Tumor Necrosis Factor-α –1031 T/C Polymorphism Is Associated with Smaller and More Proatherogenic Low Density Lipoprotein Particles in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To study, in patients with rheumatoid arthritis (RA), the association between the tumor necrosis factor (TNF)-α –1031 T/C polymorphism and quantitative and qualitative low density lipoprotein (LDL) characteristics.

Methods. From a sample of 100 patients with RA and 100 controls, we used the strategy of "recruit by genotype" to select 30 patients with RA: 15 carriers of the rare allele (C) and 15 homozygous for the more frequent allele (T). These were matched with 30 controls. Plasma lipoprotein profile including size distribution of lipoproteins was determined using nuclear magnetic resonance spectrometry. LDL susceptibility to oxidation was assessed by diene formation. The LDL affinity for extracellular matrix was determined using electrophoretic mobility shift assay. Genotyping was performed by SnapShot.

Results. Compared to TT patients with RA, carriers of the C allele had (1) LDL particles significantly smaller [20.74 (0.68) nm vs 21.18 (0.52), $p < 0.02$]; (2) LDL particles with a greater affinity for the proteoglycans (i.e., with a lower Kd) [197.26 (123.98) nmol/l vs 259.26 (139.31), $p = 0.05$]; and (3) LDL particles with significantly greater susceptibility to oxidation [shorter lag phase: 47 (20.01) min vs 74 (41.8), $p < 0.03$, and higher maximal rate of diene production: 3.1 (0.5) mol/min vs 2.6 (0.95), $p < 0.05$]. None of these differences was observed in the control group.

Conclusion. In patients with RA, genetic variability in the TNF-α gene is associated with smaller LDL particles that have a greater affinity for extracellular matrix and higher susceptibility to oxidation. Because these characteristics are associated with a greater risk of atherosclerosis, identification of such predisposition in patients with RA could help in implementing early preventive intervention measures against cardiovascular disease. (First Release July 15 2008; J Rheumatol 2008;35:1697–703)

Key Indexing Terms:
LIPOPROTEINS
TUMOR NECROSIS FACTOR-α
POLYMORPHISM
RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory disease with a multifactorial etiology. The initiating factors, although not well studied, are known to act on individuals who are genetically predisposed to the disease. Inflammation in these patients has been associated with a high cardiovascular disease (CVD) mortality rate[1-5] due to an accelerated atherogenesis process[6]. Although a great pool of cytokines plays a role in RA, current evidence suggests that tumor necrosis factor (TNF)-α is one of the key cytokines in inflammation associated with the disease. This is based on the observation that TNF-α concentrations are increased not only in the inflamed joints but also in plasma of patients with RA, the levels correlating positively with the activity of the disease and with the joint function impairment[7,8]. TNF-α also plays a very important role in the inflammation that accompanies atherosclerosis and clinical studies have demonstrated that elevated concentrations of TNF-α are associated with a greater risk of ischemic heart disease[9]. Clinical studies have indicated that small, dense low density lipoprotein (LDL) particles are associated with a higher risk of CVD because of their greater affinity for the proteoglycans of the extracellular matrix of the arterial intima, as well as their higher susceptibility to oxidative modification[10,11]. Oxidative stress plays a key role in the pathogenesis of arteriosclerosis[12-14], and has been shown to be increased in patients with RA and inversely correlated with antioxidant vitamins A and E[15].

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Funded in part by ISCIII-FIS PI030786 and CIBERDEM.

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Accepted for publication April 14, 2008.

Previous studies from our group have demonstrated that patients with RA have increased concentrations of small dense plasma LDL that have a high affinity for the proteoglycans of the extracellular matrix[16]. This atherogenic profile, combined with the presence of elevated markers of inflammation such as C-reactive protein (CRP), secretory phospholipase A_2 ($sPLA_2$-IIA), and TNF-α could explain, in part, the elevated CVD mortality observed in these patients with RA.

The concentrations of TNF-α are a response to chronic inflammatory stimuli and are modified by environmental as well as genetic factors. The different inflammatory responses to the same stimulus in different individuals can be explained on the basis of different genetic variants. The promoter region of the TNF-α gene contains several single-nucleotide polymorphisms (SNP) that can influence the production of TNF-α. Studies have evaluated the association between these SNP and CVD. The results have been inconclusive[17-19]. Similarly, in patients with RA, the association between different polymorphisms of TNF-α and the severity of RA, or susceptibility to the disease, has been studied, but with inconclusive results[20-24]. In patients with juvenile chronic arthritis, the C allele in position –1031 of the TNF-α gene has been observed to be associated with an increase in the production of TNF-α[21]. The association of this polymorphism with proatherogenic measures has not yet been evaluated in patients with RA.

