Surface Antigens of Mammalian Sarcoma Virus-Transformed Nonproducer Cells

(murine sarcoma virus/woolly monkey sarcoma virus/nonproducer cells/
immunoelectron microscopy)

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Communicated by Paul A. Marks, June 19, 1974

ABSTRACT *In vitro*, clonal BALB/3T3 mouse cells were transformed into nonproductive sarcoma cells by Kirsten murine sarcoma virus. The resulting cells are called K-BALB. Similarly, the Kirsten sarcoma virus and woolly monkey sarcoma virus transformed normal rat embryonic kidney (NRK) cells to nonproductive sarcoma cells K-NRK and W-NRK, respectively. The presence of sarcoma cell surface antigen (SCSA) on these three different cell lines has been examined by immunoelectron microscopy using the rabbit anti-K-BALB, anti-K-NRK, and anti-W-NRK sera preabsorbed with normal embryonic rat cells, NRK cells, and BALB/3T3 cells. Four different specific sarcoma cell surface antigens have been demonstrated: SCSAa, an antigen specific to the surface of W-NRK cells; SCSAb, a surface antigen common to K-NRK and W-NRK cells; SCSAc, a surface antigen common to K-BALB and W-NRK cells; and SCSAd, a surface antigen broadly reactive with the rabbit antisera to W-NRK, K-NRK, and K-BALB.

Cells nonproductively transformed by RNA-containing sarcoma viruses provide a model for studying the mechanisms involved in oncogenesis. Murine sarcoma virus (MSV)-transformed nonproducer cells release no detectable type C helper virus or virion antigens. In contrast to the high degree of immunogenicity of other known virus-induced solid tumors, the MSV-transformed nonproducer cell has no detectable transplantation antigens (1, 2). Recently, sensitive immunoelectron microscopy procedures provided the first evidence of an MSV-associated antigen on surfaces of these cells. Antisera from the mice hyperimmunized with syngeneic nonproducer cells transformed by the Kirsten (Ki) strain of MSV were shown to be weakly reactive against the cell surfaces of mouse or rat nonproducer cells transformed by either Ki or Moloney strains of MSV (3).

The isolation of sarcoma virus from a solid tumor of a woolly monkey (4, 5) has led to investigation of its biologic properties. Like MSV, the woolly monkey sarcoma virus (WSV) transforms cells in tissue culture without the production of infectious virus (6). In the present studies, the sarcoma virus-associated surface antigens of murine nonproducer cells transformed by MSV and WSV have been compared by im-

munolectron microscopy using antisera produced in rabbits against each sarcoma virus-transformed nonproducer cell type. The results demonstrate the presence of unique and common antigens on surfaces of WSV- and MSV-transformed nonproducer cells and the ability of rabbit antisera to discriminate additional sarcoma virus-associated cell surface antigens undetectable by mouse antisera.

MATERIALS AND METHODS

Cells and Viruses. Cells were grown in Dulbecco's modification of Eagle's medium supplemented with 10% calf serum (Colorado Serum Co., Denver, Colo.) in 100 × 20-mm petri dishes (Falcon Plastics, Div. B-D Laboratories, Inc., Los Angeles, Calif.). The continuous cell lines and the mouse and rat embryo cells used are described in Table 1. Detailed information on sarcoma viruses used to make nonproducer malignant cell lines is given in the references cited in Table 1.

Antisera. Antisera were prepared in 6- to 8-week-old New Zealand female rabbits, obtained from the Animal Production Branch, Division of Research Services, the National Institutes

TABLE 1. Summary of cells used for immunization and absorption and as target cells for immunoelectron microscopy

Designation of cells	Description	Refs.
BALB/3T3	A clonal line of BALB/c embryo cells.	7
K-BALB	A Kirsten (Ki) murine sarcoma virus (MSV)-transformed nonproducer subclone of BALB/3T3.	1, 8
NRK	A clonal line of normal O-M rat kidney cells.	9
K-NRK	A Ki-MSV-transformed nonproducer subclone of NRK.	8
W-NRK	A woolly monkey sarcoma virus (WSV)-transformed nonproducer subclone of NRK.	6
Embryo cells		
BALB/c mouse embryo	15- to 18-day-old embryo cells of BALB/c mice.	
Fischer rat embryo	15- to 18-day-old embryo cells of Fischer rats.	

Abbreviations: MSV, murine sarcoma virus; Ki, Kirsten; WSV, woolly monkey sarcoma virus; K-BALB, a Ki-MSV-transformed nonproducer subclone of BALB/3T3; K-NRK, a Ki-MSV-transformed nonproducer subclone of normal rat kidney cells (NRK); W-NRK, a WSV-transformed nonproducer subclone of NRK; CSA, cell surface antigen; SCSA, sarcoma cell surface antigen; G, Gross.

