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Case Report

Virological and immunological characteristics of fatal Epstein-Barr virus mononucleosis in a 17-year-old Caucasian male presenting with meningoencephalitis and hemophagocytic syndrome

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In this report, the authors present a detailed immunological and virological assessment of an immunocompetent 17-year-old Caucasian male with a fatal Epstein-Barr virus (EBV) infectious mononucleosis presenting with meningoencephalitis and hemophagocytic syndrome. The patient with serologically confirmed EBV infectious mononucleosis was admitted to the hospital because of 3 weeks' fever. Fine-needle aspiration of lymph nodes showed reactive hyperplasia with prominent hemophagocytosis. Percentages of intracellular interferon-gamma (IFN-γ) in $CD4^+$ and $CD8^+$ T cells in the peripheral blood progressively increased during the course of disease (10.2% and 8.5% on day 35; 30.1% and 53.2% on day 44; 42.2% and 75.2% on day 50; 36.1% and 50.6% on day 59, respectively). On day 50, the patient developed meningoencephalitis. Brain computed tomography (CT) was normal. Brain magnetic resonance imaging (MRI) showed multifocal inflammatory lesions in frontal and temporal cortex of the right hemisphere as well as severe perivascular inflammatory reaction. The patient was treated with steroids, cyclosporin A, and methotrexate intratecally. Following treatment, EBV viremia in the blood and cerebrospinal fluid (CSF) decreased from pretreatment values (54,490 copies of EBV DNA/ml and 39,500 copies/ml, respectively) to 8715 copies/ml in the blood and 14,690 in the CSF. Despite treatment, the patient remained unconscious and died of sepsis and pneumonia 3 months after initial symptoms. Immunohistochemical staining showed the presence of EBV in both perivascular infiltrates and grey matter. Enhanced Th1 response as shown by high levels of IFN-γ in peripheral blood lymphocytes may be a predictor of severe complications during acute EBV infection. Early implementation of immunosuppressive therapy in these patients should be considered. *Journal of NeuroVirology* (2007) **13,** 389–396.

Keywords: fatal EBV meningoencephalitis; hemophagocytic syndrome; infectious mononucleosis; interferon-gamma

Introduction

Acute Epstein-Barr virus (EBV)-induced infectious mononucleosis in the majority of immunocompetent hosts presents as a benign self-limiting disease. However, uncommon and sometimes fatal cases of infectious mononucleosis in patients with hepatic, renal, neurological, autoimmune, and lymphoproliferative

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complications have been described (Majid *et al*, 2002; Zephir *et al,* 2002; Khamma and Kumar, 2003; Hiroshima *et al*, 2003; Kobbervig *et al*, 2003; Sato *et al*, 2004). It is estimated that 1.0% to 5.0% of patients with EBV-induced infectious mononucleosis experience central nervous system (CNS) symptoms, but the exact incidence is unknown. A number of case reports described patients with infectious mononucleosis and CNS complications (meningitis, encephalitis, myelitis, neuropathy, myeloradiculitis, and encephaloradiculitis) (Majid *et al*, 2002; Zephir *et al*, 2002).

Severe or fatal infectious mononucleosis is commonly accompanied by virus-associated hemophagocytic syndrome (Kumakura, 2005). Hemophagocytic syndrome is caused by the abnormal activation of macrophages or histiocytes accompanied with intensive hemophagocytosis in the bone marrow, spleen, liver, and lymph nodes.

Virus-associated hemophagocytic syndrome is usually caused by EBV and is frequently described in immunosuppressed adults. Clinical presentation of virus-associated hemophagocytic syndrome usually includes high fever, constitutional symptoms, liver dysfunction, coagulation abnormalities, peripheral blood cytopenias, hepatosplenomegaly, and lymphadenopathy. Although the clinical presentation of virus-associated hemophagocytic syndrome can be benign, fatal outcome have also been described (mostly in individuals of Asian descent) (Kumakura, 2005). The current hypothesis holds that overexpression of proinflammatory cytokines functionally related to the abnormal activation of macrophages and possibly EBV-infected memory T cells play a major role in the pathogenesis of this disease.

