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Cytokine Profiles in Children with Primary Epstein-Barr Virus Infection

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Abstract

Primary Epstein-Barr virus (EBV) infection causes infectious mononucleosis and hemophagocytic lymphohistiocytosis (HLH) in children, where EBV infects B and $CD8^+$ T cells, respectively. We measured pro-inflammatory and anti-inflammatory cytokines in both diseases. Significantly higher concentrations of various mediators, including interferon-γ, neopterin, interleukin (IL)-6, IL-10, IL-18 and heme oxygenase-1, were observed in EBV-HLH. Because of their similarity to the profile of familial HLH, this profile was likely a consequence of HLH, but not ectopic infection. TNF-α levels were elevated in both diseases. Elevation of those mediators may contribute to the disease pathogenesis of EBV-HLH by activating and inhibiting host immune responses.

Introduction

Epstein-Barr virus (EBV) is a ubiquitous virus that infects the majority of the world's population [1]. Primary infection of EBV is usually asymptomatic but may cause infectious mononucleosis (IM). EBV infects B cells in EBV-associated IM (EBV-IM) and persists in B cells for the lifetime of the seropositive normal host. However, primary EBV infection has also been linked with hemophagocytic lymphohistiocytosis (HLH), which is an aggressive lymphoproliferative disorder [2,3]. Several reports have demonstrated that EBV infection is characterized by an increase in $CD8^+$ T cells in EBV-HLH, although the mechanism underlying ectopic EBV infection remains unclear [4-7].

The symptoms of EBV-IM and EBV-HLH are associated with T-cell activation and cytokine production [8]. In EBV-IM, proliferation of activated antigen-specific and nonspecific cytotoxic $CD8^+$ T cells leads to release of interferon (IFN)-γ, interleukin (IL)-2, and tumor necrosis factor (TNF)-α [9,10]. Studies of cytokines from HLH patients have also demonstrated elevated concentration of many pro-inflammatory cytokines, such as IFN-γ, TNF-α, and IL-6 [11-13]. However, because HLH is a heterogenous group of disorders, most reports have contained subgroups with different disease etiologies [11-13]. Moreover, the cellular targets and clonality of EBV have not been well characterized, even in reports describing EBV-HLH [11-13]. It is therefore unknown whether the differential cellular targets of EBV lead to different cytokine profiles. In this report, we analyzed markers considered to be key in patients with EBV-IM and EBV-HLH.

Methods

Study population

We studied 22 patients with primary EBV infection: 11 with EBV-HLH and 11 with EBV-IM (Table I). All patients were previously healthy. The data of 6 patients with EBV-HLH have been reported elsewhere [5]. The remaining patients with EBV-HLH also showed typical clinical features of HLH and exhibited high viral copy numbers. It was noted that $CD8^+$ T cells were the major cellular targets of EBV in all EBV-HLH patients by *in situ* hybridization for EBV-encoded small RNA1 in the available samples [5]. The diagnosis of EBV-IM was clinically determined. All patients with EBV-IM exhibited self-limited disease. Primary EBV infection was serologically confirmed for all cases of EBV-HLH and EBV-IM. We also investigated 4 cases of familial HLH (FHL) due to perforin deficiency as a disease control. Samples were collected during the acute phase of the diseases. The study was approved by the Human Research Committee of Kanazawa University Graduate School of Medical Science, and informed consent was obtained according to the Declaration of Helsinki.

Cytokine determination

Serum or plasma concentrations of cytokines were determined using the following ELISA kits: neopterin (IBL, Hamburg, Germany); IFN-γ, IL-6, TNF-α, soluble TNF receptor type 1 (sTNF-RI) and type 2 (sTNF-RII), and soluble CD163 (sCD163) (R&D systems, Minneapolis, MN); IL-18 (MBL, Nagoya, Japan); IL-10 (eBioscience, San Diego, CA); and heme oxygenase-1 (HO-1; Enzo, Ann Arbor, MI) [14,15]. Analysis of

significant differences among groups was performed using the Mann-Whitney *U* test. Differences with *p*-values less than 0.05 were considered significant.

Results

Patients with EBV-HLH exhibited high levels of IFN-γ, neopterin, IL-6, IL-18, TNF-α, sTNF-RI and sTNF-RII (Figure 1), all of which were statistically significant. Patients with EBV-IM also showed high concentrations of pro-inflammatory cytokines, albeit at lower levels than those of EBV-HLH. Six of 11 patients with EBV-IM showed high TNF-α levels similar to those of EBV-HLH, which was consistent with a previous report [9]. IL-10 was markedly elevated in patients with EBV-HLH, whereas patients with EBV-IM exhibited only modestly elevated IL-10 levels. The levels of sCD163 were elevated in both EBV-HLH and EBV-IM. Patients with EBV-HLH exhibited higher levels of HO-1 compared with those of EBV-IM.

