

Anti-Tumor Necrosis Factor- α Treatment Improves Endothelial Function in Patients With Rheumatoid Arthritis

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Background—Rheumatoid arthritis (RA) is associated with accelerated atherosclerosis and increased cardiovascular morbidity and mortality. Striking similarities exist in the inflammatory and immunologic response in RA and atherosclerosis. Indeed, adhesion molecules and cytokines, tumor necrosis factor (TNF)- α in particular, are key mediators of joint inflammation and of vascular dysfunction and progression of atherosclerotic vascular disease. Hence, the aim of the present study was to assess the effect of chronic antiinflammatory treatment with the anti-TNF- α antibody infliximab on disease activity and endothelial function in patients with active RA.

Methods and Results—Eleven RA patients (mean age 46 ± 5 years; disease duration 9 ± 2 years) with high disease activity despite treatment with stable doses of methotrexate (≤ 25 mg/wk) and prednisone (≤ 10 mg/d) were investigated. Clinical status and endothelium-dependent and -independent vasodilation of the brachial artery as assessed by high-resolution ultrasound were measured before and after 12 weeks of infliximab therapy. Flow-mediated vasodilation improved from $3.2\pm 0.4\%$ to $4.1\pm 0.5\%$ ($P=0.018$), whereas endothelium-independent vasodilation with nitroglycerin and baseline diameter remained unchanged ($13.6\pm 1.2\%$ versus $12.8\pm 1.4\%$, $P=0.98$, and 3.74 ± 0.15 versus 3.66 ± 0.11 mm, $P=0.54$, respectively). Disease activity score (DAS28) was significantly reduced, from 5.6 ± 0.3 to 3.5 ± 0.6 ($P=0.002$). Erythrocyte sedimentation rate and C-reactive protein were lowered from 34 ± 7 to 19 ± 5 mm/h ($P=0.04$) and from 38 ± 11 to 15 ± 10 mg/L ($P=0.08$), respectively.

Conclusions—This is the first study to show that anti-TNF- α treatment improves endothelial function in RA. The data suggest that in RA, endothelial dysfunction is part of the disease process and is mediated by TNF- α . (*Circulation*. 2002; 106:2184-2187.)

Key Words: endothelium ■ inflammation ■ atherosclerosis ■ risk factors

Similarities exist between the paradigm of inflammation in the pathogenesis of both atherosclerotic vascular disease and rheumatoid arthritis (RA).^{1,2} The shared features include involvement of cytokines such as tumor necrosis factor (TNF)- α and interleukin-6²; raised concentrations of C-reactive protein, fibrinogen, and amyloid-A²; increased local expression of adhesion molecules and endothelin-1;¹ and neoangiogenesis and collagen degradation via activation of macrophages and mast cells.³ These similarities raise the possibility that inflammatory mechanisms responsible for synovial lesions in patients with RA might also involve the vessel wall and facilitate the development of atherosclerotic lesions. This may explain the excess in cardiovascular disease in patients with RA.

TNF- α is a cytokine with a wide range of proinflammatory activities; it occupies a pivotal role in the initiation and

amplification of the inflammatory cascade, both in RA and in atherogenesis.^{4,5} Because RA patients show up to a 5-fold increased cardiovascular mortality, and anti-TNF- α treatment has been shown to improve the clinical course and outcome in RA,⁶ the aim of the present study was to evaluate the effect of TNF- α inhibition on endothelium-dependent vasodilation in the context of systemic inflammation.

Methods

Patients

Eleven patients with RA according to the criteria of the American Rheumatism Association⁷ (mean age 46 ± 5 years; disease duration 9 ± 2 years) with high disease activity despite treatment with methotrexate (≤ 25 mg/wk) and prednisone (≤ 10 mg/d) were included. Patients had to be taking a stable dose of methotrexate (≤ 25 mg/wk, mean 21 ± 2 mg) for at least 4 months and a stable dose of prednisone (≤ 10 mg/d, mean 6 ± 1 mg) for at least 1 month before entering the

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study. Exclusion criteria were age <18 years, uncontrolled arterial hypertension (>160/90 mm Hg), insulin-dependent diabetes mellitus, and total cholesterol >6.5 mmol/L. In these patients with a high disease activity, the clinical benefit of established anti-TNF- α therapy could not be withheld, and a placebo-controlled study design was not considered ethically appropriate.

