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BK Virus-Transformed Inbred Hamster Brain Cells

I. Status of the Viral DNA and the Association of BK Virus Early Antigens with Purified Plasma Membranes

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Inbred LSH hamster brain cells were transformed in vitro by the GS strain of BK virus (BKV), and transplantable tumors classified as undifferentiated glioblastomas were induced in the syngeneic host. The viral status in the transformed cells, designated LSH-BR-BK, was established. About 46 genome equivalents per cell of viral DNA was detected, with the majority of sequences in a free form. The transformed cells expressed large quantities of tumor (T) antigen as well as surface (S) antigen as demonstrated by indirect immunofluorescence. Sixty-three percent of tumor-bearing hamsters produced high-titer antibodies against T, whereas 3 of 14 (21%) hamsters also produced antibodies against the BKV-specific S antigen. Furthermore, the relatedness of BKV early gene products, including T, S, and tumor-specific transplantation antigen, was established by the production of a rabbit antiserum against highly purified plasma membranes of LSH-BR-BK cells and by the induction of a BKV-specific tumor-specific transplantation antigen response by these plasma membranes in the syngeneic host.

The human papovavirus, BK virus (BKV), which can be isolated from the urine of immunodeficient patients (12, 32, 41) is highly oncogenic in rodents. A high incidence of malignant tumors in hamsters has been reported after intracerebral (43) or intravenous (13) inoculation of the purified virus. BKV has a broad cell tropism, probably reflecting its broad oncogenic potential, i.e., the induction of ependymomas, choroid plexus papillomas, malignant insulinomas, osteosarcomas, and fibrosarcomas (13–15, 22, 36, 45).

We have developed an experimental system to study the oncogenicity of BKV. The results comprise: (1) in vitro transformation of inbred LSH hamster brain cell cultures which were shown to contain predominantly free viral DNA, (ii) their transplantation into the syngeneic host, (iii) analysis of sera from tumor-bearing hamsters for antibodies to early gene products, and (iv) the demonstration of BKV-determined tumor-specific transplantation antigen (TSTA) activity of highly purified plasma membranes (PM) from BKV-transformed cells. Using unfixed LSH-BR-BK cells, we have compared the cell surface reactivity of a rabbit antiserum against plasma membranes with that of sera from tumor-bearing hamsters.

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MATERIALS AND METHODS

Virus and cells. The GS strain of BKV (6386/72) was isolated and kindly provided by S. D. Gardner. The virus was propagated in primary human embryonic brain (PHEB) cells at low multiplicity of infection (0.01 PFU/cell). After concentration and purification by two cycles of equilibrium centrifugation in CsCl (17), complete particles, sedimenting at a density of 1.34 g/cm^3, were lyophilized and stored at 4°C until use for transformation. Human embryonic brain tissue (from 12- to 16-week old embryos) and newborn hamster brain tissue (inbred Syrian hamsters, LSH strain; Lakeview Hamster Colony, Wilmington, Mass.) were dispersed into single cells and grown in Eagle minimal essential medium containing 10% fetal bovine serum and antibiotics. PHEB cultures were selected for virus propagation, and secondary LSH-BR cells with comparable cell morphology were used for transformation with BKV. Normal LSH-BR cells and HSV-2-transformed hamster cell line LVG-333 (18) were used as controls.

Transformation of LSH-BR cells. At 48 h after passage, subconfluent secondary cultures of LSH-BR cells were infected with BKV (3×10^3 hemagglutinating units per 3×10^5 cells). Five days later cultures were divided at a ratio of 1:3, and fetal bovine serum was reduced to 5%. Thereafter, they were passaged whenever they reached confluency and were moni-

tored for appearance of rapidly growing, dense colonies. Expression of viral (V) and tumor (T) antigens was monitored by immunofluorescence (3, 4). The resulting BKV-transformed cell line, designated LSH-BR-BK, was passaged at a split ratio of 1:20 and grown in minimal essential medium–5% fetal bovine serum. Light and electron microscopic analyses were performed on cell cultures and on tumors grown in LSH hamsters after subcutaneous transplantation. Colony formation of the transformed cells in soft agar was determined by the method of Macpherson and Montagnier (28). The tumorigenicity of LSH-BR-BK cells was tested by their transplantation into syngeneic hosts. A transplantation line was established as described elsewhere (3).

