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Active Immunization of Sarcoma-Bearing Mouse with Tumor Cell-Diazo-Human-Gamma-Globulin Compound

(transplantable sarcoma/active immunization)

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Using a transplantable sarcoma already established in mouse, we carried out an active immunization by giving a mixed injection of a tumor cell-diazo-human-gamma-globulin compound and Freund's incomplete adjuvant, examined the life-prolonging effect and histology of transplanted tumor and reticulo-endothelial system and obtained findings to show that the anti-tumor activity was successfully established in the host.

Various attempts to strengthen tumor antigenicity such as early resection of tumor tissue, electric coagulation method, ligation or ligation opening technique (1, 2), anti-cancer drugs, treatment with radiation (3, 4) and a method using virus infected tumor cells are being made to induce immunological anti-tumor activity.

Injections such as oleic acid, B. C. G. (5—7) and Corynebact paruum were also given as a means to activate the reticulo-endothelial system and increase the immunological anti-tumor activity on the part of the tumor-bearing host.

Furthermore, methods such as transplantation of normal or sensitized medullary cell, transfusion of lymphocytes (8—10) and transfusion of leukocytes are being tried in an attempts at resolution of the problem.

Czajkowski *et al.* (11) carried out an active immunization by giving a mixed injection of Freund's complete adjuvant and a tumor-cell-diazo-protein compound (TDPC) prepared by gamma-globulin from a cell suspension of animal tumor and utilizing bisdiazo-benzidine as a coupling agent.

A marked immunizing effect was observed against C_3H mice bearing spontaneous mammary adenocarcinomas and methylcholanthrene-induced squamous cell carcinomas.

Czajkowski *et al.* (12) also reported that active immunization proved markedly effective for malignant tumor in humans.

MATERIALS AND METHODS

Tumor Transplantation

Methylcholanthrene-induced sarcoma was transplanted subcutaneously into

C_{57}BL mouse.

The Coupling Method of Tumor Cell and Diazo-Human-Gamma Globulin Compound (TDHC)

After tumor tissue was isolated by aseptic procedures, blood and other debris were removed by washing in 0.9 % saline solution. A tumor cell suspension was prepared by forcing the tissue through a stainless steel mesh (diameter 500 μ). The suspension then was centrifuged three times each at 5,000 rpm for 10 min and washed with 0.9 % saline solution. Then, 0.9 % saline solution (1.5 ml), human-gamma-globulin (0.1 ml) and BDB 1.5-fold dilution (0.5 ml) were added to precipitated cells (0.5 ml), and incubation was carried out at room temperature for 10 min. The mixture was then centrifuged twice each at 5,000 rpm for 10 min and washed with 0.9 % saline solution. The supernatant fluid was removed and 0.9 % saline solution was added to the precipitated cells to make up 1.0 ml and incomplete Freund's adjuvant 1.0 ml to make water in oil emulsion. We used these mixtures as reinforced antigen for the experiments. The procedure is summarized in Table I.

TABLE I. *The Coupling Method of TDHC*

After mincing a section of tumor tissue (in 0.9 % saline solution), let the solution go through a stainless steel mesh (diameter 500 μ) to make a cell suspension
↓
Centrifuge the suspension three times each at 5000 rpm for 10 min and wash with 0.9 % saline solution
↓
Add 0.9 % saline solution 1.5 ml, human-gamma-globulin 0.1 ml and BDB 1.5-fold dilution 0.5 ml to precipitate 0.5 ml of cells, and incubate the mixture at room temperature for 10 min
↓
Centrifuge twice each at 5000 rpm for 10 min and wash with 0.9 % saline solution
↓
Add 0.9 % saline solution to precipitate the cells to make 1.0 ml and add to incomplete Freund's adjuvant 1.0 ml to make water in oil emulsion and reinforced antigen

Process of Immunization

Mix TDHC antigen and Freund's incomplete adjuvant in equal amounts to make water in oil emulsion and inject this emulsion (0.1 ml) subcutaneously into the hind leg of C_{57}BL mouse at the 5th and 10th day after transplantation of tumor.

