Cytokine Responses in a Severe Case of Glandular Fever Treated Successfully with Foscarnet Combined with Prednisolone and Intravenous Immunoglobulin

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INTRODUCTION

Acute, primary Epstein–Barr virus (EBV) infection is often an asymptomatic or mild illness in young children, but can be more severe in teenagers and young adults as infectious mononucleosis or glandular fever [Straus et al., 1993; Macsween and Crawford, 2003]. This is due to clinical disease caused by acute EBV infection being mainly immune-mediated, with older children and teenagers having a more exaggerated response to primary EBV infection than younger children [Williams et al., 2004; Hislop et al., 2007]. Severe (life-threatening) complications usually only arise if they have some primary immunodeficiency, the most serious of which is X-linked lymphoproliferative disease [Williams et al., 2004; Milone et al., 2005], or if they have acquired some form of immunosuppression later in life, e.g. human immunodeficiency virus (HIV) infection or require chemotherapy or organ transplantation, when their EBV may reactivate, in the absence of effective T cell immunosurveillance, to cause various forms of B cell lymphoproliferative disease [Macsween and Crawford, 2003].

In the UK and USA, infectious mononucleosis is not uncommon where perhaps 20–30% of young adults are susceptible to primary EBV infection [Kangro et al., 1994; Martro et al., 2004], but this is rare in Hong Kong, where virtually all children have been infected with EBV by the age of 10 years [Kangro et al., 1994]. Interestingly, one previous study of acute EBV infection in young Hong Kong children (<2–15 years old) have demonstrated that their clinical manifestations are different from similar Western paediatric populations,
in that more Chinese children tend to become symptomatic with typical features of infectious mononucleosis [Chan et al., 2000]. However, another study suggested that primary EBV infection in younger Hong Kong infants may be mild or asymptomatic due to the presence of anti-EBV maternal antibodies, at least, up to the age of 8 months [Chan et al., 2001].

In this report, an unusually severe case of primary EBV infection in an 18-year-old Hong Kong Chinese boy is presented. Functional hepatic and haematological abnormalities were present as well as a significant EBV viral load. Combination treatment with intravenous immunoglobulin (IVIG), steroids and antiviral therapy successfully controlled the patient's disease allowing a full recovery. His cytokine profile was monitored throughout his acute illness and these results guided in part the clinical management of his disease.

MATERIALS AND METHODS

Case Report

An 18-year-old previously fit and healthy boy, with no past evidence of immunodeficiency and no recent travel history, was admitted with 10-day history of fever (39.5°C on admission), myalgia, sore throat and malaise. On examination, he had a congested throat, bilateral cervical lymphadenopathy, a fine, generalized macular rash, and mild hepatosplenomegaly. His blood pressure was 105/75 mmHg with a pulse of 80 bpm. On admission, his routine blood test results showed a mild bicytopenia with severe hepatitis, with a total white cell count of 6.9 x 10^9 L^-1 (neutrophils 31%, lymphocytes 60–3% atypical lymphocytes), platelets 118 x 10^9 L^-1, hemoglobin (Hb) 13.2 g/dL, alanine aminotransferase (ALT) 1600 IU/L, alkaline phosphatase (ALP) 302 IU/L, bilirubin 26 μmol/L, lactic dehydrogenase 1415 IU/L, prothrombin time 14.5 sec and activated partial thromboplastin time 47.5 sec. A rapid monospot and confirmatory Epstein–Barr virus (EBV) virus capsid antigen (VCA) IgM tests were positive, consistent with a diagnosis of infectious mononucleosis due to primary, acute EBV infection. Serological and polymerase chain reaction (PCR) tests were performed to rule out other possible viral causes of severe hepatitis and an abdominal ultrasound scan was also performed, several days after admission.

His condition continued to deteriorate and by the third week of his illness he had developed severe jaundice, progressive leuko- and thrombocytopenia, a purpuric skin rash and a persistent, swinging fever. Low haptoglobin levels (<0.2 g/L) and a positive Coomb's test, with a raised total bilirubin (up to 285 μmol/L on day 28 post-illness onset), suggested a hemolytic anemia. This deterioration was thought to be due to an EBV-induced immunopathological response, so the antiviral drug foscarnet (intravenous, 4.5 g every 12 hr) was started on day 21 of illness. Drug response was monitored using an in-house quantitative real-time EBV DNA PCR assay.

