

Research article

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Autoantibody profile in rheumatoid arthritis during long-term infliximab treatmentFrancesca Bobbio-Pallavicini¹, Claudia Alpini², Roberto Caporali¹, Stefano Avalu², Serena Bugatti¹ and Carlomaurizio Montecucco¹¹Department of Rheumatology University of Pavia, IRCCS Policlinico S. Matteo, Pavia, Italy²Clinical Chemistry Laboratories University of Pavia, IRCCS Policlinico S. Matteo, Pavia, Italy

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Arthritis Res Ther 2004, **6**:R264-R272 (DOI 10.1186/ar1173)© 2003 Bobbio-Pallavicini *et al.*; licensee BioMed Central Ltd. This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original URL.**Abstract**

The aim of the present study was to investigate the effect of long-term infliximab treatment on various autoantibodies in patients with rheumatoid arthritis. Serum samples from 30 consecutive patients, who were prospectively followed during infliximab and methotrexate therapy for refractory rheumatoid arthritis, were tested at baseline and after 30, 54 and 78 weeks. At these points, median values of the Disease Activity Score were 6.38 (interquartile range 5.30–6.75), 3.69 (2.67–4.62), 2.9 (2.39–4.65) and 3.71 (2.62–5.06), respectively. Various autoantibodies were assessed by standard indirect immunofluorescence and/or ELISA. Initially, 50% of patients were positive for antinuclear antibodies, and this figure increased to 80% after 78 weeks ($P = 0.029$). A less marked, similar increase was found for IgG and IgM anticardiolipin antibody titre, whereas the frequency of anti-double-stranded DNA antibodies (by ELISA) exhibited a transient rise (up to

16.7%) at 54 weeks and dropped to 0% at 78 weeks. Antibodies to proteinase-3 and myeloperoxidase were not detected. The proportion of patients who were positive for rheumatoid factor (RF) was similar at baseline and at 78 weeks (87% and 80%, respectively). However, the median RF titre exhibited a progressive reduction from 128 IU/ml (interquartile range 47–290 IU/ml) to 53 IU/ml (18–106 IU/ml). Anti-cyclic citrullinated peptide (CCP) antibodies were found in 83% of patients before therapy; anti-CCP antibody titre significantly decreased at 30 weeks but returned to baseline thereafter. In conclusion, the presence of anti-double-stranded DNA antibodies is a transient phenomenon, despite a stable increase in antinuclear and anticardiolipin antibodies. Also, the evolution of RF titres and that of anti-CCP antibody titres differed during long-term infliximab therapy.

Keywords: anti-citrullinated peptide antibodies, anti-dsDNA antibodies, antinuclear antibodies, infliximab, rheumatoid factor**Introduction**

Tumour necrosis factor (TNF)- α inhibitors have proven to be highly effective in the treatment of rheumatoid arthritis (RA); they reduce disease activity and delay radiographic progression, with quite a good safety profile [1,2]. Side effects of anti-TNF- α treatment include an increased risk for infection and induction of autoantibodies such as antinuclear antibodies (ANAs) and anti-double-stranded (ds)DNA antibodies [3,4]. In particular, anti-dsDNA antibodies were found in 5–20% of RA patients treated with either infliximab (anti-TNF- α chimeric monoclonal antibody) or etanercept (human soluble TNF- α receptor p75 fusion protein), even

though development of a lupus-like illness was encountered rarely [3-8].

The mechanism responsible for the production of these autoantibodies during anti-TNF- α therapy has not been clearly defined. Treatment with TNF- α inhibitors dramatically reduces levels of C-reactive protein, which is involved in the clearance of apoptotic bodies [9,10]. There is evidence that apoptosis is among the most influential factors in autoimmunity [11], and TNF- α plays an important role in apoptosis [12]. Furthermore in Crohn's disease it has recently been shown that infliximab can bind activated T

aCL= anticardiolipin; ACR = American College of Rheumatology; ANA = antinuclear antibody; ANCA = antineutrophil cytoplasmic antibody; CCP = cyclic citrullinated peptide; ds = double-stranded; DAS 28 = Disease Activity Score; DMARD = disease-modifying antirheumatic drug; ELISA = enzyme-linked immunosorbent assay; EMA = antiendomysial antibody; ENA = extractable nuclear antigen; IF = indirect immunofluorescence; MPO = myeloperoxidase; PBS = phosphate-buffered saline; PR3 = serine protease 3; RA = rheumatoid arthritis; RF = rheumatoid factor; SLE = systemic lupus erythematosus; TNF = tumour necrosis factor.

cells and monocytes, inducing apoptosis [13,14]. Finally, inhibition of TNF- α – a pivotal T-helper-1 cytokine – could favour a T-helper-2 response, leading to an increased (auto)antibody production.

Although many studies have investigated the ANA and anti-dsDNA antibody profile in RA, as well as in other chronic inflammatory diseases, after anti-TNF- α treatment [15,16], only few data are available regarding the behaviour of these antibodies after the first 6 months of treatment in RA. Furthermore, no data are currently available in RA patients regarding the long-term effect of anti-TNF treatment on other autoantibodies, including rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) antibodies, levels of which are related to the severity of the rheumatoid process [17-20] and could be reduced by an effective antirheumatic therapy [21].

The present study was conducted to evaluate a large panel of autoantibodies, including RF and anti-CCP antibodies, in a cohort of RA patients prospectively followed during 78 weeks of treatment with infliximab.

Materials and methods

Patients

Thirty-nine consecutive patients fulfilling the American College of Rheumatology (ACR) classification criteria for RA [22] started treatment with infliximab plus methotrexate between June 2000 and June 2001 at the Department of Rheumatology of the Pavia University Hospital and were prospectively followed up.

