Proc. Natl. Acad. Sci. USA
Vol. 75, No. 7, pp. 3055–3059, July 1978
Biochemistry

Tumor-specific transplantation antigen: Use of the $Ad2^+ND_1$ hybrid virus to identify the protein responsible for simian virus 40 tumor rejection and its genetic origin

(adenovirus 2–simian virus 40 hybrid/papovavirus early antigens)

GILBERT JAY*, FRANCIS T. JAY†, CHUNGMING CHANG‡, ROBERT M. FRIEDMAN†, AND ARTHUR S. LEVINE*

* Pediatric Oncology Branch and ‡ Macromolecular Biology Section, National Cancer Institute, and † Laboratory of Experimental Pathology, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Maryland 20014

Communicated by Robert J. Huebner, March 30, 1978

ABSTRACT **Cells transformed by simian virus 40 (SV40) possess a tumor-specific transplantation antigen (TSTA) that has the property of immunizing animals against syngeneic tumor challenge. We find that the early SV40 DNA segment present in the human adenovirus 2 (Ad2)–SV40 hybrid, $Ad2^+ND_1$, is sufficient to induce this SV40-specific TSTA in BALB/c mice. Moreover, studies on the intracellular distribution of TSTA activity in $Ad2^+ND_1$-infected cells, as determined by the ability of various subcellular fractions to immunize mice against syngeneic tumor challenge, have suggested a correlation between this biological activity and the presence of the SV40-specific 28,000 M_r protein coded by this hybrid virus. Both the TSTA activity and the 28,000 M_r protein are found in the plasma membrane fraction and in the perinuclear region of infected cells but are virtually undetectable in the cytoplasmic fraction. Using a hamster antitumor antiserum that can specifically immunoprecipitate the 28,000 M_r protein, we are able to demonstrate a loss of TSTA activity concomitant with the removal of this SV40-coded protein. Thus, it appears that antigenic determinants responsible for SV40-specific tumor rejection in mice are contained within the 28,000 M_r protein coded for by the early SV40 DNA segment that extends from 0.17 to 0.28 map unit.**

Transformation of normal cells to malignancy is accompanied by changes in the cell surface. Because many of the regulatory signals that control the growth and multiplication of normal cells operate at the cell surface, such alterations have been postulated to play a key role in the behavior and development of tumor cells (1).

One such surface alteration in cells transformed by simian virus 40 (SV40) is the acquisition of a tumor-specific transplantation antigen (TSTA) (2, 3). The fact that mice immunized with SV40 become resistant to subsequent challenges with transplantable SV40-induced tumors has led to the hypothesis that acquisition of SV40 TSTA by cells infected or transformed *in vivo* provokes an immune response leading to the destruction of these cells and to the rejection of any challenging tumor cells bearing this transplantation antigen (4–7). Although this rejection activity is well defined biologically, the antigen responsible for the activity has not been identified biochemically.

Because an immunologically identical transplantation antigen is present in cells of different species transformed by SV40 (8), it seems likely that TSTA is virus-induced. However, it is not clear whether this antigen is in fact coded for by SV40 or represents a cellular protein derepressed by the virus.

In an attempt to identify the protein containing the determinants for this SV40-induced antigenic activity, we have used the nondefective hybrid virus, $Ad2^+ND_1$, which contains a short segment of the early region of SV40 DNA (from 0.17 to 0.28 map unit) covalently integrated into the human adenovirus 2 (Ad2) genome. In this report, we present evidence that the single 28,000 M_r protein coded for by this SV40 DNA segment contains antigenic determinant(s) responsible for the rejection of SV40-specific tumors in mice.

MATERIALS AND METHODS

Assay for TSTA Activity. Female BALB/c mice, 4–6 weeks old, were immunized with the appropriate virus or subcellular fraction in two equal intraperitoneal injections, 1 week apart. The second intraperitoneal injection was followed in 10 days by an intramuscular injection of 10^4 mKSA (ASC) cells, a SV40-transformed fibroblastic line of BALB/c origin (9), kindly provided by L. W. Law. Mice were observed for at least 5 weeks for tumor development at the site of challenge.