TABLE 2. Specificities of cell surface antigens of K-BALB, K-NRK, and W-NRK cells recognized by antisera prepared in rabbits

Target cell	Cells used for absorption*	Test sera: rabbit anti-		
		K-BALB	K-NRK	W-NRK
W-NRK	NRK	+	+	++
	K-BALB + NRK	-	+	+
	K-NRK + NRK	+	-	+
	K-BALB + K-NRK	-	-	+
	W-NRK + NRK	-	-	-
K-NRK	BALB/3T3 + NRK	+ to ++	+ to ++	ND
	K-BALB + NRK	-	+	ND
	K-NRK + BALB/3T3	-	-	ND
	K-BALB + K-NRK	-	-	ND
	W-NRK + BALB/3T3	-	-	ND
NRK	BALB/3T3 + NRK	-	-	-
K-BALB	BALB/3T3 + NRK	+	+ to ++	ND
	K-BALB + NRK	-	-	ND
	K-NRK + BALB/3T3	+	-	ND
	K-BALB + K-NRK	-	-	ND
	W-NRK + BALB/3T3	-	-	ND
BALB/3T3	BALB/3T3 + NRK	-	-	-

* Sera were preabsorbed three times with equal packed cell volumes of both Fischer rat and BALB/c mouse embryo cells and then three times further with each of the indicated cells. Absorptions were performed as described in *Methods*.

-, no labeling; +, three or four small labeled sectors around the perimeter of the cell; ++, five to seven small labeled sectors; ND, not done.

of Health, Bethesda, Md. Tissue culture cells were harvested by scraping, washed twice, and resuspended in phosphate buffered saline at 10^9 cells per ml. Groups of three rabbits were immunized seven to nine times at weekly or biweekly intervals by multiple subcutaneous inoculations of 1.0 ml of cell suspension per rabbit. Antisera were obtained 10–14 days after the last immunization. The undiluted antisera against rat or mouse transformed cells were preabsorbed three times with equal volumes of freshly packed Fischer rat and BALB/c mouse embryo cells and then another three times with either BALB/3T3, NRK, a mixture of both, or test cell lines to remove xenogeneic antibodies to calf serum and the normal components of cells used for immunization, and possible fetal antibody, or to remove specific antibodies to sarcoma virus-transformed cell surface antigens. For each absorption, the mixture of antiserum and cells were incubated at 0° for 60 min with periodic agitation, and then centrifuged at $300 \times g$ for 15 min. Absorptions were similarly performed in testing the specificities of positive reactions.

Immunoelectron Microscopy. The sheep IgG anti-rabbit IgG was conjugated to six times crystallized horse ferritin with *meta*-xylylene diisocyanate according to Hsu's modification of Singer's method (10). Viable cells were gently scraped from monolayers with a rubber policeman, washed twice in serum-free medium 199 by centrifugation at 0° and $300 \times g$, and distributed to small centrifuge tubes at approximately 3×10^6 cells per tube. Cells were incubated with 0.05 ml of preabsorbed, undiluted antiserum, washed twice with serum-free medium 199, and then incubated with 0.05 ml of ferritin reagent. All incubations were performed at 0° for 30 min with periodic agitation to avoid as much as possible pinocytosis, phagocytosis, and synthesis of new cell membranes. Washed cell pellets were treated for electron microscopy as described previously (11, 12). Thin sections of at least 50 cells from each sample were observed to determine whether antigens were present on or absent from cell surfaces. To eliminate

observing the same cell in different sections of one embedded sample, thin sections were obtained from six blocks of each sample.

RESULTS

The results of the IEM studies, summarized in Table 2, indicate the presence of several different cell surface antigens (CSAs) specific for sarcoma virus-transformed cells.

Common Cell Surface Antigen(s) of K-BALB, K-NRK, and W-NRK Cells. Sera from rabbits hyperimmunized with each of the nonproducer cell lines, K-BALB, K-NRK, and W-NRK, were preabsorbed with Fischer rat and BALB/c mouse embryo cells and analyzed by immunoelectron microscopy for reactivity with K-BALB and K-NRK target cells. Furthermore, the anti-W-NRK serum similarly preabsorbed was tested for reactivity with W-NRK cells. These preabsorbed antisera showed positive reactivity with respective test cells but not with nontransformed BALB/3T3 and NRK cells. Labeling was restricted to small areas on the cell surface (Fig. 1b, c, and d). In addition to reacting with the homologous nonproducer cells, each serum also reacted with each of the other sarcoma virus-transformed cells tested, as shown in Table 2. The activity of all antisera to all target transformed nonproducer cell lines was completely absorbed by W-NRK (Fig. 1a). These results provide evidence for the presence of a broadly reactive CSA shared by each of the three sarcoma virus-transformed nonproducer cell lines examined.

