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BK Virus T Antigens Induce Kidney Carcinomas and Thymoproliferative Disorders in Transgenic Mice

STACIE A. DALRYMPLE AND KAREN L. BEEMON*

Department of Biology, the Johns Hopkins University, Baltimore, Maryland 21218

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Renal adenocarcinomas and/or extremely enlarged thymuses (up to 250 times normal size) were observed in 60 of 78 mice in a transgenic line containing a single copy of the BK virus (BKV) early region. Enlarged thymuses from different mice displayed thymoproliferative disorders of varying severity, ranging from extreme hyperplasia to thymomas and lymphomas. All kidney tumor DNAs analyzed contained highly amplified BKV sequences with multiple rearrangements in cellular DNA flanking the transgene, whereas amplification and rearrangement were observed only in some enlarged thymus DNAs. Expression of BKV T antigens was restricted to epithelial cells of kidney tumors and enlarged thymuses and was not detected in any normal tissues. Although thymocytes proliferated to numbers much greater than normal in the enlarged thymuses, no T antigen expression was detected in thymocytes.

BK virus (BKV) is a human papovavirus which has been isolated from the urine of immunosuppressed individuals, including renal and bone marrow transplant patients, and from pregnant women (10, 18). BKV is widespread, with approximately 75% of the adult human population testing seropositive (18). Infection appears to occur early during childhood and to persist in a latent state, probably in the kidneys (11). Reactivation of BKV can occur when the immune system is weakened (11). Although BKV DNA has been found in a variety of human tumors, including those of the kidney, brain, liver, and lung (7, 20), a causal linkage to human disease has not been established. BKV can, however, transform numerous types of cells in culture and is known to be oncogenic in rodents (18, 22).

The genomes of the human papovaviruses, BKV and JC virus (JCV), are 70 to 80% homologous to that of simian virus 40 (SV40), and their large tumor antigens cross-react serologically (9, 18, 23). However, the regulatory regions of these viruses are only about 40% homologous. This difference in homology may contribute to the differences in host range and pathogenicity of these viruses (21). Transgenic mice harboring the SV40 early region develop choroid plexus tumors (3, 26). Linking the SV40 T-antigen gene to various promoters directs tumors to other tissues (14, 17).

In order to compare the tissue specificity and pathology of BKV and JCV with those of SV40, transgenic mice containing the BKV or JCV early region, including the promoter, enhancer, and coding sequences for the small and large tumor antigens, were generated (24). Four of the five JCV early region transgenic mice that survived to maturity developed metastatic adrenal neuroblastomas (24). Both surviving BKV transgenic animals developed primary renal and hepatocellular tumors at the age of 8 to 10 months; no other obvious pathological changes were seen (24). A line was established from one of the BKV transgenic mice (BK37), whereas the other mouse was infertile.

We have studied the BK37 mouse line, which contains a single intact copy of the BKV early region, through several generations to further observe the effects of the BKV early region on these animals. We observed that these transgenic mice developed renal adenocarcinomas (40%) and/or grossly enlarged thymuses (74%) by the age of 8 to 10 months. Pathological changes of the thymus included extreme hyperplasia, thymomas, and lymphomas. All of the kidney tumor DNAs and many of the enlarged thymus DNAs contained BKV sequences that were amplified and displayed DNA rearrangements in the vicinity of the transgene. All kidney tumors and enlarged thymuses analyzed expressed BKV T-antigen mRNAs; however, no BKV RNA was detected in any normal tissues. Expression of T antigen was restricted to epithelial cells in both the kidney and thymus.

MATERIALS AND METHODS

Characterization of transgenic mice. Progeny of the BK37 founder were kindly provided by George Scangos. The transgenic founder had been generated by microinjection of a 3.2-kilobase (kb) *Pvu*II fragment containing the entire BKV (Dunlop) early region, as previously described (24). The transgenic progeny were outbred with CD1 mice (Charles River Breeding Laboratories, Inc.), and the BK37 line has been carried for seven generations. Most mice were sacrificed between 8 and 10 months of age, when they manifested breathing difficulties. All remaining animals were sacrificed at 12 months of age. Tissues for DNA and RNA extraction were frozen on dry ice and stored at −80°C. Tissues for histological analysis were fixed in Formalin, paraffin embedded, sectioned at 5 μm, and stained with eosin and hematoxylin.

RNA isolation and RNase protection assay. Tissues were homogenized in RNAzol (Cinna/Biotecx) using a Tekmar Tissuemizer (Tekmar Company), and RNA was isolated as described by Chomczynski and Sacchi (6). Plasmid pBKV, which originated in the laboratory of Peter Howley, was obtained from Judy Small. The BKV 3′ riboprobe template was constructed by inserting a 576-base-pair (bp) *Xba*I-*Bgl*II fragment of pBKV into pGEM-1 (Promega Biotec). The 5′ riboprobe template was similarly constructed by inserting a 1,446-bp *Mbo*I fragment of pBKV into pGEM-1. The 3′ plasmid construct was linearized with *Xba*I, and the 5′ plasmid construct was linearized with *Mst*II. The plasmid T7β2 used to generate the antisense β2 microglobulin RNA was a gift from Susan Mango. Antisense riboprobes were transcribed from the templates with T7 polymerase (Pharmacia, Inc.) and hybridized in excess to 20 μg of total tissue

* Corresponding author.

