

Hemophagocytic Lymphohistiocytosis and Other Hemophagocytic Disorders

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Hemophagocytic disorders result when critical regulatory pathways responsible for the natural termination of immune/inflammatory responses are disrupted or overwhelmed. Hemophagocytic disorders reflect pathologic defects that alter the normal crosstalk between innate and adaptive immune responses and compromise homeostatic removal of cells that are superfluous or dangerous to the organism. Although hemophagocytic disorders are considered rare, increased awareness of these conditions has led to more frequent diagnoses, more rapid initiation of life-saving treatments, and new insights into the molecules and pathways involved in natural immune down-regulation. Furthermore, improved understanding of the immunologic abnormalities revealed by hemophagocytic disorders informs potential new treatments for life-threatening multisystem organ dysfunction related to sepsis in the ICU setting, and severe cases of viral infections in previously healthy individuals.

The term hemophagocytic lymphohistiocytosis (HLH) was formally adopted by the international Histiocyte Society to describe a lethal autosomal recessive defect associated with overwhelming systemic inflammation [1]. Characteristic features of HLH were described as hectic or prolonged fevers, hepatosplenomegaly, and cytopenias, typically occurring in infancy or early childhood [2]. Farquhar and Claireux [3], at the University of Edinburgh, are credited with the first description (in 1952) of HLH, which they named “familial hemophagocytic reticulosis.” Before the description of specific genetic defects underlying HLH, sporadic cases of HLH, particularly in older children and adults, were often referred to as “secondary.” Secondary cases of HLH were attributed to infectious agents identified at the time of disease, to other circumstances such as immune suppression for management of rheumatoid disorders, or to malignancies. In recent

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years, many more cases of hemophagocytic disorders have been identified, especially in the context of severe inflammatory reactions to viral exposure, including HIV [4] and avian influenza [5].

Currently, HLH (FHL:OMIM 267,700, 603,553) includes the autosomal recessive genetic disorders, with an estimated prevalence of 1/50,000 live births, as well as the secondary forms.

Pathogenesis of hemophagocytic lymphohistiocytosis and other histiocytic disorders

HLH is characterized by multisystem inflammation, a reactive process resulting from prolonged and intense activation of antigen-presenting cells (macrophages, histiocytes) and CD8 + T cells, and excessive proliferation and ectopic migration of T cells.

Normal functions of histiocytes, a major population of cells within the innate immune system, include phagocytosis, antigen presentation, and activation of the adaptive immune system through contact and cytokine signaling.

Abnormalities in the function (but rarely the quantity) of natural killer (NK) cells have been observed in patients with all forms of HLH. NK cells play significant roles at many stages of the immune response to pathogens. They are a frontline of defense against intracellular pathogens such as viruses, which infect nonlymphoid tissues early, on entry into the organism (cytotoxic and cytokine-mediated mechanisms). NK cells modulate the initial responses of antigen-presenting cells to incoming pathogens (likely through cytokine signaling), attenuating the signal communicated to antigen-specific T cells. NK cells likely also play a role in culling activated T cells and histiocytes in the later stages of antigen-driven activation, contributing to the natural contraction of the immune response.

NK cells, and natural killer T (NKT) cells in particular, play a major role in maintaining a healthy threshold of immune responsiveness to noxious external stimuli, and are critical to the prevention and control of autoimmune conditions and severe reactions to viral infections.

Also critical to the contraction process of active T-cell populations is the mechanism of activation-induced apoptosis. Like NK cell cytotoxicity, this process involves granule-mediated cytotoxicity.

Studies of cytokine levels in blood and tissues have indicated persistently elevated circulating levels of multiple proinflammatory cytokines during symptomatic disease [6]. Published results vary from study to study, and levels of proinflammatory cytokines appear to be particularly elevated in Epstein-Barr virus (EBV)-driven HLH in Asian populations. It is currently believed that “hypercytokinemia,” and possibly “hyperchemokineemia,” generated by uncontrolled activation of histiocytes (antigen-presenting cells and T cells), underlies the progressive organ dysfunction that eventually leads to death in affected patients. Symptoms and signs of HLH include

fevers, hyperlipidemia, endothelial activation/coagulopathy, hepatic triaditis, central nervous system (CNS) vasculitis and demyelination, inflammatory lung disease with acute respiratory distress syndrome, and marrow hyperplasia or aplasia. Hemophagocytosis is a hallmark of cytokine-driven macrophages/histiocytes.

Ongoing hypercytokinemia is a reflection of the failure of natural immune down-regulation due to defective NK and cytotoxic T lymphocyte (CTL) function.

Clinical presentation of hemophagocytic lymphohistiocytosis

Until recently, it was widely believed that the symptoms of familial HLH (also referred to as FHL in the literature) generally arose during infancy and early childhood. With the more widespread availability of genetic testing, it is apparent that the first significant episode of HLH can occur throughout life [7,8], including in utero [9]. HLH should be considered as part of the differential diagnosis of nonimmune hydrops fetalis [9]. In infants, significant symptomatic overlap has been reported with neonatal sepsis [10,11], acute hepatic failure, and neonatal hemochromatosis [12].

