Acquired lipidosis of marrow macrophages

Birefringent blue crystals and Gaucher-like cells, sea-blue histiocytes, and grey-green crystals

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SUMMARY Three varieties of compound lipid inclusions occurring as a secondary phenomenon in marrow macrophages are detectable and distinguishable by Romanowsky staining, ultraviolet fluorescence, and polarised light. Birefringent blue crystals and Gaucher-like cells form one variety, sea-blue granules another, and grey-green crystals a third. All occur chiefly in myeloid leukaemias, either acute or chronic.

Normally, macrophages in the bone marrow may contain cellular remnants, lipid globules, granules of iron, and other ingested material, resulting from phagocytic activity. The appearance of these cells is well recognised and amply illustrated in haematological atlases.

Inherited disorders of lipid metabolism, usually associated with specific enzyme deficiencies, may induce storage of lipids such as sphingolipids in histiocytes in the reticuloendothelial system. In Gaucher's disease, for example, a deficiency of β-glucocerebrosidase leads to accumulation of glucosyl ceramide, whereas in Niemann Pick disease deficiency of sphingomyelinase causes sphingomyelin (phosphoryl choline ceramide) to accumulate. Other storage diseases with typical clinical syndromes and inheritance are recognised. In most of these conditions the marrow histiocytes containing the storage material concerned present a characteristic cytological and cytochemical appearance at both light microscope and ultrastructural levels (Stanbury et al., 1972). Among these inherited disorders is primary sea-blue histiocytosis, a condition in which the pathogenesis and the chemistry of the stored material are less clearly established, although phospholipid, glycosphingolipids, and ceroid have been identified (Sawitsky et al., 1972; Silverstein and Ellefson, 1972).

Sea-blue histiocytes occur in the marrow not only in the primary genetic syndrome but also in other disorders of diverse form and aetiology. Secondary sea-blue histiocytosis is an example of what has been called acquired lipidosis, in which inclusions of differing morphology, but probably all of lipid character, may accumulate in histiocytes in the bone marrow in diseases such as chronic myeloid leukaemia (CML) (Dosik et al., 1972) and dyserythropoiesis (Lewis and Verwilghen, 1977). Whereas the cytological appearance of sea-blue histiocytes is now well recognised, the various other crystalline or discrete inclusion bodies found as a secondary phenomenon in certain blood dyscrasias are less well known. They include material having a fibrillary disposition in the cytoplasm so as to give a resemblance to Gaucher cells, first described as a secondary phenomenon in CML by Albrecht (1966), and various kinds of crystals, such as the 'light green' crystals described by Stavem et al. (1977) in both acute and chronic myeloid leukaemias.

During the past few years we have looked specifically for acquired lipidosis of marrow histiocytes when studying bone marrow aspirates and have noted that some inclusions are strikingly birefringent in polarised light whereas others are not. The object of this paper is to describe and illustrate the distinguishing features of the three main varieties of secondary lipidosis which we have observed during a survey of some 1000 bone marrow samples, to indicate the conditions in which they may be found, and to make some assessment of their frequency and significance.

Material and methods

The marrow aspirates reviewed came mostly from patients under our care over the last five years but...
Acquired lipidosis of marrow macrophages

included about 100 specimens referred to us from elsewhere. During this period approximately 1000 aspirates were studied, including 367 original diagnostic aspirates from patients with haematological malignancies, aplastic anaemia, or idiopathic thrombocytopenic purpura, conditions in which acquired lipidosis of histiocytes has previously been recorded. Aspirates were all smeared directly without use of anticoagulants. They were stained by Romanowsky methods—either Leishman or May-Grünwald-Giemsa stain (Hopkins and Williams, Revector brand).

The inclusions in histiocytes were classed according to their morphology as seen in Romanowsky stains, but all histiocytes showing visible inclusions and many without were also observed under polarised light for the existence of birefringence. These observations were made with a Zeiss photomicroscope fitted with polarising filters. Examples of each type of inclusion recognised by these methods were also observed under ultraviolet (UV) light using a Zeiss fluorescence microscope and transillumination for autofluorescence.

