ANTIGENIC PROPERTIES OF MURINE SARCOMA VIRUS-TRANSFORMED BALB/3T3 NONPRODUCER CELLS

BY JOHN R. STEPHENSON AND STUART A. AARONSON

(From the Viral Leukemia and Lymphoma Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014)

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There are many biologic and biochemical similarities between spontaneous tumor cells and those induced in model systems by oncogenic viruses. However, in contrast to most naturally occurring tumor cells, cells transformed by known oncogenic viruses are generally highly antigenic and consequently are rejected by an immunologically competent host. Strong virus-specified cellular transplantation antigens, induced by DNA viruses such as polyoma, SV40, and adenovirus, have been clearly demonstrated by in vivo and in vitro techniques (see references 1–5). In the case of RNA-containing tumor viruses, there have been a number of reports of virus-induced transplantation antigens in murine sarcoma virus (MSV)1-transformed cells (6–12). However, these latter studies have been performed with MSV-transformed cells which chronically release both MSV and murine leukemia virus (MuLV). It has, therefore, been difficult to distinguish antigenic changes associated with virus replication from those due to virus-coded, nonvirion transplantation antigens.

It has recently been shown that MSV can transform mouse cells in vitro in the absence of added MuLV but requires MuLV for its replication (13, 14). It was, thus, possible to isolate MSV-transformed cell lines which lacked any evidence of virus production or antigens but from which the sarcoma genome could be rescued by infection with MuLV (13, 14). The fact that these lines were derived from a parent clone of contact-inhibited, nontumorigenic, syngeneic mouse cells, BALB/3T3 (15), made it possible to determine whether MSV, in the absence of virus production, induces detectable virus-specified transplantation antigens in transformed cells.

**Materials and Methods**

**Mice.**—6–8-wk old female BALB/c mice were obtained from the National Institutes of Health (NIH) breeding colony, Bethesda, Md.

**Cells and Viruses.**—Cells were grown in Dulbecco’s modification of Eagle’s medium supplemented with 10% calf serum. From a clonal line of BALB/3T3, clone A31 (15), a Kirsten (Ki)-MSV transformed subclone, K-234, was isolated which produced no detectable virus (16). Similar BALB/3T3 nonproducer transformants of the Moloney (M) strain of MSV have been previously described (13). A line of MSV- and MuLV-producing transformed cells was obtained by infecting K-234 cells with Rauscher (R)—MuLV. The K-234(R) subclone chronically released around 2 × 10^6 focus-forming units (FFU)/ml of MSV and 3 × 10^6 infectious

1Abbreviations used in this paper: FA, fluorescent antibody; FFU, focus-forming units; Ki, Kirsten; M, Moloney; MSV, murine sarcoma virus; MuLV, murine leukemia virus; NRK, normal rat kidney; R, Rauscher.
units/ml of R-MuLV. An SV40-transformed BALB/3T3 subclone, SV-T2, has previously
been described (17). Other cells included a normal rat kidney (NRK) cell line (18) and a
KlMSV-transformed nonproducer clone of NRK cells, K-NRK (16). R-MuLV, twice-
banded in sucrose and concentrated 1000-fold, was obtained from Electro-Nucleonics, Inc.
Bethesda, Md. Other viruses used included KlMSV(KiMuLV) and KlMSV(R) (16). For
some studies, viruses were inactivated by ultraviolet irradiation for 15 min as previously
described (19).

Immunizing Procedure.—Cells were trypsinized, washed twice by centrifugation, and re-
suspended in serum-free medium at the appropriate concentration. Injections were made sub-
cutaneously in the thigh in a volume of 0.2 ml. For some immunizations cell suspensions main-
tained on ice were ultrasonically disrupted for 60 sec at 60 w with a ½ inch sonic probe (Sonifer
Cell Disruptor Model W185D, Branson Sonic Power Co., Danbury, Conn.).

