Efficiency of antiviral therapy plus IVIG in a case of primary EBV infection associated PTLD refractory to rituximab, chemotherapy, and antiviral therapy alone

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LETTER TO THE EDITOR

Efficiency of antiviral therapy plus IVIG in a case of primary EBV infection associated PTLD refractory to rituximab, chemotherapy, and antiviral therapy alone

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Dear Editor,

Epstein–Barr virus (EBV) incompatibility in transplantation or EBV infection early after transplantation indicated by posttransplant seroconversion is considered a significant risk factor for the development of posttransplant lymphoproliferative disorder (PTLD). About 50% of patients with PTLD that do not respond to an initial reduction of immunosuppression and that are treated first-line with single-agent rituximab require additional treatment. CHOP salvage therapy after upfront treatment with rituximab achieves high remission rates. Antiviral therapy is a further treatment approach in the primary infection setting but little is known on the additional use of polyvalent intravenous immunoglobulins (IVIG).

We report on a young liver transplant recipient presenting with PTLD with high fever, anemia, thrombopenia, hypofibrinogenemia, high lactate dehydrogenase (LDH) levels, and gastrointestinal lymphoma manifestation resulting in massive hemorrhagic diarrhea. Rituximab, CHOP-based chemotherapy, and antiviral therapy alone were not successful to treat PTLD, but the additional therapeutic use of IVIG combined with antiviral therapy resulted in long-lasting control of PTLD. The patient is a 28-year-old male who underwent orthotropic liver transplantation for primary sclerosing cholangitis in October 2004. The transplantation was preemptive and time to reperfusion was 10 h 28 min. For induction, the patient received 20 mg anti-CD25 antibody basiliximab at days 0 and +4, followed by a baseline standard immunosuppression of tacrolimus (FK506) 6 mg/day and prednisolone 25 mg/day. Cytomegalovirus (CMV) serology was negative for donor and recipient. The recipient was also negative for EBV, but as data on the EBV status of the donor were unavailable, prophylactic antiviral therapy (acyclovir) was administered for 6 months. There were no infections or rejection episodes.

After 16 months, the patient was admitted with a 4-week history of fever, night sweats, abdominal pain, weight loss (3 kg), and hemorrhagic diarrhea. At this time, immunosuppression comprised tacrolimus 3 mg/day and prednisolone 7.5 mg/day. LDH levels were elevated (339 U/l), but liver function was normal. Hemoglobin was slightly

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decreased (10.2 g/dl), but platelet and total white blood cell counts were normal, as were the CD4, CD8, and CD19 cell counts (359, 1,042, and 65 per microliter, respectively) and the serum immunoglobulin levels (IgG 1,041 mg/dl, IgA 73 mg/dl, IgM 55 mg/dl). Ultrasound showed thickening, hyperperfusion, and structural transformation of the colon and terminal ileum, corresponding with inflammatory changes in colonoscopy. EBV titers indicated a primary infection (positive EBV-IgM and EBV-IgG and negative EBNA1-IgG). Quantitative EBV polymerase chain reaction (PCR) in peripheral whole blood showed an extremely high viral load (900,000 copies EBV per milliliter), but CMV-PCR and CMV serology remained negative. A whole-body computed tomography (CT) scan showed some mesenterial lymphadenopathy at the right colon flexure without any other abnormalities. Bone marrow biopsy, cerebral CT scan, cerebrospinal fluid cytology, and EBV-PCR were unremarkable. Histopathological examination of colonic mucosa biopsies revealed an EBV- and CD20-positive polymorphic B-cell PTLD (Fig. 1). At diagnosis of PTLD, immunosuppression was reduced by reduction of tacrolimus to 1 mg/day; prednisone was continued with 7.5 mg/

