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Search for Virus-Induced Antigens in Human Tumors Using the SV40-Hamster System as a Model.* (29907)

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Hamsters bearing tumors induced by either SV40 or cells transformed by this virus develop antibodies capable of reacting with a new and specific tumor antigen. This virus-induced antigen, present in both hamster(1-3) and human(2) cells transformed by the virus, can be detected by complement-fixation (1) or immunofluorescence techniques(2,3). Presumably the two tests are measuring the same antigen(4).

These observations suggested that the techniques developed for detection of this tumor antigen might be employed to determine whether human neoplasias stimulated similar antibodies in patients. Based on the SV40 model system, such antibodies would be expected to react with a new antigen in the cancer cells. This report describes the results of such an exploratory study with human cancer.

Materials and methods. Specimens from a variety of human neoplasias were obtained from (a) Ben Taub General Hospital,† Houston, Texas and from (b) Roswell Park Memorial Institute,‡ Buffalo, N. Y. The material received from the local hospital was obtained within a few hours after surgery as unfrozen tissue and was processed immediately. Specimens from Roswell Park were received in duplicate in both wet ice and frozen in liquid nitrogen (in 10% dimethylsulfoxide). They usually arrived 2-3 days after surgical removal and were processed immediately upon receipt. The frozen tissue was thawed rapidly in a 37°C water bath and washed with warm tris saline (pH 7.4).

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All tissues were accompanied by clotted blood or serum from the patient.

The tissues were repeatedly treated with 0.2% trypsin for 5-10 minute intervals with a magnetic stirrer to disperse the cells. Cells collected after trypsinization were sedimented by low speed centrifugation and resuspended in a nutrient fluid consisting of Eagle's basal medium supplemented with 10% calf serum. The pH was adjusted to 7.4 with $NaHCO_3$. All fluids contained 500 units of penicillin, 500 μg of streptomycin, and 100 units of mycostatin per ml. The cells were grown in 16 oz bottles and on 15 mm coverglasses in 60 mm petri dishes at 37°C. The cells were tested within the first passage by immunofluorescence and within the first to third tissue culture passage by complement-fixation.

The immunofluorescence technique as used in this laboratory for the SV40 system has been described(2). Cells on coverglasses were washed 3 times with warm tris saline, air dried, and fixed for 5 minutes in acetone at room temperature. They were then treated with the autologous human serum (both undiluted serum and a dilution of 1:2 were tested) for 30 minutes, washed thoroughly, and reacted for 30 minutes with an anti-human globulin prepared in rabbits and labeled with fluorescein isothiocyanate. After another washing, the cells were air-dried and mounted on slides in elvanol. The labeled anti-human globulin is the same as that employed in previous studies with measles(5), herpes simplex(6) and herpes zoster viruses (7) and is known *not* to react with human, monkey, rabbit, hamster, or mouse cells. All tests included a cell control consisting of human embryonic lung cells in early passages in culture.

A micro-complement-fixation test was carried out in plastic cups as described(8). Antigens for the test were prepared from the bottle cultures and concentrated by taking up the contents of a 16 oz bottle in 1 ml(8). All tests included complement, serum, antigen, and cell controls.

Results. Tissue from a variety of neoplasias was received (Table I). Diagnosis was made by the pathologists at the institutes in which the surgery was performed.

TABLE I. Specimens Received from Ben Taub General Hospital.

Specimen No.	Diagnosis	Cell growth
64-106	Myoma of uterus	—
64-108	Cancer of rectum	+
64-109	Mesothelioma of lung	+
64-112	Squamous cell cancer	+
64-113	Cancer of stomach	+
64-114	Ovarian tumor	+
64-115	Peritoneal sarcoma	+
64-116	Malignant schwannoma	+
64-126	Cancer of breast	—
64-129	" " "	+
64-140	Metastatic cancer of testicle	+
64-141	Kidney tumor	+
64-155	Lipoma	+
64-156	Neurofibroma	+
64-157	Lymph node–Hodgkin's disease	+
64-166	Myoma of uterus	+
64-167	Sarcoma of uterus	—
64-168	Dermoid cyst of ovary	—
64-174	Fibroids of uterus	—
64-181	" " "	—
64-184	Multiple tumors of liver and pylorus	+

+ indicates successful cultivation.
— indicates no growth in culture.

