

The expanding spectrum of hemophagocytic lymphohistiocytosis

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Purpose of review

Hemophagocytic lymphohistiocytosis (HLH) is more widely recognized by clinicians. No longer viewed as a disorder of young children, adult patients are now being identified and treated. In this review, I summarize clinical features of patients with recently identified genetic causes, discuss a new paradigm for understanding the clinical evolution of HLH, and update current results with hematopoietic cell transplantation.

Recent findings

The list of genetic defects underlying HLH continues to grow. Among the autosomal recessive defects underlying HLH, we add STX11 (Syntaxin 11) – a snare protein, and MUNC18-2 (also known as STXBP2 – Syntaxin-binding protein). These two proteins now join MUNC 13-4 as components of the degranulation machinery in cytotoxic lymphocytes, responsible for the delivery of Perforin and Granzyme B to selectively kill target cells. The mechanism of action in the newest X-linked disorder associated with HLH, XIAP deficiency (also termed XLP 2), is currently unknown. Treatment of HLH has also improved in recent years, at least in experienced centers where a significant number of patients are seen. Clinicians who are familiar with the dynamic evolution of the disease are learning how to modify treatment when initial or continuation therapy fails to achieve a stable clinical status, preferably clinical remission. Use of reduced intensity conditioning protocols pretransplant has resulted in superior short-term and long-term survival rates of greater than 85%.

Summary

Substantial progress continues to be made in exploring the complex cause and pathophysiology of HLH. Hand in hand, a greater recognition of the condition has led to improved treatments.

Keywords

cytotoxicity, hemophagocytic lymphohistiocytosis, MUNC 13-4, MUNC 13-4 (STXBP2), NK cells, perforin, SAP, STX11, XIAP deficiency

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a group of immunodeficiencies characterized by clinical signs and symptoms of extreme inflammation. It was first published in 1952 as an autosomal recessive immune dysregulatory disorder of childhood, called ‘familial hemophagocytic reticulosis’ [1]. In following decades, HLH was described as both a familial disorder and as a sporadic one, in association with infections, malignancies, or rheumatologic disorders (often referred to as secondary HLH). The disorder is characterized by severe pathologic immune activation and, in many cases, cytotoxic deficiencies. The first genetic cause of HLH, discovered in 1999, was the finding of pathologic mutations in the perforin gene, *PRF1*, in a cohort of affected patients [2]. Subsequently, animal studies demonstrated that cytotoxic deficiency leads to abnormal T cell activation

and inflammatory cytokine production, which drive disease development [3,4].

The evolution of clinically serious HLH progresses through several steps. Underlying the susceptibility to HLH is a predisposing immunodeficiency which may be identified by the finding of a genetic defect associated with HLH, and/or low or absent natural killer (NK) cell function, a family history of HLH or individual history of prior episodes of suspected HLH.

Significant immune activation is the next step, with features of fever and splenomegaly. T-cell activation is seen through immunophenotyping indicating activated and expanded CD8 T cells, as well as marked elevations in sIL2Ra (sCD25 released by activated and dividing T cells). Macrophage (histiocyte) activation is reflected in elevated ferritin levels and sCD163 deriving from these

cells. Levels of plasma cytokines including IL-1, IL-8, IL-6, gamma interferon and others are frequently elevated with active HLH and may decline with response to therapy.

The final stage is immunopathology which can affect almost any tissue. In the bone marrow, hemophagocytosis is the hallmark of activated histiocytes and is accompanied by destructive cytopenias. Decreased fibrinogen and increased triglycerides are seen, along with hepatitis and central nervous system inflammation and symptoms in at least 50% of cases.

Recent work with whole-genome expression analyses during the acute phase of HLH suggest paradoxical downregulation of various aspects of the immune response, including B cell development and function, toll-like receptor (TLR) expression and signaling, and apoptosis induction [5].