Thirty patients completed 78 weeks of therapy, and their autoantibody profiles were evaluated after informed consent, according to the local ethical committee recommendations, had been obtained. Four patients dropped out because of side effects; in three patients infliximab was stopped between 14 and 30 weeks because of lack of clinical response; one patient was lost to follow up because of change of residence; and one was lost to follow-up after 14 weeks because of unsatisfactory response and fear of potential side effects (information obtained by telephone contact). The demographical and clinical characteristics of the 30 patients studied are shown in Table 1.

Before infliximab treatment was begun, all patients had a Disease Activity Score (DAS 28) [23] greater than 4.9 despite combination therapy with at least two conventional disease-modifying anti-rheumatic drugs (DMARDs), including methotrexate. No patient had an infectious disease, active or latent tuberculosis, neoplastic disease, heart failure, cytopenia, or a demyelinating disorder.

Infliximab (3 mg/kg) was administered intravenously at 0, 2 and 6 weeks, and then every 8 weeks along with methotrex-

Table 1

Main demographic and clinical characteristics of the present series (30 patients)

Parameter	Value
Age (years)	57.13 \pm 9.21
Female/male	24/6
Duration of disease (years)	9.43 \pm 8.97
Number of tender joints	16.46 \pm 9.1
Number of swollen joints	6.83 \pm 4.814
RF positivity	86.70%
Joint erosions	100%
Serum CRP (mg/dl)	3.85 \pm 2.74
DAS 28	6.1 \pm 1.025
HAQ	1.78 \pm 0.422
Previous DMARDs	
Methotrexate	30
Hydroxychloroquine	26
Sulfasalazine	18
Cyclosporin A	19
Gold (intramuscular)	3
Leflunomide	1
Azathioprine	1

Where applicable, values are expressed as \pm standard deviation. CRP, C-reactive protein; DAS 28, Disease Activity Score; DMARD, disease-modifying antirheumatic drug; HAQ, Health Assessment Questionnaire; RF, rheumatoid factor.

ate (15–20 mg/week), according to the ATTRACT protocol [3]. Nonsteroidal anti-inflammatory drugs and oral prednisone (= 7.5 mg/day) were also permitted. A careful clinical evaluation was conducted in all patients just before each infliximab infusion. Response to therapy was evaluated according to the ACR response criteria [24], as well as by changes in DAS 28 [23] and serum C-reactive protein levels.

Serum samples for detection of autoantibodies were collected, and stored at -70°C, just before the first infliximab infusion and at 30, 54 and 78 weeks of therapy. Serological investigations were carried out at the end of the study in all serum samples taken at the different time points. Thirty age-matched and sex-matched healthy blood donors were investigated as a control group.

Antinuclear antibodies

ANAs were tested by a standard indirect immunofluorescence (IIF) technique as previously described [25], using a BX 51 Olympus fluorescence microscope (Olympus Optical Co., Hamburg, Germany) at 40 \times power. Serum was

first diluted 1:80 in phosphate-buffered saline (PBS) and overlaid onto fixed Hep2 cell slides (Immuno Concept, Sacramento, CA, USA) in a moist chamber for 30 min at room temperature. Slides were then rinsed and washed twice in PBS for 10 min. A fluorescein-labelled antibody specifically directed toward human IgG (γ chains; Delta Biologicals, Pomezia, Italy) was used as fluorescence conjugate. The positive samples (titer = 1:80) were then evaluated at increasing dilutions in PBS up to 1:640.

Anti-double-stranded DNA antibodies

Anti-dsDNA antibodies were determined both by IIF and quantitative ELISA. IIF was performed at 1:10 serum dilution in PBS using *Crithidia luciliae* as substrate (INOVA, San Diego, CA, USA) and antihuman IgG (γ chain specific) as fluorescence conjugate (Delta Biologicals). ELISA was performed using a commercially available kit (Axis-Shield, Dundee, UK) according to the manufacturer's recommendations. Alkaline phosphatase-labelled murine monoclonal antibodies to both human IgG and IgM (heavy and light chains) were used. The absorbance was read at 550 nm. Serum samples were evaluated in triplicate and the median value was considered. The upper normal limit, according to the recommendations of the manufacturer, was 30 UI/ml.

Anticardiolipin antibodies

Commercially available ELISA kits (Orgentec Diagnostika, Mainz, Germany) were used to detect IgG anticardiolipin (aCL) and IgM aCL, by means of a peroxidase conjugate solution of either polyclonal rabbit antihuman IgG (heavy and light chains) or polyclonal rabbit antihuman IgM (heavy and light chains) according to the manufacturer's instructions. The absorbance was read at 450 nm. Serum samples were evaluated in triplicate; the upper normal limits were 10 U/ml for IgG aCL and 7 U/ml for IgM aCL.

Rheumatoid factor and anti-cyclic citrullinated peptide antibodies

IgM RF was measured by immunonephelometry using the quantitative N Latex RF system (Dade Behring, Marburg, Germany). RF concentrations higher than 15 IU/ml were considered positive.

