

Acta Medica Okayama

Volume 30, Issue 1

1976

Article 3

FEBRUARY 1976

Macrophage migration inhibition-activity after
implantation of methylcholanthrene-induced
sarcoma, Ehrlich ascites cancer or mouse
ascites hepatoma-134 cancer cells in mice

Masahito Yumura*

*Okayama University,

Copyright ©1999 OKAYAMA UNIVERSITY MEDICAL SCHOOL. All rights reserved.

Macrophage migration inhibition-activity after implantation of methylcholanthrene-induced sarcoma, Ehrlich ascites cancer or mouse ascites hepatoma-134 cancer cells in mice*

Masahito Yumura

Abstract

Cells from methylcholanthrene-induced tumor (MC-tumor), Ehrlich ascites cancer or mouse ascites hepatoma (MH-134) were subcutaneously implanted in dorsal area of mice to examine the specific cell mediated immunity following implantation. The migration index (MI) of lymphocytes was determined at various time periods after cell transplantation. The MI-activity increased under all three implantations, reached maximum at a certain period, decreased gradually and disappeared. The maximum MI-activity coincided with the proliferation period of the implanted tumor cells. This peak occurred on the tenth postimplantation day with MC-tumors, on the fifth day with Ehrlich ascites cancer and on the sixth day with MH-134 cancer. In lymphoid tissues of animals with MC-tumor and Ehrlich ascites cancer, strong MI-activity appeared early in the regional axillary lymph nodes, while weak activity was observed consistently in the distant mesenteric lymph nodes. The MI-activity of the splenic lymphoid cells resembled the axillary lymph nodes cell activity. The MI-activity of venous blood lymphoid cells was parallel to the average value of lymphoid cells of the spleen and axillary and mesenteric lymph nodes.

*PMID: 184687 [PubMed - indexed for MEDLINE] Copyright ©OKAYAMA UNIVERSITY MEDICAL SCHOOL

Acta Med. Okayama 30, 37—48 (1976)

**MACROPHAGE MIGRATION INHIBITION-ACTIVITY
AFTER IMPLANTATION OF METHYLCHOLANTHRENE-
INDUCED SARCOMA, EHRLICH ASCITES CANCER
OR MOUSE ASCITES HEPATOMA-134 CANCER
CELLS IN MICE**

Masahito YUMURA

*Department of Surgery, Okayama University Medical School,
Okayama City 700, Japan (Director: Prof. S. Tanaka)*

Received for publication, November 14, 1975

Abstract: Cells from methylcholanthrene-induced tumor (MC-tumor), Ehrlich ascites cancer or mouse ascites hepatoma (MH-134) were subcutaneously implanted in dorsal area of mice to examine the specific cell mediated immunity following implantation. The migration index (MI) of lymphocytes was determined at various time periods after cell transplantation. The MI-activity increased under all three implantations, reached maximum at a certain period, decreased gradually and disappeared. The maximum MI-activity coincided with the proliferation period of the implanted tumor cells. This peak occurred on the tenth postimplantation day with MC-tumors, on the fifth day with Ehrlich ascites cancer and on the sixth day with MH-134 cancer. In lymphoid tissues of animals with MC-tumor and Ehrlich ascites cancer, strong MI-activity appeared early in the regional axillary lymph nodes, while weak activity was observed consistently in the distant mesenteric lymph nodes. The MI-activity of the splenic lymphoid cells resembled the axillary lymph nodes cell activity. The MI-activity of venous blood lymphoid cells was parallel to the average value of lymphoid cells of the spleen and axillary and mesenteric lymph nodes.

In both implanted and spontaneous tumors, the host lymphocyte anti-tumor activity appears first at the regional lymph nodes. The antitumor activity of the regional lymph nodes decreases as the tumor reaches a certain size. The antitumor activity then appears in more distant lymph nodes. (20, 21, 22, 27, 28)

Free target tumor cells used for the lymphocytotoxicity test are difficult to prepare from solid tumor tissue obtained during surgery. For this reason, the macrophage migration inhibition test (MI-test) which requires no primary culture of target tumor cells is useful for examining the parameters of specific concomitant immunity. In previous studies the lymphoid cell MI-activity was correlated to the tumor stage in cancer-bearing animals, and this data was consistent with the lymphocytotoxicity test (20, 21, 22, 23, 24). Other

reports (1-8) have demonstrated that the MI-test indicates delayed hypersensitivity specifically and sensitively and that this test is applicable to cell-mediated tumor immunity. (9)

MATERIALS AND METHODS

Experimental animals. The animals used were C3H and CBA strain mice, 4 to 6 weeks of age, weighing about 20g (research colony of Okayama University Medical School). They were fed on Solid Feed MF (Oriental Yeast Co.). Guinea pigs employed were a hybrid strain, weighing 300 to 500g and were maintained on Solid Feed MF.

