Hemophagocytic lymphohistiocytosis (HLH) and related disorders

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Hemophagocytic lymphohistiocytosis (HLH), which has many genetic causes, is characterized by multisystem inflammation. HLH is a reactive process resulting from prolonged and excessive activation of antigen presenting cells (macrophages, histiocytes) and CD8⁺ T cells. Hemophagocytosis, which is mediated through the CD163 heme-scavenging receptor, is a hallmark of activated macrophages/histiocytes and is the characteristic finding for which the disorder was named. The majority of genetic causes identified to date affect the cytotoxic function of NK and T cells, crippling immunologic mechanisms that mediate natural immune contraction. The predominant clinical findings of HLH are fevers (often hectic and persistent), cytopenias, hepatitis and splenomegaly. Due to the life-threatening implications of the diagnosis of genetically determined HLH, antiinflammatory therapy, often consisting of steroids, etoposide or antithymocyte globulin (ATG), should be instituted promptly, followed by curative hematopoietic cell transplantation. Secondary HLH, associated with autoimmune disorders or viral infections in teens and adults, also carries a significant mortality rate and should be managed in consultation with specialists familiar with the diagnosis and treatment of such disorders.

The predominant clinical findings of hemophagocytic lymphohistiocytosis (HLH) are fevers (often hectic and persistent), cytopenias, hepatitis and splenomegaly. Until recently, it was widely believed that symptoms of HLH due to genetic causes 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, from prenatal presentations through the seventh decade. Distinctions between primary (genetically determined) and secondary (acquired) forms of HLH have become increasingly blurred together as new genetic causes are identified.

Patients who develop HLH beyond early childhood or in the context of Epstein-Barr virus (EBV) infection or autoimmune diseases are being found to share some of the same genetic etiologies as infants with documented familial disease.

HLH results when critical regulatory pathways responsible for the natural termination of immune/inflammatory responses are disrupted or overwhelmed. In HLH, pathologic genetic defects alter normal crosstalk between innate and adaptive immune responses in a manner that compromises homeostatic removal of cells that are superfluous or dangerous to the organism. The result is excessive and persistent activation of antigen-presenting cells (histiocytes) and T lymphocytes. The clinical findings associated with systemic inflammation such as prolonged high fevers and hepatitis reflect this immunologic perturbation.

Currently, HLH (FHL:OMIM 267700, 603553) includes the autosomal recessive genetic disorders as well as the "secondary" forms. In recent years, many more cases of hemophagocytic disorders have been diagnosed, especially in the context of severe inflammatory reactions to viral exposure including, in addition to EBV, HIV and Avian influenza.

Pathogenesis of HLH and Other Histiocytic Disorders

HLH is characterized by multisystem inflammation—a reactive process resulting from prolonged and excessive 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 NK cells have been observed in a proportion of patients with all forms of HLH.

NK and NKT cells play a major role in maintaining a healthy threshold of immune responsiveness to noxious external stimuli and are critical to prevention and control of autoimmune conditions and severe reactions to viral infections. NK cells form a frontline of defense against intracellular pathogens, such as viruses, which infect non-lymphoid tissues early upon entry into the patient. NK cells modulate the initial responses of antigen-presenting cells to incoming pathogens (likely through cytokine signaling), thus attenuating the subsequent activation of antigen-specific T cells. NK cells likely also play a role in culling activated T cells and histiocytes in later stages of antigen-driven activation, contributing to the natural contraction of the immune response.

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

Studies of cytokine levels in blood and tissues have indicated persistently elevated circulating levels of multiple proinflammatory cytokines during symptomatic disease. Recently, gene expression analysis of mononuclear cell samples from patients with several different genotypes of HLH have consistently demonstrated highly increased expression of interleukin (IL)1b, tumor necrosis factor (TNF)α, IL-6 and IL-8. Elevations in plasma levels of interferon-γ have been previously published in EBV-driven HLH in Asian populations, as well as in a murine model of HLH triggered by LCMV.[1] It is currently believed that "hypercytokinemia" and possibly "hyperchemokinemia" generated by uncontrolled activation of histiocytes and T cells underlies the progressive organ dysfunction that eventually leads to death in affected patients. These symptoms and signs include fevers, hyperlipidemia, endothelial activation/coagulopathy, hepatitis triaditis, central nervous system (CNS) vasculitis and demyelination, inflammatory lung disease with acute respiratory distress syndrome (ARDS) and marrow hyperplasia or aplasia. Hemophagocytosis, the characteristic finding for which the disorder was named, is a hallmark of activated macrophages/histiocytes.

