CLINICAL VIGNETTE

A Blurry Picture: Hemophagocytic Lymphohistiocytosis (HLH)

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a rare, life-threatening group of syndromes characterized by overstimulation of the immune system leading to systemic inflammation, cytokine storm and multi-organ failure. HLH has a high mortality rate and prompt treatment is critical.

HLH is both a familial[1] and a sporadic disorder associated with a variety of triggers including infections, rheumatological diseases and malignancies. Diagnostic criteria for the 2004 HLH trial which are commonly used for clinical diagnosis[2] include:

A. Molecular diagnosis consistent with HLH: Pathologic mutations of *PRF1*, *UNC13D*, *STXBP2*, *Rab27a*, *STX 11*, *SH2D1A* or *XIAP* OR
B. 5 out of 8 criteria below are fulfilled:
   1. Fever > 38.3 C
   2. Splenomegaly
   3. Cytopenias (affecting 2 out 3 cell line lineages)
   4. Hypertriglyceridemia and/or hypofibrinogenemia
   5. Hemophagocytosis in bone marrow, spleen, lymph node or liver
   6. Low or absent natural killer (NK) cell activity
   7. Ferritin > 500 ng/ml
   8. Elevated soluble CD 25 or soluble IL- 2 receptor

Historically, HLH has been classified as either a primary/ familial form (F-HLH) based on pathogenic genetic mutations thought to manifest in early childhood or a secondary/reactive form associated with an underlying inflammatory, malignant or infectious condition. This classification is falling out of favor as it fails to meaningfully assess the cause, prognosis or therapy of HLH. It is now recognized that both forms of HLH can be triggered by immune-activating events and that pathogenic genetic mutations may be present in patients who develop HLH at any age.

Based on these concerns, the North American Consortium for Histiocytosis (NACHO) recommends a new classification system.[3] This revised classification proposes that HLH is considered a syndrome which comprises both "HLH disease," conditions in which immune dysregulation is central to pathogenesis and which meet consensus diagnostic criteria, as well as "HLH disease mimics," conditions including certain malignancies and infections which have an HLH-like presentation but would not benefit from HLH-directed therapy. "HLH disease" is subdivided into the following categories:

- Familial HLH (F-HLH)
- HLH associated with malignancy (M-HLH)
- HLH associated with rheumatic conditions (Rh-HLH, also called macrophage activation syndrome or MAS)
- HLH occurring with immune compromise, either primary or acquired (IC-HLH)
- HLH occurring after certain immune-activating therapies or with drug hypersensitivity (Rx-HLH)
- HLH not associated with other specific conditions (HLH-NOS)

Currently, standard of care treatment for HLH disease is an etoposide and dexamethasone-based regimen per the revised HLH-94 protocol.[2,4] Recently, the FDA has also approved emapalumab, an IFN-g blocking monoclonal antibody, for use in refractory disease. For clinically stable patients with a presumed HLH disease trigger, therapy directed at the underlying trigger alone can be considered.

We present an eighteen-year-old female with systemic juvenile idiopathic arthritis (sJIA) who developed HLH and was also found to have a variant of unknown significance in a gene associated with familial HLH. This case illustrates the challenges in diagnosis of HLH when there is overlap of multiple HLH disease subcategories in a single patient.

Case Summary

The patient was initially diagnosed with sJIA at age 17 after presenting to an outside hospital with quotidian fevers, polyarticular arthritis, transient rash, serositis, lymphadenopathy and elevated liver tests. Over the next several months, she was treated with glucocorticoids, methotrexate and tocilizumab. Methotrexate was discontinued due to worsening rashes and lab abnormalities. Tocilizumab was discontinued after her disease relapsed. She was started on anakinra which was initially effective.

