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ORIGINAL ARTICLE

Immunogenicity, efficacy and adverse events of adalimumab in RA patients

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Abstract To assess the immunogenicity of adalimumab, a human anti-TNF-α mAb, we evaluated the formation of antibodies to adalimumab, efficacy and adverse events among 15 patients with highly active rheumatoid arthritis. Four patients were treated with adalimumab as monotherapy, and 11 patients with concomitant DMARDs. Disease activity was measured by DAS28. The antibodies were detected by ELISA. Thirteen (87%) patients withdrew from therapy within 45 weeks and overall 13 (87%) patients showed antibodies to adalimumab including 11 patients who withdrew from therapy. In four patients without concomitant DMARDs and in nine patients with concomitant DMARDs, we detected anti-adalimumab antibodies. Overall, five of seven patients with adverse drug reactions and all nine patients with lack of efficacy were associated with the formation of antibodies. Two antibody-positive patients developed an exantheme. The results indicate that adalimumab is, in spite of its fully human sequences, immunogenic and induces antibodies in a high rate of adalimumab-treated patients.

Keywords Immunogenicity · Anti-adalimumab antibodies · Adverse events · Clinical response

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Introduction

The tumor necrosis factor α-blocking monoclonal antibody adalimumab is described as safe and well tolerated and shows clinical efficacy with or without the concomitant use of methotrexate in controlled clinical trials among patients with RA. Adalimumab is genetically engineered by phage-display technique and because of its fully human sequences it is supposed to be less immunogenic than murine or chimeric monoclonal antibodies. However, the formation of human anti-human antibodies (HAHA) has been reported by several authors [1–3]. It still remains unclear which part of adalimumab induces HAHA response. Immunogenicity of adalimumab and its clinical significance in daily clinical practice is poorly investigated compared to the immunogenicity of infliximab and may differ from that shown in previous controlled studies [1–3]. The aim of this trial was to assess the immunogenicity of adalimumab and the correlation of efficacy and adverse events among a small number of patients in regular clinical settings.

Patients and methods

Patients

Fifteen eligible patients had rheumatoid arthritis (RA) diagnosed according to the 1987 revised critieria of the American College of Rheumatology [4] with a disease activity score (DAS 28) of more than 4.0 who were taking adalimumab with or without concomitant methotrexate or leflunomide. All participants were treated with corticosteroids and had previously received more than one DMARD without clinical efficacy.

Exclusion criteria consisted of the British Society for Rheumatology guidelines for prescribing TNFα blockers in adults with RA [5].

Study protocol

We assessed 15 patients between November 2003 and January 2006 at a single center in Germany. Eleven patients were taking adalimumab with concomitant methotrexate or leflunomide and four patients were taking adalimumab as monotherapy. Informed consent was obtained from all patients enrolled. After screening for tuberculosis, which included chest radiographs and tuberculin skin testing, baseline assessment which included measurement of DAS28 and medical history were performed. Study visits were conducted every month to every 3 months in most cases. Every patient received adalimumab at a dosage of 40 mg subcutaneously every other week. Patients were instructed about self-injection techniques.

Assessment

DAS28 and morning stiffness were measured at baseline, after 3, 6, 12 and 18 months thereafter. A change in the DAS 28 of >1.2 (twice the measurement error) is a clinically significant change [6].

Safety was assessed on the basis of adverse events reported by patients and findings on physical examination and laboratory evaluations.

Immunogenicity assessment

Serum levels of anti-adalimumab antibodies were monitored at baseline and every other time on follow-up (i.e., in most cases between 4 weeks and 3 months thereafter). According to the optical density (OD) of the HAHA serum level, we divided the antibody-positive patients into three groups: patients with low serum levels ($0.02 \leq OD < 0.2$), intermediate levels ($0.2 \leq OD < 1.0$), and high levels of antibodies against adalimumab ($OD \geq 1.0$). Serum levels of anti-adalimumab antibodies were measured by sandwich enzyme linked immunosorbent assay (ELISA; Immundiagnostik AG, Bensheim, Germany). Ninety-six-well microtiter plates were coated with 100 µl/well of solution containing 3 µg/ml $F(ab')_2$ fragments of adalimumab (Abbott, Wiesbaden, Germany) buffered with 50 mM of sodium carbonate (pH 9.6). After incubation overnight at 4°C, the plates were blocked with 2% BSA in PBS for 1 h at room temperature. Patient serum and control samples diluted 1:200 in 2% BSA in PBS, pH 7.2, were added to the appropriate well in duplicate and incubated overnight at 4°C. After washing, horseradish peroxydase-conjugated adalimumab was added and the preparations were incubated for 1 h at room temperature. Color reaction was induced by incubation with the substrate solution (TMB) for 30 min. The reaction was stopped by addition of 0.4 M sulfuric acid and a microplate reader was used to measure the OD at a wavelength of 450 nm. The results were determined by cutoff.

