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Case report

Enteropathy-associated T-cell lymphoma in a renal transplant patient with evidence of Epstein-Barr virus involvement*

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Summary. The clinical and histological findings in a 54-year-old patient with enteropathy-associated T-cell lymphoma (EATL) occurring 18 years after renal transplantation are presented. Ten years after adult-onset coeliac disease the patient developed medium to large T-cell non-Hodgkin's lymphoma of the small intestine. Epstein-Barr virus (EBV) genome was detected by polymerase chain reaction in the lymphoma tissue and localized via Epstein-Barr virus RNAs in situ hybridization to some of the tumour cells. This is the first case report of EBV-positive EATL occuring in the setting of immunosuppression.

Key words: Enteropathy-associated T-cell lymphoma – Epstein-Barr virus – Epstein-Barr virus RNA in situ – Polymerase chain reaction – Renal transplantation

Introduction

Patients with coeliac disease have a 50- to 100-fold greater risk of developing malignant lymphoma of the gut than the general population (Swinson et al. 1983). Histologically, these lymphomas have been characterized as "malignant histiocytosis of the intestine" (Isaacson and Wright 1978). The neoplastic cells later turned out to be of T-lymphocytic rather than histiocytic origin (Isaacson et al. 1985).

The Epstein-Barr virus (EBV) has generally been found in B-cells exclusively (Pagano and Shaw 1979) and T-lymphocytes and T-cell lymphomas have been thought not to be associated with EBV. Recently, reports from different groups have documented the presence of EBV DNA in T-cell lymphomas (Jones et al. 1988; Su et al. 1990, 1991). In another group of patients with an increased risk of malignant lymphoma (secondary immunodeficient organ transplant recipients) the neoplasms are frequently EBV-associated (Hanto et al. 1981).

We present an enteropathy-associated T-cell lymphoma (EATL) in a renal transplant recipient. The association of this neoplasm with EBV is shown by polymerase chain reaction (PCR) and EBV RNAs (EBER) in situ hybridization. We are not aware of previous reports of a similar association.

Case report

The patient was subjected to renal transplantation for membranoproliferative glomerulonephritis at the age of 36 years. She was hospitalized for anaemia and intestinal symptoms 8 years later. The results of biopsy as well as clinical tests led to the diagnosis of coeliac disease, which responded well to gluten-free diet. At the age of 54 years she developed persisting abdominal pain, culminating in an acute abdomen that required hospitalization. The laboratory findings on admission were as follows: haemoglobin 10.4 g/dl, peripheral blood leucocyte count 6.2×10^9/l; atypical lymphocytes were not noted. The platelet count was 401×10^9/l. At laparotomy, an ulcerating jejunal tumour mass involving the entire wall was found. The patient is well without evidence of disease 3 years after surgical treatment of the tumour.

Methods

For immunohistochemistry paraffin sections were stained by a standard avidin-biotin method using the primary antibodies shown in Table 1. Avidin-biotin reagents were obtained from Dako, Copenhagen, Denmark. Diaminobenzidine was used as a chromogen and the sections were counterstained with haemalum. For negative controls, primary antibodies were replaced by buffer solution.

Genes for RNAs to be analysed were cloned into SP6 vectors. The pJJJ1 vector, used for EBER 1, was digested with *Mst* II/*Puv* II, and the fragment containing 89% of the *EBER 1* gene was treated with Klenow fragment and ligated into pSP65 digested with *Sma*I. After determining the orientation, the pSP65/EBER 1 plasmids (both orientations) were digested with *Bam*HI and transcribed to obtain RNAs of 175 nt (83% EBER 1 specific). The RNA was labelled with digoxigenin-dUTP [RNA labelling kit (SP6/T7);

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Table 1. Monoclonal antibody panel used for the immunohistological investigation of formalin-fixed, paraffin-embedded tissues

Antigen (CD group)	Antibody	Source
B-cell associated antigens		
CD 45R	4KB5	Dakopatts
CD 45R	KIB3	Dr. Wacker, Kiel
CD 20	L26	Dakopatts
T-cell-associated antigens		
CD 45RO	UCHL1	Dakopatts
CD 43	MT1	Biotest
CD 3	CD 3	Dakopatts
CD 4	OPD 4	Dakopatts
Miscellaneous antigens		
CD 45	LCA	Dakopatts
CD 30	Ber-H2	Dakopatts

Boehringer Mannheim]. Probes already prepared and labelled were kindly provided by Prof. H. Wolf, Regensburg, FRG.