Anti-CCP antibodies were tested using a new, second generation, commercially available ELISA kit (Axis-Shield). Briefly, 100 μ l anti-CCP standards (0, 2, 8, 30 and 100 U/ml), controls and patient samples (1:100 in PBS) were distributed into the appropriate wells. The microtitre plates were coated with a highly purified synthetic cyclic peptides containing modified arginine residues. After incubation for 60 min, the wells were washed three times with 200 μ l wash buffer (borate buffer, 0.8% [weight:volume] sodium azide). The microplates were then incubated for 30 min at room temperature with alkaline phosphate-labelled murine monoclonal antibody to human IgG and washed again

three times. A chromogenic substrate solution (Mg^{2+} phenolphthalein monophosphate buffered solution) was added to each well. After 30 min the reaction was stopped using sodium hydroxide-EDTA-carbonate buffer. The absorbance was read at 550 nm. Serum samples were evaluated in triplicate, and the upper normal limit (5 UI/ml) was assumed according to the manufacturer's recommendations. In order to follow the changes in antibody levels during therapy, all serum samples exhibiting a high concentration (= 100 U/ml) were evaluated after a further 10 \times dilution and then corrected for this additional dilution factor.

Other autoantibodies

Anti-extractable nuclear antigen (ENA) antibodies were evaluated in triplicate using commercially available ELISA kits (Axis-Shield) according to the manufacturer's recommendations. The following single ENA specificities were investigated: Sm, RNP, SSA(Ro), SSB(La), Scl-70 and Jo1.

Antineutrophil cytoplasmic antibodies (ANCA) directed toward serine protease 3 (PR3) and myeloperoxidase (MPO) were also assessed using commercial ELISA kits (Axis-Shield). Serum samples were evaluated in triplicate, and the upper normal limit was assumed according to the recommendations of the manufacturer.

Anti-endomysial antibodies (anti-EMAs) were detected by IIF at 1:10 serum dilution in PBS using monkey oesophagus as a substrate (INOVA) and antihuman IgA fluorescence conjugate (INOVA).

Statistical analysis

Fisher's exact test was run to evaluate the differences between the number of patients with a positive result before and after therapy. Wilcoxon's test was used to analyze variation in continuous variables. Statistical analysis was conducted using INSTAT2 software (Graphpad Inc. San Diego, CA, USA.)

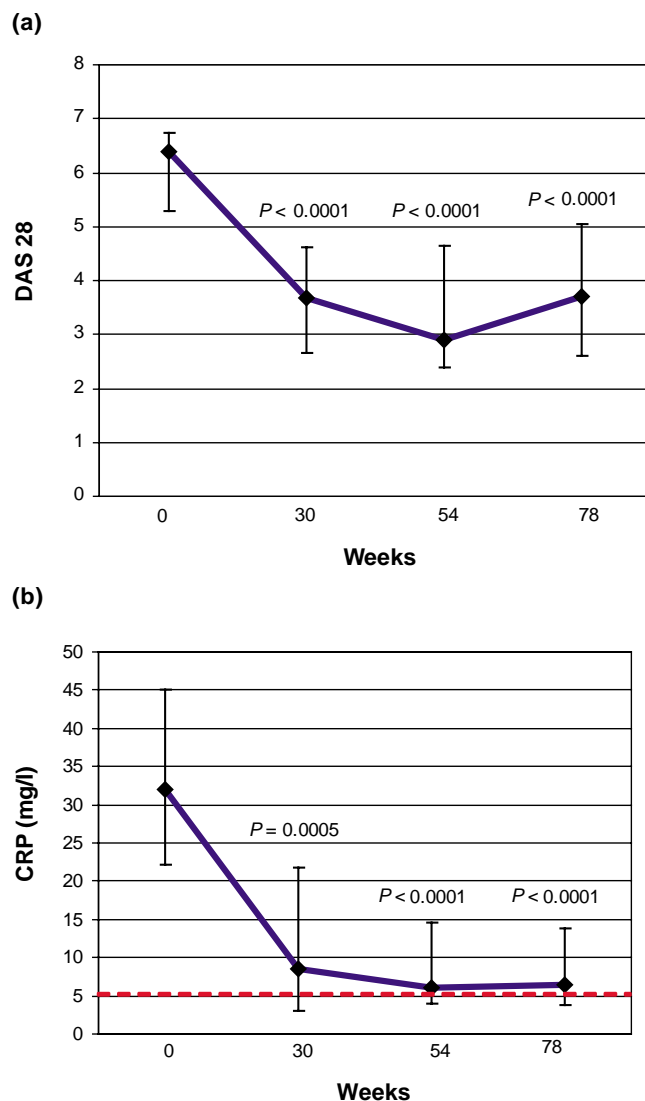
Results

Response to therapy

ACR20 response was attained by 87% of patients at 30 weeks, by 80% at 54 weeks, and by 63% at 78 weeks. ACR50 percentages were 60% at 30 and 54 weeks, and 47% at 78 weeks. ACR 70 percentages were 33%, 43% and 30%, respectively.

During the course of the study the median DAS 28 value significantly decreased from 6.38 (interquartile range 5.30–6.75) to 3.71 (interquartile range 2.62–5.06), and the serum C-reactive protein levels from 3.2 mg/l (interquartile range 2.23–4.5 mg/l) to 0.65 mg/l (interquartile range 0.38–1.38 mg/l; Fig. 1).

Figure 1



Changes in disease activity in 30 patients with rheumatoid arthritis at different times during 78 weeks of infliximab therapy as determined by (a) Disease Activity Score (DAS 28) and (b) serum C-reactive protein (CRP) levels. The diamonds indicate median values, vertical bars indicate the interquartile range, and dotted lines indicate the upper normal limit.

Frequency of autoantibodies

ANAs were found in 15 patients (50%) at baseline and in 24 patients (80%) at 78 weeks ($P < 0.0292$). Fourteen patients were ANA positive at baseline as well as at all follow-up points, whereas 1 patient with a 1:80 ANA titre at baseline became ANA negative from 30 weeks. Seven patients became ANA positive at 30 weeks, and two additional patients did so at 54 weeks. All these patients were still positive at 78 weeks. Only one patient became positive

at 78 weeks. The fluorescence ANA pattern was homogeneous in 75% of cases after infliximab treatment.

