CASE REPORT

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Hemophagocytic syndrome triggered by intense physical activity and viral infection in a young adult female with three heterozygous mutations in Munc-18-2

Hemofagocitni sindrom izazvan intenzivnom fizičkom aktivnošću i virusnom infekcijom kod mlade odrasle ženske osobe sa tri heterozigotne mutacije u Munc-18-2

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Abstract

Introduction. Hemophagocytic lymphohistiocytosis (HLH) is a rare, potentially life-threatening, hyperinflammatory syndrome caused by severe hypercytokinemia due to a highly stimulated, but ineffective immune response. **Case report**. We reported a 19-year-old woman presenting with fever, muscle and joint pain and sore throat. After diagnostic procedures we made the diagnosis of hemophagocytic lymphohistiocytosis (7 of 8 HLH-2004 diagnostic criteria) caused by Ebstein-Barr viral infection and trigerred by the intense physical activity. Genetic analysis showed three different sequence changes in Munc-18-2, two splice acceptor side mutations/changes affecting exon 10 (c.795–4 C > T) and exon 15 (c.1247–10 C > T) and a missense mutation c.1375 C > T; p.Arg 459 Trp. All mutations were in heterozygous state and their significance in pathogensis of HLH is not clear. After treatment with corticosteroids and cyclosporin A complete clinical remission was achieved. **Conclusion.** The presented case history suggests the possibility that mutations of undetermined clinical significance in a gene associated with primary HLH may underlie some cases of secondary HLH, probably by causing a partial, rather than total or subtotal, impairment of encoded protein function. Our case also suggests that strenuous physical activity (in apparent synergy with viral infection) can trigger HLH.

Key words:
lymphohistiocytosis, hemophagocytic; inflammation; immunologic factors; physical exertion; ebstein-barr virus infections; mutation; diagnosis, differential; drug therapy.

Apstrakt

Uvod. Hemofagocitna limfohistiocitoza (HLH) je redak, moguće i životno ugrožavajći, upalni sindrom izazvan povećanom citokinskom aktivnošću nastalom na terenu izuzetno stimulisanog, ali neefikasnog imunskog odgovora. **Prikaz bolesnika.** Prikazali smo 19-godišnju bolesnicu koja je hospitalizovana zbog povišene temperature, bolova u mišićima i zglobovima i bolova u grlu. Nakon kompletne dijagnostičke obrade utvrđeno je da se radi o hemofagocitnoj limfohistiocitozi (7 od 8 HLH-2004 dijagnostičkih kriterijuma) uzrokovanoj infekcijom Ebstein-Barr-ovim virusom i intenzivnom fizičkom aktivnošću. Genetska analiza pokazala je tri heterozigotne mutacije u genu Munc-8-2, dve *splice* mutacije u egzonu 10 (c.795–4 C > t) i egzonu 15 (c.1247–10, C > T) i jednu *missence* mutaciju c. 1375 C > T; p.Arg 459 Trp, čiji patogenetski značaj nije jasan. Posle lečenja sa kortikosteroidima i ciklosporinom A ostvarena je kompletna klinička remisija. **Zaključak**. Ovaj prikaz bolesnice ukazuje na to da otkrivene heterozigotne mutacije (čiji patogenetski značaj nije utvđen) u genu Munc-18-2, inače povezanom sa primarnom HLH, mogu biti u osnovi i nekih sekundarnih HLH, izazivanjem delimičnog oštećenja funkcije kodiranog proteina. Ovaj slučaj takođe sugeriše da naporna fizička aktivnost (u očiglednoj sinergiji sa virusnom infekcijom) može biti pokretač HLH.

Ključne reči:
limfohistiocitoza, hemofagocitna; zapaljenje; imunski faktori; napor, fizički; ebstein-bar virus, infekcije; mutacija; dijagnoza, diferencijalna; lečenje lekovima.