Results

Antibodies to adalimumab

The baseline characteristics of the patients are shown in Table 1. Overall, 13 (87%) patients had low serum levels up to high serum levels of antibodies against adalimumab (Fig. 1). All four patients with adalimumab monotherapy and nine patients with additional DMARDs had elevated serum levels (Fig. 1). Three patients had high levels of antibodies ($OD \geq 1.0$), two patients had intermediate levels, and eight patients had low levels ($0.02 \leq OD < 0.2$). One patient with high levels of anti-adalimumab antibodies was taking adalimumab without concomitant DMARD and withdrew from therapy owing to the development of a drug-related exantheme. The other two patients dropped out owing to lack of efficacy—one taking concomitant leflunomide, one concomitant methotrexate.

The two patients with intermediate serum levels of anti-adalimumab antibodies had to discontinue owing to adverse events, including one with exantheme and one with herpes zoster. Among the eight patients with low levels, six had to discontinue treatment, four owing to lack of efficacy, two owing to adverse drug reactions, and two patients are still taking adalimumab without lack of efficacy or adverse events.

Dropouts and antibody formation

In 13 (86%) patients adalimumab treatment failed and led to withdrawal from therapy (Table 2). Six patients discontinued treatment owing to lack of efficacy, six owing to adverse drug reactions, one because of bone surgery (Table 2). Of those patients with side effects, three discontinued treatment owing to hypersensitivity reactions (two with monotherapy), one owing to the development of herpes zoster, and one owing to shortness breath, both were taking adalimumab with concomitant methotrexate (Table 3). Of three RA patients who did not reach the third month of therapy we had no further DAS28 after baseline. Only one patient

Table 1 Baseline characteristics of 15 patients with RA treated with adalimumab

Variables	Mean ± SEM (range)/*n* (%)
Age, years	55.9 ± 8.1 (34–73)
Female sex	10 (67)
Disease duration, years	12.2 ± 8.2 (2.5–40)
Number of previous DMARDs	3.1 ± 1.1 (1–5)
DAS 28 at baseline	6.5 ± 1.2 (4.0–8.5)
Morning stiffness, minutes	132 ± 121 (0–480)
Seropositivity	9 (60)
Indication for adalimumab	*n* (%)
High disease activity	14 (93)
AEs to other biologicals	1 (7)
Refractory to other biologicals	6 (40)
Concomitant medication	*n* (%)
Corticosteroids	15 (100)
Methotrexate	10 (67)
Leflunomide 20 mg/day	1 (7)
Dosage (mg)	Mean ± SEM (range
Prednisone, mg/day	7.7 ± 2.7 (2.5–15)
Methotrexate, mg/week	13.1 ± 2.3 (7.5–20)

SEM standard error of the mean, *n* number of patients

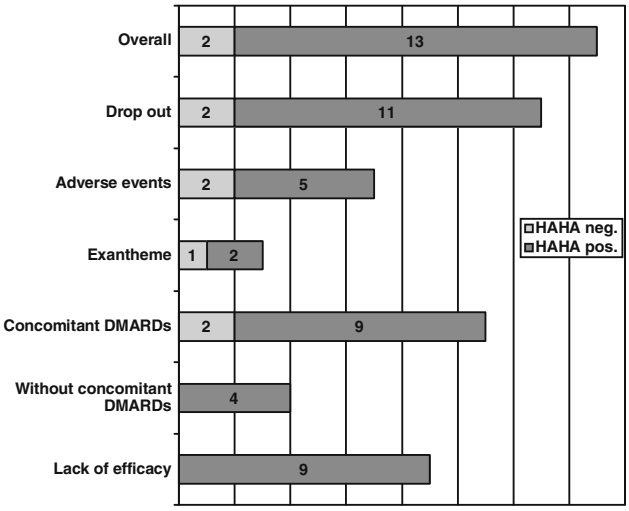

Fig. 1 Number of patients with/without HAHA response in relation to clinical parameters among 15 adalimumab-treated patients

reached 45 weeks of treatment and one reached 28 weeks of treatment before dropout. The other 11 patients discontinued adalimumab treatment within 15 weeks including three patients with adverse events who had to drop out after 3 weeks.

