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Hemophagocytic lymphohistiocytosis

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Hemophagocytic lymphohistiocytosis (HLH), although rare, is prominent among the genetically determined human immunologic disorders resulting from defects in the regulatory pathways that normally maintain homeostasis within the immune system and ensure the natural termination of effector immune responses. The dramatic clinical presentation of multisystemic inflammation and rapidly fatal course of untreated HLH have earned the respect of specialists in hematology, infectious diseases, and immunology who have had personal experience with affected patients. Although HLH remains a daunting clinical challenge, the disorder affords immunologists and geneticists the opportunity to discover the factors and pathways critical to the normal genetic program for down-regulation of immune inflammatory processes.

During the past decade, clinicians and scientists who participated in the international Histiocyte Society and representatives of the family organization, Histiocytosis Association of America, have devoted considerable efforts to raising awareness about the diagnosis and treatment of HLH among physicians and other medical practitioners worldwide. Funds raised by the family organizations have supported small grants that have led to significant breakthroughs in the rapid diagnosis and definitive treatment of HLH during the past decade.

Overview of histiocytic disorders

The histiocytoses are a heterogeneous group of disorders resulting from abnormal activation and proliferation of dendritic cells and monocyte and macrophage derivatives, historically referred to as histiocytes.

Normal functions of histiocytes, which are a major component population of the innate immune system, include phagocytosis, antigen presentation, and

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activation of the adaptive immune system through contact and cytokine signaling. Histiocytes normally participate in wound and tissue repair and maintenance and regulation of inflammation.

The classification of histiocytic disorders that is used most widely was proposed by the Histiocyte Society in 1987 and was refined in a 1998 publication [1]. The two most commonly diagnosed disorders are (1) Langerhans' cell histiocytosis (LCH) and (2) HLH.

Langerhans' cell histiocytosis is believed to occur at least five times more frequently than HLH does. LCH is a highly variable disorder characterized by the proliferation of activated Langerhans' dendritic cells (LCs), which can be clonal or oligoclonal in nature. LCH is not considered a malignant disorder. LCs are normally resident in tissues such as the skin. LC infiltrates can be diagnosed by the findings of cell-surface expression of CD1a antigen, positive S100 histochemical staining, and identification of cytoplasmic Birbeck granules (characteristic cytoplasmic membrane infoldings) by electron microscopy. Symptomatic disease is caused by local and distant consequences of chronic and abnormal cytokine release by the LCs and other bystander cells. LCH may be localized to a patch of skin or single bony lesion, or it may have systemic manifestations. Historical terms include eosinophilic granuloma of bone (unifocal LCH), Hand-Schüller-Christian disease (multifocal LCH), Letterer-Siwe disease (systemic LCH), and histiocytosis X.

An estimated 4 to 5 million persons are diagnosed with LCH each year. Twice as many men as women are diagnosed, with a peak age at onset of 1 year. LCH is distributed worldwide. Multifocal and systemic LCH more commonly is seen in patients younger than 5 years, whereas isolated bone lesions and skin disease often have a later onset in childhood. LCH can present in adulthood and can involve the lungs and central nervous system (CNS) in protean manifestations. Familial LCH is rare, occurring in approximately 2% of cases. A genetic predisposition to LCH has been proposed based on the higher than expected association with subsequent hematologic malignancy and on reports of cases in which LCH and HLH have been documented in the same individual [2].

Hemophagocytic lymphohistiocytosis encompasses several entities, including the primary or familial form and the secondary forms associated with infections or malignancies. In all its forms, HLH is believed to be a reactive process that results from hyperactivation, proliferation, and metastatic migration of macrophages and type 1 T cells.

Malignant histiocytoses, the third distinct category of histiocytic disorders, are represented by true neoplasms of monocytes and dendritic cells, typically accompanied by cytogenetic abnormalities.

Clinical features of HLH

Farquhar and Claireux, at the University of Edinburgh, are often credited with the first published description of the lethal hereditary syndrome in children,

which they named familial hemophagocytic reticulosis in 1952 [3]. They reported that a brother and sister, both healthy at birth, had developed unexplained fevers, “progressive panhematopenia,” hepatosplenomegaly, and terminal bruising at 9 weeks of age. Autopsies revealed “inflammatory cell infiltration . . . mainly lymphocytes and plasma cells” in the liver and a “highly reactive marrow” in the brother. In the sister’s spleen and other tissues, “a great proliferation of histiocytes” was found. The authors stated that “many of these [histiocytes] showed erythrophagocytosis and others contained lymphocytes and polymorphs.” This report accurately identified the key clinical, laboratory, and histologic criteria that still serve as the diagnostic guidelines for HLH as adopted by the international Histiocyte Society [4].

Hemophagocytic lymphohistiocytosis has been subdivided into two categories: (1) primary, or genetically determined, and (2) secondary, or acquired. Assignment to the autosomal recessive form, often referred to as FHL or FHLH, synonymous with familial erythrophagocytic lymphohistiocytosis, usually requires a family history of the disease, parental consanguinity, or both. FHL has an estimated incidence of 1 out of 50,000 live births and equal gender distribution [1]. Symptoms of FHL are usually evident within the first 2 months of age but can develop in utero. Most patients demonstrate a specific defect of cellular cytotoxicity in which natural killer (NK) cell function is absent [5]. In 1999, the first report appeared that linked mutations in the perforin gene (*PRF1*, which encodes the perforin protein required for normal NK cell function) with the pathogenesis of HLH (see later in article) [6]. Approximately one quarter of cases of FHL in North America can be attributed to biallelic mutations in the *PRF1* gene, similar to results reported from Europe [7]. HLH caused by *PRF1* mutations can have a delayed and unusual symptomatic onset that is as late as the third decade, and affected individuals within the same family may not develop evidence of disease at similar ages [8]. The genes responsible for the remaining approximately 75% of familial cases remain to be described.