Based on these data, and taking into account that atherosclerosis as well as RA are diseases of multifactorial origin and are the result of a process of chronic inflammation, we planned to investigate, in patients with RA, the association between the –1031 C polymorphism of the TNF-α gene and the proatherogenic measures such as LDL particle size and the susceptibility of these particles to oxidation.

MATERIALS AND METHODS

Patients. A sample of 100 patients diagnosed as having RA according to the 1987 criteria of the American College of Rheumatology and attending the outpatient clinic of the Hospital del Mar de Barcelona was studied. Due to methodological complexity, we used the strategy of "recruit by genotype"[25,26] to select from among these 100 patients with RA 15 patients who were carriers of the rare (C) allele of the –1031 T/C polymorphism of the TNF-α gene and 15 patients homozygous for the more frequent (T) allele. For comparisons, we selected 30 matched control subjects from a population of 100 unrelated individuals. No patient with RA or control subject had any clinical or laboratory evidence of renal insufficiency, chronic hepatic disease, thyroid disease, concomitant infection, diabetes mellitus, CVD, or neoplasia. Patient medications are summarized in Table 1. Our study was approved by the local ethics committee for clinical investigation.

Lipid profile and plasma lipoprotein subclasses. Fasting venous blood samples were taken into plastic tubes containing EDTA (1 mg/ml) as anticoagulant. The plasma was obtained immediately by centrifugation (10 min, 2000 rpm, 4˚C) and 10 μM of BHT was added to the plasma to protect the sample from lipid peroxidation. Aliquots were stored at –80˚C until required for analyses.

Total plasma cholesterol and triglycerides (TG) were measured by enzymatic methods (Roche Diagnostics Scandinavian AB, Bromma, Sweden). The concentrations of apolipoprotein B (apo B) and apolipoprotein A1 (apo A1) were determined by immunological methods. The size distributions of the lipoprotein subclasses were determined using nuclear magnetic resonance (NMR) spectrometry. Termed the NMR LipoProfile, the technique simultaneously quantifies the subclasses of lipoproteins, the lipid content, and the size of the particle[27]. In brief, each lipoprotein subclass is quantified using the NMR signals, which differ in frequency and shape depending on the diameter of the lipoprotein particles. The individual signals are derived from the total recorded signal using data from previously modeled reference lipoprotein subclasses. The intensity of each signal is proportional to the quantity of the subclass, which is reported in particle concentration units. NMR spectroscopy identified 6 very low density lipoprotein (VLDL) subclasses, with VLDL-1 being the smallest size particle and VLDL-6 being the largest, 3 LDL subclasses, LDL-1 the smallest and LDL-3 the largest, and 5 HDL subclasses, HDL-1 the smallest and HDL-5 the largest. Lipoprotein nomenclature is based on the methodological procedure for determining the particle size distribution, and is not related to the physiology of the lipoproteins.

Table 1. Clinical and biochemical characteristics of controls and patients with rheumatoid arthritis (RA).

	Controls, n = 30	RA Patients, n = 30
Characteristic		
Disease duration, yrs	—	10 (7.9)
Rheumatoid factor, % positive	—	93.3
Rheumatoid factor, value	—	242.0 (56.4)
HAQ, range 0–3	—	1.7 (0.6)
Swollen joint count, range 0–66	—	13.2 (5.6)
Tender joint count, range 0–68	—	18.4 (8.0)
Treatment		
Patients taking DMARD, %	—	86.6
Patients taking NSAID, %	—	93.3
Patients taking corticosteroids, %	—	90.0
Doses of prednisone equivalent, mg/day	_	6.5 (2.8)
Inflammation markers		
CRP, mg/l	0.16 (0.2)	3.7 (2.1)*
ESR, mm/h	14.9 (10.5)	67.7 (25.6)*

Values are expressed as mean (SD). * $p < 0.001$ versus controls. HAQ: Health Assessment Questionnaire; DMARD: disease modifying antirheumatic drugs; NSAID: nonsteroidal antiinflammatory drugs; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

Interaction between LDL and proteoglycans. LDL was obtained by sequential preparative ultracentrifugation[28] in a Kontron 45.6 rotor (Teknico 1055, Kontron Instruments, Sweden). The interaction between LDL and chondroitin-6-sulfate (C6S) glycosaminoglycan (GAG; Seikagaku Corp., Tokyo, Japan) in physiological saline was determined using electrophoretic mobility shift assay (EMSA) according to the method of Hurt-Camejo, *et al*[29]. This technique is based on the observation that LDL particles freed from GAG units have different migrations in agarose gels and, as such, have different banding patterns.

