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BRIEF REPORT

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Post-transplant lymphoma in a liver allograft

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Abstract We describe the development of a lymphoma in a liver allograft shortly after orthotopic liver transplantation. Aspiration and core biopsies of the nodule were persistently negative so that a diagnosis could not be made until the patient underwent retransplantation, when examination of the liver resection specimen revealed a B-cell lymphoma. Using a rapid technique based on the polymerase chain reaction, we were able to demonstrate that the tumor was of donor origin.

Key words Liver transplantation, B-cell lymphoma · B-cell lymphoma, liver transplantation

Introduction

Post-transplant lymphoma is a well recognized complication of organ transplantation [7, 14, 17]. Lymphoproliferative disorders have been reported in association with a variety of immunosuppressive regimens [6, 7, 17, 23]. They have also been described as accidentally transplanted cancers [14]. We report a case of post-transplant lymphoma that presented in a liver allograft shortly after transplantation. Polymerase chain reaction studies demonstrated that the tumor was of donor origin, although there was no evidence of lymphoma in the donor study.

Materials and methods

Case report

A 51-year-old male underwent orthotopic liver transplantation (OLT) due to posthepatitis C cirrhosis in April 1993. The liver was procured from a healthy, 43-year-old male donor who had died of brain damage after an accident. Sonographic study of the donor liver showed no abnormal masses within the porta hepatis.

The immunosuppressive regimen after OLT included therapy with antithymocyte globulin (ATG, 2 mg/kg; Fresenius) for 7 days, followed by cyclosporin (CyA), prednisone, and azathioprine at low doses [2, 3]. Fever and a progressive cholestasis led to a percutaneous liver biopsy on day 11 that revealed an acute rejection. Methylprednisolone (500 mg) bolus therapy was initiated for 3 days and serum bilirubin and transaminases gradually returned to normal.

Two months after OLT, the patient was admitted to the hospital again because of cholestasis, jaundice and pruritis. Doppler sonography showed slightly dilated intrahepatic ducts, a patent portal vein, anomalous flow within the hepatic artery, and a hypoechoic nodule of 35 mm intrahepatically between segments IV and V of Couinaud's anatomy. A fine needle biopsy of the nodule was performed but the material obtained was insufficient. Percutaneous transhepatic cholangiography was normal. Another liver biopsy showed an acute rejection that required methylprednisolone (500 mg) bolus therapy again for 3 days.

The patient was followed up by sonography, computed tomography, and magnetic resonance imaging that revealed the same size nodule and no other abnormalities. Another fine needle biopsy and a core biopsy were carried out in order to determine the presence of malignant cells, and these repeatedly revealed necrotic material.

Another episode of acute rejection was diagnosed 8 months after OLT that improved again after steroid therapy. However, a severe hepatic artery stricture was diagnosed by angiography, suggesting that the nodule was in fact a liver infarction. A progressive increase in bilirubin (with a peak value of 643 μmol/l for direct bilirubin and 1040 μmol/l for total bilirubin), urea (peak value of 48.1 mmol/l), and creatinine (peak value of 390 μmol/l) was registered. Immunosuppression was reduced to 10 mg prednisone. A

Table 1 Data regarding the microsatellites

Microsatellite	Chromosome location	Repeat	PCR Primers
ACTB	6	$(AAAG)_n$	5'AAT CTG GGC GAC AAG AGT GA 5'ACA TCT CCC CTA CCG CTA TA
D6S89	6p	$(AC)_n$	5'CTT GTT CAT CTG CCT TGT GC 5'ACC TAA GCG ACT GCC TAA AC

Table 2 Genotypes obtained for both microsatellites

	Blood recipient	Liver donor	Lymphoma
ACTB	b/c	a/f	a/f
D6S89	b/b	a/g	a/g

situation of jaundice, hepatic insufficiency and renal failure requiring hemodialysis led to retransplantation in January 1994.

Histopathological examination of the liver resection specimen revealed a B-cell lymphoma of the porta hepatis with an intense necrosis of the tumor (about 80%). Hepatic parenchyma were free of tumor and showed a chronic rejection in addition to a necrosis of the bile ducts.

Serologies of the donor and recipient before and after retransplantation for Epstein-Barr virus (EBV) were negative using an immunofluoresecence test for the detection of antibodies against EBV viral capsid antigen.

After retransplantation, immunosuppression was begun at low doses (ATG at 1 mg/kg per day for 7 days, prednisone i.v. at 20 mg/day, and CyA p.o. at 8 mg/kg per day). The post-transplant course was marked by febrile illness associated with respiratory insufficiency. A pneumonia due to herpes simplex and cytomegalovirus was diagnosed by a culture of bronchoscopic specimens and therapy with ganciclovir was begun. There was no evident clinical response to a withdrawal of azathioprine and CyA or to a reduction of steroids to 5 mg/day in addition to therapy with ganciclovir and other antibiotic, antiviral, and antifungal therapies that were introduced empirically due to persistent fever and respiratory insufficiency. A CT scan revealed bilateral lung infiltration and bronchoscopy showed diffused alveolar damage.

The patient died 3 months after retransplantation due to respiratory insuffiency. An autopsy revealed severe, diffused, alveolar damage, chronic lung fibrosis, and chronic rejection in the liver study. A careful examination of the liver failed to find evidence of lymphoma.