The frequency of the other autoantibodies did not change significantly from baseline to 78 weeks (Table 2). The numbers of patients who were positive for anti-dsDNA antibodies at baseline, 30, 54 and 78 weeks were 2, 3, 5 and 0 by ELISA and 1, 1, 2 and 1 by IIF, respectively. The two anti-dsDNA antibody positive patients at baseline by ELISA remained positive at 30 weeks of study, but only one of them was still positive at 54 weeks and none were at 78 weeks. The only anti-dsDNA antibody positive patient by IIF at baseline was also positive by ELISA and remained positive during the follow-up period. Regarding anti-ENA antibodies, three patients were positive for anti-SSA(Ro) before infliximab and one additional patient became positive during treatment.

At the end of the study, one patient was found to have low levels of IgG aCL, two were found to have low levels of IgM aCL, and one patient was positive for both. No patient was positive for anti-MPO or anti-PR3 antibodies, or EMAs either before treatment or during follow up.

Twenty-six patients (87%) were positive for RF at the beginning of the study and 24 (80%) at 78 weeks, because two patients became RF negative during the course of therapy. Twenty-five patients were positive for anti-CCP antibodies (83.3%), and this figure did not change at 78 weeks. Two patients with borderline values at baseline had a negative test at 30 and 54 weeks, and a low-level positive test at 78 weeks.

Only one out of 30 healthy control individuals had positive ANA test (1:160) and one additional individual had borderline (15 IU/ml) RF levels.

Autoantibody titre

ANA titre significantly increased during infliximab treatment. Also, a progressively increased concentration was found for aCL (either IgG or IgM), whereas anti-dsDNA antibody concentration, measured by ELISA, exhibited a transient rise at 30 and 54 weeks and returned to baseline at 78 weeks (Fig. 2).

The RF titres exhibited a progressive and significant reduction from baseline to 30, 54 and 78 weeks. However, anti-CCP antibody titres exhibited a significant reduction only at 30 weeks (Fig. 3). After 78 weeks reduction by 50% or more in RF titre with respect to baseline was found in 15 patients, whereas such a reduction in anti-CCP antibody titre was noted only in two patients. No difference was found among responders and non-responders (according to ACR 20 criteria) in RF and anti-CCP antibody titres either at baseline or at 78 weeks.

Table 2**Frequency of the autoantibodies assessed before therapy and at different times after initiation of infliximab treatment**

Antibody	Positivity (n [%])				P value ^a
	Before therapy	From start of infliximab treatment			
		30 weeks	54 weeks	78 weeks	
ANA	15/30 (50)	21/30 (70)	23/30 (76.7)	24/30 (80)	0.0292
Anti-dsDNA (ELISA)	2/30 (6.7)	3/30 (10)	5/30 (16.7)	0/30 (0)	0.4915
Anti-dsDNA (IIF)	1/30 (3.3)	1/30 (3.3)	2/3 (6.7)	1/30 (3.3)	-
Anti-ENA ^b	3/30 (10)	4/30 (13.3)	4/30 (13.3)	4/30 (13.3)	0.999
aCL IgG	0/30 (0)	1/30 (3.3)	2/30 (6.7)	2/30 (6.7)	0.4915
aCL IgM	0/30 (0)	1/30 (3.3)	2/30 (6.7)	3/30 (10)	0.2373
ANCA	0/30 (0)	0/30 (0)	0/30 (0)	0/30 (0)	-
RF	26/30 (86.7)	27/30 (90)	25/30 (83.3)	24/30 (80)	0.7306
Anti-CCP	25/30 (83.3)	22/30 (73.3)	22/30 (73.3)	25/30 (83.3)	-
EMA (IIF)	0/30 (0)	0/30 (0)	0/30 (0)	0/30 (0)	-

^aComparison between 0 and 78 weeks. ^bOnly anti-SSA(Ro) reactivity was found. ACL, anticardiolipin; ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibody; CCP, cyclic citrullinated peptide; DAS 28, Disease Activity Score; ds, double-stranded; ELISA, enzyme-linked immunosorbent assay; EMA, antiendomysial antibody; ENA, extractable nuclear antigen; IIF, indirect immunofluorescence; RF, rheumatoid factor.

Autoantibody associated clinical manifestations

A 34-year-old woman developed malar rash and a mild arthritis flare along with the appearance of anti-dsDNA antibodies (detected by both IIF and ELISA) after 30 weeks of therapy. No other signs of systemic lupus erythematosus (SLE) were present, complement levels were normal, and hemoglobin, blood cell counts and urinalysis were normal. Infliximab therapy was not stopped; both malar rash and anti-dsDNA antibodies disappeared after a few weeks and did not recur thereafter. No other clinical signs of SLE or antiphospholipid syndrome were found in the patients studied.

