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HISTOCHEMICAL AND ULTRASTRUCTURAL OBSERVATION OF THE PIGMENT IN PIGMENTED LIPID HISTIOCYTES OF CHRONIC GRANULOMATOUS DISEASE*

TOMOYUKI HARADA, HAJIME SUGIHARA, HIDEO TSUCHIYAMA AND HIROYUKI NODA**

*Department of Pathology, Nagasaki University School of Medicine, Nagasaki 852 and Department of Pediatrics,** Nagasaki University, School of Medicine, Nagasaki 852*

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Pigmented lipid histiocytes (PLH) of chronic granulomatous disease (CGD) were examined by histochemistry and electron microscopy. The yellowish brown pigments were stainable with Sudan III and IV even in paraffin sections, and the sudanophilia was kept after oxidation with peracetic acid followed by methylation. In addition, the granules were positive to Gomori's chromium hematoxylin stain, 0.02% Nile blue sulfate stain and leuco-malachite green stain. Ultrastructural observation showed numerous intracytoplasmic granules in variable shapes and sizes measuring up to 4.8 μ in diameter, with various electron densities. From these results, the pigment in PLH of CGD is comparable to a ceroid-like substance. The nature of the pigment and the pathogenesis of PLH are also discussed.

Chronic granulomatous disease (CGD) results from a congenital bactericidal defect within phagocytic cells including neutrophils (3, 9, 17, 18), and has been characterized by the formation of granulomas in generalized organs and by the appearance of histiocytes containing pigmented lipid materials mainly in the reticulo-endothelial system (1, 2). Although one histochemical and ultrastructural report with regard to the yellowish brown pigment of pigmented lipid histiocytes (PLH) has been issued (1), the exact nature of this granule has not yet been examined in detail.

In the present study, we focussed mainly on the pigment of PLH, which was found in an autopsy case of CGD (7).

MATERIALS AND METHODS

An autopsy case of a 2-year-8-month-old boy who was diagnosed to have CGD was used for this study. The peripancreatic and hepato-hilar lymph nodes were fixed in 10% formalin solution. Besides the ordinary stainings for routine study, various histochemical procedures were performed on the paraffin and frozen sections

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of these lymph nodes (14, 15, 16), as shown in Table 1. For electron microscopical investigation, small pieces of formalin-fixed materials were processed to refixation with glutaraldehyde and osmic acid, then prepared by routine procedures. The ultra-thin sections after double staining with uranyl acetate and lead citrate were observed with an electron microscope.

RESULTS

1. *Light microscopy*

In sections stained with hematoxylin and eosin, histiocytes containing yellowish brown pigments were obvious because of frequent clusters in the lymph-sinuses (Fig. 1). No specific relationship between PLH and granulomas was found. Occasionally, the phagocytosis of erythrocytes in PLH was noted.

2. *Histochemistry*

Histochemical findings on the pigments are presented in Table 1. In frozen sections, the pigment granules were all positive to common methods for fatty substances. Even in paraffin sections, Sudan III and IV stainings gave positive results, with the sudanophilia well preserved after oxidation by peracetic acid followed by methylation.

Whereas staining for cholesterol proved negative, positive results were obtained with fatty acid stain.