System of Experimentation

Three $C_{57}BL$ mice bearing established methylcholanthrene induced sarcoma were used for the system of experimentation. When each transplanted tumor grew to thumb-size, tumor tissues were aseptically isolated. Each tumor tissue was used to prepare TDHC and successive cultivations.

Mice were separated into three groups, namely, Group A consisted of 4 controls and the immunized group of 6, Group B 3 controls and the immunized group of 3 and Group C 3 controls and the immunized group of 5. (Fig. 1)

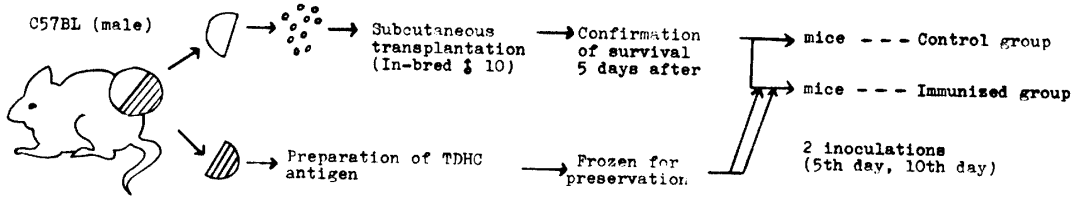

Fig. 1. Active immunization experimental system with TDHC antigen after confirming survival of a transplanted section of transplantable tumor.

Studies were conducted on the response of tumor tissue in the immunized group of mice subjected to active immunization with TDHC antigen and non-immunized group.

RESULTS

Changes in Body Weight and General Conditions

As shown in Figs. 2, 3, 4, animals in each group showed a tendency toward decrease in body weight in the early stage but lost body weight without exception and died with a tumor in the latter stage.

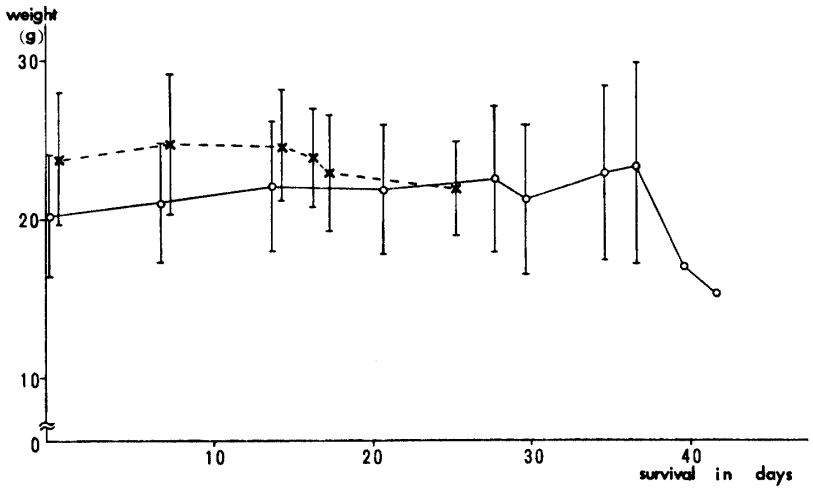

Fig. 2. Survival in days and variations in body weight (g) of immunized group and control group of group A.
○—○ immunized group average survival 34.8 days
×- - -× control group average survival 22.0 days Vertical bars indicate S. D. ($P<0.005$)

