Activation of the Immune System and Inflammatory Activity in Relation to Markers of Atherothrombotic Disease and Atherosclerosis in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To measure markers of atherogenesis and thrombogenesis in patients with rheumatoid arthritis (RA) and in matched controls, and to relate these variables to markers of inflammation and endothelial activation, and to the presence of atherosclerosis.

Methods. Thirty-nine patients with RA with disease onset between 1974 and 1978, who were younger than 65 years at the present study in 1997, were investigated together with 39 age and sex matched controls. IgG, IgA, and IgM antibodies against oxidized low density lipoprotein (oxLDL ab), malondialdehyde LDL (MDA-LDL ab) and cardiolipin (aCL) were measured by ELISA, circulating immune complexes (CIC) were isolated by precipitation, and homocysteine was measured with HPLC. Hemostatic factors of endothelial origin, i.e., plasminogen activator inhibitor-1 (PAI-1 mass), von Willebrand Factor (vWF), and D-dimer were analyzed by ELISA, and tissue plasminogen activator (tPA) activity was analyzed by a chromogen method. The products analyzed in the RA group correlated with soluble adhesion molecules (sICAM-1, sE-selectin), acute phase reactants, interleukin 6 (IL-6), and IL-2sRα, all measured by ELISA, and with accumulated disease activity. The factors were also related to the presence of plaque measured by duplex scanning. Factor analysis was performed to subgroup data in order to find latent variables.

Results. Patients with RA had significantly higher levels of oxLDL ab (IgM), MDA-LDL ab (IgA, IgM classes), aCL (IgG, IgA, IgM classes), CIC, homocysteine, PAI-1 mass, and D-dimer than controls. Patients also had significantly higher levels of sICAM-1, sE-selectin, IL-6, and IL-2sRα. PAI-1 mass, D-dimer, vWF, CIC, and aCL (IgM, IgA) correlated with erythrocyte sedimentation rate (ESR), and, with the exception of vWF, to accumulated disease activity. CIC correlated significantly with IgM antibodies against oxLDL and aCL. ESR, IL-2sRα, PAI-1, tPA activity, vWF, D-dimer, homocysteine, aCL (IgA), and MDA-LDL ab (IgA) correlated with sICAM-1. ESR, haptoglobin, IL-2sRα, PAI-1 mass, D-dimer, aCL (IgM), and MDA-LDL ab (IgM) correlated with sE-selectin. sICAM-1 was significantly higher in patients with plaque. In factor analysis, presence of atherosclerotic plaque had loadings of one latent variable together with adhesion molecules and IL-2sRα and together with antibodies of, in particular, IgM type of another one.

Conclusion. Several different etiopathogenic mechanisms for increased cardiovascular mortality in RA are implicated. Continuous endothelial activation is suggested by increased levels of adhesion molecules sICAM-1 and sE-selectin, which correlate with hemostatic factors of endothelial origin and with T cell activation as measured by IL2sRα. That factor analysis showed loadings of one variable on antilipid antibodies and plaque and another on T cell activation and plaque indicates that the immune system is involved in the development of atherosclerosis in RA. (J Rheumatol 2002;29:875–82)

Key Indexing Terms:

RHEUMATOID ARTHRITIS | IMMUNE ACTIVATION | INFLAMMATION
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An increased death rate due to cardiovascular disease (CVD) in patients with rheumatoid arthritis (RA) has been reported in several studies[1-4], although the underlying pathogenic mechanisms have not been identified. We previously found a significant relationship between markers of inflammatory activity, particularly erythrocyte sedimentation rate (ESR) and haptoglobin, and progression of CVD, as measured by the first cardiovascular event[5,6].

Atherosclerosis has recently been recognized as an inflammatory disease[7] that can cause a systemic acute phase response[8,9]. Immunocompetent cells such as macrophages and specific subtypes of T lymphocytes participate in the formation of atherosclerotic plaque[10]. A large number of immune factors, including cytokines, adhesion molecules, and receptors have been identified in plaque[10]. Several antigens have been suggested to be associated with the induction of atherosclerosis. According to a leading hypothesis, oxidative modification of low density lipoprotein (LDL) renders it immunogenic, leading to formation of autoantibodies[11]. The presence of antibodies to oxidized LDL (oxLDL ab) has been reported to relate to atherosclerosis and to predict myocardial infarction (MI)[12,13]. Antibodies to the epitope malondialdehyde LDL (MDA-LDL ab), one of the major end products of lipid peroxidation[11], are predictive of the development of atherosclerosis in the general population[14]. In patients with early RA, oxLDL ab were reported to be raised and to correlate with ESR and C-reactive protein (CRP)[15].