Further examples were stained by cytochemical methods standard in this laboratory—peroxidase, Sudan black, periodic acid Schiff (PAS), acid and alkaline phosphatase, and a range of non-specific esterases (Hayhoe and Flemans, 1969; Hayhoe and Cawley, 1972; Higgy et al., 1977).

Electron microscopy (EM) was carried out on glutaraldehyde-fixed material by standard methods (Cawley and Hayhoe, 1973).

Results

The inclusion material observed in histiocytes was of three types separable by Romanowsky stains, polarised light, UV autofluorescence, and EM (Table 1).

**Birefringent Blue Crystals and Gaucher-Like Cells**

The typical appearance of these inclusions in Romanowsky preparations is illustrated in Figures 1-5. The crystals stain a blue-grey colour in Romanowsky preparations and in any individual cell may be few in number or may occupy the bulk of the cytoplasm. They are more readily seen as the cell breaks up. They are generally lanceolate and 6-12μ in length, often arranged in bundles (Fig. 1). When the cell is disrupted the crystals lie free on the slide (Fig. 2), and when they are numerous, isolated crystals may be seen some distance from a disrupted cell. The crystals are birefringent, and Fig. 3 illustrates the same cell as in Fig. 2 using polarised light and showing the birefringence. They are frequently not obvious in a Romanowsky-stained preparation, and this is illustrated in the large, binucleated, phagocytic histiocyte in Fig. 4, which also shows the faint striations of the cytoplasm which may give a pseudo-Gaucher cell appearance. This same cell with polarised light contained many birefringent

![Fig. 1 Blue crystals (type I) in a histiocyte showing slight fragmentation from a patient with a typical chronic myeloid leukaemia.](http://jcp.bmj.com/)

**Table 1 Cytological findings distinguishing inclusions in histiocytes**

<table>
<thead>
<tr>
<th>Type of inclusion and size</th>
<th>Cytochemistry</th>
<th>Birefringence</th>
<th>Autofluorescence</th>
<th>EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue-grey crystal lanceolate 6-12μ long</td>
<td>Peroxidase, PAS, Sudan black, and phosphatases usually negative. Esterases may be positive</td>
<td>Consistently present</td>
<td>Absent</td>
<td>Microtubular structure in crystal-shaped structure</td>
</tr>
<tr>
<td>Sea-blue bodies 1-3μ diameter</td>
<td>Peroxidase, PAS, phosphatases, esterases variable. Sudan black may be positive</td>
<td>Absent</td>
<td>Present</td>
<td>Usually structureless osmiophilic inclusions. Occasionally fingerprint pattern</td>
</tr>
<tr>
<td>Grey-green crystal cigar-shaped 8-20μ long</td>
<td>Peroxidase, PAS, Sudan black, phosphatases, and esterases usually negative</td>
<td>Absent</td>
<td>Absent</td>
<td>Crystal-shaped with homogeneous ultrastructure</td>
</tr>
</tbody>
</table>
coarser and less densely packed than those of a true Gaucher's disease histiocyte, as illustrated in Figure 6.

Fig. 2 Blue crystals (type 1) showing fragmentation of a histiocyte from the same patient as in Fig. 1.

Fig. 3 The same field as in Fig. 2 under polarised light showing birefringent crystals.

Fig. 4 A pseudo-Gaucher cell containing phagocytosed cell debris from a patient with aplastic anaemia.

Fig. 5 The same field as in Fig. 4 showing many rather coarse birefringent crystals.

F. G. J. Hayhoe, R. J. Flemans, and D. C. Cowling
**Acquired lipidosis of marrow macrophages**

**Fig. 6** Gaucher disease marrow histiocyte showing many bundles of slender birefringent crystals.

**SEA-BLUE GRANULES**
These are small, rounded bodies of 1-3μ diameter which stain with a varying degree of intensity a sea-blue colour, their number ranging from a few to many in any affected histiocyte. If a Perls' stain is carried out subsequently, the cell frequently contains no stainable iron (Figs 7 and 8), but in some histiocytes with sea-blue material stainable iron may also be present. Sea-blue inclusion material in UV light may show a pink-coloured autofluorescence but we have not found it to be birefringent.