Despite attempts to differentiate primary from secondary HLH, or the reactive forms, the symptomatic presentations are highly overlapping. In the most typical form of primary HLH, the clinical course is characterized by prolonged fever (> 7 days) and hepatosplenomegaly. Neurologic symptoms may dominate the initial clinical course, with seizures or ataxia. Neurologic findings may be highly variable and can include irritability, hypo- or hyper-tonia, cranial nerve palsies, meningismus, and signs of increased intracranial pressure and altered consciousness.

Rash, lymphadenopathy, and diarrhea are less frequently observed. Standard blood testing typically reveals cytopenias, especially anemia and thrombocytopenia, liver dysfunction, hypofibrinogenemia, hypertriglyceridemia, hypoalbuminemia, and hyponatremia. In the early days to months of the disease, symptoms may improve spontaneously, followed by clinical exacerbations. Importantly, frank hemophagocytosis may not be obvious on bone marrow biopsy examination early in the course of the disease [13].

Genetics of hemophagocytic lymphohistiocytosis and other histiocytic disorders

To date, all described genetic defects associated with HLH and other histiocytic disorders appear to be related to one another in the pathway of granule-mediated cytotoxicity. These genetic defects interrupt the mechanisms responsible for triggered apoptosis (mediated by cytotoxic cells on the target cell) or activation-induced apoptosis (putative suicide of activated T cells). The first gene linked to HLH was perforin [14], a soluble, pore-forming cytolytic protein synthesized in cytolytic lymphocytes and

sequestered, along with Granzyme serine proteases, in secretory cytotoxic granules. When cytotoxic cells contact their targets, the intracellular cytoskeletal scaffold (the microtubule organizing center [MTOC]) is rotated to focus on the contact site where the cytotoxic immunologic synapse forms. Cytotoxic granules are carried along the MTOC toward the immunologic synapse, where they degranulate, allowing perforin and Granzyme B to enter the contact zone, permeabilization of the target membrane, and delivery of Granzyme B into the target cell. Once internalized, Granzyme B initiates caspase-dependant and caspase-independent apoptotic pathways, killing the target cell.

A murine model of perforin deficiency published in 1994 revealed that perforin was the crucial effector molecule in T- and NK-cell-mediated cytotoxicity [15]. This model, in a specific murine strain infected with lymphocytic choriomeningitis virus (LCMV), has been used to recapitulate the HLH scenario, revealing a key role of high gamma-interferon production and excessive activation of antigen-presenting cells in the symptomatic evolution of murine disease [16]. Perforin deficiency accounts for 15% to 20% of HLH cases in several geographic areas (also referred to as FHL2). HLH due to perforin deficiency has been reported to occur at a few weeks of age, and in older children and adults (49- and 62-years-old) [17]. Patient series have been examined to identify putative genotype/phenotype correlations [18]. These indicate that the most deleterious mutations associated with virtually no production of protein are typically identified in patients affected during infancy, whereas compound heterozygous missense mutations are more commonly identified in older patients. Mutant perforin sequences capable of generating near-normal quantities of mature perforin protein result in milder clinical phenotypes [19]. In contrast, nonrandom homozygosity of severe PRF1 mutations, such as 50delT, L17fsX22, observed in patients of African descent, is associated with mean age at onset of 2 months [20]. Heterozygosity for C272T (A91V) in PRF1 is the most frequently observed missense mutation in Caucasian patients who had FHL2, and was found to exceed the prevalence in the general population by threefold in patients from Italy [21] and North America [22] who had HLH. Once considered a benign polymorphism, A91V has more recently been proposed as a disease-modifying gene. Structural and functional studies of the human A91V perforin protein indicate decreased activity compared with native perforin [19,23]. In the North American experience, a substantial proportion of patients who have HLH and who carry this genotype have also been found to have either other biallelic disease-causing mutations in PRF1, biallelic or heterozygous deleterious mutations in MUNC 13-4, or absent NK function, suggesting the presence of other gene defects that contribute to the pathogenesis of HLH [22].

No defects in Granzyme B have been identified in association with human HLH.

A second gene responsible for HLH, MUNC 13-4, was reported in 2003 [24]. MUNC 13-4 was described as essential for cytolytic granule fusion with

other structures related to the cytoplasmic membrane in the process of degranulation. Recently, a murine model of this disease has been developed, *Jinx*, a murine cytomega 6 virus (MCMV) susceptibility phenotype caused by disruption of the murine homolog, *Unc13d* [25]. As with perforin-deficient mice, *Jinx* mice do not spontaneously develop HLH, but require infection with LCMV, which stimulates hyperactivation of CTLs and antigen-presenting cells in the context of inadequate restriction of viral proliferation. MUNC 13-4 deficiency, also referred to as FHL3, has a worldwide distribution and accounts for 15% to 20% of HLH cases.

The gene defect responsible for FHL4 is syntaxin 11 [26], which has been shown, as in MUNC 13-4 deficiency, to result in defective degranulation [27]. Syntaxin 11 is a member of the SNARE protein family that facilitates fusion in intracellular membrane trafficking events. It was recently shown to be expressed in NK cells and activated CTLs [28]. To date, the few HLH cases attributed to syntaxin 11 deficiency have been found almost exclusively in Turkish families. In some families, a more indolent course, and findings consistent with myelodysplasia and myeloid malignancy, have been seen [29,30]. Extensive study of patients from the Far East and North America has not revealed additional similar patients.

