Induction of Simian Virus 40-specific Tumour Rejection by the Ad2+ND2 Hybrid Virus

By GILBERT JAY, FRANCIS T. JAY, CHUNGMING CHANG, ARTHUR S. LEVINE AND ROBERT M. FRIEDMAN

Pediatric Oncology Branch and Macromolecular Biology Section, National Cancer Institute, and Laboratory of Experimental Pathology, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Md. 20205, U.S.A.

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SUMMARY

Immunization of BALB/c mice with Ad2+ND2, a non-defective hybrid virus containing about half of the early region of simian virus 40 (SV40) DNA covalently integrated into the human adenovirus 2 (Ad2) genome, can confer protection against subsequent challenge by syngeneic SV40 tumour cells. Analysis of subcellular fractions from Ad2+ND2-infected cells shows a close correlation between the tumour rejection activity and the presence of the two SV40-specific proteins induced by this hybrid virus. These two proteins, with mol. wt. of 56,000 (56K) and 42,000 (42K), can be specifically immunoprecipitated using sera obtained from hamsters bearing SV40-induced tumours. Such immunoprecipitates, which contain no detectable contaminating components as determined by polyacrylamide gel electrophoresis, can efficiently immunize mice against SV40 tumour challenge, suggesting that the 56K and 42K proteins are directly responsible for the induction of tumour rejection. Moreover, we have found, by immunoprecipitation, a novel antigen in SV40-transformed BALB/c cells, also of 56,000 mol. wt.; possibly, this 56K protein is responsible for induction of transplantation immunity in SV40-transformed cells.

INTRODUCTION

Tumour-specific transplantation antigens (TSTAs) induced by DNA oncogenic viruses have the capacity to induce tumour rejection in syngeneic animals (for review, see Law & Appella, 1975). By virtue of their apparent localization on the cell surface (Coggin et al. 1969; Tevethia & Rapp, 1965), it has been assumed that TSTAs play a key role in the regression of virus-induced neoplasms. Because the presence of TSTA in virus-induced tumour cells might allow the opportunity to modulate the growth of antigenic tumours, the biochemical characterization of TSTAs is of particular importance.

In an attempt to identify the protein(s) directly responsible for the rejection of simian virus 40 (SV40)-induced tumours, we have made use of the non-defective human adenovirus 2 (Ad2)-SV40 hybrid, Ad2+ND2, which has previously been shown to possess sufficient genetic information for the induction of SV40 TSTA in hamsters (Lewis et al. 1973). The advantage of using this hybrid virus in lieu of wild-type SV 40 stems from the observation that, like wild-type Ad2, Ad2+ND2 infection inhibits cellular protein synthesis while directing the host cell to synthesize primarily virus-coded polypeptides (Walter & Martin, 1975). It seems likely that this property of the hybrid virus can be utilized for the identification of the SV40-specific protein(s) responsible for transplantation rejection.
METHODS

Subcellular fractionation. Ad2 and Ad2+ND2 stocks were grown in KB cells, purified by equilibrium centrifugation in CsCl, and plaque-titred on primary human embryonic kidney cells (Jay et al. 1978a). All experiments were conducted in a P3 biohazard containment facility. For studies on subcellular fractionation (Jay et al. 1977), monolayers of human KB cells infected with either Ad2 or Ad2+ND2, at a multiplicity of 20, were harvested 24 h after infection. Where indicated, infected cells were radiolabelled by incubation in Earle’s balanced salt solution containing 10% Eagle’s minimum essential medium, 2% foetal calf serum and 20 μCi/ml L-35S-methionine (800 to 1000 Ci/mmol) for the appropriate period of time immediately before harvest. Cells were disrupted by Dounce homogenization, followed by centrifugation at 30000 g for 30 min. The supernatant containing the cytoplasmic fraction (S-30) was saved, while the pellet was treated with dextran-polyethylene glycol to effect separation of plasma membranes from intact nuclei (Brunette & Till, 1971). The crude plasma membrane fraction was treated for 60 min at 4 °C with tris-buffered saline (pH 7.4) containing 1% (v/v) Triton X-100 and the washed plasma membranes were separated from the plasma membrane wash by centrifugation at 20000 g for 20 min. The cytoplasmic fraction was further fractionated by centrifugation for 4 h at 100000 g. The resulting supernatant (S-100) was removed and adjusted to 30% saturation with ammonium sulphate. After stirring at 4 °C for 30 min, the precipitate was collected by centrifugation at 30000 g for 20 min and resuspended in tris-buffered saline (pH 7.4), before dialysis against the same buffer. All fractions containing Triton X-100 were treated with Biobeads SM2 to remove the detergent (Holloway, 1973).

