Published in final edited form as:
Pediatr Blood Cancer. 2011 May ; 56(5): 856–858. doi:10.1002/pbc.22846.

Hemophagocytic Lymphohistiocytosis with *MUNC13-4* Gene Mutation or Reduced Natural Killer Cell Function Prior to Onset of Childhood Leukemia

Tamara Y. Chang, MD[1,2,*], **Julie Jaffray, MD**[1,*], **Bruce Woda, MD**[3], **Peter E. Newburger, MD**[1,4], and **G. Naheed Usmani, MD**[1,†]

[1]Department of Pediatrics, University of Massachusetts Medical School, Worcester, MA, USA

[2]Department of Medicine, University of Massachusetts Medical School, Worcester, MA, USA

[3]Department of Pathology, University of Massachusetts Medical School, Worcester, MA, USA

[4]Department of Cancer Biology, University of Massachusetts Medical School, Worcester, MA, USA

Abstract

Hemophagocytic lymphohistiocytosis (HLH) is a rare histiocytic reactive process due to mutations in the perforin, *MUNC13-4* or syntaxin 11 genes, or secondary to malignancy, infection or autoimmune disorder. HLH as a preceding diagnosis to leukemia is rare. We report two cases with progression to acute leukemia, one heterozygous for *MUNC13-4* and the other with reduced NK cell function and perforin expression. These defects may predispose to a secondary HLH-like presentation of pre-clinical leukemia or confer increased susceptibility to malignancy. HLH patients with genetic mutations or NK cell function abnormalities need monitoring for future malignancy even if the HLH resolves.

Keywords

histiocytosis; leukemia; NK cell; mutation

Background

Hemophagocytic lymphohistiocytosis (HLH) is a rare multisystem inflammatory process due to cytokine activation from overly-stimulated lymphocytes and macrophages [1,2]. The etiology of HLH is either primary, due to a genetic mutation, or secondary from over-activation of the immune system [3]. In the familial form of HLH, mutations in three genes – perforin (*PRF1*), mammalian homolog of uncoordinated (*MUNC13-4*), and syntaxin (*STX11*) – have been linked to HLH [2–4]. Impairment of these gene products causes disruption of granule mediated toxicity, which can lead to ineffective cytolytic activity. Secondary HLH can be due to an infection, autoimmune process or malignancy which causes excessive stimulation of the immune system [5]. Usually, in the case of malignancy, HLH is a complication of the primary disease, not a preceding diagnosis [6].

†Correspondence to: G. Naheed Usmani, MD, Department of Pediatrics, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, USA. Tel. 1-508-856-4225, Fax 1-508-856-4282 (Naheed.Usmani@umassmemorial.org).
*Contributed equally to this work

Conflict of interest: nothing to declare

Current diagnostic guidelines [7] for HLH include: (1) molecular diagnosis consistent with HLH, or (2) five out of following eight diagnostic criteria: fever; splenomegaly; cytopenias affecting ≥ 2 lineages; hypertriglyceridemia or hypofibrinogenemia; hemophagocytosis in bone marrow, spleen, or lymph nodes with no evidence for malignancy; low or absent NK cell activity; ferritin ≥ 500 μg/L; and soluble CD25 (IL-2 receptor) ≥ 2,400 U/ml.

We report two unique cases in which secondary HLH was the initial diagnosis, with progression to acute leukemia. In the first case, the child was diagnosed with HLH, found to be heterozygous for *MUNC13-4* mutation, and later progressed to acute monoblastic leukemia with a MLL-AF9 gene fusion. A second child was diagnosed with HLH and later progressed to acute lymphoblastic leukemia; further investigation revealed decreased natural killer (NK) cell function and perforin expression. To our knowledge, these are the only cases of HLH with progression to leukemia with an underlying *MUNC13-4* mutation or decreased perforin expression.

