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Chédiak-Higashi and Griscelli syndromes

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Chédiak-Higashi syndrome (CHS) and Griscelli syndrome (GS) are two hereditary disorders that associate a pigmentary dilution with an immune impairment, which leads to a similar T-lymphocyte and macrophage activation syndrome called hemophagocytic syndrome (HPS), or the accelerated phase of the disease. This hemophagocytic syndrome is generally fatal if not treated by allogenic bone marrow transplantation and determines the real severity of these conditions. The molecular defects leading to these two disorders have been characterized and were shown to affect the intracellular trafficking of several lysosomal proteins, including the lytic enzymes. Together with the demonstration that HPS that occurs in familial hemophagocytic lymphohistiocytosis originates from a genetically determined perforin defect, these disorders demonstrate the importance of the secretory cytotoxic pathway in the regulation of the immune system.

Features of HPS

The HPS observed in CHS and GS does not differ from that described in familial hemophagocytic lymphohistiocytosis (FHL) and in the X-linked lymphoproliferative syndrome (XLP) [1]. HPS is characterized by lymphoid organ and extranodal infiltration by polyclonal T cells, mostly of the CD8 subset, in association with fever, edema, hepatosplenomegaly, pancytopenia, coagulation abnormalities, and liver dysfunction [2,3]. Involvement of the CNS is frequent, with symptoms ranging from confusion to severe seizures and neurologic impairment (Table 1) [4]. T cells exhibit activation markers (CD25, HLA class II). Activated macrophages that phagocytize blood cells are associated with

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Table 1

Clinical and biologic features of hemophagocytic syndrome (HPS)

Clinical manifestation	Biologic manifestation
High fever	Pancytopenia
Edema	Low fibrinogen level
Hepatosplenomegaly	Hypertriglyceridemia
Neurologic symptoms	Hyponatremia, hypoprotidemia (hemodilution)
Skin rash	Elevated liver enzymes
	Elevated LDH and ferritinemia
	Cerebrospinal fluid pleocytosis
	Defective NK cell activity
	Activated T lymphocytes and macrophages infiltrating various organs (hemophagocytosis)

activated T cells. Markers of T-lymphocyte and macrophage activation in HPS include the following:

T-lymphocyte activation

Expression of HLA Dr⁺, CD25⁺, Fas⁺

High level of soluble CD8 and CD25

High level of interferon γ (IFN γ) secretion

Macrophage activation

High level of interleukin-1 (IL-1), tumor necrosis factor α (TNF α), IL-6, neopterin

Hemophagocytosis

This condition, in most cases, is spontaneously unremitting. In the brain, inflammatory cells form perivascular foci, suggestive of infiltration of blood-derived tissue. Immune-cell infiltration results in massive tissue necrosis, organ failure, and death in the absence of immunosuppressive treatment. Massive cytokine release is another hallmark of the condition, including detection of high plasma levels of IL-1, IL6, TNF α , and IFN γ [5]. It is believed that macrophage activation is the consequence of massive TNF α production leading to hemophagocytosis, high triglyceride levels, and coagulation disorders. In most cases, the HPS is triggered by viral infections, especially those from the herpes group (Epstein-Barr virus, cytomegalovirus). No evidence exists that indicates inappropriate B-cell activation or autoimmune manifestations.

Impairment of granule-dependent cytotoxic activity causes HPS

A perforin deficiency has been identified as an underlying cause of FHL, a hereditary disorder characterized by the occurrence of a HPS early in life [6]. As a consequence of gene mutations, perforin expression barely is detected in patients' cytotoxic granules. Consequently, the cytotoxic activities of T cells and natural killer (NK) cells are impaired, demonstrating for the first time

Table 2

CHS and GS genetic defects in humans and murine counterparts

Disease	Phenotype	Gene
CHS	Partial albinism Giant granulation Defective cytotoxic function HPS	<i>CHS1/LYST</i>
Beige mice	Coat-color dilution Giant granulation Defective cytotoxic function	<i>lyst</i>
Griscelli disease 1	Partial albinism Neurologic impairment	<i>MYOVA</i>
Dilute mice	Coat-color dilution Neurologic involvement	<i>MyoVa</i>
Griscelli disease 2	Partial albinism Defective cytotoxic function HPS	<i>RAB27A</i>
Ashen mice	Coat-color dilution Defective cytotoxic function	<i>Rab27a</i>

in humans the critical role of this cytotoxic pathway in the regulation of lymphocyte homeostasis [6,7]. Similarly, the molecular defects that have been identified as causing a HPS in CHS and GS disrupt the secretory cytotoxic pathway (Table 2).

