Limitations and Utility of a Cytolytic Assay for Measuring Simian Virus 40-induced Cell Surface Antigens

Samuel J. Pancake and Peter T. Mora

SUMMARY

Antisera were produced against an SV40-transformed cell line in the syngeneic AL/N mouse. With a microcytolytic assay, the specificity of antisera produced by various immunization schedules and their ability to lyse numerous SV40-transformed cell lines were determined. Various AL/N mouse cell lines, newly transformed by SV40 and cloned, were found to be lysed by the antisera. When tumors were induced by SV40-transformed cells in the syngeneic mouse and cell lines were reestablished from tumors and such procedures were repeated, the susceptibility to serum-mediated cytolysis of the sublines was the same as that of the original SV40-transformed cell line, in spite of differences in tumorigenicity. Polyoma virus-transformed AL/N cell lines were also lysed while AL/N embryo cells, untransformed by SV40 or by polyoma, were not. SV40-transformed T-antigen-positive BALB/c mouse or hamster cell lines or a T-antigen-positive tissue cultured human cell were also resistant to lysis. A competition type of microassay demonstrated specific inhibition of the serum-mediated cytolysis by all of the SV40 T-antigen-positive cell lines tested. Thus, the lack of lysis of cells did not necessarily indicate the absence of SV40-induced surface antigens. The polyoma-transformed AL/N cell line also inhibited the antisera, but to a lesser extent, suggesting the possibility that SV40 and polyoma virus transformation may result in the appearance of partially common cell surface antigens.

INTRODUCTION

Numerous in vitro assays are being used to detect cell surface antigen changes in tumorigenic transformation. Whether these in vitro methods detect tumor-specific transplantation antigens remains to be demonstrated. In each type of assay and in each animal system, the degree of correlation can be ascertained only when the in vitro assay is correlated with appropriate transplantation experiments. However, in vitro assays are often useful when cell surface antigens are to be compared in a reproducible, rapid, and quantitative fashion, such as in the comparison of cell lines grown in tissue culture with derivative lines produced by transformation with tumorigenic viruses. The use of in vitro assays, based on the lysis of SV40-transformed strain mouse cell lines by antisera prepared in the syngeneic mouse, has implied specificity of lysis for SV40-transformed cells lines (16, 18, 19). A micromodification of a cytolytic assay based on the release of $^{31}$Cr from labeled cells (15) was used in the present study.

This study reinvestigates whether only SV40-transformed cell lines are subject to lysis by antisera or whether non-SV40-transformed cell lines may also be lysed, and whether all types of SV40-transformed cell lines are susceptible to lysis. A competition absorption modification of the cytolytic microassay is also given. A preliminary report summarized some of the results (12). In this communication antisera prepared by various immunization schedules are also compared.

MATERIALS AND METHODS

Cell Lines. The AL/N mouse cell lines (SV-AL/N), polyoma-transformed (PY-AL/N), and the spontaneously transformed (T-AL/N) lines have been described before (15). These are uncloned cell lines of a fibroblastic type (17). The SV-AL/N line had been in continuous culture for over 3 years, corresponding to more than 300 tissue culture passages. A micromodification of a cytolytic assay based on the release of $^{31}$Cr from labeled cells (15) was used in the present study.

Received January 17, 1975; accepted September 16, 1975.

1 To whom requests for reprints should be sent.

The last injection the mice were bled, but only serum from the group of mice was divided into subgroups of 5 each, designated as A, B, C, and D. The injection schedule was as follows: 3 x 10^10 cells/mouse; 4 days later, 1 x 10^9 cells/mouse; 4 days after that, 1 x 10^8 cells/mouse; 4 days after that, 1 x 10^7 cells/mouse. One week after the last injection the mice were bled, but only serum from Group C (Serum VC1) had significant lytic activity against SV-AL/N-ME and PV-AL/N cells.

Cells were grown in Eagle's or in Dulbecco-Vogt medium containing 10% fetal calf serum in a humidified CO_2 incubator.

