(Anti-)TNF alpha matters in rheumatoid arthritis

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Chapter 6

Sustained changes in lipid profile and macrophage migration inhibitory factor (MIF) levels after anti-TNF therapy in rheumatoid arthritis.

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ABSTRACT

Background.

Macrophage migration inhibitory factor (MIF) has recently emerged as an important cytokine possibly linking rheumatoid arthritis (RA) and atherogenesis. Because atherogenesis is accelerated in RA we investigated whether anti-TNF therapy could lead to sustained downregulation of systemic MIF levels and improvement of lipid profiles.

Methods.

Fifty RA patients with active disease (disease activity score 28 (DAS28 ≥ 3.2)), who started adalimumab therapy 40 mg every other week, were included. At baseline, week 16 and 52 serum levels of MIF and lipids were assessed. In addition, the DAS28 and serum C-reactive protein (CRP) levels and erythrocyte sedimentation rate (ESR) were determined.

Results.

After 16 weeks of adalimumab therapy, both DAS28 and MIF levels were significantly decreased (P < 0.001 and P = 0.020, respectively). This was sustained up to week 52 (P < 0.001 and P = 0.012, respectively). CRP levels and ESR were significantly reduced after 16 and 52 weeks of adalimumab therapy (P < 0.001). HDL cholesterol levels increased at week 16 (P < 0.001), but returned to baseline at week 52. Apo A-I levels increased at week 16 (P < 0.001) and remained stable (P = 0.005). This resulted in an improved apo B/ A-I ratio.

Conclusions.

Our results underline sustained downregulation of MIF as a potential new mechanism by which anti-TNF therapy might reduce vascular inflammation, and as such perhaps cardiovascular morbidity in RA patients. This hypothesis is supported by an improved apo B/ A-I ratio as well as reduced CRP levels in our patients.
The atherosclerotic process is accelerated in patients with rheumatoid arthritis (RA), resulting in increased cardiovascular mortality when compared to the general population. It has been suggested that the chronic systemic inflammatory state in RA enhances atherogenesis (reviewed in [1]) over and above the presence of traditional risk factors (e.g. diabetes, smoking, obesity, dyslipidemia). Inflammatory mediators from the synovium and perhaps other sites can be released into the circulation where they can alter the function of various tissues, such as skeletal muscle, liver, and vascular endothelium. This in turn may induce an array of pro-atherogenic changes, including insulin resistance, characteristic dyslipidemia, and endothelial dysfunction.

Moreover, circulating inflammatory mediators may also stimulate leukocytes and smooth muscle cells within the atherosclerotic plaque thereby promoting plaque growth or rupture.[3]

Macrophage migration inhibitory factor (MIF) has emerged as a cytokine linking RA and atherogenesis.[4] The association of coronary heart disease with a haplotype containing the rs755622C allele, which has been reported before to increase the susceptibility for various inflammatory conditions, supports the notion that MIF plays a role in inflammation and atherogenesis, although there was no difference in MIF serum levels between patients with incident coronary heart disease and individuals without such disease during follow-up in a population-based case-cohort study.[5] However, in another prospective population study in apparently healthy volunteers elevated levels of MIF were associated with an increased risk of future coronary artery disease.[6] The receptors CXCR2 on monocytes and CXCR4 on T cells have been identified as the functional receptors for MIF.[7] Interaction of CXCR2 with MIF on aortic endothelial cells was shown to induce monocyte arrest. Similarly, the interaction of CXCR4 with MIF resulted in the arrest of T cells. MIF can also induce the secretion of tumor necrosis factor (TNF) by macrophages and, conversely, TNF is able to augment MIF production.[8] In an animal model of atherosclerosis MIF blockade reduced plaque infiltration by monocytes and T cells, and even led to plaque regression.[7] Recent studies have demonstrated that MIF secretion by dendritic cells can be regulated by Toll-like receptors (TLR).[9] In the atherosclerotic lesion, especially TLR4 has been shown to be expressed by residing macrophages and dendritic cells.[10, 11] When TLR4 is triggered by its ligands (for instance lipopolysaccharide (LPS)), various cytokines, including TNF, IL-12, IL-23 and MIF, can be secreted, hereby further enhancing the inflammatory response.[9, 10] Together, the available data indicate that MIF exerts chemokine-like functions and is an important regulator of inflammatory cell recruitment and atherogenesis. Thus, it is conceivable that reducing MIF might be a potential therapeutic target for patients with atherosclerosis.