In this report, we present a detailed immunological and virological assessment of an immunocompetent young Caucasian male with EBV-induced fatal meningoencephalitis and hemophagocytic syndrome during infectious mononucleosis.

Results

Quantification of EBV DNA in the blood and cerebrospinal fluid

We used real-time polymerase chain reaction (PCR) to quantify EBV DNA in peripheral blood (PB) and cerebrospinal fluid (CSF) of the patient before and after steroid treatment. Acute EBV infection in the patient was previously confirmed by detection of immunoglobulin M (IgM) anti-VCA (viral capsid antigen) antibodies. Pretreatment, EBV viremia (day 50 of hospitalization) in the PB was 54,490 copies/ml and 39,500 copies/ml in the CSF. Upon steroid treatment, EBV DNA viral load decreased to 8715 copies/ml in the PB and 14,690 in the CSF (day 64 of hospitalization). CSF of the patient was tested and found negative for the presence of herpes simplex virus (HSV)-1/2 DNA by real-time PCR. Human immunodeficiency virus (HIV)-1 RNA and cytomegalovirus (CMV) DNA were not detected in the plasma.

Immunohistochemistry

Brain biopsy revealed intensive prerivascular infiltration with lymphoid cells around the blood vessels. The infiltration was composed of small lymphocytes and irregular big lymphoid cells with cleaved and irregular nucleus. Neuronophagia was common, such as reactive microglial infiltration through brain tissue (Figure 1A). Ratio between T and B cells (Figure 1B) was approximately 1:1. The majority of infiltrating T cells expressed CD8 (Figure 1C). Perivascular infiltration of CD68 reactive macrophages was present in both grey and white matter. Immunohistochemical staining showed the presence of EBV in perivascular lymphoid infiltrate (Figure 1D).

CSF lymphocyte subpopulations

The majority of CSF T cells (85.1% and 95.6% on days 50 and 64, respectively) were cytotoxic-suppressor $CD8^+$ T cells (60.6% and 59.2% on days 43 and 64, respectively; Table 1). All CSF $CD4^+$ T cells (24.5% and 36.4% on days 50 and 64, respectively) expressed a memory marker CD45RO. Surface markers associated with naive/resting phenotype (CD45RA and CD62L) were not detected on CSF $CD4^+$ T cells.

Peripheral blood lymphocytes

Distribution of T cells, $CD4^+$ and $CD8^+$ T cells, B cells, and activated HLA-DR^+ T cells was monitored on days 25, 37 and 59 of hospitalization (Table 1). Increased percentages of T cells, $CD8^+$ T cells, activated HLA-DR^+ T cells, as well as $CD38^+CD8^+$ T cells compared to normal values were observed in

Table 1 Peripheral blood lymphocyte subpopulations in the patient with fatal Epstein-Barr virus infectious mononucleosis presenting with meningoencephalitis and hemophagocytic syndrome

Lymphocyte subpopulation	*Day 25*	*Day 37*	*Day 59*	*Normal values**
T cells (%)	88.9	82.4	93.1	65–80
B cells (%)	4.7	2.1	1.2	5–15
$CD4^+$ T cells (%)	21.2	19.8	21.8	35–60
$CD8^+$ T cells (%)	65.8	60.6	71.7	10–30
Absolute $CD4^+$ T-cell count (cells/μl of blood)	687	339	553	500–1500
CD4/CD8 ratio	0.3	0.3	0.3	1.0–3.6
HLA-DR^+ T cells (%)	56.8	39	57.7	0–10
$CD38^+CD8^+$ T cells (%)	75.5	71.3	70.1	0–10

*Normal values from the Division of Cellular Immunology, University Hospital for Infectious Diseases, Zagreb, Croatia.