The majority of specimens received locally were successfully grown in culture; thus 15 of 21 (71%) of locally obtained tissues could be further tested. A total of 25 specimens was received from Roswell Park of which 12 arrived in liquid nitrogen and 13 were shipped in wet ice (Table II). Only 2 of 12 (17%) tissues sent at —190°C grew successfully while 6 of 13 (46%) of the specimens sent at 4°C were successfully cultivated. In only one instance (specimen no. 64-179) was growth obtained from the liquid nitrogen material, in the absence of growth from the material shipped in wet ice (specimen no. 64-172). The higher percentage of successful growth from locally obtained tissue might well have been due to the larger size of each specimen.

Each culture was tested by the immunofluorescence method against serum of the patient whose neoplasm initiated the culture. Those which grew sufficiently to permit the preparation of 16 oz bottle cultures were also tested by complement-fixation against their own serum. In addition, a few antigens were tested against heterologous human sera. Table III summarizes the results obtained. None of the sera reacted in any way with

TABLE II. Specimens Received from Roswell Park Memorial Institute.

Specimen No.	Shipping temp (°C)	Diagnosis	Cell growth
64-127	+ 4°	Cancer of breast	—
64-128	—190°	" " "	—
64-131	+ 4°	Lung tumor	+
64-137	—190°	" "	—
64-134	+ 4°	Cancer of rectum	—
64-135	—190°	" " "	—
64-144	+ 4°	Cancer of colon & ileum	—
64-148	—190°	" " " " "	—
64-145	+ 4°	Bronchogenic cancer of lung	—
64-149	—190°	Bronchogenic cancer of lung	—
64-150	+ 4°	Cancer of rectum	—
64-151	—190°	" " "	—
64-152	+ 4°	Chondrosarcoma	+
64-163	—190°	"	+
64-160	+ 4°	Rectal sigmoid cancer	+
64-164	—190°	" " "	—
64-162	+ 4°	Lymphosarcoma & hypersplenism	+
64-165	—190°	Lymphosarcoma & hypersplenism	—
64-170	+ 4°	Squamous cell carcinoma	+
64-178	—190°	" " "	—
64-172	+ 4°	Multiple polyps of G.I. tract	—
64-179	—190°	Multiple polyps of G.I. tract	+
64-176	+ 4°	Inguinal nodes of leg	—
64-180	—190°	" " " "	—
64-182	+ 4°	Mixed tumor of parotid gland	+

TABLE III. Results of Immunofluorescence and Complement-Fixation Tests.

	No. tested	No. positive	No. negative
Immunofluorescence	20	0	20*
Complement-fixation	16	0	16

* One serum reacted with intranuclear antigens of cancer cells, normal human cells and hamster embryo fibroblasts.

their own cells or with antigens in the cells as measured by the technics employed. One serum reacted with intranuclear material in a reaction typical of lupus erythematosus(9); this serum also reacted in the same fashion with normal human embryonic lung cells and with hamster cells. No other reactions, either with cytoplasmic or nuclear components, were observed. Complement-fixation tests similarly failed to detect any reactions.

Discussion. Studies to demonstrate the viral etiology of human solid tumors have usually been concerned with the inoculation of animals or tissue cultures with extracts prepared from the human cancer tissue. While suggestive results have sometimes been obtained, carefully controlled experiments have not confirmed the increased incidence of neoplasias in animals(10-12) nor the replication of "human tumor viruses" in tissue culture (13) following inoculation of cell-free extracts from human cancer material.

SV40, a known tumor virus, induces a new tumor antigen both in cells supporting the cytolytic cycle of the virus(8,14) as well as in human and hamster cells transformed by the virus(2,3). Hamsters bearing tumors induced by the virus or by virus-transformed cells regularly produce antibodies against this antigen(1-4). These observations on the SV40-hamster tumor system suggested that it might be used as a model for technics that might yield information concerning the presence of such antigens in human neoplasias. The failure to find such an antigen may be due either to (a) nonexistence of a specific new antigen in the tumors tested or (b) lack of antibody against a new antigen in the patients examined. In the hamster, the larger the tumor and the longer its residence in the animal, the more likely the formation of an antibody against a tumor specific antigen(1-4, 15). This aspect was not considered in selecting the patients for study.

