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Identification, Expression, and Immunogenicity of Kaposi's Sarcoma-Associated Herpesvirus-Encoded Small Viral Capsid Antigen

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We describe a recombinant antigen for use in serologic tests for antibodies to Kaposi's sarcoma (KS)-associated herpesvirus (KSHV). The cDNA for a small viral capsid antigen (sVCA) was identified by immunoscreening of a library prepared from the BC-1 body cavity lymphoma cell line induced into KSHV lytic gene expression by sodium butyrate. The cDNA specified a 170-amino-acid peptide with homology to small viral capsid proteins encoded by the BFRF3 gene of Epstein-Barr virus and the ORF65 gene of herpesvirus saimiri. KSHV sVCA was expressed from a 0.85-kb mRNA present late in lytic KSHV replication in BC-1 cells. This transcript was sensitive to phosphonoacetic acid and phosphonoformic acid, inhibitors of herpesvirus DNA replication. KSHV sVCA expressed in mammalian cells or *Escherichia coli* or translated in vitro was recognized as an antigen by antisera from KS patients. Rabbit antisera raised to KSHV sVCA expressed in *E. coli* detected a 22-kDa protein in KSHV-infected human B cells. Overexpressed KSHV sVCA purified from *E. coli* and used as an antigen in immunoblot screening assay did not cross-react with EBV BFRF3. Antibodies to sVCA were present in 89% of 47 human immunodeficiency virus (HIV)-positive patients with KS, in 20% of 54 HIV-positive patients without KS, but in none of 122 other patients including children born to HIV-seropositive mothers and patients with hemophilia, autoimmune disease, or nasopharyngeal carcinoma. Low-titer antibody was detected in three sera from 28 healthy subjects. Antibodies to recombinant sVCA correlate with KS in high-risk populations. Recombinant sVCA can be used to examine the seroepidemiology of infection with KSHV in the general population.

The several distinct clinical and epidemiological variants of Kaposi's sarcoma (KS) may differ in their modes of transmission and pathogenesis. Among homosexual men with human immunodeficiency virus type 1 (HIV-1) infection, the tumor seems to result from sexual transmission of an infectious agent (4). The high frequency of KS among adults of both sexes in Africa is consistent with heterosexual transmission of an infectious agent; however, since KS also occurs in African children, there are likely to be other routes of person-to-person transmission (42). The occurrence of classical KS in elderly men of Eastern Europe or Mediterranean origin is not consistent with the hypothesis that development of KS is associated with primary infection with the etiologic agent; rather, reactivation of a latent virus is more likely. The development of KS following renal transplantation could be explained by opportunistic de novo infection or by reactivation of a latent agent.

Sequences of the newly discovered gammaherpesvirus KS-associated herpesvirus (KSHV) are detectable in KS tissue from all variant forms of the disease (10, 17, 31, 35, 42). Moreover, the KSHV sequences are found in tissue from patients with body cavity lymphoma and those with multicentric Castleman's disease (6, 34). These sequences are part of a herpesvirus genome that, at least in certain body cavity lymphoma cell lines, can give rise to virions (22, 23, 30). KSHV genomes have also been found in non-KS skin lesions from renal transplant recipients and in semen from healthy individuals (20, 25, 29).

The discovery of KSHV raises several questions concerning the association of the virus with KS and the distribution of the virus in human population. (i) Is the association specific for KS? (ii) Does the association represent de novo infection or reactivation of a silent latent infection? (iii) Is KSHV infection ubiquitous in the human population or distributed only within selected high risk populations?

The development of serological tests to measure antibodies to KSHV should help to clarify the significance of the association of the virus with Kaposi's sarcoma and body cavity lymphoma. Seroepidemiological tests may also help to determine whether the infection is primary or reactivated. Several different serological tests with KSHV-infected body cavity lymphoma cells measure antibodies to lytic- or latent-cycle antigens of the virus. One test detects antibodies to a lytic-cycle polypeptide, p40, induced by butyrate (24). Two other tests measure antibodies to latent nuclear antigens detected by immunofluorescence and immunoblotting (14, 18). The general conclusions from these three seroepidemiological analyses are similar. There is high concordance between the presence of antibodies to KSHV antigens and the presence of KS (13, 14, 18, 24). Antibodies are found less frequently among high-risk populations, for example, HIV-infected patients without KS, than among patients with KS. Antibodies are generally lacking among people who are healthy or at low risk for KS, such as HIV-infected hemophiliacs (13, 14, 18). However, a recent fourth study, measuring antibody to phorbol-induced lytic-cy-

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cle antigens detectable by indirect immunofluorescence, has found antibody in about 25% of the U.S. general population (19).

Until now, all serological tests have detected antibodies to antigens present in KSHV-infected cells. Here we describe the cloning and expression of a small viral capsid antigen (sVCA) of KSHV. Using recombinant sVCA in serological tests, we found that antibodies to sVCA were present in nearly all patients with KS but less common in other population groups including HIV-infected patients without KS.

MATERIALS AND METHODS

Cell culture. BC-1 cells (7) were grown at 37°C in RPMI 1640 supplemented with 15% fetal bovine serum in the presence of 5% CO_2. HH514-16 cells, derived from the African Burkitt's lymphoma line, P3J-HR-1, were grown at 37°C in RPMI 1640 supplemented with 8% fetal bovine serum (28). Both Epstein-Barr virus (EBV) and KSHV genomes are present in BC-1 cells, while only EBV DNA is present in HH514-16 cells. To induce lytic-cycle gene expression, cells were harvested after exposure to 3 mM sodium butyrate and/or 20 ng of TPA (phorbol 12-myristate 13-acetate) per ml. COS-7 and 293T cells were used for mammalian cell expression (16, 27).

cDNA library construction and cloning. Total cellular RNA was extracted by standard procedures (2) from BC-1 cells which had been treated with sodium butyrate for 48 h. The poly(A)$^+$ RNA was selected on an oligo(dT) column. cDNAs were synthesized with a commercial kit (ZAP cDNA synthesis kit; Stratagene). The library was cloned in the LambdaZAPII vector (Stratagene) as specified by the manufacturer. Isolated clones were plaque purified before in vivo excision.