Moreover, performic acid-Schiff reaction was uninfluenced by acetylation but

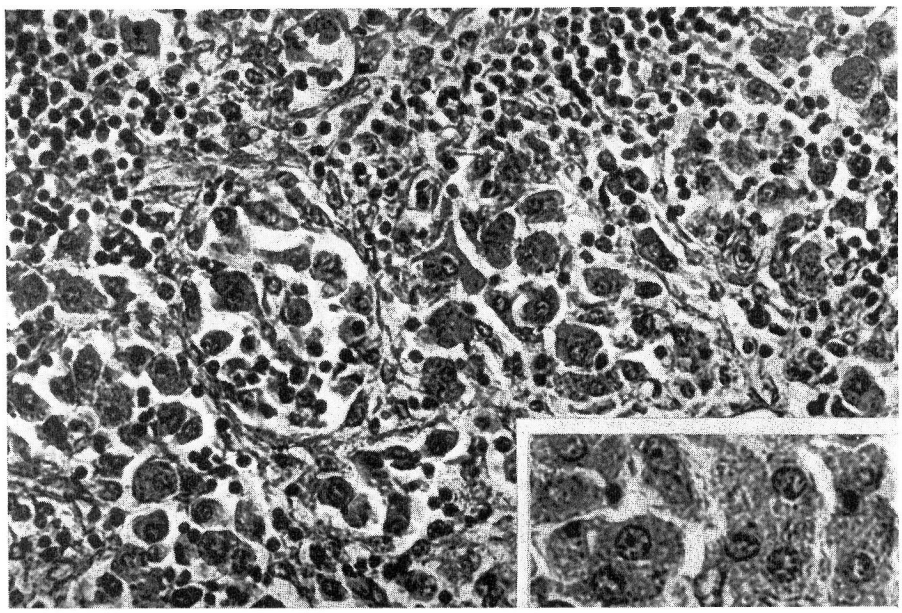

FIG. 1. Note that the distended lymph-sinuses are filled by pigmented lipid histiocytes (PLH). H. E. stain ×200 At higher magnification (inset), PLH have distinctly yellowish brown granules stained with hematoxylin and eosin. H. E. stain ×400

TABLE 1. *Histochemical findings of the pigment in PLH*

Common methods for fatty substances		
Sudan III		
frozen section		++
paraffin section		+
peracetic acid oxidation and mild methylation		+
Sudan IV		
propylene glycol-Sudan IV	frozen section	++
	paraffin section	+
alcohol-Sudan IV	frozen section	+
	paraffin section	−
Oil red 0		++
Sudan black B		++
Nile blue sulfate (Smith's method, modified by Kleeberg)		++
Methods for cholesterol ester and free cholesterol		
Schultz's method		−
Okamoto, Shimamoto and Sonoda's method		
sulfuric acid-acetic acid method		−
sulfuric acid method		±
sulfuric-iodine method		−
Methods for fatty acid		
Fischler's method		++
Holczinger's method		+
Methods for unsaturated lipid		
PFAS (performic acid-Schiff reaction)		+
acetylation		+
bromination		−
Belt and Hayes' method (ultraviolet rays)		+
Methods for compound lipid		
Smith-Dietrich's method		+
Methods for phospholipid		
Okamoto, Shimamoto, Ueda, Kusumoto and Shibata's method		
common method		+
differential method I		+
II		+
Menshik's method, modified by Dunnigan		+
Methods for glycolipid		
Okamoto, Ueda, Kusumoto and Hashimoto's method		−
Diezel's method (for ganglioside)		−
Dayan's method (for sulphalipid)		−
Methods for lipofuscin or ceroid		
Schmorl's method		+
Gomori's chromium hematoxylin stain		+
Methods for ceroid and its related pigments		
0.02% Nile blue sulfate stain (pH 2.9)		+
Leucomalachite green stain		+

Others	
PAS	+
diastase digestion	+
Prussian blue (for iron)	−
Ziehl-Neelsen (for acid fastness)	+
Gridley silver method	+
Fontana-Masson silver method (for melanin)	+
Lillie's method (for melanin)	−
H_2O_2 bleaching (for melanin)	−
Okamoto's method (for direct bilirubin)	−
Walker's method (for direct bilirubin)	−
Okamoto, Maeda and Hayashi's method (for indirect bilirubin)	−
Barret's method (for formalin pigment)	−
Luxol fast blue stain	+
Giemsa stain	+
Best carmin stain	−
Alucian blue stain	−
Toluidine blue stain (pH 2.5, 4.1, 7.0) (metachromasia)	−

was blocked by bromination, from which it is apparent that the pigments contain unsaturated fatty acids.

Among the compound lipid, all methods for phospholipid were positive, but stainings for glycolipid were negative to the granules.

Results of staining for lipofuscin, ceroid and its related pigments were all positive as shown in Table 1.

In addition, the pigments were acid fast and PAS postiive, and gave a negative reaction for iron. These findings were identical to those described by Bartman *et al.* (1) The pigment granules in PLH did not give a positive reaction with melanin, bilirubin and formalin pigments.

3. *Electron microscopy*

In the PLH there were numerous pleomorphic granules of variable shapes and sizes measuring up to 4.8 μ in diameter, with various densities (Figs. 2 and 4). Most of granules consisted of relatively electron-dense materials which were generally homogenous, and round or oval materials containing many small particles of less than 1 μ in diameter and various densities. The latter round or oval granules were usually surrounded by a limiting membrane (Fig. 3). Between them, there appeared transitional pictures, from which the speculation was made that the former developed into the latter (arrows in Fig. 2).

Although granules with irregular internal structures were seen, the concentric lamellar inclusion resembling the myelin-like configuration of phospholipid was not found. Occasionally, the phagocytized erythrocytes were noted in PLH, but no gradual transitions between intracytoplasmic granules and erythrocytes were seen (Fig. 4). Unfortunately, materials for this study had been fixed in 10% formalin, thus the intracytoplasmic organelle was indistinguishable. Other organelles showed no specific findings.