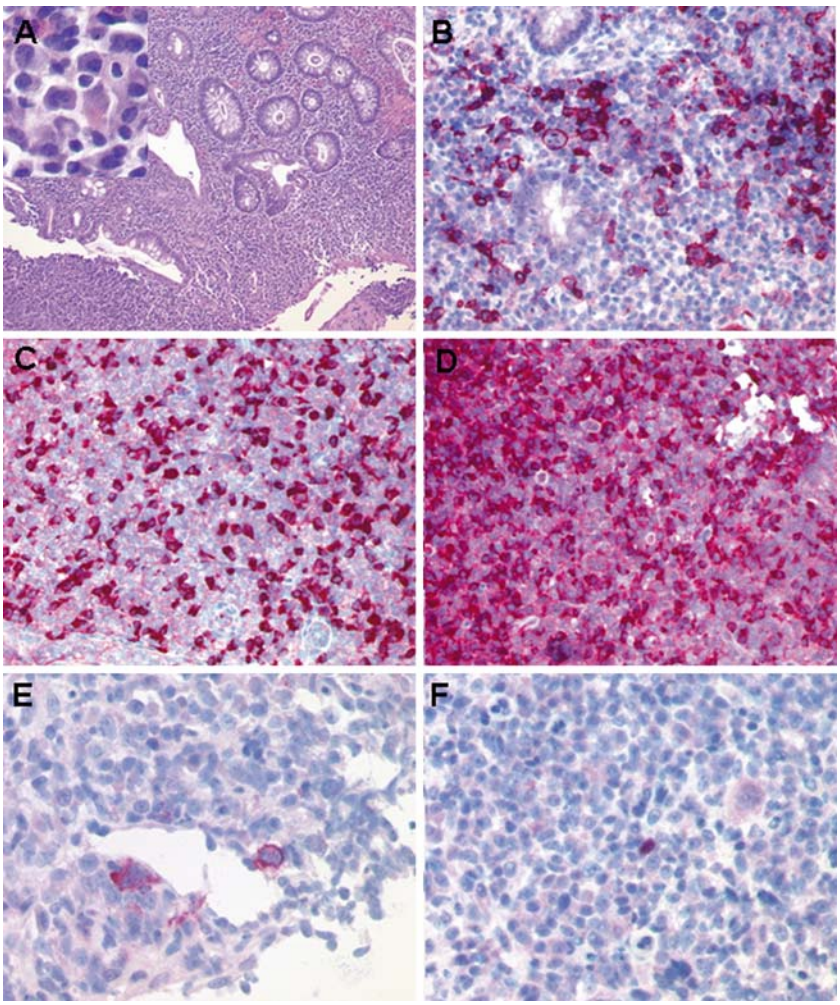

Fig. 1 Polymorphic PTLD containing CD20-positive B-blasts as well as numerous cells showing plasmacellular differentiation with a polytypic expression pattern of the immunoglobulin light chains. The infiltrate contained several LMP-1-positive B-blasts, as well as ZEBRA-expressing cells in the absence of EBNA-2 expression. Molecular analysis revealed polyclonal immunoglobulin gene rearrangements. **a** The mucosa shows a dense infiltrate composed of blasts as well as of numerous cells with features of plasmacellular differentiation (H & E stain, *insert* represents higher magnification). **b** Immunostain for CD20 (monoclonal antibody, clone L26) reveals several labeled cells frequently with large nuclei. Labeling for immunoglobulin light chains shows that the cells with features of plasmacellular differentiation express either the light chain lambda (**c**) or the light chain kappa (**d**; polyclonal antibodies). All immunostains shown employ the alkaline phosphatase anti-alkaline phosphatase system with FastRed as chromogen. **e** Immunohistological detection of the EBV-encoded latent membrane protein-1 (cocktail of monoclonal antibodies; clones CS1–4) in several cells with large nuclei corresponding to B-blasts. **f** Single small lymphoid cells expressing the ZEBRA protein of EBV (monoclonal antibody, clone BZ.1) are also detectable

day. Fever, diarrhea, and EBV viral load remained unchanged, so sequential immunochemotherapy (four doses of rituximab at days 1, 8, 15, 22 followed by four cycles of cytotoxic chemotherapy with CHOP-21) was initiated according to the PTLD-1 protocol 1 week later [1] (Fig. 2).

After rituximab was commenced, the patient's clinical status rapidly improved; diarrhea was less frequent, fever disappeared, and LDH elevation was reduced. However, at day 37 (2 weeks after the last or fourth dose of rituximab), the patient was readmitted with high fever, significant hemorrhagic diarrhea, and LDH elevation (401 U/l). Hemoglobin was significantly decreased (8.5 g/dl), as were platelet and white blood cells counts (91,000 and 1,010 per microliter, respectively). Total fibrinogen was in the lower normal range (166 mg/dl). Due to rapid clinical deterioration indicating progressive disease (PD), CHOP was started immediately with 50% dose reduction of cyclophosphamide and doxorubicin due to the patient's reduced peripheral blood count.