Discussion

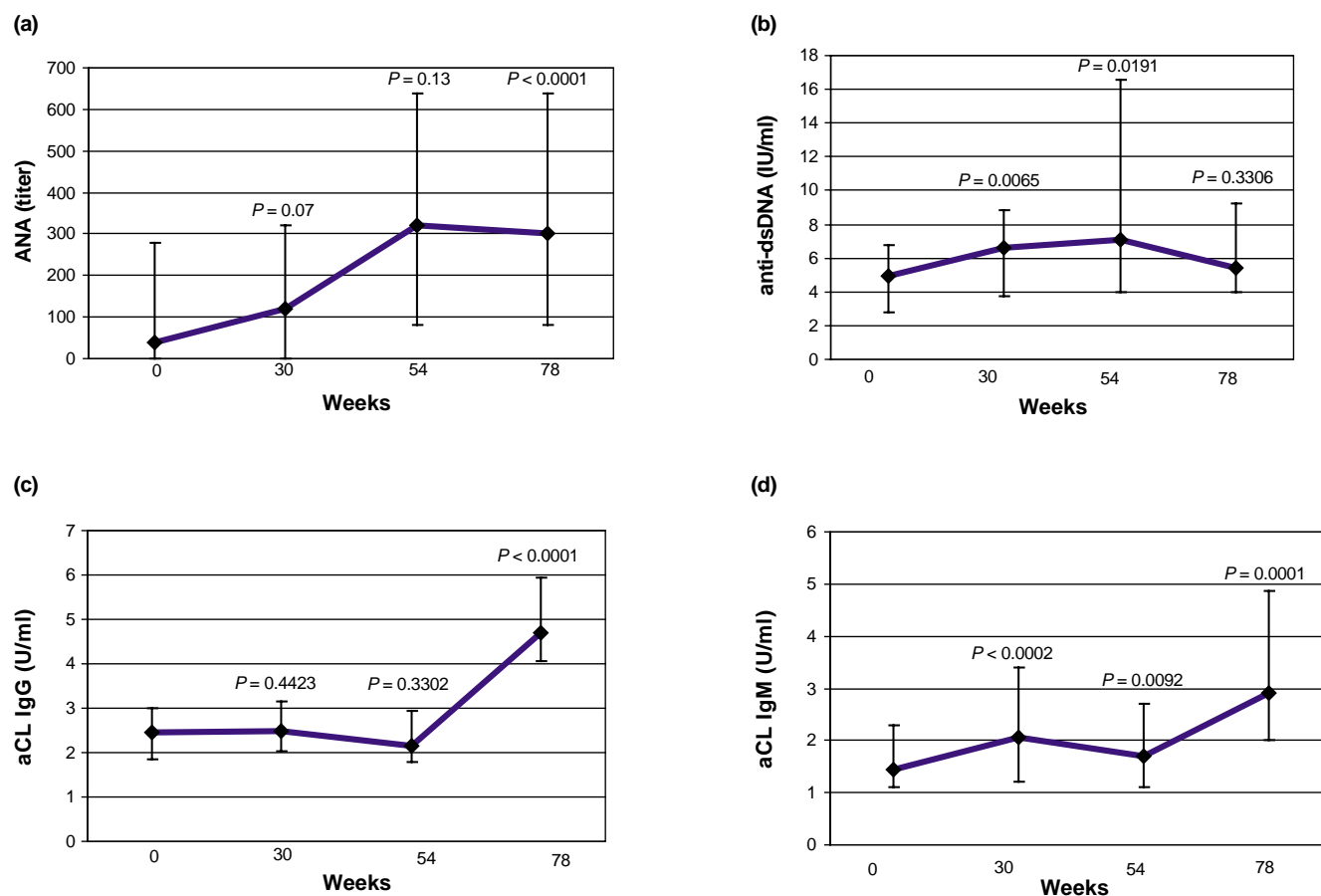
Induction of ANAs and anti-dsDNA antibodies during treatment with anti-TNF- α agents was highlighted in clinical trials and in postmarketing surveillance [3,4]. In a recent study, detection of ANAs among RA patients was reported to increase from 51.6% to 82.3% after treatment with infliximab plus methotrexate, and even higher figures were reported in patients with ankylosing spondylitis who were treated with slightly higher infliximab doses without methotrexate comedication [7]. In the same study anti-dsDNA antibodies, demonstrated by both IIF and ELISA, developed in 11.3% of RA patients after 30 weeks. Interestingly, all of the anti-dsDNA antibodies after infliximab were of IgA and/or IgM isotype, whereas it is known that lupus-associated anti-dsDNA antibodies are classically of the IgG isotype [26,27]. This may explain why the actual incidence of SLE or related disorders after infliximab treatment is very

low despite a significant rise in anti-dsDNA antibodies [3]. Nonetheless, several cases of anti-dsDNA antibodies associated with clinical manifestations of SLE have been reported in RA patients treated with infliximab or etanercept [28-35], and in all but one case [33] these clinical features disappeared completely after stopping treatment.

The results of the present study confirm induction of ANAs and anti-dsDNA antibodies after 30 and 54 weeks of treatment. Our findings are very similar to those reported by De Rycke and coworkers [7] after 30 weeks of therapy using comparable assays. ANAs were present in 50% of patients before therapy and in 76.7% after 54 weeks; anti-dsDNA antibodies were detected by ELISA in 6.7% before therapy and in 16.7% after 54 weeks. Regarding anti-dsDNA antibodies detected using IIF, we used a fluorescence-labelled conjugate specifically directed toward human γ chains in order to detect only the IgG anti-dsDNA that are strictly associated with SLE [26,27]. As expected, the frequency of these antibodies was lower, and only one patient converted from negative to positive during infliximab treatment.

At long-term analysis, up to 78 weeks, there was a progressive increase in the percentage of ANA-positive patients without any clinically relevant manifestations. Unexpectedly, however, the percentage of anti-dsDNA antibody positive patients returned to baseline, suggesting that the development of the latter autoantibodies may represent a transitory phenomenon that occurs only during the early phases of treatment. Further studies on larger series are

Figure 2



Changes in the serum concentrations of (a) antinuclear antibodies (ANAs), (b) anti-double-stranded (ds)DNA antibodies, (c) anticardiolipin (aCL) IgG and (d) aCL IgM in 30 rheumatoid arthritis patients at different times during 78 weeks of infliximab treatment. In the graphs shown in panels b-d the reported values always remained below the upper normal limits, which were 30 IU/ml for anti-dsDNA antibodies, 10 U/ml for IgG aCL and 7 U/ml for IgM aCL. The diamonds indicate median values, and vertical bars indicate the interquartile range.

