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Relationship Between T-Antigen and Tumor-Specific Transplantation Antigen in Simian Virus 40-Transformed Cells

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The simian virus 40 (SV40) tumor antigen (T-antigen) and tumor-specific transplantation antigen (TSTA) have been partially purified and studied to clarify their relationship. The T-antigen and the TSTA were partially purified from nuclei of SV AL/N cells, an SV40-transformed mouse embryo fibroblast line, by precipitation with ammonium sulfate and chromatography on DEAE- and DNA-cellulose. The T-antigen was assayed by complement fixation, and the TSTA was assayed by its ability to immunize mice against SV40-containing ascites tumor cells. When T-antigen- and TSTA-containing preparations were sedimented through sucrose gradients, each antigen had a major peak of activity at a sedimentation coefficient of 6.7 and minor peaks in other regions. Antiserum against T-antigen (from tumor-bearing hamsters) immunoprecipitated the TSTA activity. A preparation of T-antigen from human SV80 cells, which exhibited only one protein band after sodium dodecyl sulfate-polyacrylamide gel electrophoresis, had TSTA activity when as little as 0.6 μg of protein per mouse was used for immunization. These experiments demonstrate that the T-antigen, the product of the SV40 early *A* gene, is capable of inducing specific immunity against transplantation of SV40-transformed tumor cells in mice.

The simian virus 40 (SV40) tumor antigen (T-antigen) was first demonstrated by complement fixation by using sera from hamsters bearing tumors induced by SV40 (22, 26). The T-antigen is localized mainly in the nuclei of SV40-transformed cells, as demonstrated by immunofluorescent staining (35, 38).

Recent studies have identified two proteins with T-antigen activity. Their molecular weights are about 94,000 (large T-antigen) and 17,000 (small t-antigen) (1, 8, 36, 37, 40). Both of these polypeptides are products of the early half of the SV40 genome (2, 13, 19, 27, 37, 40). It has been suggested that T-antigens play a role in the stimulation of host cell DNA synthesis, the initiation of viral DNA synthesis, and the maintenance of cell transformation (5, 32-34).

The SV40 tumor-specific transplantation antigen (TSTA) is responsible for the induction of specific immunity against SV40-transformed tumor cells (15, 20, 24, 26). The SV40 TSTA can be solubilized by treatment with papain (17) or detergent (12). To understand the immune mechanism of surveillance against SV40-transformed cells, it is important to understand the nature of the SV40 TSTA.

Recently, several findings have suggested a close relationship between SV40 T-antigen and TSTA. In a series of nondefective SV40-adenovirus hybrids, the expression of TSTA is associated with the early region of the SV40 genome (29, 46) that encodes the T-antigen (2, 13, 19, 27, 37, 40). Nuclear fractions from SV40-transformed human or mouse cells are enriched in T-antigen and also in TSTA (4, 39). The expression of T-antigen and TSTA is modulated coordinately in cells transformed by temperature-sensitive SV40 viruses (3, 10, 44). Furthermore, the SV40 T-antigen and TSTA copurify during chromatography on DEAE-cellulose and phosphocellulose and both bind to double-stranded DNA (7, 11). These findings, however, do not prove that the T-antigen has TSTA activity.

In this communication we show that partially purified T-antigen and TSTA have similar sedimentation properties in solution, that anti-serum against T-antigen interacts with TSTA, and that purified T-antigen possesses TSTA activity.

MATERIALS AND METHODS

Cell lines. SV AL/N, an SV40-transformed AL/N mouse embryo fibroblast cell line, and SV80, an SV40-

transformed human fibroblast cell line, have been described previously (21, 41). The mKSA-ASC BALB/c mouse cell line, an SV40-transformed ascites tumor line derived from the mKSA-TU5 cell line (17, 25), was passed twice monthly intraperitoneally in syngeneic mice; it served as the tumor line for testing anti-SV40 TSTA activity in immunized animals. This cell line expresses strong SV40 TSTA activity, and $<10^3$ cells produced tumors in 50% of the mice within 14 days after intraperitoneal injection. The control cell line was Meth-1-A, a methylcholanthrene-induced BALB/c tumor cell line which contains its own non-SV40 TSTA (31). The line was grown in tissue culture and used as a specificity control for rejection of non-SV40 tumors in immunized mice. For this line, the dose which produced tumors in 50% of the mice was $\sim 10^3$ to 10^4 cells.