Secondary HLH more often is associated with an identifiable infectious episode, particularly involving the herpesvirus group, and tends to occur in older children, adolescents [9], and patients with autoimmune diagnoses, such as rheumatologic disorders, who have been treated with immunosuppressive agents [10,11]. The earliest attribution of acquired hemophagocytic syndrome is the publication by Scott and co-workers in 1939 [12]. Examples of secondary HLH include Epstein-Barr virus (EBV)-associated HLH, more commonly diagnosed in Asians than in children of whites or blacks [13], and the so-called “macrophage activation syndrome” associated with systemic onset juvenile rheumatoid arthritis (JRA) [10]. In some of these cases, secondary HLH seems to be a self-limited complication from which the patient is able to fully recover after receiving only supportive medical treatment such as intravenous immunoglobulin (IVIg); however, long-term remission without the use of cytotoxic and immune suppressive therapies is highly unlikely in adults with HLH and in patients with involvement of the CNS [14].

Highly lethal hemophagocytic syndromes, frequently triggered by exposure to EBV, occur in several other genetic immune disorders, including X-linked lymphoproliferative syndrome (XLP) [15], Chédiak-Higashi syndrome (CHS) [16], and Griscelli syndrome (GS) [17] (described later in article). Findings of hemophagocytosis can occur in conjunction with certain T-cell lymphomas and resolve with effective antineoplastic treatment [11].

Signs and symptoms of HLH

Despite attempts to differentiate primary cases from secondary cases of HLH, the symptomatic presentations are difficult to distinguish, and familial HLH often is associated with documented infection.

In the most typical form of HLH, the clinical course is characterized by prolonged fever and hepatosplenomegaly. Neurologic symptoms can complicate and sometimes dominate the clinical course. Rash and lymphadenopathy are observed less frequently (Table 1). In the early days of the disease, symptoms, especially fever and rash, can wax and wane spontaneously.

Although CNS involvement may be present at the time of initial clinical presentation, it develops later in the course of the disease in many patients; thus, monitoring for CNS disease must continue throughout treatment [18]. Up to 60% of patients with the familial form of HLH have CNS involvement at some point, especially if definitive treatment with hematopoietic stem-cell transplantation is delayed. Evidence for CNS involvement can range from nonspecific symptoms, such as irritability or lethargy, hypotonia or hypertonia, to seizures, ataxia, and cranial nerve involvement presenting as nerve palsy and blindness. In the most severe cases, hemiplegia, tetraplegia, and increased intracranial pressure and coma have been seen. Most of these abnormalities are reversible if appropriate anti-inflammatory treatment is instituted rapidly. Examination of the spinal fluid reveals pleocytosis with a predominance of lymphocytes and monocytes. Hemophagocytosis may be evident on microscopic examination of spun cerebrospinal fluid (CSF). CSF protein usually is elevated. MR imaging of the brain is recommended, because it is a more sensitive technique than is CT scanning for identification of areas of inflammation and demyelination, which can occur

Table 1
Initial clinical findings in familial HLH

	Literature review [51] (n = 121)	Population-based study [52] (n = 32)	Familial HLH registry [48] (n = 122)
Fever	98/108 (91%)	29/32 (91)(100%) ^a	114/12 (93%)
Splenomegaly	98/100 (98%)	27/32 (84)(100%) ^a	119/122 (97%)
Hepatomegaly	94/100 (94%)	28/31 (90)(97%) ^a	—
Lymph node enlargement	17/100 (17%)	13/31 (42)(52%) ^a	39/122 (31%)
Skin rash	7/108 (6%)	13/30 (43)(65%) ^a	30/122 (24%)
Neurological abnormalities	(20%) ^a	6/30 (20)(47%) ^a	—

^a Symptoms found at onset or at any time in the disease.

Table 2
Initial laboratory findings of familial HLH

	Literature review [51] (n = 121)	Population-based study [52] (n = 32)	Familial HLH registry [48] (n = 122)
Anemia	93/104 (89%)	23/31 (71%)(94%) ^a	—
Thrombocytopenia ($< 100 \times 109/L$)	77/94 (82%)	26/28 (93%)(100%) ^a	104/122 (85%)
Neutropenia ($< 1 \times 109/L$)	56/96 (58%)	19/28 (68%)(87%) ^a	73/122 (59%)
Leukopenia ($< 4 \times 109/L$)	38/98 (39%)	16/28 (57%)(87%) ^a	—
Hypertriglyceridemia	25/30 (83%)	4/5 (80%)(100%) ^a	78/97 (80%)
Hypofibrinogenemia	26/35 (74%)	3/3 (100%)(85%) ^a	66/101 (65%)
Elevated alanine aminotransferase	About one third 12/20 (60%)(92%) ^a		55/104 (53%)
Elevated bilirubin	About one third 6/15 (50%)(74%) ^a		—
Hyponatremia	Frequent	8/15 (53%)(79%) ^a	—
CSF pleocytosis	17/33 (52%)	6/10 (60%)(91%) ^a	—

^a Symptoms found at onset or at any time in the disease.

throughout the brain during the early phases of CNS involvement. Generalized cerebral atrophy may be apparent before the start of therapy in patients with HLH and tends to become more pronounced with prolonged use of dexamethasone (Decadron). When CNS involvement persists, bleeding and gliosis can occur. Many radiographic findings are reversible when HLH is controlled with effective systemic or intrathecal therapy early in the course.