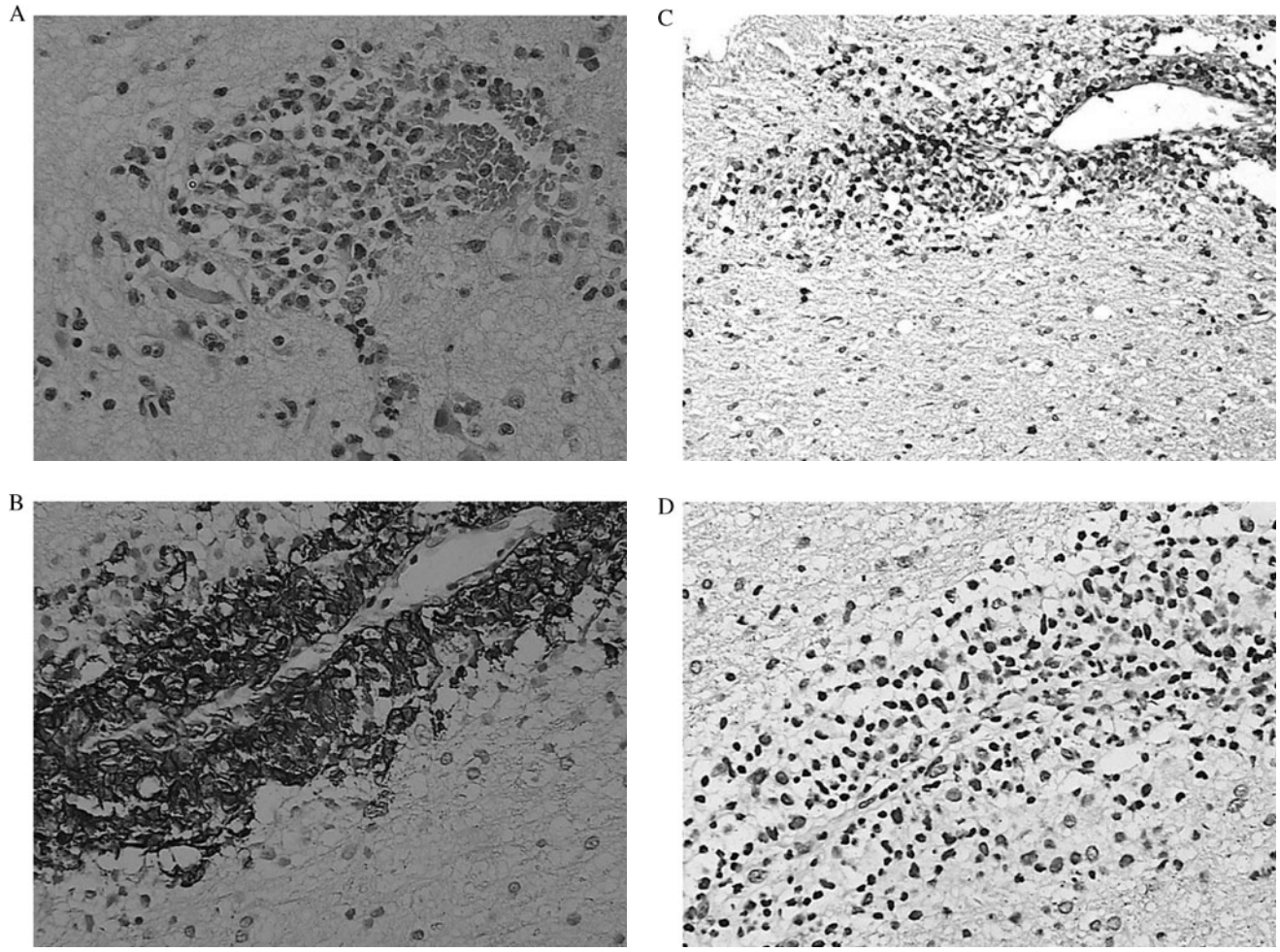

Figure 1 **(A)** Brain biopsy of the patient with EBV mononucleosis presenting with meningoencephalitis and hemophagocytic syndrome revealed reactive microglial infiltration through brain tissue with neuronophagia. **(B)** Immunohistochemical staining showed that the ratio between T and B cells in the brain was 1:1. **(C)** The majority of infiltrating T cells expressed CD8. **(D)** Immunohistochemical staining showed the presence of EBV in perivascular lymphoid infiltrate of the brain.