The notion that inflammation in RA and atherogenesis are linked is supported by data suggesting that reducing disease activity by adequate disease modifying anti-rheumatic therapy may result in a decrease in cardiovascular mortality.[12, 13] TNF blockade could diminish the
increased cardiovascular risk associated with RA by attenuating not only local but also systemic inflammation associated with atherogenesis.[14, 15]

To explore the relationship between inflammation and factors involved in atherogenesis, we investigated the early and long term effects of anti-TNF therapy on serum MIF levels and known risk factors such as C-reactive protein levels and lipid profile in RA patients.

PATIENTS AND METHODS

Patients.
Fifty RA patients with active disease (Disease Activity Score in 28 joints (DAS28)) ≥ 3.2 were included in the study. All patients received adalimumab 40 mg subcutaneously every other week in combination with methotrexate (MTX) in a stable dose for at least 8 weeks. The concomitant use of prednisone (≤ 10 mg/day) and non-steroidal anti-inflammatory drugs (NSAIDs) was allowed if stable for at least one month. Approval for this study was obtained from the institutional ethics review committee at the Academic Medical Center/ University of Amsterdam. All participants gave written informed consent.

Clinical assessments.
RA disease activity was assessed at baseline, week 16 and 52 after start of adalimumab treatment using the DAS28. Clinical response was evaluated by the EULAR response criteria. For comparison of data between responders (good and moderate) and non-responders we used response measured at week 16. In addition, the presence of extra-articular manifestations (such as vasculitis, nodules, and pleuritis) was noted before entry in the study.

Cardiovascular risk factor profiles.
In the assessment of cardiovascular risk factors the following data were recorded: medical history including cardiovascular events, smoking (current smoker, ever smoker), and current medication; hypertension; dyslipidemia; diabetes; and body mass index (kg/m²).

Lipid profiles.
Serum total cholesterol (TC), high density lipoprotein (HDL), and low density lipoprotein (LDL) cholesterol, triglyceride (TG), and lipoprotein (a) (Lp(a)) levels were assessed by standard laboratory techniques. Apo A-I and apo B levels were measured by an automated nephelometric assay using an array protein system nephelometer (Beckman, Mijdrecht, The Netherlands). In addition, erythrocyte sedimentation rate (ESR) (mm/hour) and C-reactive protein (CRP) levels (mg/L) were determined. Blood was drawn from patients while fasting at baseline and at 16
and 52 weeks after initiation of adalimumab therapy. All values were determined by the GLP certified routine clinical chemistry laboratory at the Academic Medical Center in Amsterdam.

MIF ELISA.

Natural serum MIF levels (pg/ml) were determined with a commercial quantitative sandwich-enzyme-immunoassay (human MIF, DY289, R&D systems Inc, Minneapolis, MN). The assay was performed according to manufacturer’s instructions. Fasting serum samples were stored at -80°C and analyzed all at once.

Statistical analysis.