BKV DNA analysis in LSH-BR-BK cells. High-molecular-weight cellular DNA was isolated from LSH-BR-BK cells and digested with the indicated restriction endonucleases as described elsewhere (7, 30). Electrophoresis of fractionated DNA was carried out in 1% agarose gels in TAE buffer (0.04 M Tris [pH 7.6], 0.005 M sodium acetate, 0.001 M EDTA) at 110 V overnight as previously described (30). Cellular DNA was then transferred onto nitrocellulose filters and hybridized with nick-translated BKV DNA derived from plaque-purified prototype BKV. Viral DNA sequences were then detected by autoradiography (30).

For quantitation of the number of copies the blotting method of Wahl et al. (46) was used in which cellular DNA is transferred and covalently bound to diazobenzyloxymethyl paper to allow accurate estimation of the gene copy number after hybridization with the labeled probe was used. The aminobenzyloxymethyl paper was converted to diazobenzyloxymethyl paper (Schleicher & Schuell Co.) before use for DNA transfer.

Purification of plasma membranes. Single cell suspensions were obtained from LSH-BR-BK and LSH-BR cultures by incubation in 0.13 M NaCl–0.02M sodium phosphate (phosphate-buffered saline) (pH 7.2)–0.5 mM EDTA at 36°C for 5 to 15 min. Disruption of 4×10^8 to 6×10^8 normal or BKV-transformed cells by nitrogen decompression and purification of the PM of these cells as vesicles was as previously described (33). As intrinsic and extrinsic PM markers we used Na^+-K^+-activated ATPase and lactoperoxidase-catalyzed surface radioiodination, respectively. The outer mitochondrial membranes were monitored by monoamine oxidase (33). Contamination of PM by nuclear material was determined by the content of [^{14}C]thymidine-labeled DNA. For this, 10^7 cells were pulse-labeled with [^{14}C]thymidine for 60 min (0.01 mCi of [2-^{14}C]thymidine per ml, 50 mCi/mmol; New England Nuclear Corp., Boston, Mass.), washed twice in minimal essential medium, and combined with the unlabeled cells before disruption.

Antisera. The production of antisera against structural antigens of BKV was as described elsewhere (4). Antisera against BKV T were raised in the syngeneic host by following procedures described elsewhere (3). Antiserum against purified PM of LSH-BR-BK was prepared in a rabbit by the following immunization schedule: the initial immunization consisted of PM (0.5 mg of protein) emulsified in complete Freund adjuvant (Difco Laboratories, Detroit, Mich.) and was followed by four boosters, at 2-week intervals, consisting of 0.3 mg of protein in incomplete Freund adjuvant. Two weeks after the last booster the rabbit was bled. Rabbit anti-LSH-BR-BK PM was absorbed nine times with living LSH-BR cells; equal volumes of serum and packed single cells (previously detached from the plastic by phosphate-buffered saline–0.5 mM EDTA) were incubated for 2 h at room temperature and overnight at 4°C. Antibody reactivity was tested by indirect immunofluorescence (IIF) on LSH-BR-BK cells and on LSH-BR cells as the control.

Serological tests. Hemagglutination and hemagglutination inhibition were done by standard procedures (19). The IIF test for staining of T antigens was done as described previously (4) by using goat anti-hamster IgG fluorescein conjugate (Microbiological Associates, Bethesda, Md.) at a dilution of 1:10. Immunofluorescence staining of cell surface antigens was done on living cells dispersed in phosphate-buffered saline–0.5 mM EDTA, using 5×10^5 cells per 0.05 ml in duplicates. The specificity of IIF reactions was controlled by appropriate combinations of positive and negative sera with transformed and normal cells and by a blocking test using $F(ab')_2$ fragments of rabbit hyperimmune and preimmune sera (20).

Transplantation rejection assay (TSTA activity). TSTA reactivity of PM from LSH-BR-BK cells was determined in the syngeneic host. Weanling LSH hamsters were immunized with purified PM from LSH-BR-BK cells by multiple subcutaneous injections at weekly intervals. Challenges with LSH-BR-BK cells for the tumor rejection reaction were initiated 7 days after the last immunization. Group I of the hamsters received 10^5 cells per animal subcutaneously, and group II received 10^6 cells per animal. The 50% tumor-producing dose of this passage line was determined by subcutaneous injections with 10^3 to 10^6 cells per injection site. Control groups consisted of hamsters immunized with purified PM from normal LSH-BR cells and LVG-333 cells as well as of nonimmunized animals. At 35 and 70 days after challenge, developing tumors were measured, and the mean tumor volumes were calculated (27).

