HHV-8 Subtypes in South Africa: Identifcation of a Case Suggesting a Novel B Variant

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Abstract

Human herpesvirus 8 (HHV-8) has been identified as the causative agent for all forms of Kaposi's sarcoma and is also associated with the development of body cavity-based B-cell lymphomas and multicentric Castleman's disease. HHV-8 genomes are now classified into five major subtypes (A-E) that reflect sequence heterogeneity in the highly variable open reading frame (ORF) K1. To identify HHV-8 subtypes associated with different forms of Kaposi's sarcoma, we compared the ORF 26 and ORF-K1 gene sequences from South African patients with the prototype strains of the major subtypes, as well as published sequences from other African strains. DNA prepared from Kaposi's sarcoma biopsies and/or peripheral blood lymphocytes were available from 14 patients with postrenal transplant (iatrogenic) Kaposi's sarcoma, six patients with the African endemic form, and one patient with AIDS-related body cavity-based B-cell lymphoma. We identified a B2 subtype in six patients, four of whom also had a novel B5 type ORF 26 polymorphism. Two patients had B2 type patterns for both the ORF 26 and ORF-K1 genes. The ORF-K1 subtype A5 was identified in samples from three patients with a B3/C2 type polymorphism in the ORF 26 gene. A novel ORF-K1 B variant strain was identified in a patient with African endemic Kaposi's sarcoma, who also had a B3/C2 class ORF 26 pattern. In 58.3% of iatrogenic Kaposi's sarcoma patients, a B5-type ORF 26 gene sequence pattern was identified. No association was found among particular subtypes, geographical origin of patients, or clinical presentation.

INTRODUCTION

Kaposi's sarcoma was first described in 1872. Four major clinical forms have since been described [Giraldo et al., 1989; Martin et al., 1993]: (1) epidemic or acquired immunodeficiency syndrome AIDS-associated; (2) classic; (3) African endemic; and (4) immunosuppression-associated (iatrogenic) Kaposi's sarcoma [Giraldo et al., 1989; Beral et al., 1990; Martin et al., 1993]. African endemic Kaposi's sarcoma clusters among Africans from East and Central Africa and accounts for 3-18% of all malignancies in these regions [Martin et al., 1993; Gompels and Kasolo, 1996; Lennette et al., 1996; Kasolo et al., 1997]. Since the start of the AIDS epidemic and the rapid spread of human immunodeficiency virus type 1 (HIV-1) in sub-Saharan Africa, differentiation between African endemic and epidemic Kaposi's sarcoma has become more difficult; presently the epidemic form is diagnosed predominantly in African patients [Matondo, 1995]. In South Africa, classic Kaposi's sarcoma is seen mostly in Jewish people of Eastern European (Ashkenazi) origin [Stein et al., 1994].

In 1994, human herpesvirus type 8 (HHV-8), originally called Kaposi's sarcoma-associated herpesvirus (KSHV), was identified as the etiologic agent for Kaposi's sarcoma [Chang et al., 1994; Moore et al., 1996]. HHV-8 is present in all Kaposi's sarcoma lesions and is also associated with the rare AIDS-related body cavity-based B-cell lymphoma, non-AIDS-

associated body cavity-based B-cell lymphomas, and multicentric Castleman's disease [Cesarman et al., 1995, 1996; Boshoff et al., 1995; Huang et al., 1995; Soulier et al., 1995]. HHV-8 was detected in 8% of infants<15 months of age in Zambia, where a linear increase in seroprevalence with age was noticed that reflects the endemic nature of HHV-8 infection (47% in 14-19-year-olds to 71% in persons aged≥50 years) [Kasolo et al., 1997; Olsen et al., 1998]. A high seroprevalence rate of 84-100% was also found in the non-Kaposi sarcoma endemic regions of West Africa (The Gambia and the Ivory Coast) [Lennette et al., 1996].

The diversity of HHV-8 genomes was based initially on sequence heterogeneity in the open reading frame (ORF) 26 and ORF 75 genes [Zong et al., 1997]. Only 1.5-2% sequence variations were seen in these areas, and viral strains were subsequently classified into three main groups: A, B, and C. A novel subtype (subtype N) based on the ORF 75 gene was recently identified in 20% of the circulating strains in the African Black population of South Africa [Alagiozoglou et al., 2000].