Cytotoxicity.—A modification of the method of Hellström and Sjögren (5) was used for
cytotoxicity studies. Target cells were trypsinized, washed three times, and resuspended in
medium at 10⁴ cells/ml. 0.25 ml serum was mixed with an equal volume of cells and incubated
at 37°C for 30 min, followed by the addition of 0.25 ml of guinea pig complement and incubation
for a further 60 min. The cell suspensions were then diluted 1:10 in medium and dis-
tributed to 50-mm Petri dishes (Falcon Plastics, Div. B-D Laboratories, Inc., Los Angeles,
Calif.) at 4 ml/dish. After incubation for 7 days at 37°C, the Petri dishes were stained with
hematoxylin and the colonies were counted.

Virus Neutralization.—Virus neutralization was performed by a focus reduction method.
Antisera were heat inactivated at 56°C for 30 min. Around 50 FFU of MSV were incubated
with the appropriate serum dilution for 30 min at 37°C and then assayed for focus formation
on diethylaminoethyl (DEAE)-dextran-pretreated NRK cells as previously described (13).

Fluorescent Antibody Detection of Cell Surface Antigens.—An indirect fluorescent antibody
(FA) technique was used to detect cell surface antigens. Sera from hamsters, bearing an in
vivo-induced MSV-nonproducer tumor, HT-1 (20), were a gift of G. Kelloff of NIH. Sera
from rats carrying a transplantable MSV-producing tumor were obtained from R. Wilsnak,
Huntington Research Laboratories, Baltimore, Md. Fluorescein-conjugated goat antiserum to
rat, hamster, and mouse globulins were obtained from the Resources and Logistics Segment of
the National Cancer Institute. Cells growing on cover slips were washed twice in phosphate-
buffered saline and incubated at 37°C for 30 min with antiserum, washed again, and incubated
30 min with the appropriate fluorescein-conjugated antoglobulin at a final dilution of 1:16 with
lissamine rhodamine (1:60 dilution) as counterstain. Cell surface antigens were scored with a
Leitz fluorescent microscope (X 400).

RESULTS

Comparison of the Tumorigenicity of MSV-Transformed Nonproducer and Pro-
ducer Cell Lines.—The present study was undertaken to determine whether the immunogenicity of murine sarcoma virus-transformed cells is due to anti-
gens associated with virus production, or whether MSV-transformed cells
possess new virus-specified nonvirus transplantation antigens. The tumor-
forming ability of an MSV-transformed nonproducer clonal line, K-234,
was compared with that of the identical cell line preinfected with R-MuLV
and producing both sarcoma and leukemia viruses. As can be seen in Fig. 1,
at each cell dose tested the incidence of tumor formation in mice inoculated
with K-234 was much higher than with K-234(R). Tumors induced by K-
234(R) cells grew rapidly for the first 2 wk, but only very slowly thereafter;
some tumors actually had regressed by 6 wk postinjection. On the other hand,
tumors induced by the nonproducer cell line grew progressively and eventually killed the host. This is reflected in the much higher death rate for mice injected with K-234 as compared to K-234(R) cells (Fig. 2). Clearly, the presence of replicating virus in K-234(R) cells was associated with their having a markedly reduced malignant potential. The most likely explanation was that the virus-producing cells were more antigenic than the MSV nonproducers.

To test whether this was the case, the effect of irradiation of the host on the tumorigenicity of the two cell lines was compared. Mice which received 250 rads whole body irradiation and unirradiated controls were inoculated subcutaneously with either MSV-nonproducer or virus-producing transformed cells. As shown in Table I, irradiation had little if any effect on the death rate of animals receiving MSV-nonproducer cells. However, the producer cells grew much more efficiently in immunologically suppressed mice than in the nonirradiated controls. This result further suggests that the virus-producing MSV-transformed cells were much more immunogenic than the nonproducer cells.

