Hermansky-Pudlak Syndrome and Chediak-Higashi Syndrome: Disorders of Vesicle Formation and Trafficking

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Summary
The rare autosomal recessive metabolic disorders Hermansky-Pudlak syndrome (HPS) and Chediak-Higashi syndrome (CHS) share the clinical findings of oculocutaneous albinism and a platelet storage pool deficiency. In addition, HPS exhibits ceroid lipofuscinosis and CHS is characterized by infections and an accelerated phase. The two disorders result from defects in vesicles of lysosomal lineage. Of the two known HPS-causing genes, HPS1 has no recognizable function, while ADTB3A codes for a subunit of an adaptor complex responsible for new vesicle formation from the trans-Golgi network. Other HPS-causing genes are likely to exist. The only known CHS-causing gene, LYST, codes for a large protein of unknown function. In general, HPS appears to be a disorder of vesicle formation and CHS a defect in vesicle trafficking. These diseases and their variants mirror a group of mouse hypopigmentation mutants. The gene products involved will reveal how the melanosome, platelet dense body, and lysosome are formed and trafficked within cells.

Introduction
Hermansky-Pudlak syndrome (HPS) and Chediak-Higashi syndrome (CHS), share several clinical characteristics and have piqued the interest of cell biologists focusing on the genesis and movement of intracellular vesicles (1-6). In this review, we describe these two diseases and demonstrate why they provide insight into cell biological processes. In fact, we propose that HPS and CHS represent extremes of a spectrum of disease entities that correspond to discrete steps in the genesis and movement of vesicles of lysosomal lineage.

Hermansky-Pudlak Syndrome
HPS, first reported in 1959 by two Czechoslovakian pathologists (7), consists of a collection of genetically distinct defects having common clinical and laboratory findings (3, 4). All patients have some degree of oculocutaneous albinism and platelet storage pool deficiency. Many also demonstrate ceroid lipofuscinosis, although the presence of this intracellular lipid/protein complex is no longer necessary to make the diagnosis of HPS. In our own studies, we have also arbitrarily required that dense bodies be absent from platelets for the diagnosis of HPS to be confirmed, although future clinical/molecular correlations may mandate modification of this requirement. What constitutes HPS will be discussed below, after description of the disease as we currently know it.

Clinical and Laboratory Findings
The clinical manifestations of HPS are variable (3, 4) because the physiologic processes involved are modulated by a panoply of genetic and environmental influences, and because the genetic basis of HPS is itself heterogeneous. Despite these caveats, some generalizations can be made regarding the signs and symptoms of HPS.

Oculocutaneous albinism. In HPS, tyrosinase activity remains present within melanocytes (2), but melanosome function is impaired, and pigment dilution results. This affects skin, hair, and eye color, but to variable extents. Hair color ranges from nearly normal to completely white (Fig. 1A, B). Even among the approximately 400 Puerto Rican patients with exactly the same mutation in the HPS1 gene (see below), the spectrum of hair color varies enormously (8). Typically, the hair is dirty blond or tan. HPS skin also varies from pale to brown, and often must be compared with that of an unaffected sibling to verify hypopigmentation. HPS patients are susceptible to solar damage, including actinic keratoses, nevi, and skin malignancies (9).

Ophthalmic involvement in HPS patients (10-12) includes increased crossing over of nerve fibers at the optic chiasm, typical of albinism. Iris transillumination (Fig. 1C, D) occurs because cells in the iris have reduced amounts of pigment. Loss of retinal pigment epithelium causes a pale fundus and decreased visual acuity, usually 20/200 or worse (legally blind), but sometimes as high as 20/50 (8, 12). Vision in HPS is stable, uncorrectable, and accompanied by variable degrees of congenital nystagmus.

Hematologic findings. In HPS, platelet counts are normal or elevated, but the platelets are dysfunctional because they lack dense bodies (Fig. 1E, F). These electron dense intracellular organelles, which contain ADP, ATP, serotonin, calcium, and polyphosphates (13), disgorge their contents upon activation and promote clot formation. Absence of dense bodies attenuates the secondary aggregation response and can prolong the bleeding time.

HPS patients exhibit findings typical for a platelet storage pool deficiency, i.e., bleeding of mucosal membranes and spontaneous soft tissue bruising. Major bleeds into the joints or brain are not seen, and coagulation factors, prothrombin times, and partial thromboplastin times are normal (1, 8). Excess bruising usually begins at ambulation,
and epistaxis begins in childhood. Bleeding episodes can occur during dental extractions, surgeries, acute colitis, menstrual periods, and child birth. Fatal bleeds are rare but a significant number of patients have received transfusions of packed red cells or platelets.

Interestingly, von Willebrand factor, which is stored within platelet alpha granules, is decreased in many HPS patients (14, 15). Lymphocyte and neutrophil function appear normal in HPS (16).

