Superinfection of Tumors with Viruses

The use of oncolytic viruses for the destruction of malignant tumors is based on the cytocidal effect of the virus undergoing full infectious cycles of replication within the tumor cells[1,2]. Viruses with frank or potential neurotropism have proved to be most damaging to tumors[3,4]. Viruses that replicate in the cytoplasm and leave the host cells by a budding process seldom cause immediate cell death; virus-infected cells may survive and continue to release virus. Thus, virus-carrier tumor cells may be established. The weak histoincompatibility of tumor cells may be reinforced by the added antigenicity of the superinfecting virus and an enhanced tumor rejection may thus be elicited. In the system established by LINDENMANN and KLEIN[5], a neurotropic influenza virus destroyed ascitic Ehrlich carcinoma cells in mice genetically resistant to the virus. Survivors of the oncolytic process exhibited strong immunity to challenge with tumor cells. EATON et al.[6] demonstrated oncolysis of Gross leukemia virus carrier lymphoma cells by Newcastle disease virus (NDV). Preimmunization of mice with NDV increased the rate of rejection of challenging lymphoma cells that carried NDV antigen.

Materials and methods. Malignantly transformed cultures of murine cells harboring an attenuated mouse leukemia virus were used[7,8]. These cells grow as sarcoma in mice. Tumor-bearing mice do not develop leukemia. Young adult mice may reject such tumors, if fewer than $10^{3.7}$ cells are inoculated. Mice inoculated at birth succumb to continuous tumor growth. A neurotropic variant of the RO California strain of NDV[9] was used to superinfect tumor cells. This virus undergoes full infectious cycles of replication in the brain of new-born mice causing fatal encephalitis. In adult mice inoculated intracerebrally, 'toxic encephalopathy' but no infectious replication of this virus occurs.

Results and discussion. Table I summarizes 4 experiments performed with non-inbred Timco Swiss mice. In the first experiment, malignantly transformed leukemia virus carrier cells of Timco Swiss murine tissue culture line No. 479 were superinfected with NDV and passaged once in vitro. Three groups of new-born mice were inoculated i.p. with $10^{4.7}$ live cells per mouse. The first group received the cells without superinfection with NDV. The second group received cells superinfected with NDV and suspended in heat-inactivated mouse serum containing NDV-neutralizing antibodies. The third group was given cells superinfected with NDV and suspended in normal mouse serum. The second group of mice received 2 additional doses of anti-NDV immune serum; the third group was treated twice with normal mouse serum. Mice of the second group succumbed to tumors faster than those of the first and third group. Mice of the third group displayed a prolonged course of tumor growth. A statistical analysis using the Wilcoxon test clearly shows that group 1 and group 2 differ significantly ($P = 0.00034$); also significantly different are group 1 and group 3 ($P = 0.016$) and group 2 and group 3 ($P = 0.00054$). In the second experiment, 10-day-old mice were inoculated i.p. with cultured cells of a tumor that originated in mice after inoculation with No. 479 cells (protocol No. 1078). Culture No. 1078 was passaged in vitro twice, both as superinfected with NDV and not superinfected sublines. Mice inoculated with $10^{4.3}$ cultured cells of the NDV-superinfected subline succumbed to tumors earlier than those inoculated with cells of the subline not superinfected. The blood serum of 2 mice of the group inoculated with NDV-superinfected cells fully neutralized the 10^{-1} dilution of NDV containing

[1] A. E. MOORE, Progr. exp. Tumor Res. *1*, 411 (1960).
[2] J. SINKOVICS, *Die Grundlagen der Virusforschung* (Hungarian Academy of Sciences, Budapest 1956), p. 98.
[3] W. A. CASSEL and R. E. GARRETT, Cancer *20*, 433 (1967).
[4] J. G. SINKOVICS, Arch. ges. Virusforsch. *7*, 403 (1957).
[5] J. LINDENMANN and P. A. KLEIN, *Immunological Aspects of Viral Oncolysis* (Recent Results in Cancer Research 9, Springer Verlag, New York 1967), p. 18.
[6] M. D. EATON, J. D. LEVINTHAL and A. R. SCALA, J. natn. Cancer Inst. *39*, 1089 (1967).
[7] J. G. SINKOVICS, F. GYORKEY, G. GROVES and P. GYORKEY, Arch. ges. Virusforsch. *23*, 169 (1968).
[8] J. G. SINKOVICS, B. A. BERTIN, G. F. GROVES and C. C. SHULLENBERGER, J. infect. Dis., in press, January 1969.
[9] J. G. SINKOVICS, Arch. ges. Virusforsch. *10*, 103 (1960).

Table I. Length of life and occurrence of tumor in mice inoculated with neoplastic tissue culture cells with or without superinfection with NDV

Experiment No.	Material inoculated	Mice inoculated				Average day of death
		Age	Total	Found with tumor	Found without tumor	
1	No. 479 cells	Nb	9	9	0	44.7 (42–49)
	No. 479 cells + NDV + imm serum	Nb	9	9	0	35.4 (34–40)
	No. 479 cells + NDV + norm serum	Nb	8	8	0	55 (42–71)
2	No. 1078 cells	Suckl	4	4	0	71 (24–119)
	No. 1078 cells + NDV	Suckl	6	6	0	30.5 (24–39)
3	No. 1078 cells	Nb	5	5	0	21.4 (21–23)
	No. 1078 cells + NDV	Nb	7	7	0	30.7 (22–40)
4	No. 1078 cells	Nb	10	10	0	26 (killed)
	No. 1078 cells + NDV	Nb	16	13	3	26 (killed)

Nb = new-born; Suckl = 10-day-old.
Passage 138 of culture No. 479 was used; both malignant transformation of cultured cells and attenuation of leukemia virus occurred after the 100th passage[7,8].

