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IMMUNOLOGIC DETERMINATION OF AN EPIDERMAL G_2-CHALONE-LIKE FACTOR AS A MARKER OF SQUAMOUS-CELL STRUCTURES IN BLADDER TUMORS AND URINE OF RATS

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One appraoch to the immunodiagnosis of tumors is by the detection, in neoplasms, of products of the tissues whose morphogenetic potential has not developed de novo during neoplastic transformation. For example, the epithelium of the lung and the transitional epithelium of the bladder, both in experimental animals and in man, are sufficiently often affected by metaplastic changes toward stratified squamous (keratinizing) epithelium. It is natural to suggest that such changes will be accompanied by synthesis of tissue-specific products characteristic of keratinizing epithelium.

One of these products is epidermal G_2-chalone, which the authors have isolated from rat skin [3, 6]. This antigenically active glycoprotein, with a molecular weight of about 35,000, can be detected by means of monospecific antiserum only in squamous-cell keratinizing structures. In tissues of the normal lung and unchanged mucosa of the rat urinary bladder this antigen has not been found [1].

Accordingly, in the investigation described below, an attempt was made to discover whether immunologic detection of epidermal G_2-chalone in rat bladder tumors can be used as a diagnostic test. Investigations of this sort were undertaken by the writers previously on induced tumors of the rat lung [2].

EXPERIMENTAL METHOD

Experiments were carried out on 67 male rats, obtained from the "Rappolovo" nursery, weighing 100-120 g or 150-180 g before the experiment. Some of the animals (56 rats) received 0.04% N-nitroso-N-butyl-N-hydroxybutylamine, which induces epithelial tumors of varied histological structure in the bladder of rats after a long period of administration [4, 5], throughout the experiment with their drinking water. The remaining 11 animals were kept on an ordinary diet and served as the control.

The animals were killed 4-11 months after the beginning of exposure and normal or tumor tissue from the urinary bladder was taken for investigation: some of it was used for histological examination (and, in addition, for electron-microscopic study in 20 cases), and some for immunologic study.

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TABLE 1. Determination of Antigen in Normal and Tumor Tissues of Rat Bladder

Histological structure of tumors based on data of light microscopy	No. of observations	No. of tumors in which antigen was found	
		by direct method	by immunoautoradiography
Tumors from transitional epithelium	29	0	10
Tumors from transitional epithelium with areas of squamous-cell metaplasia	13	5	8
Tumors of squamous-cell structure	14	14	—
Normal mucosa	11	0	0

TABLE 2. Concentration of Antigen in Tumors and in Autologous Urine

Histological structure of tumor	Concentration of antigen, ng/ml	
	in extract	in urine
Transitional-cell carcinoma	absent	absent
	"	"
Transitional-cell carcinoma with areas of squamous-cell metaplasia	300	"
	350	100
	700	100
	700	100
	800	300
	900	60
	350	absent
Squamous-cell carcinoma	2 000	"
	>12 000	250
	>12 000	650

In preliminary experiments to determine the antigen in urine excreted naturally by animals with and without tumors of the bladder many false positive results were obtained. This fact was interpreted as due to the presence of squamous epithelium of the urethra and, possibly, of the preputial sac, in such urine. To avoid contamination of this sort, in the present investigation a method of obtaining urine directly from the bladder was used. For this purpose, 3 ml of tap water was injected through a tube into the stomach of 12 animals of the experimental group and all animals of the control group 2 h before the material was taken. The rats were then anesthetized by intraperitoneal injection of thiopental sodium, laparotomy was performed, and urine was taken by means of a syringe through the bladder wall, and this was subsequently tested immunologically.

The presence of antigen in the tissues and urine was determined by Ouchterlony's double immunodiffusion method in gel, and by Mancini's radial immunodiffusion method in the direct or immunoautoradiographic version. The sensitivity of the methods was 2-3 μg and 60-80 ng antigen/ml of extract (100 mg tissue/ml of physiological saline buffered at pH 7.2) or urine respectively. Immunoautoradiography was used in cases when the antigen could not be detected in the samples by direct methods. Characteristics of the antigen, the method of obtaining the rabbit antibodies against it, and details of the immunologic methods with reference to determination of this antigen in rat tissues were described previously [1].

EXPERIMENTAL METHOD

The main results of the experiments are given in Table 1.

At first glance, ten cases (Table 1, top line) which, on the basis of the results of light microscopy, were classed as "pure" papillomas or carcinomas from transitional epithelium, can be regarded as false-positive results from the point of view of their containing antigen of squamous epithelium. However, on electron microscopy in all cases a moderate number of short but massive tonofibrils and (or) more massive desmosomes was found in cells of the surface and intermediate layers of the urothelium of the villi, a sign of commencing squamous-cell metaplasia.

The antigen could not be found in the urine of any of the control animals, whereas it was found in the urine of 7 animals of the experimental group, in concentrations of 60-650 ng/ml. It is interesting to compare the concentration of antigen in the urine and in extracts of tumor tissue (Table 2).

It will be clear from these results that in no case was a false positive result obtained. The concentration of antigen in the urine largely depended on that in the tumor, and it correlated basically with it, although the presence of a tumor itself did not guarantee the presence of antigen in the urine. That evidently also depended on the state of the tumor itself and, above all, on the degree of desquamation of its epithelium.

The experiment thus confirmed that epidermal G_2-chalone can be used as an immunologic marker of squamous-cell metaplasia of the epithelium of the bladder. Since such structures appear in the urothelium as a result of neoplastic transformation also, this antigen can be a reliable immunologic marker of the corresponding bladder tumors. In this connection the discovery of the antigen in the earliest stages of squamous-cell metaplasia, when such changes cannot yet be found microscopically, and also the possibility of detecting the antigens in the urine of the animals with tumors are facts of the greatest interest.

It remains only to state that, when determining epidermal G_2-chalone in tumors, we make use only of its immunochemical activity and are not concerned with its specific biological activity.

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