Sea-blue histiocytosis associated with hyperlipidaemia

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SYNOPSIS A patient with sea-blue histiocytosis in spleen and bone marrow with an accompanying hyperlipidaemia is described. The hyperlipidaemia was due to an increase in 'free' cholesterol, lecithin, and triglycerides. Despite these findings lecithin-cholesterol acyl transferase activity was normal. Although the precise biochemical defect was not identified, there was a failure of transport of cholesterol from chylomicrons *in vitro*. We propose that the sea-blue histiocyte is a marker, in some cases, of abnormal lipid metabolism.

Since the description by Moeschlin in 1947 of blue histiocytes in the spleen of a patient with unexplained splenomegaly, a number of reports have appeared concerning this cell which is large (20-60 μm in diameter) and contains numerous granules which stain blue with Romanowsky dyes. Silverstein and co-workers (1970) were the first to delineate a specific syndrome characterized by hepatosplenomegaly, mild thrombocytopenia, and frequent lung and eye abnormalities in which similar histiocytes were found in the spleen and bone marrow. Sea-blue histiocytes have also been recorded in a wide variety of conditions including such diverse conditions as chronic myeloid leukaemia (Steinberg and Dreiling, 1973) and abnormalities of lipid metabolism such as lecithin-cholesterol acyl transferase (LCAT) deficiency (Hovig and Gjone, 1973). We describe a patient in whom sea-blue histiocytes were associated with marked plasma lipid abnormalities. Although some of the biochemical findings were similar to those found in LCAT deficiency the activity of the enzyme in plasma was normal.

Case report

A 32-year-old man presented with a perianal abscess and was found on routine examination to have symptomless firm splenomegaly 4 cm below the left costal margin. There was no other clinical abnormality and no past medical history of significance. The patient had a mild thrombocytopenia (platelet

count $84 \times 10^9/\text{l}$) but otherwise full blood count and other routine investigations were normal. Splenic aspirate showed the presence of many large histiocytes which contained blue granules when stained with Romanowsky dyes. These cells were also present in the marrow in lesser numbers but were not found in the liver biopsy although small areas of fatty infiltration in the hepatic parenchymal cells were seen. Chromosomal karyotype of the patient's marrow was normal.

Electron microscopic examination of material from the patient's spleen and bone marrow showed that in both tissues there were large histiocytes (30 μm diameter) which contained numerous granules of slightly differing electron density (fig 1). Some granules were present as aggregates within vacuoles limited by a unit membrane. Others seemed free in the cytoplasmic matrix. In both situations the granules had a similar structure and were composed of an admixture of amorphous material and lamellar whorls of membranous material that tended to assume characteristic fingerprint patterns (fig 2) similar to those described in LCAT deficiency (Jacobsen *et al*, 1972). The histiocytes also contained debris derived from ingested erythrocytes.

Routine investigation, marrow morphology, ultrastructure, and chromosomal karyotype were normal in the parents of the patient.

BIOCHEMICAL INVESTIGATIONS

The patient's fasting serum was turbid and the turbidity remained when the serum had stood overnight at 4°C despite the formation of a layer of

Fig 1 Electron microscopic appearance of a sea-blue histiocyte showing numerous cytoplasmic electron dense granules and two large vacuoles, one of which contains an aggregate of granules.

'cream'. Total cholesterol concentration was raised in three of the four samples taken over 10 months, ranging from 6.2 to 10.4 mmol/l (normal range 3.6-6.7 mmol/l). The percentage of total cholesterol in the non-esterified form was persistently raised (60-70%) in comparison with normals and patients suffering from other forms of hypercholesterolaemia in which this percentage is approximately 25%. Triglyceride concentration was also raised at 4.40

to 4.55 mmol/l (normal reference level up to 1.7 mmol/l).

Thin-layer chromatography of plasma lipids confirmed the increase of free cholesterol and triglycerides. It also demonstrated raised lecithin levels and showed that lysolecithin was present.

The serum lipoproteins were studied by agarose gel (Noble, 1968) and polyacrylamide gel (Frings *et al*, 1971) electrophoresis (fig 3). Both methods

Fig 2 *Electron microscopic appearance of a granule in the cytoplasm of the sea-blue histiocyte showing the characteristic 'finger-print' arrangement of membranes.*

showed an abnormal band migrating in front of the usual α -lipoprotein band. No staining was visible in the normal α -lipoprotein position. The pre- β -lipoprotein showed a massive diffuse increase. In contrast β -lipoprotein was reduced especially on acrylamide gel. Immunoelectrophoresis against anti- α and anti- β lipoprotein antisera (Hoechst Pharmaceuticals) indicated a probable reduction in β -lipoprotein with normal or slightly increased α -lipoprotein. The lipoprotein arc showed an abnormal backward prolongation (fig 4).