The most readily identified laboratory findings based on blood testing are cytopenias, especially anemia, thrombocytopenia, and liver dysfunction, including elevated transaminases, hyperbilirubinemia, hypofibrinogenemia, hypertriglyceridemia, and hyponatremia (Table 2). When tested, the levels of ferritin and lactate dehydrogenase are markedly elevated [19], as are circulating levels of proinflammatory cytokines: tumor necrosis factor- α (TNF α), interferon- γ (IFN γ), interleukin-1 (IL-1), IL-6, IL-2 receptors (IL-2Rs) [20], and IL-18 [21]; these findings reflect the intensity of ongoing immune activation.

Histopathologic findings are consistent with the chronic activation of antigen-presenting cells (macrophages, histiocytes) and proliferation of T cells. Hemophagocytosis (Fig. 1)—the hallmark finding in HLH—may not be apparent on biopsy examination of bone marrow early in the course of the disease. If all other clinical criteria are met, however, therapy should be initiated. Hemophagocytosis may also be observed in biopsies of the lymph nodes, spleen, and spinal fluid. In the liver, hemophagocytosis is seen less often, but lymphocytic infiltration of the portal areas typically is observed. The guidelines for clinical diagnosis of HLH adopted by the Histiocyte Society are as follows [1]:

Clinical criteria

Fever

Splenomegaly

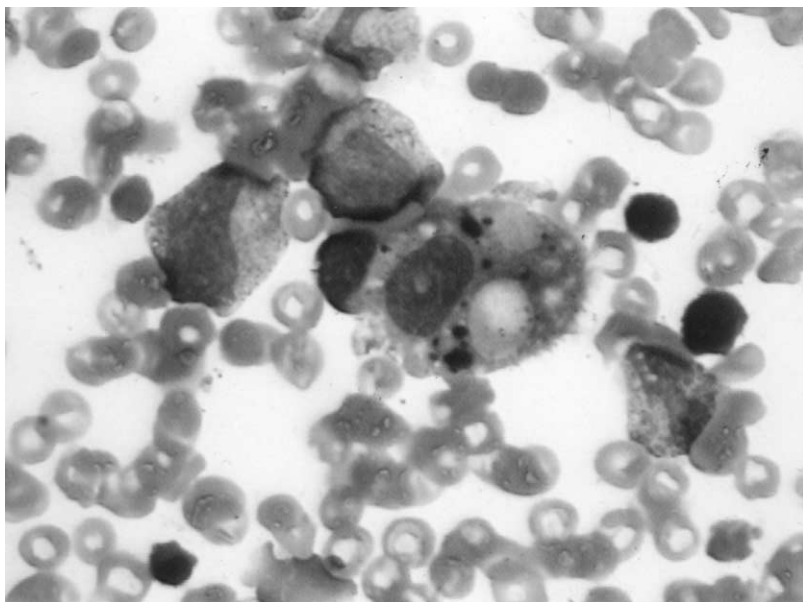


Fig. 1. A typical example of a hemophagocytosing histiocyte viewed microscopically in a bone marrow smear from a patient with HLH.

Laboratory criteria

Cytopenias (affecting two or more of three lineages in the peripheral blood):

Hemoglobin (<90 g/L)

Platelets ($<100 \times 10^9/L$)

Neutrophils ($<1.0 \times 10^9/L$)

Hypertriglyceridemia or hypofibrinogenemia (fasting triglycerides, ≥ 2.0 mmol/L or >3 SD of the normal value for age; fibrinogen ≤ 1.5 g/L or ≤ 3 SD)

Histopathologic criteria

Hemophagocytosis in bone marrow, spleen or lymph nodes

No evidence of malignancy

Notes:

All criteria are required for the diagnosis of HLH. The diagnosis of familial HLH is justified by a positive family history, and parental consanguinity is suggestive.

Comments:

1. If hemophagocytic activity is not proven at the time of presentation, further search for hemophagocytic activity is encouraged. If the bone marrow specimen is not conclusive, material can be obtained from other organs. Serial marrow aspirates over time also can be helpful.

2. The following findings may provide supportive evidence for the diagnosis:
 - a. Spinal fluid pleocytosis (mononuclear cells)
 - b. Histologic picture of the liver resembling chronic, persistent hepatitis (biopsy)
 - c. Low NK cell activity
3. Other abnormal clinical laboratory findings consistent with the diagnosis are: cerebromeningeal symptoms, lymph node enlargement, jaundice, edema, skin rash, hepatic enzyme abnormalities, hyperferritinemia, hypoproteinemia, hyponatremia, elevated spinal fluid protein, elevated very low density lipoprotein, decreased high density lipoprotein, elevated circulating soluble IL-2R.

Immunologic findings

Immunologic testing focused on assessment of NK cell function can assist in the diagnosis of HLH. In most cases of familial HLH, NK cell function in affected individuals is undetectable or markedly decreased, although the number of circulating NK cells ($CD56^{+}/16^{+}$) are generally within normal limits [6]. Absent NK cell function is a persistent finding in these patients. It can be detected before and during active disease and after remission following chemotherapy. In approximately one quarter of cases, absent NK cell function is the result of mutations in the perforin (*PRF1*) genes. In patients with *PRF1* mutations, all cytotoxic cell populations, including CD4 and CD8 T cells, are affected, although FAS-mediated killing can occur [22].