The study protocol was approved by the local ethics committee.

Study Protocol

Flow-mediated dilation and nitroglycerin-induced (0.4 mg sublingual, Nitrolingual Spray, Pohl-Boskamp) vasodilation of the brachial artery were assessed before and 12 weeks after standard treatment with infliximab (3 mg/kg at week 0, 2, and 6) by a high-resolution ultrasound vessel wall tracking device with a 10-MHz linear-array transducer (WTS-2, Pie Medical). Flow-mediated dilation of the brachial artery was induced by release of a wrist cuff inflated to suprasystolic pressure for 5 minutes. After release, the arterial diameter was recorded every 15 seconds for 3 minutes. After nitroglycerin application, the diameter was recorded every 30 seconds for 6 minutes. To minimize observer bias, ultrasound data were analyzed offline by an operator blinded as to clinical data and unaware of the patient's visit. Concomitant medication was not changed throughout the study course.

Clinical evaluation was performed on the same day as endothelial function measurements by standard assessment protocols or questionnaires. The Disease Activity Score (DAS28)⁸ assesses disease activity by including tender and swollen joint count and the erythrocyte sedimentation rate. It ranges from 0 (lowest) to 10 (highest activity). Scores below 2 were considered as remission.⁸ The Health Assessment Questionnaire completed by the patient assesses physical functional impairment and has shown to be relevant for morbidity and mortality.^{9,10} Scores range from 0 (no impairment) to 3 (maximum impairment).

Statistical Analysis

Results are presented as mean \pm SEM unless stated otherwise. Measurements of flow-mediated dilation and nitroglycerin-induced vasodilation represent the maximal increase in brachial diastolic artery diameter and are expressed as percent change from baseline.^{11,12} Differences in parameters before and after infliximab treatment were examined by 2-tailed paired Student's *t* test (StatView 3.4, Abacus Concepts). Statistical significance was accepted at $P < 0.05$.

Results

The baseline demographic and clinical characteristics of the patients are presented in the Table.

Vascular Function

Flow-mediated (endothelium-dependent) dilation improved significantly from $3.2 \pm 0.4\%$ to $4.1 \pm 0.5\%$ after 12 weeks of treatment with infliximab ($P = 0.018$). Endothelium-independent vasodilation induced by nitroglycerin remained unchanged ($13.6 \pm 1.2\%$ versus $12.8 \pm 1.4\%$; $P = 0.98$; Table and Figure) There were no significant differences in baseline brachial artery diameter between visits (3.74 ± 0.15 versus 3.66 ± 0.11 mm; $P = 0.54$). Flow-mediated dilation in RA patients at baseline was significantly impaired compared with a healthy control group ($n = 18$) matched for factors known to affect endothelial function, eg, cholesterol and blood pressure ($5.0 \pm 0.5\%$ versus $3.2 \pm 0.4\%$; $P = 0.023$; data not shown).

Inflammation Markers and Clinical Parameters

Erythrocyte sedimentation rate and C-reactive protein were lowered from 34 ± 7 to 19 ± 5 mm/h ($P = 0.04$) and from 38 ± 11 to 15 ± 10 mg/L ($P = 0.08$), respectively. Disease activity score (DAS28) improved significantly from 5.6 ± 0.3