Pulmonary fibrosis. Many HPS patients suffer a progressive restrictive lung disease beginning in their late twenties or early thirties (1, 8, 17, 18), perhaps related to accumulation of ceroid in HPS alveolar macrophages (Fig. 1G, H). Affected patients typically exhibit extensive fibrosis by the end of the fourth or fifth decade of life (Fig. 1I, J). Infection, smoking or exposure to pulmonary toxins can lead to death within two or three years. The pulmonary involvement of HPS varies considerably, and occasional patients are over 60 years old with normal lung function. Some subgroups of HPS, specifically, patients bearing mutations in the HPS1 gene (see below), are more susceptible to pulmonary fibrosis than others (8, 18). Pulmonary fibrosis has been noted in HPS patients from Czechoslovakia, Japan, Belgium, and England (3), prior to the availability of molecular genotyping.

Granulomatous colitis. This complication affects at least 15% of all HPS patients, whether or not they are of Puerto Rican heritage (8). Although the distal colon is most often involved, the entire gastrointestinal tract may be affected, with granulomatous gingivitis occurring in rare cases (1). Patients present with blood and mucus in their stools, and the colitis resembles Crohn’s disease histologically (19, 20).

Ceroid lipofuscin. This electron dense, autofluorescent aging pigment is increased in some HPS patients, who accumulate it within the kidneys, urinary sediment, lung alveolar macrophages, bone marrow, spleen, liver, and large intestine (1). Smaller amounts can be found in the heart, lymph nodes and other tissues (1). The ceroid lipofuscin of HPS appears to reside within lysosomes. Some theories blame ceroid lipofuscin accumulation for the pulmonary fibrosis, granulomatous colitis, and occasional renal impairment (1, 8) and cardiomyopathy (1) of HPS. However, no convincing evidence has been put forth to support this.

Subtypes of HPS

Two patients have been reported with mutations in the ADTB3A gene (21, 22) (see below), and we have recently identified a third. All three had persistent neutropenia and childhood infections (22). Balance problems in the two reported cases may be related to decreased visual acuity. In other respects, the clinical findings are typical for HPS, i.e., moderate visual impairment, nystagmus, hypopigmentation, and absence of platelet dense bodies.

Another subtype of HPS exists in central Puerto Rico, where affected individuals have absent dense bodies but mild hypopigmentation and visual acuity deficits. The HPS1 gene has been eliminated as a cause for the disease in this group of patients (23). As other HPS-causing genes are identified, additional clinical subtypes should emerge.

Diagnosis

The diagnosis of HPS relies on the clinical suspicion of the physician. Dirty blond hair color, excessive bruising in toddlers, and epistaxis in middle childhood provide hints, but bleeding times may remain normal. Nevertheless, virtually every patient has some degree of horizontal nystagmus present from birth (12), and every patient has a visual acuity of 20/50 or poorer.
The sine qua non of the HPS diagnosis remains demonstration of absent platelet dense bodies using the method of wet-mount electron microscopy (24). This procedure is currently performed by Dr. James White of the University of Minnesota. Studies showing reduced or absent serotonin uptake or an absent secondary aggregation response can also be helpful.

Many physicians, recognizing that HPS occurs with increased frequency among Puerto Ricans, mistakenly discount the diagnosis when caring for non-Puerto Rican patients. One HPS patient carried the diagnosis of isolated ocular albinism until her mid-twenties, when her excessive bleeding during menstrual periods prompted an examination for platelet dense bodies, which were absent by electron microscopy. We recommend consideration of platelet dense body analysis in albino patients without a molecular basis for their disorder.

Treatment

For HPS patients, sun avoidance and sunscreens are critical to prevent solar damage, and skin cancer surveillance can detect early malignancies (9). For the bleeding diathesis of HPS, thrombin and gelfoam can be used for minor wounds, and some, but not all, patients respond to 1-desamino-8-D-arginine vasopressin (DDAVP), 0.2 μg/kg in 50 ml of normal saline given intravenously over 30 minutes (25). Platelets can be either on hand or transfused prior to major procedures. The most crucial issue, however, is recognition of the functional platelet defect, which cannot be appreciated from a simple platelet count. Hence, the use of a medical alert bracelet can be extremely helpful.

Only supportive care is available for the pulmonary fibrosis of HPS, although a double-blind, placebo-controlled trial of the investigational anti-inflammatory drug, pirfenidone, is currently underway. Lung transplantation, although never performed on an HPS patient, should be feasible with close attention to coagulation, and a transplanted lung should last the natural lifetime of a patient. The granulomatous colitis of HPS generally responds to steroids and other treatments appropriate for Crohn’s disease.

Genetics

The population frequency of HPS in northwest Puerto Rico is 1 in 1800, with a gene frequency of 1 in 21 (26). All 400 affected individuals in northwest Puerto Rico are homozygous for a specific mutation, reflecting a founder effect. However, HPS has been reported throughout the globe (1), with different mutations and different genes involved (27-30). Of the approximately 60 non-Puerto Rican HPS patients examined molecularly, only approximately 25-40% have their disease due to mutations in a known gene (28, 29). In addition to the known HPS-causing genes HPS1 and ADTB3A, there is certainly another gene causing the disease in central Puerto Rico (23). The many animal models of HPS (31) also indicate locus heterogeneity. All subtypes of HPS appear to be inherited in an autosomal recessive fashion (1).

