IMMUNOLOGICAL STUDY OF TUMORS INDUCED BY HIGHLY ONCOGENIC FORMS OF ROUS VIRUS IN ADULT MICE

I. N. Kryukova, I. B. Obukh, and O. V. Babkova

UDC 616-006-092.9-097.3

Tumors induced in adult mice by strains of Rous virus highly oncogenic for mammals were compared by three immunological tests in relation to immunosensitivity and immunogenicity with tumors induced by the Schmidt-Ruppin strain in newborn mice and with a mouse tissue culture transformed in vitro and completely nononcogenic for syngeneic recipients. No difference in principle was found between the three groups of cells, suggesting that the increase in oncogenicity of the Rous virus was not due to a decrease in the synthesis of cell membrane antigen.

Variants of the Carr-Zil'ber (C-Z) strain of Rous virus (RV) with high oncogenicity for adult mice, the most resistant mammals to RV, were isolated by the writers previously [2, 5].

The present investigation was undertaken to determine whether tumors induced in adult mice by these variants contain a specific cell membrane antigen (CMA) and whether such tumors differ in their immunosensitivity and immunogenicity from tumors induced in newborn mice by one of the known strains of RV.

EXPERIMENTAL METHOD

Mouse Tumors. Tumors Af_{13}, $^{2}IaAf^{5}$, IaAf, $IIIC_3$ H/A, No. 1, $C_3H\text{-}H2^{p}$ and $C\text{-}Z_{(pl)}$ were induced in adult mice by variants of high oncogenicity for mammals, isolated by the writers from strain C-Z. Tumors C3HA, CC57W, and (B10.D2 × A)F_1 were induced in adult mice of the corresponding lines by syngeneic embryonic cultures infected with strain C-Z [3]. Tumors $S\text{-}R_{(n)}$ were induced in newborn mice of line A by virus of the Schmidt-Ruppin (S-R) strain.

The following tests of immunogenicity and immunosensitivity were used.

The Transplantation Test [6, 7]. Mice were immunized with cells of a syngeneic tumor (irradiated in a dose of 10,000 R or used in subthreshold doses) or with allogeneic Rous mouse tumors. The scheme of the experiment was described previously [6, 7]. Mice immunized with syngeneic cultures transformed and infected with RV and with OV-40 virus, and also unimmunized mice, were used as the control. Immunity against 1-10 threshold doses (TD) of the test tumor was determined.

Adoptive Transfer of Immunity by Lymphocytes. Mice were immunized and the test carried out as described previously [3]. The results of these experiments showing the duration of the latent period and weight of the tumors by the end of the experiment were subjected to statistical analysis by the Fisher–Student method with $P = 0.05$ as the level of significance.

Indirect Immunofluorescence Method on Living Cells. The modification of Lezhneva [4] was used. Three series of sera were prepared in $C3H\text{-}H2^{p}$ mice by the use of identical immunization cycles: against tumors $^{2}IaAf$, $C\text{-}Z_{(pl)}$, and $S\text{-}R_{(n)}$. The scheme of immunization and the methods used to test the sera for specificity, to exhaust them, and to perform the experiments were described previously [1].

Division of Virology and Immunology of Tumors, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 9, pp. 83-86, September, 1972. Original article submitted December 6, 1971.

TABLE 1. Use of the Transplantation Test to Study Mouse Tumors Induced by Different Variants of RV

Line of mice immunized	Material used for immunization	Number of injections	Test tumor	Number of tumors arising			P					
							latent period			weight of tumors		
				1 TD	3–5 TD	10 TD	1 TD	3–5 TD	10 TD	1 TD	3–5 TD	10 TD
Af	Living cells of tumors C_3HA and B10. D2	2	$S\text{-}R_{(n)}$	$^{7}/_{8}$	$^{7}/_{8}$	—	>0,1	>0,1	—	<0,05	<0,05	—
	Culture of Af cells transformed by RV, strain 5D	2	$S\text{-}R_{(n)}$	$^{5}/_{7}$	$^{7}/_{7}$	—	>0,1	>0,1	—	<0,05	<0,05	—
	Control: culture of CC57Br cells infected with RV, strain K-3 (4th day)	2	$S\text{-}R_{(n)}$	$^{8}/_{8}$	$^{8}/_{8}$	—		—			—	
Af	Living cells of tumors C3H-H2P and C3HA	4	$S\text{-}R_{(n)}$	$^{2}/_{6}$	$^{3}/_{6}$	$^{6}/_{6}$	>0,1	>0,1	>0,1	<0,01	<0,01	>0,1
	Culture of Af cells transformed by RV, strain 5D	4	$S\text{-}R_{(n)}$	$^{3}/_{6}$	$^{5}/_{6}$	$^{5}/_{6}$	>0,1	>0,1	>0,1	<0,05	>0,1	>0,1
	Control: Af cells infected with OV40	4	$S\text{-}R_{(n)}$	$^{6}/_{6}$	$^{6}/_{6}$	$^{6}/_{6}$						
Af	Spontaneous regression of tumors induced by variant ^{5}/IaAf	—	^{2}IaAf	$^{2}/_{5}$	$^{3}/_{5}$	—	>0,1	>0,1	—	<0,05	>0,1	—
	The same mixed with anti-mouse rabbit serum	—	^{2}IaAf	$^{2}/_{5}$	$^{3}/_{5}$	—	>0,1	>0,1	>0,1	<0,05	<0,05	—
	Control: untreated mice . .	—	^{2}IaAf	$^{6}/_{6}$	$^{6}/_{6}$	—						
C3H-H2P	Living cells of C3H-H2P tumor in subthreshold doses	2	C3H-H2P	$^{2}/_{5}$	$^{3}/_{5}$	—	>0,1	>0,1	—	<0,05	<0,05	—
	Control: untreated mice	—	C3H-H2P	$^{5}/_{5}$	$^{5}/_{5}$	—		—			—	

Note. Here and in Table 2 numerator gives number of mice with tumors, denominator gives total number of mice.

