IDENTIFICATION OF THE ABNORMAL CHOLESTATIC LIPOPROTEIN (LP-X) IN FAMILIAL LECITHIN:CHOLESTEROL ACYLTRANSFERASE DEFICIENCY

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Virtually all lipids of human plasma circulate in association with specific proteins to yield lipid–protein complexes or lipoproteins. The lipoproteins are conventionally classified into four main groups based on ultracentrifugal flotation or electrophoretic mobility: chylomicrons, very low density lipoprotein (VLDL) or pre-β-lipoprotein, low density lipoprotein (LDL) or β-lipoprotein, and high density lipoprotein (HDL) or α_1-lipoprotein. According to the chemical classification system [1], plasma lipoproteins consist of a mixture of polydisperse lipoprotein families each of which is characterized by the presence of a single, distinct apolipoprotein or its constitutive polypeptides: lipoprotein family LP-A is characterized by apolipoprotein A, lipoprotein family LP-B by apolipoprotein B and lipoprotein family LP-C by apolipoprotein C.

In sera of normal subjects and most patients with hyperlipoproteinemia of different types, 60–80% of the cholesterol is esterified. However, the levels of esterified cholesterol have been reported to be very low in obstructive jaundice [2] and in familial lecithin:cholesterol acyltransferase (LCAT) deficiency [3].

The plasma lipids of patients with biliary obstruction are characterized by an elevation of unesterified cholesterol and of phospholipids. The concentration of HDL is very low. In the LDL fraction as prepared in the ultracentrifuge an abnormal lipoprotein has been found [4]. This lipoprotein, designated LP-X, does not react with antibodies to normal LDL. Its protein moiety consists of 40% albumin and 60% apolipoprotein C. The apolipoprotein C contains its three characteristic polypeptides, C-I, C-II and C-III [5] which are normally present in VLDL and HDL. The antigenic sites of albumin are masked, but may be revealed by partial or total delipidation [6]. The average flotation rate (S_f) of LP-X is 15.9 svedbergs in NaCl solution of density 1.063 g/ml [7]. In electron micrographs LP-X appears as disc-shaped particles with a major axis of 400–600 Å [8]. According to the chemical classification systems the LP-X is classified as a subfamily of LP-C [1].

The activity of the plasma enzyme lecithin:cholesterol acyltransferase (LCAT) accounts for the intravascular esterification of cholesterol in human plasma [9]. Familial LCAT deficiency is characterized by

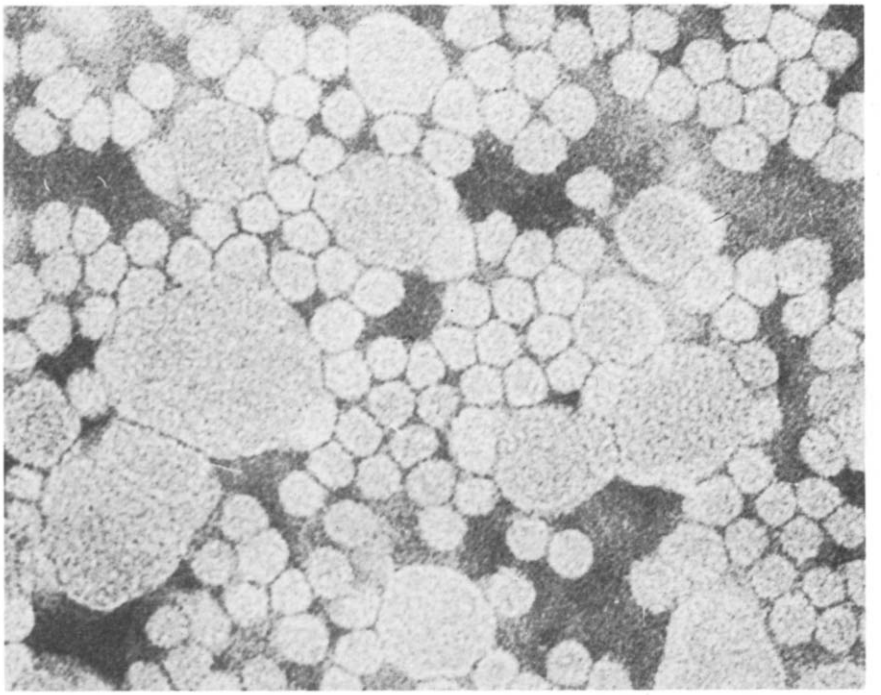

Fig. 1. Electron micrography of the low density (d 1.019–1.063 g/ml) lipoprotein fraction from plasma of a patient with familial LCAT deficiency, showing two populations of particles. The smaller particles correspond to normal LDL, the larger to the abnormal lipoprotein (× 320,000, negative staining).

proteinuria, anemia, corneal opacities, and several plasma lipid and lipoprotein abnormalities [3]. We report here the presence in plasma from patients with familial LCAT deficiency of an abnormal lipoprotein identical to LP-X.