The ORF-K1 gene has since been identified as the area with the highest sequence variability [Nicholas et al., 1998]. So far four subtypes (A, B, C, and D) and at least 13 distinct variants were identified on the basis of sequence heterogeneity. More recently, it was found that a novel subtype (subtype E), closest to the D subtype, was circulating in an isolated population of the Brazilian Amerindians [Biggar et al., 2000]. Subtypes A, B, and C correspond to those of the initial classification based on the ORF 26 gene heterogeneity [Zong et al., 1999]. Subtype D was identified in samples from Taiwan and New Zealand, while individuals from sub-Saharan Africa are infected predominantly with subtype B genomes [Zong et al., 1999].

The ORF-K1 gene contains two variable regions: variable region 1 (VR1) and variable region 2 (VR2). The first B subtypes identified were from patients in the United States and had characteristic MAVKL-like sequences in the VR2, whereas B subtypes identified in samples from sub-Saharan Africa had characteristic TAFKT-like sequences. These variants were subsequently called B1 and B2 variants, respectively (G.S. Hayward, personal communication).

In order to identify the circulating strains of HHV-8 in South Africa, we analysed the ORF-K1 and 26 regions of the virus genome from patients with different clinical presentations and geographical origins.

MATERIALS AND METHODS

Patients and Samples

Formalinfixed, paraffin-embedded tissue blocks were available from 14 patients with postrenal transplant (iatrogenic) Kaposi's sarcoma seen at Tygerberg Hospital in the Western Cape [Moosa et al., 1998]. Fresh-frozen tissue samples and peripheral blood lymphocytes collected after remission of Kaposi's sarcoma lesions were also available from these patients. The presence of HHV-8 in these patients' samples was confirmed in a previous study [Moosa et al., 1998]. Fresh-frozen Kaposi's sarcoma biopsy samples were also available from six individuals with African endemic Kaposi's sarcoma seen at the Kalafong and Themba Hospitals in Gauteng and Mpumalanga Provinces, respectively. The mean age of the patients with African endemic Kaposi's sarcoma at diagnosis was 64.5 years (Table I). Peripheral blood lymphocyte and body cavity-based B-cell lymphoma samples were also available from a patient with AIDS seen at Tygerberg Hospital.

TABLE I. Demographic and Clinical Data of South African Patients With HHV-8-Associated Diseases

Patient	Age (yr)	Sex	Race	Clinical disease	Geographic origin
TBF1	27	Male	Mixed ancestral origin	IKS	Western cape
TBF2	44	Female	Mixed ancestral origin	IKS	Western cape
TBF3	50	Male	African Black	IKS	Western cape
TBF4	48	Female	Mixed ancestral origin	IKS	Western cape
TBF5	56	Male	African Black	IKS	Western cape
TBF6	50	Female	African Black	IKS	Western cape
TBF8	36	Female	Mixed ancestral origin	IKS	Western cape
TBF10	41	Male	Mixed ancestral origin	IKS	Western cape
TBF11	30	Male	Mixed ancestral origin	IKS	Western cape
TBF12	39	Male	Mixed ancestral origin	IKS	Western cape
TBF13	43	Female	Caucasian	IKS	Western cape
TBF14	40	Female	Mixed ancestral origin	IKS	Western cape
TBB1	52	Male	African Black	AIDS-BCBL	Western cape
MP1	57	Male	African Black	AKS	Mpumalanga
MP3	76	Male	African Black	AKS	Mpumalanga
MP4	81	Male	African Black	AKS	Mpumalanga
MP5	67	Male	African Black	AKS	Mpumalanga
MP6	79	Female	African Black	AKS	Mpumalanga
MP10	27	Male	African Black	AKS	Gauteng

TBF, Tygerberg Hospital renal transplant patients; TBF1–TBF14 correspond to patients 1–14 in Moosa et al. [1998]; TBB1, Tygerberg Hospital body cavity-based B-cell lymphoma patient; MP, African endemic KS (AKS) cases from Themba and Kalafong Hospitals; IKS, iatrogenic KS; AIDS-BCBL, AIDS-associated body cavity-based B-cell lymphoma.

