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LETTER TO THE EDITOR

Dear Sir,

Epstein-Barr virus in Hodgkin's disease: frequency of a 30-bp deletion in the latent membrane protein 1 (LMP-1) oncogene in South African patients

In recent years, substantial evidence has accumulated implicating Epstein-Barr virus (EBV) in the pathogenesis of Hodgkin's disease (HD) and some non-Hodgkin's lymphomas (Pallesen et al.*, 1993; Weiss* et al.*, 1989). It has been postulated that EBV in HD interferes with the pathways of cell differentiation, through the expression of an integral membrane EBV-protein, latent membrane protein 1 (LMP-1) (Dawson* et al.*, 1990). Recent studies have identified mutations and deletions in the* LMP-1 *gene* (BNLF-1) *amplified from some cases of HD (Knecht* et al.*, 1993*b*, 1995; Sandvej* et al.*, 1994). This supported earlier work by Hu* et al. *(1991) who reported a 30-bp deletion (del-LMP-1) and 7 single-base mutations in the carboxy-terminal region of the* BNLF-1 *gene in nasopharyngeal carcinoma (NPC). It has therefore been proposed that alterations within this region of the* BNLF-1 *gene could render an EBV genome more aggressive and confer a growth advantage to infected cells (Knecht* et al.*, 1993*b; *Sandvej* et al.*, 1994).*

Our aim was 2-fold, i.e., *(1) to determine the frequency of del-LMP-1 in EBV-positive HD in South African patients, and (2) to assess whether this deletion might be associated with aggressive clinical behaviour.*

A total of 132 HD cases were included in the study (Departments of Pathology, Groote Schuur and Red Cross Hospitals). All cases of HD were analysed for the presence of EBV by: (1) immunohistochemical analysis for EBV LMP-1, and (2) RNA in situ *hybridisation to detect EBERs. Purified DNA from EBV$^+$ cases were analysed by polymerase chain reaction (PCR), using oligonucleotide primers directed at a 316-bp fragment in the C terminal region of the* BNLF-1 *gene (within which the 30-bp deletion occurs) (Knecht* et al.*, 1993*b*). In negative cases (positive for* β*-globin and EBER-1), a 156-bp fragment of the amino terminal part of the* BNLF-1 *gene was amplified to show the presence of the gene.*

Thus, 64/132 (48%) HD cases were positive for EBV; 29/51 (57%) cases were EBV$^+$ childhood cases and 35/81 (43%) were EBV$^+$ adults. A total of 24 cases were amplifiable by PCR and del-LMP-1 was detected in 21% (5/24) EBV$^+$ cases. In the remaining EBV$^+$ cases, no PCR products were detected when using primers 9 and 11, and these were analysed for the presence of the 156-bp fragment of the BNLF-1 *gene. This fragment was detected in 21 cases, whereas in the remaining 18 cases there was no amplification. The latter is probably due to the paraffin-embedded nature of the samples, in which DNA is prone to degradation and in which it is difficult to amplify using templates greater than 300 bp.*

Del-LMP-1 status was then compared in patients with regard to clinical stage, age, sex and HD subtype. Such information were recorded only for cases in which data was available (20 cases) (Table I). The 4 cases with the deletion were all of the nodular sclerosis (NS) subtype, with 3 of these being the less aggressive NS type I and the remaining case of the more aggressive NS type II. Of the 16 deletion-negative cases, 3 were of the mixed cellularity (MC) subtype and 16 of the NS subtype.

Several findings support the hypothesis that del-LMP-1 plays a role in oncogenesis. Previous results have indicated that del-LMP-1 is sometimes associated with histologically aggressive tumours that often display morphology such as giant anaplastic HRS cells, the presence of a large number of HRS cells and areas of necrosis (Kingma et al.*, 1996; Knecht* et al.*, 1993*a*). It has also been shown that transfectants (from Chinese and Taiwanese NPC) carrying del-LMP-1 were more tumorigenic when injected into nude and severe combined immunodeficiency disorder (SCID) mice (Chen* et al.*, 1992; Hu* et al.*, 1993). Also, Knecht* et al. *(1995) studied 2 angio-immunoblastic lymphadenopathy (AILD) patients to investigate whether del-LMP-1 plays a role in the progression of AILD into B immunoblastic lymphoma (B-IBL). They found del-LMP-1 and 6 identical point mutations (identical to that reported for NPC and HD) in both cases of B-IBL, and concluded that del-LMP-1 might be associated with the evolution of AILD and could therefore be clinically relevant. Also, when the normal 30-bp sequence was inserted into the Taiwanese 1510 cell line (which contains the deletion), it resulted in a loss of its transforming capacity, whereas its deletion from the prototype B95–8 LMP-1 (wt-LMP-1), resulted in transformation of BALB/3T3 cells (Li* et al.*, 1996). Sandvej* et al. *(1994) also found that del-LMP-1 occurred significantly more frequently (>60%) in Danish PTL than HD (30%) and background population as defined by acute infectious mononucleosis tonsils (30%).*

