A STUDY OF SPECIES-SPECIFIC AND SPECIFIC TUMOR ANTIGENS IN CELLS OF INTERSPECIFIC SOMATIC HYBRIDS

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A strain of interspecific somatic hybrid cells (between the cells of a hamster tumor induced by SV_{40} virus and normal kidney cells of a green African guenon) was obtained and its antigenic structure studied. It was found to contain not only species-specific hamster and monkey antigens, but also three different tumor antigens specific for SV_{40} virus (S-antigen, T-antigen, and specific transplantation antigen). The combination of species-specific antigens of monkey and hamster in the cells of the culture studied makes them incompatible with either host; the presence of transplantation antigen specific for SV_{40} virus means that these cells can be used as a new and effective material for immunization against tumors induced by SV_{40} virus.

Somatic cell hybrids are widely used today to study the genetics of somatic cells. One of the main methods of their investigation is by studying the antigenic structure of the hybrid cells. In the authors' experiments cells of a hamster sarcoma induced by SV_{40} virus were cultivated together with kidney tissue cells from a green African guenon and a strain of interspecific somatic hybrid cells was produced. Hybridization took place under the conditions of spontaneous infection of the parental cultures by syncytium-forming virus isolated from the original culture of Syrian hamster sarcoma. Barski [2] has repeatedly postulated that spontaneous hybridization of cells in culture can take place through contamination of one of the parental strains with syncytium-forming virus. The strain of hybrid cells obtained in the present authors' experiments as a result of this "spontaneous" hybridization has been cultivated successfully for more than three years.

The paper describes the results of a study of the preservation of species-specific and specific tumor antigens in the cells of interspecific somatic hybrids.

TABLE 1. Detection of Species-Specific Hamster and Monkey Surface Antigens in Hybrid Cells of Strain PZM-211

Immune serum	Titer of immune sera in MHT with cells of tissue cultures			
	test strain PZM-211	monkey kidney	hamster embryo	mouse embryo
Against sheep's red cells	1:512	1:2048	<1:4	—
Against hamster thymocytes	1:1024	<1:4	1:1024	—
Against cells of strain PZM-211	1:2048	1:512	1:1024	<1:4

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TABLE 2. Use of Blocking MHT to Detect Surface S-Antigen Specific for SV_{40} Virus in Hybrid PZM-211 Cells

Sera blocking MHT (rabbit)	Culture of cell tested in MHT	Titer of guinea pig sera tested in MHT		
		anti-PZM-211[3]	anti-hamster[3]	anti-monkey[4]
—	PZM-211	1:2048	1:1024	1:512
Anti-hamster + anti-monkey	PZM-211	1:256	<1:64	<1:32
	HEC[1] uninfected	1:1024	1:2048	—
Anti-hamster		<1:64	<1:64	—
Anti-hamster	HEC transformed by SV_{40}	128	<1:64	—
Anti-hamster	HEC infected with polyoma virus	<1:64	<1:64	—
—	MEC[2], uninfected	0	—	—
—	MEC transformed by SV_{40}	1:128	—	—

[1]Embryonic hamster culture.
[2]Embryonic mouse culture.
[3]Anti-PZM-211 and anti-hamster sera were used in dilutions of between 1:64 and 1:2048.
[4]Anti-monkey serum was used in dilutions of between 1:32 and 1:512.

TABLE 3. Immunogenic Properties of Cells of Test Strain PSM-211 (in experiments with immunization of adult Syrian hamsters)

Group No.	Immunizing material	No. of test tumor cells (SV_{40}) injected				Index of resistance
		8	8×10	8×10	8×10	
1	PZM-211 cells, 54th passage, $3.0 \cdot 10^7$ once only	0/5[1]	0/5	0/5	2/5	>3,5
2	Control	0/5	4/5	5/5	5/5	—
		No. of test tumor cells (VP) injected[2]				
		4×10^1	4×10^2	4×10^3	4×10^4	
3	PZM-211 cells, 54th passage, $3.0 \cdot 10^7$ once only	0/5[1]	0/5	3/5	4/5	0,2
4	Control	0/5	0/5	2/5	5/5	—

[1]Numerator gives number of animals in which inoculated test tumor grew; denominator gives number of animals receiving injection of that dose of test tumor cells.
[2]Hamster tumor induced by polyoma virus.