**Tumorigenicity of MSV-Nonproducer and Producer Cells in Preimmunized BALB/c Mice.**—In order to resolve whether the nonproducer cells possessed detectable virus-induced transplantation antigens, in vivo transplantation
immunity experiments were performed. BALB/c mice were immunized subcutaneously with K-234, K-234(R), or the parent clone of BALB/3T3 with four successive weekly doses of $5 \times 10^4$ cells/animal. 1 wk after the last immunizing injection, the animals were challenged with either K-234 or K-234(R) cells. As shown in Table II, preimmunization with the producer cells markedly reduced the tumor incidence in mice subsequently challenged with the homologous cells. Only one tumor developed in 60 animals preimmunized with K-234(R) as compared to 26/50 in mice pretreated with control BALB/3T3 cells. In contrast, there was no significant reduction in tumorigenicity of K-234 cells in mice preimmunized with K-234 as compared to those preimmunized with BALB/3T3. A small reduction in tumor-forming ability of both K-234 and K-234(R) was seen in mice preimmunized with BALB/3T3. This may be due to antigenic changes in the BALB/3T3 cells after prolonged passage in culture (21).

In another study, animals were preimmunized with BALB/3T3, K-234, or K-234(R) at $5 \times 10^4$ cells/injection; here, cells were first sonicated to reduce tumor induction during immunization. From the results in Table III, it is clear that preimmunization with sonicated K-234(R) cells effectively prevented challenge by the homologous cells. There were no tumors in 60 K-234(R)-preimmunized animals compared to 27/53 in BALB/3T3-preimmunized controls. Again, the nonproducer cells were no more effective than BALB/3T3 itself in protecting mice from challenge with either K-234 or K-234(R).
Tumorigenicity of Nonproducer Cells in BALB/c Mice Preimmunized with Xenogeneic Nonproducer Cells.—Transplantation immunity studies were also performed by preimmunizing BALB/c mice with a KiMSV rat nonproducer cell line, K-NRK. By use of this xenogeneic cell line, it was possible to immu-

| Clone   | Cell dose | Control | Preirradiated |
|---------|-----------|---------|---------------|
| K-234   | $10^3$    | 0       | 0             |
|         | $10^4$    | 0       | 5             |
|         | $10^5$    | 20      | 25            |
| K-234(R)| $10^3$    | 0       | 52            |
|         | $10^4$    | 0       | 84            |
|         | $10^5$    | 4       | 96            |

* 25 mice in each group were challenged at the indicated cell doses with the appropriate cells injected subcutaneously in the interscapular region.
† Determined at 4 wk after inoculation.

| Clone   | Pretreatment |
|---------|--------------|
|         | Untreated    | BALB/3T3 | K-234(R) | K-234 |
| K-234   | 2 x $10^4$   | 5/18     | 2/19     | N.D. § 1/15 |
|         | 1 x $10^4$   | 11/19    | 5/18     | N.D. 2/13 |
|         | 5 x $10^5$   | 18/18    | 11/17    | N.D. 7/13 |
| K-234(R)| 2 x $10^4$   | 17/17    | 12/17    | 0/20 N.D. |
|         | 1 x $10^5$   | 12/17    | 7/16     | 0/20 N.D. |
|         | 5 x $10^5$   | 12/17    | 7/17     | 1/20 N.D. |

* Mice were preimmunized weekly for 3 consecutive wk with 2.5 x $10^5$ viable cells injected subcutaneously in the thigh region.
† Mice were challenged at the indicated cell doses subcutaneously in the interscapular region 1 wk after the final preimmunizing injection. Results are expressed as the fraction of tumor-bearing mice at 5 wk.
§ Not done.

munize with very high cell doses without producing tumors during immunization. If virus-specific transplantation antigens were present on the MSV-nonproducer rat cells, these might be capable of immunizing mice against subsequent challenge by MSV-nonproducer mouse cells. It can be seen from the results in Table IV that K-NRK cells were no more effective than NRK cells in protecting mice from subsequent challenge with K-234. These findings
along with the results in Tables II and III demonstrate that the MSV-nonproducer cells clearly lacked evidence of virus-specific transplantation antigens detectable by transplantation immunity studies.