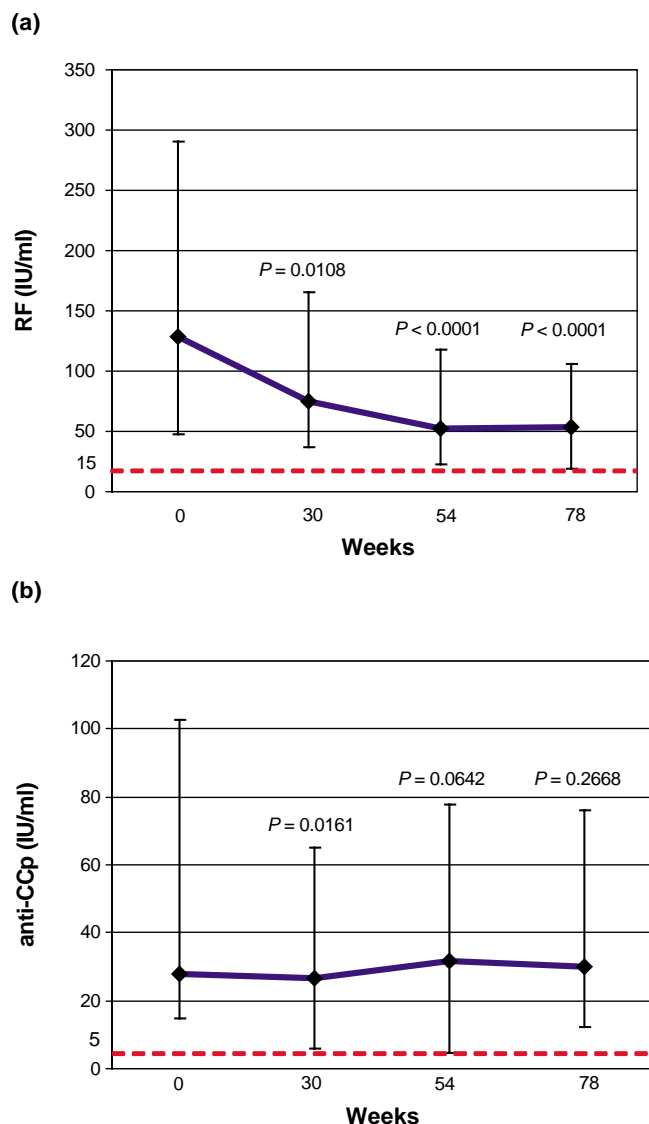
needed to confirm this. However, it is interesting that almost all of the anti-TNF therapy-induced lupus syndromes were reported within the first year of treatment [2,6,28-35]. Furthermore, the one patient in the present study who developed lupus-like clinical features along with anti-dsDNA antibodies exhibited a spontaneous regression of both clinical symptoms and anti-dsDNA antibodies, even though infliximab treatment were not stopped.

Transient spikes in aCL antibody levels were occasionally reported following bacterial infections in RA patients treated with infliximab [36]; however, the aCL antibody profile has not previously been systematically studied in relation to anti-TNF therapy [37]. We observed a significant increase in aCL antibody titres, starting from 30 weeks for IgM antibodies and at 78 weeks for IgG antibodies. However, in most cases the levels did not exceed normal limits, even after 78 weeks, and none of the patients exhibited any clinical feature related to the antiphospholipid syndrome.

Longer follow up will clarify whether aCL antibody titres may further increase and whether antiphospholipid related disorders, such as thrombosis, may develop during long-term treatment.

The development of new anti-ENA reactivity, namely anti-SSA(Ro) and SSB(La), has occasionally been reported in previous studies [7]. About 10% of the patients we studied were positive for anti-SSA(Ro) at baseline (a percentage that is to be expected in RA patients from our country [38]), and one additional patient developed anti-SSA(Ro) during treatment without associated clinical features. Anti-ENA reactivity other than anti-SSA(Ro) was not detected.

We also analyzed other autoantibodies that have not been thoroughly investigated in RA in relation to anti-TNF- α treatment until now. The most relevant findings pertain to RF and anti-CCP antibodies, because no reactivity at all was found for MPO-ANCA and PR3-ANCA or for EMAs.

Figure 3

Changes in the serum titre of **(a)** rheumatoid factor (RF) and **(b)** anti-cyclic citrullinated peptide (CCP) antibodies in 30 patients with rheumatoid arthritis at different times during 78 weeks of Infliximab treatment. The diamonds indicate median values, vertical bars indicate the interquartile range, and dotted lines indicate the upper normal limit.

At baseline RF and anti-CCP antibodies were present in most cases, as expected in patients with severe, erosive RA refractory to conventional DMARD therapy [17-19]. Although we did not identify any change in the number of positive patients, in the present study we found a significant decrease in the titres of both anti-CCP antibodies and RF after 30 weeks of infliximab therapy. These findings suggest that serial evaluations of these antibodies could be useful in monitoring the clinical course of RA patients undergoing treatment with infliximab. Decreased production of RF has been reported in association with successful

treatment with conventional DMARDs such as methotrexate and gold salts [21]. However, we observed a different evolution of RF titres with respect to anti-CCP antibody titres during long-term therapy. At 54 and 78 weeks, RF titres exhibited a progressive decrease whereas no decrease was observed for anti-CCP antibody titres, despite the persistence of clinical improvement as indicated by DAS 28.

