Increased Levels of Anti-oxidized Low-Density Lipoprotein Antibodies Are Associated With Reduced Levels of Cholesterol in the General Population

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Autoantibodies against epitopes of oxidized low-density lipoprotein (LDL), initially shown in human sera, were later related with the atherosclerotic process, although recent studies have questioned this association. Moreover, their association with total cholesterol and plasma LDL, or with the other lipoproteins, is not clear. We studied the relation between the levels of autoantibodies to oxidized LDL and lipoproteins in a population of 400 subjects from the lower Guadalhorce area in Malaga, Spain. Anti-oxidized LDL antibodies were measured by enzyme-linked immunosorbent assay (ELISA), and total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and lipoprotein(a) [Lp(a)] were measured with commercial kits. Subjects who were positive for anti-oxidized LDL antibodies had significantly lower levels of total cholesterol ($P < .01$) and LDL cholesterol ($P < .01$). There was a negative correlation between titers of anti-oxidized LDL antibodies and levels of total cholesterol ($P = .007$) and LDL cholesterol ($P = .024$). This inverse relation between the levels of anti-oxidized LDL antibodies and the levels of total cholesterol and LDL cholesterol in a large population study, together with the discordances already published, suggests that the relation between anti-oxidized LDL antibodies, arteriosclerosis, and lipids is more complex than initially thought.

AUTOANTIBODIES AGAINST epitopes of oxidized low-density lipoprotein (LDL) have been reported in human sera.[1] These autoantibodies were later related with the atherosclerotic process, with specific immunoglobulins against oxidized LDL being found in atherosclerotic lesions.[2] Patients presenting a progression of carotid atherosclerotic lesions have high titers of anti-oxidized LDL antibodies,[3] and case-control studies have shown that elevated levels of antibodies against oxidized LDL are predictive of myocardial infarction.[4,5] Elevated levels of anti-oxidized LDL antibodies have been found during the first 48 hours after an infarction, decreasing during the subsequent weeks and months.[6] They have also been related with essential hypertension,[7] non–insulin-dependent diabetes mellitus,[8] and chronic renal failure.[9] These autoantibodies have been found to be increased in autoimmune diseases such as systemic lupus erythematosus and the antiphospholipid syndrome, being associated with an increased presence of arterial thrombosis.[10,11]

However, recent studies have questioned the close relation between anti-oxidized LDL antibodies and atherosclerosis. It has been noted that patients with familial hypercholesterolemia have similar antibody values to a control population,[12] and in patients with insulin-dependent diabetes mellitus it has been shown that longer duration of diabetes, higher hemoglobin A_{1c} levels, and microangiopathy are associated with low levels of antibodies to oxidized LDL.[13] On the other hand, their association with levels of total cholesterol and LDL in plasma, or with the other lipoproteins, is not clear. Thus, as a consequence of this recent discrepancy in the scientific literature, we determined the relation between levels of autoantibodies to oxidized LDL and levels of plasma lipoproteins.

MATERIAL AND METHODS

Population and Measurements

A total of 400 people (160 men and 240 women) between the ages of 35 and 65 years were included in the study. They were selected randomly from the population census of Pizarra (Malaga, Spain), a village of 6,500 inhabitants. The participation rate was 65%. After obtaining written, informed consent from all the subjects, clinical and anthropometric data were taken, as well as a sample of blood, which was extracted after a minimum of 10 hours fasting. The serum was separated immediately after extraction and later frozen at -80°C until analysis.

Measurements were made of total cholesterol and triglycerides by enzymatic method (Ecoline 2S, Merck, Darmstadt, Germany), high-density lipoprotein (HDL) cholesterol by phosphotungstic acid precipitation (HDL-C, Boehringer Mannheim, Mannheim, Germany), lipoprotein(a) [Lp(a)] by immunoprecipitin analysis (antibody reagent set for Lp(a) SPQ Test System, DiaSorin, Stillwater, MN), and uric acid by enzymatic method (Boehringer Mannheim).

LDL Isolation

LDL was isolated from pooled plasma of healthy fasting human donors by density gradient ultracentrifugation. The LDL was then dialyzed against phosphate-buffered saline (PBS; 4°C for 30 hours; 0.14 mol/L NaCl/0.01 mol/L phosphate buffer).