The HPS1 gene. The first HPS-causing gene was mapped to chromosome 10q23.1-23.3 in 1995 using Puerto Rican and Swiss families (32). Subsequently, the gene (GenBank accession #1654350), called HPS and later HPS1, was sequenced and shown to contain an open reading frame of 2100 bp (27). The genomic structure was then determined (33). HPS1 consists of 20 exons spanning approximately 30.5 kb. Its standard transcript, 3.0 kb in size, is expressed in most tissues (27). Minor 3.9-kb, 4.4-kb, and 1.5-kb mRNAs also occur (34). Four alternative splices of HPS1 have been described (27, 33). A pseudogene of HPS1 (GenBank accession #6707403) exists on chromosome 22q12.2-12.3 and contains complete exons 3, 4, and 6 (35).

The 16-bp duplication in exon 15 of HPS1 is present in the homozygous state in all northwest Puerto Rican patients but not in any other patient group (27-29). This frameshift mutation produces no mRNA, and is easily detected by PCR amplification (27). Other mutations in HPS1 include T322insC and T322delC, indicating that the region of codons 321-324 represents a mutation hot spot (28). Codon 396 may be another area subject to recurrent mutation, since S396delC has appeared in several other patients (28, 29). In all, 12 different mutations, including deletions, insertions, nonsense mutations, and splice junction mutations, have been reported for HPS1 (3, 5, 30).

Most of the HPS1 gene mutations, as well as the frameshift mutation of the mouse homologue of HPS1, pale ear (36, 37) (see below), are predicted to result in a protein with a truncated carboxy terminus and, presumably, no residual function. This points to the critical nature of the terminal portion of the HPS1 protein. To date, no missense mutations in HPS1 have been found, although at least 24 nonpathologic DNA sequence polymorphisms have been reported (29, 30, 33).

The ADTB3A gene. ADTB3A (GenBank accession #1923267), whose coding sequence has 3281 bp, produces a 4.2-kb mRNA present in all tissues and cell lines examined (38). The product is β3A, a subunit of adaptor complex-3 (AP3), which functions to form vesicles from existing membranes such as the trans-Golgi network, or TGN (39). The only reported cases of mutations in ADTB3A are those found in the compound heterozygous state in two brothers affected with HPS (22). The mutations are a 21-amino acid deletion (A390-410) and a single amino acid substitution, L580R (21). Mutations in the pearl mouse, which is the murine equivalent of the humans with ADTB3A mutations, include a 793-bp internal tandem duplication and a 107-bp deletion (40).

Cell Biology

HPS has long been considered a defect of vesicular membranes, in part because the intracellular compartments causing the clinical manifestations of the disease share integral membrane proteins. Specifically, the melanosome’s ME491 protein is the same as the platelet’s CD63 or granulophysin which is the same as the lysosome’s LAMP-3 or LIMP-1 (3). In addition, two lysosomal membrane proteins, LAMP-1 and LAMP-2, are also found on platelet dense body membranes (41). The hypothesis that HPS results from dysfunctional membrane trafficking has been strongly supported by the finding that AP3 deficiency can cause HPS, and by the fact that several genes responsible for HPS in murine and Drosophila models are involved in vesicle formation and trafficking (see below).

The HPS1 protein. The HPS1 gene product has 700 amino acids and a predicted molecular weight of 79.3 kDa (27), with no apparent glycosylation (42) and no homology to proteins with a known function. It does contain the sequence DKF(L/V)KNRG, which resembles a region of the CHS protein (27, 43) (see below), and the carboxy terminus of the HPS1 protein contains a putative melanosomal localization signal, PLL (44). The amino acid sequence of the HPS1 protein is 81% conserved between human and mouse (36), with similar conservation between human and rat (5). A predicted Drosophila protein of unknown function has high sequence identity to the carboxy terminal portion of HPS1, but no homologues exist in lower species.

A role for the HPS1 protein in vesicle formation is suggested by the His-Leu-Leu sequence near its carboxy terminus. This recognition marker could serve as a sorting signal to target the protein to compartments of lysosomal lineage, such as the melanosome and dense granule (45, 46), although the HPS1 protein does not appear to be directly associated with lysosomes (42).
The HPS1 protein is a component of two high molecular weight complexes, a cytosolic complex of approximately 200 kDa in nonmelanotic cells and a larger, 500-kDa complex in melanotic cells (42). The large complex is perinuclear and associated with tubulovesicular structures, small noncoated vesicles, and nascent and early-stage melanosomes but not with later stage melanosomes (42). Other studies place the HPS1 protein in the perinuclear region of normal melanocytes, possibly associated with a cisternal network outside of the Golgi zone (47). This suggests that the protein is a part of the premelanosome as it forms from the smooth endoplasmic reticulum.

The HPS1 gene product appears to influence trafficking of melanocyte-specific proteins from the TGN to preformed premelanosomes (48). Specifically, the pigment-forming proteins TRP-1 (for tyrosinase related protein-1) and granulophysin displayed a large granular pattern of expression in HPS-1 melanocytes, consistent with localization to large membrane complexes visible ultrastructurally in the mutant cells (48). The appropriate location for these proteins would be within melanosomes.

The HPS1 protein is present on normal platelets but not on platelets from patients with null mutations in HPS1. Other studies have shown that fibroblasts do not require the HPS1 protein to grow, and that HPS1-deficient cells display a normal distribution and trafficking of the lysosomal membrane proteins CD63 and LAMP-1. HPS1 does not appear to interact with AP3 (46).