DNA Preparation

DNA was prepared from paraffin-embedded tissue sections as described previously [van Rensburg et al., 1996, Moosa et al., 1998]. The QIAamp tissue and QIAamp blood kits (Qiagen GmbH, Germany) were used to isolate DNA from fresh skin biopsies, peripheral blood lymphocytes, and body cavity-based B-cell lymphoma cells recovered from pleural fluid.

Polymerase Chain Reaction

Patient DNA samples were screened for HHV-8, using a nested polymerase chain reaction (PCR) method to amplify a 172-bp fragment of ORF 26 as described previously [Engelbrecht et al., 1997]. The following primers described previously [Kasolo et al., 1998; Cook et al., 1999], were used in various combinations to amplify the highly variable ORF K1 region from the different patient samples: the K1N pair, K1EX pair, K1/408/1, PMC20, and PMC21. The primer R408 (5'-GTA TTT AGT TTG TGA CAC GG) is the reverse complement of the K1/408/1 primer described previously [Cook et al., 1999]. The oligonucleotide sequences for three additional primers used [K1M1 (5'-GTG TGG AAC AAT CTG GG), OUGO1 (5'-CCT GTA CAA TCA AGA TGT TCC), and OUGO2 (5'-GTC AGT ACC AATC CACT G)] were kindly provided by T.F. Schulz, Department of Medical Microbiology, University of Liverpool.

Gene fragments containing the VR1 region were amplified by hemi- and nested PCR with the following primer sets: K1N forward/OUGO2 (outer pair) and K1N forward/R408 (inner pair); K1N forward/OUGO2 (outer pair) and K1N forward/K1EX reverse (inner pair); K1N forward/K1EX reverse (outer pair) and K1EX pair (inner pair); and OUGO1/OUGO2 (outer pair) and K1EX pair (inner pair). The following primer combinations were used to amplify gene fragments containing the VR2 region: K1M1/OUGO2 (outer pair) and K1/408/1/OUGO2 (inner pair); OUGO1/OUGO2 (outer pair) and K1/408/1/OUGO2 (inner pair); and PMC20/PMC21 and K1/408/1/PMC21 (inner pair) or K1/408/1/OUGO2 (inner pair). A single gene fragment containing both the VR1 and VR2 regions was amplified with the following primer sets: K1N forward/OUGO2 (outer pair) and OUGO1/OUGO2 (inner pair); OUGO1/OUGO2 (outer pair) and K1M1/OUGO2 (inner pair) or K1EX forward/OUGO2 (inner pair); and PMC20/PMC21 (outer pair) and OUGO1/OUGO2 (inner pair). The Expand High Fidelity enzyme system

(Roche Diagnostics GmbH, Germany) was used for all amplifications with 2.5-mM $MgCl_2$ concentration and an annealing temperature of 45°C.

DNA Sequencing and Phylogenetic Analysis

PCR products or product bands purified from agarose gel by the QIAEXII method (Qiagen) were directly sequenced using the ABI Prism Dye Terminator Cycle sequencing kit (Perkin Elmer, CA). The nested primers were used as sequencing primers. Sequence data were captured and processed with the DNAMAN (Lynnon BioSoft, Quebec, Canada) software program. Nucleotide sequences were aligned with the CLUSTAL X software program [Thompson et al., 1997]. Kimura-2- parameter distance calculation, neighbour-joining tree construction, and bootstrap analysis were done with TREECON V1.36 software program [Van de Peer and De Wachter, 1994, 1997]. The sequences were deposited in the GenBank database under accession numbers AF387367-AF387378.

RESULTS

Classification of ORF 26 Polymorphisms Subgroups identified by distinct ORF 26 polymorphisms patterns are summarised in Table II. The ORF 26 gene could be sequenced in 12 of 14 iatrogenic Kaposi's sarcoma samples, and the polymorphisms seen were briefly reported previously [Moosa et al., 1998]. Only one patient of Caucasian origin, TBF13, had an ORF 26 prototype A pattern. Patients TBF6, MP4, MP6, and MP10 had ORF 26 group B3/C2 patterns. Viral strains from eight patients' samples showed a distinct B5 subgroup pattern. The B5 subgroup had a characteristic G!T change at position 1055 in addition to A!G and A!C changes at positions 1132 and 1139, respectively. Seven of 12 patients (58.3%) with iatrogenic Kaposi's sarcoma and only one of six (16.7%) patients with African endemic Kaposi's sarcoma had ORF 26 type B5 patterns. TBF11 demonstrated a B1 pattern, while TBF4, TBB1, MP3,and MP5 had B2-type patterns. Patient TBF3 had an unclassified variant B pattern. This patient showed the same characteristic pattern of polymorphisms as the B5 subgroup but had an additional C→T change (silent mutation) at position 1077.