In situ hybridization studies were done on formalin-fixed, paraffin-embedded tissues. Sections were cut onto glass slides coated with Cementit, baked overnight and deparaffinized in xylene, hydrated through a series of graded ethanol baths and treated with proteinase K for 15 min. After this treatment the sections were fixed in 4% paraformaldehyde for 15 min and then prehybridized for 15 min. Hybridization was done by applying 5–10 µl of the probe-cocktail consisting of formamide, 20 × SSC, Denhard's solution, dextran sulphate, distilled water and EBER(-)digoxigenin-labelled probe. The sections were coverslipped. The DNA was denatured by placing sections on a heating plate at 90° C for 3–5 min. The sections were then briefly cooled on ice and the DNA hybridized overnight at 42° C. Serial washings in each of 6×, 2× and 0.2 × SSC were followed by the detection of hybrids by use of the Boehringer Mannheim DIG system. The sections were counterstained with haematoxylin and mounted.

Cells were scored positive for viral RNA if they showed the characteristic, predominantly nuclear localization of the label in the absence of nucleolar labelling and if hybridization with the control probes was negative. Sections of formalin-fixed, paraffin-embedded Raji, B95.8 and EBV-negative cells were used as positive and negative controls, respectively. Sections of EBV-positive lymphomas (as shown previously by Southern-blot hybridization, PCR and in situ hybridization) were included in each batch of experiments as positive tissue controls.

For PCR paraffin sections were cut with new disposable blades that were changed between different samples. Two slides were placed in a microcentrifuge cup, deparaffinized with two changes of octane at 65° C, followed by centrifugation. The pellet was washed with two changes of ethanol 100% and dried. Proteinase K digestion was done overnight at 48° C followed by phenol-chloroform extraction and ethanol precipitation.

Oligonucleotide primers were synthesized according to published sequences (genebank) on an automated oligonucleotide synthesizer (Applied Biosystems, 380A). PCR primers precipitated with ethanol were dissolved in water and diluted to 20 pm/µl. For the detection of EBV-DNA we used a PCR with primers W1-S (GGTCCCCTCGGACAGCTCCTAAGA) and W1-A (CTGAAGGTGAACCGCTTACCACCT) for the amplification of a 130 bp fragment from the multicopy *Bam*HI W1 repeat of the EBV genome. This PCR detects 10 pg Namalwa DNA (two EBV copies/genome) in the presence of 0.2 µg BJAB (EBV negative) (not shown). For EBNA-2, one common sense primer was used (CTATCTTGCGTTACATGGGGGACA) and two different antisense primers for amplification of a 177 bp EBNA-2A (GGGAGTGGTGGGGGCACCCCC) and 177 bp EBNA-2B (TGGGGCGGTGCGGGTGCCCCA) fragment. To check for amplifiable cellular DNA, a 210 bp fragment was amplified with primers BCL2-S (CGACGACTTCTCCCGCTACCGC) and BCL 2-A (GGCGATGTTGTCCACCAGGGGCGAC) for detection of *bcl-2* gene.

The PCR was performed as follows. The reaction volume of 50 µl contained the template in 50 mM potassium chloride, 10 mM TRIS-HCl (pH 8.3), 1.5 mM magnesium chloride, 0.001% gelatin, each primer at 0.6 µM, each dNTP at 250 µM and 1.5 units of Taq DNA polymerase (Amplitaq, Perkin-Elmer Cetus). The samples were overlaid with 50 µl mineral oil, denatured for 7 min at 94° C, and subjected to 35 cycles of amplification in an automated thermal cycler. Cycling conditions were 1 min 94° C, 1 min 55° C, 1 s 72° C for EBNA-2, 1 min 94° C, 1 min 60° C, 1 s 72° C for W1 and 1 min 94° C, 1 min 60° C, 1 min 72° C for *bcl-2* fragments. The 72° C step was lengthened 8 s per cycle for all fragments with a final 10 min incubation step at 72° C. The amplified product (15 µl) was subjected to electrophoresis on 2% agarose gels. The gels were stained in ethidium bromide and photographed on a UV transillumination lamp with polaroid 667 films. DNA was transferred in 0.4 M sodium hydroxide to Gene Screen Plus (Dupont) Nylon Membrane probes. Amplified fragments were verified by restriction analysis of hybridization with a ^{32}P-gamma ATP (Dupont) endlabelled oligonucleotide (TGGAAACCCGTCACTCT) complementary to an internal sequence common to EBNA-2A and B. Hybridization was performed in 6 × SSPE, 7% SDS and 0.1% dry milk overnight at 42° C. Stringent washes were done with 0.1 × SSC and 0.1% SDS for 2 × 15 min at 60° C followed by exposure on Kodak XR10 film.

