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Cellular antibody in mice bearing Ehrlich cancer. II. Properties of lymphoid cells from sensitized animal*

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Abstract

In the experiments conducted with cells from the spleen and regional lymphoid cells derived from the axillary and regional lymph nodes of the mice receiving subcutaneous transplantation of Ehrlich tumor cells, it has been demonstrated that these lymphoid cells possess an ability to inhibit the proliferation of the subtrain of Ehrlich tumor cells (JTC-11), and that there is a parallel relationship between the rate of appearance of immunologicallly competent cells among these sensitized lymphoid cells and the inhibitory effect of these cells on JTC-11. In the experiment to reinforce the potency of sensitized cells conducted by means of the diffusion chamber technique, it has been proven that, while it is not an absolute prerogative, it is necessary to have the sensitized lymphoid cells come in contact with the target cells is order to wield the power of the former to the fullest extent. In the tissue cultures of lymphoid cells, it has been shown that sensitized lymphoid cells has a shorter life span than non-sensitized ones.

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CELLULAR ANTIBODY IN MICE BEARING EHRLICH CANCER
II. PROPERTIES OF LYMPHOID CELLS FROM SENSITIZED ANIMAL

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It was demonstrated in a previous report[1] that the lymphoid cells from the mouse bearing Ehrlich tumor possess an ability to inhibit the proliferation of the substrain (JTC-11) derived from Ehrlich tumor cells. However, it was not clarified in what proportion immunologically competent cells in these sensitized lymphoid cells would appear and in what manner these cells would act on the target cells, JTC-11. In order to solve these problems a series of experiments was conducted: (1) After dividing lymph nodes and spleen into halves, lymphoid cells were made to diffuse out and staining the cells with methylgreen-pyronine or May-Giemsa stain the mode of appearance of immunologically competent cells was studied every week, (2) by applying the diffusion chamber technique *in vitro* observations were made to see whether or not the contact between target cells and lymphoid cells is necessary for the inhibition of the target cell proliferation, and (3) by culturing lymphoid cells alone in the same culture medium the number of lymphoid cells after the culture was studied.

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 when grown up to about 20 g in body weight, they were used for experiment. These mice received the transplantation of Ehrlich ascites tumor cells subcutaneously. Each 5×10^6 of Ehrlich ascites tumor cells were implanted between the shoulder bone and scapula on both sides. The substrain, JTC-11 cells, were used for tissue culture. Both of Ehrlich tumor cells and JTC-11 cells were donated by the courtesy of Cancer Institute of Okayama Univeristy Medical School. The animals receiving Ehrlich tumor transplantation were sacrificed two weeks after the transplantation. From these animals lymph nodes of the axilla and cervical regions were obtained and these samples were 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

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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 cultured in the YLE medium containing 20% bovine serum. Cell counts were taken on the lymphoid cells surviving through 24 — and 48—hour cultures at 37°C. For the counting of cell number citric acid treatment, a modified method by KATSUTA[3] after SANFORD's principle, was employed, i. e. the number of stained nuclei was computed on the calculating plate of BURGER-TURK, avoiding inclusion of the number of erythrocytes. For the determination of viability of the cells, the "unstained cell count method" of SCHREK[4] was employed, i. e. after supravital staining with 1% Eosin Y solution, the number of the stained cells was substracted from the whole cell number counted previously. As for the counting of cells in culture the cell number was determined by the average count from 3—4 vessels.

Next, lymph nodes from axillary and cervical regions and the spleen were removed once a week for the period of 4 weeks after the implantation of Ehrlich tumor cells. Then smears were prepared with these tissues, fixed, and stained with methylgreen-pyronine or May-Giemsa stain. Subsequently, by counting 1,000 nucleated cells of each sample cell classification was made. This classification was done following the standard, of immunologically competent cells set at Meeting on Mechanism of Antibody held at Prague[2].

Diffusion chamber is constructed by pasting millipore filter (HAWP 04700, 25 ea, HA 0.45 μ) on the both ends of vinyl water tube of 2.2 cm in outer diameter, 1.6 cm in inner diameter, and 8 mm in thickness. In the diffusion chamber so prepared the medium mentioned in the foregoing and lymphoid cells are placed, and this chamber is suspended by silk thread in the culture vessel in which JTC-11 are growing, and the incubation is carried on at 37°C. In this instance, regional lymph-node cells two weeks after the implantation of Ehrlich tumor cells are used as sensitized cells. Both the combinations of 2×10^5 and 1×10^6 of lymphoid cells against 1×10^4 of JTC-11 cells are cultured. For the counting of the number of JTC-11 after culture "citric acid treatment technique" cited already is employed.

