IMMUNOLOGICAL DETERMINANTS OF POLYOMA VIRUS ONCOGENESIS

By KARL HABEL, M.D.

(From the United States Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Biology of Viruses, Bethesda)

(Received for publication, September 13, 1961)

It has been demonstrated that adult mice and hamsters, after an inapparent infection with polyoma virus, are resistant to the transplantation of an isologous polyoma tumor even though the challenge tumor no longer yields the original inducing virus (1, 5). It was shown that this resistance was specific, being effective against only those tumors originally produced by inoculating newborns with polyoma virus, and evidence was presented suggesting that serum antibodies, either antiviral or anticellular, did not seem to be responsible for the resistance. To explain this phenomenon, it was postulated that the polyoma virus–induced tumor contains an antigen which is different from the normal cellular antigens of the animal involved. On this basis, polyoma virus when inoculated into adult mice or hamsters causes a transformation of some normal cells to tumor cells containing a new antigenic component which the immunologically competent adult recognizes as a foreign antigen and rejects. In so doing, tumor development due to the virus is suppressed and the adult becomes sensitized to the tumor antigen. A later challenge with tumor cells causes an accelerated rejection, manifested by resistance to the tumor transplant.

The studies reported here present direct and indirect evidence for the existence of the previously postulated "foreign" antigen in the polyoma tumors, and describe some of the characteristics of the development of the resistance.

Materials and Methods

Transplantable Tumor Lines.—Two transplantable fibrosarcomas originally induced in C57B1/6JN mice by inoculation of newborns with polyoma virus and a similarly induced hamster sarcoma have been previously described (2, 3). Most of the experiments now to be described used the 695 mouse tumor between its 20th and 30th transplant passages. This tumor grows more rapidly than the 1923 mouse tumor line. At the time of the resistance experiments, none of these tumors contained demonstrable virus or viral antigens, nor could virus production be induced in them by x-ray or ultraviolet irradiation. Trypsinized cell suspensions containing a determined number of viable tumor cells were inoculated subcutaneously (SC) in the interscapular area, and animals were observed for palpable tumors during 60 days. Female mice were used in all the experiments.

Virus Immunization.—Mouse embryo tissue culture grown polyoma virus, diluted to contain 10^4 TCID of virus (0.1 ml of a 1/100 dilution), was inoculated intraperitoneally at least 4 weeks prior to challenge with tumor.

X-ray Irradiation.—Whole body irradiation with 400 to 425 r was carried out, as in previous experiments (1). When cell suspensions were irradiated, they received 15,000 r.

Serological Tests.—Hemagglutinin inhibition (HI) tests against virus were carried out, as previously described (1).

Ultraviolet Irradiation.—Ultraviolet-inactivated vaccine was prepared by a graded series of exposures of a mouse embryo tissue culture supernate containing 10^7 TCID of virus per ml in a Habel-Sockrider type of apparatus. Samples were tested for residual infectious virus by inoculation of mouse embryo tissue culture, and the indirect HI production test in mice (4). That sample, showing no viable virus at the shortest exposure, was used as vaccine.

TABLE I

Lack of Transplacental Transfer of Resistance to Polyoma Tumor

Litters from	No. of challenge tumor cells		
	10^3	10^4	10^5
Immune mothers	3/6*	6/6	5/5
Normal mothers	4/8	7/8	8/8

* No. developing tumors/No. inoculated.