Antisera and Complement. Antisera were produced by the i.p. injection of the fibroblastic SV-AL/N cell line into groups of 15 young adult male AL/N mice. Sera were collected by bleeding through the tail vein. For Antiserum I through IV (Table 1) cells (10^6) were injected weekly, a total of 6 times; except for Antiserum I and II, where there was a 3-week pause half-way through the injection series. The animals were bled at weekly intervals starting 1 week after the last injection. Cells for injection were collected either by trypsinization or by dispersion with pipetting, and before injection were washed 3 times in Tris-buffered isotonic saline (containing 9 g NaCl, 0.38 g KCl, 0.1 g Na_2HPO_4, 1 g dextrose, and 3 g Tris base per liter and adjusted to pH 7.4 with HCl) to remove fetal calf serum. As shown in Table 1, a lower cross-reactivity against AL/N-ME and PV-AL/N cells was evident when antisera was prepared against dispersed SV-AL/N cells as compared to trypsinized cells (compare type II with type I and type IV with type III).

The antisera used in the remainder of the study (Group V; Tables 2, 3, and 4) were prepared using untrypsinized cells dispersed by pipetting. The SV-AL/N cells used in this immunization had been recently restarted in culture from cells frozen away when last used for antisera production (15). The group of mice was divided into subgroups of 5 each, designated as A, B, C, and D. The injection schedule was as follows: 3 x 10^6 cells/mouse; 4 days later, 1 x 10^6 cells/mouse; 4 days after that, 1 x 10^5 cells/mouse. One week after the last injection the mice were bled, but only serum from Group C (Serum VC1) had significant lytic activity against SV-AL/N cells. A 2nd bleeding 1 week later produced Serum VC2, after which the animals were given injections of a single dose of 1 x 10^6 cells and then bled weekly producing Antisera VC3, VC4, and VC5. After the 1st injection schedule the mice from Groups A, B, and D were again given injections, 1 week apart, 2 more times with 1 x 10^6 cells, and then bled at weekly intervals. In the designation of antisera, the last number indicates the bleeding number (Table 2). All sera were incubated for 45 min at 56°C to inactivate complement and stored at -70°C.

Rabbit serum was used as a source of complement. Two batches of rabbit serum (Microbiological Associates, Bethesda, Md.) were selected for use after testing several batches and finding these 2 least toxic to SV-AL/N cells (release of 51Cr by complement alone being approximately 20% of that released by rabbit anti-mouse serum; see below). Rabbit antisera against mouse lymphocytes was obtained commercially (Microbiological Associates).

51Cr Microlyticolytic Assay. The assays were carried out in the wells of the small Terasaki microtissue culture plates (Falcon Plastics, Oxnard, Calif.) as described (15). Differences are noted as follows: the whole microtest plate was conveniently flooded with a dilution of target cells diluted in HEPES-Eagle's. After the excess cell suspension was removed from the tilted plates by suction, each well retained approximately 12 μl of suspension (determined by micropipets) containing 300 to 500 cells (determined by prior counting). Cell attachment and growth was then allowed to proceed for 24 hr or longer if necessary to achieve the desired cell density (see below). To remove unattached cells, the plates were flooded with HEPES-Eagle's and the excess was sucked off again from the tilted plates. Next, the cells were labeled overnight at 37°C with 51Cr by adding 1 μl of 5 x diluted sodium [51Cr]chromate (Amersham-Searle, Arlington Heights, Ill.; 250 μCi/μl in 0.9% NaCl solution) to each well. The plates were washed twice, using the same technique as before to eliminate free 51Cr. At this stage, well-attached cells should cover 30 to 50% of the area of the bottom (flat) portion of the microwells.