**GREY-GREEN CRYSTALS**
The third type of lipid inclusion recognised in our laboratory consists of large, greyish crystals, usually thicker and longer than the birefringent blue crystals, measuring 8-20μ long and having a cigar shape. They seem to be identical with the 'light green' crystals described by Stavem et al. (1977), and we accordingly describe them as 'grey-green' crystals. The cells containing them are generally disrupted, and it seems likely that the size and rigidity of these crystals cause fragmentation of the cell during the spreading of the marrow smear so that free lying crystals are also seen. These crystals show neither birefringence nor autofluorescence, nor do they react with Perls' stain. Figures 9 and 10 show a histiocyte stained first with Leishman and subsequently with Perls' stain. This cell contained very few stainable iron granules. Other histiocytes from the same marrow aspirate from a patient with acute myeloid leukaemia showed a few grey-green crystals together with other inclusion material.

**Fig. 7** A sea-blue histiocyte in marrow from a patient with subacute myeloid leukaemia.

**Fig. 8** The same field as in Fig. 7 after restaining with Perls' stain.
Grey crystals in a macrophage.

Fig. 9

The same field as in Fig. 9 stained with Perls' stain.

Fig. 10

which, when stained by Perls' reaction, was shown to be stainable iron (Figs 11 and 12).

CYTOCHEMICAL AND ELECTRON MICROSCOPIC FINDINGS

Normal marrow histiocytes show variable cytochemical findings; they may show diffuse PAS staining and may contain some granules of PAS-positive material. They may contain granules staining with Sudan black. They are usually strongly positive for acid and alkaline phosphatase. Of the esterase stains, the $\alpha$-naphthyl butyrate esterase method usually gives a strong reaction, whereas the $\alpha$-naphthyl AS-D-chloroacetate esterase is usually negative or only weakly positive. When interpreting cytochemical reactions in and around the inclusion material of histiocytic lipidosis the background reactivity of the histiocyte cytoplasm must be taken into account. Apart from occasional staining of sea-blue material by Sudan black B all other cytochemical reactions seemed probably negative in the inclusion material, although, when the reaction was positive in the histiocyte cytoplasm, some condensation or localisation of reaction product at the surface of the inclusions was commonly noted.

Thus the blue crystals and Gaucher-like inclusion material did not give any positive cytochemical reactions although the $\mu$-naphthyl butyrate reaction was positive elsewhere in the histiocyte cytoplasm with some apparent concentration of reaction product at the surface of the crystals. Under EM these crystals contain numerous microtubules with an arrangement similar to that seen in Gaucher's disease (Fig. 13).

Sea-blue histiocytes gave variable Sudan black staining of inclusions, some staining strongly, others not staining. Other cytochemical methods gave generally negative results although PAS, acid and alkaline phosphatase, and $\alpha$-naphthyl butyrate reactions, which give variable to strong cytoplasmic staining in the histiocytes, also gave a suggestion of occasional concentration of reaction product at the granule surface.

EM showed the presence of rounded osmiophilic inclusions, sometimes in groups in vacuoles. In two marrows studied we were unable to find the 'fingerprint' pattern inclusions described by Parker et al. (1976) resembling the appearance seen in Niemann-Pick disease.

Grey-green inclusions were apparently themselves unstained by the cytochemical methods although the strongly positive $\alpha$-naphthyl butyrate stain
Acquired lipidosis of marrow macrophages

Fig. 11  Grey crystals in a histiocyte containing many other inclusions.

Fig. 12. The same field as in Fig. 11 showing much stainable iron in particles and condensation of stain about crystals.

appeared condensed around their edges (Fig. 14), as also did stainable iron (Fig. 12). EM showed a homogeneous, structureless appearance similar to that described by Stavem et al. (1977).

AUTOFLUORESCENCE

Sea-blue histiocyte material frequently showed a pink or yellow coloured autofluorescence in ultraviolet illumination, whereas neither the birefringent blue crystals, the pseudo-Gaucher cells, nor the grey-green crystals showed autofluorescence.