Eleven dropout patients had elevated serum levels of anti-adalimumab antibodies; two patients were negative (Fig. 1).

Table 2 Dropouts, treatment duration and disease activity in the course of treatment

Dropout patients (week 2–45)	Results *n* (%)
All	13 (87)
Owing to lack of efficacy	6 (40)
Owing to AEs	6 (40)
Exantheme	3 (20)
Other AEs	3 (20)
Needed surgery	1 (7)
Treatment duration	Mean ± SEM (range)
Number of injections until drop out	7.8 ± 4.2 (2–23)
Number of weeks until drop out	13.2 ± 7.8 (2–45)
Mean disease activity	Mean ± SEM (range)
DAS28 at baseline	6.5 ± 1.2 (4.0–8.5)
DAS28 three months later	6.1 ± 0.6 (4.5–7.2)

Table 3 Adverse events among 15 adalimumab-treated patients

Variable	Results *n* (%)
Patients with adverse events	7 (47)
Adverse events leading to withdrawal	6 (40)
Exantheme	3 (20)
Herpes zoster	1 (7)
Edema	1 (7)
Nausea	1 (7)
Headache	1 (7)
Dyspnea	2 (13)
Abnormal menses	1 (7)
Diarrhea	1 (7)

Efficacy and antibody formation

At baseline we measured a mean DAS28 of 6.5 and 3 months thereafter a mean DAS28 of 6.1 (Table 2). No clinically significant change in the DAS28 was observed in nine (60%) patients. These nine patients showed elevated levels of anti-adalimumab antibodies. The duration of morning stiffness after 3 months could not be evaluated in five patients because they dropped out before the 3-month follow-up.

The two antibody-negative patients showed clinical response to adalimumab.

Adverse events and antibody formation

Seven (47%) patients showed adverse drug reactions (Table 3). Five patients with adverse events were antibody positive (Fig. 1). Three patients developed an

adalimumab-related exantheme, including two patients with antibodies to adalimumab in their serum (Fig. 1). Two patients without anti-adalimumab antibodies in their serum showed adverse events, including one case with exantheme and one with local inflammation at the injection site.

Methotrexate and antibody formation

All four patients without concomitant DMARDs had to discontinue adalimumab treatment and all were tested positive on antibodies against the TNF-α-blocker (Fig. 1). One patient dropped out owing to lack of efficacy, one owing to lack of efficacy and drug-related exantheme, one owing to drug-related exantheme, and one owing to herpes zoster. Of the 11 patients with concomitant DMARDs, nine were tested positive for antibodies against adalimumab (Fig. 1). In this group nine patients had to discontinue the anti-TNFα therapy, including seven patients with antibodies against adalimumab. All seven antibody-positive patients were associated with an inadequate response to adalimumab and two patients showed adverse drug reactions.

Antibody titers in the course of treatment

Seven patients with a negative baseline titer of anti-adalimumab antibodies showed an increasing OD of the antibody titer after the first adalimumab injections. After withdrawal from adalimumab treatment we observed a decrease of the OD to baseline antibody serum levels in most cases. The other six patients showed antibodies to adalimumab in the further course of therapy. Examples are shown in Figs. 2 and 3.

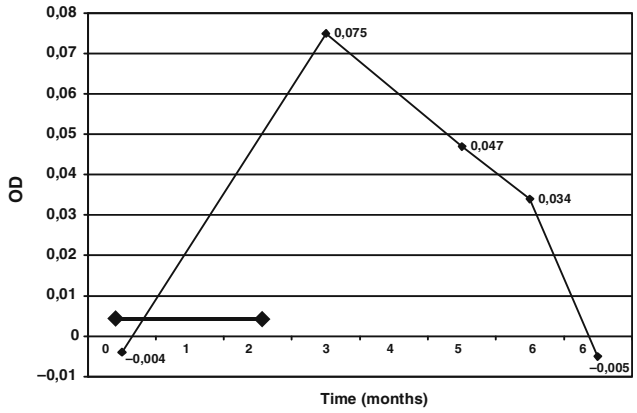

Fig. 2 Optical density (OD) of the antibody serum level (*thin line*) in the course of treatment with adalimumab (*thick line*) of a 54–year-old female patient who had to stop adalimumab-monotherapy within 2 months owing to lack of efficacy

