The Chediak-Higashi Syndrome: A Possible Lysosomal Disease

By James G. White

Large anomalous granulations in circulating leukocytes and cells of other tissues are a characteristic feature of Chediak-Higashi (C.H.) syndrome. In addition to unusual intracellular particles, patients with this disease manifest partial albinism, photophobia, neurologic deficits, hepatosplenomegaly, lymphadenopathy, increased susceptibility to viral and bacterial infections, and early death due frequently to malignant lymphoma. The giant granules in the white blood cells have been the focus of several investigations. Because the massive particles resemble normal leukocyte granules, except for their size, at least some of the anomalous granules of C.H. leukocytes may be lysosomes.

In the present study the technics of ultrastructural histochemistry have been used to identify the sites of acid phosphatase activity in the circulating white blood cells of two patients with the C.H. syndrome. The enzyme was localized to the giant granules and to degenerating remnants of massive particles in the cytoplasm of C.H. cells. The nature of enzyme staining in these subcellular organelles, and results of other investigations, including the demonstration of virus-like particles in giant granules, indicate that the C.H. syndrome may be a lysosomal disease.

Materials and Methods

Blood samples were obtained for study from normal controls and from two children with the clinical stigmata of the C.H. syndrome. A 10-year-old girl with the disease has been

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Abbreviations Used in Illustrations

P.M.N. = Polymorphonuclear cell
Fix. = Fixative
O.A. = Osmic Acid
Chlt. = Chlortaldehyde
U.A. = Uranyl Acetate
Fix. = Fixative
L.C. = Lead Citrate
O.A. = Osmic Acid
Mag. = Final print magnification

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Fig. 1.—P.M.N. leukocyte from the peripheral blood of child with the Chediak-Higashi syndrome. Large granules characteristic of this disorder are indicated by single arrows. The massive particles are many times larger than the normal neutrophil granules distributed throughout the cytoplasm of the cell. Double arrows indicate a number of large C.H. granules which appear to be in various stages of internal disintegration. Fix.: O.A.; Post-Stain: U.A. and L.C. Reduced 25 per cent from Mag. ×24,900.

previously described.6,17 She has the peculiar grey hair, nystagmus, photophobia, partial albinism, and granulation anomaly characteristic of the syndrome. The second patient is a pale, blond-haired 2-year-old male without the morbid clinical symptoms of the older child but with the typical leukocyte granulation anomaly. Both children will be part of a more extensive report in preparation.18

Blood samples of the patients and controls were transferred immediately to precooled, siliconized tubes containing 3.8 per cent sodium citrate in a ratio of 9 parts blood and 1 part anticoagulant. White cells separated from the blood were washed 6 times with 0.08 M cacodylate buffer containing 0.18 M sucrose. The washed cells were fixed for 1 hour in 3 per cent glutaraldehyde in 0.05 M cacodylate buffer with 1 per cent sucrose. The fixed cells were again washed 6 times in fresh buffer and then suspended in incubation media. The Comori media, as modified by Barka and Anderson,20 using sodium β glycerophosphate as substrate, was utilized to demonstrate enzyme activity. The cells were maintained in the media at 37
Fig. 2.—Another P.M.N. leukocyte, similar to that shown in Figure 1. The nucleus has not been cut in this section. Numerous large pale particles are evident, as well as degenerating granules which honeycomb the cell. Fix.: O.A.; Post-Stain: U.A. and L.C. Reduced 25 per cent from Mag. x 33,000.

C., at pH 5, for 1 hour. Following this incubation, the cells were washed twice in cacodylate buffer and refixed in 1 per cent veronal buffered osmium tetroxide, pH 7.3, for 1½ hours. The cells were then washed twice with veronal buffer, dehydrated in acetone and embedded in Vestopal. Thin sections were examined in the Phillips 200 electron microscope. Poststaining with uranyl acetate, lead citrate, or both was performed after ascertaining the presence of enzyme reaction product on unstained sections. Control preparations in which individual components of the substrate media were left out, or the β-glycerophosphate replaced by adenosine triphosphate, were also studied.