Experimental tumors. Three kinds of tumors were used. (a) Methylcholanthrene induced sarcoma (MC-tumor). One milligram of 20-methylcholanthrene (Wako Co.) suspended with 0.1ml of arabian gum was injected subcutaneously into the dorsal region of C3H mice. The tumor was allowed to grow for about three months and was transplanted to mice of the same strain successively for at least the third generations. (b) Ehrlich ascites cancer. Samples were obtained from the Section of Pathology, Okayama University Cancer Institute and maintained in the peritoneal cavity of Cb strain mice by successive passages. (c) C3H mouse ascites hepatoma 134 (MH-134). Samples were supplied by Dr. G. Fujii of the Medical Laboratory, Tokyo University and maintained in the peritoneal cavity of C3H mice by successive passages.

Tumor implantation. The MC-tumor was allowed to grow to about 1cm diameter and removed for implantation. A tumor mass of about 1mm³ was implanted subcutaneously between the scapulas dorsally on another mice of the same strain. Such implantations were conducted on 30 C3H mice. Four mice each were sacrificed at 3, 7, 10, 14, 21 and 28 days after tumor iso-transplantation (six animals died during the study). The axillary and mesenteric lymph nodes, spleen and sometimes blood from the inferior caval vein were collected. The axillary lymph nodes of six normal mice not transplanted with tumor were examined as controls. Ehrlich ascites cancer cells (5×10^6) suspended in 0.1 ml of 0.9% NaCl solution were injected subcutaneously into the dorsal site of 20 CBA strain mice. Four mice each were sacrificed at 3, 5, 7, 10 and 14 days after homo-transplantation. The four axillary lymph nodes, two mesenteric lymph nodes and spleen were collected. MH-134 cancer at 2×10^4 cells was suspended in 0.1 ml of 0.9% NaCl solution. This suspension was iso-transplanted subcutaneously into the dorsal region of 20 C3H mice. Two mice each were sacrificed at 2, 4, 6, 8 and 10 days after implantation. The axillary and cervical lymph nodes were collected. Such implanted tumor masses and tumor cell numbers were sufficient for tumor development and eventual host death.

Crude antigen. The collected tumors were stored at -20°C and were used in the preparation of antigens. The tumor was cut into fine pieces in 0.9% NaCl solution and homogenized by supersonication for 10 minutes at 20 kc, 7 tip, 150mA. After the homogenate was clarified by centrifugation at 3000rpm

for 30 min, the supernatant was used as the test solution containing soluble antigen. On the basis of preliminary experiments, the protein concentration was adjusted so that the migration of macrophage was specifically inhibited.

Peritoneal exudative cells. Peritoneal exudates were induced in guinea pigs by intraperitoneal injection of 20 ml of liquid paraffin. The exudates were harvested 4 to 6 days later, and after washing 3 times in Hanks' solution, peritoneal cells were suspended in TC-medium 199 and adjusted to a concentration of $2-5 \times 10^6$ cells/ml.

Lymphoid cells. The collected lymphoid tissues were sliced into fine pieces with ophthalmic scissors and passed through 80-mesh filter. The filtrate was washed with cold Hanks' solution by centrifugation at 2000rpm for 5 minutes. The washings were repeated 3 times. The final precipitate was suspended in TC-medium 199 containing 20% fetal calf serum. Lymphoid cells were prepared from the spleen by a similar method, and red cells were destroyed in 0.35% hypotonic saline solution. The isolation of venous blood lymphoid cells was performed by density gradient centrifugation using Ficoll and Conray 400.

