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Antibodies to BK Virus Structural and Tumor Antigens in Human Sera from Normal Persons and from Patients with Various Diseases, Including Neoplasia

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Sera from 1,279 patients with various diseases were examined for the presence of antibodies to BK virus (BKV) capsid antigens. The percentage of positive sera was comparable in all the diseases except rheumatoid arthritis and chronic nephropathies, where a slightly higher prevalence was found. Sera from 952 patients with tumors were examined for the presence of antibodies to BKV tumor and capsid antigens in comparison with a matched control group of 501 blood donors. Sera from 11 tumor patients (1.15%) and from 4 normal controls (0.80%) had antibodies to BKV tumor antigen. No higher prevalence of antibodies to BKV capsid antigens was found in any cancer type except in carcinomas of the urinary bladder, where the percentage of positive sera and of sera with high titers was higher than in other groups. BKV infection is discussed in relation to its possible connection with human non-neoplastic diseases as well as with human tumors and to its activation under conditions of immunosuppressive therapy.

BK virus (BKV) is a new human papovavirus recently isolated by Gardner et al. (9) from the urine of a patient receiving immunosuppressive therapy after renal transplantation. Subsequent reports have confirmed the original isolation (5, 7, 14, 35), suggesting that the frequency of activation of BKV infection is related to the degree of immunosuppression (15). Serological evidence accumulated in England (8), in the United States (34), and in Italy (26) indicates that infection by BKV is common and widespread in normal human populations and that a majority of primary infections occurs in childhood.

Primary infection by BKV in children does not seem to produce any illness (8, 19). We also found a significant increase in antibody titer to BKV capsid antigens in only one of 82 paired sera from children with acute respiratory or intestinal diseases of probable viral etiology. Nevertheless, the presence of antibodies in adult human sera suggests that primary, persistent, or recurrent infections with BKV occur in adults. Therefore, it was of interest to investigate whether BKV might be related to some disease of adults. If so, it should be possible to detect a high prevalence and high titers of antibodies to BKV capsid antigens in sera from patients affected by a specific disease.

Another problem deserving attention is the possible correlation of BKV with human neoplasia. The question is pertinent for several reasons: (i) BKV belongs in the papovavirus group and most papovaviruses are oncogenic (3); (ii) the incidence of malignant tumors is high in renal transplant recipients (21-23); (iii) BKV transforms in vitro hamster cells (18, 25) as well as mouse and simian cells (G. Barbanti-Brodano, manuscript in preparation) and is oncogenic in vivo for newborn hamsters (33); (iv) antibodies to BKV tumor (T) antigen have been detected in human sera (32); (v) BKV has been recently isolated from a reticulum cell sarcoma of the brain (41).

In the experimental models of the oncogenic papovaviruses polyoma (12), simian virus 40 (SV40) (4), and BK (25, 33), the tumor antigen is generally associated with virus transformation and oncogenicity. Moreover, a high proportion of animals developing tumors induced either by virus or by transformed cells produce antibodies to T antigen (3, 4, 11, 12, 25, 33). Consequently, if human tumors etiologically related to BKV infection exist, antibodies to BKV T antigen could be detected in the patients' sera.

This paper deals with an epidemiological study of the prevalence of antibodies to BKV

capsid antigens in sera from patients affected by various diseases. Furthermore, a survey on the prevalence of antibodies to BKV T and capsid antigens in sera from cancer patients and from normal controls is presented.

MATERIALS AND METHODS

Sera and cerebrospinal fluids (CSFs). Sera from patients with various diseases were collected in the Department of Medicine, University Hospital, Bologna. These sera corresponded to a sample of patients observed during a time interval of several months and included both the common and the rare inflammatory, degenerative, and endocrine-dismetabolic diseases of the different apparatus and organs.

Sera from cancer patients, affected by almost all types of common and rare human tumors, were collected in the Institutes for the Study and the Therapy of Tumors in Rome, Milan, and Ancona and in the University Hospital, Ferrara. As serological evidence has been supplied that meningiomas are related to SV40 (45), whose structural and T antigens are similar to those of BKV (9, 20, 24, 40), 12 sera from patients with meningioma and 24 sera from patients with other tumors of the nervous system were examined. Since antiviral antibodies in CSF have been detected in virus diseases of the central nervous system (16, 42), CSFs from several patients with brain tumors were tested for the presence of antibodies to BKV T and capsid antigens.