The results are expressed as the affinity (Kd) of LDL for C6S-GAG in nmol/l, a low Kd indicating high affinity and vice versa.

Susceptibility of LDL to oxidation. LDL samples were desalted and EDTA removed by filtration in PD10 columns. The samples were equilibrated in phosphate buffered saline (PBS). To study the susceptibility of LDL to oxidation, the kinetics of conjugated diene formation induced by hemin were used[30]. Hemin, a product of *in vivo* hemoglobin degradation, binds and oxidizes lipoproteins. The kinetics of LDL oxidation (concentration of 0.1 mg apo B/ml) were determined by monitoring the change in absorbance at 250 nm at 30˚C in an ultraviolet (UV) spectrophotometer (Uvikon 922, Kontron) in the presence of hemin (2.5 μM) and H_2O_2 (10 μM) over 4 h. Oxidation of LDL is measured with 3 characteristic indices: (1) the lag phase (LP) is defined as the time interval (min) in which endogenous antioxidants are con-

sumed; (2) the maximal rate (MR; mol dienes/mol LDL x min) is an index derived from the propagation phase that begins with the acceleration of oxidation of the polyunsaturated fatty acids; (3) the maximum diene production (MDP; mol dienes/mol LDL) represents the maximal amount of dienes produced during LDL oxidation, and is a function of the content of peroxidation substrates. These 3 variables (LP, MR, and MDP) provide more information on the susceptibility of LDL to oxidation, and their uses are widespread.

Inflammation markers. Plasma concentrations of TNF-α were measured using specific ELISA kits (R&D Systems, Abingdon, UK). CRP (mg/l) was measured using a commercial kit (Medix Biochemica, Finland). Plasma concentrations of $sPLA_2$-IIA were determined by ELISA using a monoclonal antibody (Cayman Chemical, Ann Arbor, MI, USA) for capture, together with a polyclonal antibody for colorimetric detection. Other variables such as fibrinogen (mg/l), hemoglobin, rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), and renal and hepatic function were determined using standard methodology.

Plasma concentrations of vitamins A and E. Concentrations of vitamins A and E were determined by high pressure liquid chromatography (Hewlett Packard 1050) equipped with a UV-visible detector, using retinol acetate and tocopherol acetate as internal standards. The column was a Spherisorb ODS (125 x 4 mm, 5 µm) and the mobile phase was methanol:water (100% for vitamin E and 98% for vitamin A)[31].

Genetic analyses. Genomic DNA from each subject was obtained from peripheral blood leukocytes using QIAmpBlood kits (Qiagen, Barcelona, Spain). Starting with 100 ng of DNA, the region that contained the polymorphism was amplified by polymerase chain reaction (PCR) using oligonucleotide TNF-α-1 (5' TGA TGG ACT CAC CAG GTG AGG 3') as forward primer and oligonucleotide TNF-α-2 (5' GGC TCT TTC ACT CCC TGG G3') as reverse primer. Amplification was performed using universal conditions in a thermocycler 2400 (Applied Biosystems, Madrid, Spain) and, once the process was concluded, the PCR product was purified with QIAquick PCR Purification Kit (Qiagen). The –1031 T/C polymorphism was detected by SnapShot[32] in an ABI-Prism 310 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions, and using the genotyping primer 5' AAG GAG AAG CTG AGA AGA 3'.

Statistical analyses. The results are presented as means (standard deviation). The differences between groups were compared using analysis of variance with the variables adjusted for age, body mass index (BMI), and sex. The categorical variables were analyzed using the chi-squared test. All p values < 0.05 were considered statistically significant. The SPSS statistical package (version 13.0, SPSS, Chicago, IL, USA) was used throughout.

RESULTS

The clinical and biochemical characteristics of patients with RA and controls are presented in Table 1.