Macrophage migration inhibition test. Migration inhibition factor was produced by incubating lymphoid cells at 3×10^6 /ml mixed with crude soluble antigen. After incubation for 24 hours at 37°C in 5% CO₂ containing air, the cells were sedimented by centrifugation at 3000rpm for 30 min, and supernatant assayed for migration inhibition factor. The suspension containing peritoneal exudative cells was drawn into capillary tubes (1.3 to 1.5 mm in diameter, 75 mm long, closed at one side) and centrifuged at 800rpm for 5 min. The tubes were cut at the cell-medium interphase and the part containing the cells was fixed on a cover slip in a small culture chamber of 1 ml capacity with a drop of silicon grease (High Vacuum, Dow-Corning). Four chambers were divided into two groups; one group was filled with the supernatant assayed for MIF, and the other group was filled with the supernatant without antigen. After incubation for 24 hours at 37°C in 5% CO₂ containing air, the migrated area was measured by projecting the microscopic image of cell growth from each capillary onto cellophane paper. The outline of the migration was drawn and measured on graph paper. The average area was calculated with at least four capillary tubes. The following formula was used to calculate the macrophage migration inhibition activity.

$$\frac{\text{Average area of migration with antigen}}{\text{Average area of migration without antigen}} \times 100 = \text{Migration Index (MI) (\%)}$$

From our preliminary data, 80% MI was designated as borderline of MIF positive and negative.

RESULTS

MI-activity of lymphoid cells from the axillary lymph nodes from MC-tumor bearing mice turned positive on posttransplantation day 7, reached maximum on day 10, decreased gradually thereafter and disappeared on day 21. The MI-activity of the spleen showed changes similar to the axillary

lymph nodes, reaching maximum during days 7 to 14 and disappeared by day 21. On the other hand, the MI-activity of the mesenteric lymph nodes was generally weak and disappeared later than the regional lymph nodes. The MI-activity of peripheral blood lymphocytes was parallel to the average value of the lymphoid cells of the spleen and the collected lymph nodes. (Table 1, Fig. 1) The MI-activity of the regional lymph nodes was maximum on the 10th day after transplantation, when tumors were palpable. The MI-activity decreased thereafter. Most mice had tumor death by 5 to 7 weeks after transplantation. (Fig. 2)

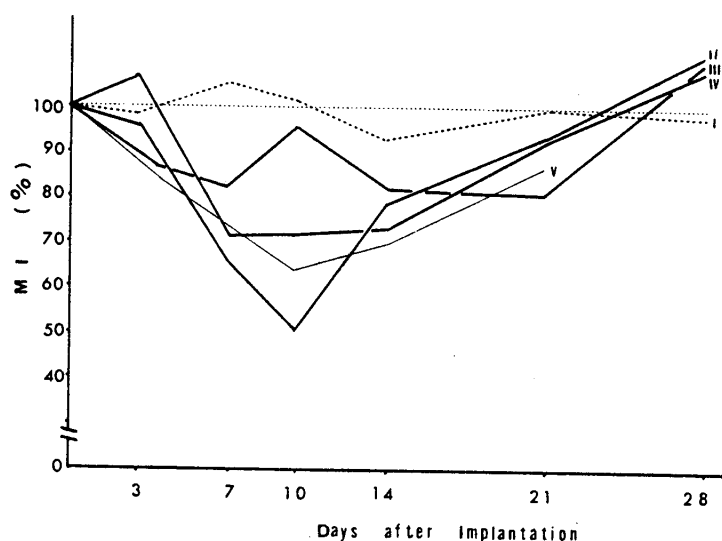


Fig. 1. Time-lapse changes of MI-activity after methylcholanthrene-induced sarcoma (MC-tumor) cell iso-transplantation. MI-activity of lymphoid cells derived from normal mice (I), and from the axillary lymph nodes (II), mesenteric lymph nodes (III), spleen (IV) and venous blood (V) of MC-tumor transplanted mice.

