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Associations between inflammatory cytokines and organ damage in pediatric patients with hemophagocytic lymphohistiocytosis

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ABSTRACT

Hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal disease characterized by overwhelming inflammation response and multiple organ damage. Most of the clinical and laboratory manifestations of HLH are thought to be related to hypercytokinemia and organ infiltration with lymphocytes and histiocytes. The aim of this study was to investigate the associations between cytokines and various manifestations of HLH. A total of 105 patients diagnosed with HLH were enrolled in this retrospective study. The information including the patients' demographic characteristics, clinical and laboratory findings at presentation and cytokine data were collected. The median age at diagnosis was 2.8 years, with 74 patients (70.4%) documented Epstein-Barr virus infection. Hepatomegaly (88.6%), splenomegaly (81.9%), cytopenia (68.6%), elevated ferritin level (93.3%), hypofibrinogenemia (61.9%) and hemophagocytosis (77.3%) were found in more than half of the patients. Interleukin (IL)-6, IL-10 and interferon (IFN)-γ were found to be moderately or significantly elevated in most patients. In the correlation analysis, IFN-γ was closely related to the concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, lactate dehydrase (LDH), triglyceride and fibrinogen, while IL-10 was associated with platelet count. When split the patients into two groups according to the cytokine levels, patients with high IFN-γ presented higher level of ALT, AST, bilirubin, LDH, triglyceride, and fibrinogen, while patients with high IL-10 presented much lower hemoglobin and platelet count. In conclusion, the present study put forward clinical evidence that hypercytokinemia is related to organ damage in HLH. IFN-γ may contribute to liver impairment and coagulation disease, while IL-10 is a cytokine related to cytopenias.

1. Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal disease characterized by dysregulated immune responses to antigens, overwhelming immune activation and life-threatening cytokine storm. Patients with HLH frequently present prolonged high fever, progressive cytopenias, liver dysfunction, coagulopathy, and neurologic symptoms [1,2]. The defects of *PRF1* and several genes encoding proteins in degranulation pathway (*MUNC13-4*, *STX11*, *STXBP2*, *LYST*, *AP3B1* and *RAB27A*) underlie the development of HLH [1,2]. The deficiency of granule-mediated cytotoxicity in NK cells and cytotoxic T cells lead to persistent antigen exposure, prolonged cytotoxic T-cell activation and cytokine storm. Marked elevation of various cytokines, such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, IL-10, IL-12 and IL-18, are documented either in animal models or clinical observation [3,4]. All of the clinical and laboratory findings of HLH are thought to be related to hypercytokinemia and organ infiltration with lymphocytes and histiocytes [5]. For example, IFN-γ directly activates macrophages and triggers hemophagocytosis, which leads to the development of severe consumptive anemia and other cytopenias [6]. However, direct clinical evidence is lacking to illustrate most of the relationships between the cytokines and the clinical and laboratory presentations.

We have begun to measure the serum levels of Th1/Th2 cytokines including IFN-γ, TNF-α, IL-10, IL-6, IL-4 and IL-2 by the cytometric bead array (CBA) technique in pediatric hematology/oncology patients with fever since 2005 [4,7,8], and found that the pattern of significant increase of IFN-γ and IL-10 combined

Abbreviations: HLH, hemophagocytic lymphohistiocytosis; ALT, alanine aminotransferase; AST, asparate aminotransferase; LDH, lactate dehydrase; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; EBV, Epstein-Barr virus; CBA, cytometric bead array.

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with a slightly increased level of IL-6 was specific for childhood HLH. In this study, we retrospectively analyzed the relationship between the above cytokines and the clinical presentations including cytopenias, liver impairment and coagulopathy, aiming to illustrate the role of hypercytokinemia in the pathophysiology of HLH.

2. Patients and methods

From July 2009 through June 2014, a total of 118 patients were initially diagnosed as HLH in the Children's Hospital of Zhejiang University School of Medicine, and 105 of them underwent cytokine measurement at diagnosis. The information including the patients' demographic characteristics, clinical and laboratory findings at presentation was collected for analysis in this study.

The diagnosis of HLH was based on HLH-2004 protocol, which mainly included the following items: (1) fever; (2) splenomegaly; (3) cytopenia $\geqslant 2$ cell lineages; (4) hypertriglyceridemia and/or hypofibrinogenemia; (5) serum ferritin $\geqslant 500$ μg/L; (6) hemophagocytosis in bone marrow, CSF or lymph nodes; (7) decreased or absent NK-cell activity. The sCD25 detection was unavailable during the period of this study and NK-cell activity was done only in part of patients.

The Th1/Th2 cytokines were measured at admission, when the diagnosis of HLH made, and at the follow-up time points. Concentrations of IFN-γ, TNF-α, IL-10, IL-6, IL-4, and IL-2 were quantitatively determined with use of the CBA kit (CBA Human Th1/Th2 Cytokine Kit II; BD Biosciences, San Jose, California) as described previously. The cytokine concentrations at the time of diagnosis were used for analysis in this study.

The comparisons of various laboratory test results in different cytokine groups were performed using the Mann-Whitney *U* test. The correlation between laboratory tests and various cytokines were determined with Spearman rank correlation analysis. All statistical analyses were performed using SPSS version 20.0 (IBM, Armonk, NY, USA). $P < 0.05$ were considered statistically significant.