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal condition of immune system dysregulation characterized by severe inflammation and uncontrolled activation of T-cells and macrophages [1–3]. When the immune system is activated in a healthy person, histiocytes, natural killer (NK) cells and cytotoxic T lymphocytes (CTL) are all activated, further stimulating each other by receptor interactions as well as by secretion of inflammatory cytokines and chemokines [2, 3]. This leads to killing of infected cells, removal of antigen, and subsequent termination of the immune response. However, in HLH, there is a defect of NK and CTL function. Activated T lymphocytes and macrophages infiltrate organs and tissues and impair their function, with the occurrence of chronic inflammation caused by hypercytokinemia [3].

Depending on the presence or absence of an underlying condition, HLH can be either primary (genetic) or secondary (acquired) [4]. Primary HLH may be familial (FHLH) – or associated with some primary immunodeficiency [4]. Familial HLH is associated with mutations of genes involved in secretory pathways of cytotoxic lymphocytes, either through inactivation of granule contents or impairment of fusion, transport or delivery of such granules through the plasma membrane [5, 6]. Acquired HLH occurs with infections, lymphoproliferative disorders, metabolic and autoimmune diseases [7].

We presented a young adult female with HLH triggered by intense physical activity and viral infection, characterized by abnormal tests of T and NK cell degranulation and three heterozygous mutations in MUNC-18-2 gene.

Case report

A 19-year-old woman presented with a five-day history of fever (39ºC), muscle and joint pain and sore throat. A day before the appearance of symptoms the patient underwent an intense physical effort in the gym. She had no history of previous disease that could be the trigger for HLH. Three days after the initiation of symptoms antibiotic therapy started in primary care. As the fever persisted and the patient's condition deteriorated, the patient was admitted to the Clinic for Infectious Diseases (April 2012). Her personal and familial medical histories were unremarkable. Physical examination showed fever, a hyperemic pharynx, and cervical lymphadenopathy (maximal diameter 15 mm). Her blood count showed leukopenia and thrombocytopenia (hemoglobin – Hgb 134 g/L, white blood cell – WBC 3.8 × 10^9/L, platelet count – PLT 94 × 10^9/L) with normal leukocyte differential (neutrophils – Neu 54%, lymphocytes – Ly 40%, monocytes – Mon 6%). Laboratory analyses showed accelerated sedimentation of erythrocytes (SE) (24 mm/1h), elevated C-reactive protein – CRP (27.2 mg/L), ferritin (578 μg/L), aspartate aminotransminase – AST (718 U/L), alanine aminotransaminase – ALT (369 U/L), lactate dehydrogenase – LDH (2,086 U/L), creatine-kinase (3,423 U/L), normal holesterol (3.41 mmol/L) and triglyceride level (1.14 mmol/L). Haptoglobin was immeasurable. Tests of hemostasis showed normal prothrombin time – PT, activated partial thromboplastin time – aPTT and fibrinogen level, and increased D-dimer (4.37 μg/mL). Serum soluble interleukin-2 receptor and interleukin-6 were increased (4.86 ng/mL and 21 pg/mL, respectively; reference range 0.2–2.0 ng/mL, and 1.9–6.5 pg/mL, respectively). Serological analyses for human immunodeficiency virus – HIV, hepatitis B virus antigen – HbsAg, hepatitis C virus – HCV, hepatitis A virus – HAV and *Mycoplasma pneumoniae* were negative. Influenza A (H1, H3), Influenza B and respiratory syncytial virus (RSV) infections were not detected by polymerase chain reaction (PCR) analysis. Tumor markers alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) were normal. Epstein-Barr virus (EBV) serology showed positive IgM antibodies and gradual increase in the titer of IgG from < 1/40 in the first sample to 1/160 in the third sample. EBV was not detected by PCR analysis, performed at two occasions. Immunological analyses: antinuclear antibodies (ANA), human epithelial line type 2 (HEp2), extractable nuclear antigens (ENA), circulating immune complexes (CIC), complement 3 (C3), complement 4 (C4), anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies (ASMA), anti-neutrophil cytoplasmatic antibodies (ANCA) and anti-liver kidney microsomal antibodies (anti-LKM) were negative. Chest radiography revealed normal finding. Computed tomography scan visualized bilateral axillary lymphadenopathy (15–20 mm) and hepatosplenomegaly (liver 178 mm, spleen 185 mm). Bone marrow aspirate examination showed discrete hemophagocytosis. Morphological examination of the bone marrow trephine biopsy showed reactive changes. Pathohistological findings of an extirpated axillar lymph node showed sinus histiocytosis. Immunophenotyping of peripheral blood lymphocytes showed increased amount of activated (HLA-DR+) CD4+ (41.05%) and CD8+ (87.41%) T cells. As hemophagocytic syndrome was suspected, functional analysis of NK cells and CTL was performed (Centre for Chronic Immunodeficiency, University of Freiburg, Germany). NK cell cytotoxicity assay showed normal findings. Flow cytometry showed normal expression of perforin in NK cells. Analysis of activation-induced degranulation performed by detection of surface expression of CD107 on NK cells showed low, but not absent degranulation of NK cells after stimulation with target cells on two occasions (8.69%; 7.0%). No recovery of NK cell degranulation after stimulation with IL-2 was detected (11.09%). This finding was compatible with familial HLH. Repeated analysis of NK cell degranulation (one month later) showed a better result than in the first assay, but it was still not normal (28.95%). Degranulation of CTL after activation with anti-CD3/CD28 antibodies was normal in the first analysis (16.27%) and reduced in the second one (7.71%). According to all these findings and revised diagnostic guidelines for HLH (Table 1) [8] the diagnosis of HLH was established. Genetic analysis (performed at University Medical Center Hamburg, Germany) did not show changes in MUNC 13-4 gene, but three different sequence changes in