In CHS, a defect in the lysosomal trafficking regulator (*CHS1/LYST*) [8,9], a large cytosolic protein with no clear homology with other known proteins [10], affects the sorting and trafficking of several lysosomal proteins, among them the lytic enzymes, granzymes, and perforin [11]. As a consequence of *CHS1/LYST* mutation, lytic enzymes accumulate in giant intracellular granules that cannot be released, accounting for the defective secretory cytotoxic activity of T cells and NK cells in patients with CHS.

In GS, mutations in the small GTPase *RAB27A* gene, a regulator of intracellular vesicular traffic, leads to defective cytotoxic granule exocytosis [12]. This defect also impairs secretory cytotoxic activity [12] and is associated with occurrence of HS in this disorder.

Regulation of lymphocyte homeostasis by granule-dependent cytotoxic activity

There are several hypotheses for cytotoxic-granule regulation of T-cell proliferation. In normal situations, in response to a viral infection, antigen-specific cytotoxic T cells (CTLs) expand, secrete soluble mediators (eg, IFN γ) that enhance immunity, and interfere with viral replication, and mediate lysis of infected cells by way of release of cytotoxic granules. These various mechanisms participate in the down-regulation of the immune response. Although T-cell mediated recognition leads to T-cell activation in situations that are characterized

by defective lymphocyte cytotoxic activity, the resulting cells fail to kill the antigen-presenting cells and to remove the source of antigen stimulation. Persistent activation of T cells, which produce large quantities of cytokines (eg, IFN γ), leads to macrophage activation. Sustained macrophage activation results in tissue infiltration and production of high levels of TNF α and other proinflammatory cytokines, which have a major role in the clinical and biologic symptoms and in tissue damage observed in HPS.

Such trans-action of cytotoxic granules is supported by studies that used the perforin knock-out mice model [13–17] and by the observation that following allogeneic stem cell transplantation, the presence of a few donor cells in the recipient is usually sufficient to durably control HPS [18]. Trans-action of cytotoxic activity also may be exerted against neighboring T cells that transiently have acquired MHC-I molecules by way of contact with target cells, leading to fratricidal lysis; however, this mechanism was not proved to be physiologically relevant [19,20]. The hypothesis of a cis-action (suicide) of the secretory cytotoxic pathway following the killing process of the target cells is less likely given the low sensitivity of CTLs to their own lytic enzymes; however, it remains possible that at some stage or after a certain number of divisions, CTLs become sensitive to the action of secreted or intracellular perforin. These various mechanisms are not mutually exclusive and may contribute in the sustained expansion of CD8⁺ lymphocytes and to a lesser extent in the presence of CD4⁺ lymphocytes observed in cytotoxic activity-deficient conditions. In addition to its role in the resistance to infection, cytotoxic activity directly or indirectly functions as a regulator for T-cell activation [7,13,14].

Chédiak-Higashi syndrome

Clinical phenotype and pathologic manifestations

Chédiak-Higashi syndrome is a rare disease in most populations, although a cluster of cases has been described in the Venezuelan Andes [21]. CHS is a complex condition that is inherited as an autosomal recessive disease [22]. Diagnosis of CHS usually is first suspected because of the coexistence of a partial oculocutaneous albinism, accompanied by signs of HPS. In some patients, the diagnosis is considered after the observation of giant intracellular granules as an incidental finding on a peripheral blood smear. Most patients are diagnosed during the first decade of life, but in some patients with mild expression, who frequently were referred for peripheral neuropathy, first diagnosis was made at adult age.

