Cellular antibody in mice bearing Ehrlich cancer. I. A quantitative study on antitumor activity of cellular antibody in vitro

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Abstract

For the purpose to clarify whether or not the cells of regional lymph nodes and spleen of the tumor bearing individual develop the antitumor activity the author observed the proliferation of JTC-11 cells in vitro by mixing with the lymph-node and spleen cells from the mice bearing Ehrlich ascites tumor in solid form. After 24- to 48 -hour incubation the antitumor activity was estimated from the number of proliferated JTC-11 cells. As the result, it has been found that one week after implantation of tumor the regional lymph-node cells acquire the inhibitory activity against the proliferation of JTC-11 cells. The spleen cells also show a marked inhibitory effect on the turner cell proliferation but two weeks after implantation these inhibitory activities of the cells both from lymph node and spleen are largely retarded three to four weeks when the host is emanciated by the growing tumors. Discussions are made on the inhibitory mechanisms from the viewpoint of immune reaction and on the transplantability of tumor cells without any rejection.

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In spontaneous cancer, especially in human cancer, there is yet no definitive evidence that the host would present immunological reaction against the tumor recognizing it as not-self. In the past several decades many attempts have been made to detect immuno-antibodies in the serum of cancer patients but no convincing evidence seems to be presented up to date. Nonetheless, at the present stage in the light of studies on autoimmune diseases and homotransplantation immunity this problem appears to be understood somewhat on the concept of tissue immunity or delayed type of allergy-like immunity. Actually, KLEIN\(^3\), in his autoimmune experiments with mice by inducing methylcholanthrene carcinoma in animals, has reported that for the antitumor activity of the tumor bearing mice the lymphoid cells are responsible. TAKEDA\(^2\) likewise has found the antitumor activity in lymphoid cells from F\(_1\) strain mice sensitized with MH 134 tumor cells (originating from C\(_3\)H strain mouse) killed by irradiation with x-ray. As has been reported repeatedly, even in human cancer the tumors having intense infiltration of round cells in the surrounding interstitium proves to be good in prognosis. By means of tissue culture method HANAOKA\(^4\) and ROSENAU\(^6\) have observed mutual cell damaging reaction between target cells and lymphoid cells from the host receiving homotransplantation in which the grafted tissue is rejected. These results seem to suggest the possibility that the cellular immune reaction between the tumor cells and the host lymphocytes can be seen directly \textit{in vitro} by applying the tissue culture method. In view of this, the author carried out a series of experiments by means of tissue culture technique in order to see the reaction of the lymphoid cells of cancer bearing mice to the tumor cells. In this paper it is reported that the antitumor activity of lymphoid cells can be also evidenced \textit{in vitro} experiment.

**MATERIALS AND METHODS**

Cb strain male mice of 4 weeks old were obtained from The Mouse Colony
of Okayama University. The animals were fed on solid feed, MF of Oriental Yeast Co. with fresh vegetables and after growing up to about 20 g in body weight, they were used for experiment. These mice received the transplantation of tumors, Ehrlich ascites tumor cells or JTC-11 cells, subcutaneously. Each $5 \times 10^6$ cells of Ehrlich ascites tumor were implanted in between the shoulder bone and scapula on both sides. JTC-11 cells in culture grown in the medium composed of 80% YLE solution and 20% bovine serum were also transplanted subcutaneously between the shoulder bone and scapula on both sides as in the case of Ehrlich ascites tumor cells. Each animal received 3 injections of the cell at the intervals of one week, 2,000 cells at one time. Both of Ehrlich tumor cells and JTC-11 cells were donated by the courtesy of Cancer Institute of Okayama University Medical School. The animals receiving Ehrlich ascites cells’ transplantation were sacrificed one, two, three, and four weeks after the transplantation and these treated with JTC-11 cells one week after the last injection. From these animals lymph nodes of the axilla and cervical regions and the spleen were obtained and from each organ the tissue extract was prepared separately i.e. each of these samples was cut into small pieces with ophthalmic scissors in cold Hanks solution, and centrifuged at 1,000 r.p.m. for 10 minutes. The sediment was washed with cold Hanks solution three times by repeated centrifugation at 1,000 r.p.m. for 10 minutes each. With this sediment cell suspension was prepared. This lymphoid cell suspension was mixed with the substrain of Ehrlich tumor cells, JCT-11, and cultured in the YLE medium containing 20% of bovine serum, i.e. $1 \times 10^4$ JTC-11 cells were mixed with $2 \times 10^4$~$1 \times 10^5$ cells of lymphoid cells in 1.5 ml of the medium in a short culture vessel. Cell counts were taken on the tumor cells surviving 24- and 48-hour cultures at 37°C. As the control groups for each of the above test groups mixed cultures were carried out with JTC-11 cells and lymph-node cells or spleen cells obtained from untreated mice, and the cell number of JTC-11 was counted at the same intervals as in the case of test groups. Prior to the counting of cells, the cells growing on the wall of culture vessel were gently scraped off with rubber cleaner. For counting cell number citric acid treatment, a modified method by Katsuta after Sanford’s principle, was employed, i.e. the number of stained nuclei was computed on the calculating plate of Bürger-Türk, avoiding inclusion of the number of erythrocytes. For the determination of viability of the cells the “unstained cell count method” of Schrek was employed, i.e. after supravital staining with 1% Eosin Y solution, the number of the stained cells was subtracted from the whole cell number counted previously. The rate at which cells are stained was 30% at maximum and 12% at minimum, showing a variety of percentage. For the present experiment those that showed over 80% viable cells were used. As for counting cells in culture the cell number
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was determined by the average count from 3-4 vessels.