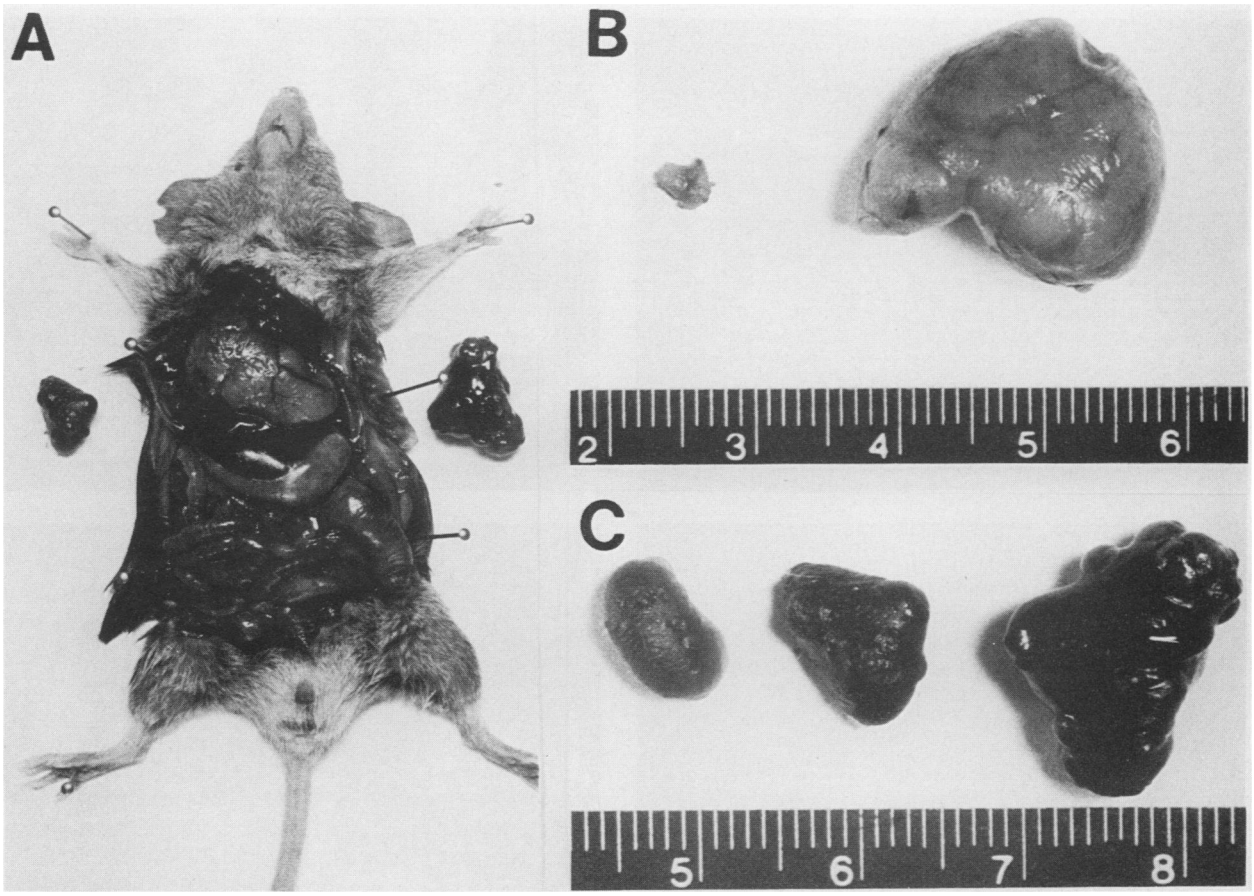

FIG. 1. Pathological changes of BK37 transgenic mice. (A) Dissection of a BK37 mouse revealed bilateral kidney tumors and a grossly enlarged thymus. (B) Comparison of a grossly enlarged thymus (right) with a normal thymus (left) from a CD1 mouse of similar age. (C) Comparison of bilateral kidney tumors (center and right) from a BK37 mouse with an undiseased kidney (left) from a CD1 mouse of similar age. Scale is in centimeters.

RNA as described previously (16). Unhybridized RNAs were eliminated by digestion at 37°C with 0.1 μg of RNase A (Boehringer Mannheim Biochemicals) per ml and 10 U of RNase T_1 (Calbiochem-Behring) per ml. The protected fragments were ethanol precipitated, denatured at 90°C in 95% formamide, and electrophoresed on a 6% acrylamide–8 M urea sequencing gel (15).

Analysis of DNAs. DNA was isolated from various tissues as previously described (13), and 15 μg of DNA was digested overnight with 30 to 40 U of restriction enzyme (New England BioLabs, Inc. Boehringer Mannheim) according to the specifications of the manufacturers. Samples were then electrophoresed for 5 h on either 1 or 2.5% agarose gels, blotted according to Southern (25), and hybridized at 42°C as described by Wahl and Berger (28) with oligo-labeled probes (8). The probes, isolated from pBKV, were a 3.2-kb *Pvu*II fragment, a 1,443-bp *Hin*dIII fragment, and a 757-bp *Stu*I fragment. Crude thymus fractionation was achieved by teasing the thymus in phosphate-buffered saline (PBS) with a razor blade, and filtering it through a Nitex membrane.

Indirect immunofluorescence of thymic and renal sections. Enlarged thymuses and tumor-bearing kidneys were harvested from BK37 mice and frozen in 2-pentanone. Tissues were subsequently embedded in O.C.T. Compound (Tissue-Tek), and 6-μm sections were cut on a cryostat. Thymic sections were fixed for 30 min in absolute methanol at 4°C and then made permeable with acetone for 20 min at 4°C. Primary antibodies, diluted in PBS with 10% calf serum (PBS/10), were bound for 1 h. After several rinses in PBS/10, fluorescein- or rhodamine-conjugated second antibody, diluted in PBS with 50% calf serum, was bound for 30 min. Antibodies used were a guinea pig anti-keratin (Dako), a fluorescein-conjugated rabbit anti-hamster immunoglobulin G (Cappel), a rhodamine-conjugated rabbit anti-guinea pig immunoglobulin G (Cappel), and serum from a hamster bearing an SV40-induced tumor (a gift from Gilbert Jay). This serum had previously been shown to cross-react with BKV T antigen. In the kidney sections, the guinea pig anti-keratin antibody, diluted in PBS/10, was bound to unfixed kidney tumor sections for 1 h. After being washed, the section was fixed in absolute methanol for 15 min at 4°C and made permeable by incubation for 15 min in acetone at 4°C. The hamster tumor serum, diluted in PBS/10, was bound for 1 h. Secondary antibodies were bound as described above. Sections were also stained with Hoechst 33258 (Boehringer Mannheim). The punctate nuclear-staining pattern observed with the tumor serum was identical to that observed with Hoechst 33258.

RESULTS

Incidence of pathological changes in BK transgenic mice. A majority (74%) of the transgenic mice harboring the BKV