3. Results

3.1. Patients' characteristics

A total of 105 patients with newly diagnosed HLH were included for retrospective analysis. Of these patients, 53 were male and 52 were female, with a male to female ratio of 1:1. The median age at diagnosis was 2.8 years (range, 8 days to 14.6 years), with 42 patients younger than 24 months (40.0%) and 10 patients older than 10 years old (9.5%). As to the possible conditions associated with HLH, infection was documented in 80 patients, including Epstein-Barr virus (EBV) in 74 (70.4%), cytomegalovirus in 7, and other viruses in 3, bacteria in 3, mycoplasma and legionella in 4, fungi in 2 and rickettsia in 1. Rheumatological disorders were documented in 2 patients, and tumor (leukemia and lymphoma) was diagnosed in 3 patients.

3.2. Clinical manifestations and laboratory findings

All patients presented high fever at admission, with the longest duration of 25 days. Signs of reticuloendothelial system activation were common, including hepatomegaly (93/105, 88.6%), splenomegaly (86/105, 81.9%), and lymphadenopathy (44/105, 41.9%). Central nervous system disorders such as altered mental status and seizure were found in 11 (10.5%) patients. At the time of diagnosis, cytopenia was documented in about two thirds of patients (72/105, 68.6%). Neutropenia ($<1.0 \times 10^9$/L), anemia (<90 g/L) and thrombocytopenia ($<100 \times 10^9$/L) occurred in 57.1% (60/105), 41.0% (43/105), and 74.3% (78/105) of cases, respectively. Most patients had some degree of impaired liver function, with elevated alanine aminotransferase (ALT) (>50 U/L, 82/105, 80.0%), aspartate aminotransferase (AST) (>50 U/L, 94/105, 89.5%), total bilirubin (>17.1 mmol/L, 51/105, 48.6%) and lactate dehydrase (LDH) (>300 U/L, 103/105, 98.1%). Hypertriglyceridemia ($\geqslant 3.0$ mmol/L) and hypofibrinogenemia ($\leqslant 1.5$ g/L) were found in 44.7% (47/105) and 61.9% (65/105) of the patients, respectively. Significantly increased serum ferritin level (>500 μg/L) was widely documented in 93.3% (98/105) of the patients, including 75.2% (79/105) with ferritin concentration higher than 1500 μg/L. 77.3% (75/97) of the patients were found to have hemophagocytosis in their bone marrow smears.

3.3. Th1/Th2 cytokines in HLH

The distribution of Th1/Th2 cytokines at the time of diagnosis was shown in Fig. 1. The normal reference ranges for IL-2, IL-4 IL-6, IL-10, TNF-α and IFN-γ were 1.1–9.8 pg/mL, 0.1–3.0 pg/mL, 1.7–16.6 pg/mL, 2.6-4.9 pg/mL, 0.1–5.2 pg/mL and 1.6–17.3 pg/mL, respectively. The median and range of IL-2, IL-4 and TNF-α were 2.35 (0.1–12.8) pg/mL, 2.10 (0.1–23.6) pg/mL, 2.7 (0.1–26.2) pg/mL, respectively, with >90% of the results were in normal range. On the contrary, IL-6, IL-10 and IFN-γ were found to be moderately or significantly elevated in most patients. The median and range of IL-6, IL-10 and IFN-γ were 37.4 (1.1–2432.3) pg/mL, 473.9 (1.6 to >5000) pg/mL and 701.6 (3.4 to >5000) pg/mL, with 83 (79.0%), 103 (98.1%) and 94 (89.5%) patients presenting cytokine levels higher than upper limit of the normal reference range, respectively. Nearly one third of patients presented IL-6 higher than 75 pg/mL (29.5%), IL-10 higher than 800 pg/mL (30.4%) and IFN-γ higher than 1000 pg/mL (35.2%), and were assigned into high cytokine groups, respectively; while the patients with IL-6 less than 75 pg/mL, IL-10 less than 800 pg/mL or IFN-γ less than 1000 pg/mL were assigned into low cytokine groups. The IL-6 (27.6 pg/mL vs. 51.0 pg/mL, $P = 0.136$), IL-10 (451.6 pg/mL vs. 493.1 pg/mL, $P = 0.736$) and IFN-γ (733.2 pg/mL vs. 649.8 pg/mL, $P = 0.961$) distribution were comparable between patients with EBV related HLH and non-EBV related HLH. The gender distribution and the neutrophil count were not related to the cytokine levels as well (data not shown).

3.4. Associations between cytokines and blood count

We first investigated the relationship between the cytokines and blood count. As shown in Table 1, IL-6 and IFN-γ had no relationship with blood count, while IL-10 was associated with platelet count. We then compared the blood count in low and high cytokine groups. As shown in Table 2, similarly, the blood counts were

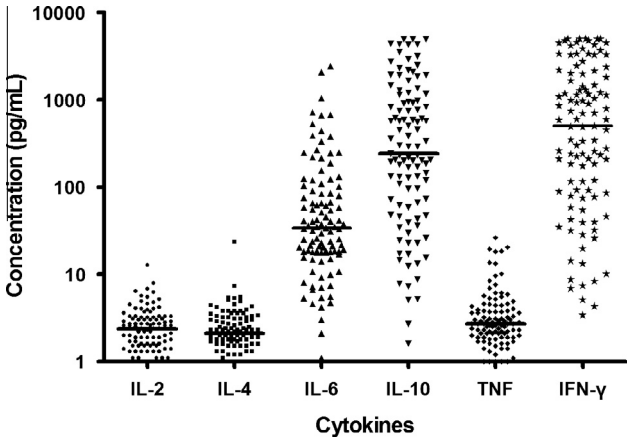

Fig. 1. Distribution of Th1/Th2 cytokines in pediatric patients with HLH.