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Abstract

For the purpose to study in vivo changes of the mice bearing Ehrlich ascites tumor with special emphasis on the problems of cellular antibody and serum antibody, a series of experiments such as neutralization tests in vivo and in vitro study of the effect of lymph-node cells from the tumor bearing animals on target cells were carried out, and the findings thus obtained are briefly summarized as follows : 1. Regional lymph-node cells from the mouse transplanted with Ehrlich ascites tumor cells show a marked cytotoxic action on their cultured target cells, JTC-11, synergistically with serum from mouse bearing Ehrlich cancer. 2. The tumor cells inoculated with lymph-node cells from the tumor bearing animals showed a retardation in growth and finally regressed. 3. Spleen and lymph nodes of tumor bearing animals showed a marked increase in weight.

CELLULAR ANTIBODY IN MICE BEARING EHRLICH CANCER

III. RELATIONSHIP OF CELLULAR ANTIBODY TO SERUM ANTIBODY

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In the previous papers^{1,2} it was demonstrated that the lymphoid cells from the mice bearing Ehrlich cancer inhibit the proliferation of target cells *in vitro* and that it is necessary for these cells to come in contact with the target cells in order to wield a sufficient inhibitory effect on the proliferation. Consequently, it was assumed that the antibodies are adhered to the surface of these lymphoid cells. It is known³ that aside from the antibody bound to these lymphoid cells the serum antibody also plays an important role in the inhibition of tumor growth. However, there is a report⁴ to the contrary that the serum antibody weakens the ability of the cellular antibody, bringing about an enhancement of tumor proliferation, and also another opinion⁵ that the survival of the tumor transplant, which is destined to be discarded by the host, is prolonged by the serum antibody. Therefore, there still remain many unsolved problems about the relationship between the cellular antibody and the serum antibody. For the purpose to clarify these problems the following experiments were conducted: 1) to see the effect of the cellular antibody to which serum antibody was bound previously *in vitro* on target cells; 2) to see whether or not the lymphoid cell that showed anti-tumor effect *in vitro* would also reveal the same effect *in vivo*; and 3) to see changes in the weights of various organs *in vivo* weekly after the tumor transplantation.

MATERIALS AND METHODS

The male adult mice of Cb strain, 4 weeks old, were used. They were fed on solid food MF (Oriental Yeast Co.) with fresh vegetables. These mice received the transplantation of Ehrlich ascites tumor cells subcutaneously to the total of 5×10^6 each in between the shoulder bones and scapula on both sides. After 14 days the animals belonging to the first group were sacrificed by decapitation and the lymph nodes of axillary and cervical regions were obtained.

These lymph nodes were then broken into small pieces with ophthalmic scissors in cold Hanks solution, centrifuged at 1,000 rpm for 10 minutes, 3 times each, and the sediment so obtained served as the lymph-node cells from "sensitized" animals. Other animals served as control, and without any pretreatment the lymph nodes from the similar regions as in the former group were obtained and the lymph-node cells were prepared in the same fashion as just mentioned. The animals belonging to the second group were inoculated with the tumor cells as in those of the first group. The blood was obtained by heart puncture two weeks after the tumor inoculation. The serum was separated by centrifugation, inactivated by being heated at 56°C for 30 minutes and used for the test of antitumor activity. In the test, fresh guinea-pig serum served as the complement. It was used after being absorbed by washed, packed spleen cells of mouse at 4°C for one hour. To determine the antitumor effect of mouse-antiserum, following MÖLLER's method⁶, the mixture of 0.05 ml cell suspension containing 1×10^7 JTC-11 cells/ml of medium and 0.05 ml guinea-pig serum was incubated at 37°C for 90 minutes, and after staining by SCHREK's method the number of stained cells was taken for this purpose. The culture medium was YLE solution containing 20% bovine serum. The target cells used in tissue culture were a substrain of Ehrlich cancer cells (JTC-11, SATO). For the test of antitumor activity of the lymph-node cells, 2×10^4 of the tumor cells were cultured with 4×10^5 lymph-node cells. And both antiserum and complement were used in the proportion of 0.12 ml each for each 1.5 ml of the culture medium. After incubating for 24 or 48 hours at 37°C, the cell counts were taken by KATSUDA's method⁷, which is a modification of SUNFORD's principle, and the average number from 3-4 culture vessels was recorded.

For the determination of living cell number the so-called "unstained cell count method" of SCHREK was used, i. e. by the supravital staining with 1% Esosin-Y solution the living cell number was calculated by subtracting the stained cell number from the whole cell number and it was used for lymph-node cells.

As for determining the anti-tumor effect of lymph-node cells *in vivo*, the cell mixture of 5×10^4 Ehrlich ascites tumor cells and 5×10^6 lymph-node cells was immediately transplanted subcutaneously on the back of mice.