_Tumorigenicity of MSV-Transformed Producer and Nonproducer Cells in Rauscher Virus Preimmunized Mice._—From the above studies, it seemed likely that the transplantation antigens of K-234(R) cells were primarily due to maturing virus at the cell surface. To directly test this possibility, mice were immunized weekly for 3 consecutive wk with UV-irradiated R-MuLV (approximately $10^8 - 10^9$ particles per injection) and then challenged 1 wk later with the K-234 and K-234(R) lines at $5 \times 10^4$ cells/animal. As shown in Table III, R-MuLV was able to prevent subsequent challenge by K-234(R) but had no effect on the tumorigenicity of K-234 cells. These results demon-

### TABLE III

**Tumorigenicity of MSV-Producer and Nonproducer Cells in Mice Preimmunized with Sonicated Cells or UV-Inactivated R-MuLV***

| Tumor challenge | Pretreatment | Untreated | BALB/c T3 | K-234(R) | K-234 | R* |
|-----------------|--------------|-----------|-----------|-----------|-------|----|
| Clone           | Cell dose    | 3/6       | 0/5       | N.D.§     | 0/3   | N.D.|
| K-234           | $2 \times 10^4$ | 3/6       | 0/5       | N.D.§     | 0/3   | N.D.|
|                 | $1 \times 10^6$ | 6/6       | 2/5       | N.D.      | 2/5   | N.D.|
| K-234(R)        | $2 \times 10^4$ | 17/17∥    | 8/17      | 0/21      | 5/9   | N.D.|
|                 | $1 \times 10^6$ | 12/17∥    | 9/18      | 0/20      | 3/7   | N.D.|
|                 | $5 \times 10^5$ | 12/17∥    | 10/18     | 0/19      | 2/5   | 1/10|

* Preimmunizations and challenge injections were carried out as described in the legend to Table II with the exception that the cell dose for preimmunizations was increased to $5 \times 10^4$ cells/animal and the cells were sonicated for 60 sec before injection. Results are expressed as the fraction of mice bearing tumors at 5 wk.

† Preimmunizations were carried out as above, but with twice-banded, UV-inactivated Rauscher leukemia virus at a dose of approximately $10^6 - 10^9$ physical particles/injection.

§ Not done.

∥ Same data as in Table II.

### TABLE IV

**Tumorigenicity of MSV-Producer and Nonproducer Cells in Mice Preimmunized with Xenogeneic MSV-Nonproducer Cells***

| Tumor challenge | Pretreatment | Untreated | NRK | K-NRK |
|-----------------|--------------|-----------|-----|-------|
| Clone           | Cell dose    | 9/10      | 12/20 | 14/20 |
| K-234           | $1 \times 10^6$ | 9/10      | 12/20 | 14/20 |

* Mice were preimmunized weekly for 3 consecutive wk with $5 \times 10^4$ viable cells injected subcutaneously in the thigh region.

† Tumor challenges were performed as described in Table II. Results are expressed as the fraction of mice bearing tumors at 5 wk.
strate that the transplantation antigens detected in virus-producing MSV-transformed cells are to a large extent due to viral structural proteins at the cell surface.

**Cytotoxicity and Fluorescent Antibody Studies.**—It was possible that in vivo methods were not sufficiently sensitive to detect weakly antigenic cell surface changes in the MSV-nonproducer cells. For this reason, a series of in vitro cytotoxicity and fluorescent antibody studies were performed. Sera from BALB/c mice bearing tumors induced by the producer cell line, K-234(R), and from untreated mice were tested for cytotoxic activity against K-234, K-234(R), and BALB/3T3. As shown in Table V, there was a decrease of more than 50% in the colony-forming ability of K-234(R) cells exposed to serum from K-234-preimmunized mice. In contrast, there was only a small decrease in the colony-forming ability of either K-234 or BALB/3T3 cells. From these studies, it is concluded that K-234(R) tumor-bearing mice had circulating antibodies which were cytotoxic to K-234(R) cells but not more toxic to K-234 than to BALB/3T3 target cells.

The MSV-producer, nonproducer, and control BALB/3T3 cells were examined by a fluorescent antibody method which detects cell surface antigens. With sera obtained from BALB/c mice preimmunized with each cell line, specific staining of the cell surface was observed only when sera from mice preimmunized with K-234(R) cells were tested against the same cell line. As shown in Table VI, the surface antibody titer was 1:50 before and 1:30 after adsorption with BALB/3T3 cells. A small but definite reaction was seen with antisera to MSV-producer cells against both BALB/3T3 and K-234 cells, but this was eliminated by adsorption of the serum with control BALB/3T3 cells. The reactivity of sera from mice preimmunized with MSV-nonproducer cells was not specific for the MSV-transformed cells and was eliminated by adsorption with BALB/3T3.