Circulating immune complexes (CIC) have been reported to occur in increased concentrations in young survivors of MI[16] and are strong and independent risk factors for development of MI in healthy individuals[17]. Antibodies to cardiolipin (aCL) are associated with venous and arterial thrombosis in the antiphospholipid antibody syndrome[18] and have been shown to predict MI in healthy subjects[13,19]. Homocysteine has atherogenic properties[20], and further is a marker of arterial thrombogenic complications, particularly in systemic lupus erythematosus[21]. Increased levels of homocysteine have been reported in RA[22], but the significance of this finding has yet to be elucidated.

In contrast to the established action of immunological and inflammatory mechanisms in atherosclerosis *per se*, knowledge about the atherosclerotic process and its relationship to these mechanisms in RA is sparse. We measured antibodies against modified lipoproteins and phospholipid cardiolipin, i.e., ox LDL ab, MDA-LDL ab, and aCL, in addition to CIC, homocysteine, and hemostatic factors, in patients with medium term RA and in age and sex matched controls. In the RA group, the atherogenic and/or thrombogenic variables were considered in relation to markers of endothelial activation, i.e., soluble adhesion molecules. The variables were also correlated with acute phase reactants, with markers of immune activation including the proinflammatory cytokine interleukin 6 (IL-6), and the soluble α-chain of IL-2 receptor (IL-2sRα; CD25) and with accumulated disease activity scores. Further, the relationship of immunological and inflammatory activity with atherosclerosis, as measured by ultrasound registered atherosclerotic plaque, was investigated in the patient group.

MATERIALS AND METHODS

Subjects. All patients registered at the Department of Rheumatology, University Hospital, Umeå, between 1974 and 1979 (n = 211) with early (i.e., duration ≤ 1 year) seropositive RA[23] were included in an earlier study on predictors of CVD and overall mortality[5]. Of these, all patients younger than 65 years in 1997 were invited to participate in the present study. Seven patients declined and 3 patients of the original cohort had died before initiating the study. Thirty-nine patients (30 female, 9 male) with a mean age of 51.6 years (range 37–65) and a disease duration between 19 and 23 years were admitted to the study.

Controls were 39 age and sex matched individuals randomly assembled from the population register of the same region. Of the original control group, one was excluded and replaced, since she had been subjected to a prosthetic operation of the iliac artery and thus, in part, lacked a normal endothelium.

At the time of blood sampling, 10 study patients were receiving corticosteroids, and 26 patients were taking various disease modifying antirheumatic drugs, i.e., methotrexate (n = 8), sulfasalazine (n = 4), myocrisine (n = 7), chloroquine/hydroxychloroquine phosphate (n = 5), azathioprine (n = 5), and penicillamine (n = 1). Additionally 7 patients with RA and 4 control subjects were receiving hormone replacement therapy.

The presence of established CVD risk factors in patients and controls is presented in Table 1. There were no significant differences between the groups.

Clinical disease activity data accumulated longitudinally was assessed retrospectively according to recorded information as described by Baeckman, *et al*[24]. The activity score takes into account number of swollen and tender joints, ESR, and global disease activity, as judged by the clinician, at presentation, after one year and subsequently every 2 years after disease onset.

The study was approved by the local ethics committee and all participants gave their informed consent.

Blood sampling. All blood samples were drawn between 8:00 and 11:00 AM within a 6 week period. ESR was performed on whole blood. Non-anticoagulated blood was collected, after an overnight fast, for analysis of cholesterol, while EDTA plasma was collected for protein analysis by electrophoresis, without prior freezing. For analysis of hemostatic factors, blood was collected into Stabilyte tubes for tissue plasminogen activator (tPA) activity and plasminogen activator inhibitor-1 (PAI-1) activity and into citrate tubes for von Willebrand factor (vWF) and PAI-1 mass. Serum was used for all other analyses. After centrifugation at 2000 × g for 20 min plasma and serum were stored at –80°C until assayed.

Table 1. Established cardiovascular risk factors in 39 RA patients with 19–23 years of disease duration, and 39 age and sex-matched controls. Results are presented as median and range in parentheses when appropriate.

Variable	RA, n = 39	Controls, n = 39
Hypertension, treated	9	5
Diabetes mellitus	1	0
Smoking, current or former	18	22
Cardiovascular event ever	1	1
Body mass index	22.68 (18.59–36.18)	23.74 (18.07–34.21)
s–Cholesterol mmol/l	5.20 (3.70–8.14)	5.80 (4.70–7.40)

Isolation and oxidative modifications of lipoproteins. Serum lipoproteins were isolated by preparative ultracentrifugation as described[25]. Oxidative modifications of LDL were done using cupric sulfate and malondialdehyde-*bis*-dimethylacetal according to published protocols[26,27].

Determination of antibodies against cardiolipin, oxLDL, and MDA-LDL. Autoantibodies against cardiolipin, oxLDL, and MDA-LDL were determined using slight modifications of published protocols[13,26]. After incubation and washing with phosphate buffered saline (PBS), 50 μl of secondary antibody diluted 1/1000 in PBS-1% bovine serum albumin were added. Secondary antibodies were alkaline phosphatase labeled affinity purified anti-human IgG (Sigma A-3150), IgM (A-3275), or IgA (A-3400).

