Case Report

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Cytomegalovirus reactivation after low-dose steroid treatment for hemolytic anemia in a patient with primary Epstein-Barr virus infection

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Reaktivierung von latentem Cytomegalovirus nach niedrigdosierter Kortikosteroidtherapie einer hämolytischen Anämie bei einem Patienten mit primärer Epstein-Barr-Virus-Infektion

Zusammenfassung. Bei Cytomegalie-seropositiven Patienten kann eine intensive therapeutische Suprimierung der Immunabwehr zur Reaktivierung einer latenten Cytomegalovirus-Infektion führen. Viele dieser immunsuppressiven Therapie-Protokolle enthalten auch hochdosierte Kortikosteroide. Nicht bekannt ist hingegen, ob eine Behandlung mit niedrigen Dosen von Kortikosteroiden bei sonst gesunden Personen eindeutig mit einer Reaktivierung von Cytomegalovirus verbunden ist. Wir beschreiben die Reaktivierung einer Cytomegalovirus-Infektion mit bei einem 21-jährigen immunkompetenten Mann, der einen Monat lang mit niedrigen Dosen von Kortikosteroiden behandelt wurde. Die Indikation zur Steroidtherapie war eine ausgeprägte hämolytische Anämie, ausgelöst durch eine primäre Mononukleose (Epstein-Barr).

Summary. Cytomegalovirus reactivation is a well described event occurring after intensive therapeutic suppression of the immune function in patients with latent infection. Treatment protocols for suppression of the immune response often include high-dose steroids. However, it is not known whether even a low-dose steroid treatment can reactivate latent cytomegalovirus in otherwise healthy persons. We documented cytomegalovirus reactivation after low-dose steroid treatment for autoimmune hemolytic anemia as a complication of Epstein–Barr virus mononucleosis in an immunocompetent 21-year-old man.

Key words: Cytomegalovirus reactivation, steroid therapy, Epstein-Barr mononucleosis.

Introduction

Cytomegalovirus (CMV), as a member of the gammaherpesvirus family, remains after primary infection in a clinically silent condition of latency. When the host's immune system becomes compromised, CMV reactivation results in productive infection and shedding of the virus, with or without accompanying disease [1]. The occurrence of CMV disease after viral reactivation is common in settings after organ transplantation, as a consequence of the profound immunosuppression. The type and duration of immunosuppressive treatment directly correlate with the frequency of CMV reactivation, therefore protocols for organ transplant patients include different kinds of prophylaxis against CMV disease in selected groups of patients [2]. Although CMV is a known pathogen in immunosuppressed transplant patients, the role and frequency of reactivation in an immunocompetent host are not clearly defined. Epstein-Barr virus (EBV) is another member of the herpesvirus group and shares certain features of CMV, such as similar clinical presentation, latency and a deleterious disease in patients who have received transplants [3]. Little is known about the influence of acute EBV infection on CMV reactivation. In addition to causing a clinical syndrome known as infectious mononucleosis, EBV infection can provoke different autoimmune complications. One of these is autoimmune hemolytic anemia, which occurs in about 0.5–3% of patients [4] and, if severe, should be treated with systemic steroids. Corticosteroids are immunomodulatory drugs and are frequently used in treatment of various immune disorders. In serious conditions, such as graft rejection or severe inflammatory connective tissue disease, high doses of steroids are used, often in conjunction with other immunosuppressants. Such augmented immunosuppressive therapy can reactivate latent viruses in seropositive patients by interfering with the immune functions responsible for controlling latent viral infection [5]. We present a case study of a patient in whom latent CMV was reactivated after a month of low-dose steroid treatment for severe autoimmune hemolytic anemia, which complicated the otherwise benign course of EBV mononucleosis.

Case report

A 21-year-old man was admitted to hospital because of dark urine and jaundice. The anamnestic data revealed a two-week fever with cervical lymphadenopathy. He had previously been in good health without a history of drug abuse, blood transfusions or any evidence of autoimmune diseases in the family. The physical examination revealed bilateral cervical lymph nodes 3 cm in size and submandibular nodes 5 cm in size, as well as hepatomegaly and splenomegaly. The patient was febrile with no signs of tonsillopharyngitis.

