Establishment of a Bovine Adenovirus Type 3 (BAV3)-Transformed Hamster Cell Line and Characterization of BAV3-Tumor Associated Surface Antigens

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ABSTRACT. A clonal cell line of transformant, BHKT73, was isolated from BHK21/13 cells infected with bovine adenovirus type 3 (BAV3). BHKT73 cells showed characteristics typical of transformed cells, including the ability to grow in 1.3% methylcellulose, high agglutinability with lectins, and unclear cytoplasmic microtubular fibers. Cot analysis showed that BHKT73 cells were contain BAV3 DNA.

Tumor-associated surface antigens (TASAs) of BHKT73 cells, and TrD-5 and TrJE-6 cells (A31 cells transformed by BAV3 DNA fragments with respective map units of 3.6-19.7 and 0-11.9) were studied by the protein A binding assay with rabbit anti-BHKT73 IgG, BALB/c mice anti-BHKT73 antiserum and BALB/c mice anti-TrD-5 and TrJE-6 antisera. All these antisera bound to the surfaces of BHKT73, TrD-5 and TrJE-6 cells, but not to the surfaces of SV40- and murine sarcoma virus-transformed A31 cells. They also bound to the surface of polyomavirus-transformed cells (pyBHK and PV-4). After absorption with pyBHK cells, these antisera showed decreased ability to bind to the surfaces of BHKT73, TrD-5, PV-4 and pyBHK cells. These results indicate that all the genetic information of BAV3 that is required for the expression of BAV3-TASAs is included between the map units of 3.6 and 11.9 on the BAV3 gene, and that BAV3-TASAs may have antigenic determinant site(s) common to one of the TASAs on polyomavirus-transformed cells.

Transformed cells have characteristics that differ from those of their normal counterparts. These characteristics include 1) alteration of morphology; 2) change in cytoskeletal system, including the decrease of cytoplasmic microtubular fibers and the disappearance of actin cables or stress fibers (3, 8, 17, 33); 3) increase in the degree of agglutination with lectins (6, 21, 29); 4) release of a plasminogen activator (31, 39); 5) loss of anchorage-dependent growth control; 6) increase in the nutrient uptake (13); 7) blockage of glycolipid synthesis (12); 8) decrease of large external transformation-sensitive protein (LETS protein) (16) [also called cell surface protein (CSP)

Abbreviations used: BAV3, bovine adenovirus type 3; TASAs, tumor-associated surface antigens; MuSV, murine sarcoma virus; MEM, minimum essential medium; FBS, fetal bovine serum; Con A, concanavalin A; WGA, wheat germ agglutinin; RCA, castor bean agglutinin; WFA, *Wistaria floribunda* agglutinin; PBS, phosphate-buffered saline; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; DOC, sodium desoxycholate; NP-40, nonidet P-40; SDS, sodium dodecyl sulfate.

(42) or galactoprotein A (10); 9) increase of 100K protein or glucose regulated protein (2, 35) and 10) expression of tumor-associated surface antigens (TASAs) including viral specific antigens serologically distinguishable from each other and detected among different species, and the host-derived antigens on their surface (14, 24, 41).

The majority of these characteristics associated with transformation seem to be related to alterations in the cell surface. Therefore, a comparison of the characteristics of the cell surface of virus-transformed cells and parental normal cells is important for determining the functions and roles of cell surface components, including TASAs. It is also important to know the nature of the TASAs in order to determine the mechanism of transformation so that cancers can be treated immunologically. Thus, the TASAs of SV40-, polyomavirus-, human adenovirus- and Rous sarcoma virus-transformed cells have been studied (14, 24, 41).

The characters of the TASAs on bovine adenovirus type 3 (BAV3)-transformed cells, however, have not been studied. Therefore, we obtained a BAV3-transformed hamster cell line (BHKT73) and prepared antisera against the transformant. Other antisera also were obtained from syngeneic BALB/c mice by immunizing them with TrD-5 and TrJE-6 cells (A31 cells transformed by BAV3 DNA fragments with respective map units of 3.6–19.7 and 0–11.9). Using these antisera, we analyzed the characters of BAV3- and its DNA fragment-transformed A31 cells (19).