The majority of the patients with RA had positive RF (93.3%) and had radiologically-identified erosions (90%). The mean age of the patients and controls was 53.2 (12.6) and 53 (13.1) years, respectively. The mean duration of disease in patients with RA was 10 years (range 1–30). Patients had been treated with nonsteroidal antiinflammatory drugs (NSAID) in 93.3% of cases, corticosteroids in 90%, and disease modifying antirheumatic drugs (DMARD) in 86.6%. There were no significant differences between the groups with respect to age, sex, and BMI. Patients had significantly higher levels of inflammation markers (CRP and ESR) compared to controls ($p < 0.001$).

Table 2 summarizes the associations of the TNF-α polymorphism with the measures of inflammation in patients and controls.

In patients with RA, carriers of the C allele had TNF-α concentrations that were 1.6-fold higher than carriers of the TT genotype (nonsignificant, $p = 0.096$). No statistical associations between the other measures of inflammation studied and the polymorphism were observed in patients with RA. The controls with the C allele had significantly lower BMI than the homozygous TT [mean 26.10 (3.57) vs 29.75 (4.95); $p < 0.027$]. In controls, the C allele was associated with significantly lower $sPLA_2$-IIA [mean 11.72 (6.04) ng/ml vs 18.19 (11.02); $p < 0.05$]. A similar tendency was observed in patients with RA, but did not reach statistical significance.

Concentrations of vitamin E were significantly elevated in the control carriers of the C allele [mean 13.21 (3.54) mg/l vs 10.92 (1.6); $p < 0.05$] compared to TT controls (Table 3). Although we did not observe significant differences in the patients with RA, the carriers of the C allele had a tendency towards lower concentrations of the vitamins studied.

RA patients with the C allele had LDL particles that were significantly smaller than the carriers of the TT genotype, even when normalized for TG concentration [mean 20.74 (0.68) nm vs 21.18 (0.52); $p < 0.02$] (Figure 1). None of the other lipid measures studied in patients and controls (lipid profile; Table 3) showed significant differences between the carriers of the different allelic genotypes. Medications (prednisone, NSAID, or DMARD) that might have affected lipid levels were not different between the genotypes. LDL from the RA subjects with the C allele had a greater affinity for the proteoglycans, i.e., with a lower Kd [197.26 (123.98) nmol/l vs 259.26 (139.31); $p = 0.05$] (Figure 2). Figure 3 depicts the association between the measures of oxidation and the TNF-α –1031 polymorphism in patients with RA. RA patients with the C allele had significantly greater LDL oxidation potential as characterized by a shorter LP (less resistance to oxidation; Figure 3A) and an elevated MR (greater rate production of oxidized LDL; Figure 3B) than the patients homozygous for the T allele [mean 47 (20.01) min vs 74 (41.8); $p < 0.03$, and mean 3.1 (0.5) mol/min vs 2.6 (0.95); $p < 0.05$, respectively]. None of these differences was observed in the control group.

DISCUSSION

To our knowledge, this is the first study showing a significant association between the –1031 T/C polymorphism of the TNF-α gene and atherogenic lipid markers in patients with RA. TNF-α plays a key role in inflammation not only in RA but also in arteriosclerosis. Previous studies suggest that the elevated concentration of TNF-α is an independent marker of the clinical evolution of CVD, not only in individuals with myocardial infarction but also in those with known coronary artery disease[33,34]. In our study, patients with RA who were carriers of the C allele of the –1031 TNF-α polymorphism had a mean concentration of plasma TNF-α that was 1.6-fold that of the RA patients with the TT genotype, although this was statistically nonsignificant. The lack of significance might be due to the relatively small number of patients selected for our

Table 2. Clinical features and inflammatory markers in controls and patients with RA segregated by TNF-α –1031 T/C polymorphism. Values are expressed as mean (SD).

