ONCOLOGY

THE INHALATIONAL METHOD OF TRANSPLANTATION OF TUMORS

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The problem of lung cancer in man has recently been widely discussed in the oncological literature. The interest in this problem is due to the increase in the number of cases of this disease among the population in various countries, especially in large cities and industrial centers [4, 6, 7]. In connection with the increasing attention which is being paid to lung cancar in man, several questions arise, which call for experimental investigation.

It is known that patients with cancer of the lungs may excrete into the external environment isolated tumor cells or groups of cells, which have become detached from tissues that are being destroyed [1, 2]. Is it possible for "infection" (homotransplantation) of these tumor cells to take place in persons near the patient?

N. N. Petrov [5], describing some unsuccessful experiments on inoculating human subjects with tumors from other patients, pointed out that "it would be quite untrue to conclude from these solitary facts that inoculation of human tumors cannot succeed in man" (p. 270). In numerous experiments carried out at the beginning of the experimental era of oncology, transplantations were usually done in animals whose origin was unknown and which had different hereditary features. In some cases, few it is true, these experiments were successful, especially if the transplantations were made from parents to offspring, if the recipients were young and the tumor possessed powers of malignant, infiltrative and metastatic growth (N. N. Petrov, 1947). Is it therefore possible to completely exclude "infection" (homotransplantation) of members of a family, especially the children of a patient, by inhalation of lung cancer cells which have been excreted into the external environment? Is the inhalational transplantation of tumors generally possible? These problems formed the background to our experiments on the study of the inhalational method of transplantation.

There are indications in the literature of the successful transplantation of tumors by the inhalational route [3, 9, 10], but the tumors studied in these investigations were transplanted tumors. M. M. Maevskii and T. M. Maevskaya [3], for example, used Ehrlich's carcinoma — an old laboratory strain of the tumor — repeatedly transplanted intraperitoneally in mice, and Schmidt [9, 10] used several laboratory strains of tumors. The relative ease of transplantation of these strains in the lung may be due to the adaptation of these tumors to animals in the course of prolonged transplantation.

We decided to test the possibility of inhalational transplantation of a spontaneous tumor located in the lungs. So far as we know, such an investigation has never been made before. Nevertheless it would most closely reproduce the conditions appertaining to cancer of the lung in man. By using material from a recent case of malignant disease, and not a laboratory strain of tumor which had undergone prolonged passage, we might, to some extent, obtain the answer to our question. By chance we were able to select some suitable material.

EXPERIMENTAL METHOD

A spontaneous tumor of the mammary gland was found in female mouse No. 675, belonging to the high-cancer C_3HA line, in the 12th month of life. At autopsy it was found that, besides the tumor of the mammary gland, the mouse had multiple tumor nodules in both lungs (Fig. 1).

Histological examination showed that the mammary gland tumor was an adenocarcinoma and that the tumors in the lungs were metastases of this adenocarcinoma. This was thus a tumor, arising in natural conditions and having, as a result of metastasization, the situation which we needed — in the lungs.

A finely minced suspension of tissue in physiological saline was made from the nodules of this tumor, and injected intranasally into 4 10-week-old mice of the same line C_3HA in which the tumor had developed. Inhalation was carried out under ether anesthesia. The dose of tumor suspension administered was 0.03-0.05 ml.

EXPERIMENTAL RESULTS

One of the experimental mice died from an accidental cause 2 weeks after transplantation; no tumor was found in this animal's lung. In 3 mice, 10 weeks after transplantation, we found quickened breathing, and 11 weeks from the beginning of the experiment they were sacrificed. In all 3 animals well-developed tumors were seen in the lungs, and in two of them more than half the lung tissue was replaced by tumor. Histologically these tumors duplicated the structure of the original tumor. The lung tumor of one of these mice was transplanted by inhalation to 4 fresh mice of the C_3HA line. Two weeks later, one mouse developed a tumor of the lung. Futher transplantations of this tumor led to the development of similar tumors in the third and fourth generations.