Assay for TSTA activity. Female BALB/c mice, 4 to 6 weeks old, were immunized with the appropriate virus or subcellular fraction in two equal intraperitoneal (i.p.) injections, one week apart. The second i.p. injection was followed 10 days later by an intramuscular (i.m.) injection of 10^4 mKSA (ASC) cells, kindly provided by Dr L. W. Law (Appella et al. 1976). Mice were observed for at least 5 weeks for tumour development at the site of challenge.

Immunoprecipitation. Immunoprecipitations were carried out in tris-buffered saline (pH 7.4) containing 0.5% Nonidet P-40 and 2 mM-phenylmethyl sulphonylfluoride, as described previously (Jay et al. 1978a). Inactivated Staphylococcus aureus (strain Cowan I) was used to adsorb the antigen-antibody complexes, as described by Kessler (1975).

Polyacrylamide gel electrophoresis. All samples for analysis contained 62 mM-tris-hydrochloride (pH 6.8), 5% (v/v) 2-mercaptoethanol, 2% (w/v) SDS, 2 mM-phenylmethyl sulphonylfluoride and 10% (v/v) glycerol, and were heated at 100 °C for 2 min. The SDS-polyacrylamide gel system used was that of Laemmli & Maizel, described by Laemmli (1970) and electrophoresis was performed by the procedure of O’Farrell et al. (1973). Radiolabelled protein bands on the gels were detected by fluorography (Laskey & Mills, 1975).

RESULTS

Induction of SV40 TSTA in BALB/c mice by Ad2+ND2

When an established line of SV40-transformed BALB/c cells, mKSA (Appella et al. 1976) was injected i.m. into 4-week-old syngeneic mice at a dose of 10^4 cells per animal, tumour could be detected at the site of inoculation in 20 out of 20 animals as early as 10 days after challenge (Table 1). Previous studies have shown that immunization of animals with SV40 prior to challenge with SV40 tumour cells will induce the rejection of such tumours (Defendi, 1963; Habel & Eddy, 1963; Khera et al. 1963; Koch & Sabin, 1963). These observations
Adz+NDz-induced SV4o-specific TSTA

Table 1. Virus induction of SV4o-specific TSTA

<table>
<thead>
<tr>
<th>Inoculum*</th>
<th>Dose† (p.f.u.)</th>
<th>Tumour bearing mice/total mice‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated</td>
<td>—</td>
<td>20/20</td>
</tr>
<tr>
<td>Ad2</td>
<td>2 x 10⁷</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>2 x 10⁸</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>2 x 10⁹</td>
<td>10/10</td>
</tr>
<tr>
<td>Ad2+NDz</td>
<td>2 x 10⁷</td>
<td>9/10</td>
</tr>
<tr>
<td></td>
<td>2 x 10⁸</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>2 x 10⁹</td>
<td>1/10</td>
</tr>
</tbody>
</table>

* Mice were inoculated twice (days 0 and 7).
† Total dosage given in two equal injections.
‡ Mice were challenged with 10⁴ mKSA(ASC) tumour cells 10 days after the second inoculation (day 17).

suggest that injection of SV4o induces a virus-specific transplantation antigen in the infected cells, resulting in an immune response directed against these cells as well as against all subsequent challenges with cells bearing the same transplantation antigen.