Dermatologic manifestations in CHS include hair color varying from blond to dark but exhibiting a silvery tint, particularly noticeable in strong light. Microscopic examination of hair under oil immersion has revealed the presence of giant melanin granules [23,24]. An increased red reflex, photophobia, and nystagmus in bright light, which represent expressions of the ocular pigmentary dilution,

also can be observed [25,26]. The skin of patients is less pigmented than that of their parents and siblings and is susceptible to severe sunburns. This pigmentary disturbance results from the abnormal aggregation of melanin into giant melanosomes and the inability of the melanosomes to transfer melanin to the adjacent keratinocytes [24,27–29].

Giant intracytoplasmic granulations are the hallmark of this disorder and occur in all granulated cells including hematopoietic cells, melanocytes, fibroblasts, renal tubular cells, neurons, and Schwann cells [23,24,30]. They display endosomal and lysosomal characteristics and appear preferentially distributed to the perinuclear region of the cells [31,32].

Most patients exhibit an increased susceptibility to bacterial infections. T cells and NK cells have defective cytotoxic activity, which results from the inability to secrete cytolytic proteins stored in giant granules [33–36]. Some patients who survive childhood, with or without complications of HPS, often develop neurologic manifestations that can include seizures, mental deficiency, or progressive intellectual decline and peripheral neuropathy [37,38]. It is not clear whether these neurologic manifestations, which are the first recognized manifestation of the disease in some adult patients, are primary or result from brain infiltration during previously undiagnosed HPS.

Animal models

Disorders similar to human CHS occur in many mammalian species, of which the best known and most extensively studied is the beige mouse [39–41]. The phenotype of the beige mouse, long considered to be a homologue of human CHS, corresponds to human CHS in most aspects, except *beige* mutants and no other CHS animal model develop accelerated phases [41]. The *beige* gene was localized to chromosome 13 by traditional mouse breeding studies [42]. The human CHS gene was mapped to chromosome 1q42–q43 in a region syntenic to the beige locus [43,44]. The mouse gene was cloned by complementation of lysosomal morphologic defect using yeast artificial chromosome and by more traditional positional cloning methods. The beige gene *lyst* (for lysosomal trafficking regulator) was used to isolate the human CHS gene, and pathologic mutations in each of the disorders were characterized [8,9,45].

Molecular defects

The human and mouse genes for CHS are enormous (13.5 kilobases [kb]), code for a protein of 3801 amino acids, with a predicted molecular mass of 429,153 kDa; there is 88% homology between the two species' proteins. The mRNA, expressed in all cells, is produced in low levels and is difficult to detect by northern analysis. Several minor, smaller mRNA isoforms, resulting from alternative splicing, have been described, but only the full-length protein isoform seems relevant to CHS, because in some patients, the base changes are present only in the largest transcript [46,47].

Functional aspects

Until recently, the sequence of the gene has not been informative about protein function. The size of the protein makes the protein difficult to study by standard techniques. The CHS1/LYST protein shows no significant homology to any other known protein or protein family; however, it contains several architectural features that may provide clues to its biologic function. Overall, the amino-terminus part of the protein contains a series of α helices called Armadillo (ARM) [48] and Huntington, EF₃, PP₂A regulatory A subunit, TOR (HEAT) repeat motifs believed to mediate membrane associations and vesicle transport [49]. The carboxy-terminal half portion of the CHS1/LYST protein has two domains that are conserved among CHS1/LYST homologues: The beige and CHS (BEACH) domain has several conserved amino acids that define members of the beige and CHS families, but its function is unknown. A second domain is formed by several consecutive so-called “WD40 motifs” that are believed to mediate protein–protein interactions [50,51]. This arrangement of protein motifs also is found in the yeast serine–threonine protein kinase Vps15, which is associated with vacuolar protein trafficking [52]. Although Vps15 is not a homologue of CHS1/LYST, a defect in protein sorting that characterized the Vps15 yeast mutant has been demonstrated in the B lymphocytes of patients with CHS, suggesting a defect in transport from the trans-Golgi network or from early endosomes into late endosomes. This missorting affects MHC class II molecules in particular [53,54]. The *CHS1/LYST* gene may have a role in regulation of organelle fusion and fission, because CHS mutant cells have enlarged, centrally localized lysosomes and melanosomes, whereas cells that overexpress *CHS1/LYST* have abnormally small and peripherally localized lysosomes [10]. These defective functions affect the proper release of the content of cytotoxic granules, the lytic enzymes [11,55], and the membrane expression of the regulatory molecule cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) in T cells [56]. These results support the theory that the CHS1/LYST protein is involved in intracellular trafficking along the secretory pathway.