RESULTS

In the combination culture with regional lymph-node cells (Fig. 1) just as in the case of spleen cells (Fig. 2) the appearance of immumologically competent cells like hemacytoblasts reached the peak two weeks after the sensitization and it decreased as the death from tumors approached. This finding coincides with the inhibitory effect of lymphoid cells on the proliferation of JTC-11 as reported in a previous paper[1]. In the case where 2×10^5 lymphoid cells were placed in

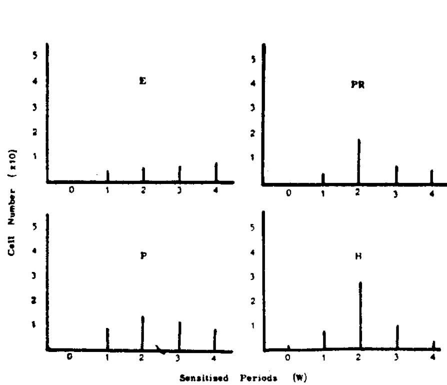

Fig. 1 Appearance of immunologically competent cells in mice sensitized with Ehrlich cancer cells (lymph node)
H: Haemocytoblast R: Reticulum cell
E: Eosinophil P: Plasma cell
Pr: Proplasma cell Method: see text.

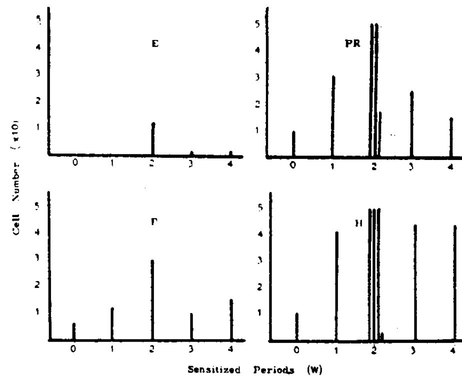

Fig. 2 Appearance of immunologically competent cells in mice sensitized with Ehrlich cancer cells (spleen)
H: Haemocytoblast R: Reticulum cell
E: Eosinophil P: Plasma cell
Pr: Proplasma cell Method: see text.

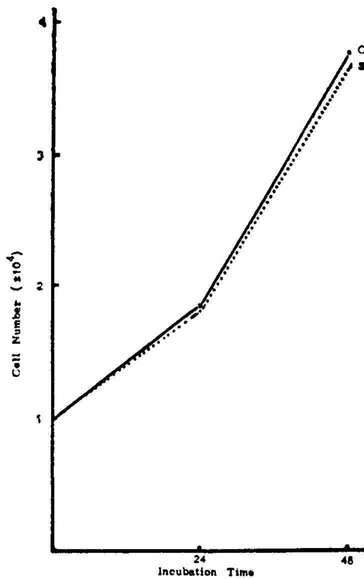

Fig. 3 Inhibition of JTC-11 in diffusion chamber method *in vitro* (lymph-node cells $=2\times10^5$) S: sensitized lymph-node cells in chamber C: non-sensitized lymph-node cells in chamber Method: see text.

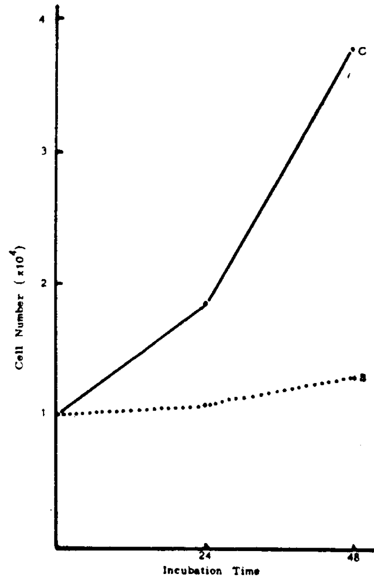

Fig. 4 Inhibition of JTC-11 in diffusion chamber method *in vitro* (Lymph-node cells $=1\times10^6$) S: sensitized lymph-node cells in chamber C: non-sensitized lymph-node cells in chamber Method: see text.