Observations

Case 1

A three year-old Hispanic female was admitted with three days of irritability and left leg pain. Initial laboratory evaluation revealed thrombocytopenia with platelet count 86,000/mm^3 and elevated c-reactive protein of 71 mg/L (normal <10 mg/L). Physical examination, as well as radiographs and ultrasound of the hip and leg, were unremarkable. Four days after discharge, she presented with increased irritability, leg pain and emesis. CBC revealed WBC 4,800/mm^3, hgb 10g/dL, platelets 100,000/mm^3 and absolute neutrophil count (ANC) 1,700/mm^3. Two days later the cytopenias were more pronounced: WBC 2,400/mm^3, ANC 700/mm^3, hgb 7.6 g/dL. Other laboratory findings included erythrocyte sedimentation rate (ESR) 32 mm/hr, ferritin 686 ng/mL, and lactate dehydrogenase (LDH) 767 IU/L. Peripheral blood flow cytometry detected no abnormal lymphoid or myelomonocytic population. Bone marrow biopsy revealed numerous macrophages with hemophagocytosis without evidence of acute leukemia. Cytogenetic analysis revealed no chromosomal abnormalities. Serological studies were negative for hepatitis viruses, Epstein-Barr virus (EBV), cytomegalovirus, HIV, parvovirus B19, and human herpesvirus 6. Serum immunoglobulin levels were normal.

Gene mutation analysis revealed heterozygosity for a 753(3+) g>a splice site mutation in *MUNC13-4* [8], elevated soluble IL-2 receptor (3,352 U/ml) and decreased numbers of NK cells. The diagnosis of HLH was thus based on five diagnostic criteria. Her blood counts and clinical status recovered spontaneously and she was discharged with presumed resolution of HLH. After four months of clinical remission, with normal peripheral blood counts and ferritin levels, but mildly elevated LDH, she presented with left arm pain and hepatosplenomegaly. CBC showed pancytopenia with WBC 4,400/mm^3, hgb 7.9 g/dL, platelets 131,000/mm^3, ANC 900/mm^3, reticulocyte count 2.7%, ESR 73 mm/hr, LDH 1,470 IU/L, and ferritin 1,829 ng/mL. Bone marrow biopsy revealed replacement by immature hematopoietic cells with 80% monoblasts, but no hemophagocytosis. Monoblasts were positive for CD13, CD33, CD64, CD18, HLA-DR, myeloperoxidase, CD11b, CD15, and CD4 (dim). Karyotyping showed 46, XX, t(9;11) (p22;q23); fluorescence in situ hybridization (FISH) analysis revealed rearrangement of the AF9 and MLL genes, but no FLT3 rearrangement. Spinal fluid showed WBC 858/mm^3 with 95% blasts. She was diagnosed with acute monoblastic leukemia and enrolled in Children's Oncology Group protocol AAML 0531 [9]. At the end of induction, her bone marrow had 7% blasts and her CNS was negative for leukemic cells. After two more cycles of chemotherapy, a lumbar puncture revealed monoblasts in the CSF. After one year in morphological remission, with

persistent MLL rearrangement detected by FISH, she had a CNS relapse, with bone marrow positive for minimal residual disease. She is currently receiving ongoing treatment.

Case 2

A 4 year old, previously healthy Caucasian female initially presented in 1993 with a fever and reticular rash, diagnosed as a viral illness. One week later, she returned with continued fever and headache, accompanied by splenomegaly, hepatomegaly, lymphadenopathy and pancytopenia: WBC 1,000/mm^3, hgb 5.3 g/dL, platelets 190,000/mm^3, reticulocte count 0.6%. Other laboratory data included LDH 1,822 IU/L, ESR 65 mm/hr, decreased fibrinogen (138 mg/dl) and elevated fasting triglycerides (290 mg/dL). Viral studies for cytomegalovirus, herpes simplex virus, EBV and parvovirus were negative, and serum immunoglobulin levels were normal. A bone marrow biopsy showed a hypercellular marrow with erythroid and megakaryocytic hyperplasia and myeloid hypoplasia with left-shifted maturation; there was no evidence of leukemia. She was diagnosed with viral-induced hemophagocytic syndrome and treated with ceftazidime and intravenous gamma globulin. The pancytopenia slowly improved, and all other signs and symptoms of hemophagocytic syndrome resolved.