The genetic defect responsible for FHL1 linked to chromosome 9 [31] in a study of two extended Pakistani families has not yet been discovered.

Related hemophagocytic disorders occur with significant frequency in five other genetic diseases that have been linked with defective cytotoxic function. Three distinct immunodeficiencies associated with pseudoalbinism due to defects in lysosomal trafficking have been associated with life-threatening episodes of HLH: Chediak Higashi syndrome (LYST, or CHS1) [32], Griscelli syndrome (Rab27A) [33], and Hermansky-Pudlak syndrome type II (AP3B1) [34]. Rab27a, a small Rho GTPase, interacts directly with MUNC 13-4 and is thought to play a role in docking the cytotoxic granules on the MTOC. These diagnoses can be suspected on physical examination in the presence of very fair or grayish hair, and when distinctive laboratory abnormalities of the neutrophil and platelet compartments have been detected.

HLH following exposure to EBV and, less commonly, to other viruses, termed fulminant infectious mononucleosis [35], is the most frequent life-threatening complication of X-linked lymphoproliferative syndrome (XLP). XLP is caused by hemizygous mutations in SH2D1A encoding SLAM-associated protein (SAP), which lead to abnormal NK cell responses and NKT cell deficiency. XLP2, due to hemizygous mutations in X-linked inhibitor-of-apoptosis (XIAP or BIRC4), was recently described in three families with multiple related males who developed EBV-associated HLH [36]. Similar to XLP, marked decreases in NKT cell numbers have been demonstrated in patients who have XIAP deficiency. In addition to HLH complications, patients who have XLP and XLP2 may demonstrate features of common variable immunodeficiency, autoimmune disease, and lymphoproliferative disorders, even without prior exposure to EBV. Patients who

have XLP and XLP2 may survive into adulthood in good health before succumbing to a serious complication of their underlying disease. Thus, lack of prior significant medical history should not exclude these diagnoses. Several murine models of XLP have been created [37]. Following infection with LCMV, but also leishmania and toxoplasma, SAP-deficient mice develop aggressive CD8 T-cell populations, generating high levels of gamma-interferon, leading to life-threatening tissue inflammation. As with other murine populations harboring defects in genes that affect cytotoxicity or apoptosis, noninfected XIAP-deficient mice do not express a pathologic phenotype.

Taken together, the nine genetic disorders described above still account for less than one half of the diagnosed cases of HLH in children, including many familial cases still awaiting molecular definition.

Hemophagocytic disorders associated with other immunodeficiencies and infections

The clinical syndrome of HLH has occasionally been observed in patients who have other underlying primary immunodeficiencies, including some with restricted T repertoires and function, such as the del22q11 syndrome, or DiGeorge syndrome [38], and severe combined immunodeficiency. Sporadic cases of hemophagocytic complications in patients who have chronic granulomatous disease [39] and X-linked agammaglobulinemia, and the X-linked NEMO [40] defect have also been reported. In these case reports, various infections, including *Burkholderia cepacia* and adenovirus, have been temporally associated with the development of the hemophagocytic symptomatic complex.

Although it is clear that many genetic forms of HLH may be triggered by viral or parasitic infections, leishmaniasis, in particular, has been cited as an HLH mimic. HLH associated with leishmaniasis is curable by specific treatment of the infection itself without need for anti-inflammatory or cytotoxic therapies (see later discussion), but is potentially fatal without antibiotics [41,42]. Visceral leishmaniasis is a strongly immunosuppressive, generalized infection of the reticuloendothelial system manifested by intermittent fevers, massive hepatosplenomegaly, pancytopenia, fatigue, and hepatitis. Hemophagocytosis is often obvious on bone marrow biopsy. Leishmanial amastigotes may also be seen on the marrow biopsy. Today, molecular diagnostics to identify this infection are widely available, and should be applied to individuals originating from endemic areas bordering the Mediterranean basin, as part of the differential diagnosis of HLH. Although the genetic make-up of patients who develop life-threatening leishmaniasis has received little study, it appears that this condition represents a clear example of secondary HLH.

In contrast, other infection-associated cases of HLH should remain suspect for underlying genetic predispositions. Most commonly, viral infections have been reported in association with HLH, especially involving the herpes virus group. EBV has long been recognized in Asia as a major instigating

infection. EBV-associated HLH in Japanese series appears to differ from typical EBV infections and HLH associated with EBV in the West, in two regards [43,44]. Principally, in Japanese cases of HLH, EBV is commonly found as an ectopic infection of NK and T cells, often associated with clonal expansion. In contrast, this phenomenon has been reported to a much lesser extent in the West. Indeed, EB viral load typically decreases with anti-B-cell therapy (eg, Rituxan) when used in combination with cytotoxic therapy outside of Asia, suggesting that the major niche for EBV (B cells) is being destroyed. Secondly, many cases of HLH in Japan respond to a short course of therapy and do not require hematopoietic cell transplant to achieve prolonged remission or cure. This result is less commonly seen in the West.

