

Gene Abnormalities in Patients with Hemophagocytic Lymphohistiocytosis

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Abstract

Hemophagocytic lymphohistiocytosis is thought to occur as a primary (familial) form or secondary to infection or malignancy. Recently, several defects in genes important for immune functions were identified in patients with HLH. These include mutations in perforin, the gamma common chain of the receptor for interleukin-2, Slap and purine nucleoside phosphorylase. Since abnormal function of these genes is associated with a wide clinical spectrum, HLH is probably another manifestation of immune deficiency and a thorough immune evaluation should be done in all such patients.

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Hemophagocytic lymphohistiocytosis, a non-Langerhan's cell histiocytosis, is a rare disease that affects mostly young children. HLH is suspected when prolonged fever, hepatosplenomegaly and pancytopenia are associated with liver and coagulation abnormalities, elevated triglyceride and ferritin levels and reduced fibrinogen [1]. Neurologic involvement may also occur, manifesting in irritability, meningitis, convulsions and altered consciousness [2]. The hallmark of HLH is lymphohistiocytic accumulation in the reticuloendothelial system and hemophagocytosis [3]. The diagnosis of HLH is difficult as there are no specific clinical markers or laboratory tests, however guidelines [Table 1] have facilitated the identification of these patients [4].

Traditionally, HLH is thought to include two different conditions that may be difficult to distinguish from each other. The first is primary or familial HLH, which typically affects young infants, with

parental consanguinity or another family member affected by HLH; the other is secondary HLH. The latter group is also referred to as infection-associated HLH or malignancy-associated HLH in which a strong immune activation may result in a lymphohistiocytic proliferation with hemophagocytosis [3]. In familial HLH, the recommended treatment is immunotherapy combined with steroids and a T lymphocyte suppressor to achieve clinically stable remission, and ultimately a cure by bone marrow transplant provided an acceptable donor is available. The non-familial HLH group is frequently associated with malignancies and infections particularly Epstein-Barr virus, which may cause an uncontrolled immune activation; thus the mainstay of treatment is immune suppression [6].

In many patients there is evidence for immune dysfunction [Table 2]. These include impaired monocyte-mediated antibody-dependent cellular cytotoxicity [7], T cell hyperactivation with cytokine overproduction [8,9], reduced T cell number and response to mitogens [10,11], and decreased T cell cytotoxic function [10]. A low number of natural killer cells with reduced NK cell activity that persist during remission is commonly found [12,13], as are other abnormalities [14]. Abnormal thymus architecture and thymocyte depletion even prior to treatment has been documented [15]. Some researchers have also recognized an association of immune deficiencies with HLH [12,16].

The frequent immune abnormalities described among patients with HLH have stimulated research for defects in genes involved in immune functions. Indeed, in the last two years several mutations in such genes were found [Table 3]. This review summarizes these

HLH = hemophagocytic lymphohistiocytosis

NK = natural killer

Table 1. Diagnostic guidelines for hemophagocytic lymphohistiocytosis [4]

Clinical criteria

- Fever
- Splenomegaly

Laboratory criteria

- Cytopenia (Affecting 2 of 3 lineages in the peripheral blood)
 - Hemoglobin (<90 g/L)
 - Platelets (<100 X10⁹/L)
 - Neutrophils (<1.0 X10⁹/L)
- Hypertriglyceridemia and/or hypofibrinogenemia

Histopathologic criteria

- Hemophagocytosis in bone marrow or spleen or lymph nodes
- No evidence of malignancy

Table 2. Immunologic abnormalities in HLH

- Impaired monocyte-mediated antibody-dependent cellular cytotoxicity [7]
- T cell hyperactivation [8]
- Overproduction of interferon- γ , interleukin-2, tissue necrosis factor- α
- Reduced number of CD8+ T lymphocytes [10]
- Decreased T cell cytotoxic function [10]
- Decreased T lymphocyte response to mitogens [11]
- Reduced NK cell activity with low or normal number of NK cells [12]
- Persistence of diminished NK activity during remission [13]
- Plasma factor able to inhibit lymphocyte proliferation [14]
- Decreased CD45RA (memory) and increased CD45RO (activated) T cells numbers [8]

Table 3. Gene abnormalities in HLH

Perforin [17,18]
Interleukin-2 receptor γ chain [19]
SH2D1A [20]
Purine nucleoside phosphorylase [unpublished]

recent publications; recognition of these novel defects is tremendously important for patient management as well as for family genetic counseling.