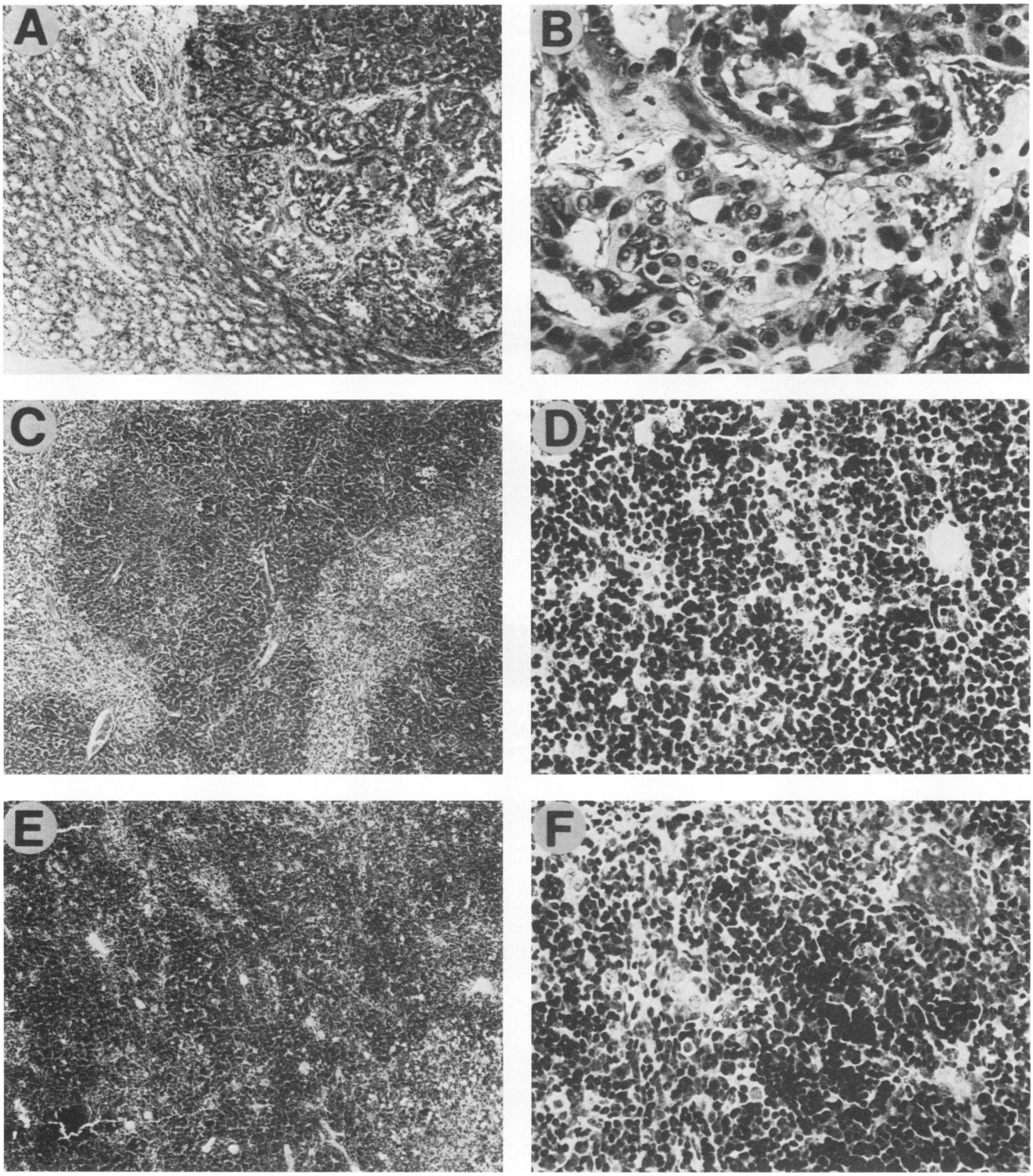

FIG. 2. Histological study of kidney and thymus changes in BK transgenic mice. (A) BK37 renal adenocarcinoma. Note the prominent tumor junction. Invasive neoplastic epithelial cells (right) were infiltrating the normal kidney tissue (left). Original magnification, ×90. (B) The same kidney tumor showing many giant tumor cells. Original magnification, ×400. (C and E) Two grossly enlarged thymuses of similar size. The thymus pictured in panel C, classified as extreme hyperplasia, showed intact basic architecture, but the aggregate amount of medullary tissue was increased, so the cortex was not clearly definable. The thymoma shown in panel E displayed abnormal thymic architecture. The neoplastic component was epithelial; however, there was a moderately large component of nonneoplastic lymphocytes. Original magnification, ×40. (D and F) Higher magnifications of the thymuses shown in panels C and E. Note the preponderance of neoplastic epithelial cells (light colored) in the thymoma (F). Original magnification, ×400.

early region displayed respiratory difficulties at approximately 8 to 10 months of age. These mice tended to weigh 20 to 30% less than nontransgenic littermates, and dissection revealed that their thoracic cavities were entirely occluded by their thymuses, which frequently reached 7 to 10% of total body weight (Fig. 1A). A typical enlarged thymus weighed 2.0 to 2.5 g, while that of a normal 8- to 10-month-old mouse weighed approximately 0.01 g (Fig. 1B). These thymuses, despite their large sizes, usually appeared morphologically normal upon gross inspection, although sometimes the two lobes of a thymus were enlarged to very different degrees. In a few rare cases, the thymuses appeared to contain nodules. Occasionally, enlarged lymph nodes and spleens were also observed in conjunction with thymic enlargement.

On the basis of histological analysis of thymic sections from BK37 mice, veterinary pathologists diagnosed thymoproliferative disorders of varying severity. In some enlarged thymuses, classified as extreme hyperplasia, the basic thymic architecture was intact (Fig. 2C and D). Thymomas, in which the normal thymic architecture was altered by invasive neoplastic epithelial cells, were also observed (Fig. 2E and F). Additionally, thymic lymphomas, in which the cell

