ARTHRITIS & RHEUMATISM
Vol. 42, No. 2, February 1999, pp 373–383

ASSOCIATION OF THE INFLAMMATORY STATE IN ACTIVE JUVENILE RHEUMATOID ARTHRITIS WITH HYPO–HIGH-DENSITY LIPOPROTEINEMIA AND REDUCED LIPOPROTEIN-ASSOCIATED PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE ACTIVITY

ALEXANDROS D. TSELEPIS, MOSES ELISAF, SOTIRIS BESIS, SONIA-ATHENA P. KARABINA, M. JOHN CHAPMAN, and ANTIGONI SIAMOPOULOU

Objective. **To investigate the relationship between the quantitative and qualitative abnormalities of apolipoprotein B (Apo B)– and Apo A-I–containing lipoproteins and between lipoprotein-associated platelet-activating factor acetylhydrolase (PAF-AH) activity in patients with juvenile rheumatoid arthritis (JRA) as a function of the inflammatory state.**

Methods. **Twenty-six JRA patients and 22 age- and sex-matched control subjects with normal lipid levels participated in the study. Fourteen patients had active disease, and 12 had inactive disease. Plasma lipoproteins were fractionated by gradient ultracentrifugation into 9 subfractions, and their chemical composition and mass were determined. The PAF-AH activity associated with lipoprotein subfractions and the activity in plasma were also measured.**

Results. **Patients with active JRA had significantly lower plasma total cholesterol and high-density lipoprotein (HDL) cholesterol levels as compared with controls, due to the decrease in the mass of both the HDL2 and HDL3 subfractions. Patients with active JRA also had higher plasma triglyceride levels, mainly due to the higher triglyceride content of the very low-density lipoprotein plus the intermediate-density lipoprotein subfraction. The plasma PAF-AH activity in patients with active JRA was lower than that in controls, mainly due to the decrease in PAF-AH activity associated with the intermediate and dense low-density lipoprotein subclasses. The lipid abnormalities and the reduction in plasma PAF-AH activity were significantly correlated with plasma C-reactive protein levels and were not observed in patients with inactive JRA.**

Conclusion. **This is the first study to show that patients with active JRA exhibit low levels of HDL2 and HDL3 and are deficient in plasma PAF-AH activity. These alterations suggest that active JRA is associated with partial loss of the antiinflammatory activity of plasma Apo B– and Apo A-I–containing lipoproteins.**

Dyslipoproteinemia is a feature of certain rheumatic diseases, mainly systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (1). Indeed, lipid abnormalities, such as decreased levels of cholesterol and triglycerides in very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) have been described in adults with RA (2). Dyslipoproteinemia has also been reported to occur in children with rheumatic diseases, mainly those with juvenile rheumatoid arthritis (JRA) (3,4) and SLE (5–7). Lipid abnormalities in JRA are characterized by low serum levels of LDL and HDL cholesterol, as well as by elevated concentrations of VLDL cholesterol and triglycerides (3,4). The dyslipoproteinemia in children with active SLE is characterized by elevated plasma triglycerides (5–7) and VLDL cholesterol (7), as well as depressed plasma HDL cholesterol and apolipoprotein A-I (Apo A-I) levels (7).

It is well known that plasma LDL and HDL are distributed as a continuum of particles over the density ranges of 1.019–1.063 gm/ml and 1.063–1.210 gm/ml, respectively. Substantial evidence has now accumulated

Alexandros D. Tselepis, MD, PhD, Moses Elisaf, MD, Sotiris Besis, MD, Sonia-Athena P. Karabina, PhD, Antigoni Siamopoulou, MD: University of Ioannina, Ioannina, Greece; M. John Chapman, PhD: Hôpital de la Pitié, Paris, France.

Address reprint requests to Antigoni Siamopoulou, MD, Associate Professor of Pediatrics, Department of Pediatrics, Medical School, University of Ioannina, 45110 Ioannina, Greece.

Submitted for publication February 23, 1998; accepted in revised form August 4, 1998.

indicating that there is qualitative heterogeneity of lipoprotein particles within these density intervals (8,9). Indeed, multiple subspecies of LDL as well as HDL particles that vary markedly in their physicochemical, hydrodynamic, functional, and structural properties are present in both normolipidemic and dyslipidemic subjects (9,10). The qualitative heterogeneity of LDL particles appears to be intimately linked not only to the metabolism of individual LDL subspecies, but also to their potential atherogenicity (11,12). Thus, small dense LDL particles are considered to be more atherogenic than larger, light LDL particles, and their predominance is positively correlated with the risk of coronary artery disease (13).

One of the functional features of LDL and HDL particles is their ability to degrade phospholipids that have a short acyl chain residue at the *sn*-2 position. This reaction is catalyzed by a specific Ca^{2+}-independent phospholipase A_2, called platelet-activating factor acetylhydrolase (PAF-AH) (14,15). PAF-AH (EC 3.11.48) hydrolyzes and inactivates PAF, the potent lipid mediator in inflammatory reactions that may also play an important role in atherogenesis (16,17). PAF-AH has been reported to be a potent antiinflammatory enzyme, since it plays a major role in the regulation of the pathophysiologic effects of PAF (18). Furthermore, this enzyme can effectively hydrolyze oxidized phospholipids (19) that exhibit a wide spectrum of biologic activities resembling those of PAF (PAF-like phospholipids) (20). Such oxidized phospholipids are formed on LDL during oxidation, and may play an important role in the inflammatory and atherogenic effects of oxidized LDL, whereas the LDL- as well as HDL-associated PAF-AH play a major role in degrading and inactivating these inflammatory oxidized phospholipids (19,21). Recently, we showed that in the plasma of subjects with normal lipid levels, the distribution of PAF-AH among the lipoprotein subfractions is heterogenous and is preferentially associated with the small, dense LDL and small HDL particles (i.e., very high-density lipoprotein [VHDL]) (22–24).