Mutations in perforin

Perforin is an important mediator of lymphocyte cytotoxicity. When perforin knockout mice are infected with lymphocytic choriomeningitis virus they develop a CD8+ T cell, interferon-gamma and tumor necrosis factor-alpha dependent fatal disease similar to that found in patients with HLH. This led Stepp et al. [17] to investigate for mutations in the perforin gene located at 10q21-22 chromosome for mutations. Nine independent mutations were detected in exons 2 and 3 of the perforin gene among eight unrelated patients with HLH; five were from consanguineous families and three from non-consanguineous families. These mutations putatively caused premature stop codons or amino acid substitutions, and were associated with complete or nearly complete absence of perforin in the patient's cytotoxic CD8+ T cell granules. The significance of these mutations was proved by demonstrating greatly reduced perforin-mediated cytolytic capability of the patients' cells. It is thought that the absence of perforin-competent cells prevents the elimination of antigen-presenting cells, which continue to produce activation and proliferation signals to cytotoxic cells. This also explains why donor bone marrow engraftment that contains perforin-competent cells can cure these patients. Further studies have indicated that mutations in the gene for perforin may account for 20–40% of familial HLH [18].

Mutation in IL-2 receptor gamma chain

Defects in the gamma chain of the IL-2 receptor are the most frequently recognized cause of severe combined immunodeficiency. The gamma chain is also known as the common gamma chain due to its participation in the IL-4, IL-9, IL-12 and IL-15 receptors, which may explain why abnormal gamma chain affects T lymphocytes as well as NK cells. Patients with SCID usually present with recurrent infections and failure to thrive in the first year of life and will die without bone marrow transplant [21].

We recently described a 2 month old boy, born at term, who had prolonged fever and generalized convulsions [19]. On admission the patient's weight was at the 10th percentile, significantly lower than his birth weight. Physical examination revealed enlarged liver and spleen, while laboratory evaluation indicated pancytopenia, abnormal coagulation and liver function, low fibrinogen and high triglycerides and ferritin. The cerebrospinal fluid examination was normal and no infectious organisms were cultured from it, or from

the blood, urine and stool. There was no evidence for a viral infection. Chest X-ray demonstrated bilateral interstitial pneumonia, while a thymus was not evident. Bone marrow biopsy showed diffuse infiltration of histiocytes with hemophagocytosis, lack of lymphocytes, but no morphologic signs of malignancy. Flow cytometry of peripheral blood revealed markedly diminished NK and T lymphocyte populations. Proliferative responses of peripheral T cells to mitogens indicated deficient T cell function, while B cell responses were relatively preserved. This suggested a defect in the IL-2R gamma chain, which was confirmed by Western blotting of peripheral blood lymphocyte and evaluation of IL-2R gamma expression by flow cytometry. Sequencing of the IL-2R gamma gene of the patient revealed a point mutation in the fifth exon, causing an amino acid change at the extracellular domain of the gamma chain molecule, near a "hot spot" area [22]. The mother's DNA was heterozygous for the mutation. Because of the diagnosis of SCID due to IL-2R gamma deficiency, the patient was placed in an isolated environment, and only irradiated blood products were used. Unfortunately, during preparations for bone marrow transplant the infant died of an uncontrolled infection.

HLH and IL-2R gamma deficiency are both very rare conditions, and this association by mere coincidence seems unlikely. The reduced T and NK cell number and function found in patients with IL-2R gamma defects may cause abnormal cytotoxic activity, which under certain conditions predisposes for uncontrolled response to infections culminating in HLH.