Since a coincidence between high frequency of malignancy and of BKV activation was observed in renal transplant patients, 113 sera from renal transplant patients, collected in the University Hospital, Milan, were analyzed for the presence of antibodies to BKV T and capsid antigens. Sera of normal controls were obtained from healthy blood donors, matched in age and sex with the patients, in the Blood Centers of the University Hospitals in Ferrara and Bologna.

HAI test for detection of antibodies to BKV capsid antigens. The hemagglutination inhibition (HAI) test was carried out in disposable plates by the microtiter method (29) as previously described (26). Sera were pretreated with sodium periodate to remove nonspecific inhibitors. Only HAI titers equal to or higher than 128 were considered positive, because only above this serum dilution was a strict correlation found between the results of the HAI test and those of the more specific immunofluorescence (IF) test for BKV capsid antigens (26).

IF for detection of antibodies to BKV T antigen. Hamster kidney and rhesus monkey kidney cells transformed by BKV, 100% positive for T antigen (25), were used to detect antibodies to BKV T antigen. The indirect technique was used throughout. BKV-transformed cells were cultured on cover slips, fixed in acetone, and exposed first to human serum (diluted 1:2 in phosphate-buffered saline) and then to fluorescein-conjugated goat immunoglobulin G (IgG) to human immunoglobulins (IgM, IgA, IgG, Hyland, Cosa Mesa, Calif.; diluted 1:5 in phosphate-buffered saline). Cells were never stored, and the IF test was always run immediately after fixation. The continuous presence of T antigen in BKV-transformed cells was monitored periodically by IF staining with a hamster serum to BKV T antigen, obtained from animals bearing tumors induced by BKV-transformed cells (25). To test the specificity of the positive reaction to BKV T antigen in human sera, a blocking test was performed. BKV-transformed cells were first reacted with a human serum found positive in a previous normal IF test. Hamster serum to BKV T antigen was then added, followed by fluorescein-conjugated antibodies to hamster IgG.

RESULTS

Antibodies to BKV capsid antigens in sera of patients with various diseases. HAI antibodies to BKV capsid antigens were detected in 417 of 1,279 sera (32.7%). Of the patients with HAI antibodies, 234 (56.1%) were males and 183 (43.9%) were females. They were distributed in all age groups between 20 and 70 years. There was no significant difference in the prevalence of HAI antibodies in different age groups and in different disease groups. Also, the range of titers and percentage of sera with HAI titers equal to or higher than 512 were not significantly different in the various groups (Table 1). No specific disease in each disease group showed a prevalence of positive sera or of titers equal to or higher than 512. The only diseases showing a slightly higher incidence of positive sera and of titers equal to or higher than 512 were rheumatoid arthritis and chronic nephropathies. The latter also showed a high limit in the range of titers (four sera had an HAI titer higher than 8,192; Table 1).

Antibodies to BKV T and capsid antigens in sera and CSFs of tumor patients and of normal controls. When the antibody prevalence was examined by the site or type of tumor, antibodies to BKV T antigen were detected in sera from 11 of 952 neoplastic patients (1.15%). The tumors of the T antibody-positive patients were three lung carcinomas, two rectum carcinomas, one stomach carcinoma, one kidney carcinoma, one carcinoma of the urinary bladder, one lymphosarcoma, one acute leukemia, and one cerebellar astrocytoma (Table 2). Antibodies to BKV T antigen were detected also in sera from 4 of 501 normal controls (0.80%; Table 2). The four T antibody-positive controls were healthy at the moment of giving serum and did not declare any significant diseases in their past history, except one who had suffered from malaria and another who had undergone an operation for a cyst of the kidney. The positive sera from both cancer patients and normal controls gave a typical T antigen staining on BKV-transformed cells (Fig. 1), did not stain normal cells, and were able to block a specific IF stain-

TABLE 1. *Prevalence of antibodies to BKV capsid antigens in sera of patients affected by various diseases*