all tests. Percentages of $CD4^+$ T cells and B cells were decreased compared to normal values. Absolute counts of $CD4^+$ T cells were within normal values on days 25 (687 cells/μl) and 59 (553 cells/μl), with a temporary decrease on day 37 of hospitalization (339 cells/μl).

Intracellular IFN-γ expression

Intracellular expression of Th1 cytokine interferon (IFN)-γ and immunosuppressive cytokine IL-10 in the PB T cells was analyzed on days 35, 44, 50, and 59. Percentages of IFN-γ^+CD4^+ and IFN-γ^+CD8^+ T cells progressively increased from 10.2% and 8.5% (day 35, respectively) to a maximum of 42.2% and 75.2% (day 50, respectively). On day 59 of hospitalization, a slight decrease in the percentages of IFN-γ^+CD4^+ and IFN-γ^+CD8^+ T cells was observed (36.1% and 50.6%, respectively). Intracellular IFN-γ synthesis in PB T cells on days 44, 50, and 59 of hospitalization was higher compared with normal values (<30% of IFN-γ–producing $CD8^+$ or $CD4^+$ T cells). It appears that steroid administration was unable to fully reverse IFN-γ overexpression in peripheral blood T cells of our patient.

The expression of interleukin (IL)-10 was increased in both $CD4^+$ and $CD8^+$ T cells (2.6% and 2.4%, respectively) on day 44 as well as in $CD8^+$ T cells on days 50 (3.4%) (normal values 0.7% to 2.2% of IL-10–expressing T cells).

Hemophagocytic syndrome

Fine-needle aspiration of the lymph node showed reactive hyperplasia with prominent hemophagocytosis. The majority of cells in the aspirate were lymphocytes and immunoblasts, but macrophages, hemocytophages, and large cells of reticuloendothelial system were also found. Bone marrow aspirate smear revealed maturation arrest in nonsegmented

granulocytes and enlarged number of reactive lymphocytes in the bone marrow.

Discussion

The aim of this case report was to present immunological and virological assessment of an immunocompetent young male with fatal EBV infectious mononucleosis presenting with meningoencephalitis and hemophagocytic syndrome. EBV DNA was quantified in the whole blood and CSF of the patients on two occasions, supporting the serological and clinical diagnosis of EBV-induced infectious mononucleosis. A rapid increase in intracellular IFN-γ expression in the PB $CD4^+$ and $CD8^+$ T cells was documented between days 35 and 50 of hospitalization. Although EBV DNA viremia in the PB and CSF as well as intracellular IFN-γ T-cell expression slightly decreased upon steroid treatment, the patient died because of disease complications (sepsis and pneumonia).

The ability of EBV to induce usually nonfatal neurological manifestations such as meningitis, encephalitis, myelitis, and neuropathy during acute infection in both children and adults has been well recognized (Doja *et al*, 2006; Majid *et al*, 2002; Tselis *et al*, 1997; Merelli *et al*, 1997). However, rare cases of fatal EBV-meningoencephalitis and encephalitis during acute infection/or mononucleosis in young immunocompetent adults have also been described. Clinical presentation of these rare cases included necrotizing hemorrhagic encephalitis (Francisci *et al*, 2004), encephalitis with involvement of basal ganglia and limbic structure (Riemer *et al*, 2001), or meningoencephalitis in combination with a lymphoma-like B-lymphocyte response (Schellinger *et al*, 1999). A young immunocompetent adult with fatal infectious mononucleosis described in our report also developed meningoencephalitis. However, the clinical presentation of our patient also included hemophagocytic syndrome. EBV-associated hemophagocytic syndrome frequently occurs in apparently immunocompetent children and adults with infectious mononucleosis, chronic active EBV infection, familial HLH, X-linked lymphoproliferative disease, peripheral T-cell lymphoma, as well as natural killer (NK) cell leukemia (Imasuku, 2002; Kumakura, 2005). Most frequent clinical symptoms of EBV-associated hemophagocytic syndrome are fever, liver dysfunction, coagulation abnormalities, peripheral blood cytopenias, hepatosplenomegaly, and lymphadenopathy.

