Chapter 18
Hemophagocytic Lymphohistiocytosis and EBV Susceptibility

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a severe systemic inflammatory syndrome that can be fatal. HLH occurs when histiocytes and lymphocytes become overactive, and they attack the body instead of bacteria and viruses. Histiocytes, like macrophages, are phagocytes—cells that ingest and destroy pathogens, as well as cellular debris. When histiocytes and lymphocytes are overactive in HLH, they overproduce cytokines which can lead to a cytokine storm. These cytokines also attack blood cells and bone marrow in particular, as well as the spleen, liver, lymph nodes, skin, and even the brain. Initial clinical features are typically high and unremitting fever, rash, hepatitis (liver inflammation), jaundice, an enlarged liver and spleen, pancytopenia (low counts of all blood types), and lymphadenopathy (enlarged lymph nodes). Neurological components such as confusion, seizures, and even coma may also occur.

HLH occurs in someone because they have specific genetic defects that directly cause HLH, in which case it is called Primary HLH. Primary HLH is also sometimes called Familial HLH. The genetic mutations that cause Primary HLH are present at birth. Individuals with Primary HLH often become ill in the first few years of life, and if not diagnosed and treated, this condition is usually fatal. For Primary HLH, treatment typically includes hematopoietic stem cell transplantation (HSCT).

HLH sometimes occurs in people with other medical problems that cause a strong activation of the immune system, such as infection or cancer. HLH in these settings is called Secondary HLH. Secondary HLH may also be associated with medical conditions such as autoimmune diseases, in which HLH is often called Macrophage Activation Syndrome (MAS). It can also be associated with primary immunodeficiency diseases (PI). If secondary HLH and its underlying medical condition are detected promptly and then treated aggressively, the prognosis is improved.

Causes of Primary HLH

Primary HLH is a rare disease, reported in about 1 per 50,000 births worldwide per year. These numbers seem to be increasing slightly, possibly due to increased success in detecting the disease. It is caused by defects in several genes, including *PRF1, UNC13D, STXBP2, STX11, RAB27A, LYST, AP3B1, SH2D1A, MAGT1* and *XIAP/BIRC4*. Individuals have mutations in RAB27A, are said to have Griscelli syndrome, while individuals with mutations in *LYST* are said to have Chediak-Higashi syndrome. When individuals have mutations in *SH2D1A*, and *XIAP/BIRC4*, they are usually classified as having X-linked Lymphoproliferative Disease Type 1 or 2, respectively (XLP1 and XLP2). XLP1 and XLP2 usually occur only in boys as they are X-linked disorders, as is HLH due to *MAGT1* mutations (Table 18:1). All of these genes normally produce proteins that regulate immune cells, but when these are absent or don't work correctly, the cells of the immune system become overactive, expand unchecked, and create the harmful cytokine storm.

Susceptibility to Epstein-Barr virus (EBV) infection

EBV is one of the most common human viruses, infecting most people at some point in their lives. EBV infections in children usually do not cause symptoms, or the symptoms are not distinguishable from other mild, brief childhood illnesses. People who get symptoms from EBV infection, usually teenagers or adults, have

infectious mononucleosis ("Mono"), which usually improves in two to four weeks. However, EBV can cause serious complications in individuals with PI. In particular, for individuals who have genetic defects which predispose them to HLH, EBV infection can become a life-threatening problem due to the development of EBV-driven HLH. Additionally, XLP1 is characterized by extreme vulnerability to EBV infection, and HLH in these individuals is nearly always associated with EBV. Male individuals with *MAGT1* mutations are also highly susceptible to developing HLH from EBV infection.

EBV is also a major triggering factor for Secondary HLH. In this setting, individuals have often been disease-free for most of their lives, but then HLH manifests when EBV infection triggers an immune response that cannot be shut off. In other words, what started as infectious mononucleosis progresses into HLH. Individuals with XLP1, *MAGT1* mutations and other select forms of PI are also prone to develop lymphoma (cancer involving lymph nodes) as a result of EBV infection, and this can occur with or without the development of HLH.

Chronic active EBV infection (CAEBV)

EBV typically infects B cells (the lymphocytes that develop into plasma cells, the antibody producing cells). In individuals without PI, T cells and NK cells mount an immune response against EBV infected B cells thereby controlling EBV infection. In some individuals, EBV predominantly infects T cells or NK cells, causing an EBV related illness lasting less than 3 months known as chronic active EBV (CAEBV) that is characterized by high levels of EBV in the blood. CAEBV occurs in apparently healthy children and adolescents and has been most commonly described in individuals of East Asian or Hispanic origin. In CAEBV, EBV infection causes T cells or NK cells to multiply rapidly and overproduce cytokines, leading to the development of HLH and a lymphoproliferative disorder that can be detected with specialized imaging (e.g., CAT scans). This lymphoproliferative disorder can progress into (EBV-driven) lymphoma. In terms of treatment, special measures are required to control the cytokine storm generated by EBV and to suppress proliferating EBV-containing T or NK cells, because the clinical course often results in a poor outcome, unless HSCT is successful.

