Critical Factors in Successful Recovery of Rous Sarcoma Virus from Turkey Tumors

Abstract. Studies of tumor cell cultures with antiviral antibody showed that most of the cell-associated virus and viral antigen were found at the cell membrane and were thus accessible to the effect of neutralizing antibody. Humoral antibody present in tumor tissue neutralized cell-associated virus in vivo and thus rendered the tumor cells temporarily noninfectious. When these cells were grown in vitro in the absence of antibody, virus eventually reappeared. However, the time of reappearance, amount of virus produced, and persistence of virus in such cultures depended upon the amount of virus used to produce the tumor.

Electron microscope studies (*1*) have shown that virus particles are extracytoplasmic and are located at the cell membranes and in vacuoles of chicken sarcoma cells. When such cells were stained with fluorescent antiviral antibody, viral antigen was located mainly at the cell membrane and in the cytoplasm (*2*). Our studies with fluorescein-labeled (*3*), as well as unlabeled, turkey antiviral antibody revealed the following picture with turkey sarcoma cells in culture: In fluorescent antibody stained cells the bulk of the antigen was found at the cell membrane with much less in the cytoplasm and vacuoles. However, when cells were grown in the presence of antiviral antibody and washed extensively with saline, only a few cells showed finely granular fluorescence in the cytoplasm and none at the cell membrane. In addition, such cells, when sonically disrupted, always contained less than 10 percent of the amount of infectious virus found in comparable numbers of tumor cells grown in the absence of antiviral antibody—for example, 1.7 log ED_{50} as compared to 4.0 log ED_{50}. Thus, infectious virus associated with tumor cells is mainly extracytoplasmic and consequently is accessible to circulating antibody.

The amount of virus recoverable from homogenates of tumor tissue from both chickens (*4, 5*) and turkeys (*6*) was found to be directly related to the infecting dose and varied greatly even within the same dose group. In the latter instance, failure to recover virus was frequently associated with the presence of antiviral antibody (*6*). The studies described below (*7*) show that virus can be successfully recovered from such noninfectious tumors when the tumor cells were grown in tissue culture in the absence of antibody. Briefly, our procedure was as follows: 0.2-ml amounts of suitably diluted standard virus (*8*) were injected into the wing web of turkeys 5 to 7 days old. At various times after infection, the birds were bled and their tumors were dissected. Each tumor and serum was processed individually. All infectivity titrations and serum neutralization tests (*6*) were carried out in eggs, by using an improved pock-counting technique (*9*). Each serum was inactivated (56°C for 30 min) and assayed for the presence of antiviral antibody. The tumor was washed and minced. Part of the tissue was homogenized as a 10-percent suspension and titrated for infectivity. The remainder of the tumor tissue was washed thrice with balanced salt solution and exposed to 0.25 percent trypsin at 37°C for 1 hr. The resulting cell suspension was washed thrice and resuspended in complete medium (*10*) to a concentration of 1×10^6 cells per milliliter. A sample was removed for sonic oscillation and subsequent infectivity titration. The cells were then distributed into bottles and incubated at 40°C. The cultures were fed twice a week, and culture supernatants were collected at intervals and assayed for infectivity. Under these conditions, the cells of most tumors grew well for as long as 2 to 3 mo and were subcultured at intervals of 1 to 3 wk.

Table 1. Selected examples of the effect of antibody and infecting dose on recovery of virus from turkey tumors. All numbers are logarithms.

Infecting dose*	Serum† neutralization titer	Infectivity titer* of						
		Tumor‡ homogenate	Washed tumor cells§	Tissue culture supernatant collected on day:				
				2	8	22	36	50
4.3	0.1	>5.7	4.4	3.7	4.0	3.0	3.8	3.2
4.3	2.9	<0.3	<0.3	<0.3	4.0	3.0	3.0	2.8
0.3	2.5	<0.3	1.1	<0.3	3.9	<0.3	2.0	<0.3
0.3	2.3	<0.3	0.4	<0.3	2.4	<0.3	<0.3	<0.3
0.03	3.4	<0.3	<0.3	0.6	<0.3	<0.3	<0.3	1.0
0.3	2.5	<0.3	<0.3	<0.3	<0.3	<0.3	1.1	0.6

* ED_{50} per milliliter. † Diluted 1:10. ‡ 10 percent tumor tissue extract. § Sonically disrupted cells obtained from trypsinized tumor tissue.

The data in Table 1 show that, even when tumors were produced with large amounts of virus, circulating antibody, when present, not only rendered tumor homogenates noninfectious but also neutralized virus associated with intact cells. Large numbers of such cells, when washed free of antibody and sonically disrupted, were noninfectious. Inoculation of the same numbers of intact cells gave similar results. The data further show that several days in an antibody-free environment were required for the cells to produce sufficient quantities of new virus to permit successful recovery. When small amounts of virus were used to produce tumors, virus was eventually released in low titer and in an unpredictable and intermittent fashion in cell cultures from each of 14 turkey tumors and three chicken tumors analyzed. Several cultures of trypsinized normal wing web cells from tumor-bearing turkeys were carried in tissue culture along with the tumor cell cultures and were tested for the presence of virus at the same time as the latter. None were positive, although these cultures were fully susceptible to infection. The previous report (*5*) of failure to recover virus in vitro from noninfectious chicken tumors was probably due to suboptimal conditions of cell cultivation. It is hoped that these findings will be of value in efforts to isolate oncogenic viruses from naturally occurring tumors in man and other animals.

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References and Notes

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7. These studies were supported by grants from the American Cancer Society and the National Cancer Institute.
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10. The medium consisted of the following: Scherer's maintenance solution, 68 percent; tryptose phosphate broth (Difco), 20 percent; inactivated calf serum, 10 percent; beef embryo extract, 2 percent; penicillin G, 100 units per milliliter; streptomycin, 100 μg/ml; *p*H adjusted to 7.6 with 7.5-percent sodium bicarbonate solution.

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