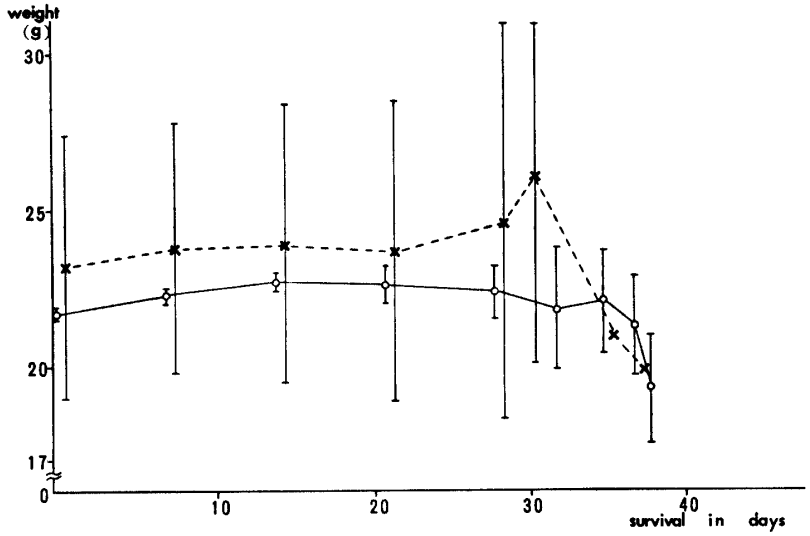

Fig. 3. Survival in days and variations in body weight (g) of immunized group and control group of group B.
○—○ immunized group average survival 36.6 days
×---× control group average survival 31.6 days Vertical bars indicate S. D. ($P<0.25$)

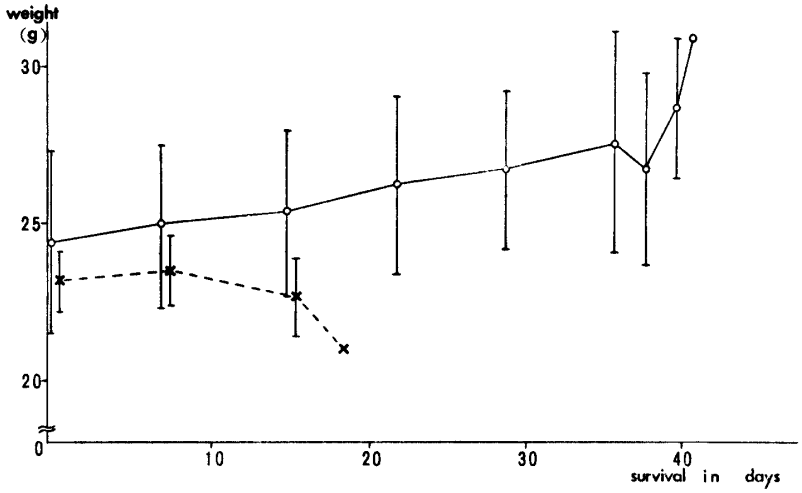

Fig. 4. Survival in days and variations in body weight (g) of immunized group and control group of group C.
○—○ immunized group average survival 39.0 days
×---× control group average survival 16.0 days Vertical bars indicate S. D. ($P<0.005$)

Life-prolonging Effect

The number of survival days after subcutaneous transplantation of transplantable tumor in each group is shown in Figs. 2, 3 and 4.

Histopathological Findings of Mice Subjected to Active Immunization with TDHC Antigen

As to tumor tissue in the group of mice immunized after survival of subcutaneously transplanted section, there was no particular difference in the

center of tumor between the immunized group and the control group; however, in the area around the tumor there was a tendency toward a localized encapsulation of tumor tissue by fibrous connective tissue in addition to marked infiltration of lymphocytes and histiocytes in the immunized group (Fig. 5) compared with the control group (Fig. 6).