Subcellular Fractionation. Ad2 and $Ad2^+ND_1$ stocks were grown in KB cells, purified by equilibrium centrifugation in CsCl, and plaque-titered on primary human embryonic kidney cells (10). All experiments were conducted in a P3 biohazard containment facility. For studies on subcellular fractionation (10, 11), monolayers of human KB cells infected with either Ad2 or $Ad2^+ND_1$, at a multiplicity of 20, were harvested 24 hr after infection. Where indicated, infected cells were radiolabeled by incubation in Earle's balanced salt solution containing 10% Eagle's minimum essential medium, 2% fetal calf serum, and 20 μCi of L-[^{35}S]methionine (800 Ci/mmol) per ml, for the appropriate period of time immediately before harvest (11). Cells were disrupted by Dounce homogenization and then centrifuged. The supernatant containing the cytoplasmic fraction was stored; the pellet was treated with dextran/polyethylene glycol to effect separation of plasma membranes from intact nuclei (12). The nuclear fraction was treated for 10 min at 4° with 10 mM Tris·HCl, pH 6.8/150 mM KCl/5 mM $MgCl_2$/1% Triton X-100, and the washed nuclei were separated from the nuclear wash by centrifugation at 2000 × *g* for 15 min. The plasma membrane fraction was treated for 60 min at 4° with Tris-buffered saline (pH 7.4) containing 1% Triton X-100, and the washed plasma membranes were separated from the plasma membrane wash by centrifugation at 20,000 × *g* for 20 min. All fractions containing Triton X-100 were treated with Biobeads SM2 to remove the detergent (13).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: SV40, simian virus 40; TSTA, tumor-specific transplantation antigen; Ad2, adenovirus 2; PFU, plaque-forming units; $NaDodSO_4$, sodium dodecyl sulfate.

RESULTS

Induction of SV40-Specific Transplantation Antigen by Ad2$^+$ND$_1$. When 4-week-old BALB/c mice were given intramuscular injections of 10^4 mKSA (ASC) cells, tumors could be detected at the site of inoculation in 20/20 animals as early as 10 days after injection (Table 1). However, if mice were first immunized with SV40 [10^6 plaque-forming units (PFU) per animal] before challenge with mKSA cells, only 1/10 mice developed any tumor over an observation period of at least 5 weeks. Whereas a 10-fold higher dose of SV40 conferred complete protection against tumor challenge, a 10-fold lower dose failed to offer any detectable protection.

In contrast to SV40, inoculation of Ad2 at doses from 2×10^7 to 2×10^9 PFU did not confer any detectable protection against SV40 tumor challenge (Table 1). However, inoculation of Ad2$^+$ND$_1$, a nondefective hybrid virus containing a segment of the early region of SV40 DNA covalently integrated into the Ad2 genome, did confer a dose-dependent protection. At 14 days, 8/10 inoculated mice had responded to the tumor challenge at 2×10^7 PFU, 2/10 at 2×10^8 PFU, and 0/10 at 2×10^9 PFU. At that time, all of the control animals that had not been inoculated or had been inoculated with equivalent doses of Ad2 had already developed tumors. Unlike the protection afforded by SV40, that provided by Ad2$^+$ND$_1$ was relatively durable only at high virus concentrations. At 2×10^8 PFU, the protection observed represented only a delay in the appearance of tumors; however, at 2×10^9 PFU, the protection appeared to be long-lasting.

Subcellular Localization of TSTA Activity. Early studies have shown that the SV40 insertion in Ad2$^+$ND$_1$ results in the induction of only one detectable polypeptide, of 28,000 M_r, not found in wild-type Ad2-infected cells (14). To determine whether a correlation exists between the 28,000 M_r protein and the antigenic determinants for SV40-specific TSTA, we attempted to identify both protein and TSTA activity in various fractions from infected cells.

Subcellular fractions from cells infected with either Ad2 or Ad2$^+$ND$_1$ were tested for their ability to confer protection against SV40 tumor challenge. Human KB cells in monolayer cultures were infected with the appropriate virus, and the cells were harvested 24 hr later. In order to determine the amount of the 28,000 M_r protein present in each of the subcellular fractions to be tested for tumor rejection activity, half of each set of infected cells was labeled with [^{35}S]methionine, beginning 2 hr before the time of harvest, and the labeled and unlabeled cells were subsequently processed in parallel.

