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CASE REPORT

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Detection of Epstein Barr virus in an hepatic leiomyomatous neoplasm in an adult human immunodeficiency virus 1-infected patient

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Abstract We report the first case of a human immunodeficiency virus (HIV)-related primary hepatic leiomyoma in an adult patient. The diagnosis was made at autopsy and confirmed by immunohistochemistry. Epstein Barr virus (EBV) was identified in tumour cells by in situ hybridization. Review of the literature revealed 13 cases of visceral myogenic tumours occuring in acquired immunodeficiency syndrome children, and only 2 cases in adults. One was a spinal epidural leiomyoma, the other multiple smooth muscle tumours of the colon and adrenal gland. This is the first report of EBV in smooth muscle neoplastic cells in an HIV-infected adult patient.

Key words HIV 1 infection · Malignant lymphoma Hepatic leiomyoma · Epstein Barr virus

Introduction

Van Hoeven et al. [18] recently described a case of a human immunodeficiency virus (HIV)-infected child who developed a smooth muscle tumour within the liver. Two cases of smooth muscle tumours in HIV-infected adult patients have been already published in the literature [13, 17]. In this report, we describe the case of a young HIV 1-infected homosexual man, having the acquired immunodeficiency syndrome (AIDS) for two years, who developed a hepatic leiomyomatous neoplasm hybridization positive for Epstein Barr virus (EBV).

Clinical history

A 33 year-old-man entered Saint-Antoine's hospital in August 1992 with left hypoacusia, unsteadiness in walking and balance and left lateropulsion, rotary vertigo, headhache, nausea and vomiting, lasting for 2 months. This young homosexual man had been treated for pleural tuberculosis for 10 months in 1981. His HIV 1 sero-positivity was diagnosed in 1986, and he developed AIDS status in January 1990. Thereafter, he was seen for several opportunistic infections: recurring anal herpes; hairy leucoplakia of the tongue; pulmonary pneumocystosis in January 1990, treated with Bactrim; molluscum contagiosum and Campylobacter diarrhoea in August 1991; and digestive microsporidiosis in January 1992.

When he was hospitalized, disorders of swallowing, vertical diplopia and impairment of motor function appeared. The patient was apyrexial; upright stance was impossible. He had a left hemiparesis brisk left tendon reflexes, hypoaesthesia of the left upper limb, dysmetria of left hemicorpus, and paralysis of left abducens. Fundoscopy was normal.

Brain magnetic resonance imaging revealed a tumour in the posterior cranial fossa, within the left medium cerebellar peduncle, extending to the pons and the left cerebellar hemisphere. The mass showed low signal intensity on T1 weighted images, high signal intensity on T2 weighted images, and strong homogenous enhancement after injection. Cerebrospinal fluid was clear with less than 1 cell/mm^3, and no abnormal cells. Lactate dehydrogenase level was low, and the CD4 lymphocyte count was 9/mm^3. Cultures for viruses on blood and cerebrospinal fluid were negative but *mycobacterium avium-intracellulare* was isolated from blood and stools, and *Candida albicans* in the oral cavity.

The digestive candidiasis was resistant to fluconazol. Despite treatment of the mycobacterium septicemia and a test therapy for toxoplasmosis, the brain tumour did not descrease in size. The patient developed a paralysis of the left trigeminal nerve, and finally died six weeks after his hospitalization. An autopsy was performed 24 h after death.

Materials and methods

Autopsy specimens were fixed in 4% formalin. Paraffin embedded sections were stained with haematoxylin and eosin, giemsa, Ziehl-Neelsen, periodic acid-Schiff, and Gordon-Sweet silver impregnation.

Immunohistochemistry was performed on paraffin embedded sections, using a three-step indirect immunoperoxidase technique, with 3 amino ethyl carbazole as detection agent. The monoclonal and polyclonal antibodies used are presented in Table 1.