A paired t-test or the Wilcoxon signed ranks test, whichever was appropriate, was used to determine significant changes from baseline. Probability values < 0.05 were considered statistically significant in a 2-tailed test. This exploratory study was not powered to correct for multiple comparisons by Bonferroni correction. Independent samples t-tests were used for sub-analysis to detect differences in baseline values or changes after treatment between groups. Correlations were assessed with the Pearson product-moment or Spearman rank-order correlation coefficients, whichever was appropriate. Stepwise backward multivariable linear regression analysis was used to identify possible baseline predictors of change in MIF level at week 16 and 52. Because delta MIF levels had a skewed distribution, values were rank-transformed before linear regression analysis. Baseline variables included in the analysis were sex, BMI, MIF, CRP, triglyceride, total cholesterol and HDL levels. Values are expressed as the mean ± standard deviation (SD) or median and interquartile range (IQR), whichever was appropriate. SPSS 12.0.2 for Windows (SPSS, UK) was used.

RESULTS

Patients and clinical response.

The baseline patient characteristics of 50 patients are shown in Table 1. The DAS28 score decreased significantly after 16 weeks (DAS28 3.7 ± 1.2) and 52 weeks (3.4 ± 1.4) of adalimumab therapy compared to baseline (5.6 ± 1.1) (both P < 0.001). At week 16 all patients were evaluable for clinical response: 11 (22%) patients were EULAR non-responders, 25 (50%) moderate responders and 14 (28%) good responders. At week 52 there were 44 patients with an evaluable response of whom 6 (14%) were non-responders, 18 (41%) moderate responders, and 20 (45%) good responders. Six patients dropped out of the study between 16 and 52 weeks follow-up due to lack of efficacy in 5 patients and a serious adverse event in 1 patient.
Pre-treatment serum MIF levels.

Large variability in MIF levels was observed between patients varying from the lowest detectable concentration of 60 pg/ml up to 6571 pg/ml. There was no significant relationship with use of low dose corticosteroids or dosage of MTX, nor with clinical measures of disease activity at baseline (data not shown).

Pre-treatment apo A-I and HDL levels are inversely correlated with systemic inflammation.

There was a negative correlation between pre-treatment apo A-I levels and CRP levels (r = -0.338, P = 0.017) as well as ESR (r = -0.347, P = 0.014). Similarly, pre-treatment HDL cholesterol correlated inversely with CRP levels and ESR (r = -0.290, P = 0.041 and r = -0.340, P = 0.016, respectively).

Interestingly, higher pre-treatment HDL levels correlated with lower MIF levels before initiation of adalimumab therapy (r = -0.294, P = 0.040). As expected, baseline LDL cholesterol was significantly lower in the 6 patients who used statins compared to the other 44 who did not use a statin (P = 0.007); other lipoproteins did not differ between these groups. All patients who used statins were known with a history of hypercholesterolaemia. Four of the 6 patients had a prior cardiovascu-
lar event, and all 6 were on concomitant anti-hypertensive drugs. One patient also had type 1 diabetes.

Sustained downregulation of MIF and inflammatory parameters, but not of HDL cholesterol after adalimumab therapy.

Serum MIF levels were significantly decreased 16 weeks after initiation of adalimumab therapy (median 171 pg/ml, IQR 60-444) compared to baseline (median 333 pg/ml, IQR 93-1544, P = 0.020). This effect was sustained up to week 52 (median 145, IQR 60-335, P = 0.012) (Figure 1).

CRP and ESR levels decreased significantly after 16 and 52 weeks of adalimumab therapy (both P < 0.001, Table 2).

The mean HDL cholesterol levels increased at week 16 compared to baseline (P < 0.001). However, HDL levels returned to nearly baseline at week 52 (Table 2). LDL cholesterol levels did not change after adalimumab treatment. Furthermore, Lp(a) levels decreased significantly at week 16 up to week 52 after treatment (both P = 0.001, Table 2).

Figure 1. Serum MIF levels before, and 16 and 52 weeks after adalimumab therapy. The median values and range are shown for each time point. A large variability in MIF concentration was observed between patients. Some high pre-treatment MIF concentrations may be due to the presence of MIF promoter polymorphisms in certain patients. The presence of such polymorphisms was not analyzed in this study, the data however show that even high baseline MIF concentrations diminish significantly after anti-TNFα therapy.
Table 2. Changes in MIF levels and lipid profile over time.