In the determination of antibodies against oxLDL and MDA-LDL, plates were coated with antigens at a final concentration of 5 μg/ml per well. Positive and negative control samples were included on each assay plate. Optical density (OD) was read at 405 nm until positive control value reached OD of 1.0 ± 0.1. Antibody levels were expressed as OD units.

Isolation of circulating immune complex by precipitation. CIC were isolated by precipitation of 200 μl of serum with an equal volume of 5% polyethylene glycol 6000 and the protein concentration subsequently measured by the Lowry method[28,29].

Hemostatic factors. vWF was determined by ELISA using antibody and conjugated-second antibody (Dako, Gentofte, Denmark). ELISA methods were also used for analyses of PAI-mass (Immulite PAI-1; Biopool, Umeå, Sweden) and for the fibrin split product D-dimer (NycoCard D-dimer, Nycomed, Lidingö, Sweden). Functional activity of PAI-1 and tPA was analyzed by biofunctional immunosorbent assay and chromogen methods, respectively (Chromolize PAI-1 and Spectrolyse tPA, Biopool, Umeå, Sweden). Homocysteine was determined by high performance liquid chromatography. Haptoglobin and CRP were determined by immunoturbidimetry. IL-6, IL-2sRα, soluble intercellular adhesion molecule-1 (sICAM-1), and soluble E-selectin (sE-selectin) were measured using ELISA (R&D Systems, Minneapolis, MN, USA). Cholesterol was determined by dry chemistry on a Vitros 950 IRC multianalyzer (Johnson & Johnson, Clinical Diagnostics Inc., New York, NY, USA).

Ultrasound measurements. The presence of plaque was identified by B-mode ultrasound as described[30]. Briefly, the presence of plaque of the common carotid and the common femoral arteries of the right side were determined by a semiquantitative method.

Statistics. Differences in continuous data between groups were analyzed statistically with either the Wilcoxon signed rank test or the Mann-Whitney U test. Category data were analyzed by chi-square test. Correlations between variables were analyzed by Spearman's rank correlation. These calculations were performed using the StatView 4.51 program (Brain Power, Calabasas, CA, USA). Factor analysis, i.e., explorative data analysis, was performed to find patterns among data that could be interpreted meaningfully. Briefly, in factor analysis, observations are examined to see if data could have been generated by a model involving a number of unobservable, latent variables, fundamental to the data generating process. The best variables, and linear combinations of them, are used to "explain" the observed data. The present factor analysis was based on Spearman correlation, and factor loadings > 0.3 were considered. The factor analysis was performed by SPSS with Varimax, with Kaiser normalization as a rotation method. All p values refer to 2 sided tests; $p < 0.05$ was considered significant. Formation of clusters of variables with high loadings of the same latent variable in the factor analysis, and with a hypothetical pathogenetic relationship (i.e., acute phase reactants, IgM antibodies, and immune factors) was performed. Canonic correlation between these clusters of variables, based on Spearman rank correlation, was evaluated.

RESULTS

Markers of immune activation. Patients with RA had significantly higher levels of acute phase reactants, i.e., ESR, CRP, and haptoglobin, as well as of IL-6 and IL-2sRα, compared with controls (Table 2). The levels of soluble adhesion molecules, sICAM-1 and sE-selectin, were also significantly higher in patients with RA compared with controls. The mean accumulated disease activity score was 4.5 (range 3.0–6.1).

Table 2. Acute phase reactants, IL–6, IL–2sRα and adhesion molecules (sICAM–1, sE-selectin), in 39 RA patients with 19–23 years of disease duration, and in 39 age and sex-matched controls. Results presented as median (range).

Variable	RA	Controls	p<
ESR, mm/h	22.0 (2.0–90.0)	6.0 (2.0–22.0)	0.001
Haptoglobin, g/l	1.56 (0.1–5.83)	1.01 (0.38–1.96)	0.001
CRP, mg/l	10 (5–129)	5 (5–5)	0.001
IL–6, pg/ml	7.20 (3.12–102.50)	3.12 (3.12–3.12)	0.001
IL-2sRα pg/ml	1516 (784–2597)	1007 (664–2096)	0.001
sICAM–1, ng/ml	364 (181–1369)	247 (133–407)	0.001
sE-selectin, ng/ml	67.1 (24.5–191.5)	53.7 (20.9–116.7)	0.01

Factors of potential atherogenic and thrombogenic importance. As presented in Table 3, patients with RA had significantly higher levels of IgM oxLDL ab and of IgM and IgA MDA-LDL ab compared with controls. Patients with RA also had significantly higher levels of IgG, IgM and IgA aCL, CIC, homocysteine, PAI-1 mass, and D-dimer.

The CIC level correlated significantly with IgM antibodies against oxLDL ($r_s = 0.36$, $p < 0.05$) and CL ($r_s = 0.49$, $p < 0.01$) in patients with RA (data not shown).