In situ hybridization was performed as previously described by Audouin et al. [1]. Briefly, sections of paraffin embedded buffered formalin fixed tissue were spread out on silane coated slides. After permeabilization of the sections, in situ hybridization at 37° C was carried out overnight. To detect the EBV encoded small RNA (EBER), we used as specific probe, the Dakopatts fluorescein-conjugated EBV (EBER) oligonucleotides (Y017), with nitroblue tetrazolium – 5 bromo 4 chloro 3 indolphosphate as detection agent. Finally, sections were stained with fast red K699 Dakopatts.

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Table 1 Immunohistochemical analysis of both neoplasms

Central nervous system neoplasm		Immunoblasts
PD7/26-2B11, Dakopatts	Anti-CD 45, monoclonal leucocyte common antigen	+
L26, Dakopatts	Anti-CD 20, monoclonal B lymphocytes	+
UCHL 1, Dakopatts	Anti-CD 45 RO, monoclonal T lymphocytes	–
Liver neoplasm		**Spindle cells**
V9, Dakopatts	Anti-vimentin, monoclonal, mesenchymal cells	–
1A4, Sigma	Anti-α smooth muscle actin, monoclonal, smooth muscle cells	+
KD1, Dakopatts	Anti-CD 68, monoclonal, macrophages	–
anti-PS 100, Dakopatts	Polyclonal, Schwann cells	–
F8/86, Dakopatts	Factor VIII-associated antigen, monoclonal, endothelial cells	–
QBEnd/10, Biogenex	Anti-CD 34, monoclonal, endothelial cells	–
BNH 9, Dakopatts	Monoclonal, endothelial cells	–

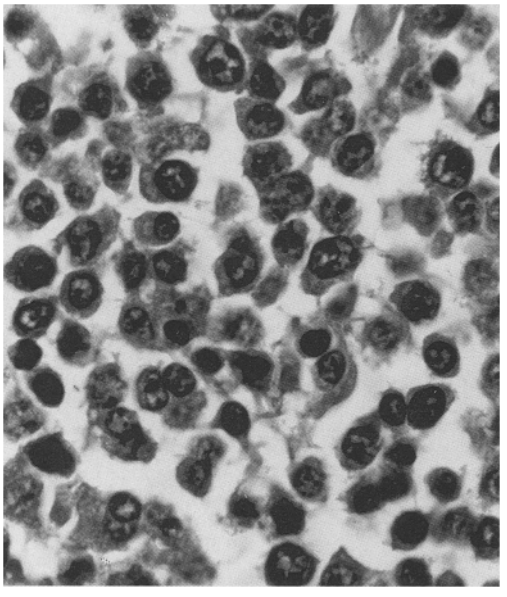

Fig. 1. Central nervous system immunoblastic malignant lymphoma, with plasmacytic differentiation. (Haematoxylin and eosin, ×3000)

Pathological findings

Autopsy revealed disseminated atypical mycobacteriosis (small mesenteric lymphadenopathy with partial necrosis; splenic micronodules without splenomegaly; microscopic liver granulomas; numerous yellowish bulges of the mucosa of the small intestine, corresponding to bacteria laden macrophages in lamina propria). Atypical mycobacteria in the cytoplasm of the histiocytes were Ziehl-Neelsen positive. There was a necrotizing adrenalitis with cytomegalovirus, and suppurative bronchiolitis (without any necrotizing lesions on gross examination, the lungs were oedematous and congested). A large ill-defined tumour was found in the brain stem. It extended from the top of the left cerebral peduncle to low on the left side of the spinal bulb. The left medial cerebellar peduncle and dentate nucleus were totaly infiltrated. On light microscopic examination, the tumour showed large areas of ischaemic necrosis; the well preserved territories were predominantly perivascular. The neoplasm was composed of large cells having features of immunoblasts with plasmacytic differentiation on morphological examination (Fig. 1), positive on immunohistochemical analysis with the anti-CD 45 and anti-CD 20 antibodies and negative with the anti-CD 45 RO antibody. They were mixed with numerous histiocytes and small reactive lymphocytes. Lastly, a solitary 2 cm diameter firm, white and fasciculated tumour was found beneath the capsule in the right hepatic lobe (Fig. 2). On histopathological examination, this tumour was composed of a highly cellular population of spindle cells arranged in interwoven fascicles (Fig. 3). The cells had abundant eosinophilic cytoplasm and regular spindle nuclei with blunt ends. No mitotic figures were observed, nor tumour necrosis. Immunohistochemically, the tumour cells were positive for α smooth muscle actin. They were negative with all the other antibodies tested, as shown in Table 1.