Using sodium dodecyl sulfate (NaDodSO$_4$)/polyacrylamide gel electrophoresis (15), we found that the plasma membrane fraction from Ad2$^+$ND$_1$-infected cells (Fig. 1, track c) was significantly enriched for the 28,000 M_r protein, representing about 80% of the total of this protein when compared to the whole cell extract (Fig. 1, track a; ref. 10). Ad2-infected cells demonstrated an almost identical distribution of radiolabeled proteins (Fig. 1, track b), except for the absence of the 28,000 M_r protein which is specific to Ad2$^+$ND$_1$-infected cells. In order to enrich further for the Ad2$^+$ND$_1$-specific protein, the plasma membrane fraction was treated with 1% Triton X-100. Densitometric comparison of the resulting plasma membrane wash (Fig. 1, track e) with the insoluble washed plasma membranes (Fig. 1, track g) showed that more than 90% of the 28,000 M_r protein could be released from the plasma membrane by treatment with this nonionic detergent.

When injected into mice, the plasma membrane wash from Ad2$^+$ND$_1$-infected cells conferred protection against subsequent challenge by mKSA cells (Table 2). At 60 μg of protein, 6/10 mice developed tumors, and at 300 μg of protein, 0/10. In contrast, virtually all mice immunized with the plasma membrane wash from Ad2-infected cells developed tumors at either 60 or 300 μg of protein. That the protection conferred by the plasma membrane wash from Ad2$^+$ND$_1$-infected cells was specifically directed toward SV40 tumor cells is shown by the fact that, whereas a dose of 300 μg of protein offered complete protection against challenge with mKSA cells, an identical dose offered no protection against subsequent challenge with Meth A cells (TD_{50} ~10^3 cells) derived from a chemically induced tumor.

The detergent-washed plasma membrane fraction from Ad2$^+$ND$_1$-infected cells was also tested for TSTA activity (Table 2). Whereas 7/10 mice developed tumors after receiving

Table 1. Virus induction of SV40-specific TSTA activity

Inoculum	Dose,* PFU	Tumor-bearing mice/total mice at various times after challenge with mKSA tumor cells			
		Day 14	Day 21	Day 28	Day 35
Uninoculated	—	20/20	20/20	20/20	20/20
SV40	1×10^5	10/10	10/10	10/10	10/10
	1×10^6	1/10	1/10	1/10	1/10
	1×10^7	0/10	0/10	0/10	0/10
Ad2	2×10^7	10/10	10/10	10/10	10/10
	2×10^8	10/10	10/10	10/10	10/10
	2×10^9	10/10	10/10	10/10	10/10
Ad2$^+$ND$_1$	2×10^7	8/10	10/10	10/10	10/10
	2×10^8	2/10	8/10	9/10	9/10
	2×10^9	0/10	1/10	3/10	3/10

* Total dosage given in two equal injections, 7 days apart.

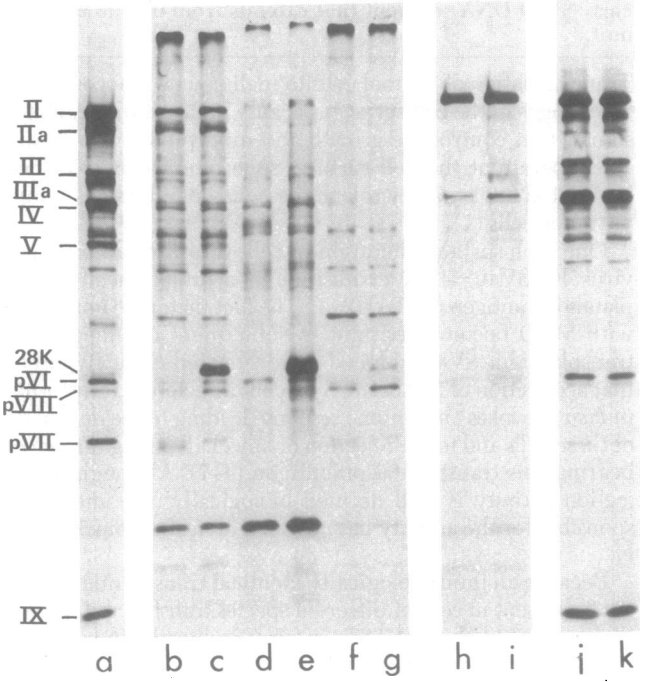

FIG. 1. Subcellular distribution of the Ad2$^+$ND$_1$-specific 28,000 M_r protein. Autoradiogram of a 12.5% NaDodSO$_4$/polyacrylamide gel (11, 15) displaying the [^{35}S]methionine-labeled proteins found in the various subcellular fractions from KB cells infected with either Ad2 (tracks b, d, f, h, and j) or Ad2$^+$ND$_1$ (tracks c, e, g, i, and k). Tracks: a, whole cell extract from Ad2$^+$ND$_1$-infected cells; b and c, plasma membrane fraction; d and e, plasma membrane wash; f and g, washed plasma membranes; h and i, nuclear membrane wash; j and k, cytoplasmic fraction.

