Henry Ford Hospital Medical Journal

Volume 27
Number 3 *John W. Rebuck Testimonial Issue*

Article 13

9-1979

The Chediak-Higashi Syndrome: Spectrum of Giant Organelles in Peripheral Blood Cells

James G. White

C. C. Clawson

Henry Ford Hosp Med Journal
Vol 27, No 3, 1979

The Chediak-Higashi Syndrome: Spectrum of Giant Organelles in Peripheral Blood Cells

James G. White, MD* and C. C. Clawson, MD*

The presence of giant organelles in the cytoplasm of peripheral blood elements is diagnostic for the Chediak-Higashi syndrome. These abnormal cytoplasmic inclusions are found within neutrophils, eosinophils, basophils, lymphocytes, monocytes, and platelets. Their fine structural characteristics differ. Neutrophils demonstrate striking variability in the size, shape, and internal features of their giant organelles. Lymphocytes contain two different types of giant inclusions, one with a smoothly homogeneous substructure, the other with structures resembling microtubules. Emphasizing the ultrastructural similarities and differences of these anomalous granulations may provide valuable clues for understanding the basic defect of this inherited disorder.

Introduction

Despite the extreme rarity of patients with the Chediak-Higashi syndrome (CHS), this disorder has attracted intense interest since it was initially described (1-4). Pigmentary dilution resulting in pseudo-albinism, an increased susceptibility to pyogenic infections, an accelerated phase characterized by lymphadenopathy, heptosplenomegaly, pancytopenia, and a terminal process resembling a lymphoreticular malignancy (5,6) represent its typical clinical features. Most patients die in early childhood from infection, complications of the accelerated phase, or from the unusual lymphoreticular malignancy (7).

Giant organelles, present in the cytoplasm of most peripheral blood cells, are diagnostic of CHS. The massive leukocyte inclusions have been particularly intriguing. They have been subjected to many biochemical, histochemical, cytochemical, and immunochemical procedures and explored in detail by light, phase contrast, and electron microscopy in attempts to define their nature, origin, and role in the disease (8-22). Because the patients are prone to bacterial infection, most studies have focused on the polymorphonuclear leukocyte (PMN). Investigations of neutrophil precursors in bone marrow from patients and animal models suggested that azurophilic lysosomes developing in promyelocytes undergo fusion during maturation to form the giant inclusions observed in peripheral blood cells (10,11,15,17,23-29). Consequently, various investigators concluded that the process of fusion was exclusively concerned with the azurophilic organelles in the promyelocytes and myelocytes, that it was complete before the cells entered the peripheral blood, and that the nonlysosomal organelles, called specific or secondary granules, were not involved in the evolution of giant organelles (17,18,25,26,28). This view of giant CHS inclusions has been used to explain the development of giant granules in other CHS leukocytes, tissue cells, and organs of the body.

However, early studies in our laboratory did not agree with this prevailing concept (11,12). Although azurophilic granules were involved in the formation of the large inclusions, the process was not complete in the bone marrow. Rather, fusion between giant organelles and normal-sized granules continued in the mature PMN, and nonlysosomal organelles as well as azurophilic lysosomes were involved. In addition, we demonstrated other manifestations of the disease in CHS leukocytes, including a process resembling cytoplasmic sequestration (12). Recent immunochemical studies have confirmed and extended our early findings by demonstrating that markers for both primary azurophilic lysosomes and specific secondary granules are present in the giant CHS inclusions (30).

As a result of these studies (11,12,31), it is clear that the prevailing concept of giant granule formation in CHS neutrophils needs to be modified. Furthermore, based on our

* Department of Pediatrics, University of Minnesota Health Sciences Center, Minneapolis, MN 55455

Address reprint requests to Dr. White, Department of Pediatrics, University of Minnesota Health Sciences Center, Minneapolis, MN 55455

Supported by USPHS grants HL-11880, AM-06317, HL-06314, CA-12607, CA-08832, CA-11996, GM-AM-22167, HL-20695, HL-06833, and a grant from the Leukemia Task Force.

recent investigations, the use of this concept to explain the development of giant granules in other blood cells, tissues, and organs in patients with CHS is no longer tenable. The purpose of this report is to demonstrate that in each type of blood cell there are unique features of giant granule formation that do not conform to the prevailing concept.

Materials and Methods

Patients

The present study is based on the examination of blood samples from three patients with the characteristic clinical and laboratory features of CHS, although two of the patients are no longer available for study. The third, a thirteen-year-old boy currently in excellent health, has been evaluated annually for the past nine years. The diagnosis of CHS was made shortly after his birth. He developed a parotid abscess in the newborn nursery, and blood smears revealed cytoplasmic inclusions in his leukocytes typical of CHS. Infections were frequent during the first few years of his life, but in the past eight years they have been no more frequent than in normal children of comparable age. He has no signs of the accelerated phase of the disease. Giant organelles typical of CHS have been observed in keratinocytes, melanocytes, peripheral blood cells, and bone marrow. His mother and father are unrelated, and his only sibling, a sister, has no evidence of CHS. Most of our recent studies have been carried out on blood samples from this patient.

Preparation of white blood cell samples

Several different procedures have been used to collect leukocytes from patients with CHS. In early studies we depended on buffy coat samples of whole blood collected in a ratio of 9:1 with 3.8% tri-sodium citrate anticoagulant and sedimented at room temperature under a force of 200 x g (11,12). Subsequently, we have separated CHS leukocytes from whole blood, which was collected in heparinized syringes, by dextran sedimentation and by a sequential procedure involving dextran sedimentation, isolation on ficoll-hypaque density gradients, and subsequent washing and resuspension in Hanks' balanced salt solution (31,32). All methods have yielded excellent white cell preparations, but each is employed to provide samples enriched in a particular cell type. Buffy coats and samples obtained by dextran sedimentation yield all varieties of leukocytes and platelets. Ficoll-hypaque provides samples rich in lymphocytes, monocytes, platelets, and basophils, while the combination of dextran sedimentation and ficoll-hypaque isolation provides nearly pure samples of granulocytes (33).

Preparation of samples for cytochemistry and electron microscopy

The basic procedure we developed for fixation of blood cells initially requires combining the leukocytes with an equal volume of 0.1% glutaraldehyde in White's saline, pH 7.4, followed by sedimentation of the cells to a pellet and subsequent fixation in 3% glutaraldehyde buffered in White's saline, pH 7.4, for 30-60 minutes at 4°C. For cytochemical studies, the second phase of fixation utilizes 0.5% glutaraldehyde and 2.5% paraformaldehyde in 0.1M cacodylate buffer. The procedures for demonstrating acid phosphatase (12) and myeloperoxidase (35) in leukocytes have been described in detail in previous publications. After initial fixation in glutaraldehyde or after incubation for enzyme activities, the samples were post-fixed in either 1% osmic acid in Zetterquist's buffer, pH 7.3, 1% osmic acid in 0.1M phosphate buffer, or 1% osmic acid in distilled water containing 1.5% potassium ferrocyanide (36). All fixed samples were dehydrated in a graded series of alcohols and embedded in Epon 812. Thin sections cut on an ultra-microtome were either stained with lead citrate and uranyl acetate or examined unstained if enzyme activities were to be localized. The sections were studied in a Philips 301 electron microscope.

Results

Polymorphonuclear leukocytes (PMN)

Thin sections of well-preserved CHS neutrophils reveal a striking variability in the size, shape, and internal structure of giant organelles in their cytoplasm (Figs. 1, 2). Some of the enlarged bodies have rounded contours or are spindle shaped. Their enclosing membranes are sharply demarcated, and the material inside is homogenous, often with a periodic, linear substructure. This type of giant organelle most closely resembles the azurophilic granule, or primary lysosome, of the mature normal PMN and its bone marrow precursors. When viewed in the light microscope on Wright-stained blood smears, this form is sharply demarcated, usually spherical in shape, and stains a darker purple color than other large masses in the CHS neutrophil. It is reasonably well established that this type of massive inclusion arises through fusion of smaller, perhaps normal-sized, primary lysosomes during maturation of CHS neutrophils in the bone marrow (Fig. 3).