EBV was sought in these two neoplasms, and in situ hybridization was performed both on the malignant lymphoma of the central nervous system and on the leiomyomatous tumour of the liver. EBV RNAs were detected in tumour cell nuclei of both neoplasms. More than 50% of the tumour immunoblasts were hybridized in the central nervous system malignant lymphoma, while almost 50% of the spindle cells of the liver neoplasia presented a strong nuclear hybridization signal (Fig. 4), which was not observed with the control probe tested

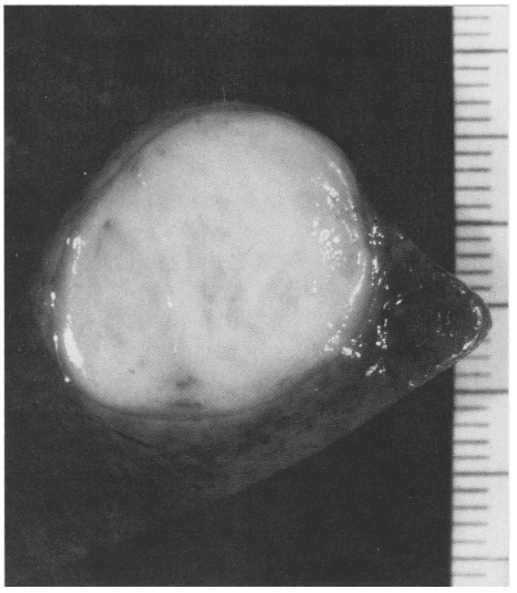

Fig. 2. Gross photograph of solitary sub-capsular leiomyoma of the liver seen at autopsy. (Metric scale)

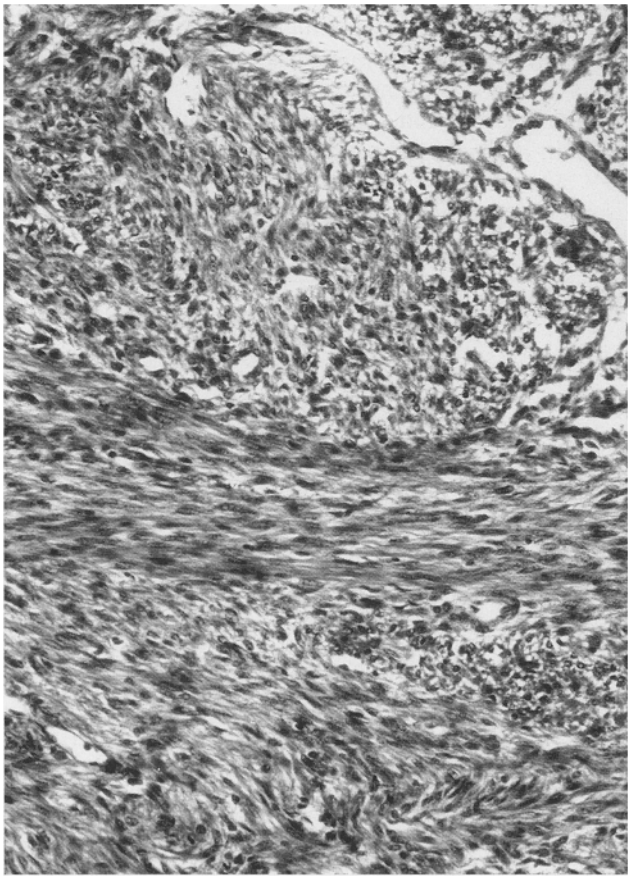

Fig. 3. Hepatic leiomyoma shows interwoven bundles of spindle shaped cells with regular neuclei, and no mitoses. (Haematoxylin and eosin, ×1200)