The results of laboratory tests on admission revealed: red blood cell count (RBC) 2.15×10^{12}/l (normal range – NR $4.20–6.30 \times 10^{12}$/l); hemoglobin 98 g/l (NR 120–160 g/l); white blood cell count 17.8×10^9/l (NR $4.0–10.0 \times 10^9$/l), with 47% lymphocytes (NR 30–50%), 31% polymorphonuclear leukocytes (NR 45–70%), 4% monocytes (NR 2–10%) and 18% atypical cells (NR 1–5%); platelet count 151×10^9/l (NR 150–350×10^9/l); reticulocytes 12.2% (NR 0.5–2.5%); total bilirubin 111 μmol/l (NR < 17.5 μmol/l); unconjugated bilirubin 83 μmol/l, conjugated 28 μmol/l (NR < 4 μmol/l); alanine aminotransferase (ALT) 192 IU/l (NR 12–48 IU/l); aspartate aminotransferase (AST) 239 IU/l (NR 11–38 IU/l); lactate dehydrogenase (LDH) 1371 IU/l (NR < 241 IU/l); gamma glutamyl transferase (γGT) 257 IU/l (NR 11–55 IU/l). The urine analysis showed dark colored urine with positive bilirubin and normal urobilinogen. Erythrocyte sedimentation rate (ESR), coagulation studies, serum copper, ceruloplasmin, total protein, albumin, and IgA, IgM and IgG immunoglobulin levels were within normal limits. Antinuclear antibodies, antimitochondrial antibodies, liver/kidney microsomal smooth-muscle antibodies were negative. Serology was negative for hepatitis A, B, C and E viruses, parvovirus B 19, toxoplasmosis and HIV infection. Heterophilic antibodies were present. Acute EBV infection was confirmed by the presence of EBV viral capsid antigen (VCA) IgM and EBV early antigen (EA) IgG antibodies in enzyme-linked immunosorbent assay (ELISA) of two consecutive serum samples. Epstein-Barr nuclear antigen (EBNA) IgG and VCA IgG were not detected. Serology of CMV revealed previous infection: IgM was negative and IgG positive. Qualitative PCR tests detected EBV DNA in serum; CMV DNA and human herpes virus-6 (HHV-6) DNA were not detected. Abdominal ultrasonography showed hepatosplenomegaly.

On the third day of hospitalization the patient developed severe anemia (hemoglobin 91 g/l) with RBC agglutination in vitro (peripheral blood smear indicated spherocytosis, polychromatophylia, RBC agglutination and rouleaux formation), thrombocytopenia (platelet count 130×10^9/l), lymphocytopenia (lymphocytes 19%) and an increase in total bilirubin (131 μmol/l), unconjugated bilirubin (92 μmol/l), ALT (338 IU/l), AST (390 IU/l), LDH (1584 IU/l), γGT (262 IU/l) and reticulocytes (14.1%). A qualitative hemoglobin test of the urine was positive. The indirect Coombs test was negative but the direct test was positive and IgG auto-antibodies were detected. Cold IgM antibodies were present in the patient's serum within a thermal range of 4–20 °C (anti-i antibody in dilution 1:1024, anti-I in 1:256). The serum was positive for polyspecific anti-HLA lymphocytotoxic antibodies which reacted with all cells of the lymphocyte panel. HLA tissue typing was as follows: HLA-A1, A24, B8, B50, DRB1*15, DRB1*07, DQB1*0202. Immunophenotyping of blood cells did not detect any markers of leukemic cells.

Oral methylprednisolone in doses of 120 mg daily was initiated. At the end of the second week of steroid treatment the clinical and laboratory parameters markedly improved. The patient was discharged and the steroid treatment was tapered over the next two weeks. After four weeks of treatment all laboratory results returned to normal values. Shortly after discontinuation of the corticosteroid he was readmitted to hospital because of high fever and polymyalgias. No abnormality could be detected on physical examination. Except for slightly elevated CRP and LDH and moderate thrombocytopenia, biochemical and hematological tests were normal. A recurrence of EBV infection was suspected but EBV DNA was detected only in polymorphonuclear cells, not in the serum. The serologic profile of EBV showed late acute infection (disappearance of IgM anti-VCA, presence of IgG anti-VCA), whereas the serology of CMV was unchanged compared to previous results (anti-IgG positive, anti-IgM negative). Unexpectedly, CMV DNA was present in urine and serum, and on that basis productive CMV infection was diagnosed. The time course of the diseases and the PCR results are shown in Fig. 1. Stored serum samples collected at check-up visits one and three weeks prior to readmission were retrospectively tested for CMV DNA with qualitative PCR; however, both samples were negative. In order to detect CMV DNA we used a qualitative nested PCR test amplifying a region of the IE 2 gene coding for gp 107. The patient received symptomatic treatment and fully recovered six days later. We concluded that the flu-like illness was caused by CMV reactivation triggered by the prolonged corticosteroid treatment. This diagnosis was further strengthened on testing a serum sample a month later: PCR for CMV DNA was negative but the serologic profile of CMV