Genomic library. Total genomic DNA prepared from BC-1 cells by standard procedures (2) was partially digested with *Sau*3A so that the DNA fragments were 20 to 40 kb in size. DNA was then ligated to a cosmid vector, Supercos-1 (Stratagene), or to a lambda phage vector, λFix (Stratagene), and packaged with Gigapack LX (Stratagene).

DNA sequence analysis. The sequences of cDNA clone CA20 and its gene were determined in both directions via primer walking. DNA sequence data were compiled and analyzed by using GELASSEMBLE, TESTCODE, BLAST, and FRAMES of Wisconsin sequence analysis package GCG, version 8 (Genetics Computer Group, Madison, Wis.).

RNA preparation and Northern blot analysis. Each sample contained total RNA prepared from 2×10^6 cells; the RNA was fractionated on 1% formaldehyde–agarose gels and transferred to a nylon membrane (Nytran; Schleicher & Schuell) by standard procedures (2). The DNA probe for Northern blotting was a 620-bp PCR product of CA20, amplified by primers 17809 (5′-tggaccatggccaa ctttaaggtgagagacc-3′) and 17810 (5′-ggaattcaacaaaaagtggccgccctatcgg-3′). Hybridization was carried out in 50% formaldehyde–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt's solution–1% sodium dodecyl sulfate (SDS)–100 μg of salmon sperm DNA per ml at 45°C overnight. The filters were washed with 3× SSC–0.1% SDS twice for 15 min each and then with 0.1× SSC–0.1% SDS once for 30 min at 68°C. For quantitation of RNA loading, blots were stripped and reprobed with genes for RNase P H1 RNA (3). The band intensity was quantitated with a PhosphorImager (Molecular Dynamics).

Expression of KSHV sVCA in mammalian cells. A 10-μg portion of each plasmid DNA was transfected into 75% confluent COS-7 or 293T cells by the calcium phosphate precipitation method for 5 h followed by a 10% glycerol shock for 2 min at the end of transfection (2). At 3 days after transfection, total cellular proteins were prepared for Western blotting.

In vitro translation and immunoprecipitation. A commercial in vitro transcription and translation system, TNT Coupled Reticulocyte Lysate System (Promega, Madison, Wis.), was used to generate the in vitro translation product of KSHV sVCA. A 1-μg portion of plasmid DNA containing the CA20 cDNA downstream of a T3 promoter in pBK-CMV (Stratagene) was added to 50 μl of TNT reaction mixture in the presence of [^{35}S]methionine at 800 μCi/ml. The reaction mixtures were incubated at 30°C for 90 min. Translation products were analyzed on by SDS-polyacrylamide gel electrophoresis (PAGE) (12% acrylamide) and visualized by autoradiography or used directly in immunoprecipitation. For immunoprecipitation, 2 μl of TNT product, 5 μl of serum, and 1.5 mg of protein A-Sepharose CL-4B (Pharmacia) were added to 400 μl of radioimmunoprecipitation/bovine serum albumin buffer (10 mM Tris-HCl [pH8.0], 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 100 mM NaCl, 1% bovine serum albumin) and incubated at 4°C for 2 h. Protein A-Sepharose beads were washed thoroughly three times in 1× RIP buffer and once in 10 mM Tris-HCl (pH 6.8). The immunoprecipitation complex was resolved on by SDS-PAGE (12% polyacrylamide) and detected by autoradiography.

Expression of KSHV sVCA in *E. coil* and partial purification. The open reading frame (ORF) of KSHV sVCA was amplified by PCR from CA20 with primers 17809 (5′-tggaccatggccaactttaaggtgagagacc-3′) and 17810 (5′-ggaattcaa caaaaagtggccgccctatcgg-3′). The resulting PCR product was subcloned into the pET30b vector (Novagen) with *Nco*I and *Eco*RI sites (underlined), yielding the recombinant plasmid pET30b-sVCA. *E. coli* BL21(DE3)pLysS harboring pET30b-sVCA in logarithmic-phase growth was induced by treatment with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 2 h at 37°C. A crude extract of the induced cells was prepared by 1:20 concentration of the cell pellet in 50 mM Tris-HCl (pH 8.0). KSHV sVCA was purified by chromatography of crude extracts onto two consecutive affinity columns, a Nickel column and S · Tag agarose (Novagen). The elution buffer used in the Nickel column was a gradient of 0.1 to 1.0 M imidazole in 50 mM NaCl–20 mM Tris-HCl (pH 8.0). The elution buffer used for S · Tag agarose was 0.5% SDS in 20 mM Tris-HCl (pH 7.5)–0.15 M NaCl–0.1% Triton X-100.

Immunization of rabbits. Nickel column eluents containing the sVCA protein were electrophoresed on a preparative SDS–10% polyacrylamide gel. The gel was stained with Coomassie brilliant blue, and strips containing the sVCA were ground and emulsified in complete Freund adjuvent (0.5 ml per rabbit). Rabbits were immunized by subcutaneous injection and boosted every 2 weeks with an equivalent amount of protein emulsified in incomplete Freund adjuvent (0.5 ml per rabbit). Serum was collected 1 to 2 weeks following each boost.