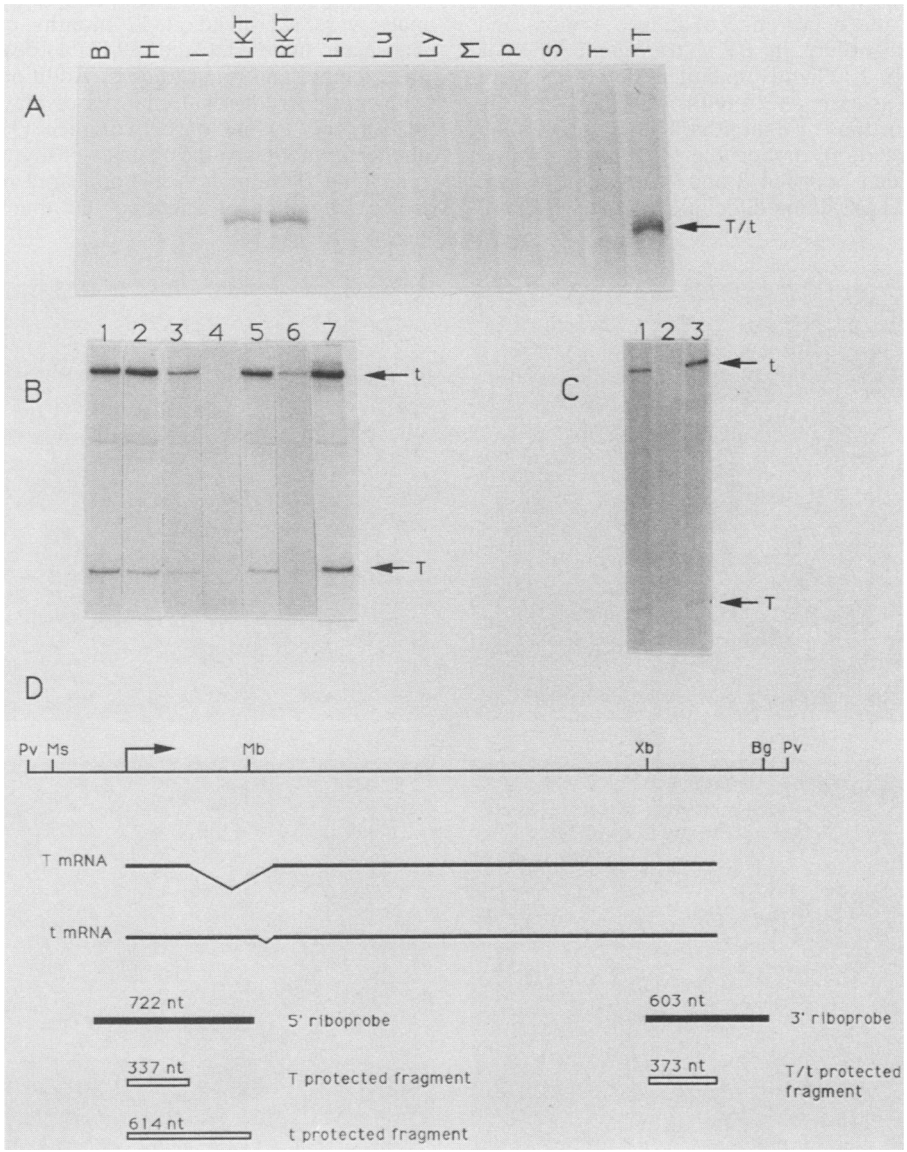

FIG. 3. Expression of T-antigen mRNAs in BK37 transgenic mice. (A) RNA from various tissues of a BK37 transgenic mouse was analyzed by an RNase protection assay, using the 3′ riboprobe. Lanes: B, brain; H, heart; I, intestine; LKT, left kidney tumor; RKT right kidney tumor; Li, liver; Lu, lung; Ly, lymph node; M, muscle; P, pancreas; S, spleen; T, testes; TT, enlarged thymus. (B) RNA from various tumors of transgenic mice was analyzed by an RNase protection assay, using the 5′ riboprobe. Small and large T-antigen-protected fragments are labeled as t and T, respectively. Lanes: 1 to 3, RNA from three different enlarged thymuses; 4, RNA from a normal-appearing BK37 kidney; 5 to 7, RNA from three different kidney tumors. (C) RNase protection assay of crudely fractionated thymus. Lanes: 1, total thymus; 2, filtrate (thymocytes); 3, residue (stromal components). (D) Diagram of the BKV early region showing the large T- and small t-antigen mRNAs, as well as the riboprobes used, and the protected fragments generated upon hybridization of the riboprobes to BKV mRNAs. Abbreviations: Pv, *Pvu*II; Ms, *Mst*II; Mb, *Mbo*I; Xb, *Xba*I; Bg, *Bgl*II.

type appeared to be a poorly differentiated or undifferentiated lymphoid stem cell, were seen (data not shown).

Half of the mice exhibiting thymic abnormalities also developed unilateral or bilateral kidney tumors. Renal tumors ranged in severity from kidneys of normal size with a few nodules to grossly misshapen nodular masses weighing up to 5.1 g. However, a typical tumor-bearing kidney contained many nodules and weighed 0.5 to 1.0 g compared with 0.2 g for a normal kidney (Fig. 1C). One mouse had bilateral kidney tumors that amounted to 20% of its total body weight. The kidney tumors were diagnosed as renal adenocarcinomas of epithelial origin. These adenocarcinomas affected the tubular epithelium and contained many multinucleated, giant tumor cells (Fig. 2A and B).

The frequency of the occurrence of kidney tumors and thymoproliferative disorders in BKV transgenic mice is summarized in Table 1. Slightly enlarged thymuses were sometimes observed as early as 3 months of age and usually reached severe proportions by 8 months. Kidney tumors, on the other hand, were rarely noticeable at 6 months. Most animals were sacrificed between 8 and 10 months of age, when they manifested breathing difficulties. Otherwise, animals were sacrificed at 12 months of age. Of 78 BKV transgenic mice analyzed, 29 (37%) developed both thymic enlargement and renal tumors. Additionally, 29 mice (37%) with no apparent renal changes displayed abnormalities of the thymus, while only two mice (3%) developed renal adenocarcinomas in the absence of thymic enlargement. The remaining 18 animals (23%) appeared morphologically normal at the time of sacrifice. Of the 96 BKV transgenic

TABLE 1. Frequency of kidney tumors and enlarged thymuses in BK37 mice

Pathological change	No. of affected mice (% of total)[a]		
	Female	Male	Total
Kidney tumor only	2 (6)	0	2 (3)
Enlarged thymus only[b]	6 (18)	23 (52)	29 (37)
Both changes	16 (47)	13 (30)	29 (37)
No change	10 (29)	8 (18)	18 (23)

[a] A total of 34 female and 44 male mice were analyzed at 8 to 12 months of age.

[b] Includes extreme hyperplasia, thymomas, and lymphomas.

