CONCISE REPORT

Anticardiolipin antibodies in rheumatoid patients treated with etanercept or conventional combination therapy: direct and indirect evidence for a possible association with infections

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Objective: To assess the occurrence of anticardiolipin antibodies (ACA) (as well as of anti-DNA antibodies) in patients with rheumatoid arthritis treated with etanercept or combination therapy.

Methods: Eight patients treated with etanercept 25 mg twice weekly were studied for a period of 85 weeks. A control group of 39 patients with rheumatoid arthritis undergoing combination treatment (methotrexate (MTX) + cyclosporin A or MTX + chloroquine) were studied for the same period of time. The occurrence of anticardiolipin antibodies (ACA-IgG) and anti-DNA was examined, together with the possible occurrence of infections due to bacteria capable of inducing B cell activation.

Results: In 5/8 patients receiving etanercept an increase of ACA-IgG was seen, while anti-DNA became positive in 3/8 patients. A nasal or bronchial infection due to *Staphylococcus aureus* (*Staph aureus*) or a urinary tract infection due to *E coli*, occurred in all five cases. Antibiotic treatment produced a return to normal of ACA-IgG, and also of anti-DNA, in all cases except one. The infectious agent was eradicated in all subjects but one. In the control group *Staph aureus* was found in the nasal swab in 10/39 subjects; ACA-IgM (followed by ACA-IgG) appeared at the same time as infection occurred in 6/10, while no infection related to the increased ACA-IgM was recorded in the other four.

Conclusions: Bacterial DNA, especially that enriched in CpG motifs, is a powerful immunostimulant that may, in some cases, lead to ACA or anti-DNA positivity, once tumour necrosis factor α is blocked. Eradication of the infections leads to a rapid decrease of ACA-IgG and of anti-DNA levels.

Treatment with biological agents directed against tumour necrosis factor α (TNF α) has been shown to be safe and to have significant clinical benefits.^{1–3} Among the side effects, infections and injection site reactions are by far the most common adverse clinical events, and the occurrence of anti-DNA and anticardiolipin antibodies (ACA) is the most interesting biological reaction. While taking part in the European etanercept study, we realised that some of our patients developed strong positivities against cardiolipin; the levels were clinically significant.⁴ This prompted us to consider why such an immunological reaction should occur and to attempt to clarify the reasons for this.

PATIENTS AND METHODS

Eight patients, refractory to conventional treatments, entered into a long term open study with etanercept. Conditions for

inclusion were (a) >6 swollen joints at entry; (b) refractory to one or more disease modifying antirheumatic drugs (DMARDs); (c) a disease duration of <10 years; and (d) age >18 or <75 years at entry. All had severe erosions of hands and feet. After a wash out period from the previous DMARDs, the patients received 25 mg etanercept subcutaneously twice weekly. In addition to the protocol guidelines, we assessed at each visit pharyngeal and nasal swabs for bacteria and fungi, together with urine cultures and bronchial sputum cultures obtained through vaporised aerosol. A chronic nasal carriage or a clinically relevant bronchial carriage of infection was defined when a swab was positive for a high number of colonies (>1000 colony forming units) of *Staph aureus* on two consecutive visits, accompanied by a low grade fever. Initially, the clinical visits were set up at weeks 3, 5, 9, 13, and 25, then every three months. Biochemistry, haematology, and immunology assessments were made at the time. We checked antinuclear antibodies (ANA; HEp-2 cell), antibodies to DNA (enzyme linked immunosorbent assay (ELISA): normal values <30 IU/ml tested against normal and reference sera), and ACA (ELISA: normal values <15 GPL or MPL, tested against normal and reference sera) at each visit, up to the 85th week of follow up. When positive swabs with a high number of colonies were found, the antibiotic amoxycillin sulbactam was given. Thirty nine patients with active rheumatoid arthritis despite treatment with methotrexate (MTX; 10–20 mg/week) underwent combination treatment with cyclosporin A (CsA; 3–5 mg/kg/day; 26 patients) or with chloroquine (4 mg/kg/day; 13 patients). They were followed up for the same period of 85 weeks. In all patients we evaluated ACA, ANA, anti-DNA and rheumatoid factor. Mean values, standard deviations, and significance were calculated with the PRISM software for McIntosh (Graph-Pad Software, Inc San Diego, CA 92121, USA).