To our knowledge, there is a paucity of data on the distribution of plasma lipoproteins in relation to plasma PAF-AH activity in children with inflammatory diseases, as well as in healthy children. In the present study, we evaluated children with JRA, an inflammatory disease that is associated with dyslipidemia, in an attempt to define the dyslipoproteinemic state observed in JRA patients and to correlate the inflammatory state with PAF-AH levels and with lipoprotein subfraction phenotype.

PATIENTS AND METHODS

Study population. Twenty-six children (12 boys, 14 girls) were studied. Each was diagnosed as having JRA according to the criteria established by the American College of Rheumatology (25). All were attending the juvenile rheumatology clinic at the university hospital (Ioannina, Greece). The mean age of the patients at the time of the study was 11 years (range 3–14 years), and the mean duration of disease was 4.5 years (range 6 months to 10 years). Patients were categorized as having polyarticular-onset ($\geq$5 involved joints), pauciarticular-onset ($\leq$4 involved joints), or systemic-onset JRA, as defined within the first 6 months of disease onset.

Clinical activity was assessed in a standard manner by findings on history and physical examination of each patient, as performed by 1 physician (SB). A disease activity score was assigned based on the presence of joint swelling, warmth, redness, range of motion, muscle weakness, pain, morning stiffness, and the use of antiinflammatory medications. The score ranged from 0 to 5, with 0 representing no complaints or physical findings of active disease and no use of antiinflammatory medication, and 5 representing very active clinical disease and the use of antiinflammatory medication. The disease was defined as active if the score was $\geq$3 and inactive if the score was $\leq$2. None of the patients had evidence of any endocrine disorder or liver or renal dysfunction.

Twenty-two age- and sex-matched healthy children who had normal lipid levels (8 boys, 14 girls) were included in the study as controls. None of them was receiving any medication.

The functional class of all children was estimated according to the Steinbrocker functional criteria (26). Three-day dietary intake records were obtained for each child, and parents were instructed in the proper recording of dietary records. The records were analyzed in terms of calorie, protein, fat, and carbohydrate content.

Analytical methods. All subjects participating in our study followed their normal diet until the day of the study. Blood samples were obtained after an overnight fast for evaluation of lipid and lipoprotein profiles. The measurement of plasma lipid parameters was performed by standard, commercially available techniques as previously described (24). LDL cholesterol levels were calculated using the Friedewald formula. The cholesterol, phospholipid, and triglyceride content of each lipoprotein subfraction was determined enzymatically using BioMerieux kits (Marcy l'Etoile, France). The protein content of the gradient fractions and lipoprotein subfractions was determined by the bicinchoninic acid (BCA) method. Lipoprotein mass in each subfraction was calculated as the sum of the concentrations of the individual components (cholesterol, triglyceride, phospholipid, and protein) and allowed determination of the percent chemical composition (23). C-reactive protein (CRP) was measured by nephelometry, and erythrocyte sedimentation rate (ESR) was determined by the Westergren method.

Fractionation of plasma lipoproteins. Venous blood was collected into glass tubes containing EDTA (3 m*M*), from which plasma was rapidly separated by low-speed centrifugation (1,000*g*) for 20 minutes at 4°C. Immediately after collection of plasma, gentamicin (50 μg/ml) and EDTA (0.3 m*M*) were added. Lipoproteins were fractionated by isopyknic

Table 1. Morphometric and clinical characteristics of control subjects and JRA patients*

Characteristic	Controls (n = 22)	JRA patients (n = 26)
Sex, no. male/no. female	8/14	12/14
Age, mean ± SD years (range)	11 ± 4 (7–14)	11 ± 3 (3–14)
Body mass index, mean ± SD kg/m^2	18 ± 2.4	18.9 ± 3.3
Functional class, no. in class I/II	22/0	17/9
CRP, mean ± SD mg/liter		
Active JRA	–	91.9 ± 33.2†
Inactive JRA	–	8.2 ± 4.3
ESR, mean ± SD mg/liter	15.1 ± 5	
Active JRA	–	77.1 ± 31.4‡
Inactive JRA	–	16.3 ± 7.8
Disease duration, mean (range) years	–	4.5 (6 months–10 years)
No. with active/inactive JRA	–	14/12
No. with onset type, poly/pauci/systemic disease	–	15/8/3
Medication, no. of patients (active/inactive JRA)		
NSAIDs	–	23 (13/10)
Methotrexate	–	10 (6/4)
Methylprednisolone	–	6 (5/1)

* JRA = juvenile rheumatoid arthritis; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; poly = polyarticular; pauci = pauciarticular; NSAIDs = nonsteroidal antiinflammatory drugs.
† $P < 0.0001$ versus inactive JRA group.
‡ $P < 0.005$ versus controls and versus inactive JRA group.

density-gradient ultracentrifugation using a Beckman SW41 Ti rotor at 40,000 revolutions per minute for 44 hours in a Beckman L7 centrifuge at 15°C, as described previously (23,27). Briefly, plasma density was increased to 1.21 gm/ml by addition of dry, solid KBr. Construction of a discontinuous density gradient at ambient temperature was initiated by pumping 2 ml of an NaCl–KBr solution of d 1.24 gm/ml into the bottom of the tube. The following solutions were then layered above: 3 ml of plasma at 1.21 gm/ml; 2 ml of a NaCl–KBr solution of d 1.063 gm/ml; 2.5 ml of d 1.019 gm/ml, and 2.5 ml NaCl solution of d 1.006 gm/ml. All density solutions contained 0.3 m*M* EDTA and 50 μg/ml gentamicin at pH 7.4.

After ultracentrifugation, 30 fractions (0.4 ml each) were collected by successive aspiration with a precision pipette from the meniscus downward. All fractions were analyzed for their protein content by the BCA method, and their purity was evaluated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Subsequently, equal volumes of certain gradient fractions were pooled to constitute the lipoprotein subfractions, as follows: fractions 1 and 2 (VLDL + IDL d <1.019 gm/ml), fractions 3 and 4 (LDL1 d 1.019–1.023 gm/ml), fractions 5 and 6 (LDL2 d 1.023–1.029 gm/ml), fractions 7 and 8 (LDL3 d 1.029–1.039 gm/ml), fractions 9 and 10 (LDL4 d 1.039–1.050 gm/ml), fractions 11 and 12 (LDL5 d 1.050–1.063 gm/ml), fractions 13–16 (HDL2 d 1.063–1.100 gm/ml), fractions 17–22 (HDL3 d 1.100–1.167 gm/ml), and fractions 23 and 24 (VHDL d 1.167–1.190 gm/ml). Fractions 25–30 did not contain lipoproteins and were therefore discarded.