Table 1
Revised diagnostic guidelines for hemophagocytic lymphohistiocytosis (HLH)

The diagnosis HLH can be established if one the 2 below is fulfilled:
The molecular diagnosis consistent with HLH or
Diagnostic criteria for HLH fulfilled (five out of the eight criteria below)
Fever
Splenomegaly
Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood):
Hemoglobin < 90 g/L (in infants < 4 weeks: hemoglobin < 100 g/L)
Platelets < 100 × 109/L
Neutrophils < 1.0 × 109/L
Hypertriglyceridemia and/or hypofibrinogenemia:
Fasting triglycerides ≥ 265 mg/100 mL
Fibrinogen ≤ 1.5 g/L
Hemophagocytosis in bone marrow or spleen or lymph nodes
No evidence of malignancy (low or absent NK cell activity)
New diagnostic criteria
Ferritin ≥ 500 mg/L
Soluble CD25 (i.e., soluble IL-2 receptor) > 2,400 U/mL

NK – Natural killer.

Munc-18-2 were detected: two splice acceptor site mutations/changes affecting exon 10 (c.795–4 C > T) and exon 15 (c.1247–10 C > T) and a missense mutation c.1375 C > T; p.Arg459Trp, each of them in heterozygous state. The patient's mother was the carrier of the exon 10 splice site mutation and her father the carrier of the remaining two mutations. A splice site prediction program revealed no changes in splicing. The missense mutation had a low conservation grade. The overall pathogenetic significance of the detected mutations was unclear. At first, the patient was treated with symptomatic therapy. However, the patient's condition steadily deteriorated and laboratory analyses showed further aggravation. Therefore, the treatment consisting of corticosteroids, intravenous immunoglobulins, albumins and intravenous acyclovir was initiated. Soon after the initiation of treatment, the patient's condition gradually improved. However, as any attempt to reduce the dose of corticosteroids resulted in a drop of hemoglobin level, leukocyte and thrombocyte values, cyclosporin A (6 mg/kg) was introduced and the patient was transferred to the Hematology Department. In a few weeks fever subsided, her general condition improved, and all laboratory findings became normal. On the day 78 of hospitalization, the patient was discharged in good clinical condition. She thereafter remained, up to the time of writing (two years later) in excellent health.