In addition, for the purpose to pursue *in vivo* changes after the implantation of tumor cells such organs as liver, kidneys, spleen, lungs, thymus and axillary lymph nodes were taken out of mice. Once every week for the period of four weeks their weights were compared with respective organs obtained from non-sensitized groups of mice. In this instance, organ enlargement assay was represented by the percentage of body weight according to the method of SIMONSEN and others⁸.

RESULTS

The blood serum obtained from the animals two weeks after the tumor inoculation showed a slight antitumor activity, i.e. by incubating for 90 minutes 18 per 300 tumor cells were stained with Eosin-Y stain, showing 6% cytotoxic titer, while the titer with the serum from control animals proved to be 0%. In the cell culture with addition of antiserum some anti-tumor effect was observed, but when lymph-node cells were further added to such a culture, a marked anti-tumor effect was observed (Fig. 1).

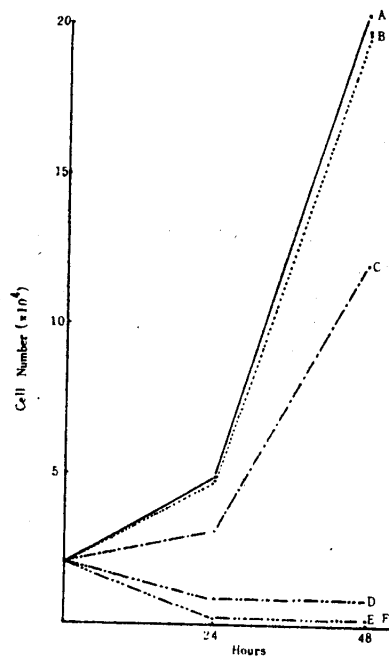


Fig. 1 Growing Curve of the JTC-11 Cells Cultured with or without Lymph-node Cells and Serum.

A: control without lymph-node cells and serum B: cultured with lymph-node cells from control mice C: cultured with lymph-node cells from sensitized animals. D: cultured with the serum from tumor bearing animal. E: cultured with serum from tumor bearing animals and lymph-node cells from control mice. F: cultured with serum and lymph-node cells from tumor bearing animals. Method: see text.

In the *in vivo* experiment the tumor formation after the implantation with lymph-node cells from tumor bearing animal was delayed and tumors themselves did not grow much in contrast to that with non-sensitized ones, but later they became smaller, showing regression (Fig. 2). On the 28th day after implantation

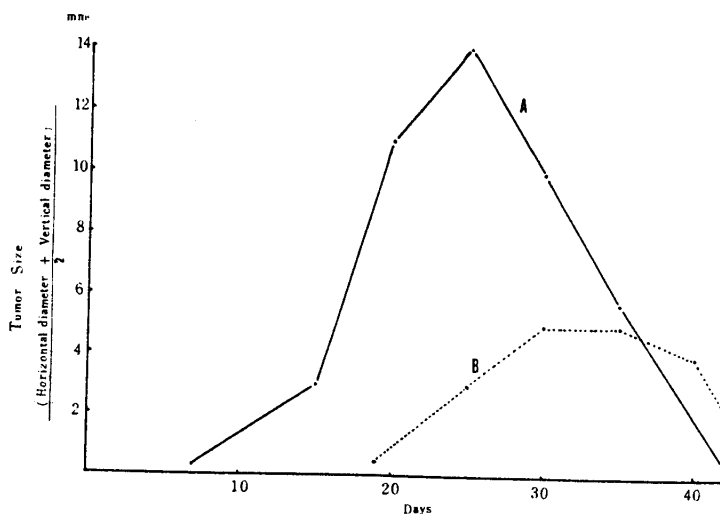


Fig. 2 Growth of Ehrlich Cancer Inoculated with Lymph-node Cells on the Back of Mice.

A: control group with lymph-node cells from non-sensitized animals. B: experimental group with lymph-node cells from tumor-bearing animals. Method: see text.

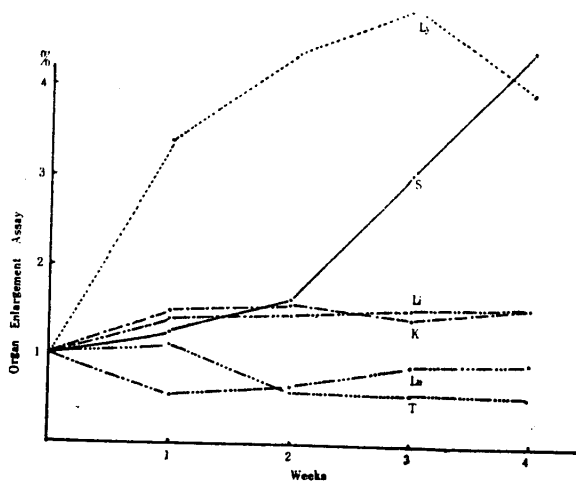


Fig. 3 Changes in Organ Weight after Tumor Inoculation (organ enlargement assay).

