Hemophagocytosis in a case with Crimean-Congo hemorrhagic fever and an overview of possible pathogenesis with current evidence

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Hemophagocytic lymphohistiocytosis (HLH) is a clinicopathologic condition characterized by high fever, hepatosplenomegaly, cytopenia, hyperferritinemia, and increased hemophagocytic macrophage proliferation and activation in the reticuloendothelial system. Primary HLH is familial and is a fatal disease that begins during early childhood. Secondary HLH may be acquired after intense activation of the immune system due to infection. Clinical and biologic symptoms result from cytokines secreted by T-lymphocytes and macrophages. Subtypes of primary HLH are caused by genetic defects in several cell types, including perforin-dependent cytotoxic T-lymphocytes and natural killer (NK) cells. Secondary HLH is often associated with intracellular pathogen infections. Crimean-Congo hemorrhagic fever (CCHF) is caused by a tick-borne virus, Nairovirus, from the Bunyaviridae family. It is characterized by a poor prognosis and has a high mortality. We report the case of a 14-year-old boy living in a CCHF-endemic area with no history of tick exposure. He presented with fever, and laboratory tests showed bicytopenia and hemophagocytosis in the bone marrow aspiration. Blood samples were polymerase chain reaction (PCR)-negative for CCFH but immunoglobulin (Ig)M-positive. In conclusion, patients with hemophagocytosis should be assessed for CCHF during the evaluation of cytopenia.

Key words: hemophagocytosis, Crimean Congo hemorrhagic fever (CCHF), natural killer (NK), cytotoxic T cells, perforin/granzyme deficiency.

Hemophagocytic lymphohistiocytosis (HLH) is a rare syndrome characterized by systemic proliferation and reactivation of benign histiocytes throughout the entire reticuloendothelial system. Some gene defects related to the perforin/granzyme system in natural killer (NK) and CD8 cytotoxic T-lymphocytes (cTL) are responsible for this syndrome. In HLH, NK and CD8 cTL cells become dysfunctional, increase activation of histiocytes and lymphocytes, and initiate phagocytosis of hematopoietic cells. The primary form of HLH is familial; this autosomal recessive condition is fatal and starts during infancy or early childhood. Untreated patients die in less than two months. Secondary HLH (SHLH), however, is not caused by genetic mutations. Immune system activation due to infection, malignancy and/or autoimmunity triggers SHLH. SHLH may result from intense immune system activation following severe infections. A number of viral pathogens, in concert or independently, can also cause SHLH, including Epstein-Barr virus, cytomegalovirus, parvovirus, herpes simplex virus, varicella zoster virus, human herpes virus 8, and human immunodeficiency virus. SLH may also coincide with bacterial, parasitic or fungal infections[1].

Symptoms common for both HLH types include fever, cytopenia affecting at least two of three lineages in peripheral blood, hypertriglyceridemia and/or hypofibrinogenemia, hyperferritinemia, hemophagocytosis in bone marrow, spleen or lymph node, high serum free interleukin (IL)-2 receptor (CD25) levels, absent or decreased activity of CD8 cTL and NK cells, and

splenomegaly, while no evidence of malignancy is present[2].

Crimean-Congo hemorrhagic fever (CCHF) is a viral disease that infects humans via Hyalomma ticks or direct contact with the blood of infected animals (including domestic animals). Sporadic occurrences as well as outbreaks have been reported in certain areas of the world, including Africa, Asia, Southeast Europe, and the Middle East. In Turkey, CCHF primarily occurred during the spring and summer between 2003-2012, and had an estimated 5% mortality. Symptoms include acute fever, nausea, vomiting, headache, myalgia, increased liver enzyme levels, as well as hematologic manifestations ranging from mucocutaneous bleeding to excessive intravascular coagulation and massive hemorrhages with hemophagocytosis. As with other viral hemorrhagic disorders, lymphocytes, monocytes, macrophage activation, and excessive cytokine secretion play key roles in the pathogenesis and prognosis[3].

Crimean-Congo hemorrhagic fever (CCHF) is a major healthcare burden due to its high mortality rates. The disease is often described as tick-borne, but in many cases, CCHF-seropositive patients present without a history of tick exposure. Clinical symptoms and a history of visiting endemic areas may indicate CCHF. Definitive diagnosis requires virus isolation and molecular studies, such as immunologic studies and/or real-time polymerase chain reaction (RT-PCR)[4]. In this report, we present the case of a 14-year-old boy living in a CCHF-endemic area with no history of tick exposure. Hemophagocytosis was observed in the bone marrow aspirate, and blood samples were PCR-negative, but immunoglobulin (Ig)M-positive, for CCHF.