Sera obtained from two other species immunized with MSV tumors were tested for reactivity against the mouse cell lines by the fluorescent antibody
technique. Sera from rats bearing a transplantable MSV-producing tumor, M-MSV(R), had a surface antibody titer of over 1:200 against K-234(R) cells (Table VI). In striking contrast, this serum was not reactive against either K-234 or BALB/3T3 cells. Sera from hamsters immunized with an MSV-nonproducer hamster tumor, HT-1 (20), showed no reactivity with any of the mouse cell lines. Thus, while in vitro cytotoxicity and fluorescence antibody tests readily detected surface antigens on virus-producing MSV-transformed BALB/3T3 cells, neither method allowed detection of MSV-induced surface antigens on the MSV-nonproducer cells.

**Virus Neutralization Studies.**—It was of interest to determine whether virus neutralizing antibodies could be detected in sera of mice immunized with either the MSV-producer or nonproducer cell lines. Each was tested against

| Species | Cells used for immunization | Cells used for absorption | Fluorescent antibody titer* | Target cells |
|---------|-----------------------------|---------------------------|-----------------------------|-------------|
|         |                             | BALB/3T3                  |                             |             |
| Mouse   | K-234                       | -                         | 2 2 2                       | KiMuLV      |
|         | K-234                       | BALB/3T3                  | Neg Neg Neg                 | KiMuLV      |
|         | K-234(R)                    | -                         | 2 3 50                      | K-234       |
|         | K-234(R)                    | BALB/3T3                  | Neg Neg 30                  | K-234       |
|         | BALB/3T3                    | -                         | 2 2 2                       | KiMuLV      |
| Rat     | M-MSV(R)                    | -                         | <2 <2 200                   | KiMuLV      |
| Hamster | HT-1                        | -                         | <2 <2 <2                   |             |

* The titer is the reciprocal of the highest serum dilution which gave a positive reaction.
† Sera were preabsorbed by incubation with an equal volume of packed cells for 30 min at 4°C.

The virus stock originally used to transform the MSV-nonproducer cells, KiMSV(KiMuLV), and against the virus produced by K-234(R) cells. As is shown in Table VII, the sera from K-234(R)-inoculated mice contained neutralizing antibodies to the R-MuLV pseudotype of KiMSV with a titer of 1:20. Neither this serum nor sera from mice immunized with the MSV-nonproducer cells inhibited focus formation by KiMSV(KiMuLV).

**Comparison of the Tumorigenicity of MSV- and SV40-Transformed Cells.**—Previous studies have demonstrated that the malignant potential of in vitro cultivated inbred mouse cell lines is related to their lack of contact inhibition of cell division. Thus, under experimental conditions where the host’s immune defenses were suppressed by total body irradiation, SV40 transformed-BALB/3T3 cells which grew to a very high saturation density in culture were very tumorigenic (17). The ability of this previously reported SV40-transformed BALB/3T3 subclone, SV-T2, to form tumors in normal, unirradiated BALB/c
mice was compared with the tumorigenicity of the MSV-transformed cells of
the present study. As shown in Table VIII, SV-T2 was at least 3 log units less
tumorigenic in unirradiated animals than the MSV-nonproducer cells, even
though the SV40-transformed cells achieved a higher saturation density in
culture. The tumorigenicity of K-234(R) was approximately 50-fold less
than that of K-234 but still far greater than that of SV-T2. These findings indi-
cate that the transplantation antigens of even the virus-producing MSV-

**TABLE VII**

Neutralization of Focus Formation by KiMSV(KiMuLV) and KiMSV(R) with Sera from
Immunized BALB/c Mice

| Virus            | Neutralizing titer* |
|------------------|---------------------|
|                  | BALB/3T3 | K-234 | K-234(R) |
| KiMSV(KiMuLV)    | <2        | <2    | <2       |
| KiMSV(R)         | <2        | <2    | 20       |

* Approximately 50 FFU of each virus were incubated with an equal volume of the appro-
priate serum for 30 min at 37°C and then assayed for focus formation on NRK cells. The
end-point titer was the highest serum dilution giving more than 70% inhibition of focus
formation.