<table>
<thead>
<tr>
<th></th>
<th>Week 0 (n = 50)</th>
<th>Week 16 (n = 50)</th>
<th>P-value</th>
<th>Week 52 (n = 44)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIF (pg/ml)</td>
<td>333 (90-1544)</td>
<td>171 (60-444)</td>
<td>0.020</td>
<td>145 (60-335)</td>
<td>0.012</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.86 ± 1.07</td>
<td>5.06 ± 1.16</td>
<td>0.053</td>
<td>4.98 ± 1.13</td>
<td>0.301</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.52 ± 0.38</td>
<td>1.66 ± 0.38</td>
<td>&lt;0.001</td>
<td>1.62 ± 0.39</td>
<td>0.061</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.88 ± 0.95</td>
<td>2.96 ± 1.01</td>
<td>0.392</td>
<td>2.93 ± 1.02</td>
<td>0.577</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.01 ± 0.51</td>
<td>0.99 ± 0.54</td>
<td>0.513</td>
<td>0.94 ± 0.43</td>
<td>0.350</td>
</tr>
<tr>
<td>Apo A-I (mmol/L)</td>
<td>1.46 ± 0.25</td>
<td>1.56 ± 0.22</td>
<td>&lt;0.001</td>
<td>1.56 ± 0.21</td>
<td>0.005</td>
</tr>
<tr>
<td>Apo B (mmol/L)</td>
<td>0.99 ± 0.25</td>
<td>1.00 ± 0.27</td>
<td>0.465</td>
<td>0.99 ± 0.28</td>
<td>0.816</td>
</tr>
<tr>
<td>Lp (a) (mmol/L)</td>
<td>198 (65-356)</td>
<td>175 (65-377)</td>
<td>&lt;0.001</td>
<td>171 (47-375)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol/HDL</td>
<td>3.33 ± 0.93</td>
<td>3.15 ± 0.85</td>
<td>0.034</td>
<td>3.19 ± 0.84</td>
<td>0.272</td>
</tr>
<tr>
<td>Apo B/Apo A-I</td>
<td>0.70 ± 0.21</td>
<td>0.65 ± 0.20</td>
<td>0.014</td>
<td>0.65 ± 0.20</td>
<td>0.050</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.13 ± 1.97</td>
<td>4.95 ± 1.14</td>
<td>0.847</td>
<td>5.04 ± 1.34</td>
<td>0.930</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>8.8 (4.6-19.6)</td>
<td>3.8 (1.6-9.1)</td>
<td>&lt;0.001</td>
<td>2.7 (1.1-5.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Values are represented as mean ± standard deviation, or median and interquartile range. P values < 0.05 (two-sided) are significant and shown in Italic.

Chapter 6

Improvement of the atherogenic index after adalimumab therapy.

The mean apo A-I levels (high levels are thought to be cardioprotective) were significantly increased to 1.56 ± 0.22 mmol/L at week 16 compared to 1.46 ± 0.25 mmol/L at baseline (P < 0.001) and remained significantly elevated up to week 52 (1.56 ± 0.22 mmol/L) (P = 0.005). Of interest, apo B levels (an indicator of the total number of atherogenic particles) did not change over the course of 52 weeks adalimumab therapy. Thus, there was a significant decrease in the apo B/A-I ratio at week 16 (P = 0.014), which remained lowered up to week 52 (P = 0.050). The total cholesterol/HDL ratio showed temporary improvement (P = 0.034), due to the increase in HDL levels at week 16, which had diminished again one year after start of treatment (Table 2).

Baseline MIF concentrations and gender predict changes in MIF after adalimumab treatment.