Table 2. Subcellular distribution of TSTA activity

Inoculum	Dose,* μg protein	Challenging tumor cells	Tumor-bearing mice /total mice, inoculum from cells infected with Ad2	Ad2+ND1
Plasma	60	mKSA	9/10	6/10
membrane	300	mKSA	10/10	0/10
wash	300	Meth A	—	10/10
Washed	500	mKSA	10/10	7/10
plasma	2000	mKSA	10/10	0/10
membrane				
Nuclear	200	mKSA	6/7	2/7
membrane	300	mKSA	9/10	0/8
wash	900	mKSA	10/10	0/8
	300	Meth A	—	10/10
Cytoplasmic	500	mKSA	10/10	10/10
fraction	1500	mKSA	10/10	7/10

* Total dosage given in two equal injections, 7 days apart.

a dose of 500 μg of protein, 0/10 developed tumors after receiving 2 mg of protein, suggesting that this subcellular fraction also contained tumor rejection activity. However, the specific activity of this fraction was only 1/10 that of the plasma membrane wash, because 10 times as much protein was required to confer equivalent protection against tumor challenge. This observation correlated well with the amount of the 28,000 M_r protein present in the two fractions: there was also 10 times as much Ad2+ND1-specific protein in the plasma membrane wash as in the washed plasma membranes (Fig. 1, tracks e and g).

We have also observed that about 20% of the intracellular content of the 28,000 M_r protein is associated with the nuclear membrane (10) and that this protein can be quantitatively removed from the nuclei by treatment with 1% Triton X-100. The resulting nuclear membrane wash (Fig. 1, track i) represented a significant enrichment for this Ad2+ND1-specific protein when compared to the whole cell extract (Fig. 1, track a). Of injected mice, 2/7 receiving 200 μg of protein developed tumors upon subsequent challenge with mKSA cells, whereas 0/8 receiving either 300 or 900 μg of protein developed tumors (Table 2). The specific activity of this fraction was about equivalent to that of the plasma membrane wash. This protection was again specific to Ad2+ND1: equivalent protein doses of a parallel fraction from Ad2-infected cells (Fig. 1, track h) failed to induce significant tumor rejection. Again, this TSTA activity was directed against challenge by SV40 tumor cells but not by Meth A cells.

Analysis of the cytoplasmic fraction from Ad2+ND1-infected cells failed to reveal the presence of the 28,000 M_r protein (Fig. 1, track k). At a dose of 500 μg of protein from this fraction, 10/10 mice developed tumors in response to subsequent challenge with mKSA cells, and at 1.5 mg of protein, 7/10 developed tumors (Table 2). This finding suggests that the TSTA activity in the cytoplasmic fraction is quite low, consistent with our inability to detect the 28,000 M_r protein.

Effect of Treatment with Antitumor Serum on TSTA Activity. When the nuclear membrane wash from KB cells infected with Ad2+ND1 and labeled with [35S]methionine for 4 hr before harvesting was incubated with antitumor serum obtained from a hamster bearing an SV40-induced tumor, the 28,000 M_r protein was immunoprecipitated along with a trace amount of the Ad2-specific polypeptide II (Fig. 2 *left*, track b). When an equivalent amount of normal hamster serum was used in place of the antitumor serum, the 28,000 M_r protein was not detected, but a trace amount of polypeptide II was again nonspecifically precipitated (Fig. 2 *left*, track d).