Diagnosis of hemophagocytic lymphohistiocytosis

Despite increasing insights into its genetic and immunologic basis, HLH remains a syndrome, defined and diagnosed by a unique pattern of clinical findings. Although the individual signs or symptoms of HLH may occur in a variety of clinical circumstances, the combination of these features, caused by pathologic inflammation, forms the pattern of HLH. Though disease-associated genetic defects are a part of common diagnostic criteria, genetic testing is most useful to confirm a clinical diagnosis, predict the risk of future recurrence in affected patients, and to define HLH predisposition in asymptomatic family members. As part of the HLH-94 clinical trial, The Histiocyte Society proposed defining clinical characteristics of HLH in 1994. This set of criteria was later revised for the HLH-2004 trial and serves as a current *de facto* diagnostic definition of HLH [6]. In addition to the criteria listed below [7], evidence of inflammatory cells in the spinal fluid (lymphocytes and macrophages) as well as an elevated CSF protein and/or characteristic findings on the brain MRI is highly suspicious for HLH. Neurologic symptoms observed in patients with HLH are broad-ranging varying from lethargy to nerve palsies, seizures and coma [8]. The diagnosis of HLH may be established by (additionally, in the case of familial HLH, no evidence of malignancy should be apparent)

- (1) molecular diagnosis consistent with HLH: pathologic mutations of *PRF1*, *UNC13D*, *Munc18-2*, *Rab27a*, *STX11*, *SH2D1A* or *BIRC4*; or
- (2) five out of the eight criteria listed below are fulfilled:
 - (a) fever of at least 38.3 °C;
 - (b) splenomegaly;

Key points

- Discovery of additional genetic defects involved with pathogenesis of hemophagocytic lymphohistiocytosis (HLH) supports the central role for defective immune cytotoxicity in the development of the disease.
- A greater recognition of HLH has led to more diagnoses in affected adults.
- Use of more immunomodulatory, reduced intensity chemotherapy (RIC) prior to hematopoietic cell transplantation leads to improved long-term survival and cure of HLH.

- (c) cytopenias (affecting at least two of three lineages in the peripheral blood): hemoglobin less than 9 g/dl (in infants <4 weeks: hemoglobin <10 g/dl); platelets less than $100 \times 10^3/\text{ml}$; neutrophils less than $1 \times 10^3/\text{ml}$;
- (d) hypertriglyceridemia (fasting >265 mg/dl) and/or hypofibrinogenemia (<150 mg/dl);
- (e) hemophagocytosis in bone marrow or spleen or lymph nodes or liver;
- (f) low or absent NK-cell activity;
- (g) ferritin greater than 500 ng/ml (while the HLH-2004 protocol uses ferritin >500 ng/ml in practice, ferritin >3000 ng/ml is concerning for HLH and ferritin >10 000 as highly suspicious [9]);
- (h) elevated soluble CD25 (soluble IL-2 receptor alpha) (elevations above age-adjusted, laboratory-specific normal levels (defined as >2 SD from the mean) appear more meaningful than the original designation of '>2400 U/ml', because of variations between laboratories [10]).

In addition to the signs and symptoms described above, other clinical features may be prominent in distinct genetic forms. In patients with STXBP [9,11,12], chronic and severe colitis is present in a significant proportion of patients [11] as is hypogammaglobulinemia. With greater awareness of B cell dysfunction in several forms of HLH, hypogammaglobulinemia is more frequently diagnosed even in the absence of apparent protein loss from the bowel. MUNC 18-2 and MUNC 13-4 deficiency often result in platelet dysfunction with consequences ranging from easy bruising to excessive bleeding with surgical procedures.

Men with XIAP deficiency also have a significant incidence of colitis, which may be the most serious complication in a given patient; diagnosis is often delayed as patients may be managed primarily by gastroenterologists. XIAP deficiency is described in association with chronic splenomegaly and recurrent, self-remitting bouts of active HLH [13].

'Secondary' HLH can occur in the setting of a growing variety of well defined primary immunodeficiencies and generally responds to the same therapy recommended for familial HLH.