Mutation analysis

Considering the length of the *CHS1/LYST* gene, mutation analysis in patients with CHS is a difficult task. The mutations that have been reported lead to truncated proteins by nonsense mutation or frameshift mutations [8,9,47,57]. Although this finding reflects a relatively small sample size, it alternatively might indicate that missense mutations in *CHS1/LYST* lead to a different phenotype or to a nonrecognizable phenotype. There is no obvious correlation between the length of the predicted residual CHS1/LYST protein and the severity of the disease. Similar homozygous mutations have been found to be associated with a typical course and less commonly with a milder clinical course, even within a same family [57]. It seems likely that factors other than mutations in the *CHS1/LYST* gene may influence clinical expression of CHS. The tight linkage of the CHS locus to several polymorphic markers on chromosome 1 and the iden-

tification of the *CHS1/LYST* gene allow early prenatal diagnosis on chorionic villus samples.

Griscelli syndrome

Griscelli syndrome is an autosomal recessive genetic disorder that clinically is characterized by partial pigmentary dilution or albinism with silvery gray hair, frequent infections, cellular immunodeficiency, neurologic abnormalities, and fatal outcome caused by an uncontrolled T-lymphocyte and macrophage activation syndrome, the so-called “accelerated phase” of the disorder. GS first was described in 1978 by Griscelli and colleagues in two patients [58]. Since then, more than 40 cases have been described in the literature [12,59–69]. GS is rare in almost all populations, although most reported cases are from the Turkish and Mediterranean populations. The age at diagnosis of 20 reported cases of patient with GS ranges from 1 month to 8 years, with a mean of 17.5 months. Both genders equally are affected.

Clinical and pathologic manifestations

Albinism

The most consistent cutaneous expression of albinism in patients with GS is a silvery gray sheen to their hair [12,64]. This symptom is more obvious in patients with black hair but is visible in patients with blond hair. Patients generally have lighter hair than their unaffected family members. Hyperpigmentation in sun-exposed areas has been noted in a few cases. Hypopigmented spots on the retinas have been described [64]. Light-microscopic examinations and electron microscopic findings of hair and skin are characteristic. Hair shafts contain a typical pattern of uneven accumulation of large pigment granules instead of the homogeneous distribution of small pigment granules in normal hair. Fontana-stained silver sections of the skin show hyperpigmented melanocytes, contrasting the poorly pigmented adjacent keratinocytes, instead of the homogeneous distribution of melanin granules that are observed in melanocytes and in surrounding keratinocytes in a normal epidermis. Electron microscopy shows that the cytoplasm of melanocytes is filled with numerous mature, stage IV melanosomes, predominantly around the nucleus, but has normal dendritic processes [58,59,64,70]. These findings are observed constantly and are identical in both groups of patients (ie, patients with *MYOVA* or with *RAB27A* mutations).