Yamashita *et al* (1998) reported a case of a 26-year-old man with chronic active infection presenting with acute cerebellar ataxia, encephalitis, and multiple abscesses that failed to respond to treatment with antivirals (acyclovir, gancyclovir), prednisolone, and interleukin-2. Mizuno *et al* (2003) described neurological symptoms (relapsing-remitting multifocal clinical and magnetic resonance imaging [MRI] abnormalities) in a patient with serology compatible with acute EBV infection that evolved over several years into interstitial pneumonitis and hemophagocytic syndrome and subsequent lymphomatoid granulomatosis. Brain MRI of our patient showed multifocal inflammatory lesions and severe perivascular inflammatory reaction. However, unlike the patient described by Mizuno *et al* (2003), the patient described in our study did not exhibit relapsing-remitting course of disease and failed to respond to treatment.

To our knowledge, this is the first description of a fatal EBV mononucleosis during acute infection in an immunocompetent young Caucasian adult presenting with both meningoencephalitis and hemophagocytic syndrome.

In our previous studies we described the distribution of lymphocyte subpopulations in the PB of patients with self-limiting EBV-infectious mononucleosis (Židovec Lepej *et al*, 2003). Distribution of PB lymphocytes in the patient presented in this report (increased percentages of total T cells, $CD8^+$ T cells, activated $HLA\text{-}DR^+$ T cells, and $CD38^+CD8^+$ T cells accompanied with decreased percentages of B cells and $CD4^+$ T cells) is similar to that previously described in patients with nonfatal disease (Židovec Lepej *et al*, 2003).

The analysis of intracellular cytokine synthesis in patients with uncomplicated infectious mononucleosis showed a gradual decrease in the percentage of IFN-γ–producing $CD8^+$ T cells over time (Attarbaschi *et al*, 2003). However, intracellular cytokine synthesis in fatal infectious mononucleosis with hemophagocytic syndrome and EBV-induced meningoencephalitis has not been previously described. Our report showed a gradual increase in the percentage of IFN-γ–producing $CD8^+$ T cells between days 35 and 50 of hospitalization. Additionally, we showed an increase in the percentage of IFN-γ–producing $CD4^+$ T cells in our patient as well.

It has been proposed that systemic cytokine overexpression causes the abnormal activation of macrophages and lymphocytes in hemophagocytic syndrome and is indirectly responsible for the subsequent phagocytosis of blood cells. Our results showing increased percentages of IFN-γ– and IL-10–producing $CD4^+$ and $CD8^+$ T cells in the patient with fatal EBV-induced meningoencephalitis and hemophagocytic syndrome during infectious mononucleosis support this hypothesis. In this case, as shown by histology, meningoencephalitis could be a part of hemophagocytic syndrome as well. We believe that a possible association between an enhanced Th1-type of response and unfavorable disease outcome in fatal infectious mononucleosis is worth investigating.

B cells are the main cell type infected with EBV in infectious mononucleosis. However, in

EBV-associated hemophagocytic syndrome infection of T cells and NK cells becomes relatively prominent. Kasahara *et al* (2001) reported infection of $CD8^+$ T cells in EBV-associated hemophagocytic syndrome. It is reasonable to assume that both T cells and NK cells of the patient reported in this study were also infected with EBV. Interestingly, we observed a gradual increase in the percentage of IFN-γ–producing $CD8^+$ T cells during disease progression in our patient. This finding strongly supports the concept proposed by Imashuku (2002) that, due to the fulminant and unfavorable clinical course, patients with EBV-associated hemophagocytic syndrome require special therapeutic measures in order to control the EBV-induced cytokine storm and to suppress the proliferation of EBV genome–containing cells.

