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EXPERIMENTAL CARCINOGENESIS OF THE BLADDER FOLLOWING ADMINISTRATION OF FREUND'S ADJUVANT AND LEVAMISOLE

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KEY WORDS: bladder; tumors; Freund's adjuvant; levamisole.

The object of this investigation was a histological and electron-microscopic study of the effect of Freund's complete adjuvant (FCA) and of levamisole on the early and late stages of experimental carcinogenesis in the urinary bladder.

EXPERIMENTAL METHOD

Experiments were carried out on 120 male Wistar rats weighing 150-180 g. Throughout the experiment the animals were given a 0.05% aqueous solution of N-butyl-N-butanol(4)-nitrosamine, a known urotropic carcinogen, to drink. The animals were divided into six groups, with 20 rats in each group.

The animals of the first three groups were used to study the action of FCA and levamisole in the early stages of carcinogenesis, and those of the remaining three groups for the same purpose in the late stages.

The animals of group 1 (control) received the carcinogen only. In group 2, twice during the 10 days before the beginning of the experiment and twice during the 20th-30th days of carcinogenesis, the rats received a subcutaneous injection of 0.1 ml FCA in the plantar surface of the hind limbs. The rats of group 3 received an intraperitoneal injection of 3 mg/kg levamisole (Decaris, from Richter, Hungary), diluted in sterile physiological saline, daily for the 10 days before the beginning of the experiment and during the 20th-30th days of carcinogenesis. Five rats from each group were killed 5, 12, 20, and 40 weeks after the beginning of injection of the carcinogen. The animals of groups 4 and 5 received carcinogen only until they developed a carcinoma of the bladder after 32-34 weeks. At the 34th and 37th weeks of carcinogenesis the rats of group 4 were then

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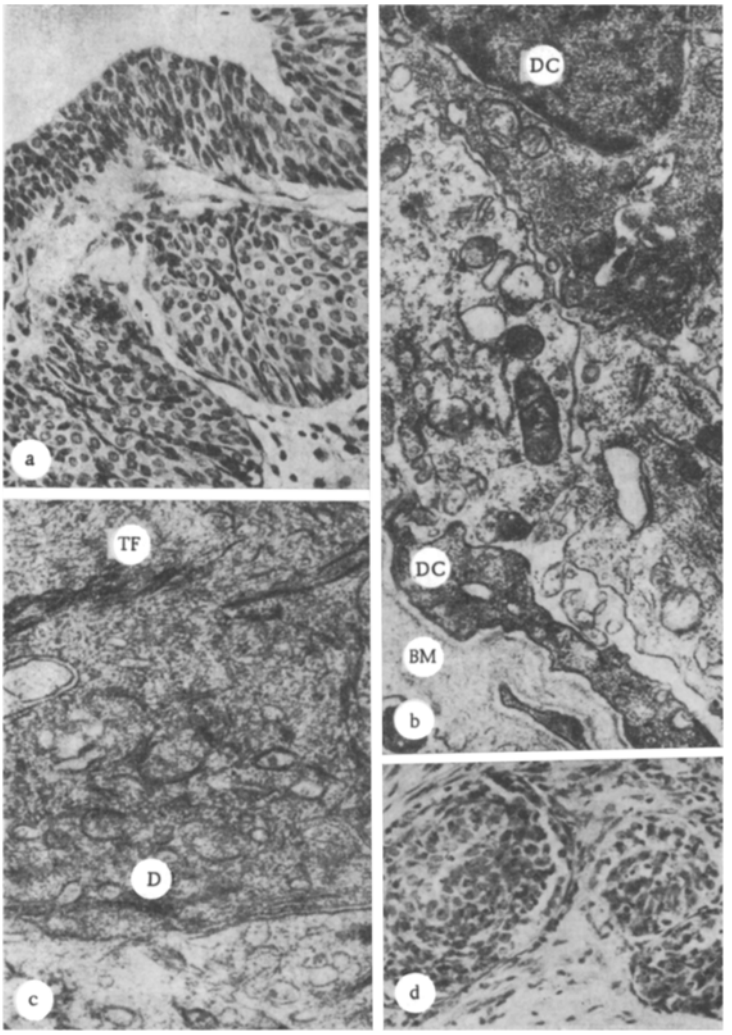

