Reaction of Certain Mouse and Hamster Tumor Tissue Cultures to Polyoma Virus.* (27782)

HAROLD E. PEARSON

Department of Public Health, University of Southern Calif. School of Med., and Laboratory Division, Los Angeles County Hospital, Los Angeles

Polyoma virus grows readily in cultures of various mouse tissues(1). The virus may induce necrosis or there may be no observed histologic reaction associated with a virus carrier state of variable duration; for reasons unknown, some cells are converted to neoplasia. In hamster cell cultures necrosis does not occur; virus persists for variable, usually relatively short periods, and some cells may become neoplastic. Virus is recovered irregularly or not at all from mouse or hamster tumors induced either *in vitro* or *in vivo* by the polyoma agent(2); tissue cultures of some polyoma induced mouse tumors are susceptible to infection with the virus and undergo cytolysis(3). The virus has been demonstrated in mouse tumors thought not to have been induced by it, such as Sarcoma 180(4) and mouse mammary carcinoma(5). Polyoma virus is cytopathic for some hemic mouse tumors, P329, MBIII and in addition may become chronically carried by lymphoma $P388D_1$(1). It also is cytopathic for cultures of a spontaneous mouse mammary carcinoma (6). The present work describes some reactions of certain mouse and hamster tumor tissue cultures to the presence of polyoma virus.

Materials and methods. The following tumors were used: L2 and 1210 lymphomas, Gardner 6C3HED lymphosarcoma, RC carcinoma(7), HT6 hamster fibrosarcoma (induced by us with polyoma virus *in vivo*), Chemically induced EpO ependymoma(8), P815 (Dunn-Potter) mast cell tumor(9), WT adenocarcinoma, spontaneous in our Swiss mice(10), unclassified WM tumor, rapidly developed in one of our Swiss mice which had been inoculated intracerebrally with the above named WT adenocarcinoma. The WM tumor may be simply a cell variant of the WT tumor; it grew as spindle cells both in the mouse and in tissue culture and the latter reacted differently than WT cells to polyoma virus. WT solid tumor (but not tissue culture) cells contained electron-dense particles similar to those seen in mouse mammary carcinoma; such particles were not found in WM solid tumor cells. All tissue cultures were maintained at 37°C in Leighton tubes with Eagle's medium containing 10% fetal calf serum, penicillin and streptomycin; mast cell culture fluid had 4 μg/ml folic acid added(9). Fluids were changed twice weekly. Cells were examined unstained in the tubes and on slips stained with hematoxylin-eosin or acridine orange. Tests for polyoma virus were done by use of mouse embryo fibroblast tube tissue cultures; these were observed during cultivation for 2 weeks for the presence of cell necrosis. Cells to be tested were first frozen and thawed twice, sonicated in a Raytheon 250 W. sonic oscillator for 10 minutes and frozen overnight at –20°C. Before they were put in tissue culture, all tumors used were originally tested for the presence of any agent cytolytic for mouse fibroblasts. None was found. A polyoma virus clone grown in mouse embryo fibroblasts and originally from Dr. Dulbecco's laboratory, Caltech, was used for all experiments. It was added to cultures in a final concentration of 10^{-1} (10^4 cytolytic doses).

Results. Tumors could be separated into 4 groups according to their reaction to polyoma virus (Table I). *Group 1* were those without cytolysis and virus could be recovered for several weeks from the cells. RC, EpO and HT6 tumors were listed here. No histologic changes were seen in the virus infected cells. Virus was not recovered for longer than 3 weeks from RC cells. In HT6 virus was found after 1 month, but not after 6 weeks. Little virus proliferation in HT6 cells occurred as judged from titers of 10^{-2} at 1 day, 10^{-3} at 2 weeks and 10^{-2} at 1 month. The response of EpO cells was similar with a virus titer of 10^{-3} at 1 month and no virus was detected after 7 weeks. *Group 2* tumor cells

* Aided by grants from Nat. Cancer Inst.

TABLE I. Reaction of Tumor Tissue Cultures to Polyoma Virus.

Group	Tumor	Tissue culture reaction	Persistence of virus	Remarks
1	RC carcinoma	No cytolysis	No virus after 3 wk	
	EpO ependymoma	"	Virus present 1 mo; none at 2 mo	Low grade virus product; titer 10^{-3} at 1 mo
	HT6 fibrosarcoma hamster, polyoma induced (Pearson)	"	*Idem*	Low grade virus product; titer 10^{-2} at 1 mo
2	P815 mast cell (Dunn-Potter)	Some cytolysis	"	
	1210 lymphoid leukemia	Variable cytolysis	"	
	L2 lymphocytic lymphoma			
	6C3HED lymphosarcoma (Gardner)			
3	WT adenocarcinoma (Pearson)	No cytolysis	Virus persisted at least 6 mo	Electron-dense bodies in original tumor similar to those in mouse mammary carcinoma
4	WM tumor (Pearson)	Variable cytolysis	Virus persisted at least 3 mo	May be variant of WT tumor; no electron-dense bodies in original tumor

had variable degrees of cytolysis and cell alteration; virus persisted for several weeks. Included here were L2, 1210, 6C3HED and P815 tumors. In the first 3 of these there was some cytolysis and the surviving cells were smaller with less cytoplasm; the nuclei were slightly shrunken and more darkly stained than in noninfected cells. After 7-10 days the cells regained their original appearance; virus was harbored by the cells for 1 but not for 2 months. Virus considerably altered the P815 cells. After 1 week the infected cells had less cytoplasm which stained paler red with acridine orange than in uninoculated control cells. The culture medium of the infected cells was more alkaline than that of control cells. After 11 days relatively few virus-infected cells survived; the pale cytoplasm of some contained relatively large vacuoles within which there was fairly uniform, finely granular material stained light green with acridine orange. Some nuclei also had relatively dense, polymorphous inclusions similarly stained. Cells surviving 2-3 weeks had less, relatively pale cytoplasm with less prominent cytoplasmic processes. These morphologic changes were similar to those described by others(11) for mouse fibroblasts. After 1 month there were scattered cells of abnormal appearance as well as groups of cells that appeared to be normal; virus was recovered from cells after 1 but not 2 months.