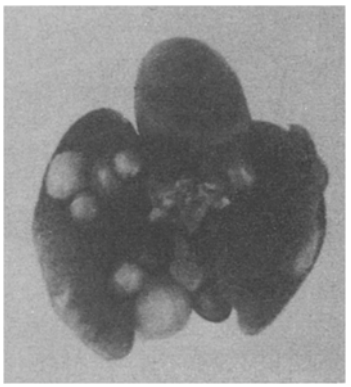

Fig. 1. Metastases of a spontaneous adenocarcinoma of the mammary gland in the lung of female mouse No. 675.

The experiments thus gave a convincing answer to the question which had been asked on the possibility of homotransplantation of cells of a spontaneous tumor by the inhalational method. In these experiments, however, the conditions, for such a transplantation had been the most favorable possible: a relatively large dose of tumor suspension was inhaled directly into the respiratory tract of the animals.

The question naturally arose whether it was possible for tumors to developed in the lung by inhalation of tumor cells in ordinary conditions. On a priori grounds it might be thought that the probability of such "infection" was infinitesimally small. Nevertheless we carried out an appropriate experiment. Young mice of the same pure line were reared together with mice affected by tumors of the lungs. Observations were made on both the newly introduced young mice and on the offspring of these mice and those with the tumors. We considered the observations on the offspring to be interesting in view of the fact that young, and especially newborn mice are more susceptible to homotransplantation [8, 9, 10]. In the course of 2 years of observation we found no case of "contact infection": no tumor was found in the lungs of a single newly introduced mouse or of the offspring of the experimental mice. It must be remembered that in our experiments the mice with tumors of the lungs and the mice in contact with them belonged to the same inbred line.

Thus although our experiments showed that by inhalation of a suspension of cells from a spontaneous tumor, homotransplantation of the inhaled tumor cells is possible and tumors may develop, situated in the lungs, the chances of success in homotransplantation of this sort in normal conditions are infinitesimally small.

In natural conditions, patients with malignant neoplasms of the lungs excrete into the external environment cells detached from disintegrating tumors. Under these circumstances it is not impossible for a viral agent to be liberated and spread. The extensive clinical material, however, points to the absence of cases of contact infection of cancer of the lung. It is probable that this path of spread of the carcinogenic agent is of no practical importance although, of course, much of the material must be reexamined in the light of modern knowledge of the greater susceptibility of young animals (especially newborn) to cancer-producing virus and of the long latent period of these tumors.

During transplantstion of pulmonary metastases of spontaneous adenocarcinoma of the mammary gland in a mouse into the lung, we noted a number of peculiarities of the growth of these tumors. They increased in size much more slowly than did the same tumors transplanted subcutaneously. In the case of primary transplantation, for instance, when subcutaneous transplantation was carried out at the same time as transplantation into the lung, large tumors, suitable for transplantation, developed in 3 weeks, whereas such tumors developed in the lungs in $2\frac{1}{2}$ months. In the subcutaneous tumors areas of extensive necrosis were observed, but these were few in the lung tumors. In mice sacrificed in the initial stage of growth of the tumor (on the 20th-25th day after transplantation), there were small, greyish nodules of tumor tissue, the size of a pin head, situated in the lung, in areas of lung tissue which were macroscopically unchanged. In mice dying from lung tumors, or sacrificed when signs of tumor were present — severe dyspnea and wasting ($2-2\frac{1}{2}$ months after inhalation) — in the lungs there were either

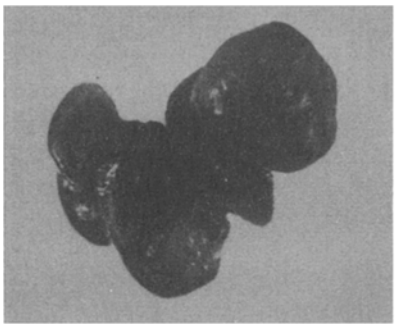

Fig. 2. Tumor of the lung developing after intranasal inhalation of a suspension of adenocarcinoma of the mmary gland. Large tumor nodules, almost completely replacing lung tissue.