After initiation of chemotherapy at day 38, the clinical status improved rapidly again, with still frequent but nonhemorrhagic diarrhea, disappearance of fever (at day 40), and reduction of LDH (179 U/l at day 51). Platelet and white blood cell counts increased to 251,000 and 3,070 per microliter, respectively, at day 44, and total fibrinogen was 568 mg/dl at day 50. Prior to the planned second application of CHOP, the patient presented with neutropenic fever and rising LDH levels. LDH peaked at 707 U/l on day 56 when the second cycle of CHOP chemotherapy (25% dose-reduced) was initiated prematurely. Again, fever resolved quickly and LDH levels reduced to a nadir of 217 U/l but increased to 582 U/l at day 73 when the third cycle of CHOP was administered prematurely. Although there was again an initial response to chemotherapy, fever reached 40°C at day 84 and was accompanied by hemorrhagic diarrhea, increasing LDH levels, marked tachycardia, and tachypnea. Ultrasound and CT scans demonstrated progressive lymphadenopathy and colonic infiltration. EBV viral load in peripheral blood was 400,000 copies per milliliter (Fig. 2). On the basis of previous experience of antiviral therapy in ZEBRA-positive PTLD [2], antiviral therapy with cidofovir was commenced.

Two days after commencing cidofovir 5 mg/kg at day 86, the patient was febrile (41°C) and had elevated LDH (2,003 U/L) and critical gastrointestinal bleeding due to hyperfibrinolysis (fibrinogen <40 m/dl, anti-thrombin-III activity 82%), requiring multiple daily erythrocyte transfusions. Secondary consumption of platelets and coagulation factors made intensive care management necessary. Antibiotics had no evident effect. Because colonoscopy and ultrasound examinations performed on days 91 and 92 confirmed a dramatic improvement of PTLD-associated lesions, cidofovir was continued at day 94. The patient recovered quickly and hyperfibrinolysis resolved, with fibrinogen levels reaching 153 and 397 mg/dl at days 95 and 105, respectively. Platelet counts stabilized (206,000 and 270,000 per microliter at days 96 and 103, respectively) and LDH decreased (328 U/l at day 105). From day 98 onwards, the patient was afebrile and diarrhea was less frequent. Monitoring of EBV DNA in peripheral blood showed an initial increase from 403,000 copies per milliliter prior to cidofovir to 956,000 and 1,400,000 copies per milliliter at days 92 and 96, respectively, with a final drop to 3,475 copies per milliliter at day 104 when the third course of cidofovir was administered (Fig. 2). However, from a nadir of 328 U/L at 105 day, LDH levels increased to 1,664 U per 763 l within 15 days. With increasing LDH levels, the patient again experienced clinical deterioration with high fever, tachycardia, tachypnea, and a second hyperfibrinolytic syndrome with severe gastrointestinal bleeding. Fibrinogen levels decreased from 475 mg/dl on day 110 to 84 mg/dl on day 124 and abdominal ultrasound on day 124 did not indicate PTLD within the colon, but peritoneal lymphomatosis (peritoneal thickening and presence of ascites) indicative of PD was suspected. EBV DNA in peripheral blood was 400,000 copies per milliliter on day 122.

As a last resort, we initiated IVIG 0.6 g/kg followed by 0.2 g/kg every 3 weeks in addition to cidofovir treatment at day 125, which successfully terminated the second hyperfibrinolytic syndrome. From day 131 on, i.e., 6 days after the first application of IVIG + cidofovir, the patient's clinical status rapidly improved; gastrointestinal bleeding resolved, diarrhea was less frequent, fever disappeared, and LDH elevation was reduced to 884 U/l at day 128 and to 236 U/l at day 138, respectively. When EBV DNA levels increased in parallel with LDH levels after further applications of cidofovir at days 131, 141, 148, and 155, antiviral therapy was switched to foscarnet (180 mg/kg per day) at day 156. LDH peaked at 356 U/l at day 159 but normalized with foscarnet treatment within 6 days. There was no worsening of PTLD symptoms. At day 169, antiviral therapy was stopped and maintenance immunosuppression was reduced to prednisolone 15 mg/day only. The patient was discharged from hospital and continued IVIG applications every 3 weeks on an outpatient basis. With a follow-up of more than 19 months, the patient stayed in complete response and is still in an excellent condition, although immunosuppression had to be increased to transplant rejection and the patient's underlying autoimmune disease (graft rejection at approximately day 190, activity index according to Banff 3 of 9; histologically proven inflammatory bowel disease without evidence of PTLD on day 243). EBV-PCR remained positive during follow-up, but quantitative EBV-PCR of whole blood and plasma confirmed lytic EBV replication only until day 332, when the initially