Study of tumor specimen

DNA was isolated from peripheral blood of the recipient and from paraffin blocks from both the liver donor and the lymphoma. DNA extraction from blood was carried out by salt precipitation [11]. The paraffin was removed from the blocks with xylene and the tissue was digested with proteinase K.

The DNA obtained was amplified by the polymerase chain reaction (PCR) [19] using five microsatellites [22]; of these ACTB [18] and D6S89 [8] were the most informative (Table 1).

PCR was performed for each locus using 200 ng DNA, 200 μM each dATP, dCTP, dGTP, and dTTP, 10 pmol of one primer, 1.5 pmol of the other primer, end-labelled with [γ ^{32}P] ATP, 50 mM KCl, 10 mM TRIS (PH 8.3), 1.5 mM Mg Cl_2, and 1 unit of Taq DNA polymerase (Boehringer Mannheim) in a total volume 25 μl.

The reaction was performed for 30 cycles at 94 °C for 20 s, annealing at 61 °C for 40 s and extension at 74 °C for 1 min.

Results

The genotypes obtained for the two markers ACTB and D6S89 are shown in Table 2. Results demonstrated that the genotype of the lymphoma was the same as that of the liver donor, indicating that the lymphoma was of donor origin.

Considering that the PCR is an extremely sensitive technique that is able to amplify very small amounts of DNA, it would be very easy to detect the existence of contamination or chimeras. For the ACTB and D6S89 markers, the recipient alleles – b/c and b/b, respectively – were not detected in the lymphoma, demonstrating that DNA from the recipient was not present (Fig. 1). If contamination or chimerism had been present, we would have expected to find four alleles: a/b/c/f and a/b/g, respectively. Thus, the results obtained clearly demonstrate that the lymphoma originated in the donor.

Discussion

Lymphoproliferative disorders (LPD) are a potential complication of organ transplantation. Compared with the general population, there is a disproportionately high incidence of solid lymphomas in transplant recipients [12, 14, 16]. In contrast to the usual predominance of Hodgkin's disease, non-Hodgkin lymphoma of B-cell origin is the most common form in transplanted patients [12, 14–16]. Most of the reported cases of LPD have been related to immunosuppressive therapy [6, 7, 17, 23], but a number of cases arising in the allograft from donor cells have also been described [1, 4, 5, 10, 20]. Reduction or withdrawal of immunosuppression has been recommended as a part of lymphoma therapy [1, 7, 12, 21], although many lymphomas do not respond to a reduction in the dosage [5, 20].

A B-cell lymphoma of the porta hepatis developed in our patient 2 months after OLT. No evidence of Epstein-Barr virus infection was observed, in contrast to most LPD in transplanted patients [6, 9, 13, 21]. What is original about our case is that we were able to demonstrate that the tumor was of donor origin using the PCR in order to amplify the DNA. In our patient, a diagnosis could not be made before retransplantation because of persistent negative results of aspiration biopsies and

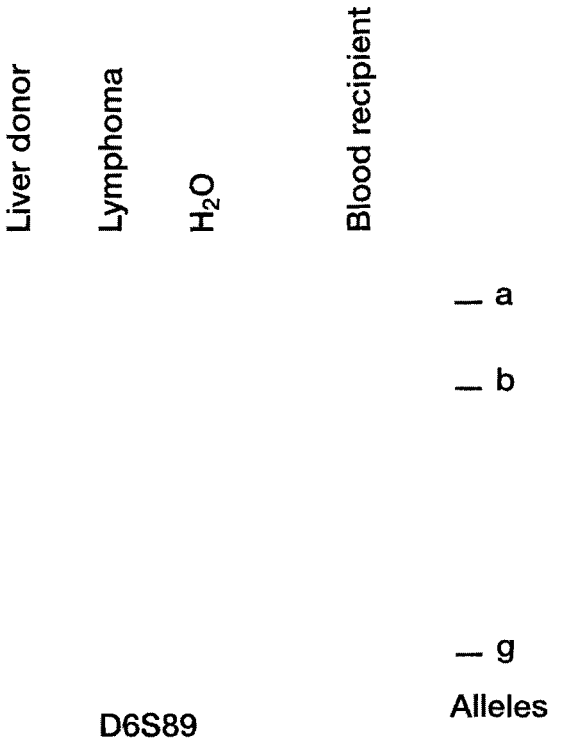

Fig. 1 Amplification of the DNA of the blood recipient, liver donor, and lymphoma

core biopsies. Yet, the study of the liver resection specimen revealed an intense necrosis of the lymphoma.

It is worth noting that both kidneys were also harvested from the same donor. We checked to see whether any LPD had arisen in either of the recipients of the kidneys but both are free of lymphoma in radiological studies.

In conclusion, we would like to emphasize the following points:

1. The possibility of lymphoma must always be considered when sonography shows a portal mass, even if the sonographic study of the donor is normal.
2. Negative core biopsies for malignant cells do not rule out the presence of lymphoma, especially when material obtained is necrotic.
3. In our patient, the tumor was already necrotic, but additional necrosis might have occurred after withdrawal of immunosuppression. No evidence of lymphoma was found after retransplantation; yet, the patient died of infectious complications.

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