TABLE 2. Adoptive Transfer of Immunity by Lymphocytes of Unimmunized Mice

Expt. No.	Donors of lymphocytes immunized with tumor cells	Number of injections	Test tumor	Number of tumors arising 1 TD	5 TD	10 TD	P latent per. 1 TD	5 TD	10 TD	wt. of tumors 1 TD	5 TD	10 TD
1	(B10.D2×A) F_1	1	CC57W	5/5	5/5	5/5	0,1	0,1	0,1	0,1	0,1	0,1
	C3H=H2pandC3HA	5	CC57W	0/5	0/5	1/5				0,01	0,01	0,05
	Embryonic C3H = $H2^p$ + RV cells	5	CC57W	5/5	5/5	—	0,1	0,1	—	0,1	0,1	—
	Lymphocytes of unimmunized mice	—	CC57W	5/5	5/5	5/5		—			—	
2	^{2}IaAf	3	Af_{13}	—	5/5	5/5	—	0,1	0,1	—	0,05	0,1
	Lymphocytes of unimmunized mice	—	Af_{13}	5/5	5/5	5/5		—			—	
3	C3H=H2p	5	^{2}IaAf	4/5	4/5	5/5	0,1	0,1	0,1	0,01	0,01	0,05
	Embryonic C3H = $H2^p$ + RV cells	5	^{2}IaAf	5/5	5/5	—		—			—	

TABLE 3. Indirect Immunofluorescence Test with Sera of Immunized Mice

Serum against tumor cells	IF tested with different tumors ^{2}IaAf	№ 1	Af_{13}	ShC3H/A	K=3(pl)	CC57W	C3HA	Sh-$R_{(n)}$
^{2}IaAf	0,2	0,3—0,2	0,4—02	0,24	0,27	—	0,4	—
№ 1	0,22	0,22—0,18	0,22	0,39	0,25	—	—	—
Sh-$R_{(n)}$	—	—	0,25	0,3	0,28—0,39	0,26	0,52	0,28—0,3

EXPERIMENTAL RESULTS

The Transplantation Test. In the experiments of series I the intensity of immunity against mouse tumors was tested in relation to S-$R_{(n)}$ tumors. The presence of CMA in its cells had been shown by the writers previously [1].

Double immunization with cells of tumors C3HA and B10.D2 and also by cells of a culture transformed in vitro by strain 5D, gave weak immunity against 1-5 TD of cells, as shown by an increase of only 2.5-3 times in the size of the tumors in the control group compared with those in the experimental group (Table 1). Quadruple immunization with the same tumors gave clear protection agains 2 TD and reduced the number of of tumors after injection of 10 TD, although the tumors in the experimental and control series were equal in weight ($P > 0.1$).

In the next experiments immunity was tested against tumors induced in adult mice by highly oncogenic variants.

Double immunization with cells of C3H-$H2^p$ tumors in subthreshold doses delayed the growth of 2-3 TD of cells of the same tumor by comparison with growth in untreated mice. Single immunization with massive doses of irradiated tumor Af_{13} cells had the effect of accelerating growth of 3.5 and 10 TD of cells of the same tumor. The difference between the sizes of the tumors and their latent periods was significant ($P = 0.001$).

Spontaneous regression of tumors induced by variant ^{5}IaAf gave rise to marked resistance to growth of 2 and 5 TD of cells of the analogous tumor ^{2}IaAf (Table 1).

Adoptive Transfer of Immunity by Lymphocytes. Immunization of the mice and testing of immunity were carried out with tumors induced by highly oncogenic variants (Table 2). Single immunization was ineffective, but triple immunization produced resistance to 5 TD of cells of tumor Af_{13}, and five cycles of immunization completely prevented growth of 5 TD of CC57W cells and delayed growth of ^{2}IaAf cells, and led to statistically significant resistance to 10 TD of cells of both tumors. Five cycles of immunization with the tissue culture on the 4th day after infection with RV were ineffective even against 1 TD of cells of the same tumors.

The Immunofluorescence Test. In each experiment two indices of fluorescence (IF) were distinguished: for normal cells treated with immune serum and for test cells treated with normal mouse serum. The two indices were virtually identical. The arithmetic means of both IF for several experiments are given in Table 3. All three test sera exhibited virtually identical activity, and this test gave results in agreement with those described above: weak but clearly defined immunosensitivity and immunogenicity were found in practically equal degrees in all viruses studied and in the tissue culture transformed in vitro by RV and completely nononcogenic for mice of all ages [1].

Absence of correlation between the transforming activity of the virus and ability to synthesize CMA has been demonstrated on different virus models. The results in this paper confirm this principle.

LITERATURE CITED

1. I. Kryukova, O. Babkova, and I. Obukh, J. Nat. Cancer Inst., 47, 819 (1971).
2. I. Kryukova, I. Obukh, and F. Toth, J. Nat. Cancer Inst., 45, 49 (1970).
3. I. Kryukova, I. Obukh, O. Babkova, et al., Internat. J. Cancer, 6, 275 (1970).
4. O. M. Lezhneva, in: Immunochemical Analysis [in Russian], Moscow (1968), p. 189.
5. I. B. Obukh and I. N. Kryukova, in: The Spread, Virology, and Immunology of the Leukemias of Man and Animals [in Russian], Riga (1970), p. 213.
6. P. Koldovsky and J. Bubenik, Folia Biol. (Prague), No. 10, 81 (1964).
7. H. Sjögren and N. Lonsson, Exp. Cell Res., 32, 618 (1963).