	Controls		RA Patients	
TNF-1031	TT, n = 11	CC/CT, n = 19	TT, n = 15	CC/CT, n = 15
Age, yrs	51.09 (12.88)	54.36 (16.69)	49.93 (10.97)	56.06 (14.71)
Sex, female/male	11/0	16/3	14/1	13/2
BMI, kg/m^2	29.75 (4.95)	26.10 (3.57)*	25.77 (5.09)	26.21 (5.41)
Inflammation markers				
CRP, mg/l	0.2 (0.28)	0.13 (0.16)	3.59 (2.31)	3.9 (2.01)
ESR, mm/h	11.72 (10.72)	16.78 (10.37)	68.2 (25.62)	66.2 (26.37)
Fibrinogen, mg/dl	358.18 (65.87)	379.27 (76.1)	503.2 (94.42)	548.42 (94.74)
sPLA$_2$-IIA, ng/ml	18.19 (11.02)	11.72 (6.04)*	110.44 (91.5)	81.86 (56.88)
TNF-α, pg/ml	11.05 (1.9)	11.9 (3.2)	16.36 (4.38)	24.9 (23.84)

* $p < 0.05$ versus homozygotes for the TT genotype. BMI: body mass index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; sPLA$_2$-IIA: secretory group IIA phospholipase A$_2$; TNF-α = tumor necrosis factor-α.

Table 3. Lipid profile and vitamins in controls and RA patients segregated by TNF-α –1031 T/C polymorphism. Values are expressed as mean (SD).

	Controls		RA Patients	
TNF-1031	TT, n = 11	CC/CT, n = 19	TT, n = 15	CC/CT, n = 15
Lipid profile				
Chol, mg/dl	214.87 (40.93)	212.65 (42.8)	210.4 (54.18)	194.75 (30.75)
TG, mg/dl	106.67 (35.07)	107.34 (44.89)	123.79 (78.46)	86.60 (38.39)
Apo B, g/l	1.19 (0.25)	1.11 (0.27)	1.16 (0.32)	1.06 (0.15)
Apo A-I, g/l	1.42 (0.29)	1.49 (0.16)	1.4 (0.24)	1.43 (0.26)
Lp (a), mg/dl	15.75 (11.84)	23.94 (20.45)	36.79 (32.3)	44.15 (49.14)
LDL-chol, mg/dl	148.05 (44.04)	146.26 (40.86)	138.9 (43.19)	132.14 (27.05)
HDL-chol, mg/dl	49.32 (12.37)	52.17 (12.7)	54.19 (15.2)	50.84 (14.27)
VLDL size, nm	41.63 (7.14)	41.77 (3.96)	42.39 (6.27)	42.26 (6.42)
VLDL-2 TG, mg/dl	11.63 (7.55)	10.56 (9.27)	14.08 (9.93)	6.88 (5.71)
VLDL-4 TG, mg/dl	25.94 (19.89)	31.01 (21.88)	29.11 (19.55)	20.12 (24.40)
LDL-1 chol, mg/dl	13.21 (20.05)	6.07 (10.14)	18.75 (31.95)	18.73 (16.9)
LDL-3 chol, mg/dl	77.30 (54.13)	76.69 (38.86)	53.94 (25.16)	71.91 (50.80)
HDL size, nm	9.05 (0.44)	9.08 (0.52)	9.42 (0.52)	9.37 (0.55)
HDL-2 chol, mg/dl	15.38 (4.79)	15.83 (4.17)	7.66 (5.76)	8.35 (5.01)
HDL-5 chol, mg/dl	8.64 (7.16)	6.71 (7.44)	10.82 (9.41)	8.62 (9.81)
Vitamins				
Vitamin A, µg/l	480.78 (81.31)	449.67 (106.2)	467.55 (199.0)	381.15 (132.2)
Vitamin E, mg/l	10.92 (1.6)	13.21 (3.54)*	14.21 (4.11)	11.93 (3.4)

* $p < 0.05$ vs homozygotes for the TT genotype. TNF: tumor necrosis factor; TG: triglyceride; LDL: low density lipoprotein; HDL: high density lipoprotein; VLDL: very low density lipoprotein; Apo: apoliprotein.

study. No association was observed in the control group. We have shown as well that the RA patients with the C allele of the TNF-α –1031 polymorphism had smaller size LDL than RA patients with the TT genotype. Plasma TG concentrations are often the most important factors influencing LDL size. But in our group of patients with RA there were no significant differences in mean TG in carriers of the TT genotype compared with carriers of the C allele. Further, no significant correlation of LDL size with TG concentrations was observed in patients with RA, and there were no significant differences with respect to genotype. As such, there might be differences in the pathogenesis of small dense LDL particles specific to patients with RA. For instance, our patients with RA had high concentrations of sPLA$_2$-IIA (7.5-fold higher than controls) and it has been shown that sPLA$_2$ hydrolyzes the surface phospholipids of LDL, causing a reduction in the size and an increase in the density of these lipoproteins[35]. However, more studies are warranted to clarify the factors that most influence LDL size in patients with RA.

