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Development of Antiinfliximab Antibodies and Relationship to Clinical Response in Patients With Rheumatoid Arthritis

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Objective. **Treatment of patients with infliximab, a chimeric monoclonal IgG1 antibody against tumor necrosis factor, may result in the formation of infliximab-specific IgG antibodies. This study evaluated the clinical significance of these antibodies in patients with rheumatoid arthritis (RA).**

Methods. **Antiinfliximab antibodies were measured using a newly developed radioimmunoassay in a cohort of 51 consecutive patients with RA treated with infliximab, with a followup of 1 year. In addition, serum infliximab levels were determined by enzyme-linked immunosorbent assay. The results were analyzed in relation to the clinical response to treatment according to the European League Against Rheumatism criteria.**

Results. **Antibodies against infliximab were detected in 22 patients (43%). Patients without detectable antiinfliximab antibodies (n = 29 [57%]) were significantly more often classified as responders (20 of 29 [69%]) compared with patients with detectable antiinfliximab antibodies (8 of 22 [36%]; $P = 0.04$). Three patients had an infusion-related allergic reaction, all of whom had detectable antiinfliximab antibodies.**

Conclusion. **In this study, nearly half of the RA patients treated with infliximab developed antiinfliximab antibodies within the first year of treatment. This seems to be clinically relevant, since development of antiinfliximab antibodies is associated with a reduced response to treatment.**

Treatment with infliximab provides great benefit to many patients with rheumatoid arthritis (RA) (1–3). However, some patients have persistent active disease and others show loss of efficacy after prolonged treatment. Infliximab can induce the formation of antibodies to infliximab that may lead to side effects and loss of efficacy. Development of antibodies to infliximab is related to the dose of infliximab and is diminished by concomitant treatment with methotrexate (MTX) (1). To what extent formation of antibodies to infliximab plays a role in clinical practice is unknown.

The Anti–Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy (ATTRACT) study group reported that 8% of patients receiving infliximab plus MTX developed antibodies to infliximab during the 102-week study period of the trial. However, despite the presence of antibodies, similar proportions of patients with antibodies to infliximab and without antibodies to infliximab achieved a 20% improvement response according to the American College of Rheumatology response criteria (ACR20) (4). We have recently shown that a good response to treatment is associated with high serum trough levels of infliximab (5). Conversely, serum trough levels of infliximab were below the limit of detection in patients prior to development of infusion-related allergic reactions. These findings suggest that formation of antibodies against infliximab might play a role in the efficacy of infliximab therapy in patients with RA.

To investigate the formation of antibodies against infliximab, we developed an antigen binding assay and measured levels of antiinfliximab antibodies in the serum of a cohort of 51 RA patients treated with inflix-

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imab, with a followup of 1 year. The presence of antiinfliximab IgG antibodies as well as trough levels of infliximab in the serum were related to clinical response and allergic reactions.

PATIENTS AND METHODS

Patients. Consecutive patients with RA who were receiving infliximab for RA at the Department of Rheumatology of Slotervaart Hospital in Amsterdam from April 2000 to January 2002 were included in this open, prospective observational study. Treatment of these patients was in accordance with the consensus statement on the initiation and continuation of tumor necrosis factor (TNF)–blocking therapy in RA (6). All patients fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria for RA (7), and all had evidence of active disease, as indicated by a Disease Activity Score in 28 joints (DAS28) of >3.2 despite earlier treatment with 2 disease-modifying antirheumatic drugs (DMARDs), including MTX, at a dosage of 25 mg weekly or at the maximum tolerable dosage (based on the Dutch guidelines for starting anti-TNF treatment).

All patients were given intravenous infusions of 3 mg/kg of infliximab at weeks 0, 2, and 6, and every 8 weeks thereafter. After 14 weeks of treatment, the treating rheumatologist was free to increase the dosage of infliximab to 7.5 mg/kg body weight in patients whose condition exhibited an inadequate response. Moreover, after 14 weeks, dosing intervals were kept stable at 8-week intervals. Concomitant medication, including MTX, was continued.

Disease activity was assessed using the DAS28 (8) before each infusion. For assessment of clinical response, we used the European League Against Rheumatism (EULAR) response criteria (9). Patients who stopped treatment or who needed a dosage escalation were regarded as nonresponders. An infusion reaction was defined as any significant adverse event that occurred during the infusion or within 2 hours after the infusion.