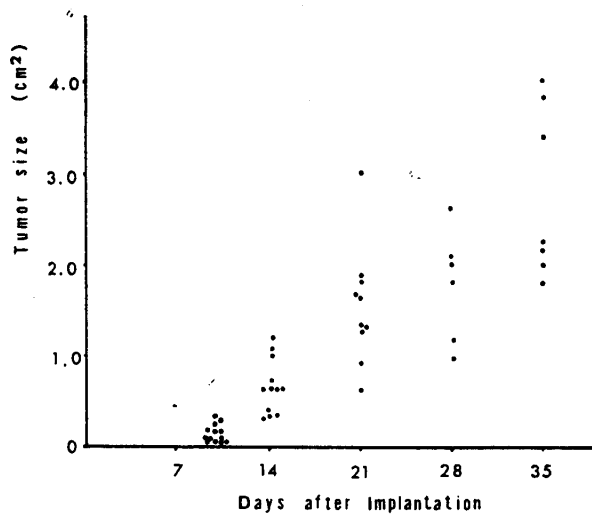
TABLE 1 TIME-LAPSE CHANGES IN MI-ACTIVITY AFTER MC-TUMOR IMPLANTATION IN MICE

	Days after transplantation (Mean M.I. %±S.E.)					
	3	7	10	14	21	28
Axillary lymph node	96.7±16.4	66.4±18.0	51.7±11.3	78.6±6.7	93.0±13.6	112.5±15.6
Spleen	106.9±22.9	71.8±11.1	72.1±14.0	72.4±11.0	92.2±28.4	108.0±23.6
Mesenteric lymph node	87.7±22.7	83.0±18.9	95.8±15.1	82.9±7.1	81.9±8.2	110.8±18.6
Venous blood	84.2	73.9	64.5	69.8	84.3	Not tested

Each value represents the mean of 10 experiments except for the venous blood values which were based on 4 mice at each time period.

MI-Activity of Cancer-bearing Animals

41

Fig. 2. Growth curve of tumor after iso-transplantation of 1mm³ MC-tumor.

MI-activity of lymphoid cells from the axillary lymph nodes from Ehrlich ascites cancer bearing mice reached maximum at posttransplantation day 5, and thereafter the MI-activity decreased and disappeared by day 14. Spleen cells from these mice showed positive MI-activity from day 5 to 7 and thereafter disappeared gradually in a pattern similar to the regional lymph nodes. In the distant mesenteric lymph nodes, MI-activity was maximal though weak on day 10. (Table 2, Fig. 3) The tumor was palpable on about day 4, and by 3 to 4 weeks the tumor led to host death. (Fig. 4)

In lymphoid cells isolated from the axillary and cervical lymph nodes after implantation of MH-134 cancer, the MI-activity appeared strongest on

TABLE 2 TIME-LAPSE CHANGES IN MI-ACTIVITY AFTER EHRlich ASCITES CANCER CELL TRANSPLANTATION

		Days after transplantation (MI %)				
		3	5	7	10	14
Axillary lymph node	Exp. 1	92.8	70.0	78.4	83.2	104.7
	Exp. 2	90.2	67.4	73.9		
Spleen	Exp. 1	97.5	74.5	72.3	89.1	101.4
Mesenteric lymph node	Exp. 1	87.4	105.9	84.5	85.7	109.3
	Exp. 2	98.6				

Each value represents the mean of 4 mice. The axillary lymph node and mesenteric lymph node studies were conducted twice.

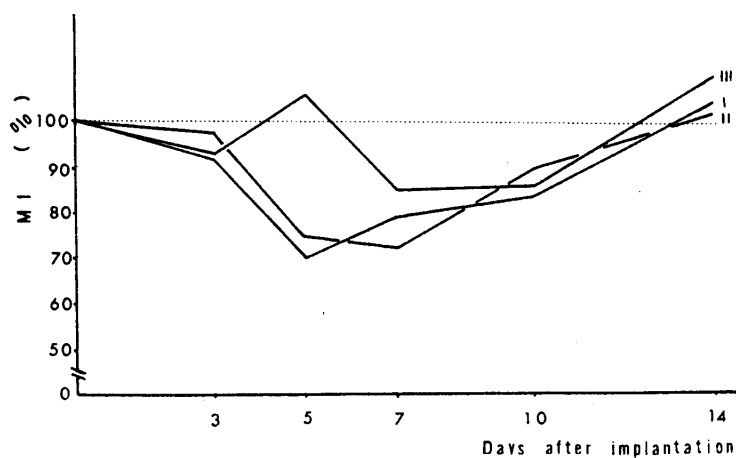


Fig. 3. Time-lapse changes in MI-activity after Ehrlich ascites cancer homo-transplantation. MI-activity of lymphoid cells from axillary lymph nodes (I), spleen (II) and mesenteric lymph nodes (III) from Ehrlich ascites cancer transplanted mice.