RESULTS

Establishment and characterization of BKV-transformed cells. Duplicates of secondary cultures from newborn hamster brain cells (LSH-BR) infected with BKV and control cultures were maintained simultaneously. Cultures were passaged 5 days postinfection at a ratio of 1:3 with 5% fetal bovine serum. Immunofluorescence staining with serum of tumor-bearing hamsters revealed 2 to 5% of the cells positive for BKV T antigen. Structural viral antigens were not detected. At confluency, 6 days later, a second passage was initiated. Within the next 13 days one to two rapidly growing colonies of presumed transformed cells per culture flask developed. They were selected from the monolayer by brief trypsinization (day 25 postinfection). Cells derived from a single colony were further cultured and designated as LSH-BR-BK.

The cells grew rapidly and were passaged in weekly intervals at a 1:20 ratio. Of the cells, >95% were positive for nuclear T antigen, whereas V antigens were not detected, and electron microscopy did not reveal any virus particles (Fig. 1c). Overlaying of permissive PHEB cells with lysates of LSH-BR-BK cells did not result in the expression of V antigens or a cytopathic effect. When single LSH-BR-BK cells were plated in soft agar (10^2 to 10^3 cells per 60-mm petri dish) colonies developed within 12 days. In contrast, normal LSH-BR cells failed to form colonies. LSH-BR-BK cells of passage 5 when inoculated into weanling LSH hamsters (4.5×10^6 per animal) produced tumors after about 9 weeks. The tumor cells expressed BKV