RESULTS

Table 1 shows the clinical, demographic, and immunological data at entry of the patients receiving etanercept. All patients had clear evidence of active disease at the start of the study, despite the combination treatments. In the control group 26 patients already receiving MTX, were given CsA, 13 patients already receiving chloroquine were given MTX. Data for the control group in comparison with the group receiving etanercept were as follows: mean (SD) age was similar (etanercept

Abbreviations: ACA, anticardiolipin antibodies; ANA, antinuclear antibodies; CRP, C reactive protein; CsA, cyclosporin A; DMARDs, disease modifying antirheumatic drugs; MTX, methotrexate; TNF α , tumour necrosis factor α

Table 1 Clinical and laboratory parameters of each patient treated with etanercept, at entry into the study group

Patient No	SJC (n)	CRP (mg/l) (nv 0–5)	ACA-IgM* (nv <15)	ACA-IgG* (nv <15)	Anti-DNA* (nv <30 IU)	Previous treatment
1	10	105	<15	<15	Neg	MTX, CsA, SSZ
2	51	88	<15	<15	Neg	MTX, SSZ
3	27	60	40	23	Neg	CH, CTX
4	41	39	16	<15	Neg	MTX, CH
5	49	137	<15	<15	Neg	MTX, GS
6	32	120	<15	<15	Neg	MTX, GS
7	32	35	33	37	30	MTX, AZA
8	6	20	112	<15	Neg	MTX, SSZ, CH

*Units: ACA-IgM, MPLU, international ELISA units for IgM; ACA-IgG, GPLU, international ELISA units for IgG, β_2 glycoprotein I dependent; anti-DNA, international ELISA units.
 MTX, methotrexate; CsA, cyclosporin A; SSZ, sulfasalazine; CH, chloroquine; CTX, cytoxan; GS, gold salts; AZA, azathioprine; SJC, swollen joint count (66 joints).

Figure 1 Behaviour of ACA-IgM up to the 85th week of follow up (normal value 0–15 U/ml). Positivity of culture swabs from nasal discharge or from bronchial sputum, or of urine cultures are reported.

group 61.3 (10.6) v controls 62.2 (13.4)), the percentage who were rheumatoid factor positive was similar (etanercept group 75% v controls 74.5%), ANA was positive in one of the etanercept group and in 38.4% of the controls, and the C reactive protein (CRP) levels were much higher in the etanercept group (75.5 (43.2) mg/l) than in the controls (29.1 (39.1) mg/l). In the etanercept group the best clinical result was obtained at the 12th month. In particular, 4/8 patients had an improvement of >50% ACR, 7/8 had a >50% improvement in the number of swollen joints, and 8/8 had a >50% improvement of CRP levels. At the 85th week three patients showed a further improvement in the number of swollen joints, but no further improvement was seen in CRP levels. It should be noted that all patients were refractory to conventional DMARDs. Among patients receiving MTX and CsA 75% attained the 50% ACR improvement at the 12th month, while among those receiving chloroquine and MTX 76% obtained the ACR 50%. In the etanercept treated group 5/8 (63%) patients had a clinically relevant nasal discharge (defined as the simultaneous presence of a high number of colonies >1000 colony forming units, accompanied by low grade fever) that was positive for *Staph aureus* infection.