Other herpes group viruses that appear to trigger HLH include cytomegalovirus [45], varicella zoster virus [46], human herpesvirus (HHV) 6 [47], and HHV8 [48]. Anecdotally, Varivax has been described to precipitate HLH in genetically susceptible individuals. Avian influenza is a particularly potent stimulus for hemophagocytic reactions in Asia, which appear to be associated with the high mortality seen in that infection. Rubella [49], adenovirus [50], parvovirus [47], and hepatitis B virus [51] have all been reported in connection with HLH.

HLH is a recognized complication of HIV infection. In some cases, it is diagnosed in the context of associated opportunistic infections or lymphoma. It can present at almost any time along the course of the HIV disease, from acute seroconversion to end-stage disease, or during highly active antiretroviral therapy. In some cases, it has been hypothesized that a strong antiviral cytotoxic response may be responsible for the symptoms of HLH.

Other microbial pathogens include *Mycobacterium tuberculosis* [52], *Serratia marcesans* [11], *Burkholderia cepacia* [39], and fungal infections such as *Candida* [53], *aspergillus* [54], and histoplasmosis [55].

In most cases of infection-associated HLH, cytotoxic therapy in combination with antimicrobial therapy is required to control the disease process.

Hemophagocytic disorders associated with autoimmune disorders

The most commonly recognized association of hemophagocytic syndrome with rheumatoid disorders is the macrophage activation syndrome (MAS) [56,57]. MAS, most often associated with systemic onset juvenile inflammatory arthritis (soJIA), can be the first presenting feature of soJIA. MAS, and particularly recurrent MAS, bears a close resemblance symptomatically and immunologically to HLH. Many cases of MAS are characterized by low NK-cell function and deficient perforin expression in cytotoxic cells [58]. More recently, mutations in MUNC 13-4 have been identified in MAS associated with soJIA [59].

MAS is less frequently reported in other rheumatologic disorders such as pauciarticular rheumatoid arthritis and systemic lupus erythematosus [60]. MAS has also been described in association with Behcet's syndrome [61].

An HLH-like syndrome can complicate inflammatory bowel disease [62], especially when immune suppressive therapy precedes activation of EBV or histoplasmosis, especially in the setting of EBV or histoplasma infections during immune suppression of affected individuals.

Hemophagocytic disorders associated with malignancies

The association of hemophagocytic complications with malignancies, typically lymphoid malignancies, including T-cell and NK-cell tumors, peppers the literature [44]. In many cases, HLH represents a proximate life-threatening complication of the tumor or its therapy, and is associated with a poor prognosis. Until recently, these malignancies and hemophagocytic reactions have been viewed as side effects, perhaps the consequence of abnormal cytokine generation by the malignant cells. However, more recent findings linking underlying genetic defects (such as PRF1 and SH2D1A) suggest that pre-existing genetic susceptibility, as in murine models, which predict increased risk of lymphomagenesis [63], may play a role in the evolution of lymphoid tumors in humans and a predisposition to severe inflammatory reactions subsequent to therapy.

A retrospective study of 29 archival lymphoma samples revealed that tumors from eight individuals harbored mutations in PRF1 (four biallelic, four heterozygous) [64]. Furthermore, in a sample of 100 cases of childhood acute lymphoblastic leukemia (ALL), the incidence of heterozygosity for A91V mutation in perforin was more than twice as frequent in children with leukemias as in the general population [65]. A much larger study prompted by the occurrences of HLH during remission of childhood ALL with the current treatment protocol in the United States, examined 2272 children with ALL and 655 normal controls. Allele frequencies in Caucasian controls and patients who had ALL were similar, except for a significantly higher frequency in the small subgroup of BCR-ABL-positive ALL [66]. The study of correlations and causality between mutations in perforin and malignancies is still nascent.

Diagnosis of hemophagocytic lymphohistiocytosis

To assist with the rapid diagnosis of HLH, the Histiocyte Society has developed a set of diagnostic guidelines that encompass clinical and laboratory findings (Box 1) [2]. In the absence of a family history or specific genetic diagnosis, an assemblage of five or more of the eight diagnostic criteria are needed for a provisional diagnosis of HLH and initiation of therapy. These include persistent fevers without clear cause, splenomegaly, bicytopenia, hypertriglyceridemia or hypofibrinogenemia, and hemophagocytosis on bone marrow biopsy. Hemophagocytosis may not be clearly apparent in the initial bone marrow biopsy early in the disease process (Fig. 1). However, autopsy studies of more advanced cases repeatedly show that

Box 1. Diagnostic guidelines for hemophagocytic lymphohistiocytosis (patterned after the HLH 2004 protocol)

