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Dissociation of Multiple Polyoma Virus Functions by Ultraviolet Irradiation (33902)

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The polyoma virus has been associated with several activities. It produces cytopathic effects in mouse embryo tissue cultures (1), produces tumors in newborn mice and hamsters (2) and transforms tissue culture cells (3), induces tumor-specific transplantation antigens (4, 5) and complement-fixing antigens (6) in these cells and agglutinates erythrocytes (7). Most of these functions have been shown to be intimately associated with each other (7–9). Recent reports, however, have suggested that some of these functions may be partially dissociated from each other (10–15).

The results of the present study indicate that the individual functions of polyoma virus can be further separated by ultraviolet irradiation. It was found that the ability of polyoma virus to affect tumor-specific transplantation resistance in mice persisted at a dose of ultraviolet irradiation which produced loss of infectivity and oncogenicity.

Materials and Methods. Dulbecco's large plaque polyoma virus, PyD, was used in these studies. The source of ultraviolet irradiation was a Westinghouse germicidal lamp, G30T8, which delivered at least 95% of energy at 2537 Å. Five ml of the virus suspension (10^6 pfu/ml) was placed in a 100-mm petri dish on a mechanical shaker, 21.2 cm from the lamp. At this distance, the energy at the surface of the petri dish was 3900 ergs/mm^2/min. Infectivity of the polyoma virus was determined by inoculating 0.1 ml of dilutions of the virus suspensions into culture tubes containing mouse embryo cells. The cultures were observed for cytopathic effect (CPE) at 2-day intervals and the tissue culture infective dose (TCID) was determined. Tumorigenicity was determined by inoculating litters of golden Syrian hamsters with 0.1 ml of virus preparations 24 hr after birth. As an assay for immunogenicity, 8-week-old C57Bl/6 JN and C3Hf/HeN mice

TABLE I. Effect of Ultraviolet Irradiation on Infectivity and Tumorigenicity of Polyoma Virus.

UV Radiation		Infectivity[a]	
(min)	Energy (erg/mm²/min)	(TCID in 0.1 ml)	Tumor incidence[b]
0	0	10^5	15/15
0	0	10^{3c}	8/9
10	3.9×10^4	10^2	6/9
30	1.2×10^5	0	0/14

[a] Infectivity assayed with mouse embryo tissue culture cells.

[b] Ratio of the number of hamsters with tumors to the number inoculated.

[c] Unirradiated virus suspension tested at 1:100 dilution.

were inoculated with 0.1 ml of virus preparations. After 28 days, the animals were bled and the sera tested for hemagglutination inhibiting (HI) antibody (7). The animals were then tested for transplantation resistance by challenge with syngeneic polyoma tumor cells. The transplantable 695 polyoma tumor (16) was used for challenge of the C57Bl/6 mice. (This tumor has shown a recent change in our laboratory, with a decrease in the TD_{50} from 5×10^4 cells to 1×10^3 cells. The polyoma tumor specific transplantation and T antigen have been demonstrated to be still present.) Subcutaneous tumors were removed, finely minced, and then pressed through a 60-mesh stainless steel screen to obtain a single cell suspension. The transplantable 4198 tumor (17) of C3H was maintained in tissue culture and harvested with 0.25% trypsin. The viability of the cell suspensions was determined by trypan blue dye exclusion and varying doses of viable cells were inoculated subcutaneously. The animals were observed for tumor development for 2 months.

Results. Infectivity of the polyoma virus was markedly decreased by 10 min of UV irradiation and was undetectable after 30 min of irradiation (Table I). Tumorigenicity declined in parallel with the decreased infectivity (Table I). The tumor incidence was reduced after 10 min of radiation and the latency period for tumor appearance was prolonged (Fig. 1). Virus irradiated for 30 min produced no tumors.

The ability of the irradiated suspensions to induce HI antibody in mice was also determined (Table II). Virus irradiated for 10 min produced markedly reduced titers and virus irradiated for 30 min produced insignificant titers in most animals.

Challenge of these mice with transplantable syngeneic polyoma tumors gave a different pattern. Transplantation resistance was significantly affected by the irradiated virus, even at the radiation dose which produced complete inactivation by all the other parameters studies (Table III). In the C3H mice, the virus which had received the 10-min UV dose gave comparable protection as the unirradiated virus. The virus which had been irradiated for 30 min still gave protection at two challenge levels, but the effect was considerably weaker. In the C57Bl animals, a different effect on transplantation resistance was observed. Pretreatment with irradiated polyoma virus resulted in an increased incidence of tumor growth compared to the controls. Since the observed effect on transplantation resistance in these experiments was small, the experiments with both the C3H and C57Bl animals were repeated. Pretreatment with virus which had been irra-

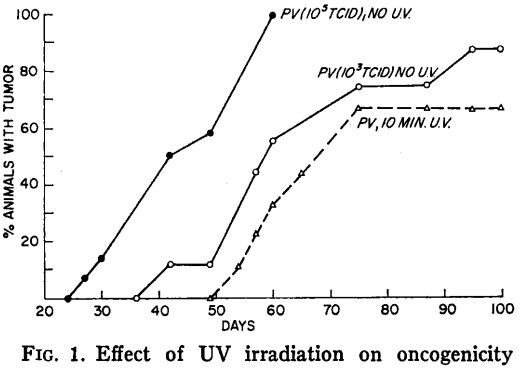

FIG. 1. Effect of UV irradiation on oncogenicity of polyoma virus, PyD, for newborn Syrian hamsters; percentage of tumor incidence is plotted against the number of days after virus inoculation. Two doses of unirradiated PV were given [10^5 TCID (●); 10^3 TCID (○)]. PV suspension, originally 10^5 TCID, was given 10 min UV irradiation (△); and 30 min UV irradiation (not shown; zero tumor incidence).