Oxidation of LDL

Oxidized LDL was prepared by incubating the LDL for 3 hours at 37°C with 0.5 mol/L malonyldialdehyde (MDA) at a constant ratio of 100 μg/1 mg LDL. The reaction was stopped by adjusting the pH to 7.4 with 1 mol/L NaOH. After conjugation, MDA-LDL was extensively dialyzed against PBS.

Anti-oxidized LDL Aantibodies

Microtiter plates for determination of anti-oxidized LDL antibodies were coated with either native LDL or with MDA-LDL, both at 10 μg/mL in PBS. The plates were incubated for 2 hours at 37°C and overnight at 4°C. After washing 4 times with PBS, plates were blocked

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Table 1. Mean ± SD Values of Plasma Lipids, Uric Acid, and Blood Pressure in Subjects Positive for Anti-oxidized LDL Antibodies Compared to Those Negative

	Negative	Positive
Total cholesterol (mg/dL)	224.2 ± 43.8	211.1 ± 40.2*
LDL cholesterol (mg/dL)	148.2 ± 39.2	138.8 ± 37.1*
HDL cholesterol (mg/dL)	49.5 ± 12.2	48.4 ± 14.3
Lp(a) (mg/dL)	30.1 ± 24.6	26.5 ± 24.0
Total triglycerides (mg/dL)	132.9 ± 89.6	119.2 ± 73.9
Uric acid (mg/dL)	5.2 ± 4.1	4.6 ± 1.4
Systolic blood pressure (mm Hg)	140.5 ± 20.9	138.3 ± 21.6
Diastolic blood pressure (mm Hg)	87.8 ± 11.2	86.2 ± 10.8

*$P < .01$.

with 1% bovine serum albumin (BSA)/PBS for 2 hours at room temperature. Serum samples were diluted 1:100 in 1% BSA/PBS and incubated for 3 hours at room temperature. After washing, an alkaline phosphatase–conjugated anti-human IgG (Sigma Immuno Chemicals, St Louis, MO) was diluted 1:1,000 in 1% BSA/PBS and added. It was then left for 3 hours at room temperature. A 1-mg/mL quantity of *p*-nitrophenyl-phosphate (Sigma) in 500 mmol/L carbonate buffer containing 1 mmol/L $MgCl_2$ (pH 9.8) was used as substrate. The reaction was stopped with 1 mol/L NaOH after 60 minutes. The absorbance was read at 405 nm. Binding of antibodies to oxidized LDL was calculated by subtracting the native LDL from the MDA-LDL. Optic density values above the mean plus 2 SD of the values of a group of 65 healthy young persons aged 20 to 24 years were considered positive. The inter- and intra-assay coefficients of variation of the technique ranged from 5% to 15%.

Statistical Study

Plasma lipoprotein values of the positive subjects were compared with those of the negative subjects by means of Students *t* test for independent data. Linear correlation coefficients (Pearson's *r*) were made between the titers of anti-oxidized LDL antibodies (expressed as optic density) and the various lipid parameters. Multiple regression analysis was made with the dependent variable being the titers of anti-oxidized LDL antibodies and the independent variables including all those that could be related with the antibodies.

RESULTS

Of the total population, 40.5% were positive for anti-oxidized LDL antibodies. Forty-one percent were men, in whom the positivity rate was 37.3%, with women comprising 59% of the population and having a positivity rate of 42.2%. There were no significant differences between subjects who were positive for anti-oxidized LDL antibodies and those who were negative in mean age (51.02 ± 9.19 years in the negative *v* 49.29 ± 9.32 in the positive subjects), or in the levels of glucose, insulin, insulin/glucose ratio, or uric acid. Blood pressure was similar in both groups (Table 1). Table 1 also shows the plasma lipid values of the positive patients versus the negative. There were significantly lower levels of total cholesterol ($P < .01$) and LDL cholesterol ($P < .01$) in the subjects who were positive for anti-oxidized LDL antibodies. There were no statistically significant differences in the levels of HDL cholesterol, Lp(a), or total triglycerides. Results were similar when analyzed according to sex.

