statistical analysis
The last observation carried forward (LOCF) method by intent-to-treat (ITT) analysis was used for the evaluation of the DAS28 (ESR), serum CRP level and EULAR response. For differences between groups, we used the Fisher’s exact test or Mann-Whitney U-test as appropriate. We used Wilcoxon signed-rank tests to evaluate the change between pre- and post-treatment. Values of \( p \leq 0.05 \) were considered to be significant.

Results
Patient characteristics
Most of the 51 patients who entered the study were female (78%), and the mean (SD) age was 61.7 (13.7) years (Table I). At baseline, patients had active disease, as indicated by a mean (SD) DAS28-ESR score of 5.54 (1.04). Most of the patients had established disease, with a mean disease duration of 9.1 years at baseline. Most patients administrated concomitant methotrexate (61%) with a mean dose of 7.2 (1.7) mg/week, and 35% were administrated prednisone at a mean dose of 7.0 (5.3) mg/day. Eight patients were administered concomitant non-methotrexate non-biologic DMARDs including leflunomide (n=1), tacrolimus (n=2), salazosulapyridine (n=2) and mizoribine (n=3). Twelve (24%) patients received adalimumab monotherapy. The concomitant treatments (prednisolone and/or synthetic DMARDs) were maintained at least 4 weeks before introduction of adalimumab in all the subjects.

Table I. Baseline characteristics, including age, gender, duration of disease at baseline, MTX use at baseline, prednisolone use and DAS28 (ESR) at baseline, were not different between non-responders (n=9) and good responders (n=18). The distribution is characterised by disease duration (years) at baseline was significantly different between groups, we used the Fisher’s exact test or Mann-Whitney U-test as described in the text. The mean reduction in the DAS28-ESR score after 8 weeks of ADA therapy was 1.5 points. Eleven patients stopped treatment before week 8, four for lack of efficacy (as concluded by the treating rheumatologist), five because of side effects (chronic coughing, palpitations and liver dysfunction) and two because of financial limitations.

Clinical response
We examined the changes of DAS28-ESR, CRP, and EULAR response criteria (Fig. 1). At the 8-week follow-up, 40 of 51 patients were still receiving adalimumab treatment. Of the remaining 40 patients, thirty-one (78%) were classified as responders according to the EULAR response criteria, including 18 (45%) good responders and 13 (33%) moderate responders. Nine patients were considered non-responders to ADA therapy. None of the baseline variables was different between good responders and non-responders (Table I). The mean reduction in the DAS28-ESR score after 8 weeks of ADA therapy was 1.5 points. Eleven patients stopped treatment before week 8, four for lack of efficacy (as concluded by the treating rheumatologist), five because of side effects (chronic coughing, palpitations and liver dysfunction) and two because of financial limitations.

Cytokine and chemokine assays
We initially examined whether administration of ADA reduces inflammatory mediators in patients with RA using an antibody array to compare good responders with non-responders. Data from a representative experiment are depicted in Fig. 2. Macrophage migration inhibitory factor (MIF), macrophage stimulating protein-\( \alpha \) (MSP-\( \alpha \)), vascular endothelial growth factor (VEGF) and uPAR decreased only in the good responders. These proteins were not changed in the non-responders.

Clinical response and the change of soluble urokinase plasminogen activator receptor
The change of serum uPAR concentration was confirmed by ELISA. As shown in Fig. 3A, uPAR was significantly decreased after treatment in good responders (Wilcoxon signed-rank test, \( p=0.007 \)) whereas the decrement was not found in non-responders. Furthermore, serum uPAR concentrations at baseline were significantly different...
Discussion

This is the first report to indicate that serum uPAR may be predictive of the primary response of ADA in patients with RA. Changes of uPAR detected by antibody array was considered plausible since other inflammatory mediators of MIF and VEGF had shown reasonable alteration in previous studies (11, 12). The reduction of serum MSP-α after treatment with TNF inhibitor has not yet reported. However, Park et al. (13) showed that MSP induces uPAR expression via MAPK, AP-1 and NF-κB signalling pathways and, in turn, stimulates cell invasiveness in human gastric cancer AGS cells. Our present data indicate the in vivo interaction of uPAR and MCP-α in RA. Synergistic interplay of uPAR and MSP-α may explain the activation of MAPK, AP-1 and NF-κB found in rheumatoid synovitis (14, 15).

Slot et al. found that uPAR concentration was increased in RA compared with other inflammatory rheumatic disorders. They concluded that the difference may be related with the destructive joint changes specific to RA, and that serum uPAR could reflect the erosive activity in RA (6). The present data in conjunction with that of Slot et al. (6) indicate that uPAR may be released from the actively proliferating rheumatoid synovial tissues.
Available data have also shown that the expression of uPA/uPAR could be induced by various inflammatory factors. Higher levels of uPA could be detected in cell supernatant from CT-26 murine colorectal carcinoma cells stimulated with lipopolysaccharide (LPS), tumour necrosis factor alpha (TNF-alpha) and IL-6 (16). Consistent with these data, the results of our study suggest that elevated serum uPAR, which had perhaps been induced by certain cytokines such as TNF-alpha and IL-6 etc., might be associated with the response to ADA in patients with RA.

In summary, our data show a clear association between the uPAR concentration and primary clinical response to ADA. Supposing that hypervascularity of the synovial tissues represents the severity of RA (17), the present results may reflect the increment of fibrinolysis due to rheumatoid synovial inflammation. Although larger studies, including studies of therapy with other drugs, are warranted to confirm our observation, our data will be beneficial for clinical practices that prescribe ADA.

References