The Distribution of Human Tissue Antigens in Five Human Tumors Grown in Rats or Hamsters*

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In a previous report (3) it was shown that two human tumors, Toolan's HS #1 and HEp #3, still produced human tissue antigens after having grown for many generations in rats and hamsters treated with cortisone. In the meantime, several antisera to human tissues and tumors, each of which could detect a different antigen, have been prepared. It was, therefore, decided to compare the distribution of these antigens in five human neoplasms that are now grown in cortisone-treated rats or hamsters with their distribution in surgical specimens of tumors and tissues.

MATERIALS AND METHODS

Human tumors grown in rats or hamsters treated with cortisone.1—The human tumors grown in cortisone-treated rats or hamsters were:

1. HS #1: 38th generation of a sarcoma grown in rats.
2. HEp #3: 19th generation of an epidermoid carcinoma of the buccal mucosa, grown in rats.
4. HEp #1: 74th generation of a metastatic epidermoid carcinoma of the cervix, grown in rats or hamsters. The samples used in this experiment were grown in hamsters.
5. Melanoma #1: Originally isolated and grown in cortisone-treated hamsters by Dr. A. H. Handler and maintained for twenty generations in cortisone-treated hamsters and rats by Dr. Toolan. The samples used for this experiment were all grown in rats.

The characteristics of tumors 1, 2, 3, and 4 have been described (6).

Human tumors and normal tissues freshly obtained at surgery. —The surgical specimens used for comparison with the transplanted tumors were: a melanoma, a carcinoma of the buccal mucosa, four carcinomas of the cervix, and a sarcoma of the mesentery. Other surgical specimens used in this study are listed in Table 1.

Antigenic tests.—The tissue antigens were prepared as described previously (3). Forty gm. of tissue in 100 ml. cold saline was homogenized in a Waring Blender; the insoluble material was removed by centrifugation at 12,000 g for 30 min., and the supernate was dialyzed against cold distilled water. The water-soluble fraction was lyophilized and used for the precipitin tests or for immunization.

Antiserum reagents.—Anti-ovarian carcinoma serum I was prepared by injecting a rabbit with the saline-insoluble sediment of the homogenized carcinoma of the ovary #1 (3).

The other antisera were prepared as follows: Rabbits were injected weekly with the lyophilized tissue extracts described above (100 mg/injec-

* The numerater indicates the number of tissues containing the antigen; the denominator, the number of tissues analyzed.

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The concentrations of 40 mg/ml. Antigen 2 still produced one or more fainter lines with other antisera that were prepared against: (a) carcinoma of the cervix (anti-ca-cervix 1), (b) ovarian cyst (anti-ov-cyst), and (c) normal uterine tissue (anti-N-uterus). All antisera were absorbed with lyophilized pooled plasma until no precipitate was formed with human plasma.

Immunological tests.—The immunological tests were performed according to the modified (5) gel diffusion technic of Ouchterlony (4). All reservoirs were filled twice with 0.2 ml. reagent, and the plates were kept at 25°C.

RESULTS

All antisera reacted strongly with at least one antigen of the homologous tissue and frequently produced one or more fainter lines with other antisera. Since none of the antisera reacted with human plasma (Fig. 4), it must be concluded that the precipitates were due to tissue antigens.

Antigen 1 was absent most frequently. Approximately 50 per cent of the tissues contained all four antigens; 25 per cent lacked all reference antigens, it contained another antigen detectable by anti-ca-cervix serum, which was present in the usual concentration (Fig. 10). Although HEp #1, a carcinoma of the cervix, lacked all reference antigens, it contained another antigen detectable by anti-ca-cervix serum, which finding indicated that human antigens are produced by this tumor (Fig. 10).
Antigen 2 was only present in HS #1 (Fig. 2), but some of the other antigens detectable by anti-ca-ov. The sera were present in the other tumors grown in rats or hamsters (Fig. 3). Antigens 1 and 3 were absent from all five tumors (Figs. 6-8). In other words, HS #1 contained two, the melanoma contained one, and the other two tumors contained none of the four reference antigens.

Table 2

The Antigen Content of the Surgical Specimens

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Antigens containing</th>
<th>Tumors containing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma, ovary</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Ovarian cyst</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal ovary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, cervix</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Carcinoma, uterus</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Normal uterus</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Uterine fibroma</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Carcinoma, breast</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Carcinoma, buccal mucosa</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Carcinoma, tongue</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Carcinoma, bladder</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Carcinoma, stomach</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Reticulum-cell sarcoma</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Normal spleen</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Normal stomach</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sarcoma, mesentery</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sarcoma, pelvis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Tissues from the same person.
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Discussion

The study of tissue and tumor antigens has been hampered by two technical difficulties: (a) the large number of antigens in tissues and (b) the difficulty of obtaining potent precipitating antisera. The development of the gel diffusion technique, which facilitate analysis of systems containing more than one antigen, has given new impetus to the study of tissue antigens. The double diffusion technic of Ouchterlony (4) is of special value, because it not only permits the direct comparison of several systems but allows identical antigens to be identified. Björklund applied this technic to the immunological study of rabbit bone marrow (3) and human tissues (1). Antisera were obtained from hyperimmunized horses. The antibodies against plasma proteins were removed by absorption with lyophilized plasma (1) without much dilution of the antiserum. The immunization of horses requires large amounts of immunizing antigen, and even under these conditions antibodies against few tissue antigens are formed.