TABLE II. Analysis of Sequence Polymorphisms in the ORF 26 Gene of HHV-8

	ORF 26 polymorphisms									
Patient	1032	1055	1077	1086	1094	1103	1122	1132	1139	Class[b]
BCBL-R[a]	C	G	C	C	G	C	G	A	A	A
TBF13	—	—	—	—	—	—	—	—	—	A
MP4	—	—	—	T	—	—	—	—	C	B3/C2
MP6	—	—	—	T	—	—	—	—	C	B3/C2
TBF6	—	—	—	T	—	—	—	—	C	B3/C2
MP10	—	—	—	T	—	—	—	—	C	B3/C2
MP1	—	T	—	—	—	—	—	G	C	B5
TBF1	—	T	—	—	—	—	—	G	C	B5
TBF2	—	T	—	—	—	—	—	G	C	B5
TBF5	—	T	—	—	—	—	—	G	C	B5
TBF8	—	T	—	—	—	—	—	G	C	B5
TBF10	—	T	—	—	—	—	—	G	C	B5
TBF12	—	T	—	—	—	—	—	G	C	B5
TBF14	—	T	—	—	—	—	—	G	C	B5
TBF3	—	T	T	—	—	—	—	G	C	?
TBF11	—	—	—	—	—	—	—	G	C	B1
TBF4	A	—	—	—	—	—	—	G	C	B2
TBB1	A	—	—	—	—	—	—	G	C	B2
MP3	A	—	—	—	—	—	—	G	C	B2
MP5	A	—	—	—	—	—	—	G	C	B2

[a]Reference sequence BCBL-R were described by Zong et al. [1997].
[b]Class refers to the ORF 26 classification subgroup.

ORF-K1 Subgroups

The ORF-K1 VR1 and VR2 amino acid sequence data from this study as well as the prototype and previously identified African strains are summarised in Figure 1. HHV-8 genomes from

patients MP4, MP6, and TBF6 belong the ORF-K1 subtype A major subgroup with amino acid patterns characteristic of the A5 variant group. In the phylogenetic tree (Fig. 2), South African A5 variants cluster with those identified in patients from Northern Italy (IFe1, IFe5) and Uganda (Ug374). A novel genome type was identified in the viral strain from patient MP10. This strain's VR1 region of the ORF-K1 protein was almost identical to that of the B1 prototype strain except for one amino acid change near both ends. In the VR2 region, it had the characteristic MAVKL-like sequence at the beginning of VR2 and was essentially homologous to the B1 prototype strain, except for an 11-amino acid deletion from position 201- 211, which suggests its classification as a novel B variant (Fig. 1).

Three patients with post-transplant Kaposi's sarcoma (TBF2, TBF5, TBF10), one with AIDS-associated body cavity-based B-cell lymphoma (TBB1), and two patients with African endemic Kaposi's sarcoma (MP1 and MP5), had the characteristic TAFKT-like sequence characteristic of the B2 variant type genomes at the beginning of VR2 (Fig. 1). In the phylogenetic tree analysis, the two South African strains (MP5 and TBB1) are similar and cluster together with Ugandan (Ug81 and Ugd1) strains (Fig. 2). The HHV-8 B2 genomes from TBF5, TBF10, and MP1 form a separate cluster from the other B2 type genomes (bootstrap value of 100%). These B2 type genomes (TBF5, TBF10, MP1) all have a B5 type ORF 26 polymorphism. Only the VR2 region excluding the last five amino acids could be amplified and sequenced from patient TBF2, who also had a B5-type ORF 26 polymorphism.