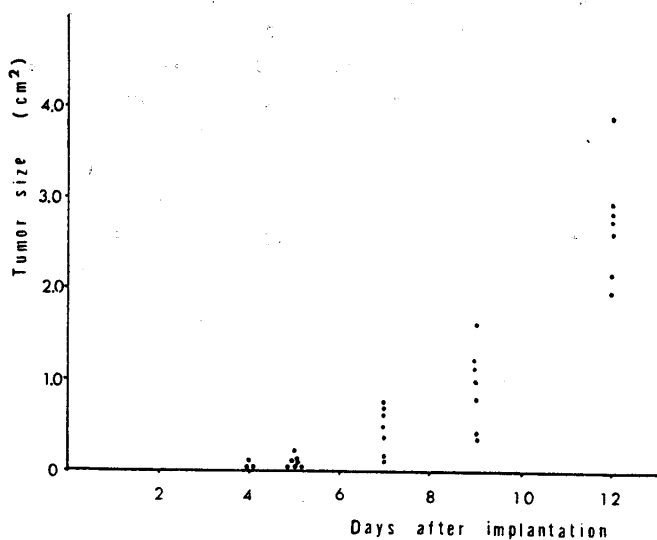


Fig. 4. Growth curve of tumor after homo-transplantation of 5×10^6 Ehrlich ascites cancer cells.

day 6 after implantation and decreased gradually thereafter (Table 3, Fig. 5). The tumor proliferation after implantation of 2×10^4 MH-134 cancer cells is shown in Fig. 6.

TABLE 3 TIME-LAPSE CHANGES IN MI-ACTIVITY AFTER MH-134 CANCER CELL TRANSPLANTATION IN THE REGIONAL AXILLARY AND CERVICAL LYMPH NODES OF MICE

	Days after transplantation (MI %)				
	2	4	6	8	10
Exp. 1	91.6	76.3	66.4	79.1	75.5
Exp. 2	101.0	72.3	54.6	69.8	88.2

The experiment was repeated twice.

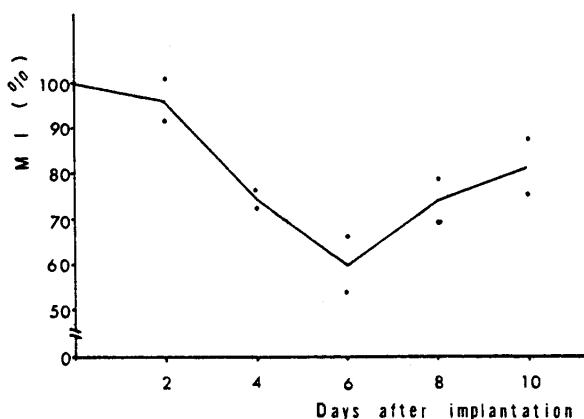


Fig. 5. Time-lapse changes of MI-activity after MH-134 cancer cell iso-transplantation. The lymphoid cells used were prepared from regional axillary and cervical lymph nodes of mice.

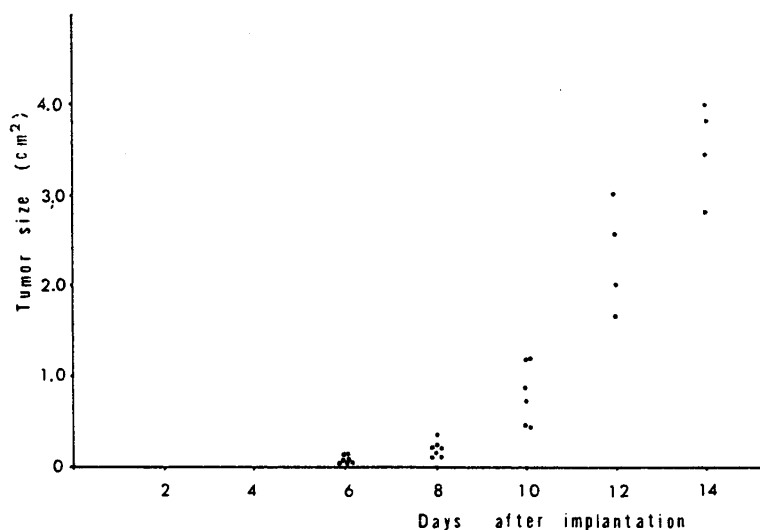


Fig. 6. Growth curve of tumor after iso-transplantation of 2×10^4 MH-134 cancer cells.

In all three implanted experimental tumors lymphoid cell MI-activity increased in the regional lymph nodes to a maximal point, and as this activity began to diminish, tumor growth was initiated.