Results

Histological examination revealed a widely ulcerated, medium to large cell pleomorphic lymphoma (Fig. 1) infiltrating all coats of the jejunal wall. The tumour cells, positive for leucocyte common antigen, were negative with all B-cell markers tested. Some tumour cells yielded a positive reaction with the CD3, CD43 and CD45RO markers, whereas CD4 was negative in all tumour samples tested. The adjacent jejunal mucosa presented an inflammatory reaction. No villous atrophy or increased number of intraepithelial lymphocytes (IEL) was noted and no lymphoepithelial lesions were found.

Re-examination of the jejunal biopsies taken in 1979 showed partial villous atrophy, relative crypt hyperplasia, decrease in the number of goblet cells and increased numbers of IEL and plasmocytes in the lamina propria (Fig. 2). Some of these IEL were CD3, CD43 and CD45RO positive; none was CD4 positive (Table 2). The PCR findings demonstrated EBV type A in tumour samples. There was insufficient amplifiable DNA for PCR in the jejunal biopsy specimens taken in 1979. The in situ findings done with EBER in situ hybridization indicated the presence of EBV genome in a few very sparsely distributed tumour cells. The morphology of EBER-positive cells is obviously that of malignant lymphoma (Fig. 3), although the identity of the cells could not be visualized by double labelling with EBER and markers for T-cells. No small lymphocytes were EBER positive. In adjacent sections from the tumour margins, mucosal lymphocytes showed no label. EBER in situ hybridization of the mucosal biopsies taken in 1979 showed EBV genome present in lymphoid cells of the lamina propria. No IEL were labelled (Fig. 4). The phe-

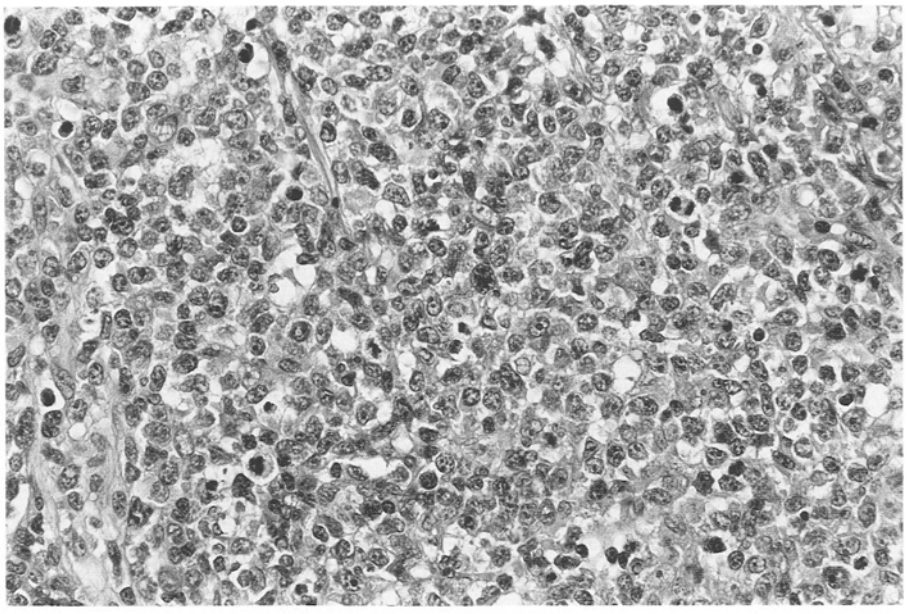

Fig. 1. Medium to large cell pleomorphic lymphoma, small bowel resection (1989). H & E, ×50