INCIDENCE OF ACQUIRED LIPIDOSIS OF MARROW HISTIOCYTES

The incidence of these three types of lipid inclusion material within marrow histiocytes in the main haematological disorders where this phenomenon occurs—myeloid leukaemias, ITP, and aplastic anaemia—is set out in Table 2 and contrasted with their incidence in lymphoid leukaemias and myeloma. The numbers of readily recognisable marrow histiocytes vary between the different forms of leukaemia, as among other blood diseases, and a comparison between their ease of identification in acute leukaemias of myeloid and lymphoid origin shows that only 11 of 30 cases of ALL (37%) had obvious histiocytes, while 38 of 46 cases of AML (83%) showed them. Even allowing for this difference Table 2 shows that secondary histiocytic lipidosis of every kind is much commoner in myeloid proliferations than in lymphoid, where this type of inclusion material seems to occur only very rarely. More than one type of inclusion may be found in the same marrow aspirate; in six cases blue crystals and sea-blue histiocytes were both found, and in two cases inclusions of all three types were present.

Among the non-leukaemic marrows studied, inclusion material of one or another kind was found in histiocytes from two of 14 cases of ITP and from

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. examined</th>
<th>Birefringent blue crystals and Gaucher-like cells</th>
<th>Sea-blue histiocytes</th>
<th>Grey-green crystals</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>142</td>
<td>4</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>CML</td>
<td>38</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>ALL</td>
<td>65</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>CLL</td>
<td>44</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Myeloma</td>
<td>58</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>ITP</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Aplastic anaemia</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
four of six cases of aplastic anaemia, and in isolated cases of hereditary dyserythropoietic anaemia, β-thalassaemia, hypersplenism, β-lipoproteinaemia, and angioimmunoblastic lymphadenopathy. Histiocytes with these types of inclusion were not found in any of several hundred marrow samples, mostly within normal limits cytologically, from patients with Hodgkin's disease or other lymphomas, or in marrows from patients with anaemias secondary to deficiency of haematinic factors or to haemolysis.

Discussion

Earlier observations on acquired lipidosis of marrow histiocytes have been reviewed by Dosik et al. (1972), who noted particularly the occurrence of Gaucher-like cells and sea-blue histiocytes in chronic myeloid leukaemia. A range of cytological appearances transitional between normal marrow macrophages and classical Gaucher cells had been described by Kattlove et al. (1969). These cells, and also sea-blue histiocytes, have been reported to occur occasionally in small numbers in the marrow from patients with polycythaemia, rheumatoid arthritis, thrombocytopenic purpura, and hyperlipoproteinaemia (Dosik et al., 1972; Sawitsky et al., 1972). They have also been observed in thalassaemia and in congenital dyserythropoietic anaemia. A further type of inclusion was described by Stavem et al. (1977) as light green crystals; this was found in six of 11 randomly selected marrow aspirates from patients with acute or chronic myeloid leukaemia.

The present study leads us to believe that the Gaucher-like cells of other writers are one cytological manifestation of the accumulation of birefringent crystalline material in marrow macrophages. These inclusions may be invisible with light microscopy of Romanowsky-stained slides and revealed only by polarised light. The composition of the inclusion material is probably the same as in true Gaucher's disease, namely, glucosyl ceramide; certainly the ultrastructure shows a similar pattern of parallel bundles of microtubules which probably account for the birefringence noted.

This type of acquired birefringent inclusion in myeloid leukaemias may arise from increased breakdown of granulocytes with overproduction of glucosyl ceramide, which the marrow macrophages,

Fig. 13  EM photograph of bundles of microtubules in a blue crystal which would be birefringent.
Acquired lipidosis of marrow macrophages

despite increased glucocerebrosidase activity, are unable to metabolise. A similar mechanism has been postulated in dyserythropoietic states (Van Dorpe et al., 1973).