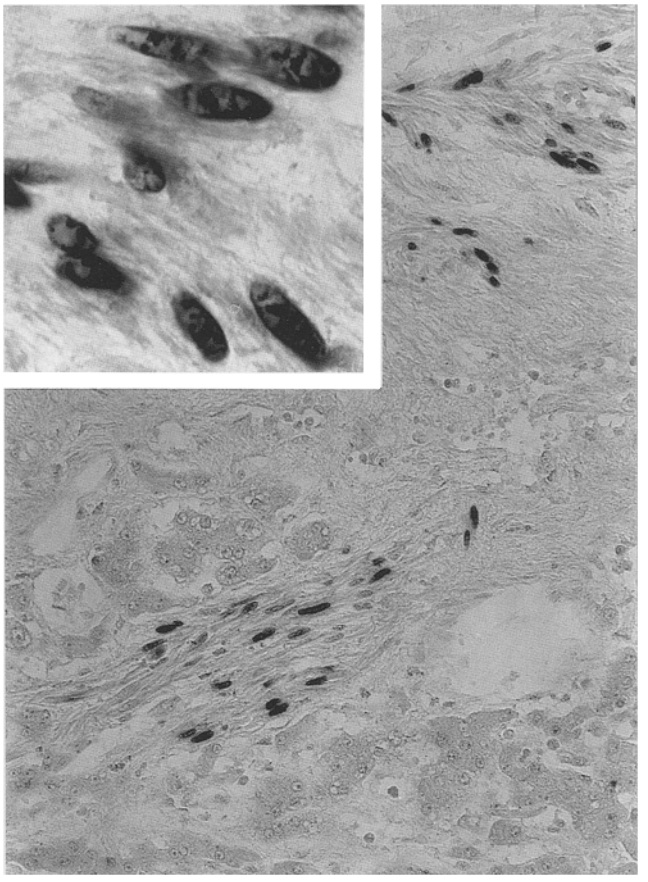

Fig. 4. In situ hybridization with the Epstein Barr virus probe. The hybridization signal is present only within the neoplastic smooth muscle cells; hepatocytes are not hybridized (×1200). *Inset:* EBER RNAs are detected only in the nuclei of the neoplastic cells, and the intersity of the reaction varies from one cell to the other. (×3000)

on adjacent sections. Adjacent hepatocytes were negative, as were muscle cells around blood vessels.

Discussion

Primary smooth muscle tumours of the liver are rare. They probably arise from smooth muscle of the vessels or biliary tree. Less than twenty cases of primary leiomyosarcoma of the liver have been published and benign tumours are rarer still. However criteria of malignancy are not well defined because of the rarity of these neoplasms [15], and some authors prefer to interpret the bland looking smooth muscle tumours as tumours of uncertain malignancy [18].

Chronic immunodeficiency, either congenital or iatrogenic, is associated with an increased risk of malignancy [2, 6, 11, 16]. But soft tissue tumours are not usually associated with immunodeficiency disorders [7]. Leiomyosarcomas have been reported in renal transplant recipients, after therapeutic irradiation, and leiomyoma in individuals with congenital immunodeficiency [6]. It is now well established that adult patients with acquired