ORF-K1 PROTEIN'S VARIABLE REGIONS

Sample	VR1 (52-92)	VR2 (191-231)	K1	ORF26
A1	CNNTRLFRP TETTLFPVTI ACNFTCVEQSGHRQSIWITW HA	TGFRTFSTNS LVNIIHATTH DVVVVKEAKS THFHIELHFLV	A1	
A4	CNDTRLFRL TERTLFPVTI PCNFTCVEQSGHRQSIWITW HA	TGFRTFSTNS LVNIIH---- DVVVVKEAKS THFHIELHFLV	A4	
IFe1	CNDTRLLRL TDQSFTVDTI TCNFTCVEQS GHRQSIWITW NA	TGFRTVSTNS LVNIIHATNH DVVVVKEAKS TNPHIEVPFLV	A5	
IFe5	CNDTRLWRL TDQSFTVATI TCNFTCVEQS GHRQSIWITW NA	TGFRTFSTNT LVNIIHATTH HVVVVKEAKS TNPHIEVPFLV	A5	
Ug374	CNDTRLWRL TDQSFTVATI TCNFTCVEQS GHRQSIWITW NA	TGFRTFSTNS LVNIIHATTH DVVVVKEAKS TNPHIEVPFLV	A5	
MP4	**CNNTRLWRL TKQLFTVAGI TCNFTCVEQS GHRQSIWITW NA**	**TGFRTFSTNS LVNIIHATTH DVVVVKEAKS TNPHIEVPFLV**	**A5**	**B3/C2**
MP6	**CNDTRLWRL TNQSFTVANI TCNFTCVEQS GHRQSIWITW NA**	**TGFRTFSTNS LVNIIHATTH DVVVVKEAKS TNPHIEVPFLV**	**A5**	**B3/C2**
TBF6	**CNDTRLWRL TSQSFTVATI TCNFTCVEQS GHRQSIWITW NA**	**TGFTTFSTNS LVNIIHATTH DVVVVKEAKS TNPHIEVPFLV**	**A5**	**B3/C2**
B1	CNGTQLHRI TASNLTVSSL TCNFTCMTTS GPTHSIWIQW YT	MAVKLLRTNG LLKIIPATTH AAVAVEEVKS TNTHIQVPFLV	B1	
MP10	**CNGTLLHRI TASNLTVSSL TCNFTCMTTS GPTHSIWIEW YT**	**MAVKVVRTNG L---------- --VAVEEVKS TNTHIQVPFLV**	**NB**	**B3/C2**
UgD1	CNGTQLRRI RGSNLTVSLL TCNFTCMTAS GPTHSIWIEW YT	TAFKMSRTNG LLKIIPATTH AAVAVEEVKS TNPHIQVPFLV	B2	
Ug81	CNGTRLHRI TTSNLTVSLL TCNFTCMTTS GPTHSIWIEW YT	TTFKMSRTNG LLKIIPATTH AAVAVEEVKS TNPHIQVPFLV	B2	
G51	CNGTRLRRI TASNLTVSGL TCNFTCMTTS GPTHSIWIEW YT	TAFKTLRTNG LLKIIPATTH AAVAVEEVKS TNPHIQVPFLV	B2	
G71	CNGTRLWRI TASNLTVSSL SCNFTCMTTS GPTHSIWIEW YT	TAFKKLRNNG LLKIIPATTH AAVAVEEVKS TNPHIQVPFLV	B2	
G413	CNGTRLLRI TASNPTVCSL TCNFTCMTAS GPTHSIWIEW YT	TAFETLRTNG LLKIIPATTH AAVAVEEVKS TNPHIQVPFLV	B2	
MP1		**TAFKTLTTNG LLKIIPATTH AAVAVEEVKS TNPHIQVPFLV**	**B2**	**B5**
MP5	**CNGTQLWRI RESTLTVSSL TCNFTCMTAS GPTHSIWIEW YT**	**TAFKISRTNG LLKIIPATTH AAVAVEEVKS TNPHIQVPFLV**	**B2**	**B2**
TBB1	**CNGTQLWRI RESTLTVSSL TCNFTCMTAS GPTHSIWIEW YT**	**TAFKISRTNG LLKIIPATTH AAVAVEEVKS TNPHIQVPFLV**	**B2**	**B2**
TBF2		**TAFKTLTTNG LLKIIPATTH AAVAVEEVKF TNSQIQ**	**B2**	**B5**
TBF5		**TAFKTLTTNG LLKIIPATTH AAVAVEEVKS TNPHIQVPFLV**	**B2**	**B5**
TBF10		**TAFKTLRTNG LLKIIPATTH AAVAVEEVKS TNPHIQVPFLV**	**B2**	**B5**
C1	CNDTRLFRL THDTFTVVNF ICNFSCVGQS GHRHSLWMTW YG	TGFRTFSTNS -----HATTH DVV--KEAKF TNPHIEVPFLV	C1	
C3	CNDTRLLRL TQQTFTVVAL ICNFSCVGQS GHRHSLWITW YP	TGFRTFSTNS -----AATTH DVLVMKEAKS TNLHIQVHFIV	C3	
D1	CNGTRLLRI TGATLTIPSL TGNFTCVDHS GLSHSIWIQR YP	TGFTTFSTNR LVNIIPATTH AVVVVEKVKS LHPHIEVPFLV	D1	