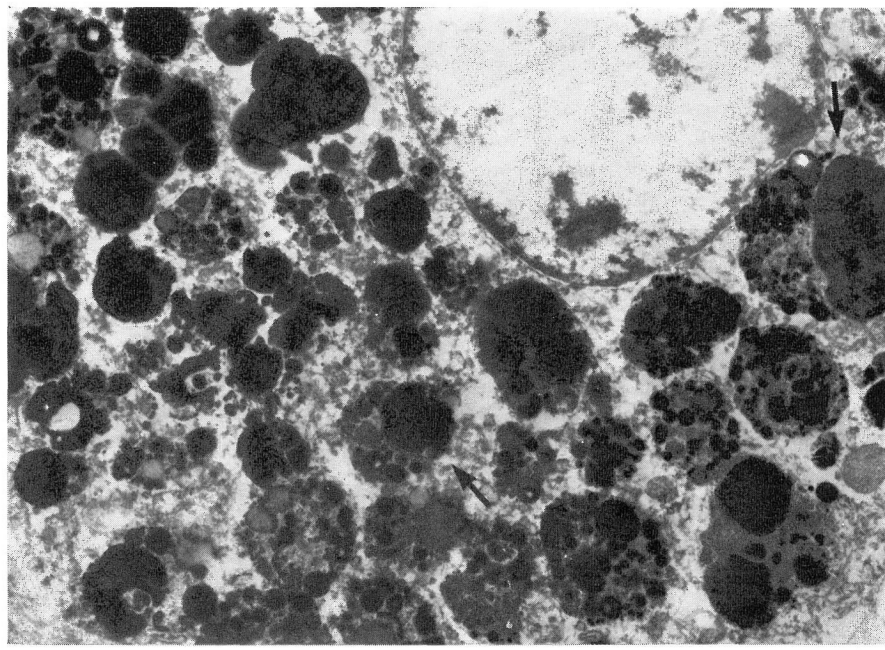

FIG. 2. Numerous intracytoplasmic granules are seen in PLH. There are transitional features between the relative homogenous materials and the granules consisting of small particles (arrows). ×6,700

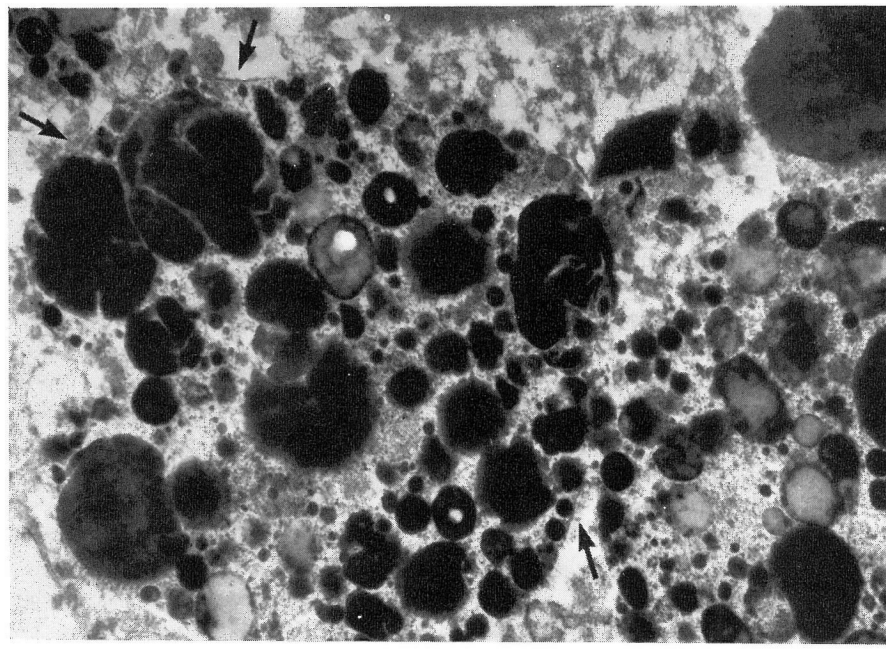

FIG. 3. A limiting membrane (arrows) is visible at several points on the border of this granule. ×16,600