The approach reported here represents another way to examine the problem of the viral etiology of human neoplasias. Extension of the work to include other types of neoplasias and to include those of large size and long residence would be useful but our results suggest that many specimens would need to be surveyed. It is noteworthy, however, that non-specific reactions were not encountered and positive results, even if encountered only infrequently, would therefore presumably be significant.

Summary. Cell cultures derived from various types of human cancers were examined for the presence of tumor specific antigens by both immunofluorescence and complement fixation methods, in which the serum of the patient was tested against cells grown from

his cancer. The methods, previously used successfully to detect a new intranuclear antigen induced by the papovavirus SV40 in transformed human and hamster cells, and in hamster tumors, failed to reveal the presence of immunologically reactive components.

1. Black, P. H., Rowe, W. R., Turner, H. C., Huebner, R. J., Proc. Nat. Acad. Sci., U. S., 1963, v50, 1148.

2. Rapp, F., Butel, J. S., Melnick, J. L., Proc. Soc. Exp. Biol. and Med., 1964, v116, 1131.

3. Pope, J. H., Rowe, W. P., J. Exp. Med., 1964, v120, 121.

4. Kitahara, T., Butel, J. S., Rapp, F., Melnick, J. L., Nature, 1965, in press.

5. Rapp, F., J. Bact., 1964, v88, 1448.

6. Rapp, F., Hsu, T. C., Virology, 1965, in press.

7. Rapp, F., Vanderslice, D., ibid., 1964, v22, 321.

8. Rapp, F., Kitahara, T., Butel, J. S., Melnick, J. L., Proc. Nat. Acad. Sci., U. S., 1964, v52, 1138.

9. Rapp, F., J. Immunol., 1962, v88, 732.

10. Girardi, A. J., Hilleman, M. R., Zwickey, R. E., Proc. Soc. Exp. Biol. and Med., 1962, v111, 84.

11. Moore, A. E., Caparo, A. C., Cancer Res., 1964, v24, 765.

12. Girardi, A. J., Hilleman, M. R., Zwickey, R. E., Proc. Soc. Exp. Biol. and Med., 1963, v114, 609.

13. Girardi, A. J., Slotnick, V. B., Hilleman, M. R., ibid., 1962, v110, 776.

14. Sabin, A. B., Koch, M. A., Proc. Nat. Acad. Sci., U. S., 1964, v52, 1131.

15. Huebner, R. J., Rowe, W. P., Turner, H. C., Lane, W. T., ibid., 1963, v50, 379.

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Immunofluorescence of Newcastle Disease Virus in Paraffin Embedded Tissues of Early Chick Embryos.* (29908)

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Three members of the myxovirus group (mumps, influenza A, and Newcastle disease virus) have been studied previously in early chick embryos and each was observed to produce developmental defects, differing somewhat for each of the viruses(1-6). Essentially, however, the defects occurred in tissues which were in early stages of differentiation and which were directly exposed to the inoculated virus. Although increase in virus titer was observed in all 3 instances it was not known whether the defects were caused by selective replication of virus in the tissues affected or whether the virus replicated in all cells but caused damage only in certain tissues which were vulnerable because of a particularly sensitive phase in their developmental process. To study the distribution of the replicating virus in the early chick embryo, a method was necessary which would allow identification of the virus in serially sectioned embryos. The successful use of a paraffin embedding technique for immunofluorescent staining has been reported(7) by Guy Ste.-Marie using several types of antigen including influenza A virus. These techniques have been adapted for staining Newcastle disease virus in serially sectioned tissues of the early chick embryo.

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Methods and materials. Preparation of the conjugate. Viral antigen to be used for antibody production was grown in the allantoic cavity of the chick embryo. Young adult chickens were immunized by intranasal instillation of the Blacksburg strain of Newcastle disease virus (NDV), and this was followed in about 5 weeks by intramuscular injections of 0.4 ml of the Roakin strain of NDV of ID_{50} $10^{10.9}$ (2 injections 3 days apart). Ten to twelve days after the final injection, the chickens were bled. Hemagglutination-inhibition titers of the sera ranged between 1-2000 and 1-4000. The globulins were extracted and conjugated to fluorescein isothiocyanate by the method of Coons(8) modified by Riggs *et al*(9). Instead of the dialysis techniques to remove the excess dye, the conjugate was cleared by passing through