As shown in Table 1, inoculation with Ad2 at doses ranging from 2 x 10⁷ to 2 x 10⁹ p.f.u. does not protect mice against subsequent challenges with SV4o-transformed mKSA cells. This result is consistent with the finding that the transplantation antigen responsible for SV4o-tumour rejection is virus-specific (Girardi, 1965; Law et al. 1977). However, unlike Ad2, the non-defective Ad2+NDz hybrid, which contains a segment of the early region of SV4o DNA covalently integrated into the Ad2 genome, does confer a dose-dependent protection against mKSA challenge (Table 1). Nine out of ten mice immunized with Ad2+NDz respond to SV4o tumour challenge at a dose of 2 x 10⁷ p.f.u., 5 out of 10 at 2 x 10⁸ p.f.u., and 1 out of 10 at 2 x 10⁹ p.f.u. These results suggest that the presence of the SV4o DNA segment has conferred on Ad2+NDz the ability to induce SV4o TSTA in BALB/c mice.

Intracellular distribution of TSTA activity in Ad2+NDz-infected KB cells

When ³⁵S-methionine-labelled lysates of human KB cells that had been infected with either Ad2 or Ad2+NDz were analysed on an SDS-polyacrylamide slab gel (Fig. 1a, b), two proteins with mol. wt. of 56000 (56K) and 42000 (42K) were found to be specific to Ad2+NDz and absent from Ad2-infected cells (Walter & Martin, 1975; Jay et al. 1977). These two proteins have been shown to share extensive amino acid sequence homology and to be coded for by the SV4o DNA segment present in Ad2+NDz (Deppert & Walter, 1976). In order to determine whether the 56K or 42K proteins are responsible for the TSTA activity, we have attempted to correlate the presence of these two proteins in the various subcellular fractions with the tumour rejection activity.

Human KB cells that had been infected with either Ad2 or Ad2+NDz for 24 h were harvested and subjected to subcellular fractionation. In order to quantify the amounts of the 56K and 42K protein present in each of the subcellular fractions to be tested for tumour rejection activity, half of each set of infected cells was incubated with ³⁵S-methionine beginning 2 h before the time of harvest; the labelled and unlabelled cells were subsequently fractionated in parallel.

We have shown (Jay et al. 1977) that a major portion of both the 56K and 42K protein is physically associated with the membrane fraction from Ad2+NDz-infected cells (Fig. 1d). A parallel fraction from Ad2-infected cells contains an almost identical distribution of radiolabelled proteins (Fig. 1c), except for the absence of the two Ad2+NDz-specific polypeptides. When injected into mice, the crude membrane fraction from Ad2+NDz-infected cells conferred protection against subsequent challenge by mKSA cells (Table 2). At 100 µg
Fig. 1. Subcellular distribution of the Ad2*ND2-specific 56K and 42K proteins. Autoradiogram of a 12.5% SDS-polyacrylamide gel displaying the 35S-methionine-labelled proteins found in the various subcellular fractions from KB cells infected with either Ad2 (tracks a, c, e, g, i and k) or Ad2*ND2 (tracks b, d, f, h, j and l). Tracks a and b are whole cell lysates; tracks c and d, crude membranes; tracks e and f, membrane washes; tracks g and h, washed membranes; tracks i and j, cytoplasmic S-30; tracks k and l, 0 to 30% ammonium sulphate fractions of cytoplasmic S-100.