Small, dense LDL particles play a key role in the development of the atheromatous plaque because of the ease in crossing the vascular endothelium. Once within the intima, the small dense LDL particles have a greater affinity for the proteoglycans of the extracellular matrix. Our results showed that the LDL from RA patients with the C allele of the –1031 TNF- α polymorphism were not only smaller and denser but

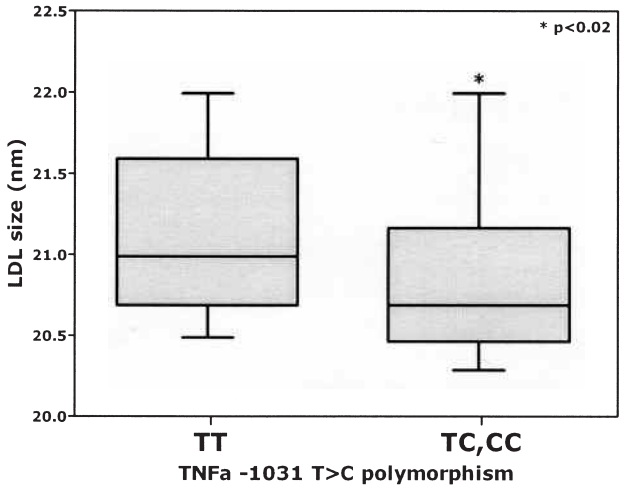


Figure 1. LDL particle size in patients with RA segregated by TNF-α –1031 T/C polymorphism.

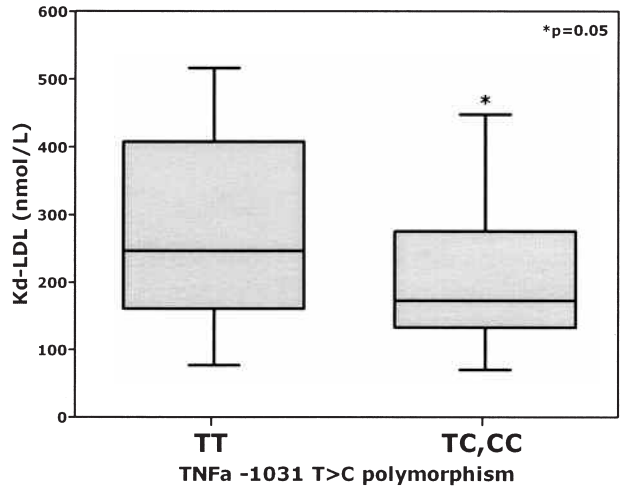
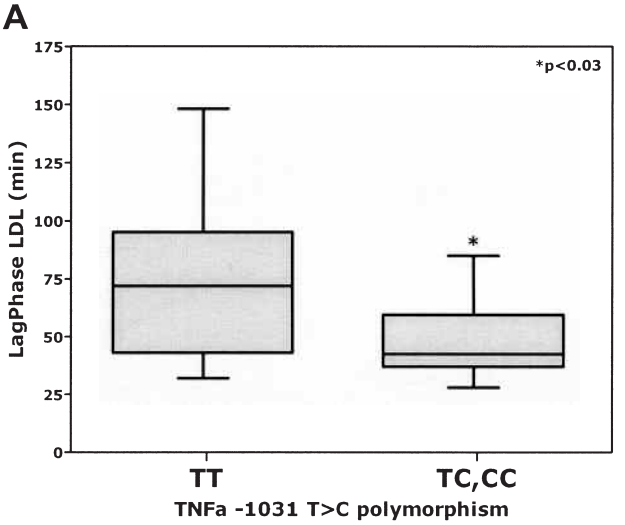


Figure 2. Kd-LDL in patients with RA segregated by TNF-α –1031 T/C polymorphism. The results are expressed as the affinity (Kd) of LDL for C6S-GAG in nmol/l, low Kd indicating high affinity and vice versa. C6S-GAG: chondroitin-6-sulfate glycosaminoglycan.