The simple linear correlations between the titers of anti-oxidized LDL antibodies and the different plasma lipids studied are shown in Table 2. There was a negative correlation between the titers of anti-oxidized LDL antibodies and the levels of total cholesterol ($P = .007$) and LDL cholesterol ($P = .024$).

Multiple linear regression analysis was perfromed with the anti-oxidized LDL antibodies as the dependent variable and the independent variables of weight, age, sex, and levels of LDL cholesterol, total cholesterol, HDL cholesterol, insulin, total triglycerides, and Lp(a). Only the cholesterol levels entered the model significantly (.0076), having a negative association with the titers of anti-oxidized LDL antibodies (multiple $R = 0.13706$).

DISCUSSION

We are unaware of any other population studies of the prevalence of anti-oxidized LDL antibodies. In this study there was a high prevalence of these autoantibodies in persons ranging in age from 35 to 65 years. Although during the initial phases of the study of the role of these autoantibodies they were suggested as a marker of atherosclerosis,[2-5] there are at present discordant opinions.[12,13]

Our study showed a clear inverse relation between the levels of plasma cholesterol and LDL and the levels of these autoantibodies. Although some studies relate autoantibody titers with atherosclerosis, in most no direct relation has been found between the level of these autoantibodies and cholesterol levels,[3,4,10,11] nor was a statistically significant relation found in

Table 2. Simple Linear Correlations Between the Titers of Anti-oxidized LDL Antibodies (expressed as optical density) and the Levels of Total Cholesterol, Total Triglycerides, LDL Cholesterol, HDL Cholesterol, and Lp(a)

	Lp(a)	Triglycerides	Anti-Oxidized LDL	HDL Cholesterol	Cholesterol
LDL cholesterol	0.20	0.10	−0.11	−0.10	0.92
	$P = .000$	$P = .046$	$P = .024$	$P = .045$	$P = .000$
Lp(a)		−0.16	−0.06	0.092	0.15
		$P = .002$	$P = .2129$	$P = .074$	$P = .004$
Triglycerides			−0.07	−0.41	0.37
			$P = .150$	$P = .000$	$P = .000$
Anti-oxidized LDL				−0.017	−0.14
				$P = .740$	$P = .007$
HDL cholesterol					0.048
					$P = .343$

NOTE. Results are expressed as Pearson's *r* value and the levels of statistical significance.

one study of a healthy population.[14] However, there is disagreement about how autoantibody levels are affected by decreases in cholesterol levels. After hormone-replacement therapy in menopausal women there is a reduction in cholesterol levels, which in some studies was accompanied by a drop in levels of anti-oxidized LDL antibodies,[15] but not in others.[16] On the other hand, some investigations have tied the modifications in levels of anti-oxidized LDL antibodies to the changes in the LDL oxidation capacity,[17] although others have not.[15]

Not only do the studies fail to find a statistically significant relation between cholesterol levels and levels of anti-oxidized LDL antibodies, but in subjects with hypercholesterolemia some investigators have found familial levels of autoantibodies similar to the controls, and in these same subjects, patients with a history of myocardial infarction had significantly lower IgM titers against oxidized LDL compared with patients without a history of myocardial infarction and with controls.[12] In diabetic patients, in whom the oxidative capacity is greater,[18] those with a longer history of diabetes, with higher levels of glycosylated hemoglobin, and with microangiopathy had lower levels of anti-oxidized LDL antibodies,[13] although this study found oxidized LDL immune complexes in some patients with microangiopathy who were negative for anti-oxidized LDL antibodies and the authors speculated that oxidized LDL immune complexes mask free antibodies.[19] This is an attractive hypothesis to explain the results of our study. A negative relation has already been found between immunocomplexes formed by LDL and free antibodies.[20] No differences were found either in autoantibody levels between normoalbuminuric and albuminuric diabetics.[21] In this relationship between free antibodies and cholesterol it should be remembered that we are dealing only with plasma levels and not with their concentration in the subepithelium. Although their presence in the arteries is known, it is not clear whether a greater concentration of free antibodies is associated with a greater deposit in the endothelium.

The existence of this inverse relation between levels of anti-oxidized LDL antibodies and levels of cholesterol and LDL cholesterol in a large population study, together with the discordance in the literature, suggest that the relation between anti-oxidized LDL antibodies, arteriosclerosis, and lipid parameters is more complex than initially suspected.

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