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Circulating Epstein-Barr virus DNA to monitor lymphoproliferative disease following pediatric liver transplantation

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Abstract Epstein-Barr virus (EBV) infection can induce uncontrolled lymphocyte B proliferation in immunosuppressed transplant patients. Monitoring circulating EBV-infected lymphocytes can help in identifying patients at risk of posttransplant lymphoproliferative disease (PTLD). Circulating EBV genome levels were determined in 54 liver transplant pediatric recipients. Ten patients had more than 500 EBV genome/10^5 peripheral blood lymphocytes (PBL) and exhibited clinical manifestations of EBV infection; three developed PTLD. To treat EBV infection, the level of immunosuppression was reduced and acute rejection developed in 4 patients. Three were treated with steroid and one had to be switched from cyclosporine to tacrolimus. Treatment of acute rejection was associated with increases in circulating EBV genome. None of the patients with less than 500 EBV genome/10^5 PBL developed PTLD or EBV infection. Monitoring of EBV DNA is useful in the management of EBV infection and PTLD following pediatric liver transplantation. EBV infection should be treated in ways which do not expose patients to the risk of rejection.

Key words Liver transplantation · Posttransplant lymphoproliferative disease · Epstein-Barr virus · Humans · Children

Introduction

Epstein-Barr virus (EBV) infection can induce uncontrolled lymphocyte B proliferation in immunosuppressed transplant patients. The risk of developing EBV-related posttransplant lymphoproliferative disease (PTLD) is particularly high in children who contract primary EBV infection after the transplant operation [1]. The incidence of PTLD in the pediatric population following liver transplantation (LTx) can exceed 10% [2]. It has been reported that the monitoring of circulating EBV-infected lymphocytes in solid organ and stem cell transplant patients can help in identifying patients at risk of developing PTLD [3, 4]. To further confirm this observation and to reduce the risk of PTLD by decrease of immunosuppression, we studied a group of pediatric patients who underwent LTx.

Patients and methods

A new PCR method for quantification of EBV DNA in peripheral blood lymphocytes (PBL) was developed according to a previously reported method [5] based on internal amplification control and a series of external standards which are amplified in parallel with test samples [6]. EBV nuclear antibody (EBNA) levels were determined using the ELISA technique. Starting in February 1998, serial EBV genome determinations were performed in two groups of LTx pediatric patients: group 1, 23 patients transplanted before February 1998, in whom circulating EBV genome monitoring started at variable times after LTx, and group 2, 31 patients transplanted after February 1998 who underwent prospective monitoring of circulating EBV genomes every 3 months after LTx, or whenever symptoms which could be attributed to EBV infection developed.

The immunosuppression regimen was based on the combination of steroids and cyclosporine; patients were switched from cyclosporine to tacrolimus to treat steroid-resistant rejection or cy-

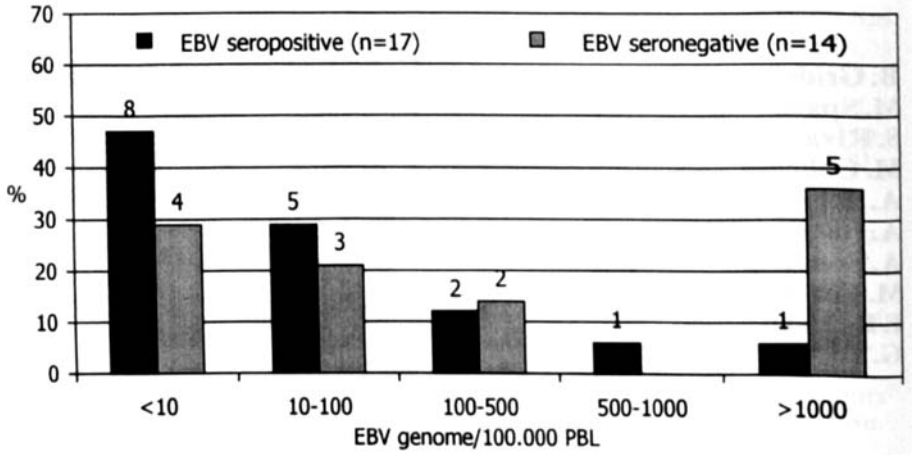

Fig. 1 Distribution of Epstein-Barr virus (EBV) genome/10^5 peripheral blood lymphocytes (PBL) in the patients prospectively monitored for EBV infection