Baseline Characteristics and Results

	Baseline	After Infliximab	<i>P</i>
Age, y	46 \pm 5
Women, %	82
Rheumatoid factor positive, %	91
Disease duration, y	9 \pm 2
Smoking, %	18
Medication	
Methotrexate, mg/wk	21 \pm 2
Prednisone, %	64
NSAID, %	36
Folic acid, %	91
Mean arterial pressure, mm Hg	91	93	0.28
Cholesterol, mmol/L	4.8 \pm 0.3	5.0 \pm 0.4	0.77
C-reactive protein, mg/L	38 \pm 11	15 \pm 10	0.08
Erythrocyte sedimentation rate, mm/h	34 \pm 7	19 \pm 5	0.04
Homocysteine, mmol/L	16.0 \pm 3.7	15.3 \pm 3.1	0.48
Baseline artery diameter, mm	3.74 \pm 0.15	3.66 \pm 0.11	0.54
Flow-mediated dilation, %	3.2 \pm 0.4	4.1 \pm 0.5	0.018
Nitroglycerin-induced dilation, %	13.6 \pm 1.2	12.8 \pm 1.4	0.98
Disease activity score*	5.6 \pm 0.3	3.5 \pm 0.6	0.002
HAQ score*	1.8 \pm 0.1	1.2 \pm 0.3	0.054

NSAID indicates nonsteroidal antiinflammatory drug; HAQ, Health Assessment Questionnaire.

Results are expressed as mean \pm SEM, except for percentages.

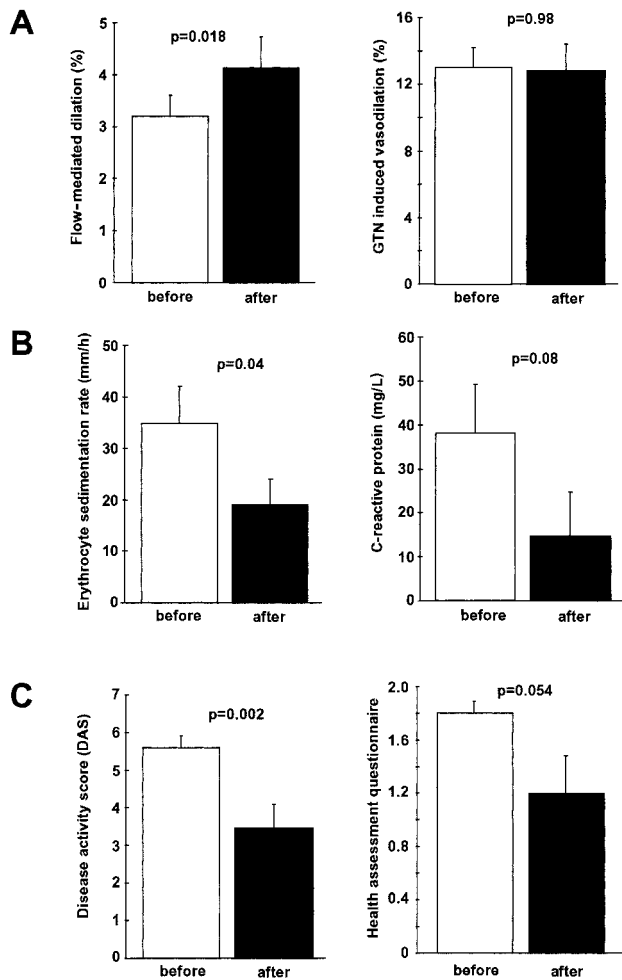
*Disease Activity Score ranged from 0 (lowest activity) to 10 (highest activity); Health Assessment Questionnaire score ranged from 0 (no impairment) to 3 (maximum impairment).

to 3.5 ± 0.6 ($P = 0.002$) during treatment. Improvement in the Health Assessment Questionnaire Score did not reach statistical significance (1.8 ± 0.1 versus 1.2 ± 0.3 ; $P = 0.054$).