Type of disease	No. (%) of sera with antibodies/ no. of sera tested		Range of titers	No. (%) of sera with HAI titer of 512 or higher
Hemopathies	9/49	(18.3)	128-1,024	2 (22.2)
Heart diseases	77/276	(27.8)	128-2,048	23 (29.8)
Vascular diseases	29/118	(25.4)	256-2,048	6 (20.7)
Digestive diseases	20/72	(27.7)	128-2,048	7 (35.0)
Endocrine diseases	42/100	(42.0)	128-4,096	13 (30.9)
Rheumatic diseases	65/213	(30.5)	128-4,096	23 (35.3)
Liver diseases	21/85	(24.7)	128-2,048	6 (28.6)
Acute pneumopathies	7/18	(38.8)	128-512	1 (14.3)
Chronic pneumopathies	4/24	(16.6)	128-1,024	1 (25.0)
Diseases of the central nervous system	3/7	(42.8)	256-8,192	1 (33.3)
Undetermined febrile states	19/64	(29.6)	128-2,048	2 (10.5)
Chronic compensated nephropathies	12/43	(27.9)	128-2,048	9 (75.0)
Chronic decompensated nephropathies [a]	105/190	(55.2)	128->8,192	49 (46.6)
Miscellaneous diseases	4/20	(20.0)	256-2,048	1 (25.0)
Total	417/1,279	(32.7)	128->8,192	144 (34.5)
Rheumatoid arthritis [b]				
Group 1	18/31	(58.1)	128-4,096	10 (55.5)
Group 2	36/115	(31.4)	128-1,024	12 (33.3)

[a] Patients treated with hemodialysis.

[b] Cases of rheumatoid arthritis were taken from the 213 cases of rheumatic diseases. Group 1, Patients subjected to immunosuppressive therapy with prednisone and azathioprine; group 2, patients subjected to non-immunosuppressive therapy.

ing produced by a hamster serum to BKV T antigen, thereby proving the specificity of the reaction.

Antibodies to BKV structural antigens were detected in sera from 197 cancer patients (20.7%; Table 2). Positive sera were found in 131 of 617 patients affected by epithelial tumors (21.2%) and in 66 of 335 patients affected by mesenchymal and miscellaneous tumors (19.7%). No significant differences between the various types of tumors were found regarding the percentage of positive sera, the range of titers, and the percentage of sera with HAI titers equal to or higher than 512. The only exceptions were the patients with carcinomas of the urinary bladder, who showed the highest percentage of positive sera (45.1% in comparison to 20.7% for all categories), the highest upper limit in the range of titers (two sera had HAI titers higher than 8,192), and the highest percentage of sera with titers of 512 or higher (57.1%; Table 2). Antibodies to BKV capsid antigens were detected in 201 of 501 sera from normal controls (40.1%; Table 2).

Eight of the 11 sera from tumor patients containing T antibodies had a low titer (128 to 256) and three were negative for antibodies to BKV capsid antigens. The four sera from normal controls positive for T antibodies had a high titer (1,024 to 2,048) of antibodies to BKV capsid antigens (Table 3). Of all tumor patients, 523 (55.0%) were on radiotherapy and/or treatment with antiblastic drugs (cyclophosphamide, cytosine arabinoside, adriamycin, vincristine, methothrexate, and prednisone) and 201 (21.2%) had diffused or metastasized tumors (Table 4). Nine of the 11 patients whose sera were positive for T antibodies were on treatment and had diffused tumors or metastasis (Table 4). Serum from the T antibody-positive patient with kidney carcinoma was tested and found positive again 5 months after removal of the tumor, when metastases were detected. No T antibodies were detected in sera and CSFs from patients with meningiomas, and no antibodies to BK virus capsid antigens were found in CSFs of the same patients. CSFs from 10 patients, 6 with gliomas and glioblastomas, 2 with medulloblastomas, and 2 with astrocytoma (including the one positive for T antibodies in serum), were negative for antibodies both to T and structural antigens of BKV, even in two cases showing a high titer (512) of antibodies to structural antigens in serum.

Five of 113 sera (4.4%) from renal transplant patients contained antibodies to BKV T antigen and 48 (42.4%) had antibodies to BKV capsid antigens (Table 2).

