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Specificity of Resistance to Tumor Challenge of Polyoma and SV 40 Virus-Immune Hamsters. (28259)

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Zilber and his associates(1) were the first to demonstrate an abnormal antigen in a virus-induced tumor when they worked with Rous sarcoma. More detailed studies of the relationships between the inducing virus and the new cellular antigen in the transformed cells were later made by Klein and his associates(2), and by Habel(3), using polyoma virus-induced tumors in the mouse and the hamster. Since that time there has rapidly appeared published evidence for the existence of new cellular antigens in Gross leukemia cells(4), Shope papilloma virus tumors(5), and Moloney virus leukemia(6). The biological method which most readily demonstrated the new antigen in polyoma tumors, tested the resistance of virus immunized adult animals to challenge with isologous transplantable polyoma tumor. This same technic has been used to demonstrate new cellular antigens in SV 40 virus induced hamster tumors in the experiments reported here.

The original work with polyoma tumors had demonstrated the specificity of the new antigen insofar as there was no cross resistance by polyoma immune mice against challenge with tumors induced by other oncogenic agents. SV 40 and polyoma viruses have several physical and chemical characteristics in common(7). They are both DNA viruses of the same size, both have the same number of capsomeres, and both produce sarcomas on inoculation of newborn hamsters. For this reason it was felt that a cross-immunity test, in which animals immunized with each of these 2 viruses were challenged with the corresponding tumors, would be a more severe and direct test of the specificity of the new virus-induced cellular antigens. It will be shown that in such a cross-immunity test each virus appears to induce a resistance to tumor challenge which is quite specific.

Materials and methods. Viruses. Polyoma virus was grown in mouse embryo tissue cul-

TABLE I. Results of Quantitative Subcutaneous Challenge of Virus Immunized and Normal Hamsters with Tumors Induced by the Homologous and Heterologous Virus.

Groups of hamsters	No. of tumor cells in challenge			
	10^2	10^3	10^4	10^5
Challenged with polyoma tumor				
Normal	2/5*(0)†	5/5 (28.0)	5/5 (29.4)	—
Polyoma virus immune	0/5	0/5	0/5	—
SV 40 virus immune	5/5 (2.8)	5/5 (34.6)	4/4 (33.7)	—
Challenged with SV 40 tumor				
Normal	—	4/5 (17.0)	3/4 (21.0)	4/4 (33.5)
Polyoma virus immune	—	4/5 (14.5)	5/5 (20.7)	4/4 (32.2)
SV 40 virus immune	—	0/5	0/5	1/5 (27.0)

* Fraction represents No. of animals developing tumors out of total inoculated.
† Avg diameter in mm of tumors present 1 mo from date of challenge.

ture from random-bred Swiss mice and a standard pool had a titer of 1.5×10^7 pfu/ml. SV 40 virus was grown in primary green monkey kidney tissue cultures and had a titer of 4×10^7 $TCID_{50}$ per ml. *Antiviral antibody.* Polyoma hemagglutination-inhibition (HI) titers were tested against 8 hemagglutination units of standard virus using pattern agglutination of guinea pig red blood cells at 4°C as the indicator system. SV 40 antibodies were determined by a neutralization test against 1000 $TCID_{50}$ of virus in primary green monkey kidney cultures. *Tumors.* Transplantable hamster polyoma sarcoma and SV 40 sarcoma were used at the indicated passage levels. The polyoma tumor line (HE 11 C) had been originated by inoculation of adult hamsters with hamster embryo tissue culture cells transformed by polyoma virus *in vitro*(8). The SV 40 tumor (A 426) had been initiated by inoculation of newborn hamsters with the virus(9). Challenge with tumors consisted of a subcutaneous inoculation into the interscapular area of 0.1 ml containing the indicated number of viable tumor cells obtained by trypsinization of the tumor. Animals on test were observed for 2 months and diameters of tumors were measured at one month from challenge.

Results. In the first experiment, groups of adult Syrian hamsters were inoculated intraperitoneally with 0.1 ml of either polyoma or SV 40 standard virus. One month later the virus immunized groups along with normal control hamsters were divided into 2 subgroups, one of which was quantitatively challenged with transplant passage 6 of the polyoma tumor and the other with the second transplant passage of the SV 40 tumor. At the time of challenge several animals in each group were bled. The sera of the polyoma immunized hamsters had a polyoma HI antibody titer greater than 1/1280, while the SV 40 immune and control animals' sera were negative. On the other hand, only the SV 40 immune animals had demonstrable neutralizing antibodies against SV 40 virus in their sera (1/80 dilution of serum neutralized 1000 $TCID_{50}$ of virus). Table I shows the results of the tumor challenge. On challenge with the polyoma tumor, the animals immunized with polyoma virus resisted at least 100 times the minimum number of cells required to produce tumors in control hamsters, whereas the SV 40 immunized group were just as susceptible as the controls. Exactly opposite results were obtained on challenge with the SV 40 tumor. The SV 40 immunized animals resisted at least 100 minimum tumor-producing doses of cells but the polyoma immune hamsters were as susceptible as the controls.