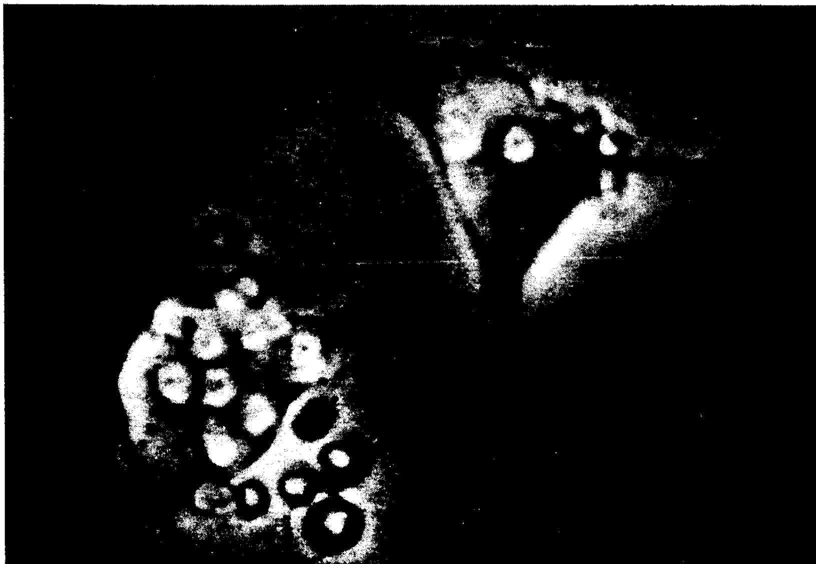

Fig. 5. Sensitized lympdoid cells attach around the JTC-11 cell
Method: see text.

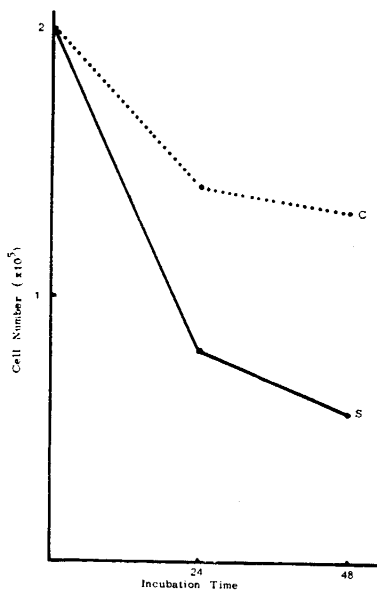

Fig. 6 Culture of lymphoid cells alone
S: sensitized lymphoid cells C: non-sensitized lymphoid cells Method: see text.

the diffusion chamber, there was observed no inhibitory effect (Fig. 3) on the growth of JTC-11 outside the chamber, whereas with 1×10^6 cells, showing a signifficant difference from the control, there could be observed an inhibitory effect on the proliferation of JTC-11 (Fig. 4). When the sensitized lymphoid cells are mixed and cultured with JTC-11, lymphoid cells become attached around the JTC-11 cells (Fig. 5). When lymphoid cells alone are cultured, the decrease in the number of cells is marked in the case of sensitized ones as compared with the case of non-sensitized ones (Fig. 6).

DISCUSSION

It has long been known that lymphocytes play an important rôle in the rejection of tumor and homograft transplants. MEDAWAR[5] has observed a marked infiltra-

tion of lymphocytes in the rejected homograft. TOOLAN[9] has found that lymphocytes were infiltrated around the rejected tumor transplant and that regional lymph nodes were hyperplastic, showing the appearance of numerous large lymphoid cells in the cortex of such lymph nodes. Later, SCOTHORNE and others[7], RADICI and others[8], and MASSE and his coworker[9] have observed in their transplantation experiments of skin homograft that regional lymph nodes became hyperplastic and large pyroninophilic lymphoid cells appeared in these nodes but no such changes in distant lymph nodes. MEDAWAR after transplanting skin graft on one ear of rabbit has found aside from the topical lymph node, the lymph node on the opposite side also reacted, and in the subsequent experiments using dye he considers that this morphological activation is due to the systemic circulation of antigen. ANDRE and others[11] in their investigation with stamp specimens of local and distant lymph nodes and spleen of the rabbit transplanted with skin homograft have observed that with lapse of time changes observable in local lymph nodes similarly occur in distant lymph nodes and in the spleen though the extent of changes is lesser. In other words, it has been verified that immune reaction appears in between lymph tissues as systemic reaction, and there are observed the appearance of large pyroninophilic lymphoid cells and the proliferation of primitive reticular cells. These large pyroninophilic lymphocytes are also variously called as transitional cells[12], basophilic lymphoid cells[13], primitive reticular cells[14], hemocytoblasts[15], immunoblasts[15], acute splenic tumor cells[16], lymphoblasts[17], reticular cells[18], large lymphatic reticular cells[19], lymphogonia[10], etc.