1. A molecular diagnosis consistent with HLH
2. Diagnostic criteria for HLH fulfilled (five or more out of the eight criteria below)
 - Fever
 - Splenomegaly
 - Cytopenias (affecting two of three lineages in the peripheral blood: hemoglobin <90 g/L (in infants <4 weeks: hemoglobin <100 g/L), platelets <100 10⁹/L, neutrophils <1.0 10⁹/L)
 - Hypertriglyceridemia or hypofibrinogenemia: fasting triglycerides >3.0 mmol/L (>265 mg/dL), fibrinogen <1.5 g/L
 - Hemophagocytosis in bone marrow, spleen, lymph nodes, or cerebrospinal fluid: no evidence of malignancy
 - Low or absent NK cell activity (according to local laboratory reference)
 - Elevated ferritin (>500 mg/L)
 - Soluble CD25 (ie, soluble interleukin-2 receptor) above normal limits for age

The diagnosis of HLH can be established by one or both of the criteria described.

hemophagocytosis is present throughout the organs of the reticuloendothelial system, and most other organs, including the lung, testes, pancreas, kidney, intestine, and even brain [67]. Diagnostic liver biopsies, often performed early in the disease for diagnosis of hepatitis, rarely reveal hemophagocytosis; rather, perivascular lymphoid infiltrates and triaditis with lymphoid infiltration are commonly seen. This latter finding should not decrease suspicion for HLH if other clinical findings point to the diagnosis.

Symptoms of CNS dysfunction, cerebrospinal fluid pleocytosis, or findings of foci of inflammation by CNS MRI scanning, are found in more than one half of patients who have HLH at initial clinical presentation. Characteristic findings on MRI, as summarized recently [68], include

- Multiple nodular or ring-enhancing parenchymal lesions
- A laminated pattern of nodular parenchymal lesions on T2-weighted images
- Leptomeningeal enhancement
- Confluent parenchymal lesions
- Mild ventriculomegaly
- Brain edema

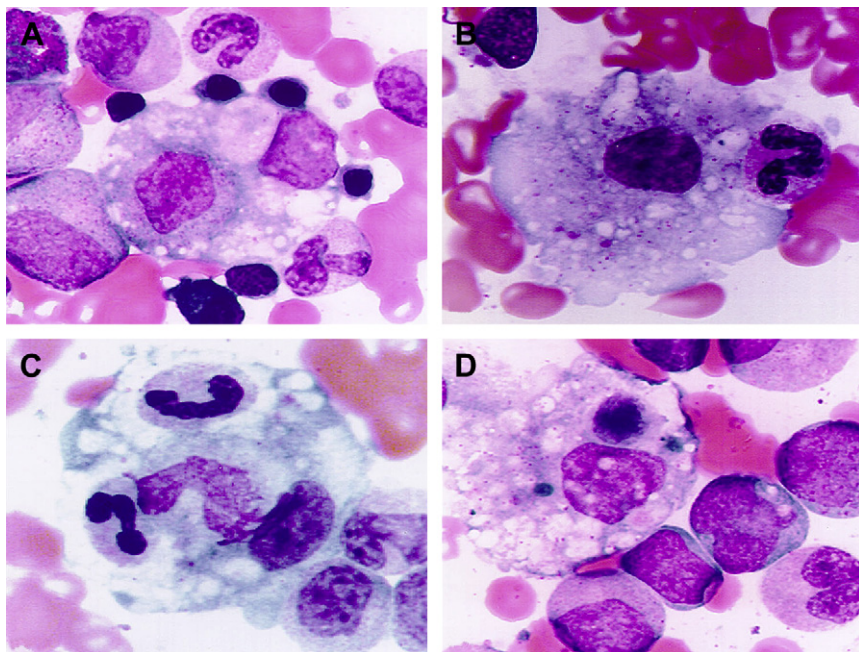


Fig. 1. Several examples of hemophagocytosis observed in the bone marrow of patients with HLH. (A) Attachment of hematopoietic cells to the surface of an activated histiocyte prior to internalization. This attachment is believed to be facilitated by the up-regulation of heme receptors CD163. (B–D) Clear examples of hemophagocytosis, showing polymorphonuclear cells, red cell precursors, and platelet debris in the cytoplasm of activated histiocytes. (Courtesy of Dr. A. Grom, Division of Rheumatology, Cincinnati Children's Hospital, Cincinnati, OH.)

Examination of the spinal fluid typically reveals a predominance of lymphocytes and monocytes, elevated protein content, and sometimes, hemophagocytosis on cytopspin. Generalized cerebral atrophy may be apparent on brain scans at diagnosis and can worsen with prolonged steroid treatment.

Additional criteria for provisional diagnosis of HLH include elevated levels of ferritin [69,70] and soluble IL2R α (sCD25) [71], both markers of generalized inflammation. A recent review of charts from patients who had elevated ferritin results from a large pediatric hospital concluded that a ferritin level of more than 10,000 mcg/dL was 90% sensitive and 96% specific for HLH [69]. However, this measurement does not discriminate between genetic or secondary forms of HLH. Synthesis of ferritin and interleukin (IL)-10 is induced during the protective anti-inflammatory process of macrophage scavenging of heme through the CD163 receptor [72].