One month later, she returned with fever, hepatosplenomegaly, and worsening pancytopenia: WBC 1,600/mm^3 with no peripheral blasts, hgb 8.9 g/dL, platelets 121,000/mm^3. Bone marrow biopsy showed complete replacement with lymphoblasts, positive for CD10, CD19, and HLA-DR, indicating a pre-B cell phenotype. Cytogenetic analysis revealed clonal hyperdiploidy with tetraploidy 21 and X and trisomies 4, 6, 10, 14, 15, 17, and 18. She was treated on Dana-Farber Cancer Institute protocol 91-001 for standard risk pre-B cell acute lymphoblastic leukemia [10] and has remained in continued clinical remission, currently 15 years off therapy.

To determine a possible underlying cause for her HLH, additional testing was performed, including NK cell function, which was revealed to be defective at all effector-to-target ratios. The studies also demonstrated a reduced percent of cytotoxicity of NK cells, measured in lytic units. Perforin expression was reduced in NK and CD8 cells, and markedly reduced in NKT cells. Granzyme B expression and soluble IL-2 receptor levels were normal. By current diagnostic guidelines [7], her case meets five criteria for HLH.

Conclusions

None of the eight previously reported cases of HLH-like clinical presentations in children with subsequent progression to myelodysplasia or leukemia [5,12–14] have had either *MUNC13-4* gene mutations or abnormal NK cell and perforin function. However, only half of these case reports mentioned molecular testing of their patients. Two cases of myelodysplasia/AML developed in familial HLH patients with homozygous syntaxin 11 mutations after treatment with etoposide [12]. Two other patients presented with clonal cytogenetic abnormalities of unknown significance [11,14].

The current cases suggest that decreased perforin function or underlying genetic mutation in *MUNC13-4* may confer increased susceptibility to develop not only HLH, but also malignancy. Clementi *et al.* proposed a similar scenario when evaluating the effects of perforin gene mutations on the immune system, noting a possible correlation between reduced perforin activity and an increased susceptibility to lymphoma [15–17]. They hypothesized that perforin may play a role in immune surveillance preventing tumor growth, the decreased expression of which may cause an increased susceptibility to both secondary HLH and neoplastic transformation.

Alternatively, underlying defects in lymphocyte secretory granule function may predispose patients to an HLH-like presentation of pre-clinical leukemia. Both current patients progressed to acute leukemia within one to four months of initial presentation, after a brief period of apparent resolution. It is possible that this temporal relationship between the initial HLH presentation and subsequent rapid progression to acute leukemia represents an unusual pre-clinical manifestation of leukemia related to the underlying defect. Heterozygosity for a known HLH genetic mutation has also been hypothesized to confer a predisposition to HLH in an inflammatory disorder. Van Montfrans *et al.* reported a case of fatal HLH in a child with chronic granulomatous disease and a heterozygous substitution in the *PRF1* gene [18].

Patients who present with HLH and have abnormalities in NK cell function or genetic markers of HLH, even in a single allele, should have continued monitoring for the development of malignancy even if the HLH resolves. Further studies are necessary to determine whether the presentation of HLH with progression to leukemia with such underlying defects may predict long-term prognosis or response to therapy, or whether such individuals may benefit from more intensified treatment, such as hematopoietic stem cell transplantation.

Acknowledgments

Grant sponsor: NIH; grant number: R01 DK054369.