The B3A subunit of AP3. The B3A protein is a subunit of AP3, one of four known adaptor complexes (AP1, AP2, AP3, and AP4). Adaptors are components of cytosolic protein coats that mediate vesicle formation and incorporation of cargo proteins into the nascent vesicle’s membranes (39). AP3 apparently produces vesicles from the TGN and/or endosomes. It interacts with clathrin (49), which provides structure to the newly formed vesicle. AP3 consists of a 160 kDa subunit, a 47-kDa μ subunit, a 23-kDa σ subunit, and the 140 kDa B3A subunit (38, 50-52). The B3A subunit is phosphorylated on serine residues (38). The μ subunit of AP3 recognizes tyrosine-based (50, 53) and dileucine-based (54) sorting signals of cargo proteins targeted for incorporation into the membranes of new vesicles.

The 3A subunit of AP3 (40). The 3A gene encodes a 25-kDa protein, RABGGTA (58), that interacts with syntaxin 13, part of the membrane integral membrane proteins use AP3 for targeting to lysosome-related organelles. Trafficking of MHC class II molecules and the associated invariant chains to their intracellular compartments appeared normal in AP3-deficient cells (56).

In B3A-deficient human melanocytes, TRP-1 localization is normal, but tyrosinase expression is restricted to the perinuclear region (Fig. 2) and localized in large vesicles resembling late endosomes (57). Moreover, the abnormal tyrosinase distribution is corrected by transfection with the normal B3A gene, supporting the concept that tyrosinase trafficking is regulated by AP3 (57).

Fibroblasts from patients with ADTB3A mutations show reduction of the μ, σ, and δ subunits of AP3 as well; apparently the B3A subunit stabilizes the entire AP3 complex against degradation (21).

Models of Pigment Dilution in Other Organisms

The earliest and closest models for HPS consist of inbred mice having variable degrees of pigment dilution combined with a platelet storage pool deficiency (31) (Table 1). This group of mutants actually represents a spectrum of disease ranging from HPS to CHS and reflecting various human conditions (see below). Several of the model mice exhibit increased urinary excretion of lysosomal enzymes. All the HPS mice inherit their disease in an autosomal recessive fashion.

Six mouse models have had their causative genes isolated. Two of these, pale ear and pearl, represent the murine counterparts of humans with mutations in HPS1 and ADTB3A, respectively. In fact, the human ADTB3A mutations were discovered because the murine pearl gene was found to code for the B3A subunit of AP3 (40). The mocha gene encodes the δ subunit of AP3 (55) and pallidin encodes a 25-kDa protein, pallidin (58), that interacts with syntaxin 13, part of the membrane.
The gunmetal gene codes for the α subunit of rab geranylgeranyltransferase, an enzyme that adds 20-carbon prenyl groups to cysteine residues on the carboxy termini of rab proteins to make them membrane-bound (60). Rab proteins are key to vesicle transport and organelle dynamics (61), and the gunmetal mutation results in decreased prenylation and decreased membrane association of rab27a (60). The ashen mouse, which recently became a model of HPS based upon the finding of absent platelet dense bodies, codes for rab27a itself (62). Mutations in rab27a result in Griscelli syndrome (63), which falls within the HPS-CHS spectrum of disorders (64) (see below).

There also exist at least 11 Drosophila melanogaster models of HPS and related disorders. These flies have mutations at eye color loci and are members of the “granule group” of mutants (65). The 7 granule group genes isolated to date are identical to genes involved in lysosomal trafficking in other organisms. For example, all four subunits of AP3 have a Drosophila mutant model. The garnet gene produces a δ subunit of AP3 (66), while ruby codes for a β3 subunit (67), carmine codes for µ3 (68), and orange for δ3 (69). Three other genes of the granule group encode Drosophila homologues of the yeast VPS (Vesicle Proteins for Sorting) genes. The light fly has mutations in VPS41, whose protein product interacts with the δ subunit of AP3 (70). Deep orange and carnation have mutations in VPS18 and VPS33, respectively (71, 72). The products of these genes interact in a large complex, and mutations in deep orange cause accumulation of multivesicular bodies (72).

In yeast, the VPS18 and VPS33 proteins form a multisubunit complex with VPS11 and VPS16 (73). Moreover, VPS33 interacts with the t-SNARE VAM3, indicating that these proteins might act together to direct docking and/or fusion of transport intermediates with the yeast vacuole (74). VAM3 is transported via an AP3-dependent pathway (75). There are more than 40 different yeast VPS genes which influence trafficking of proteins destined for the lysosome-like vacuole (76). Mutants in any of these genes could be primitive models for human HPS.

**Chediak-Higashi Syndrome**

CHS (1, 2, 77, 78) was first described by Beguez-Cesar in 1943 (79), but acquired its eponym a decade later from Moises Chediak (80) and Ototaka Higashi (81). It is characterized by an infectious diathesis, giant intracellular granules, variable degrees of oculocutaneous albinism, and a platelet storage pool deficiency. The infections are generally fatal in the first decade of life, but CHS patients can also succumb to a chronic lymphohistiocytic infiltration known as the accelerated phase during the second or third decades of life.

