Anti-nuclear antibodies, anti-DNA and C4 complement evolution in rheumatoid arthritis and ankylosing spondylitis treated with TNF-α blockers

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Abstract

Objective

To investigate autoantibody induction in rheumatoid arthritis (RA) and ankylosing spondylitis (AS) in a cohort of French patients treated with TNF-α blockers.

Methods

We tested the serum of patients for antinuclear antibodies (ANA), anti-DNA antibodies and C4 complement at baseline, and for each infusion for infliximab, and at month 3, 6 and 12 for etanercept. We looked for all signs suggesting a drug-induced lupus. We tried to correlate ANA and anti-DNA development with various clinical data, especially the response to treatment.

Results

229 patients were included in the study. 159 were treated with infliximab (98 RA and 61 AS) and 125 with etanercept (116 RA and 9 AS). In the infliximab group, 43.6% of RA patients and 27.1% of AS had significant levels of ANA at baseline. This proportion increased during the follow up to 73% in RA patients and 52% in AS patients. The proportion of patients positive for anti-DNA antibodies increased from 0% to 9.5% in RA group, and from 0% to 2% in AS group. In the etanercept group, 58.5% of these patients had significant levels of ANA at baseline; this proportion raised to 63.3% in patients previously treated with infliximab, and fell to 20.6% in the patients who never received TNF-α blockers. No significant variation of ANA, anti-DNA and C4 levels was observed in the etanercept group. Only three patients developed clinical manifestations (chilblain lupus) possibly related to these auto-antibodies, two with infliximab and one with etanercept.

Conclusion

The ANA induction was only observed under infliximab therapy. Thus, ANA induction seems not to be a therapeutic class effect. This difference between infliximab and etanercept treatment may be the consequence of differential capacity of a monoclonal antibody and a soluble receptor in inducing apoptotic cell death of the cells expressing TNF on their membrane.

Key words

Auto-antibodies, drug-induced lupus, rheumatoid arthritis, TNF-α blockers, spondylarthropathy.

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Introduction

The chronic inflammatory joint diseases rheumatoid arthritis (RA) and ankylosing spondylitis (AS) are the most two frequent types of autoimmune arthritis with a prevalence rate of 1-2% (1). The classical treatment schemes for these two pathologies consist of a combination of disease-modifying antirheumatic drugs (DMARDs), nonsteroidal anti-inflammatory dugs (NSAIDs), in combination with methotrexate (MTX) or sulfasalazine. But in numerous cases, these therapies are not sufficient to control inflammation and structural damage, and consequently to reduce disease activity and delay radiographic progression (2-4).

Based on these insights, biological agents have been introduced for the treatment of chronic inflammatory disease such as RA, spondylarthropathy, Crohn's disease and recently psoriatic arthritis, with very good results (5-10). Headache, upper respiratory tract infections and injection-related reactions are the most commonly observed adverse events with infliximab (a chimeric anti-TNF-α monoclonal Ig1 antibody) and etanercept (soluble TNF-α receptor). However, serious adverse events, such as opportunistic infections, have been reported.

Clinical trials have shown that selective tumor necrosis factor (TNF)-α inhibitors induce autoantibodies, such as antinuclear antibodies (ANA), and anti-DNA antibodies (5, 11, 12), but rare cases of lupus-like syndrome have been described (12). Biological markers of systemic lupus are antinuclear antibodies, anti-DNA antibodies, hypocomplementemia (C4) and hematologic abnormalities (haemolytic anemia, thrombopenia, leucopenia …). Despite some limited clinical similarities, the drug-induced lupus syndromes are now recognised as being distinct from the classic genuine Systemic Lupus Erythematosus (SLE). For many years, numerous therapies such as anti-rheumatic drugs have been implicated in the induction of lupus-like disease (13, 14). Characteristically, the clinical symptoms resolve upon withdrawal of treatment and the serological abnormalities return to normality. Serological abnormalities such as ANA can be found in a variety of other systemic autoimmune disorders like rheumatoid arthritis. This phenomenon has been shown with infliximab, as well as etanercept, but with differences (15-18) and it seems that it was more important with infliximab than etanercept. The mechanism responsible for the production of autoantibodies during anti-TNF-α therapy has not been clearly defined and several hypotheses have been suggested: clearance of apoptotic bodies by reducing levels of C-reactive protein, apoptosis, or inhibition of TNF-α could favour T-helper 2 response leading to an increased antibody production. Thus, the study of antibodies induction during anti-TNF-α therapy remains an important topic in patient follow-up.