RESULTS

Lack of Resistance to Tumor Challenge in Newborn Mice of Immune Mothers.—All virus-inoculated adult mice at the time of demonstrable resistance to the transplanted polyoma tumor have circulating antibodies against the polyoma virus, and will be referred to as "virus-immune." This raises the question whether the antiviral antibodies are responsible for the resistance even though the challenge tumor contains no virus or viral antigens. In our preliminary report, hyperimmune antipolyoma rabbit serum given passively to normal adult mice conferred no resistance to tumor challenge. However, since that experiment involved heterologous species serum given over a period of only 4 days, this question was now tested in the homologous species. Adult female C57B1 mice were made immune to polyoma virus, and 1 month later were bred with normal male C57B1. Litters from these immune mothers with similar litters from non-immune mothers were challenged with 695 tumor, when 1 or 2 days old. Results of one of two experiments showing the same lack of evidence of protection by antiviral antibody derived transplacentally from immune mothers is given in Table I. On the day of tumor challenge, the newborns of immune mothers had a 1/100 titer of polyoma HI antibodies in their sera. Had some other serum antibody, such as anticellular antibody, been present in the immune mothers and responsible for their resistance, it likewise had not been transferred to the newborns.

Lack of Resistance Following Immunization with Inactivated Virus and Mouse Embryo Cell Antigen.—Further evidence concerning the basis of resistance was sought to rule out any possible anticellular reactivity caused by the small amount of mouse embryo cell antigens in the virus used to immunize the mice. Adult C57B1 mice were inoculated with either undiluted, ultraviolet-inactivated polyoma tissue culture vaccine or the supernate from normal, uninfected mouse embryo cultures. Doses of 0.1, 0.2, and 0.5 ml at 2 week intervals were given intraperitoneally. All vaccinated mice and controls were challenged with the 695 tumor, 1 week after the last dose of vaccine. At that time, sera from both vaccinated groups were negative for HI antibodies against polyoma virus. Table II shows that the two vaccinated groups of mice were just as susceptible to tumor transplant as the controls, even though they had received 700 times as much embryo tissue culture material as mice made resistant by a single dose of virus.

TABLE II

Lack of Resistance to Tumor Challenge after Immunization with Inactivated Virus

Immunization	No. of challenge tumor cells			
	10^3	10^4	10^5	10^6
Ultraviolet vaccine	—	1/5	5/5	5/5
Normal METC*	—	1/5	5/5	5/5
Control	0/5	2/5	5/5	5/5

* Supernatant fluid from normal mouse embryo tissue cultures.

Immunological Incompetence Gives Enhanced Susceptibility to Transplant Tumor.—If the hypothesis that the polyoma tumor contains a foreign cellular antigen were true, then this should be a factor in the relative susceptibility of the normal adult mouse to tumor challenge unrelated to previous immunization with virus. This was indeed suggested by the fact that it usually required 10^5 tumor cells to produce a take in the normal adult. For direct evidence the immunological capability of mice was eliminated in two ways: by whole body x-irradiation of adults, and by using newborn animals. In Table III are the results of two experiments. Whole body x-irradiation of 400 r was given several hours before challenge. X-rayed normal adults were 10 times more susceptible to tumor challenge than un-x-rayed adults, and normal newborns were 100 times more susceptible than adults. Even mice already immunized with virus, then x-rayed at time of challenge, showed less resistance than un-x-rayed immunes, suggesting that the virus-immune animal already sensitized to the tumor antigen receives an antigenic booster effect from the challenge transplant. The basic resistance persists, but the booster effect is eliminated by irradiation.

Immunological Tolerance Produced by Tumor Cells.—Further evidence for the existence of a different antigen in tumor came from immunological tolerance experiments. Newborn C57B1 mice received 4 intraperitoneal inoculations of 0.05 ml of a cell-free 10 per cent extract of the 695 tumor on days 1, 2, 3, and 6 after birth. In two experiments, the mice were challenged with various numbers of 695 tumor cells on day 35 or 49, along with controls of the same age. There not only was no evidence of tolerance but some moderate degree of resistance in the inoculated, as compared with the control mice. On the other hand, newborn C_3H mice that had received the same treatment and were challenged on the 36th day with the C57B1 tumor rejected the transplant in 27 days, while normal C_3H mice required only 13 days for complete rejection. More definite results with a direct test were obtained when viable but non-

