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Comparison of the Rates of Ultraviolet Inactivation of the Capacity of Type 12 Adenovirus to Infect Cells and to Induce T Antigen Formation

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Active infection of various mammalian cells with the oncogenic adenovirus type 12 leads to the early production of a new antigen which has been termed T (or tumorlike) antigen. This antigen is similar, immunologically, to a new antigen found in tumors induced by type 12 adenovirus, as well as in cells transformed in vitro. The antigen is distinct from the viral structural proteins and is made when synthesis of host or new viral deoxyribonucleic acid (DNA) is blocked by 5-fluorodeoxyuridine (FUDR); it can be detected by complement fixation with sera from hamsters bearing virus-induced tumors free from detectable virions (Z. Gilead and H. S. Ginsberg, J. Bacteriol. **90**:120, 1965).

TABLE 1. *Effect of ultraviolet irradiation on viral infectivity and capacity to induce synthesis of T antigen*

Length of irradiation[a]	Survivors	Corresponding T antigen titer
min	*PFU/ml*	
0	3×10^{10}	1:32
0.5	6×10^{9}	—
1	1.2×10^{9}	1:16
2	1.2×10^{8}	1:8
3	1×10^{7}	—
4	4×10^{5}	1:2
6	2×10^{5}	<1:2

[a] Irradiated at a distance of 20 cm with a Westinghouse germicidal lamp.

The data currently available imply that the information for production of the T antigen resides in the infecting adenovirus genome. To test this hypothesis, further experiments were devised to determine whether the virus's capacity to induce the formation of the T antigen was restricted when the integrity of the viral template was damaged. Ultraviolet irradiation was employed, since studies with highly purified P^{32}-labeled virus (W. C. Lawrence and H. S. Ginsberg, *unpublished data*) indicate that ultraviolet-irradiated virus adsorbs to cells and uncoats normally. The experimental results to be described suggest that the ability to induce the T antigen is sensitive to ultraviolet irradiation, presumably through damage to the viral DNA.

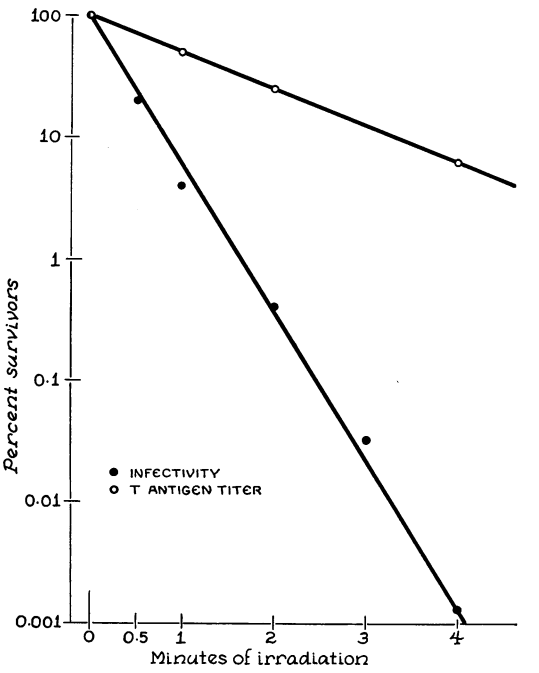

FIG. 1. *Comparison of the rates of ultraviolet inactivation of viral infectivity and the capacity to produce T antigen. Purified type 12 adenovirus was irradiated at a distance of 20 cm with a Westinghouse germicidal lamp. The infectivity of each sample was assayed by plaque assay. Production of T antigen upon infection with the irradiated virus was tested in spinner cultures of KB cells and was assayed by complement fixation.*

Methods for purification of virus, the infectivity assay, and complement-fixation titration of the T antigen were described previously (Z. Gilead and H. S. Ginsberg, J. Bacteriol. **90**:120, 1965). A

freshly purified type 12 adenovirus preparation was passed through a Sephadex G-25 column to free it from CsCl. It was then diluted with phosphate-buffered saline so as to obtain an approximate titer of 2×10^{10} plaque-forming units (PFU) /ml, based on absorbance at 260 mμ, with the use of the previously established value of 1 optical density unit at 260 mμ = 2×10^{10} PFU/ml. Samples (0.5 ml) of this preparation were stirred in a 60-mm petri dish with a magnetic flea and were irradiated at a distance of 20 cm with a Westinghouse germicidal lamp for various lengths of time. The infectivity of each sample was determined by plaque assay. All samples were diluted similarly to contain approximately 4×10^8 viral particles (infectious plus inactivated for irradiated samples) for the infection of 50-ml spinner cultures containing 10^7 KB cells. (Care was taken to use low multiplicities so as to avoid multiplicity reactivation.) The cells were cultured in 10% dialyzed chicken serum in the presence of 10^{-6} M FUDR. At 18 hr after infection, when the synthesis of T antigen had stopped, the cultures were centrifuged, and were washed once in phosphate-buffered saline. The pelleted cells were suspended at a concentration of 5×10^6 cells per milliliter; they were then treated in an MSE ultrasonic disintegrator at 0 C, and the titer of T antigen was measured (Table 1).

The two inactivation rates are plotted in Fig. 1 as percentage of the control. Both curves obey single-hit kinetics. A precise comparison cannot be made between the size of the genome locus required for viral multiplication and that essential for production of the T antigen, owing to (i) the small range in which the T antigen could be measured, and (ii) the quantitative limitations of the complement-fixation technique employed. Nevertheless, a comparison of the inactivation rates of viral infectivity and T antigen production indicates that the target size of the genome locus responsible for the synthesis of the T antigen is significantly smaller than that which is essential for viral replication, and suggests that their sizes differ by a factor of the order of 4. Similar results were reported for the target size of the locus responsible for the formation of the simian virus 40 (SV 40) T antigen (R. I. Carp and R. V. Gilden, Virology **27**:639, 1965).

Studies with inhibitors such as actinomycin D, puromycin, and cycloheximide (Z. Gilead and H. S. Ginsberg, *unpublished data*) indicate that the production of the adenovirus T antigen, similar to SV 40, requires synthesis of ribonucleic acid and protein. These results and the data reported in this communication strengthen the hypothesis that the T antigen is a product of genetic information contained in the viral DNA.

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