A second variety of giant organelle is usually larger than the first, very irregular in surface contour, and highly variable in the organization, structure, and electron density of its internal content (Figs. 1, 2). This type is encountered more freqently in mature CHS neutrophils than the first variety and appears to occupy larger volumes of the cell cytoplasm. These giant inclusions correspond to the irregu-

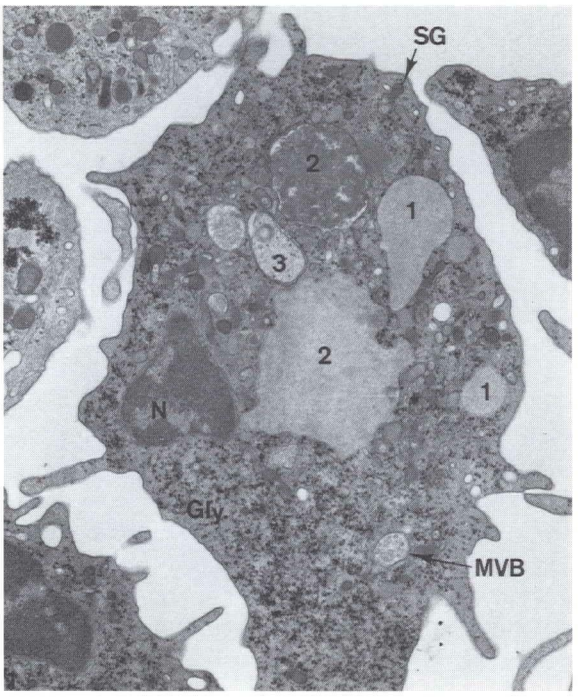

Fig. 1. PMN from a patient with CHS

Cell is irregular in form with a few short pseudopods evident on its surface. A large number of organelles occupy the cytoplasm, but only a single lobe of the nucleus (N) is evident. Dark particles of glycogen (Gly) are spread evenly throughout the cell and a single multivesicular body (MVB) is apparent. Normal-sized specific or secondary granules (SG) are dispersed throughout the cytoplasm. Three types of giant CHS organelles are evident: 1) sharply defined borders; generally round or spindle-shaped; it closely resembles azurophilic granules in promyelocytes and myelocytes; 2) irregular in form; electron density varies considerably; some appear swollen or in stages of degeneration, while others are intact; 3) double-ringed organelle in which cytoplasmic constituents are sequestered (X19,500).

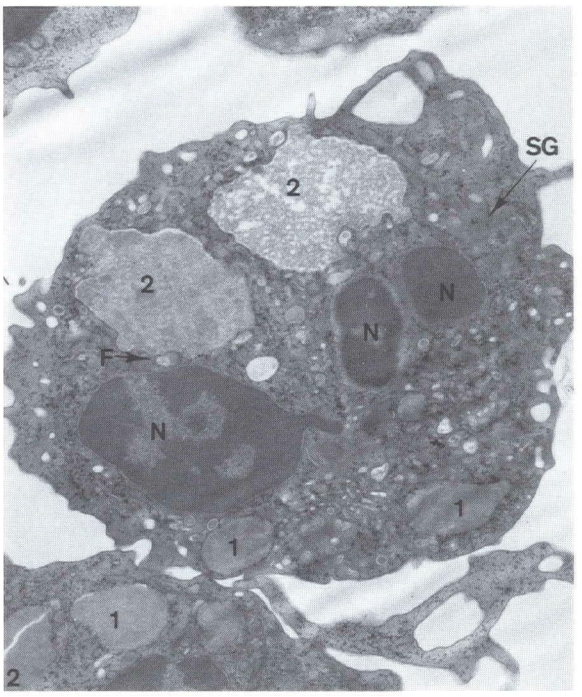

Fig. 2. CHS neutrophil

Several lobes of the nucleus (N) are evident. Giant granules of the first type (1) are apparent in this and an adjacent cell. The second variety (2) is present in two forms, intact and diluted. Sites where fusion (F) appears to be occurring between small granules (SG) and the massive inclusions can be identified around the irregular borders of both giant organelles (X17,000).

lar, gray-blue structures present in the cytoplasm of CHS neutrophils viewed in the light microscope. In thin section, the internal matrix of the giant bodies displays a range of appearances. At one end of the spectrum the basic substance is similar to the matrix material of the first form of massive particle and may include areas in which a periodic linear substructure can be defined. For the most part, however, the matrix is mottled with zones of differing density which often surround masses of ill-defined membranous residues. On occasion the membrane residues are in parallel alignment, producing the appearance of myelin configurations. Previous studies have shown that these giant inclusions are unusually permeable to vital dyes (11). Increased permeability could permit water to leak into the massive bodies before or during fixation, resulting in a swollen appearance and separation of the contents present in some inclusions. The similarity of some of the enlarged structures to bags of degenerating debris was far more common when osmic acid was used as the only fixative (11) but can still be observed in well-preserved cells after glutaraldehyde-osmium fixation.

Although the variable content and substructural organization of this form of giant organelle has attracted a great deal of attention, the tortuous border is the most interesting feature of the inclusion. Careful examination of enclosing membranes reveals multiple sites of interaction with normal-sized organelles in the surrounding cytoplasm. In some areas, the membrane of an adjacent organelle, whether a primary or secondary granule, fuses with the surface of the giant body. In a few zones, an entire small organelle appears to have been incorporated. Nucleoids from the smaller organelles are dense and contribute to the opaque areas after fusion. Membranous residues in the large, irregular organelles may come from the nucleoids of small organelles but may also derive from surfaces of normal-sized granules.

When it was first proposed that giant granules continue to fuse with small organelles, even in mature PMN, and that secondary granules as well as azurophilic lysosomes (11,12) were incorporated, considerable controversy resulted (37). Yet, even a cursory examination of well-preserved CHS

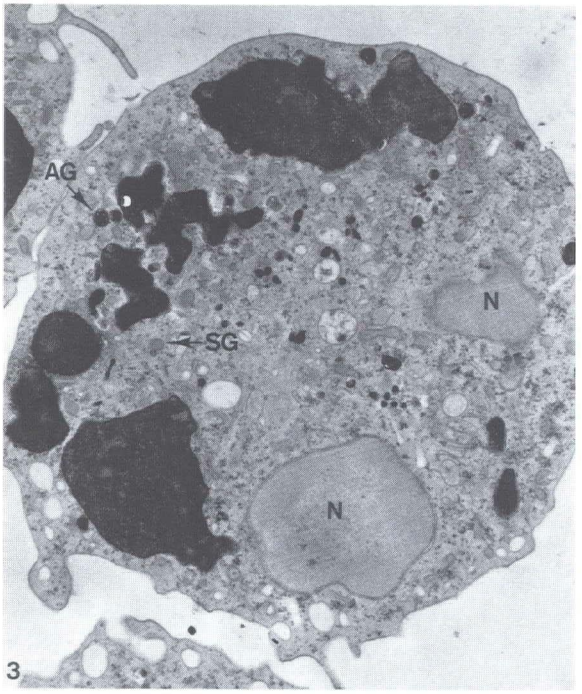

Fig. 3. CHS neutrophil reacted for peroxidase

Nuclear lobes (N), specific granules (SG), and glycogen particles are distributed throughout the cytoplasm. Dense reaction product specific for peroxidase is confined to massive organelles as well as small azurophilic granules (AG). Some peroxidase positive organelles have smooth surface contours, while most are irregular. The possibility that fusion continues between large and small organelles in peripheral CHS neutrophils is strongly suggested (X17,000).

peripheral blood cells in thin section reveals that this concept is reasonable. The granules are huge, often with five or more giant inclusions in the plane of sectioned cytoplasm in a single cell. Also, all CHS neutrophils appear to contain several of the massive bodies. Considering the sheer mass of material in the giant inclusions and the large volume of cytoplasm they occupy, it would be virtually impossible for them to represent the normal amount of azurophilic granule substance produced during maturation of PMN. However, the concept that the giant organelles interacted with both primary and secondary granules in mature CHS neutrophils has failed to gain acceptance until recently. Rausch, et al have recently demonstrated by employing fluorescent tagged antibodies specific for components of either primary lysosomes or secondary granules, but not both, that the giant organelles of CHS neutrophils contain both azurophilic and specific granule markers (30). Their findings corroborate previous ultrastructural studies which lacked a specific marker for secondary granules.