The aim of this study was to describe the effect of infliximab and etanercept on ANA, anti-DNA antibodies and C4 complement. We correlated these results with the quality of clinical response, drug used and the disease of interest (RA versus AS).

Patients and methods

Study cohort and treatment protocol

It was a prospective study which included patients who attended the Department of Rheumatology of Pellegrin University Hospital (Bordeaux, France), between April 2000 and December 2004. These patients received infliximab or etanercept, and were treated for RA or AS.

All RA patients included were aged ≥18 years and met the ACR criteria for RA diagnosis (19), and all AS patients fulfilled the modified New York criteria diagnosis for axial forms (20), and ESSG criteria for peripheric forms (21). At each follow-up visit, the disease activity scores were measured (Disease Activity Score for 28 joints for RA and Bath Ankylosing Spondylitis Disease Activity Index for AS). The French version of the Health Assessment Questionnaire (F-HAQ) was also taken into consideration.

Infliximab was administered at week 0, then at week 2 and 6 regardless of the type of arthritis treated; the patients with RA received an infusion of 3 mg/kg every each 8 weeks following while

the patients with AS received an infusion of 3 or 5 mg/kg every each 6 or 8 weeks.

Etanercept was administered twice a week, and the patients received 25 mg by injection. They were followed up at time 0, at month 3, month 6 and each 6 months. Blood sampling was performed at baseline and at every follow-up visit. Patients entered the study at different time points; therefore the duration of follow-up was not the same for all patients. Blood sampling consisted of: ANA, anti-DNA, C4 complement, C-reactive protein, ESR, lymphocyte and CD4-CD8 cell count.

Each individual signed an informed consent form after receiving additional verbal information regarding the study. The protocol was approved by the committee for the protection of persons participating in biomedical research (French law 88-1138; December 20, 1988).

Detection of autoantibodies and C4 complement

ANA levels were determined at the Laboratory of Immunology of the Pellegrin University Hospital, by an indirect immunofluorescence technique (IIF) using Hep-2 cells (Menarini kit). A level equal to or greater than 1:250 was interpreted as a positive result. For positive sera that had nuclear granular or cytoplasmic staining, the identification of autoantibodies against (ENA) was further investigated by enzyme-linked immunoabsorbent assay. Anti-DNA antibodies were determined at the same laboratory, by an ELISA technique (enzyme-linked immunosorbent assay, Biomedical Diagnostic kit). The upper normal limit was 20 UI/ml. The anti-DNA determined was only anti-double DNA and only IgG isotype was found (not IgM, IgA). The C4 complement was determined by nephelemetry (Behring), the normal values were 0.15-0.35g/l.

Systematic recording of clinical adverse events

All patients were asked about all events that occurred since the first injection, and a careful clinical examination was performed at every follow-up visit. Specific symptoms known to be associated with systemic lupus were looked for. If dermatologic signs appeared, a specific dermatology consultation was carried out.

Statistical analysis

For each parameter of interest, we differentiated between the infliximab-treated patients and those treated with etanercept, and also between RA and AS patients.

First, we performed a descriptive analysis at the inclusion of patients. We then studied ANA, anti-DNA antibodies and C4 complement evolution between baseline and the last follow-up visit available for each patient.

Concerning the etanercept-treated sample, we later decided to differentiate between patients who had received infliximab previously, and those who had not.

Infliximab-treated and etanercept-treated groups were compared for ANA induction. The average levels of antibodies and C4 complement were compared using Student *t*-test for paired data.

The proportion of newly developed antibodies and C4 complement in patients, who were negative at baseline, were compared using the χ^2 test or Fisher's exact test where appropriate.

The threshold of tests was determined using a 0.05 alpha risk. Statistical analyses were performed on STATA 7.0 SE Software (Stata, College Station, TX).