Genetic testing

A logical search for the genetic cause of HLH should reflect an understanding of the most likely candidates based on sex and ethnic background of the patient. Several HLH screening tests using flow cytometry are clinically available in the USA, Japan, China, Australia, Viet Nam and several European countries. Clinically approved flow cytometry assays include those for the two X-linked forms of HLH: SAP and XIAP deficiencies, and Perforin/Granzyme B [14]. Although no defects in Granzyme B have been described in association with HLH, the assay can reveal activation of T cells even in the absence of perforin staining. Degranulation of NK cells is also detected by flow cytometry by quantitating the expression of CD107a (a structural element of the membrane of cytotoxic granules in NK cells) [14]. Abnormalities in CD107a detectable degranulation may suggest defects in MUNC 13-4, MUNC 18-2 and STX11 [15–17]. Screening flow cytometry should be

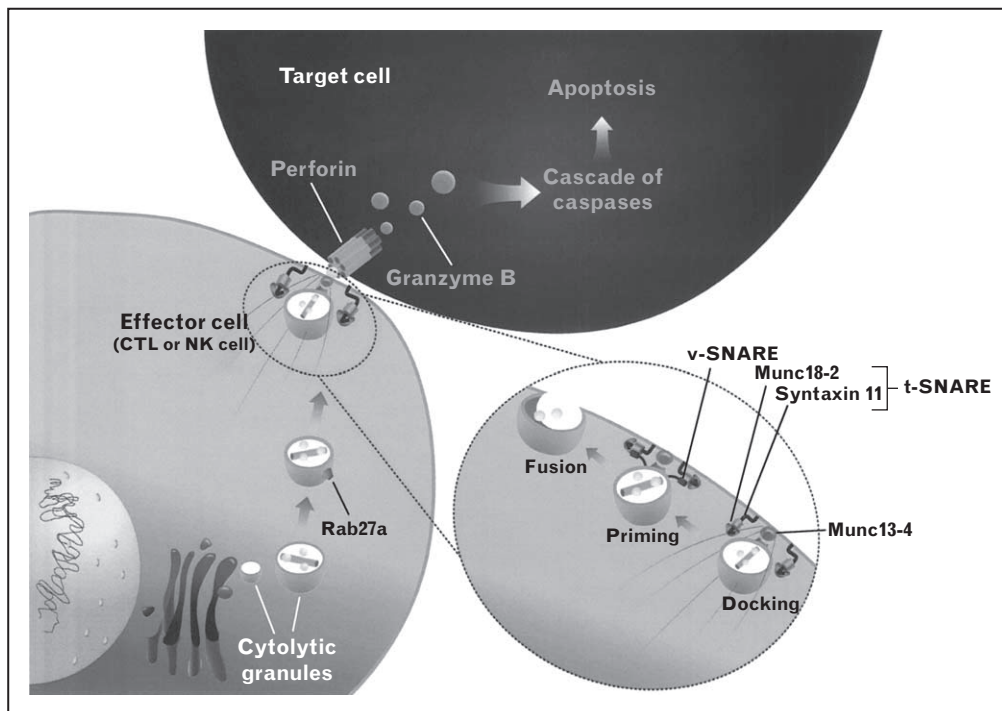
performed in a credentialed laboratory where age specific normal ranges have been determined. Assays in research laboratories comparing a patient's cells to the 'control of the day' have been misleading.

Definitive mutational analysis currently may take weeks, although much less time-consuming techniques are being developed (Fig. 1) [18].

Triggers of hemophagocytic lymphohistiocytosis

A wide variety of pathogens have been identified as triggers of HLH disease. Of note, Epstein–Barr virus (EBV) is a particularly potent activator of T cells and is frequently identified in patients with many genetic forms of the disease. Men with XLP are particularly vulnerable to EBV-driven HLH, as their genetic defect interferes with the activation of NK cells and viral clearance through the EBV-specific receptor (2B4) resulting in the persistence of EBV-infected B cells which activate CD8 T cells driving HLH [10,19•]. Asian patients of both sexes are prone to EBV-associated HLH, wherein the EBV infects T and NK cells and leads to clonal proliferation and persistence of those cells. Many patients