The third variety of aberrant inclusion in PMN resembles an autophagic vacuole. Cytoplasmic contents enclosed within double rings of membrane react for both acid phosphatase and myeloperoxidase. We have previously described stages in the degradation of cytoplasmic constituents enclosed by the double ring confining lysosomal enzymes (12), and the precise relationship of these areas of cytoplasmic sequestration to the two major giant inclusions will be the subject of a separate report.

It is surprising that PMN from patients with CHS filled with several varieties of giant lysosomes appear to survive normally once they reach the peripheral circulation, since patients usually have a neutropenia attributed to destruction of maturing precursors in the bone marrow or a mechanical inability to leave it (22,38). Nevertheless, one might question whether the cells survive normally because frequently we have found what appear to be dead or dying PMN in peripheral blood samples (Fig. 4). The dead cells have rounded contours, subsurface membrane vesiculation, condensed nuclei and cytoplasm, and sequestered masses of glycogen. We have never encountered such cells in the blood of normal individuals or of patients with other blood diseases.

Eosinophils

Eosinophils on peripheral blood smears of patients with CHS are characteristic of the disease and confirm the diagnosis (Fig. 5). While giant granules are always present in CHS eosinophils and occur in no other human disease, little attention has been paid to their development. It is reasonably clear that the giant eosinophil granules develop by fusion of smaller organelles (Fig. 6). Normal eosinophil granules contain a single crystalloid in their substructure, but the enlarged granules usually contain several of the typical crystalloids. The larger they are, the more crystals they may contain. Yet, among the massive inclusions in CHS eosinophils are smaller granules similar in size and appearance to those in normal cells. The cells vary considerably in the balance between very large inclusions and more normal-sized granules, but the CHS eosinophils with large inclusions have fewer individual storage granules than those with generally smaller organelles (Fig. 7). It seems reasonable that the larger organelles evolve at the expense of the smaller and that the fusion process continues in mature cells.

In contrast to the PMN, there is little evidence of cytoplasmic injury in CHS eosinophils. Irregular, giant inclusions similar to the second variety observed in every neutrophil are rarely evident. It is surprising that they ever occur in eosinophils, which lack secondary granules of the type found in PMN, but their rarity offers further support for the concept that secondary granules are involved in forming giant inclusions in PMN.

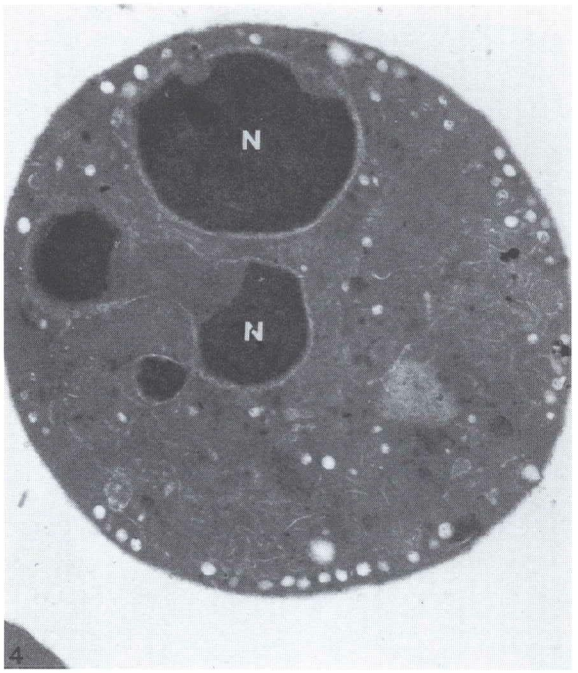

Fig. 4. CHS neutrophil

This type of cell is rare in peripheral blood of patients with CHS but more frequent in patient samples than in normal blood. It has no pseudopods and is nearly spherical. Vesiculation is apparent just under the surface membrane. Nuclear (N) lobes and the entire cytoplasm appear condensed. PMN of this type do not appear to be functional and may be in an advanced stage of cell death (X25,000).

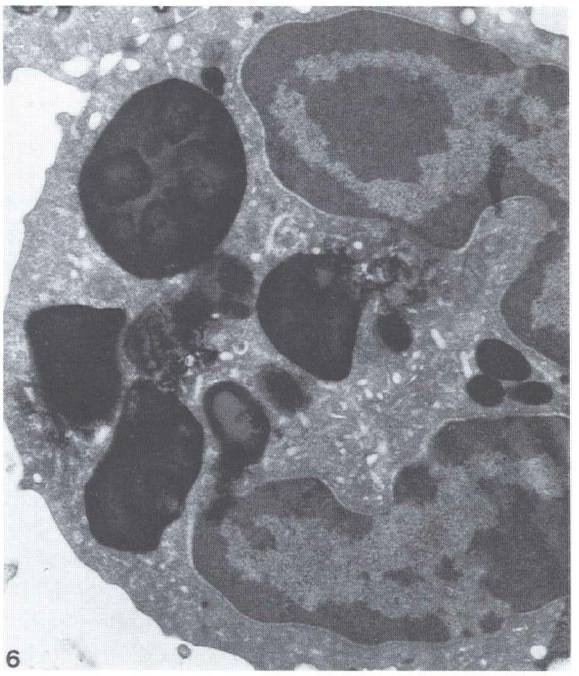

Fig. 6. CHS eosinophil reacted for peroxidase

Huge organelles containing several crystalloids as well as normal-sized granules with dense reaction product are apparent (X17,700).

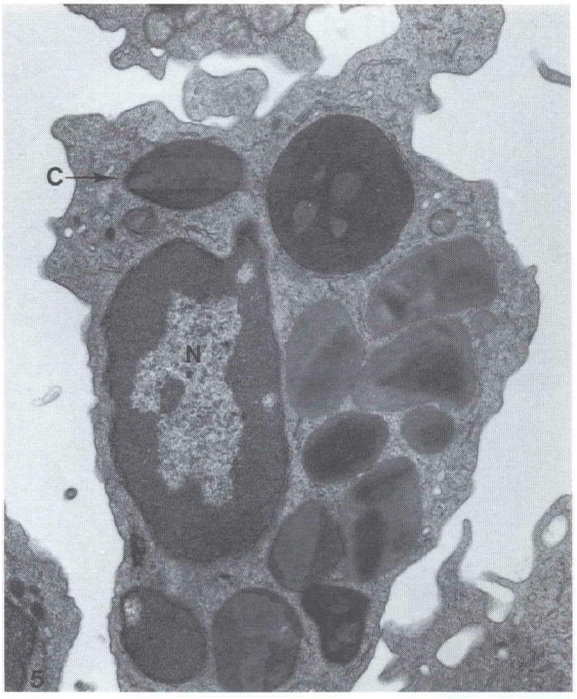

Fig. 5. CHS eosinophil

Nucleus (N) is eccentric. Cytoplasm contains organelles ranging from normal sized to huge; large organelles may contain a single crystal (C) or many crystalloids (X18,200).