High levels of sIL2Ra are almost never seen outside HLH. Normal ranges for levels of sIL2Ra vary with age (highest in infants and lower in teens and adults) (Fig. 2A, B). When interpreting results of sIL2Ra levels, it is important to know the relevant normal age ranges and the method

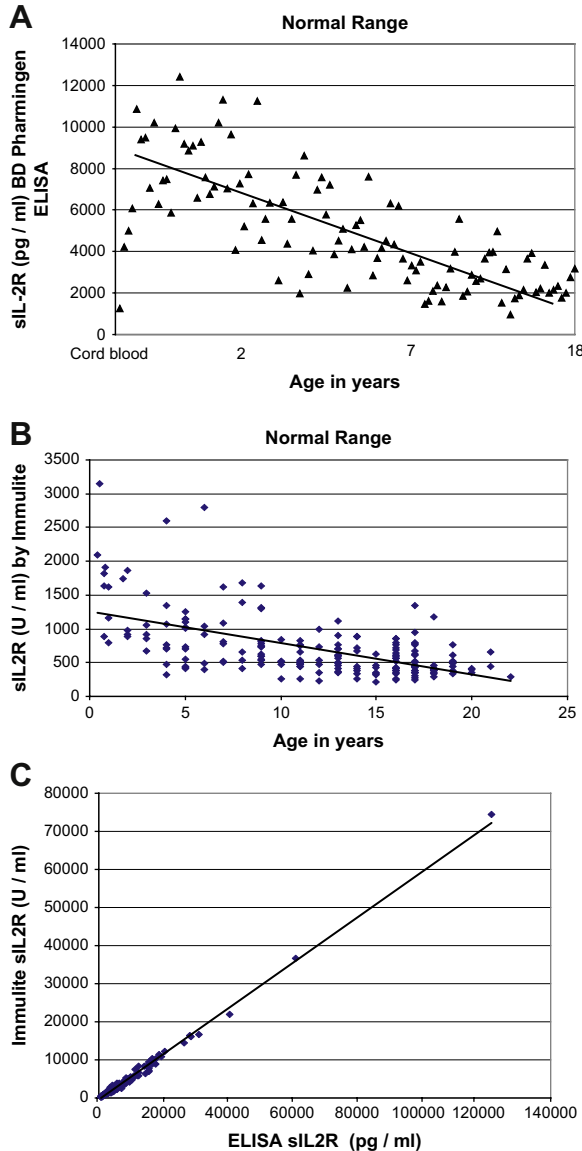


Fig. 2. (A) Plasma levels of sIL2R from normal controls, as determined by the BD Pharmingen ELISA method, displayed according to age of subjects. Note that plasma levels in younger subjects are higher than in adults. (B) Plasma levels of sIL2R from normal subjects, as determined by the Immulite chemiluminescent method, displayed according to age of subjects. (C) Plasma levels of sIL2R from the same control samples, analyzed by two different methods (BD Pharmingen ELISA and Immulite). The levels reveal different absolute values, but perfect correlation between the two methods.

used for analysis. Although trends of sIL2Ra levels are correlated, the quantities reported can vary, depending on the method used (Fig. 2C). Another disease marker is soluble CD163, an anti-inflammatory molecule shed on engagement of Toll-like receptors on monocyte/macrophages [72]. CD163 attaches hematopoietic cells to monocyte/macrophages, facilitating hemophagocytosis. Examination of bone marrow and liver biopsies for CD163 expression in hemophagocytic syndromes frequently reveals extensive activation of resident or infiltrating monocyte/macrophages, far exceeding the microscopic findings of hemophagocytosis (Fig. 3). In some cases, plasma proinflammatory cytokines, such as tumor necrosis factor (TNF)- α , interferon- γ , IL-1, IL-6, and IL-18, are also elevated, but these are not specific for the diagnosis of HLH; rather, trends in circulating cytokine levels may be more informative in predicting response to therapy. Recently, soluble TWEAK (a TNF-related inducer of apoptosis) has also been suggested as a biomarker for HLH [73].

In most familial cases of HLH, NK function is low or absent, although the numbers of circulating NK cells (CD56+/16+) are generally normal. However, the finding of NK function within normal limits, especially during active symptomatic disease, should not preclude a diagnosis of primary or secondary HLH. A recent review of patients diagnosed with HLH due to mutations in perforin or MUNC 13-4, through the Molecular Genetics Laboratory at Cincinnati Children's Hospital Medical Center, found that 24% of patients who had perforin deficiency (17/69) and 35% of patients who had MUNC 13-4 defects (14/41) demonstrated normal NK function in a standard chromium release assay. In another group of patients who had HLH but normal sequences of PRF1 and MUNC 13-4, only 59% demonstrated decreased NK function.

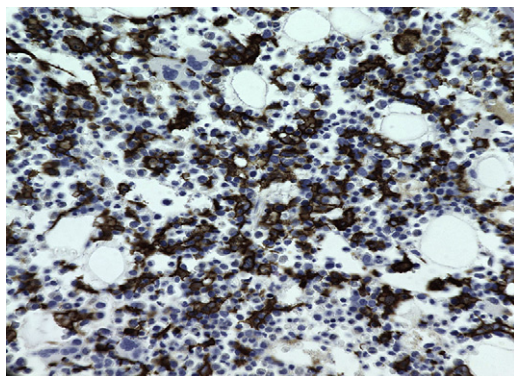


Fig. 3. In situ staining for CD163 expression on cells from a bone marrow biopsy of a patient with HLH. Abundant histiocytes expressing CD163 are seen. (Courtesy of Dr. J. Mo, Division of Pathology, Cincinnati Children's Hospital, Cincinnati, OH.)