closporine toxicity. Diagnosis of acute rejection was always made on the basis of histology. The histological diagnosis of PTLD was based on the criteria of Frizzera et al. [7]. EBV early RNA oligonucleotide probes were used for the demonstration of EBV by in situ hybidrization and antibodies to EBV LMP1 were also used for immunohistochemical demonstration of EBV.

Results

At the time of LTx, group 1 patients had a median age of 2.1 years (range 0.5–15 years) and group 2 patients, 2.3 years (range 0.2–15). The median follow up is 38 months (range 11–101 months) and 12 months (range 6–19 months) for groups 1 and 2, respectively. At the time of this report, 31 (57%) patients were still receiving cyclosporine as the main immunosuppressant, while 19 (36%) had been switched to tacrolimus; 4 patients (7%), transplanted elsewhere, were also receiving tacrolimus. The EBNA status at the time of LTx was not known for all patients in group 1 and therefore it is not being reported. Out of this group, 3 patients (13%) had more than 500 circulating EBV genome/10^5 PBL and 2 of them developed EBV infection-related manifestations. One patient had a mononucleosis-like syndrome which regressed with reduction of tacrolimus therapy; at the onset of fever, lymphoadenopathy and tonsil enlargement, the EBV genome/10^5 PBL was 5000 and decreased to less than 1000 in 6 months. Another patient, 13 months after LTx, developed a polymorphous monoclonal PTLD which was localized in the liver graft and cervical lymph nodes. Tacrolimus therapy was stopped and PTLD regressed over 3 months. The patient is alive and well over 1 year after the appearance of PTLD and withdrawal of immunosuppression; the liver function is normal.

In group 2, 17 patients (54.8%) were EBNA positive at the time of LTx and 14 (45.2%) were EBNA negative. The median ages of ENBA-positive and EBNA-negative patients were 4 (range 0.2–13) and 2.2 (range 0.5–10) years, respectively. In Fig. 1, the distribution of EBV genome/10^5 PBL levels is depicted. Five of the 7 patients, with more than 500 EBV genome/10^5 PBL, were EBNA negative at the time of LTx. One EBNA-positive patient developed mononucleosis-like syndrome localized at the tonsils, which were removed. The immunosuppression was reduced and the EBV genome blood level decreased from 3000 to undetectable in 3 months. This patient was 6 months old at the time of LTx and became EBNA negative at the time of overt EBV infection. It is possible that the EBNA was of maternal origin and its loss was associated to primary EBV infection. Another patient, EBNA negative, is currently showing evidence of a possible PTLD 8 months after LTx.

In Table 1, the symptoms of the 10 patients who had EBV genome/10^5 PBL level above 500 are reported. In order to treat EBV infection, in all patients the level of immunosuppression was reduced and episodes of acute rejection developed in 4 of them. Three patients were successfully treated with steroid boluses and 1 had to be switched from cyclosporine to tacrolimus for steroid-resistant rejection. Treatment of acute rejection was associated with variable increases in circulating EBV genome levels.

One patient in group 2, developed fever and enlargement of abdominal lymph nodes around the celiac axis at CT scan, 3 months after LTx. Repeated EBV genome determinations were negative; on the basis of positive

Table 1 Clinical manifestations in patients with more than 500 Epstein-Barr virus genome/10^5 peripheral blood lymphocytes

Symptoms	Number/total	(%)
Fever	4/10	40
Lymphoadenopathy	4/10	40
Rash	1/10	10
GI symptoms	5/10	50
None	2/10	20

IgM anti-Bartonella, a diagnosis of cat scratch disease was made and the patient was successfully treated with the proper antibiotic therapy.