TABLE III

Immunological Basis for Resistance to Polyoma Tumor Transplant

Pretreatment of mice	No. of challenge tumor cells						
	Experiment 1				Experiment 2		
	10^3	10^4	10^5	10^6	10^4	10^5	10^6
Normal adult	0/5	1/5	5/5	5/5	0/5	5/5	5/5
Normal adult x-rayed*	0/5	4/5	5/5	5/5	3/4	5/5	5/5
Immune adult	0/5	0/5	1/5	4/5	0/5	0/5	3/5
Immune adult x-rayed*	0/5	—	4/5	5/5	0/5	3/5	5/5
Normal newborn	2/4	7/8	6/6	7/7	—	—	—
Tumor extract immunized	0/5	0/5	3/5	5/5	—	—	—

* 400 r whole body irradiation.

dividing tumor cells were used to inoculate the newborns. A trypsinized suspension of 695 tumor cells was given 15,000 r x-irradiation and 10^6 cells inoculated subcutaneously or intraperitoneally at 1 and 3 days after birth. Challenge with tumor at 31 days gave results shown in Table IV in which the inoculated mice required only one-tenth the number of tumor cells as controls to establish a positive transplant.

One attempt to demonstrate tolerance in hamsters by inoculation of newborns with x-rayed hamster tumor cells on days 1 and 3, and challenge with the hamster transplantable tumor on day 59 showed partial resistance rather than tolerance. The tumors of inoculated animals after challenge were less than half the size of those in the controls.

Resistance to Transplant Produced by Immunization with Tumor Cells.—The last and most direct test for the presence of a non-C57B1 antigen in the polyoma tumors, involved immunizing normal adult C57B1 mice with tumor cells and

later challenging with transplant. At the same time an attempt was made to see whether the same antigen existed in the two different mouse polyoma tumors and in the hamster tumor. The results of the first experiment are given in the last line of Table III. Adult mice were immunized with a 20 per cent cell-free extract of 695 tumor by one inoculation of extract mixed with Freund's incomplete adjuvant followed at 3 and 6 weeks with extract in saline. They were challenged at 7 weeks and showed only suggestive evidence of resistance. At the time of challenge sera were negative for polyoma HI antibodies.

An attempt was made to immunize adult mice with viable tumor cells given intraperitoneally in 3 doses, 2 weeks apart. Groups were inoculated with 10^3 cells of 695 tumor, 10^5 of 1923 tumor or 10^6 cells of the hamster tumor, and were challenged with 695 tumor cells 6 weeks from the start of immunization.

TABLE IV

Susceptibility of Mice to Tumor Challenge as Adults after Either Polyoma Virus or Tumor Inoculation at Birth

Pretreatment of mice	695 Challenge			1923 Challenge		
	10^3	10^4	10^5	10^4	10^5	10^6
Tolerant*	2/5	5/5	5/5	—	—	—
Virus infected‡	0/5	0/5	3/4	0/5	0/5	1/4
Normal	0/5	3/4	5/5	0/5	1/5	5/5

* 10^6 x-rayed 695 tumor cells subcutaneously on day 1 and intraperitoneally on day 3 after birth. Challenge at 31 days.

‡ 10^4 to 10^5 TCID of polyoma virus subcutaneously when 1 day old. Challenge at 37 days.

In spite of the immunizing doses of the mouse tumors being given intraperitoneally, very few receiving the 1923 cells survived for the challenge without developing intraperitoneal tumors. However, on the basis of the size of the tumor resulting from challenge, there was some resistance in the 695 immunized group. Evidence for resistance in the 1923 immunized group was equivocal, owing to the small numbers available, yet both tumor incidence and the size of the single tumor measured suggested increased resistance. There was no evidence of resistance resulting from immunization with the hamster tumor. (Table V). At the bottom of this table are the results of a different experiment in which C57B1 mice received a single dose of 15×10^6 cells from a parotid tumor produced by virus in a C_3H mouse. This tumor was also virus-free at the time of harvest. On challenge with 695 tumor, again the degree of resistance was of questionable statistical significance but fewer tumors were produced and they were much smaller in the immunized group. All animals at challenge were negative for HI antibodies.