Measurement of serum infliximab and antiinfliximab antibody levels. Serum samples were collected 1 hour prior to each infusion, for the assessment of serum infliximab and antiinfliximab antibodies. Infliximab levels in the serum were determined by enzyme-linked immunosorbent assay, as described elsewhere (5). Antiinfliximab was detected with a newly developed radioimmunoassay. Infliximab-specific IgG was measured by an antigen binding test, essentially according to the procedure described by Aalberse et al (10). Briefly, serum (1 μl/test) was preincubated with agarose-immobilized protein A (1 mg/test; Pharmacia, Uppsala, Sweden) in Freeze buffer (CLB Sanquin, Amsterdam, The Netherlands). Nonbound serum components were removed by washing before ~1 ng of ^{125}I-labeled pepsin-treated infliximab was added. After overnight incubation, nonbound radiolabel was washed away and agarose-bound radioactivity was measured. High-radioactivity samples were retested at the appropriate dilutions. Test results were converted into arbitrary units (AU) per milliliter by comparison with dilutions of a reference serum. The cutoff level for a positive signal was set at 12 AU (mean +3 SD of the pretreatment values).

Statistical analysis. Differences between patient groups were analyzed by chi-square test or Mann-Whitney U test, as appropriate. The threshold for significance was set at a P value of less than 0.05. To analyze the change in the DAS28 among patients with and without antiinfliximab antibodies after 1 year of treatment, we used the last observation carried forward for patients who stopped treatment or who had received an increased dosage of infliximab.

RESULTS

Characteristics of the cohort. The majority of the 51 patients who entered the study were women (82%). The mean ± SD age of the patients was 56 ± 13 years and the mean ± SD disease duration was 12 ± 9 years. Seventy percent of the patients were rheumatoid factor positive. The mean number of DMARDs received before infliximab treatment was 3.7. Forty-four patients (86%) were receiving concomitant MTX, with a mean dosage of 15 mg/every week, while 3 patients were receiving azathioprine and 1 patient was receiving concomitant cyclosporine. The remaining 3 patients were not taking concomitant immunosuppressive drugs. At study entry, all patients had active disease, as indicated by a mean ± SD DAS28 of 6.0 ± 1.3.

Clinical response. After 1 year of followup, 28 patients (55%) were classified as treatment responders according to the EULAR response criteria, without having needed an increase in the infliximab dosage. After 14 weeks of treatment, the mean ± SD DAS28 had improved to 4.7 ± 1.7. Eight patients stopped treatment before the end of 1 year of therapy. Two patients stopped receiving infliximab after developing an infusion-related allergic reaction. Two patients stopped treatment because of skin problems, 1 patient because of edema, and 3 patients because of treatment inefficacy. Six patients received a dosage escalation.

Detection of antiinfliximab antibodies. Prior to treatment, the antiinfliximab concentration was below 10 AU/ml in all 51 samples. At followup, serum samples from 22 patients were found to be positive for antiinfliximab antibodies. One patient was antibody positive at week 6 after start of treatment, 6 patients at week 14, 6 patients at week 22, 2 patients at week 30, 4 patients at week 38, and 3 patients at week 46. In most of these patients, the titer of antiinfliximab antibodies increased during continuation of treatment. In 5 patients with relatively low titers of antiinfliximab (25 AU/ml, 93 AU/ml, 18 AU/ml, 25 AU/ml, and 64 AU/ml), the antiinfliximab levels became undetectable during fol-

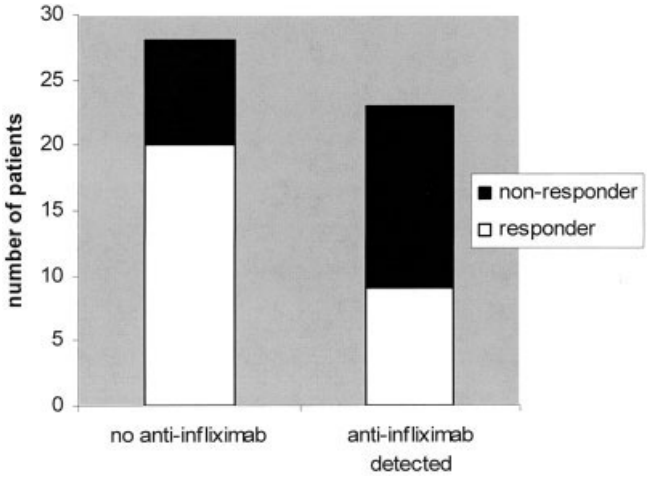

Figure 1. Presence or absence of detectable antiinfliximab antibodies in relation to response to treatment (according to the European League Against Rheumatism criteria) with infliximab. Patients without detectable antiinfliximab were significantly more often classified as responders compared with patients with detectable antiinfliximab (20 of 29 [69%] versus 8 of 22 [36%]; $P = 0.04$).

lowup, while in 3 patients, antiinfliximab antibodies fell below the level of detection after dosage escalation. In these latter 3 patients, we observed an increase in the serum trough levels of infliximab just above the limit of detection (0.1 mg/liter) that coincided with an improvement in the DAS28.