Discussion

The results of the present study demonstrate for the first time that anti-TNF- α treatment not only ameliorates the disease process but also substantially improves vascular function in RA. Patients with RA show an increase in both cardiovascular morbidity and mortality.¹³ Interestingly, methotrexate reduces cardiovascular morbidity and mortality in RA compared with other disease-modifying antirheumatic drugs, such as sulfasalazine, penicillamine, and gold.¹⁴ The mechanisms by which cardiovascular protection is provided remains to be determined, but suppression of systemic and vascular inflammation may be involved. Because impaired endothelial function is a key event in the progression of atherosclerotic vascular disease and is associated with an increased cardiovascular event rate,^{15,16} the results of the present study strongly suggest that TNF- α -mediated inflammatory changes play an important role in vascular dysfunction in RA.

TNF- α is an important mediator of systemic and vascular wall inflammation, both in RA and in atherosclerotic vascular disease. Indeed, plasma concentrations of TNF- α are directly associated with the degree of early carotid atherosclerosis¹⁷ and the impairment of vascular function in systemic lupus



A, Endothelial function measurements. Flow-mediated dilation improved significantly from $3.2 \pm 0.4\%$ to $4.1 \pm 0.5\%$ after infliximab ($P=0.018$), whereas nitroglycerin-induced vasodilation was unchanged ($13.6 \pm 1.2\%$ versus $12.8 \pm 1.4\%$; $P=0.98$). B, Plasma markers of inflammation. Erythrocyte sedimentation rate and C-reactive protein were lowered from 34 ± 7 to 19 ± 5 mm/h ($P=0.04$) and from 38 ± 11 to 15 ± 10 mg/L ($P=0.08$), respectively. C, Clinical assessment. Disease activity score (DAS28) and Health Assessment Questionnaire (HAQ) scores were lowered from 5.6 ± 0.3 to 3.5 ± 0.6 ($P=0.002$) and from 1.8 ± 0.1 to 1.2 ± 0.3 ($P=0.054$), respectively. GTN indicates nitroglycerin.

erythematosus.^{18,19} Interestingly, short-term administration of TNF- α severely depresses endothelium-dependent relaxation in rats.²⁰ The beneficial effects of anti-TNF- α treatment on endothelial function in the present study are in line with data from Fichtlscherer et al²¹ demonstrating improved endothelium-dependent vasodilation by TNF- α antagonism with etanercept in patients with chronic heart failure. Whereas these effects were only transient because of the short half-life of etanercept, infliximab at the currently applied dosage ensures therapeutically relevant plasma levels for as long as 8 weeks.⁶

Furthermore, various effects of TNF- α are mediated by the induction of a cellular state consistent with oxidative stress.²² Moreover, TNF- α blocks the activation of endothelial nitric oxide synthase (eNOS) by interfering with the phosphorylation of Akt,²³ which is essential for flow-dependent and acetylcholine-dependent relaxation of blood vessels.²⁴ In

addition to the posttranscriptional inactivation of eNOS, TNF- α directly degrades eNOS mRNA.²⁵

The results of the present study are of particular interest, because only patients with high disease activity despite at least 4 months of intensive disease-modifying antirheumatic drug treatment concomitant with methotrexate were included, and still a beneficial effect on vascular function was observed.

Conclusions

The present study provides the first evidence that TNF- α antagonism not only reduces RA activity but also improves endothelial function. These results add to the growing amount of literature confirming atherosclerosis as an inflammatory condition and showing that inflammatory diseases such as RA are associated with an increased risk and therefore incidence of atherosclerotic disease. In view of the proatherogenic effects of intravascular inflammation, it remains to be determined whether innate immunity is involved in atherogenesis, because an efficient innate immune defense may offer an early advantage at the expense of chronic vascular damage in later years.²⁶ In view of the beneficial effects of methotrexate on prognosis in RA, the results of the present study suggest that TNF- α antagonism may provide additional benefit in these patients at cardiovascular risk. Whether and to what degree amelioration of endothelial dysfunction found in the present study translates into clinical benefit needs to be determined in large-scale clinical trials. Future research in this area could lead to improvements in the understanding and management of cardiovascular complications associated with RA and other chronic inflammatory diseases, as well as to potential new antiinflammatory strategies for atherosclerosis in general.

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