



## Autoantibodies and thrombophilia in rheumatoid arthritis : TNFalpha and TNF alpha blockers.

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## Autoantibodies and thrombophilia in RA: TNF $\alpha$ and TNF $\alpha$ blockers

G F Ferraccioli, E Gremese

Subtitle.

Patients with rheumatoid arthritis (RA), an autoimmune chronic-relapsing inflammatory disease affecting joints and internal organs, present in several studies a median survival much shorter than that of the general population.<sup>1</sup> The main cause of early premature death is cardiovascular morbidity. Solomon *et al* clearly showed that women with RA (aged 30–55) have an increased risk of myocardial infarction (MI), a relative risk (RR) of 2, compared with women without RA, increasing according to disease duration up to an RR of 3.1 after 10 years of disease.<sup>2</sup> Del Rincon *et al* had previously recognised that the incidence rate ratio of having MI compared with patients without RA of similar age and sex was 2.65, and therefore substantially increased.<sup>3</sup> In addition to the increased risk of MI, patients with RA have an increased risk of carotid disease and of peripheral arterial disease, only partially explained by the previous use of corticosteroids.<sup>4</sup> More recently, data from the National Data Bank for Rheumatic Diseases confirm that patients with RA have an increased prevalence of congestive heart failure (CHF) compared with patients with osteoarthritis (3.5% v 2.2%), that their risk factors are those of the general population (male sex, hypertension, diabetes, age, body mass index), and that the Health Assessment Questionnaire, an index of overall health, is strongly predictive of future CHF.<sup>5</sup>

All these data clearly show that RA represents a population at high cardiovascular risk. Overall, the cardiovascular risk of RA has been estimated to be around 3.96,<sup>3</sup> and therefore very similar to the overall risk of non-insulin dependent diabetes, which has been estimated by Laakso to be two- to fourfold higher than in non-diabetic subjects.<sup>6</sup> It is very similar to non-insulin dependent diabetes in adult men in which the RR of cardiovascular mortality and MI was found to be 2.8 in diabetic men (age 28–61) presenting signs of inflammation.<sup>7</sup>

“RA presents a cardiovascular risk similar to non-insulin dependent diabetes”

### RA, VASCULAR AND MYOCARDIAL DISEASE

Tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin (IL)1 $\beta$  have emerged as crucial mediators of the inflammatory process leading to atherosclerosis.<sup>8,9</sup> In RA TNF $\alpha$  and IL1 $\beta$  are considered to be the key driving cytokines.<sup>10,11</sup> TNF $\alpha$  could increase the risk of atherogenesis by decreasing gene expression of lipoprotein lipase (thus increasing very low density lipoprotein and triglyceridaemia),<sup>12</sup> of scavenger receptor A,<sup>13</sup> which might predispose, as in the ApoE3Leiden transgenic mice, to accelerated atherosclerosis (and diet induced hypercholesterolaemia might increase the degree of the atherosclerotic process), and of the PAI-1 gene, indirectly, through an increase of C reactive protein (CRP) levels.<sup>14,15</sup> To account for the possible endothelial dysfunction, TNF $\alpha$  also inhibits endothelial nitric oxide synthase gene promoter activity.<sup>16,17</sup> In addition, TNF $\alpha$  increases gene expression of low density lipoprotein receptor 1 (LOX-1),<sup>18,19</sup> a receptor that can support adhesion of Gram negative and Gram positive bacteria, is involved as a cell adhesion molecule in endotoxin induced inflammation, and capable of binding, internalising, and degrading oxidised low density lipoprotein. TNF $\alpha$  may also induce gene expression of vascular cell adhesion molecule-1,<sup>20</sup> which has a fundamental role in rolling-type mononuclear cell adhesion, neointima formation in the case of injury, and smooth muscle cell proliferation. IL1 $\beta$  exerts very similar effects at all levels.<sup>21</sup> In addition, both cytokines increase secretory sphingomyelinase, which generates ceramide,<sup>22</sup> a crucial inducer of apoptosis, cellular differentiation, and cellular senescence, processes strongly involved in atherogenesis. Of interest the Janus face of TNF $\alpha$  has been further demonstrated by showing in an experimental model in mice that continuous infusion of TNF $\alpha$  exerts a strong antithrombotic activity through TNF receptors and the rapid production of nitric oxide in vascular smooth muscle cells.<sup>23</sup>