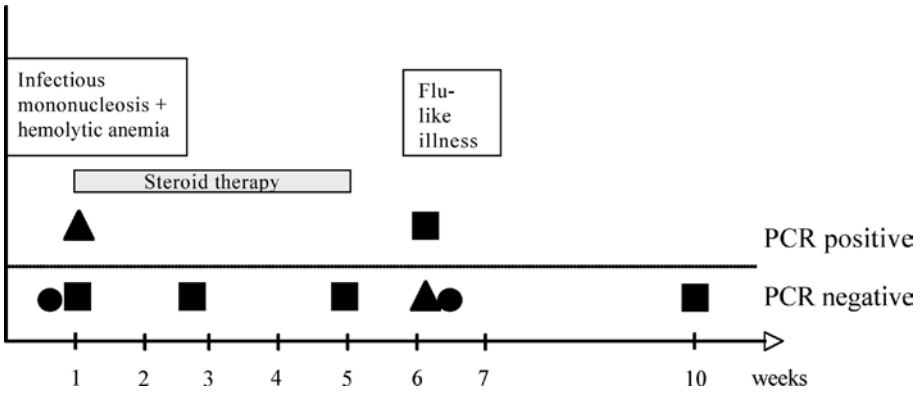

Fig. 1. The time course of the disease and steroid therapy is indicated in boxes. The result of each qualitative PCR for EBV (▲), CMV (■) and HHV-6 (●) in serum samples is marked with symbols

antibodies showed a fourfold rise of IgG, with IgM antibodies remaining negative.

Discussion

Primary CMV infection in healthy adults is usually asymptomatic or follows a course of a mild mononucleosis syndrome. CMV disease caused by reactivation in immunocompetent individuals is rarely described, probably because symptoms are minor and consequently little relevance is attached to them on the part of physicians. Reactivation of latent human CMV usually occurs after profound suppression of the immune system, either by acquired infection (in AIDS, for example) or through medication (steroids, cytostatics, immunosuppressive agents) [6, 7]. Herpesviruses can interfere with normal immune function but the intensity of immune dysfunction during acute infection is rarely profound enough or sufficiently prolonged to cause reactivation of other latent viruses [8]. Studies examining direct herpesvirus-herpesvirus interaction are limited but they indicate that a correlation does exist, mostly described as the activation of HHV-6 and HHV-7 in association with CMV infection in post-transplant patients [9]. However, it is not known whether EBV infection can trigger CMV reactivation. EBV interferes with the immune system by stimulating excessive and nonfunctional B-cell proliferation, whereas control of CMV latency depends mostly on cellular immune defense. Aalto et al. indicated that the interplay of EBV and CMV is unidirectional. Thus, immunoreactivation of EBV may occur in an EBV-seropositive patient with proven primary CMV infection, but primary EBV infection has no influence on CMV reactivation [10]. This observation is supported by experimental data showing that CMV superinfection leads to reactivation of EBV in an EBV-positive Burkitt's lymphoma B-cell line [11].

Steroids are often used as anti-inflammatory or immunosuppressive drugs and their main effect is suppression of T cells [12]. Cumulative doses of steroids correlate with an increased number of reactivated CMV diseases, as shown in patients after kidney transplantation [13]. However, it has not been convincingly demonstrated that low doses of steroids can reactivate previously controlled latent viral infections, even though such doses are being widely used in current medical practice for disorders of moderate severity.

The productive infection of CMV in the presented case was detected after a month of low-dose steroid therapy. The patient received a total of 3 g of methylprednisolone, a dose often used as a single-pulse dose for treatment of graft rejection or severe connective tissue disease where such doses result in more frequent CMV reactivations only if additional immunosuppressive treatment is given [5, 14].

Nevertheless, the reported case indicates that even low doses of steroids given as the only immunosuppressive agent can reactivate CMV. In our patient, a flu-like self-limited disease coincided with detection of CMV DNA in urine and serum. This could simply have been a co-finding, but no other clinical reason or laboratory result explained the second febrile disease, and we therefore concluded that the patient developed a mild CMV disease as a consequence of low-dose steroid therapy.

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