Results

The demographic characteristics and clinical data on the study population are given in Table I. A cohort of 229

Table I. Demographic characteristic of patients included.

	RA (n=98)	SA (n=61)	RA (n=116)	SA (n=9)
TNF-α blocker	infliximab	infliximab	etanercept	etanercept
Median age (year)	54.85 ± 12.6	45.8 ± 13.8	54.7 ± 14.3	39.1 ± 11.6
Sex ratio (male/female)	32/66	48/13	34/82	6/3
Disease duration	14.9 ± 8.1	18 ± 12	14.4 ± 9.4	15.8 ± 12.5
DAS 28 or BASDAI	DAS 28 = 4.81	BASDAI = 53.77	DAS 28 = 3.77	BASDAI = 51.35*
HLA	DR1 = 23 DR4 = 42 DR1, DR4 = 10	HLAB27 = 42	DR1 =54 DR4 = 44 DR1, DR4 = 13	HLAB27 = 7
DMARDs	4.16	1.69	4.51	1.78
CRP (mg/l)	36.38	29.44	34.46	20.89
ESR (mm/h)	45.79	37.13	45.28	31.78
Concomittants treatments:				
-MTX (≥ 7.5mg/s)	80	33	60	4
-corticoides	59	10	85	2
-AINS	51	48	48	7
Follow-up	Baseline, W2, W6, every 8 weeks		Baseline, M3, M6, M12	
TNF-α blocker received before	9 (etanercept)	3 (etanercept)	40 (infliximab)	3 (infliximab)

*This data is explained by missing and inadequate values for 2 peripheric spondylarthropathies.

patients were included, 165 RA and 64 AS. 159 patients received infliximab treatment, 125 etanercept and 55 patients received both.

Infliximab-treated RA patients (n=98)

Figures 1 and 2 shows the ANA and anti-DNA antibodies evolution in the whole RA patients group.

At the entry, before receiving any infliximab treatment, 43.6% of RA patients (n=41/94) were ANA positive, with titers ≥1:250. This prevalence rose to 73.01% at the last visit available observed with a maximal follow-up of four years. The average ANA values increased significantly during therapy from 1:500 to 1:1500 (p=7.10^{-4}), but remained moderate, being inferior to 1/1000. Figure 1 shows that this variation depends on the ANA initial value. The progression of ANA levels for patients who were ANA positive at baseline was much more important than that for the ANA negative patients (progression mean of 1:1545 [ANA+ group] *vs.* 1:631 [ANA- group], p=0.02). We could observe a significant ANA induction from the fifth visit.

No patient presented anti-DNA antibodies positive ratio at inclusion, and 9.52% patients had positive values at the last visit available. Regarding C4 complement, we could observe that 4.6% (n=4/86) of the patients had an hypocomplementemia at the onset versus 18.6% (n=16/86) at the last visit available (p=4.10^{-3}).

Infliximab-treated AS patients (n=61)

27.1% of AS patients (n=16/59) were ANA positive at the baseline of the study, and 52% (n=26/50) at the last follow-up visit. No statistical difference could be observed between baseline and the last visit, and also according to ANA baseline values. In our cohort, 31 were axial forms, 5 peripheral and 25 were axial and peripheric forms. At baseline, the ANA positive patients were divided as 10/31 for axial forms (32.25%), 0/5 for peripheral forms (0%) and 3/25 for axial and peripheral forms (12%).

In this group, no difference was observed between positive anti-DNA antibody patients at baseline (0%) and at the last follow-up visit (2%).