Partial purification of T-antigen and of TSTA from SV AL/N cells and preparation of purified T-antigen from SV80 cells. The T-antigen and the TSTA were solubilized from a nucleus-enriched fraction of SV AL/N cells by Triton X-100 detergent and further purified by ammonium sulfate precipitation and chromatography on DEAE-cellulose and DNA-cellulose as previously described (11). The purified T-antigen from SV80 cells was prepared as previously described (43).

Sucrose gradient ultracentrifugation. The fractions with T-antigen and TSTA activity eluted from the DNA-cellulose column in a sharp peak (peak III, Fig. 2 in reference 11). From the pool of these fractions 300 μg of protein (30) was layered on a linear 5 to 20% sucrose gradient containing 10 mM Tris, pH 8.0, 0.6 M NaCl, 100 μM L-1-tosylamide-2-phenyl-ethylchloromethyl ketone, 100 μM phenylmethylsulfonylfluoride, and 10% glycerol and centrifuged for 20 h at 4°C and 40,000 rpm in a Spinco SW41 rotor. Fractions of 0.3 ml each were collected from the bottom of the gradient. Each fraction was tested for T-antigen activity by using 25 μl per complement fixation assay; the rest of the fractions were pooled as indicated in Fig. 1. The pooled fractions were divided into two aliquots and injected 1 week apart into mice for the determination of TSTA activity.

Complement fixation assay. The complement fixation assay was performed by the standard microtiter method with 5 U of guinea pig complement (9). The hamster anti-T serum (lot 2x0857) was obtained from Jack Gruber, National Cancer Institute; the same lot was used for our previous study of the T-antigen (11).

Immunoprecipitation. The partially purified T-antigen and TSTA preparations were added to appropriate amounts of normal hamster serum or hamster anti-T antiserum (see Table 2). The mixtures were incubated for 30 min at 4°C with constant shaking. For each 1 μl of serum, 10 μl of *Staphylococcus aureus* cowan I, prepared as reported previously (14), was added to precipitate antigen-antibody complexes, and shaking was continued for 1 h. The mixture was then centrifuged at 5,000 rpm for 10 min. The supernatant solutions were saved for TSTA tests. The pellets were washed three times with Tris-buffered saline (TBS) and suspended in TBS for TSTA tests.

Assay for cell surface antigens. The assay for cell surface antigens was a modification of the method of Dorval et al. (16), which detects cell surface antigens by allowing them to react with antiserum and then detecting the complex by its ^{125}I-labeled protein A from *S. aureus*. SV AL/N cells were trypsinized and grown overnight in Eagle spinner culture medium. The cells were washed three times with TBS, with centrifugation at 1,200 rpm for 5 min. Cells were resuspended, and 10^6 cells in 1 ml of TBS were put in each of several tubes. Next, 2, 5, or 20 μl of normal hamster serum, hamster anti-T serum, rabbit anti-mouse spleen serum, or normal rabbit serum was added to each tube, and the resulting mixtures were shaken at 4°C for 1 h. After the cells were washed three times in TBS, they were resuspended in 1 ml of

