IMMUNOLOGIC ANALYSIS OF RAT SARCOMAS CAUSED BY INJECTION OF HUMAN TUMOR EXTRACTS

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In the course of systematic experiments to study the virus etiology of human tumors undertaken in the last few years the authors have studied the possible carcinogenic action on rats of certain benign and malignant (epithelial and connective-tissue neoplasms). Of 20 human sarcomas investigated, three were found to possess the strongest carcionogenic actions reticulosarcoma, chondromyxosarcoma, and myxosarcoma. The experimental method and results obtained are described in previous publications [1, 2, 4].

We previously showed that a cell-free agent possessing the properties of viruses and capable of inducing malignant neoplasms, mainly sarcomas of different histological structure and location, in Wistar rats, can be isolated from human sarcoma tissues. Furthermore, a carcinogenic factor similar in its physicochemical characteristics to the virus agent from human sarcoma tissue could be isolated from the tissue of these sarcomas induced in rats [1, 2]. Five transplantable strains were obtained, two of which were transplanted by cell-free extracts.

The study of the antigenic structure of the induced rat sarcomas showed that they contain a specific tissue antigen, different from the antigens of normal rat organs. This antigen was closely similar in its electrophoretic mobility to serum β-globulins. The results of experiments with enzymes showed that the antigen consists of protein, possibly with admixture of polysaccharides [4].

In this investigation an immunochemical analysis was made of rats tumors induced by extracts of human sarcomas in order to determine the nature of the neoplasms produced.

EXPERIMENTAL

The test object consisted of three groups of rat tumors induced by a saline extract of human sarcomas.*

The tumors of group 1 were serial passages of a primary reticulosarcoma KRS-321 induced by injection of a saline extract of a human reticulosarcoma. Tumors of the 1st, 5th, 7th, 10th, 11th, 16th, and 24th generations in rats were used in the experiments. Histologically they were all reticulosarcomas. The neoplasms of group 2 had developed in different rats after administration of the same extract of human reticulosarcoma: KRS-321 (reticulosarcoma), 357 (myogenic sarcoma), 358 and 375 (fibrosarcomas), 362 and 344 (polymorphocellular sarcomas), 373 (spindle-cell sarcoma), and 403 (leiomyosarcoma).

The tumors of group 3 had developed in different rats after injection of saline extracts of human sarcomas of different histological structure. The following rat neoplasms were taken: tumor 364 (peritoneal mesothelioma), 368 and 382 (fibrosarcomas), 399 (fibroadenoma of the breast), 413 (polymorphocellular sarcoma).

The immunologic analysis was made with rabbit antisera against rat sarcoma 321, absorbed with saline extracts of normal organs and serum of Wistar rats in gel-microdiffusion experiments. The technique of absorption was described earlier [3, 4]. Altogether 7 immune rabbit sera, against the saline extract of rat sarcoma 321, were obtained and tested. High specificity was detected in two of these sera (105 and 104) and they were used in the present investigation.

*The method of preparing the extract and of the transplantations and the morphology of the developing neoplasms were described previously [1, 2].

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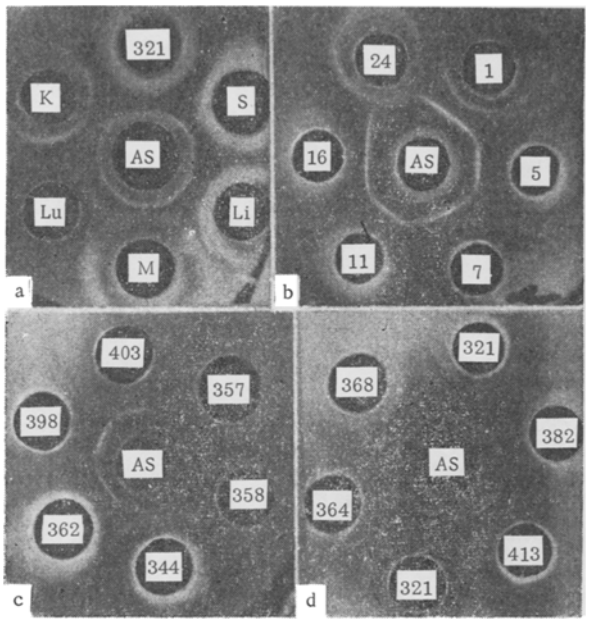