Nine months after initial diagnosis, the patient was admitted to our hospital for suspected relapse of her disease. She presented with quotidian fever, fatigue, new onset Raynaud phenomenon and perniosis. Testing elevated aminotransferases, leukocytosis, ferritin 6,275 ng/mL (reference 8 – 180), triglyceride 701

mg/dL (reference < 90), splenomegaly, mediastinal lymphadenopathy and trace pericardial effusion. Serological testing included positive anti-Ro/SSA antibodies, anti-La/SSB antibodies and rheumatoid factor (RF), which were unexpected given her previous diagnosis of sJIA. Her antinuclear antibody (ANA), anti-double stranded DNA antibody (anti-dsDNA) and cyclic citrullinated peptide (CCP) antibody tests were negative. The diagnosis of HLH was confirmed after further testing revealed decreased NK cell activity, elevated soluble IL-2 receptor at 890 unit/mL (reference 137 – 838) and a bone marrow biopsy showing increased hemophagocytosis. In addition, IL-18 level came back significantly elevated at 615,192 pg/mL (reference range 89 - 549).

Whole exome sequencing and Cincinnati Children's Hospital HLH Gene Sequencing Panel were sent during the hospitalization. She was found to have a variant of unknown significance in *UNC13D*, a gene with bi-allelic variants known to be associated with autosomal recessive familial HLH type 3.

Given the patient's underlying inflammatory disease, the long-term immunomodulatory therapy she had received and the findings on genetic testing, this patient's HLH could be considered to be an overlap presentation of familial HLH, HLH associated with rheumatic conditions and HLH occurring with immune compromise under the North American Consortium for Histiocytosis classification system. Even though the underlying rheumatologic diagnosis was brought into question given the unexpected finding of several positive serologies, her clinical presentation was still thought to be most consistent with sJIA, so the decision was made to target this underlying condition as treatment for her HLH. She received intravenous glucocorticoids and an increased dose of anakinra. She developed drug-induced liver injury on the anakinra and was transitioned to canakinumab. Her clinical condition improved and she was discharged from the hospital on oral prednisone and canakinumab.

Discussion

Familial HLH: F-HLH is sub-classified based on the underlying genetic mutation. These mutations can result in impaired CD8+ T cell and NK cell cytotoxicity or altered lymphocyte activation and survival.[5] In the setting of a trigger such as a viral infection or tissue inflammation, CD8+ T cells and macrophages drive an amplification loop of inflammatory cytokine production. CD8+T cells, unable to kill target cells owing to defective cytolysis, are subject to sustained cell-cell contact, antigen presentation, and stimulation.[6] Sustained activation of CD8+ T cells results in the release of large amounts of IFN-g, a potent activator of macrophages. In response, macrophages produce high levels of IL-1b, IL-6, IL-18, and TNF-a, which may account for many clinical features of HLH.[7] IL-18, in conjunction with IL-12, is believed to drive the further activation of CD8+ T cells and their production of IFN-g.

About 70% of F-HLH is caused by loss of function mutations in *PRF1*, the gene that encodes perforin, which is critical for the release of cytolysis vesicles from T cells and NK cells. Mutations in other genes involved in cytolysis, such as *STX 11* and *STXP 2*, are associated with F-HLH as well.[8,9] These genetic mutations have high penetration, and in F-HLH types 1-5, disease develops within the first year of life.[10] Other familial causes include Primary Immunodeficiency Syndromes (PID) such as Chediak-Higashi syndrome and EBV-triggered X-linked lymphoproliferative disorder type 1 (XLP1).

HLH associated with rheumatic conditions (Rh-HLH): Heterozygous mutations in F-HLH associated genes occur in up to 40% of sJIA patients who develop HLH, a condition also known as macrophage activation syndrome (MAS).[3,11,12] These mutations may lower the threshold for HLH disease, particularly in the setting of chronic inflammation, tissue damage or infection.