TABLE II. Polyoma Hemagglutination-Inhibiting (HI) Antibody Titer in C3H/HeN and C57Bl/6JN Mice.

Strain	Treatment	No. of mice with HI antibody titers: <100	100	200	400	800	1600	3200	Mean HI titer
C3H	None	5	1						100
	Virus						1	4	2680
	Virus, UV 10 min			1	2	1	1		680
	Virus, UV 30 min	2	2	1					130
C57Bl	None	5							<100
	Virus						4	1	1520
	Virus, UV 10 min		1	2	1	1			340
	Virus, UV 30 min	5							<100

diated for 30 min again produced some protection in C3H mice and enhanced tumor growth in C57Bl.

Discussion. The finding that ultraviolet irradiation of polyoma virus caused a decrease in oncogenicity is consistent with the known effects of UV radiation on transformation *in vitro* (10–12). The decreased oncogenicity, however, is quite different from the results of Defendi and Jensen (15). They found that UV irradiation produced an increased incidence of tumors. The reasons for these apparently contradictory results are not clear. The amount of UV irradiation used in the present study was higher than that used by Defendi and Jensen. The polyoma virus strain used here was more oncogenic for newborn hamsters (100% incidence at 10^5 TCID) than the strain used in their study (60% incidence at $10^{7.5}$pfu). One hypothesis proposed by Defendi and Jensen to explain their results was that irradiated virus produced tumors in hamsters which may have been defective for the specific transplantation antigen and that growth would therefore be less impaired by the host's immune response. Enhanced tumor growth due to such a mechanism may have masked the partial inactivation of the viral oncogenicity. The strain of polyoma virus used in the present study has been poorly immunogenic in hamsters (18) and immunologic interactions may have had less of an effect on the results.

The marked decrease in the titers of HI antibody induced in mice by the irradiated virus is of interest. The hemagglutination property of polyoma virus is quite stable (7, 12, 15) and it is very unlikely that doses of UV radiation used caused alterations of this structure in the viral coat. The probable explanation is that the polyoma virus may have to retain infectivity and be able to replicate in mice to present an adequate immunogenic dose. Indeed, the studies of Rowe *et al.* (8) indicated that mouse antibody titers paralleled infectivity titers.

The persistent ability of the irradiated polyoma virus to affect transplantation resis-

TABLE III. Resistance to Tumor Challenge after Immunization with Irradiated Virus.

Immunization	No. of challenge tumor cells: C3H mice: 4198 tumor 5×10^3	5×10^4	5×10^5	C57Bl/6 mice: 695 tumor 3×10^2	1×10^3
Polyoma virus	0/5[a]	0/5	0/5	0/5	0/6
Polyoma virus, 10 min UV irradiation	0/5	0/5	0/5	3/5	3/6
Polyoma virus, 30 min UV irradiation	1/5	2/5	4/5	4/5	4/5
Control	3/4	3/6	4/5	1/5	2/6

[a] Number developing tumors/number inoculated.

tance represents the greatest dissociation of viral functions found in this study. After 30 min of UV irradiation, this was the only function detected in the virus preparation. The results indicated that transformation of cells *in vivo* may not be an essential event in the induction of the polyoma-specific transplantation antigen (19). Habel postulated that infectious multiplying virus was required to produce resistance. This hypothesis was based partially on the observation that repeated inoculations with UV inactivated virus were not effective in producing transplantation resistance to challenge by 695 tumor in C57Bl/6 mice (9). In the present study, one inoculation of inactivated virus was effective in producing transplantation resistance, but only in C3H mice and not in C57Bl/6 mice. The UV-inactivated virus produced apparent enhancement of tumor growth at two tumor challenge levels of 695 tumor cells in C57Bl mice. One possible explanation is that the inactivated virus was capable of inducing the specific transplantation antigen in C57Bl and that enhancing antibodies were produced. The presence of enhancing serum antibodies could account for the lack of protection observed by Habel and the increased tumor incidence observed here. This enhanced incidence of tumors produced by the irradiated virus emphasizes the potential hazard of the use of inactivated tumor virus vaccines for immunoprophylaxis. These results also emphasize the usefulness of ultraviolet irradiation in dissociating the multiple functions of an oncogenic virus.

Summary. Ultraviolet irradiation of polyoma virus produced differential inactivation of individual viral functions. Infectivity, oncogenicity, and ability to induce HI antibody declined in parallel. The ability of polyoma virus to affect tumor-specific transplantation resistance persisted at a dose of ultraviolet irradiation which resulted in loss of the other viral functions.

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