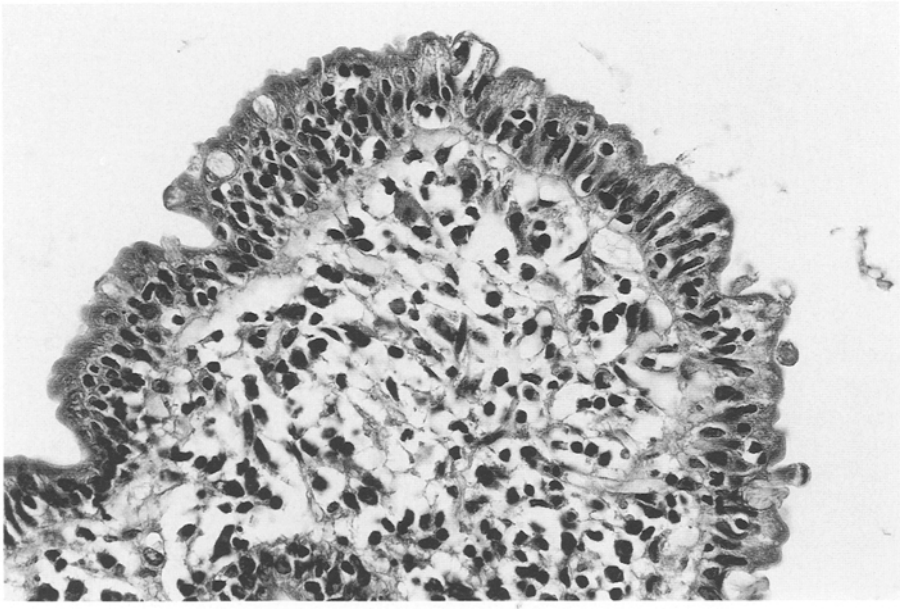

Fig. 2. Jejunal biopsy (1979) with partial villous atrophy, relative crypt hyperplasia, decrease in numbers of goblet cells and increased numbers of intra-epithelial lymphocytes and plasmocytes in the lamina propria, H & E, ×100

Table 2. Results of T-cell phenotyping in jejunal biopsies (1979) and malignant lymphoma (1989)

	CD 3	CD 4	CD 43	CD 45RO
Intraepithelial lymphocytes	Some	–	Some	Some
Lamina propria lymphocytes	+	+	+	+
Non-Hodgkin lymphoma	Some	–	Some	Some

notype of positive cells could not be characterized as B- or T-cell type. The number of positive cells in this biopsy clearly outnumbered that of normal small lymphocytes which could be detected using EBER in situ hybridization. We have studied cases of reactive lymphadenopathy and other reactive conditions such as lymphoid gastritis with EBER in situ hybridization. Extremely few lymphoid cells were labelled (data not shown). Compared with these cases, large numbers of EBV-RNA-containing cells were present in the lamina propria of our patient.

Discussion

The clinical and histopathological criteria required for the diagnosis of an enteropathy-associated intestinal lymphoma are fulfilled in this patient. Only a few lymphoma cells were positive for EBV genome, but many lymphoid cells in the lamina propria of intestinal mucosa biopsies, taken years before the development of the lymphoma, yielded a positive reaction for EBER. Further, the patient had been treated with immunosuppressive

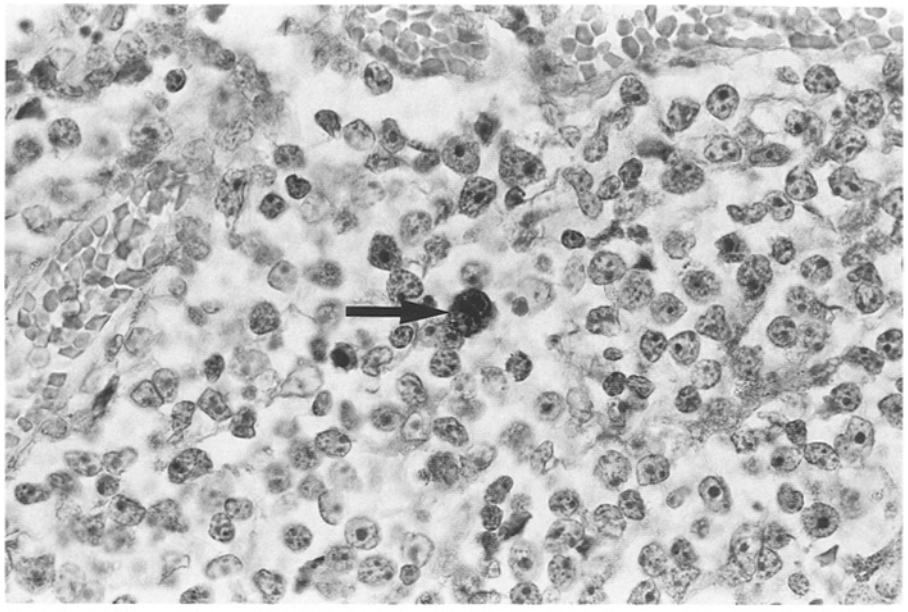

Fig. 3. EBER in situ hybridization on lymphoma tissue reveals very few Epstein-Barr virus (EBV)-positive tumour cells (*arrow*). In situ hybridization with EBER (–), haemalum counterstain, ×158