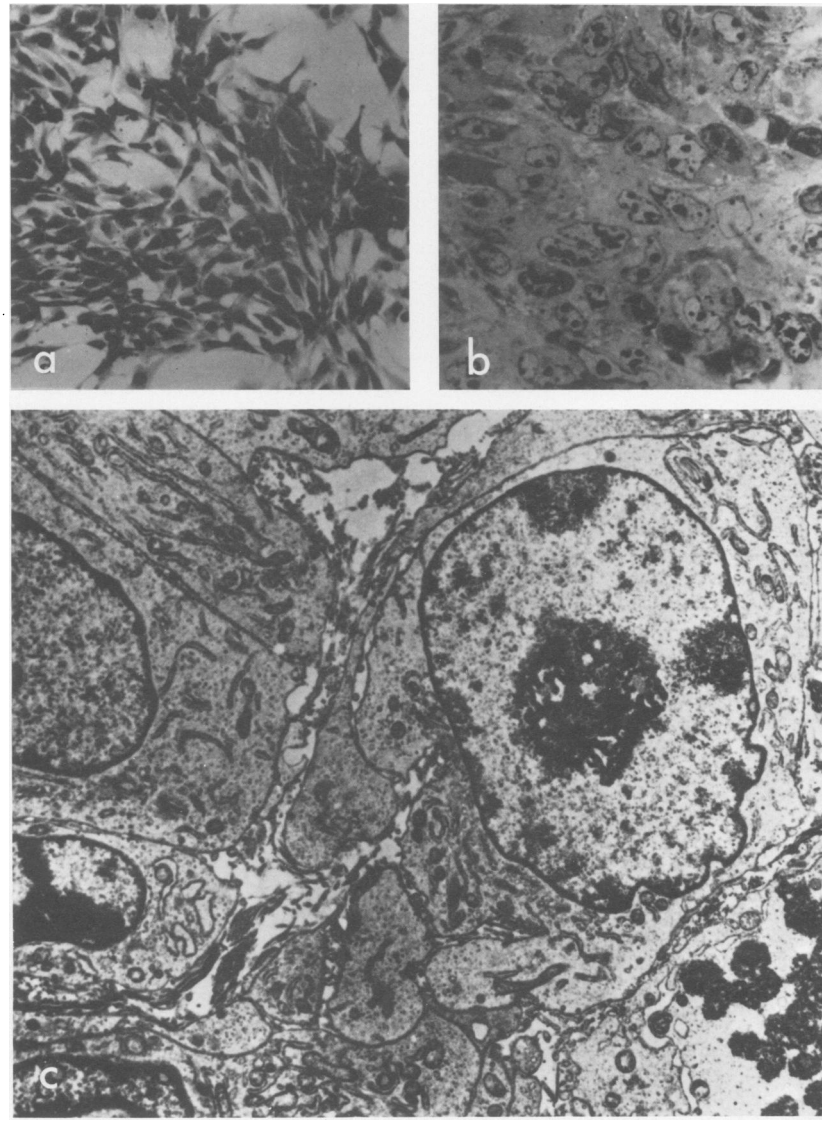

FIG. 1. *Light and electron microscopic examination of LSH-BR-BK cells obtained from cultures or tumors. (a) Specimen from tissue culture, demonstrating an abundant outgrowth of multipolar stellate cells; haematoxylin and eosin stain (micrograph, ×100). (b) Cells from a tumor subcutaneously grown in LSH hamsters, forming characteristic sheets of tumor cells with large irregular nuclei and coarse chromatin granules; toluidine blue stain (micrograph, ×630). (c) Cells from subcutaneous tumors as under (b) with interdigitating processes and clusters of mitochondria and granular endoplasmic reticulum; uranyl acetate, lead citrate stain (electron micrograph, ×5,600).*

T, and sera of tumor-bearing hamsters tested for anti-BKV T were strongly positive. Light and electron microscopic examination of both transformed cultured cells and tumor tissue demonstrated several morphological features, suggesting their astrocyte or spongioblast origin (Fig. 1). The cells had stellate contours and formed a cytoplasmic feltwork. Unusual for astrocytes, but typical for poorly differentiated, actively proliferating cells, were the sheets of cells observed in subcutaneous tumors and the high nucleus-cytoplasm ratio, the abundance of Golgi apparati, endoplasmic reticulum, and mitochondria and the paucity of microfilaments (Fig. 1c). Histochemical analysis with phosphotungstic acid-hematoxylin staining revealed gliofibrils in some of these cells. Therefore, subcutaneously transplantable tumors were classified as undifferentiated glioblastomas.

Status of viral DNA sequences in LSH-BR-BK cells. In experiments reported here LSH-BR-BK cells were analyzed in passage 98. The status of viral DNA was determined by the blotting method (7, 30, 38, 44). Total cellular DNA was fractionated by electrophoresis through agarose gels, blotted onto nitrocellulose filters, and then hybridized to ^{32}P-labeled BKV DNA. The rationale was that if viral DNA were integrated, it should not enter the gel due to its high molecular weight. As shown in Fig. 2, bands in the LSH DNA hybridizing to BKV probe comigrated with forms II, III, and I of BKV DNA present in the reconstruction experiment. This suggested that viral DNA in this transformed cell line was present predominantly in a free form. Then, total LSH DNA digested with the restriction endonuclease *Hin*dII, which does not cleave BKV DNA, should contain forms I, II, and III of viral DNA. Most of the BKV DNA in both LSH and in the reconstruction experiment were in forms II and III (form I DNA was converted to forms II and III due to nicking activity in the enzyme; Fig. 2). In addition, very faint bands, both larger and smaller than linear viral DNA, could be detected. These bands might represent residual integrated sequences. Only one band, comigrating with the linear BKV DNA, could be detected when LSH DNA was digested with either of the restriction endonucleases *Eco*RI or *Hpa*II (Fig. 2).

*Hin*dIII cleavage of LSH DNA produced two large fragments, A and B, which were the same as those of BKV DNA (Fig. 2). However, the two smaller fragments, D and C, were replaced by a single fragment (C-D) whose molecular weight was the same as the combined molecular weights of D and C fragments. It should be noted that the strain of BKV used for transformation was also missing this *Hin*dIII site (29).