Western immunoblot analysis. After SDS-PAGE, proteins were transferred to nitrocellulose filters (Schleicher & Schuell) by standard procedures (2). The filters were incubated with blocking buffer (5% nonfat dried milk, 10 mM Tris-HCl [pH 7.4], 30 mM NaCl) for 1 h at room temperature. A 1:150 dilution of serum was added for 2 h at room temperature or overnight at 4°C. The filters were subsequently washed in Tween 20-Tris buffer (0.2% Tween 20, 0.15 M NaCl, 10 mM Tris-HCl [pH 7.0]) three times for 10 min each. After the washing step, ^{125}I-labeled protein A was added at a 1:2,000 dilution to trace the bands by autoradiography. A cassette miniblot system (Immunetics) was used to screen 25 sera at one time. A 1.5-μl volume of serum diluted 1:33 in blocking buffer was used in each channel of the miniblot apparatus.

Sera. Sera were collected from HIV-infected patients in Connecticut, New York, and California (24). Other sera from patients with hemophilia, autoimmune disease, febrile illness, or nasopharyngeal carcinoma, children born to HIV-seropositive mothers, or healthy subjects were obtained from serum collections in our laboratory.

Nucleotide sequences accession number. The cDNA sequence reported in this study has been deposited into GenBank under accession number U50141.

RESULTS

Molecular cloning of KSHV sVCA. Sera from KS patients recognize KSHV-associated polypeptides in butyrate-treated BC-1 cells. These polypeptides were assumed to represent the lytic-cycle gene products of KSHV (23, 24). To clone lytic-cycle antigens, about 10^6 plaques of a cDNA library constructed from BC-1 cells induced by sodium butyrate for 48 h were immunoscreened with KS patient serum 0103. Positive phage clones were identified and subjected to in vivo excision. A partial nucleotide sequence of the insert in each plasmid was determined from both ends; homologs were sought in the database. Among 54 clones analyzed, 11 showed significant homology to gammaherpesvirus ORF which encode sVCA components, namely, EBV BFRF3 and herpesvirus saimiri (HVS) ORF65 (1, 40). After alignment of these 11 clones, clone CA20 was chosen for further study since it was likely to represent a full-length cDNA. The genomic counterpart of CA20 was obtained from cosmids which contained other KSHV sequences including KS631Bam (8). DNA sequences of this 716-bp cDNA as well as its gene are summarized in Fig. 1A. The putative TATA box (TATTAAA) was located 50 bp upstream of the start of CA20 cDNA; the polyadenylation signal (AATAAA) was located 22 bp upstream of the polyadenylation site. The ORF of CA20 encodes a 170-amino-acid (aa) polypeptide, which was designated KSHV sVCA (Fig. 1A). Amino acid sequences of KSHV sVCA shared 48% similarity and 27% identity with EBV BFRF3 and 60% similarity and 40% identity with HVS ORF65, suggesting that these three ORFs may possess related biological functions (Fig. 1B).

Detection of KSHV sVCA transcripts in BC-1 cells. To study the kinetics of expression of KSHV sVCA, a PCR product representing the coding region of CA20 was used as a probe to detect mRNAs prepared from chemically treated BC-1. As shown in Fig. 2A, expression of KSHV sVCA was detected after chemical treatment of KSHV-positive cells (lanes 5 to 7 and 13 to 15) but not of KSHV-negative EBV-positive HH514-16 cells (lanes 2 and 10). The size of the major tran-

