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Studies on Active Immunization to Sarcoma-carrying Mouse

Establishment of the Clone of Transplantable Sarcoma

(immunization/transplantable sarcoma/clone)

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Sarcoma induced after Methylcholanthrene intramuscular administration to $C_{57}BL$ mouse was transplanted in the same inbred mouse. Tissue cultures of the above established sarcoma were then prepared.

When the colony grew to an appropriate size (the number of cells being 1×10^2 or more per colony) in the Petri dish, a sterile cylinder was set up surrounding one colony and pipetting was done to pick up the tumor cells in the colony. After that, the tumor cells were cultured in a CO_2 incubator.

A clone system of the transplantable sarcoma was established.

In pursuing studies on immunity to tumors, there are many fields yet to be explored such as the question of antigenicity of the tumor itself as discussed by Fairley (1), Old (2), Klein (3) and Hudson (4) and a decline in the immune capacity on the part of the host (5—8).

Moreover, the immune tolerance and enhancement (9—11) to tumor antigen of the cancer-carrying host makes immunological treatment of the tumor difficult.

There is a growing understanding that strengthening of antigenicity on the part of tumor and reactivity of abnormal defense mechanism of the living body on the part of the host, that is, activation of immunological response to the two are indispensable for an increase in the immune effect on the tumor.

We succeded in establishing a transplantable tumor strain in $C_{57}BL$ male mouse which is considered suitable for experiments in active immunity, using a combination of tumor cell-diazo-human-gamma-globulin for sarcoma-carrying mouse.

MATERIALS AND METHODS

Experimental Animals

Inbred C_{57}BL male mice were used.

With C_{57}BL mouse, the involvement of virus and appearance of spontaneous malignant tumor are extremely rare unless treatment such as X-Ray radiation and drugs have been given.

Experimental Tumor

When the survival time of the host is not over two weeks after transplantation, the period is too short to acquire anti-tumor activity.

When the host survives over a long period of time, it is inconvenient to make observations on the life-prolonging effect. Thus, a transplantable tumor stain with a survival time of 4—5 weeks after transplantation showed facilitate research projects.

Methylcholanthrene 0.25mg was dissolved in Freund's incomplete adjuvant 0.5ml ; the solution was mixed well with 0.5ml of 0.9% saline solution; 0.25ml of the mixture (containing methylcholanthrene 0.0625mg) was injected intramuscularly into the femoral region of mouse hind leg in 20 C_{57}BL male mice.

The strain obtained from the tumor 180 days after intramuscular injection of methylcholanthrene was judged to be the most suitable for the experiment.

Culture solution used 15% calf serum added to the Eagle's minimum essential medium. Removing blood and other impurities, initial cultivation produced tumor tissue isolated by the aseptic procedure was minced in PBS (pH 7.4) supplemented with penicillin and streptomycin. The solution was replaced 2—3 times, 0.25% trypsin was added and pipetting as usual ; lastly, cells were counted in solution prepared by dissolving 0.05% crystal violet in 0.1M citric acid solution and were suspended in culture solution ; the solution was then distributed into culture jars and cultivation was carried out at 37°C.

The cells in the above-mentioned culture jars became a full sheet 4 to 5 days later, and the cultivation was then repeated. To free the cells from the glass wall, 0.05% trypsin solution was used.

Cultivation was done in a Petri dish and when the colony grew to an appropriate size (the number of cells being 1×10^2 or more per colony), the colony for cloning was examined under a microscope, and a sterile cylinder (5—10mm in diameter) was set up in such a way as to surround the colony examined. In doing so, vaseline was applied to the bottom of the cylinder so that the bottom of the cylinder adhered closely to that of the Petri dish. With a pipette, 2—3 drops of 0.05—0.01 % trypsin were added to the cylinder, then the trypsin was drawn up gently with a pipette for removal. After that, fresh culture solution was added, pipetting was done and the colony was transferred to a culture solution in another Petri dish, and cultivation carried out in a CO_2 incubator.