Although the pathogenesis of acute fulminant EBV-associated hemophagocytic syndrome is not yet fully understood, cytokine-mediated activation of macrophages probably predisposes to the development of this syndrome (Kumakura, 2005). *In vivo* and *in vitro* studies showed that infection of T cells by EBV can selectively up-regulate TNF-α expression, which, in combination with IFN-γ, is able to activate macrophages (Lay *et al*, 1997). We observed the progressive increase in the percentage of IFN-γ–positive $CD8^+$ T cells in the blood of our patient with fatal outcome. These findings suggest that the role of increasing concentrations of IFN-γ and tumor necrosis factor (TNF)-α as predictors of fatal outcome in EBV-associated hemophagocytic syndrome should be evaluated.

The predominant lymphocyte subpopulation in the CSF of patients with inflammatory and noninflammatory diseases is memory $CD4^+$ T cells (Kivisäkk *et al*, 2002). Contrary to this, $CD8^+$ T cells were the predominant T-cell population in the CSF of our patient. This finding is consistent with the study by Lehrnbecher *et al* (1996) that showed activated $CD8^+$ T cells in the CSF of a patient with EBV meningoencephalitis. Similarly, activated $CD8^+$ T cells were described in the CSF of untreated HIV patients (Neuenburg *et al*, 2005). Further investigations are needed to determine whether the predominance of $CD8^+$ T cells is a typical finding in patients with meningoencephalitis and EBV DNA in the CSF.

Acute infectious mononucleosis in immunocompetent persons is often characterized by a transient self-limited elevation of liver enzymes. Severe hepatitis during EBV infectious mononucleosis is rarely observed in immunocompetent adults (Negro, 2006). However, clinically significant liver damage is usually observed in immunocompromised patients (HIV-infected persons, transplanted patients, X-linked lymphoproliferative disorder) or in sporadic fatal infectious mononucleosis of immunocompetent patients (Pelletier *et al*, 1976; Hinedi and Koff, 2003; Markin, 1994; Negro, 2006). Pelletier *et al* (1974) estimated that liver failure represented a direct cause of death in about 50% of patients suffering from fatal infectious mononucleosis. Severe hepatitis and long high-grade fever could have been the first predictors of unfavorable outcome in our patient.

The mechanism of EBV-related liver damage has not been yet elucidated. The current hypothesis holds that cytotoxic T cells cause hepatocyte injury by indirect mechanisms such as cytokine synthesis and expression of perforin cytotoxic granules (Drebber *et al*, 2006). Increasing levels of IFN-γ in our patients could have significantly contributed to the hepatic injury in our patient as well.

Recent studies demonstrated the indirect involvement of EBV in the pathogenesis of multiple sclerosis (MS). The most conclusive evidence comes from epidemiological studies linking the EBV reactivation with diseases activity in MS patients in early phase of disease and showing that late EBV infection carried a higher risk of developing MS (for review see Christensen, 2006). Analysis of antigen-specific cellular immunity by major histocomaptibility complex (MHC) multimers revealed a molecular mimicry between EBV and myelin epitopes, suggesting that EBV-specific $CD8^+$ and $CD4^+$ T cells might cross the blood-brain barrier and, by targeting myelin antigens, contribute to MS pathology (Holmoy *et al*, 2004). Percentages of total $CD8^+$ T cells in the blood and CSF of the patient we described in this report have been significantly increased. MHC-tetramer studies have shown that CD8+ T cells specific for lytic epitopes of EBV account for between 5% and 50% of the total CD8+ T cells in the blood of patients with acute infectious mononucleosis (Hislop *et al*, 2002). Although we did not perform the MHC-tetramer analysis of CD8+ T cells in the blood and CSF of our patient, it is reasonable to assume that at least a part of these cells were EBV-specific and able to target myelin antigens as well.