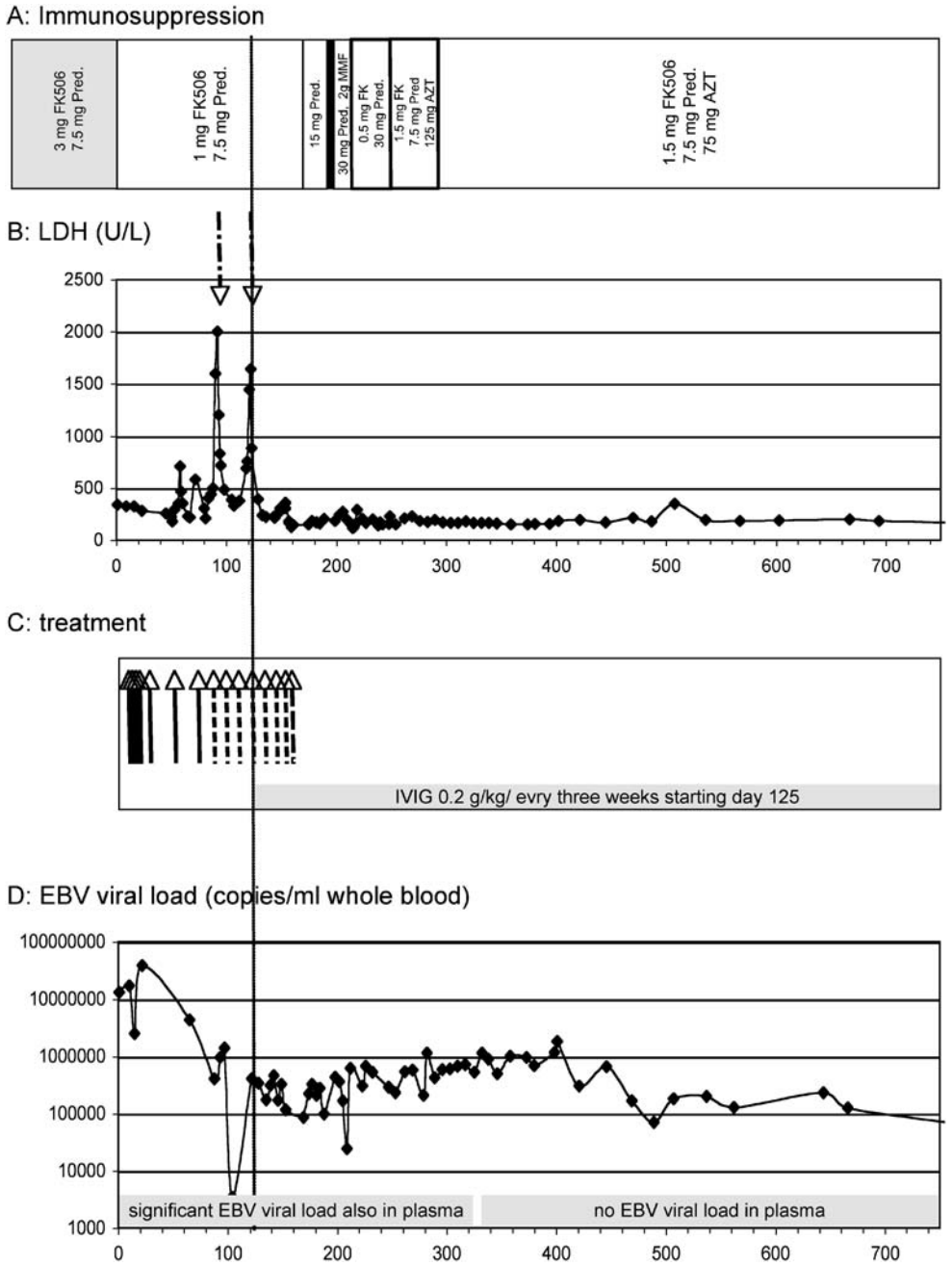

Fig. 2 **a** Immunosuppressive therapy consisted of 3 mg/day FK506 and 7.5 mg/day prednisolone at diagnosis of PTLD, of 1 mg/day FK506 and 7.5 mg/day prednisolone from days −7 to +169, of 15 mg/day prednisolone from days +169 to +189, of high-dose methylprednisolone from days +190 to +195 (*black bar*), of 30 mg/day prednisolone and 2 g/day MMF from days +196 to +217, of 0.5 mg/day FK506 and 30 mg/day prednisolone from days +218 to +249, of 1.5 mg/d FK506, 7.5 mg prednisolone and 125 mg/day AZA from days +250 to +295, and of 1.5 mg/day FK506, 7.5 mg/day prednisolone and 75 mg/day AZA from days +296 to +767. **b** LDH values from start of therapy (day 1: first application of rituximab) to end of follow-up (day +750). *Arrows* indicate hyperfibrinolytic syndrome due to tumor lysis (*first arrow*) or due to PTLD progression (*second arrow*). LDH values below 245 U/l were regarded within the normal range. **c** Treatment: *solid arrows* indicate the application of rituximab (375 mg/m^2) at days +1, +7, +14, and +21. *Dotted arrows* indicate application of CHOP (750 mg/m^2 cyclophosphamide, 2 mg vincristine, 50 mg/m^2 doxorubicin, 100 mg prednisolone) at days +38, +55, and +72 (information on individual dose reductions given in the text). *Small-dashed arrows* indicate applications of cidofovir (5 mg/kg) at days +86, +94, +104, +125, +131, +141, +148, and +155. The *large-dashed arrow* indicates the start of foscarnet therapy (180 mg/kg per day, days +156 to +169). The *light gray bar* indicates continuous application of IVIG 0.2 g/kg every 3 weeks starting at day +125, indicated by a *light gray line*. **d** EBV viral load (whole blood) in copies per milliliter in a logarithmic scale from start of therapy to end of follow-up

elevated viral load in plasma became constantly negative but remained very high in whole blood.

Due to an interval of more than 1 year between transplantation and PTLD in this formerly EBV-seronegative transplant recipient, we suspected community acquired primary infection 4 to 8 weeks prior to clinical presentation rather than a transplant-associated transmission. The first approach of treatment in PTLD usually is to reduce immunosuppression. For patients that do not respond to a reduction of immunosuppression, many will go on with rituximab monotherapy [3–5] and/or CHOP-like chemotherapy [1]. However, early treatment algorithms for PTLD also included antiviral therapy in an attempt to control EBV infection [6–8] and polymorphic and monomorphic PTLD with expression of ZEBRA—an early marker of EBV's lytic replication—do respond to foscarnet [2]. Thus, immunologic and antiviral therapy may be moderately successful for treating