After repeating the above-mentioned procedure three times,the colony was

subjected to successive cultivation, and some preparations were freeze-dried for preservation.

RESULTS

The tumor strain was transplanted subcutaneously into male $C_{57}BL$ mouse of the same line, and we confirmed the survival of the transplant 5—7 days after transplantation.

We then prepared a clone system of transplantable sarcoma by means of tissue cultures of the isolated tumor.

The rate of successful transplantation was 100 percent.

The histologic findings showed undifferentiated spindle cell sarcoma (Fig. 1), the infiltrative development was seen at the margin of the tumor (Fig. 2). Viral particles were not observed under the electromicroscope. The survival time for the host was not over 4—5 weeks.

When cultured tumor cells were injected into the tail vein of $C_{57}BL$ male mouse, transplantation was established 100 percent under the skin and in the lung (Fig. 3). The histologic findings were the same as mentioned earlier.

The cultured tumor cells showed a slender spindle-shape (Fig. 4) and formed a piled-up colony in a culture jar.

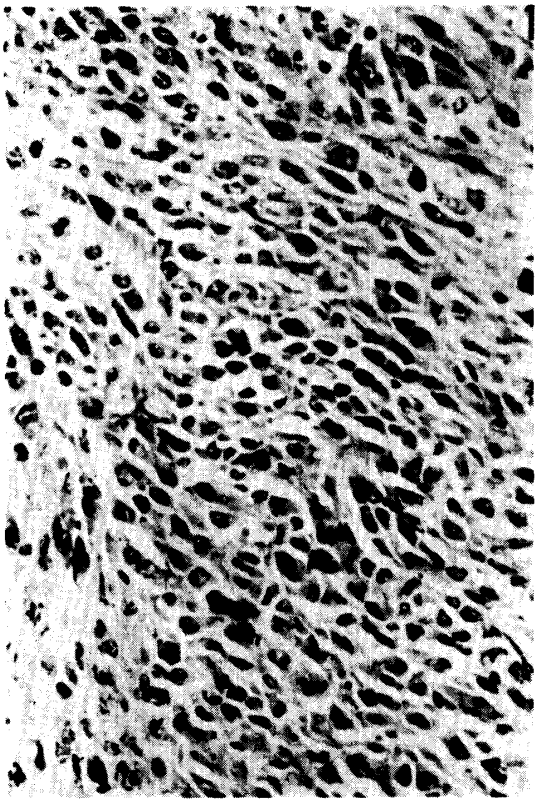

Fig. 1. Experimental transplantable tumor. H-E stain ×200. Spindle-shaped cell sarcoma of the undifferentiated type.

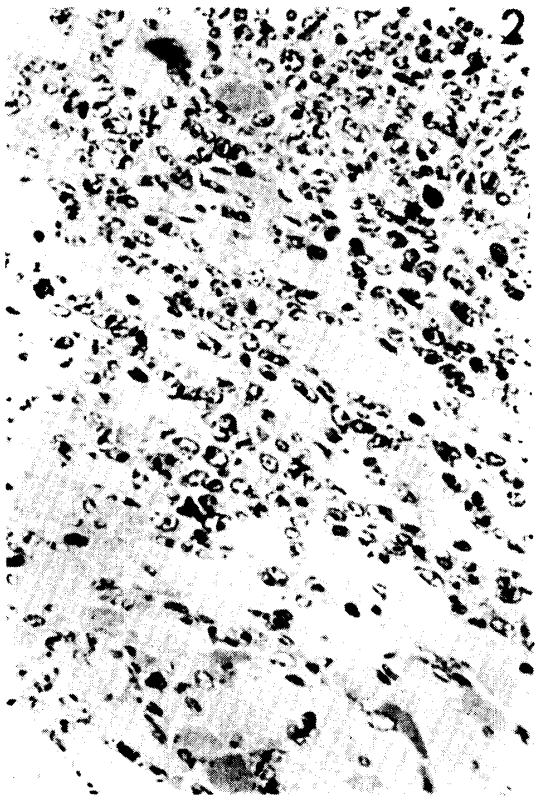

Fig. 2. Histology depicting the margin of experimental transplantable tumor. H-E stain ×200. Infiltration into the connective tissue and adipose tissue.