For cytoly sis, 1 μl of diluted antiserum was added to the wells and, after 1 hr of incubation at 37°C, the plates were washed once again. One μl of diluted complement was then added, the final concentration of complement being at least twice that necessary to produce maximum release of 51Cr as determined by previous titration. The antisera produced no release of 51Cr in the absence of complement. For each value on a titration curve the combined aspirated supernatants from 3 wells were counted. Duplicate titrations within a single assay generally gave values that differed by less than ±20%. In each assay all counts were corrected for the counts released by complement alone. The value of “100% chromium release” was obtained from wells where rabbit antisera against mouse lymphocytes were added and the released counts were corrected for complement toxicity. The percentage of chromium release shown in the charts is a percentage taking this value as 100%. Total uptake of 51Cr into the cells was determined by adding 10 μl NCS tissue solubilizer (Amersham-Searle) to each of 3 untreated wells and then collecting and combining the solutions. The procedure was repeated 3 times, and the total solubilized radioactivity was determined by counting. The 100% chromium release value was usually approximately 70% of the total chromium uptake value. All counting was done using an Aquasol scintillation mixture (New England Nuclear, Boston, Mass.) and a Packard Tri-Carb liquid scintillation counter. Chart 1 and the duplicate titrations for Antiserum VC4 in Chart 2 illustrate the reproducibility of the microlytic assay and the general significance of all further
values expressed as antisera titers. Antiserum titer was defined as the final dilution of antiserum in the well which gives a 50% chromium release; i.e., the antiserum releases one-half as many counts as is released by the rabbit antiprotein serum in the same assay. The average deviation of the 50% chromium release value from 1 independent experiment to the next is shown in Table 2.

Following the removal of the supernatants, the cells in the wells were always examined visually by means of a trypan blue dye exclusion test (15). This provided an independent check on the extent of cell lysis and cell attachment at the end of the assay. Results from plates with damaged or large numbers of detached cells during any part of the assay were not included in collating data. This problem seldom occurred (less than 5% of the assays) and was usually the result of using plates with too high a cell density, resulting in their detachment as a single sheet before the completion of the assay. With appropriately seeded microplates essentially all cells remained attached subsequent to lysis. No definite difference could be detected between the percentage of cells permeable to trypan blue and the percentage of 51Cr released, indicating that the release of 51Cr correlates with the degree of cytolysis.

Cell Competition Assay. To estimate the number of cells that inhibit lysis by the anti-SV-AL/N sera by 50%, varying numbers of unlabeled competing suspended cells were included in the microwells during the incubation with antisera. The cells were obtained from growing monolayers after 1 hr washing in situ with the Tris-buffered isotonic saline, followed by incubation at 37°C for 15 min in the same solution. The detached cells were then centrifuged and resuspended by gentle pipetting in the solution and appropriately diluted. One μl of diluted cell suspension was added per well to wells containing the already labeled SV-AL/N target cells. This was immediately followed by addition of a quantity of specific antisera known to release about 50% of the chromium counts in the absence of added competing cells. After 1 hr the plates were washed, removing the unlabeled cells. Complement was added and the released counts were determined as before. Percentage of inhibition of lysis was plotted against the number of competing cells added per well and the 50% inhibition of lysis values were estimated from such plots.

T-Antigen Assay. Cells were grown on coverslips for 48 hr and then fixed in acetone and reacted with serum from hamsters bearing tumors induced by SV40-transformed hamster cells (H-50 cells). After washing, the cells were reacted with fluorescein-conjugated anti-hamster γ-globulin (Antibodies Inc., Davis, Calif.)

RESULTS

Factors Influencing Reproducibility of the Cytolytic Assay. With each cell line the attachment and the growth in the microwells was different. Number of cells plated and growth period before the assay were selected accordingly as described in “Materials and Methods.” In general, the non-viral-transformed cell lines, such as SV-AL/N and PY-AL/N and BALB 3T3 SV-T2. At the time of addition of antiserum, cells must be preconfluent in the microwells to avoid detachment before the assay can be completed. Chromium toxicity was minimized by using low concentrations during overnight incubation. Plates should be exposed minimally to the open air due to the pH changes which result. Antisera titration curves carried out in HEPES-Eagle’s or HEPES-Dulbecco-Vogt medium gave similar results (Chart 1). HEPES-Eagle’s was used in all subsequent experiments since it showed smaller pH changes when exposed to air than HEPES-Dulbecco-Vogt.