**RESULTS**

In the JTC-11 cells incubated with the regional lymph-node cells from the animal having the implanted Ehrlich tumor cells the number of JTC-11 cells showed a marked suppressed proliferation already one week after sensitization, most markedly after two weeks, and three to four weeks after the implantation when the death from tumors approached, the proliferation rate recovered to some extent (Fig. 1).

In the JTC-11 cells incubated with the regional lymph-node cells obtained two weeks after the tumor inoculation the JTC-11 proliferation was markedly inhibited, and the suppressing effect became more marked with the increase in...

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**Fig. 1** Inhibition of the JTC-11 cell growth by the regional lymph-node cells from the animals implanted with Ehrlich cancer cells. O; control, 1; 1 week after implantation, 2; 2 weeks after implantation, 3; 3 weeks after implantation, 4; 4 weeks after implantation, Method; see text. lymph-node cells; $2 \times 10^6$

**Fig. 2** Inhibition of the JTC-11 cell growth by the regional lymph-node cells from the animals implanted Ehrlich cancer cells obtained two weeks after the tumor inoculation. A; incubated with $5 \times 10^6$ lymph-node cells, B; incubated with $1 \times 10^6$ lymph-node cells, C; control, Method; see text.
the number of lymphoid cells and even by 48-hour incubation the cells did not proliferate exceeding the original level (Fig. 2).

As in the case of lymph-node cells the spleen cells showed also a fairly marked inhibitory effect on the proliferation of JTC-11. That is, the spleen cells from the animal one week after the tumor inoculation showed hardly any inhibition on the tumor cell growth but those affected with the spleen cells taken two weeks after the tumor inoculation showed a marked inhibitory effect as in the case of lymph-node cells from the tumor bearing animals. Such an inhibitory effect of the spleen cells was also diminished 3 to 4 weeks after the tumor inoculation at the time when the death from tumors approached (Fig. 3). The inhibitory effect of spleen cells on the proliferation of JTC-11 cells was stronger than that of lymph-node cells at the corresponding stage.

In the case where the regional lymph-node cells or spleen cells from the animals transplanted with JTC-11 cells there was only a slight inhibitory effect on

Fig. 3 Inhibition of the JTC-11 cell growth of the spleen cells from the animals implanted Ehrlich cancer cells. O; control, 1; 1 week after implantation, 2; 2 weeks after implantation, 3; 3 weeks after implantation, 4; 4 weeks after implantation, Method; see text.

Fig. 4 The growth of JTC-11 cells incubated with the lymphoid cells from the animals receiving the transplantations of 3 times with the intervals of one week. SP; incubated with spleen cells, LY; incubated with lymph-node cells, C; control (JTC-11 cells alone), Method; see text.
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the JTC-11 cells. After 24-hour inoculation the cell proliferation seemed to be inhibited to some degree but they increased markedly after 48 hours.

