ALIMENTARY TRACT

Epstein-Barr virus associated oesophageal ulcers in AIDS

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Abstract

Epstein-Barr virus (EBV) associated ulceration has not previously been included in the differential diagnosis of oesophageal ulcers in AIDS. We report five cases of oesophageal ulceration in homosexual men with advanced human immunodeficiency virus infection in whom this was considered to be the most likely cause. DNA in situ hybridisation studies showed EBV in biopsy material from three of four patients with oesophageal ulcers and in none of three controls. Of other viruses studied, only human papillomavirus was present, and this was found in both patients and control subjects. These findings support the hypothesis that EBV is an aetiological factor in some cases of AIDS-associated oesophageal ulceration.

Epstein-Barr virus (EBV) is a member of the herpesviridae family, and therefore characteristically establishes latent infection which may be reactivated in immunosuppression. The pathogenicity of EBV is related to its ability to infect certain cell types. It is well established that EBV can infect cells of the B lymphocyte lineage.¹ More recently, evidence has emerged to suggest that the prime target for infection is the epithelial cell and that infection of B lymphocytes is of secondary importance with regard to the viral life cycle.²⁻⁴

EBV infection has been found in association with oral hairy leucoplakia, a condition associated with the human immunodeficiency virus (HIV),⁵⁻⁷ and has also been implicated in the development of some AIDS associated lymphomas.^{4,8,9} We describe atypical oesopha-

geal ulceration occurring in HIV seropositive patients, together with the results of in situ DNA hybridisation studies that show EBV to be present in three of the four lesions from which material was available. We suggest that this may represent a further manifestation of EBV reactivation in AIDS.

Methods

Of the five patients, all homosexual men, three had AIDS related complex and two had AIDS at the time oesophageal ulceration developed (see Table I). The three control oesophageal biopsy specimens were obtained from HIV seropositive homosexual men aged between 24 and 37, who underwent endoscopy for dyspepsia. In each, the findings were those of mild gastritis in the absence of any oesophageal abnormality.

Tissue obtained at biopsy was routinely processed, sectioned at multiple levels, and stained as previously described.¹⁰ In addition, all cases were stained for cytomegalovirus early and late antigens with a mouse monoclonal antibody (DAKO CMV/M757), and avidin-biotin immunoperoxidase technique.¹¹ DNA in situ hybridisation was performed on biopsy materials from four of the five cases and from the three control subjects. The method used has been described elsewhere.¹² The probes used were as follows: complete human papillomavirus 2, 6, 11, 16, 18, 31, and 32 cloned in pBR322; the EBV probe was the 3.3 kb Bam HI fragment cloned in pBR322 and the cytomegalovirus probe was the 21 kb Hind III fragment cloned in pH1E2. The negative control was pBR322 and the positive control was total genomic DNA.

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TABLE 1 Clinical and endoscopic features

Case No	Age (years)	HIV status (CDC)	Symptoms	Treatment on presentation	Mouth	Pharynx	Oesophagus (distance from incisors)
1	54	IV A	Dysphagia, sore throat, weight loss	None	N	0.5 cm uvular ulcer	Solitary ulcer (36 cm)
2	29	IV C ₂	Dysphagia, sore throat, weight loss	Ketoconazole	N	2.0 cm tonsillar ulcer	Not known
3	27	IV C ₁	Dysphagia, weight loss	Zidovudine, ketoconazole, loperamide, codeine phosphate	N	N	Multiple linear ulcers (26/35 cm)
4	43	IV C ₂	Chest pain, dysphagia	Zidovudine, acyclovir, phenytoin	OHL	N	Solitary ulcer (30 cm)
5	40	IV C ₁	Chest pain, dysphagia, weight loss	Zidovudine, sulfadoxine and pyrimethamine (Fansidar), folic acid	N	N	Solitary linear ulcer (29 cm)

CDC=Centers for Disease Control. N=normal appearance. OHL=oral hairy leucoplakia.

Koilocytes with multinucleated cell (arrow) and individual cell keratinisation (arrow head). (Haematoxylin and eosin; original magnification $\times 383$.)