To be able to immunize mice with large amounts of the viable mouse tumor cells, but still not produce tumors in the immunization process, tumor cells were given 15,000 r x-irradiation. Mice received 1.7×10^6 x-rayed cells of 695 tumor, or 3.4×10^5 x-rayed cells of 1923 tumor intramuscularly, and were challenged 4 weeks later. A second attempted method of immunizing with viable cells involved the inoculation of 10^5 cells of each of the two mouse tumors into the lower thigh of adult C57B1 mice. At 2 to 3 weeks when a definitely palpable tumor appeared, the involved leg was amputated. These mice were also challenged at 4 weeks. The results of these two types of experiment are given in Table VI. It is apparent that 695 x-rayed cells produced definite resistance against challenge with 695, and also to 1923. The 1923

TABLE V

Cross-Immunity to Polyoma Mouse Tumor After Immunization with Viable Tumor Cells

Immunized with	No. of 695 cells in challenge			
	10^3	10^4	10^5	10^6
Experiment 1				
695 mouse tumor	—	1/3	5/5 (9.6)*	5/5
1923 mouse tumor	—	0/3	1/2 (6.5)	3/3
Hamster tumor	—	4/4	5/5 (15.0)	5/5
Controls	1/5	2/5	5/5 (15.2)	5/5
Experiment 2				
C_3H parotid tumor	—	1/5 (1.0)	3/5 (1.4)	3/4 (8.5)
Controls	—	2/5 (1.4)	4/5 (7.4)	5/5 (15.0)

* Average diameter of tumors in millimeters at 1 month after challenge.

x-rayed cells on the other hand produced no evidence of resistance against itself or 695. Mice whose 695 tumors had been removed by amputation showed no resistance to either tumor, whereas the 1923 amputated mice showed borderline evidence of resistance to both challenges. These differences might very well be related to the fact that 5 times more x-rayed 695 cells than 1923 cells were given as the immunizing dose, and that 695 tumors develop much more rapidly after transplantation than do the 1923 tumors. No HI antibodies were present at the time of challenge.

Although control groups immunized with normal mouse cells were not included as a part of these experiments, it has been shown in several other similar experiments that this gives no evidence of protection against challenge. This would be expected since the C57B1 mice are highly inbred and accept skin grafts from each other.

One attempt was made to immunize adult hamsters with a cell-free extract of hamster tumor. A 10 per cent homogenate of hamster tumor was frozen

and thawed 3 times, then mixed with Freund's incomplete adjuvant. An intramuscular dose of 0.5 ml was followed 1 month later by 0.2 ml. Seven weeks from the first immunizing dose, the hamsters were challenged with hamster tumor. Table VII shows that none of the immunized hamsters completely resisted the challenge, but the degree of tumor growth was definitely retarded as compared to control tumors.

TABLE VI

Cross-Immunization between Two Mouse Polyoma Tumors: Immunization with Viable Cells

Immunization	No. of challenge tumor cells					
	695				1923	
	10^3	10^4	10^5	10^5	10^5	10^6
695 x-rayed*	—	—	3/5 (6.8)	4/5 (12.2)	—	4/4 (4.7)
695 amputated‡	—	—	4/4 (17.0)	4/4 (20.0)	—	4/4 (22.5)
1923 x-rayed	—	—	6/6 (15.8)	—	—	6/6 (22.8)
1923 amputated	—	—	2/3 (13.0)	—	—	3/3 (15.3)
Control	0/5	2/5 (3.8)	5/5 (16.2)	—	4/4 (15.0)	4/4 (21.2)

Figures in parentheses show average diameter in millimeters of tumors 1 month after challenge.

* Cell suspension irradiated with 15,000 r.

‡ 10^5 tumor cells inoculated into thigh muscles and leg amputated when tumor palpable.