Results

The circulating leukocytes of patients with the C.H. syndrome contain one or several large particles in addition to normal small granulations (Figs. 1–4). The enlarged particles are grossly similar to normal white cell granules in that they are surrounded by a unit membrane and have a relatively homogeneous substructure.

Degenerative changes in the C.H. particles can readily be detected. The internal substructure of the granules is often converted into membranous, or myelin-like configurations (Fig. 3A). Many of the particles appear to undergo internal destruction (Fig. 3B). The degenerating granules may fuse to form
large, membrane-bound areas honeycombing the cells (Figs. 1, 2). Lymphocytes also contain the massive granules typical of the C.H. syndrome (Fig. 4).

Cells from the children with C.H. syndrome incubated in the modified Gomori media contained discrete deposits of lead phosphate indicative of acid phosphatase activity. The enzyme reaction product was localized to the massive particles and degenerating remnants derived from giant granules (Fig. 5). Both neutrophils and lymphocytes contained focal deposits of acid phosphatase activity (Figs. 5, 6A and 6B). The specific localization to the characteristic C.H. particles is shown in Figures 6B and 7A. The peculiar cobblestone substructure of the matrix of the large granule stained for acid phosphatase in Figure 7A is the same as that of the particle shown in Figure 7B, which was similarly fixed in gluteraldehyde before osmic acid, but was not incubated for enzyme activity. This unusual internal structure is not seen in the matrix of normal-sized particles.
The normal controls had only irregular staining of their leukocyte granules by this technic. Enzyme reaction product was also absent from the small, normal neutrophil granules of C.H. cells. Control preparations in which the substrate incubation media was modified yielded no staining of the anomalous granules.

**DISCUSSION**

The distinctive granulations of the Chediak-Higashi syndrome are apparent in neutrophils, basophils, eosinophils, and lymphocytes of circulating blood. The physical similarity of giant granules to the small particles of normal granulocytes has been noted in both light and electron microscopic studies.\(^1\) Cytochemically, the large aberrant granules also resemble normal granulocyte particles. The Sudan black B reaction for lipid and the peroxidase reaction are positive in C.H. particles and normal leukocyte granules, while the P.A.S. (periodic acid Schiff) stain for carbohydrate and the Fuelgren reaction for DNA are negative in both. The histochemical and cytochemical findings have led investigators to conclude that the anomalous granules are qualitatively, if not quantitatively, similar to normal granulocyte particles.\(^3,7-11,17\)

The nature of normal leukocyte cytoplasmic particles has been clarified by
Fig. 4.—Lymphocyte from a Chediak-Higashi patient. A massive granule is present in the cytoplasm of this cell. Two relatively smaller, but still large, organelles are apparent above the giant granule. The internal structure of the latter particles suggests that they are multivesicular bodies. Fix.: O.A., Post-Fix.: U.A. and L.C. Reduced 35 per cent from Mag. ×64,500.

histochemical\textsuperscript{22} and biochemical\textsuperscript{14,23,24} studies demonstrating that they contain digestive enzymes, and are, therefore, lysosomes.\textsuperscript{25-28} The fine structural demonstration of the reaction product of the digestive enzyme, acid phosphatase, in giant C.H. granules and their degenerating remnants is consistent with the biochemical and histochemical findings in small normal leukocyte granules. The results of the present study, therefore, indicate that C.H. granules may be giant lysosomes.