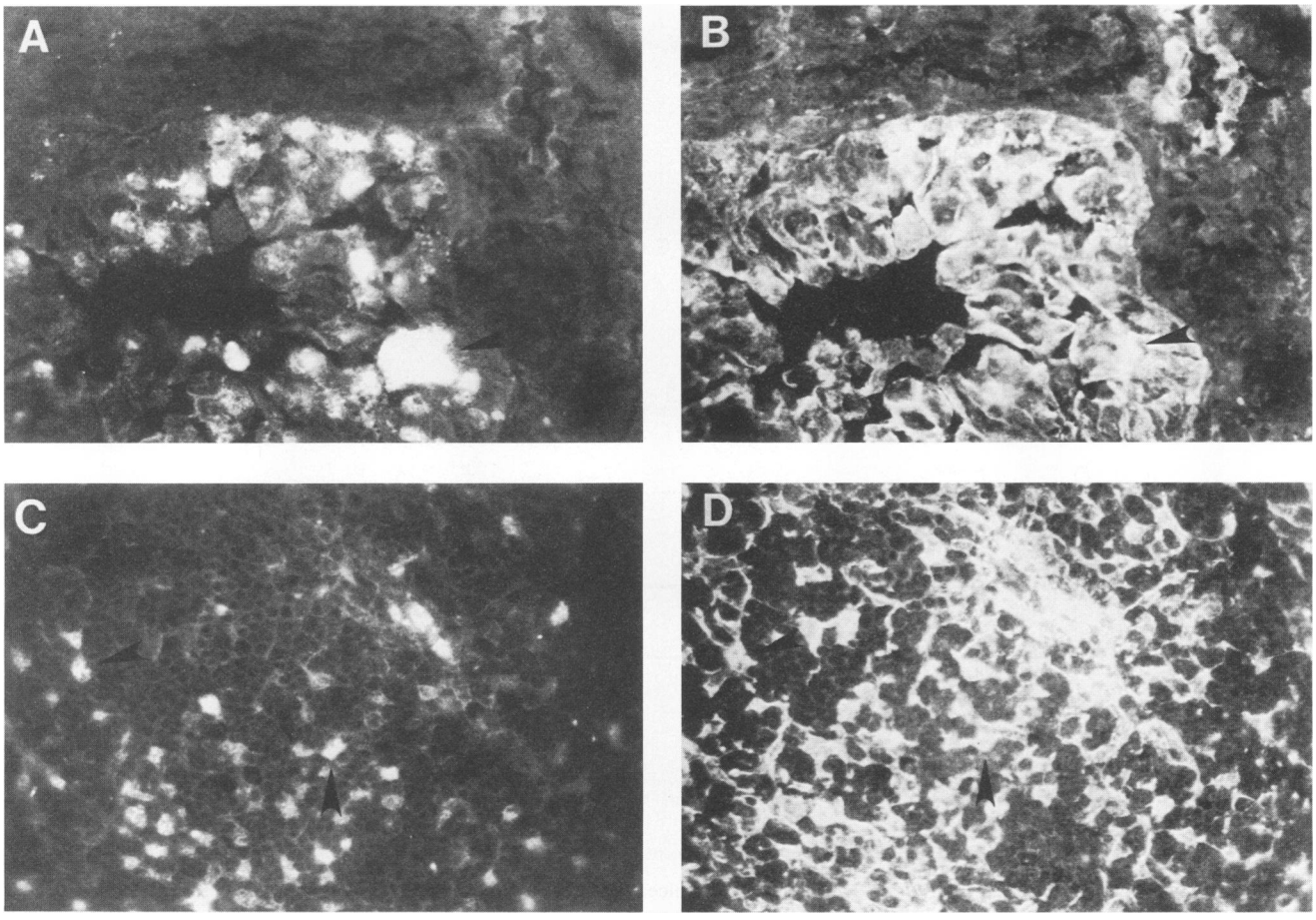

FIG. 4. Immunohistology of BK37 renal tumors and enlarged thymuses. (A and B) Double staining of a kidney tumor section with a hamster SV40 tumor serum (1:80) (A) and a guinea pig anti-keratin antibody (1:20) (B). All T-antigen-positive cells also contained keratin, suggesting that T antigen was being expressed in epithelial cells. The histologic evidence also suggested that these cells were tubular epithelium. The arrowhead shows a giant nucleus from a tumor cell. (C and D) Double staining of a section from an enlarged thymus labeled with the same antibodies as above. All of the cells expressing T antigen (C) were keratin positive (D), although not all keratin-positive cells expressed T antigen. The arrowheads point to two examples each of double-stained cells. In both the kidney and the thymus, T antigen was localized to a subset of epithelial cells. Staining was as described in Materials and Methods. Original magnification, ×400.

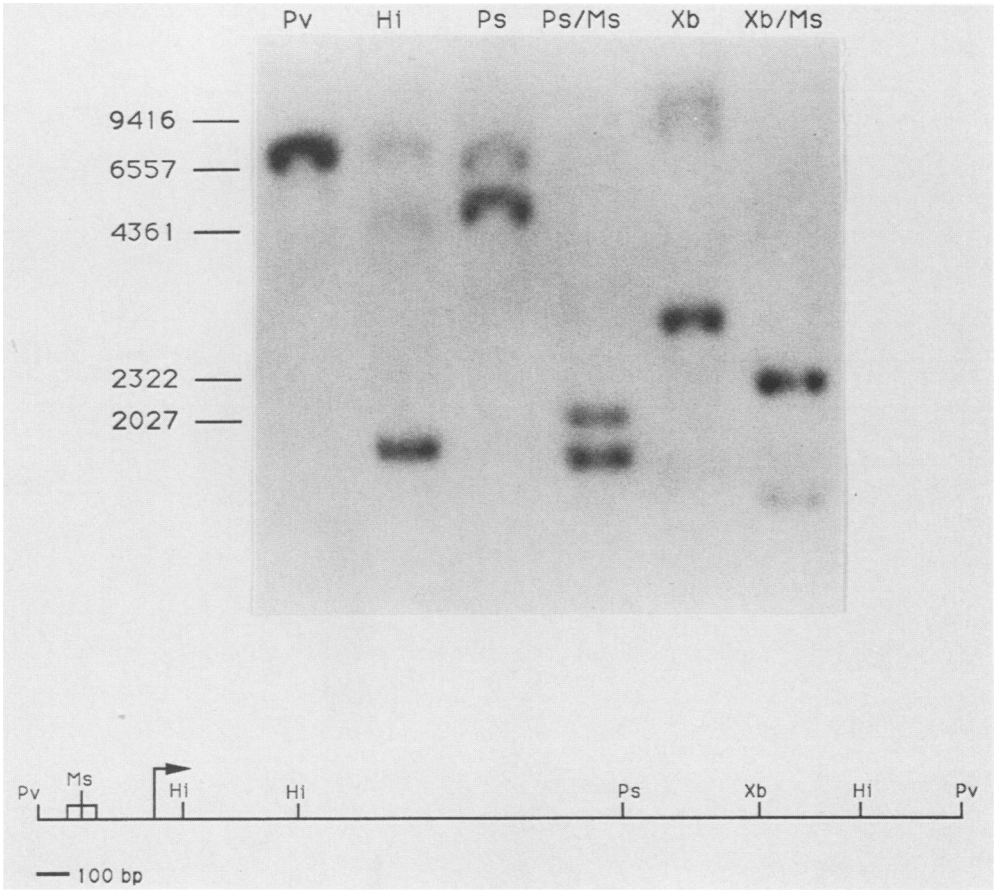

FIG. 5. Integrity of the transgene. Southern blot hybridization of BK37 tail DNA digested with various restriction enzymes. The probe used was the 3.2-kb *Pvu*II fragment which contained the entire BKV early region (shown at bottom). Digestion of BK37 tail DNA with *Pvu*II and Southern blot hybridization yielded a 7-kb fragment which included the BKV early region and cellular flanking sequences. Analysis with other restriction enzymes suggested a single intact copy of the transgene. Lanes: Pv, *Pvu*II; Hi, *Hin*dIII; Ps, *Pst*I; Ms, *Mst*II; Xb, *Xba*I. Lambda *Hin*dIII DNA markers are shown in base pairs to the left of the gel.

offspring generated, 18 died before they reached 8 months of age and were not analyzed. Interestingly, female mice displayed a higher proportion of kidney tumors than did the males, whereas male mice developed a slightly higher proportion of enlarged thymuses than did the females (Table 1). The higher incidence of kidney tumors in the female mice did not appear to be related to immunocompromised states due to pregnancy, since the females that were bred did not display phenotypic distributions that were altered from those of unbred females.