TABLE VII

Immunization of Adult Hamsters with Hamster Tumor Extract

	No. of challenge tumor cells			
	10^3	10^4	10^5	10^6
Immunized with tumor extract	—	2/2 (20)	2/2 (35)	2/2 (31)
Controls	2/2 (8)	3/3 (33)	3/3 (63)	2/2 (62)

Figures in parentheses show average largest diameter of tumors 1 month after challenge.

An attempt was made to demonstrate a possible relationship between polyoma hamster tumor and mouse tumor antigens by challenging virus-immune and normal adults with the heterologous tumor and determining the time required for rejection of the transplant. When immune and normal adult C57B1 mice were challenged with 3×10^6 hamster tumor cells or normal hamster embryo cells, the heterologous transplants were rejected in the same period of time by both groups. The same was true when normal and virus-immune adult hamsters were challenged with 695 mouse tumor cells. However, when virus-immune and normal C_3H mice were challenged with 4×10^6

cells of 695 tumor, the polyoma-immune mice rejected the transplant in 11 days, as compared with 25 days in normal mice. On the other hand, virus-immune and normal C_3H mice both rejected a transplant of normal adult C57B1 lung cells equally well in 11 days.

Resistance to Tumor Challenge in Virus-Immune Mice is Cell-Mediated.— Since passive transfer of antiviral serum antibodies did not induce resistance to tumor challenge in normal mice, the source of the resistance was investigated in the immunologically competent cells. Adult C57B1 mice which had been immunized with an inoculation of polyoma virus were challenged with 10^5 or 10^6 cells of 695 tumor. Three months later those that had resisted the challenge along with normal mice of the same age were bled out. The spleens and axillary lymph nodes were removed and passed through a tissue sieve and the cells inoculated intraperitoneally into normal adult C57B1 mice. Each recipient

TABLE VIII

Transfer of Resistance to Tumor Challenge by Spleen, Thymus, and Bone Marrow Cells of Virus-Immune Mice

Cells transferred from	Recipients	No. challenge tumor cells		
		10^4	10^5	10^6
Virus-immune	X-irradiated*	0/3	2/2	—
None	Unirradiated	3/5	5/5	5/5

* 400 r whole body irradiation.

received the cells derived from one donor. Two days later the recipients were challenged with 10^5 cells of the 695 tumor. All mice developed tumors, but those that had received cells from immune mice had tumors progressing much more slowly than the tumors in recipients of normal cells.

A similar experiment used a sieved cell preparation of spleen, thymus, and bone marrow from C57B1 mice 5 weeks after being immunized with polyoma virus. The cells were given intraperitoneally to adult C57B1 mice that had received 400 r whole body x-irradiation. The controls were normal un-x-rayed adults. Table VIII shows some protection against the tumor challenge given 5 days after the cell transfer.

One further experiment involved the incubation of a mixture of lymph node cells from virus-immune or normal C57B1 mice with 695 cells, and subsequent inoculation of normal mice with these mixtures. Lymph node cell suspensions were prepared by trypsinization of the nodes from 10 virus-immune and 10 normal mice. 2×10^6 node cells from the virus immunes and 8×10^6 normal node cells were mixed with 6×10^4 cells from a 695 tumor. The cell mixtures suspended in 5 per cent calf serum–Eagle's medium were incubated with shaking

at 37°C for 2 hours, then the indicated numbers of cells were inoculated subcutaneously into 4 mice each. All 4 mice in each group developed tumors, but Fig. 1 shows that the tumor progression was slower in the virus-immune node recipients.