Results

All biopsies were assessed for the presence of particular histological features characteristic of viral infection. All cases showed epithelial hyperplasia and koilocytes indicative of viral infection (Figure). Surface irregularity, hyper and parakeratosis, and individual cell keratinisation were seen less frequently (Table II). None of the five case biopsy specimens showed multinucleated cells characteristic of herpes simplex virus infection, nor was there any histological or immunocytochemical evidence of cytomegalovirus infection. Results of in situ hybridisation for EBV and human papillomavirus are shown in Table III. EBV was detected in three of four cases but in no control specimens, whereas human papillomavirus was detected in three of four cases and in two of three control specimens.

Discussion

Dysphagia is a frequent complaint in patients with AIDS. A recent study has found oesophageal candidiasis to be present in up to 54% of cases and discrete ulceration in 25%.¹³ Dysphagia may also be associated with cytomegalovirus and herpes simplex virus infections and with non-Hodgkin's lymphoma and Kaposi's sarcoma.^{14 15} Our five cases of atypical oesopha-

geal ulceration were found during the investigation of 46 patients with dysphagia, 18 of whom had discrete ulceration. The endoscopic features of these ulcers were different from those usually associated with cytomegalovirus or herpes simplex virus infections, in that they were deep, often linear, and found in the mid-oesophagus. Aphthous ulceration in the oesophagus has been described in association with HIV disease.¹⁶ These ulcers have a very similar macroscopic appearance to the EBV associated ulcers we describe, but apparently lack any microscopic features suggestive of a specific aetiology. Oesophageal ulcers occurring in association with electron microscopic evidence of HIV infection have previously been described by Rabeneck *et al.*¹⁷ But five of the eight patients they reported had clinical features of an HIV seroconversion illness and six showed a reversible lymphopenia, also suggestive of this condition. As mucosal ulceration has previously been described as a feature of HIV seroconversion illness, self limiting oesophageal ulceration may also occur as a feature of this phenomenon.¹⁸ In view of the widely diverse causes of oesophageal ulceration in HIV disease we would recommend that the

TABLE II Histological features of EBV associated oesophageal ulceration

Histological features	Case No							
	1		2		3		4	
	Bx1	Bx2	Bx1*	Bx2†	Bx1	Bx2	Bx1	Bx1
Epithelial hyperplasia	+	+	+	+	+	-	+	+
Hyper/parakeratosis	-	+	+	+	+	-	-	+
Individual cell keratin	-	+	+	-	-	-	+	+
Surface irregularity	-	+	-	-	+	-	-	-
Koilocytosis	+	+	+	+	+	+	+	+
Candida on surface	-	-	-	-	-	-	-	+

*Tonsillar fossa biopsy; †oesophageal biopsy.
Bx1 = 1st biopsy; Bx2 = 2nd biopsy.

TABLE III Results of DNA in situ hybridisation studies on biopsies of EBV associated ulcers in patients and control subjects

Case No	Human papillomavirus						
	EBV	2	6	11	16	18	31
1	+++	-	+	++	++	-	-
2	+	-	-	+	+	-	-
3	insufficient tissue for analysis						
4	+	-	-	-	-	-	-
5	-	-	-	-	+	+	+
Control							
1	-	-	-	-	-	-	-
2	-	-	-	-	-	+	-
3	-	-	-	-	-	+	-

EBV = Epstein-Barr virus.

macroscopic and microscopic features of the ulceration and the stage of disease are considered in the diagnosis.

The histological features seen in our patients included koilocytosis, epithelial thickening, individual cell keratinisation, and cell multinucleation. These are all features of viral epithelial infection and are the same as those previously described in oral hairy leukoplakia. In addition, using high stringency DNA in situ hybridisation techniques we showed EBV DNA in epithelial cells from the ulcerated areas but not from the control specimens. Our failure to detect EBV in one of the four cases may have been due to a sampling error as biopsy of the ulcers often caused severe discomfort. In contrast, human papillomavirus was detected both in areas of oesophageal ulceration and in control epithelium. The role of human papillomavirus DNA detection at this site is therefore unclear.

It has recently been suggested that the permissive cell type for EBV replication lies primarily within the stratified squamous epithelium, and that intact host T cell immunity is required to control viral replication at this site.⁴ The impaired cellular immunity seen in advanced HIV infection may allow EBV replication to proceed unchecked in infected oesophageal epithelial cells, with resulting cell lysis and ulceration. Local Langerhans cell depletion has previously been shown in association with oral hairy leukoplakia and remission of both oral and oesophageal lesions may be related to spontaneous improvement in Langerhans cell function or number.¹⁹

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