The apolipoprotein content of each gradient fraction was evaluated by SDS-PAGE (5–19% gradient) as previously described (28).

PAF-AH assay. PAF-AH activity in the lipoprotein subfractions and in plasma was measured by the trichloroacetic acid precipitation procedure (23), with some modifications. Four micrograms of protein from each lipoprotein subfraction or 50 μl of plasma (diluted 1/50, volume/volume, with HEPES buffer) was mixed with HEPES buffer, pH 7.4 (4.2 m*M* HEPES, 137 m*M* NaCl, 2.6 m*M* KCl, and 2 m*M* EDTA), in a final volume of 90 μl. After preincubation at 37°C, the reaction was initiated and performed for 10 minutes at 37°C by the addition of 10 μl of a 1 m*M* ^{3}H-labeled PAF solution (100 μM final concentration in the reaction mixture) prepared as previously described (23). The results, after correction for nonenzymatic degradation of ^{3}H-labeled PAF, are expressed as nanomoles of PAF degraded each minute per milliliter of lipoprotein protein or per milliliter of plasma (23).

Statistical analysis. Values are expressed as the mean ± SD, except for the plasma Lp(a) levels, which are expressed as median and range. One-way analysis of variance, followed by the least significant difference method, was used to assess differences in lipid parameters. Statistical analysis of the plasma Lp(a) levels was performed by nonparametric statistics (Kruskal-Wallis test) because of the skewed distribution of Lp(a) levels. Two-tailed significance values are given, and a P value less than 0.05 was considered statistically significant. Correlation coefficients were determined according to Pearson. The proportions of each chemical component in a corresponding lipoprotein subfraction among the 3 groups were compared by Student's t-test.

RESULTS

Population characteristics. As shown in Table 1, there were no significant differences in the number, sex, age, and body mass index between JRA patients and control subjects. During the study, 14 JRA patients had active disease and 12 had inactive disease. As expected,

Table 2. Fasting lipid and lipoprotein levels in the plasma of JRA patients and control subjects with normal lipid levels*

Parameter	Control population (n = 22)	Active JRA (n = 14)	Inactive JRA (n = 12)
Total cholesterol, mg/dl	146 ± 18	122 ± 17†	154 ± 21
Triglycerides, mg/dl	43 ± 9	59 ± 14‡	38 ± 14
LDL cholesterol, mg/dl	102 ± 10	103 ± 25	106 ± 30
HDL cholesterol, mg/dl	42 ± 7	30 ± 6§	41 ± 16
LDL:HDL cholesterol ratio	2.43 ± 0.7	3.43 ± 0.9§	2.58 ± 1.0
Lp(a), mg/dl	10 (0.8–28)	13 (0.8–40)	8 (0.8–30)
Apo A-I, mg/dl	127 ± 15	103 ± 20§	139 ± 16
Apo B, mg/dl	65 ± 10	70 ± 20	71 ± 22

* Values are the mean ± SD, except for the Lp(a) values, which are expressed as the median (range). JRA = juvenile rheumatoid arthritis; LDL = low-density lipoprotein; HDL = high-density lipoprotein; Apo = apolipoprotein.
† $P < 0.05$ versus controls and versus inactive JRA group.
‡ $P < 0.003$ versus controls and versus inactive JRA group.
§ $P < 0.005$ versus controls and versus inactive JRA group.

CRP levels were significantly higher in the patients with active JRA than in those with inactive JRA ($P < 0.0001$), and the ESR values were significantly higher in patients with active disease compared with both the controls and the patients with inactive JRA ($P < 0.005$).

Fifteen children had polyarticular-onset, 8 had pauciarticular-onset, and 3 had systemic-onset JRA. All patients were able to perform usual activities of daily living (self-care, vocational). According to the Steinbrocker classification, 17 patients were categorized as functional class I and 9 as class II. At the time of the study, 23 children were taking nonsteroidal antiinflammatory drugs, and 10 were receiving oral methotrexate once a week. Six patients, mostly with active systemic or polyarticular JRA, received small doses of oral methylprednisolone (<6 mg/day). None of the patients had ever taken hydroxychloroquine.

There was no history of weight loss in the JRA patients. All children who participated in the study had a mean ± SD total caloric intake of 2,600 ± 300 cal/day. The mean percentage of calories consumed as proteins was 17 ± 3%, as carbohydrates 50 ± 7%, and as fat 33 ± 5%, with a mean (±SD) fatty acid distribution as follows: saturated fat 15 ± 3%, monounsaturated fat 13 ± 2%, and polyunsaturated fat 5 ± 2%. The mean cholesterol intake was 350 ± 15 mg/day.

Lipid profile. The lipid profile of the total JRA population was not significantly different from that of the control group. When patients were categorized according to the type of disease onset, there was no difference in the lipid profile between the control group and each patient group or among the patient groups (results not shown). In contrast, when patients were categorized according to disease activity, the patients with active JRA had significantly lower plasma levels of total and HDL cholesterol, as well as Apo A-I, compared with the control group and with the patients with inactive JRA (Table 2). Additionally, the atherogenic ratio of LDL cholesterol:HDL cholesterol and the total plasma triglyceride levels were significantly higher in the active JRA group compared with the other 2 groups. No difference was observed among the 3 groups in plasma levels of LDL cholesterol, Apo B, and Lp(a) (Table 2). The same results were obtained when the patients receiving methotrexate or methylprednisolone were excluded from the statistical analysis (results not shown).