References

1. Filipovich A. Hemophagocytic lymphohistiocytosis and other hemophagocytic disorders. Immunol Allergy Clin North Am. 2008; 28:293–313. [PubMed: 18424334]
2. Janka G. Hemophagocytic syndromes. Blood Rev. 2007; 21:245–253. [PubMed: 17590250]
3. Verbsky J, Grossman W. Hemophagocytic lymphohistiocytosis: diagnosis, pathophysiology, treatment, and future perspectives. Ann Med. 2006; 38:20–31. [PubMed: 16448985]
4. Santoro A, Cannella S, Bossi G, et al. Novel Munc 13-4 mutations in children and young adult patients with haemophagocytic lymphohistiocytosis. J Med Genet. 2006; 43:953–960. [PubMed: 16825436]
5. O'Brien M, Lee-Kim Y, George T, et al. Precursor B-cell acute lymphoblastic leukemia presenting with hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2008; 50:381–383. [PubMed: 16856156]
6. Garcia-Suarez J, Bañas H, Krsnik I, et al. Hemophagocytic syndrome associated with retinoic acid syndrome in acute promyelocytic leukemia. Am J Hematol. 2004; 76:172–175. [PubMed: 15164385]
7. Henter J, Horne A, Arico M, et al. HLH-2004: Diagnostic and Therapeutic Guidelines for Hemophagocytic Lymphohistiocytosis. Pediatr Blood Cancer. 2007; 48:124–131. [PubMed: 16937360]
8. Yamamoto K, Ishii E, Sako M, et al. Identification of novel MUNC13-4 mutations in familial haemophagocytic lymphohistiocytosis and functional analysis of MUNC13-4-deficient cytotoxic T lymphocytes. J Med Genet. 2004; 41:763–767. [PubMed: 15466010]
9. Gamis A. A Phase III randomized trial of gentuzumab ozogamicin (Mylotarg®) combined with conventional chemotherapy for de novo acute myeloid leukemia (AML) in children, adolescents, and young adults. Cure Search Children's Oncology Group. 2009:1–141.
10. Silverman L, Stevenson K, O'Brien J, et al. Long term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985–2000) Long term results from DFCI ALL Consortium. Leukemia. 2009; 24:320–334. [epub ahead of print]. [PubMed: 20016537]
11. Kumar M, Boggino H, Hudnall D, et al. Acute myeloid leukemia associated with hemophagocytic syndrome and t(4;7)(q21;q36). Cancer Genet Cytogenet. 2000; 122:26–29. [PubMed: 11104028]

12. Rudd E, Ericson K, Zheng C, et al. Spectrum and clinical implications of syntaxin 11 gene mutations in familial haemophagocytic lymphohistiocytosis: association with disease-free remissions and haematopoietic malignancies. J Med Genet. 2006; 43:14–20.

13. Suzuki T, Mugishima H, Yamada A, et al. Development of acute lymphoblastic leukemia following hemophagocytic lymphohistiocytosis: is it secondary leukemia? Int J Hematol. 1999; 70:58–59. [PubMed: 10446498]

14. Tadmor T, Vadazs Z, Dar H, et al. Hemophagocytic syndrome preceding acute myeloid leukemia with t(7;17)(q12;q11), monosomy 17 and 5p- J Pediatr Hematol Oncol. 2006; 28:544–546. [PubMed: 16912598]

15. Clementi R, Emmi L, Maccario R. Adult onset and atypical presentation of hemophagocytic lymphohistiocytosis in siblings carrying PRF1 mutations. Blood. 2002; 100:2266–2267. [PubMed: 12229880]

16. Clementi R, Dagna L, Dianzani U, Dupre L, et al. Inherited perforin and Fas mutations in a patient with autoimmune lymphoproliferative syndrome and lymphoma. N Engl J Med. 2004; 351:1419–1424. [PubMed: 15459303]

17. Clementi R, Locatelli F, Dupré L, et al. A proportion of patients with lymphoma may harbor mutations of the perforin gene. Blood. 2005; 105:4424–4428. [PubMed: 15728124]

18. van Montfrans JM, Rudd E, van de Corput L, et al. Fatal hemophagocytic lymphohistiocytosis in X-linked chronic granulomatous disease associated with a perforin gene variant. Pediatric blood & cancer. 2009; 52(4):527–529. [PubMed: 19058215]