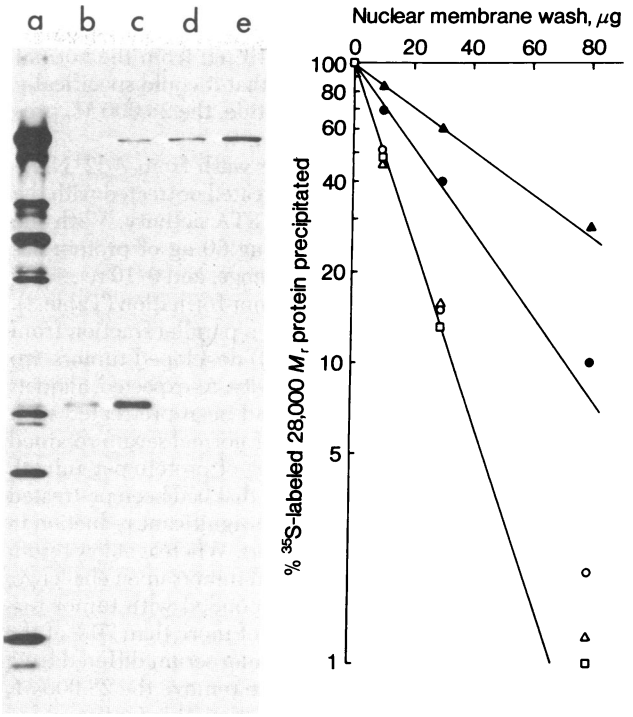

FIG. 2. Removal of the 28,000 M_r protein by immunoprecipitation using an SV40-specific antitumor serum. (*Left*) Autoradiogram of a 12.5% NaDodSO4/polyacrylamide gel displaying the immunoprecipitates from the [35S]methionine-labeled nuclear membrane wash of Ad2+ND1-infected cells, with different concentrations of either normal or antitumor serum (10, 16). Tracks: a, whole cell extract from Ad2+ND1-infected cells used as a marker to identify the position of the 28,000 M_r protein; b, low concentration of antitumor serum (12 μl of serum/100 μg of protein in nuclear membrane wash); c, high concentration of antitumor serum (24 μl/100 μg of protein); d, low concentration of normal serum (12 μl/100 μg of protein); e, high concentration of normal serum (24 μl/100 μg of protein). (*Right*) Competition-immunoprecipitation curves for estimation of the amount of 28,000 M_r protein present in the unlabeled nuclear membrane wash from Ad2+ND1-infected cells after treatment with the appropriate serum. Antitumor serum (2 μl) was incubated with 10 μg of [35S]-methionine-labeled nuclear membrane wash from Ad2+ND1-infected cells, either in the absence or in the presence of varying amounts of unlabeled serum-treated nuclear membrane wash. The amount of [35S]methionine-labeled 28,000 M_r protein immunoprecipitated was determined by NaDodSO4/polyacrylamide gel electrophoresis, followed by densitometric analysis of the autoradiogram. The competing unlabeled fractions were: □, untreated; ●, treated with a low concentration of antitumor serum (12 μl of serum/100 μg of protein); ▲, treated with a high concentration of antitumor serum (24 μl/100 μg of protein); ○, treated with a low concentration of normal serum (12 μl/100 μg of protein); △, treated with a high concentration of normal serum (24 μl/100 μg of protein).

In order to demonstrate unequivocally that the antigenic determinant(s) responsible for the induction of tumor rejection is contained within the Ad2+ND1-specific 28,000 M_r protein, we studied the effect of the antitumor serum on the TSTA activity of a subcellular fraction from Ad2+ND1-infected cells. The nuclear membrane wash from Ad2+ND1-infected cells was incubated with either antitumor serum or normal serum. Two antibody concentrations were chosen, both reactions occurring in the presence of antigen excess (as determined by titration with a parallel 35S-labeled fraction) in order to avoid any nonspecific binding that might occur in the presence of antibody excess. NaDodSO4/polyacrylamide gel analysis of the immu-

noprecipitates from the ^{35}S-labeled nuclear membrane wash indicated that at either serum concentration the antitumor serum (Fig. 2 *left*, tracks b and c) differed from the normal serum (Fig. 2 *left*, tracks d and e) in that it could specifically immunoprecipitate only one polypeptide, the 28,000 M_r protein.

Aliquots of the nuclear membrane wash from $Ad2^+ND_1$-infected cells that had been either untreated or treated with the appropriate serum were tested for TSTA activity. With the untreated sample, 6/10 mice receiving 60 μg of protein developed tumors upon subsequent challenge, and 0/10 receiving 300 μg of protein responded with tumor formation (Table 3). All of the control mice (injected with a parallel fraction from Ad2-infected cells or not immunized) developed tumors, indicating the lack of any protective activity. As expected, aliquots from $Ad2^+ND_1$-infected cells that had been pretreated with either a low or a high concentration of normal serum retained their TSTA activity at a dose of 300 μg of protein per animal. On the other hand, equivalent aliquots that had been pretreated with antitumor serum demonstrated a significant reduction in their ability to induce rejection of tumors. Whereas at low serum concentration only 1/7 mice developed tumors upon challenge, at high serum concentration 5/7 responded with tumor formation. The latter represented a loss of more than 70% of the protection activity. Because the antitumor serum differed from the normal serum only in its ability to remove the 28,000 M_r protein from a parallel ^{35}S-labeled fraction, the significant loss of tumor rejection activity upon treatment of the infected cell fraction with this serum is most likely explained by the loss of the $Ad2^+ND_1$-specific polypeptide.