It is noteworthy that plasma triglyceride levels were positively correlated with the CRP levels ($P < 0.05$) (Figure 1A), whereas an inverse correlation was observed between HDL cholesterol and CRP levels ($P < 0.005$) (Figure 1B).

Characterization of lipoprotein gradient fractions. The Apo B and Apo A-I contents of individual gradient fractions were evaluated by SDS-PAGE. Fractions 1 through 12 (d <1.063 gm/ml) contained almost exclusively Apo B, whereas fraction 13 (d 1.063–1.072 gm/ml) contained a mixture of Apo B and Apo A-I. In contrast, Apo A-I was the major apolipoprotein in gradient fractions 14 through 23 (d 1.072–1.179 gm/ml). Fraction 24 (d 1.179–1.190 gm/ml) contained traces of albumin. Gradient fractions were pooled to constitute the major lipoprotein subfractions as described in Patients and Methods.

Mass profile and chemical composition of lipoprotein subfractions. As shown in Table 3, the lipoprotein mass of the VLDL + IDL subfraction (gra-

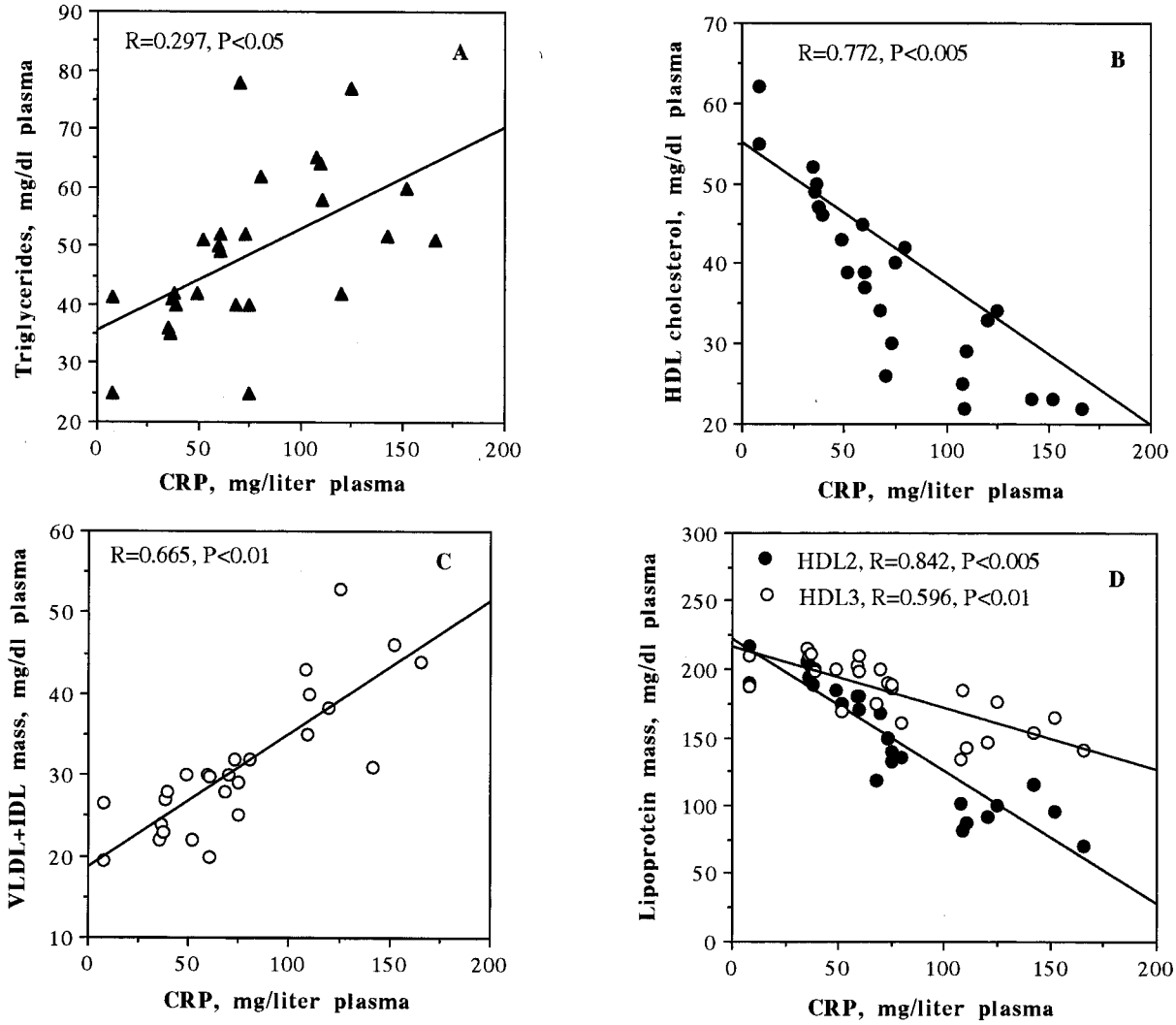

Figure 1. Correlations between plasma levels of C-reactive protein (CRP) and lipids as well as lipoprotein concentrations in patients with juvenile rheumatoid arthritis. HDL = high-density lipoprotein; VLDL = very low-density lipoprotein; IDL = intermediate-density lipoprotein.

dient fractions 1 + 2) in patients with active JRA was significantly higher compared with either the controls or the patients with inactive JRA ($P < 0.01$). The triglyceride content (weight %) of this subfraction was also significantly higher in the patients with active JRA ($P < 0.01$), whereas the protein content (weight %) was significantly lower compared with the control and the inactive JRA groups ($P < 0.05$). As a consequence, the triglyceride:protein ratio was significantly higher in the active JRA group ($P < 0.05$). No difference was observed among the 3 groups in the weight % content of phospholipids and cholesterol of this subfraction. It is important to note that the VLDL + IDL mass was positively correlated with the CRP levels ($P < 0.01$) (Figure 1C).