Immunologic features

Most patients develop recurrent HPS (or accelerated phases) with features that are identical to those in CHS, FHL, and XLP (described earlier). Immunologic abnormalities are restricted to the patients with the *RAB27A* defect. The capacity of lymphocytes and NK cells to lyse target cells is impaired or absent in these patients. This decrease in T- and NK cell cytotoxicity, which results from an

inability to secrete cytotoxic granules when *RAB27A* is not functional, is a consistent feature in these patients [12]. Patients have normal numbers of T, B, and NK cells and normal B and neutrophil function, although decreased chemiluminescence and chemotactic responses have been reported [64]. The lymphocytes proliferate in vitro in response to phytohemagglutinin, purified protein derivative, or candida, and in mixed lymphocyte reaction. No immunologic abnormalities have been observed in patients with GS and myosin-Va defects; T lymphocytes and NK cells from these patients exhibit a normal cytotoxic activity. The myosin-Va defect does not affect cytotoxic granule secretion, and these patients do not develop an accelerated phase [12].

Neurologic features

In GS, neurologic manifestations are frequent and are observed in association with immunologic features or in isolation. In association with a HPS, patients frequently develop progressive symptoms caused by activated lymphocytes and macrophages that infiltrate the CNS [59,63,68], a development that is similar to that in patients with FHL. Manifestations include hyperreflexia, seizures, signs of intracranial hypertension (vomiting, altered consciousness), regression of developmental milestones, hypertonia, nystagmus, and ataxia. Psychomotor development is normal at onset, and regression of the signs, in part, can be observed during the remission phase, although some sequelae may be irreversible. Neurologic manifestation may be the first sign of an accelerated phase. In contrast, in a few patients, severe neurologic symptoms are isolated, are noticeable since birth without any sign of accelerated phase, and consist of hypotonia, absence of coordinated voluntary movements, and severe psychomotor developmental delay [71], similar to the neurologic signs in the syndrome described by Elejalde [72]. The CNS disorder is static and does not improve with time. These two types of neurologic expression rely on two different genetic defects. Similarly, cranial CT scan and MR imaging are different in the two groups of patients with GS. Isolated congenital cerebellar atrophy was observed in one studied patient with the myosin-Va defect [71]. In contrast, in patients with *RAB27A* mutations, cerebellar hypodense areas, ventricular dilatation, or hyperdense areas compatible with inflammatory changes were observed. Further findings included generalized changes in white matter and periventricular calcifications [62, 63,68]. Biopsy specimens showed cerebral lymphohistiocytic infiltration with occasional erythrophagocytosis.

Molecular basis

Albinism in GS has some phenotypic resemblance to a murine mutant, the dilute mouse (*d*), which results from defects of myosin Va [73], an atypical myosin that acts as a molecular motor involved in transport of melanosomes and other cytoplasmic organelles [74]. Genetic linkage analysis to candidate regions of the human genome that are believed to possibly be homologous to the murine dilute region was performed in several families with GS. This study localized the

candidate gene on chromosome 15q21 in a region that contains the human gene for myosin Va (*MYOVA*). Mutations in several patients with GS were identified in this gene, demonstrating the relationship of myosin Va to this disorder; however, in other patients with GS, myosin Va normally was expressed and no mutation was detected in the coding sequence of the gene, even in the alternatively spliced region for which exon–intron boundaries were characterized. Further linkage analysis that was performed in these families with GS confirmed the original localization to 15q21, but fine haplotype analysis defined a second locus for GS that is adjacent to the *MYOVA* gene (Fig. 1) [66]. The *RAB27A* gene, a member of the Rab GTPase family which is recognized as a key player in vesicular transport and organelle dynamics, is found in this genetic region. Mutations in *RAB27A* were detected in all patients with GS who have been analyzed so far who did not display *MYOVA* mutations [12]. Ashen mutant mice were shown to be homologous to this form of GS, although this mutant spontaneously does not develop the accelerated phase of the disease [75]. Mutations in *MYOVA* or *RAB27A*, two genes that are separated by less than 1.6 centimorgans at 15q21, can lead to GS (see Table 2 and Fig. 1).