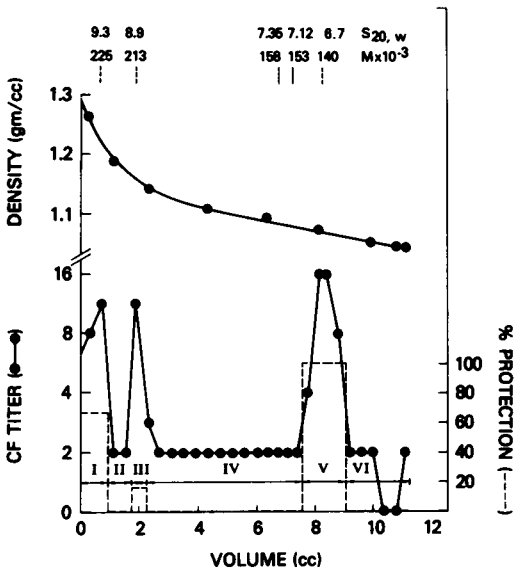

FIG. 1. *Sedimentation velocity analysis of T-antigen and TSTA activities on a sucrose gradient. The preparation from SV AL/N cells, containing 300 μg of protein (30) and obtained from combined T-antigen-active fractions after the DNA-cellulose chromatography (11), was layered on a linear 5 to 20% preformed sucrose gradient and centrifuged for 20 h at 4°C and 40,000 rpm. Fractions of 0.3 ml each were collected from the bottom; 25 μl of each fraction was tested by the complement fixation (CF) test for T-antigen (11); the fractions were pooled as indicated and injected in two doses for the TSTA test. Because 10 of 10 animals in the control group died of ascites tumors, the TSTA activity is presented as the percentage of animals protected; e.g., of the mice inoculated with pool I, 67% were protected (2/6 died of ascites tumor; Table 1). In parallel separate gradients protein standards were run (aldolase and immunoglobulin G, with sedimentation coefficients of 7.35 and 7.12, respectively); sedimentation coefficients, corrected to water at 20°C ($S_{20,w}$) (and molecular weights, $M \times 10^{-3}$), calculated from their positions, are indicated at the top of the figure (dotted lines) for the peaks obtained.*

TBS. Protein A from *S. aureus* (10^6 cpm), labeled with ^{125}I and prepared as reported previously (16), was added to each tube, and the mixtures were incubated at 4°C for 1 h. Finally, the cells were washed three times with TBS, and the radioactivity in the cell pellet was counted with a gamma counter.

Assay for TSTA activity. The TSTA assays were performed as described previously (12). Adult BALB/c mice, 5 to 8 weeks old, were obtained from the National Institutes of Health breeding colony. The antigen samples were injected twice intraperitoneally at 1-week intervals to immunize the animals; control animals received TBS alone. At 10 days after the second injection, some of the mice were injected intraperitoneally with 10^4 SV40-transformed BALB/c mKSA-ASC cells. As another control, other mice were injected intramuscularly with methycholanthrene-transformed Meth-1-A BALB/c cells, which produce a non-SV40 TSTA. Inoculated mice were followed for 5 weeks. Ascites tumors appeared by about 2 weeks after injection with tumor cells; the mean time to death was 20 days.

SDS-gel electrophoresis. Approximately 5 μg of purified T-antigen from SV80 cells, purified as described earlier (43), was applied to a 7.5% sodium dodecyl sulfate (SDS)-polyacrylamide gel tube and subjected to electrophoresis by the method of Laemmli (28). The gel was stained with Coomassie brilliant blue (0.2% in methanol-acetic acid-water, 5:1:5) and destained in methanol-acetic acid-water (5:1:5).

RESULTS

Sucrose gradient ultracentrifugation of partially purified T-antigen and TSTA. T-antigen and TSTA activities were purified as described above. A sample containing 300 μg of protein was overlaid on a 5 to 20% sucrose gradient. The results in Fig. 1 and Table 1 show that the T-antigen activity appeared in multiple peaks. Most of the activity had a sedimentation coefficient of approximately 6.7, whereas the rest appeared in minor peaks that were apparently aggregated forms of the main component. This result was similar to those obtained with less purified preparations (7). The TSTA activity was similarly distributed across the gradients. Mice infected with the fraction having a sedimentation coefficient of 6.7 were completely protected against the challenge with SV40-transformed tumor cells. The similarity in the sedimentation profiles of T-antigen and TSTA further supports the suggestion that both activities reside in the same protein. To extend this hypothesis further, antibody studies were undertaken.