In conclusion, fatal infectious mononucleosis with EBV-induced meningoencephalitis and hemophagocytic syndrome might be associated with IFN-γ overexpression. Early implementation of steroid treatment and the subsequent decrease in the number of EBV-producing B cells, plasma viremia, as well as $CD8^+$IFN-γ–producing cells could be of benefit in these patients.

Materials and methods

Case report

A previously healthy 17-year-old male without a remarkable medical history was admitted to the hospital because of 3 weeks fever (up to 39.5°C) and pharingitis (for 7 days).

On admission, the patient's temperature was 39.5°C. Examination showed hepatospenomegaly and pharingitis. Cardiovascular, respiratory and central nervous systems appeared to be normal. Laboratory tests revealed a leukocyte count of 7.5×10^9/L

with 23% of reactive lymphocytes, increased sedimentation rate (35), and increased levels of liver enzymes: aspartate aminotransferase (ASAT) 482 U/L (normal range 11–38 U/L); alanine aminotransferase (ALT) 562 U/L (normal range 12–48 U/L); γ-glutamyltransferase (GGT) 961 U/L (normal range 11–55 U/L); lactate dehydrogenase (LDH) 1338 U/L (normal range 0–460 U/L) and gamma-globulins 32.6% (normal range 14.5–19.5%).

Initial diagnosis of EBV-induced mononucleosis was confirmed by detection of IgM anti-VCA antibodies in serum and high level of EBV viremia (54,490 copies of EBV DNA/ml of blood, real-time PCR). Serological test for HIV-1, hepatitis A, B, and C viruses were negative. The patient was also tested and found negative for CMV DNA and HIV-1 RNA in the plasma. Abdominal ultrasound and CT showed hepatosplenomegaly. Tests for antinuclear factor (1:16), anti-neutrophilic cytoplasmic antibodies (ANCA), anti-mitochondrial antibody (AMHA), anti-smooth muscle antibody (ASMA), liver-kidney microsomal autoantibodies (LKM) IIF, and antids (double-stranded) DNA antibodies were normal. Fine-needle aspiration of lymph node revealed reactive lymphocytic hyperplasia with hemophagocytic macrophages. Bone marrow aspirate showed infiltration of reactive lymphocytes.

On day 50, the patient developed meningoencephalitis. Lumbar punctures on days 50, 60, 64, and 67 of hospitalization revealed a pleocytosis of 250, 586, 93, 80 cells/μl of CSF, respectively. Cytological analysis of four CSF samples showed similar cellular composition. The majority of CSF cells were lymphocytes (62%, 76%, 89%, and 90%, respectively) and monocytes (11%, 8%, 4%, 6%, respectively). Biochemical analysis of the CSF showed increased protein content (3515, 897, 897 mg/L, respectively, normal range 150–450 mg/L) and increased lactate concentrations (7.3 and 3.8 mmol/L, respectively, normal range 1.2–2.8 mmol/L). Chloride concentrations and glucose blood/CSF ratios were normal. EBV viremia in the CSF was 39,500 copies of EBV DNA/ml. HSV-1/2 DNA was not detected in the CSF.

Brain computed tomography (CT) was normal. Brain MRI showed multifocal inflammatory lesions in frontal and temporal cortex of the right hemisphere as well as severe perivascular inflammatory reaction. Stereotactic biopsy of the brain was performed to exclude lymphoma. The presence of EBV in perivascular infiltrates and grey substance was documented immunohistochemically.

The patient was treated with steroids, cyclosporin A, and methotrexate intrathecally. EBV DNA viral load decreased upon treatment to 8715 copies/ml in the blood and 14,690 in the CSF. The patient remained unconscious and died of sepsis and pneumonia (*Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* [MRSA]) 99 days after initial symptoms. The autopsy was not performed.