The total LDL mass, calculated as the sum of the mass of the 5 LDL subfractions, constituted from gradient fractions 3–12 (as described in Patients and Methods), was not significantly different among the 3 groups (mean ± SD 242.8 ± 28.4 mg/dl of plasma for the controls, 226.4 ± 22.3 mg/dl for the active JRA group, and 239.5 ± 19.8 mg/dl for the inactive JRA group). Similarly, no significant difference was observed in the mass of each LDL subfraction between the control and the active JRA group (Table 4) as well as between those groups and the inactive JRA group (results not shown).

Table 3. Chemical composition of the VLDL + IDL subfraction isolated from control subjects with normal lipid levels and JRA patients by isopyknic density gradient ultracentrifugation*

	VLDL + IDL d <1.019 gm/ml		
	Controls	Active JRA	Inactive JRA
Component, %			
Cholesterol	13.9 ± 1.9	12.2 ± 2.5	14.6 ± 3.2
Triglyceride	43.8 ± 7.5	52.8 ± 6.3†	44.1 ± 5.6
Phospholipid	17.4 ± 5.3	15.8 ± 3.4	18.1 ± 2.1
Protein	24.8 ± 3.6	19.2 ± 2.5‡	23.2 ± 6.3
Triglyceride:protein ratio	1.77 ± 0.8	2.75 ± 0.9‡	1.90 ± 0.6
Lipoprotein mass, mg/dl of plasma	26.6 ± 9.1	35.4 ± 8.3†	28.1 ± 10.3

* Values are the mean ± SD of duplicate analyses of each component and are in weight percent. Lipoprotein mass corresponds to the sum of all lipid and protein components. VLDL = very low-density lipoprotein; IDL = intermediate-density lipoprotein; JRA = juvenile rheumatoid arthritis.
† $P < 0.01$ versus controls and versus inactive JRA group.
‡ $P < 0.05$ versus controls and versus inactive JRA group.

Therefore, the mass distribution profiles were not different between the control and the active JRA group (LDL1 9.0 ± 2.2%, LDL2 20.6 ± 3.5%, LDL3 40.3 ± 9.6%, LDL4 17.2 ± 3.8%, and LDL5 12.9 ± 3.1% in the control group, and LDL1 10.7 ± 2.3%, LDL2 19.1 ± 2.8%, LDL3 41.0 ± 5.6%, LDL4 18.4 ± 4.2%, and LDL5 10.8 ± 3.1% in the active JRA group). No difference also was observed between these groups and the inactive JRA group (results not shown).

However, the weight % triglyceride content in all LDL subfractions, except LDL5, was significantly higher ($P < 0.01$) and the weight % protein content was significantly lower ($P < 0.05$) in the active JRA group compared with the controls. As a consequence, the triglyceride:protein ratio was significantly higher in the active JRA group ($P < 0.01$) (Table 4). No difference was observed in the weight % content of cholesterol and phospholipids of all LDL subfractions between the 2 groups (Table 4). The weight % values for all the components in the 5 LDL subfractions of the patients with inactive JRA were similar to those of the controls (results not shown).

The total HDL mass in the active JRA group (400 ± 24 mg/dl of plasma) was significantly lower ($P < 0.01$) compared with either the controls (475 ± 33 mg/dl) or the inactive JRA group (481 ± 40 mg/dl). This phenomenon is due to the significant decrease ($P < 0.03$) in the mass of HDL2 and HDL3 subfractions in the active JRA group (HDL2 156 ± 34 mg/dl and HDL3 182 ± 33 mg/dl in the control group; HDL2 114 ± 27 mg/dl and HDL3 156 ± 23 mg/dl in the active JRA group). In contrast, the mass of VHDL was similar among the 3 groups (137 ± 57 mg/dl in the control group, 130 ± 57 mg/dl in the active JRA group, and 138 ± 40 mg/dl in the inactive JRA group). The decrease in the mass of both HDL2 and HDL3 subfractions was due to the significant decrease in the mass of all the lipid and protein constituents ($P < 0.01$), although the weight % content of each constituent in these subfractions was

Table 4. Mean weight % chemical composition of LDL subfractions from control subjects with normal lipid levels and patients with active JRA*

	LDL subfraction									
	1 (d 1.019–1.023 gm/ml)		2 (d 1.023–1.029 gm/ml)		3 (d 1.029–1.039 gm/ml)		4 (d 1.039–1.050 gm/ml)		5 (d 1.050–1.063 gm/ml)	
	Controls	Active JRA	Controls	Active JRA	Controls	Active JRA	Controls	Active JRA	Controls	Active JRA
Component, %										
Cholesterol	36.7 ± 1.3	37.0 ± 1.6	38.1 ± 2.4	38.7 ± 3.9	40.7 ± 2.5	39.0 ± 2.7	39.9 ± 1.2	42.1 ± 3.2	32.0 ± 2.0	35.1 ± 2.4
Triglyceride	12.6 ± 2.2	19.2 ± 2.9†	10.2 ± 1.8	16.9 ± 2.1†	7.4 ± 1.1	12.7 ± 2.0†	5.6 ± 0.9	9.8 ± 1.3†	6.5 ± 1.0	6.8 ± 1.4
Phospholipid	23.5 ± 2.1	21.8 ± 2.1	23.8 ± 4.7	22.9 ± 2.7	24.5 ± 3.0	25.7 ± 1.5	23.0 ± 2.5	22.5 ± 1.6	22.7 ± 1.9	22.9 ± 1.9
Protein	27.2 ± 3.0	21.2 ± 2.8†	27.9 ± 3.6	21.5 ± 2.5‡	27.5 ± 2.2	22.6 ± 2.1‡	30.9 ± 1.4	25.6 ± 3.0‡	38.5 ± 1.8	35.2 ± 4.1
Triglyceride: protein ratio	0.46 ± 0.1	0.90 ± 0.06†	0.36 ± 0.09	0.79 ± 0.09†	0.27 ± 0.04	0.56 ± 0.04†	0.18 ± 0.03	0.38 ± 0.04†	0.17 ± 0.02	0.19 ± 0.03
Lipoprotein mass, mg/dl plasma	21.8 ± 10.2	24.3 ± 8.9	50.1 ± 18.1	43.2 ± 19.0	97.8 ± 26.5	92.8 ± 29	41.8 ± 17.3	41.7 ± 14.3	31.3 ± 7.6	24.4 ± 8.6

* Values are the mean ± SD of duplicate analyses of each component in each low-density lipoprotein (LDL) subfraction and are in weight percent.
† $P < 0.01$ versus controls.
‡ $P < 0.05$ versus controls.