Fig. 1. Comparison of the ORF-K1 VR1 and VR2 region amino acid sequences from South African samples (in bold) with the consensus sequences for the various groups and subgroups. The VR1 region from MP1, TBF2, TBF5, and TBF10 could not be amplified. Sequences of the reference strains were obtained from Genbank database: A1 (AF133038), A4 (AF133039), B1 (AF133040), C1 (AF133041), C3 (AF133042), D1 (AF133043) [Zong et al., 1999]; Ug81 (AF130291), Ugd1 (AF130292), Ug374 (AF130289), IFe1 (AF130282), IFe5 (AF130284), G51 (AF130264), G71 (AF130265), G413 (AF130262) [Cook et al., 1999]. Dashes indicate amino acid deletions. NB, novel B variant.

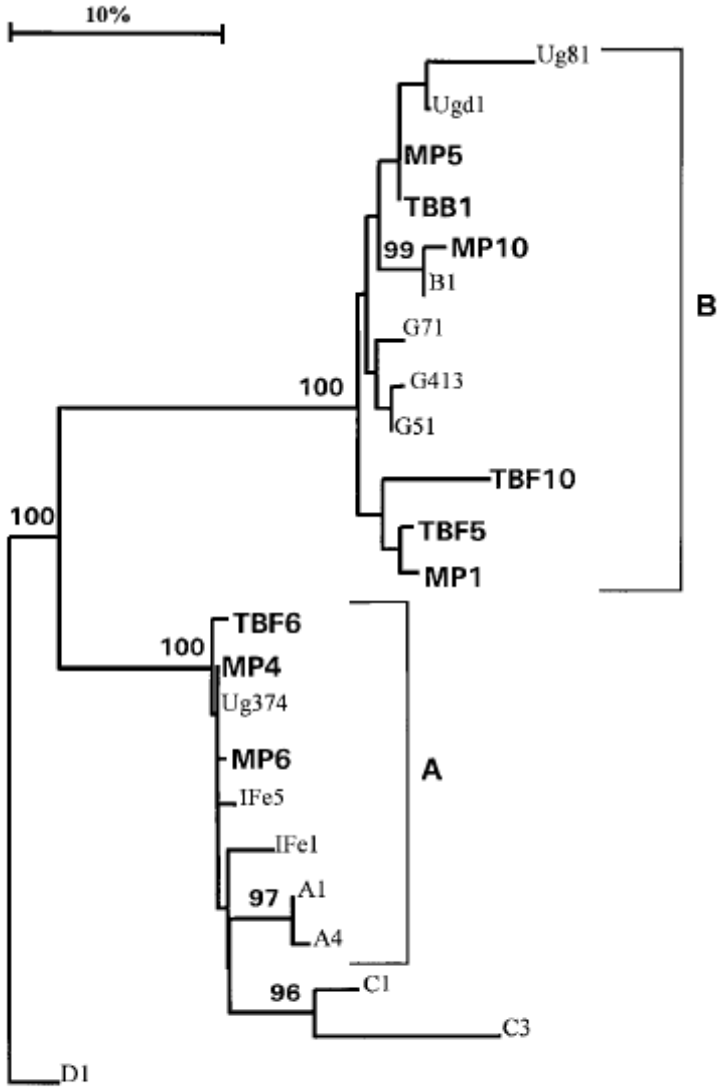

Fig. 2. Rooted phylogenetic tree analysis of partial ORF-K1 nucleotide sequences that include the VR2 region and transmembrane domain of HHV-8 genomes from South African patients. The neighbour-joining method for tree construction is based on 395-bp sequences (insertions and deletions were not taken into account). Bootstrap values of > 90% are indicated at the branch nodes considered. The bar scale calculating sequence dissimilarity on the horizontal axis is shown. TBF2 was not included for phylogenetic analysis, as it did not meet the required sequence.