TABLE 1. *TSTA activity of fractions from sucrose gradient*[a]

Immunization fraction	Tumor cell challenge (no. of mKSA-ASC cells)	Tumor incidence[b]
Pool I	10^4	2/6 ($P < 0.003$)
Pool II	10^4	6/6
Pool III	10^4	5/6
Pool IV	10^4	8/8
Pool V	10^4	0/7 ($P < 0.001$)
Pool VI	10^4	7/7
None	10^4	10/10

[a] Adult BALB/c mice were immunized with fractions from the sucrose gradient (Fig. 1) and then injected intraperitoneally with 10^4 mKSA-ASC tumor cells.

[b] Number of mice developing tumors/number of mice inoculated. All tumors were lethal in ~20 days.

Immunoprecipitation of TSTA with anti-T serum. TSTA was immunoprecipitated with the hamster anti-T serum. Immunization of mice with 5 or 15 μg of the partially purified protein (after the DNA-cellulose chromatography) protected them against an otherwise lethal dose of SV40-tumor cells (Table 2, first section). Normal hamster serum (2 to 20 μl) mixed with the TSTA preparation and *S. aureus* bacterium did not precipitate TSTA activity from the supernatant; the inoculated supernatant contained nearly its initial level of TSTA activity (Table 2, second section). In contrast, increasing volumes of anti-T serum in the presence of *S. aureus* precipitated proportionally increasing amounts of TSTA activity from the supernatant; 5 μl of anti-T serum removed 57 to 85% of the original TSTA (15 μg), and 20 μl of anti-T serum removed 85 to 100%. There was no TSTA activity in the precipitate formed by incubation of 15 μg of the TSTA preparation with 20 μl of normal hamster serum and *S. aureus*, but TSTA activity was recovered from the precipitate formed with 20 μl of the anti-T serum. The TSTA activity was SV40 specific; it did not protect the mice against a lethal dose of methylcholantrene-induced tumor cells (Table 2, fourth section).

Cell surface antigens. To determine whether the anti-T serum possessed reactivity against cell surface components of SV AL/N cells, a sensitive assay with ^{125}I-labeled protein A from *S. aureus* was carried out as described above; the results are shown in Table 3. The anti-T serum behaved like normal hamster serum and did not bind to the SV AL/N cells. As a positive control, rabbit anti-mouse serum or normal rabbit serum was allowed to bind to the SV AL/N mouse cells. The anti-mouse serum bound eight times more ^{125}I-labeled protein A to the SV AL/N cells than did the normal rabbit serum.

TSTA activity of purified T-antigen from SV80 cells. The T-antigen, purified from SV80 cells as previously described (43), was homogeneous on SDS gels (Fig. 2) and had an apparent

TABLE 2. *Interaction of TSTA with anti-T serum*

Immunization protein (μg)	Incubation with serum (μl)	Tumor cell challenge (no. of cells)	Tumor incidence[a]		*P* value[b]	
			Expt 1	Expt 2	Expt 1	Expt 2
DNA-cellulose-bound fraction[c]						
0 (TBS)		10^4 mKSA[d]	12/12	14/14		
15		10^4 mKSA	0/7	0/7	<0.001	<0.001
5		10^4 mKSA	0/6	2/7	<0.001	<0.001
1		10^4 mKSA	6/8	6/7		
Supernatant[e]						
15	NHS (2)	10^4 mKSA	0/6	1/8		
15	NHS (5)	10^4 mKSA	1/7	1/8		
15	NHS (20)	10^4 mKSA	2/7	3/8		
15	Anti-T serum (2)	10^4 mKSA	2/7	3/7		
15	Anti-T serum (5)	10^4 mKSA	4/7	6/7	≤0.03	<0.002
15	Anti-T serum (20)	10^4 mKSA	6/7	7/7	<0.002	<0.001
Precipitate[f]						
15	NHS (20)	10^4 mKSA	7/7	8/8		
15	Anti-T serum (20)	10^4 mKSA	0/7	1/8	<0.001	<0.001
Precipitate[f]						
15	Anti-T serum (20)	10^4 Meth-1-A[g]		3/6		
TBS		10^4 Meth-1-A		4/7		

[a] Number of mice developing tumors/number of mice inoculated.