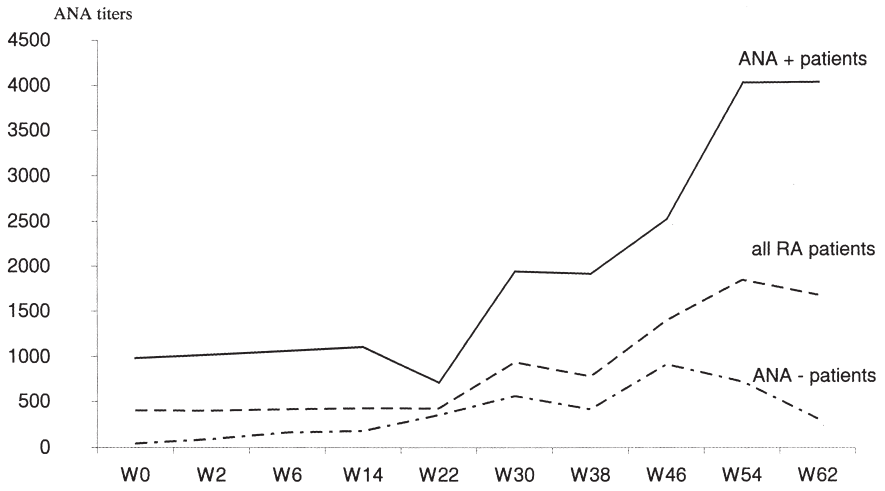

Fig. 1. Evolution of ANA titre means during the 62 first weeks of follow-up in RA patients treated with infliximab.
Distinction has been performed between two groups of patients:
(—) represents patients who were ANA positive at the onset.
(-·-·-) represents patients who were ANA negative at the onset.

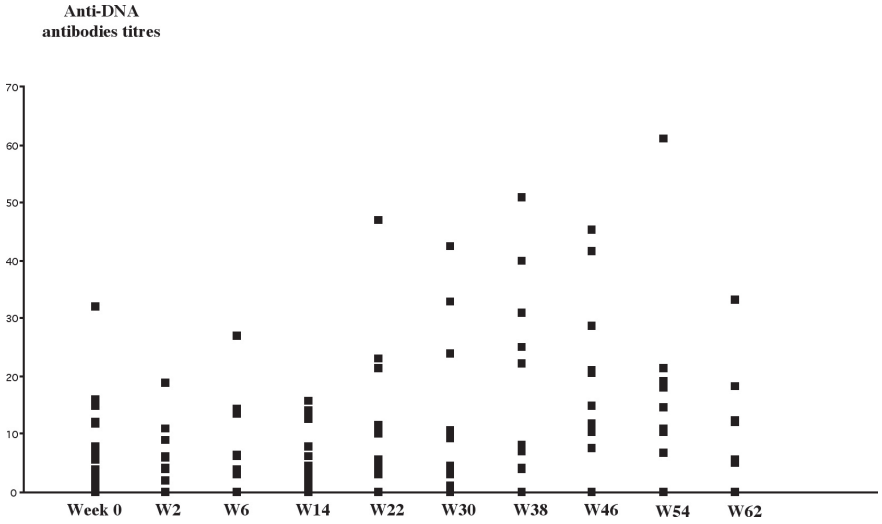

Fig. 2.

2.27% (n=1/44) of AS patients were C4 complement positive at baseline, compared to 20.45% (n=9/44) at the last visit available (p<0.01).

Etanercept-treated RA patients

At entry, there was a high proportion of patients with ANA positive patients at the onset (58.6%, n=65/111), with differences observed between two classes: patients who had received infliximab before and those who had not. When we look at this first category of patients, the rate of patients positive for ANA was 63.33% at baseline versus 21.31% in the group who had not received infliximab (p<10^{-4}). Regardless of who had received infliximab before or not, we could not observe an increase in the proportion of ANA positive patients and in ANA levels in each group of treated patients. Anti-DNA and C4 complement results are stacked.

Mean progression of the ANA ratio in the infliximab group was about 1:923±2142 *vs.* 1:89±1278 in the etanercept group (p=0.01). Thus, we clearly described that there is a significant difference between infliximab and etanercept in ANA induction.

Etanercept-treated AS patients

Only nine spondylarthropathies were included, of whom three had been

previously treated with infliximab and, for this reason, we decided not to evaluate this group.

ANA induction and correlation with clinical response

It seems that being a good TNF-α blocker responder is associated with ANA induction. In the infliximab treated patients, 70.2% of good responders were ANA positive *vs*. 43.8% in the weak responders group of patients (p=0.026). The same observation was made in the etanercept group of patients who had not received infliximab before, with 45.8% (n=11/24) of good responders being ANA positive *vs*. 0% in the weak responders group (p=0.007). Thus, ANA appeared significantly much more in good responder patients with a DAS 28 score <2.8, than in the weak responder group of RA patients. No correlation was observed between ANA ratio increase and clinical results in the AS patients group.