Patients with familial HLH that is not caused by mutations in *PRF1* also show decreased NK cell function, which is usually a permanent feature of their disorder before bone marrow transplantation. These patients usually have normal numbers of circulating NK cells, but patients with onset of HLH in infancy may lack NK cells in their peripheral blood. Parents of children with HLH and siblings who are presumed heterozygous carriers of HLH often demonstrate decreased NK cell function when they are tested while healthy [23]. Decreased NK cell function in a sibling of a patient with HLH is not a reliable means of presymptomatic diagnosis.

In patients with secondary EBV-associated HLH studied in North America, NK cell function often is decreased during the acute phase of symptomatic disease, coincident with a near total disappearance of circulating NK cells; function typically recovers after HLH resolves spontaneously or after chemotherapy, when the proportion of circulating NK cells normalizes (Kogawa, MD, Filipovich, MD, personal communication). It is not known whether the same is true for cases of EBV-associated HLH in Asia, where HLH has been associated with EBV infection of T cells [24], and clonal T-cell proliferation is suggestive of an EBV-driven neoplastic process [25].

Table 3

Comparison of NK cell numbers and NK cytotoxicity between patients with different HLH phenotypes and normal pediatric controls

	Primary HLH (n = 8) ^a	Primary HLH (n = 7) ^b	EBV-associated HLH (n = 5)	Normal control range
NK cells (CD56 ⁺ /16 ⁺ cells) (%)	4.2 (0–9)	12.2 (1–39)	1.5 (0–3)	4–21
NK function (lytic units)	0.05 (0–0.4)	0.06 (0–0.4)	1.59 (0–3.6)	>3.2

Abbreviations: EBV, Epstein-Barr virus; HLH, hemophagocytic lymphohistiocytosis; NK, natural killer.

^a Patients with biallelic pathologic mutations in the *PRF1* genes.

^b Patients with normal *PRF1* genes.

A summary of findings related to NK cell number and function in a group of children who were studied prospectively by the North American reference laboratory for the HLH-94 protocol (Children's Hospital Medical Center, Cincinnati, OH) is shown in Table 3.

A rapid screening assay using flow cytometry for detection of HLH cases caused by mutations in *PRF1* has been developed [22]. Subsequent molecular confirmation by *PRF1* gene sequencing indicates that this approach is highly reliable. Using four-color immunofluorescent staining, the proportions of NK and other T-cell populations expressing intracellular perforin can be quantitated. Normal, age-related ranges have been established to analyze results from patients and family members. The percentages of perforin-positive NK cells do not vary much with age and are usually greater than 85%. In contrast, proportions of CD8 T cells that express perforin are low at birth and gradually increase with age, concordant with the rising proportion of CD28-CD8⁺ T cells—the effector cytotoxic population [22]. Fig. 2 shows characteristic histogram patterns obtained from a normal subject, patients with HLH and complete and partial perforin defects, a heterozygote carrier of a perforin mutation, and a patient with EBV-associated HLH. Table 4 summarizes the results of perforin analyses of NK and T cells in patients with primary and EBV-associated HLH.

Results of mutational analyses in North American families with HLH caused by perforin defects suggest that approximately half of affected patients carry homozygous mutations 50delT (amino acid change Leu17X), resulting in

Fig. 2. Sample histograms of perforin staining in cytotoxic cells. (a) Normal adult control. A normal percentage of perforin expression in NK cells in adults is 92% ± 6%; for CD8⁺ T cells: range, 8% to 28%; for CD56⁺ T cells: range, 30% to 77%. (b) Complete perforin deficiency in a 10-year-old patient with primary HLH (116 C>A; 445 G>A). The perforin staining pattern showed no difference from the isotype control in all cell types. (c) Partial perforin deficiency in a patient with primary HLH (133 G>A; 160 C>T). Perforin expression in NK cells is decreased moderately, and no perforin is detected in CD8⁺ and CD56⁺ T cells. (d) The mother of patient in panel b (heterozygous 445 G>A), demonstrating abnormally decreased perforin expression in CD8⁺ T cells and NK T cells. (e) EBV-associated HLH, demonstrating increased proportions of perforin expressing CD8⁺ and CD56⁺ T cells. TCR = T-cell receptor.