In patients with RA, IgM aCL showed significant correlation with IgM oxLDL ab ($r_s = 0.61$, $p < 0.001$) and with IgM MDA-LDL ab ($r_s = 0.59$, $p < 0.001$). The IgA aCL also correlated with IgA antibodies against oxLDL and MDA-LDL ($r_s = 0.41$, $p < 0.05$ and $r_s = 0.36$, $p < 0.05$, respectively). Oxidized LDL ab of the 3 isotypes correlated significantly with MDA-LDL ab (IgG: $r_s = 0.42$, $p < 0.05$, IgM: $r_s = 0.57$, $p < 0.001$, IgA: $r_s = 0.57$, $p < 0.001$).

Correlation of atherothrombogenic factors with markers of inflammatory activity. The levels of aCL (IgA and IgM classes) and CIC correlated with ESR in patients with RA (Table 4). The CIC level correlated significantly with IL-6. Only homocysteine was significantly correlated with IL-2sRα. The hemostatic factors PAI-mass, vWF, and D-dimer correlated significantly with ESR. The PAI-1 mass level and D-dimer correlated significantly with haptoglobin, while PAI-1 mass also correlated with IL-6.

The levels of IgM aCL, CIC, homocysteine, PAI-1 mass, and D-dimer all correlated significantly with the accumulated disease activity score.

IgG MDA-LDL ab showed an inverse correlation with IL-6 ($r_s = -0.32$, $p < 0.05$, data not shown). Oxidized LDL ab did not correlate with any of the measured markers of inflammation in univariate analysis.

Correlation of atherothrombogenic factor and markers of inflammation with adhesion molecules. When the associa-

Table 3. Levels of factors of importance for atherothrombogenesis in 39 patients with RA and 39 age and sex matched controls. Results presented as median (range).

Variable	RA, n = 39	Controls, n = 39	p
OxLDL			
IgG	0.441 (0.191–1.045)	0.423 (0.112–0.915)	NS
IgA	0.524 (0.113–1.476)	0.440 (0.153–1.046)	= 0.07
IgM	0.509 (0.091–1.666)	0.250 (0.070–1.180)	< 0.001
MDA-LDL			
IgG	0.609 (0.264–1.280)	0.514 (0.211–1.139)	= 0.06
IgA	0.359 (0.146–1.780)	0.278 (0.97–0.518)	< 0.05
IgM	0.896 (0.238–2.680)	0.423 (0.167–1.831)	< 0.001
aCL			
IgG	0.903 (0.335–1.536)	0.751 (0.425–1.210)	< 0.05
IgA	0.451 (0.172–1.157)	0.357 (0.169–0.664)	< 0.01
IgM	0.417 (0.131–1.900)	0.203 (0.075–0.621)	< 0.001
CIC, μg/ml	136.41 (5.74–223,29)	114.71 (2.94–170.29)	< 0.01
Homocysteine, μmol/l	8.0 (4.0–28.0)	7.0 (4.0–13.0)	< 0.01
PAI-1 mass, ng/ml	34.50 (10.50–260.00)	17.75 (3.50–76.00)	< 0.01
tPA-activity, IU/ml	0.2 (0–0.9)	0.1 (0.0–0.8)	NS
D-dimer, mg/l	0.30 (0.05–3.70)	0.10 (0.05–0.30)	< 0.001
vWF, IE/ml	1.34 (0.80–2.96)	1.23 (0.51–2.1)	NS

NS: not significant.

tion with markers of atherothrombogenesis was analyzed, sICAM-1 showed a correlation with MDA-LDL ab (IgA), aCL (IgA), homocysteine, PAI-1 mass, tPA activity, vWF, D-dimer, and an inverse correlation with PAI-1 activity (Table 5). Soluble ICAM-1 did not correlate with oxLDL ab of any isotype.

Soluble E-selectin correlated significantly with PAI-1 mass, D-dimer, aCL (IgM), and with MDA-LDL ab (IgM).

Soluble ICAM-1 levels correlated with ESR and with IL-2sRα but not with haptoglobin or IL-6 in RA. Soluble E-selectin correlated with ESR, haptoglobin, and IL-2sRα (Table 5).

There was a strong correlation between sICAM-1 and sE-selectin ($r_s = 0.48$, $p < 0.01$).

Relation to the presence of plaque. Soluble ICAM-1 was significantly higher in those patients (18/38; 47%) with evidence of atherosclerotic plaque ($p < 0.05$, data not shown). The presence of plaque did not correlate with oxLDL ab, MDA-LDL ab, or aCL of any isotype nor with CIC in univariate analysis.