**Clinical and Laboratory Findings**

As for HPS, the clinical findings in CHS are variable but include several characteristic manifestations. Heterozygotes for CHS are always completely normal.

**Oculocutaneous albinism.** Hair color in CHS patients can be blond to light brown, but the truly characteristic color is metallic silver. Giant hypomelanized melanosomes in CHS melanocytes cannot be transferred to surrounding keratinocytes (82), resulting in skin color that varies from white to gray. Sun-exposed areas may pigment, and nevi and lentigines do arise with some frequency. The eyes of CHS patients have reduced pigment in the retina, iris, choroid and ciliary epithelium. On electron microscopy, giant aggregates of melanin appear in the melanosomes of these tissues (83). Irids can be gray, blue, or even brown. Patients can also have nystagmus, photophobia, and reduced...
visual acuity. Electroretinograms and visual evoked potentials show progressive abnormalities (84).

**Bleeding.** CHS patients have the same diathesis toward easy bruising, mucosal bleeding, and epistaxis that HPS patients exhibit, but some CHS patients have reduced or irregular platelet dense bodies rather than total absence of these intracellular organelles. CHS platelets do have low levels of ATP, ADP, calcium, and serotonin, and secreted dense body constituents (i.e., nucleotides and calcium) are lacking as well (85). Aggregation studies performed on CHS patients (85, 86) reveal an absent or minimal second wave of aggregation following stimulation with epinephrine or other agonists. Alpha granules have been normal in number and morphology in CHS patients.

**Infections.** Children with CHS suffer from recurrent infections beginning in infancy. Typical organs affected include the skin and respiratory systems. Periorbital cellulitis, otitis media, pneumonias, pyoderma, abscesses, sinus infections, and dental caries are prominent types of infections, and *Staphylococcus aureus* and *β-hemolytic Streptococcus* are the primary organisms, although gram negative organisms, *Candida*, and *Aspergillus* are also important (87). Response to antibiotics is slower than expected.

Although the bone marrow appears normal to hypercellular in CHS, neutropenia is common. CHS neutrophils, as well as lymphocytes, eosinophils, and platelets, contain giant granules, up to 4 µm in diameter (Fig. 3). The granules contain acid hydrolases, myeloperoxidase, and other proteins necessary for bactericidal activity (88), and exist alongside normal-sized primary lysosomes (89). The giant granules impair leucocyte migration, perhaps by inhibiting cell deformability. Phagocytosis appears normal in CHS cells (90), with intracellular bactericidal activity delayed but improving to near normal over time. This suggests that the primary defect in bacterial killing resides in the early stage of the process, while later functions remain intact. In CHS patients, natural killer (NK) cells are present in normal or slightly elevated numbers, but their function is impaired (91), contributing to the susceptibility to defective cellular immunity and to development of the accelerated phase.

A number of parameters are normal in CHS, including immunoglobulins, antibody production, delayed hypersensitivity, reticuloendothelial clearance, monocyte number and phagocytic function (90), levels of complement (92), suppressor cells, response to interferon, target cell recognition, and antibody-dependent, cell-mediated cytolyis (93, 94).

**Accelerated phase.** Approximately 85% of CHS patients experience the accelerated phase sometime between a few months and several years of age. The accelerated phase involves fever, anemia, neutropenia, and occasionally thrombocytopenia, hepatosplenomegaly, lymphadenopathy, and jaundice (87). Liver function tests are elevated, cellular immunity is decreased, and pancytopenia presents due to splenomegaly and hemolysis. A coagulopathy may also develop due to liver dysfunction and thrombocytopenia (87). The accelerated phase represents a reactive process with perivascular lymphohistiocytic infiltrates that are benign histologically (87, 95, 96) and resemble infectious mononucleosis (95). Lack of NK cell function has been theorized to allow the accelerated phase to become fulminant.

**Neurologic manifestations.** CHS patients can experience a peripheral or cranial neuropathy, autonomic dysfunction, weakness and sensory deficits, loss of deep tendon reflexes, clumsiness, a wide-based gait, seizures, abnormal electromyograms, and decreased motor nerve conduction velocities or spinocerebellar degeneration (77, 97). On pathological examination of four CHS patients, lymphohistiocytic infiltrates were evident throughout the nervous system (98). Cytoplasmic inclusions, resembling lysosomes or large, irregular lipofuscin granules, were present in astrocytes, choroid plexus epithelial cells, Schwann cells, and satellite cells of the dorsal spinal ganglia.

**Diagnosis**

The hallmark of CHS is the presence of giant peroxidase-positive granules in polymorphonuclear leucocytes, megakaryocytes, neurons, conjunctival fibroblasts, and cultured lymphoblasts (1, 2, 77, 78). Other clinical characteristics, such as mild ocularcutaneous albinism, silvery hair, bleeding problems, provide clues to seek a definitive diagnosis. Prenatal diagnosis has been achieved by examination for large, acid phosphatase-positive lysosomes in amniocytes and chorionic villus cells (99). The molecular diagnosis of CHS remains difficult because of the large size of the *LYST* gene and because several different mutations have been identified. Molecular diagnosis is not commercially available.