Group 3 tumor cells had no observed changes in cell morphology but did have persistent virus. The WT adenocarcinoma cells had no cytologic alterations after infection with 10^{-1} to 10^{-5} dilutions of virus. The cells persistently shed virus for at least as long as 6 months.

Group 4 tumor cells had cytoloysis and virus persisted for months in surviving cells. The unclassified WM mouse tumor reacted in this way. The cells had changes similar to those described for P815 infected cells. These developed slowly and were first seen approximately 1 week after infection. Scattered cells were involved and became necrotic during the next week. Surviving cells continued to harbor virus for at least 3 months. The persistently infected cells did not differ morphologically from noninfected cells.

Discussion. Tumor cells react to polyoma virus in ways superficially similar to those of non-tumor cells. One might expect that involvement of some as yet unknown key metabolic reactions in the cell is responsible for cytolysis of certain cells. Species differences are known in that hamster cells in culture are

resistant to polyoma virus cytolysis. Temporary virus carriage by cells may be mechanical in that the virus survives in an inert state, or it may be metabolic in that the virus participates biochemically in cell reactions. A cell-virus relationship that temporarily results in fewer mitoses and some cell loss occurs with polyoma and other viruses and is a factor in the selection of surviving cells. The persistent carrier state may involve the nuclei, the cytoplasm or both. It has not been possible to determine this by use of the fluorescent antibody technic probably because of the small amount of virus present. What effect the virus has, under this circumstance, on the behavior of the tumor is not known. In the case of a DNA virus such as polyoma, which has not been shown to affect the genome of cells (12), perhaps a persistent carrier state has relatively little effect on the cell population. Environmental effects on the virus such as changes in oncogenicity(13) require further study.

Summary. Tissue cultured cells of certain mouse and hamster tumors were classified according to their reactions to polyoma virus. *Group 1* cells had no morphologic changes and virus persisted only a few weeks in the culture. Murine RC carcinoma and ependymoma and HT6 fibrosarcoma (hamster) behaved in this way. *Group 2* cells had variable degrees of cytolysis and morphologic changes; virus persisted for several weeks. L2 and 1210 lymphomas, 6C3HED lymphosarcoma (Gardner) and the P815 mast cell (Dunn-Potter) gave this response. *Group 3* cells had no changes in cell morphology with persistent virus. A spontaneous adenocarcinoma (WT, Pearson) gave this response. *Group 4* cells had cytolysis and virus persisted for months in surviving cells. The unclassified mouse tumor (WM, Pearson) reacted in this way.

1. Dawe, C. J., *Nat. Cancer Inst. Monograph,* 1960, No. 4, 67.
2. Ham, A. W., Siminovitch, L., *Progr. Exp. Tumor Res.,* 1961, v2, 68.
3. Hellström, I., Hellström, K. E., Sjögren, H. O., *Virol.,* 1962, v16, 282.
4. Li, C. P., Jahnes, W. G., *Virol.,* 1959, v9, 489.
5. Howatson, A. F., *et al., J. Nat. Cancer Inst.,* 1960, v24, 1131.
6. Wallace, R., Davies, M. C., Pease, B., Englert, M., Moyer, A. W., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 845.
7. Dunham, L. J., Stewart, H. L., *J. Nat. Cancer Inst.,* 1953, v13, 1299.
8. Pearson, H. E., Baker, R. F., *ibid.,* 1961, v27, 793.
9. Schindler, R., Day, M., Fischer, G. A., *Cancer Res.,* 1959, v19, 47.
10. Pearson, H. E., Baker, R. F., *J. Nat. Cancer Inst.,* 1962, v29, 491.
11. Bereczky, E., Dmochowski, L., Grey, C. E., *ibid.,* 1961, v27, 99.
12. Vogt, M., Dulbecco, R., *Virol.,* 1962, v16, 41.
13. Law, L. W., Rabson, A. S., Dawe, C. J., *Nature,* 1961, v190, 97.

Received August 20, 1962. P.S.E.B.M., 1962, v111.

Suppression of the Immune Response by Drugs in Combination. (27783)

SAMUEL BIEBER, GERTRUDE B. ELION, GEORGE H. HITCHINGS, DONA C. HOOPER, AND HENRY C. NATHAN
Wellcome Research Laboratories, Burroughs Wellcome and Co. Inc., Tuckahoe, N. Y.

A search for agents capable of suppressing the immune response led to the adoption of a test system based on the formation of hemagglutinins to sheep red blood cells in mice(1). The demonstration that combinations of antimetabolites which affect biochemical systems related sequentially or concurrently show potentiation *in vitro*(2,3,4) and *in vivo*(5) suggested that the same principle might be applied to suppression of the immune response. The results of the experiments described herein demonstrate this to be the case.

Materials and methods. Charles River CD_1 male mice weighing 19-21 g were used for these experiments. Antigen administration, hemagglutinin determinations and scoring