Time of Appearance of Resistance after Virus Immunization.—Groups of 10 adult C57B1 mice each were given 10^4 TCID of polyoma virus intraperitoneally on the indicated number of days before all were challenged with 695 tumor. The results given in Table IX indicate that solid resistance developed

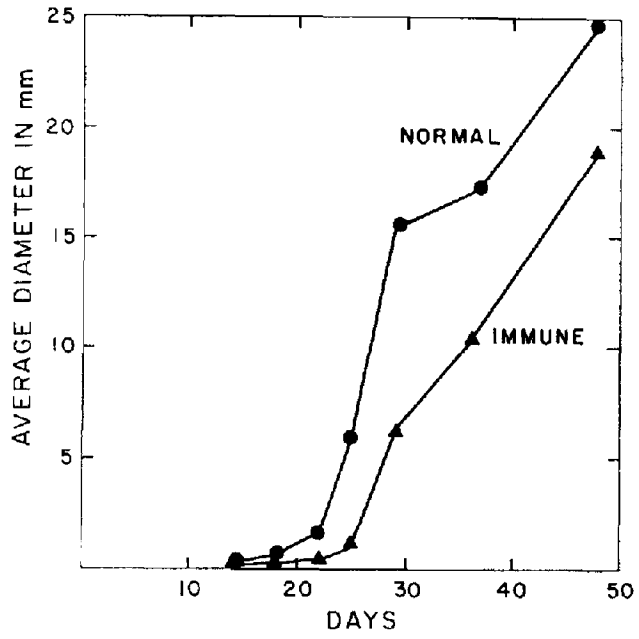

FIG. 1. Tumor cells incubated with lymph node cells from normal or virus-immune mice, then inoculated into adult mice. Rate of tumor growth.

between the 6th and the 10th day after virus inoculation. Some mice receiving virus on the same day as the tumor challenge also were resistant and one wonders if the virus may have multiplied in and destroyed the tumor cells, as we know from previous studies (2) it is capable of doing this *in vitro*. Two months following the first challenge, all survivors without tumors were rechallenged with 695 tumor. Those that had resisted a 10^4 challenge were inoculated with 10^5 cells, and those resistant to 10^5 were now given a 10^6 tumor cell challenge. In general, and especially in those given the 10^6 second challenge, it would appear that those groups most resistant to the first challenge were less resistant to the second. This is logical if we assume that at the time of original challenge these groups permitted little growth of the tumor before total rejec-

tion, and therefore, less opportunity for a booster antigenic effect. In these same groups, it is interesting that those mice that did resist the second challenge of 10^6 cells all developed small but definite tumors at the end of the 1st week which subsequently disappeared. The fact that there was increased resistance to the second challenge is further evidence for the existence of a tumor antigen to which the mice have reacted.

Resistance to Tumor Challenge in Mice Inoculated with Virus at Birth.—C57B1 newborn mice were inoculated intraperitoneally with 5×10^4 TCID of polyoma virus, and at 5 weeks of age were challenged with both 695 and 1923 tumors.

TABLE IX

Time of Appearance of Resistance to Tumor Transplant After Immunizing Inoculation of Polyoma Virus

Virus inoculation days before first challenge	No. of challenge tumor cells			
	First challenge		Second challenge*	
	10^4	10^5	10^5	10^6
0	0/5	2/5	0/5	1/3
1	0/5	5/5	0/5	—
3	0/5	4/5	4/5	0/1
6	0/5	2/5	1/5	2/3
10	0/5	0/5	3/5	3/5
15	0/5	0/5	2/5	4/5
20	0/5	0/5	0/5	3/5
30	0/5	1/5	1/5	1/4
Control—no virus	0/5	5/5	5/5	—

* Given 2 months after first challenge to those surviving without tumors. New Controls at this time had 5/5 at 10^5 and 2/5 at 10^4 challenge.

The results are given under "Virus-infected" group, included in Table IV. With both tumors there was some protection as compared with controls, but not the degree of resistance shown by virus-immunized adults.