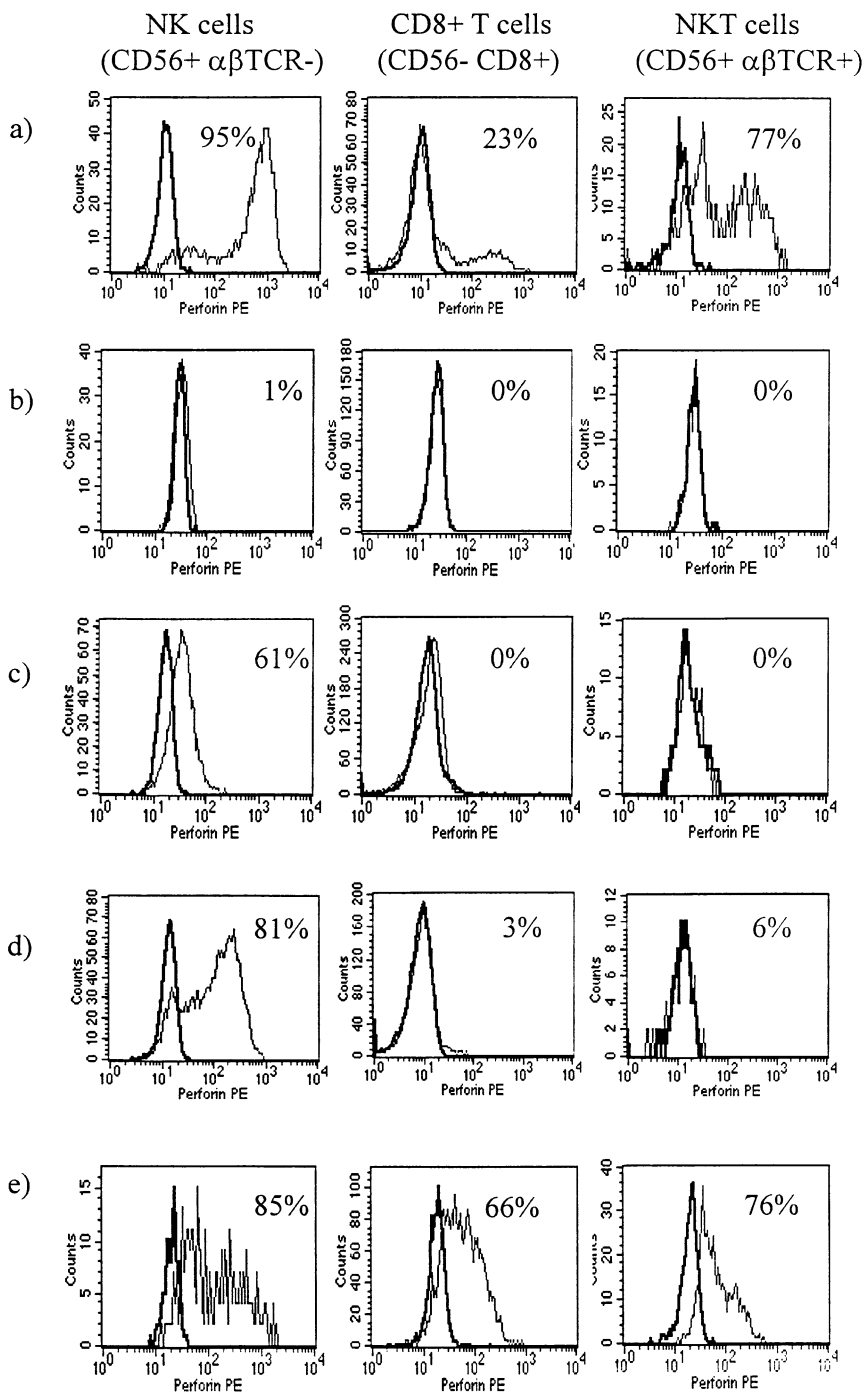


Table 4

Comparison of the proportions of perforin-expressing NK cells and CD8⁺ T cells between patients with different HLH phenotypes and normal pediatric controls

	Primary HLH (n = 8) ^a	Primary HLH (n = 6) ^b	EBV-associated HLH (n = 6)	Normal control range
Perforin-expressing NK cells (%)	0.13 (0–1)	91.8 (83–97)	86.7 (82–96)	age, <1 y 94 ± 3 age, 1–15 y 86 ± 5
Perforin-expressing CD8 ⁺ T cells (%)	0	3.3 (0–10)	39.7 (5–81)	age, <1 y 1.1 ± 0.5 age, 1–15 y 7 ± 5

Abbreviations: EBV, Epstein-Barr virus; HLH, hemophagocytic lymphohistiocytosis; NK, natural killer.

^a Patients with biallelic pathologic mutations in the *PRF1* genes.

^b Patients with normal *PRF1* genes.

a premature stop codon. In the rest of the families, a heterogeneous assortment of unique heterozygous mutations is described (Lee, Sumegi, MD, PhD, and Filipovich, MD, personal communication).

Other quantitative and qualitative studies of immune function in familial HLH are largely unrevealing. Proportions of lymphocyte subsets are often normal, especially during the early phases of symptomatic disease, although T cells can be highly activated (eg, HLA DR⁺). Mitogen responses are generally within normal limits. T-cell cytotoxic function can be reduced, and hypergammaglobulinemia is seen in some cases. All of these findings are relatively nonspecific and cannot confirm or exclude the diagnosis of HLH.

Differential diagnosis

Hemophagocytic lymphohistiocytosis can occur as a complication of XLP, CHS, and Griscelli syndrome, which are distinct, prematurely lethal immunodeficiencies that are curable by bone marrow transplantation [26–28]. In these patients, fatal infectious mononucleosis caused by EBV may be pathologically indistinguishable from HLH.

Hemophagocytic lymphohistiocytosis also has been reported in patients with decreased T-cell function, such as the CATCH 22 (deletion of chromosome 22q11) syndrome [29], and in association with systemic onset JRA—in which case it is usually called macrophage activation syndrome (MAS). MAS is the most serious, and often life-threatening, complication of systemic onset JRA. It was first described by Hadchouel and co-workers in 1985 [30] as a hemorrhagic syndrome associated with mental status changes, hepatosplenomegaly, increased serum levels of liver enzymes, and a sharp fall in blood counts; the term MAS was coined by Stephan and co-workers in 1993 [31] in reference to the histologic findings on bone marrow aspiration of numerous, well-differentiated macrophages (or histiocytes), and actively phagocytosing hematopoietic elements. As in HLH, such cells can infiltrate many organs in MAS. MAS responds to

therapies that are similar to therapies typically effective in HLH. Studies have shown that the amount of perforin expressed in NK cells from patients with severe systemic onset JRA is reduced when compared with quantities measured in lymphocytes from normal subjects [32], suggesting a mechanism of immune susceptibility to hemophagocytic syndrome that is similar to that identified in one form of hereditary HLH.

