Induction of autoantibodies in refractory rheumatoid arthritis treated by infliximab

Y. Allanore[1]*, J. Sellam[1]*,
F. Batteux[2], C. Job Deslandre[1],
B. Weill[2], A. Kahan[1]

[1]Rheumatology A Department and [2]Biological Immunology Department, Paris V University, Cochin Hospital, AP-HP, Paris, France.

*These authors contributed equally to the study.

Please address correspondence to:
Dr Yannick Allanore, Cochin Hospital, Department of Rheumatology A, 27, rue du Faubourg Saint-Jacques, 75014 Paris, France.
E-mail: yannick.allanore@cch.ap-hop-paris.fr

Abbreviations:
RA: rheumatoid arthritis;
RF: rheumatoid factor;
ANA: antinuclear antibodies;
anti dsDNA: anti double-stranded DNA antibodies;
aENA: anti-extractable nuclear antigen antibodies;
NS: not significant.

ABSTRACT

Objectives. *To investigate autoantibody induction in rheumatoid arthritis (RA) patients treated with infliximab. Methods. We included 59 refractory RA patients treated with infliximab in combination with low-dose prednisone and methotrexate or leflunomide. We tested the sera of the patients for antinuclear antibodies (ANA), rheumatoid factor (RF), anti double-stranded DNA antibodies (anti dsDNA), anti-histone and anti-extractable nuclear antigen antibodies (aENA) at baseline and before infusion at weeks 6 and 30. Infliximab, initiated at a dose of 3 mg/kg, was increased to 5 mg/kg if insufficient improvement was observed after three infusions.*

Results. *At week 6, only the frequency of anti-histone IgM antibody-positive patients had significantly increased (19 vs 42%, p = 0.009). At week 30, the frequency of patients with ANA had increased from 29% to 69% (p < 0.001), that of patients with anti-dsDNA antibodies had increased from 0% to 3% for IgG (NS) and from 0% to 32% for IgM (p < 0.001); the frequency of anti-histone IgG detection had increased from 22% to 32% (p = 0.04) and that of IgM detection, from 18% to 79% (p < 0.001). No lupus-like syndrome was observed. RF decreased significantly (87 IU to 52.5 IU, from baseline to week 30; p < 0.001). No significant difference was observed between the 16 non-responders and the responders, in terms of autoantibody status at baseline and changes with infliximab therapy.*

Conclusion. *Infliximab therapy lead to the selective and delayed induction of autoantibodies. This induction was not associated with clinical symptoms until week 30 and did not differ between responders and non-responders.*

Introduction

The development of anti-TNF therapy represents a milestone in the treatment of rheumatoid arthritis (RA). Infliximab (Remicade®; Centocor®), a chimeric, monoclonal anti-TNF IgG1 antibody has proved effective against active RA refractory to methotrexate (MTX) (1-3). In RA, anti-TNF therapy, including infliximab, induces antinuclear antibodies (ANA) and anti double-stranded DNA antibodies (anti-dsDNA), but rarely lupus-like syndromes (3-5). According to safety data from infliximab trials, 63.8% of RA patients develop ANA and 13% develop anti-dsDNA antibodies during infliximab treatment (6). With the exception of clinical trials, only one study has investigated the effect of infliximab on autoantibodies in RA(7).

The aim of this prospective study was to describe the autoantibody induction profile and to assess its clinical impact on the response to infliximab.

Patients and methods

Patients and medication regimen

We included 62 patients (54 women and 8 men; mean age at baseline 54.6 years [range 41-68]) with RAfulfilling American College of Rheumatology criteria (8) and refractory to disease-modifying antirheumatic drugs (DMARDs) including MTX. The mean duration of RAwas 14.3 years [range 5.6–23]. Patients received infliximab at weeks 0, 2, 6, and every 8 weeks thereafter, in combination with MTX (7.5 to 20 mg per week) in 53 patients or leflunomide (20 mg per day) in 9 patients. All patients received prednisolone (less than 10 mg per day). The initial dose of infliximab was 3 mg/kg. After the third infusion, the dose could be increased to 5 mg/kg in cases in which the clinician judged that insufficient improvement had occurred. We collected the following data: patient global assessments on a 10-cm visual analogue scale (VAS), Ritchie's index, swollen joint count, erythrocyte sedimentation rate (mm/h) and the disease activity score (DAS) (9, 10). None of the patients received premedication before infliximab infusion.