Screening assays using flow cytometry of cytotoxic cells have been developed to assist the rapid diagnosis of distinct genetic subtypes of HLH. Intracellular staining for perforin [74] and SAP [75] and BIRC4 (Filipovich, personal observation) can identify patients who have FHL2, XLP, and XIAP deficiency, respectively. Recently, it has been proposed that decreased surface expression of CD107a (LAMP 1) [27,76] on NK cells can predict the presence of mutations in MUNC 13-4 and syntaxin 11 genes. CD107a is a molecule expressed on the membrane of the secretory granules in cytotoxic T cells and NK cells. When degranulation occurs, after fusion of the granules' molecular structures with the cytoplasmic membrane of the cytotoxic cell, CD107a is transiently detectable on the surface of the cytotoxic cell and can be quantitated by surface staining. This approach can serve as an initial screen for FHL3 and FHL4, for which no rapid screening tests have yet been developed, and may potentially help identify patients who have related genes causal for HLH (Fig. 4).

The goals of the diagnostic evaluation are

- To exclude other underlying conditions (eg, malignant disease)
- To identify coexisting infections
- To establish the extent of the disease (eg, CNS involvement)
- To collect materials for future studies (eg, genetic testing)

Treatment of hemophagocytic lymphohistiocytosis and related disorders

A retrospective review of HLH 25 years ago described mean survival of less than a month after symptomatic onset and 5% overall survival at 1 year

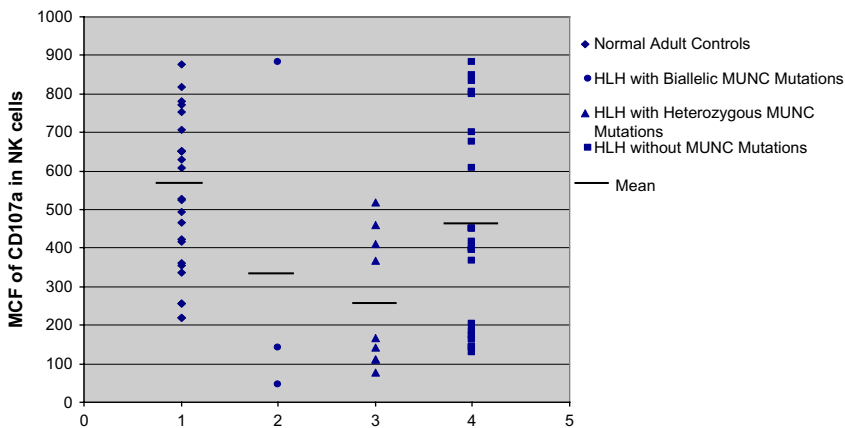


Fig. 4. Results of studies examining the proportion of NK cells expressing CD107a by flow cytometry 6 hours after exposure to K562 target cells. Results from normal controls on the left are compared with results observed with NK cells from patients who have HLH, patients who carry biallelic or heterozygous mutations in MUNC 13-4, and patients who have HLH for whom the genetic cause has not been determined.

after diagnosis [77]. Today, effective initial therapy of HLH consists of combinations of proapoptotic chemotherapy and immunosuppressive drugs targeting the hyperactivated T cells and histiocytes. Currently, definitive treatment of, and a potential cure for, FLH is only achieved by hematopoietic cell therapy (HCT). Projected survival rates, 5 years from diagnosis, range from 50% to 70% [44].

Because HLH can be rapidly fatal without specific intervention, it is recommended that treatment be started when a high clinical suspicion exists, even when results of diagnostic studies are still pending. Effective treatments for HLH have included therapies that target activated macrophages/histiocytes (etoposide, steroids, high-dose intravenous IgG) or activated T cells (steroids, cyclosporine A, antithymocyte globulins, 2 CdA). Generally, combinations of drugs that interrupt the “vicious cycle” of immune activation in HLH are most effective. Anecdotal reports of the use of anti-TNF antibodies, Campath-1H (a humanized anti-CD52 antibody that targets antigen-presenting cells and T cells), and abatacept (CTLA4 agonist) have claimed benefit in symptom control. The need to treat coexisting infections, potential triggers of HLH, is obvious.

Results of the international consensus protocol sponsored by the Histiocyte Society for treatment of patients newly diagnosed with HLH (HLH-94) were published in 2005 [78]. The goals of the trial were to achieve clinical remission of the life-threatening inflammation and to provide potentially curative therapy with allogeneic HCT. Initial treatment combined intravenous etoposide and intravenous or oral dexamethasone (for CNS penetration), with or without intrathecal methotrexate, followed by a maintenance therapy with the addition of cyclosporine A. Approximately 75% of eligible patients who had *de novo* HLH who started HLH-94 therapy achieved satisfactory clinical remission after 8 weeks of treatment. However, nearly a quarter of patients on protocol experienced persistent or recrudescing symptoms and ultimately died from complications of HLH. Overall, results with HLH-94 show a 55% HLH-free survival at 3 years. A minor proportion of patients came “off therapy” after 8 weeks because they were symptom free and lacked features of familial disease; some have remained healthy for several years off therapy. HLH-94-like therapy has been effective in control of hemophagocytic reactions in patients who have XLP, CHS, Griscelli syndrome, viral-associated HLH (especially EBV), and MAS.