Functional aspects

Myosin Va is an unconventional myosin heavy chain that is implicated in vesicle transport in cells and is abundant in neurons and melanocytes. It has the

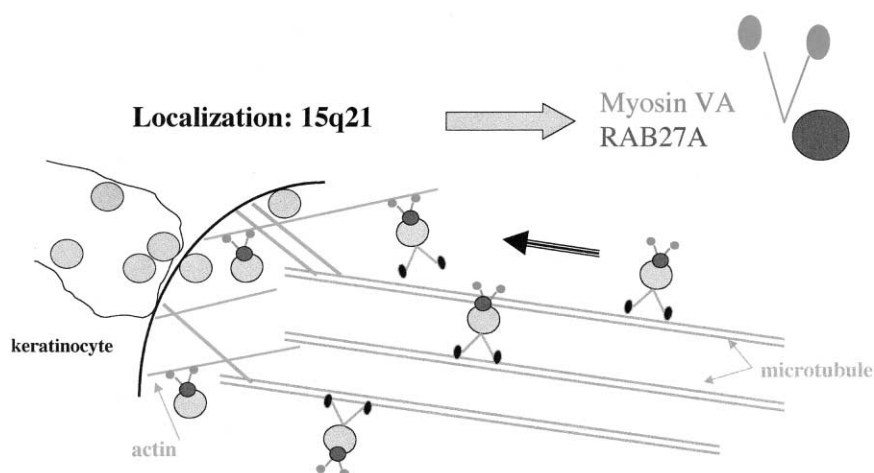


Fig. 1. Melanosome transport in melanocytes. Melanosomes move along microtubules using specific microtubule motor proteins (such as kinesin), moving from the perinuclear region to the periphery of the cell. At the periphery, melanosomes shift from microtubules to actin filaments by way of the molecular motor, myosin Va. This association requires the presence of the RAB27A protein. This step retains melanosomes at the periphery of the cell, where they can be transferred to adjacent keratinocytes. Without functional myosin Va or RAB27A, the melanosomes are transported back to the perinuclear region of the melanocytes. In cytotoxic cells, RAB27A, but not myosin Va, is required for peripheral transport and secretion of cytotoxic granules.

expected structure for a member of this family (ie, a globular head domain containing the ATP- and actin-binding sites; a neck domain, which is the site of calmodulin [light-chain] binding; and a tail domain, which is believed to represent the cargo-binding domain). Myosin Va acts as a dimer and moves cargo along actin filaments in a plus-end-directed manner, allowing the capture and accumulation of the cargo at the periphery of the cells (see Fig. 1). There is evidence of a role for myosin Va in structural and functional cross-talk between the actin- and microtubule-based cytoskeletons. Myosin Va binds to melanosomes in melanocytes [76] and to synaptic vesicles in nerve terminals [77]. Studies of dilute mice showed that the defect in myosin Va leads to the concentration of melanosomes in the center of the melanocytes; however, in wild-type mice, the melanosomes were concentrated in the dendrites and dendritic tips of the melanocytes. This defect also impairs the processing of presynaptic vesicles in the peripheral regions of neurons and the distribution of smooth endoplasmic reticulum in Purkinje cell neurons [70].

RAB27A is expressed in melanocytes, peripheral leukocytes, platelets, and other cells and tissue types, except in brain cells [78]. Each member of the Rab protein family has characteristic intracellular distribution pattern, suggesting a unique function in transport. Similar to myosin Va, RAB27A colocalizes in part with melanosomes in melanocytes (see Fig. 1), and its defect leads to the abnormal melanosome distribution observed in GS [79]. RAB27A was shown to be necessary for cytotoxic granule exocytosis and cytotoxic activity of T and NK cells, which were shown to be defective in this group of patients [12]. In GS, *MYOVA* and *RAB27A* mutations lead to the same defective transport of melanosomes, which are not transferred properly to surrounding keratinocytes, accounting for the pigmentary dilution characteristic of this disorder. Pigmentary dilution is indistinguishable between these two molecular defects; however, because of the role of myosin-Va in brain tissue, only patients with the myosin-Va defect develop a primary and severe neurologic impairment without immunologic expression. In contrast, defective RAB27A expression always is associated with abnormal lymphocyte cytotoxic activity, which results in a lymphoproliferative syndrome. Neurologic signs in patients with *RAB27A* mutations are the consequence of an accelerated phase and seem secondary to perivascular lymphohistiocytic organ infiltration.

