

# Post-transplant malignant lymphoma with monoclonal immunoglobulin gene rearrangement and polyclonal Epstein-Barr virus episomes

Y Sadahira, K Kumori, Y Mikami, T Otsuki, T Manabe, K Aoyama

## Abstract

**This report describes the case of an 8 year old boy who developed ileocecal B cell lymphoma after liver transplantation. The patient underwent orthotopic liver transplantation for biliary atresia and had been given immunosuppressive drugs—cyclosporin A and tacrolimus hydrate. Six years after the liver transplantation, the patient had a sudden onset of fever and abdominal pain. Necropsy revealed an ileocecal mass that was a B cell lymphoma. Epstein-Barr virus (EBV) encoded RNA 1 was demonstrated in lymphoma cells and hyperplastic follicular germinal centre cells in various tissues. Although monoclonal immunoglobulin gene rearrangement was detected in the liver, EBV episomes were of polyclonal origin and lytic forms of EBV were also demonstrated by Southern blotting. Immunohistochemically, lymphoma cells were positive for p53 but negative for latent membrane protein 1 and EBV nuclear antigen 2. These findings suggested that this B cell lymphoma might have occurred sporadically, regardless of EBV infection.**

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Post-transplant lymphoproliferative disorder (PTLPD) is a well recognised complication of organ transplantation.<sup>1-3</sup> Histologically, PTLPDs represent a disease spectrum ranging from benign polyclonal, polymorphic B cell proliferations to obviously malignant lymphomas, which are generally associated with Epstein-Barr virus (EBV) infection.<sup>1,2</sup> EBV associated PTLPDs usually occur within one year after transplantation and show type III latency; namely, they express virus encoded latent proteins, EBV nuclear antigen 1 (EBNA-1), EBNA-2, and latent membrane antigen 1 (LMP-1).<sup>4</sup> In B cell lymphomas, EBV episomes are of monoclonal origin, which suggests that EBV is involved directly in the lymphomagenesis, rather than being a passenger as a result of the immunocompromised status.<sup>3,5</sup> However, it has been reported recently that half of the tumours seen after kidney transplantation are not associated with EBV.<sup>6,7</sup> The interval between grafting and the diagnosis of EBV negative PTLPDs was significantly longer than that of EBV positive PTLPDs. Thus, it is possible that the causes of lymphoma in transplants are multiple. Here, we report an unusual

case of EBV positive PTLPD occurring in an 8 year old boy six years after liver transplantation. This was a histologically apparent malignant lymphoma, but displayed polyclonal EBV episomes, despite the monoclonal nature of the immunoglobulin gene rearrangement. This lymphoma might have occurred sporadically, without an association with the EBV infection.

## Clinical history

An 8 year and 5 month old boy was admitted to Kawasaki Medical School on the 4 March 1999 because of jaundice and high fever. He had received a liver allograft (left outer area) from his mother in August 1992 at Okayama National Hospital (Okayama, Japan). He had undergone hepatopertoenterostomy four times, but progression of liver disease to liver cirrhosis mandated liver transplantation. Since the transplant, he had been given the immunosuppressive drug, cyclosporin A (25-75 mg/day) for one month and then tacrolimus hydrate (FK506) (1.5 mg/day) for six years. Serum titres of antibodies to EBV and human immunodeficiency virus had not been examined. On admission, hepatosplenomegaly and liver function test abnormality were noted. Scintigraphy disclosed intrahepatic bile duct dilation but no obstruction. The patient had severe abdominal pain on 15 April 1999. Emergency computed tomography disclosed an abnormal mass below the right kidney. On 20 April, his condition suddenly became worse and he died.