DISCUSSION

Through this experiment it has been clarified that regional lymph-node cells and spleen cells of the mice receiving the implantation of Ehrlich ascites tumor cells acquire an inhibitory activity to the proliferation of the tumor cells. This antitumor activity increases with the growth of the tumor, reaching maximum after a certain period of time and then reduced with emancipation of the animals.

Ehrlich cancer cells originally developed in hybrid mice and successively maintained for several decades have proven that they grow in almost all strains of mice when implanted. Generally, normal mouse tissue cells transplanted to the other strain of mouse is rejected after a certain period of time by immune reaction. Here arises a problem why the tumor cells are not rejected even at the stage where the host cells have acquired a marked antitumor activity.

The first problem to be solved is whether or not the antitumor activity presented by the regional lymph-node cells or spleen cells is due to immune reaction. Concerning the facts suggesting that lymphocyte is responsible for tissue immunity many observations have been reported; HARRIS and HARRIS, and HARRIS et al. have demonstrated a rapid appearance of agglutinins in the rabbit transplanted with cells of regional lymph nodes removed from another rabbit previously sensitized by injection of dysentery or typhus organisms into its foot pad. POTTER et al. (1938) succeeded in transfer of immunity to mouse transplantable leukemia as target cells, by passive transfer of the spleen cells from sensitized mice. MITCHISON also has succeeded in passive transfer with homologous tumor transplantation. BILLINGHAM et al. in their study on homologous skin graft transfer, having found that the transplanted skin graft is readily rejected in the mice previously receiving transfer of cells from regional lymph nodes.

Concerning in vitro studies, ROSENAU et al. have found that such lymph-node cells induce lysis of L cells in the absence of complement, and HANAOKA et al. have observed an inhibition of proliferation of SCL leukemic cells in vitro. KOPROWSKI injected guinea-pig bone marrow along with adjuvant into rat and during tissue culture of sensitized lymph-node cells from the rat, he made brain tissue come in contact with the cells in culture and observed that the lymph-node cells reacted specifically with glia cells, bringing about a contactual agglutination phenomenon. He also observed that non-treated lymph-node
cells coming in contact with sensitized serum in vitro elicited a similar phenomenon. Wilson observed that the lymphoid cells from the mice or rat that received skin homograft transfer aggregated in an orderly manner onto target cells, kidney cells in vitro, and on comparing the number of target cells with that of non-sensitized kidney cells under similar conditions, he found a decrease in the number of target cells in the former. In this instance, it was observed that the greater the number of sensitized lymphoid cells, the more marked was the decrease in the number of kidney cells. Similar phenomena have been observed in the present experiments.

The stage where the antitumor activity reaches the maximum, also coincides with that of maximal immune activity. In immunological study of Ehrlich cancer in vivo Numata proved that immuno-antibody reaches its peak about two weeks after subcutaneous transfer and one week after intraperitoneal implantation. Concerning the delay in the appearance of antitumor activity of spleen cells, the observations of Medawar may be helpful for the analysis. By unilateral transplantation of skin graft on rabbit ear, he observed the regional lymph nodes and those on the opposite side which showed some delay in response. By injecting dyes into the ears, he observed the dye does not reach directly to the lymph nodes on the opposite side but indirectly through circulating blood. This explains the delayed immune response of the opposite side lymph node. The delayed antitumor activity of the spleen seen in the present experiment may be well explained by the delayed intake of antigen by spleen.

Thus it is clear that the antitumor activity of lymph node and spleen is solely due to the immune reaction. For the question why the tumor cells are not rejected as in the case of normal tissue cells, it may be considered that the immune reaction may be too weak to reject the transplanted tumor. Generally, the antibody or tissue immunity seems to appear marked against the somatic protein, which is produced by the differentiated cells. Undifferentiated tumor cell does not develop somatic proteins and may be weak in antibody formation, and this is the problem to be settled in future.

SUMMARY

For the purpose to clarify whether or not the cells of regional lymph nodes and spleen of the tumor bearing individual develop the antitumor activity the author observed the proliferation of JTC-11 cells in vitro by mixing with the lymph-node and spleen cells from the mice bearing Ehrlich ascites tumor in solid form. After 24— to 48—hour incubation the antitumor activity was estimated from the number of proliferated JTC-11 cells. As the result, it has been found that one week after implantation of tumor the regional lymph-node cells acquire
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