Mutation analysis

The *RAB27A* gene comprises six exons; only exons two to six are translated [78]. Ten different mutations have been reported in 16 independent families [12]. Three single, homozygous, missense mutations were identified: a W73G transition that lies within the conserved GTP-binding motif; and L130P and A152P transitions that affect residues that are highly conserved among Rab proteins. The other, nonsense mutations (deletions or splicing-site alterations) predict early protein truncation. In each case, location of the stop codon predicts a truncation of the consensus carboxy-terminal motif of the protein involved in

Rab protein geranylgeranylation and should leave the RAB27A protein in an inactive state.

The *MYOVA* gene contains an open reading frame of 1855 codons that encodes a protein of 215kD [73]. MyosinVa is a member of the molecular motor molecules with structurally conserved heads followed by a neck domain, tail region and globular C-terminal domain [80]. Alternatively spliced transcripts can be generated within the tail region of myosin Va [66,81,82]. Four different mutations in *MYOVA* have been identified, each of which lead to a predicted truncated protein. One patient was homozygous for a C2332T transition in codon 779, resulting in a nonsense mutation that predicted a protein that lacked the last 1078 residues. A second patient had a homozygous insertion of 47 bp at position 4634 (residue 1545), which predicted a protein that lacked most of the globular domain. A third patient was heterozygous for a 1-bp deletion at codon 4950 and a C2893T transition in codon 1650 and 965, respectively, each leading to early protein truncation (G. de Saint Basile, unpublished data).

As discussed earlier, there is a strict correlation between the type of gene affected (*RAB27A* or *MYOVA*) and the phenotype displayed by patients with GS. Although pigmentary dilution is identical in these two groups of patients, only patients with *RAB27A* mutations have a decreased cytotoxic activity resulting in the development of a HPS, whereas primary, severe, and irreversible neurologic impairment characterized patients with *MYOVA* mutations [12]. These data provide information on the essential role of each of these proteins regarding the tissues that are affected by their anomalies.

Animal models

Two naturally occurring mutants in mice, the dilute (*d*) mice and the ashen (*ash*) mice, are the ortholog for the two forms of GS, which result from myosin-Va and *Rab27a* deficiencies, respectively (see Table 2). Both murine mutants present with identical pigmentary dilution that is characterized by normal synthesis of pigment granules but abnormal melanosome transport (the melanosomes are localized mainly in the perinuclear region of the melanocytes) [71,76,83]. Dilute null mice also show a neurologic defect that is characterized by opisthotonus and ataxia, resulting in death 2 to 3 weeks after birth [70,84]. The neurologic defect is believed to result from the failure to transport smooth endoplasmic reticulum from the dendritic shaft to the dendritic spines of cerebellar Purkinje cells, which probably affects the regulation of intracellular Ca^{2+} and the postsynaptic signal transduction. The retroviral integration into the *d* gene in mice (*dv*) allowed the gene's cloning and the demonstration that it encodes an unconventional myosin heavy chain, myosin Va, expressed in nearly all tissues in the adult [73,85]. Neurologic impairment and pigmentary dilution can vary with the type of myosin-Va defect [81]. Murine and human myosin Va display the same dimeric structure function and share 93% homology.

In contrast, ashen mice exhibit the same pigmentary dilution as dilute mice, but in association with a defective cytotoxic activity [86,87]. As in GS, this

defective function results from the role of Rab27a in cytotoxic granule exocytosis. Ashen mice also have been described with increased bleeding times and a reduced number of platelet dense granules [75]. Meiotic and physical linkage allowed the mapping of an ashen-critical region on murine chromosome 9 that is tightly associated with dilute locus. A splicing defect, which results from a single transversion in exon 4 donor splice site, activation of a cryptic downstream sites, and abnormal Rab27a transcript, was identified in these mice [75]; however, no signs of an accelerated phase (a condition that may require viral challenge) have been observed in ashen mice.