X-linked lymphoproliferative syndrome is caused by hemizygous mutation on the X chromosome, expressed in males, in the genetic sequence of SH2D1A, also known as SAP (signaling lymphocyte activation molecule [SLAM]-associated protein; CD150)—a short SH2 domain containing protein that normally is expressed only in lymphoid tissues [33]. Males who are affected with XLP are at risk for development of fulminant and fatal infectious mononucleosis when infected with EBV and are highly predisposed to development of lymphomas with or without previous EBV exposure. Average life expectancy without curative bone marrow transplantation is less than 10 years. SH2D1A shares extensive homology with the SH2 domain of SH₂ domain-containing inositol 5-phosphatase (SHIP) that functions as a negative regulator of lymphocyte activation [34]. SH2D1A has been reported to associate with two members of the CD2 subset of the immunoglobulin superfamily of cell-surface receptors: (1) SLAM, which contributes to activation of T cells, and (2) 2B4, which contributes to activation of NK cell cytotoxicity [35]. In both scenarios, SH2D1A seems to function as a constitutively expressed signal adaptor protein that competes with the SH2 domain of the protein tyrosine phosphatase-2 (SHP-2). In the case of its interaction with 2B4, mutations in SH2D1A interfere with cytotoxicity that normally is enhanced through ligation of this NK cell receptor by CD48, an antigen characteristically expressed on EBV-infected B cells [36]. The precise function of SH2D1A in T-cell activation is not known, but a role in orchestrating the extent of interactions between T cells and antigen-presenting cells is postulated. Findings suggest a direct association between SH2D1A and Dok1, which links to the Ras-GAP pathways, and other cell-cycle regulatory proteins [37].

Decreased expression of transcripts of genetically normal SH2D1A, determined by real-time polymerase chain reaction (PCR), has been reported in peripheral blood T cells from patients with JRA, compared with expression in normal subjects and in patients with other autoimmune conditions [38]. This finding, along with the reduced levels of perforin expression in lymphocytes from severely affected children with systemic onset JRA, may contribute to the prevalence of HLH-like MAS in systemic onset JRA.

Chédiak-Higashi syndrome is an autosomal recessive disorder with an estimated incidence of one case per million population; it usually is caused by biallelic truncation mutations of the lysosomal-trafficking regulator genes encoded on chromosome 1 [39]. An analogous disorder is seen in beige mice. Patients with CHS express variable degrees of oculocutaneous albinism, easy bruisability as a result of deficient platelet dense bodies, recurrent infections with neutropenia complicated by impaired chemotaxis and bactericidal activity, and

abnormal NK cell function. Neurologic impairment is also variable and can include progressive peripheral neuropathy. Presumptive diagnosis is straightforward, based on examination of a peripheral blood smear that reveals huge cytoplasmic granules (in reality, enormous lysosomes) in neutrophils and lymphocytes. On skin biopsy, melanocytes contain giant melanosomes. The most deadly complication of CHS is the so-called “accelerated phase” that usually is triggered by EBV infection and is symptomatically indistinguishable from other forms of HLH. The median life expectancy of patients with CHS, unless corrected with allogeneic bone marrow transplantation, is approximately 10 years. As in HLH, NK cells in patients with CHS do not kill K562 cells, a process that is perforin-dependent, although IL-2–induced cytotoxicity mediated through Fas-FasL and other mechanisms can occur [40]. In CHS, there is evidence that membrane targeting of proteins normally present in secretory lysosomes is defective. This finding seems to occur with the cytoplasmic T-lymphocyte–associated antigen CD152, which normally has a significant role in down-regulation of T-cell activation [41]. As speculated in the pathogenesis of XLP, studies indicate that antigen presentation may be perturbed in CHS, because the intracellular peptide loading of class II molecules may be limited by defects in lysosomal biogenesis, reducing effective responses to viral challenges [42].

A disorder that is related to CHS, that is also distinguishable from primary HLH by the clinical findings of pseudoalbinism, is GS. GS is a rare, prematurely lethal autosomal recessive disorder that is associated with biallelic mutations in one of the two closely situated genes on chromosome 15: (1) the myosin V gene and (2) the Rab27a gene [43]. Both genes encode proteins that form part of the overlapping schemes of the eukaryotic cytoskeleton—the underpinning of the intracellular transport systems for products that are transferred through vesicles (lysosomes) and organelles to their terminal effector sites, often at or near the cytoplasmic membrane. As in CHS, GS affects the metabolism of lymphocytes and melanocytes. The severe hemophagocytic syndrome may be related more closely to the Rab27a gene defect, consistent with *in vitro* findings of reduced T-cell cytotoxicity in patients with GS [44].

Pathogenesis

The clinical findings in HLH largely can be explained as the consequence of the prolonged production of extremely elevated levels of circulating cytokines and chemokines, presumably originating from activated histiocytes, and, probably, T cells. Excess circulating IL-1 β , TNF α , IL-6, and IFN γ likely contribute to the early and persistent clinical findings of fevers, hyperlipidemia, and endothelial activation, responsible in part for coagulopathy and later sequelae that include tissue infiltration by lymphocytes and histiocytes, hepatic triaditis, CNS vasculitis and demyelination, marrow hyperplasia, and aplasia. Hemophagocytosis is a hallmark of cytokine-driven overactivated macrophages. Understanding the

unrelenting release of proinflammatory and type 1 cytokines may depend on the histologic findings of histiocytic and lymphocytic proliferation in lymphoid tissues and ectopic locations, associated with permanent or transient defects in immunologic cytotoxicity.

Cytotoxic immune cells, including NK cells, NK T cells, and cytotoxic CD8 and CD4 T cells, have a major regulatory role in maintaining the threshold of responsiveness to external noxious stimuli that is required of a healthy immune system and organism. By triggering apoptosis (programmed cell death), cytotoxic cells signal the rapid removal of other immune cells (antigen-presenting histiocytes and lymphocytes), which are abnormal or misplaced, senescent, or potentially dangerous to the host. This cytotoxic mechanism is critical to the prevention and control of autoimmune conditions, chronic viral infections, and other pathologic immune and inflammatory disorders (such as HLH).