With stepwise backward multivariable linear regression analysis we identified baseline predictors for change in MIF levels after treatment. Included in the analysis were the following baseline variables: sex, BMI, MIF, CRP, triglyceride, total cholesterol and HDL levels. No baseline predictors for change in MIF concentration over time were identified other than patient gender and pre-treatment MIF concentrations. Baseline MIF concentration in combination with gender predicted 45 % of the variance in change of MIF concentration at week 16 (adjusted $R^2 = 0.453$). MIF concentration at baseline alone predicted 38% of the variance in change of MIF concentration at week 52 (adjusted $R^2 = 0.383$). A significantly larger decrease in MIF concentration was seen in female compared to male patients at week 16 (P = 0.011), but no gender difference was observed at week 52. We observed no association between baseline levels of inflammation or lipid profile and change in MIF levels after treatment.

Changes in MIF levels and lipid profile in relationship to clinical response.

We analyzed whether changes in MIF concentration differ between EULAR responders versus non-responders. We found no relationship between clinical response and changes in MIF.
change in lipid profile and MIF levels after TNF blockade

1. concentration at week 16 nor at week 52. However, the decrease in Lp(a) concentration was
greater in responders than in non-responders at week 16 (P = 0.018), with a similar trend at
week 52 (P = 0.087). Furthermore, in accordance with previous data, an increase in HDL levels
at week 16 was associated with a decrease in DAS28 score at the same time point (r = -0.308, P =
0.030).[16] Similarly, HDL levels increased more in EULAR responders than in non-responders,
although this difference did not reach statistical significance (P = 0.068). The increase in apo A-I
was not different between response groups.

10. DISCUSSION

12. Both RA and atherosclerosis are related to chronic inflammation. There is increasing evidence
that TNF and MIF are involved in these conditions and that the role of these cytokines is linked.
[4, 8, 17] In both RA and atherosclerosis enhanced MIF levels have been observed at the site of
inflammation [17, 18] and MIF was shown to mediate leukocyte recruitment into the inflamed
joint and vessel wall.[19, 20] Furthermore, MIF can mediate integrin activation and induce
expression of other inflammatory cytokines, such as IL-6, and TNF, and matrix metallopro-
teinases (MMP) associated with joint damage in RA and plaque instability in atherosclerosis.
[20-22] The role of TNF is supported by the observation that anti-TNF therapy may reduce the
increased cardiovascular risk associated with RA by decreasing systemic inflammation. Previ-
ous work has shown that TNF blockade may influence lipid levels, insulin resistance, vascular
adhesion molecule expression, and endothelial function.[16, 23-26] We performed the present
study to provide more insight into the mechanisms that could be involved in the effects of
anti-TNF therapy on cardiovascular risk. The results confirm our hypothesis that adalimumab
treatment leads to downregulation of MIF with potential beneficial consequences for vascular
inflammation. Moreover, we show for the first time that long term TNF blocking therapy with
adalimumab has a favorable influence on the lipid profile of RA patients.