DISCUSSION

The search for antibodies to BKV structural antigens in sera from patients with various diseases did not reveal any significant correlation between a specific disease or disease group and BKV infection, since no higher incidence of antibodies was found in comparison to normal controls. Also, sera with HAI titers equal to or higher than 512, probably indicating acute infection by BKV, were found at a comparable

TABLE 2. *Prevalence of antibodies to BKV T antigen and capsid antigens in sera from cancer patients, renal transplant recipients, and normal controls*

Site or type of tumor	No. of sera tested	No. (%) of sera with antibodies		Range of HAI titers	No. (%) of sera with HAI titers of 512 or higher
		T[a]	V[b]		
Lung	56	3(5.3)	13(23.3)	128–512	3 (23.1)
Breast	209	0	46(22.0)	128–1,024	12 (26.0)
Sigmoid colon and rectum	73	2(2.7)	13(17.9)	128–1,024	3 (23.1)
Colon	8	0	2(25.0)	128–256	0
Stomach	50	1(2.0)	7(14.0)	256–1,024	2 (28.5)
Esophagus	10	0	2(20.0)	128–256	0
Prostate	6	0	1(16.7)	128	0
Kidney	31	1(3.2)	5(16.2)	128–512	1 (20.0)
Pancreas	14	0	3(21.5)	128–512	1 (33.3)
Ovary	18	0	5(27.8)	128–1,024	1 (20.0)
Liver	12	0	3(25.0)	128–512	1 (33.3)
Skin, lip, vulva	49	0	9(19.4)	256–2,048	3 (33.3)
Uterus	32	0	5(15.5)	128–1,024	1 (20.0)
Larynx	30	0	5(16.7)	128–256	0
Urinary bladder	31	1(3.2)	14(45.1)	128–>8,192	8 (57.1)
Thyroid	24	0	5(20.9)	128–2,048	2 (40.0)
Fibrosarcoma	32	0	5(15.7)	128–256	0
Osteosarcoma	11	0	3(27.3)	128	0
Lymphosarcoma	15	1(6.6)	3(20.0)	128–512	1 (33.3)
Reticulum cell sarcoma	16	0	2(12.5)	128–256	0
Melanoma	45	0	8(17.8)	128–1,024	3 (37.5)
Lymphoma	32	0	7(21.9)	128–1,024	3 (42.8)
Hodgkin's disease	52	0	12(23.7)	128–1,024	5 (41.6)
Leukemia	40	1(2.5)	4(10.0)	128–256	0
Plasmacytoma	3	0	0	0	0
Nervous system[c]	24	1[d](4.2)	7(29.1)	128–512	2 (28.6)
Meningioma	12	0	4(25.0)	128–1,024	1 (25.0)
Seminoma	5	0	1(20.0)	128	0
Wilms tumor	3	0	1(33.3)	128	0
Pleural mesotelioma	3	0	1(33.3)	128	0
Salivary glands	6	0	1(16.7)	256	0
Total	952	11(1.15)	197(20.7)	128–>8,192	53 (26.9)
Renal transplant recipients	113	5(4.40)	48(42.4)	128–2,048	18 (37.5)
Normal persons	501	4(0.80)	201(40.1)	128–8,192	86 (42.7)

[a] Antibodies to BKV T antigen detected by IF on BKV-transformed cells.
[b] Antibodies to BKV capsid antigens detected by HAI.
[c] Tumors of the nervous system: 1 oligodendroglioma, 4 medulloblastomas, 8 gliomas and glioblastomas, 7 astrocytomas, and 4 neurinomas.
[d] Cerebellar astrocytoma.

frequency in each disease group and in normal controls (see also references 8, 26, and 34). The significance of the slightly higher prevalence of positive sera among patients affected by rheumatoid arthritis and chronic nephropathies, as compared to patients affected by other diseases and to normal controls, is not clear at present. Further investigation should establish whether BKV infection bears some relationship to the pathogenesis of these diseases or is merely activated by the immunosuppressive therapy. Our results tend to support the latter hypothesis. Indeed, in the group of patients affected by rheumatoid arthritis the higher prevalence of positive sera was associated with the immunosuppressive therapy (prednisone and azathioprine). In addition, most of the patients affected by chronic nephropathies were on treatment with cyclophosphamide, and patients subjected to hemodialysis were immunosuppressed as a consequence of the toxic state, determined by uremia. In this connection it has been shown recently that BKV infection is activated by immunosuppressive therapy in situations other than renal transplantation (28).