Variability of the Antisera. Although all sera were prepared by immunization of AL/N mice with the SV-AL/N cell line, their cytolytic titers on SV-AL/N target cells and the extent of their cross-reactivity against non-SV40-transformed AL/N cell lines differed markedly. As indicated in Table 1, the degree of cross-reactivity may be influenced by both the method of harvesting of the SV-AL/N cell line used for immunization and by the immunization schedule. When immunization was interrupted for 3 weeks, higher-titer antisera were produced, but these sera also possessed higher cross-reactivities (compare Antisera I and II with Antisera III

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Antiserum dilutions producing 50% chromium release from target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>T-AL/N</td>
</tr>
<tr>
<td>I</td>
<td>Trypsinization</td>
</tr>
<tr>
<td>II</td>
<td>Dispersion</td>
</tr>
<tr>
<td>III</td>
<td>Trypsinization</td>
</tr>
<tr>
<td>IV</td>
<td>Dispersion</td>
</tr>
</tbody>
</table>

* See “Materials and Methods.”
and IV (Table 1). Only low cytolytic activity was detected against the T-AL/N cell line, but strong cross-reactivities were seen with the PY-AL/N and AL/N-ME cell lines (Table 1). Since antisera of high titer showed a disproportionate increase in cross-reactivity (compare, for example, Antiserum II with Antiserum IV), smaller cell doses and reduced numbers of injections were used in immunization Series V. Also, in the immunization Series V only nontrypsinized cells were injected (cf. "Materials and Methods").

A representative titration is shown in Chart 2, using the SV-AL/N cell line and several antisera from Series VC. In general, serum strength was observed to increase with bleeding as indicated in the series 2, 3, 4, and 5. Serum titers were of the order of 100 to \( \sim 300 \).

The titer of Serum VC4 on the newly established normal cell line from AL/N mouse embryo (AL/N-ME) was quite low (<20), as was that of Serum VC5 on the T-antigen-negative cloned AL/N-ME lines, (Table 2). With the previous antisera, AL/N-ME was the most cross-reactive of the non-viral-transformed control cell lines tested (see Table 1), indicating that in this respect the VC antisera are relatively specific. However, they still possessed substantial cytolytic activity against PY-AL/N cells (titers of 50 to 80 (Chart 3)).

Testing of Antisera on SV40-transformed T-Antigen-positive Cell Lines Other than SV-AL/N. In further testing of Series V antisera, the SV40-transformed BALB 3T3 cell lines were found to be not susceptible; i.e., in several independent titrations, the BALB 3T3 SV-T2 cell line was not lysed by

---

**Chart 2.** Titration of cytolytic activity of various antisera. Release of chromium from labeled SV-AL/N target cells. The immunization schedule in the preparation for these antisera (Group V) is given in "Materials and Methods." Antisera designated by the letter "C" refer to a subgroup of animals that had been bled 2, 3, 4, or 5 times, as indicated by the included number.

**Chart 3.** Titration of serum cytolytic activity of antisera using chromium labeled PY-AL/N and AL/N-ME cell lines. The activities of the same antisera against the SV-AL/N cell line are given in Chart 2. For designation of antisera see legend of Chart 2 and also "Materials and Methods."

**Table 2**

<table>
<thead>
<tr>
<th>Competing Cells</th>
<th>T-Antigen</th>
<th>Dilution of Antisera* that lysed 50% cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV-AL/N</td>
<td>+</td>
<td>80 ± 5* (2)</td>
</tr>
<tr>
<td>AL/N-ME</td>
<td>-</td>
<td>&lt;5* (2)</td>
</tr>
<tr>
<td>SV40-infected clone</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>178 ± 118 (4)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>34 ± 6 (2)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>&lt;20 (4)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>&lt;20 (4)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>&lt;20 (2)</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>&lt;20 (4)</td>
</tr>
</tbody>
</table>

* Antisera were prepared and coded as given in "Materials and Methods."