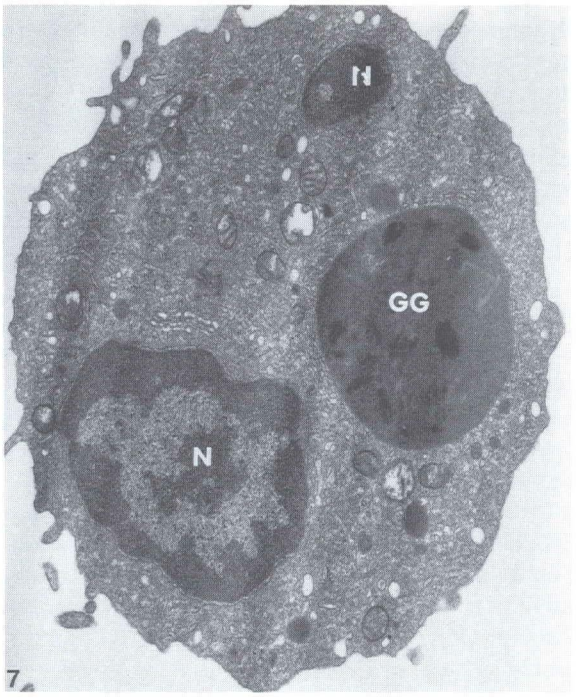

Fig. 7. CHS eosinophil

This cell contains one giant granule (GG) almost as large as a nuclear lobe (N). Small granules are also apparent in the cytoplasm (X19,000).

Occasional giant granules in CHS eosinophils are poorly preserved in thin sections. The apparent swelling and dissolution of internal contents may be a fixation artifact. However, since it has been shown that the massive inclusions are more permeable to vital dyes (11,39), this defect may result in the swollen appearance which resembles the changes observed in some of the massive inclusions of PMN. Despite this, right-shaped, double membrane-enclosed areas resembling autophagic vacuoles, which are common in CHS neutrophils, have rarely been observed in eosinophils from CHS patients.

Basophils

All CHS basophils examined in thin sections have contained massively enlarged organelles (Figs. 8, 9). It is possible that they contain an admixture of giant and more normal-sized granules, but too few have been observed to permit such a conclusion. Many giant basophil inclusions appear to be massively enlarged granules of the type seen in normal cells. Others seem swollen, as if they had taken up water before or during fixation, and their contents appear diluted. The resemblance of the swollen giant inclusions to similarly altered massive granules in eosinophils and PMN is striking and suggests that huge organelles in the circulating granulocytes may be subject to mechanical injury in vivo or may be damaged during separation of the cells in vitro. Giant granules of CHS basophils often contain small crystalline inclusions which are most easily recognized in the swollen organelles but can also be observed in giant granules with intact structure. Crystals of the type found in CHS basophil inclusions do not appear in normal basophil granules and may develop in the giant organelles after fusion has occurred. Their origin is unknown. In some giant basophil granules the crystalline structures are quite large, resembling the multiplex crystals of the huge eosinophil granules.

Lymphocytes

Giant granules are found in about 40% of CHS lymphocytes in thin sections, which agrees well with the 50-90% found by light microscopy. Two major types of giant inclusion are present in the cytoplasm of CHS lymphocytes. One appears to be smoothly homogenous in substructure or to contain two or more zones of differing composition (Fig. 10). Droplets resembling lipid and very dense deposits associated with them are often present, suggesting the appearance of ceroid or lipofuchsin. Lipid droplets and dense material may be surrounded by a matrix of protein or be complexed to a large organelle within the same enclosing membrane. Cytochemical studies (11) have shown that this type of organelle is acid phosphatase positive (Fig. 11). Reaction product of acid phosphatase may fill the matrix or be deposited only in a peripheral ring or on one side of the

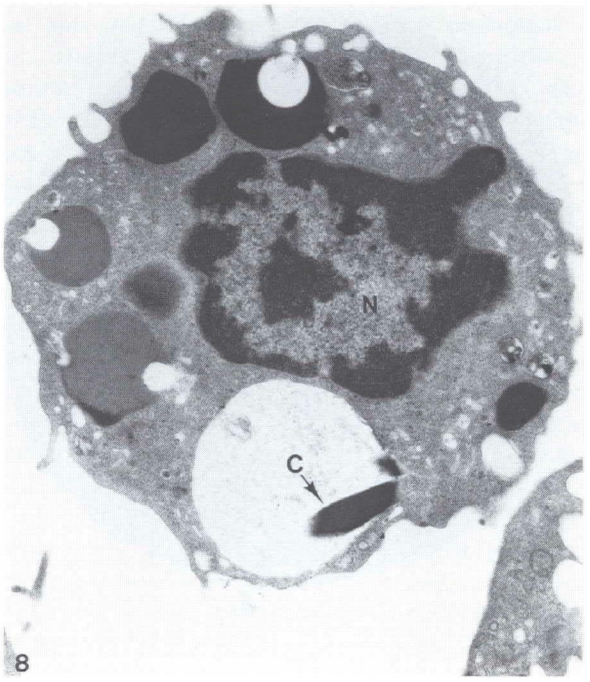

Fig 8. CHS basophil
Several giant granules surround the nucleus (N); one appears swollen but retains a crystalloid element (C) (X17,000).

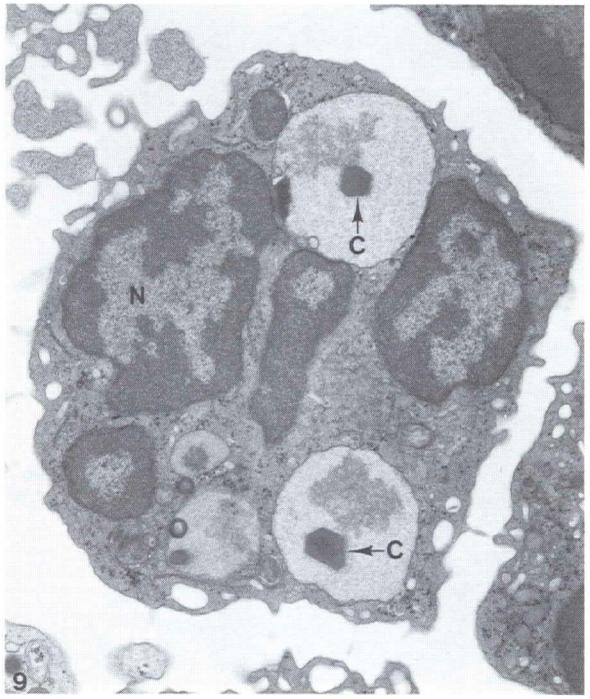

Fig. 9. CHS basophil
Crystalline (C) structures are evident in several giant granules (X15,500).

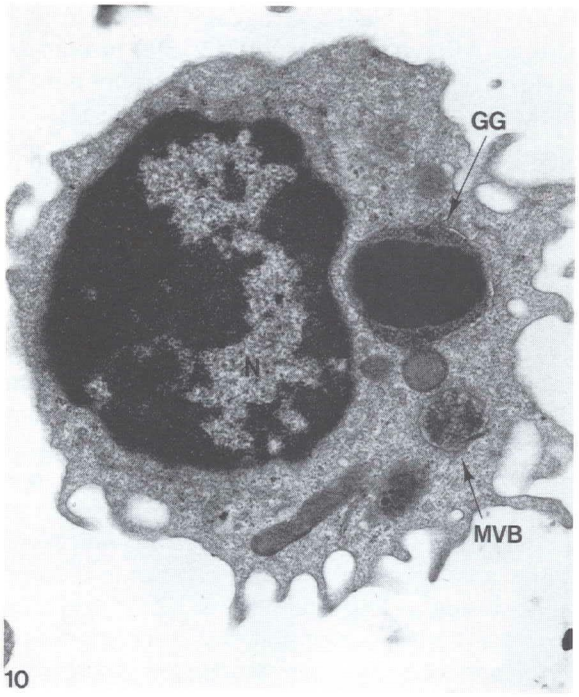

Fig. 10. CHS lymphocyte
Giant granule (GG) is clearly larger than multivesicular body (MVB) in the cytoplasm. The huge inclusion contains a dense core and less dense material around the periphery (X19,000).