“The biological data strongly highlight the pro-atherogenic milieu of RA inflammation”

### RA, THROMBOPHILIA, AND ISCHAEMIC MYOCARDIAL DISEASE

Published data on the thrombophilic status are conflicting,<sup>23</sup> mediated by predisposing factors, among which are antiphospholipid antibodies (aPL).<sup>24–27</sup> There is no doubt that aPL are markers of an autoimmune thrombophilia. In RA anticardiolipin antibodies (aCL) have been reported in percentages ranging from 11.4% (IgG isotype)<sup>24</sup> to 17.8%,<sup>25</sup> up to 32%.<sup>26</sup> Moreover, both venous and arterial thrombosis (venous/arterial ratio: 2:1) were found to be associated with aCL positivity, at least in one study,<sup>25</sup> even though this was not confirmed in a smaller series.<sup>27</sup> None of the previous studies focused specifically on the moderate to high levels that might be of clinically relevant significance as recently demonstrated by Galli in her meta-analysis.<sup>29</sup>

“A subset of RA presents a thrombophilic milieu possibly related to aCL antibodies”

However, no data are available on the relationships existing between the reported increased risk of myocardial infarction in RA and the pro-atherogenic or the thrombophilic status related to the levels of aPL.

### TNF BLOCKERS AND CARDIOVASCULAR RISK

These issues become even more critical after the introduction of TNF $\alpha$  blockers in clinical practice. These drugs are extremely efficacious in controlling inflammation. In fact they enable CRP levels to be markedly controlled, yet their ultimate effect on the cardiovascular risk is completely unknown. Data in rheumatoid patients have shown that etanercept (TNF RII 75.Fc-IgG fusion protein) in the short term has positive effects on the physiology of the endothelium,<sup>30</sup> but not in those with longstanding disease.<sup>31</sup> On the other hand, in a long term follow up of eight patients treated with etanercept we observed acute spikes of positivity of aCL at biologically relevant levels (>40 MPL and GPL units) occurring mainly during upper respiratory tract infections, and returning to the basal levels after eradication of the infections.<sup>32</sup> In another study, during treatment with infliximab (mouse  $\times$  human monoclonal chimeric IgG1k), we detected the occurrence of two spikes of aCL



(MPL>40 units), both complicated by events possibly related to the thrombophilic biology: in one case a femoral head osteonecrosis in a 29 year old woman with no other risk factor (no steroids, no smoking habit, no dislipidaemia, etc), in the other case, acute myocardial infarction occurring after the positivity of aCL and lupus anticoagulant (LAC), without any relationship with infections.<sup>33</sup> All these events occurred while the systemic inflammation was clearly better controlled as demonstrated by the CRP levels. Trials with etanercept and infliximab,<sup>34, 35</sup> have clearly shown the occurrence of aPL. Even though no prospective studies strictly related to the possible pro-atherogenic or thrombophilic milieu in patients developing cardiovascular side effects have been published, a recent report has described both in Crohn's disease and in RA the development of new-onset heart failure or an exacerbation of heart failure after infliximab treatment and in some patients an improvement after treatment was stopped, thus lending credit to the causal relationship.<sup>36</sup> Of interest, congestive heart failure is a contraindication to infliximab as well as to etanercept treatment in RA, after trials demonstrated clear harm in class III-IV heart failure.<sup>37, 38</sup>

On the other hand, the Wichita Arthritis Center has demonstrated that the rate of occurrence of CHF is reduced by anti-TNF $\alpha$  treatment, thus suggesting that control of the inflammation is probably the most important driver of heart damage and that its control by treatment clearly improves the outcome.<sup>5</sup>

Targeting IL1 $\beta$ , appears less problematic from the autoimmunity viewpoint.

The experience in patients with RA receiving IL1 receptor antagonist (ana-

kinra) disclosed no changes in the overall autoimmune setting (no increase of antinuclear antibodies (ANA), DNA antibodies, aCL, or rheumatoid factor).