To estimate the number of viral DNA copies

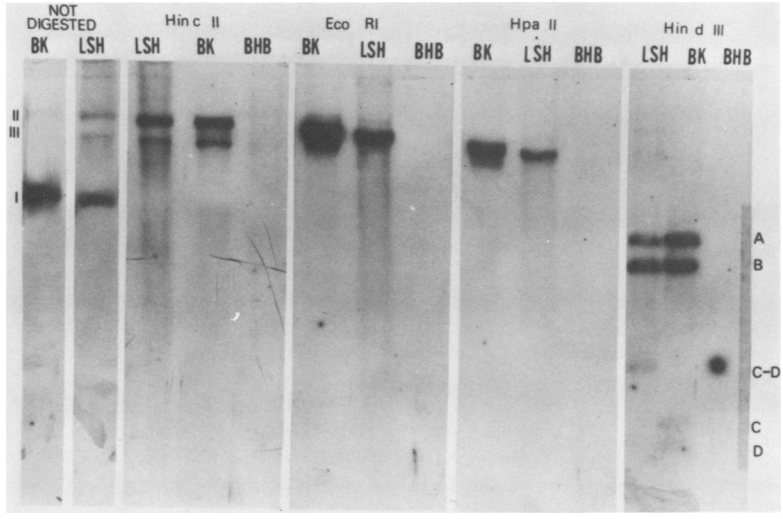

FIG. 2. *Detection of BKV DNA sequences in LSH-BR-BK cells. High-molecular-weight cell DNA (0.01 mg) was cleaved with each of the restriction endonucleases HindII, EcoRI, HpaII, and HindIII. The digested DNA samples (and one undigested DNA sample) were fractionated on 1% agarose gels and hybridized with ^{32}P-labeled BKV DNA as described in the text. In parallel with the DNA from transformed LSH-BR-BK cells, DNA was analyzed from normal LSH-BR cells as well as LSH-BR cell DNA plus 560 pg of unlabeled BKV DNA. The bands above the two larger bands in the HindIII-digested DNA samples are due to incomplete digestion, and the faint bands in the EcoRI-digested LSH and reconstruction DNA samples are an artifact of sample application.*

in the LSH-BR-BK cells, 0.01 mg of high-molecular-weight DNA from LSH-BR cells was mixed with either 56 pg (7.4 copies per cell; 21), or 112 pg (14.8 copies per cell) and cleaved with the restriction endonuclease *Eco*RI. High-molecular-weight DNA (0.01 mg) from LSH-BR-BK cells was also cleaved with this enzyme. The samples were fractionated by electrophoresis, blotted, and hybridized to ^{32}P-BKV DNA as described above. The band containing labeled BKV DNA in each filter was visualized by autoradiography, and the amount of radioactivity in each band was measured by counting the region of the paper containing the band. It should be noted that, as reported first by Wahl et al. (46) and confirmed by our reconstruction experiment (Table 1), this method of filter hybridization shows a linear relationship between the number of copies in the DNA covalently bound to the filter and the number of counts hybridized. The number of counts in the sample containing 14.8 copies of viral DNA was about twice the number of counts in the sample containing 7.4 copies (Table 1). The number of copies in LSH-BR-BK cells was determined by extrapolation to be 46 per cell.

Analysis of LSH-BR-BK cells with sera from tumor-bearing hamsters. LSH-BR-BK cells tested for T reactivity, whether with sera deriving from syngeneic tumor-bearing hamsters or with sera from another transplantable line (3), revealed an intensive nuclear immunofluorescence in >95% of the cells (Fig. 3a). LSH-BR-BK cells were also transplanted in syngeneic LSH hamsters (four passages in 52 animals). Subcutaneous injection of ~10^5 LSH-BR-BK cells resulted in tumors that killed the animals after 6 to 12 weeks; the dose that produced tumors in 50% of the animals was about 10^4 cells. In agreement with our previous results (4), sera containing anti-BKV T IIF titers of 1:320 were obtained from 69% (35 of 52) of tumor-bearing hamsters. Three animals developed antinuclear antibodies detected in LSH-BR-cells, reminiscent of findings previously reported (40). Fourteen sera with high anti-T reactivity were tested for the presence of antibodies to BKV-induced surface (S) antigen (Fig. 3c). Three sera were found to be positive as identified by an intense, speckled fluorescence staining of the cell surface. These sera failed to react with normal LSH-BR cells or LVG-333 cells.

Purity of plasma membranes. Using the strategy of subcellular fractionation described before (34), we isolated PM of LSH-BR-BK and LSH-BR cells with similar yields and of comparable purities. The specific activity of the Na^+-K^+-activated ATPase and ^{125}I was at least 25-fold higher in the PM fraction when compared with the cell homogenate. The yield of both PM markers was ≥60%. As nitrogen decompression of cells prevented concomitant destruction of the nuclei, <0.005% of the total cellular, [^{14}C]-thymidine-labeled DNA was associated with purified PM (34). A minimum lysis of mitochondria was indicated by a contamination of PM by about 0.4% of the total cellular monoamine oxidase.