The findings presented here show that good precipitating antisera may be obtained in rabbits. However, the relatively small number of tissue antigens that may be detected with any one antiserum necessitates the immunization of many rabbits.

Another problem in the study of tissue antigens is a quantitative one. Our finding that organ and tumor extracts, if prepared in a similar fashion, contain a given antigen in approximately the same concentration suggests that the extraction procedure is reproducible and that it is possible to obtain comparable results on a dry-weight basis. The reproducibility of these data indicates that failure to detect an antigen is owing to its absence.

The finding that some antisera contain antibody against “group-specific” antigens like antigen 1 suggests that discretion be used in the interpretation of data obtained with such antisera. For example, if an antiserum is prepared against a tumor and the normal control tissue is from a different person, failure to find an antigen in the control tissue is not in itself sufficient evidence for the tumor-specific nature of the antigens.

The results presented here show that some tissue antigens are widely distributed in human tissues and tumors. Antigen 2 was present in all tissues, and antigens 3 and 4 were present in the majority of tissues. Why these latter two antigens were absent from some tumors is not easily explained. One out of five carcinomas of the ovary

Fig. 1.—Center reservoir: Anti-ov.-ca. I serum. A, carcinoma, ovary #2; B, uterine fibroma; C, uterus; D, carcinoma, breast.

Fig. 2.—Center reservoir: Anti-ov.-ca. I serum. A, carcinoma, ovary #3; B, ovary; C, carcinoma, ovary #4; D, HS #1.

Fig. 3.—Center reservoir: Anti-ov.-ca. I serum. A, carcinoma, ovary #2; B, carcinoma, ovary #2; C, carcinoma, ovary #2; D, HS #1.

Fig. 4.—Center reservoir: Anti-ov.-cyst serum. A, ovarian cyst; B, ovary; C, carcinoma, ovary #2; D, plasma.

Fig. 5.—Center reservoir: Anti-ov.-cyst serum. A, ovarian cyst; B, uterus; C, carcinoma, cervix; D, carcinoma, cervix.

Fig. 6.—Center reservoir: Anti-ov.-cyst serum. A, melanoma; B, carcinoma, uterus; C, melanoma (grown in rats); D, HEP #1.

Fig. 7.—Center reservoir: Anti-uterus serum. A, uterus; B, H.emb.Rh. #1; C, sarcoma, mesentery; D, reticulum-cell sarcoma.

Fig. 8.—Center reservoir: Anti-uterus serum. A, carcinoma, cervix II; B, HEP #2; C, carcinoma, buccal mucosa; D, HEP #3.

Fig. 9.—Center reservoir: Anti-ca.-cervix I serum. A, carcinoma, cervix I; B, ovarian cyst; C, carcinoma, ovary 5; D, carcinoma, ovary 4.

Fig. 10.—Center reservoir: Anti-ca.-cervix I serum. A, carcinoma, cervix II; B, HEP #1; C, Melanoma (grown in rats); D, HS #1.
lacked these antigens, and one wonders whether the other tissues from the same person were deficient in these substances. One reticulum-cell sarcoma lacked antigens 1 and 3, but these antigens were present in the spleen and stomach from the same person and in another reticulum-cell sarcoma.

Antigen 1 occurred in carcinomas of the cervix and uterus but was absent from the normal uterine tissues of the same persons. In this case, the difference is probably owing to the fact that the tumor is histologically different from the normal tissue.

Antigen 4 was absent from only one of the many uterine fibromas and uteri examined. This indicates that the tissues from some individuals may be deficient in certain widely distributed antigens, and consequently this must be taken into consideration with reference to reports that some malignant cells are antigenically deficient (5).

The observation that human tumors, when grown in foreign hosts, continue to synthesize human antigens even after 3 years of transplantation, is confirmed by the present findings. However, the absence of some antigens from these tumors was unexpected. Antigen 2, which was present in all normal tissues, sarcomas, and carcinomas, was only present in HS #1. Since this antigen could be detected in as little as 1 mg/ml of lyophilized carcinoma or melanoma, but not in 40 mg of rat-grown carcinomas or melanomas, it must be concluded that the transplantable tumors produce very little if any of this antigen. Antigen 3 occurred in all surgical carcinomas of the cervix, yet was absent from HEp #1 (a cervical carcinoma) and the other transplanted tumors.

The absence of antigens 1 and 3 from the transplantable human tumors suggests the following possibilities:

1. These tumors were deficient before transplantation or contained a few cells that were deficient and that were then biologically selected.

2. Growth in the foreign host results in the loss of these antigens.

These alternatives cannot be resolved experimentally, since the original surgical specimens are no longer available for analysis. The findings that some tumors and tissues obtained immediately after surgery were antigenically deficient and that HEp #3 was already deficient in some antigens after only a few generations in the foreign host (3) suggest that the first possibility is not improbable.

If these transplanted human tumors were antigenically deficient before transplantation, the question arises whether there is a correlation between a tumor's heterotransplantability and its antigenic deficiency. Studies designed to solve some of these problems are now in progress.

**SUMMARY**

Antisera against human tumors and tissues were used to analyze the antigenic composition of approximately 40 surgical specimens of normal and neoplastic tissues and five human tumors grown in rats and hamsters.

It was found that, of the four reference antigens studied, one occurred in all and two in the majority of the surgical specimens, whereas a fourth antigen was present in only 50 per cent of the cases.

All human tumors grown in foreign hosts lacked two or more of the reference antigens; one tumor lacked three of the antigens, and two tumors lacked all four.

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