Gel microdiffusion reaction between antiserum and tumor extracts. a) Specificity of antiserum against rat sarcoma 321 after absorption with extracts of normal rat organs. 321) sarcoma; S) spleen; Lu) lung; M) muscle; Li) liver; K) kidney; b) preservation of tumor antigen of sarcoma 321 during passages in Wistar rats. 1, 5, 7, 11, 16, 24) Generations of sarcoma 321; c) common serologic properties of tumor antigen of sarcomas induced by extract of the same human sarcoma (explanation in text); d) common serologic properties of tumor antigen of tumors induced by extracts of different human sarcomas (explanation in text); As) absorbed rabbit antiserum against rat sarcoma 321, specificity of which is indicated in the figure, a.

The protein concentration in the antigens was determined by Kjeldahl's method for the biuret reaction and equalized.

EXPERIMENTAL RESULTS

After absorption with saline extracts of normal organs, the antiserum against rat sarcoma 321 (see figure, a) gave a precipitation line only with the sarcoma antigen. Consequently, this antiserum revealed a specific tissue antigen of the tumor which was absent from normal tissues or present in them in extremely small concentrations.

The fate of this tumor antigen was next studied during prolonged passages of the primary induced sarcoma 321 in adult rats. It is clear from the figure, b that the antigens of all the tested generations of sarcoma 321 gave a clear line of identity with the original sarcoma against the antiserum specific to this sarcoma (KRS-321). Preservation of the tissue-specific antigen until the 24th generation during these passages showed that the malignant transformation in this type of carbinogenesis is highly stable.

In the next series of experiments rat tumors induced by injection of extract from the same human reticulosarcoma were studied. The antigens of all these tumors gave clear lines of identity with the antigen of rat sarcoma 358 induced by the same extract (see figure, c). Consequently, all the tumors induced by the same tumor extract contained a common specific tissue antigen.

In the last series of experiments rat tumors arising after injection of extracts of human tumors of different histological type. Rat tumors 368, 382, and 413 were induced by an extract of chondromyxosarcoma, tumor 364 by an extract of myxosarcoma, and tumor 399 by an extract of reticulosarcoma.

It will be seen from the figure, d that antigens of tumors 382, 368, 413, and 364 reacted with antiserum specific to sarcoma 321. The character of the lines demonstrates the serologic homogeneity of the tissue antigens of these tumors and the tumor antigen of sarcoma 321.

The antigen of rat tumor 399 gave no reaction. Evidently, the fibroadenoma of the breast at this stage did not contain any detectable quantity of tumor antigen.

The serologic methods used have not yet answered the question whether the immunologic difference discovered are qualitative or quantitative. In the present experiment, within the limits of sensitivity of the method, the difference appeared to be qualitative. However, both explanations can be considered as equally valuable for judging the mechanism of genetic transformation of the cell during malignant change. The only difference is whether this is a case of the depression of a completely inactive gene or of stimulation of a weakly active gene producing the specific tissue antigen.

Malignant transformation first involves the genes concerned in synthesis of the cell globulins. These globulins evidently help to maintain the normal morphophysiological state of the cell. Indirect immunochemical evidence [4] indicates a disturbance of the synthesis of the cell polysaccharides.

It follows from the results described above that the sarcoma tissue antigen was preserved for more than one year until the 24th passage of the tumor in rats. This demonstrates the stability of the immunochemical transformation of the tissue under the influence of human sarcoma extract.

All tumors of rats developing after injection of the extract of the same human sarcoma contained one common tissue antigen serologically identical with the tumor antigen of rat sarcoma 321 (see figure, c). This suggests that malignant change under the influence of human tumor extract takes place by a mechanism of virus type.

All malignant tumors of rats induced by extracts of different human sarcomas, likewise contained a tissue antigen with properties common to the specific antigen of rat sarcoma 321 (see figure, d). The serologic homogeneity of the specific antigens of the tumors induced by extracts of different human sarcomas demonstrated by these experiments may be explained by the fact that malignant transformation of the cell probably takes place by the same biochemical mechanism. The carcinogenic factor directly or indirectly sets in motion the same pathological chain of biochemical processes, leading to malignant anaplasia of the cell. The possibility is not ruled out that in these experiments the first link of this chain of processes was activation of a latent oncogenic rat virus by the human tumor extract.

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