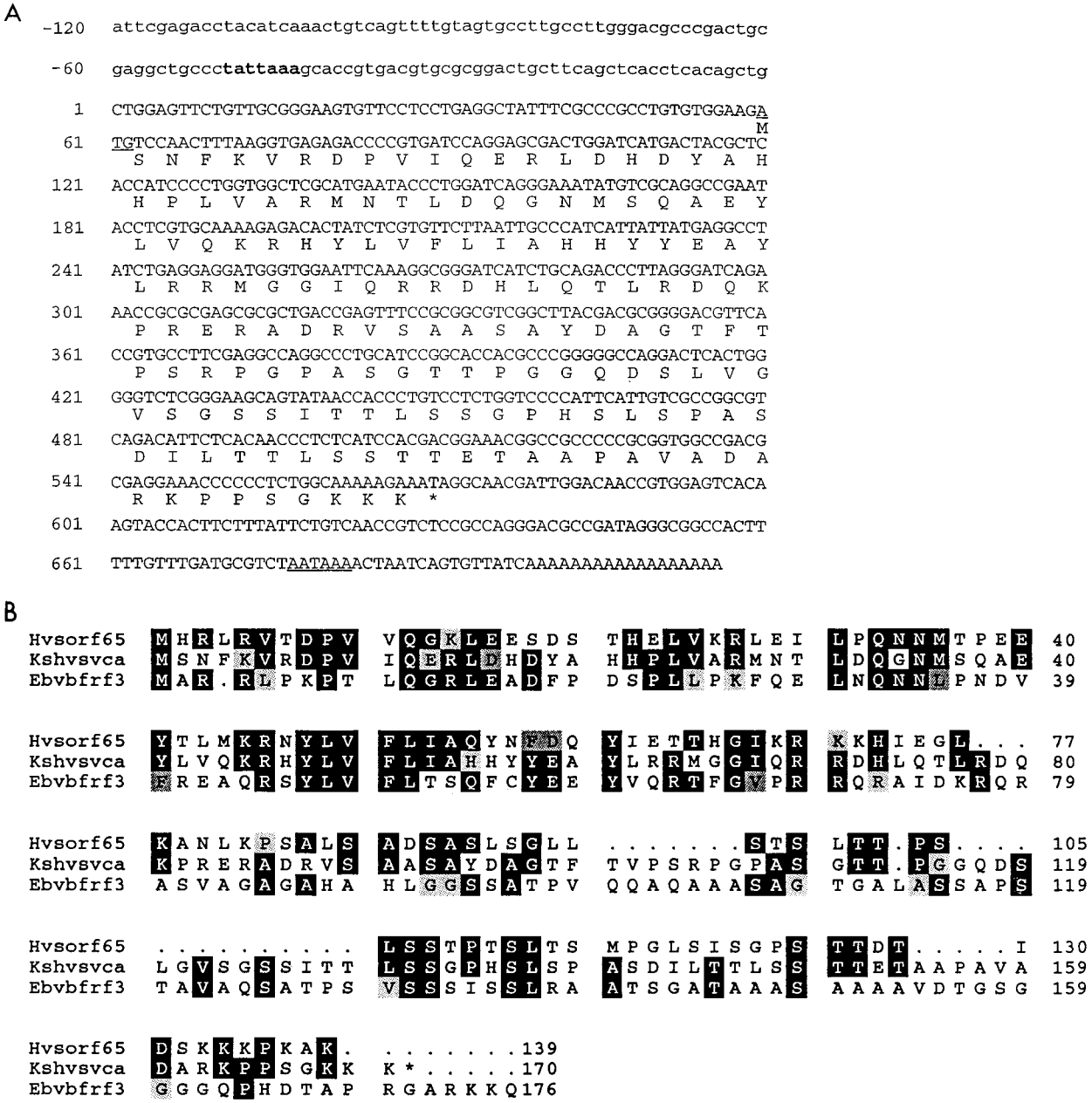

FIG. 1. Sequence analysis of the KSHV sVCA gene. (A) Nucleotide and predicted amino acid sequence of sVCA. cDNA sequences are shown in capital letters; 5′-flanking genomic sequences are shown in lowercase type. The 170 amino acids of the ORF deduced from the sequence are depicted beneath each line of nucleotide sequence. The first nucleotide of the cDNA was arbitrarily assigned as position 1. The putative TATA element tattaaa is shown in boldface type, and the polyadenylation recognition sequence AATAAA is underlined. (B). Amino acid sequence comparison among KSHV sVCA, EBV BFRF3, and HVS ORF65. Sequences were aligned with the GCG PILEUP program. The gap weight is 3.00, and the gap length weight is 0.1. The percent similarity and identity are 48 and 27% between KSHV sVCA and EBV BFRF3 and 60 and 40% between KSHV sVCA and HVS ORF65.

script, 0.85 kb, was consistent with the size of the cDNA clone, which was 716 nucleotides. This stable transcript was detectable between 12 and 18 h after chemical induction (data not shown); its expression was sustained for at least 48 h (Fig. 2A). Sodium butyrate induced a higher level of expression of the 0.85-kb sVCA mRNA than did TPA, as previously shown for the mRNA detected by KS330Bam, which represents the gene for another capsid component (23).

KSHV sVCA is encoded by a viral lytic late gene. To determine the stage of the viral life cycle in which KSHV sVCA was expressed, BC-1 cells were pretreated with a protein synthesis inhibitor, cycloheximide, or with one of the herpesvirus DNA polymerase inhibitors phosphonoacetic acid (PAA), phosphonoformic acid (PFA), or acycloguanosine (ACG) before addition of butyrate as the inducing agent. Expression of the 0.85-kb sVCA mRNA was dramatically inhibited by the addition of either cycloheximide, PAA, or PFA (Fig. 2B). Since protein synthesis and viral DNA replication were required for its expression, KSHV sVCA could be classified as a late-gene product. sVCA expression was not inhibited by ACG. It is not

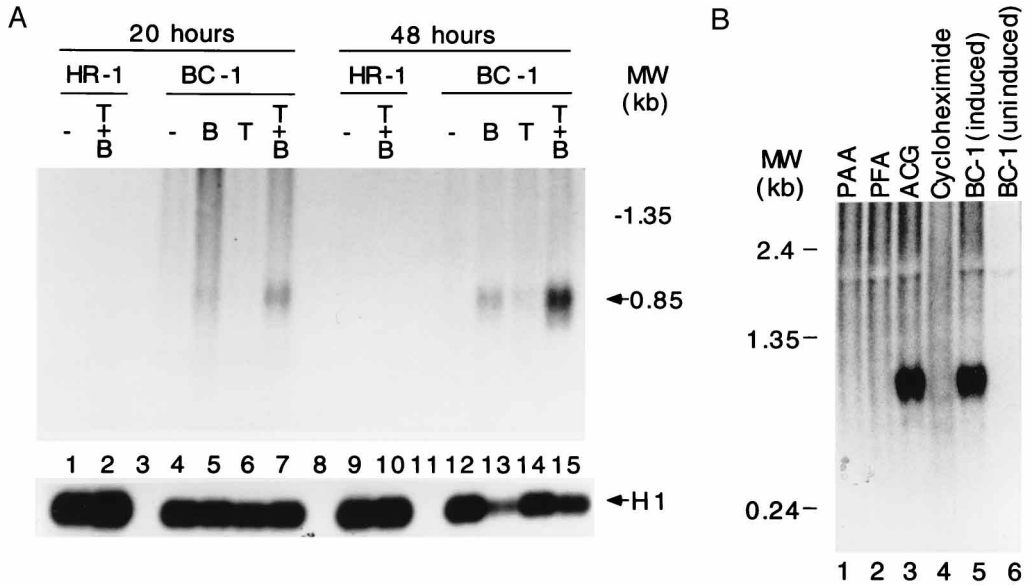

FIG. 2. Transcription of sVCA in BC-1 cells. (A). sVCA mRNA expressed upon chemical induction of BC-1 cells. U, B, T, and B+T represent cells that were untreated, induced with butyrate, induced with TPA, or induced with butyrate and TPA, respectively. No RNA was loaded in lanes 3, 8, and 11. The probe was a PCR-amplified product of sVCA ORF labeled with [α-^{32}P]dCTP by the random-prime method. RNA loading was quantitated by hybridizing the same filter with DNA encoding H1 RNA of human RNase P. (B). Sensitivity of sVCA expression to inhibitors of viral DNA synthesis and protein synthesis. Cells were pretreated with 500 μM PAA, 500 μM PFA, or 100 μM ACG for 10 h or with cycloheximide (100 μg/ml) for 6 h before induction with sodium butyrate for 18 h. RNAs prepared from each sample were analyzed by Northern blot analysis. MW, molecular size.

known whether ACG resistance resulted from insensitivity of the BC-1 KSHV thymidine kinase or DNA polymerase (12, 26, 38).