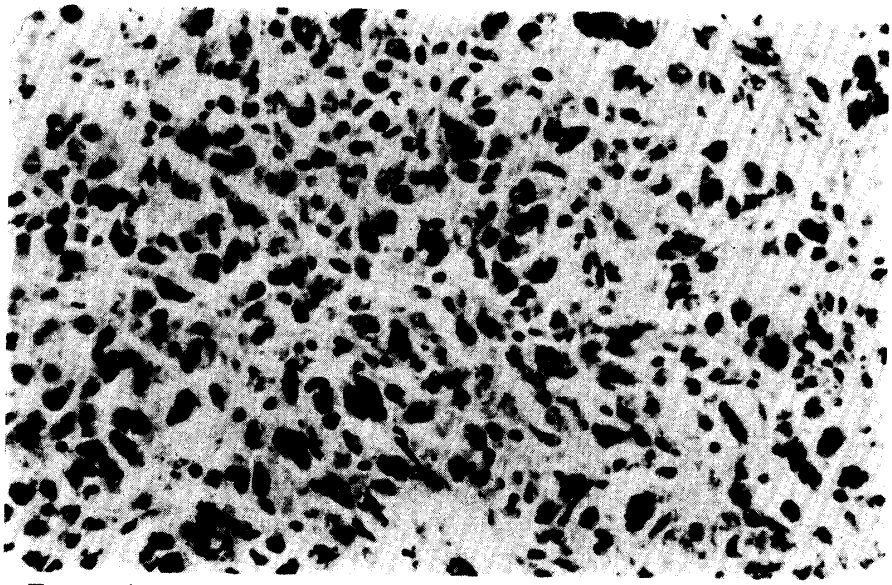

Fig. 5. Tumor tissue from a mouse immunized with TDHC antigen after confirming the survival of transplanted tissue. H. E. stain ×200. Infiltration by lymphocytes, histiocytes and neutrophils is evident. The tumor is relatively localized.

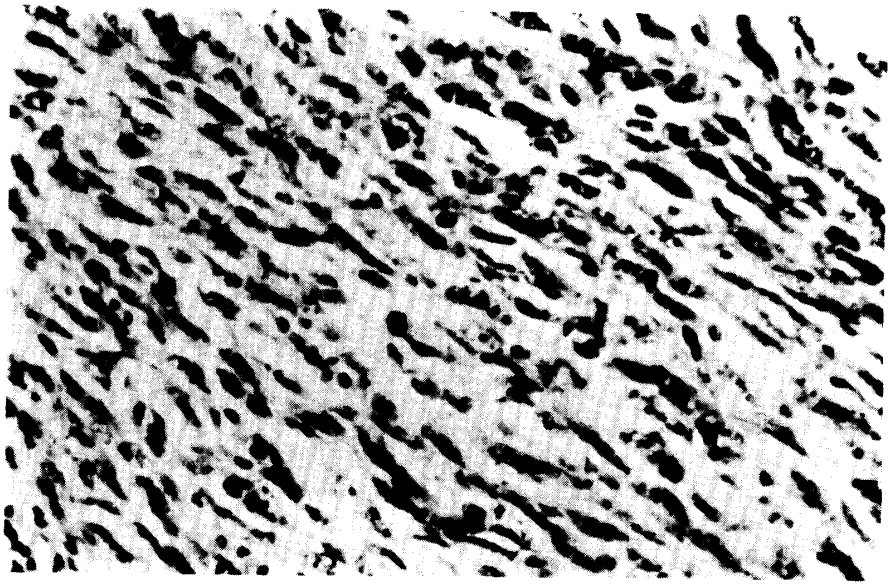

Fig. 6. Histology of tumor tissue from a control mouse not given an injection of antigen after confirming the survival of a transplanted tumor section. H. E. stain ×200. The infiltration of the tumor tissue is evident.

DISCUSSION

In the present experiment, incomplete adjuvant was used in order to

eliminate the reticulo-endothelial system stimulating action and the anti-tumor factor of the complete adjuvant as well as constituents of tubecule bacillus contained in the complete adjuvant (13).

With this method it is difficult to determine whether the disappearance of tumors is due to the anti-tumor activity generated by the immunity treatment or technical failure in the tumor transplantation.

Therefore, we began the immunization from the 5th day after transplantation when survival of the transplanted section could be confirmed.

As to changes in body weight of immunized group, there was an increase independent of an increase in the transplanted tumor up to 7—8 days before death, then a sharp decrease. This finding warrants further study.

Other findings of immunologically anti-tumor activity include cell infiltration of the lymphocytic system, fibrous cytotropic reaction in tumor and sinus histiocytosis at local lymph nodes (14—16).

Histological findings showed the cellular reaction of the lymphatic system and the fibrous cytotropic reaction.

Regarding immune mechanism by TDPC antigen, Czajkowski *et al.* (11) investigated the anti-tumor activity in the production of serum immunity on the basis of results of experiments using spontaneous cancer of the breast in C_3H mouse.

Immunological induction of anti-tumor activity by TDHC antigen is a promising method which should elucidate the mechanisms of tumor immunization and should pave the way for immunological treatment of tumors (17).

In clinical application of this method, hazardous side-effects such as allergy or anaphylaxy due to protein contained in the compound used are expected to appear, therefore further studies are in progress.

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