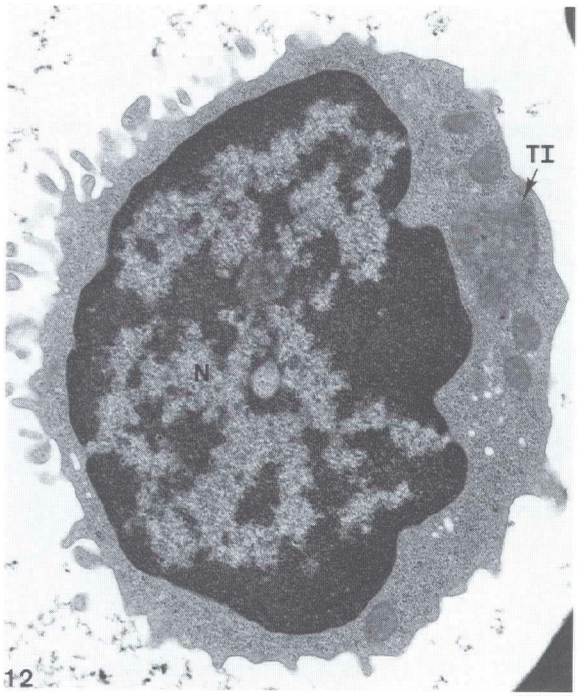

Fig. 12. CHS lymphocyte
Giant organelle in the cytoplasm of this cell contains large numbers of circular profiles resembling microtubules and is referred to as a tubular inclusion (TI) (X22,000).

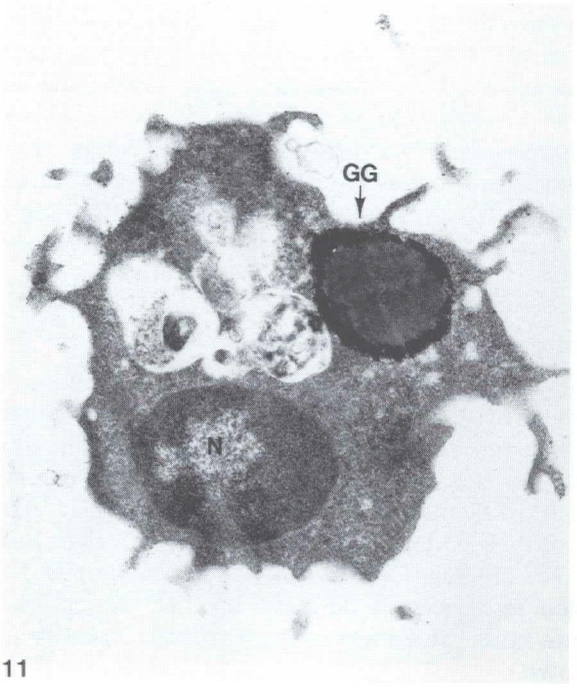

Fig. 11. CHS lymphocyte reacted for acid phosphatase activity
Dense reaction product is localized to a rim around the unreactive core of the giant granule (GG) (X27,000).

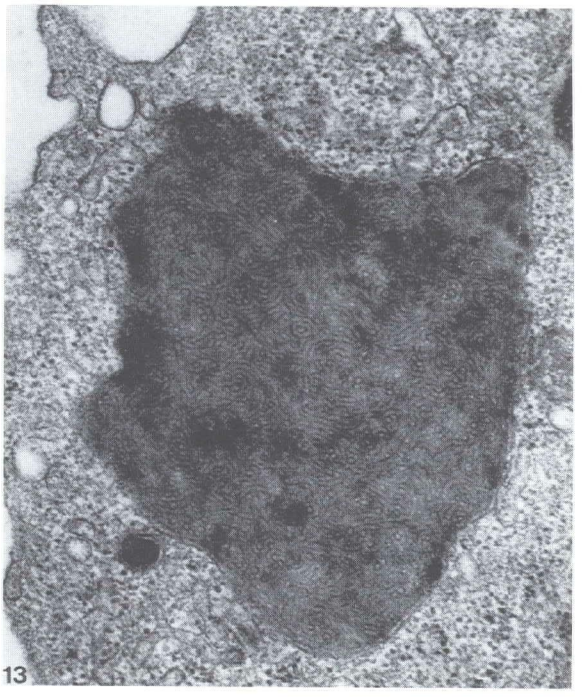

Fig. 13. CHS lymphocyte
Giant tubular inclusion shown here contains innumerable circular profiles resembling microtubules (X49,500).

massive inclusion. The distribution of reaction product suggests that the giant organelles have developed by fusion and that lysosomal enzymes may be sequestered as a result.

The second variety of giant inclusion (Fig. 12) is filled with structures resembling microtubules (34). Although small organelles containing tubules are also present in about 5% of normal lymphocytes, they reach massive proportions in CHS lymphocytes (Fig. 13). Normal cells may have five or six of the tubule-filled organelles, whereas CHS lymphocytes usually reveal only one or two. The mechanism involved in their evolution is still being evaluated. In our original study they appeared to arise from the cytoplasm and to be sequestered within membranes, i.e., the groups of tubules formed first and were then surrounded by a membrane. This finding was supported by the observation that small granules and cytoplasmic debris were often enclosed by the same membrane as the tubular elements. Although the tubular elements themselves were cytochemically inert, they were occasionally surrounded by a ring of acid phosphatase (34). Either the enzyme was enclosed with masses of tubules at the time the surrounding membrane developed, or granules subsequently fused with the tubules to form secondary lysosomes. While the latter possibility seemed more likely, the initial mechanism whereby giant tubular inclusions develop did not appear to involve fusion of smaller organelles. Rather, the masses of tubular material developed in the cell cytoplasm before enclosure. If this suggested mechanism proves to be accurate, then fusion may not be the only process whereby giant granules can evolve, even though they may continue to expand by fusion with lysosomal organelles after initial formation.

Monocytes

Monocytes from the peripheral blood of patients with CHS represent an enigma. The few investigators who have recorded the frequency of enlarged granules indicate that less than 10% of the cells contain giant inclusions (40-43). Their size is not very impressive, ranging from areas with four or five azurophilic granules close together to single inclusions 0.1 to 4.6 μ in diameter. Particles 0.1 μ in diameter cannot be distinguished from granules in normal monocytes, and cells with bigger inclusions may actually be large CHS lymphocytes containing giant granules mistaken for monocytes. In our experience, giant organelles of the types found in granulocytes or lymphocytes are extremely rare in monocytes from human patients with CHS. Yet, recent publications (30) would indicate that CHS monocytes are commonly involved in the disease process and often contain giant organelles. Studies in animal models of the human disease indicate that 10% of the monocytes in mink and 28% in cattle with CHS have abnormal granules (44). Although precise figures have not been provided for the monocytes of mice with CHS, the literature suggests that enlarged lysosomes appear frequently (23, 24, 27, 28). Insofar as the monocyte is concerned, the human disease does not appear to conform with the animal models of CHS.

Nevertheless, there is some evidence that human CHS monocytes may be involved in the disease process. In thin sections, over 30% of the cells do have manifestations of the disorder. However, the nature of the changes differs significantly from those observed in granulocytes and lymphocytes. Instead of forming massive inclusions of fused granules typical of those observed in PMN and eosinophils, the monocyte develops, almost exclusively, the double-ringed organelles discussed above as the third form of inclusion occurring in PMN (Figs. 14, 15). The steps in formation of the ring-like structures were described in detail in an earlier publication (12) and will be discussed in a later report. The central space of the double-ringed organelle is not empty, and, in many examples, it forms a complete sphere enclosing a few granules or other constituents. Cytochemical studies demonstrated that the substance between the inner and outer membranes reacts for both acid phosphatase and myeloperoxidase. In other examples, small granules and other cytoplasmic contents trapped inside the double ring also stain for the two enzymes and undergo degenerative changes. Thus, the major alteration in CHS monocytes is the development of areas of cytoplasmic sequestration rather than formation of giant organelles consisting of fused granules. While the light microscope might show the structures as giant masses of fused granular material, the electron microscope demonstrates that they form in a very different manner from most massive inclusions in CHS neutrophils, eosinophils, and lymphocytes. Yet, double-ringed lysosomes also form in neutrophils and lymphocytes and thus are not unique to CHS monocytes. However, because they are the major aberration in monocytes from CHS patients, their formation deserves further study.

