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

G Ferraccioli, F Mecchia, E Di Poi, M Fabris

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.

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 61.4±13.8 years; control group 61.2±13.8 years). There were no significant differences in baseline serum levels of C reactive protein (CRP) (etanercept 0.5±0.6 mg/l; control group 0.5±0.8 mg/l) or ESR (etanercept 23±4 mm/hr; control group 19±6 mm/hr).

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

CONCISE REPORT

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group 61.3 (10.6) vs controls 62.2 (13.4), the percentage who were rheumatoid factor positive was similar (etanercept group 75% vs 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

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

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

*Units: ACA-IgM, MPLU, international ELISA units for IgM; ACA-IgG, GPLU, international ELISA units for IgG; β2 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).
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-TNFα 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 related to the anti-TNFα treatment which blocked by the biological agent. The prompt decrease of ACA-IgG synthesis after bacterial eradication strongly supports the blocked by the biological agent. The prompt decrease of ACA-IgG synthesis after bacterial eradication strongly supports the involvement of infection in our patients.

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 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.

It is known that nasal colonisation with Staphylococcus aureus is usually without ill effects. 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, eradication of Staphylococcus 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. CpG motifs can activate CD86 expression, interleukin 6 synthesis by B cells, and interferon γ by natural killer and T helper 1 cells. These motifs expressed by Staphylococcus DNA (as well as by E coli DNA) may well act as activators of B cells once TNFα 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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