Presence of a Specific WSV-Associated CSA on W-NRK Cells. The existence of additional CSAs was indicated by the results of cross-absorption studies with K-NRK and K-BALB cells. While the anti-W-NRK serum reactivity with W-NRK cells was completely absorbed by W-NRK cells (Fig. 1a), the reactivity was only partially absorbed by either K-BALB or K-NRK cells or by a mixture of both (Fig. 1b). These results indicate that W-NRK cells possess a specific CSA not shared with K-NRK and K-BALB cells.

FIG. 1. (a) W-NRK cells were incubated with rabbit anti-W-NRK serum preabsorbed by W-NRK cells, and then with ferritin reagent. No labeling was observed on the surface of W-NRK cells. The same negative reaction was found on the surface of K-NRK and K-BALB cells. (b) W-NRK cells were incubated with rabbit anti-W-NRK cells preabsorbed using K-BALB and K-NRK cells, and then with ferritin reagent. Several labeled areas forming a small sector were observed on the surface of target cells as shown in this figure. A similar reaction was also found with the same antiserum preabsorbed by either K-BALB or K-NRK cells. (c) K-BALB cells were incubated with rabbit anti-K-BALB cells preabsorbed by K-NRK cells, and then with ferritin reagent. The same reaction observed in (b) was found. A similar reaction was also demonstrated using the same preabsorbed antiserum with W-NRK cells. (d) W-NRK cells were incubated with rabbit anti-K-NRK cells preabsorbed by K-BALB cells, and then with ferritin reagent. The same reaction observed in (b) and (c) was found. A similar reaction was also demonstrated between this preabsorbed serum and K-NRK cells. All immunologic procedures performed at 0° resulted in the strong aggregation of ferritin as observed in (b), (c), and (d). $\times 50,000$.

Evidence for Two Additional CSAs of Sarcoma Virus-Transformed Nonproducer Cells. The results of absorption of the anti-K-BALB and anti-K-NRK sera by K-BALB and K-NRK cells were studied next. K-BALB but not K-NRK cells completely absorbed the reactivity of the anti-K-BALB serum against W-NRK cells (Fig. 1c). In contrast, the reactivity of the anti-K-NRK serum with W-NRK cells was fully absorbed by K-NRK (Fig. 1d) but not by K-BALB cells. These results indicate the existence of a CSA common to K-NRK and W-NRK cells and a second CSA shared by W-NRK and K-BALB cells. Further evidence that the rabbit antisera used in this study were able to detect a difference between the sarcoma virus-associated CSAs of K-BALB and K-NRK cells was derived from the findings that the reactivity of the anti-K-BALB serum with K-BALB cells was absorbed by K-BALB but not by K-NRK cells (Fig. 1c). Conversely, the reactivity of the anti-K-NRK serum with K-NRK cells was absorbed by K-NRK but not K-BALB cells (Fig. 1d).

Classification of Cell Surface Antigens of Sarcoma Virus-Transformed Cells. The four CSA specificities of sarcoma virus-transformed cell lines demonstrated in the present

study are summarized in Table 3. These are tentatively designated as sarcoma cell surface antigens (SCSAs). The antigen unique to W-NRK cells is designated SCSAa. The antigen specificities common between K-NRK and W-NRK cells and between K-BALB and W-NRK cells are termed SCSAb and SCSAc, respectively. The fourth specificity shared by each of the three sarcoma virus-transformed nonproducer cell lines examined is designated SCSAd.

DISCUSSION

Infection of cells in tissue culture with MSV remarkably alters their biologic properties. The cells become hyperrefractive and spindle-shaped, and exhibit loss of normal growth control *in vitro* and *in vivo* (13, 14). Infection with WSV also results in loss of growth regulation. The finding that the morphology of WSV-transformed nonproducer cells is different from that of nonproducer cells transformed by MSV (6) has suggested that there are differences in the genetic information of these two mammalian sarcoma viruses. In biochemical studies of mammalian sarcoma viruses, a major portion of the Ki-MSV genome has been shown to be genetically unrelated to its helper leukemia virus, Ki-MuLV (15). Subsequent studies have indicated that the nonhelper virus

TABLE 3. Summary of antigenic specificities detected on Ki-MSV- and WSV-transformed cells

Antigenic specificity	Detected on cell surface of:			Interpretation
	K-BALB	K-NRK	W-NRK	
SCSAa	—	—	+	WSV-induced specific CSA in W-NRK.
SCSAb	—	+	+	A common Ki-MSV- and WSV-induced specific CSA in K- and W-NRK.
SCSAc	+	—	+	A common Ki-MSV- and WSV-induced specific CSA in K-BALB and W-NRK.
SCSAd	+	+	+	A common specific CSA induced by Ki-MSV and WSV in K-BALB, K-NRK, and W-NRK.