MATERIALS AND METHODS

Cells and virus. A31 (a clone derived from mouse BALB/3T3 cells); SA-26 (A31 cells transformed by SV40); N-7 [A31 cells transformed by murine sarcoma virus (MuSV)]; PV-4 (A31 cells transformed by polyomavirus) and pyBHK (BHK21/13 cells transformed by polyomavirus) cells were obtained from Dr. A. Hakura (Osaka University, Osaka, Japan). BHK21/13 (an established cell line from syrian hamster kidney) cells were purchased from Flow Laboratories Inc. (Rockville, Md., U.S.A.). WT-51, TrD-5 and TrJE-6 cells (A31 cells transformed by BAV3 or its DNA fragments) were established in our laboratories (19). A clonal virus, BAV3-1, which originated from a BAV3 prototype (WBR-1) (7) was purified by CsCl density gradient centrifugation (26). Cells were cultured in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS; Flow Laboratories Inc.) and 100 µg/ml of kanamycin (Takeda Chemical Industries, Ltd., Osaka, Japan).

Colony formation in methylcellulose. The method of Risser and Pollack (34) was used to examine the growth of cells in methylcellulose. Briefly, cells were suspended in MEM containing 10% FBS and 1.3% methylcellulose (4,000 cps, Wako Pure Chemical Industries Ltd., Osaka, Japan) at 5×10^2 to 10^5 cells per ml. This suspension was overlaid on a 1.0% agar layer (purified agar, Difco Laboratories, Detroit, U.S.A.) containing MEM supplemented with 10% FBS. After 9 days of incubation at 37°C, the number of colonies were counted.

Cell agglutination by lectins. Concanavalin A (Con A), wheat germ agglutinin (WGA), castor bean agglutinin (RCA) and *Wistaria floribunda* agglutinin (WFA) were purified by the established methods (1, 23, 37). The method of Tsuda *et al.* (37) was used in the cell agglutination assays.

Morphology of cytoplasmic microtubules. The method of Weber *et al.* (40) was used to stain BHK21/13 and BHKT73 cells with anti-porcine tubulin rabbit IgG and fluorescein-conjugated anti-rabbit IgG goat antibodies (Microbiological Associates Inc., U.S.A.) as described previously (20, 30).

Cot analysis. The method of Britten and Kohne (4) was used in the reassociation kinetics

experiments as described previously (27). The method of Gelb *et al.* (11) was used to determine the copy number of viral DNA in a cells.

Preparation of antibodies. 1) Rabbit anti-BHKT73 IgG. BHKT73 cells harvested with a 0.02% EDTA solution were washed with phosphate-buffered saline (PBS), pH 7.2, then injected into rabbits intravenously at a dose of 2×10^7 cells per rabbit. Injections were made 4 to 5 times at one-week intervals. One week after the last injection, the animals were bled to obtain anti-BHKT73 antisera. The purified IgG of the antiserum was prepared by DEAE-cellulose column chromatography (9). The IgG was passed through a sterile Millipore filter (HA type, Niohn Millipore Ltd., Osaka, Japan).

2) BALB/c mice anti-BHKT73 antiserum. BHKT73 cells (1 to 2×10^6) also were injected into 9-week-old male BALB/c mice intraperitoneally at intervals of 3 to 5 days for 4 weeks. One week after the last injection, the animals were bled to obtain the antiserum. Antisera from 4 to 6 mice were pooled and passed through a sterile Millipore filter.

3) BALB/c mice anti-TrD-5 and TrJE-6 antisera. The antisera were obtained from BALB/c mice immunized by subcutaneous injections of fixed transformed cells or by subcutaneous implantation of viable transformed cells. Hyperimmunization of mice was carried out as follows. Confluent or subconfluent TrD-5 cells were killed by treating them with $30 \mu\text{g/ml}$ of mitomycin C at 37°C for 40 min, after which they were harvested with a 0.02% EDTA solution and washed with PBS. The treated cells were fixed with 0.5% paraformaldehyde in PBS at 37°C for 20 min then washed with PBS and injected into 8–9-week-old male mice. Injections (2×10^6 cells) were made 6 to 8 times at intervals of 3 to 7 days, and the mice were bled one week after the last injection. BALB/c mice (9–10-week-old male) were implanted subcutaneously with viable TrJE-6 cells (10^6 cells). After 4 weeks, these tumor-bearing mice were bled.

Absorption of antisera. Antisera diluted with medium A [MEM containing 4% FBS and 5 mM HEPES buffer (pH 7.2)] was absorbed prior to the binding assay by incubating the antisera at 37°C for 15 min with monolayer cells grown on a Falcon dish (Oxnard, U.S.A.), then the absorbed antisera were passed through Millipore filters. Large scale absorption with BHK21/13 cells was performed with cells grown on a roller bottle.