DISCUSSION

It is generally agreed that experimental tumors induced by chemical carcinogens and oncogenic viruses as well as spontaneous tumors and human cancers all possess tumor-associated antigen on the cell surface. This finding was first definitively demonstrated by Klein, *et al.* (10) using MC-tumors. Despite the possession of tumor specific transplantation antigen (TSTA) by tumor cells, cancer ultimately led to host death without resistance to cancer. However, it is generally recognized that cancer-bearing bodies identify TSTA and try to eliminate the cancer cells. This reaction is known as concomitant immunity, in which lymphocytes play a principal role. Since the presence of TSTA and the cell-mediated immunity by lymphocytes are now well established, immunological examinations of cancer-bearing subjects are being conducted on specific cell-mediated immunity with the cytotoxicity test, the colony inhibition test, the mixed lymphocyte-target cell interaction, the adoptive transfer and the neutralization test, and on non-specific cell-mediated immunity with blastformation against PHA.

The present MI-test is one method for studying specific cell-mediated immunity. In 1932 Rich and Lewis (11) found when antigen was added to spleen tissue cultures of sensitized animals, cell migration from spleen tissue were inhibited. These investigators pointed out that such a phenomenon can be used to test for delayed hypersensitivity reaction. George and Vaughan (12) in 1962 found that the addition of purified protein derivative (PPD) to culture medium inhibited the migration of peritoneal exudative cells enclosed in capillary tubes prepared from tuberculin positive guinea pigs. They found that the degree of migration inhibition correlated with the delayed type skin reaction. Bloom and Bennett (13) divided peritoneal exudative cells of guinea pigs sensitized with bacillus Calmette-Guerin (BCG) into lymphocytes and macrophages. Each cell type was placed into separate capillary tubes and similar experiments were conducted. These investigators demonstrated that macrophages under such conditions exist as indicator cells. In addition, they reported a soluble factor produced from sensitized lymphocytes during contacts with antigen inhibited the migration of macrophages prepared from nonsensitized guinea pigs. This phenomenon was confirmed by David (14), and this soluble factor was later designated as the macrophage migration inhibition factor (MIF). The results of MI-test using PPD (12, 16), ovalmin (1), diphtheria toxoid (1), histoplasmin (15) or streptokinase-strepto-

dornase (16) as antigens coincided closely with the results of the delayed type skin reaction using these same antigens. In humans the tuberculin test results agree with those of the MI-test. The MI-test with tumor had its origins in the tests of Bloom and Bennett (19) on tumor homogenate as antigen. In MI-tests on MC-sarcoma induced in C3H mice as well as on spontaneous carcinoma, Vaage, David and Brown (18) stated that the test correlated closely with the antitumor activity of lymphocytes and that the activity was specific to the tumor antigen. In the present study the indirect method of MI was determined in which the soluble factor produced when lymphoid cells incubated with tumor homogenate inhibited the migration of nonsensitized guinea pig macrophages. It has also been shown that the MI time-lapse changes occurred after iso-transplantation of methylcholanthrene induced sarcoma and MH-134 ascites tumor and after homo-transplantation of Ehrlich ascites cancer. The MI-activity of the regional lymph nodes reached maximum at a time coinciding with the start of tumor growth, and the activity gradually decreased as the tumor grew. The MI-activity finally disappeared. The course of this MI-activity was invariably related to tumor growth irrespective of the tumor type. It is not clear whether the decreased MI-activity accompanying tumor growth is induced by a toxohormone-like substance produced from the tumor or by a humoral factor as a blocking antibody. Cerilli and Smith (19) reported that MI-activity increased linearly to the eleventh post-transplantation day with MC-tumor. This data coincides with the present experiment. Kashihara (20) observed a strong lymphoid cell antitumor activity on the first and second week after transplantation in neutralization test transplanting a mixture of tumor cells and lymphoid cells from regional lymph nodes obtained after subcutaneous transplantation of MC-tumor cells in the dorsal regions of untreated mice. Ohsugi (21) likewise found at the 14th post-transplantation day of MC-tumor iso-transplantation that lymphoid cells from the regional lymph nodes markedly inhibited the *in vitro* proliferation of MC-tumor cells. The same author (22) also observed the strongest antitumor activity when mammary tumors grew to about 10% of body weight in *in vitro* experiment with splenic lymphoid cells of C3H and RIII strain mice bearing spontaneous mammary carcinoma. Hara (23) observed strong cytotoxic activity in the first week when JTC-11 cells derived from Ehrlich ascites cancer were incubated with lymphoid cells from mice transplanted with Ehrlich ascites cancer. Satoh (24) conducted similar experiments with A cells derived from C3H mouse mammary cancer and observed strong antitumor activity on day 10 or 14 after iso-transplantation. The activity then decreased and disappeared. Deckers *et al.* (25) iso-transplanted MC-tumor subcutaneously in mice. Nontreated mice transplanted i. p. with