## Pathological findings

A complete necropsy was performed. In the ileocecal region, a raised lesion (5 × 4 × 1 cm) was found. Continuous with this lesion, mesenteric lymph nodes were enlarged and formed a tumour mass (6 × 4.5 × 4 cm). Histologically, monotonous atypical lymphoid cells proliferated in mucosa, effacing the intestinal glands and some mesenteric lymph nodes. The nuclei of the lymphoid cells were a little smaller than those of the endothelial cells, and the cells had one or two small inconspicuous nucleoli. Mitotic activity was clearly seen and exceeded 10 for each high power field. The histology was consistent with a PTLPD malignant lymphoma according to Knowles's classification.<sup>1</sup> Reactive lymphoid follicles were formed adjacent to the ileocecal tumour. The allografted liver, which weighed 350 g, was grey/white in the portal area. Microscopically, atypical lymphoid cells resembling those seen in the ileocecal lesion densely infiltrated into

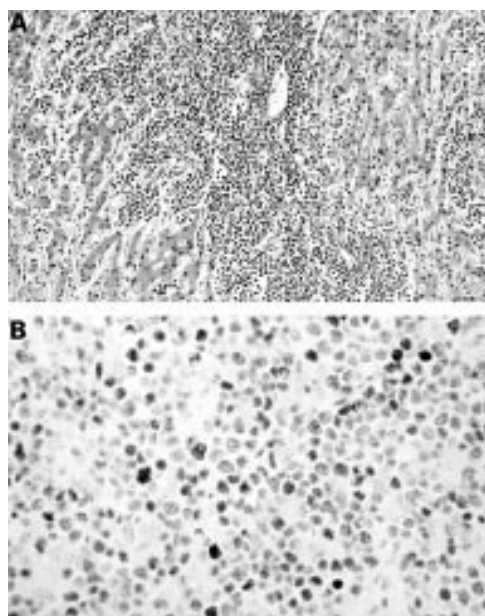
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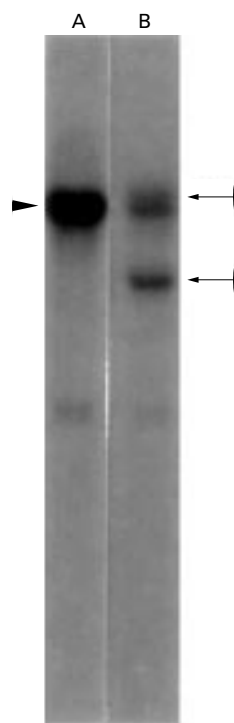
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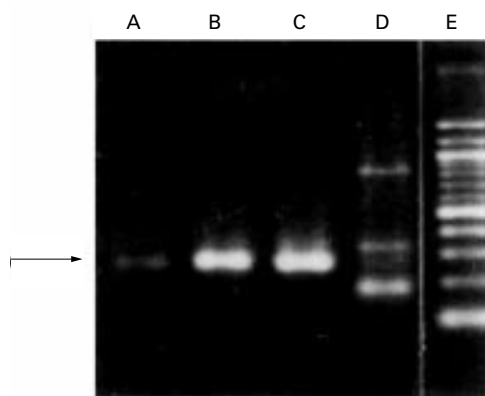
**Figure 1** (A) Lymphoma cell infiltration of the liver. Haematoxylin and eosin staining; original magnification,  $\times 35$ . (B) Immunostaining for p53. The nuclei of lymphoma cells are positive. Original magnification,  $\times 140$ .

the portal area (fig 1A). They formed small nodules in some places, which were up to 2 cm in diameter. They showed monotonous proliferation and extended to the sinusoid. There was no evidence of cirrhosis. The bone marrow showed hypercellularity with atypical lymphoid cell proliferation. The spleen, which weighed 350 g, showed atypical cells infiltrating the white pulp. The pancreas head lymph nodes showed follicular and paracortical hyperplasia.