Table 2. Subcellular distribution of TSTA activity

<table>
<thead>
<tr>
<th>Inoculum*</th>
<th>Dose† (µg protein)</th>
<th>Challenging tumour cells‡</th>
<th>Tumour-bearing mice/total mice, with inoculum obtained from cells infected with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ad2</td>
<td>Ad2*ND2</td>
</tr>
<tr>
<td>Crude membranes</td>
<td>100</td>
<td>mKSA</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>mKSA</td>
<td>10/10</td>
</tr>
<tr>
<td>Membrane wash</td>
<td>150</td>
<td>mKSA</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>mKSA</td>
<td>10/10</td>
</tr>
<tr>
<td>Washed membranes</td>
<td>125</td>
<td>mKSA</td>
<td>9/10</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>mKSA</td>
<td>10/10</td>
</tr>
<tr>
<td>Cytoplasmic S-30</td>
<td>50</td>
<td>mKSA</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>mKSA</td>
<td>10/10</td>
</tr>
<tr>
<td>Cytoplasmic S-100</td>
<td>100</td>
<td>mKSA</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>mKSA</td>
<td>10/10</td>
</tr>
</tbody>
</table>

* Mice were inoculated twice (days 0 and 7).
† Total dosage given in two equal injections.
‡ Mice were challenged with 10⁴ tumour cells 10 days after the second inoculation (day 17).
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protein, only 4 out of 10 mice developed tumours and at 250 µg protein, 0 out of 10. In contrast, all of the mice injected with the crude membrane fraction from Adz-infected cells developed tumours at either 100 µg or 250 µg protein. That the protection conferred by the membrane fraction from Adz + NDz-infected cells was specifically directed against SV40 tumour cells is shown by the fact that while a dose of 250 µg protein offered complete protection against challenge with 10⁴ mKSA cells (TD₅₀ 10² to 10³ cells), an identical dose offered no protection against challenge with 10⁴ Meth A cells (TD₅₀ 10⁹ cells), derived from a chemically induced tumour (McCollester, 1970).

As a further step to enrich with the membrane-bound Adz + NDz-specific proteins, we have treated the crude membrane fraction with 1% (v/v) Triton X-100. Analysis of the resulting ³⁵S-methionine-labelled membrane wash (Fig. 1 e, f) and washed membranes (Fig. 1 g, h) shows that only a small fraction of the 56K and 42K proteins was solubilized by Triton X-100 (Fig. 1 f), while the greater part was tightly bound to the membrane fraction (Fig. 1 h). When parallel samples were tested for tumour rejection activity, both fractions were found to be positive (Table 2). However, the specific activity of the washed membrane fraction was considerably higher than that of the membrane wash fraction, in agreement with the amounts of 56K and 42K protein present. When mice were immunized with the membrane wash from Adz + NDz-infected cells, 9 out of 10 receiving 150 µg protein developed tumours after mKSA challenge and 5 out of 10 at 500 µg protein (Table 2). In contrast, with the washed membranes from Adz + NDz-infected cells, only 3 out of 10 receiving 125 µg protein developed tumours and 0 out of 10 at 500 µg (Table 2). Such protection was not seen with parallel fractions from Adz-infected cells.

The 56K and 42K proteins have also been detected in the cytoplasmic fraction of infected cells (Fig. 1 i, j). When injected into mice, this fraction was found to possess TSTA in high specific activity (Table 2). At 50 µg protein, only 6 out of 10 mice developed tumours, and at 150 µg protein, 0 out of 10 responded. When the cytoplasmic S-30 fraction was centrifuged at 100000 g for 4 h and the resulting supernatant S-100 adjusted to 30% saturation with ammonium sulphate, the precipitate was found to contain a significant enrichment for the 56K and 42K proteins, relative to other ³⁵S-labelled components (Fig. 1 k, l). At 100 µg protein, only 3 out of 10 mice developed tumours and at 250 µg, 0 out of 10 (Table 2). Again, no protection could be detected with a parallel fraction from Adz-infected cells.