---

\* Mean ± S.D.

\^ Number in parentheses, number of independent titrations performed where >1.

\* No significant lysis at lowest serum dilution used.

\^ Cytolysis for 50% lysis is less than the lowest dilution tested.

---

Downloaded from cancerres.aacrjournals.org on August 15, 2017, © 1976 American Association for Cancer Research.
10× dilutions of Antiserum VC3 and Antiserum VC5, while the titers of these antisera on SV-AL/N are 130 and 433, respectively. Similarly, the cloned BALB 3T3 SV40-transformed reestablished cell line was not lysed by a 10× dilution of Antiserum VC2 or VC5. Also the BALB TU-5 cell line, the tumor induc-
ying SV40 T-antigen-positive cell line, was not lysed by Anti-
serum VC3 (20× dilution) or Antiserum VC5 (40× dilution).

The SV40 T-antigen-positive hamster H-50 cell line and the T-antigen-negative hamster MSV cell line were not lysed by Antiserum VC1 (10× dilution). The SV40 T-antigen-po-

Cell Lines in Which SV40 Transformation Results in Sus-
ceptibility to Lysis by the Antisera. The T-antigen-positive
and T-antigen-negative clones derived from a newly estab-
lished embryo cell line (AL/N-ME) after infection by SV40
(see "Materials and Methods") were grown and tested in the
cytolytic assay following cloning as soon as sufficient cells
were available. Table 2 summarizes the results obtained
with the various antisera. The parent AL/N-ME cell line and
the T-antigen-negative clones were not lysed significantly
by the highest concentrations of antisera tested. Thus,
clones 11 and 13 were not lysed with the strong Antiserum
VC5 (20× dilution), while this antiserum lysed 50% of the T-

Retention of Susceptibility to Lysis by SV40-transformed
AL/N Cell Lines After Repeated Transplantation in the
Syngenic Host. Table 3 shows no differences in suscepti-
bility to lysis between the parent SV-AL/N and all the deri-

Table 3

<table>
<thead>
<tr>
<th>Target cell line</th>
<th>Passage through host</th>
<th>Method of reestablishing cell culture</th>
<th>Dilution of antiserum VC3 that lysed 50% of cells</th>
<th>TDM±</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV-AL/N</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Derivative A</td>
<td>1st</td>
<td>Trypsinization</td>
<td>160 (3)</td>
<td>5×10⁶</td>
</tr>
<tr>
<td>Derivative B</td>
<td>1st</td>
<td>Mincing</td>
<td>160 (2)</td>
<td>10⁶</td>
</tr>
<tr>
<td>Derivative from A</td>
<td>2nd</td>
<td>Trypsinization</td>
<td>163 (3)</td>
<td>5×10⁶</td>
</tr>
<tr>
<td>Derivative from B</td>
<td>2nd</td>
<td>Trypsinization</td>
<td>140 (2)</td>
<td>&lt;10⁶</td>
</tr>
</tbody>
</table>

∑ Described in Ref. 7.

a The 1st in vivo passage was in 300-rad-irradiated AL/N mice, the 2nd was in immuno-
logically competent mice.

b Cell number that when implanted i.m. into immunocompetent weanling mouse,
causes large (≥1-cm diameter) tumor in less than 4 weeks in ~50% of 10 animals tested
(7).