Case Report

A 14-year-old boy admitted to our clinic because of a six-day persistent high fever. He had a history of watery diarrhea with mucus, which began 3-5 days after the initiation of the fever. The diarrhea was self-limited and the patient did not observe blood. The patient was diagnosed with tonsillopharyngitis by the local physician. He was prescribed antibiotics, but since his fever persisted, he was referred to another hospital, at which thrombocytopenia and leukopenia were detected. The patient was then referred to our hospital. The patient's history did not include tick exposure, and his family history was also insignificant. The physical examination revealed a temperature of 37.5°C; respiratory rate, 20/min; heart rate, 76/min; blood pressure, 100/60 mmHg; weight, 44 kg (10-25th percentile); and height, 153 cm (10-25th percentile). No abnormalities were apparent during the systemic examination. His thrombocyte count was 61,000/mm^3; white blood cell count, 1,300/mm^3; prothrombin time (PT), 2 seconds; C-reactive protein (CRP), negative; aspartate aminotransferase (AST), 115 U/L; alanine aminotransferase (ALT), 61 U/L; lactate dehydrogenase (LDH), 416 U/L; D-dimer, 5 μg/dl; triglycerides, 201 mg/dl; creatinine phosphokinase, 509 U/L; ferritin 1601 ng/ml; and hemophagocytosis in the bone marrow smear. Blood, throat, urine, and stool cultures were negative for pathogen microorganisms. The chest X-ray was also unremarkable. Blood samples were negative for brucella, salmonella, leptospira, toxoplasmosis, rubella, cytomegalovirus, and herpes simplex type 1 and type 2. The patient's temperature fluctuated between 36.5°C and 37.5°C during the follow-up. Antibiotic treatment was initiated for febrile neutropenia (sulperazone plus amikacin). The patient had no history of tick exposure but lived in a CCHF-endemic area (Kizilcahamam). Laboratory values also suggested the presence of a viral agent (CRP negativity and normal sedimentation). Therefore, we evaluated the patient for CCHF, and blood samples were PCR- negative for CCHF, but IgM-positive.

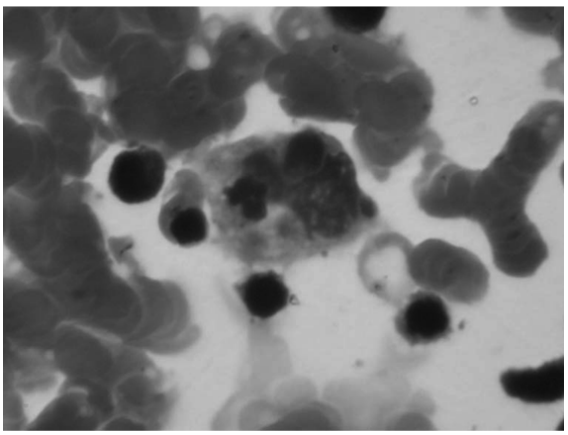

Fig. 1. A macrophage that performed hemophagocytosis.

Discussion

Clinical and biologic symptoms of HLH are due to excessive cytokines secreted by activated T-lymphocytes and macrophages. Genetic defects related to perforin-dependent T lymphocytes and NK cells have been established in the primary HLH group. Intracellular microbial pathogenic infections often accompany SHLH[5]. Macrophages and dendritic cells produce IL-12 in response to many microorganisms. Secreted IL-12 then stimulates NK cells and T cells to produce interferon-gamma (IFN-ɣ), which activates macrophages to kill microorganisms. Therefore, the natural immune response through increased cytokines takes place as macrophage and dendritic cell response to microorganisms, which is followed by secretion of IL-12 then IFN-γ, which initiates macrophage activation and elimination of microorganisms[6]. IFN-γ also plays a role in hemophagocytosis in severe influenza cases caused by highly pathogenic avian H5N1 viruses[7]. Studies have demonstrated that if dendritic cells and CD8 T cells are exposed to a specific fragment of influenza hemagglutinin protein (H5), the survival time of CD8 cTLs and NK cells increases, and their interactions are prolonged. Other studies have reported that intracellular levels of perforin protein within the CD8 cTLs decrease and IFN-γ secretion increases[8]. Introduction of such specific proteins like H5 may increase IFN-γ levels and may induce hemophagocytosis.

In a Turkish study of 14 CCHF patients, half (n=7) presented with reactive hemophagocytosis[9]. The authors of the study suggested that cytopenia may have developed due to hemophagocytosis. In another Turkish study on CCHF cases, all of the patients (n=5) had hemophagocytosis[10]. In another study from our country, it was reported that patients who died from CCHF had higher IL-1, IL-6 and tumor necrosis factor (TNF)-α levels. Disseminated intravascular coagulation (DIC) scores were also higher in these patients. DIC scores were positively correlated with IL-6 and TNF-α levels and negatively correlated with IL-10 levels[11]. In a case report, IL-6, IL-10, TNF-α, and soluble TNF receptor levels were higher in a patient with severe CCHF[12]. Macrophages and dendritic cells have been found to secrete IL-1, IL-6, IL-12, and TNF-α, inducing IFN-γ secretion from TH1 cells, which activates macrophages6. In addition, the absence of IL-12 is reported to lead TH2 cells to secrete decreased levels of IL-10, the cytokine that inhibits macrophages6. Due to a partial deficiency of the perforin/granzyme system in NK and T cells, failure to eliminate the targeted antigen presented by antigen presenting cells (APCs) may occur. APCs may then stimulate IFN-γ secretion from NK cells and T cells, activating hemophagocytosis and related complications.