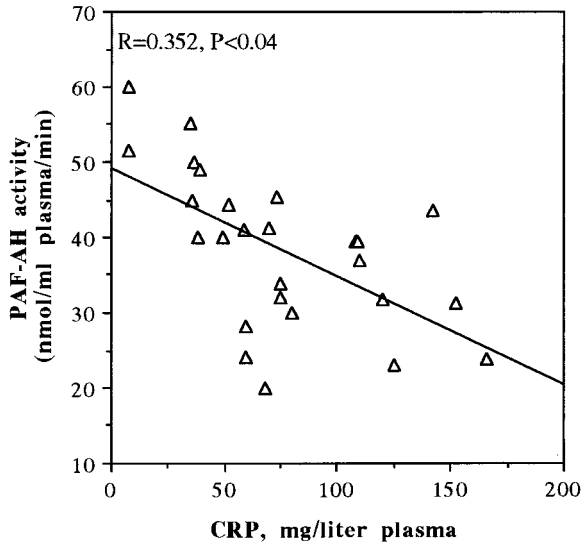

Figure 2. Correlation between plasma levels of C-reactive protein (CRP) and total plasma platelet-activating factor acetylhydrolase (PAF-AH) activity in patients with juvenile rheumatoid arthritis.

not different compared with the control and the inactive JRA group (results not shown).

The distribution profile of total HDL mass among the 3 subfractions as a function of density revealed a significant decrease, by 4.8 ± 0.4%, in HDL2 and a significant increase, by 4.1 ± 0.3%, in VHDL mass ($P < 0.05$) in the active JRA group compared with the control group and with the inactive JRA group. It is important to note that the lipoprotein mass of both HDL2 and HDL3 was inversely correlated with plasma CRP levels ($P < 0.005$ and $P < 0.01$, respectively) (Figure 1D). The mass profile, as well as the chemical composition, of all the above lipoprotein subfractions in all JRA patients studied was not significantly different from those of the control group. Also, no difference was observed between the control group and each patient group or between patient groups when the patients were subgrouped according to the disease-onset type (results not shown).

PAF-AH activity in plasma and lipoprotein subfractions. Mean (±SD) plasma PAF-AH activity (in nmoles/milliliter/minute) was significantly lower in patients with active JRA (37.3 ± 6.6) than in either the controls (51.3 ± 8.2) or the patients with inactive JRA (47.1 ± 9.4) ($P < 0.03$). No difference in the enzyme activity was observed between controls and the patients with inactive JRA. Plasma PAF-AH activity was inversely correlated with the plasma CRP levels ($P < 0.04$) (Figure 2).

To further investigate the decrease in plasma PAF-AH activity in the active JRA group, we measured the enzyme activity associated with each lipoprotein subfraction. The proportion of total plasma activity associated with the total LDL fraction in the control group was 61.1 ± 3.1% (mean ± SD) and with total HDL, 38.4 ± 1.9%. This proportion was similar to that in the inactive JRA group, whereas in the active JRA group, there was a significant decrease in the enzyme activity associated with LDL (49.3 ± 1.1%; $P < 0.02$) and a significant increase in the enzyme activity associated with total HDL (47.9 ± 2.2%; $P < 0.03$), mainly due to the increase in PAF-AH activity associated with VHDL. In all groups, PAF-AH activity was primarily associated with the dense LDL5 subfraction, representing 48–51% of total LDL-associated activity and 22–29% of total plasma–associated activity in all groups.

As shown in Figure 3A, patients with active JRA had significantly higher PAF-AH activity in the VLDL + IDL subfraction and in the large light LDL subfractions LDL1 and LDL2 compared with either the controls or the patients with inactive JRA ($P < 0.02$ for all comparisons). In contrast, enzyme activity in the intermediate and small dense LDL subfractions (LDL3, LDL4, and LDL5) was significantly lower in the active JRA group than that in the control group or in the inactive JRA group ($P < 0.01$ for all comparisons). No difference was observed among the 3 groups in PAF-AH activity associated with HDL2 and HDL3, whereas enzyme activity associated with the VHDL subfraction in the active JRA group was significantly higher than that in the controls or the inactive JRA group ($P < 0.05$ for both comparisons) (Figure 3A). Similar results for the enzyme activity associated with the VLDL + IDL, as well as with the LDL, subfractions were observed when the parameter was expressed as a percentage of the total plasma activity (Figure 3B). In contrast, the percentage of the PAF-AH activity associated with the HDL2 and HDL3 subfractions in the active JRA group was significantly lower compared with the control group or the inactive JRA group ($P < 0.03$ for both comparisons). Additionally, the percentage of the enzyme activity associated with the VHDL subfraction was significantly higher in the active JRA group compared with the other 2 groups ($P < 0.002$) (Figure 3B).