Detection of autoantibodies

Serum samples were obtained at baseline and before the infusions at weeks 6 and 30. RF was determined by ELISA (IgM isotype, positive 20 IU). ANA were detected by indirect immunofluorescence on HEp2 cells (positive 1/80). If a result of at least at 1/160 was obtained for ANA, sera were tested by ELISA for anti-dsDNA IgM and IgG (positive 20 IU), positive results were

Autoantibodies in RA with infliximab therapy / Y. Allanore et al.

BRIEFPAPER

also tested and verified by immunofluorescence on chritidia cells, and counterimmunoelectrophoresis for the following anti-extractable nuclear antigen antibodies (anti-ENA): anti-Sm, anti-RNP 70, anti-Ro/SSA, anti-La/SSB, anti-Scl-70 (topoisomerase I) was performed. Anti-histone IgM and IgG antibodies were detected by ELISA (positive 50 IU).

Statistical analysis

McNemar's test, a chi^2 test for paired analysis, was used to compare the frequency of autoantibody detection before and after treatment with infliximab. We compared various sets of unpaired qualitative data by the chi^2 test. We used the non-parametric Wilcoxon's test for comparisons of quantitative paired data. The threshold for statistical significance was set at = 0.05.

Results

Efficacy and adverse effects of infliximab

Three patients stopped infliximab before the third infusion because of adverse effects (two infections, one serious hypersensitivity reaction). These three patients were not included in further analysis, and results are therefore presented for 59 patients. Between weeks 0 and 30, mean tender joint count (18 ± 10 at baseline vs 6 ± 6 at week 30; $p < 0.0001$), mean global assessment by VAS (64 ± 18 vs 31 ± 25; $p < 0.0001$), mean ESR (34 ± 27 vs 19 ± 14; $p = 0.0006$), mean CRP (28 ± 37 vs 10 ± 15; $p = 0.0007$) and mean DAS

score (7.9 vs 2.5; $p < 0.0001$) decreased significantly. Sixteen patients with disease insufficiently improved by infliximab at a dose of 3 mg/kg were treated with 5 mg/kg from the third infusion: these patients were considered to be insufficient-responders (at 3 mk/kg).

Effect of infliximab treatment on autoantibodies

Autoantibody status is reported in Table I for baseline, week 6 and week 30. RF concentrations decreased significantly from baseline to week 30 ($p < 0.001$). At week 6, the only significant change from baseline was an increase in the frequency of patients with anti-histone IgM antibodies. By week 30 (before the 6th infusion), the frequency of patients positive for ANA, anti-dsDNAIgM, anti-histone IgG and IgM antibodies had significantly increased with respect to baseline measurements. None of positive patients for ANA at baseline became negative during the follow-up. Anti-dsDNA IgG appeared between baseline and week 30 in two patients, but titres remained low (24 and 25 IU). No aENA were observed at weeks 0, 6 and 30. There was no difference for antibody status and changes between patients receiving methotrexate and those treated by leflunomide in association with infliximab. No clinical lupus-like syndrome was observed between the start of the study and week 30.

Autoantibody profile according to clinical response

The responder (infliximab dose kept constant at 3 mg/kg) and insufficient-

responder (infliximab dose increased to 5 mg/kg) groups did not differ significantly in RF concentration or the frequency of detection of ANA, anti-dsDNA IgM and IgG, anti-histone IgG and IgM antibodies at each evaluation. No autoantibody determination at baseline or at week 6 had significant predictive value for clinical infliximab response.

Discussion

These results suggest that the induction of autoantibody production by infliximab treatment in combination with methotrexate or leflunomide is selective and subject to a time lag: ANA, anti-dsDNA IgM, anti-histone IgG and IgM antibodies increased significantly from baseline to week 30, whereas only anti-histone IgM antibodies had already significantly increased by week 6. Anti-ENA antibodies were not influenced by the treatment and RF decreased with infliximab treatment. A time lag (mean of 6.5 weeks after treatment initiation) in the induction of autoantibodies has been reported before (11).