Removal of TSTA activity by treatment with antitumour serum

Antisera from hamsters bearing SV40-induced tumours have been shown to cross-react with Adz + NDz-infected cells (Deppert & Walter, 1976). When the cytoplasmic fraction from ³⁵S-methionine-labelled Adz + NDz-infected cells was incubated with an antiserum from a tumour-bearing hamster, the 56K and 42K proteins were the only two components that were immunoprecipitated (Fig. 2 d). These two proteins can be identified by their presence in whole cell lysates of Adz + NDz-infected cells (Fig. 2 b) but not in lysates of Adz-infected cells (Fig. 2 a). Unlike the antitumour serum, normal hamster serum did not immunoprecipitate these Adz + NDz-specific proteins (Fig. 2 c).

In order to show definitively whether the 56K and 42K proteins do contain antigenic determinants responsible for tumour rejection, we have determined whether immunoprecipitates containing only the 56K and 42K proteins possess any TSTA activity. The cytoplasmic fraction from Adz + NDz-infected cells was chosen for this study because a major portion of the 56K and 42K protein in this fraction has previously been shown to exist in a relatively free form, unassociated with any fast-sedimenting cellular components (Jay et al. 1977) and because these two proteins can be specifically immunoprecipitated from this fraction free of any contaminating components (Fig. 2 d). It is of interest to note that the small amount of 56K and 42K protein solubilized from the membrane fraction by
Fig. 2. Immunoprecipitation of SV40-specific proteins using antiserum obtained from hamster bearing an SV40-induced tumour. Autoradiogram of a 9% SDS-polyacrylamide gel showing immunoprecipitates from 35S-methionine-labelled extracts of either Adz+ND2-infected KB cells (tracks c and d) or SV40-transformed BALB/c cells (tracks e and f), using either serum from a normal hamster (tracks c and e) or serum from a hamster bearing an SV40-induced tumour (tracks d and f). 35S-methionine-labelled extracts from KB cells infected with either Ad2 (track a) or Adz+ND2 (track b) were run in parallel as mol. wt. markers.

Two antibody concentrations were used: either 8 µl or 20 µl of antitumour serum or normal serum were incubated with each 300 µg of protein in the cytoplasmic S-30 fraction of Adz+ND2-infected cells. The previous experiment (Table 2) had shown that 300 µg of protein from this fraction will suffice to confer complete protection against SV40 tumour challenge. As determined by titration with a parallel 35S-methionine-labelled cytoplasmic fraction, the low and high concentrations of antitumour serum used for the immunoprecipitation represented, respectively, sub-saturating and saturating levels of antibodies against the 56K and 42K proteins present in this subcellular fraction. In other words, while the low antitumour serum concentration would remove only a fraction of the immunoprecipitable 56K and 42K proteins, the higher antitumour serum concentration would remove all protein that could be immunoprecipitated. At the end of the incubation, immune complexes were recovered using protein A-containing Staphylococcus aureus and were resuspended in tris-buffered saline.
Table 3. Immunoprecipitation of TSTA activity with antitumour serum

<table>
<thead>
<tr>
<th>Inoculum*</th>
<th>Treatment with serum</th>
<th>Tumour bearing mice/total mice†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated</td>
<td>Untreated</td>
<td>10/10</td>
</tr>
<tr>
<td>Ad2+ND2 cytoplasmic S-30 (300 µg)</td>
<td>Untreated</td>
<td>0/10</td>
</tr>
<tr>
<td>Immune complexes</td>
<td>Low antitumour serum</td>
<td>6/10</td>
</tr>
<tr>
<td>Immune complexes</td>
<td>High antitumour serum</td>
<td>4/10</td>
</tr>
<tr>
<td>Immune complexes</td>
<td>Low normal serum</td>
<td>10/10</td>
</tr>
<tr>
<td>Immune complexes</td>
<td>High normal serum</td>
<td>10/10</td>
</tr>
</tbody>
</table>

* Total dosage given in two equal injections (days 0 and 7).
† Mice were challenged with 10⁴ mKSA(ASC) tumour cells 10 days after the second inoculation (day 17).