Discussion

In this report, we describe cytokine profiles from children affected with EBV-HLH and EBV-IM. While both diseases are characterized by acute systemic inflammatory responses induced by primary EBV infection, the clinical courses are quite different. EBV-HLH is a potentially fatal disease that often requires immune-modulating

chemotherapy, whereas EBV-IM is a benign self-limited disease. This difference may be fundamentally due to the differential cellular targets of EBV infection; EBV ectopically infects $CD8^+$ T cells in EBV-HLH, while B cells are the target in EBV-IM [4-6]. Alternatively, the patients may develop HLH based on the ability of the immune system to cope with this pathogen. However, it remains unknown whether the differential cellular targets of EBV lead to different cytokine profiles in the peripheral blood. To address this issue, serum or plasma concentrations of various cytokines were determined.

Hypersecretion of pro-inflammatory cytokines produced by activated T cells and macrophages accounts for the severe systemic manifestations in HLH [2,3]. In fact, our patients with EBV-HLH exhibited profound hypercytokinemia. The high concentration of neopterin might indicate pathological activation of macrophages and involvement in the disease development of EBV-HLH [16]. These cytokine profiles were similar to those found in FHL caused by perforin deficiency, which is the most common form of genetic HLH [2,3]. The cytokine profile of EBV-HLH is therefore likely a general consequence of HLH, rather than a result of clonal proliferation of EBV-infected $CD8^+$ T cells. Some patients with EBV-IM exhibited marked immune responses to regulate EBV-infected B cells and shared typical findings of HLH, such as cytopenia and hemophagocytosis. Because such patients tended to exhibit higher levels of pro-inflammatory cytokines, it was difficult to clearly distinguish EBV-HLH from EBV-IM based solely upon the cytokine profiles. However, we have used flow cytometry to detect EBV-infected cells in EBV-HLH as clonally-expanded and highly-activated $CD8^+$ T cells with CD5 downregulation [5,6]. Since suppression of severe hyperinflammation and eradication of EBV-infected cells are crucial in treatment of EBV-HLH [17], the combined analysis of

cytokine profiles and our flow cytometry approach may be useful in follow-up of patients with EBV-HLH.

To evaluate the anti-inflammatory responses in EBV-HLH and EBV-IM, we also analyzed circulating mediators, including IL-10, sCD163 and HO-1 (Figure 1). IL-10 plays multiple roles in immunosuppression and contributes to maintaining the balance of the immune system in inflammation. Consistent with previous reports [11-13], IL-10 was markedly elevated in EBV-HLH patients. EBV-infected cells in EBV-HLH secrete viral IL-10, a product of the EBV replication gene *BCRF1*, which shares structural and functional similarity with human IL-10 [18]. However, patients with FHL exhibited levels of IL-10 that were comparable to EBV-HLH, suggesting that the high IL-10 levels in EBV-HLH were likely a consequence of HLH itself. Further studies using ELISA that can distinguish between human and viral IL-10 will be necessary. A soluble hemoglobin scavenger receptor (sCD163) is also shed from the monocyte-macrophage membrane upon inflammatory stimuli and reflects anti-inflammatory processes [15]. Interestingly, our results for sCD163 indicate alternative activation of macrophages, in addition to classical activation, in primary EBV infection. HO-1 is the rate-limiting enzyme in the heme breakdown pathway, and has potent anti-inflammatory, anti-apoptotic, and anti-oxidative effects [19]. It has recently been reported that HO-1 is useful in the differential diagnosis of secondary HLH [20]. Indeed, our EBV-HLH patients exhibited higher levels of HO-1. Taken together, the profiles of anti-inflammatory mediators among EBV-HLH, EBV-IM, and FHL were largely similar to the profiles of pro-inflammatory cytokines.

In summary, our studies demonstrate significantly higher concentrations of both pro-inflammatory cytokines and anti-inflammatory mediators in EBV-HLH compared

with EBV-IM. These profiles may contribute to the disease pathogenesis of EBV-HLH by activating and inhibiting host immune responses.

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Conflict of interest

Nothing to declare.