Discussion

Early diagnosis and decrease in immunosuppression are important steps in the management of EBV infection and PTLD. Circulating EBV genome level determination has been previously reported to be useful in identifying organ and stem cell transplant recipients at increased risk of PTLD development [3, 4]. We studied 54 children who received a LTx with serial EBV genome level determinations. None of the patients with less than 500 EBV genome/10^5 PBL developed PTLD or had clinical manifestation compatible with EBV infection, except 1 who had cat scratch disease. Out of 10 patients who had more than 500 EBV genome/10^5 PBL, 80% had clinical manifestationsof EBV infection and 30% had PTLD which regressed with immunosuppression reduction in two patients and withdrawal in 1. This last patient has apparently developed tolerance to his liver graft.

In 4 patients, the reduction of immunosuppression resulted in regression of EBV infection clinical manifestations and/or reduction of the circulating level of EBV genomes at the expense of episodes of acute rejection; treatment was associated with a new increase in circulating EBV genomes. This suggests that the relationship between immunosuppression and circulating EBV genomes is dynamic and that, if immunosuppression has to be increased to treat rejection, the risks associated with EBV infection in pediatric LTx recipients probably increase again. Ideally, EBV infection in solid organ transplant recipients should be treated in ways which do not expose patients to the risk of rejection. Reconstitution of EBV immunity with preparation and infusion of EBV-specific autologous cytotoxic lymphocytes (CTL) seems a promising way to decrease the risk of PTLD in solid organ transplant recipients [5, 8]. We are planning a trial of pre-emptive CTL infusion in LTx pediatric recipients with circulating EBV genomes above 500/10^5 PBL.

References

1. Newell KA, Alonso EM, Whitington PF, Bruce DS, Millis JM, Piper JB, Woodle ES, Kelly SM, Koeppen H, Hart J, Rubin CM, Thistlethwaite JR (1996) Posttransplantation lymphoproliferative disease in pediatric liver transplantation. Transplantation 62: 370–375
2. Malatack J, Gartner J, Urbach A, Zitelli B (1991) Orthotopic liver transplantation, Esptein-Barr virus, cyclosporine and lymphoproliferative disease: a growing concern. J Pediatr 11: 667–673
3. Riddler SA, Breinig MC, McKnight JLC (1994) Increased levels of circulating Epstein-Barr virus infected lymphocytes and decreased EBV nuclear antigen antibody response are associated with the developement of posttransplant lymphoproliferative disease in solid organ transplant recipients. Blood 84: 972–984
4. Lucas KG, Burton RL, Zimmerman SE, Wang J, Cornetta KG, Robertson KA, Lee CH, Emanuel DJ (1998) Semiquantitative Epstein-Barr virus (EBV) polymerase chain reaction for the determination of patients at risk for EBV-induced lymphoproliferative disease after stem cell transplantation. Blood 91: 3654–3661
5. Gerna G, Furione M, Baldanti F, Sarasini A (1994) Comparative quantitation of human cytomegalovirus DNA in blood leukocytes and plasma of transplant and AIDS patient. J Clin Microbiol 32: 2709–2717
6. Baldanti F, Sarasini A, Simoncini L, Percivalle E, Grossi P, Comoli P, Dossena L, Zavattoni M, Gerna G (1997) Quantification of EBV DNA in blood and bone marrow and solid organ transplant recipients by PCR. 22nd International Herpesvirus Workshop, San Diego, Calif., USA, p 148
7. Frizzera G, Hanto DW, Gajl-Peczalska KJ (1997) Polymorphic diffuse B-cell hyperplasia and lymphomas in renal transplant recipients. Cancer Res 41: 4262–4279
8. Haque T, Amlot PL, Helling N, Thomas JA, Sweny P, Rolles K, Burroughs AK, Prentice HG, Crawford DH (1998) Reconstitution of EBV-specific T cell immunity in solid organ transplant recipients. J Immunol 160: 6204–6209