EBV-associated PTLD especially PTLDs with a significant lytic replication of EBV, which may have been a critical point to success in the patient described. Fever, cytopenia, coagulopathy, and high LDH levels combined with a considerable increase of serum ferritin levels (42 μg/l at day 21, 1,334 μg/l at day 110, 13,620 μg/l at day 128) further evokes macrophage activation syndrome (hemophagocytic syndrome; HPS) in this patient. HPS is classically associated with EBV primary infection [9]. Although not present at diagnosis of PTLD, HPS may have been induced by lysis of EBV-infected lymphoma cells at day 88 when cidofovir was commenced and by progression of PTLD at day 124. Application of IVIG in combination with prolonged antiviral therapy (and IR) terminated these processes inducing a significant and long-lasting response, possibly by enabling EBV-specific T cell control. (EBV-specific T cell counts were not monitored during the patients clinical course). The resumption of cidofovir therapy alone was accompanied by a rapid decrease in viral load (Fig. 2d). This is compatible with recent data suggesting that the resumption of ganciclovir therapy in primary CMV viremia can induce CMV-specific CD4 T-cells that remain stable for a period extending beyond several years [10]. Conversely, another study that analyzed patients during and after primary CMV infection in the absence of antiviral treatment demonstrated an initial rise in specific CD4 T-cells but that after a subsequent, rapid decrease, CMV-specific CD4 T cell counts remained low or undetectable in the face of detectable viral load [11]. Similarly, other groups have reported in HIV infections that even transient administration of highly active antiretroviral therapy prior to seroconversion can preserve HIV-specific immunity, enabling the host immune system to subsequently control viral replication, preserving HIV-specific CD8 T cell number and function while HIV-specific T cell help is sustained [12]. While the mechanism of immunomodulation by IVIG (and/or anti-CMV-specific IG) in PTLD still remains to be determined, recent reports suggest significant action in the prophylaxis of PTLD [13] via increased humoral EBV immunity [14] and IVIG may be active even in patients that do not respond to upfront antiviral therapy [15].

With respect to monitoring therapy, in our patient, progression of PTLD was not accompanied by increasing EBV loads and nor were increasing EBV loads, in response to intensified immunosuppression after day 190, associated with a PTLD relapse (Fig. 2a–c). These findings are in accordance with previous observations in EBV-associated PTLD with upfront rituximab treatment [16].

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