Fig. 1. Carcinogenesis in bladder of rats receiving Freund's complete adjuvant: a) many spindle-shaped dark cells in hyperplastic urothelium and Brunn's cell nests, 5 weeks after beginning of carcinogenesis, hematoxylin-eosin, 220×; b) two dark cells (DC) in thickness of hyperplastic urothelium; one of them is spread out on basement membrane (BM), 16,400×; c) epithelial dark cell with tonofibrils (TF) in cytoplasm and with desmosomes (D) in region of junction with neighboring epithelial cells, 34,400×; d) layers of transplanted transitional-cell carcinoma beneath skin of recipient rat, hematoxylin-eosin, 220×.

given FCA and those of group 5 were given levamisole. The intervals between injection of the dose of the two preparations were the same as in groups 2 and 3. In group 6 (control for the late stages of carcinogenesis) only the carcinogen was injected into the rats. Animals of groups 4, 5, and 6 were killed five at a time 40, 48, and 50 weeks after the beginning of the experiment.

The bladders of all the killed rats were studied in the light and electron microscope. Acid phosphatase (AcP) and alkaline phosphatase (AlP) activity was estimated visually in frozen sections of the bladder.

In all groups pieces of carcinoma of the bladder, freed from keratinizing masses and urinary calculi, were taken from each rat killed 40 weeks after the beginning of the experiment for transplantation. They were then carefully cut into small pieces in Hanks's solution. The resulting suspension from 30 donors was injected through a needle 2 mm in diameter in a dose of 2 ml subcutaneously into three intact Wistar recipient rats. For each of the six groups there were thus five recipients. Skin biopsy was carried out on two of them 1 week, and on the other three, 3 weeks, after transplantation. The skin was examined under the light microscope.

EXPERIMENTAL RESULTS

In the rats of the first three groups (early stages of carcinogenesis) a carcinoma of the bladder was obtained by the 40th week of the experiment. The course of carcinogenesis was morphologically identical in all animals in groups 1 (control) and 3 (with levamisole).

In the rats of group 2 receiving FCA, in the initial stages of carcinogenesis certain differences were observed. Hyperplasia of the transitional epithelium (urothelium), which developed 5 weeks after the beginning of the experiment, was less marked and focal in character. Brunn's cell nests were fewer in number. The urothelium and Brunn's nests were penetrated by a larger number than in the rats of groups 1 and 3 of spindle-shaped cells, whose cytoplasm was darker than that of epithelial cells. They were arranged singly or in small groups, mainly in the basal layers and often on the basement membrane. Whereas in the rats of groups 1 and 3 the number of these cells varied from 10 to 30‰, in the rats of group 2 they varied from 60 to 90‰ (Fig. 1a).

Macrophages, with comparatively dark cytoplasm, lymphocytes, and mast cells were seen in the stroma of the submucosal layer more frequently than in material taken from the rats of groups 1 and 3. AcP activity in the cytoplasm of the spindle-shaped dark cells of the urothelium and in the stromal histiocytes was considerably higher than in the epithelial cells, whereas AlP, the activity of which in the urothelium was low, could not be detected at all in them.

The electron-dense cytoplasm of the spindle-shaped dark cells lying in the urothelium was distinguished by its relatively smooth surface and by the absence of desmosomes and of the characteristic deep interdigitations with neighboring urothelial cells. Compared with the latter, free ribosomes, lysosomes, and vacuoles were often far more numerous in the dark cells. The rough endoplasmic reticulum was more powerfully developed. Spindle-shaped cytoplasmic vesicles, characteristic of the urothelium, were absent in the dark cells lying in the surface layers of the urothelium. Nuclei of the dark cells, with a festooned shape and with denser, irregularly distributed chromatin, also were distinguished (Fig. 1b). Macrophages located beneath the basement membrane or in immediate contact with it on the side of the submucosal layer were exactly similar in their ultrastructure.

Papillomatous hyperplasia was found in only one rat 12 weeks after the beginning of the experiment. By contrast with the rats of groups 1 and 3, in the remaining four animals the hyperplasia was still of the initial diffuse character. The number of spindle-shaped dark cells was reduced about by half. Their structure was unchanged. Tumors, found in all rats on the 20th and 40th weeks of the experiment, were indistinguishable from those in the animals of groups 1 and 3. The dark cells described above were far less frequently seen. Besides them, individual epithelial cells also had cytoplasm of increased electron density. However, the latter were distinguished by the presence of desmosomes, tonofibrils (Fig. 1c), and other features of epithelium. The characteristics of these dark cells of the urothelium have been described by Romanenko.