The decrease in RF titre roughly paralleled DAS 28 values, suggesting that this measure could be regarded as an additional marker of disease activity, although we did not find significant correlations between changes in RF and ACR response. This lack of correlation might be due to a type 2 error related to the small sample size and, at least in part, to the fact that four patients who stopped therapy before 34 weeks because of inefficacy had to be excluded from the analysis. A further prospective study in a larger series of RA patients comparing responders and nonresponders is now in progress.

The mechanisms by which infliximab could lead to a decrease in titres of autoantibodies such as RF and anti-CCP are not understood and any explanation remains speculative. Infliximab therapy has proven to reduce the amount of synovium infiltrating cells, including plasma cells [39]. Because RF-producing cells are present in inflamed rheumatoid synovium and the local environment may favour synovial RF production [40], we can speculate that the reduction in inflammatory lymphoplasmacytic infiltrate in rheumatoid synovium will lead to a reduced production of RF. Our data also suggest that generation of RF and anti-CCP antibodies may be controlled in a different manner in RA, because inhibition of RF appears to be more dependent on TNF- α blockade and more persistent than inhibition of anti-CCP antibodies.

Conclusion

In conclusion, several autoantibodies can be induced by infliximab therapy; however, levels of these autoantibodies may evolve differently during long-term follow-up in RA. The development of ANAs was persistent up to 78 weeks of therapy, as was the rise in aCL antibody titre, without any related clinical manifestations. On the contrary, the presence of anti-dsDNA antibodies appeared to be an early but transient phenomenon, lasting about 1 year. This might explain why almost all of the infliximab-induced lupus syndromes were reported within the first year of treatment in RA.

No differences in RF and anti-CCP antibody positivity were observed even though a significant reduction in RF and anti-CCP antibody titres was found during the course of treatment. The reduction in RF titre was more pronounced and persistent than that of anti-CCP antibodies, suggest-

ing that the production of these antibodies may follow different regulatory pathways.

Competing interest

None declared.

References

- Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden Jr, Smolen JS, Weisman M, Emery P, Feldmann M, Harriman GR, Maini RN, Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group: **Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy Study Group.** *N Engl J Med* 2000, **343**:1594-1602.
- Genovese MC, Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, Wasko MC, Moreland LW, Weaver AL, Markenson J, Cannon GW, Spencer-Green G, Finck BK: **Etanercept versus methotrexate in patients with early rheumatoid arthritis: two-year radiographic and clinical outcomes.** *Arthritis Rheum* 2002, **46**:1443-1450.
- Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P: **Infliximab (chimeric anti-tumor necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group.** *Lancet* 1999, **354**:1932-1939.
- Moreland LW, Schiff MH, Baumgartner SW, Tindall EA, Fleischmann RM, Bulpitt KJ, Weaver AL, Keystone EC, Furst DE, Mease PJ, Ruderman EM, Horwitz DA, Arkefeld DG, Garrison L, Burge DJ, Blosch CM, Lange ML, Mc Donnell ND, Weinblatt ME: **Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial.** *Ann Intern Med* 1999, **130**:478-486.
- Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, Antoni C, Leeb B, Elliott MJ, Woody JN, Schaible TF, Feldmann M: **Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor α monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis.** *Arthritis Rheum* 1998, **41**:1552-1563.
- Charles PJ, Smeenk RJ, De Jong J, Feldmann M, Maini RN: **Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor alpha: findings in open-label and randomized placebo-controlled trials.** *Arthritis Rheum* 2000, **43**:2383-2390.
- De Rycke L, Kruithof E, Van Damme N, Hoffman IE, Van den Bossche N, Van den Bosch F, Veys EM, De Keyser F: **Antinuclear antibodies following infliximab treatment in patients with rheumatoid arthritis or spondylarthropathy.** *Arthritis Rheum* 2003, **48**:1015-1023.
- Antivalle M, Marazza MG, Randisi G, Sarzi-Puttini P, Dell'Acqua D, Carrabba M: **Long-term treatment of rheumatoid arthritis with infliximab: efficacy, side effects and autoantibodies induction [abstract].** *Arthritis Rheum* 2002, **46**:s534.
- Gershov D, Kim S, Brot N, Elkon KB: **C-Reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity.** *J Exp Med* 2000, **192**:1353-1364.
- Pisetsky DS: **Tumor necrosis factor alpha blockers and the induction of anti-DNA autoantibodies.** *Arthritis Rheum* 2000, **43**:2381-2382.
- Lorenz HM, Herrmann M, Winkler T, Gaipi U, Kalden JR: **Role of apoptosis in autoimmunity.** *Apoptosis* 2000, **5**:443-449.
- Schultz DR, Harrington WJ jr: **Apoptosis: programmed cell death at a molecular level.** *Semin Arthritis Rheum* 2003, **32**:345-369.
- Van den Brande JM, Braat H, van den Brink GR, Versteeg HH, Bauer CA, Hoedemaeker I, van Montfrans C, Hommes DW, Peppelenbosch MP, van Deventer SJ: **Infliximab but not Etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease.** *Gastroenterology* 2003, **124**:1774-1785.
- Di Sabatino A, Ciccocioppo R, Cinque B, Millimaggi D, Morera R, Ricevuti L, Cifone MG, Corazza GR: **Defective mucosal T cell death is sustainably reverted by infliximab in a capsase dependent pathway in Crohn's disease.** *Gut* 2004, **53**:70-77.
- Vermiere S, Noman M, Van Assche G, Baert F, Van Steen K, Esters N, Joossens S, Bossuyt X, Rutgeerts P: **Autoimmunity associated with anti-tumor necrosis factor alpha treatment in Crohn's disease: a prospective cohort study.** *Gastroenterology* 2003, **125**:32-39.
- Garcia-Planella E, Domenech E, Esteve-Comas M, Bernal I, Cabre E, Boix J, Gassull MA: **Development of antinuclear antibodies and its clinical impact in patients with Crohn's disease treated with chimeric monoclonal anti-TNF α antibodies (infliximab).** *Eur J Gastroenterol Hepatol* 2003, **15**:351-354.
- Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van't Hof M, van de Putte LB, van Rijswijk MH, van Venrooij WJ, van Riel PL: **The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis.** *Arthritis Rheum* 2000, **43**:1831-1835.
- Meyer O, Labarre C, Dougados M, Goupille P, Cantagrel A, Dubois A, Nicaise-Roland P, Sibilia J, Combe B: **Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five years radiographic damage.** *Ann Rheum Dis* 2003, **62**:120-126.
- Bas S, Genevay S, Meyer O, Gabay C: **Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis.** *Rheumatology* 2003, **42**:677-680.
- Visser H, le Cessie S, Vos K, Breedveld FC, Hazes JM: **How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis.** *Arthritis Rheum* 2002, **46**:357-365.
- Olsen NJ, Callahan LF, Pincus T: **In vitro rheumatoid factor synthesis in patients taking second-line drugs for rheumatoid arthritis. Independent associations with disease activity.** *Arthritis Rheum* 1988, **31**:1090-1096.
- Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS: **The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis.** *Arthritis Rheum* 1988, **31**:315-324.
- Prevo ML, van't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL: **Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis.** *Arthritis Rheum* 1995, **38**:44-48.
- Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, Katz LM, Lightfoot R Jr, Paulus H, Strand V: **American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis.** *Arthritis Rheum* 1995, **38**:727-735.
- Brito J, Biamonti G, Caporali R, Montecucco C: **Autoantibodies to human nuclear lamin B2 protein. Epitope specificity in different autoimmune diseases.** *J Immunol* 1994, **153**:2268-2277.
- von Muhlen CA, Tan EM: **Autoantibodies in the diagnosis of systemic rheumatic diseases.** *Semin Arthritis Rheum* 1995, **24**:323-258.
- Bootsma H, Spronk PE, Hummel EJ, de Boer G, ter Borg EJ, Limburg PC, Kallenberg CG: **Anti-double stranded DNA antibodies in systemic lupus erythematosus: detection and clinical relevance of IgM-class antibodies.** *Scand J Rheumatol* 1996, **25**:352-359.
- Cairns AP, Duncan MK, Hinder AE, Taggart AJ: **New onset systemic lupus erythematosus in a patient receiving etanercept for rheumatoid arthritis.** *Ann Rheum Dis* 2002, **61**:1031-1032.
- Shakoor N, Michalska M, Harris CA, Block JA: **Drug-induced systemic lupus erythematosus associated with etanercept therapy.** *Lancet* 2002, **359**:579-580.
- Debandt M, Vittecoq O, Descamps V, Le Loet X, Meyer O: **Anti-TNF-alpha-induced systemic lupus syndrome.** *Clin Rheumatol* 2003, **22**:56-61.
- Favalli EG, Sinigaglia L, Varena M, Arnoldi C: **Drug-induced lupus following treatment with infliximab in rheumatoid arthritis.** *Lupus* 2002, **11**:753-755.
- Ali Y, Shah S: **Infliximab-induced systemic lupus erythematosus.** *Ann Intern Med* 2002, **137**:625-626.
- Carlson E, Rothfield N: **Etanercept-induced lupus-like syndrome in a patient with rheumatoid arthritis.** *Arthritis Rheum* 2003, **48**:1165-1166.