Patients without detectable antiinfliximab antibodies were significantly more often classified as responders when compared with patients with detectable antiinfliximab antibodies (20 of 29 without antibodies [69%] versus 8 of 22 with antibodies [36%] considered responders; $P = 0.04$) (Figure 1). Patients without detectable antiinfliximab antibodies had significantly more improvement in the DAS28 than did patients with detectable antiinfliximab antibodies (mean ± SD decrease in the DAS28 1.9 ± 1.2 versus 0.9 ± 1.8; $P = 0.02$). The maximum antiinfliximab titer was significantly higher in patients classified as nonresponders (median 42 AU/ml, interquartile range 8–310 AU/ml) compared with patients classified as responders (median 9 AU/ml, interquartile range 6–17 AU/ml) ($P = 0.025$) (Figure 2).

In 3 patients, there was an infusion-related allergic reaction. In all 3 of these patients, the infusion reactions consisted of tachycardia, erythema, and shortness of breath. These patients were treated with antihistamines, and full recovery occurred within a few hours. Two of the patients stopped further treatment, whereas 1 patient continued treatment without further infusion reactions. Antiinfliximab antibodies were detected in the serum of all 3 of these patients (786 AU/ml, 748 AU/ml, and 64 AU/ml, respectively). However, most of the patients with antiinfliximab antibodies, including 2 patients with the highest titers of antiinfliximab (1,359 AU/ml and 29,133 AU/ml), did not develop an allergic infusion reaction.

The development of antiinfliximab antibodies coincided with a decrease in serum trough levels of infliximab prior to the detection of antiinfliximab. Detection of antibodies against infliximab occurred only in samples with undetectable serum trough levels of infliximab. An example is shown in Figure 3. Mean serum trough levels of infliximab at 8 weeks after administration of 3 mg/kg infliximab were significantly lower in patients with antiinfliximab antibodies compared with patients without such antibodies (0.2 mg/liter versus 1.5 mg/liter; $P < 0.001$).

Baseline characteristics of the patients with and without antiinfliximab antibodies were similar. The mean MTX dose in patients with and without antiinfliximab antibodies was similar. None of the 3 patients receiving azathioprine as concomitant therapy had detectable antiinfliximab antibodies. Antibodies to inflix-

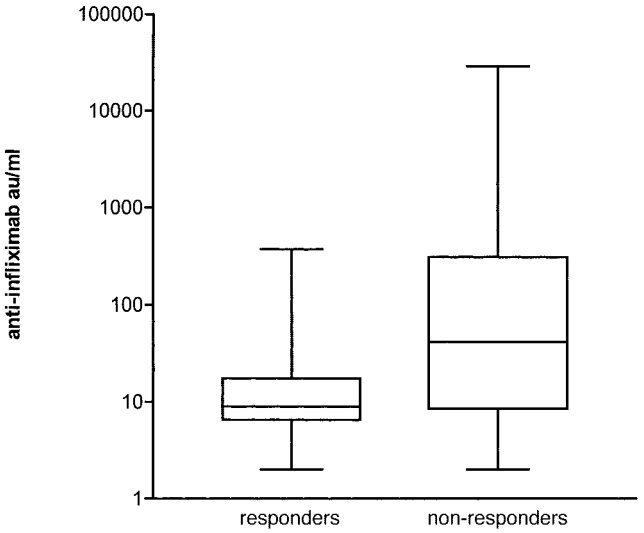

Figure 2. Maximum antiinfliximab titers in patients classified as responders versus patients classified as nonresponders to infliximab therapy. The maximum antiinfliximab titer was significantly higher in nonresponders (median 42 arbitrary units [AU]/ml, interquartile range 8–310) compared with responders (median 9 AU/ml, interquartile range 6–17) ($P = 0.025$). Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles.