Binding assay of ^{125}I -labeled protein A. Protein A (Pharmacia, Uppsala, Sweden) was labeled with Na^{125}I by the chloramin-T method (5). The labeled protein A was separated from excess reagents by gel filtration on a Sephadex G-25 column. The specific radioactivity was 12,500 cpm/ng protein. The binding assay was performed with monolayer cells grown on a flat-bottomed microtest plate (Microtest II, Falcon) by the method of Brown *et al.* (5).

Thirty thousand cells were seeded in each well and the plate was incubated at 37°C for one day in a humidified incubator under 5% CO_2 in air. The medium then was removed by aspiration, and $40 \mu\text{l}$ of antiserum, control serum, or medium A was added to each well. After incubation at 37°C for 1 h, the cells were washed twice, each time with $200 \mu\text{l}$ of medium B [MEM containing 1% BSA and 5 mM HEPES buffer (pH 7.2)]. ^{125}I -labeled protein A (5×10^4 cpm in $50 \mu\text{l}$ of medium B) then was added to each well. After incubation at 37°C for 1 h, these cells were washed twice, each time with $200 \mu\text{l}$ of medium B. The cells then were dissolved in $100 \mu\text{l}$ of 2 N NaOH and transferred to a tube for measurement of radioactivity.

The binding assay was carried out in quadruplicate. Values for the radioactivities were corrected for nonspecific binding by subtracting the mean cpm of the ^{125}I -protein A bound to target cells incubated without antiserum.

Radiolabeling and preparation of cell extracts. Cells grown on a dish (Falcon, #3002) were radiolabeled by adding 1 ml of fresh leucine-depleted MEM containing [^3H]leucine ($50 \mu\text{Ci/ml}$, 130–190 Ci/mmol, RCC Amersham, England). After 3 h of incubation, the cells

were rinsed 3 times with cold Tris-buffered saline (TBS) and made soluble on ice by the addition of 0.5 ml of cell extraction buffer [0.15 M NaCl, 1% DOC, 1% NP-40, 0.1% SDS, 10 mM sodium phosphate (pH 7.0) and 1% Trasylol] (15).

Immunoprecipitation. Immunoprecipitation of radiolabeled cell extracts was carried out by the method of Hutchinson *et al.* (15). The polyoma anti-T antiserum obtained from hamsters inoculated with polyomavirus-transformed cells was the gift of Dr. A. Hakura. The radiolabeled cell extract (190 μ l) was incubated for 2 h with 10 μ l of polyoma anti-T antiserum in an ice bath, then it was mixed with formalin-fixed *Staphylococcus aureus* bacteria (5 μ l packed cell volume) (22) and incubated for 16 h. The precipitate obtained after centrifugation (Eppendorf model 5412 centrifuge) for 1 min was washed four times with 400 μ l of cell extraction buffer. Radiolabeled proteins were released from the precipitate by boiling it for 5 min in 40 μ l of buffer [0.0625 M Tris-HCl buffer (pH 6.8) containing 2% SDS, 10% glycerol, 5% dithiothreitol and 0.001% bromophenol blue]. The bacteria then were removed by centrifugation. The supernatant (20 μ l) was electrophoresed on a SDS-polyacrylamide slab gel according to the method of Laemmli (25). The concentration of acrylamide was 11%. The gel was treated with EN³HANCE (New England Nuclear, U.S.A.) and exposed to XS film (Kodak, U.S.A.) after drying.

RESULTS

Isolation of BAV3-transformed BHK21/13 cells. BHK21/13 cells grown on a plastic dish were infected for 3 h with purified BAV3 at a multiplicity of 50 plaque-forming units per cells, then they were harvested with trypsin. A sample of the infected cells were reseeded on a plastic dish and cultured for 23 days. Based on morphology and the ability to grow in methylcellulose, eight clonal lines of transformants were isolated and selected. Of these isolates, BHKT73 cells differed most morphologically from the parental BHK21/13 cells (Fig. 1), and they grew well in methylcellulose. The detailed characteristics of BHKT73 cells were

1) Growth in methylcellulose. A comparison of the ability of BHK21/13 and BHKT73 cells to grow in methylcellulose was made at low cell density because BHK21/13 cells grew in a medium containing 1.3% methylcellulose when seeded at a high density. When seeded at 5×10^2 cells per ml, BHKT73 cells formed colonies well, but BHK21/13 cells formed scarcely any colonies (Table 1).