Expression of KSHV sVCA in mammalian cells. sVCA was transiently expressed in COS-7 or 293T cells transfected with the pBK-CMV vector containing the CA20 cDNA. A protein band estimated at 30 kDa was recognized by KS patient sera on immunoblots prepared from extracts of both cell types (Fig. 3A). The 30-kDa protein represents a fusion between 56 aa of β-galactosidase encoded in the vector and the 170 aa of the sVCA ORF. These results suggested that CA20 encoded an antigen recognized by KS sera.

In vitro translation of KSHV sVCA. To determine the intrinsic molecular weight of the KSHV sVCA ORF product, the protein was synthesized in a rabbit reticulocyte lysate (Fig. 3B). In this experimental system, the sVCA protein was not fused to another peptide; the first ATG of KSHV sVCA was used as the initiation codon. A predominant 22-kDa protein was seen when plasmid DNA containing the sVCA gene served as a template for in vitro transcription and translation (Fig. 3B, lane 2). As with the sVCA encoded by BFRF3 of EBV, the migration was slightly slower than predicted (32). The size of in vitro translated sVCA corresponded to that expressed in chemically treated BC-1 cells (Fig. 3D). The 22-kDa in vitro-translated product was immunoprecipitated by sera from patients with KS (lanes 3 to 6) but not by control sera (lanes 7 to 10). These results provided further evidence that KSHV sVCA was a potential serological marker for KS.

Expression of KSHV sVCA in *E. coli*. To obtain large quantities of protein for further serological studies, sVCA ORF was expressed in *E. coli* with the pET30b system (Novagen). A 44-aa peptide containing His · Tag and S · Tag encoded in the vector was fused to the N terminus of sVCA. A protein band with an estimated molecular mass of 29 kDa was detected by immunoblotting in extracts from *E. coli* harboring pET30b-sVCA (Fig. 3C, lane 3) but not in extracts of control cells containing vector alone (lane 1) or plasmid containing an unrelated insert (lane 2). These results suggested that immunoreactive epitopes of KSHV sVCA were retained when expressed in *E. coli.*

KSHV sVCA was detected as a 22-kDa viral lytic protein in BC-1 cells. Antisera to sVCA were raised in rabbits immunized with the protein expressed in *E. coli*. These antisera recognized a 22-kDa protein in BC-1 cells treated with sodium butyrate (Fig. 3D, lane 2) or TPA plus sodium butyrate (lane 4) but only faintly detected the protein in cells treated with TPA (lane 3). The size of the polypeptide corresponded to the in vitro translation product (Fig. 3B); its pattern of expression following chemical induction corresponded to that of the sVCA 0.85-kb mRNA (Fig. 2A). Therefore, the 22-kDa immunoreactive protein in BC-1 cells is KSHV sVCA.

KSHV sVCA does not share cross-immunogenicity with EBV BFRF3. EBV infection is widespread in the general population; antibodies to EBV BFRF3 represent an immunodominant serological marker for the past EBV infection (33, 39). EBV BFRF3 and KSHV sVCA are 48% similar at the level of the primary amino acid sequence. If KSHV sVCA was to be used as a serological marker, it was essential to determine whether any cross-reactivity existed between these two related proteins. Therefore, 10 EBV-positive sera were tested for antibodies to KSHV sVCA by a Western immunoblot analysis (Fig. 4). Protein extracts prepared from *E. coli* containing either pET30b alone or recombinant pET30b-sVCA were run in parallel in each test. The five sera from KS patients reacted with KSHV sVCA; none of the other EBV-positive sera recognized the 29-kDa sVCA fusion protein. In an independent experiment, a healthy donor serum which has been used as a reference positive control for antibodies to EBV BFRF3 failed to detect KSHV sVCA (data not shown). Moreover, rabbit antibodies to KSHV sVCA did not react with BFRF3 expressed in mammalian cells (data not shown). These results indicate that EBV BFRF3 and KSHV sVCA, although similar