TABLE 1. *Determination of the number of copies in LSH-BR-BK cells*

Sample	No. of counts	No. of copies[a]
LSH-BR plus 56 pg of BKV DNA	128	7.4
LSH-BR plus 112 pg of BKV DNA	245	14.8
LSH-BR-BK	762	46.0

[a] The number of copies was determined from the equation: [(micrograms of BKV DNA)/(micrograms of cellular DNA)] × 1.33 × 10^6 = copies per cell (36).

Reactivity of rabbit antiserum against plasma membranes of LSH-BR-BK cells. After five immunizations and extensive absorptions of the rabbit hyperimmune serum with living LSH-BR cells, a membrane immune fluorescence specific for LSH-BR-BK cells could be demonstrated (Fig. 3e). This serum did not react with the surfaces of LSH-BR cells (Fig. 3f). This heterologous serum did not yield a reaction with the nuclear T antigen. This most likely indicated that the BKV-specific protein in the plasma membrane expressed antigenic sites different from those of the nuclear T antigen, an observation reported for the nuclear and membrane-associated simian virus 40 M_r 90,000 to 100,000 T antigens (33, 37). However, the reactivity of tumor-bearing hamster sera and the rabbit hyperimmune serum with identical site specificities on LSH-BR-BK cells was indicated because F(ab')$_2$ fragments from the rabbit antiserum inhibited the reaction of the three hamster sera which had antibodies against S antigen (Table 2). No blocking was obtained with F(ab')$_2$ fragments from preimmune rabbit serum (Table 2).

TSTA activity of purified PM from LSH-BR-BK cells. In weanling hamsters the 50% tumor-producing dose was found to be 10^4 LSH-BR-BK cells per animal yielding tumors of an average size of 0.5 cm in diameter after 7 weeks. None of the hamsters immunized with PM from LSH-BR-BK cells had measurable tumors 5 weeks after transplantation, whereas hamsters in the control group had developed progressively growing tumors at the same time (Table 3). Ten weeks after challenge, tumors developing in immunized hamsters of group I and group II rep-