**TABLE VIII**

Comparison of the Tumorigenicity of MSV Transformed and SV40-Transformed BALB/3T3
Cells*

| Challenge cells | Saturation density in culture | Cell number required for 50% tumor incidence |
|-----------------|-------------------------------|---------------------------------------------|
| K-234           | $3.5 \times 10^5$            | $1 \times 10^4$                            |
| K-234(R)        | $3.7 \times 10^5$            | $5 \times 10^3$                            |
| SV-T2           | $>10 \times 10^6$            | $>7 \times 10^5$                           |

* Tumor challenges were carried out as described in Table II.
† The maximum cell number attained when $5 \times 10^3$ cells/cm² were inoculated into 20-
cm² Petri dishes under conditions where medium containing 10% calf serum was changed
every 3 days.

transformed cells are far less immunogenic than those of cells transformed
nonproductively by SV40.

**DISCUSSION**

Mouse cells that are infected by MSV in the absence of added MuLV be-
come strikingly altered in their morphology in tissue culture and attain the
ability to form tumors in vivo. MSV-transformed clonal lines of this nature
have so far been found to lack any evidence of virus production or viral-
specified cellular antigens (13, 16). Superinfection of such MSV nonproducer
cells with MuLV results in rescue of the MSV genome. In the present study, we compared the antigenicity of virus-producing and MSV nonproducer transformed cells in order to determine whether virus-coded, nonvirion transplantation antigens could be detected. The system was very well suited for these studies because: all cell lines were derived from a clonal line of nontumorigenic, syngeneic mouse embryo cells; the MSV- and MuLV-producing transformed line was obtained by MuLV infection of the nonproducer clonal line so that otherwise they were identical; and neither the producer nor the nonproducer cell lines had been preselected by in vivo passage.

In the present studies, MSV-transformed virus-producing cells had clearly demonstrable virus-specific transplantation antigens. The number of deaths due to progressive tumor growth when mice were injected with K-234(R) cells was greatly increased when mice were irradiated before tumor challenge. Furthermore, preimmunization with K-234(R) cells considerably reduced the fraction of mice developing tumors after challenge with the homologous cell line. In in vitro studies, surface antigens were detected on K-234(R) cells by fluorescent antibody and cytotoxicity tests. Finally, preimmunization of syngeneic mice with UV-inactivated, purified R-MuLV effectively protected them from subsequent challenge with K-234(R). It is concluded from these findings that the immunogenicity of virus-producing MSV-transformed cells is to a large extent attributable to surface antigens associated with virus production.

In striking contrast, the MSV-transformed nonproducer cells were found by each of the above procedures to be no more immunogenic than control BALB/3T3 cells. One possible explanation for our results is that MSV does not induce cell surface alterations. However, it has been demonstrated that MSV-transformed cells have a markedly altered glucose transport (22), and recent studies indicate a small but significant increase in agglutinability of MSV-transformed compared to normal cells in response to plant lectins such as concanavalin A (B. Ozanne, personal communication). These findings provide biochemical evidence that surface alterations may be present in MSV-transformed cells.

If MSV does in fact induce cell surface changes, these clearly were not detectable by the immunologic techniques used in the present report. To explain these results, host factors such as blocking antibodies which have been described for RNA tumor virus-producing cells (23) or antigenic modulation (24) could be invoked. However, the fact that sera from mice preimmunized with virus-infected, transformed cells appeared to lack both cytotoxic and FA surface staining antibodies against in vitro-cultured nonproducer cells argues against both of these possibilities. Further considerations are that cell surface alterations induced by MSV are not immunogenic because they represent either: (a) modified production of normal cell components; (b) virus-coded antigens which are poorly, if at all, immunogenic because of either their struc-
ture or location; (c) derepression of embryonic antigens; or (d) virus-coded antigens to which the animal is tolerant. The last possibility is unlikely in view of inability of sera from MSV-immunized animals of two other species, the rat and hamster, to detect antigens in MSV-nonproducer mouse cells. A further resolution of these possibilities should increase our understanding of the mechanism of transformation by MSV.