As shown in Table 3, while all of the control mice that were not inoculated developed tumours after challenge, none of the mice receiving 300 µg of the untreated cytoplasmic S-30 fraction from Ad2+ND2-infected cells developed tumours. The number of responding mice inoculated with immune complexes derived from an equivalent amount of the same fraction, using either low or high antitumour serum, was 6 out of 10 and 4 out of 10, respectively. This suggests that more than 50 % of the animals receiving the immune complexes had acquired resistance to challenge by SV40 tumour cells. In contrast, mice inoculated with immune complexes obtained with either a low or high concentration of normal hamster serum all developed tumours.

Since the only detectable difference between immune complexes obtained with antitumour serum or normal serum is the presence of the 56K and 42K proteins in the former but not in the latter (Fig. 2c, d), we conclude that these Ad2+ND2-specific proteins contain the antigenic determinants for TSTA. Our inability to induce tumour rejection in all of the mice using immune complexes obtained with a saturating amount of antitumour serum from a subcellular fraction that has been shown to contain sufficient TSTA activity to confer complete protection, may indicate that only a fraction of the 56K and 42K protein can be immunoprecipitated under the conditions used in our studies. This is consistent with our earlier finding that a fraction of the 56K and 42K protein in the cytoplasmic fraction is tightly complexed to ribosomes (Jay et al. 1977) and hence may not be immunoprecipitated with high efficiency although it does contain TSTA activity. Alternatively, it is possible that the binding of antibodies to the 56K and 42K proteins alters or blocks the TSTA determinants, resulting in a decrease in the biological activity of the antigens.

Detection of a 56K protein in SV40-transformed cells

To determine whether there are proteins in SV40-transformed cells that are analogous in size to the Ad2+ND2-specific proteins which may function as TSTA, we have used our antitumour serum which has a high titre for the 56K and 42K polypeptides to immunoprecipitate 35S-methionine-labelled proteins from an SV40-transformed BALB/c cell line, SVR12-2 (Risser & Pollack, 1974). The immunoprecipitates were analysed on a 9 % SDS-polyacrylamide gel (Fig. 2e, f). Apart from the large T-antigen, with a mol. wt. of 94000 (Prives et al. 1975; Tegtmeyer et al. 1975), our antitumour serum also specifically immunoprecipitated a protein of 56000 mol. wt. (Fig. 2f) that co-electrophoresed with the 56K protein of Ad2+ND2 (Fig. 2d). The small t-antigen, with a mol. wt. of 17000 (Prives et al. 1977; Crawford et al. 1978), migrated with the solvent front and was not resolved on this gel. The 56K protein from SV40-transformed cells is not recognized by normal hamster serum (Fig. 2e). Furthermore, the fact that this protein is not found in normal BALB/c cells (data not shown) suggests that it is SV40-specific.
**DISCUSSION**

Ad2\textsuperscript{+}ND\textsubscript{2} is a non-defective hybrid virus that contains a segment of SV40 DNA (extending from 0.11 to 0.44 map units from the unique endonuclease \textit{R.EcoRI} cleavage site) covalently integrated into the Ad2 genome (Morrow et al. 1973). As a result of this insertion, Ad2\textsuperscript{+}ND\textsubscript{2} has acquired the SV40 function of inducing in hamsters the rejection of SV40-specific tumours (Lewis et al. 1973).

We show here that the tumour rejection activity associated with Ad2\textsuperscript{+}ND\textsubscript{2} can also be demonstrated in BALB/c mice (Table 1). It is notable that while less than $1 \times 10^6$ p.f.u. of SV40 will suffice to confer complete protection against SV40 tumour challenge in BALB/c mice (Anderson et al. 1977b), more than $2 \times 10^6$ p.f.u. of Ad2\textsuperscript{+}ND\textsubscript{2} is required to confer equivalent protection. This dramatic difference in efficiency between the two viruses may be due to the fact that while SV40 can transform mouse cells in culture, human Ad2 does not generally induce transformation nor propagate lytically in these cells. The need for a high dose of Ad2\textsuperscript{+}ND\textsubscript{2} to induce sufficient SV40-specific TSTA in BALB/c mice such that the animals mount an immune response against SV40 tumour cells may therefore be explained by the probability that only a single round of virus macromolecular biosynthesis occurs \textit{in vivo} and/or by the possibility that this virus has a low transforming efficiency in mouse cells \textit{in vivo}.