BKV T antigens are expressed only in kidney tumors and enlarged thymuses. The BKV large T- and small t-antigen mRNAs are differentially spliced from the same precursor RNA and share 5′ and 3′ ends and a splice acceptor site (23). The 3′ ends and splice sites of the mRNAs have been defined unambiguously; however, two possible sites for the common 5′ end were previously suggested (23). To determine in which tissues the BKV early region was expressed, total RNA was isolated from various organs of BK37 mice and analyzed by an RNase protection assay. A representative RNase protection assay is shown in Fig. 3A. The antisense riboprobe used spanned the common 3′ end of the T-antigen mRNAs and yielded the expected protected fragment of 373 nucleotides (Fig. 3D). The kidney tumors and enlarged thymus of this mouse were found to express T-antigen mRNAs, but no transgene expression was detected in any of the other tissues examined, including the enlarged spleen and lymph nodes of this animal (Fig. 3A).

In order to distinguish large T-antigen from small t-antigen mRNA, a riboprobe spanning the 5′ end and the splice donor site of the BKV large T-antigen mRNA was also used in RNase protection assays. Depending which of the two possible 5′ start sites is used (23), the predicted sizes for the protected fragments are 614 or 619 nucleotides for small t mRNA and 337 or 343 nucleotides for large T mRNA (Fig. 3D). Both large T- and small t-antigen mRNAs were detected in all kidney tumors and enlarged thymuses analyzed, while no T-antigen expression was seen in normal-appearing BK37 kidney (Fig. 3B). The total amount of T-antigen mRNA expressed, as well as the ratio of small t to large T mRNA, varied among the different tumors (Fig. 3B).

Expression of T-antigen mRNAs was observed exclusively in kidney tumors and enlarged thymuses in all mice analyzed. In kidneys that appeared morphologically normal and thymuses that appeared normal or only two to three times normal size, we could not detect BKV mRNAs even after long exposure times of the autoradiograms (data not shown). No truncated or other unusual RNAs were detected with either probe. To check for the presence of intact

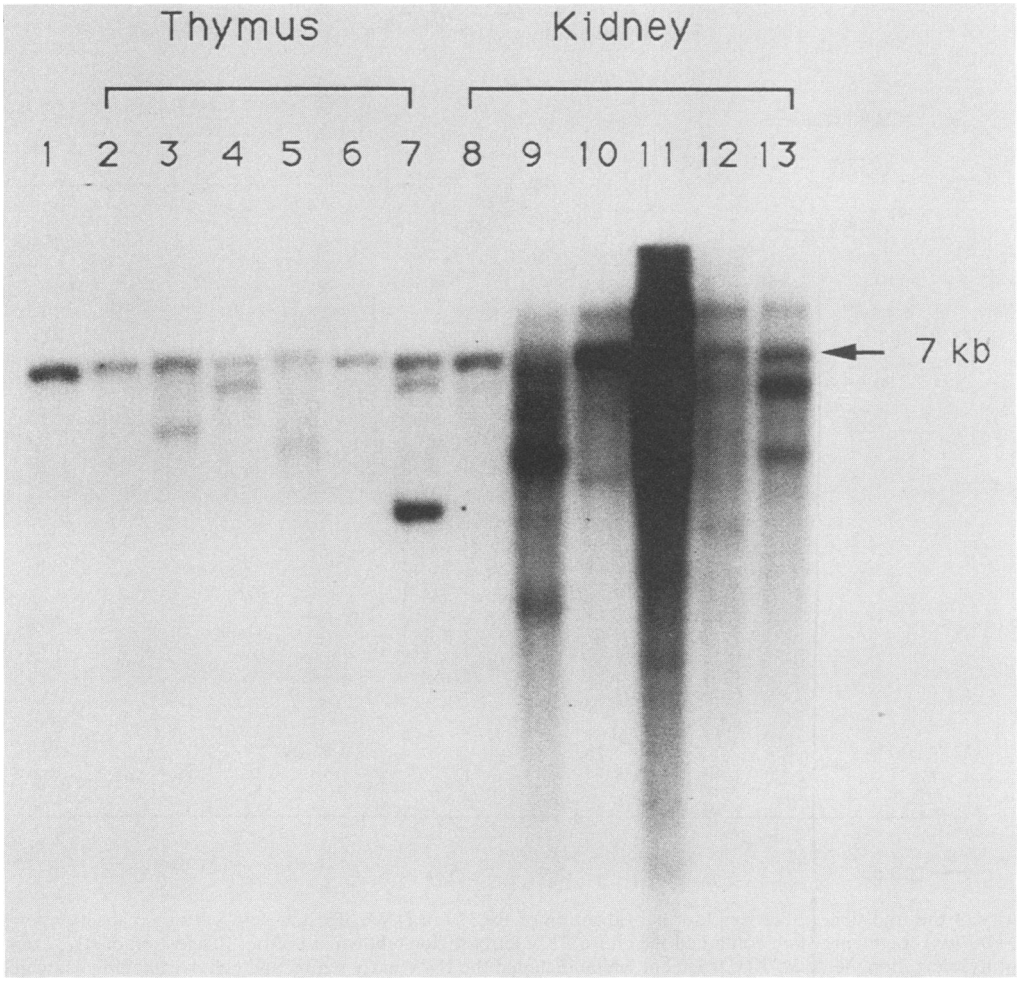

FIG. 6. Rearrangement and amplification of the *Pvu*II fragment in BK37 tumors. DNAs from various affected tissues were restricted with *Pvu*II, Southern blotted, and hybridized with the early region probe. The expected 7-kb band was seen in all tumors. Additional bands of various sizes and intensities indicated multiple rearrangements and amplifications. Lanes: 1, tail DNA; 2 to 7, various DNAs from grossly enlarged thymuses; 6 and 7, DNAs from the left and right lobes, respectively, of the same thymus; 8, DNA from a normal-appearing BK37 kidney; 9 to 13, DNAs from various kidney tumors; 12 and 13, DNAs from the left and right kidney tumors of the same mouse.

mRNAs, we included a probe for β2 microglobulin in our RNase protection assays (data not shown).

Localization of BKV T antigen to epithelial cells. To determine which thymic cell types were expressing BKV T antigens, RNase protection assays using the 5′ riboprobe were performed on RNA from crudely fractionated enlarged thymuses. When the thymus was teased apart with a razor blade and subsequently filtered through a Nitex membrane, the filtrate contained predominantly a single-cell suspension of thymocytes, while the residue remaining on the filter contained predominantly cells from the stroma of the thymus. In an RNase protection assay using the 5′ riboprobe, BKV T-antigen mRNAs were detected only in the stromal fraction, suggesting that thymocytes are unlikely to express the transgene (Fig. 3C).