In our study, the frequency of patients positive for ANA (29% at baseline) increased to 69%, with 24 newly positive patients (41%) at week 30. These results are similar to safety data reporting the detection of ANA in 63.8% of patients following infliximab therapy (6). However, the pattern of autoantibody induction in our study differed from that reported by De Rycke et al. These authors studied 62 RA patients treated with infliximab in combination with

Table I. Autoantibody profile before and after infliximab treatment.

	Week 0		Week 6		W0 vs W6	Week 30		W0 vs W30
RF; median (range)	87; (0-300)		74; (0-300)		p = 0.05	52.5; (0-300)		p < 0.001
Negative patients (n)	9/59		8/59		NS	12/59		NS
ANA¶	17/59	29 %	20/59	34 %	NS	41/59	69 %	p < 0.001
Anti-histone IgM ¶	11/59	19 %	25/59	42 %	p = 0.009	46/59	78 %	p < 0.001
Anti-histone IgG ¶	13/59	22 %	22/59	37 %	p = 0.23	19/59	32 %	p = 0.04
Anti-dsDNAIgM ¶	0/59	0 %	3/59	5 %	NS	19/59	32 %	p < 0.001
Anti-dsDNAIgG ¶	0/59	0 %	1/59	2 %	NS	2/59	3 %	NS
Anti-ENA¶	0/0	0 %	0/0	0 %	NS	0/0	0 %	NS

¶: positive patients/59; frequency positive patients (%).
SD: standard deviation; NS: non-significant; RF: rheumatoid factor (Ig M); ANA: antinuclear antibodies; Anti-dsDNA: anti double-stranded DNAantibodies; Anti-ENA: anti-extractable nuclear antigen antibodies.

methotrexate, and reported a higher frequency of patients positive for ANA at baseline (32/62; 51.6%) and at week 30 (51/62; 82.3%) than observed in our study (7). The higher frequency of ANA at baseline in De Rycke's study may be noted and should have influenced the autoantibody changes observed in this study which differ from our results. De Rycke *et al.* observed the production of anti-dsDNAIgM or IgA by 17% of patients at week 30, but no anti-dsDNA IgG; in our study, the frequency of non-IgG anti-dsDNA antibodies was higher (32%) and we observed the production of low levels of anti-dsDNAIgG in two patients at week 30. This isotype is more specifically associated with lupus erythematosus. We observed no lupus-like syndrome in the 30 weeks of follow-up, even in these two patients. Although these results are reassuring, the long-term follow-up of patients positive for anti-dsDNA antibodies is recommended.

The frequency of anti-histone antibodies increased significantly from baseline to week 30 for both isotypes: IgM and IgG. The frequencies of these antibodies — 78% for IgM and 32% for IgG at week 30 — were higher than those reported by De Rycke (9.7%). In this study, the frequency of detection of the IgM isotype increased more than that of the IgG isotype. These autoantibodies are usually observed in drug-induced lupus; they were not associated with clinical symptoms in our cohort at any point in the 30-week follow-up period. The production of anti-histone antibodies may reflect immunological stimulation by infliximab. The mechanism leading to autoantibody induction by infliximab remains unclear, however, it has been suggested that infliximab induces monocyte apoptosis in Crohn's disease (12), which may release nucleosomes leading to lupus syndrome; nevertheless, this would not be associated with clinical implication because of the absence of prevailing B lymphocytes apoptosis (13).

RF concentration decreased significantly from baseline to week 6 (p = 0.05) and week 30 (p < 0.001). Charles *et al.* reported similar results in 22 RA patients treated with infliximab (14). RF concentration is recognised as a prognostic factor but not as a factor associated with disease activity. Further investigation is required to determine the physiological significance of this decrease in RF concentration with infliximab treatment.

Autoantibody status at baseline and changes in response to infliximab therapy were not predictive of or associated with clinical response. The ATTRACT Study previously reported there to be no clear relationship between infliximab dose and antibody development (2).

Conclusion
Our results show the selective induction of autoantibodies, especially ANA, anti-dsDNA IgM, anti-histone IgM and IgG antibodies after infliximab therapy in patients with refractory RA. This induction of autoantibodies was not associated with clinical symptoms at any point in the 30 weeks of follow-up and did not differ between responders and non-responders. Long-term follow-up is however necessary, especially for patients positive for anti-dsDNA antibodies.

Acknowledgements

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