In order to determine the residual amount of 28,000 M_r protein present in each of the serum-treated fractions tested for TSTA activity, we performed competition-immunoprecipitation experiments (Fig. 2 *right*). We used a fixed amount of a parallel ^{35}S-labeled nuclear membrane wash fraction from $Ad2^+ND_1$-infected cells and $NaDodSO_4$/polyacrylamide gel electrophoresis, followed by densitometric analysis, to estimate the amount of labeled 28,000 M_r protein that could be specifically immunoprecipitated with a limiting amount of antitumor serum. The immunoprecipitation was carried out in the presence of an increasing amount of various unlabeled serum-treated fractions. Whereas treatment with normal serum, at either low or high concentration, resulted in no decrease in the amount of immunoreactive 28,000 M_r protein, treatment with antitumor serum resulted in a loss of 50% of the protein at low serum concentration and 75% at high serum concentration (as determined from relative amounts of protein required for 50% competition; Fig. 2 *right*).

The residual amount of 28,000 M_r protein present in fractions treated with either a low or high concentration of antitumor serum was equivalent to that present in 150 and 75 μg of protein, respectively, of the original untreated fraction. The extent of protection expected for 150 or 75 μg of protein is in close agreement with the TSTA activity observed for fractions that had been treated with low or high concentrations of the antitumor serum. This result indicates that removal of the 28,000 M_r protein from the cell extract resulted in a proportional loss of TSTA activity.

Table 3. Effect of removal of the 28,000 M_r protein

Nuclear membrane wash	Dose,* μg protein	Treatment with serum†	% 28,000 M_r protein remaining‡	Tumor bearing mice/ total mice
Uninoculated	0	—	—	10/10
Ad2	300	—	—	10/10
$Ad2^+ND_1$	60	—	—	6/10
	300	—	100	0/10
	300	TS,L	50	1/7
	300	TS,H	25	5/7
	300	NS,L	100	1/7
	300	NS,H	100	0/7

* Total dosage given in two equal injections, 7 days apart.
† TS, antitumor serum; NS, normal serum; L, low serum concentration; H, high serum concentration.
‡ As determined by competition-immunoprecipitation.

DISCUSSION

Studies with nondefective Ad2–SV40 hybrid viruses containing varying but overlapping DNA segments from the early region of the SV40 genome have led to the conclusion that the segment between 0.28 and 0.44 unit from the unique *Eco*RI cleavage site on the SV40 map is responsible for the induction of the SV40-specific TSTA (17, 18). This conclusion was based solely upon the observation that one of the hybrid viruses studied, $Ad2^+ND_1$, seemed to lack the property of inducing a functional SV40-specific TSTA in either hamsters (17) or mice (19). Our finding that $Ad2^+ND_1$ does in fact induce a functional SV40-specific TSTA in BALB/c mice (Table 1) allows us to reassign that region on the viral genome sufficient for the induction of TSTA to the segment covering 0.17–0.28 unit on the SV40 map. With the knowledge that the entire early region of the SV40 genome is transcribed with the same polarity (20, 21), our results suggest that some or all of the sequences present in the extreme 3′ terminus are responsible for the induction of some or all of the TSTA determinants. This conclusion is predicated upon the purity of the $Ad2^+ND_1$ stock used in our experiments; were other SV40-containing hybrids present in this stock, the assignment of the TSTA coding region might be in error. However, the nondefective hybrids with SV40 segments larger than that contained in $Ad2^+ND_1$ code for SV40-specific proteins other than the 28,000 M_r protein (22). Our finding that $Ad2^+ND_1$ as used in the present studies only induces the 28,000 M_r polypeptide confirms the purity of the $Ad2^+ND_1$ stock.

Our observation that a large inoculum of $Ad2^+ND_1$ (2×10^9 PFU per animal) is required to confer relatively durable protection in mice against SV40 tumor challenge could explain the apparent absence of SV40-specific TSTA previously reported in the case of this hybrid virus (19). Because human Ad2 does not generally transform or propagate lytically in cultured mouse cells, our inability to detect TSTA activity unless large inocula of $Ad2^+ND_1$ are used in BALB/c mice may reflect the possibility that only one round of viral macromolecular biosynthesis occurs in the infected cells *in vivo*.