Large acid phosphatase positive granules were observed in many C.H. lymphocytes. Normal lymphocytes ordinarily have little histochemical evidence for acid phosphatase containing organelles in their cytoplasm.\textsuperscript{22,29} Phytohemagglutinin stimulation of normal lymphocytes in vitro, however, will markedly increase the number of cytoplasmic particles with acid phosphatase activity.\textsuperscript{29,30} The massive granules with acid phosphatase in C.H. lymphocytes may, therefore, represent an aberration of the latent capacity of lymphocytes to form lysosomes. Massive granules which occur in tissues other than blood may also prove to be large lysosomes.\textsuperscript{7,31}
Fig. 5.—Sample of white blood cells from Chediak-Higashi patient incubated in modified Gomori media. Specific deposits of lead phosphate indicating the sites of acid phosphatase are seen in the anomalous large granules and in the walled-off areas of degenerating granules. Both the lymphocytes and neutrophils contain areas stained by the reaction product of acid phosphatase. Fix.: Glut.; Post-Fix.: O.A.; Post-Stain: U.A. and L.C. Reduced 30 per cent from Mag. ×8,450.

The ultrastructural demonstration of acid phosphatase in C.H. granules by the technic used in this study suggests that the large particles are abnormal. The high concentration and strict localization of lead phosphate in C.H. granules resulted not only from the acid hydrolase activity of the particles, but by the ready entrance of substrate and capture ions into these sites of reaction. Penetration of reagents into normal-sized leukocyte granules appears to be impaired. In all control studies with normal granulocytes, and in the normal-sized granules of C.H. leukocytes, evidence for acid phosphatase activity by the technic of Gomori, modified for ultrastructural histochemistry, has been extremely irregular or absent. This is not the case in the cytochemical method adapted for light microscopy where damage to lysosomes and long incubation times permit staining of normal leukocyte granules. Since the small particles are known to contain acid hydrolases, the difference in enzyme staining observed at the ultrastructural level indicates a fundamental difference between normal and C.H. lysosomes. The rapid penetration and brilliant color
reaction of large granules in C.H. bone marrow—compared to the slow, less intense reaction of normal bone marrow cell particles when both are exposed to supravital dyes probably—reflects the same phenomenon. Thus the unit membranes surrounding the large granules appear to be more permeable than the walls surrounding normal sized lysosomes, and this difference may be of fundamental importance. The “structure-linked latency” of hydrolytic enzymes in lysosomes has been attributed to a lack of permeability of the surrounding membrane both to internal enzymes and external substrates. Integrity of normal lysosomal membranes has been demonstrated by histochemical, as well as biochemical, technics and is most likely the basis for the difficulty in staining normal leukocyte granules for acid phosphatase by the technics of ultrastructural histochemistry. The rapid accumulation of substrates and dye substances in C.H. lysosomes suggests that the membranes of the giant particles may lack properties necessary to maintain “latency” of enclosed digestive enzymes.

In the electron microscope the unit membranes of massive particles are entirely similar to the membranes of normal granules. The internal matrix of the giant granules, however, is significantly different. Gluteraldehyde-fixed cells, postfixed in osmic acid, reveal a tiny cobblestone reticular substructure in large particles not evident in the normal-sized lysosomes. The large particles in
the cytoplasm of osmic acid-fixed leukocytes manifest various degrees of internal destruction, including development of myelin configurations. The degeneration of the massive C.H. granules may be related to external influences acting through a more permeable membrane, or to autoactivation of contained enzymes due to faulty construction of the internal matrix. The disorganization of internal structure manifested by the degenerative changes may convert the massive particles to functionally ineffective lysosomes.

The internal breakdown of C.H. granules probably represents a form of autodigestion, but should not be confused with autophagy. Walling off of undesirable cellular components into digestive vacuoles does occur in C.H. leukocytes, but the massive granules are not part of the cellular components enclosed in autophagic vacuoles. Rather, it is suggested that hydrolytic enzymes may leak through the permeable membranes of degenerating large granules and sufficiently damage cytoplasmic constituents, such as mitochondria and ribosomes, to require their removal by autophagocytosis.

The demonstration that C.H. granules may be abnormal lysosomes im-
Fig. 7A.—A characteristic Chediak-Higashi granule specifically stained by lead phosphate, the reaction product of acid phosphatase-β glycerophosphate interaction. Note the fine substructure of this particle. The tiny rectangular cobblestone framework differs from the coarse irregular matrix of normal leukocyte particles. Fix.: Glut.; Post-Fix.: O.A.; Post-Stain: U.A. and L.C. Reduced 20 per cent from Mag. x 102,500.