SCSA, sarcoma cell surface antigen.

portion of Ki-MSV consists of genetic information of the rat, the species from which Ki-MSV was initially isolated (16). A similar pattern is now emerging with other mammalian sarcoma viruses. Both MSV and WSV appear to contain only a portion of the genetic information of their respective helper type C viruses (16, 17). If the origin of these viruses is analogous to that of Ki-MSV, their genomes may also contain non-type C genetic information of the species from which each was isolated. The recent finding that the woolly helper leukemia virus may not be indigenous to the woolly monkey (17) does not exclude the possibility that a portion of the WSV genome is homologous to genetic information of woolly monkey cells.

The present studies have utilized antisera to either MSV- or WSV-transformed nonproducer clones derived from the same parent clone of untransformed cells to compare antigenic changes in the cell surfaces associated with transformation by either virus. By immunoelectron microscopy, it has been possible to detect four distinguishable antigenic specificities on murine cells containing these two sarcoma viruses. These new antigens could result directly from translational products of virus-coded information. Alternatively, they may be due to indirect effects of the virus on cellular functions, either causing transcription and/or translation of unexpressed cellular information or altering the information of the cell surface by exposing preexisting antigenic specificities. Or it is possible that rat and mouse cellular genomes may express respectively different phenotypic cell surface antigenic changes after infection with Ki-MSV or WSV. In this case, the viral genome itself might indirectly affect the expression of CSAs through the cellular genome.

The finding of an antigen (SCSAa) specific for MSV-transformed cells is consistent with the known biologic and biochemical differences between the WSV and Ki-MSV. An antigen shared by WSV- and Ki-MSV-transformed rat cells but not Ki-MSV-transformed mouse cells (SCSAb) is likely

to be due to a change of cell surface construction induced by each virus. Similarly, an antigen shared by Ki-MSV-transformed mouse and WSV-transformed rat cells (SCSAc) also may be induced through indirect interaction of the viruses with the two host cells. Consequently, SCSAc existing on K-BALB and W-NRK cells may also be induced by the co-operation of WSV genome with a genetic function common to BALB/3T3 and NRK cells. The lack of cross-reactivity of Ki-MSV-transformed rat cells with SCSAc would argue that the effect of Ki-MSV on the rat cell must not produce this antigen in sufficient quantity to permit its detection.

There is precedent for the interaction between viral and cellular genes in the expression of cell surface antigens. For example, in the Gross (G) murine leukemia virus-associated surface antigen system, immunogenetic analyses demonstrate Mendelian inheritance of G_{IX}, indicating that the phenotypic expression of G_{IX} may be controlled by the effect of G-murine leukemia virus-coded functions on cellular genes (18, 19). Thus, the strength of G_{IX} expression in different mouse strains is variable and genetically determined. While G_{IX} is expressed on normal thymocytes of several G_{IX}⁺ mouse strains as well as on the cell surface of some leukemias from G_{IX}⁺ mouse strains, in the present SCSA system, sarcoma virus-related antigens were only detected in transformed cells. This suggests that in contrast to the G_{IX}, the sarcoma viral genome plays a more dominant role in the induction of SCSAs.

In our previous report, mouse antisera were shown to recognize a single antigen common to both MSV-transformed mouse and rat cells. The antisera produced in rabbits in the present studies contain more specific antibodies and are quantitatively stronger in their reactivities. The patterns of surface reactivity observed were similar, with small sectors of the cell surface showing reactivity in both cases. Differences in the reactivities of antisera prepared in different species have also been reported with cell surface antigens in other systems (20). C57BL/6 mice recognize only G cell surface antigens GCSAa and c (21, 22), while rats produce specific antibodies against GCSAa, b, c, and G_{IX} on the cell surface and against subgroup-specific envelope antigen(s) (sub-gsVEA) of type C viruses (18, 20, 22, 23). The question, as to whether one or more of the SCSAs recognized by the antisera prepared in rabbits is identical to the single antigen previously detected using antisera from hyperimmunized mice, requires further study.

The detection of a surface antigen (SCSAd) cross-reactive among nonproducer cells transformed by sarcoma viruses of two mammalian species suggests the possibility that a viral gene common to MSV and WSV may induce this broadly reactive antigen. Alternatively, the antigen might be induced by the malignant transformation process itself. In any case, this reactivity may be common to other classes of tumor cells as well. It should be possible to examine spontaneous and chemically-induced mammalian tumors, under conditions where the test antisera can be appropriately absorbed, to test for the presence of sarcoma virus-associated antigen(s). Their detection in a specific class of tumors might provide evidence for a common underlying mechanism.

We thank Misses Macie M. Sturm, Margaret Liu, and Roberta K. Reynolds for their excellent technical assistance. This work was supported in part by Contract no. N01-CP-4-3207, N01-CP-3-3212, and N01-CP-4-3429 within the Virus Cancer Program of the National Cancer Institute, and NIH Grant AM-13200.

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