[b] *P* values given when significant (18).

[c] BALB/c mice were immunized intraperitoneally twice with the indicated dose of the DNA-cellulose-bound fraction (T-antigen and TSTA) from SV AL/N cells as described in the text.

[d] mKSA, mKSA-ASC tumor cell line.

[e] Mice were immunized with the supernatants obtained after the immunoprecipitation of 15 μg of antigens with either normal hamster serum (NHS) or anti-T serum and *S. aureus*.

[f] Mice were immunized with pellets obtained from immunoprecipitation as described in footnote *e*.

[g] Meth-1-A is a chemically induced tumor cell line which has its own TSTA (31). It served as specificity control, as described previously (12).

TABLE 3. *^{125}I-labeled protein A cell surface antigen test of anti-T serum against SV AL/N cells*[a]

Serum	Dose (μl)	Amt of ^{125}I-labeled protein A[b] (cpm)	Ratio of Immune serum to normal serum
Normal hamster	2	640 ± 12	
	5	785 ± 5	
	20	1,210 ± 20	
Anti-T	2	658 ± 5	1.0
	5	734 ± 16	0.94
	20	1,199 ± 12	0.98
Normal rabbit	2	805 ± 7	
Rabbit anti-mouse	2	6,477 ± 35	8.1

[a] The test was carried out as described in the text.

[b] Amount of ^{125}I-labeled protein A bound to the serum-treated SV AL/N cells.

[c] Ratio was calculated by dividing the counts per minute in the immune serum-treated SV AL/N cells by the counts per minute in the normal serum-treated SV AL/N cells, both at the same dose.

molecular weight of 94,000. This T-antigen preparation was tested for its TSTA activity; BALB/c mice were immunized with 0.024, 0.12, or 0.6 μg of the purified protein and later injected with SV40-transformed mKSA-ASC cells (~10 to 100 times the dose which produced tumors in 50% of the mice). An immunizing dose of 0.6 μg protected the mice against the SV40-transformed tumor cells (Table 4).

DISCUSSION

The sedimentation pattern of our partially purified T-antigen contained a number of peaks of T-antigen activity, similar to the pattern reported previously (8). The pattern suggests that the protein has a tendency to aggregate. The T-antigen and TSTA activities had similar sedimentation patterns.

The relationship of T-antigen and TSTA was further demonstrated by immunoprecipitation

FIG. 2. *SDS-polyacrylamide gel electrophoresis of the purified T-antigen from SV40 cells. Approximately 5 μg of T-antigen from SV40-transformed human SV80 cells, purified as described previously (43), was applied to a 7.5% SDS-polyacrylamide tube gel and subjected to electrophoresis by the method of Laemmli (28). The gel was stained with Coomassie brilliant blue (0.2%, in methanol–acetic acid–water, 5:1:5) and destained in methanol–acetic acid–water (5:1:5).*

TABLE 4. *TSTA activity in purified SV80 large T-antigen*

Immunization dose[a] (μg)	Tumor cell challenge (no. of mKSA-ASC cells)	Tumor incidence[b]	*P* value[c]
0	10^4	10/10	
0.024	10^4	8/8	
0.12	10^4	6/8	
0.6	10^4	2/7	<0.003

[a] Adult BALB/c mice were immunized intraperitoneally twice with purified SV80 large T-antigen with the indicated dosage.

[b] Number of mice developing tumor/number of mice inoculated.

[c] See reference 18.

of TSTA with anti-T serum. No measurable antibodies against the cell surface antigens of the SV40-transformed SV AL/N cells were found in our anti-T serum, even though a sensitive ^{125}I assay was used. This result confirms previous findings, which used a less sensitive immunofluorescent assay (6, 45). Anti-T serum in the presence of *S. aureus* did, however, quantitatively precipitate the solubilized TSTA, whereas normal hamster serum did not remove significant amounts of TSTA. Conversely, the TSTA activity was found in the precipitated complex formed with anti-T serum, but not with normal hamster serum. These results indicate that anti-T serum interacted with TSTA. A low dose of purified T-antigen from human SV80 cells, homogeneous by SDS-polyacrylamide gel electrophoresis, was able to induce immunity against SV40-transformed mKSA tumor cells. We believe that these results prove that the same polypeptide product of the SV40 *A* gene contains both T-antigen and TSTA activities.