**Treatment**

The only curative therapy for CHS is bone marrow transplantation, which has also reversed the leucocyte defect in the mouse model of CHS, *beige* (100). Seven of 10 children from two separate institutions who received related donor bone marrows had a successful transplantation (101). None of the children had recurrence of the accelerated phase, and all had improved NK cell activity. However, this therapy does not prevent the progressive neuropathy of CHS.

Other therapeutic modalities for CHS are symptomatic (77). Childhood immunizations are well-tolerated and should be provided (87). Antibiotics will treat the infections, although with a slow response. Aspirin-containing products should be avoided. Desmopressin and ε-amino-caproic acid can be effective for treatment and prophylaxis, while platelet transfusions may be needed for major procedures or bleeds. Corticosteroids, chemotherapeutics, intravenous immunoglobulin, and splenectomy may occasionally induce a temporary remission of the accelerated phase. Ascorbic acid treatment has not proven clinically effective in preventing either infections or the accelerated phase in CHS patients (102, 103).

**Genetics**

CHS is an extremely rare, autosomal recessively inherited disorder that has been found in countries all over the world. Although more than 200 cases have been reported over time (1, 77), many fewer are likely to be alive today.

Unlike HPS, CHS does not display locus heterogeneity. However, it remains possible that some CHS patients, particularly clinical variants, might have their disease due to mutations in genes not yet discovered. The clinical variability observed in the animal models of CHS, i.e., the Alaskan mink, cattle, cats, killer whales, and rats (77), supports the possibility of locus heterogeneity. Presently, the one gene proven to cause CHS is *LYST*, which was discovered with the assistance of extensive investigations into the best animal model of CHS, the *beige* mouse (77).

**The LYST gene.** The *lyst* gene in the *beige* mouse, on chromosome 13, is homologous to the human *LYST* (for lysosome trafficking regulator), on chromosome 1q42.1-42.2 (104-106). *LYST* (107-109) has a coding region of 11,403 bp and is expressed in all cell types. The 13449-bp mRNA (GenBank accession #4502838) is present in low levels and is difficult to detect by Northern blot analysis. A large, 11.4 kb transcript appears to be responsible for gene function, while the
function of a smaller, 5.8 kb transcript derived from the 5' terminus of \( LYST \) is unknown (107, 108). The human \( LYST \) gene has homologues in all mammalian species (109). The human and mouse proteins are 82% identical and 88% homologous. High degrees of identity are also seen between the human and the rat and cow CHS genes.

To date, \( LYST \) mutations have been identified in 13 CHS patients (107-110). All human mutations, as well as those in the mouse (111) and rat (112) beige models, have been nonsense or frameshift mutations with premature termination. There is no obvious “hot spot”, and no correlation between severity of disease and residual length of the truncated \( LYST \) protein.

The \( LYST \) protein. The \( LYST \) gene encodes a large cytosolic protein with 3801 amino acids and a molecular mass of 429 kDa (109). The \( LYST \) protein contains several phosphorylation sites and myristylation sites, indicating a capacity for regulation and interaction with other proteins or membranes. In addition, the amino-terminus of the \( LYST \) protein contains a series of hydrophobic helices resembling HEAT and ARM domains (109). HEAT repeat proteins are involved in vesicle transport, and ARM domains are thought to mediate membrane associations (113). The N-terminus of \( LYST \) also shows low homology to stathmin, a phosphoprotein involved in regulation of microtubule polymerization (109, 114).

The central region of \( LYST \) contains two leucine zippers and an 8-amino-acid domain homologous to a sequence in the HPS1 protein (27). HPS1 and \( LYST \) may interact through this homologous domain, possibly via another linker protein (115, 116).

The carboxy-terminus of \( LYST \) contains seven consecutive WD40 motifs that form beta sheets (109). WD40 motifs are considered to mediate protein-protein interactions (117). Another motif in the C-terminus, conserved among \( LYST \) homologues in other species, is a 345-amino-acid domain called BEACH, for “beige” and “CHS” (109). The BEACH domain contains a consensus “WIDL” amino acid stretch as well as other conserved amino acids. The combination of BEACH and WD-40 domains are found in several mammalian proteins including FAN, CDC4L, and neurobeachin (78, 118). FAN links TNFα to neutral sphingomyelinase (119), and neurobeachin anchors protein kinase A in the trafficking of neuronal post-Golgi membranes (118). The function of CDC4L (120) is unknown. The exact function of \( LYST \) cannot be predicted from homology searches, but these structural studies do suggest that it functions in vesicle trafficking.

**CHS Cell Biology**

Mutations in \( LYST \) result in giant granular inclusions (78, 88) in many different cell types, including platelets (Fig. 3), which are also deficient in dense bodies (121). To understand why giant granules form and some normal vesicles do not, several aspects of CHS cell biology must be addressed.

**Endosomal transport in CHS.** Secretory lysosomes are synthesized normally in CHS cells (122), and both early (recycling) and late (multi-vesicular) endosomes display normal morphology and accessibility to endocytic tracers (123). However, some downstream functions are impaired. For example, peptide loading and antigen presentation, parts of the immunologic process, are delayed in CHS B cells (123), and melanosome, secretory granule, and lysosome release are functionally impaired. Other endocytic functions, such as protein degradation and recycling of transferrin receptors, are not affected.