Despite effective viral suppression and regression of his lymphadenopathy and splenomegaly, his fever persisted and he developed bilateral pleural effusions with ECG abnormalities (T-wave inversion over lateral leads). By day 26 his thrombocytopenia reached a nadir of 26 x 10^9 L^-1 with an Hb of 9.4 g/dL. A bone marrow examination showed normocellular marrow with reactive hemophagocytosis.

Intravenous immunoglobulin (IVIG, Intragam P, CSL Ltd., Australia, 400 mg/kg/day for 5 days) and oral prednisolone (1 mg/kg/day for 7 days) were started on days 27 and 29 of illness, respectively. By day 31 of illness, his fever began subsiding with recovery of his Hb and platelet counts. Foscarnet was stopped after 17 days once EBV suppression was achieved, due concerns about a potential neutropenic adverse drug reaction. Despite this, 5 days later, the patient developed a severe neutropenia (other cell lines preserved), with the absolute neutrophil count dropping to <0.5 x 10^9 L^-1. He was treated with G-CSF (30 MU/day, subcutaneously) for 3 days, with a good response and subsequent normalization of the cell counts. He was finally discharged well after 48 days of illness, then followed up in the infectious diseases clinic 2 weeks later, where he reported having remained well and asymptomatic.

Assay of Serum Concentrations of Cytokines and Chemokines

Serum was prepared from clotted blood by centrifugation (2000g for 10 min). Concentrations of cytokines and chemokines IL-12p70, IFN-γ, TNF-α, IL-1β, IL-10, IL-4, IL-6, CXCL8, CCL3, CCL5, CXCCL10 were measured using cytometric bead array (CBA) with flex set reagents (BD PharMingen, San Diego, CA, USA) on a 4-color FACScalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) located in a biosafety level-2 laboratory [Wong et al., 2004; Wong et al., 2007]. These were chosen to measure both the Th1 and Th2 immune responses to acute EBV infection. In CBA, different bead populations with distinct fluorescence intensities had been coated with capturing antibodies specific for different cytokines or chemokines. After incubating with 50 μL of serum, the cytokine/chemokine captured beads were mixed with phycoerythrin-conjugated detection antibodies to form sandwich complexes. Fluorescence flow cytometry of the beads provided simultaneous quantification of a panel of cytokines and chemokines. Serum concentrations of soluble tumor necrosis factor receptor1 (sTNFR1) were assayed using a human TNF R1 quantitative colorimetric sandwich ELISA kit (R&D systems Corp., MN, USA). The coefficients of variation for all cytokines and chemokines assays were less than 10%. Their respective normal ranges have been derived from measurement of ≥100 healthy subjects [Wong et al., 2004, 2007].

Assay of Plasma EBV DNA Levels

Plasma was prepared from EDTA blood after centrifugation (2000g for 10 min). DNA was extracted from
800 μL of plasma using the QIAamp Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the "blood and body fluid protocol" as recommended by the manufacturer and eluted with 50 μL of water. The plasma EBV DNA levels were measured using a real-time quantitative PCR system targeting the BamHI-W fragment region of the EBV genome using the primers W-44F (5'-CCCAACACTCCACCACCC-3') and W-119R (5'-TCTTAGGAGCTGTCCGAGGG-3') and the dual-labeled fluorescent probe W-67T [5'-(FAM)CACAACACACCCACCCGTCTC(TAMRA)-3'] [Lo et al., 1999]. Fluorogenic PCR reactions were set up in a reaction volume of 50 μL using components (except for the fluorescent probes and amplification primers) supplied in a TaqMan PCR Core Reagent Kit (Applied Biosystems, Foster City, CA, USA). Each reaction contained 5 μL of 10X buffer A; 300 nM of each of the amplification primers; 25 nM of the fluorescent probe; 4 mM MgCl2; 200 mM each of dATP, dCTP, and dGTP; 400 mM dUTP; 1.25 units of AmpliTaq Gold; 0.5 unit of AmpErase uracil N-glycosylase, and 5 μL of plasma DNA amplifications were carried out in a 96-well reaction plate format in an Applied Biosystems 7300 Sequence Detector. Each sample was analyzed in duplicate and the mean of the two results was taken.