Table II. Oncolytic effect of VSV on a murine lymphoma

Experiment No.	Tumor cells	No. of cells inoculated per mouse	Superinfecting virus	Mice dead with lymphoma (day of death of individual mice)	Average day of death	Mice rejecting lymphoma (day individual mice found healthy)	Total: Lymphoma inoculated
1	No. 620	approx. 10^6		19 20 20 21 47 69	32.6	–	6/6
	No. 620	approx. 10^6	NDV	19 28 47 47	35.2	218	4/5
2	No. 620	approx. 10^6		27 28 29 30 30	26.8	–	5/5
	No. 620	approx. 10^6	VSV	29 30	29.5	188 188 188	2/5
3	No. 818	$10^{5.3}$		17 17 17 17 18 34	20	–	6/6
	No. 818	$10^{5.3}$	VSV	17 17 28	20	216 216	3/5
	No. 818	$10^{5.3}$	NDV	4 13 18 20 54	21.8	–	5/5
4	No. 818	$10^{5.7}$		15 15 15 15 15 17 18	15.7	–	7/7
	No. 818	$10^{5.7}$	VSV	17 22 23 41	25.6	224 224 224	4/7

brain extract of newborn mice (approximately $10^{2.7}$ LD_{50} of NDV), whereas serum of 2 mice of the group inoculated with cells of the not superinfected culture No. 1078 failed to neutralize NDV. In the third experiment, new-born mice were inoculated i.p. with 10^5 cells of culture No. 1078, either with or without superinfection with NDV. 4 mice succumbed presumably to encephalitis within 3 days after inoculation in the group receiving NDV-superinfected cells. Death from tumors was delayed in the group inoculated with NDV-superinfected cells. The statistical difference between these 2 groups is significant ($P = 0.006$). In the fourth experiment, new-born mice were inoculated with $10^{3.3}$ NDV-superinfected and non-superinfected cells of culture No. 1078, respectively. Six 2-day-old mice died presumably from encephalitis, in the group inoculated with NDV-superinfected cells. 26 days later, when the first deaths from tumor growth occurred, all mice were killed. Mice inoculated with not superinfected cells all had large tumors. 3 of 16 mice inoculated with cells superinfected with NDV were free of tumors; 13 mice had tumors but the neoplasms were smaller than those in the control group.

These experiments suggest that (1) slower tumor growth in vivo caused by superinfection of the inoculated cells with NDV may occur; and (2) accelerated tumor growth in mice inoculated with NDV-superinfected neoplastic cells also may occur. The latter effect may be correlated with the presence of antibodies directed against NDV; these antibodies either may be added to the system or produced by the host. The faculty of specific antibody production may not be fully operative in mice at birth; cell-mediated immune reactions, however, may be activated already at birth, because these latter faculties of immunity appear earlier both phylo- and ontogenetically than those of specific antibody production[10,11]. Tumor growth facilitation in the presence of anti-NDV antibody may be explained by 'immunological enhancement', a phenomenon thought to be antagonistic to cell-mediated tumor rejection[12].

Table II shows that a leukemia virus (Rauscher) producing murine lymphoma (protocol No. 620)[7,8] and an established culture of lymphoblasts deriving from this lymphoma (protocol No. 818)[7,8] were not inhibited significantly by NDV when the virus was incubated for 1 h at 37 °C with the cells before inoculation into 3-week-old mice. However, vesicular stomatitis virus (VSV) exerted detectable oncolytic effect. All those mice found without tumor at 188, 216 and 224 days after inoculation of lymphoma cells and VSV did show tumor growth 1 month after inoculation. These tumors were rejected, whereas mice receiving only lymphoma cells, failed to reject the tumor.

These studies remain incomplete, because as yet no answer has been provided to the following questions: (1) Does NDV replicate in the cultured malignant cells? (2) Does the decreased growth rate of some NDV-superinfected tumors occur on account of an immune rejection by the host, or is it due to subtle cytopathic damage by the superinfecting virus? (3) Was the accelerated growth of some NDV-superinfected tumors due to 'immunological enhancement'? Indirect evidence indicates NDV antigens present in tumor cells, because tumor-bearing young adult mice were shown to produce antibody neutralizing NDV. Conversely, release of neurotropic virus from these cells should be minimal, because only a few new-born mice died, presumably because of encephalitis, within the first few days after inoculation. Neither inhibition of growth nor cytopathic effects were observed in vitro in cultures superinfected with NDV[13].

Zusammenfassung. Neoplastische Gewebskulturen, infiziert mit einem Mäuseleukämievirus, wurden entweder mit «Newcastle Disease Virus» oder mit «Vesiculare Stomatitis Virus» superinfiziert; die Zellen zeigten beschleunigte oder verlängerte Wachstumsraten, wenn sie in den ursprünglichen Mäusestamm injiziert wurden.

J. G. Sinkovics and C. D. Howe

Department of Medicine,
The University of Texas M. D. Anderson
Hospital and Tumor Institute,
Houston (Texas 77025, USA), 20 November 1968.

[10] R. A. Good and B. W. Papermaster, Adv. Immunol. *48*, 1 (1964).
[11] J. G. Sinkovics, Exp. med. Surg. *21*, 251 (1963).
[12] N. Kaliss, Ann. N.Y. Acad. Sci. *129*, 155 (1966).
[13] This work was supported by U.S. Public Health Service grants No. CA-7923 and No. CA-5831. The authors thank Mrs. Glenda F. Groves for technical assistance and Dr. B. W. Brown (Department of Biomathematics) for statistical analysis.