The characteristic macrolysosomes of CHS cells are acidic, acquire endocytic tracers such as bovine serum albumin-conjugated colloidal gold with normal kinetics (123, 124), and occasionally fuse with the plasma membrane. The lysosomal membrane markers HLA-DM, LAMP-1, LAMP-2, CD63, CD82, and β-hexosaminidase all accumulate in the macrolysosomes, as expected, but these proteins also accumulate at the cell surface in CHS cells (123, 125). The mannose-6-phosphate receptor (MPR), normally excluded from lysosomes, is very abundant in the macrolysosomes in CHS. In contrast, late endosomes

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**Fig. 3** Giant granules (arrows) in CHS cells. A. Neutrophil with several giant lysosomes. (×3300) B. Lymphocyte with a single giant granule. (×3300) C. Eosinophil with several large granules of different electron density. (×2600). D. Platelet showing many normal sized lysosomes and one giant lysosome. (×5500). All micrographs courtesy of Dr. James White, University of Minnesota.
Fig. 4 Hypothetical model of LYST protein function in protein sorting and vesicle formation and trafficking. A. Schematic drawing of the motifs found in the LYST protein. B. Late Golgi processes. Proteins destined to be transported to lysosomes bind to receptor molecules in the trans-Golgi membrane (1). This results in activation of the “VPS15 like” C-terminal portion of LYST (2). VPS15 activation provides a binding site for the VPS34-like PI3K (3), which then translocates to the membrane (4). Since the PI3K can be replaced by another specific PI-kinases in this LYST-mediated process, it is termed PI(x)K. Inset: PI(x)K generates PtdIns(x)P_n (phosphatidylinositol [x]-phosphate) from PtdIns and free phosphate. PtdIns(x)P_n is a source for the supply of DAG (diacylglycerol) to the Golgi complex. DAG is formed by phospholipase C, and activates PKC. C. Early vesicle formation. The C-terminus of LYST activates the specific local phosphorylation of membrane phospholipids, resulting in recruitment of coat proteins (5) and specific guanine nucleotide exchange factors (GEF) required for vesicle formation and transport. The GEFs stimulate activation of members of the rab family of small GTPases (6). Meanwhile, the N-terminal portion of LYST connects to microtubule structures (7). D. Early vesicle movement. The N-terminal portion of LYST “pulls” the vesicle out of the late Golgi membrane towards the microtubule structures (8). Once the vesicle is formed and starts moving along the microtubule, the LYST protein is released from the vesicle (9), probably recycling to the late Golgi for a new round of vesicle formation.
contain a paucity of the normally abundant membrane markers HLA-DR, CD63, and CD82 (116).

Fagile et al. (116) concluded that defective lysosomal fusion with the plasma membrane is not the primary defect in CHS cells. Furthermore, the lack of T-cell and NK cell toxicity in CHS occurs because cytolytic enzymes are missorted and thus absent from the granules, which release these enzymes upon fusion with the plasma membrane. Finally, the giant granules of CHS result from endosomal missorting of proteins involved in the regulation of lysosomal homeostasis.

**Association of LYST with microtubules.** The macrolysosomes of CHS cells are located in the perinuclear region, consistent with a defect in the microtubular network. Furthermore, the LYST protein has homology to the microtubule polymerization protein, stathmin (119, 114), although CHS cells have normal numbers and distribution of microtubules themselves (126-128). We propose that the N-terminus of LYST interacts with microtubules, but truncation of the C-terminus of the LYST protein, which occurs in all human LYST mutations, causes malfunctioning of the microtubular moving system. In this case, the microtubule-associated portion of LYST will remain present in a microtubular distribution.

**PKC activity and granule formation.** Evidence indicates a correlation between decreased protein kinase C (PKC) activity and increased lysosome size. Specific PKC inhibitors such as chelerythrin and calphostin C induce giant granule formation in normal fibroblasts (129), and inhibition of calpain, which inactivates PKC, prevents giant granule formation in beige fibroblasts (129). These findings are important because PKC activity is down-regulated in CHS cells (129-131). Moreover, decreased PKC activity in polymorphonuclear leukocytes (PMNs) and natural killer (NK) cells from beige mice appears responsible for the impaired immune functions in CHS. The down-regulation of PKC activity in CHS cells can be corrected by calpain inhibition (129-130), which also corrects the NK cell dysfunction of beige mice (131).

**Phosphoinositide kinases.** Phosphoinositol-3 kinase (PI3K) plays an important role in the regulation of protein sorting (132). The localized generation of PI-lipids by PI kinases (PI(x)K) acts to target proteins critical for cellular trafficking, such as FYVE proteins (133, 134) to the appropriate location on a membrane that will become a vesicle. PI-lipids also relocate specific guanine nucleotide exchange factors to the vicinity of new vesicle formation. These exchange factors are needed to activate members of the rab family of small GTPases which facilitate the docking of vesicles with target membranes. Inhibition of PI3K with wortmannin impairs transport of membrane proteins from endosomes to lysosomes (135), and causes accumulation of mannose-phosphate receptors in swollen lysosomal compartments of normal fibroblasts (136), an effect similar to that seen in CHS cells.

