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Rabbits as a Source of Antisera Against SV40 Virus-Induced Cell Surface Antigens (39144)

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Current immunotherapy studies in our laboratory have led us to seek an abundant source of antibody directed against surface antigens on SV40 virus-transformed tumor cells. Most previous studies of such antibodies have used hamster or mouse sera, which are impractical when large volumes are required. The present study indicates that rabbits can also yield potent antisera against antigens on the surface of SV40-transformed cells, including SV40-specific antigens.

Materials and methods. Cells were grown in Dulbecco's modified Eagle's Medium with 10% fetal calf serum. SV40-transformed cells included the TT101 hamster sarcoma line (Flow Laboratories), SV3T3 Swiss mouse fibroblasts (from Dr. H. Green), and TRK73 rabbit kidney cells (from Dr. P. Black, 1). Untransformed 3T3 cells, two untransformed hamster fibroblast lines (Nil-2 and BHK21/13), a hamster sarcoma line transformed by Herpesvirus type 2 (333-8-9, from Dr. F. Rapp), and a dimethylnitrosamine-transformed line of BHK cells (DMN4B, from Dr. G. DiMayorca) were used as controls.

TRK73 cells were scraped from glass bottles into phosphate-buffered saline (PBS), washed three times in PBS, suspended to 8×10^7 cells/ml in Hanks Salt Solution (HSS), and stirred for 1 hr at 36° with Vibrio Cholera neuraminidase (General Biochemicals) at 25 units/ml. After two washes in HSS, 8×10^7 cells in HSS were injected intravenously (iv) into New Zealand White rabbits. All rabbits were reimmunized intraperitoneally (ip) 3 weeks later, and then received three further injections ip at 2-week intervals of cells not treated with neuraminidase. Sera were harvested 10 days after the last booster, heat inactivated, and stored frozen.

Antisera were assayed by a mixed hemadsorption (MHA) technique (2–4). As a reagent for this test, rabbit antiserum against sheep red blood cells (SRBC) was obtained by immunizing rabbits iv with eight injections, at 2-day intervals, of boiled SRBC stroma from 10 ml of sheep blood, giving a ninth injection 3 weeks later, and bleeding the rabbits 5 days after the last injection. After heat inactivation, the serum had a hemagglutination titer for SRBC of $^1/_{160}$. Goat antiserum against rabbit γ-globulins (Cappel Laboratories) had a hemagglutination titer of $^1/_{320}$ against SRBC coated with rabbit antiserum. Indicator cells were prepared by washing SRBC twice in PBS, and then mixing equal volumes of 2% SRBC and a $^1/_{20}$ dilution of rabbit anti-SRBC serum for 1 hr. After three PBS washes, the coated SRBC were further coated in a similar manner with undiluted goat anti-rabbit γ-globulin, washed three times with PBS, and resuspended in PBS to 0.4% concentration.

The MHA test was performed on duplicate cultures of cell monolayers in glass tubes. The cells were washed twice with HSS. One milliliter of test serum diluted in HSS was then added. After incubation for 1 hr at room temperature, the cultures were washed three times with HSS, and 1 ml of coated SRBC was added. After a further hour of incubation, the tubes were placed vertically to permit nonadherent SRBC to run off the monolayers, and examined under the microscope for adsorbed SRBC.

In absorption experiments, cells pelleted by centrifugation were mixed with an equal volume of rabbit antiserum diluted $^1/_8$ in HSS, and the mixture was stirred gently in an ice bath for 1 hr. The cells were then

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removed by centrifugation, and the antibody activity remaining in the supernatant was assayed in cultures of TT101 cells by the MHA technique.

Results. Antisera from nine rabbits were first screened in cultures of TT101 cells at a single dilution of $^1/_{160}$, chosen because normal rabbit sera were always unreactive at this dilution, although occasionally positive at lower dilutions. Six of the nine antisera were positive. Three of these that appeared to give particularly strong reactions were then selected for further testing. Table I shows that each of these sera was highly reactive against all three SV40-transformed cell lines. The specificity of this result was demonstrated for two of these antisera (TRK1-3 and K62) by their failure to react against any of the five lines of control cells. The third antiserum (K3) appeared to lack this specificity, since it reacted against three of the control lines. Two normal rabbit sera were unreactive against all cell lines tested.

The reactivity of serum K62 (MHA titer = 640) was removed almost completely by absorption with the SV40-transformed lines SV3T3 and TT101, leaving residual titers of 80 and <80, respectively. Absorption with the control lines BHK and 3T3 reduced titers only to 320, a result consistent with nonspecific adsorption of γ-globulin. An intermediate reduction in titer to 160 was observed after absorption with the SV40-transformed line TKR73 and the control line 333-8-9, suggesting that each of these lines is partially cross-reactive with TT101 cells. Absorption with SRBC failed to reduce antibody titers, excluding Forssman antigen (5) as a major factor in the reactivity of the rabbit serum against the hamster cells.

Discussion. Rabbits immunized with SV40-transformed rabbit cells responded with antibodies which reacted against SV40-transformed hamster, mouse, and rabbit cells. Two sera (TRK1-3 and K62) failed to react against control cell lines, although further studies with K62 showed partial loss of antibody activity after absorption with one of the control lines. A third antiserum reacted directly against three control lines. These results are probably best explained by the presence of both SV40-specific antibodies and antibodies directed against antigens shared by cells not transformed by SV40. This complex antibody response resembled data obtained with other species (6). The addition of rabbits to the list of species which appear to share SV40-specific surface antigens detectable by serologic tests lends weight to the presumption that such antigens are virus-coded. The results also add to the evidence that surface antigens not specific for SV40 (presumably coded for by the host cell) can also appear in some cell lines as a result of SV40 transformation (1, 7, 8).

The MHA titers of our rabbit sera are comparable to those obtained with sera from SV40-immune hamsters (4; and Mool-

TABLE I. MHA TITERS[a] OF RABBIT SERA TESTED IN SV40-TRANSFORMED AND CONTROL CELL LINES.

		Antisera from rabbits immunized with SV40-transformed rabbit cells			Normal rabbit sera	
	Cell line	TRK1-3	K62	K3	NRS1[b]	NRS2
SV40-transformed	TT101	320–640	640	640	<80	<80
	SV3T3	320–640	320–640	320–640	<80	<80
	TRK73	1280	640	640	<80	<80
Untransformed	3T3	<80	<80	<80	<80	<80
	Nil-2	<80	<80	>160[c]	<80	<80
	BHK	<80	<80	<80	<80	<80
Chemically transformed	DMN4B	<80	<80	>160[c]	<80	<80
Herpesvirus transformed	333-8-9	<80	<80	>160[c]	<80	<80

[a] Titer = reciprocal of highest dilution positive for adsorption of SRBC. Duplicate tests always agreed within one twofold dilution.

[b] Preimmunization serum from rabbit which supplied serum TRK1-3 after immunization.

[c] The highest dilution tested was 1/160.

ten, unpublished data), but rabbit sera are conveniently obtainable in much larger quantity. Rabbits therefore constitute a potentially important resource for investigations that require large amounts of antibody. These may include immunotherapy studies, and use of antibody in immunoadsorbents for the isolation and purification of SV40-specific surface antigens.

Summary. Rabbits immunized with SV40 virus-transformed rabbit cells yielded antisera highly reactive in a mixed-hemadsorption assay against SV40-transformed hamster, mouse, and rabbit cells. Such antisera may prove of value in studies requiring large amounts of antibody against SV40-induced cell surface antigens.

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