immunodeficiency syndrome (AIDS) have an increased risk of developing tumours, especially malignant lymphoma and Kaposi's sarcoma [8, 11, 14]. In keeping with this, our own patient developed an immunoblastic malignant lymphoma in the central nervous system, of B phenotype assessed by the positivity of the neoplastic cells with the L26 (anti-CD20) antibody. But adult patients with HIV infection do not appear to have an increased incidence of soft tissue tumours other than Kaposi's sarcoma [7]. Isolated cases of spindle celled lesions have been recently reported in AIDS patients. Among these, thirteen cases of visceral myogenic tumours occuring in AIDS children [2, 3, 4, 6, 8, 10, 11, 14, 16, 18], and two cases in adults [13, 17], have been published. Moreover, about ten other cases of smooth muscle tumours in AIDS patients, either children or adults, are also mentioned in these reviews [3, 9, 13, 18]. These smooth muscle tumours involve visceral sites preferentially: only one case mentioned by Van Hoeven et al. [18] arose in the soft tissues of the neck. Five of the paediatric cases (but none of the adult cases) were located in the liver [8, 10, 14, 18]. Two more cases mentioned in the literature were also located in the liver [9, 18]. Two out of these five paediatric cases were discovered only at autopsy as solitary small spherical tumour masses within the liver [8, 18]. This is also the case of our own AIDS-related hepatic spindle cell tumour. It had the classical morphological features of a leiomyoma, including well circumscribed nodule although not encapsulated (Fig. 4), less than 3 cm diameter, without necrosis; regular spindle shape cells, no atypical nuclei, less than one mitotic figure per ten high power fields and high immunopositivity with anti-α specific smooth muscle actin antibody. The autopsy revealed no other smooth muscle tumour which could have been the primary site of an hepatic metastasis.

Chadwick et al. [3] suggested that the association of smooth muscle cell neoplasms and HIV infection in children may not be fortuitous. McLoughlin et al. [6] have discussed the possible direct or indirect action of the retrovirus in the development of such neoplasms. Ross et al. [14] failed to detect HIV in smooth muscle cells by means of in situ hybridization, using a biotinylated nucleic acid probe for HIV. This result does not favour direct effect of HIV in the development of these tumours. However numerous indirect mechanisms have already been proposed [2, 3, 14] such as: defective host immunosurveillance, chronic antigenic stimulation and unregulated polyclonal B cell expansion. HIV-infected T lymphocytes may elaborate growth factors and cytokines which stimulate tumours. In addition, anti-retroviral therapy could have a tumorigenic role in treated HIV-infected patients [6, 8]. Other possible promoting factors could be the multiple co-infecting viral and non-viral micro-organisms. Among them, EBV has already been associated with nasopharyngeal carcinoma, Burkitt's lymphoma [12] and virus-induced B cell polymorphic lymphoproliferations. Ross et al. [14] failed to detect EBV nucleic acids in smooth muscle neoplastic cells using in situ hybridization and a biotinylated labelled specific probe. Conversely, Lee et al. [5] were able to detect EBER RNAs in the nuclei of the neoplastic smooth muscle cells in the three cases of post-transplant spindle cell tumour they tested. However, immunohistochemistry for latent membrane proteins was negative in the two cases they studied. Van Hoeven et al. [18] were also able to detect EBER RNAs with in situ hybridization in a case of intrahepatic myogenic tumour in an AIDS child. This case was first reported by Ninane et al. [10] as a fibrosarcoma. Von Hoeven et al. [18] had the opportunity to test the original material: immunohistochemistry suggested the smooth muscle differentiation of the tumour, and in situ hybridization the presence of EBV in the spindle cells. In our own case, EBER RNAs are also detected in more than 50% of the neoplastic cells, located in the nuclei. However, there was no in situ hybridization signal in the nuclei of blood vessel smooth muscle cells, and hepatocytes were not hybridized. This patient presented also an immunoblastic malignant lymphoma of the central nervous system and EBER RNAs were also observed in the nuclei of the majority of the immunoblastic lymphomatous cells. At the same time, we had the opportunity to diagnose another case of myogenic tumour in an HIV-infected young adult. It was a basi-thoracic neoplasia of uncertain malignant potential, but we could not observe any positive cell for EBER RNAs with in situ hybridization. We therefore cannot conclude as Lee et al. [5] did for post-transplant spindle cell tumours that myogenic tumours in HIV-infected patients are always associated with EBV, and resemble the EBV-related lymphoproliferative disorders occuring in transplanted and AIDS patients.

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