Chronic stimulation of TLR 9, along with increased IL-1b and IL-18 signaling, may play important roles in the development of HLH in sJIA, systemic lupus erythematosus (SLE), and with Epstein-Barr virus (EBV) infection.[3,13] HLH may develop in children with sJIA shortly after starting targeted blockers of IL-6, IL-1b or TNF-a suggesting that acute alterations to the inflammatory environment, and not just inflammation itself, is an important factor in disease development.[14] The role of IFN-g in Rh-HLH pathogenesis is not as clear as it is for F-HLH. Some studies have found significant elevations of IFN-g, whereas others have not and in this latter group macrophage activation may rely on TLR stimulation.[15]

HLH occurring with immune compromise, either primary or acquired (IC-HLH): HLH may occur in patients with severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD), Wiskott-Aldrich syndrome, DiGeorge syndrome, X-linked agammaglobulinemia, and autoimmune lymphoproliferative syndrome. HLH can also occur in adults undergoing chemotherapy for cancer or immunosuppressive therapy for inflammatory disease.[5]

In adults, viral infection (primary or reactivation in the setting of immunosuppression) is the most common trigger of IC-HLH, with EBV being the most commonly identified culprit. HLH driven by primary EBV infection occurs mostly in children and adolescents, often in the context of F-HLH and primary immune deficiency syndromes. In adults, HLH is more often triggered by EBV reactivation in the setting of immune compromise. A unifying process in EBV-HLH appears to be the failure to control viral replication in B cells. In all these scenarios, uncontrolled viral replication and sustained antigenic stimulation of T cells appear to drive HLH.[16-18]

Table Comparing Features of F-HLH, IC-HLH and Rh-HLH

	F-HLH	IC-HLH	Rh-HLH
Demographics	Age under 1	Immunocompromised children or adults Children with PID	JIA, Kawasaki's disease, SLE Adults: SLE, Still's disease
Risk factor	Genetic defect	Immunosuppression Defective cytotoxicity	Chronic inflammation Elevated IL-18
Trigger	Viral infection	Viral infection	Unknown
Unique features	Family history Consanguinity CNS disease Severe disease	Children with PID	Falling WBC and platelet count Fever transforms from quotidian to persistent

IL-18 and its significance: Besides the HLH-2004 criteria and the H-Score, different biomarkers have been proposed to help with the diagnosis of HLH. IL-18 levels are being used more frequently for this purpose. Weiss et al. found that a total IL-18 level >24,000 pg/mL distinguished sJIA-associated Rh-HLH from F-HLH with 83% sensitivity and 94% specificity. In addition, a ratio of total IL-18/CXCL9 of <2.3 was found to effectively identify patients with F-HLH, distinguishing them from patients with MAS. The authors also demonstrated that IL-18 binding protein (IL-18BP), the natural inhibitor of IL-18, was more elevated in F-HLH and malignancy-associated HLH, while a higher free IL-18 level was associated with Rh-HLH susceptibility in an experimental mouse model.[19]

These data support the notion that Rh-HLH derives from a primary inflammasome/macrophage activation state with secondary T-cell activation, whereas F- HLH may be more driven by pathologic T-cell activation. Nonetheless, it has been repeatedly observed that heterozygous F-HLH mutations, while common in databases of healthy volunteers, are particularly enriched in Rh-HLH populations.[12]

Conclusion

Evolving insight into the pathogenesis of HLH disease has led to a more nuanced understanding of what was once dichotomized as either "primary" or "secondary" HLH. It is now recognized that HLH disease comprises a spectrum of conditions that arise from a background of varying degrees of pathogenic genetic mutations as well as immune-stimulating environmental triggers. This case demonstrates that mutations in familial HLH-associated genes may be involved in the pathogenesis of some forms of HLH associated with rheumatic conditions and that this distinction can be blurry. The evolution of our understanding of HLH is leading away from a single disease with two separate etiologies, and more towards a state of sustained immune system activation triggered by several different pathways, depending on an individual's genetic predispositions and environmental triggers.

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