Plasma cells

Although plasma cells are rare in peripheral blood from patients with CHS, they occur more commonly than in normal blood samples (Fig. 16). Giant organelles, though infrequent, have been observed previously in CHS plasma cells (14). Their most striking feature is the peculiar arrangement of their endoplasmic reticulum, which often resembles cells in stages of Russell body formation (45). The tortuous arrangements observed are not seen in normal plasma cells, and their relationship to the disease is unknown.

Platelets

Platelets from patients with CHS have been virtually ignored

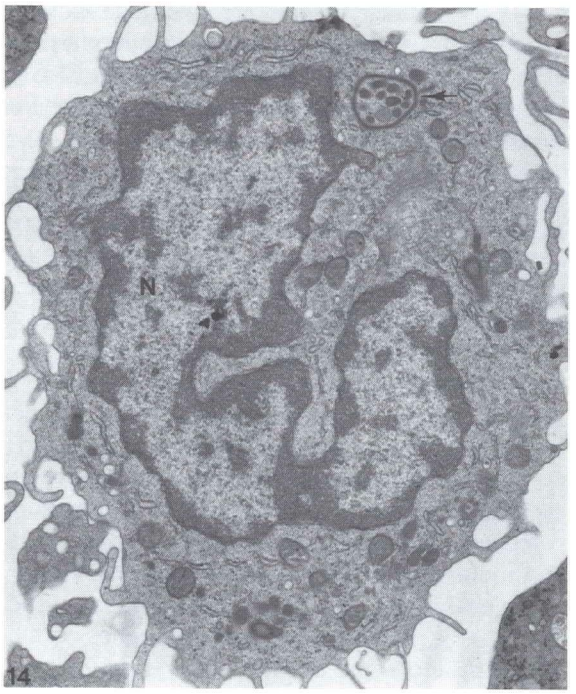

Fig. 14. CHS monocyte

CHS monocytes are comparable in structure to normal monocytes. The only manifestations of CHS are double-ringed organelles (↑) containing granules and other cytoplasmic constituents (X16,000).

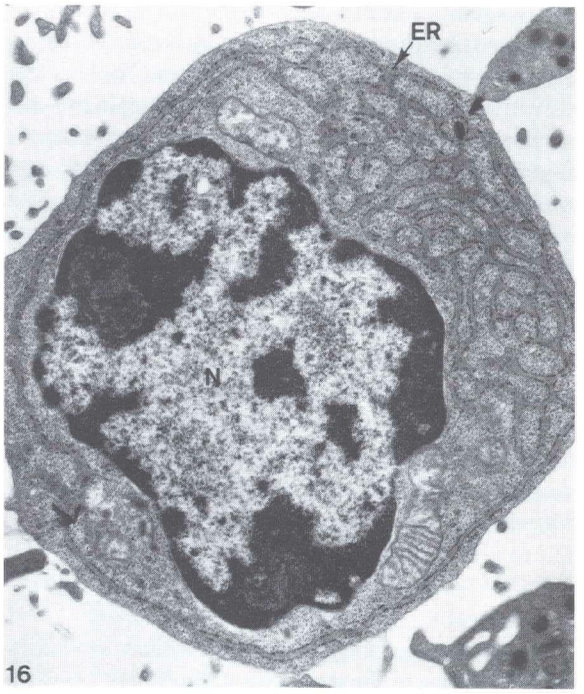

Fig. 16. CHS plasma cell

Rough endoplasmic reticulum (ER) filling the cytoplasm of the cell has a racemous pattern not evident in normal plasma cells (X22,500).

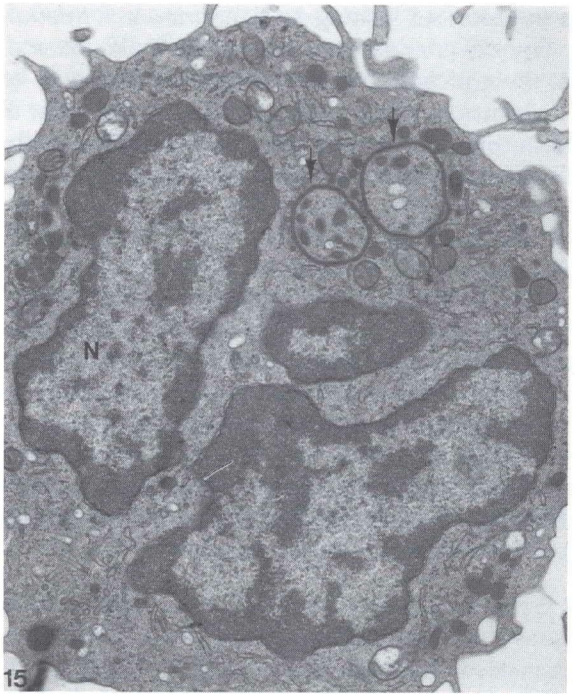

Fig. 15. CHS monocyte

This example contains two unusual double-ringed organelles (↑) sequestering cytoplasmic constituents (X15,500).

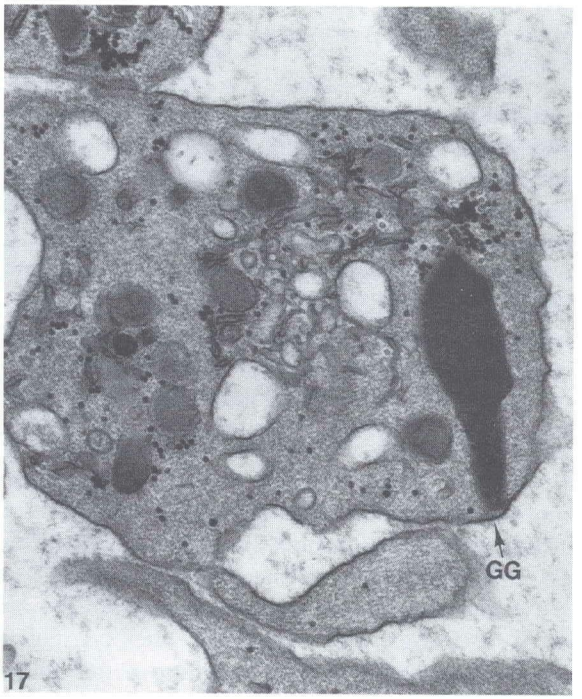

Fig. 17. CHS platelet

Giant granule (GG) in the cytoplasm of this example is very unusual. Organelles of this type have not been observed in normal platelets or in cells from patients with other inherited platelet disorders (X50,000).

in morphological studies, since it had been assumed that they do not exhibit the giant granule anomaly. The impetus for their closer examination came from recent studies suggesting that CHS platelets are very deficient in the storage pool of adenine nucleotides and serotonin confined to a specialized organelle in the cell referred to as the dense body (46,48). In the course of reevaluating CHS platelets for this deficiency, we found (Fig. 17) that rare platelets do contain giant organelles (49). By careful search of peripheral blood smears in the light microscope, we have also been able to identify giant organelles in platelets, but they occur so infrequently it is doubtful they influence platelet function. Still, their presence in platelets indicates that the basic CHS defect exists in this cell line and may influence hemostatic function. Precisely how the basic pathologic process results in platelet storage pool disease remains to be determined.

Discussion

The characteristic giant granulations found in nearly all types of leukocytes in the peripheral blood of CHS patients have fascinated investigators since the disease was first described (1-4). Of the more than two hundred publications devoted to the disorder, most have focused on the blood cell defect, with emphasis on giant granules in PMN. The emphasis is well placed, for a major problem of children with CHS is their susceptibility to infection, and neutrophil dysfunction may play a principal role in failure of patient host defense (7).

