Propagation of Columbia SK Virus in Ehrlich Ascites Tumor Cells with Oncolysis. (25614)

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Virus propagation in Ehrlich ascites tumor cells first reported by Koprowska and Koprowski(10) included the Mengo and EMC viruses of the Columbia SK group. The severe oncolysis was confirmed by Flanagan and Colter(3), and Levy and Snellbaker(11). Growth of these viruses and the F virus(8) and the MM virus(12) in cell culture also produced cell damage. Only Columbia SK virus of this group has failed to reproduce or to show cytopathogenic effects in tissue culture, though it may interfere with growth of other viruses(9). We now report the propagation of the SK virus in tumor cells with severe oncolysis.

Materials and methods. Ehrlich ascites cells were inoculated with Columbia SK virus from mouse brain passage. A mixture of 0.1 cc of 10^{-2} dilution of original brain suspension containing about 10^6 LD_{50} of virus and 0.3 cc of Ehrlich ascites fluid (cells) was inoculated into the peritoneal cavity of 3 to 5 mice. Before the mice died 3 to 5 days after inoculation, the infected ascites were aspirated, the cavity washed with 2 cc of saline, and 0.1 cc of ascites-saline fluid was inoculated, together with 0.3 cc of fresh Ehrlich cells, into new mice. This procedure was repeated through 4 generations. In fifth and subsequent generations the procedure was altered as follows: Infected ascites were centrifuged and 0.1 cc of 10^{-3} dilution of the supernatant containing no cells used as the next inoculum. Transmission was followed for 27 generations. Control mice were similarly inoculated with 0.1 cc of saline mixed with 0.3 cc of Ehrlich cells in place of the virus-Ehrlich cell mixture. Smears were prepared of each ascitic fluid, fixed with methanol, then stained with Giemsa solution and degree of cell damage observed. Some preparations were stained with acridin orange and studied in the fluorescent microscope(7). At each generation number of cells in the ascitic fluid was counted, and titer of virus and hemagglutinin calculated. For the hemagglutination and antihemagglutination tests, 1% of sheep red cells suspended in Mayer's barbital buffered saline was used(6). Anti-SK serum was made in rabbits by several injections of the source virus from mouse brain.

Results. 1. Oncolytic effect of Columbia SK virus on Ehrlich tumor cells. At the first inoculation with the virus from mouse brain, there was moderate cell degeneration, chiefly an increase of vacuoles in the tumor cells. This was much less pronounced in the second, third and fourth generations, but during these generations blind passages were continued. At the fifth generation the infected ascites became semi-transparent, with a decrease in cell count, and in smear preparations severe cell damage to most of the cancer cells was seen. The nuclei were pycnotic and karyorrhectic, the nucleoli had disappeared and the chromatin was rough. Many cells were of irregular shape, and in others the cell wall disrupted with freed nuclei. Many vacuoles were observed in the nuclei and cytoplasm of the cells considered infected. Cytoplasm of the damaged cells stained poorly and very little orange fluorescence, specific for ribonucleic acid, could be seen after staining with acridin orange. No specific findings such as inclusion bodies or cytoplasmic masses could be observed. From the 5th to the 27th (present) generation, except for the 10th, destruction of tumor cells mentioned continued in from moderate to severe degree (Table I). Cell counts made at the 9th and at a number of subsequent generations showed a marked diminution in number of cells (Table I). The mice were usually paralyzed by the virus infection by the 4th day and dead on the 4th to 5th day. Fig. 1 shows the typical cytopathogenic changes.

2. Hemagglutination of infected ascites. Agglutination of sheep red cells by ascitic

TABLE I. Oncolytic and Hemagglutination Effects of Columbia SK Virus on Ehrlich Tumor Cells.

No. of passage	Oncolytic effects 3-4 days after infection: Cell destruction	Oncolytic effects 3-4 days after infection: Total No. of cells in fluid	HA titer of cell-free ascitic fluid
Control	—	10^{7-8}	0
1	—		
2	—		
3	—		4
4	—		4
5	+		128
6	+	10^{6}	32
7	+	10^{6}	64
8	+		
9	+	3×10^{6}	
10	—	10^{8}	8
11	+	2×10^{6}	
12	+		32
13	+		16
14	+	5×10^{5}	16
15	+	10^{6}	256
16	+	4×10^{6}	
17	+	5×10^{5}	16
18	+		
19	+	10^{5}	256
20	+	5×10^{5}	
21	+	10^{5}	
22	+		
23	+	3×10^{6}	
24	+	3×10^{6}	
25	+	10^{5}	256
26	+	10^{6}	

fluid was titrated by the method of Horvath and Jungeblut(6); no hemagglutinin was detected with either the cell-free portion of normal ascitic fluid or the resuspended cancer cells. Hemagglutinins were detectable in varying titer in the infected ascitic cell-free fluid (Table I). Maximum titer was 1:256, noted in the 16th, 19th and 25th generations. Hemagglutinin titer in the tumor cells (when liberated by cell destruction using dry ice and acetone) was higher than in the cell-free fluid: 1:128 as compared with 1:32 at the 12th generation, and 1:32 as compared with 1:12 at the 13th generation.