To further study the cell types expressing T antigen, indirect immunofluorescence was performed on sections of affected tissues. Both thymic and renal sections were analyzed by using both a guinea pig anti-keratin antibody as an epithelial cell marker and a BKV T-antigen cross-reacting serum from a hamster bearing an SV40-induced tumor. Results of double labeling with the two antibodies suggested that all cells expressing T antigen were epithelial in both the kidney and thymus; however, not all keratin-containing cells in affected kidneys and thymuses were found to express T antigen (Fig. 4). In kidney sections, T-antigen expression was frequently seen in the epithelium of the tubules (Fig. 4A). In some cases, the lumens of the tubules were completely infiltrated by the T-antigen-positive cells (data not shown).

Tissues expressing T antigen exhibited DNA amplification and rearrangement. The BK37 founder animal was originally reported to have one to two copies of the BKV early region and to be missing an undetermined amount of transgenic DNA at the 3′ end, including part of the large T-antigen-coding region (24). However, several different restriction analyses of tail DNA from BK37 mice suggested the presence of an intact, single copy of the BKV early region (Fig. 5). In addition, our RNase protection assays suggested that the 3′ ends of the T-antigen mRNAs were intact (Fig. 3A).

To investigate the possibility of rearrangement or amplification of the transgene, DNA was extracted from both tumor

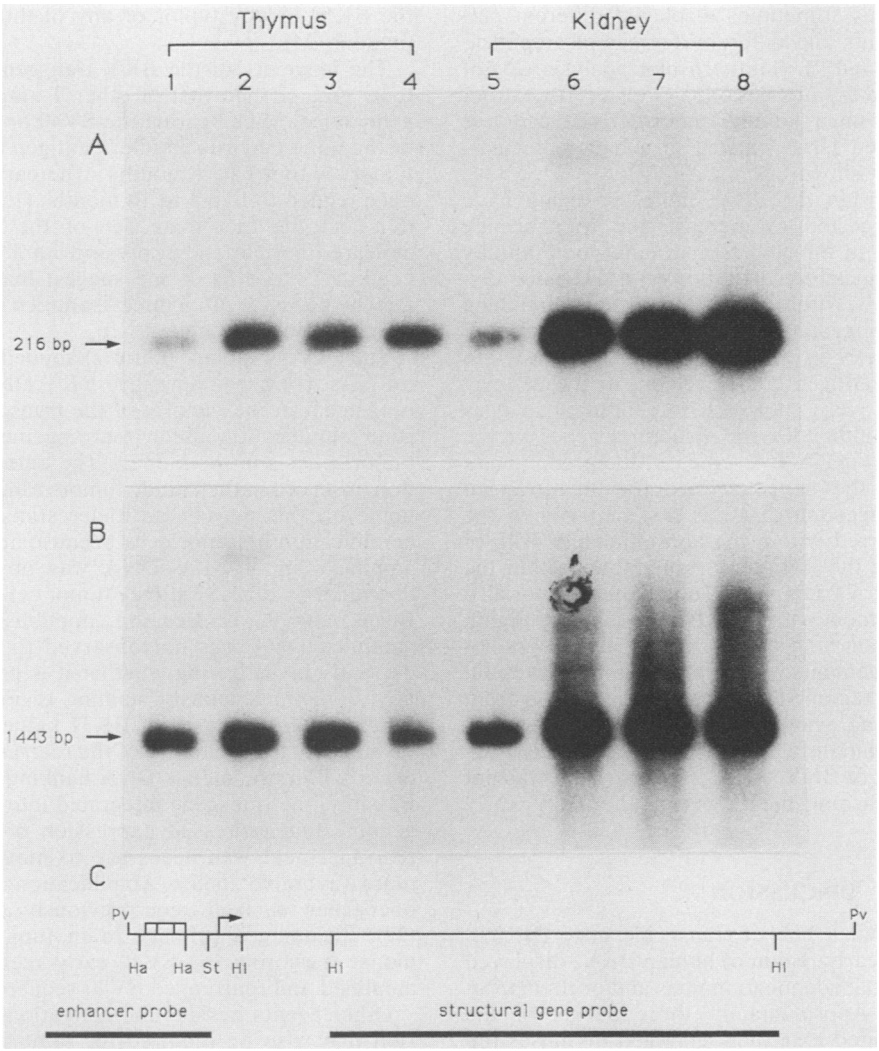

FIG. 7. DNA rearrangements in BK37 tumors occurred predominantly within the cellular DNA flanking the transgene. (A) BK37 DNAs were digested with *Hae*III, electrophoresed through a 2.5% agarose gel, blotted, and probed with the enhancer probe (*Stu*I fragment). The *Hae*III digest yielded a 216-bp fragment, plus a number of smaller fragments that were not detected and a number of fragments within the structural gene that did not hybridize with the *Stu*I probe. Only the *Stu*I site used to isolate the probe is shown. Lanes: 1, enlarged thymus DNA that did not show rearrangement when digested with *Pvu*II; 2 to 4, various thymus DNAs that displayed rearrangements upon *Pvu*II digestion; 5, normal-appearing kidney DNA that did not show rearrangements when digested with *Pvu*II; 6 to 8, various kidney tumor DNAs that were highly rearranged and amplified upon *Pvu*II digestion. (B) The same DNAs as in panel A were restricted with *Hin*dIII, electrophoresed through a 1.0% agarose gel, blotted, and hybridized with the structural gene probe (*Hin*dIII fragment). This digest yielded a 1,443-bp fragment. (C) Map of the BKV *Pvu*II fragment showing the restriction sites and probes used. Boxes represent the enhancer direct repeats. Pv, *Pvu*II; Ha, *Hae*III; St, *Stu*I; Hi, *Hin*dIII. For the sake of clarity, not all *Hae*III and *Stu*I sites are shown.

and normal tissues and analyzed by Southern blot hybridization (25), using a probe specific for the entire BKV early region. The BKV early region is contained within a 3.2-kb *Pvu*II fragment, which had been injected into fertilized eggs to generate the BKV transgenic mice (24). *Pvu*II digestion and Southern blot hybridization of BK37 tail DNA yielded one band approximately 7 kb in length (Fig. 5). The 7-kb band was detectable in all tissue DNAs analyzed and was the only band present in DNAs from unaffected tissues (data not shown). However, DNAs from all of the kidney tumors and many of the enlarged thymuses showed, in addition to the 7-kb band, other bands of various intensities, indicating multiple rearrangements and amplifications (Fig. 6). Kidney tumor DNAs contained BKV sequences that were amplified as much as 50-fold, whereas BKV DNAs from enlarged thymuses were amplified less frequently and less intensely (two- to fourfold). Although many unique rearrangements were detected within the kidney tumor DNAs, particular rearrangements could be favored since tumor DNAs from different animals often displayed rearranged bands of the same apparent size (Fig. 6). Left and right kidney tumor DNAs from the same mouse, as well as DNAs isolated from individual nodules on the same kidney, usually contained both unique rearrangements and rearranged bands of similar sizes (Fig. 6, lanes 12 and 13). In contrast to the kidney tumor DNAs, not all of the enlarged thymus DNAs displayed rearrangements. The thymic DNAs that were rearranged (38%) contained only one or two new bands, which were not observed to be common to DNAs from different animals (Fig. 6). DNA extracted from the left and right lobes

of an enlarged thymus sometimes displayed different patterns of rearrangements and different degrees of amplification (Fig. 6, lanes 6 and 7). Southern blot analysis (25) of undigested kidney and thymus DNAs, as well as Hirt extraction (12) of DNA from a kidney tumor-derived cell line indicated the amplified DNA existed in a high-molecular-weight form (data not shown).