"TNF $\alpha$  blocker treatment in a minority of patients with RA leads to heart failure or heart failure exacerbation"

These reported data highlight the need for a careful analysis of the presence of ischaemic myocardial disease when planning a long course with TNF $\alpha$  blockers, because no proof of an improvement of endothelial function currently exists in patients with a long history of RA, and although some recent very preliminary data suggest a reduction of the overall cardiovascular risk in patients treated with anti-TNF $\alpha$  biological agents, a better definition of all the risk factors underlying the appearance of cardiovascular events is needed.

#### AUTOIMMUNITY DURING TREATMENT WITH TNFA BLOCKERS

The etanercept packaging states that 11% of patients receiving the drug developed ANA with a titre  $\geq$ 1/40, compared with 5% of placebo treated patients, and developed DNA antibodies as assessed by radioimmunoassay in 15% compared with 4% and as assessed by *Crithidia* in 3% compared with 0% in placebo treated patients. On the other hand, the infliximab packaging states that 62% of patients treated with the drug developed ANA compared with 27% in placebo treated patients, and developed DNA antibodies assessed by radioimmunoassay in 15% compared with 0% of placebo treated patients. Trials also showed that aCL occur during both infliximab and etanercept treatment. The question is why these auto-

antibodies develop during treatment. Several possible pathogenetic pathways may be envisioned (table 1).<sup>39-45</sup> Although no strict relationship between the pathogenetic pathways leading to DNA antibodies and atherogenesis has so far been demonstrated,<sup>46</sup> strong evidence suggests that all the events leading to the development of aPL (aCL, anti- $\beta_2$ -glycoprotein I, or LAC) all have a strong impact on the endothelial damage, on foam cell development, and eventually on plaque formation.

"aPL may be predisposing risk factors for the occurrence of cardiovascular side effects"

#### CONCLUSIONS

Rheumatoid arthritis is a chronic autoimmune inflammatory disease predisposing to a high cardiovascular morbidity, at least similar to that of non-insulin dependent diabetes. Inflammation certainly represents the major driving force to accelerated atherosclerosis.<sup>46</sup> TNF $\alpha$  blockers might be useful to control such comorbidity. A small percentage of patients seem to have a thrombophilic milieu at baseline that might be increased by two possible events, theoretically controllable infections and autoimmunity appearing when TNF $\alpha$  blockers are used. Both events are identifiable, infections can be generally eradicable, autoimmunity can be monitored through specific assays, and once identified it can be carefully assessed over time and might also be given appropriate treatment. The message therefore is the need for careful assessment of infections and autoimmunity and careful monitoring of both features during the follow up.

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**Table 1** Pathogenetic pathways thought to lead to anti-DNA and antiphospholipid (thrombophilic) autoantibodies in patients with RA receiving TNF $\alpha$  blockers

##### DNA antibodies

1. Apoptosis induced by TNF $\alpha$  leads to increased tissue and systemic levels of chromatin-DNA complexes<sup>39</sup>
2. Blockade of TNF $\alpha$  leads to a fall in the acute phase reactants serum amyloid P, as well as in CRP<sup>40</sup>
3. Low levels of serum amyloid P decrease the clearance of chromatin-DNA complexes<sup>41</sup>
4. High antigen levels (DNA, chromatin, etc) may induce loss of tolerance and the development of autoantibodies in genetically susceptible hosts<sup>42</sup>

##### aPL (aCL, anti- $\beta_2$ -glycoprotein I, or LAC)

1. TNF $\alpha$  mediates CD8+cytotoxic T lymphocyte capable of eliminating autoreactive B cells. Blockade of TNF $\alpha$  allows survival of autoreactive B cell clones<sup>43</sup>
2. TNF $\alpha$  protects against infections. Blockade of TNF $\alpha$  leads to more infections, more lipopolysaccharide stimulation of IL6 by monocytes/macrophages and through the bacterial CpG DNA, more activation of B cells<sup>44</sup>
3. Infectious agents, through a molecular mimicry mechanism, have been shown to lead to anti- $\beta_2$ -glycoprotein I, to aCL, and to clinical and biological manifestations of the antiphospholipid syndrome in experimental animals<sup>45</sup>

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