Figure 1 The steps involved in perforin-dependent cytotoxicity



Cytotoxic granules in the effector cells harbor preformed perforin and granzyme B. When the cytotoxic cell is activated, the microtubule organizing center (MTOC) focuses on the point of contact with the target cell where the cytotoxic immunologic synapse will form. The granules are tethered to the MTOC by Rab 27a and dragged to the synapse where the degranulation process takes place involving snare proteins, MUNC 13-4, STX11 and STXBP2 ultimately resulting in release of perforin and Granzyme B into the cleft between the effector and target cell. Perforin polymerizes into a pore which inserts into the membrane of the target cell allowing Granzyme B entry to the cytoplasm of the target cell. Granzyme B then initiates activation of caspases leading to apoptosis of the target cell. See review in [18].

in Asia resolve their EBV-driven T/NK cell associated HLH with treatment protocols similar to those effective in familial HLH and do not require hematopoietic cell transplantation (HCT) [20].

Regrettably, past literature on secondary HLH has often cited association with a viral trigger as a favorable finding with respect to ultimate prognosis, delaying in some cases a genetic diagnosis which would better guide management.

Another important trigger of HLH is leishmaniasis, a secondary form responsive to appropriate antibiotics. This differential diagnosis should be kept in mind within endemic areas for sand flies and in individuals who have traveled to such areas.

Hemophagocytic lymphohistiocytosis in adults

Late-onset familial hemophagocytic lymphohistiocytosis occurs more commonly than previously suspected. A retrospective analysis of genetic and immunological test results of 1531 samples from patients with a suspected diagnosis of HLH identified 175 patients 18 years or older. Missense and splice site sequence variants in PRF1, MUNC 13-4 and MUNC 18-2 genes were found in 25 adults (14%) [21]. The A91V-PRF1 genotype was identified (biallelic or monoallelic) in half the patients. Although this mutation has been argued to be a SNP, cumulative evidence suggests that it is not biologically neutral; indeed reports of lymphoid malignancies associated with this mutation are increasing [22]. Older adults with HLH may benefit by treatment with steroids alone – although steroid therapy can exacerbate or induce comorbidities.

Advances in treatment of hemophagocytic lymphohistiocytosis

The combination of etoposide and decadron proposed in HLH 1994 remains a useful frontline therapy available in virtually all of the developed world, benefitting 60–70% of children and adults with HLH by inducing initial clinical remission of inflammatory symptoms. However, HLH may persist or recur as immune suppression is reduced. The use of Campath, a humanized monoclonal anti-CD52 antibody, as salvage therapy prior to HCT is being analyzed; this treatment is logical as both activated T cells and activated macrophages (histiocytes) are targeted through CD52.

Induction therapy appears curative for Asian forms of EBV-driven HLH infecting T and NK cells. However, patients harboring pathologic mutations in HLH-associated autosomal or X-linked recessive genes

require allogeneic HCT to achieve durable remission. Historically, the 3-year survival after HCT in patients treated with HLH 94 was 62%; best results (approximately 70% long-term survival) were achieved with matched family or unrelated donors [23]. More recently, use of reduced intensity conditioning protocols, especially Campath based, have led to improved results after HCT. In the context of HCT, Campath targets both host antigen-presenting cells and donor T cells reducing the incidence of clinical graft-versus-host disease and the dampening the overall inflammatory environment which often follows myeloablative pretransplant chemotherapy. In a recent contemporaneous series of HCT with, predominately, unrelated donor HCT, 3-year posttransplant survival improved from 43 to 92% (with no early transplant-related mortality) [24[•],25[•]].

Current clinical research efforts focus on the discovery of additional causal genes, development of rapid HLH diagnostic tools, and improvements in initial induction therapy and more efficacious/less toxic curative HCT approaches.

Conclusion

In the years to come, we anticipate that additional genetic causes of HLH and related conditions will be discovered. With the closing of the HLH 2004 study protocol shortly, diagnostic criteria for future clinical trials will be re-examined, perhaps more in accordance with the stages described above: genetic predisposition, evidence of excessive immune activation, and finally immunopathology. As a better understanding of the pathogenesis of HLH emerges, it is likely that additional existing and emerging biologic pharmaceuticals will enter the compendium of effective treatment for the disease.

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Conflicts of interest

There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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