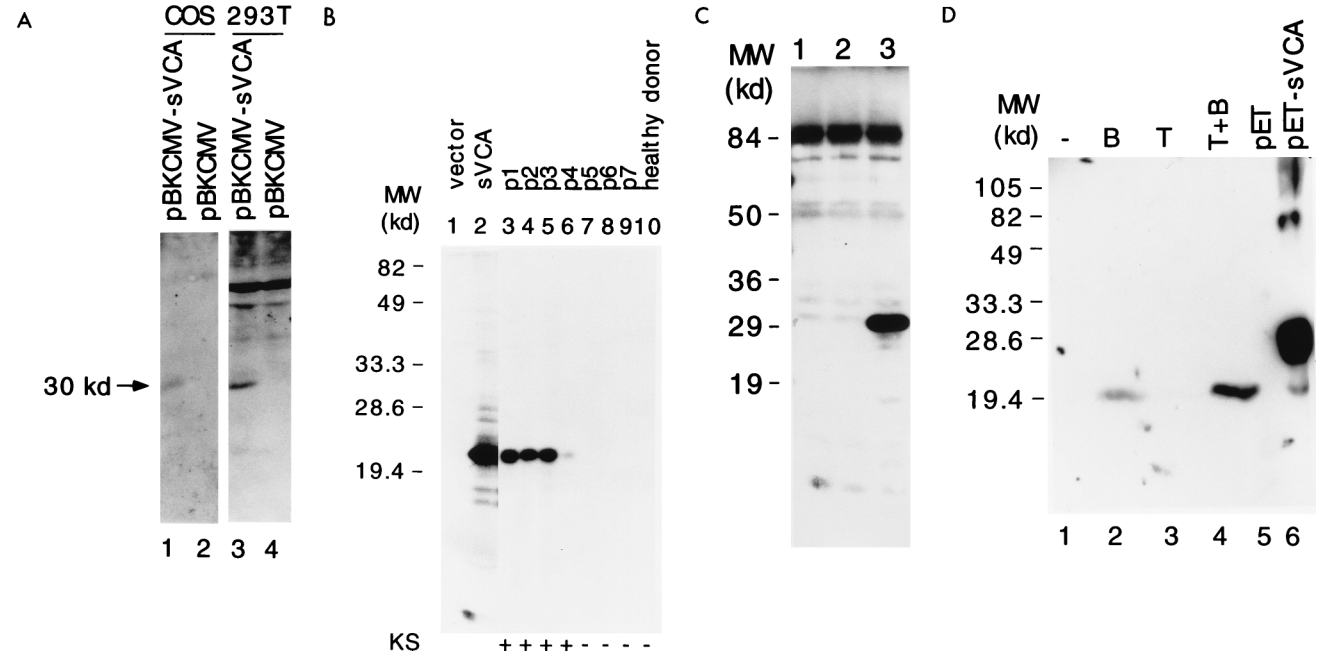

FIG. 3. Expression of sVCA polypeptide. (A) Expression of sVCA in COS-7 and 293T cells. Cells were transfected with pBK-CMV-sVCA (lanes 1 and 3) or with vector control pBK-CMV (lanes 2 and 4). An immunoblot was probed with human serum 0103 from a patient with KS. (B) In vitro translation and immunoprecipitation of KSHV sVCA. Lanes 1 and 2 represent proteins synthesized in the TNT system from the pBK-CMV vector control and pBK-CMV-sVCA. Lanes 3 to 10 represent immunoprecipitation of the in vitro-translated product by patient sera. p1 and p2, AIDS patients with KS; p3, Mediterranean KS patient; p4, transplant KS patient; p5, AIDS patient without KS; p6, Mediterranean patient without KS; p7, transplant patient without KS. (C) Expression of sVCA in *E. coli*. Lanes: 1, vector control (pET30b); 2, pET30b-vMIP1 (a KSHV-encoded chemokine); 3, pET30b-sVCA. An immunoblot containing bacterial extracts was probed with KS patient serum 0103. (D) Expression of sVCA from the KSHV genome in BC-1 cells. Lane 1 to 4 contain extracts from BC-1 cells untreated, induced with butyrate, induced with TPA, and induced with TPA plus butyrate, respectively. Lane 5 and 6 contain extracts from bacteria transformed with pET30b vector or pET30b-sVCA. An immunoblot was probed with serum from a rabbit which was immunized with purified sVCA expressed in *E. coli*. MW, molecular mass in kilodaltons.

at the level of the primary amino acid sequence, do not present cross-reactive epitopes in the milieu of the human immune system.

Purification of KSHV sVCA. With the aid of two tags at its N terminus, His · Tag and S · Tag, KSHV sVCA was partially purified from *E. coli* extracts using two consecutive affinity columns (Fig. 5). A highly purified soluble form of KSHV sVCA was present in fractions 3 to 7 eluted from the S · Tag agarose affinity column. The antigenicity of the purified protein was preserved on immunoblot analysis and provided material for large-scale screening (Fig. 6).

Prevalence of antibodies to KSHV sVCA. There was a high correlation between the presence of antibody to p40 antigen in butyrate-treated KSHV-infected cells and the presence of an-

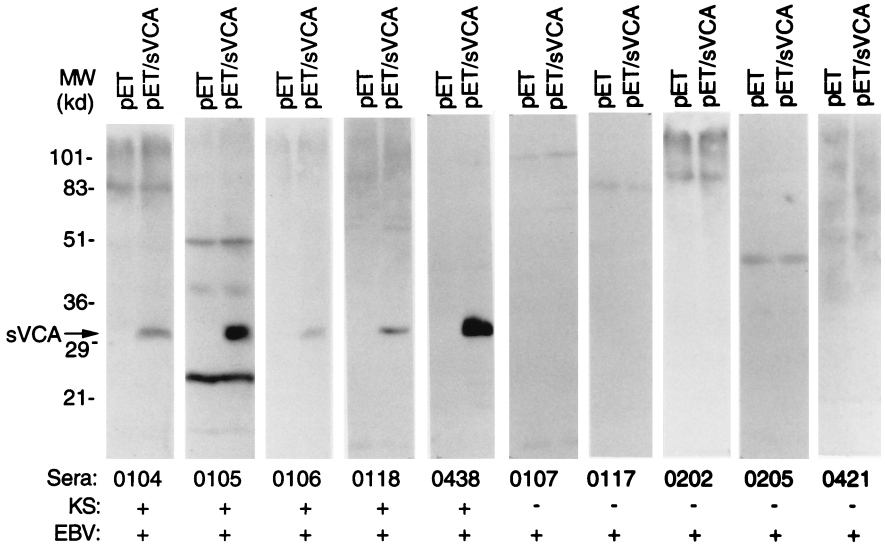

FIG. 4. Recognition of KSHV sVCA expressed in *E. coli* by sera from KS patients. Extracts from *E. coli* transformed with pET30b or pET30b-sVCA were used to detect specific antibodies by immunoblotting. Sera 0104, 0105, 0106, 0118, and 0438 were from patients with KS. Sera 0107, 0117, 0202, 0205, and 0421 were from patients without KS. MW, molecular mass in kilodaltons.