DAMESHEK[15] states that from this cell two cells of the antibody producing cells, lymphocyte and plasmacyte, are derived, but AMANO[10] has a different opinion, claiming that the cell of the plasma cell series is derived from the vascular endothelial cell. ANDREINI and others[20] have observed an increase of nitrogen, DNA and RNA in local lymph nodes of the animal receiving heterograft, and the large pyroninophilic cell contains a large amount of RNA, and it is considered that this is immunologically the most active cell. In the present experiment many large pyroninophilic lymphocytes appeared in the lymph nodes and the spleen two weeks after the implantation of Ehrlich tumor cells in mouse, and in this instance, there is observed a marked inhibitory effect on the substrain of Ehrlich tumor cells (JTC-11).

Diffusion chamber method was devised by ALGIRE[21], which is sealed with millipore filters on the both ends so that it passes only the humor but no cellular components. After placing the material with culture medium into this diffusion chamber, it is inserted into the peritoneal cavity of a mouse previously immunized with isologous normal tissue or with malignant tumor and the fate of the cells within the chamber has been observed. As the result, it has been found

that the cells continue to proliferate, maintaining viablity for a long period of time. WEAVER[22] and coworkers by means of this diffusion chamber technique have demonstrated that the fluid antibody has not so much significance on the destruction of homogranft transplant. ROSENAU and coworkers[23] have observed that the sensitized lymphoid cells enclosed within the diffusion chamber cannot induce lysis of target cells. They state that in order to effect the destruction or growth inhibition of target cell it is necessary for the sensitized cell to come in contact with the target cell. However, there are opinions opposed to this concept. That is, according to the recent report by NAJAIRIAN and FELDMAN[24] when the cells immunized by homologous tissue are labeled with tritiated thymidine and passive transfer of homograft immunity is effected to a recipient of the same species, these cells hardly appear at the site of homotransplant. This seems to indicate that sensitized lymphoid cells, being unable to come in contact with the transplant at the time of homograft transplantation, cannot reject the transplant. This can be also assumed from the fact that when the sensitized lymphoid cells are transplanted after being kept in the diffusion chamber, homograft transplant is rejected. In the present experiment likewise when many sensitized lymphoid cells were placed in the diffusion chamber, and transplanted, they acted inhibitorily on the growth of JTC-11. In other words, while it is not absolute prerogative for both of these cell groups to come in contact for sensitized lymphoid cells to wield their power on target cells, in order to effect the potential to the fullest extent it is necessary for the two groups to come in contact.

When lymphoid cells alone are cultured, the life span of sensitized lymphoid cells is longer than non-sensitized ones, though it cannot be said definitely because tissue culture method of lymphoid cells has not yet been established. A similar phenomenon can be observed in the case where lymphoid cells are cultured along with target cells. It is assumed that a certain factor in the component that has been released by the cell demolition acts upon the target cell. According to MIHASHI[25] it is said that when the filtrate of monocytes obtained from the ascites of the mouse previously immunized with strong viable bacilli of *S. enteritis* is made to react on non-sensitized monocytes *in vitro*, these monocytes acquire immunity, and the immunity transporting factor in this culture medium filtrate loses its activity in the presence of RNase.

SUMMARY

In the experiments conducted with cells from the spleen and regional lymphoid cells derived from the axillary and regional lymph nodes of the mice receiving subcutaneous transplantation of Ehrlich tumor cells, it has been demonstrated that these lymphoid cells possess an ability to inhibit the proliferation of the

subtrain of Ehrlich tumor cells (JTC-11), and that there is a parallel relationship between the rate of appearance of immunologicallly competent cells among these sensitized lymphoid cells and the inhibitory effect of these cells on JTC-11.

In the experiment to reinforce the potency of sensitized cells conducted by means of the diffusion chamber technique, it has been proven that, while it is not an absolute prerogative, it is necessary to have the sensitized lymphoid cells come in contact with the target cells is order to wield the power of the former to the fullest extent.

In the tissue cultures of lymphoid cells, it has been shown that sensitized lymphoid cells has a shorter life span than non-sensitized ones.

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