It should be noted that the PAF-AH activity in plasma as well as in individual lipoprotein subfractions in all JRA patients studied was not significantly different from that in the control group. There was no difference in the enzyme activity between controls and each patient

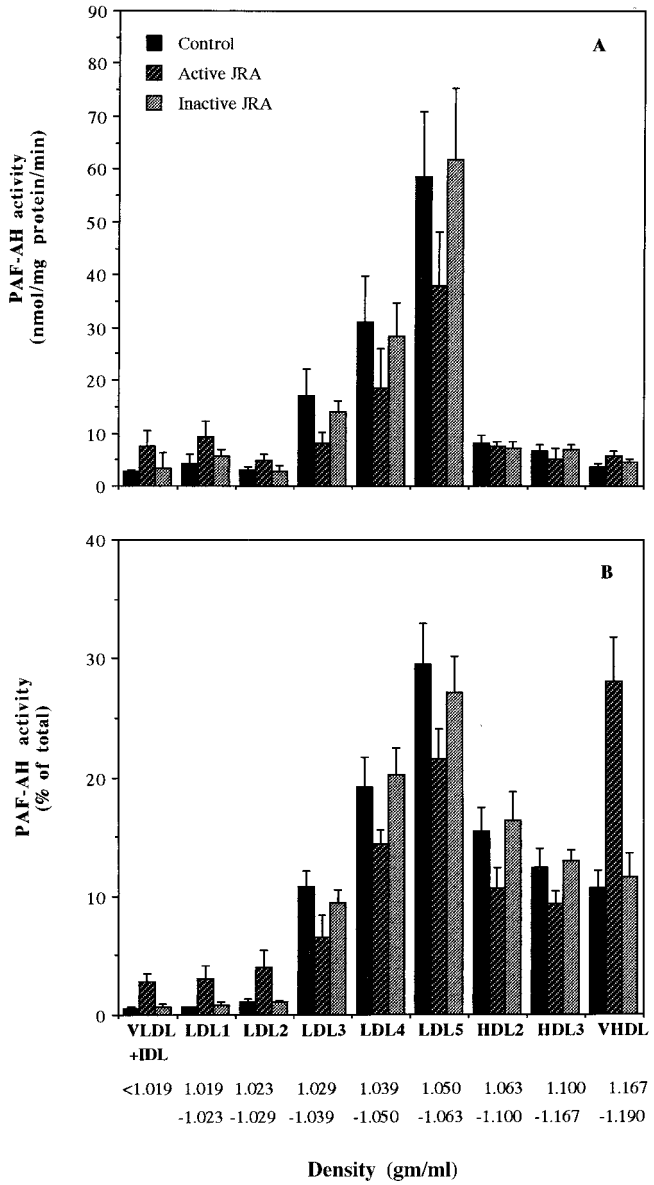

Figure 3. Distribution of plasma platelet-activating factor acetylhydrolase (PAF-AH) activity in the major lipoprotein subfractions from patients with juvenile rheumatoid arthritis (JRA) and in age- and sex-matched control subjects with normal lipid levels. Lipoprotein subfractions were isolated from plasma by isopyknic gradient ultracentrifugation as described in Patients and Methods. PAF-AH activity was determined by the trichloroacetic acid precipitation method with 4 μg of protein from each lipoprotein subfraction as the source of the enzyme and 100 μM ^{3}H-labeled PAF as the substrate. PAF-AH activity is expressed as nanomoles per milligram of protein per minute (**A**) and as a percentage of total plasma enzyme activity (**B**). Values are the mean and SD.

group, as well as between the groups when the patients were subgrouped according to the disease-onset type (results not shown).

Finally, similar results for all lipoprotein subfractions were obtained when the patients receiving methotrexate or methylprednisolone were excluded from the statistical analysis. Additionally, no correlation between the methylprednisolone dose (up to 6 mg/day) in the 6 JRA patients and any of the above lipoprotein and enzyme parameters was observed (results not shown).

DISCUSSION

In the present study, we have shown for the first time that the plasma lipoprotein subfraction profile and lipoprotein-associated PAF-AH activity are significantly altered in JRA patients and are correlated with the inflammatory phase of the disease. Plasma HDL levels in our patients with active JRA were significantly decreased compared with those in patients with inactive JRA and with those in controls with normal lipid levels, mainly due to the decrease in HDL2 and HDL3 subfractions. Furthermore, a significant reduction in plasma PAF-AH activity due to a decrease in enzyme activity associated with the intermediate and dense LDL subfractions was observed in the active JRA group. Since such lipoprotein alterations have not been detected in patients with inactive disease, and they are strongly correlated with plasma CRP levels, we suggest that the degree of inflammatory activity is intimately associated with partial loss of the antiinflammatory role of lipoprotein-associated PAF-AH activity in JRA patients.

Similar lipid abnormalities closely related to disease activity in JRA patients have been previously described (3,4). However, our data show that this dyslipidemia is not present in patients with inactive JRA, unlike previously published data which showed that dyslipidemia is also present in patients with inactive JRA, although to a lesser extent (3). Interestingly, no difference in the observed lipid and lipoprotein abnormalities was found when patients were categorized according to disease-onset type, a finding which is not in agreement with previously published data (3,4). Further studies involving large numbers of JRA patients with each disease-onset type are needed for the complete elucidation of the possible relationship between the disease-onset type and the dyslipoproteinemia observed in JRA patients.

Increased mortality due to atherosclerotic cardiovascular disease has been reported in adults with RA (29–31). The lipid abnormalities found in the present study could be associated with an increased risk of atherosclerosis in patients with active JRA (32–34). Beyond the dyslipidemia observed, another factor con-

tributing to an increased risk of premature atherosclerosis in patients with active JRA is the lower PAF-AH activity level. It has been suggested that PAF-AH can inhibit the oxidative modification of LDL and can decrease the biologic activity of oxidized LDL, playing an important antiatherogenic role (35). Since PAF-AH is preferentially associated with the most atherogenic, dense LDL subfractions (22–24) exerting a protective role, the levels of PAF-AH associated with dense LDL under native conditions in plasma may be a crucial factor which determines its atherogenic potency during oxidation (22). Our present results show that the intermediate and dense LDL subfractions (i.e., LDL3–5) contain significantly lower enzyme activity in patients with active JRA compared with controls and with patients with inactive JRA. Consequently, these LDL subspecies may exhibit less antiinflammatory potency in their native form and become more atherogenic in the artery wall during oxidation.

Several lines of evidence suggest that the protective role of HDL against LDL oxidation (33) is at least partly due to the HDL-associated PAF-AH (36). According to our results, the plasma PAF-AH activity associated with the most functional HDL subfractions (HDL2 and HDL3) was significantly lower due to the lower plasma levels of those subfractions. Thus, it can be an additional factor contributing to a higher atherogenicity of the intermediate and dense LDL subfractions in our patients with active JRA. The lower plasma PAF-AH levels in patients with active JRA could also contribute to the disease activity, since an enhanced production and secretion of PAF has been reported in patients with autoimmune diseases, including RA and SLE (37).