Genetics of HLH and Other Hemophagocytic Disorders

To date, autosomal recessive genetic defects associated with HLH (**Table 1**) are related to one another in the pathway of granule-mediated cytotoxicity. These genetic defects interrupt mechanisms responsible for triggered apoptosis (mediated by cytotoxic cells upon the target cell) or activation-induced apoptosis (putative suicide of activated T cells). The first gene reported in 1999 to be a cause of HLH (FHL2) was perforin, PRF1, a soluble, pore-forming cytolytic protein synthesized in cytotoxic lymphocytes and sequestered, along with Granzyme serine proteases, in secretory cytotoxic granules.[2] When cytotoxic cells contact their targets, an intracellular cytoskeletal scaffold (the microtubule organizing center, or 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, permeabilize the target cell membrane and enable delivery of Granzyme B into the target cell. Once internalized, Granzyme B initiates both caspase-dependant and caspase-independent apoptotic pathways, thus killing the target cell. No defects in Granzyme B have been identified in association with human HLH.

A second gene responsible for HLH (FHL3) was reported in 2003: MUNC 13-4.[3] MUNC 13-4 was described as essential for cytolytic granule fusion with other structures related to the cytoplasmic membrane in the process of degranulation. The gene defect responsible for FHL4 is Syntaxin 11,[4] which has been shown, as in MUNC 13-4 deficiency, to result in defective degranulation. Syntaxin 11 is a member of the SNARE protein family, which facilitates fusion in intracellular membrane trafficking events. It was recently shown to be expressed in NK cells and activated CTLs.[5] Although apparently far less common than defects in PRF1 and MUNC 13-4, cases attributed to STX11 deficiency have a worldwide distribution. The genetic defect responsible for FHL1 linked to chromosome 9 in a study of two extended Pakistani families has not yet been discovered.

Related hemophagocytic disorders occur with low frequency in five other genetic diseases that have been linked with defective cytotoxic function. Three distinct immunodeficiencies that are typically associated with

Table 1. Genetic causes of hemophagocytic lymphohistiocytosis (HLH).

HLH related to defects in the perforin/granule-mediated pathway of cytotoxicity
FHL 2 – Perforin (PRF1) AR
FHL 3 – MUNC 13-4 AR
FHL4 – STX11 AR
Griscelli s. type 2 – Rab27A AR
Chediak Higashi s. – LYST1 AR
Hermansky Pudlak s. type II - AP3B1 AR
X-linked syndromes associated with HLH
XLP1 – SH2D1A (SAP) X
XLP2 – BIRC4 (XIAP) X

AR indicates autosomal recessive; X, X-linked.

pseudoalbinism due to defects in lysosomal trafficking have been associated with life-threatening episodes of HLH: Chediak-Higashi syndrome (LYST, or CHS1),[6] Griscelli syndrome (Rab27A),[7] and Hermansky-Pudlak syndrome type II (AP3B1).[8] Rab27a, a small Rho GTPase, interacts directly with MUNC 13-4 and is thought to play a role in docking of the cytotoxic granules on the MTOC. These diagnoses can be suspected on physical examination due to the presence of very fair or grayish hair in many affected patients and by detection of distinctive laboratory abnormalities of the neutrophil and platelet compartments.

HLH following exposure to EBV and, less commonly, other viruses, termed fulminant infectious mononucleosis, is the most frequent life-threatening complication of X-linked lymphoproliferative syndrome (XLP1). XLP1 is caused by hemizygous mutations in SH2D1A encoding SAP (SLAM-associated protein), which lead to abnormal NK cell responses and iNKT cell deficiency. Recent research suggests that lymphocytes from patients with XLP1 demonstrate decreased activation-induced apoptosis, which contributes to the lymphoproliferative clinical phenotypes. X-linked lymphoproliferative syndrome 2 (XLP2) due to hemizygous mutations in X-linked inhibitor-of-apoptosis (XIAP, or BIRC4) has been described in males who develop sporadic as well as EBV-associated HLH.[9] Patients with 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.

Taken together, the nine genetic disorders described above still account for less than half of the diagnosed patients with HLH in North America who have been tested at the reference laboratory in Cincinnati Children's Hospital Medical Center, including many familial cases still awaiting molecular definition.

Clinical Presentation of HLH

Until recently, it was widely believed that symptoms of familial hemophagocytic lymphohistiocytosis (FHL) generally arose during infancy and early childhood. With the more widespread availability of genetic testing, it is apparent that the first significant episode of FHL can occur throughout life,[10] including in utero.