Virus Challenge of Adults Made Tolerant to Tumor.—According to our original hypothesis of the immunological basis for resistance in virus-immune mice, an adult mouse made tolerant to the foreign antigen contained in the polyoma tumor should develop tumors when infected with virus. Six newborn mice made tolerant by inoculation of x-irradiated 695 cells at birth were inoculated intravenously with 5×10^6 TCID of polyoma virus when 32 days old. Six normal mice of the same age were similarly inoculated. Other mice inoculated with irradiated cells at the same time had been shown to be more susceptible to tumor challenge (Table IV). The mice inoculated intravenously with virus have been observed for 3 months to date with no evidence of tumors.

DISCUSSION

Adult animals that have been given an immunizing inapparent infection by inoculation with viable polyoma virus are subsequently resistant to a transplant of an isologous transplantable polyoma tumor. This phenomenon has been found by us in the case of three different polyoma tumors in one inbred strain of mice and in hamsters, and it has likewise been reported with 10 tumors in 4 strains of mice by Sjögren *et al.* (5) in Stockholm. In both laboratories, the transplantable polyoma tumors used as challenge were free of demonstrable polyoma virus. Furthermore, we have shown that this resistance is specific for tumors resulting from polyoma virus infection. In interpreting these findings, we have put forward the hypothesis that as the result of the inapparent virus infection of adult animals some normal cells are transformed to tumor cells just as in newborns, and that these transformed cells in both age groups contain a new "foreign" cell antigen. The new antigen is not recognized as foreign by the immunologically immature newborn so a tumor develops, but the immunologically capable adult recognizes the foreign antigen and rejects it, thus preventing tumor development and becoming sensitized to tumor antigen.

Evidence to support this hypothesis has been developed in the experiments reported here. The existence of a different or foreign cellular antigen in the polyoma tumors has been shown by the increased susceptibility to tumor challenge in animals that are immunologically incompetent; by the ability of tumor cell antigens to create tolerance in newborn mice to later tumor challenge; and, by the production of resistance to challenge after immunization with tumor antigens. Furthermore, it has been shown that the resistance of virus-immune animals is not mediated by serum antibodies, that antiviral antibodies are probably not involved and that this resistance like other transplantation immunity is cell-mediated by the immunologically competent spleen, bone marrow, and lymph node cells.

The experiments reported here and experiments in Dr. George Klein's laboratory in Stockholm (6) show that the "foreign" antigen present in polyoma tumors is similar from tumor to tumor in one inbred strain of mice, and also from strain to strain. However, our experiments have failed to show any antigenic relationship between polyoma tumors of mice and those of the hamster. This would suggest that by whatever intracellular mechanism the virus causes a normal cell to transform to a tumor cell, it permanently alters the genome to direct the production of a new and immunologically different cell antigen, and that the immunological structure of the tumor antigen is specific within a species as well as being specific for tumors produced by polyoma virus.

Although the tumor antigen must represent an *addition* of an antigen not present in the normal cell, it could still be the result of a *loss* of a genetic character such as the ability to form a certain enzyme, which loss is then

reflected in the production of a cell component of biochemical structure sufficiently different to represent a new antigen. However, once the virus has been responsible for the original transforming event, it may no longer be necessary or involved. From then on immunological factors may be limiting. Our present findings in part confirm a recently proposed hypothesis of Zilber (7), although he has suggested that the new tumor antigen caused by a virus might be the cause of increased proliferation.