A previous study (25), in which MSV-transformed nonproducer hamster tumor cells were found to be ineffective in protecting mice from subsequent challenge with virus-producing sarcoma cells, supports the present findings that MSV nonproducer cells lack detectable surface antigens. In contrast, there have been two reports of virus-specific “transplantation antigens” in MSV-transformed, supposedly virus-free cell lines. In one study, Ting (9) demonstrated transplantation resistance in inbred rats to a “nonproducer” tumor cell line designated MSB-1. However, this cell line was later shown to release both a focus-forming virus, MSV(0) (26), and a rat-tropic helper leukemia virus (27). In light of the present studies, it is likely that the transplantation antigens associated with these cells were in large part due to replicating virus. In a more recent report, transplantation resistance against a mouse tumor line, XM-1, was reported in mice immunized either with XM-1 cells or with MSV(MuLV) (11). However, these cells, too, were later found to produce C-type virus particles (Ting, personal communication).

The morphologic and growth properties of MSV-producing and nonproducer transformed mouse cells are indistinguishable in tissue culture (13, 16). Yet, the present report shows that the tumor-forming ability of nonproducer cells is far greater in an immunologically competent host. The results demonstrate that MSV nonproducer cells lack detectable virus-specified surface antigens and that the immunogenicity of virus-producing MSV-transformed cells is due to the presence of virion antigens at the cell surface. If RNA tumor viruses have an etiologic role in spontaneous tumors, it is apparent from the present studies that the selective growth advantage of an in vivo transformed nonproducer cell over its virus-producing counterpart might well account for the difficulty in detecting virus production in naturally occurring neoplasms.

SUMMARY

The isolation of clonal lines of murine sarcoma virus-transformed, nonproducer BALB/3T3 cells has provided a model system for determining whether RNA tumor virus-transformed cells possess virus-specific transplantation antigens. MSV nonproducer cells (K-234) were clonally derived from an inbred mouse cell line, BALB/3T3. A parallel virus-producing cell line was obtained by infection of the MSV nonproducer cells with Rauscher leukemia virus. K-234 was much more tumorigenic than K-234(R). Preimmunization of syngeneic mice with either K-234(R) or with UV-inactivated Rauscher leukemia virus induced transplantation resistance to subsequent challenge with
K-234(R), but not with K-234. In contrast, mice preimmunized with nonproducer cells were not made resistant to subsequent challenge with the homologous cells. Antisera prepared from mice immunized with K-234(R) were specifically cytotoxic and positive by fluorescent antibody staining for K-234(R) target cells, but not to either BALB/3T3 or K-234. The results show that MSV nonproducer cells lack detectable transplantation antigens and suggest that the transplantation resistance to the producing cells is attributable to maturing virus at the cell surface.