3. Virus titration of infected ascites. Infected fluids diluted to 10^{-3} at the 5th, 8th and 9th generations were inoculated intraperitoneally without cancer cells, with survival of all the mice. However, when the inoculations were accompanied by uninfected cancer cells, all the mice became paralyzed within 5 days. The titer of active virus contained in cell-free fluid of the 7th generation was 10^{5} LD_{50} per 0.1 ml when it had been inoculated with Ehrlich cells, but only 10^{2} LD_{50} when inoculated intraperitoneally without cancer cells. This phenomenon was presumably due to adaptation of virus to the Ehrlich cells and to its more rapid propagation in them. When the virus was injected intraperitoneally and the cancer subcutaneously, or conversely, evidence of virus infection, *i.e.*, paralysis, was delayed for 3 to 5 days. Results with disrupted cells were the same as with the cell-free ascitic fluid. Amount of intracellular virus in both the 7th and the 15th generations was 10^{5} LD_{50} per 0.1 ml after cell destruction using dry ice and acetone.

4. Antihemagglutination tests. The antigenicity of the adapted strain was tested with

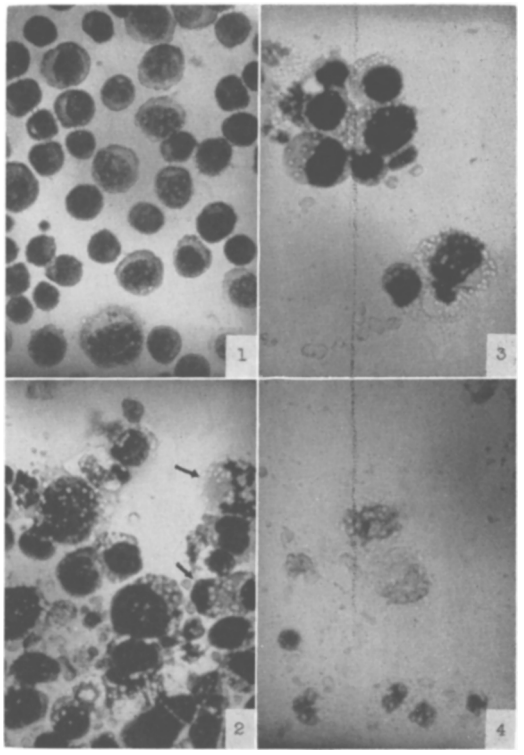

FIG. 1. Oncolytic changes produced in Ehrlich ascites cells by infection with adapted Columbia SK virus. 1. Normal Ehrlich ascites cells. 2. Infected cells (11th generation, 3 days after infection). Many vacuoles in most of the cells, and even in the mitotic cells (arrows). 3. Infected cells (27th generation, 3 days after infection) showing vacuolar degeneration. 4. Infected cells (27th generation, 4 days after infection) showing oncolysis.

TABLE II. Effect of Immune Serum on Oncolysis, Neutralization and Anti-Hemagglutination.

Serum	Oncolysis	Total cell count	Anti-HA titer†	Virus titer neutralized
Normal*	+	3×10^6	1:2	0
Immune*	—	3×10^7	1:640	10^{-3}‡

* Using 10^{-1} dilution of sera adsorbed with Ehrlich cells.

† Using 4 units of hemagglutinin of 19th infected ascites.

‡ 0.1 cc of 10^{-3} dilution of 19th passage ascites, containing about 10^2 LD_{50} of virus, was neutralized by 0.1 cc of 10^{-1} dilution of immune serum.

anti-SK rabbit serum made by the subcutaneous infections of mouse brain virus once a week for 4 weeks. In the last inoculum the virus was mixed with Freund adjuvant. The anti-HA titer of the serum from rabbits inoculated with the adapted strain was 1:64 after the third inoculation (preceding the Freund adjuvant); after the fourth inoculation it was 1:1280.

5. Neutralization tests. After adsorption (using the Ehrlich cells) of non-specific antibody against mouse tissues, a 10^{-1} dilution of the immune serum was mixed with a 10^{-3} dilution of infected fluid from the 19th generation plus Ehrlich cells. This mixture was then inoculated into mice. Control mice were injected with the same dilution of virus and Ehrlich cells in normal rabbit serum. After 3 days all control mice became paralyzed, and the cancer cells showed great destruction, while mice inoculated with the immune serum mixture were healthy and the cancer cells escaped oncolysis (Table II). Ehrlich cells mixed with the infected ascitic fluid (21st generation) were free of oncolysis when inoculated into the abdominal cavities of mice immunized by injection of ultraviolet-inactivated brain virus once a week for 4 weeks, showing similar antigenicity in the original brain virus and the adapted strain.