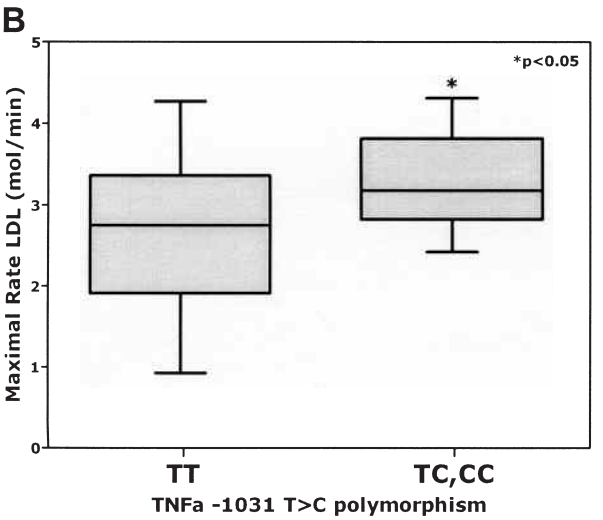


Figure 3. A. Lag phase (LP); B. Maximal rate (MR) of LDL oxidation in patients with RA segregated by TNF-α –1031 T/C polymorphism. LP results are expressed in min and MR results in mol dienes/mol LDL x min.

also had a greater affinity for the proteoglycans of the extracellular matrix. *In vitro* studies have demonstrated that LDL from the intima are bound to proteoglycans of the extracellular matrix, and have increased susceptibility to oxidation, to a higher rate of oxidation, and as well to increased uptake by macrophages. In confirmation of these findings, we observed that the C allele is associated with LDL particles that are more susceptible to oxidation (shorter LP) and greater oxidation rate (MR) than RA patients with the TT genotype. The retention of these particles by matrix proteoglycans may not be the only mechanism responsible for the increase in LDL oxidation. Oxidative stress has been shown to be inversely correlated with antioxidant vitamin A and E concentrations, and we have seen in our patients with RA that carriers of the C allele have a tendency towards lower concentrations of vitamin A and E. Thus, in addition to smaller LDL with greater affinity for the matrix proteoglycans, lower vitamin A and E concentrations could contribute to the increased susceptibility to LDL oxidation seen in patients with RA who are carriers of the C allele.

Small, dense LDL particles have an important role in the atherogenesis process because they are the principal targets, in the intima of the artery, of free radical oxidation. The outcome of LDL oxidation is 2-fold. First, when the LDL particle is oxidized and modified, it is not recognized by the normal LDL receptor and instead is taken up by other nonregulated scavenger receptors of the macrophages, which then proceed to form foam cells. Second, a series of products derived from lipid peroxidation is formed. These are essentially oxysterols, hydroperoxides, and aldehydes. All these substances have

considerable biological reactivity in the dysfunctional endothelium and in the inflammatory response and, as well, in the proliferation of the cells that are specific in the atheromatosis process[36-38]. In clinical studies in different populations, small, dense LDL particles that are more easily oxidized have been shown to be associated with a greater risk of CVD. Patients with RA carrying the –1031 C allele have qualitative alterations in lipid profile that make them more atherogenic compared to non-carriers of this allele. This suggests that there is a genetic modification of CVD risk in these patients.

The associations observed in patients with RA who are carriers of the C allele were not observed in our control group. On the other hand, control group carriers of the C allele were associated with significantly lower BMI, significantly lower concentrations of $sPLA_2$-IIA, and significantly higher concentrations of vitamin E. Controls were selected from a population of 100 unrelated individuals. Although the 30 control subjects selected were matched with RA patients, the genotypes did not match, i.e., 11 (TT) and 19 (TC+CC). As such, there could have been a bias towards the C allele. Although we cannot rule out a possible bias, we believe our results suggest that the genetic susceptibility needs other additional factors, in the present case, an increase in the proinflammatory status of the patient, for the clinical conditions to be fully expressed. The gene-environment interaction and/or gene-gene interaction is a constant in the susceptibility of an individual to any disease that has a multifactorial etiology such as, for example, RA and arteriosclerosis.

Our results show that, in patients with RA, the genetic variability in the TNF-α gene is associated with smaller, denser LDL particles that have a greater affinity for extracellular matrix and higher susceptibility to oxidation. Because these characteristics are associated with a greater risk of atherosclerosis, identification of such patients with RA could help in implementing early preventive intervention measures against CVD in this subset of patients.

ACKNOWLEDGMENT

We are grateful to Prof. Pere Benito, the clinical laboratory, and the patients from the Hospital del Mar for making this study possible. We thank the members of the Unitat de Recerca de Lípids i Arteriosclerosi (Reus, Catalonia, Spain) and the Wallenberg Laboratory (Göteborg, Sweden) for expert technical assistance.

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