Tissue Antigens of Human Tumors Grown in Rats, Hamsters, and Eggs*

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It was recently shown that human cancers could be grown as transplantable tumors in the subcutaneous tissues of cortisone-treated animals of various species (9—11). These tumors are now being studied in many laboratories on the assumption that they have not changed their original characteristics. It is therefore important to ascertain the validity of this assumption.

Two human tumors which had been grown for many generations in rats were used in the studies to be reported here. These tumors were analyzed for the presence of human and rat tissue antigens, using the agar-diffusion technic. Rat-grown human tumors which had been grown for one generation in hamsters or for several generations in eggs were tested also to ascertain whether they had adapted themselves to synthesizing rat antigens.

MATERIALS AND METHODS

Antisera.—Antisera were prepared against the Murphy-Sturm lymphosarcoma of the rat and against a human carcinoma of the parotid. The immunizing antigens were prepared by homogenizing the tissues in a blender and washing the saline-insoluble sediments until the supernates were clear. These sediments were then lyophilized and injected into rabbits. A more detailed description can be found in (3). Each rabbit received several series of eleven injections (100 mg/injection) given 3 times a week for 4 weeks, and was bled 1 week after the last injection. Antibodies against human plasma proteins were removed by adding 500 mg. pooled human plasma to 10 ml. of the antihuman carcinoma serum. The antirat tumor serum was similarly absorbed with lyophilized pooled rat plasma.

Test antigens.—Two rat-grown human tumors, Toolan’s HS#1 and HEp#8, were used (11). HS#1, a sarcoma, had been carried through 81 transfers in rats. The 81st rat-grown generation was used for immunological analysis. After 85 generations, it was transferred to eggs, and the sixth and seventh egg-grown generations were pooled for immunological studies. HEp#8, an epidermoid carcinoma, was studied after the third, fifth, sixth, seventh, and thirteenth generations in rats. After the sixteenth generation in rats, it was grown for 10 days (one generation) in the cheek pouch of hamsters. After ten generations in the rat and one in hamsters, it was grown in chicken eggs, and the fifth, sixth and seventh generations were pooled for immunological analysis. A human carcinoma of the cervix and the Murphy-Sturm lymphosarcoma of the rat were used as control antigens.

Each tissue was homogenized in saline with a blender in a ratio of 100 ml. saline to 40 gm. of wet tissue. The homogenate was centrifuged at 20,000 g for 1 hour at 8°—5° C. The supernate was then either dialyzed against saline and used as such, or dialyzed against distilled water and lyophilized. The latter procedure was used only with the third, fifth, sixth, and seventh generations of HEp#8 in rats.) The lyophilized antigens were regenerated by adding 1 ml. borate buffer (pH 8) to either 40 or 80 mg. of powder.

Gel diffusion technic.—The gel diffusion technic was a modification of the Ouchterlony procedure (6). Agar was suspended in saline buffered at pH 7 with phosphate buffer and melted. The agar was then clarified by centrifugation, remelted, and, after adding Merthiolate to a final concentration of 1 × 10⁻⁴, one layer of agar was poured into Petri dishes. After the agar solidified, four stainless steel penicillin assay cylinders were arranged in a square, a fifth one placed in the center (the distance from the center of the central cylinder to the center of the peripheral cylinders was 1.8 cm.), and another layer of agar was poured on top of the first; 0.2 ml. antiserum was then pipetted into the central cylinder, and 0.2 ml. of the various tumor
extracts or plasmas was pipetted into the peripheral cylinders. After 3 days all cylinders were refilled with 0.2 ml. of the same material. The plates were kept at a constant temperature (25°C), and shadowgraphs were made 2 weeks after the first filling.

RESULTS

When antigens and their homologous antibodies diffuse toward each other through agar, lines of specific precipitate are formed in the region of optimal proportions for each antigen-antibody system. Ouchterlony (6) showed that when the reservoirs are arranged in a triangle and the same antigen is placed in two of the reservoirs while the antibody is placed in the third, the two lines will coalesce (reaction of identity). However, if the two reservoirs contain unrelated antigens, and antibodies against these antigens are placed in the antiserum reservoir, the lines formed by each antigen-antibody system will remain independent of the others (reaction of nonidentity).

When the antiserum against the human carcinoma was tested against the rat-grown HEp#3, at least three lines appeared. These lines were present when the dilute extracts of the third, fifth, sixth, and seventh rat-grown generations were used.

When more concentrated preparations were used (80 mg/ml), these three lines tended to fuse into one dense line. When this line was compared with the lines produced with the human carcinoma of the cervix, it was found to give a reaction of identity with one of them (Fig. 1). Some of the other HEp#3 lines which became visible at this concentration had their identical counterparts in the human carcinoma of the cervix. However, the human carcinoma of the cervix contained several antigens which were absent in the rat-grown HEp#3.