Blue pigment macrophages or sea-blue histiocytes were first described in the spleen and later in bone marrow by Moeschlin (1951). The recognition of a rather benign genetic syndrome characterised by accumulation of sea-blue histiocytes in spleen, liver, and bone marrow emerged during the next 20 years, and the condition became known as the sea-blue histiocytic syndrome. There is agreement that the sea-blue material contains phospholipid and glycosphingolipid, but patients may have a raised plasma triglyceride level, and Jacobsen et al. (1972) recorded a deficiency of lecithin-cholesterol acyl transferase in some hereditary cases. Secondary sea-blue histiocytosis has been described in the spleen and marrow of a patient with hyperlipidaemia by Parker et al. (1976), who suggested that the sea-blue histiocyte might be regarded as a marker for abnormal lipid metabolism. Although this suggestion may be correct in a very general sense, the occurrence of these cells in the marrow in myeloid leukaemias and occasionally in dyserythropoietic states probably has a similar cause to that leading to the accumulation of birefringent blue crystals and Gaucher-like cells, namely, failure of the macrophage enzymes—in this case chiefly sphingomyelin— to cope with the overproduction of sphingomyelin (phosphoryl choline ceramide) resulting from increased turnover of granulocytes or erythroblasts in the marrow. The parallel with Niemann-Pick disease is apparent, and indeed sea-blue material shows weak autofluorescence in UV light and ultrastructurally may show characteristic lamellar whorls similar to those described in Niemann-Pick disease (Parker et al., 1976). In these structures the osmiophilic material is thought to be phosphoryl choline and unstained material the ceramide portion of sphingomyelin. The lamellar or ‘fingerprint’ pattern may not always be shown by sea-blue material, since we did not find it in either of two cases examined by electron microscopy in the present study.

The third type of lipid inclusions, the grey-green crystals, show no autofluorescence or birefringence. Stavem et al. (1977) found them only in patients with myeloid leukaemias and not in lymphoid leukaemias. Our experience is similar, although we have also found them in one patient with aplastic anaemia and in another with myeloma and eosinophilia. The chemical composition of these grey-green crystals is unknown, and they do not show a periodic structure in the electron microscope.

Cytochemical studies have not generally been very helpful in distinguishing these varieties of acquired lipidosis. Apart from occasional sudanophilia of sea-blue granules the intrinsic reactions of all inclusion materials appear weak or negative to peroxidase, PAS, Sudan black, B acid and alkaline phosphatase, and non-specific esterases, although a surface positivity may be seen with all inclusions, especially to the lysosomal enzymes acid phosphatase and α-naphthyl butyrate esterase, which are usually strongly positive in normal histiocytes. These variable positive reactions of inclusion material may reflect non-specific surface adsorption of reaction products or a functional localisation of enzymes to the site of the inclusion because components of the inclusion material may serve as substrates for enzyme activity.

The presence of birefringence in pathological material, including conditions associated with lipid storage, is reviewed by Wolman (1975). The differences between birefringent and non-birefringent deposits in histiocytes are not thought to be of great significance, since variations in the proportion of polar lipids to other cytoplasmic constituents and the degree of dispersal or concentration may dictate whether the lipid molecules are arranged in an orderly fashion leading to birefringence or not. Nevertheless, although we have observed variability in intensity of birefringence in the blue crystals and Gaucher-like cells, it does seem to offer a reasonably consistent and easily recognised discriminatory

Fig. 14  Marrow histioocyte containing grey crystals with strongly positive cytoplasmic staining for α-naphthyl butyrate esterase and stain outlining unstained centre of crystals.
feature separating these inclusions from the other two types.

All three inclusions appear virtually restricted to states of pathological myelopoiesis, chiefly myeloid leukaemias, dyserythropoietic or aplastic anaemias, and, much less commonly, idiopathic thrombocytopenic purpura. In lymphoproliferative states, in the common anaemias, and in normal bone marrow aspirates, they are sufficiently rare that a positive finding must cast doubt on the diagnosis. In our own series, only a single case of acute lymphocytic leukaemia showed inclusions of both blue crystals and sea-blue material, while the single case of myeloma with inclusions also showed a marked reactive eosinophilia from which the material may have been derived. Sea-blue histiocytes have been described recently in the marrow and spleen of a patient with lymphocytic lymphoma (Mason et al., 1978), but there were unusual features in this case, and the presence of two diseases could not be excluded.

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References


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