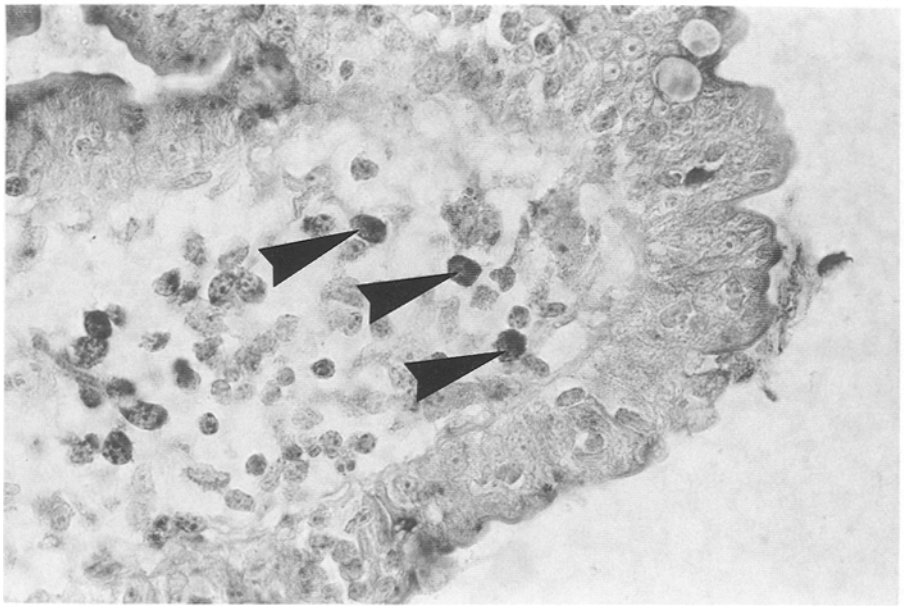

Fig. 4. EBER in situ hybridization on jejunal biopsy shows the presence of EBV in lymphoid cells of the lamina propria. In situ hybridization with EBER (–), no counterstain, ×158

drugs, azathioprine and prednisone, for 18 years after kidney transplantation. These observations raise several questions about the association of immunosuppression, enteropathy, infection by EBV, and malignant lymphoma.

The immunophenotype of IEL was identical to that of lymphoma cells. Similar observations, in combination with the occurrence of monoclonal T-cell receptor gene rearrangements of IEL in coeliac disease, led to the assumption that adult-onset enteropathy amounts to a premalignant condition (Moroz et al. 1988) or even a low-grade malignant lymphoma of IEL (Wright et al. 1991). Although most lymphoproliferative lesions that occur in the setting of immunodeficiency are of B-cell origin and T-cell lymphomas have been described only rarely (Frizzera et al. 1981; Borisch-Chappuis et al. 1990), the first description of T-cells containing EBV derived from a patient with immunologically mediated disease (Kikuta et al. 1988) and the first three cases of T-cell lymphomas associated with EBV infections have arisen in chronically ill patients, where disorders of the immune system are not completely excluded (Jones et al. 1988). More recently, larger series of EBV-positive T-cell lymphomas have been published (Su et al. 1990, 1991; Hamilton-Dutoit and Pallesen 1992), the latter occurring in apparently immunocompetent patients. The import of this positivity remains to be established. The mechanisms that result in the infection of T-cells by EBV and, possibly, in the unfolding of malignant lymphoma are not evident. T-cells transfected with EBV support sustained virus replication (Stevenson et al. 1986) and human thymocytes can be infected by EBV (Watry et al.

1991). However, in our patient the infection – if EBER positivity does not simply represent an uptake of EBV-DNA from neighbouring cells – may have taken place after clonal expansion, and not before, as noted in other lymphoid neoplasms in immunodeficiency (Borisch et al. 1992b).

We describe a case of EATL lymphoma in an immunocompromised host, presenting with evidence of EBV involvement. To our knowledge, this association has not been described previously. Our findings do not explain the aetiological impact of these associations on the genesis of the lymphoma but we suggest that a T-cell lymphoma developed from the enteropathy-asociated lymphocytes. Consecutively, some of the tumour cells have been "superinfected" with EBV or they have taken up EBV from the surrounding tissues.

The highly sensitive EBER in situ hybridization method has been used successfully in various studies (MacMahon et al. 1991; Wu et al. 1991). The probe applied in this study recognizes a region of the EBV genome which is highly expressed in latent infection (Glickman et al. 1988). This method is able to detect small EBV-positive lymhocytes, for example in tissue specimens from Hodgkin's disease and reactive conditions (Weiss et al. 1991). The number of EBV-containing lymphoid cells may increase with time in immunodeficient patients, while lymphoid B- and T-cells proliferate, perhaps as a consequence of deficient immunosurveillance. This may occur in HIV-positive patients (Borisch et al., 1992a). This would explain the high content of EBV-positive small lymphocytes in the jejunal biopsies of our patient.

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