Yilmaz et al.[13] compared the NK cell counts of patients with mild CCHF and severe CCHF, and revealed that patients with severe CCHF had more NK cells. NK cell counts were much higher in two deceased patients. A positive correlation was also found between AST, ALT and activated partial thromboplastin time (aPTT). Similarly, Akinci et al.[14] compared fatal and non-fatal CCHF cases and determined that CD8 cTL levels were higher in fatal cases. Viral load and CD8 cTL count were positively correlated, but no correlation was observed with viral load and other lymphocyte subtypes. Yilmaz et al.[13] reported that increased NK levels were responsible for natural immunity, while Akinci et al.[14] determined that increased CD8 T cells were responsible for acquired immunity. These findings suggest that an increased immune response (acquired or natural) may result from excessive cytokine secretion of macrophage/dendritic cells or a perforin/granzyme deficiency in either CD8 T or NK cells, both of which target APCs.

Mortality rates are high for both primary and secondary hemophagocytosis[15]. CCHF has been determined to cause hemophagocytosis, but hemophagocytosis rates for patients who died from CCHF have not been reported. It is also unclear whether hemophagocytosis is responsible for the poor DIC scores of CCHF patients, or which factors are responsible for the initiation, progression and termination of hemophagocytosis in CCHF.

Barut et al.[16] suggested that increased serum ferritin levels may play a role in CCHF-related hemophagocytosis pathogenesis, and therefore, ferritin may be a useful marker for determining the diagnosis and prognosis of the disease. Our patients also had high serum ferritin levels (1601 ng/ml). Ferritin is an

acute phase reactant. Production of ferritin is increased by IL-1, IL-6, TNF, and IFN-γ, all of which increase during CCHF and play a role in the hemophagocytosis pathogenesis[17-19]. We propose that hemophagocytosis may contribute to the CCHF pathogenesis.

In our patient, serum ferritin levels were increased, and hemophagocytosis was present but not fatal, perhaps due to the interaction between the virus, host, and cytokine balance. The virus is presenting cells (APCs) by host macrophages/APCs. The natural and acquired cellular responses depend on the cytokine type and the amount of cytokines secreted by the macrophages/APCs. Natural and acquired immunity (NK cells and CD8 T cells, respectively) target infected macrophages/APCs, and if the perforin/granzyme system is sufficient, the immune cells are terminated and the attack finishes. Our patient experienced hemophagocytosis, potentially because of a temporary perforin/granzyme deficiency during the NK/CD8 T cell-associated immune response, but recovered. Therefore, we recommend a thorough evaluation of viral cell, host cell and cytokine levels for treatment of CCHF.

Crimean-Congo hemorrhagic fever (CCHF) is associated with laboratory anomalies, such as anemia, leukopenia, thrombocytopenia, increased fibrin metabolites (D-dimer), and increased AST/ALT levels, as well as prolongation of PT and aPTT. Urinalysis may show proteinuria and/or hematuria. Oliguria and azotemia may develop[20,21]. In a Turkish study, 35 CCHF patients had increased AST, ALT, LDH, creatinine phosphokinase, leukopenia, and thrombocytopenia[12]. In our patient, laboratory test results were as follows: thrombocytopenia, 61,000/mm^3; leukopenia, 1,300/mm^3; PT prolongation, 2 seconds; CRP, negative; AST, 115 U/L; ALT, 61 U/L; LDH, 416 U/L; D-dimer, 5 μg/dl; triglycerides, 201 mg/dl; creatinine phosphokinase, 509 U/L; and increased ferritin (1601 ng/ml) and hemophagocytosis aspiration. These findings were consistent with both CCHF and hemophagocytosis syndrome.

Although many of the studies discussed above do not mention hemophagocytosis, CCHF has many similarities to hemophagocytosis. We found that in CCHF, NK and CD8 cTL cells, which play a role in hemophagocytosis pathogenesis, were similarly high. NK and CD8 cTL cell counts were positively correlated with IL-1, IL-6 and TNF-α and negatively correlated with IL-10, an active macrophage inhibitor. Thus, CCHF seems to resemble hemophagocytosis and hypercytokinemia. Our patient had hemophagocytosis, but it was not fatal, most likely because his immune system had regulative and regenerative capacity.

In conclusion, patients who live in CCHF-endemic locations and have hemophagocytosis but no history of tick exposure should be evaluated for CCHF.

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