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REFERENCES

1. Sjögren, H. O., I. Hellström, and G. Klein. 1961. Transplantation of polyoma virus-induced tumors in mice. Cancer Res. 21:329.
2. Habel, K., and B. E. Eddy. 1963. Specificity of resistance to tumor challenge of polyoma and SV40 virus immune-hamsters. Proc. Soc. Exp. Biol. Med. 113:1.
3. 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.
4. Trentin, J. J., and E. Bryan. 1966. Virus-induced transplantation immunity to human adenovirus type 12 tumors of hamster and mouse. Proc. Soc. Exp. Biol. Med. 121:116.
5. Hellström, I., and H. O. Sjögren. 1965. Demonstration of H-2 isoantigens and polyoma specific tumor antigens by measuring colony formation in vitro. Exp. Cell Res. 40:212.
6. Klein, G., E. Klein, and G. Haughton. 1966. Variation of antigenic characteristics between different mouse lymphomas induced by the Moloney virus. J. Nat. Cancer Inst. 36:607.
7. Fefer, A., J. L. McCoy, and J. P. Glynn. 1967. Antigenicity of a virus-induced murine sarcoma (Moloney). Cancer Res. 27:962.
8. Fefer, A., J. L. McCoy, and J. P. Glynn. 1967. Induction and regression of primary Moloney sarcoma virus-induced tumors in mice. Cancer Res. 27:1626.
9. Ting, R. C. 1967. Tumor induction in thymectomized rats by murine sarcoma virus (Moloney) and properties of the induced virus-free tumor cells. Proc. Soc. Exp. Biol. Med. 120:778.
10. Steeves, R. A. 1968. Cellular antigen of Friend virus-induced leukemias. Cancer Res. 28:339.
11. Law, L. W., and R. C. Ting. 1970. Antigenic properties of a nonreleaser neoplasm induced in the mouse by murine sarcoma virus. J. Nat. Cancer Inst. 44:615.
12. Shirai, T., H. Kaji, N. Takeichi, F. Sendo, H. Saito, M. Hosokawa, and H. Kobayashi. 1971. Cell surface antigens detectable by cytotoxic test on Friend virus-induced and Friend virus-infected tumors in the rat. J. Nat. Cancer Inst. 46:139.
13. Aaronson, S. A., and W. P. Rowe. 1970. Nonproducer clones of murine sarcoma virus transformed BALB/3T3 cells. Virology. 42:29.
14. Aaronson, S. A., J. L. Jainchill, and G. J. Todaro. 1970. Murine sarcoma virus transformation of BALB/3T3 cells: lack of dependence on murine leukemia virus. Proc. Nat. Acad. Sci. U.S.A. 66:1236.
15. Aaronson, S. A., and G. J. Todaro. 1968. Development of 3T3-like lines from BALB/c mouse embryo cultures: transformation susceptibility to SV40. J. Cell. Physiol. 72:141.
16. Aaronson, S. A., and C. A. Weaver. 1971. Characterization of murine sarcoma virus (Kirsten) transformation of mouse and human cells. J. Gen. Virol. 13:245.
17. Aaronson, S. A., and G. J. Todaro. 1968. Basis for the acquisition of malignant potential by mouse cells cultivated in vitro. Science (Washington). 68:1024.
18. Duc-Nguyen, H., E. N. Rosenblum, and R. F. Zeigel. 1966. Persistent infection of a rat kidney cell line with Rauscher murine leukemia virus. J. Bacteriol. 92:1133.
19. Aaronson, S. A. 1970. Effect of ultraviolet irradiation on the survival of simian virus 40 functions in human and mouse cells. J. Virol. 6:393.
20. Huebner, R. J., J. W. Hartley, W. P. Rowe, W. T. Lane, and W. I. Capps. 1966. Rescue of the defective genome of Moloney sarcoma virus from a noninfectious hamster tumor and the production of pseudotype sarcoma viruses with various murine leukemia viruses. Proc. Nat. Acad. Sci. U.S.A. 56:1164.
21. Pfeiffer, S. E., H. R. Herschman, J. E. Lightbody, G. Sato, and L. Levine. 1971. Modification of cell surface: antigenicity as a function of culture conditions. J. Cell. Physiol. 78:145.
22. Hatanaka, M., R. J. Huebner, and R. V. Gilden. 1969. Alterations in the characteristic of sugar uptake by mouse cells transformed by murine sarcoma viruses. J. Nat. Cancer Inst. 43:1091.
23. Hellström, I., and K. E. Hellström. 1969. Studies on cellular immunity and its serum-mediated inhibition in Moloney-virus-induced mouse sarcomas. Int. J. Cancer. 4:587.
24. Boyse, E. A., L. J. Old, E. Stockert, and N. Shigeno. 1968. Genetic origin of tumor antigens. Cancer Res. 28:1280.
25. Chuat, J., L. Berman, P. Gunvén, and E. Klein. 1969. Studies on murine sarcoma virus: antigenic characterization of murine sarcoma virus induced tumor cells. Int. J. Cancer. 4:465.
26. Ting, R. C. 1968. Biological and serological properties of viral particles from a non-producer rat neoplasm induced by murine sarcoma virus (Moloney). J. Virol. 2:865.
27. Aaronson, S. A. 1971. Isolation of a rat-tropic helper virus from M-MSV-O stocks. Virology. 44:29.