Mutations in SH2D1A

Mutations in the gene for SH2D1A were previously recognized as a cause for X-linked lymphoproliferative disease. Patients with XLP have an increased susceptibility to Epstein-Barr virus-induced disease, such as fulminant infectious mononucleosis and malignant lymphoma. The frequent association of HLH with Epstein-Barr virus infections and the similar clinical manifestations prompted Arico and colleagues [20] to evaluate possible mutations in the gene for SH2D1A (Slap). This gene encodes a protein termed SLAM-associated protein, which is expressed primarily in T and NK cells, and may regulate Epstein-Barr virus-specific immune responses [23]. Mutations were found among four unrelated males of Italian ethnicity. In two of them there was no family history suggestive of HLH, immunodeficiency or XLP, and only one had a sibling who suffered from HLH. Therefore, there is mounting evidence that many of the so-called HLH secondary to infection are patients with an underlying immune abnormality.

Mutation in purine nucleoside phosphorylase

Purine nucleoside phosphorylase is an important enzyme in purine nucleoside salvage or degradation to hypoxanthine and uric acid. The enzyme deficiency leads to abnormal accumulation of nucleotides associated with increased susceptibility to infections and early death. Some patients also suffer from early motor nervous system dysfunction [24]. Studies in PNP-deficient mice have demonstrated

IL = interleukin

SCID = severe combined immunodeficiency

XLP = X-linked lymphoproliferative disease

that increased deoxyguanosine triphosphate concentration in the mitochondria of thymocytes causes enhanced apoptosis and subsequently reduced T lymphocyte number and function [25]. Indeed without PNP supplement, these mice have significantly reduced cytotoxic activity and die at an early age.

Recently, a mutation in the gene for PNP was found in a girl, the first child of unrelated parents, who suffered from HLH (Gooi 2001, unpublished). She was investigated for dystonia and quadriplegia at the age of 9 months. At age 15 months she presented with fever, diarrhea and loss of appetite, and subsequently developed jaundice and hepatosplenomegaly. Laboratory evaluation revealed pancytopenia and abnormal liver and coagulation function. Blood, stool and cerebrospinal fluid cultures were sterile. Bone marrow aspirate showed no evidence of malignancy, however there was marked lymphocyte depletion, dyserythropoiesis and erythrophagocytosis. Liver biopsy revealed necrotic tissue with erythrophagocytic histiocytes, supporting the diagnosis of HLH. Immune investigation demonstrated low levels of immunoglobulins and depressed T lymphocyte number and response to mitogens. Genetic analysis disclosed that the patient had a compound mutation in the gene for PNP, while her parents were heterozygous to the mutations. This patient, together with the previous one described, demonstrate that HLH may be an indication of an underlying severe immune deficiency.

Despite supportive treatment, the patient developed respiratory failure and died after 3 weeks. Autopsy showed a small fibrotic thymus with only a few calcified Hassle's corpuscles and marked lymphocyte depletion. Similar depletion was noted in the tonsils, lymph nodes, spleen, and Peyer's patches. There were also numerous multinucleated cells with cytoplasmic inclusions suggestive of a viral infection in the lungs, liver, spleen and adrenals. The viral RNA extracted from these tissues identified the presence of measles virus in the tissues. Interestingly, 6 weeks prior to admission she had received her measles-mumps-rubella immunization. Wild-type measles have been suggested as a cause of fatal HLH [26,27], and progressive measles encephalitis secondary to measles vaccination has been documented in an immune-deficient patient [28]. Thus, the close temporal association of the measles-mumps-rubella immunization and HLH development is suggestive that the infection was due to the vaccine strain. This patient has also taught us once again that the presence of a viral infection cannot be used to distinguish between primary and secondary HLH [29].

Conclusion

Although the precise pathogenesis leading to HLH development is unclear, there is compelling evidence that different genetic abnormalities in the immune system that lead to immunodeficiency may underlie this syndrome. The classification of patients as either primary-familial HLH versus secondary-acquired HLH may be artificial since as yet undetermined proportion of HLH may develop in patients with a defect in the immune system. The diagnosis of an underlying severe combined immune deficiency

would clearly affect management and mandates the urgency of a bone marrow transplant as well as genetic counseling. Thus, a thorough immune evaluation should be performed in all patients with HLH prior to treatment.

Addendum: Since the submission of this manuscript, mutations in SH2D1A were identified as a cause for other immunodeficiency syndromes (Morra M, Siander O, Calpe S, et al. Alterations of the X-linked lymphoproliferative disease gene SH2D1A in common variable immunodeficiency syndrome. *Blood* 2001;98:1321-5).

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PNP = purine nucleoside phosphorylase

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