Despite our knowledge that TSTA is virus-induced, there has been no direct evidence to show that it is virus-coded. It is known that the early region of SV40 codes for, at least in part if not in whole, a protein of approximately 100,000 M_r (23–25) that is detectable using antitumor antisera obtained from hamsters bearing SV40-induced tumors. Because the entire early region of SV40 would be required to code for a protein of this molecular weight, one might anticipate that this protein, which contains the antigenic determinants for T antigen, would also contain the antigenic determinants for TSTA if the latter is indeed virus-coded. There have been attempts to correlate TSTA activity with the 100,000 M_r T antigen, which has been

shown to play a role in cell transformation (26–30). Studies with one conditional-lethal mutant of SV40, tsA28, which is temperature-sensitive in transformation, have demonstrated a concomitant loss of T antigen and TSTA activities at the nonpermissive temperature (31). Furthermore, TSTA seems to share with T antigen the ability to bind to double-stranded DNA (32). Such correlative studies are indirect, however, and complicated by the recent finding that the antitumor antisera used for the detection of T-antigen activity can specifically recognize not only the 100,000 M_r protein but also at least one other protein of about 17,000 M_r that is also coded for by SV40 (33). In fact, there is evidence to suggest that the TSTA activity does not reside in the 100,000 M_r T antigen. The T-antigen and TSTA activities exhibit differences not only in thermal stability (31) but also in apparent molecular size. Whereas TSTA has been reported to have a M_r of about 50,000 (9), the 100,000 M_r T antigen has been shown to exist in polymeric forms with sedimentation coefficients as high as 22 S (34, 35). Furthermore, TSTA has been detected in plasma membrane fractions (2, 3), which appear to be devoid of T-antigen activity (36).

Our observation that the removal of the 28,000 M_r protein from an $Ad2^+ND_1$-infected cell extract resulted in a concomitant loss of TSTA activity allows us to associate the SV40-specific tumor rejection activity with a specific polypeptide chain. Because this $Ad2^+ND_1$-specific 28,000 M_r protein can be recognized by SV40-specific antitumor antisera (10) and because the protein can be synthesized *in vitro* by using SV40 DNA-selected mRNA (37, 22), our finding provides direct evidence that the SV40-specific TSTA is virus-coded.

Although it can be inferred from our studies with $Ad2^+ND_1$ that the region between 0.17 and 0.28 map unit from the *Eco*RI cleavage site on the SV40 genome directly codes for the antigenic determinant(s) responsible for tumor rejection in syngeneic mice, it is not clear whether these antigenic determinants also reside in a polypeptide of 28,000 M_r in SV40-transformed cells. Although tryptic peptide analyses have demonstrated extensive amino acid homology between the SV40-specific 100,000 M_r protein and the $Ad2^+ND_1$-specific 28,000 M_r protein (22), it cannot be assumed that the homologous regions within the two distinct polypeptide chains have identical tertiary structure or antigenic determinants. Furthermore, even if the 100,000 M_r protein does contain the conformational determinants for TSTA (31, 32), the intracellular compartmentalization of this macromolecule (resulting from the properties conferred by the additional amino acid sequence) could physically prevent it from serving as the functional TSTA *in vivo*. Previous attempts to identify, in SV40-transformed cells, polypeptides other than the 100,000 M_r protein that are induced by the early region of SV40 have been inconclusive (38). More recent studies have demonstrated at least one additional stable polypeptide of about 17,000 M_r (33), and possibly other polypeptides (39). Our results suggest that, whatever its molecular weight in SV40-transformed cells, the polypeptide containing TSTA activity in all probability includes amino acid sequences coded for by that region of the SV40 genome present in $Ad2^+ND_1$.

We thank Ernest Winocour, Heiner Westphal, and Andrew M. Lewis, Jr., for their critical reading of this manuscript and for their valuable comments.