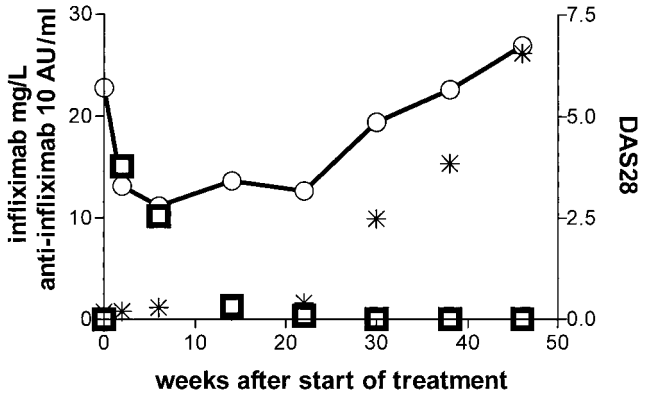

Figure 3. Trough levels of infliximab in the serum (□), levels of antiinfliximab (*), and the Disease Activity Score in 28 joints (DAS28) (○) in a rheumatoid arthritis patient treated with infliximab. After an initial improvement of disease activity, the patient had a relapse of disease activity that coincided with a decrease in the serum trough levels of infliximab and an increase in the antiinfliximab titer. AU = arbitrary units.

imab were detected in 2 of the 3 patients who did not receive concomitant medication. All of these patients who had not taken concomitant medication were considered nonresponders to the infliximab regimen.

DISCUSSION

The results presented herein show that almost half of the 51 patients with RA who were treated with 3 mg/kg infliximab every 8 weeks developed detectable antiinfliximab IgG antibodies within the first year of treatment. Moreover, the presence of these antibodies was associated with a reduced response to treatment.

In the ATTRACT study, 8% of patients receiving infliximab plus MTX developed antibodies to infliximab during the 102-week study period. It was reported that despite the presence of antibodies, similar proportions of patients with antibodies to infliximab and without antibodies to infliximab achieved an ACR20 response (2). In patients with Crohn's disease, 61% of patients treated with infliximab developed antiinfliximab antibodies (11). It has been demonstrated that RA patients who respond to therapy have higher serum trough levels of infliximab compared with patients who do not respond to therapy (5). Indeed, serum trough levels of infliximab were undetectable after an 8-week treatment interval in patients who developed an infusion-related allergic reaction.

The difference between the findings of this study and those reported by the ATTRACT study group might be explained by the fact that in our study, all patients started treatment on the 3 mg/kg dosage every 8 weeks, while 75% of the patients in the ATTRACT study received higher dosages. In addition, some of the difference might be attributable to differences in the assays used; thus, a formal comparison between the assays is warranted.

Experiments with animal models have shown that induction of an immune response to therapeutic antibodies is associated with an accelerated clearance of the antibody (12). Patients with detectable antiinfliximab have lower mean serum trough levels of infliximab compared with patients without antiinfliximab antibodies. This indicates that antiinfliximab antibody formation accelerates the clearance of infliximab from the circulation.

Interestingly, we observed that in some patients with antiinfliximab antibodies and an inadequate response to treatment, continuation of treatment with higher dosages of infliximab resulted in decreased levels of antiinfliximab antibodies. This might be attributable to induction of immune tolerance, similar to what is seen in patients with hemophilia who develop antibodies to factor VIII. Alternatively, it could be the result of overdosing the capacity of the immune system to produce antiinfliximab antibodies. It can be speculated that continuation of treatment with increased dosages of infliximab is effective in patients with low antiinfliximab antibody titers, whereas those patients with high titers of antiinfliximab antibodies probably benefit more from switching to other TNF-blocking agents.

Infliximab may induce infusion-related allergic reactions. In all 3 patients who developed an infusion-related reaction, high levels of antiinfliximab antibodies were detected. It is remarkable that most of the patients with antiinfliximab antibodies did not have a clinically overt allergic response. This is not simply related to the titer of the antibodies, since 2 patients who had the highest antibody titers did not have clinically overt infusion reactions. The fact that 2 of the 3 patients who received infliximab without concomitant immunosuppressive medication developed antiinfliximab antibodies illustrates the importance of adequate concomitant immunosuppressive therapy to prevent formation of antibodies to infliximab.

Many patients with RA exhibit persistent disease activity despite having received infusions of infliximab. The response to infliximab therapy is related to the level of infliximab in the serum and the presence of antiinfliximab antibodies, and shows a large interindividual

variation. Further investigation into the mechanisms that determine serum concentrations of infliximab and formation of antiinfliximab antibodies may help to optimize this treatment.

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