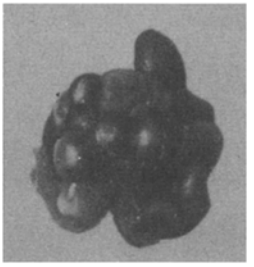

Fig. 3. Hepatoma — a tumor in the lung, developing as the result of inhalational transplantation.

solitary, large tumor nodules, occupying almost the whole of the thoracic cavity, reaching a diameter of 1.2 cm, compressing the heart and the residual lung tissue (Fig. 2), or a large number of smaller nodules, hanging in bunches from the hilum of the lung, almost completely replacing the lung tissue and also compressing the thoracic organs. The weight of the tumors, in such cases, filling the thoracic cavity, reached 1.5 g. The survival period of the animals with a tumor developing after inhalation of a suspension of a spontaneous tumor was longer than in cases where the same tumor was transplanted subcutaneously into the same mouse.

In order to ascertain whether these distinctive features depended on the transplantation of this particular spontaneous tumor of the mammary gland, we tried the method of intranasal transplantation also with certain transplanted strains of mouse tumors.

We had available a hepatoma, transplanted in mice of line C_3HA, and 2 adenocarcinomas: one derived from a spontaneous tumor of a C_3HA mouse and transplanted subcutaneously into mice of the same line, and the other derived from a spontaneous adenocarcinoma in a C57 mouse (black) and transplanted subcutaneously into mice of line C57 (black). As might have been expected, in accordance with data in the literature [3, 9, 10], all 3 tumors were readily transplanted by intranasal infection, and gave rise to growth of tumors in the lungs of the mice (Fig. 3). The transplanted tumors retained the histological structure of the original tumors.

It must be mentioned that success in intranasal infection depends to a large extent on the correct anesthesia of the animal and throughness of the infection. In deep sleep, mice readily inhale small droplets of the suspension which enables tumor cells to pass through the trachea and bronchi into the lung tissue. If the anesthesia is light or the suspension is inhaled in large drops, inhalation of the liquid is interfered with, it is scattered by the expired air and the success of the transplantation is jeopardized. These findings are in agreement with the observations of M. M. Maevskii and T. M. Maevskaya [3].

Transplanted strains of tumors, when inoculated intranasally, possess the same properties as were noted during transplantation of a spontaneous tumor into the lung: slow growth, relatively feeble development of necrotic processes, longer survival of the animals affected by the tumors. This makes the method of intranasal inoculation of animals particularly suitable for the study of certain problems of the immunology of tumors which require comparatively long periods of observation, such as for example, the increase in antibody titers during the growth of tumors. Slowly developing tumors in the lungs may be used as models for the study of the problem of vaccination and for investigating in vivo the action of specific antitumor antibodies.

The intranasal method of transplantation of tumors may be used for the heteroptransplantation of tumors in general and, in particular, of cancer of the lung in man. Finally, the tumor, growing in the lung, is a suitable model for the study of the action of chemoterapeutic drugs when given by the inhalational method.

SUMMARY

The inhalation of a cell suspension of a spontaneous tumor (metastases in the lung of spontaneous adenocarcinoma of the mammary gland in C_3HA mice) results in homotransplantation of the inhaled cells with the development of tumors in the lung. Observations of natural contacts of healthy mice with animals having transplanted pulmonary tumors for a period of 2 years failed to reveal such transplantation.

The inhalation method of tumor transplantation may also emplored for inoculating transplantable laboratory strains, as well as for transplantation of naturally occuring tumors in mice. The inhalation method is of help in maintaining the tumor strain localized in the lung during passage.

Tumors developing in the lungs as the result of inhalation transplantation have some peculiarities: a longer duration of growth in comparison with subcutaneous inoculation, slow development of necrotic processes, and longer survival of the mice suffering from this tumor. All this makes this method convenient for solving a number of experimental oncological problems.

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