DISCUSSION

This is the first study to report on the genetic diversity of HHV-8 genomes originating from different geographic areas and clinical forms of Kaposi's sarcoma in South Africa. According to the ORF-K1 classification, six patients were subtype B and three were subtype A. This is the first report of a novel B variant with an inframe deletion in VR2. Although the HHV-8 genome identified in patient MP10 showed homology to the B1 type genomes, the 33-bp deletion suggests its classification as a novel B variant. Inframe deletions have so far been described in the ORF-K1 subtype A4 variant and in all C subtype genomes [Cook et al., 1999; Zong et al., 1999]. B subtype genomes identified previously in African-Americans and individuals from African countries like Zaire, Tanzania, Uganda, and Zambia primarily belong to the B1 variant group [Poole et al., 1999; Zong et al., 1999]. It was however found that some genomes identified from Uganda and The Gambia belong to the B2 variant group [Cook et al., 1999;

Zong et al., 1999]. These data showed that most B subtypes found in the South African patients were B2 variant strains, and no B1 variants were identified.

HHV-8 subtype A genomes with the distinct A5 amino acid pattern in the K1 protein differ from other A variants by 6-8% [Cook et al., 1999; Zong et al., 1999]. A5 type genomes were previously found in African patients [Cook et al., 1999, Zong et al., 1999], and in two patients from Northern Italy [Cook et al., 1999]. The HHV-8 genomes identified in HIV-negative and -positive children from Zambia with Kaposi's sarcoma or febrile illnesses were also A5 variants [Kasolo et al., 1998, Poole et al., 1999]. Three of the South African patients belonged to this variant group; two of them cluster with HHV-8 genomes identified in patient samples from Northern Italy and Uganda [Cook et al., 1999]. A5 type ORF-K1 gene sequences were also previously documented in HHV-8 genomes from two South African patients [Poole et al., 1999].

We also found a novel B5 polymorphism in the ORF 26 gene sequences from 58.3% of our renal transplant patients. Three of these renal transplant patients, as well as two patients with African endemic Kaposi's sarcoma, had B2 type ORF-K1 genes. Distinct clustering of ORF 26 type B3/C2 polymorphisms were found in the HHV-8 genomes from Saudi Arabian renal transplant patients [Foreman et al., 1998], and they also belonged to the ORF-K1 subtype C [Poole et al., 1999]. In contrast, we identified ORF 26 type B3/C2 patterns in one patient with post-transplant Kaposi's sarcoma and two patients with the African endemic form who had A5 type ORF-K1 genes. A B3/C2 type ORF 26 gene polymorphism was also seen in the patient (MP10) with African endemic Kaposi's sarcoma who had the novel B type ORF-K1 gene. A similar B3/C2 ORF 26 and A5 ORF-K1 pattern was previously described in two African patients [Poole et al., 1999]. The ambiguity observed for the B3 and C2 type (B3/C2) ORF 26 pattern show that HHV-8 classification based on ORF 26 alone may be insufficient [Poole et al., 1999]. Linkage analysis studies involving the ORF 75, T0.7/ K12, K14.1, K15, and ORF-K1 genes of HHV-8 revealed that chimerism do exist in HHV-8 genomes from various samples [Poole et al., 1999]. Poole and colleagues suggested that the B3/C2 type pattern might represent either true B variants or mosaic genomes.

In this study, neither K1 nor ORF 26 subtypes had any characteristic geographical or clinical associations. The presence of the B3/C2 type ORF 26 pattern in our patients belonging to the A5 K1-subtype suggests the presence of chimeric HHV-8 genomes. Studies involving larger numbers of Kaposi's sarcoma patients need to be conducted to determine the incidence and significance of the novel B variant and A5 type infections in South Africa, as well as to determine the extent of chimerism in A5 type genomes.

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