RESULTS

Serological tests for acute hepatitis A, B, C and E were all negative. Polymerase chain reaction (PCR) testing in an acute serum sample was negative for human cytomegalovirus, parvovirus B19 and human herpesviruses 6 and 7. Furthermore, no virus was isolated from a throat and oral swab, urine, nasopharyngeal aspirate or bone marrow sample. These confirmed that primary EBV infection was the most likely cause of the patient's severe thrombocytopenia and hepatitis. The abdominal ultrasound showed hepatosplenomegaly (liver span 14.5 cm, spleen 15 cm), mild ascites and bilateral bulky kidneys.

Table I shows that the patient's main haematological abnormalities were a mild anaemia (Hb 8.8–11.9 g/dL), a severe thrombocytopenia (33–127) x 10^9 L^-1), a moderate to severe neutropaenia (1–40%) and mild to moderate lymphocytosis (48–83%) despite a relatively normal total white blood cell count (WCC). Derangement of his liver function tests including a mild clotting impairment (international normalized ratio, INR, up to 1.36; activated thromboplastin time, APTT, up to 50.5 sec), a low albumin (as low as 23 g/L), a raised total bilirubin (as high as 318 μmol/L), a raised alkaline phosphatase (ALP up to 635 IU/L) and grossly raised alanine transaminase (ALT, up to 1600 IU/L). It can be seen that there is a brief and rapid improvement in the patient’s haematological parameters during the period when he was on the IVIG, prednisolone or the foscarnet during days 27–39 post-illness onset.

Viral load monitoring for EBV DNA showed a rapid 3 log₈ decrease in EBV levels from 69831 to 22 copies/mL

<table>
<thead>
<tr>
<th>TABLE I:</th>
<th>Hb (g/dL)</th>
<th>WCC (x10^9/L)</th>
<th>Platelets (x10^9/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>ESR (mm/hr)</th>
<th>CRP (μg/L)</th>
<th>Total bilirubin (μmol/L)</th>
<th>Total protein (g/L)</th>
<th>Albumin (g/L)</th>
<th>INR</th>
<th>APTT (sec)</th>
<th>PT (s)</th>
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</thead>
<tbody>
<tr>
<td>16 August</td>
<td>13.2</td>
<td>38</td>
<td>251</td>
<td>25</td>
<td>635</td>
<td>5</td>
<td>0.2</td>
<td>318</td>
<td>54</td>
<td>30.4</td>
<td>1.16</td>
<td>16.5</td>
<td>11.7</td>
</tr>
<tr>
<td>17 August</td>
<td>13.4</td>
<td>105</td>
<td>105</td>
<td>32.7</td>
<td>100</td>
<td>2</td>
<td>0.2</td>
<td>318</td>
<td>54</td>
<td>30.4</td>
<td>1.16</td>
<td>16.5</td>
<td>11.7</td>
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<td>18 August</td>
<td>13.4</td>
<td>104</td>
<td>104</td>
<td>32.7</td>
<td>100</td>
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<td>0.2</td>
<td>318</td>
<td>54</td>
<td>30.4</td>
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<td>16.5</td>
<td>11.7</td>
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<tr>
<td>20 August</td>
<td>13.9</td>
<td>104</td>
<td>104</td>
<td>32.7</td>
<td>100</td>
<td>2</td>
<td>0.2</td>
<td>318</td>
<td>54</td>
<td>30.4</td>
<td>1.16</td>
<td>16.5</td>
<td>11.7</td>
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<tr>
<td>21 August</td>
<td>15.3</td>
<td>105</td>
<td>105</td>
<td>32.7</td>
<td>100</td>
<td>2</td>
<td>0.2</td>
<td>318</td>
<td>54</td>
<td>30.4</td>
<td>1.16</td>
<td>16.5</td>
<td>11.7</td>
</tr>
</tbody>
</table>

*The patient was on intravenous immunoglobulin on days 27–32, prednisolone on days 28–36, and foscarnet during days 27–39 post-illness onset.*
plasma on intravenous foscarnet during days 21–36 post-illness onset (Fig. 1, Table II).