**Structural characteristics of the LYST protein.** By virtue of its HEAT repeats, ARM repeats, and WD40 motifs, the LYST protein resembles a p150 yeast serine/threonine protein kinase, VPS15 (109). The VPS15 yeast mutant presents as a defect in sorting to the vacuole (137), causing secretion of vacuolar hydrolases. VPS15 regulates VPS34, a PI3 kinase, and in yeast and mammalian cells, VPS34/PI3K is involved in endosomal transport and sorting into late endosomes and lysosomes (138, 139). The human LYST sequence does not contain a bona fide kinase domain (140), but its carboxy terminus does resemble VPS15.

**The Function and Dysfunction of LYST**

We hypothesize that the LYST protein acts as an intermediate that affects lipid-related protein trafficking. In this model, which is based upon the various domains found in LYST (Fig. 4A), the C-terminus functions at the TGN to sort specific proteins pertinent for endosomes or lysosomes (Fig. 4B). The protein sorting is supported by lipid phosphorylation carried out by a VPS34-like PI kinase activated by the VPS15-like domain of the C-terminal region of LYST. The PI kinase stimulates PKC activity indirectly, i.e., via DAG (Fig. 4 inset). Phosphorylation of specific phospholipids in the TGN results in local recruitment of coat proteins required for vesicle formation and transport. The localized generation of PI lipids by PI(x)K also acts as exchange factors which activate members of the small GTPases (Fig. 4C). The GTP/GDP exchange factors interact with the leucine zippers and the HPS1 homology domain of the central part of the LYST protein. Rab GTP hydrolysis energizes vesicle formation or vesicle attachment to LYST.

Meanwhile, the N-terminal portion of LYST binds to microtubules via its stathmin-like domain (Fig. 4C). Once vesicles form, they continue to be transported peripherally along the microtubules, but the very large and sterically hindering LYST protein is released for recycling well before the dendrite is reached (Fig. 4D).

When LYST is mutated, PI(x)K is not activated, and decreased DAG production leads to down regulation of PKC. Pigment forming vesicles and dense bodies fail to form and do not move to the periphery.

The model is supported by the finding of a microtubule association of LYST, by the down regulation of PKC activity, and by the known structural domains of LYST. There is also evidence that rabs play a role in the process. Antisense expression of rab7 or rab9 in HeLa cells results in the formation of large vacuoles, resembling those of CHS fibroblasts (141, 142), and rab7 interacts with VPS34 (143). Finally, the large size of LYST makes it a good candidate to bind to both the TGN and microtubules.

The model does not explicitly explain the formation of giant vesicles in CHS cells. It may be that, absent the controlled, directed formation and peripheral movement of nascent vesicles, proteins accumulate within the TGN until a critical, massive size is reached, whereupon a giant vesicle breaks away. Its size would preclude movement from the perinuclear region. Alternatively, the giant vesicles may represent endosomes formed from the plasma membrane which were intended to fuse with LYST-dependent vesicles derived from the TGN. In the absence of LYST, vital proteins, crucial for maintaining the size and course of the fused vesicles, never reach the endosomes, resulting in their growth to giant granules.

**Where Does HPS Stop and CHS Begin?**

Studies of the clinical spectrum of HPS and the mouse models of combined hypopigmentation and storage pool deficiency provide insight into the relationship between HPS and CHS. HPS-2 disease, which clearly fits into the category of HPS based upon absence of platelet dense bodies and other clinical findings, manifests with neutropenia and a diathesis toward infections. The ashen mouse, now considered a model for HPS because of the recent finding of absent platelet dense bodies (62), remains a model also for Griscelli syndrome, a disorder of pigment dilution and silvery hair plus other clinical findings (64). Griscelli syndrome patients who manifest immunologic defects and hemagocytic syndrome, an uncontrolled T-lymphocyte and macrophage activation syndrome, have their disease due to deficiency of rab27a (63), as for ashen (62). Griscelli syndrome patients who exhibit neurologic impairment instead, have mutations in myosin 5a (144), the gene affected in the mouse dilute (145). (Leaden resemble ashen and dilute but its molecular defect has not been resolved.)
Clearly, the neurologic and immunological symptoms of these patients and mice bring them closer to CHS, and confirm the existence of a continuum of disease. The concept of a gradation of pathology applies not only to clinical manifestations, but to cell biological phenomena as well. HPS defects appear more involved with vesicle formation, while CHS appears due to vesicle transport defects. The Griscelli syndromes fall in between, with rab27a defects (vesicle formation/fusion) resembling HPS and myosin 5a defects (microtubular transport) resembling CHS.

The complete spectrum of disease between HPS and CHS requires some shading. This will be accomplished by the molecular description of HPS patients whose mutations have not yet been defined, and by the isolation of genes responsible for clinical variants. These genes are being sought among those responsible for animal models of the HPS/CHS spectrum, as well as genes involved in the pathways of vesicle formation and trafficking. From our model of the mechanism of LYST action, we predict that specific ras, exchange factors, and vesicle docking and fusion proteins are involved. As the gamut of human disease becomes defined, so will the pathways for the formation of vesicles of lysosomal lineage.

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