The SV40 *A* gene products have at least two properties. One property is that of the TSTA, which is responsible for transplantation immunity in mice. The other property is that of stimulating host cell DNA synthesis (47) and possibly initiating and maintaining transformation (5, 32, 33). Although it appears that one polypeptide accounts for both the T-antigen and TSTA activity, the antigenic determinants for these two activities may be different, as reported previously (3, 29). It is also possible that early gene products other than the 94,000-dalton T-antigen (e.g., small t-antigen) will also be shown to possess TSTA activity.

The T-antigen and TSTA are located predominantly inside the nucleus (4, 39). The immunogenicity of the intracellular T-antigen and TSTA activity might be explained by the disintegration

of tumor cells and subsequent exposure of the intracellular antigens to immunocytes. There must, however, be some TSTA on the cell surface which is responsible for immunosensitivity, because it is difficult with the current concepts of immune mechanisms against tumor cells to conceive of an intracellular antigen being responsible for in vivo cytotoxic or cytostatic effects on tumor cells. This SV40 cell surface TSTA, which may be then responsible for immunosensitivity, is yet to be identified. Recently, Ito et al. have identified a T-antigen on the cell surface from polyoma virus-transformed cells, with a molecular weight of 56,000 (23). An antigen of the same molecular weight which is apparently SV40 specific, has been identified in SV40-transformed cells (Chang et al., manuscript in preparation). Whether this antigen is the cell surface TSTA, however, remains to be shown.