The cytokine analysis showed significantly marked elevation (at least 10 log₁₀ above their reference ranges) in serum levels of Th1 cytokines IFN-γ and soluble TNFR1, Th1-related chemokines CXCL10, CXCL9 and CCL3 for chemotactic migration of Th1 cells and macrophages, on days 11–27 post-illness onset. An early spike of the Th1 cytokine IL-12 was observed on day 11 post-illness onset (Table II).

**DISCUSSION**

In this severe case of glandular fever in a Chinese teenager, the opportunity was taken to further investigate the immunopathogenesis of this severe EBV infection with serial cytokine studies. The most significant findings of the cytokine analysis was marked elevation in levels of IFN-γ, CXCL10, CXCL9 and CCL3 on days 11–27 after illness-onset.

Previous cytokine studies on acute EBV infection have shown that Th1 responses are prominent in glandular fever, with elevated IL-2, IL-12 and IFN-γ levels [Andersson, 1996; Corsi et al., 2004]. In this case, the hyper-Th1 response was observed with the increase in levels of IFN-γ, IL-12, sTNFR1, CXCL10, CXCL9 and CCL3. This Th1 (cell-mediated immunity) cytokine and chemokine storm was probably due to an EBV-induced Th1 immune response, via the IL-12 driven activation of IFN-γ- and CCL3-producing CD8+ cytotoxic T cells [Attarbaschi et al., 2003]. The unusual concomitant increased Th2 (humoral immunity) cytokine IL-4 could be due to a pronounced increase in IFN-γ and IL-4 co-expressing CD4+ T cells [Attarbaschi et al., 2003]. Such a response, in some immunosuppressed individuals, may potentially lead to the development of B cell lymphoproliferative disease caused by the clonal outgrowth of EBV-infected B-cells [Ohga et al., 2002].

A comparison with some of the acute phase (generally within 7–10 days of illness onset) cytokine levels measured in this and other reported cases of infectious mononucleosis can be seen in Table III. The specific cytokines listed in Table III are those that have other values for comparison, as reported in the literature for patients with infectious mononucleosis, so not all the ones listed in Table II are shown in Table III. In this study, the earliest sample was taken about 11 days after illness onset, due to the relatively late presentation of this case. Hence, only the day 11 cytokine levels are reported for this study in Table III. It can be seen that these cytokine levels vary considerably between these studies, which may be due to several differences between each study, including patient ages, days after illness onset and laboratory cytokine testing kits. Therefore, it is likely that a meaningful comparison can only be made between patients within the same study protocol. However, despite this, concentrations of the Th1 cytokine IFN-γ and the inflammatory cytokines IL-6 and CXCL8 were found to be possibly higher in this
Cytokines in Treated Glandular Fever

Foscarnet rapidly curtailed the EBV replication and gradually suppressed the accompanying Th1 and Th2 immune responses in this case. However, persistent elevation of inflammatory cytokine IL-6 and CXCL8 could only be suppressed after combined treatment with IVIG and prednisolone. Th1 response and aberrant production of IL-6 and CXCL8 could lead to the activation of macrophages and the subsequent hemophagocytic syndrome, resulting in an exaggerated host immune response and possible fatal outcome [Wong et al., 2004].

The present case illustrates the autocrine loop of both Th1 and Th2 in an acute and the subsequent persistent inflammatory immune response in prolonged EBV infection [Ohga et al., 2002]. This may provide a basis for understanding the effectiveness of the immune-modulating effects of IVIG and steroid treatment in this and other previously reported severe cases of infectious mononucleosis [Brandfonbrener et al., 1986; Dotevall and Westin, 1989; Cyran et al., 1991].

For treatment considerations, it is known that Epstein-Barr virus viral loads in blood may be higher in more severely symptomatic patients [Williams et al., 2004; Balfour et al., 2005] and that a reduction of EBV viral loads is associated with clinical benefit [Balfour et al., 2007]. Hence, in this case, the severe clinical presentation indicated that an attempt should be made to reduce the patient’s EBV viral load, which was of the order of $4 \log_{10}$ (about 70,000 EBV DNA copies/mL) in the acute phase—a similar level to some previously reported studies of infectious mononucleosis [Balfour et al., 2005, 2007].