EXPERIMENTAL METHOD AND RESULTS

Species-specific and specific tumor antigens were determined in the cells of the test hybrid strain (described as PZM-211) at the 16th-54th passage in vitro in the following tests: 1) in the mixed hemadsorption test (MHT) by the method suggested by Espmark and Fagreus [4, 5], the indirect immunofluorescence test of Coons and Kaplan [3], and the transplantation test in Murka's modification [1]. Species-specific antigens of the test strain of PZM-211 cells was carried out with the aid of a set of immune sera obtained by immunization of hamsters, rabbits, and guinea pigs with sheep's red cells and hamster's red cells and thymocytes. Immune sera were obtained in guinea pigs against the test PZM-211 cells. To detect specific (for SV_{40} tumors) surface (S-) antigen in the test cells the method of blocking species-specific surface antigens was used.

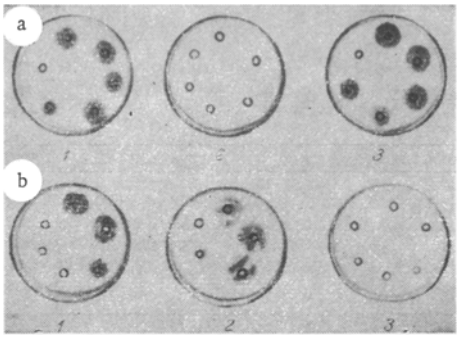

Fig. 1. The use of blocking of the MHT to study antigenic structure of strain PZM-211: a) rabbit blocking serum against monkey red cells, guinea pig testing serum against PZM-211 cells; 1) test cells of strain PZM-211 (titer of serum 1:1024); 2) monkey cells (complete blocking); 3) hamster cells (titer of serum 1:1024); b) rabbit blocking serum against hamster thymocytes, guinea pig testing serum against PZM-211 cells; 1) test cells of strain PZM-211 (titer of serum 1:256); 2) monkey cells (titer of serum 1:512); 3) hamster cells (complete blocking).

The results of the study of species-specific antigens in cells of strain PZM-211 and in the control cultures from normal monkey kidney and normal mouse and hamster embryonic fibroblasts in the mixed hemadsorption test are given in Table 1.

As Table 1 shows, on the surface of the investigated cells of strain PZM-211 there are antigens specific to both hamster and monkey. To detect surface antigen specific for SV_{40} tumors the method of blocking the species-specific antigens on the surface of the strain PZM-211 cells by means of a mixture of two antispecific sera (anti-hamster and anti-monkey), prepared by immunization of rabbits, was used. The blocking sera were applied to the test cells 24 h before the MHT was performed with immune guinea pig sera (and with the indicator system against guinea pig globulin). Completeness of blocking of the species-specific antigens with the aid of rabbit anti-sera was verified by the MHT with immune anti-hamster and anti-monkey guinea pig sera (Fig. 1). The results relating to detection of S-antigen in the PZM-211 cells in the experiments with blocking of species-specific antigens are given in Table 2. Besides specific S-antigen, nuclear T-antigen, specific for SV_{40} was found in the nuclei of 100% of the PZM-211 cells.

Finally, in the experiments in vivo to study the immunogenic activity of the hybrid cells they were found to contain the third of the group of specific tumor antigens – transplantation antigen (Table 2).

Besides species-specific hamster and monkey antigens, the cells of the strain of interspecific somatic hybrid cells obtained thus also contain the whole range of tumor antigens specific for SV_{40}. This makes these cells a new and effective material for immunization against tumors induced by SV_{40} virus.

LITERATURE CITED

1. L. M. Murka, Byull. Éksperim. Biol. i Med., No. 12, 11 (1965).
2. G. Barski, Internat. J. Cancer, 3, 320 (1968).
3. A. H. Coons and M. H. Kaplan, J. Exp. Med., 91, 1 (1950).
4. J. A. Espmark and A. J. Fagreus, J. Immunol., 94, 530 (1965).
5. A. J. Fagreus, J. A. Espmark, and J. Jonsson, J. Immunol., 9, 161 (1965).