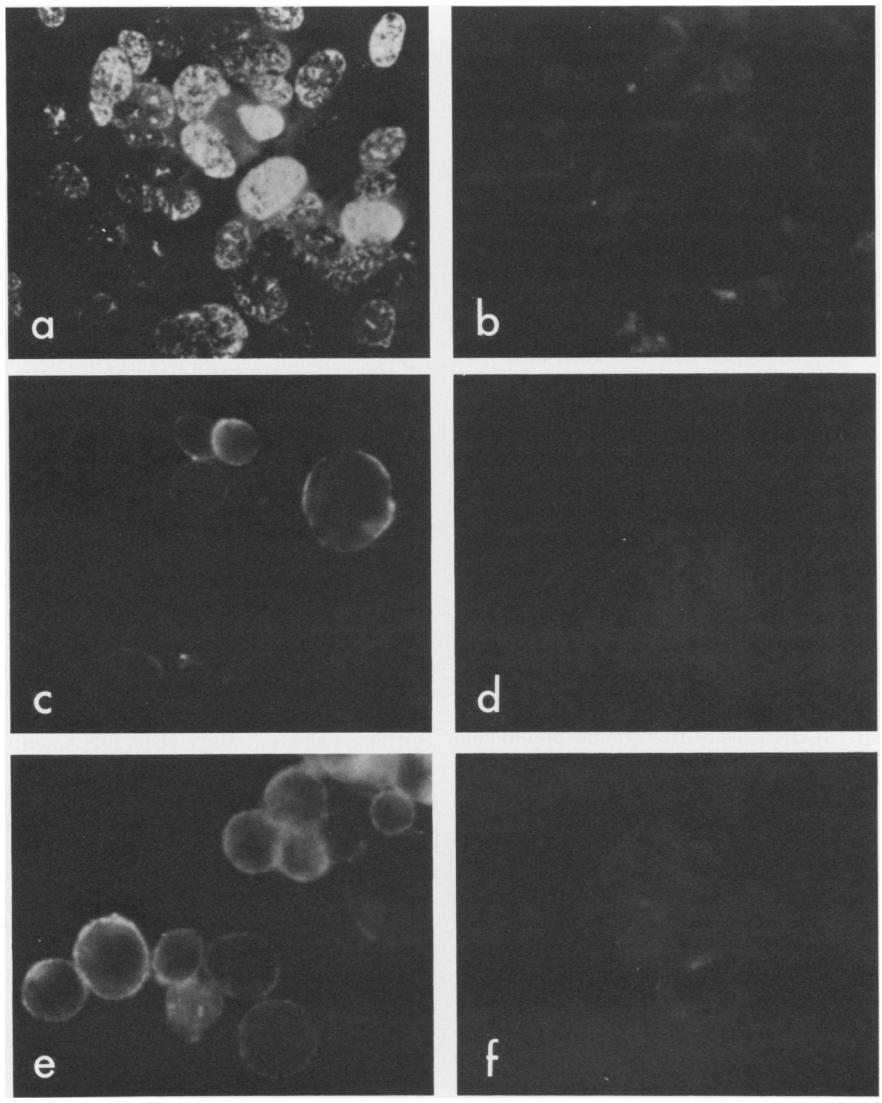

FIG. 3. *IIF patterns of LSH-BR-BK and LSH-BR cells reacted with serum from syngeneic tumor-bearing hamsters and with heterologous antiserum against purified PM of LSH-BR-BK cells. (a) LSH-BR-BK and (b) LSH-BR cells fixed in −20°C methanol-acetone for 3 min and reacted with tumor-bearing hamster serum at a 1:80 dilution (×500). (c) Live LSH-BR-BK and (d) live LSH-BR cells reacted with sera of tumor-bearing hamsters at 1:4 dilutions (×500). (e) Live LSH-BR-BK and (f) live LSH-BR cells reacted with rabbit anti-LSH-BR-BK PM serum at a 1:8 dilution (×500).*

resented 3 and 7%, respectively, of the mean tumor mass estimated in the nonimmunized hamsters. LSH hamsters immunized with PM from normal LSH-BR or LVG-333 cells did not differ significantly in their tumor rejection capacity from that of nonimmunized controls.

DISCUSSION

We have established an experimental syngeneic system to link biochemical and immunovirological studies on BKV and BKV-specific antigens with immunological responses in vivo.

Inbred LSH newborn hamster brain cells were transformed in vitro by the GS strain of BKV and yielded transplantable tumors that could be classified as undifferentiated glioblastomas. The GS strain of BKV, never passed in Vero cells, was obtained from S. D. Gardner after the fourth passage in human embryonic kidney cells. After four additional passages at low multiplicity of

infection in PHEB cells and CsCl purification only complete virions, sedimenting at 1.34 g/cm^3, were used for transformation experiments. Like the MM strain, the DNA of the GS strain contains three *Hin*dIII sites instead of four (23, 29). This results in the same regions of nonhomology of base sequences within 0.57 to 0.71 map units, which comprise the functions of the initiation and maintenance of transformation (23, 29). However, GS differs from MM in that the coding region for small t antigen is intact in GS (29), whereas the coding region for the carboxyl end of this antigen is deleted in MM (35). Viral DNA in transformed LSH-BR-BK cells was present in a large number of copies and existed predominantly in a free form. The possibility that integrated sequences are not detectable due to the presence of a large amount of copies of free viral DNA cannot be ruled out. The large numbers of free viral DNA (46 copies per cell) are not due to a virus carrier state in the LSH-BR-BK culture because (i) no viral antigen was detected by IIF, (ii) no virions were observed by electron microscopy, and (iii) >95% of the cells were exhibiting an intense T-antigen reactivity throughout all passages. Although this cell line was derived from a single colony of BKV-transformed cells, further cloning is now in progress to investigate the stability of viral DNA in subclones and to study the possible heterogeneous virus-host cell interactions (11, 24).

TABLE 2. *IIF of live LSH-BR-BK cells; blocking tests with rabbit antiserum against PM of LSH-BR-BK cells or with rabbit preimmune serum*[a]

Serum[b]	IIF titer	Dilution	IIF reaction after blocking with F(ab')$_2$ fragments[c]	
			R-PM	R-PI
TBH-1	8	2	−	+
TBH-2	8	2	−	+
TBH-3	8	2	−	+
R-PM	16	4	−	+
R-PI	2	4	−	−

[a] Live LSH-BR-BK cells were reacted with F(ab')$_2$ fragments from a rabbit antiserum against purified PM of LSH-BR-BK cells or from rabbit preimmune serum (negative control). The cells were then analyzed by IIF using three sera of tumor-bearing hamsters (TBH-1, 2, and 3).

[b] TBH, Tumor-bearing hamster serum (LSH-BR-BK transplant); R-PM, rabbit antiserum against PM; R-PI, rabbit preimmune serum.