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LITERATURE CITED

1. **Ahmed-Zadeh, C, B. Allet, J. Greenblatt, and R. Weil.** 1976. Two forms of Simian virus-40 specific T antigen in abortive and lytic infection. Proc. Natl. Acad. Sci. U.S.A. **73**:1097-1101.
2. **Alwine, J. C., S. I. Reed, J. Ferguson, and G. R. Strak.** 1975. Properties of T antigen induced by wild type SV40 and tsA mutants in lytic infection. Cell **6**: 529-533.
3. **Anderson, J. L., C. Chang, P. T. Mora, and R. G. Martin.** 1977. Expression and thermal stability of simian virus 40 tumor-specific transplantation antigen and tumor antigen in wild type- and *tsA* mutant-transformed cells. J. Virol. **21**:459-467.
4. **Anderson, J. L., R. G. Martin, C. Chang, P. T. Mora, and D. M. Livingston.** 1977. Nuclear preparations of SV40 transformed cells contain tumor specific transplantation antigen activity. Virology **76**:420-425.
5. **Brugge, J. S., and J. C. Butel.** 1975. Role of simian virus 40 gene *A* function in maintenance of transformation. J. Virol. **15**:619-635.
6. **Butel, J. C., S. S. Tevethia, and J. L. Melnick.** 1972. Oncogenicity and cell transformation by papovavirus SV40. Adv. Cancer Res. **15**:1-55.
7. **Carroll, R. B., L. Hager, and R. Dulbecco.** 1974. Simian virus 40 T antigen binds to DNA. Proc. Natl. Acad. Sci. U.S.A. **71**:3754-3755.
8. **Carroll, R. B., and A. E. Smith.** 1976. Monomer molecular weight of T antigen from SV40 infected and transformed cells. Proc. Natl. Acad. Sci. U.S.A. **73**: 2254-2258.
9. **Casey, H. L.** 1965. Standardized diagnostic complement fixation method and adaptation to microtest. Public Health Monogr. no. 74. U.S. Government Printing Office, Washington, D.C.
10. **Chang, C., J. L. Anderson, R. G. Martin, and P. T. Mora.** 1977. Expression of tumor-specific transplantation antigen in cell lines transformed by wild-type or *tsA* mutant simian virus 40. J. Virol. **22**:281-289.
11. **Chang, C., S. W. Luborsky, and P. T. Mora.** 1977. The SV40 tumor specific transplantation antigen binds to DNA. Nature (London) **269**:438-439.
12. **Chang, C., S. J. Pancake, S. W. Luborsky, and P. T. Mora.** 1977. Detergent solubilization and partial purification of tumor specific surface and transplantation antigens from SV40 transformed mouse cells. Int. J. Cancer **19**:258-266.
13. **Crawford, L. V., C. N. Cole, A. E. Smith, E. Paucha, P. Tegtmeyer, K. Rundell, and P. Berg.** 1978. Organization and expression of early genes of Simian virus 40. Proc. Natl. Acad. Sci. U.S.A. **75**:117-121.
14. **Cullen, S. E., and B. D. Schwartz.** 1976. An improved method for isolation of H-2 and Ia alloantigens with immunoprecipitation induced by protein A-bearing staphylococci. J. Immunol. **117**:136-142.
15. **Defendi, V.** 1963. Effects of SV40 virus immunization on growth of transplantable SV40 and polyoma virus tumors in hamsters. Proc. Soc. Exp. Biol. Med. **113**:12-16.
16. **Dorval, G., K. J. Welsh, and H. Wigzell.** 1975. A radioimmunoassay of cellular surface antigen of living cells using soluble protein A from *Staphylococcus aureus*. J. Immunol. Methods **7**:237-250.
17. **Drapkin, M. S., E. Appella, and L. W. Law.** 1974. Immunogenic properties of a soluble tumor specific transplantation antigen induced by Simian virus-40. J. Natl. Cancer Inst. **52**:259-264.
18. **Goldstein, A.** 1967. Biostatistics, an introductory text, p. 111. The Macmillian Co., New York.
19. **Greenblatt, J. F., B. Allet, R. Weil, and C. Ahmed-Zadeh.** 1976. Synthesis of the tumor antigen and the major capsid protein of SV40 in a cell free system derived from *Escherichia coli*. J. Mol. Biol. **108**: 361-379.
20. **Habel, K., and B. E. Eddy.** 1963. Specificity of resistence to tumor challenge of polyoma and SV40 virus immune hamsters. Proc. Soc. Exp. Biol. Med. **113**:1-4.
21. **Henderson, C., and D. M. Livingston.** 1974. Partial purification and characterization of the SV40 T antigen. Cell **3**:65-70.
22. **Huebner, R. J., W. P. Rowe, H. C. Turner, and W. T. Lane.** 1963. Specific adenovirus complement-fixing antigen in virus-free hamster and rat tumors. Proc. Natl. Acad. Sci. U.S.A. **50**:379-389.
23. **Ito, Y., J. R. Brocklehurst, and R. Dulbecco.** 1977. Virus-specific proteins in plasma membrane of cells lytically infected or transformed by polyoma virus. Proc. Natl. Acad. Sci. U.S.A. **74**:4666-4670.
24. **Khera, K. S., A. Ashkenazi, F. Rapp, and J. L. Melnick.** 1963. Immunity in hamsters to cells transformed *in vitro* and *in vivo* by SV40. Tests for antigenic relationship among the papovaviruses. J. Immunol. **91**: 604-613.
25. **Kit, S., T. Kurimura, and D. R. Dubbs.** 1969. Transplantable mouse tumor line induced by injection of SV40-transformed mouse kidney cells. Int. J. Cancer **4**: 384-392.
26. **Koch, M. A., and A. B. Sabin.** 1963. Specificity of virus induced resistance to transplantation of polyoma and SV40 tumors in adult hamsters. Proc. Soc. Exp. Biol. Med. **113**:4-12.
27. **Kuchino, T., and N. Yamaguchi.** 1975. Characterization of T antigen in cells infected with a temperature-sensitive mutant of simian virus 40. J. Virol. **15**:1302-1307.
28. **Laemmli, U.** 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London) **227**:680-685.
29. **Lewis, A. M., Jr., and W. P. Rowe.** 1973. Studies of nondefective adenovirus 2-simian virus 40 hybrid viruses. VIII. Association of simian virus 40 transplantation antigen with a specific region of the early viral genome. J. Virol. **12**:836-840.
30. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.