The entire experiment was repeated using larger numbers of animals and a wider range of tumor cell numbers in the challenge. In this experiment, the tumor challenge was made 3-4 weeks after the single immunizing dose of the 2 viruses. The HE 11 C polyoma tumor was in its eleventh passage and the SV 40 in the fifth passage when used. Again, several animals were bled at the time of challenge and their sera tested for polyoma and SV 40 antibodies. Polyoma HI antibodies were present only in the polyoma immunized and SV 40 neutralizing antibodies only in the

TABLE II. Quantitative Subcutaneous Challenge of Virus Immunized and Normal Hamsters with Tumors Induced by the Homologous and Heterologous Virus.

Groups of hamsters	No. of tumor cells in challenge					
	10^1	10^2	10^3	10^4	10^5	10^6
Challenged with polyoma tumor						
Normal	0/10	8/10 (11.3)	9/10 (13.7)	10/10 (25.0)	—	—
Polyoma virus immune	—	—	2/9 (0.)	5/9 (11.6)	9/9 (17.3)	10/10 (36.2)
SV 40 virus immune	1/10 (0.)	7/10 (18.0)	9/9 (14.4)	9/9 (23.1)	—	—
Challenged with SV 40 tumor						
Normal	1/10 (2.0)	6/10 (12.5)	10/10 (19.1)	10/10 (32.0)	—	—
Polyoma virus immune	2/9 (16.3)	6/10 (11.8)	9/9 (22.2)	9/9 (28.6)	—	—
SV 40 virus immune	—	—	4/8 (8.0)	4/9 (30.0)	8/9 (31.0)	9/9 (37.4)

SV 40 immunized animals. Evidence for homologous resistance and complete specificity was of the same order of magnitude as in the first experiment (Table II).

Discussion. Evidence in the polyoma system has established that the resistance of virus-immune animals to challenge with the isologous virus-induced tumor is probably on an immunological basis and the conclusion that the tumor contains a new cellular antigen appears justified(8). On the same basis the results reported here suggest the presence of a similar new cellular antigen in the tumors induced in the hamster by SV 40 virus. Thus, SV 40 can be added to the growing list of virus-induced tumors in which there is evidence for a cellular antigen or antigens foreign to the host. In fact all virus-induced tumors thus far investigated have shown this phenomenon so it may well be the general rule.

One of the most interesting aspects of the recent work suggesting new antigens in virus-induced tumors is the specificity of the reaction. In the case of polyoma tumors, it appears that all mouse polyoma tumors contain the same new cellular antigens even though the tumors originate in different inbred strains of mice(10,11), and *in vitro* transformed cells also contain it(8). In the experiments reported here the specificity of the resistance to challenge by tumors produced by each of 2 similar tumor viruses is quite strict. The nature of the new antigen(s) has not been determined but in polyoma tumors it appears to be cellular in origin rather than viral, yet is present only as the result of the virus transformation(10). This specificity of the resistance of virus-immune animals to challenge with the corresponding transplantable virus-induced tumor makes available a possible method for demonstrating etiological relationship between a given virus and a transplantable tumor attributed to that virus. The etiological relationship dependent on the presence of a specific cell antigen has been demonstrated by immunological methods even though the tumor no longer contains demonstrable virus. In fact the demonstration of this immunological specificity may be better

evidence of etiological relationship than is the isolation of the original inoculated virus from the tumor, since it is well known that tumors offer good sites of multiplication of unrelated viruses. Whether superinfection of one tumor by another tumor virus would induce the cellular antigen characteristic of the superinfecting virus and therefore give false evidence of an etiological relationship in the resistance type of test has not been investigated.

Evidence similar to that reported here for the presence of a new cellular antigen in SV 40 induced tumors and the specificity of SV 40 and polyoma hamster tumors has been found by Defendi(12) and Koch and Sabin (13) and is reported in this issue of the Proceedings.

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Specificity of Virus-Induced Resistance to Transplantation of Polyoma and SV 40 Tumors in Adult Hamsters.* (28260)

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Habel(1,2) and Sjögren *et al.*(3,4) independently demonstrated that a previous inoculation of adult mice with polyoma virus grown in mouse embryo tissue cultures induced a resistance to transplantation of isologous mouse tumors induced by polyoma virus but not of spontaneous or carcinogen-induced isologous tumors. Various experiments by both Habel and Sjögren led to the conclusion that this resistance was due to the presence in the mouse polyoma tumor cells of specific cellular antigen(s) that resulted from infection of the cells with polyoma virus but was unrelated to the viral antigens. Moreover, since the polyoma tumor antigen(s) was present in tumors in which neither polyoma virus antigens nor infectivity were demonstrable by the techniques employed, it appeared that the tumor specific antigen was a consequence of a genetic modification of cells in which the genome of the tumor-inducing virus was no longer demonstrable. For this reason it appeared highly desirable to carry out additional studies on the specificity of this tumor antigen to determine especially whether other DNA or RNA viruses multiplying in mouse embryo tissue cultures or in the living host would or would not give rise to a cellular antigenic modification that might also produce resistance to transplantation of polyoma virus induced tumor cells.

Most of the resistance tests in mice have been carried out with 10^4 or more tumor cells, because smaller numbers either produced no tumors or did so irregularly. The number of cells used for challenge was critical in demonstrating the resistance phenomenon since with

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