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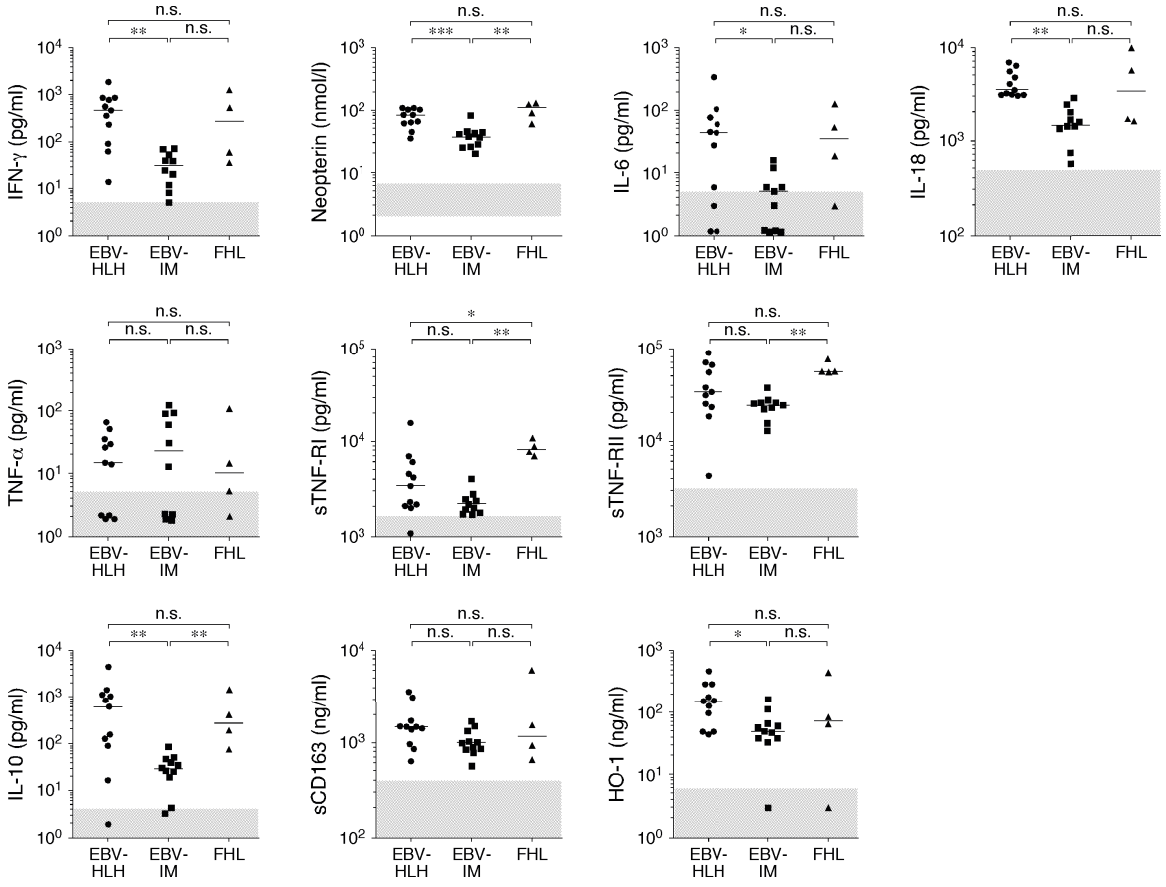
Figure Legends

Fig. 1. Cytokine profiles in patients with EBV-HLH, EBV-IM, and FHL.

Serum or plasma concentrations of interferon-γ, neopterin, interleukin (IL)-6, IL-18, tumor necrosis factor (TNF)-α, soluble TNF receptor type 1 (sTNF-RII) and type 2 (sTNF-RII), IL-10, soluble CD163 (sCD163) and heme oxygenase-1 (HO-1). Horizontal bars indicate the median values. Shaded areas represent the ranges of normal values. EBV, Epstein-Barr virus; HLH, hemophagocytic lymphohistiocytosis; IM, infectious mononucleosis; FLH, familial HLH; n.s., not significant. *$p < 0.05$; ** $p < 0.01$; *** $p \leq 0.001$.


IFN-γ (pg/ml)
Neopterin (nmol/l)
IL-6 (pg/ml)
IL-18 (pg/ml)
TNF-α (pg/ml)
sTNF-RI (pg/ml)
sTNF-RII (pg/ml)
IL-10 (pg/ml)
sCD163 (ng/ml)
HO-1 (ng/ml)
n.s.
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EBV-HLH
EBV-IM
FHL