Explorative data analysis. Factor analysis including data on levels of oxLDL ab, MDA-LDL ab, aCL, CIC, homocysteine, ESR, haptoglobin, IL-6, IL-2sRα, adhesion molecules, hemostatic factors, and presence of atherosclerotic plaque yielded 5 latent variables. These had an eigenvalue > 1.3, accounting for 65% of the total variation between these variables (Table 6). These included one latent variable showing large loadings on sE-selectin, sICAM-1, and IL-2sRα, and some on homocysteine. Further, this variable showed loading on presence of plaques. A second latent variable showed the largest loadings on the IgM isotype of aCL, oxLDL ab, and MDA-LDL ab, and on CIC, and this variable also showed loading on presence of plaques. A third variable had large loadings on the IgA isotype of oxLDL ab, MDA-LDL ab, and aCL, on D-dimer and on PAI-1 mass. A fourth latent variable showed the largest loadings on the acute phase reactants (ESR, haptoglobin, IL-6), CIC, and PAI-1 mass. The fifth factor showed its heaviest loadings on tPA activity, some on homocysteine, and inverse loading on oxLDL ab.

Canonic correlation revealed that the cluster of IgM ab (MDA-LDL, aCL) and CIC correlated with the cluster of acute phase reactants (ESR, haptoglobin; $r_s = 0.68$, $p < 0.01$). The cluster comprising factors associated with immune activation (IL-2sRα, sICAM-1, and sE-selectin) also correlated with the acute phase reactants (ESR, haptoglobin, IL-6; $r_s = 0.62$, $p < 0.05$). Further, there was a correlation between the cluster of IgM ab and CIC and immunological factors (IL-2sRα, sICAM-1, and sE-selectin; $r_s = 0.67$, $p < 0.05$).

DISCUSSION

We attempted to analyze the relationship between antibodies of potential atherogenic importance as well as hemostatic factors and markers of endothelial cell activation and of the immune system, in relation to atherosclerotic plaques, in patients with a chronic inflammatory joint disease. The patient cohort comprised virtually all patients with RA younger than 65 years and with disease onset recorded between 1974 to 1978. Thus, the cohort comprised the whole spectrum of patients from those with active disease to those in total remission, and consequently, the median

Table 4. Correlations (Spearman's rank correlation) between factors of presumed significance for atherogenesis and thrombogenesis and clinical and laboratory markers of inflammation in 39 patients with RA.

Variable	ESR	Haptoglobin	IL-6	IL-2sRα	Accumulated Disease Activity[a]
aCL					
IgG	0.00	–0.27	–0.11	–0.22	NS
IgA	0.41*	0.20	0.24	0.29	NS
IgM	0.40*	–0.03	0.04	–0.02	0.41*
CIC	0.59***	0.21	0.38*	0.01	0.50**
Homocysteine	0.29	0.08	0.13	0.39*	0.36*
PAI-1 mass	0.63***	0.43**	0.37*	0.16	0.42*
vWF	0.39*	0.21	0.28	0.26	NS
D-dimer	0.41*	0.44**	0.28	0.24	0.39*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; [a]Accumulated disease activity = Average score over time, based on ESR, number of arthritic joints and doctor's global assessment[24]. NS: not significant.

Table 5. Correlations (Spearman's rank correlation) between adhesion molecules and factors of importance for atherogenesis/thrombogenesis and inflammatory variables, respectively, in 39 patients with RA.

Variable	sICAM-1	sE-selectin
ESR	0.36*	0.34*
Haptoglobin	NS	0.34*
IL-2sRα	0.65***	0.56***
MDA LDL ab		
IgA	0.44**	NS
IgM	NS	0.32*
aCL		
IgA	0.44**	NS
IgM	NS	0.37*
Homocysteine	0.41*	NS
PAI-1 activity	–0.37*	NS
PAI-1 mass	0.54***	0.44***
tPA activity	0.38*	NS
vWF	0.37*	NS
D-dimer	0.34*	0.33*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS: not significant.

Table 6. Factor analysis with analysis of latent variables for oxLDL ab, MDA LDL ab, aCL, CIC, homocysteine, ESR, haptoglobin, IL-6 and IL-2sRα, adhesion molecules (sICAM-1, sE-selectin), hemostase factors (PAI-1 mass, tPA activity, D-Dimer, vWF), and plaque in 39 patients with RA. Numbers denote factor loadings > 0.3.

Variable	1	2	3	4	5
oxLDL ab					
IgG	—	—	—	—	–0.739
IgA	—	—	0.836	—	—
IgM	—	0.828	—	—	—
MDA LDL ab					
IgG	—	–	0.396	–0.472	–0.397
IgA	—	0.361	0.775	—	—
IgM	—	0.720	0.371	—	—
aCL					
IgG	–0.334	0.477	—	—	—
IgA	—	—	0.511	—	—
IgM	—	0.842	—	—	—
CIC	—	0.549	—	0.503	—
Homocysteine	0.493	—	—	—	0.547
ESR	—	—	—	0.782	—
Haptoglobin	—	—	0.322	0.701	—
IL6	—	—	—	0.809	—
IL2sRα	0.822	—	—	—	—
sICAM-1	0.688	—	0.364	—	—
sE-selectin	0.719	—	—	—	—
PAI-1 mass	—	0.390	0.537	0.497	—
tPA activity	—	—	—	—	0.855
D-Dimer	—	—	0.637	—	0.348
vWF	—	—	—	0.394	—
Plaque	0.433	0.435	—	—	—

values of disease activity markers (i.e., ESR, CRP, haptoglobin) are not very high. However, the cohort does exhibit an evident increase of immunological markers (i.e., IL-6, IL-2sRα) and endothelial cell derived products (sICAM-1 and sE-selectin).