c Number in parentheses, number of independent assays; value given is the median

Chart 4. Competition assay to compare cell lines in their ability to remove
antiserum activity. SV-AL/N target cells were plated into microwells and after
attachment, growth, and ¹⁴C-labeling, the indicated number of the desig-
nated unlabeled competing cells were added per microwell, followed by 1 μl
of diluted antiserum in this case, VC1 (see "Materials and Methods"). The
serum at a dilution (50×) sufficient to lyse about 50% of the labeled cells as
determined by previous titration. With the use of the extent of lysis produced
by the diluted antiserum in the absence of added unlabeled cells as 100%, the
percentage of inhibition of lysis is plotted against the number of competing
cells added.
competition experiments where increasing numbers of the unlabeled cells were added to wells, immediately followed by addition of a constant amount of antiserum. For the SV-AL/N cell line 1.8 × 10^5 cells/microwell reduced lysis by 50%, while for the PY-AL/N cell line 1.2 × 10^5 cells reduced lysis by 50%. In contrast, the addition of 2.0 × 10^5 T-AL/N cells resulted in only a 30% reduction of lysis.

By means of this type of assay, the degrees of inhibition of the cytolytic antiserum by a variety of cells and cell lines were compared (for summary of data and for the method of calculation see Table 4). All of the SV40-transformed (T-antigen-positive) cells and cell lines, including the clonal derivative line, exhibited a capacity to reduce lysis by 50% at a relatively small cell number, (generally below 6 × 10^6/microwell), while the untransformed (T-antigen-negative) cells or cell lines did not produce a 50% reduction of lysis at the highest number of cells tested (2 to 5 × 10^6/microwell), with the exception of the PY-AL/N cell line that gave 50% inhibition at 1.2 to 1.6 × 10^6 cells/microwell. The transformed and untransformed hamster and human cell lines appeared to be distinguishable using this criterion, even when no such distinction was obtainable by the direct cell lysis assay.

DISCUSSION

A serum-mediated complement-dependent microcytolytic assay, developed previously (Ref. 15, cf. also Refs. 16 and 19) was reexamined to determine whether it can be utilized to detect common and specific cell surface antigens induced in various cell lines by SV40 transformation. In the cell lines derived from AL/N mouse embryo the T-antigen-positive clones generally were found to be susceptible to lysis, while the untransformed, or T-antigen-negative, clones were not. The SV-AL/N cell line used to prepare the antiserum was consistently the most susceptible to lysis. Other SV40 T-antigen-positive cell lines from BALB/c mouse and from hamster and T-antigen-positive cells of human origin were not susceptible to lysis by these antiserum. Similar observations have been recently reported for other SV40-transformed BALB/c cell lines (4, 6). These results contrast with earlier claims regarding the susceptibility to lysis of T-antigen-positive cell lines from BALB/c mouse and hamster (15, 18, 19). A lack of correlation between Forssman antigen content and lytic susceptibility has also been observed in guinea pig hepatomas (11).

By means of the absorption or competition type of assay, SV40-specific antigens were detected on the surface of all of the SV40-transformed (T-antigen-positive) cells or cell lines (Table 4). Thus, the cytolytic assay is more stringent in its requirements than the absorption assay and SV40-specific common antigens, detectable by absorption or competition, may not necessarily be detectable by direct cytolysis.

A portion of the antibody cytolytic to the SV-AL/N cell line was inhibited by the presence of large numbers of PY-AL/N cells (Chart 3). This implies that the PY-AL/N cell line contains antigen material(s) cross-reactive with those detected in the SV-AL/N cell line. This is consistent with the observed

Table 4

<table>
<thead>
<tr>
<th>Antisera</th>
<th>VC1</th>
<th>VC2</th>
<th>VC3</th>
<th>VC4</th>
<th>VB5</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-antigen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-AL/N</td>
<td>-</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>PY-AL/N</td>
<td>-</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME-SV-AL/N-CL12</td>
<td>+</td>
<td>1.8</td>
<td>5.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-AL/N-CL3 SV40</td>
<td>+</td>
<td>0.6</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-50 hamster</td>
<td>+</td>
<td>1.7</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSV hamster</td>
<td>-</td>
<td>&lt;0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wi38 human cells</td>
<td>-</td>
<td>&lt;0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WI-VA-2 human cells</td>
<td>+</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Efficiency of inhibition is defined as the number of added unlabeled cells/microwell required for 50% inhibition of the given antiserum divided into the number of SV-AL/N cells similarly determined to be necessary for 50% inhibition. cf. Chart 4.