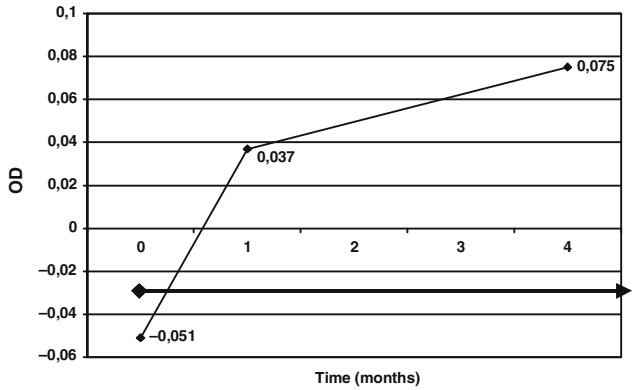

Fig. 3 Optical density (OD) of the antibody serum level (*thin line*) in the course of treatment with adalimumab (*thick arrow*) of a 56-year-old male patient. He started adalimumab treatment with concomitant methotrexate after discontinuing infliximab because of lack of efficacy. The therapy with adalimumab is still ongoing; no adverse drug reactions were observed until present

Discussion

In this regular clinical setting, our investigation indicates that in spite of its fully human sequences adalimumab induces HAHA responses in a high rate of adalimumab-treated patients with or without the concomitant use of DMARDs. Previous trials described an incidence of anti-adalimumab antibodies of about 12% among patients treated with adalimumab as monotherapy [1] and about 1% among patients who had taken concomitant methotrexate [2]. There seems to be no difference in the pattern or frequence of adverse events between patients with or without these antibodies [1]. The correlation between HAHA response and reduced clinical efficacy is reported differently [1–3]. Van de Putte et al. [1] found no statistical difference in ACR20 response rates between HAHA-positive and HAHA-negative patients treated with the recommended dose of 40 mg every other week. In the ARMADA trial no correlation between the presence of anti-adalimumab antibodies and a reduced response to treatment was observed [2] but other trials reported a lower rate in the ACR20 response among antibody-positive patients [3]. In our trial it is difficult to correlate HAHA response and clinical parameters because we observed an overall high incidence of anti-adalimumab antibodies and dropouts owing to adverse events and lack of efficacy. Moreover, we could not compare antibody-positive with antibody-negative patients because there were only two patients without detected HAHA. Furthermore, we did not evaluate the concentration of

adalimumab in the serum, so we could not assess the clearance of Humira® from the serum.

The ELISA, which used $F(ab')_2$ fragments of adalimumab for HAHA binding, is supposed to be highly specific because binding of unspecific antibodies to the Fc part of adalimumab is prevented. Antibodies against adalimumab detected in our assay could be specific to any part of this $F(ab')_2$ fragment. We assume that the HAHA reactivity is either directed against non-human glycosylated sites on the surface of the antibody expressed in CHO cells or against the antigenic determinants in VL and VH regions of adalimumab that include the CDRs (complementary determining region). The latter was reported by Ritter et al. [7] who characterized the HAHA response against a humanized monoclonal antibody for the treatment of colon cancer with the use of the highly sensitive BIACORE method. He detected, despite humanization, HAHA responses in 63% of the treated patients [7]. A further evidence for the development of anti-idiotype antibodies against adalimumab might be the very low incidence of antibodies against etanercept, a recombinant human TNF-receptor fusion protein, between 0.5 and 3% [8–12].

In contrast to adalimumab and etanercept, the clinical significance of antibodies against infliximab, a chimeric monoclonal antibody to TNFα, is well described among patients with Crohn's disease [13, 14]. Infliximab is highly immunogenic and treatment is associated with a high incidence of human anti-chimeric antibodies (HACA) between 14 and 61% [13–17]. Among patients with rheumatoid arthritis HACA incidence is lower, probably because of the concomitant use of methotrexate in all study patients, and the clinical significance is less investigated [18, 19]. Patients with detectable HACA are more likely to have infusion reactions and insufficient clinical response than those without antibodies against infliximab. The concomitant use of immunomodulators reduces the incidence of HACA formation [13–15]. Furthermore, after induction therapy at week 0, 4 and 6, scheduled treatment every 8 weeks is associated with lower HACA incidence [17]. Because infliximab in the serum interferes with the assays for HACA detection [13–19], the incidence of anti-infliximab antibodies could be underestimated in most trials described above. The interference of adalimumab with our and previously used assays is unknown and not yet investigated.

Because of the small number of patients, our data must be handled with caution and suggests that further trials of regular clinical settings with a higher number of patients have to be performed.

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