Lymphocyte count, CD4-CD8 evolution with infliximab or etanercept

At entry, lymphopenia was observed in 62% RA and 27% AS of infliximab patients treated. No inducted lymphopenia was shown in RA (p=0.9) or in AS (p=0.7). There was no CD4-CD8 rapport fall (p=0.52 for RA patients and p=0.98 for AS patients).

These results were similar to the etanercept patients treated. No lymphopenia induction existed (p=0.76), and there was no significant variation of the CD4-CD8 rapport.

Clinical manifestations

Only three patients, two women and one man, developed chilblain lupus with no major organ involvement, during the period of follow-up. These manifestations, although minor and not specific, appeared suggestive for a lupus-like syndrome in the context. Two of them were treated with infliximab and one with etanercept. All were RA patients and none of them had significant ANA levels before TNF blockers were administered. The therapy was purchased in spite of clinical symptoms without making worse, with specific treatment (calcium inhibitors, antimalaria drugs). The first patient, a woman, had been treated with infliximab for 22 months. She developed a chilblain lupus during the winter period and her ANA level was very high at 1:32000 (anti-DNA and C4 complement were normal). Infliximab treatment was not stopped, and her chilblain lupus was improved by specific treatment. The second patient had been treated with infliximab for four years, before he presented with chilblain lupus. Chilblains are cutaneous inflammatory lesions commonly occurring during cold and humid periods. Long-lasting chilblains can be either idiopathic and isolated, or associated with various connective tissue diseases, especially lupus (22). He had a low titre of ANA (1:100), no anti-DNA and normal C4 level, so we completed the immunologic investigation and found a very high titre of anti-nucleosome (90 UI/L). The latter patient had received only two infliximab infusions, but the treatment was stopped because of venous problems and was replaced by etanercept. At baseline ANA were 1:500, after one year of treatment, she developed clinical symptoms of chilblain lupus and the treatment was stopped only after three weeks, and took again because of active RA. Her ANA were 1:1000 without anti-DNA or C4 complement abnormalities. With specific treatment, clinical symptoms disappeared and etanercept purchased. Table II summarizes our observed results.

Discussion

Our study confirms that TNF-α blockers can induce the appearance of ANA or increase in both RA and AS patients. In the infliximab group, 73% of the RA patients and 52% of the AS patients had ANA at the end of the observational period, and 9.5% and 2% had anti-DNA antibodies; hypocomplementemia was also observed. These auto-antibody rates were most often moderate, and rarely associated with drug-induced lupus. During RA, the appearance of ANA was observed at moderated levels in patients who had been previously ANA negative to TNF-blocker onset, and at higher levels if they had been positive at baseline, the ANA rate was multiplied four times at the end of the evaluation (mean time from baseline of 26.7 weeks ± 3.2). We confirm that ANA induction seems not to be a therapeutic class effect, and to be an independent factor of the disease treated. In the etanercept group, there was no ANA induction with 53.3% on average of the RA patients at the end of the period of observation against 58.5% at baseline (whether they had received infliximab

Table II. Description of immunologic characteristics of patients stratified by treatments.

Cohort	TNF-α blocker	Time point	ANA positivity (%)	Anti-DNA positivity (%)	C4 positivity < 0.15 g/l (%)	Lupus-like syndrome
RA(n=98)	infliximab	Baseline	43.6%	0%	4.6%	
		Last visit	73.0%	9.5%	18.6%	2
RA (n=116)	etanercept	Baseline	58.5%	20.7%	6.4%	
		Last visit	53.3%	5.1%	8.5%	1*
RA (n=76)	etanercept without infliximab	Baseline	20.6%	0%	3.1%	
		Last visit	28.7%	2.4%	6.2%	1*
RA (n=40)	etanercept + infliximab	Baseline	63.3%	18.8%	14.3%	
		Last visit	64.3%	12.5%	14.3%	0
SpA (n=59)	infliximab	Baseline	27.1%	0%	2.3%	
		Last visit	52%	2%	20.4%	0
SpA (n=9)	etanercept	Baseline	22.2%	22.2%		
		Last visit	not realised	not realised	not realised	0

*The case in these two groups is the same, so there are three cases of lupus-like syndrome as described in the manuscript.

previously or not). The high proportion of ANA at baseline in this category could be explained by the high number of RA who had received infliximab before: nearly one third (40/116).