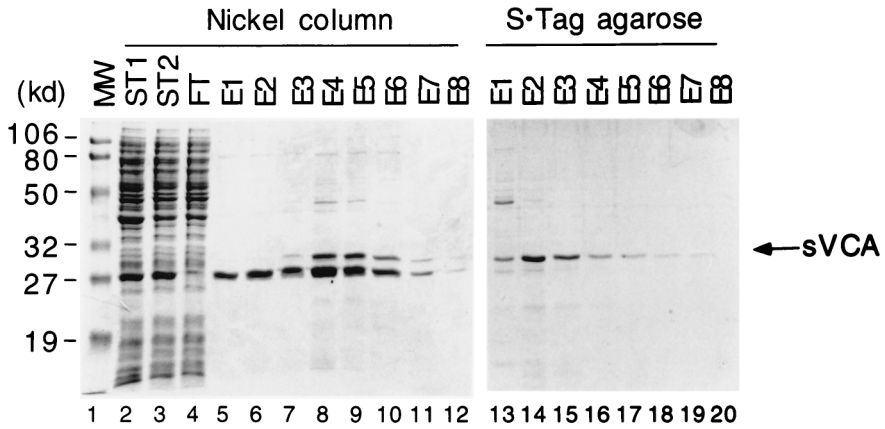

FIG. 5. Purification of KSHV sVCA expressed in *E. coli*. Lanes: 2, total extracts from *E. coli* with pET30b-sVCA induced by IPTG; 3, soluble supernatant of the total extract; 4, flowthrough of Nickel column; 5 to 12, eluate fractions from the Nickel column after washing (see Materials and Methods). Fractions E4, E5, and E6 were pooled and loaded on an S · Tag agarose column. Lanes 13 to 20 are eluate fractions from the S · Tag agarose column. The gels were stained with Coomassie blue. MW, molecular mass in kilodaltons.

tibody to sVCA in HIV-positive patients. Of 34 sera with antibody to p40, 31 (91%) also contained antibody to sVCA. However, antibody to sVCA was detected in 23 of 67 HIV-infected patients lacking antibody to p40. These included 13 of 15 patients with KS and 10 of 52 patients without KS. Thus, antibody to sVCA appeared to be a more sensitive serological marker than antibody to p40. Nearly 90% of HIV-infected patients with KS were seropositive (Table 1). In other studies, we have found that sera from more than 90% of patients with Mediterranean KS and posttransplantation KS also react to sVCA (data not shown). Of HIV-infected patients without KS, 20% were seropositive to sVCA. Antibodies to sVCA were not detected in children with hemophilia or acute viral illness and were not detected in female patients with autoimmune disease; they were not detected in children born to HIV-seropositive mothers or in sera from Chinese patients with nasopharyngeal carcinoma. Since these sera have extremely high antibody titers to EBV proteins, the results underscore the lack of immunologic cross-reactivity between sVCA and EBV proteins. Three sera from 28 healthy adults showed weak seroreactivity by comparison with six sera from AIDS patients with KS as a reference (Fig. 6C and D).