a For designation of the different antisera see "Materials and Methods."

b The < symbol indicates that the maximum number of cells added, 2 × 10^4 or more/microwell, gave substantially less than 50% inhibition of lysis, the inhibition decreasing with decreasing numbers of cells. With the T-antigen-positive cell lines (SV-AL/N, ME-SV-AL/N-CL12, T-AL/N-SV-1-CL-1, H-50 hamster, and WI-VA-2 human), near-complete inhibition was obtained with <2 × 10^4 cells. The microassay is probably not suitable for the addition of more than 2 to 5 × 10^6 cells/microwell as the added cells form layers over the labeled target cell monolayer, probably resulting in a nonspecific lowering of target cell lysis. Addition of large numbers of inadvertently damaged cells especially appeared to interfere with lysis in a nonspecific manner. In more recent experiments diluted antiserum was absorbed with attached unlabeled cells, and residual antiserum activity against SV-AL/N target cells was then determined as before. Use of this method indicated that absorption with large numbers of live T-antigen-negative cells gave no reduction in antiserum titers.

c Obtained from a clonal cell line of T-AL/N after SV40 infection and recloning (P. T. Mora and L. Couvillion, in preparation).
susceptibility of the PY-AL/N cell line to lysis by anti-SV-AL/N sera (Chart 3). There was no SV40 T-antigen detectable in the PY-AL/N cell line, eliminating the possibility that this cell line was transformed accidentally by the SV40 virus. Similar cross-reactivity of PY-AL/N cells with antisera prepared against SV-AL/N cell-induced tumors in the syngeneic mouse was reported recently by another laboratory (5) using a different absorption type assay (isotopic antiglobulin technique).

The observations that the newly prepared AL/N-ME embryonic cells and the established T-AL/N embryonic cell line are largely unlysed by the antisera against the SV-AL/N cell line (Chart 3; Tables 1 and 2), and that the T-AL/N cell line does not absorb out significant antiserum activity (Chart 4 and Table 4), suggest that the antigens detected are unrelated to embryonic fibroblastic type antigens. Even so, the antigens measured by these methods may yet represent increased quantities or increased exposure of other types of embryonic tissue or possibly Forssman-type antigens, although the latter have been reported to be absent from SV40- and polyoma virus-transformed Swiss 3T3 mouse cells (13) and to be present in decreased amounts in SV40-transformed hamster cells compared to fetal hamster cells (7).

Cell lines further derived from the SV-AL/N cell line after repeated passage through syngeneic mice retained diminished susceptibility to lysis by the antiserum while a major increase in their tumorigenicity was observed (Table 3; Ref. 9). This suggests that the cell surface antigens that appear as a consequence of SV40 transformation and that mediate serum cytolysis are not selected against during repeated transplantation. This further implies that, if the increased tumorigenicity of the derivative cell lines from the SV-AL/N cells (Table 3) is due to selective destruction of the more immunogenic and/or immunosensitive cells in vivo, then this is unlikely to occur through serum-mediated cytolysis (9).

In conclusion, in certain syngeneic systems with strong antigenic differences, such as in the SV-AL/N system, direct cytolytic microassays may be useful in providing a rapid and relatively simple procedure to detect, and to some extent to quantitate, viral-induced cell surface antigens. By means of the absorption assay, SV40-induced cell surface antigens can be measured that are not necessarily detectable by the direct cytolysis assay. The biological significance of both of these assays remains to be explored.

ACKNOWLEDGMENTS

We thank Dr. Richard W. Smith for advice on the assay and on the immunization procedures, Lynda Couvillion for the T-antigen tests, and Lorenzo Waters for assistance.

REFERENCES

Limitations and Utility of a Cytolytic Assay for Measuring Simian Virus 40-induced Cell Surface Antigens

Samuel J. Pancake and Peter T. Mora