31. **McCollester, P. L.** 1970. Isolation of Meth-A cell surface membrane possessing tumor-specific transplantation antigen activity. Cancer Res. **30**:2832-2840.
32. **Martin, R. G., and J. Y. Chou.** 1975. Simian virus 40 functions required for the establishment and maintenance of malignant transformation. J. Virol. **15**: 599-612.
33. **Martin, R. G., M. Persico-DiLauro, C. Edwards, and A. Oppenheim.** 1977. Molecular basis of transformation by simian virus 40 virus, p. 87-102. *In* J. Schultz and Z. Brada (ed.), Genetic manipulation as it affects the cancer problem. Academic Press Inc., New York.
34. **Osborn, M., and K. Weber.** 1975. Simian virus 40 gene *A* function and maintenance of transformation. J. Virol. **15**:636-644.
35. **Pope, J. H., and W. P. Rowe.** 1964. Detection of specific antigen in SV40 transformed cells by immunofluorescence. J. Exp. Med. **120**:121-127.
36. **Prives, C., and Y. Beck.** 1977. Characterization of SV40 T antigen polypeptides synthesized *in vivo* and *in vitro*. INSERM Colloq. **69**:175-188.
37. **Prives, C., E. Gilbora, M. Revel, and E. Winocur.** 1977. Cell-free translation of SV40 early messenger RNA coding for viral T antigen. Proc. Natl. Acad. Sci. U.S.A. **74**:457-461.
38. **Rapp, F., J. C. Butel, and J. L. Melnick.** 1964. Virus induced intranuclear antigen in cells transformed by papovavirus SV40. Proc. Soc. Exp. Biol. Med. **116**: 1131-1135.
39. **Rogers, M. J., L. W. Law, and E. Appella.** 1977. Subcellular distribution of the tumor specific transplantation antigen of SV40 transformed cells. J. Natl. Cancer Inst. **59**:1291-1295.
40. **Rundell, K., J. K. Collins, P. Tegtmeyer, H. L. Ozer, C. Lai, and D. Nathans.** 1977. Identification of simian virus 40 protein A. J. Virol. **21**:636-646.
41. **Smith, R. W., J. Morganroth, and P. T. Mora.** 1970. SV40 virus induced tumor-specific transplantation antigen in cultured mouse cells. Nature (London) **227**: 141-145.
42. **Tegtmeyer, P.** 1975. Function of simian virus 40 gene *A* in transforming infection. J. Virol. **15**:613-618.
43. **Tenen, D., H. Garewal, L. Haines, V. Hudson, V. Woodward, S. Light, and D. M. Livingston.** 1977. Purification of SV40 tumor antigen from a line of SV40 transformed human cells. Proc. Natl. Acad. Sci. U.S.A. **74**:3745-3749.
44. **Tenen, D. G., P. Baygell, and D. M. Livingston.** 1975. Thermolabile T (tumor) antigen from cells transformed by a temperature sensitive mutant of SV40. Proc. Natl. Acad. Sci. U.S.A. **72**:4351-4355.
45. **Tevethia, S. S., L. A. Couvillion, and F. Rapp.** 1968. Development in hamsters of antibodies against surface antigens present in cells transformed by papovavirus SV40. J. Immunol. **100**:358-362.
46. **Tevethia, M. J., and S. S. Tevethia.** 1977. Biology of simian virus 40 (SV40) transplantation antigen (TrAg). III. Involvement of SV40 gene A in the expression of TrAg in permissive cells. Virology **81**:212-223.
47. **Tjian, R., G. Fey, and E. Graessmann.** 1978. Biological activity of purified Simian virus-40 T antigen proteins. Proc. Natl. Acad. Sci. U.S.A. **75**:1279-1283.