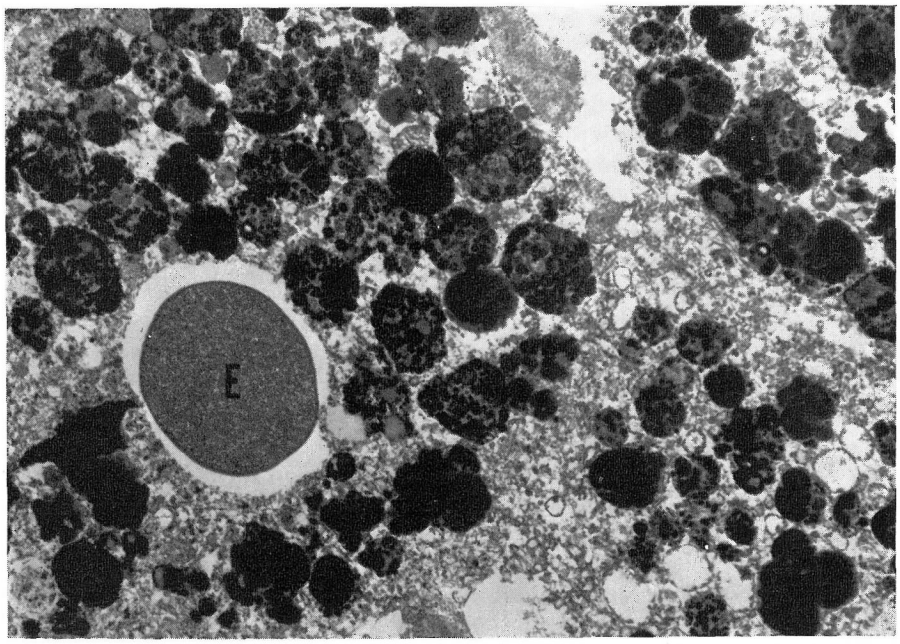

FIG. 4. No gradual transition between phagocytized erythrocyte (E) and intracytoplasmic granules. ×5,600

DISCUSSION

In this study, the yellowish brown pigments in PLH all showed positive reaction to the Gomori's chromium hematoxylin stain, 0.02% Nile blue sulfate stain (pH 2.9) and leuco-malachite green stain. From these results, it may be suggested that these granules are a ceroid-like pigment (6,12,13,20). Furthermore, because the sudanophilia was preserved after oxidation with peracetic acid followed by methylation in paraffin section (22), it is much like the lipogenic ceroid-like pigment.

Other histochemical findings indicated that the main components of these granules were lipids, which are insoluble in fat solvents such as unsaturated fatty acids and phospholipids. Although the histochemical examination for protein was not performed, the presence of a proteinous component (presumably glycoprotein) (13) may be likely because the Nile blue sulfate stain and PAS reaction were positive (6,13).

The ultrastructural features of this pigment observed in the present study are quite similar to those reported by Bartman *et al.* (1). Electron microscopically, they examined the PLH in the spleen and classified it into large, middle-sized and small granules. They presumed that the large homogeneous and intermediate-sized inclusions would be simple lipids (triglycerides), and that the more electron dense and small less homogeneous inclusions were very similar to lipofuscin or ceroid. On the basis of the histochemical results in the present study, it is that most of the granules observed in fine structure are complexes consisting of unsaturated fatty acids, phospholipids and glycoproteins. Although the phospholipid stainings were

positive, we were unable to find the myelinated structure (11). Moreover, although the demonatration of acid phosphatase was not performed ultrastructurally, those granules surrounded by a unit membrane may be lysosomal in origin.

According to the present study, the yellowish brown pigments in PLH of CGD are comparable to ceroid-like pigments. In regard to the source of ceroid, it is widely accepted that unsaturated lipids are important factors (4). Thus far, there have been many studies on the relationship between ceroid and erythrocyte (8,13,22). However, no transitional figures between granules and phagocytized erythrocytes were found. On the other hand, tissue necrosis (10) and bile juice (21) have been suggested as the source of ceroid pigment, but no such findings were seen in the present study.

It is widely known that ceroid-containing histiocytes are found in many pathologic states (19, 21). Recently Golde *et al.* reported that partial sphingomyelinase deficiency may be one cause of sea-blue histiocytosis (5). Also, in histiocytes of CGD, there may be a possible defficiency of primary lysosomal enzymes.

Initially, there was thought to be a deficiency of an intracellular bactericidal component in neutrophils of patients with CGD (9,17). Later, this was confirmed in circulating mononuclear phagocytes (3) and monocytes (18), as well as in neutrophils. This evidence suggests that the same defects may exist in other phagocytic cells including tissue macrophage, and that they may be a cause of PLH. However, in the present investigation it is difficult to determine whether the storages of yellowish brown pigments are a secondary consequence of the inability of phagocytic cells to destroy ingested bacteria and other foreign-body materials, or whether they are primary phenomenon resulting from the metabolic dysfunction of tissue macro pages. Further studies of PLH will be necessary to clarify their exact natures.

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