The increased serum levels of adhesion molecules compared with controls, particularly the raised level of sE-selectin, may reflect an upregulation of endothelial expression of adhesion molecules in patients with RA. Shedding of E-selectin is restricted to activated endothelial cells[31]. Soluble E-selectin was significantly increased in patients with incident coronary heart disease and carotid atherosclerosis[32]. ICAM-1 is a member of the immunoglobulin supergene family and a natural ligand for the lymphocyte function-associated antigen-1 molecule and is expressed on macrophages as well as on vessel endothelium after stimulation with various inflammatory cytokines[33]. The level of sICAM-1 has been shown to predict CVD in healthy individuals[34]. In the present study, sE-selectin was not significantly correlated to atherosclerotic manifestations (as assessed by univariate analysis), but correlated with hemostatic factors (i.e., PAI-1 mass, D-dimer). Soluble ICAM-1 was, however, significantly higher in the RA patients with

plaque than in those without atherosclerotic manifestations. Further, there was a significant correlation between sE-selectin and sICAM-1. The loading on adhesion molecules, as well as on the presence of atherosclerotic plaques, of the same latent variable in the factor analysis further supports the concept that presence of prolonged endothelial activation leads to the formation of atherosclerotic plaque. Our results are compatible with those of Aoki, *et al*[35], who found increased sICAM-1 mainly in RA patients with endothelial involvement in the form of vasculitis, leading them to suggest sICAM-1 to be a marker of vasculitis. We found that sICAM-1 correlated with ESR, but not with CRP or IL-6, and did not have loadings of the same factor as these variables in the factor analysis. This finding argues against sICAM-1 having a simple acute phase role that is upregulated by proinflammatory cytokines. The correlations between the adhesion molecules, particularly sICAM-1, and factors involved in hemostasis, some of which (i.e., PAI-1, tPA, and vWF) are also products of endothelial cells, is another indication that endothelial activation may contribute to the atherothrombotic process in RA. The soluble α-chain of the IL-2 receptor, a marker of T cell activation[36], had statistical loadings of the same variable as adhesion molecules in factor analysis (Table 6, variable 1). This may indicate that T lymphocytes are involved in the endothelial upregulation in RA. The level of IL-2sRα has, in some studies, been suggested to vary with disease activity[37], but the relevance of this point is contentious[38]. An association between IL-2sRα and endothelial cell processes is further supported by loadings of the same latent variable as that of homocysteine in the factor analysis. Homocysteine has been reported to exert direct as well as indirect injurious effects on the endothelium[20].

Increased production of antibodies is a consequence of B cell activation. We found significantly increased levels of IgM antibodies to oxLDL and of both IgM and IgA antibodies to its epitope, MDA-LDL, in patients with RA. In an earlier study on oxLDL ab in juvenile chronic arthritis, IgG levels were significantly higher than in controls[39]. Increased levels of antibodies to oxLDL were also reported in early RA, but without identifying which Ig subtypes were involved[15]. Contrary to that report, we found no correlation between oxLDL ab and any markers of inflammation in univariate analysis. However, we found a strong correlation between CIC, which were also significantly elevated in the RA patients, and IgM antibodies to both CL and oxLDL. The loadings of one latent variable on CIC and on the IgM isotype of CL and oxLDL/MDA-LDL in the factor analysis (Table 6, variable 2) indicate that these antibodies constitute CIC. Further, the IgM and IgA isotypes of antibodies to oxLDL and MDA-LDL and CL intercorrelated. It is possible that aCL recognizes epitopes generated during a process in which lipid peroxidation and the generation of CIC are components. Increased generation of oxLDL ab and/or CIC may damage the endothelium in the chronic inflammatory process in RA. The canonic correlation between the cluster of IgM ab and CIC on one hand (variable 2) and endothelial adhesion molecules (variable 1) on the other may further support an interaction between these processes rather than a causal relationship. IgG antibodies to MDA-LDL have been reported to predict atherosclerosis in the normal population[14], while antibodies against oxLDL and CL of both IgG and IgA isotype could predict MI in healthy men[13]. In atherosclerotic, apolipoprotein E-deficient mice, however, autoantibodies to epitopes of oxLDL were exclusively of the IgM class[40]. Whether the high levels of antibodies to oxLDL really create a risk for atherosclerosis has been debated. Some authors have reported contradictory findings and a protective role of these antibodies has been suggested, at least in the early phase of atherosclerosis[41]. In our RA group, we found loadings of the same latent variable on antibodies to oxLDL, MDA-LDL, and CL of the IgM isotype, on CIC and on presence of plaque in the factor analysis (Table 6, variable 2).