Ly: lymph-node S: spleen Li: liver K: kidney Lu: lung T: thymus Method: see text.

the weight of tumors transplanted with the lymph-node cells from tumor bearing animal was 0.061 g in the mean value while it was 0.565 g in the control.

The organs of the tumor bearing mice showed an increase in weight, especially in spleen and lymph nodes (Fig. 3).

DISCUSSION

From the experiments of ALGIRE and his co-workers⁹ using the diffusion chamber and those of BILLINGHAM and others¹⁰ in which they did passive transfer with serum, the consensus of opinions is that antiserum-antibody is not concerned with homotransplantation. However, GORER and his colleagues^{11,12} reported that a specific antibody could be detected in the serum of the mouse that rejected tumor homograft. MITCHISON¹³, WINN and others¹⁴ have also observed these phenomena. By the present experiments in Cb mice transplanted with Ehrlich cancer cells there has been observed a serum antibody that shows 6% cytotoxic titer, and this, according to HELLSTRÖM¹⁵, would fall within the limit of experimental error. Furthermore, in view of 0% cytotoxic titer of non-sensitized serum, it seems to be reasonable to assume the presence of serum antibody. According to AKIYAMA¹⁶ it is said that in the mice inoculated with AH39 cells anti-transplantability of serum commences to increase within about one week after antigenic stimulation, reaching its maximum around the second week, and thereafter the titer persists for a long period of time. In the present experiments the serum obtained in the second week after implantation of Ehrlich ascites tumor was likewise used as antiserum.

As for the relationship between serum-antibody and cellular antibody there are opinions that cellular antibody is formed by serum-antibody becoming attached to cells¹⁷. On the other hand, in the phase microscopic observations of the reaction between SCI leukemic cells and sensitized lymphocytes, HANAOKA¹⁸ has demonstrated the presence of cellular antibody on the surface of sensitized lymphocytes. He claims that this cellular antibody is present in 19S fraction and it is different from the serum antibody contained in 7S fraction and therefore is an independent one.

Furthermore, AKIYAMA¹⁶ has concluded on the study of Yoshida sarcoma that serum antibody and cellular antibody are different and independent from each other. The fact that lymph-node cells in the presence of antiserum, irrespective of whether they were previously sensitized or not, show a similar inhibitory effect on target cells, as observed in the present experiments, seems to indicate that this antiserum also contains a certain cytophilic antibody. As for the fresh guinea-pig serum to be used as a complement, since it loses its toxicity

against mouse cells when it is either absorbed by mouse tissue or by being heated at 56°C for 20 minutes according to SCHLESINGER¹⁹, in the present experiments the fresh guinea-pig serum was made to be absorbed by washed, packed spleen cells of the mouse at 4°C for one hour before the use.

Next, among those who have observed the effect of sensitized lymphoid cells *in vivo*, there is an extensive study by WINN²⁰. In his neutralization experiment where the combination of SaI and the sensitized lymph-node cells from SaI-susceptible mouse is injected to the isogenetic mouse, he has observed that there is no appreciable change in the size of tumors developed whether the mixture of sensitized lymphnode cells and SaI is or not incubated prior to injection; that the greater the number of sensitized lymph-node cells challenging against SaI the longer is the survival of recipient mouse; and that even when there is no appreciable difference in the survival time, the weight of tumor extracted at a fixed time shows a definite relationship between the inhibitory effect on tumor proliferation and the number of lymph-node cells. KIDD and others²¹ have demonstrated that when the homogenate of the lymph nodes from the mouse previously removed of its regressive lymphoma is inoculated to the cells from the mouse susceptible to lymphoma and incubated *in vitro* for 1—2 hours, tumor cell proliferation is inhibited.

Since Ehrlich ascites tumor used is a non-specific tumor, when it is transplanted to the mouse susceptible to it, tumor develops but when the number of cells transplanted is small, it gradually shows regression. However, in the neutralization tests *in vivo* there can be seen distinct differences in the action behaviors of sensitized lymph-node cells and of non-sensitized ones on recipient tissues.

Finally, it has been observed that along with the proliferation of tumors in the recipient, the function of the reticulo-endothelial system is accelerated, making the weight of spleen and lymph nodes markedly heavier. This is an interesting phenomenon when compared with the increase in immunologically competent cells as described in a previous paper². As has been stated by SIMONSEN⁴ in the transplantation of tumor to animals, the younger the host the greater is the gain in the weight of organs of the recipient.

SUMMARY

For the purpose to study *in vivo* changes of the mice bearing Ehrlich ascites tumor with special emphasis on the problems of cellular antibody and serum antibody, a series of experiments such as neutralization tests *in vivo* and *in vitro* study of the effect of lymph-node cells from the tumor bearing animals on

target cells were carried out, and the findings thus obtained are briefly summarized as follows :

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