Trivedi et al. *(1994) showed that del-LMP-1, when injected into a mouse mammary adenocarcinoma system, was non-immunogenic and suggested that it may be able to escape the host's immune surveillance system and trigger uncontrollable proliferation. The proliferation of LMP-1 expressing tumours and a high association of del-LMP-1 have been observed in patients with decreased immunocompetence,* e.g., *PTLDs and acquired immunodeficiency syndrome (AIDS)-related lymphomas, and has led to the hypothesis that impairment of the immune function may be a requirement for EBV-driven lymphomagenesis (Dolcetti* et al.*, 1997; Kingma* et al.*, 1996; Santon* et al.*, 1995; Trivedi* et al.*, 1994). Knecht* et al. *(1996) investigated the NF-*κ*B domain of the C-terminal region of the* LMP-1 *gene in immunocompromised (HIV$^+$) hosts and found that del-LMP-1 was significantly associated in immunocompromised patients compared with non-malignant reactive cases.*

On the other hand, some studies have indicated that del-LMP-1 does not play a major role in oncogenesis. Kingma et al. *(1996) and Dolcetti* et al. *(1997), for example, reported the presence of del-LMP-1 in immunosuppressed and non-immuno-*

TABLE I – COMPARISON OF CLINICAL DATA OF EBV-POSITIVE HODGKIN'S DISEASE WITH AND WITHOUT DEL-LMP-1

	LMP$^+$ cases with deletion (n = 4)	LMP$^+$ without deletion (n = 16)
Sex		
Males	4 (100%)	13 (89%)
Females	0 (0%)	3 (19%)
Age (years)		
Median	15.5	29.7
Range	(4–26)	(6–61)
Stage		
I	0 (0%)	6 (38%)
II	2 (50%)	1 (6%)
III	1 (25%)	8 (50%)
IV	1 (25%)	1 (6%)
Histology		
Nodular sclerosis	4 (100%)	13 (81%)
Mixed cellularity	0 (0%)	3 (19%)

n = 20. EBV, Epstein-Barr virus; DEL, 30-bp deletion; LMP-1, latent membrane protein 1.

suppressed patients and suggested that the impairment of the immune system alone does not appear to be a requirement for EBV-driven lymphomagenesis. Also, Smir et al. *(1996), Scheinfeld* et al. *(1997) and Tao* et al. *(1998) found no association of del-LMP-1 with PTLDs, while the former also did not find any association between del-LMP-1 and the survival of patients. Several studies have also now shown that the incidence of del-LMP-1 is similar in some of the EBV-associated malignancies and in the normal population, suggesting that the del-LMP-1 variant in those cases probably reflects the frequency of the deletion in a broad population (Chen* et al., *1996; Dolcetti* et al., *1997; Hayashi* et al., *1997, 1998; Itakura* et al., *1996; Khanim* et al., *1996; Mansoor* et al., *1997; Scheinfeld* et al., *1997; Smir* et al., *1996). Khanim* et al. *(1996) examined case material from various geographic regions (Kenya, Gambia, New Guinea, China and Europe) and found that, for all these regions, del-LMP-1 was not preferentially associated with various EBV-lymphoproliferative disorders when compared with normal patients. These findings have led to the suggestion that del-LMP-1 may in fact represent the most prevalent EBV strain in particular populations (Jenkins and Farrell, 1996).*

Previous studies have reported the presence of the 30-bp deletion in approximately 9–30% of HD cases (Khanim et al., *1996; Knecht* et al., *1993*a, *1995; Sandvej* et al., *1994; Santon* et al., *1995). We found the deletion not only in 21% of South African HD cases, which is in agreement with data from these reports, but also in reactive lymphoid tissues (data not shown). Four of the del-LMP-1 cases we analysed were of the poorly aggressive NS type I, whereas only one del-LMP-1 case was associated with aggressive morphology (NS type II) (Table I). Although our data suggest that there is no association between del-LMP-1 and aggressive HD and that del-LMP-1 does not increase the risk of developing HD, a larger number of cases needs to be analysed to reach firm conclusions.*

Yours sincerely,

M. Fadiel Essop[1], Mark Engel[2], Pauline Close[1], Colin Sinclair-Smith[2] and Gorm Pallesen[3]*

[1]*Department of Anatomical Pathology, UCT Medical School, Observatory, South Africa*

[2]*Department of Histopathology, Red Cross War Memorial Children's Hospital and Institute of Child Health, Mowbray, South Africa*

[3]*Department of Pathology, Sentralsjukehuset i Rogaland, Stavanger, Norway*

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*Correspondence to: Department of Anatomical Pathology, UCT Medical School, Observatory 7925, South Africa. Fax: (27)21–4481789. E-mail: mfessop@chempath.uct.ac.za

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