In factor analysis, one latent variable had loadings on the inflammatory markers (i.e., ESR, haptoglobin, and IL-6), on CIC (Table 6, variable 4), and on hemostastic factors of endothelial origin. From this we conclude that a continuous state of inflammatory activity, causing an abnormal presence of CIC, may contribute directly to a vascular lesion.

Several factors of potential thrombogenic importance (i.e., PAI-1 mass, vWF, D-dimer, and aCL) were significantly higher in the RA group than in controls. Despite the relatively low disease activity in the RA group, all these factors correlated with inflammatory variables, particularly ESR and the accumulated disease activity score, and this relationship was further supported in factor analysis (Table 6, variable 4). This emphasizes the close relationship between inflammatory activity in RA and alterations of hemostatic factors towards hypercoagulability, as demonstrated by ourselves and others[6,42]. In an earlier study, we found PAI-1 to be a strong predictor of cardiovascular event in active RA[6]. In addition to its direct involvement in the hemostasis, PAI-1 may, as a key component of the fibrinolytic process, play an important role in plaque formation and subsequent rupture by activating plaque destabilizing metalloproteinases[43].

These results describe increased levels of several potentially atherogenic and/or thrombogenic factors of particular importance in RA, but do not indicate a uniform etiopathogenic mechanism for the observed increased cardiovascular morbidity and mortality in RA. It is interesting, however, that despite low grade inflammatory activity and a lack of clinically overt vasculitis, a continuous activation of the endothelium is apparent. This would promote an accelerated atherosclerosis, as indicated by a relationship between atherosclerotic plaques and both adhesion molecules and specific antibodies, as well as a disturbed hemostasis.

Apparently, there are several processes of potential importance for atherogenesis and thrombogenesis involving concurrent action of T as well as of B lymphocytes.