29. It has previously been suggested that chronic systemic inflammation in RA and subsequent
atherosclerosis are in part the result of chronic cytokine overflow from the inflamed joints into
the circulation.[2] Anti-TNF therapy has been shown to diminish local inflammation in the joint
by decreasing synovial cell infiltration and expression of adhesion molecules, chemokines, and
cytokines, which coincides with a reduction of acute phase reactants.[24, 27-29] A decrease in
CRP levels was previously shown to be accompanied by a reduction in synovial MIF and TNF
expression in the same patient when disease activity was reduced by conventional disease-
modifying antirheumatic drug (DMARD) therapy.[30] In light of these data we hypothesized
that pro-inflammatory cytokine release from the inflamed joint could be diminished after adali-
mumab treatment, resulting in a decrease in systemic levels of cytokines, including MIF (Figure
2). Consistent with this notion we found serum MIF levels to be significantly downregulated
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Figure 2. Schematic view of the interaction between MIF and TNF.
Both macrophages and T cells as well as dendritic cells and fibroblast-like synoviocytes produce MIF and TNF. TNF induces the production of MIF, and vice versa TNF production can be induced by MIF. [8, 17] In RA increased levels of MIF and TNF have been found locally in the synovial fluid and synovial tissue, which perpetuate the inflammatory process not only by inducing further cytokine secretion, but also by enhancing leukocyte migration towards the site of inflammation.[19] With anti-TNF antibody therapy available bioactive TNF is neutralized. Furthermore, the infiltration of the inflamed synovium by macrophages (main producers of TNF and MIF) was shown to diminish early after treatment.[27] Hence, both the number of MIF producing cells as well as the concentration of bioactive TNF decreases after anti-TNF therapy potentially leading to a decrease in systemic MIF levels.
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within 16 weeks after adalimumab therapy, an effect that was sustained up to one year after initiation of treatment. Whether anti-TNF therapy reduces MIF in the atherosclerotic lesion in patients is as yet unknown, but beneficial effects of TNF inhibition on atherosclerotic lesions have been demonstrated in animal atherosclerosis models.[31] A decrease in MIF expression could lead to reduced monocyte and T cell influx into the inflamed vessel wall, hence arresting plaque formation.[7]

HDL levels increased temporarily after treatment, resulting in an improved atherogenic index (total cholesterol/ HDL cholesterol) at week 16, which was no longer present after 1 year of adalimumab treatment. A brief rise in HDL cholesterol, also known as an inverse phase reaction, is to be expected after reversing the inflammatory state and was previously reported in other studies with infliximab.[16, 32] In addition we observed a sustained increase in apo A-I levels and thus improvement of the apo B/A-I ratio. Based on these findings one can speculate that the apo B/A-I ratio may better reflect the cardiovascular risk profile after TNF blocking therapy than the traditional atherogenic index (total cholesterol/ HDL), as differential effects of adalimumab therapy can be observed for apo A-I and HDL levels. Of interest, serum apo-A1 has previously been shown to inhibit T-cell contact induced monocyte activation.[33] As
Change in lipid profile and MIF levels after TNF blockade

1. As a result, cytokine (TNFα and IL-1β) production by monocytes was inhibited while monocyte proliferation remained unaltered. These data indicate a novel anti-inflammatory mechanism of this apolipoprotein. Conceivably, the sustained increase of apo-A1 levels in our study might lead to inhibition of T-cell induced monocyte activation, both in the inflamed synovium and in the vessel wall.

2. Lipoprotein (a) has been demonstrated to have a spectrum of pathogenic activities among which increased vascular adhesion molecule expression, chemotaxis of monocytes, foam cell formation, smooth muscle cell proliferation, and increased platelet aggregation. Different clinical studies have shown Lp(a) levels to be an independent risk factor for developing CHD.[34, 35] Hence, the significant decrease in Lp(a) levels both 16 and 52 weeks after adalimumab therapy could contribute to a decrease in the pro-atherogenic state. This could be a direct effect of anti-TNF therapy but may also be an indirect of the overall diminishment in inflammation.

3. The open label rather than placebo-controlled design is obviously a limitation of this study, as we cannot conclude with complete certainty that the decrease in MIF levels resulting from decreased inflammation was a direct effect of TNF blockade or merely a result of regression to the mean. However, the patients had persistent disease activity in spite of at least 2 conventional DMARDs before inclusion in the study, suggesting that the reduction of inflammation was the result of TNF blockade. Thus, the data presented in this exploratory study provide the rationale for future studies with a controlled design to confirm the effects on lipid profiles and MIF levels in relationship to cardiovascular endpoints.

4. In conclusion, TNF blocking therapy reduced systemic MIF levels, possibly reflecting a reduction in atherogenic state. Apart from reduced systemic inflammation as shown by reduced CRP and ESR levels, the sustained decrease in apo B/apo A-I ratio suggests a favorable effect of adalimumab treatment on markers associated with atherogenesis.

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REFERENCE LIST


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