Fig. 1. Morphology of BHK21/13 and BHKT73 cells. Cells grown on a coverslip were stained with Giemsa solution. A) BHK21/13, B) BHKT73. Magnification, $\times 120$.

TABLE 1. GROWTH OF BHK21/13 AND BHKT73 CELLS IN METHYLCELLULOSE

Cell number (cells/plate)	Number of colonies/plate	
	BHK21/13	BHKT73
2.5×10^2	26	254
5.0×10^2	20	453
2.5×10^3	165	536

Cells were grown at 37°C in a medium containing 1.3% methylcellulose as described in the text. Colonies were counted on the 9th day of culture.

2) Agglutination with lectins. Agglutination tests were performed with Con A, WGA, RCA and WFA. BHKT73 cells were agglutinated more easily with Con A and WFA than were BHK21/13 cells (Table 2).

3) Cytoplasmic microtubules. The networks of cytoplasmic microtubular fibers in BHK21/13 and BHKT73 cells were examined by indirect immunofluorescent staining with anti-tubulin rabbit IgG and fluorescein-conjugated anti-rabbit IgG goat antibodies. The rigidly constructed network of microtubular fibers was well stained in the cytoplasm of BHK21/13 cells, but the network in the cytoplasm of BHKT73 cells only was stained weakly (Fig. 2).

4) Viral genome. The presence of viral DNA in BHKT73 cells was determined by reassociation kinetics with ^{32}P -labeled BAV3 DNA. The DNA extracted from BHKT73 cells accelerated the reassociation of ^{32}P -labeled BAV3 DNA, whereas DNA from BHK21/13 cells did not (Fig. 3). In contrast, the DNAs extracted from calf thymus and hamster embryo did not accelerate the reassociation reaction, but the DNA of BHKT73 cells accelerated the reassociation of ^{32}P -labeled *Eco*RI D-fragments (data not shown). These results mean that BHKT73 cells contained BAV3 DNA and its D-fragment, but BHK21/13 cells did not. The quantity of BAV3 DNA in BHKT73 cells was estimated from four independent experiments to be 0.83 ± 0.22 (mean $\pm$ SD) genome/diploid cell DNA.

Characterization of TASAs. 1) ^{125}I -protein A binding assay. TASAs of BHKT73 cells, BAV3- and its DNA fragment-transformed A31 cells (WT-51, TrD-5 and TrJE-6) were studied by the protein A binding assay. A31 cells transformed by SV40, polyomavirus and MuSV, and BHK21/13 cells transformed by polyomavirus were used

TABLE 2. AGGLUTINATION OF BHK21/13 AND BHKT73 CELLS WITH LECTINS

Lectin	Concentration of lectin required for half maximum agglutination ($\mu\text{g/ml}$)	
	BHK21/13	BHKT73
Con A	125	16
WFA	125	32
WGA	63	63
RCA	8	8

Cells were harvested from the bottles by treating them with a 0.02% EDTA solution, after which they were washed repeatedly with PBS.

Fig. 2. Network of cytoplasmic microtubular fibers of BHK21/13 and BHK73 cells. Cells grown on a coverslip were fixed with 3.7% paraformaldehyde in PBS and stained with rabbit anti-tubulin IgG and fluorescein-conjugated goat anti-rabbit IgG antibodies. A) BHK21/13, B) BHK73. Magnification, $\times 360$.

as the controls in the analysis of BAV3-TASAs. When rabbit anti-BHKT73 IgG was used without absorption with BHK21/13 cells, it bound to the surface of all the cells tested (data not shown). After absorption with BHK21/13 cells, it bound to the surfaces of BHKT73, WT-51 and TrD-5 cells, but not to the surfaces of SA-26, N-7 and A31 cells (Table 3, first column). The control, normal rabbit serum, showed no significant binding to the cells tested (less than 50 cpm). Although the binding amount of ^{125}I -protein A to each cell line differed slightly in different experiments, this was the result of different physiological conditions. Rabbit anti-BHKT73 IgG also bound to the surfaces of polyomavirus-transformed cells (pyBHK and PV-4). Similar results were obtained when BALB/c mice anti-BHKT73 antiserum was used (Table 3, second

Fig. 3. Kinetics of reassociation of ^{32}P -labeled BAV3 DNA in the presence of DNA obtained from BHK21/13 and BHKT73 cells. Reassociation reactions were carried out as described in the text. The reaction mixtures contained 8.4 ng of ^{32}P -labeled BAV3 whole DNA and 1 mg of BHK21/13 (○) or BHKT73 (●) DNA per milliliter.