Some patients have continued maintenance treatment with episodic etoposide for a year or more after diagnosis of HLH; such prolonged exposure to etoposide has been associated with evolution to acute myeloid leukemia [78]. Because no genetic studies related to defects potentially underlying HLH have been published in such cases, it is not clear whether predisposition to malignant transformation is inherent or acquired.

HLH 2004 (which represents a modest modification of HLH-94) is currently enrolling patients.

Patients who proceeded to HCT after initial treatment with HLH-94 experienced a 3-year disease-free survival of 63% [39]. HCT recipients of matched related or matched unrelated donor grafts achieved a 3-year disease-free survival of approximately 70%, in contrast to that of recipients of mismatched related or unrelated donor grafts (50%) [77]. Similarly, promising results with HCT have been published from centers in Europe [79–81], and with the use of reduced-intensity pretransplant conditioning [82]. Results of posttransplant chimerism studies reported in the French transplant series confirm that stable mixed chimerism can be associated with freedom from HLH relapse [80], although the relevant cell types contributed by the donor graft have not been fully characterized.

The best results with HCT have been observed in children who experienced prompt and complete response to the HLH-94 before transplant, and were free of significant CNS involvement [83].

Several posttransplant complications are reported with significant frequency, including severe inflammatory reactions during the early engraftment period, often mimicking HLH! Acute graft-versus-host disease has occurred in 30% to 60% of patients who had HLH treated with conventional chemotherapy, and posttransplant autoimmune hemolytic anemia, and, to a lesser degree, other immune cytopenias, are commonly seen. Late complications of prior CNS damage can manifest months to years after HCT with neurocognitive deficits. Fortunately, long-term follow-up of survivors of HCT for HLH indicates that most children return to a normal or near-normal quality of life [84].

Frequently asked questions about hemophagocytic lymphohistiocytosis

1. Why is the disease named hemophagocytic lymphohistiocytosis versus macrophage activation syndrome or lymphohistiocytic activation syndrome?

Hemophagocytosis (typically sought on bone marrow biopsy) is not a universal finding of this group of disorders, even in the most fulminant forms. Indeed, some controversy exists as to whether the phenomenon recognized by the findings of hematopoietic cells engulfed by activated monocyte/macrophages represents phagocytosis, pinocytosis, or some related mechanism. Perhaps in the future a new name for this group of disorders will be adopted.

2. How common is natural killer cell dysfunction in hemophagocytic lymphohistiocytosis?

The HLH 2004 protocol, developed by the working party of the Histiocyte Society, indicated that decreased or absent NK function showed high sensitivity and specificity for the diagnosis of HLH. Recent studies from North America indicate that substantial numbers of patients who have

known genetic defects associated with HLH demonstrate normal NK function during active disease (up to 40%) (see text). The likely reason for the discrepancy between this newer data and that used for the analysis to develop diagnostic markers for HLH 2004 is the greater heterogeneity in ethnic backgrounds and diversity of underlying genetic causes represented in the much larger North American series than that available at a few selected European centers for the earlier analysis.

3. Is hemophagocytic lymphohistiocytosis predominantly a disease of infants?

With the description of several genetic defects causing life-threatening histiocytic disorders, it has become clear that HLH can present at any time, from intrauterine development into the seventh decade of life. “Null” mutations of HLH-related genes are associated with younger age at symptomatic onset, whereas mutations resulting in some synthesis of mature protein, often associated with missense mutations, may result in symptomatic disease at older ages.

4. Is evidence of hemophagocytosis required for the diagnosis of hemophagocytic lymphohistiocytosis?

Although “hemophagocytosis” is a hallmark of activated histiocytes, the finding of hemophagocytosis on bone marrow biopsy (the most readily accessible tissue for differential diagnosis of pancytopenia) is not a prerequisite for the clinical diagnosis of HLH. Hemophagocytosis may not be evident even during fulminant disease. Other noninvasive assays supporting the presence of highly activated histiocytes are being developed, such as the detection of soluble CD163 in plasma samples. Recent work suggests that engulfment of hematopoietic cells by activated histiocytes requires expression of specific surface receptors, among them CD163, which is involved in the pathway of ferritin-related hemoglobin salvage, a putative homeostatic anti-inflammatory pathway. Autopsy studies indicate that hemophagocytosis is more commonly observed in the reticuloendothelial organs (liver, spleen, lymph nodes) than in bone marrow or other tissues. Hemophagocytosis can also be detected on cytopsin slides of cerebrospinal fluid, when the CNS is involved with disease. Recent studies staining bone marrow and liver biopsies for CD163 expression of histiocytes in HLH and related disorders indicate a high level of activated cell populations, even when hemophagocytosis is scant or not apparent.

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