All tumors (100%) transplanted subcutaneously into recipients from donors of groups 1 and 2 took and began to grow. Solid sheets of transitional-cell carcinoma located in the subcutaneous cellular tissue were larger 3 weeks after transplantation than after 1 week. They were surrounded by well-developed granulation tissue (Fig. 1d). Marked mitotic activity and, in some cases, foci of squamous-cells or glandular metaplasia, were found in these sheets. None of the tumors taken from group 3 donors receiving levamisole took successfully. Masses of necrosis surrounded by granulation tissue were found 1 week after subcutaneous transplantation into the recipients, and only small foci of granulation tissue were present after 3 weeks.

In all animals of groups 4, 5, and 6 (the late stages of carcinogenesis) tumors up to 3.5 cm in diameter, invading the muscular layers of the bladder wall, were obtained by the 50th week. More frequently than in animals of the first 3 groups, dark cancer cells with electron-dense cytoplasm were found in all the killed rats.

Injection of FCA and levamisole changed neither the structure of the tumors nor the character or intensity of their infiltration by mononuclear cells. Meanwhile, the results of subcutaneous transplantation of the tumors were similar to those obtained in the first stage of these investigations. Tumors taken from donors of groups 4 (FCA) and 6 (control) also took and began to grow in 100% of rats, whereas not one of the tumors taken from the animals of group 5 (after levamisole) took successfully. The histological changes were indistinguishable in principle from those found beneath the skin of the recipients of the first three groups.

Injection of FCA before the beginning of carcinogenesis and in its early period was accompanied by an increase in the number of dark spindle-shaped cells in the thickness of the hyperplastic urothelium. By contrast from the repeatedly studied dark epithelial cells of the urothelium, these cells did not possess the set

of features characteristic of epithelium, but had an ultrastructure similar to that of macrophages, located in the submucosal layer of the bladder.

The correlation found between the increase in the number of dark cells (macrophages) in the urothelium of the rats receiving FCA and delay of the development of papillomatous hyperplasia in these animals is in harmony with evidence for the carcinoinhibitory action of macrophages activated by injection of FCA [5]. Injection of FCA in the late stages of carcinogenesis was not accompanied by hyperplasia of the nonepithelial dark cells, possibly on account of the immunodepressive effect of the developed bladder carcinoma.

The results are evidence that among the dark cells found in tumors of the urothelium, epithelial and nonepithelial cells must be distinguished. Whereas the former, like dark cells of the parenchyma of many organs [3], serve as a unique indicator of the functional activity of the tissue, including tumor tissue [1], the latter evidently perform protective functions and have a different prognostic significance.

Injection of levamisole in these experiments had no morphologically detectable effect on carcinogenesis in the urinary bladder. However, it did affect the results of subcutaneous transplantation of the tumor. None of the tumors taken from animals receiving levamisole took successfully in the recipients, by contrast with the 100% take in the other groups. These results agree with evidence of the carcinoinhibitory action of levamisole and, in particular, of its action in preventing metastatic spread of the tumor [4]. The mechanism of this action requires further study.

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INHIBITION OF THE CARCINOGENIC EFFECT OF 7,12-DIMETHYLBENZ(a)ANTHRACENE IN FEMALE RATS BY BUFORMIN, PHENYTOIN, PINEAL POLYPEPTIDE EXTRACT, AND L-DOPA

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7,12-Dimethylbenz(a)anthracene (DMBA) induces adenocarcinoma of the mammary gland in female rats [11]. Meanwhile, after administration of DMBA to an animal, significant hormonal and metabolic disturbances arise [1, 3, 4, 8]; these disturbances characterize the syndrome of cancrophilia, i.e., the sum of the metabolic conditions promoting proliferation of somatic nonlymphoid cells and depressing cellular immunity [5].

The object of this investigation was to study the effect on the carcinogenic action of DMBA of the antidiabetic drug buformin, the antiepileptic drug phenytoin, pineal polypeptide extract (PPE), and the catecholamine precursor L-dopa, which exert their action on different systems of the body that may participate in the formation of the cancrophilia syndrome. However, all these substances likewise possess one common property, namely they lower the threshold of sensitivity of the hypothalamo-hypophyseal complex to homeostatic signals

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