Several treatments have been tried for severe infectious mononucleosis, including steroids either alone or in combination with acyclovir. However, neither of these regimens have been shown to be consistently effective. Steroids may produce rapid symptom relief, but may increase the risk of more severe complications such as encephalitis and myocarditis. Therefore, steroid use, alone, is generally not recommended except for severe cases, e.g. those with impending airways obstruction [McGowan et al., 1992; Straus et al., 1993; Macsween and Crawford, 2003].

The use of steroids in combination with acyclovir has also been reported, with mixed results. This class of antiviral drugs has less effect on EBV-associated disease because their inhibitory effects only act on the linear form of the viral DNA genome whereas both the linear and circular forms are used in EBV replication in acute infection [Straus et al., 1993]. Also, acute EBV disease is mainly immunopathological rather than a direct effect of the virus itself [Macsween and Crawford, 2003; Hislop et al., 2007], unlike other herpesvirus infections such as herpes simplex types 1, 2 and varicella zoster, against which acyclovir is more effective. Although acyclovir has been found to suppress oral shedding of EBV, a randomized, multi-center, double-blind study than in these previously reported studies of EBV infectious mononucleosis [Biglino et al., 1996; Wright-Browne et al., 1998; Corsi et al., 2004].

<table>
<thead>
<tr>
<th>Days post-illness onset</th>
<th>EBV load (UL-12970) (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
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<tbody>
<tr>
<td>11</td>
<td>10.2</td>
<td>2351.3</td>
<td>29.3</td>
<td>22.2</td>
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<td>17</td>
<td>14.4</td>
<td>3301.3</td>
<td>28.3</td>
<td>22.2</td>
<td>21.9</td>
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<tr>
<td>21</td>
<td>14.223</td>
<td>3197.1</td>
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<tr>
<td>27</td>
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<td>3400.6</td>
<td>29.0</td>
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<tr>
<td>48</td>
<td>14.228</td>
<td>3400.6</td>
<td>29.0</td>
<td>22.2</td>
<td>21.9</td>
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<tr>
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<td>&gt;8.8</td>
<td>&lt;10.0</td>
<td>7.2</td>
<td>7.2</td>
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</tbody>
</table>

The patient was on intravenous immunoglobulin on days 37–43, prednisolone on days 28–36 and foscarnet on days 21–28.

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TABLE III. Comparison of Some Cytokine Levels in EBV Infectious Mononucleosis Across Different Studies

<table>
<thead>
<tr>
<th>Serum cytokine/chemokine (all units pg/mL, unless stated otherwise)</th>
<th>This study n = 1, 18 years, M</th>
<th>Biglino et al., n = 18, 23 ± 6 years, 4F:14M</th>
<th>Wright-Browne et al., n = 14, 20 ± 2 years ‘college students’, NG</th>
<th>Corsi et al., n = 23, NG, NG</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12p70</td>
<td>10.2</td>
<td>—</td>
<td>7.75 (median)</td>
<td>73.8 ± 3.5 (as ‘IL-12’)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.4</td>
<td>115 ± 69</td>
<td>35.15 (median)</td>
<td>—</td>
</tr>
<tr>
<td>IL-10</td>
<td>29.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>IL-6</td>
<td>20.4</td>
<td>13 ± 12</td>
<td>4.85 (median)</td>
<td>—</td>
</tr>
<tr>
<td>IL-18</td>
<td>3.6</td>
<td>21 ± 30</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>CXCL8 (IL-8)</td>
<td>40.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>IL-4</td>
<td>96.5</td>
<td>1725 ± 1833</td>
<td>—</td>
<td>—</td>
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<tr>
<td>IFN-γ</td>
<td>231.3</td>
<td>170 ± 127</td>
<td>—</td>
<td>6.3 ± 2.7</td>
</tr>
</tbody>
</table>

n, number of patient results reported; M, male; F, female; NG, not given; —, not tested/reported.

Thus, this case provides additional data to the literature on the treatment of severe EBV infectious mononucleosis in an otherwise previously fit and healthy, immunocompetent young adult.

REFERENCES