Discussion

Primary hemophagocytic lymphohistiocytosis appears during infancy in 70–80% of the patients, usually within the first two years of life [9, 10]. Thanks to a better understanding of the genetic basis of the disease and better diagnostics, HLH has been increasingly diagnosed in patients presenting beyond infancy. These atypical presentations have been reported in adolescents and even in adults [3, 6]. Time of onset of disease and severity of clinical presentation are a reflection of underlying genetic aberrations and the intensity of triggering factors[1, 2]. Rohr et al. [9] classified a group of HLH patients presenting with HLH before two years of age as "typical FHLH" and the patients older than two years of age and survival without hematopoietic stem cell transplantation (HSCT) until at least six years of age as "atypical HLH" [10].

The presented patient met seven of the eight HLH-2004 diagnostic criteria (fever, splenomegaly, pancytopenia, hypofibrinogenemia, hemophagocytosis in bone marrow, abnormal NK cell activity, elevated ferritin and solubile IL-2 receptor). As immunological analyses were negative the diagnosis of myositis and other rheumatological diseases were excluded. Functional tests of NK cells and cytotoxic T lymphocytes showed findings that were not discriminative between primary and secondary HLH. Genetic analysis in our patient showed three different sequence changes of Munc-18-2 (syntaxin binding protein 2 – STXBP2) in heterozygous state. Syntaxin binding protein 2 (Munc-18-2) is involved in the regulation of vesicle transport to the plasma membrane [6]. According to the literature, some mutations in STXBP2 may be associated with milder and often recurrent HLH episodes and prolonged survival even without HSCT, which is unusual in the patients with the "typical" HLH [6]. However, clinical significance and

patogenetic potential of three different heterozygous sequence changes of Munc-18-2 gene detected in the presented patient is, at present, not clear (zur Stadt, personal communication). It is possible that these sequence changes decrease the threshold for the development of HLH, but to a lesser extent than severe mutations that cause HLH during the early stages of life.

When the diagnosis of hemophagocytic syndrome was established in our patient, search for an underlying infection, genetic, rheumatological, or malignant disease was undertaken. The presented patient had elevated creatin kinase which is an unusual finding in hemophagocytic syndrome, unless in the context of underlying myositis. However, the presence of rheumatological disease or malignant disease was excluded. Consequently, the diagnosis of HLH triggered by EBV infection was made. However, in addition to genetic predisposition and triggerring factors, additional factors that contribute to the development of HLH may exist. Such a factor in the presented patient was intense physical activity. Strenuous exercise induces negative changes in the cellular compartment of the immune system and suppression of NK cell cytotoxic activity [11]. Furthermore, strenuous exercise induces increased circulating levels of several cytokines [10]. Bruunsgaard et al. [12] show that post-exercise cytokine production is related to skeletal muscle damage. They found an association between increased levels of IL-6, that is locally produced in response to exercise-induced muscle damage, and the increased creatine kinase level, but it is not a typical finding in HLH.

The immediate aim of HLH treatment is the suppression of the increased inflammatory response and control of cell proliferation using immunosuppressive or immunomodulatory agents and cytotoxic drugs [4]. The treatment differs in children and adults and depends on the underlying disease, the presence of a trigger and severity of symptoms. Chemotherapy using dexamethasone, cyclosporin, and etoposide is used for severe, particularly familial and EBV-associated haemophagocytic syndrome cases [1, 4]. The presented patient was sucessfully treated by corticosteroids and cyclosporin A which corresponds to the expected clinical course of secondary HLH.

Conclusion

The case we reported illustrates the difficulties in distinguishing between primary and secondary HLH. This is very important since the preferred therapeutic approach in these two forms of the disease is quite different. Adult cases with HLH may be associated with „mild“ mutations in the primary HLH-related gene (with „weaker“ phenotypic expression), predisposing to disease initiation upon the action of common trigger(s), thus resulting in clinically secondary HLH. Further studies are needed to clarify the significance of particular gene mutations in the pathogenesis of HLH and to determine optimal therapeutic modalities in different patients suffering from HLH. This appears to be particularly true for those with “atypical” presentations of what is still generally classified as „secondary“ HLH.

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