TABLE 3. BINDING OF ANTISERA TO VARIOUS CELL LINES

Cell line	¹²⁵ I-protein A bound (mean cpm $\pm$ S.D.)			
	Anti-BHKT73 serum		Anti-TrJE-6 serum ^c	Anti-TrD-5 serum ^c
	Rabbit ^a	BALB/c ^b	BALB/c	BALB/c
BHK21/13	81 $\pm$ 10	566 $\pm$ 96	-26 $\pm$ 13	-16 $\pm$ 17
BHKT73	465 $\pm$ 30	2465 $\pm$ 182	N.T. ^d	497 $\pm$ 46
pyBHK	230 $\pm$ 37	4683 $\pm$ 155	803 $\pm$ 40	419 $\pm$ 45
A31	23 $\pm$ 19	89 $\pm$ 20	8 $\pm$ 17	-2 $\pm$ 9
WT-51	611 $\pm$ 74	4496 $\pm$ 287	1580 $\pm$ 156	N.T. ^d
TrD-5	652 $\pm$ 164	6916 $\pm$ 716	2087 $\pm$ 73	655 $\pm$ 103
TrJE-6	N.T. ^d	1632 $\pm$ 78	834 $\pm$ 41	N.T. ^d
SA-26	22 $\pm$ 3	-8 $\pm$ 16	5 $\pm$ 34	N.T. ^d
N-7	61 $\pm$ 37	42 $\pm$ 15	32 $\pm$ 34	7 $\pm$ 15
PV-4	411 $\pm$ 139	913 $\pm$ 83	1873 $\pm$ 110	N.T. ^d

^a Rabbit anti-BHKT73 IgG (10 μ g/40 μ l) was absorbed with BHK21/13 cells (3.5×10^6) at 37°C for 60 min.

^b BALB/c mice anti-BHKT73 antiserum was diluted 20-fold with medium A then absorbed with BHK21/13 cells (1.6×10^6) at 37°C for 60 min.

^c Anti-TrJE-6 antiserum (obtained from TrJE-6 tumor-bearing mice) and anti-TrD-5 antiserum were diluted 16-fold with medium A.

^d Not tested.

column). These results indicate that the BAV3-TASAs detected with xenogeneic anti-BHKT73 antiserum have an antigenic determinant site(s) common to one of the TASAs on the surface of the polyomavirus-transformed cells.

We obtained almost the same results with syngeneic antisera. BALB/c mice were immunized by subcutaneous injections of fixed transformed cells or by subcutaneous implantation of viable transformed cells. Anti-TrJE-6 (third column, Table 3) and anti-TrD-5 (last column, Table 3) antisera bound to the surfaces of BHKT73, WT-51, TrD-5 and TrJE-6 cells, but not to the surfaces of SA-26, N-7, BHK21/13 and A31 cells. They also bound to the surfaces of pyBHK and PV-4 cells.

BALB/c mice anti-BHKT73 antiserum and TrJE-6 antiserum did not bind to the

TABLE 4. BINDING OF MOUSE ANTISERUM TO EMBRYONIC CELLS

Serum	Dilution rate ^a	¹²⁵ I-protein A bound (mean cpm $\pm$ S.D.)	
		TrD-5	Embryonic cells ^b
Normal ^c	5	N.T. ^e	13 $\pm$ 13
Anti-TrJE-6 ^d	11	551 $\pm$ 49	1 $\pm$ 8
Anti-BHKT73	50	934 $\pm$ 68	11 $\pm$ 8

^a Serum was diluted with medium A.

^b Secondary cultured cells of 14-day-old embryos from BALB/c mice.

^c Normal BALB/c mice (14-week-old male) serum.

^d Serum was obtained from TrJE-6 tumor-bearing mice.

^e Not tested.

Fig. 4. Effect of absorption with pyBHK cells on binding of anti-TrJE-6 antiserum to transformed cells. Anti-TrJE-6 antiserum (obtained from TrJE-6 tumor-bearing mice) that had been diluted 16-fold with medium A was absorbed with the indicated number of pyBHK cells after which its residual binding activities to TrD-5 ($\circ$), PV-4 ($\bullet$) and pyBHK ($\square$) cells were determined.