1. Robbins, J. C. & Nicolson, G. L. (1975) in *Cancer: A Comprehensive Treatise*, ed. Becker, F. F. (Plenum, New York), Vol. 4, pp. 3–54.
2. Tevethia, S. S. & Rapp, F. (1965) *Proc. Soc. Exp. Biol. Med.* **12**, 455–458.
3. Coggin, J. H., Elrod, L. H., Ambrose, K. R. & Anderson, N. G. (1969) *Proc. Soc. Exp. Biol. Med.* **132**, 328–336.
4. Khera, K. S., Ashkenazi, A., Rapp, F. & Melnick, J. L. (1963) *J. Immunol.* **91**, 604–613.
5. Habel, K. & Eddy, B. E. (1963) *Proc. Soc. Exp. Biol. Med.* **113**, 1–4.
6. Koch, M. A. & Sabin, A. B. (1963) *Proc. Soc. Exp. Biol. Med.* **113**, 4–12.
7. Defendi, V. (1963) *Proc. Soc. Exp. Biol. Med.* **113**, 12–16.
8. Law, L. W., Takemoto, K. K., Rogers, M. J. & Ting, R. C. (1977) *J. Nat. Cancer Inst.* **59**, 1523–1526.
9. Apella, E., Law, L. W. & Henriksen, O. (1976) *Cancer Res* **36**, 3539–3544.
10. Jay, G., Jay, F. T., Friedman, R. M. & Levine, A. S. (1978) *J. Virol.*, **26**, 411–419.
11. Jay, G., Jay, F. T., Friedman, R. M. & Levine, A. S. (1977) *J. Virol.* **23**, 692–699.
12. Brunette, D. M. & Till, J. E. (1971) *J. Membr. Biol.* **5**, 215–224.
13. Holloway, R. W. (1973) *Anal. Biochem.* **53**, 304–308.
14. Lopez-Revilla, R. & Walter, G. (1973) *Nature New Biol.* **244**, 165–167.
15. O'Farrell, P. Z., Gold, L. M. & Huang, W. H. (1973) *J. Biol. Chem.* **248**, 5499–5501.
16. Kessler, S. W. (1975) *J. Immunol.* **115**, 1617–1624.
17. Lewis, A. M., Jr. & Rowe, W. P. (1973) *J. Virol.* **12**, 836–840.
18. Kelly, T. J., Jr. & Lewis, A. M., Jr. (1976) *J. Virol.* **12**, 643–652.
19. Tevethia, S. S. & Lewis, A. M., Jr. (1976) *J. Virol.* **20**, 539–542.
20. Khoury, G., Martin, M. A., Lee, T. N. H., Danna, K. J. & Nathans, D. (1973) *J. Mol. Biol.* **78**, 377–389.
21. Sambrook, J., Sugden, B., Keller, W. & Sharp, P. A. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3711–3715.
22. Mann, K., Hunter, T., Walter, G. & Linke, H. (1977) *J. Virol.* **24**, 151–169.
23. Prives, C., Aviv, H., Gilboa, E., Winocour, E. & Revel, M. (1975) *Colloq. Inst. Natl. Sante R. Med.* **47**, 305–312.
24. Anderson, C. W., Lewis, J. B., Baum, D. R. & Gesteland, R. F. (1976) *J. Virol.* **18**, 685–692.
25. Rundell, K., Collins, J. K., Tegtmeyer, P., Ozer, H. L., Lai, C.-J. & Nathans, D. (1977) *J. Virol.* **21**, 636–646.
26. Martin, R. G. & Chou, J. Y. (1975) *J. Virol.* **15**, 599–612.
27. Tegtmeyer, P. (1975) *J. Virol.* **15**, 613–618.
28. Brugge, J. S. & Butel, J. S. (1975) *J. Virol.* **15**, 619–635.
29. Osborn, M. & Weber, K. (1975) *J. Virol.* **15**, 636–644.
30. Kimura, G. & Itagaki, A. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 673–677.
31. Anderson, J. L., Chang, C., Mora, P. T. & Martin, R. G. (1977) *J. Virol.* **21**, 459–467.
32. Chang, C., Luborsky, S. W. & Mora, P. T. (1977) *Nature* **269**, 438–440.
33. Prives, C., Gilboa, E., Revel, M. & Winocour, E. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 457–461.
34. Potter, C. W., McLaughlin, B. C. & Oxford, J. S. (1969) *J. Virol.* **4**, 574–579.
35. Osborn, M. & Weber, K. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 267–276.
36. Tegtmeyer, P., Schwartz, M., Collins, J. K. & Rundell, K. (1975) *J. Virol.* **16**, 168–178.
37. Anderson, C. W., Lewis, J. B., Baum, P. R. & Gesteland, R. F. (1976) *J. Virol.* **18**, 685–692.
38. Robb, J. A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 447–451.
39. Ito, Y., Brocklehurst, J. R. & Dulbecco, R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4666–4670.