Lipoproteins of different density classes were prepared by ultracentrifugation of serum from three patients with familial LCAT deficiency. Electron microscopy of the LDL fraction (density 1.019–1.063 g/ml) revealed the presence of two populations of particles, with a particle diameter of 200–250 Å, and 400–900 Å, respectively (fig. 1). The LDL fraction was further separated by gel filtration on agarose gel columns [10] into three fractions. After re-chromatography fraction I contained large particles, 600–1100 Å, many of which appeared to be breaking up into smaller particles 100–400 Å in diameter. The material in fraction I was interpreted as a combination of aggregated and degraded particles. Fraction II contained particles corresponding to the larger particles found in the LDL fraction prior to the gel filtration, whereas the particles of fraction II corresponded to normal LDL.

The abnormal lipoprotein present in fraction II exhibited a composition similar to that of LP-X (given in parenthesis [4]): 5.0% (5.8%) protein, 60.7% (66.5%) phospholipid, 3.1% (2.9%) triglyceride, 28.3% (22.4%) unesterified and 2.9% (2.4%) esterified cholesterol. The phospholipid–protein ratio was 12.1 (11.5).

The abnormal lipoprotein did not react with antiserum to normal LDL in agar gel double diffusion experiments, but with antibodies to apolipoprotein C. After partial delipidation with n-heptane [6], it also reacted with antiserum to albumin. The polypeptides of apolipoprotein C were present in the supernatant fluid after ultracentrifugation of the partially delipidated abnormal lipoprotein at the density 1.21 g/ml. They constituted 65% of the protein moiety, whereas the remaining 35% was found as albumin in the infranatant fluid. Neither before nor after partial or total delipidation did the abnormal lipoprotein ex-

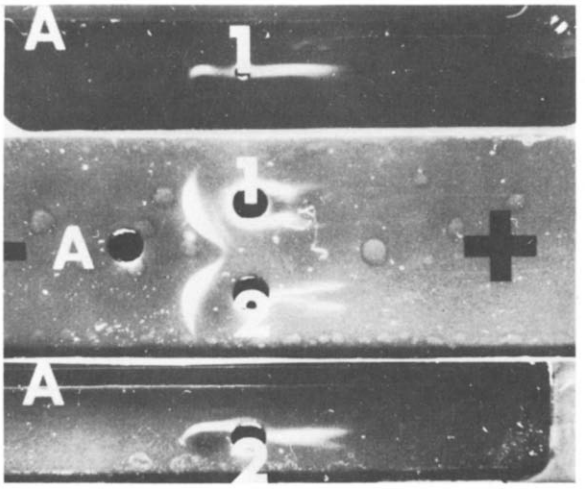

Fig. 2. Agar immunoelectrophoretic test showing cathodal migration of the abnormal lipoprotein of patients with LCAT deficiency, and identity reaction between this lipoprotein and LP-X from a patient with obstructive jaundice. Troughs and well A: antiserum to apolipoprotein C. Well 1: plasma from a patient with LCAT deficiency. Well 2: plasma from a patient with obstructive jaundice.

hibit precipitin bands with antisera to LDL or to 15 other serum proteins tested. However, a weak precipitin band developed against anti-α_1-lipoprotein serum, but not against antisera to the principal polypeptides of HDL, A-I and A-II.

The presence of C-I, C-II and C-III was verified immunologically and by analytical polyacrylamide disc gel electrophoresis in basic and acidic buffer systems after isolation of the abnormal lipoprotein by immunoprecipitation of normal LDL with goat lipoprotein-free anti-LDL serum and its total delipidation.

By agar immunoelectrophoresis of serum from patients with LCAT deficiency, the abnormal lipoprotein exhibited a cathodal migration similar to LP-X from patients with obstructive jaundice, and reactions of identity were obtained between these two abnormal lipoproteins (fig. 2). The identity reactions were verified in agar gel double diffusion tests. The abnormal lipoprotein from patients with LCAT deficiency was found only in the LDL fraction. The observations permit the conclusion that this abnormal lipoprotein is closely related to LP-X.

The amount of LP-X in plasma of patients with obstructive jaundice ranged from 40 to 1200 mg% [11], whereas plasma from three patients with LCAT deficiency contained 49, 98, and 152 mg%, respectively.

The demonstration of LP-X in plasma of patients with liver diseases is a sensitive indicator of biliary obstruction [12]. It has been suggested that the accumulation of LP-X may be the result of an impaired lipoprotein catabolism caused by the inhibitory action of increased concentrations of bile salts [6]. However, the finding of a lipoprotein belonging to the LP-X family in plasma of patients with familial LCAT deficiency suggests that also other factors might be involved in the formation of this abnormal lipoprotein. These patients have no symptoms or signs of biliary obstruction as judged by the routine liver function tests [3]. Since increased amounts of unesterified cholesterol are common to both disorders, the formation of LP-X may represent a mechanism for its removal.

Further studies on the lipoprotein abnormalities in patients with obstructive jaundice and with familial LCAT deficiency may clarify important problems related to the origin and metabolism of LP-X and its relationship to the normal lipoprotein metabolism.

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