Despite attempts to differentiate primary from secondary or reactive forms of FHL, the symptomatic presentations are highly overlapping. In the most typical form of FHL,[11] the clinical course is characterized by prolonged fevers and hepatosplenomegaly. Neurologic symptoms may dominate the initial clinical course with seizures and/or ataxia. Neurologic findings may be highly variable and can include irritability, hypo or hypertonia, cranial nerve palsies, meningismus, 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, hemophagocytosis may not be obvious on bone marrow biopsy examination early in the course of the disease.[12]

Diagnosis of HLH

To assist with the rapid diagnosis of HLH, the Histiocyte Society has developed a set of diagnostic guidelines that encompass both clinical and laboratory findings.[12] With additional experience these diagnostic criteria have been modestly modified, as shown in **Table 2**. A constellation of these features in the absence of a family history or specific genetic diagnosis can contribute to a provisional diagnosis of HLH and support the need for initiation of HLH-specific therapy.

Hemophagocytosis may not be clearly apparent in the initial bone marrow biopsy early in the disease process. 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. Immunologic criteria for provisional diagnosis include elevated levels of ferritin[13] and soluble IL2Rα (sCD25),[14] both markers of generalized inflammation. Ferritin is induced during the

Table 2. Proposed HLH diagnostic criteria, 2009.

1. Molecular diagnosis of hemophagocytic lymphohistiocytosis (HLH) or X-linked lymphoproliferative syndrome (XLP).
2. Or at least 3 of 4:
   a. Fever
   b. Splenomegaly
   c. Cytopenias (minimum 2 cell lines reduced)
   d. Hepatitis
3. And at least 1 of 4:
   a. Hemophagocytosis
   b. ↑ Ferritin
   c. ↑ sIL2Rα (age based)
   d. Absent or very decreased NK function
4. Other results supportive of HLH diagnosis:
   a. Hypertriglyceridemia
   b. Hypofibrinogenemia
   c. Hyponatremia

protective anti-inflammatory process of macrophage scavenging of heme through the CD163 receptor, as is IL-10. Upregulation of CD163 on monocyte/macrophages facilitates hemophagocytosis. Very high levels of sIL2Rα are almost never seen outside HLH. Normal ranges for levels of sIL2Rα vary with age: highest in infants, and lower in teens and adults.

Symptoms of CNS dysfunction, cerebrospinal fluid pleocytosis, or findings of foci of inflammation by CNS MRI scanning are found in more than half of patients with HLH during the first several weeks from initial clinical presentation.[15] NK function is low or absent in many patients with HLH at initial presentation, although the number 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 FHL or secondary HLH

Screening assays have been developed using intracelluar staining for relevant proteins by flow cytometry of cytotoxic cells to assist the rapid diagnosis of distinct genetic subtypes of HLH such as perforin deficiency[16] and XLP1.[17]

In summary, the goals of the diagnostic evaluation are (1) to exclude other underlying conditions (eg, malignant disease), (2) to identify coexisting infections, (3) to establish the extent of the disease, eg, CNS involvement, and (4) to collect materials for future studies, eg, genetic testing.

Treatment of HLH and Related Disorders

A retrospective review of FHL 25 years ago described mean survival of less than a month after symptomatic onset and 5% overall survival at 1 year after diagnosis. Today, effective initial therapy of HLH (FHL) consists of combinations of proapoptotic chemotherapy and immunosuppressive drugs targeting the hyperactivated T cells and histiocytes. Currently, definitive treatment and potential cure of FHL is only achieved by hematopoietic cell transplantation (HCT). Projected survival rates 5 years from diagnosis range from 50% to 70%.[18]

Since HLH can be rapidly fatal without specific intervention, it is recommended that treatment be started when there is a high clinical suspicion, even when results of some diagnostic studies are still pending. Effective treatments for HLH have included therapies that target activated macrophages/histiocytes (etoposide, steroids, high-dose IVIgG) and/or activated T cells (steroids, Cyclosporine A, antithymocyte globulins, 2 CdA, Campath 1H). The need to treat coexisting infections, potential triggers of HLH, is obvious. The results of the only international, multi-institutional, prospective study for treatment of HLH, HLH 94, have been published.[18] The best results with HCT have been observed in children who experienced prompt and complete response to induction treatment with the HLH-94 protocol prior to transplantation and were free of significant CNS involvement.[19] 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.[19]

Disclosures

Conflict-of-interest disclosure: The author declares no competing financial interests.
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