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REFERENCES

1. Allebeck P. Increased mortality in rheumatoid arthritis. Scand J Rheumatol 1982;11:81-6.
2. Wolfe F, Mitchell DM, Sibley JT, et al. The mortality of rheumatoid arthritis. Arthritis Rheum 1994;37:481-94.
3. Myllykangas-Luosujärvi R, Aho K, Kautiainen H, Isomäki H. Cardiovascular mortality in females with rheumatoid arthritis. J Rheumatol 1995;22:1065-7.
4. Wallberg-Jonsson S, Ohman M-L, Rantapää-Dahlqvist S. Cardiovascular morbidity and mortality in patients with seropositive rheumatoid arthritis in Northern Sweden. J Rheumatol 1997; 24:445-51.
5. Wallberg-Jonsson S, Johansson H, Öhman M-L, Rantapää- Dahlqvist S. Extent of inflammation predicts cardiovascular disease and overall mortality in seropositive rheumatoid arthritis. J Rheumatol 1999;26:2562-71.
6. Wallberg-Jonsson S, Cederfeldt M, Rantapää-Dahlqvist S. Hemostatic factors and cardiovascular disease in active rheumatoid arthritis — an 8 year followup study. J Rheumatol 2000;27:71-5.
7. Ross R. Atherosclerosis — an inflammatory disease. N Engl J Med 1999;340:115-26.
8. Kuller LH, Tracy R, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested control study. Am J Epidemiol 1996;144:537-47.
9. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973-9.
10. Hansson GK. Cell-mediated immunity in atherosclerosis. Curr Opin Lipidol 1997;8:301-11.
11. Witztum JL, Steinberg D. Role of oxidized low density lipolipoprotein in atherogenesis. J Clin Invest 1991;88:1785-92.
12. Puurunen M, Mänttäri M, Manninen V, et al. Antibody against oxidized low-density lipoprotein predicting myocardial infarction. Arch Intern Med 1994;154:2605-9.
13. Wu R, Nityanand S, Berglund L, Lithell H, Holm G, Lefvert AK. Antibodies against cardiolipin and oxidatively modified LDL in 50-year-old men predict myocardial infarction. Arterioscler Thromb Vasc Biol 1997;17:3159-63.
14. Salonen JT, Yla-Herttula S, Yamamoto R, et al. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. Lancet 1992;339:883-7.
15. Paimela L, Helve T, Leirisalo-Repo M, et al. Clinical significance of antibodies against oxidised low density lipoprotein in early RA. Ann Rheum Dis 1996;55:558-63.
16. Lefvert A-K, Hamsten A, Holm G. Association between circulating immune complexes, complement C4 null alleles, and myocardial infarction before age 45 years. Arterioscler Thromb Vasc Biol 1995;15:665-8.
17. Mustafa A, Nityanand S, Berglund L, Lithell H, Lefvert A-K. Circulating immune complexes in 50-year old men as a strong and independent risk factor for myocardial infarction. Circulation 2000;102:2576-81.
18. Hughes GRV. The antiphospholipid syndrome: ten years on. Lancet 1993;342:341-4.
19. Vaarala O, Mänttäri M, Manninen V, et al. Anti-cardiolipin antibodies and risk of myocardial infarction in a prospective cohort of middle-aged men. Circulation 1995;91:23-7.
20. Guthikonda S, Haynes WG. Homocysteine as a novel risk factor for atherosclerosis. Curr Opin Cardiol 1999;14:283-91.
21. Petri M, Roubenhoff R, Dallal G, Nadeau M, Selhub J, Rosenberg I. Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. Lancet 1996;348:1120-4.
22. Pettersson T, Abrahamsson L, Nilsson B, Norberg B. Serum homocysteine and methylmalonic acid in patients with rheumatoid arthritis and cobalaminopenia. J Rheumatol 1998;25:859-63.
23. Ropes MW, Bennet GA, Cobb S. Diagnostic criteria for rheumatoid arthritis. Ann Rheum Dis 1959;18:49-53.
24. Baeckman E, Ekbom A, Sparen P, Feltelius N, Klareskog L. Disease activity and risk of lymphoma in patients with rheumatoid arthritis: nested case-control study. BMJ 1998;317:180-1.
25. Carlson K. Lipoprotein fractionation. J Clin Pathol 1973;5 Suppl:32-7.
26. Wu R, Lefvert A-K. Autoantibodies against oxidized low density lipoproteins (oxLDL): characterization of antibody isotype, subclass, affinity and effect on the macrophage uptake of oxLDL. Clin Exp Immunol 1995;102:174-80.
27. Zhou X, Paulsson G, Stemme S, Hansson GK. Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice. J Clin Invest 1998;101:1717-25.
28. Creighton WD, Lambert PH, Miescher PA. Detection of antibodies and soluble antigen-antibody complexes by precipitation with polyethylene glycol. J Immunol 1973;111:1219-27.
29. Mustafa A, Hamsten A, Holm G, Lefvert A-K. Circulating immune complexes induced by food proteins implicated in precocious myocardial infarction. Ann Med 2001;33:103-12.
30. Wendelhag I, Wiklund O, Wikstrand J. Atherosclerotic changes in the femoral and carotid arteries in familial hypercholesterolemia. Ultrasonographic assessment of intima-media thickness and plaque occurrence. Arterioscler Thromb 1993;13:1404-11.
31. Gedeit RG. Tumor necrosis factor induces E-selectin expression on vascular endothelial cells. Crit Care Med 1996;24:1543-6.
32. Hwang S-J, Ballantyne CM, Sharrett AR, et al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 1997;96:4219-25.
33. Mojcik CF, Shevach EM. Adhesion molecules. A rheumatologic perspective. Arthritis Rheum 1997;6:991-1004.
34. Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma concentration of soluble intracellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet 1998;351:88-92.
35. Aoki S, Imai K, Yachi A. Soluble intercellular adhesion molecule-1 (ICAM-1) antigen in patients with rheumatoid arthritis. Scand J Immunol 1993;38:485-90.
36. Rubin LA, Kurman CC, Fritz ME, et al. Soluble interleukin-2 receptors are released from activated human lymphoid cells in vitro. J Immunol 1985;135:3172-7.
37. Matsumoto T, Iwasaka K. Clinical and laboratory parameters which affect soluble interleukin-2 receptor levels in the serum and synovial fluids of patients with rheumatoid arthritis. Ann Rheum Dis 1993;52:876-80.
38. Merkel PA, Dooley MA, Dawson DH, Pisetsky DS, Polisson RP. Interleukin-2 receptor levels in sera of patients with rheumatoid arthritis treated with sulfasalazine, parenteral gold, or placebo. J Rheumatol 1996;23:1856-61.
39. Savolainen A, von Essen R, Alfthan G, Vaarala O, Palosuo T, Aho K. Antibodies against low-density lipoprotein in juvenile chronic

arthritis. Scand J Rheumatol 1995;24:209-11.
40. Palinski W, Harkko S, Miller E, et al. Cloning of monoclonal autoantibodies to epitopes of oxidized lipoproteins from a polipoprotein E-deficient mice. Demonstration of epitopes of oxidized low density lipoprotein in human plasma. J Clin Invest 1996;98:800-14.
41 Palinski W, Miller E, Witztum JL. Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. Proc Natl Acad Sci USA 1995;92:821-5.
42. Kopeikina LT, Kamper EF, Koutsokos V, Bassiakos Y, Stavridis I. Imbalance of tissue-type plasminogen activator (t-PA) and its specific inhibitor (PAI-1) in patients with rheumatoid arthritis associated with disease activity. Clin Rheumatol 1997;16:254-60.
43. Van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 1994;89:36-44.