Research findings regarding the molecular and cellular biology of HLH and related disorders (discussed in the section on differential diagnosis) point to the central role of defects in the perforin-mediated regulatory and cytotoxic pathway in the pathogenesis of the life-threatening hemophagocytic disorders. Perforin is believed to be principally responsible for the translocation of granzyme B from cytotoxic granules in cytotoxic T and NK cells into target cells. After delivery to the target cells, granzyme B migrates to the nucleus to participate in triggering apoptosis.

Defects in the perforin pathway can result from: (1) too few effector cells (eg, NK cell deficiency), (2) failure to synthesize perforin (eg, PRF1-negative HLH), (3) failure to signal mobilization of perforin and granzyme B (eg, XLP), or (4) failure to promptly deliver the cytotoxic granules to sites of action because of sluggish cytoskeletal machinery (eg, CHS, GS). Genomic expression studies have implicated the PIK3 signaling cascade ($\text{PIK3} \rightarrow \text{Rac1} \rightarrow \text{PAK1} \rightarrow \text{MEK} \rightarrow \text{ERK}$) in the regulation of perforin and trafficking of granzyme B [45], suggesting that additional causes for HLH may be uncovered by studying this pathway and its interacting proteins.

In contrast to the mechanism of defective immune cytotoxicity among genetically determined disorders leading to hemophagocytic syndrome, it has been theorized that in some cases of EBV-associated HLH, the vicious cycle of T cell $\leftrightarrow$ antigen presenting cell (APC) activation is initiated primarily by EBV infection of T cells and their clonal expansion. Even in this setting, however, circumstantial evidence points to a transient, acquired, NK cell cytotoxic deficiency, which may perpetuate the hemophagocytic response. Preliminary findings in the author's laboratory suggest that the immune response in patients with EBV-associated HLH differs substantially from that experienced by healthy subjects with infectious mononucleosis. Patients with infectious mononucleosis maintain the circulating population of strongly perforin-positive NK cells during acute infection, whereas patients with EBV-associated HLH demonstrate a dramatic reduction in the number of circulating NK cells, and particularly the most potent (CD56bright) population. NK cell function and number returns to normal with resolution of EBV-associated HLH following chemotherapy.

Treatment

Diagnostic evaluation

Because the most effective treatment for primary and secondary HLH and for hemophagocytic syndrome associated with other genetic immunodeficiencies is the same, the goals of the diagnostic evaluation are: (1) to exclude other causes for the symptomatic complex (eg, malignant disease), (2) to identify potential infectious cofactors that would require specific treatment, (3) to establish the extent of the disease, especially the presence of CNS involvement, and (4) to collect materials for future characterization of underlying genetic defects. Given the poor prognosis of HLH without prompt and effective treatment, it is recommended that treatment be initiated when there is a high clinical suspicion, even if all recommended studies have not been completed.

In addition to the careful history and physical examination, initial studies should include a complete blood count, coagulation profile including fibrinogen level, serum electrolyte and fasting triglyceride level, diagnostic bone marrow biopsy, and lumbar puncture. Samples for infectious diagnosis should be collected from blood, marrow, CSF, urine, and stool. Quantitative PCR testing for EBV and cytomegalovirus (CMV) can be helpful when followed serially during the treatment of infection-associated HLH. If IVIgG is administered, serologic testing for infectious causes must be obtained beforehand, or preinfusion serum can be saved for future studies.

Magnetic resonance imaging of the brain is recommended, even when the initial CSF analysis is normal, because CNS involvement often is detected later in the course of the disease, and a baseline examination can be valuable later. Immunologic studies including quantitation of NK cells, studies of NK function, and perforin quantitation are reliable if obtained within a week of initiating treatment, but should be directed to laboratories that specialize in these studies, where age-appropriate normal ranges have been established. Storage of DNA, viably cryopreserved lymphocytes, and establishment of immortalized cell lines for future genetic analyses is encouraged.

Initial treatment

If left undiagnosed and untreated, primary HLH can be rapidly fatal. Historically, the average interval from diagnosis to death, even with high levels of supportive care, was reported as less than 2 weeks. It has become apparent that secondary HLH and MAS associated with systemic onset JRA have similar poor prognoses without intervention with immunosuppressive and anti-inflammatory therapies.

Effective treatments for HLH and other life-threatening hemophagocytic disorders have included the use of agents that interrupt the function of activated macrophages and histiocytes (etoposide, steroids, high-dose IVIgG) or activated T cells (steroids, cyclosporin A, antithymocyte globulins), breaking the cycle of unopposed immune activation leading to HLH. According to

some studies, adjunctive treatment with anti-TNF antibodies has improved symptomatic control. Treatments directed at eliminating infections that may have triggered the exaggerated inflammatory response are recommended where appropriate.

An international collaboration sponsored by the Histiocyte Society and led by Dr. Henter of Stockholm, Sweden, developed a consensus protocol for the treatment of newly diagnosed HLH. This protocol was based on the principal of combining antihistiocyte and anti-T cell therapies to achieve initial clinical remission of HLH symptoms [46]. The major features of the recommended treatment at outset consisted of a combination of intensive etoposide and dexamethasone (CNS-penetrating) dosing, with or without intrathecal methotrexate, followed by a maintenance course of cyclosporin A and less frequent pulses of etoposide and dexamethasone once clinical remission had been consolidated. This protocol, called HLH-94, proved to be a set of treatment guidelines for the management of what now is recognized as a genetically diverse group of patients who met the clinical diagnostic criteria for HLH as published in 1991 [4]. It was recommended that patients with presumed familial HLH and patients with relapsing symptoms after initial response to HLH-94 should proceed to definitive therapy with allogeneic hematopoietic stem cell transplantation (HSCT). For patients without evidence of familial disease (eg, presumed secondary HLH) who achieved complete clinical remission after 8 weeks of therapy, it was recommended that therapy be stopped and that the patients be observed for potential future symptomatic recrudescence.