spleen cells obtained 7 to 14 days after tumor iso-transplantation indicated growth inhibition of simultaneously implanted subcutaneous MC-tumor. From these findings it seems that tumor bearing animals have strong antitumor activity to autochthonous tumor as concomitant immunity, and the activity decreases when the tumor increased beyond a certain period, although the time period may differ in each tumor.

MI-activity appeared earliest and strongest in the regional axillary lymph nodes of mice implanted MC-tumor and Ehrlich ascites cancer cells. In the distant mesenteric lymph nodes weak MI-activity was persistently observed. This can be explained as TSTA flows out of the tumor then enters the regional lymph nodes, where concomitant immunity is first established, as stated by Fisher *et al.* (26). In subcutaneous dorsal implantation of Ehrlich ascites cancer in mice, Orita *et al.* (27) removed the regional axillary and distant mesenteric lymph nodes and spleen. They conducted cultures of mixed lymphoid cells prepared from each of these tissues and JTC-11 cells derived from Ehrlich ascites cancer. On post-transplantation day 8 antitumor activity was not observed in the spleen and mesenteric lymph nodes but the regional axillary lymph nodes indicated activity. On day 21 the mesenteric lymph nodes showed antitumor activity. A time difference was thus found in the appearance of antitumor activity depending on the site. Orita *et al.* (28) observed differences in cytotoxic activity between the regional lymph nodes and distant lymph nodes in mixed cultures of node cells and autochthonous primary tumor cells from extirpated tumor tissues, such as gastric and mammary cancer.

The MI-test originally used for evaluating delayed hypersensitivity was sensitive for measuring concomitant immunity, and it has been used clinically because of its reliability and the ease of adjusting antigen. There are many reports (29, 30, 31) of decreased lymphocyte MI-activity in various advanced cancer bearing patients. The described time-lapse observations of MI-activity changes after tumor transplantation may be a useful index for determining the advance of malignant tumor.

Acknowledgment: The author wishes to express profound thanks to Prof. S. Tanaka and Dr. K. Orita for kind guidance throughout this work. This work was supported in part by the Grant-in Aid (no. 837025) from the Research Foundation, Ministry of Education.

REFERENCES

1. David, J. R., Al-Askari, S., Lawrence, H. S. and Thomas, L.: Delayed hypersensitivity *in vitro*. The specificity of inhibition of cell migration by antigens. *J. Immunol.* **93**, 264-283, 1964.
2. Bennett, B. and Bloom, B. R.: Reaction *in vivo* and *in vitro* reduced by a soluble substance associated with delayed hypersensitivity. *Proc. Natl. Acad. Sci. U. S. A.* **59**, 756-762, 1968.