Our findings that TSTA activity in Ad2\textsuperscript{+}ND\textsubscript{2}-infected cells is highly enriched in subcellular fractions containing the SV40-specific 56K and 42K proteins and that immunoprecipitates containing only the 56K and 42K proteins are capable of inducing tumour rejection, allow us to identify the specific macromolecules induced by Ad2\textsuperscript{+}ND\textsubscript{2} that possess the antigenic determinants responsible for SV40 tumour rejection. While our data do not indicate whether the 56K or 42K protein is responsible for the TSTA activity, the previous finding that the two Ad2\textsuperscript{+}ND\textsubscript{2}-specific proteins contain extensive amino acid sequence homology (Deppert & Walter, 1976) lends support to the possibility that both proteins may possess TSTA activity, possibly with varying efficiencies.

It is of interest that a 28K protein induced by a different hybrid virus, Ad2\textsuperscript{+}ND\textsubscript{1}, has also been shown to contain TSTA activity (Jay et al. 1978b). Tryptic peptide analysis suggests that this 28K protein is contained within the larger Ad2\textsuperscript{+}ND\textsubscript{2}-specific 56K and 42K proteins (Mann et al. 1977). Our finding that the Ad2\textsuperscript{+}ND\textsubscript{2} virus dose required to confer 50\% protection against SV40 tumour challenge is only one-tenth that of Ad2\textsuperscript{+}ND\textsubscript{1} (Jay et al. 1978b) may be interpreted to suggest that the larger SV40-coded proteins induced by Ad2\textsuperscript{+}ND\textsubscript{2} contain additional antigenic determinants not found in the 28K protein of Ad2\textsuperscript{+}ND\textsubscript{1}. This is further supported by our observation that the specific activity of membrane fractions from cells separately infected by the two hybrid viruses are detectably different, with those from Ad2\textsuperscript{+}ND\textsubscript{2} being severalfold more efficient than those from Ad2\textsuperscript{+}ND\textsubscript{1}, although the amount of SV40-specific proteins accumulated in the respective cells is not significantly different (Jay et al. 1978b). It seems likely, therefore, that while the antigenic determinants present in the 28K protein are sufficient to induce tumour rejection, additional antigenic sites present in the larger 56K and 42K proteins confer added stability and increased efficiency.

Since virtually all of the methionine-containing tryptic peptides derived from the Ad2\textsuperscript{+}ND\textsubscript{2}-specific 56K and 42K proteins are also present in the SV40-coded large T-antigen (Mann et al. 1977), our observation provides direct evidence that the SV40-specific TSTA is virus-coded. However, it is not clear whether the 94K large T-antigen does in fact function as TSTA in SV40-transformed cells (Anderson et al. 1977a; Chang et al. 1977). The intranuclear compartmentalization of this virus-coded protein (Tegtmeyer et al. 1975) may prevent it.
from serving as the functional component in tumour rejection in vivo. Our ability to immuno-precipitate a novel protein of 56,000 mol. wt. from SV40-transformed BALB/c cells suggests another possible candidate as TSTA in SV40-transformed cells. Work is in progress to determine whether this 56K protein shares amino acid sequence homology with the Ad2+ND2-specific proteins which contain tumour rejection activity.

Our identification in Ad2+ND2-infected cells of the protein antigens which contain SV40 TSTA activity provides a model system for the study of the molecular mechanism of tumour rejection induced by specific virus antigens.

REFERENCES


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