No significant difference was found between the prevalence of BKV T antibodies in sera from cancer patients and from normal persons,

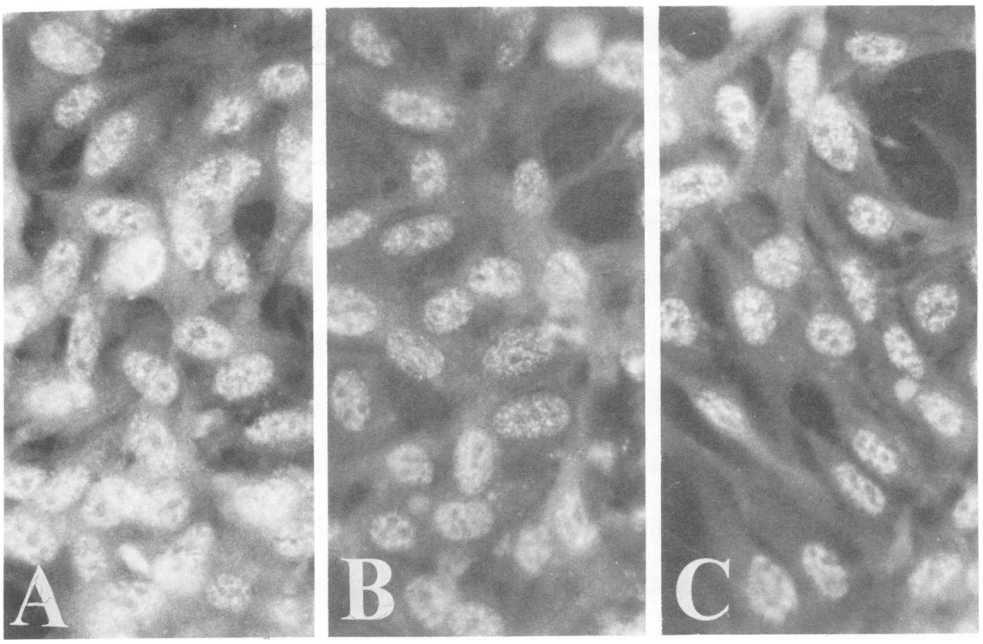

FIG. 1. *T antigen-positive staining of BKV-transformed rhesus monkey kidney cells reacted by IF with human sera from: (A) a patient with renal carcinoma; (B) a patient with acute leukemia; (C) a normal blood donor.*

TABLE 3. *Antibody titers for BKV capsid antigens in sera from T antibody-positive cancer patients and normal controls*

Antibodies to BKV T antigen in serum	Antibody titer for BKV capsid antigens
Patients	
Lung carcinoma	128
Lung carcinoma	Negative
Lung carcinoma	128
Rectum carcinoma	128
Rectum carcinoma	Negative
Stomach carcinoma	128
Kidney carcinoma	128
Urinary bladder carcinoma	Negative
Cerebellar astrocytoma	256
Acute leukemia	128
Lymphosarcoma	128
Normal controls	
1	2,048
2	2,048
3	1,024
4	2,048

although sera from cancer patients had a low titer or were negative for antibodies to BKV capsid antigens, whereas sera from normal controls had a high titer of antibodies to BKV capsid antigens. This could suggest that normal persons produced antibodies to BKV T antigen while undergoing acute infection by BKV. Production of T antibodies, always accompanied by a rise in serum titer of antibodies to capsid antigens, has been reported during acute infection of monkeys with SV40 (10, 27, 36, 43) as well as of humans with adenoviruses (17) and BKV (32). On the contrary, the presence of antibodies to BKV T antigen in sera from cancer patients, accompanied by a low level or absence of antibodies to BKV capsid antigens, might be due to the integration of the viral genome into the cell genome with consequent minimal or lack of production of infectious virus. Antibodies to virus structural antigens are barely detectable or absent in animals bearing tumors induced by polyoma (13), SV40 (4, 11), or BKV (33). Alternatively, cancer patients also may have suffered acute infection by BKV without displaying high titers of antibodies because of the immunosuppressive therapy. Indeed, 9 of the 11 positive cancer patients were on radiotherapy and/or antiblastic chemotherapy treatment. Observations, documented with virus detection by electron microscopy or virus isolation in urine, have been made on two immunosuppressed patients undergoing acute infection by BKV (5) or progressive multifocal leukoencephalopathy-SV40 (44), respectively, where serum antibodies to virus capsid antigens were undetectable. Nine of 11 tumors from T antibody-positive cancer patients were dif-

fused or metastasized, suggesting that tumor diffusion or metastasis might have contributed to antigenic stimulation for production of T antibodies.