Since there was a high level of free cholesterol and normal values of esterified cholesterol in the plasma, the plasma LCAT activity was measured. However, serum cholesterol esterifying activity (Stokke and Norum, 1971) was normal at 120 $\mu\text{mol/l}$ per hour.

In addition the pattern of isoenzymes of LCAT (Glomset and Wright, 1964) from the patient's plasma was similar to that from normal plasma and also resembled that obtained by Lacko and co-workers (1974).

The granules in the histiocytes in our case were not due to a lysosomal storage disorder, because there was no disturbance in a number of acid hydrolase activities including β -glucosidase and acid esterase in the cultured skin fibroblasts from our patient.

An attempt was made to determine the possible site of the basic biochemical defect in a series of *in vitro* incubation studies with plasma. Tritiated cholesterol was incubated for four hours with serum from the patient with sea-blue histiocytosis, from a

Fig 3 Lipoprotein electrophoresis on polyacrylamide gel (Frings *et al*, 1971) of the serum from the patient, a sample from a Frederickson and Levy (1972) type IV hyperlipoproteinaemia, and two normal controls

nephrotic child, and from a normal adult. In all sera LCAT was inhibited by Ellman's reagent (5,5-dithiobis [2-nitrobenzoic acid]). Serum lipoproteins were then separated by agar gel electrophoresis, and the amount of labelled cholesterol present in different fractions was determined using liquid scintillation counting. In these studies the

radioactivity remained in the chylomicrons in the patient's plasma to a greater extent than in normal or hyperlipaemic nephrotic plasma.

Discussion

Two groups of conditions involving the sea-blue histiocyte have been reported. The first is characterized by abundant sea-blue histiocytosis in the spleen, liver, and bone marrow and in some cases by hepatic lipid abnormalities. The second group has histiocytes confined to the spleen and bone marrow, and frequently other diseases co-exist (Silverstein and Ellefson, 1972). When these simple histological criteria are used this patient's condition corresponds to the second pattern.

Although hyperlipidaemia has rarely been recorded in association with the sea-blue histiocytic syndrome, LCAT deficiency (Jacobsen *et al*, 1972; Hovig and Gjone, 1973) shows a pattern of hyperlipidaemia somewhat similar to that of our case together with abnormal histiocytes. There was no evidence of LCAT deficiency to account for the apparent defect of cholesterol esterification in our patient (Glomset and Norum, 1973).

Transport of cholesterol to the α -lipoproteins is necessary for the esterification of cholesterol. Since in this case LCAT activity *in vitro* was normal despite the abnormality *in vivo* it seems reasonable to suggest that there may be a transport defect in this patient. This suggestion is supported by the results

Fig 4 Lipoprotein immunoelectrophoresis of serum from the patient and two normal controls using anti- α and anti- β lipoprotein

of the incubation studies with ^{14}C -labelled cholesterol which demonstrated a failure of transfer from the chylomicrons to α -lipoprotein, the preferred substrate of LCAT. Possibly the primary defect is in one of the polypeptide components of HDL_3 ,¹ since this has been shown to be involved in such transfer of cholesterol (Marcel and Vezina, 1973).

Some indication of the pathogenesis of the sea-blue histiocyte has been provided by the observation of Hovig and Gjone (1973) who found that the plasma from patients with LCAT deficiency contained abnormal particulate lamellar lipid material with an ultrastructural finger-print pattern. We suggest that the sea-blue histiocyte is merely a macrophage that has ingested large amounts of particulate lipid in the process of clearing such particles from the blood by the mononuclear phagocyte system.

Thus although the precise biochemical defect in our patient has not been identified, we propose that the sea-blue histiocyte can be a marker for abnormalities of lipid metabolism, many of which probably remain to be described.

We are most grateful to Dr S. H. Davies for his encouragement, to Dr J. Butterworth and other colleagues for their help with lysosomal enzyme estimations, and to Mrs Maureen Gilchrist for the chromosome analysis. Thanks are also due to Miss Linda Graham and Miss Kathleen Denholm for typing the manuscript.

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¹High Density Lipoprotein₃