[c] A protein concentration of 0.5 mg/ml of F(ab')$_2$ fragments was used. −, Blocking; +, no blocking.

The presence of BKV DNA in a free form in nonproducer BKV-transformed cells has been described by others (10, 11, 30, 31, 39, 42, 43, 47). Similar results were obtained from Simian virus 40 (16) and polyoma virus-transformed cells (5). The findings also parallel those on Epstein-Barr virus, as Epstein-Barr virus DNA sequences are found predominantly in a free form in tumor biopsies of nasopharyngeal carcinoma and Burkitt's lymphoma and in Burkitt's lymphoma-derived lymphoblastic cell lines (1, 25). Whether the free viral DNA is an excision product of tandem repeats of integrated sequences, as in the polyoma- (2) or simian virus 40- (6) trans-

TABLE 3. *Transplantation rejection of LSH-BR-BK cells in syngeneic weanling hamsters after immunization with plasma membranes from LSH-BR-BK cells (TSTA activity)*

LSH hamsters[a]	Immunized with:	Tumor challenge dose (cells)[b]	No. of tumor-bearing hamsters/total[c] (% tumor vol)[d] at days post challenge:	
			35	70
Group I	LSH-BR-BK PM	10^5	0/6 (0)	2/6 (3)[e]
	LSH-BR PM	10^5	2/6 (124)	6/6 (94)
	LVG-333 PM	10^5	2/8 (87)	7/8 (78)
	Phosphate-buffered saline	10^5	2/6 (100)	6/6 (100)
Group II	LSH-BR-BK PM	10^6	0/6 (0)	4/6 (7)
	LSH-BR PM	10^6	4/6 (82)	6/6 (104)
	Phosphate-buffered saline	10^6	4/6 (100)	6/6 (100)

[a] Group I LSH hamsters were immunized subcutaneously once with 40 μg and seven times with 20 μg of protein of purified PM from LSH-BR-BK cells; group II LSH hamsters were immunized once each with 5 μg and 20 μg and six times with 40 μg of protein. Control groups consisted of animals immunized with purified PM from LSH-BR and LVG-333 cells and nonimmunized animals.

[b] Hamsters were challenged 7 days after last immunization with 10^5 LSH-BR-BK cells per animal in group I and 10^6 LSH-BR-BK cells per animal in group II.

[c] Number of tumor-bearing hamsters per total number of hamsters.

[d] Mean tumor volumes were calculated at 35 days and 70 days postchallenge, and percent tumor volumes as compared with those of nonimmunized hamsters were calculated.

[e] $P = 0.005$.

formed cells, remains to be investigated.

We could demonstrate in a syngeneic system by immunization of inbred LSH weanling hamsters with highly purified PM of transformed LSH-BR-BK cells that these membranes induce a TSTA response in hamsters subsequently challenged with BKV-transformed cells. Furthermore, IIF reactivity of rabbit antiserum prepared against these PM could be demonstrated with LSH-BR-BK cells, but not with normal LSH-BR cells. In addition, analogous cell surface reactivity was found in 3 of 14 (21%) high-titer anti-BKV T sera from tumor-bearing hamsters, as defined by blocking with $F(ab')_2$ fragments of the rabbit antiserum. That certain tumor-bearing hamsters recognize not only the T antigen but also the cell surface antigens of BKV-transformed cells detectable by IIF is in agreement with a previous study reported by Lanford and Butel (26). Negative results obtained by others (9) may depend upon the anti-T serum used, although ^{125}I-labeled protein A was employed as a highly sensitive reagent for surface-bound immunoglobulin.

The conventional designation of papovavirus-induced surface antigens, TSTA and S antigen, as separate antigens has resulted from different identification techniques (8). The TSTA activity of highly purified LSH-BR-BK PM and the IIF reactivity of a rabbit antiserum against LSH-BR-BK PM with the surface of BKV-transformed cells suggest that TSTA and S antigen share common specificities, as also discussed by Lanford and Butel (26). The rabbit antiserum did not react with the nuclear antigen by IIF; however, it immunoprecipitated an M_r 96,000 and an M_r 55,000 polypeptide from purified LSH-BR-BK plasma membranes (Schmidt-Ullrich et al., submitted for publication). A host cell-modified M_r 90,000 to 100,000 T antigen appears to be expressed in plasma membranes of BKV-transformed cells (Schmidt-Ullrich et al., manuscript in preparation) as described for simian virus 40-transformed cells (33, 37).

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