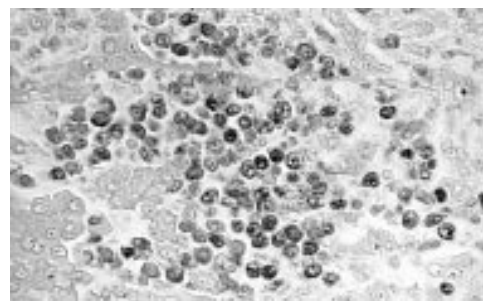
Ileocecal and liver sections were evaluated with immunohistochemistry on paraffin wax embedded sections. Lymphoma cells in both



**Figure 2** Southern blot analyses of liver with the  $\gamma$ H probe after digestion with *Hind*III. Lane A, placental DNA showing a germ line band (arrowhead); lane B, DNA from the liver. Arrows show rearranged bands.



**Figure 3** PCR amplification of the Fr2-JH region of the rearranged immunoglobulin gene in the ileocecal tumour (lane A) and the liver (lane B) using paraffin wax embedded sections. PCR primers were prepared using a DNA synthesiser, as described previously.<sup>5</sup> The PCR products were separated on an agarose gel containing 0.3 mg/ml (0.003%) of ethidium bromide, and bands were visualised and photographed using ultraviolet transillumination. The predicted sizes of the amplified products were 240–280 bp (arrow). Lane C, positive control (a paraffin wax section of diffuse large cell lymphoma); lane D, negative control (an anaplastic large cell lymphoma cell line); lane E, molecular weight markers (a 100 bp ladder marker).



**Figure 4** Epstein Barr virus (EBV) infection of the liver demonstrated using EBV encoded RNA 1 (EBER-1) *in situ* hybridisation. Nuclei of lymphoma cells were EBER-1 positive, whereas hepatic cells were negative. Original magnification,  $\times 140$ .

lesions were positive for immunoglobulin  $\lambda$  light chain, CD10, CD20, and p53 (fig 1B). They were negative for  $\kappa$  light chain, CD3, CD43, CD45RO, bcl-2, and cyclin D1. The MIB-1 index of the lymphoma cells was 90%.

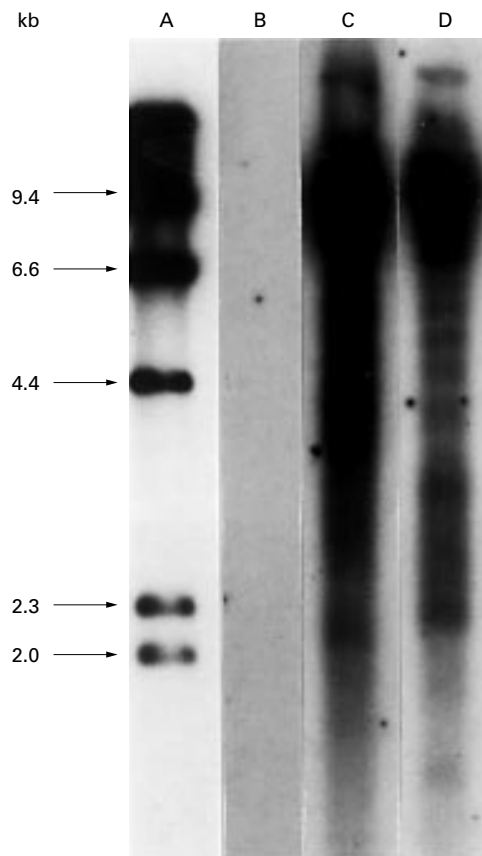
Southern blotting using DNA from fresh liver showed one or two rearranged immunoglobulin (Ig) heavy chain bands, indicating monoclonal B cell expansion (fig 2). No abnormality was found in the bcl-6 and c-myc genes. Monoclonality was confirmed in the liver and ileocecal tumours by polymerase chain reaction (PCR) analysis for DNA isolated from paraffin wax embedded sections (fig 3).