Fig. 5. SDS-polyacrylamide slab gel electrophoresis of the immunoprecipitate. Actively growing pyBHK, BHK T73 and TrD-5 cells were used for the radiolabel. The immunoprecipitates were prepared with polyoma anti-T antiserum as described in the text.

surfaces of embryonic cells from BALB/c mice (Table 4). This indicates that the BAV3-TASAs detected in this experiment are not related to embryonic antigens.

2) Absorption with pyBHK cells. BALB/c mice anti-TrJE-6 antiserum was absorbed with pyBHK cells, then its residual binding activities to TrD-5, PV-4 and pyBHK cells were determined (Fig. 4). The amount of antiserum bound to the surfaces of these cells was decreased markedly by absorption with pyBHK cells. The binding abilities of rabbit anti-BHKT73 IgG and BALB/c mice anti-BHKT73 antiserum to the surfaces of TrD-5, PV-4, BHKT73 and pyBHK cells also were decreased by absorption with pyBHK cells (data not shown).

Assay of polyoma T antigen. We investigated whether the BHKT73 and TrD-5 cells had ever been super-infected by polyomavirus. Polyomavirus small T antigen (15) was detected in the extract of pyBHK cells, but not in the extracts of BHKT73 and TrD-5 cells (Fig. 5). These results show that BHKT73 and TrD-5 cells have never been super-infected by polyomavirus.

DISCUSSION

BHKT73 cells could grow and form colonies in medium containing 1.3% methyl-cellulose (Table 1). They were agglutinated with Con A and WFA (Table 2) and showed fewer and obscured cytoplasmic microtubular fibers (Fig. 2). In addition, BHKT73 cells were proved to contain BAV3 DNA and its *EcoRI* D-fragment. These cells also released a tumor angiogenesis factor (38) and expressed T antigen (28). The appearance of all the phenotypes of BHKT73 cells thus is believed to be due to transformation by BAV3.

BAV3-TASAs on BAV3-transformed cells also were found on the surfaces of TrD-5 and TrJE-6 cells, both of which were transformed from A31 cells by the *EcoRI* D-fragment DNA (3.6–19.7 map units) and by *Hind* III JE-fragment DNA (0–11.9 map units) of BAV3 DNA (19). This locus has been shown to contain genes related to T antigen (28). Clearly, all the genetic information of BAV3 required for the transformation and expression of BAV3-TASAs is located between the map units 3.6 and 11.9 on the BAV3 genome.

All the antisera used in the experiments reported here bound to the surfaces of BAV3- and its DNA fragment-transformed BHK21/13 and A31 cells, and to the surfaces of polyomavirus-transformed BHK21/13 and A31 cells as well (Table 3). These antisera did not bind to the surfaces of SA-26, N-7, BHK21/13, A31 and BALB/c mice embryonic cells (Table 3 and 4). The abilities of these antisera to bind to TrD-5, BHKT73, PV-4 and pyBHK cells were abolished after the antisera had been absorbed with pyBHK cells (Fig. 4). Polyomavirus occasionally induces superinfection in cultured cells. The cross-reactivity of the TASAs of BAV3-transformed and polyomavirus-transformed cells, however, was not due to superinfection by polyomavirus in the BAV3-transformed cells because 1) polyomavirus and its DNA have never been used in our laboratories; 2) the chance of superinfection by polyomavirus is equal for every cell and there is no reason why only BAV3-transformed cells would be infected with polyomavirus; and 3) no polyomavirus small T antigen was detected in the extracts of BHKT73 and TrD-5 cells (Fig. 5). Therefore, we conclude that the TASAs on BAV3-transformed cells are BAV3-specific antigens which are serologically distinguishable and which have regions common to the TASAs on polyomavirus-transformed cells.

In their early work on the TASAs induced by SV40, Pancake and Mora (32) reported that antisera prepared by repeated inoculation of SV40-transformed mouse cells into syngeneic AL/N mice could kill polyomavirus-transformed AL/N mouse cells. They concluded that SV40 and polyomavirus transformation might result in the appearance of common cell surface antigens that differ from the embryonic antigens. The TASAs detected on BAV3-transformed cells in our experiments also were not the embryonic antigens described by other investigators (24, 36) (Table 4). The possibility that they are host-derived TASAs can not be completely ruled out, but our finding BAV3-TASAs with antigenic determinant site(s) common to polyomavirus-TASAs may be a clue to the function of TASAs in the process of viral transformation.

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