It has been reported that macrophages play an important role in lipoprotein metabolism (38,39), and a relationship between diseases involving monocyte/macrophages (i.e., myeloproliferative diseases) and hypocholesterolemia due to low HDL cholesterol levels has been documented (40,41). Thus, taking into account the significant activation of the monocyte/macrophages observed in patients with active disease, it is highly likely that the low HDL mass observed in our patients with active JRA could be partly ascribed to the enhanced reticuloendothelial system activity. Additionally, the inflammatory state in patients with active JRA could account for the observed decreased PAF-AH activity, since it is known that several mediators of inflammation decrease enzyme secretion from human macrophages, which are one of the main cellular sources of the plasma form of PAF-AH (42,43).

The decreased lipoprotein lipase (LPL) activity found in patients with autoimmune diseases, particularly in adults with RA, can be implicated in the lipid disturbances observed in our patients with active JRA (1,44). The reduced LPL activity could be responsible for the decreased hydrolysis of VLDL triglycerides, causing accumulation of VLDL + IDL and resulting in increased total and VLDL + IDL triglycerides, and subsequently, in decreased HDL2 and HDL3 lipoprotein mass and increased triglyceride content of most LDL subfractions (45–47).

The observed dyslipoproteinemia in our patient cohort is unlikely to be related to malnutrition or to reduced function, since despite active disease, our patients had normal dietary intake and were categorized as functional class I or II according to the Steinbrocker classification. Treatment with corticosteroids may have also had an indirect impact on the dyslipoproteinemia observed. However, only a few patients received small doses of methylprednisolone, which were not correlated with the levels of lipid and lipoprotein parameters measured. Some patients were also given methotrexate, which might have influenced serum lipids and lipoproteins. However, the exclusion of patients treated with methotrexate from the statistical analysis did not significantly influence lipid parameters or the compositional characteristics of the lipoprotein subfractions in both groups of JRA patients.

We conclude that patients with active JRA exhibit low HDL2 and HDL3 levels as well as increased plasma triglyceride levels. These lipoprotein alterations are highly correlated with the inflammatory state of the disease and may result in higher atherogenicity in JRA patients, especially those with prolonged periods of active disease. Furthermore, a significant reduction in the plasma PAF-AH activity mainly due to the reduction in enzyme activity associated with the intermediate and dense LDL subfractions was observed in the patients with active JRA and was also highly correlated with the inflammatory state of the disease. Since PAF is involved in atherogenesis and may participate in the inflammatory reactions that occur in patients with RA, the decrease in PAF-AH activity could be a factor that contributes to higher atherogenicity as well as to the inflammatory state in the patients with active JRA. However, it is highly likely that patients who have long periods of remission have a lower incidence of atherosclerotic cardiovascular disease.

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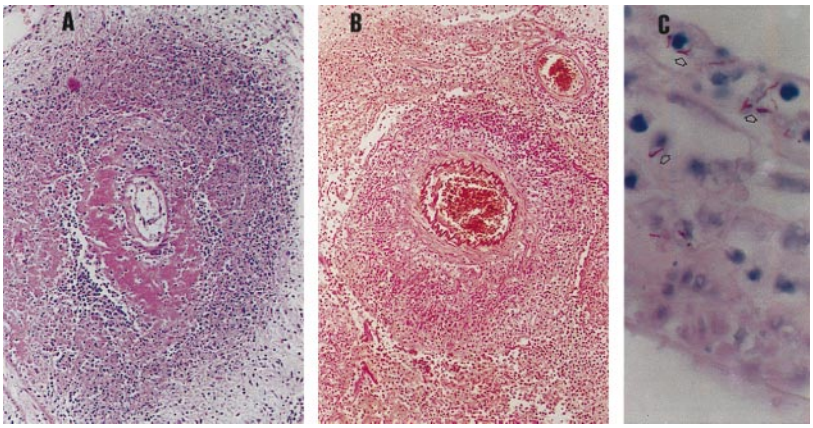
Clinical Images: Histopathologic features of cerebral vasculitis associated with Mycobacterium tuberculosis



The patient, a 37-year-old man, was diagnosed with systemic adult Still's disease in March 1997. He was initially treated with indomethacin and prednisone. Due to inadequate response, methotrexate was added to the regimen. Two months later, he was admitted to the hospital with fever, confusion, agitation, and a low level of consciousness. Cerebrospinal fluid (CSF) examination showed 20 red blood cells/mm^3, 29 white blood cells/mm^3 (42% neutrophils, 58% lymphocytes), a glucose level of 156 mg/dl (serum glucose level 250 mg/dl), and a protein level of 274 mg/dl. Stains for bacteria (Gram), mycobacteria, and fungi in the CSF were negative. Computerized tomography of the brain yielded normal results. Treatment with antibiotics, intravenous acyclovir, and intravenous methylprednisolone was instituted. Cultures for common bacteria and fungi in the CSF, blood, and urine were also negative, and aseptic meningitis was diagnosed. The patient died 2 weeks after admission. One week after his death, *Mycobacterium tuberculosis* was demonstrated in the CSF culture. The photomicrographs shown here reveal a variety of histologic patterns of angiitis in the patient. **A,** Necrotizing vasculitis with fibrinoid necrosis involving the wall of a medium-sized leptomeningeal cortical artery (hematoxylin and eosin stained; original magnification × 40). **B,** Lymphocytic vasculitis characterized by prominent infiltration of lymphocytes in the media of a cerebral artery. The internal elastic lamina is not fragmented (orcein stained; original magnification × 40). **C,** *M tuberculosis* invading the vessel wall (**arrows**) (Ziehl-Neelsen stained; original magnification × 100).

Francisco J. Blanco García, MD
Mercedes Sánchez Blas, MD
Mercedes Freire González, MD
Hospital Juan Canalejo
La Coruña, Spain