However, the preoccupation with giant PMN granules may have obscured important details of the pathologic process. The many studies of CHS neutrophils and their bone marrow precursors have resulted in the concept that the massive inclusions arise by fusion of small azurophilic granules in promyelocytes and myelocytes, a process believed to be complete by the time the cell reaches the peripheral circulation (17,18,25,26). Observations on the mechanism of giant granule formation in PMN have been employed without qualification to explain the formation of massive inclusions in other blood cells, tissues, and organs of patients with CHS.

The present study has demonstrated that the basic pathology of CHS is far more complex than can be deduced from a single cell type. Even in the PMN simple fusion of azurophilic granules cannot completely explain the origin of massive inclusions, their effects on other organelles in neutrophil cytoplasm, and their deleterious influence on cell functions. We have shown (11, 12, 31) that the process of granule fusion continues in the mature PMN and involves specific granules as well as azurophilic lysosomes. Furthermore, the influence of the disease process extends to areas of PMN cytoplasm apart from the huge inclusions, resulting in the development of zones of cytoplasmic sequestration (12). The multiple defects present in CHS neutrophils may all contribute to defective function of the cells and to their early demise.

It was both logical and fortuitous that the concept of giant granules arising solely by fusion of azurophilic lysosomes in bone marrow precursors could be extended to explain massive inclusions in eosinophils: logical because the eosinophil and PMN are similar in many respects and arise from the same stem cell in bone marrow; fortuitous because the eosinophil in humans is reputed to have only a single type of storage organelle containing a distinctive crystalloid. Electron microscopic studies demonstrated that giant granules of CHS eosinophils appeared to develop by fusion of smaller azurophilic lysosomes in promyelocytes and myelocytes, a process supported by finding multiple crystalloid inclusions in the massive inclusions. However, even for the eosinophil, the concept may be simplistic. In many eosinophils, growth of the giant organelles continues in mature cells, and some of them show signs of internal disintergration. Normal-sized azurophilic granules are present among the massive inclusions, and rare areas of the cytoplasm reveal changes suggestive of the second form of giant, irregular granule observed in PMN. The rarity of the latter form in CHS eosinophils and its high frequency in CHS neutrophils should have suggested a significant difference in processes leading to giant granule formation in the two types of granulocytes. Apparently it did not, and, as a result, the concept of azurophil granule fusion in bone marrow precursor cells is still regarded by many as the only mechanism of giant granule formation in CHS peripheral blood PMN, eosinophils, and other leukocytes.

Many workers appear to have extended the same concept to giant granules in the monocyte (28), but, as applied to CHS granulocytes, it is not tenable. Light microscopic studies have indicated that 10% or less of peripheral blood monocytes from CHS patients contain giant organelles (40-43), which consisted of four or five azurophilic granules close together in one area of the cytoplasm. Large inclusions were described in a few CHS monocytes, but their cells may have been large lymphocytes. Smears from five different patients here failed to reveal any typical giant azurophilic lysosomes in their monocytes.

The CHS monocyte, however, does show manifestations of the disease. In thin sections, more than a third reveal various stages in the formation of double-ringed structures enclosing areas of the cytoplasm or surrounding small groups of azurophilic granules. In our earlier studies, we demonstrated the sequential formation of double rings and showed that the substance between the two rings of mem-

brane was acid phosphatase positive. Recently, we have shown that both this substance and the encircled granules are peroxidase positive. While double-ringed, spherical organelles have also been identified in CHS lymphocytes and PMN, they are far more common in the monocyte. In our opinion, they constitute the major manifestation of CHS in this cell type. As shown previously, this unusual organelle arises by fusion, but the structures involved in its formation do not appear to be typical azurophilic granules, even though the substance between the double-ringed membranes stains for acid phosphatase and peroxidase, which are constituents of the lysosomal granules of the monocyte. Instead, the structural elements appear to develop from small, atypical granules, from segments of the terminal cisternae of the Golgi zone, or from elements of a specialized region of Golgi-associated, smooth endoplasmic reticulum, referred to as the Golgi-endoplasmic-reticulum-lysosome (GERL). Although further studies will be required to establish their precise origin, it is clear that giant organelle formation in monocytes does not involve a fusion process like that proposed for giant inclusions in granulocytes.

The lymphocyte also poses a problem for the accepted concept of fusion of azurophilic lysosomes, since about 75% of CHS lymphocytes on peripheral blood smears contain one or two giant inclusions. Study of thin sections of the cells has confirmed this high frequency and has also shown that there are two types of giant inclusions in CHS lymphocytes. The first variety is a complex organelle containing homogenous protein or constituents resembling lipid droplets, ceroid or lipofuchsin pigment, or debris. We have observed stages of fusion between small granules in CHS lymphocytes that resulted in this type of large inclusion. It reacts for acid phosphatase, and the asymmetric distribution of enzyme reaction product suggests that it arises by fusion of smaller granules (11). The organization of reaction product also suggests that fusion occurs between lysosomes and nonlysosomal organelles. The second variety of giant organelle is composed of tightly packed masses of circular profiles very similar in appearance to microtubules (34). It appears to result from masses of tubules aggregating in an area of the cytoplasm, followed by enclosure within a membrane. The origin of the microtubular material and the enclosing membrane are uncertain. Small granules and cytoplasmic constituents are found occasionally within the membrane, and debris surrounding the tubules has been observed to react for acid phosphatase. These giant tubular inclusions do not result by fusion of azurophilic lysosomes, but by a process resembling cytoplasmic sequestration. Even though lysosomal granules may subsequently fuse with and convert them to secondary lysosomes, the mechanism of formation is not consistent with the concept proposed to explain the formation of giant inclusions in CHS granulocytes.

Conclusion

The present study has demonstrated that the CHS anomaly of circulating blood cells is far more complex than the prevailing view would suggest. Although fusion of azurophilic granules takes place in bone marrow precursors of the granulocyte series and is the major factor contributing to the massive inclusions in CHS eosinophils, it is not limited to interactions between azurophilic granules in precursor cells. It continues at all stages of leukocyte development and involves secondary granules and other cytoplasmic components, as well as azurophilic lysosomes, in mature CHS neutrophils. Striking differences in fusion processes observed in PMN and eosinophils emphasize the importance of looking for features of the disease unique to each cell type, as well as for aspects common to all CHS cells.

Studies of CHS monocytes and lymphocytes are also pertinent, as examination of the anomaly in CHS monocytes has shown a process of producing enlarged organelles far different from the mechanism proposed for giant inclusions in granulocytes. When viewed in the light microscope, a few CHS monocytes may appear to contain masses of fused azurophilic granules, but electron microscopy reveals that the mechanism of their formation is quite distinct from the process of azurophilic granule fusion in PMN and eosinophils. Lymphocytes, which manifest two varieties of giant granule formation, are both similar to CHS granulocytes and different.

It is our hope that emphasis on the differences peculiar to each cell type, as well as the similarities, will lead to a solution to the basic defect of the CHS anomaly.