Molecular methods

Whole-blood and CSF EBV DNA viremia and the presence of HSV 1/2 DNA in the CSF were determined by real-time PCR on LightCycler by using LightCycler EBV Quantification kit and LightCycler HSV 1/2 Detection Kit, respectively (Roche Diagnostics, Mannheim, Germany). The presence of HIV-1 RNA in the plasma of our patient was analyzed by using Cobas Amplicor HIV-1 Monitor Test, version 1.5, with lower limit of detection of 50 (ultrasensitive method) copies of HIV-1 RNA/ml. CMV DNA quantification was performed by using Cobas Amplicor CMV Monitor Test with the detection range between 400 and 100,000 copies/ml (Roche Diagnostics).

Flow cytometry

Percentages of T-cell, $CD4^+$ and $CD8^+$ T cells, B cells, and NK cells in the PB were determined by using a four-color flow cytometry panel CYTO-STAT tetra CHROME (CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5) (Beckman Coulter, Fullerton, CA, USA). Percentages of $CD38^+CD8^+$ T cells and HLA-DR^+ T cells were determined by using conjugated anti-human monoclonal antibodies specific for CD38 (FITC), HLA-DR (FITC), CD8 (PE) and CD3 (RPE-Cy5) (DakoCytomation A/S, Glostrup, Denmark).

Flow cytometric determination of T cells, $CD4^+$ and $CD8^+$ T cells, truly naive ($CD45RA^+CD62L^+$), as well as memory ($CD45RO^+$) $CD4^+$ T cells in the CSF of the patient was performed as recommended by Kivisäkk *et al* (2003). CSF lymphocyte subpopulations were determined by using conjugated anti-human monoclonal antibodies specific for CD4 (FITC), CD62L (FITC), CD45RA (PE), CD45RO (PE), CD3 (RPE-Cy5), and CD45 (RPE-Cy5) (DakoCytomation, Glostrup, Denmark). Nonspecific binding to Fc receptors was blocked with 0.2 mg/ml of IgG1 (clone MCG1; IQ Products, Groningen, Netherlands).

PB and CSF samples were prepared by using a nonwash method on Multi-Q-Prep System with Imuno-Prep Reagent System and analyzed on Epics XL-MCL Flow cytometer (Beckman Coulter, Fullerton, CA, USA). Absolute counts of $CD4^+$ T cells were also determined directly on the cytometer by using Flow-Count Fluorospheres (Beckman Coulter).

Intracellular IFN-γ detection in $CD8^+$ T cells was done by using Cytodetect kit (IQ Product, Groningen, The Netherlands).

Normal values for peripheral blood lymphocyte subpopulations and intracellular cytokine detection used in this report are as recommended by the Division of Cellular Immunology, University Hospital for Infectious Diseases, Zagreb, Croatia.

Serology

Serum samples were tested for IgM and IgG antibodies to viral capsid antigen (VCA), IgG antibodies

to early antigen-diffuse (EA(D), and EBV nuclear antigen (EBNA), respectively, by using enzyme-linked immunosorbent assay (ELISA; DiaSorin, Saluggia).

Tissue processing
The biopsy after neurosurgical treatment yielded four needle core of grayish cerebral tissue. The specimens were fixed immediately in 10% buffered formalin, dehydrated in ethanol, and embedded in paraffin. Paraffin blocks were cut in seven 5-μ thin slices, deparaffinate, and hydrated on glass slides for immunohistochemistry and histochemistry. One slide of each block was stained with hematoxylin and eosin (Kemika, Croatia) and other prepared for immunohistochemistry with monoclonal antibodies against CD20, CD3, CD4, CD8, CD68, and EBV, using a step kit based on the LSAB (labeled streptavidin-bitoin) immunohistochemistry (Dako, Glostrup, Dennmark). For myelin staining (Luxol fast blue), paraffin blocks were cut in one 15-μ thin slice.

Other diagnostics
Diagnostic procedures/tests performed during the workup of our patient included: brain CT, brain MRI, brain biopsy, abdominal ultrasound, routine hematological and biochemical tests, tests for autoimmune antibodies, cytological examination of the CSF, fine-needle lymph node aspiration, bone-marrow punction, and immunohistochemistry.

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