Fig. 3. Pulmonary tumor induced by injection of experimental transplantable tumor cell into the tail vein.

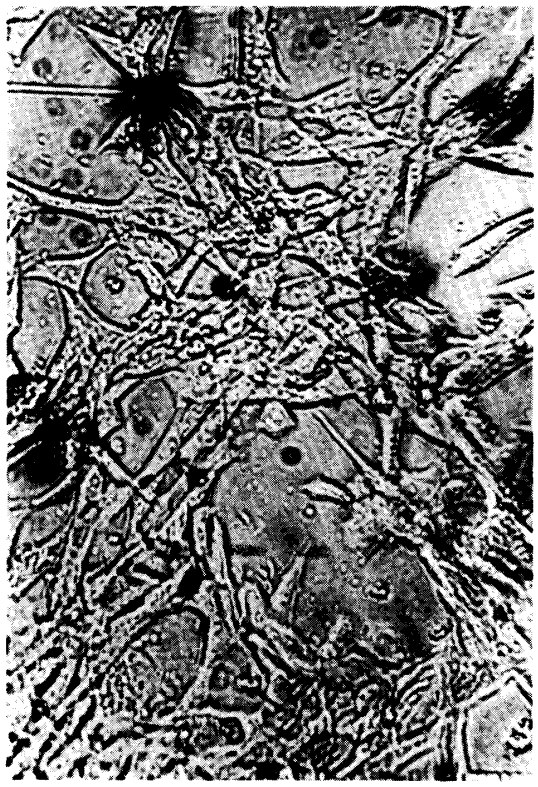

Fig. 4. Culture of tissue from an experimental transplantable tumor. ×200. Slender, spindle-shaped cells are seen forming a piled-up colony.

DISCUSSION

Studies on tumor antigen originated with Prehen and Main (12) and Klein *et al.* (13). Today, there is no doubt about the existence of tumor antigen. However, the chemical character has yet to be clarified Using methylcholanthrene-induced sarcoma in in-bred rats, Takeda and Aizawa (14) classified the antigenicity into three categories and found that there was some cross-resistance in the same category.

Yamagishi *et al.* (15) reportedly prepared methylcholanthrene-induced sarcoma in C_3H/HeJ mice, analyzed the methylcholanthrene-induced tumor thus prepared and were able to obtain a fraction which has two opposing activities, namely, the tumor facilitating fraction and the tumor protective fraction.

Using spontaneous tumor in animals enables one to set up a more approximate experimental system as a model, the objective being to obtain immunological therapy for malignant tumors in human. However, it is often difficult to obtain uniform experimental materials in large quantities at one time and there may be a complex causal relation between the host and tumor.

For the purpose of examining the active immune effect of a combination of tumor cell-diazo-human-gamma globulin on cancer-carrying mice, we established a clone system of transplantable sarcoma suitable for this experi-

ment with the survival time of 3—5 weeks after transplantation in in-bred $C_{57}BL$ male mouse using sarcoma induced by subcutaneous injection of methylcholanthrene.

Only male animals were used in order to maintain the geno-immunological uniformity between transplanted tumor and the host since an experiment using transplantable tumor was to be conducted.

The antibody producing capacity of mice is said to reach a peak two months after birth and decrease markedly at the 19th month.

This tumor was undifferentiated spindle cell carcinoma, scanty of blood vessels and fibers, grew in a way to infiltrate and destroy surrounding tissues and in a few cases there was a metastasis to the lung.

Tissue culture of this tumor can easily be carried out, it can be preserved by freezing ; transplantation back to male mice of the same line was 100% successful and the gross observations and histologic findings of the surviving tumor were the same as seen in the original tumor.

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