Next, we searched for the presence of EBV infection in the ileal tumour, liver, duodenum, pancreas head lymph node, and tonsils using *in situ* hybridisation for EBV encoded RNA 1 (EBER-1). Positive signals for EBER-1 were seen on lymphoma cell nuclei in all tissue examined (fig 4). A total of 70% of lymphoma cells were positive and this did not vary between the ileocecal and liver lesions. Immunohistochemically, the lymphoma cells did not express LMP-1 or EBNA-2. Nuclear staining of the BZLF1 protein was seen in 2% of the cells within the lesions.<sup>3</sup> EBER-1 signals were also seen in germinal centre cells in the ileum, duodenum, tonsils, and peri-pancreas head lymph nodes. Clonality of the EBV was examined in the liver using probes for the terminal repeat sequences. Not only latent, multiple circularised EBV episomes but also linear, replicating EBV were present (fig 5).

## Discussion

Knowles *et al* evaluated PTLPD in a series of patients who had received heart transplants and subclassified it into three categories: the first consists of plasmacytic hyperplasia, the second of polymorphic lymphoproliferative disorders, and the third of apparent malignant lymphoma and multiple myeloma.<sup>1</sup> Our case was an overt monoclonal malignant lymphoma. However, it contained non-clonal EBV episomes and exhibited type I latency of the viral infection.

Immunosuppressive drugs—cyclosporin A and tacrolimus hydrate—might induce the immunosuppression that allows EBV infected lymphocytes to proliferate and permits the development of EBV positive lymphoma.<sup>2</sup>



**Figure 5** Southern blot analysis of the liver. For the presence of latent episomal or linear replication Epstein Barr virus (EBV) DNA, the 7.7 kb BamHI *NheI* fused genomic terminal fragment was used as a probe. Lane A, molecular marker; lane B, negative control; lane C, positive control, containing DNA from an EBV infected polyclonal lymphoblastoid cell line (B95-8), which is permissive for lytic EBV infection. Both circular and linear forms of the virus genome are seen. Lane D, DNA from the liver, containing multiple high molecular bands consistent with episomal viral DNA and additional bands in the range of 2.5 to 3 kb consistent with linear viral DNA.

Nevertheless, our findings suggest that this case may be a sporadic tumour that developed independently of EBV infection because of the following: (1) the presence of polyclonal EBV episomal forms; (2) a long interval between transplantation and development of the lymphoma; (3) the negativity of LMP-1 and EBNA-2, which play an important role in neoplastic processes in PTLPD; and (4) the presence of EBV positive reactive follicular hyperplasia in the lymphoid tissue adjacent to the lymphoma.

Our case is not the first to show monoclonal Ig gene rearrangement and polyclonal EBV episomes. A similar case was reported by Kaplan *et al* from eight cases of PTLPD.<sup>9</sup> In these cases, EBV infection of the lymphoma cells might have occurred at some point after neoplastic transformation. Alternatively, an

admixture of EBV infected B cells could be the reason for the appearance of multiple episomal forms of EBV. The clonal analysis using the EBV terminal probe could detect minor clones missed by Ig gene analysis because of the increased sensitivity.<sup>5</sup> Although it was unsuccessful in our case, one way to confirm our finding would be to microdissect tumour cells and then determine whether or not the lymphoma cells were infected in a monoclonal or polyclonal fashion. The absence of EBNA-2 and LMP-1 does not exclude a role of EBV in tumorigenesis because EBNA1 also has oncogenic properties.<sup>10</sup>

One possible explanation for the p53 staining of the lymphoma cells was as a result of inactivation and stabilisation of p53 protein by mutation of the p53 gene. However, it is also possible that inactivation of p53 by an EBV encoded protein resulted in stabilisation and accumulation of p53 in the lymphoma cells, because EBV infection is associated with the overexpression of p53 in infectious mononucleosis tissues.<sup>11</sup>

In conclusion, we describe a case of EBV positive PTLPD occurring six years after liver transplantation. Examination of the clonality of the EBV is important in the diagnostic evaluation of EBV positive PTLPD, even in apparent malignant lymphoma, because the importance of EBV infection in the lymphoma may vary in each case.

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