Antibodies to BKV structural antigens were also not prevalent in any type of tumor. All the groups of tumor patients showed a slightly lower percentage of positive sera as compared to normal controls, probably because of immunosuppressive therapy. Acute infection by BKV seems the most likely explanation of the higher prevalence of positive sera and of high antibody titers to BKV capsid antigens in sera from patients with tumors of the urinary bladder. Indeed, BKV produces a persistent infection with viruria in humans (5, 9, 14, 15), as SV40 does in monkeys (1, 39); therefore, it is possible that the bladder mucosa, altered by the neoplasm and exposed to virus, becomes readily infected. The possibility that BKV infection may directly contribute to the production of neoplasms of urinary bladder seems to be unlikely, because only 1 patient out of 31 (3.2%) had T antibodies in his serum. It is interesting that a high prevalence of neutralizing antibodies to SV40 was found in sera from patients with cancer of the urinary bladder (30). Since BKV and SV40 are antigenically related (9, 20, 24, 40) and SV40 infection is rare in humans (30, 31, 37, 38) as compared to the high frequency of BKV infection (8, 26, 34), it is likely that antibodies to BKV capsid antigens were detected in that study. An investigation of the frequency of BKV isolation from urine of patients with cancer of the urinary bladder is underway.

TABLE 4. *Treatment and diffusion or metastasis of tumors in all the cancer patients and in the patients positive for T antibodies*

Patients	Treated[a]	Not treated	Diffused tumor or metastasis	Localized tumor
Cancer patients	523 (55.0%)	429 (45.0%)	201 (21.2%)	751 (78.8%)
Patients positive for T antibodies				
Lung carcinoma	+		+	
Lung carcinoma	+		+	
Lung carcinoma		+	+	
Rectum carcinoma	+			+
Rectum carcinoma	+		+	
Stomach carcinoma	+		+	
Kidney carcinoma		+	+	
Urinary bladder carcinoma	+		+	
Cerebellar astrocytoma	+			+
Acute leukemia	+		+	
Lymphosarcoma	+		+	

[a] With irradiation and/or antiblastic chemotherapy.

The lack of T antibodies in sera of patients affected by meningiomas and the low percentage of T antibody-positive sera among patients with other tumors of the nervous system may depend on insufficient stimulation of the immune system due to the segregated localization of tumors and would not rule out a possible correlation between these tumors and BKV infection. Indeed, it has been reported that serum antibody levels may be low in viral infections of the central nervous system (2). On the other hand, cultured cells from seven human meningiomas have been found negative when tested by IF with hamster serum to BKV T antigen.

A higher percentage of T antibody-positive sera (4.4%) was found in renal transplant patients in comparison to normal controls (0.80%). Although it is impossible at present to decide whether malignant tumors of renal transplant recipients are etiologically related to BKV, our results confirm that these patients represent a very interesting group for a prospective study of the connection between BKV and human neoplasia.

The possibility that we were detecting in human sera antibodies to SV40 T antigen, which is similar to BKV T antigen (40), is unlikely, because all the T antibody-positive cancer patients and normal controls had no neutralizing antibodies to SV40 in their sera, except one control and one patient who had a neutralizing titer of 1:8. On the contrary, since infection by JC virus, the human papovavirus most commonly associated with progressive multifocal leukoencephalopathy, is diffused as BKV infection in humans and JC virus T antigen is

closely related to that of BKV (20), it cannot be excluded that T antibodies detected in human sera had arisen from infection with JC virus.

On the basis of the foregoing results, showing a low percentage of T antibody-positive sera among cancer patients, a lack of significant difference between the percentage of positive sera of cancer patients and of normal controls, and a lack of a definite clustering of positive sera in a specific tumor type, it would be difficult to postulate an extensive etiological role of BKV in human neoplasia. It cannot be excluded, however, that BKV may be occasionally related to some human tumor. On the other hand, the search for T antibodies in sera of cancer patients is a preliminary, but by no means a conclusive, approach to the study of the BKV relationship to human tumors. Indeed, in animals bearing SV40-induced tumors that contain T antigen, T antibodies may not be produced if the tumor is small. Moreover, in some SV40-induced tumors T antigen is not demonstrable; only the surface (S) antigen is present, and animals bearing these tumors do not produce T antibodies (6). Therefore, the problem of the connection of BKV with human neoplasia will have to be solved on the basis of more direct tests, such as demonstration of BKV T and S antigens and of integrated viral genome in neoplastic cells. Experiments along these lines are underway.

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