Even though two simultaneously developing dynamic systems make interpretations of time relationships difficult, nevertheless, when adult mice were challenged with tumor transplants at various intervals after virus inoculation, resistance appeared at about the 6th day. This means that sensitization to tumor antigen develops quite rapidly in the virus inoculated adult mouse. According to our hypothesis, this would be due to the fact that transformed cells containing tumor antigen were present within a few days after virus inoculation. On the other hand, when newborn mice were inoculated with virus, then challenged with tumor as adults, they were found to be partially resistant. This appears to be inconsistent with our hypothesis. If virus-inoculated newborn mice develop tumors because they are immunologically immature at the time virus transforms normal cells, then they ought to be tolerant to the tumor antigen rather than resistant. However, this brings up some interesting questions concerning the quantitative aspects of transformation by virus in relation to time, especially in the newborn where only a few days can make a big difference in the animal's immunological capabilities. If the newborn animal has only a relatively few cells transformed by virus, the number of tumor cells, and, therefore, the antigenic mass of the tumor antigen may be too small to establish tolerance. These cells then will persist into the period of immunological maturity with eventual sensitization and rejection. Furthermore in all of our experiments, it has been apparent that the resistance of virus-immune adults is not complete, but can be overcome by a large enough challenge. Therefore, the balance between the quantitative efficiency of transformation and early tumor growth on the one hand, and the developing immunological competence on the other, will determine the fate of the tumor. This may also be the basis for differences in the ease with which tumors can be produced by polyoma virus in newborn mice of different inbred strains. It is interesting that the C57B1 mice used in our experiments are not highly susceptible to the tumor producing effects of the virus. Further timed experiments with mouse strains of high and low susceptibilities to viral oncogenesis are required to answer this question.

The demonstration of the immunological basis for the inability of polyoma virus to produce tumors in adult mice in spite of their high susceptibility to infection gives a logical explanation for the known facts concerning the ecology of polyoma virus in mouse populations under natural conditions. Although a

great deal of polyoma virus infection is spread in laboratory mouse colonies (8), as well as in wild mouse populations (9), a naturally occurring polyoma-induced tumor is an extreme rarity. Unless a newborn mouse were exposed to very high concentrations of virus, the quantitative and temporal factors discussed above might well result in the development of resistance. Perhaps the only way in which the mouse, naturally infected as an adult or infected with a small amount of virus as a newborn mouse, can develop a polyoma-induced tumor is by the chance occurrence of some event which temporarily reduces its immunological competence at the proper time after virus transformation of normal cells to tumor cells.

SUMMARY

Adult mice and hamsters can be made resistant to an isologous transplantable polyoma tumor by an inapparent infection with polyoma virus. This resistance is cell-mediated and seems not to be related to anti-viral serum antibodies. The basis of the resistance appears to be a transplantation type of cellular immunity directed against a "foreign" antigen contained in the tumor cell. Evidence has been presented to demonstrate this tumor antigen. It is possible that this phenomenon may explain the lack of oncogenesis by polyoma virus infection of adult mice, and the rarity of naturally occurring polyoma tumors.

BIBLIOGRAPHY

1. Habel, K., Resistance of polyoma virus immune animals to transplanted polyoma tumors, *Proc. Soc. Exp. Biol. and Med.*, 1961, **106,** 722.
2. Habel, K., and Silverberg, R. J., Relationship of polyoma virus and tumor *in vivo*, *Virology*, 1960, **12,** 463.
3. Habel, K. and Atanasiu, P., Transplantation of polyoma virus induced tumor in the hamster, *Proc. Soc Exp. Biol. and Med.*, 1959, **102,** 99.
4. Rowe, W. P., Hartley, J. W., Estes, J. D., and Huebner, R. J., Studies of mouse polyoma virus infection. I. Procedures for quantitation and detection of virus, *J. Exp. Med.*, 1959, **109,** 379.
5. Sjügren, H. O., Hellstrom, I., and Klein, G., Transplantation of polyoma virus-induced tumors in mice, *Cancer Research*, 1961, **21,** 329.
6. Klein, G., personal communication.
7. Zilber, L. A., On the interaction between tumor viruses and cells: A virogenetic concept of tumorigenesis, *J. Nat. Cancer Inst.*, 1961, **26,** 1311.
8. Rowe, W. P., Hartley, J. W., Law, L. W., and Huebner, R. J., Studies of mouse polyoma virus infection. III. Distribution of antibodies in laboratory mouse colonies, *J. Exp. Med.*, 1959, **109,** 449.
9. Rowe, W. P., The epidemiology of mouse polyoma virus infection, *Bact. Rev.*, 1961, **25,** 18.