References

1. Beguez-Cesar A. Neutropenia cronica maligna familiar con granulaciones atipicas de los leucocitos. Sociedad Cubana de Pediat Boletin 1943;15:900-922.
2. Steinbrinck W. Uber eine neue Granulationsanomalie der Leukocyten. Dtsch Archiv Klin Med 1948;193:577-81.
3. Chediak MM. Nouvelle anomalie leucocytaire de caractere constitutionnel et familial. Rev Hemat 1952;7:362-67.
4. Higashi O. Congenital gigantism of peroxidase granules. The first case ever reported of qualitative abnormality of peroxidase. Tohoku J Exp Med 1954;59:315-32.
5. Efrati P, Jonas W. Chediak's anomaly of leukocytes in malignant lymphoma associated with leukemic manifestations: Case report with necropsy. Blood 1958;13:1063-73.
6. Dent PB, Fish LA, White JF, Good RA. Chediak-Higashi syndrome. Observations on the nature of the associated malignancy. Lab Invest 1966;15:1634-42.
7. Wolff SM, Dale DC, Clark RA, Root RK, Kimball HR. The Chediak-Higahsi syndrome; Studies of host defenses. Ann Intern Med 1972;76:293-306.
8. Astaldi G, Strosselli E. Histochimie des granulations geantes des polynucleaires dans l 'anomalie de Chediak. Nouv Rev Franc Hemat 1961;1:626-28.
9. Bernard J, Bessis M, Seligmann M, Chassigneux J, Chome J. Un case de maladie de Chediak-Steinbrinck-Higashi: Etude clinique et cytologique. Presse Med 1960;68:563-66.
10. Bessis M, Bernard J, Seligmann M. Etude cytologique d'un cas de maladie de Chediak. Nouv Rev Franc Hemat 1961;1:422-40.
11. White JG. The Chediak-Higashi syndrome: A possible lysosomal disease. Blood 1966;28:143-56.
12. White JG. The Chediak-Higashi syndrome: Cytoplasmic sequestration in circulating leukocytes. Blood 1967;29:435-51.
13. Fortunato RA. Estudio electronimicroscopico de la anomalia de Chediak-Higashi-Steinbrink. Acta Cient Venez 1967;18:73-8.
14. Windhorst DB, White JG, Zelickson AS, Clawson CC, Dent PB, Pollara B, Good RA. The Chediak-Higashi anomaly and the Aleutian trait in mink: Homologous defects of lysosomal structure. Ann NY Acad Sci 1968;155:818-46.
15. Efrati P, Danon D. Electron microscopical study of bone marrow cells in a case of Chediak-Higashi-Steinbrink syndrome. Br J Haematol 1968;15:173-84.
16. Padgett GA. The Chediak-Higashi syndrome. Adv Vet Sci 1968;12:239-84.
17. Douglas SD, Blume RS, Wolff SM. Fine structural studies of leukocytes from patients and heterozygotes with Chediak-Higashi syndrome. Blood 1969;33:527-40.
18. Douglas SD, Blume RS, Glade PR, Chessin LN, Wolff SM. Fine structure of continuous long term lymphoid cell cultures from a Chediak-Higashi patient and heterozygote. Lab Invest 1969;21:225-9.
19. Blume RS, Glade PR, Gralnick HR, Chessin LN, Wolff SM. The Chediak-Higashi syndrome: Continuous suspension culture of peripheral blood leukocytes. Blood 1969;33:821-32.
20. Moran TJ, Estevez JM. Chediak-Higashi disease: Morphologic studies of a patient and her family. Arch Pathol 1969;88:329-39.
21. Bedaya V, Grimley PM, Duque O. Chediak-Higashi syndrome. Arch Pathol 1969;88:340-9.
22. Blume RS, Wolff SM. The Chediak-Higashi syndrome: Studies in four patients and a review of the literature. Medicine 1972;51:247-80.
23. Bennett JM, Blume RS, Wolff SM. Characterization and significance of abnormal leukocyte granules in the beige mouse: A possible homologue for Chediak-Higashi Aleutian trait. J Lab Clin Med 1969;73:235-43.
24. Lutzner MA, Tierney JH, Benditt EP. Giant granules and widespread cytoplasmic inclusions in a genetic syndrome of Aleutian mink. Lab Invest 1966;14:2063-79.
25. Davis WC, Spicer SS, Green WB, Padgett GA. Ultrastructure of bone marrow granulocytes in normal mink and mink with the homolog of the Chediak-Higashi trait in humans. I. Origin of the abnormal granules present in the neutrophils of mink with CHS trait. Lab Invest 1971;24:303-17.
26. Davis WC, Spicer SS, Green WB, Padgett GA. Ultrastructure of cells in bone marrow and peripheral blood of normal mink and mink with the homologue of the Chediak-Higashi trait of humans. II. Cytoplasmic granules in eosinophils, basophils, mononuclear cells and platelets. Am J Pathol 1971;63:411-32.
27. Oliver C, Essner E. Distribution of anomalous lysosomes in the beige mouse: A homologue of Chediak-Higashi syndrome. J Histochem Cytochem 1973;21:218-28.
28. Oliver C, Essner E. Formation of anomalous lysosomes in monocytes, neutrophils, and eosinophils from bone marrow of mice with Chediak-Higashi syndrome. Lab Invest 1975;32:17-27.
29. Rozenszajn LA, Ben David E, Bar Sela S. Large granules and lysosomal fusion in human Chediak-Higashi white blood cells. Acta Haemat 1977;57:279-89.
30. Rausch PG, Pryzwansky KB, Spitznagel JK. Immunocytochemical identification of azurophilic specific granule markers in the giant granules of Chediak-Higashi neutrophils. N Eng J Med 1978;298:693-98.
31. White JG. The Chediak-Higashi syndrome: Fine structure of giant inclusions in freeze-fractured neutrophils. Am J Pathol 1973;72:503-19.
32. Clawson CC, White JG, Repine JE. The Chediak-Higashi syndrome: Evidence that defective leukotaxis is primarily due to an impediment by giant granules. Am J Path 1978;92:745-53.
33. Craddock PR, Hammerschmidt DE, White JG, Dalmasso AP, Jacob HS. Complement (C5a)-induced granulocyte aggregation *in vitro*: A possible mechanism of complement-mediated leukostasis and leukopenia. J Clin Invest 1977;60:261-64.
34. White JG. Giant organelles containing tubules in Chediak-Higashi lymphocytes. Am J Pathol 1972;69:225-38.
35. White JG. Interaction of membrane systems in blood platelets. Am J Pathol 1972;66:295-312.
36. Karnovsky MJ. Use of ferrocyanide reduced osmium tetroxide in electron microscopy. Amer Soc Cell Biol Ann Mtg, New Orleans, 1971.
37. Klebanoff SJ, Clark RA. The neutrophil: Function and clinical disorders. New York: North-Holland Publishing Co, 1979: 735-92.
38. Blume RS, Bennett JM, Yankee RA, Wolff SM. Defective granulocyte regulation in the Chediak-Higashi syndrome. N Eng J Med 1968;279:1009-15.
39. Kritzler RA, Terner JY, Lindenbaum J, Magidson J, Williams R, Preisig R, Phillips GB. Chediak-Higashi syndrome: Cytologic and serum lipid observations in a case and family. Am J Med 1964;36:583-94.

40. Jannini P, Pinto Lima FX, Hubner Franca H, Tricta DF, Tannos D. Sobre tres casos de anomalia leucocitaria indentica a la descreita por Bequez-Cesar, Steinbrinck, Chediak, Higahsi y Sato. Sangre (Barcelona) 1963;8:138-59.

41. Saraiva LG, Azevedo M, Correa JG, Carvalho G, Prospero JD. Anomalous panleukocytic granulation. Blood 1959;14:1112-27.

42. Hansson H, Lineall F, Nilsson LR. Soderhjelm L, Undritz E. Die Chediak-Steinbrinck-anamolie respektive erblichkonstitutionelle riesengranulation (Granulagiganten) der leukozyten in Nordschweden. Folia Haematol Neue Folge (Frankfurt) 1959;3:152-96.

43. Bessis M. Living blood cells and their ultrastructure. New York: Springer-Verlag, 1973:345-50.

44. Padgett GA. The Chediak-Higashi syndrome. Adv Vet Sci 1968;12:239-84.

45. Russell W. An address on a characteristic organism of cancer. Br Med J 1890;2:1356-58.

46. Buchanan GR, Handin RI. Platelet function in the Chediak-Higashi syndrome. Blood 1976;47:941-48.

47. Bell TG, Meyers KM, Prieur DJ, et al. Decreased nucleotide and serotonin storage associated with defective function in Chediak-Higashi syndrome cattle and human platelets. Blood 1976;48:175-84.

48. Boxer GJ, Holmsen H, Robkin L, et al. Abnormal platelet function in Chediak-Higashi syndrome. Br J Haematol 1977;35:521-33.

49. White JG. Platelet microtubules and giant granules in the Chediak-Higashi syndrome. Am J Med Tech 1978;44:273-79.