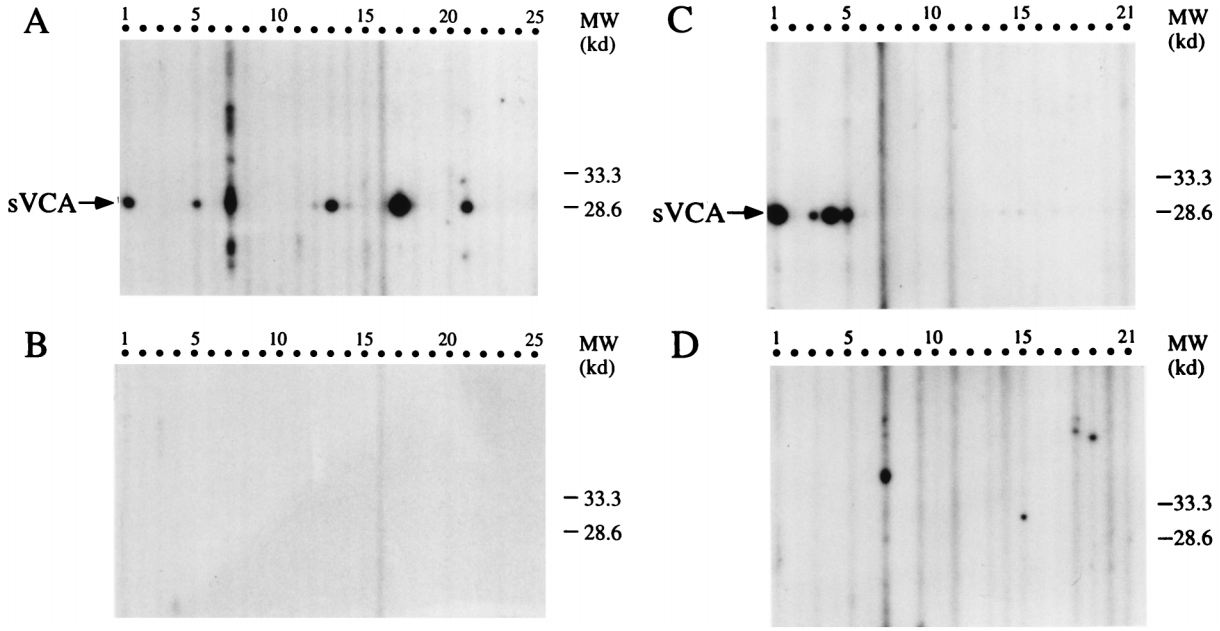

FIG. 6. Screening of human sera for antibodies to purified KSHV sVCA. A cassette miniblot system was used in the Western blot procedure. Eluate fraction E3 from the S · Tag agarose column was used in panels A and C. Comparable eluate from *E. coli* transformed with pET30b was used as a negative control antigen in panels B and D. (A and B) The same 25 sera were tested against purified sVCA (A) and control antigen (B). The signal representing specific antibody binding to KSHV sVCA is indicated by an arrow. Positive reactions are seen in lanes 1, 5, 7, 12, 13, 14, 17, and 21 in panel A. Serum 16, with a high background, is not scored as positive. (C) Reactions with sera from AIDS patients with KS are shown in lanes 1 to 6; strongly positive reactions are seen in lanes 1, 3, 4, and 5, and a weakly positive reaction is seen in lane 6. Reactions with sera from people without KS are shown in lanes 7 to 21. Lanes: 7 and 8, hemophilia; 9 to 12, autoimmune disease; 13, acute viral illness; 14 to 17, healthy adults; 18 to 21, Chinese patients with nasopharyngeal cancer. Weak reactions with sera from healthy adults are seen in lanes 14, 15, and 17. (D) The same sera were reacted with control antigen. MW, molecular mass in kilodaltons.

TABLE 1. Prevalence of antibody to KSHV sVCA in various populations

Sample profile	Source	No. (%) of patients with Ab to KSHV sVCA/total no.
HIV-positive with KS	United States	42/47 (89%)
HIV-positive without KS	United States	11/54 (20%)
HIV-positive children	United States	0/12
HIV-negative children	United States	0/10
Hemophilia patients	United States	0/25
Autoimmune patients	United States	0/25
Children with acute illness	United States	0/25
Healthy adults	United States	3/28 (11%)
Nasopharyngeal cancer	China	0/25

DISCUSSION

In this study, we identify, clone, and express a KSHV-encoded protein with homology to small virion capsid components of other herpesviruses. We show that this small viral capsid component is immunogenic in humans and can serve as a serological marker for infection with KSHV. KSHV sVCA is homologous to structural components of several other herpesviruses including VP26 of herpes simplex virus (HSV), BFRF3 of EBV, ORF65 of HVS, and the recently identified smallest capsid protein (SCP) of human cytomegalovirus (15). Despite this homology, KSHV sVCA does not appear to be antigenically cross-reactive. KSHV sVCA is similar in size to its counterparts among the gammaherpesvirus (Fig. 1B); however, the comparable proteins among alpha- and betaherpesvirus are smaller, in the range of 10 to 12 kDa (9, 21). All proteins of this group are highly basic. KSHV sVCA contains 12% arginine and lysine and has a predicted pI of 10.45. Among the gammaherpesvirus, the homology extends throughout the proteins (Fig. 1B). The smaller HSV VP26 and cytomegalovirus SCP are homologous to the C-terminal portion of KSHV of sVCA.

Among this group of related herpesvirus structural components, only HSV VP26 has been studied in any detail (5, 37, 41). Computer analysis of images collected by cryoelectron microscopy shows that each hexon tip is surrounded by six copies of VP26 (5, 37); however, VP26 does not associate with the penton (37). The in vitro assembly by HSV nucleocapsids from components purified from a baculovirus expression system does not require VP26 (36). A comparison of capsid structures with or without VP26 suggests that the small virion protein may play a role in linking the viral capsid to outer virion structures (5). The location of this group of proteins on the outer surface of the capsid may contribute to their immunodominant behavior.

This is the first report in which a recombinant viral gene product was used as an antigen to determine the prevalence of antibodies to KSHV in different populations. We have found a high seroreactivity rate against KSHV sVCA among patients with different types of KS: 89% among AIDS KS patients, 93% among posttransplantation KS patients, and 95% among classic KS patients (Table 1 and unpublished results). This rate of seroreactivity among KS patients is higher than that previously reported for antibodies to KSHV antigens present in B cells (13, 14, 18, 24). The frequency of antibodies to inducible p40 (24), latent nuclear antigens p226/234 (13, 14), and latency-associated nuclear antigen (18) detectable by immunofluorescence ranged from 67 to 83% of patients with KS. The recombinant sVCA antigen may therefore be a more sensitive serological marker than antigens present in infected cells. We found that 20% of sera from HIV-infected patients without KS contained antibodies to KSHV sVCA. This frequency is similar to that of other studies with antigens prepared from KSHV-infected cell lines. In those studies, 13 to 30% of HIV-infected homosexual patients were seropositive. Also in agreement with three other studies which used cell antigens, we did not detect a high frequency of antibodies to KSHV sVCA in patients with hemophilia, acute viral syndromes, or autoimmune disease or in healthy donors (13, 18).

The low frequency of antibodies to KSHV sVCA in the general population stands in marked contrast to the pattern of antibodies to its EBV homolog, BFRF3. Antibodies to BFRF3 are present in nearly all human adults, since EBV infection is nearly universal in humans. This comparison of seroepidemiology between EBV and KSHV suggests two alternative possibilities: (i) KSHV infection, unlike EBV infection, is not ubiquitous in the human population, or (ii) antibodies to KSHV sVCA, unlike those to EBV sVCA, do not reflect KSHV infection.

The question that remains unanswered is whether seropositivity to KSHV sVCA reflects de novo infection, reactivated latent infection, or an alteration in the host immune response which stimulates the production of detectable antibodies (11). The availability of recombinant sVCA should facilitate further seroepidemiological studies to address this question.

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