Strategies for diagnosis of CHS and GS

The diagnosis of CHS and GS must be considered in children with decreased pigmentation accompanied by neurologic abnormalities or signs of the accelerated phase, such as hepatosplenomegaly, jaundice, or pancytopenia. Microscopic examination of the hair shaft provides strong support for the diagnosis of these syndromes and allows one to distinguish between these two disorders. In CHS, the clusters of pigment are smaller than those in GS, although they remain abnormal. A hallmark of CHS is the presence of giant organelles-inclusion bodies in almost all granulated cells, which are not observed in GS. In GS, neurologic symptoms and analysis of the cytotoxic activity of lymphocytes allow the distinction of the two molecular causes that lead to this disorder. Confirmation can be provided by mutation analysis of patient DNA.

The partial albinism with immunodeficiency (PAID) syndrome is similar to GS but, with progressive demyelination of white matter in the brain, has been described in eight Saudi Arabian kindred [62]. Genetic analysis in one family with PAID syndrome identified a mutation in the *RAB27A* gene, indicating that PAID syndrome and GS probably correspond to the same disorder (G. de Saint Basile, unpublished data). Similarly, Elejalde syndrome, or neuroectodermal melanolyosomal syndrome, was described with the same albinism features as those in GS associated with early onset of severe CNS disorder, without signs and symptoms of accelerated phase [72]. This exact phenotype is displayed by four unrelated patients with GS and myosin-Va defect [66,71] (G. de Saint Basile, unpublished data), strongly suggesting that Elejalde syndrome and this form of GS are allelic.

In familial hemophagocytic lymphohistiocytosis, XLP, and virus-associated HPS, patients develop an accelerated phase that is identical to that observed in GS or CHS, and NK cytotoxic activity often is decreased or absent [88]; however, these disorders do not involve a partial albinism, and microscopic examination of the hair shaft allows differentiation of GS and CHS from these disorders.

Treatment and prognosis

The prognosis for long-term survival in CHS and GS caused by a *RAB27A* mutation is relatively poor. These disorders usually are rapidly fatal during the

accelerated phase of the disease. A number of treatments, including cytotoxic agents, initially were tried with moderate or no effect, until the use of the epipodophyllotoxin derivative etoposide (VP-16) was introduced, which is effective in most cases [89–93]. Immunotherapy using antithymocyte globulins and cyclosporin A also has achieved remissions [94,95]; however, chemotherapy or immunosuppression is sometimes ineffective and frequently fails to control relapses. Allogenic bone marrow transplantation remains the only curative treatment in this disease [96]. This treatment corrects the cytotoxic defect and prevents further accelerated phases. Partial engraftment is compatible with long-term remission, and only a small proportion of the donor cells in the patient is sufficient to prevent reactivation of the accelerated phase in most cases [18].

No specific treatment can be proposed for myosin-Va deficiency. The severe neurologic impairment and psychomotor delay do not improve with time. Recurrent infections in some of these patients were treated easily with positional changes and antibiotics.

Summary

The molecular defects that are responsible for CHS and GS affect proteins that are involved in intracellular trafficking. CHS1/LYST, RAB27A and MYOXA protein anomalies result in defective melanosome transport and melanin transfer to adjacent keratinocytes, accounting for the characteristic hypopigmentation observed in these disorders. CHS1/LYST and RAB27A proteins are necessary for proper sorting and secretion of cytotoxic granule content, which is the resulting defective lytic function that accounts for the occurrence of HPSs and determines the severity of these disorders. In contrast, MYOXA seems dispensable for secretory cytotoxic functions but not for the development and function of neurologic tissues. Secretory and nonsecretory functions of lymphocytes are key mechanisms in the regulation of lymphocyte homeostasis. It is tempting to speculate that the secretory cytotoxic pathway will be impaired in other cases of yet unexplained HPSs. Acquired forms of HPS can be observed during the course of viral infection or autoimmune diseases. The possibility that viral proteins transiently may interfere with the cytotoxic pathway to limit the pathway's effect, which already has been observed in the intracellular transport of proteins, is an attractive hypothesis.

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