In an article recently submitted for publication (Blood 2002, in press), the HLH-94 study committee reported the outcomes of the 113 patients from 21 countries who met study eligibility criteria and began therapy between July 1, 1994 and June 30, 1998. This study has the largest series of patients with HLH who were treated in a relatively uniform manner from time of diagnosis. All treated patients were under the age of 15. Most patients who were treated according to HLH-94 showed significant symptomatic response within 2 weeks of initiation of treatment. Unfortunately, approximately 25% of all patients had return of some symptoms during the 8-week initial treatment phase, and 25 patients from the total group died before HSCT, mostly from progressive disease. These results suggest the need for (1) risk stratification of future therapies or (2) more intensive front-line treatment for all patients. Patients who had recrudescent symptoms as the intensity of the recommended therapy was decreased often responded to reinitiation of the protocol. Other salvage therapies that had occasional benefit included antithymocyte globulin.

Overall results with HLH-94 show a 55% ($\pm 9\%$) survival at 3 years. Overall survival for patients who were presumed or known to have primary HLH was 46% ($\pm 11\%$). Of the patients who proceeded to HSCT, the 3-year disease-free survival was 63% ($\pm 12\%$). Twenty patients in the study, who were diagnosed in Japan, completed therapy at 8 weeks and have remained alive without therapy for at least 3 years.

Hematopoietic stem cell transplantation

Believing that familial HLH was a genetic immunologic disorder, Fischer and colleagues in France hypothesized that the disease could be corrected by replacement of the patient's immune system through bone marrow transplantation. They published the first success with bone marrow transplantation for HLH in 1986 using a healthy, matched sibling donor [47]. Ten years later, Arico and colleagues at the Histiocyte Society, using data from the International HLH Registry, showed that more than 60% of children with HLH who had a matched sibling transplant were alive 5 years after the transplant, compared with only 10% of children with HLH who had received chemotherapy only [48].

Most children with HLH who could benefit from a transplant lack matched sibling donors; thus, alternative donors have been pursued, including the options of closely matched relatives, unrelated donors of marrow or cord blood, and haploidentical-related donors whose grafts have been treated with extensive T-cell depletion to prevent graft versus host disease.

In many cases, the results of marrow transplantation using alternative stem cell donors are just as promising as those using sibling donors. The follow-up of children treated with the HLH-94 induction protocol shows comparable 3-year disease-free survival for patients who received a bone marrow transplant from a matched sibling ($n=19$; 74%) or a closely matched, unrelated donor ($n=33$; 70%). The best results were seen in children who had a prompt and complete response to the HLH-94 chemotherapy protocol before transplant, emphasizing the importance of controlling the HLH process before transplantation. These observations support those reported earlier from the National Marrow Donor Program in the US, in which the patients who had active systemic or CNS disease before transplantation had poorer long-term outcomes [49].

For patients registered on the HLH-94 protocol, survival following haploidentical family-member-donor transplantation was somewhat less favorable ($n=10$; 40%). In another limited series of children with HLH treated in Europe with T-depleted grafts from family members, the survival rate was similar ($n=11$; 54%) [50]. Three patients in that series required second grafts after initial rejection episodes. Rejections following T-cell depletion in transplantation for HLH seem to be as common as in other diseases despite the defect in NK cytotoxicity common to recipients with HLH.

In most cases, use of conventional chemotherapy, without the need to expose young children to radiotherapy, is adequate to achieve a graft take. Although patients with HLH have had successful transplants using unrelated cord blood, some rejections have occurred. This fact raises the concern that cord blood transplants are one-time treatments, because second donations cannot be obtained if the engraftment is insufficient. Little experience exists with so-called “mini-transplants,” or nonmyeloablative conditioning therapy for bone marrow transplantation of HLH, and this approach currently cannot be recommended.

Generally, use of non-T-depleted marrow has been recommended, because early recovery of NK and cytotoxic T cells seems to be important to control the

underlying HLH [49]. Approximately half of the children who have had bone marrow transplantation for HLH become mixed chimeras. Long-term follow-up indicates that persistence of a mixture of host and donor cells is not likely to result in recurrence of HLH, especially if NK cell function is restored.

Although approximately two thirds of children with HLH who undergo bone marrow transplantation can expect to be cured of their disease, a number of complications can occur, including severe inflammatory reactions, hemolytic anemia, and graft-versus-host disease. As with other rare conditions, the results of bone marrow transplantation are generally better when the procedure is performed at a major pediatric transplant center where the medical staff is familiar with the treatment of infants and patients with HLH.

Long-term follow-up of survivors of bone marrow transplantation for HLH indicates that most children return to a normal or near-normal quality of life. Changes of brain atrophy in brain scans during the acute phase of HLH and are caused by steroid therapy, gradually resolve after the transplant; however, children who experience serious involvement of the brain with HLH may have neurologic problems and learning disabilities as a consequence, despite adequate control of the disease after bone marrow transplantation. For this reason, neuropsychologic evaluation is recommended before transplant and at intervals afterward. As they mature, transplant recipients require regular follow-up by transplant specialists to prevent late complications relating to growth and hormone function.

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