6. Trials against cell cultures of Sarcoma 180. To study the cytopathogenicity of the adapted SK virus, infected ascites from the 19th, 24th and 25th generations were inoculated into established cell cultures of Sarcoma 180 growing in Medium 199 with 5% of calf serum. No cell destruction occurred. The 19th inoculum was passed at 4-day intervals through 3 generations at 10^{-1} dilution, but no cytopathogenic effect appeared. Third generation inoculum was injected into mice together with Ehrlich cells, but none of the mice became paralyzed.

Discussion. The evidence that the virus was adapted to and reproduced in the tumor cells lies principally in the fact that the adapted virus alone had a low infectivity titer, but when given with Ehrlich tumor cells was highly lethal to the mice. Thus, adapted virus showed a titer of 10^5 LD_{50} per 0.1 ml when inoculated with tumor cells, but only 10^2 LD_{50} when inoculated without such cells. This thousand-fold difference may be interpreted as indicating that 99.9% of the virus was adapted to the Ehrlich cells and was incapable of producing death without passage through such cells.

Adaptation of other viruses to Ehrlich ascites cells has been accomplished or attempted: Influenza by Ackermann(1), NDV by Flanagan, Love and Tesar(4), Mengo by Flanagan and Colter(3), and vaccinia by Cassel(2) and by Furusawa, Toyoshima and Miyagawa(5).

Summary. Propagation of Columbia SK virus in Ehrlich ascites cells *in vivo* is described. After several passages an adapted strain of SK virus having an oncolytic effect was obtained. The adapted strain was relatively non-pathogenic for mice unless accompanied by Ehrlich cells, in which it could propagate freely. This strain, after many passages, maintains ability to agglutinate sheep red cells and is neutralized by antiserum against the original brain virus. The strain shows no cytopathogenicity to Sarcoma 180 cells in tissue culture.

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Potentiation of Chlorpromazine by Insulin.* (25615)

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Soon after its tranquilizing effect had been described(1) chlorpromazine was shown to decrease significantly the oxygen consumption of the whole animal(2). This effect combined with inhibition by chlorpromazine on the heat regulating center of the hypothalamus(1), and with the increased peripheral heat lost(3) constitute the main factors responsible for the marked hypothermia in animals injected with chlorpromazine. Other studies, which have shown that chlorpromazine inhibits activities of both cytochrome oxydase and adenosine triphosphatase(4,5,6) indicate that the decrease in metabolism caused by chlorpromazine may be due to some disturbances in metabolic pathways. Similarly insulin is also known to cause some inhibition in metabolic pathways as evidenced by its effect on hexokinase (7). It is also generally accepted that the depletion of substrate for cerebral metabolism, which is caused by insulin, is the sole explanation for the neural effect of this insulin on the brain and for its use in psychiatry(8). For these reasons it was postulated that chlorpromazine and insulin could potentiate one another on their effect on oxygen consumption or body temperature and on sleeping time of short-acting barbiturates such as hexobarbital.

Materials and methods. Two experiments were performed. In the first male albino rats, weighing 200 to 250 g, were used. The criterium for evaluating response to chlorpromazine was changes in rectal temperature as estimated by inserting a thermistor to a depth of 40 mm. Blood glucose determinations were also made on some groups of the first experiment. In *this experiment* 4 groups of 6 rats were used. Group 1 received chlorpromazine† (CPZ) (1 mg/kg); Group 2, CPZ (5 mg/kg); Group 3, CPZ (10 mg/kg); Group 4, CPZ (1 mg/kg) plus insulin regular (IR) (4 I.U./kg); Group 5 CPZ (5 mg/kg) plus IR (4 I.U./kg); and Group 6, CPZ (10 mg/kg) plus IR (4 I.U./kg). In the *second experiment* 4 groups of mice weighing 20 g were used. All 5 groups received hexobarbital (100 mg/kg). In addition Group 2 received IR (4 I.U./kg), Group 3 CPZ (1 mg/kg) and Group 4 regular insulin (4 I.U./kg) plus CPZ (1 mg/kg). All substances were injected intraperitoneally. Insulin and CPZ were injected, one hour and one half-hour respectively, before injection of hexobarbital. Sleeping time was the length of time between loss and reestablishment of righting reflex(9).

Results. A. *Potentiation of chlorpromazine hypothermia by insulin.* The results obtained in the first experiment are illustrated in Fig. 1 and 2. Fig. 1 shows that if the dose of chlorpromazine is sufficient to induce hypothermia (5 and 10 mg/kg), insulin potentiates this drop in body temperature. Furthermore, a dose of chlorpromazine of 5 mg/kg is potentiated sooner than a higher dose (Fig. 2); however insulin potentiates for a longer time a larger dose of chlorpromazine (10 mg/kg). In other words insulin by itself has no effect on body temperature but it greatly potentiates the hypothermia of chlorpromazine. Blood sugar determinations indicate that

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