Immediately raises the possibility that functional impairment of the large particles could be involved in the susceptibility of these patients to infection. A functional incapacity of giant C.H. leukocyte lysosomes has been indicated by an unusual finding in two children with the syndrome. In the leukocytes of both patients tiny particles indistinguishable from viruses were observed. The virus-like particles were localized to the large degenerating C.H. granules. The size, structural appearance and distribution of the virus-like agents were similar in the leukocytes of the two children, both of whom had developed lymphoma before the blood samples were obtained. The apparent inability of giant C.H. granules to destroy virus-like particles suggests the functional ineffectiveness of the giant granules to act effectively as lysosomes.

Thus, the distinctive giant granules of circulating C.H. leukocytes contain acid phosphatase activity and are therefore similar to normal leukocyte lysosomes. However, physical, cytochemical, histochemical and physiologic differences indicate that the massive granules may fail to act as competent lysosomes. Alterations in the giant leukocyte lysosomes may be directly related to the susceptibility of children with the C.H. syndrome to infection, and possibly
Fig. 7B.—A Chediak-Higashi granule of a neutrophil prepared in a similar manner to the particle shown in Fig. 7A, except that it was not incubated in the enzyme substrate media. The substructure is similar to that of the granule of the previous illustration. Adjacent, normal-sized granules do not appear to have the same type of internal structure. Fix.: Glut.; Post-Fix.: O.A.; Post-Stain: U.A. amid L.C. Reduced 10 per cent from Mag. ×107,000.

to lymphoma. Similar degeneration of C.H. particles in cells of different tissues could result in other morbid clinical features of C.H. disease. For example, breakdown of massive granules in the central and peripheral nervous systems may cause destruction of nerve cells and result in neurologic deficits commonly observed in patients with C.H. syndrome. Investigations of the chemical structure and membrane properties of isolated C.H. leukocyte subcellular particles, and histochemical studies of massive particles in tissue sites other than circulating white cells, are in progress.

**SUMMARY**

All varieties of circulating white blood cells in patients with Chediak-Higashi syndrome characteristically contain giant granules in their cytoplasm. The similarity of the massive particles to normal-sized leukocyte lysosomes has
been previously recognized. In the present study the technics of ultrastructural histochemistry were used to demonstrate acid phosphatase activity in giant C.H. particles and their degenerating remnants in the leukocytes of two patients with the C.H. syndrome. The presence of an acid hydrolase in massive granules indicates that they are lysosomes. The selective localization of enzyme reaction product in large particles by this method, when small normal-appearing lysosomes in the same cells remained unstained, has suggested that the unit membranes surrounding C.H. granules are abnormally permeable. The increased permeability of the large particles may be related to the pathogenesis of morbid clinical features of C.H. syndrome.

**SUMMARIO IN INTERLINGUA**

Omne le varietates de leucocytos circulante in patientes con syndrome de Chediak-Higashi contine characteristicamente granulos gigante in lor cytoplasma. Le similitude del massive particulas con lysosomas leucocytic de dimensiones normal ha previamente essite recognoscite. In le presente studio, le technicas de histochimia ultrastructural esseva usate pro demonstrar be activitate de phosphatase acide in gigante particulas de Chediak-Higashi e lor degenerante residuos in le leucocytos de duo patientes con syndrome de Chediak-Higashi. Le presentia de un hydrolase acide in granulos massive indica que illos es lysosomas. Le localisation selective del producto de reaction enzymatic in grande particulas per iste metodo quando micre lysosomas de apparentia normal intra le mesme cellulas remane noninturate pare indicar que le membrana unitari inveloppante granulas de Chediak-Higashi es anormalmente permeahile. Le augmentate permeabilitate del grande particulas es possibilemente relationate al pathogenese de morbide characteristicas clinic del syndrome de Chediak-Higashi.

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