We showed that ANA induction was correlated to good clinical response in the RA patients, whatever the drug used. Thus, this notion seems to confort the TH1/TH2 concept hypothesis (23, 24), because TNF-α blockade stops TH1 cytokine induction, and benefits from TH2 cytokines (IL10, IFN-α), and pull lupus immunologic cascade and the autoantibodies production (25). It could also be explained by the down-regulation of material nuclear clearance (26, 27).

The therapeutic maintenance of TNF-α blockers in our three chilblain lupus cases did not make them worse. It asks the question of systematically TNF-α blockers interruption when lupus symptoms appear, and their direct implication in their physiopathology. The inflammatory disease could be incriminated (in fact 30% of RA patients have ANA), as other drugs used by these patients.

Our results on the RA patients treated with infliximab were similar to other reports published, with 44% to 81% of ANA at the end of the observational period. Few studies have been carried out on ANA and etanercept, only two have been published. For the first one, we did not have any results (28), and the second one by Caramashi *et al*., seemed to be confirmed by our results and showed no ANA induction. However, only 11 patients were included (28, 29). The results for AS patients were contradictory with previous reports. ANA values were higher at baseline, so different from previous reports (30-34), and this might be explained by the high proportion of peripheric spondylarthropathy versus axial forms and their immmunologic profile which is very close to that of RA. This hypothesis was not confirmed in our cohort: at baseline, the ANA positive patients were divided as 10/31 for axial forms (32.25%), 0/5 for peripheral forms (0%) and 3/25 for axial and peripheral forms (12%). Furthermore, ANA induction was less important than the previous published studies at the end of the observational period (in our study 52%, against 85 to 89% in others works) (30-32). This could be explained by the high proportion of patients treated with methotrexate therapy (33/61). Of interest in this context is the observation by Boehm *et al*. that methotrexate therapy could lead to a decrease in circulating autoantibodies in patients with cutaneous lupus (35), and by Gerards *et al*. who explained that methotrexate is able to inhibit cytokine production (36).

Antibody production is probably initiated by a double action. They reduce inflammation protein synthesis, which participates in the clearance of apoptotic blebs. Prolonged exposure to the immune system of excessive amounts of intracellular material potentially induces and maintains an ANA response by repeated stimulation. Infliximab is composed of an Fc fragment which allows the induction of Autoantibody Dependant Cell Cytotoxicity (ADCC) phenomenon and cell apoptosis with the release of apoptotic nuclear macromolecules. Etanercept is also composed of an Fc fragment, without inducing any ADCC phenomenon. It was confirmed by the differences observed between etanercept and infliximab in our cohort. This difference may be the consequence of the differential capacity of a monoclonal antibody and a soluble receptor in inducing apoptotic cell death of the cells expressing TNF on their membrane.

The idea of different action way could be confirmed by their different biochemistry, biomolecular parameters and their different efficacy in Crohn disease (CD) or others granulomatosis, as sarcoidosis. Some reports showed that infliximab could increase the number of apoptotic T lymphocytes in the lamina propria and apoptotic monocytes in peripheral blood in CD, but not etanercept. The risk of reactivation of latent Mycobacterial tuberculosis infection is greater with the TNF monoclonal antibody infliximab than with the soluble TNF receptor etanercept, and could be explained by their different effects on TNF and IFN gamma (37). Infliximab is also able to release intracellular bacillus after cell apoptosis (37-42). These differences were confirmed by a French team, who suggested that *in vivo* infliximab and adalimumab were more efficient than etanercept. They decrease the frequency of memory $CD4^+$ T lymphocyte releasing IFN-gamma upon challenge with mycobacterial antigens (41).

In conclusion, our study confirms that the induction of autoantibodies 1) is absent under etanercept therapy, 2) is independent of the arthritic disease treated, and 3) there are very few lupus syndromes associated with no severe organ damage. Further studies are needed to confirm the class effect therapeutic absence with adalimumab and the etanercept effect in spondylarthropathies.

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