34. Lepore L, Marchetti F, Facchini S, Leone V, Ventura A: **Drug-induced systemic lupus erythematosus associated with etanercept therapy in a child with juvenile idiopathic arthritis.** *Clin Exp Rheumatol* 2003, **21**:276-277.
35. Mohan AK, Edwards ET, Cote TR, Siegel JN, Braun MM: **Drug-induced systemic lupus erythematosus and TNF-alpha blockers.** *Lancet* 2002, **360**:646.
36. Ferraccioli G, Mecchia F, Di Poi E, Fabris M: **Anticardiolipin antibodies in rheumatoid patients treated with etanercept or conventional combination therapy: direct and indirect evidence for a possible association with infections.** *Ann Rheum Dis* 2002, **61**:358-361.
37. Jonsdottir T, Harju A, van Vollenhoven A, Klareskog L, van Vollenhoven R: **Treatment with TNF-antagonist is associated with an increasing frequency of anticardiolipin antibodies (ACLA)-ACLA positivity predicts worse clinical outcomes [abstract].** *Arthritis Rheum* 2002, **46**:s573.
38. Belfiore N, Rossi S, Bobbio Pallavicini F, Epis O, Caporali R, Montecucco C: **Anti-Ro(SS-A) 52 kDa and 60 kDa specificities in undifferentiated connective tissue disease.** *Joint Bone Spine* 2000, **67**:183-187.
39. Smeets TJ, Kraan MC, van Loon ME, Tak PP: **Tumor necrosis factor alpha blockade reduces the synovial cell infiltrate early after initiation of treatment, but apparently not by induction of apoptosis in synovial tissue.** *Arthritis Rheum* 2003, **48**:2155-2162.
40. Reparon-Schuijt CC, van Esch WJ, van Kooten C, Levarht EW, Breedveld FC, Verweij CL: **Functional analysis of rheumatoid factor-producing B cells from the synovial fluid of rheumatoid arthritis patients.** *Arthritis Rheum* 1998, **41**:2211-2220.