HS#1 still produced human antigens after having been grown for 31 generations in rats, and some of these antigens were absent in the HEp#3. It is of interest that the rat lymphosarcoma antigens did not react with the antisera against human tumors (Fig. 2). The antiserum against the rat lymphosarcoma produced several lines with the homologous tumor. Some of these lines were also produced by the rat-grown human tumors (Fig. 3). The lines due to rat antigens in HEp#3 remained at approximately the same distance from the antigen reservoir, regardless of how many generations the tumor had been grown in rats. The distance of these lines was also similar to those due to the identical antigens in the rat lymphosarcoma.

The effect of a foreign host or environment on a tissue or tumor has been a source of speculation ever since tissues could be grown in eggs, tissue culture, or animals of foreign strains or species. The observation that the transplanted tissue's ability to grow in the foreign environment often increased after a prolonged sojourn in the new medium led to the assumption that the cells adapted themselves either by losing some of their own antigenic components or by acquiring antigenic components of the host. Putnoky (7, 8), for example, had shown that the Ehrlich mouse carcinoma could be made to grow in the adult rat. After maintaining this mouse tumor for 7 years in rats, he showed that this rat-adapted mouse tumor was still composed of mouse cells but that some kind of antigenic change had taken place which enabled it to grow better in rats as well as to act as a "rat antigen." There are many examples of transplantable tumors which lose their strain.
FIG. 1.—Center: Antihuman carcinoma serum. Top reservoirs (Left): Human carcinoma of the cervix (40 mg/ml). (Right): HEp#3; thirteen generations in rats (80 mg/ml). Bottom reservoirs (Left): Human plasma. (Right): HEp#3; seven generations in rats (80 mg/ml).

FIG. 2.—Center: Antihuman carcinoma serum. Top reservoirs (Left): HEp#3; thirteen generations in rats (40 mg/ml). (Right): HEp#3; sixteen generations in rats, one generation in hamsters (80 mg/ml). Bottom reservoirs (Left): Rat lymphosarcoma (40 mg/ml). (Right): Human carcinoma of the cervix (40 mg/ml).

FIG. 3.—Center: Antirat lymphosarcoma serum. Top reservoirs (Left): Rat lymphosarcoma (40 mg/ml). (Right): HEp#3; thirteen generations in rats (40 mg/ml). Bottom reservoirs (Left): HS#1; thirty-one generations in rats (40 mg/ml). (Right): Rat plasma.

FIG. 4.—Center: Antihuman carcinoma serum. Top reservoirs (Left): Human carcinoma of the cervix (40 mg/ml). (Right): HEp#3; thirteen generations in rats (80 mg/ml). Bottom reservoirs (Left): HS#1; grown in eggs (40 mg/ml). (Right): HEp#3; grown in eggs (80 mg/ml).
specificity as determined by histocompatibility tests. (For a review and discussion see Hauschka [1]). However, no serological tests have as yet been applied to the analysis of the antigens involved.

A few studies have been published on the effect of tissue culture media on cellular antigens. Kimura (2) claimed that duck fibroblasts, when grown in chick plasma and chick embryo extract, could no longer synthesize duck proteins but that instead the presence of chicken proteins could be demonstrated with the use of the precipitin reaction. His interpretation, however, can be challenged on the ground that he did not take enough precautions to eliminate the carry-over of proteins from the medium. Landsteiner and Parker (4) showed that growth of chick fibroblasts for 55 generations in rabbit plasma had no measurable effect on the cell's capacity to synthesize chicken serum proteins. Langman (5) transferred rabbit embryo gonads, which had first been grown in rabbit plasma, to a medium containing cat plasma and cat spleen extract. He was able to demonstrate the presence of cat proteins in these tissues only after they had been grown for at least 18 days in the heterologous medium, but not if grown for only 3 days in the cat medium. Since these tissues were also grown for some time in rabbit plasma after their sojourn in the heterologous plasma, Langman assumed that carry-over of cat proteins due to absorption had been ruled out in these experiments. However, the possibility exists that the cells which were grown for a longer period had ingested more cat proteins than those which had grown for 3 days in this medium. It may have been more difficult to eliminate these ingested proteins from the old cultures than from those which had been grown for only 3 days in cat medium.

Our own results show that the growth of human tumors for many generations in rats, hamsters, and eggs did not diminish their ability to synthesize the same human tissue antigens.2

Efforts to demonstrate changes in protein synthesis were negative, i.e., no rat proteins could be detected in the rat-grown human tumors after transfer to eggs. However, negative results are always difficult to interpret, and as yet it cannot be ruled out that a tissue grown in a foreign environment adapts itself by synthesizing the foreign proteins.

2 It is of interest that the human tumors grown in rats did not produce any human serum albumin, gamma globulin, fibrinogen, or beta lipoprotein even though the antisera used for their detection gave reactions with very small amounts of these plasma proteins.

SUMMARY

A human epidermoid carcinoma (HEp#3) and a human sarcoma (HS#1) which had been grown for many generations in rats and eggs as well as in hamsters (HEp#3 only) were analyzed by the method of Ouchterlony for the presence of human antigens. The same human antigens were always detected, regardless of the number of generations that the tumor had been grown in the foreign hosts studied.

Attempts to demonstrate that the human tumor might synthesize the foreign host's (rat) tissue proteins were negative.

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