Figure 1 shows that some of the patients were positive for ACA-IgM at entry and demonstrated changes in the levels of the antibody. In particular, one patient already IgM positive had a dramatic drop of the levels after antibiotic treatment. Two patients had a clear increase by the time a positive culture was demonstrated. Figure 2 shows the behaviour of ACA-IgG. Data show that all IgG increments except one could be related to nasal or bronchial culture positive for *Staph aureus*. In one patient a urinary tract infection due to *E coli* and in another one a pharyngitis without any isolated organism was demonstrated. In patients 7, 4, and 3, anti-DNA (64 IU, 68 IU, 72 IU) rose simultaneously with ACA-IgG. Eradication of the infection led to a sharp decrease of the antibody level on all occasions, even though in patient 5 no definite eradication could be obtained. Among the controls 7/26 (27%) in the MTX + CsA subset and 3/13 (23%) in the chloroquine + MTX subset developed nasal discharge with *Staph aureus* during treatment: once in six patients, three times in four patients. In three patients treated with MTX + CsA and in three treated with chloroquine + MTX we noted a simultaneous increase of ACA-IgM, followed the next month or later by the ACA-IgG. In none of these cases did IgG or IgM levels exceed 40 IU/ml. In

Figure 2 Behaviour of ACA-IgG up to the 85th week of follow up (normal value 0–15 U/ml). Positivity of culture swabs from nasal discharge or from bronchial sputum, or of urine cultures are reported.

a single patient treated with MTX + CsA, ACA-IgG appeared but were not related to infection. In four patients ACA-IgM appeared but no carriage of infection was found.

In conclusion in 60% of the control cases we observed the simultaneous positivity of a nasal swab for *Staph aureus* and of ACA-IgM/IgG. In all cases where infections occurred we noticed an increase in the number of swollen joints (range 1–3 joints more) both in the group receiving etanercept and in the group receiving combination treatment. Antibiotic treatment led to a return to normal of ACA in all cases and did not modify the basic treatment. None of the patients had lupus anticoagulant positivity at any time.

DISCUSSION

In clinical practice we consider that when ACA-IgG levels reach >40 GPL an increased risk of a thrombotic event has to be taken into account. The discovery that some patients developed such high levels raised some concern about whether we should have treated these patients or not with antiaggregants. No clinical manifestations related to antiphospholipid antibody positivity were observed. More importantly we noted a real variation of the antibody levels over time. This behaviour might have been related to the anti-TNF α treatment which was given continuously, but a temporary activation of the B cells is a more realistic explanation. This led us to assess whether microbes or fungi might have been involved. It is well known that TNF α is crucial for the clearance of infectious agents.^{5–6} Our data suggest that this was indeed the case. In fact a temporal relationship was seen on all occasions but one in the etanercept group, and eradication of the infection led ACA levels to drop in the great majority of cases. In the control group a smaller percentage of patients developed an infection, and only in 60% could a temporal relationship between the appearance of ACA and infections be shown. This suggests that other immunological processes may favour the appearance of the antibodies during combination treatment. Certainly, in patients infected, antibiotics led to a normalisation of the antibodies.

It is known that nasal colonisation with *Staph aureus* is common, it can occur in 15–44% of the general population and

is usually without ill effects.^{7–8} In our patients with rheumatoid arthritis the incidence of positive carriage of infection fell within this range. All the patients with a high number of colonies and a low grade fever, had improved overall health (no more fever, improvement of the number of swollen joints) and improved laboratory parameters after treatment with the antibiotics. These results suggest that in our patients, as found in Wegener's disease,⁹ eradication of *Staph aureus* infection had a significant effect.

A possible explanation of why the infection can lead to the appearance of ACA might be found in the bacterial DNA, which is enriched in unmethylated CpG motifs.¹⁰ CpG motifs can activate CD86 expression, interleukin 6 synthesis by B cells, and interferon γ by natural killer and T helper 1 cells.^{11–13} These motifs expressed by *Staphylococcus* DNA (as well as by *E coli* DNA) may well act as activators of B cells once TNF α is blocked by the biological agent. The prompt decrease of ACA-IgG synthesis after bacterial eradication strongly supports the involvement of infection in our patients.

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