Diagnosis of HLH

When most of the typical clinical signs are present and HLH is suspected, blood tests can help confirm the diagnosis by measuring the levels of blood cells, as well as various markers that indicate excessive immune activity. A bone marrow biopsy may be done to look for evidence of hemophagocytosis (bone marrow phagocytic cells that ingest red cells), and a spinal tap may also be performed. Once the diagnosis of HLH is made, it is important to determine whether HLH is Primary or Secondary, because Primary HLH will eventually need HSCT. Genetic testing can look for mutations in genes known to be involved in Primary HLH. There are also special screening tests that can be done to evaluate for genetic causes of HLH. In addition to causing Secondary HLH, infections, particularly viral infections, can also trigger primary HLH. Hence, it is important to screen for viral infections, including EBV. Blood tests can help detect an active infection. In boys with EBV infections, special screening tests can be done to look for XLP1 and XLP2. In individuals of East Asian or Hispanic ethnicity, CAEBV should be considered, and special tests can help determine if EBV is in the T cells or NK cells. Primary HLH often presents very early in life or in in very young children. If HLH occurs for the first time in older children, it is important to consider Secondary HLH due to infection, cancer or autoimmune causes e.g. rheumatologic, but one should not discard the possibility of Primary HLH in older children, or even young adults. In some cases, it may be necessary to complete detailed evaluations for lymphoma or leukemia before starting treatment for HLH.

Treatment of HLH

Once HLH has been diagnosed, therapy should start as soon as possible. HLH therapy includes aggressive courses of immunosuppressants and anti-inflammatory agents such as corticosteroids, chemotherapeutic agents, and anti-cytokine agents. High doses of dexamethasone (corticosteroid) and etoposide (a chemotherapy drug) are the mainstay of treatment for HLH. The cytokine storm can be targeted with drugs such as anakinra or emapalumab, an anti-interferon gamma antibody. If individuals have EBV related HLH, rituximab may be used to deplete B-cells, which are the cells commonly infected with EBV. Other agents used against HLH might include anti-thymocyte globulin (ATG), a T cell depleting antibody, or alemtuzumab, a lymphocyte depleting antibody. In CAEBV, treatment with rituximab is not effective as EBV infects T cells or NK

cells and rituximab targets only the B cells. Special measures that include combinations of the above drugs are required to control the cytokine storm generated by EBV, and to suppress EBV containing T cells or NK cells. Antibiotics, antiviral drugs, anti-fungal drugs, as well as immunoglobulin replacement therapy are often administered to combat infections and provide protection during HLH therapy that invariably is immunosuppressive.

Despite effective treatment of HLH with the above immune suppressive and anti-inflammatory agents, recurrence of HLH is to be expected in individuals who have Primary HLH. HSCT is the only therapy with a possibility of permanently restoring normal immune function. If a genetic defect is identified, curative treatment with HSCT should be considered. The earlier a transplant can be done, the better are its chances of success. In the absence of a genetic defect, HSCT should also be considered for young individuals, those with a family history of bad inflammation or death, recurrent HLH, or laboratory data suggesting an inherited defect in NK cell and T cell cytotoxic function. Individuals with CAEBV also have recurrent episodes of HLH, are at risk for developing lymphoma and invariably need to undergo stem cell transplant to get rid of EBV infection. Without HSCT, CAEBV is often fatal. EBV-driven lymphoma typically responds well to standard lymphoma (chemo) therapy, but depending if an underlying PI is present, may require curative HSCT (timed in conjunction with lymphoma therapy) as well.

Expectations

Primary HLH and HLH related to EBV or other infections remain a life-threatening problem, particularly for individuals with genetic disorders that cause HLH. Understanding of HLH and the role of EBV infection have significantly improved in the past couple of decades. Early recognition and initiation of treatment for HLH is essential for favorable outcome. The next decade promises to yield even further advances in diagnostics and treatment breakthroughs that will continue to improve outcomes. Increased knowledge regarding genetic defects that underlie HLH and PI may help with better separation between Primary and Secondary forms of HLH. Advances in HSCT also make possible transformative treatments for individuals with both Primary and Secondary forms of HLH.

Genetic Defects Associated with Primary HLH

Table 18:1

Primary Immune Deficiency	Gene Mutation	Clinical Features
Familial HLH 2	PRF1	Onset of HLH at a very early age Blacks most likely (98%) to have mutations in PRF1
Familial HLH 3	UNC13D	Neurologic involvement common
Familial HLH 4	STX11	More common in Kurdish, Turkish, and Lebanese ethnic background
Familial HLH 5	STXBP2	Severe diarrhea, bleeding, low Immunoglobulin levels
Chediak-Higashi Syndrome	LYST	Albinism Frequent bacterial infections Progressive neurologic dysfunction that is not corrected by HSCT
Griscelli Syndrome type 2	RAB27A	Partial albinism Silvery grey hair Variable neurological involvement
Hermanski-Pudlak syndrome types 2 and 9	AP3B1 BLOC1S6	Albinism, platelet function defect Bleeding due to defect in platelet function
X-linked lymphoproliferative disease type 1	SH2D1A	Extreme vulnerability to EBV infection Lymphoproliferative disease, including lymphoma Low immunoglobulin levels
X-linked lymphoproliferative disease type 2	BIRC4	Inflammatory bowel disease
XMEN (**X**-linked immunodeficiency with **m**agnesium defect, **E**BV infection, and **n**eoplasia) Disease	MAGT1	Persistent high level EBV infection Frequent sinus/ear/lung infections EBV associated lymphoma
Lysinuric Protein Intolerance	SLC7A7	Episodes of vomiting, diarrhea, stupor or coma after a protein-rich meal, muscle weakness