Rearrangements within the BKV enhancer region have been previously suggested to increase the transforming capacity of the virus in infected human embryonic kidney cells (29). DNAs from affected tissues of BK37 mice displayed rearrangements within a 7-kb transgene-containing *Pvu*II fragment. To determine whether the DNA rearrangements in the BK37 DNAs occurred within the BKV enhancer region, the T-antigen coding region, or the flanking mouse DNA, DNAs were subjected to Southern analysis with probes specific either for the structural genes or the enhancer sequences (Fig. 7). In each case, the predominant band observed was the expected size for unrearranged transgenic DNA, suggesting that the vast majority of the rearrangements occurred within the approximately 4 kb of mouse DNA flanking the transgene rather than within the BKV insert itself (Fig. 7). DNA from one kidney tumor also contained a rearrangement within the BKV regulatory region (data not shown). Upon longer exposures of the autoradiograms, minor rearrangements were detected in the structural gene and regulatory region of several of the DNAs from affected tissues (data not shown). However, since the transgene appeared predominantly unrearranged, the results suggested that activation of BKV T-antigen expression was not likely because of rearrangements within the BKV early region.

DISCUSSION

We have shown that a line of transgenic mice (BK37), containing the entire early region of human BKV, displayed a high incidence of renal adenocarcinomas and/or thymoproliferative disorders. Approximately three-fourths of the BK37 offspring exhibited extremely enlarged thymuses (up to 250 times the weight of a normal adult thymus), some of which had progressed to thymomas or lymphomas. Pathological changes involving other tissue types, with the exception of occasional enlarged spleens and lymph nodes in conjunction with thymic enlargement, were never observed. In contrast, the BK37 founder, as well as a second BKV transgenic mouse (BK50), which carried about 10 copies of the BKV early region and was infertile, developed primary renal and hepatocellular carcinomas (24). Thus, renal carcinomas have been observed in two independently derived BKV transgenic mice, and this phenotype has been transmitted to the offspring of one of these mice. Since BKV is thought to persist in human kidneys and to replicate in epithelial cells, this mouse line may be a good model system for studying the effects of BKV expression in vivo (10, 11).

RNase protection assays revealed that BKV T-antigen mRNAs were present in all renal tumors and grossly enlarged thymuses examined. In contrast, no BKV T-antigen transcripts were detected in morphologically normal kidney and thymus or in any other tissue type assayed. The precise correlation of T-antigen expression with tissue pathology strongly suggests that BKV T antigens are involved in pathogenesis in these animals. In contrast, the BK50 mouse expressed T-antigen mRNAs at a high level in liver tumor and muscle tissue and at a lower level in brain, heart, and lung tissue (24). No expression data were reported for either the BK50 kidney tumor or any of the tissues of the BK37 founder (24).

The longevity of the BKV transgenic mice was substantially greater than that of other T-antigen-containing transgenic mice. Mice bearing the SV40 or JCV large T antigens or the polyomavirus middle T antigen tended to succumb to tumors within 1 to 4 months, whereas the BK37 transgenic mice tended to live 8 to 10 months (1, 2, 24). This observation and the fact that 23% of the BK transgenic mice appeared normal and expressed no T antigens when sacrificed at 12 months of age suggest that an activation event may be necessary to induce T-antigen expression and tumor formation in these mice.

Since every kidney tumor examined expressed T-antigen mRNAs, contained amplified BKV DNA, and showed rearrangement in the vicinity of the transgene, we suggest that gene amplification and/or rearrangement may contribute to tumorigenesis in the kidney. The extent of DNA amplification observed in the kidney tumors ranged from 5- to 50-fold; however, this may be an underestimation of the extent of amplification in tumor cells because approximately 100-fold amplification of BKV DNA was observed in a cell line derived from a BK37 kidney tumor cell (S. A. Dalrymple, A. Beggs, and K. L. Beemon, unpublished data). Since the amplified DNA was not observed to be episomal, we are currently investigating whether it is present in double minutes or homogeneously staining regions of chromosomes. Southern blot analyses of BK37 kidney and thymus DNAs revealed that the majority of the rearrangements appeared to occur within the mouse DNA flanking the BKV sequences. Possibly, the transgene integrated into an area of the mouse genome that repressed expression of the T antigens, and rearrangement was necessary to move the transgene to a more favorable locus. Amplification or rearrangement of oncogenes has been seen previously in a variety of tumors (27), including a primary brain tumor from a transgenic mouse harboring the SV40 early region, which contained amplified and rearranged SV40 sequences (3).

Other events besides the activation of T-antigen expression may also be required for tumorigenesis in the BK37 mouse line. Transfection of BKV DNA into human embryonic kidney cells generated transformed foci, but cotransfection with an activated *ras* oncogene was necessary for anchorage-independent growth (19). This finding suggests that cooperation between multiple oncogenes could be necessary to promote tumorigenesis in BK37 mice.

Rearrangement and amplification of BKV sequences in the enlarged thymuses were observed less frequently than those in the kidney tumors, although T-antigen expression was detected in all cases examined. This observation and the fact that not all of the enlarged thymuses contained neoplastic cells, suggested that pathogenesis in the thymus may have occurred by a different mechanism from that in the kidney. We suggest that while BK T-antigen expression may have been sufficient to induce thymic hyperplasia, a second event may have been required for thymic neoplasms. Thymic hyperplasia and lymphomas in conjunction with T-antigen expression have also been observed in SV40 T-antigen transgenic mice having several different promoters, suggesting that the expression of T antigens in the thymus and the thymus pathologies observed may be independent of the promoter used (2, 3, 5, 26). In the BK37 line, as well as in mice in which the SV40 T antigen was driven by a neuronal promoter, T antigen was expressed in a subset of the epithelial cells of the thymus (2). Although the thymocytes did not appear to express T antigen, they were present in

much higher than normal numbers. Since thymic epithelial cells are known to secrete factors that influence the differentiation and development of thymocytes (4), the expression of T antigen in the epithelial cells may affect the proliferation or targeting of thymocytes.

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