3. Folk, R. E., Collste, I. and Möller, G.: *In vitro* detection of transplantation immunity. The inhibition of migration of immune spleen cells and peripheral blood lymphocytes by specific antigens. *J. Immunol.* **104**, 1287-1292, 1970.
4. Al-Askari, S., David, J. R., Lawrence, H. S. and Thomas, L.: *In vitro* studies of homograft sensitivity. *Nature* **205**, 916-917, 1965.
5. Thor, D. E. and Dray, S.: The cell-migration-inhibition correlate of delayed hypersensitivity. *J. Immunol.* **101**, 469, 1968.
6. Rocklin, R. E., Meyers, O. L. and David, J. R.: An *in vitro* assay for cellular hypersensitivity in man. *J. Immunol.* **104**, 95-104, 1970.
7. Wolberg, W. H.: Inhibition of migration of human autogenous and allogenic leucocyte by extracts of patient's cancer. *Cancer Res.* **31**, 798-802, 1971.
8. Anderson, V., Bendixen, G. and Schiødt, T.: An *in vitro* demonstration of cellular immunity against autologous mammary carcinoma in man. *Acta Med. Scand.* **106**, 101-103, 1969.
9. Bloom, B. R., Bennett, B., Oettgen, H. F., McLean, E. P. and Old, L. J.: Demonstration of delayed hypersensitivity to soluble antigens of chemically induced tumors by inhibition of macrophage migration. *Proc. Natl. Acad. Sci. U.S.A.* **64**, 1176-1180, 1969.
10. Klein, G., Sjögren, H. O., Klein, E. and Hellström, K. E.: Demonstration of resistance against methylcholanthrene induced sarcoma in the primary autochthonous host. *Cancer Res.* **20**, 1561, 1960.
11. Rich, A. R. and Levis, M. R.: The nature of allergy in tuberculosis as revealed by tissue culture studies. *Bull. Johns. Hopkins Hosp.* **50**, 115, 1932.
12. Geoge, M. and Vaughan, J. H.: *In vitro* cell migration as a model for delayed hypersensitivity. *Proc. Soc. Exp. Biol. Med.* **111**, 514-521, 1962.
13. Bloom, B. R. and Bennett, B.: Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. *Science* **153**, 80-82, 1966.
14. David, J. R.: Delayed hypersensitivity *in vitro*. Its mediation by cell-free substances formed by lymphoid cell-antigen interaction. *Proc. Natl. Acad. Sci. U. S. A.* **56**, 72, 1966.
15. Thor, D. E., Veach, S. R. and Jurezic, R. E.: Cell migration inhibition factor released by antigen from human peripheral lymphocytes. *Nature* **219**, 755-757, 1968.
16. Rocklin, R. E., Meyers, O. L. and David, J. R.: An *in vitro* assay for cellular hypersensitivity in man. *J. Immunol.* **104**, 95-102, 1972.
17. Lockshin and Bombardieri, S.: *In vitro* delayed hypersensitivity in normal and hyporeactive patients. *Immunology* **20**, 641-648, 1971.
18. Vaage, J., David, J. R. and Brown, B. W.: Tumor specific resistance in mice detected by inhibition of macrophage migration. *Cancer Res.* **32**, 680-687, 1972.
19. Cerilli, J. and Smith, M. C.: The development of cellular immunity to tumor cells. *Surg. Gynecol. Obst.* **134**, 739-745, 1972.
20. Kashihara, E.: Inhibitory effect of isologous regional lymphnode cells from the methylcholanthrene induced sarcoma bearing mice on the tumor *in vitro*. *Acta Med. Okayama* **24**, 161-173, 1970.
21. Ohsugi, M.: *In vitro* studies on the inhibitory effect of lymph node cells. Antitumor activity of regional lymph node cells from methylcholanthrene-induced sarcoma bearing mice on the same primary culture sarcoma cells. *Acta Med. Okayama* **24**, 447-456, 1970.
22. Ohsugi, M.: *In vitro* studies on the inhibitory effect of lymphoid cells. Antitumor activity of lymphoid cells from spontaneous mammary tumor-bearing mice on the autochthonous primary culture tumor cells. *Acta Med. Okayama* **25**, 229-235, 1971.
23. Hara, S.: Cellular antibody in mice bearing Ehrlich cancer. 1. A quantitative study on antitumor activity of cellular antibody *in vitro*. *Acta Med. Okayama* **19**, 91-98, 1965.

24. Satoh, K.: *In vitro* studies on tumor specific immunity by using C3H mammary cancer-A cells. 1. Inhibitory effect of lymph node cells from the tumor bearing isologous C3H mouse on the proliferation of the tumor cells. *Acta Med. Okayama* **20**, 261-268, 1966.
25. Deckers, P. J., Edgerton, B. W., Thomas, B. S. and Pilch, Y. H.: The adoptive transfer of concomitant immunity to murine tumor isografts with spleen cells from tumor-bearing animals. *Cancer Res.* **31**, 734-742, 1971.
26. Fisher, B. and Fisher, E. R.: Studies concerning the regional lymph node in cancer. 1. Induction of immunity. *Cancer* **27**, 1001, 1971.
27. Orita, K., Tomoyasu, S., Kaneda, S., Miwa, H. and Konaga, E.: Time-lapse changes in the concomitant immunity of lymphoid cells from different regions of Ehrlich tumor bearing mice and alterations in blastformation rate by PHA-stimulation. *Acta Med. Okayama* **26**, 81-88, 1972.
28. Orita, K., Kobayashi, M., Konaga, E., Kokumai, Y., Hiramatsu, M. and Tanaka, S.: Growth-inhibition effect of lymph node cells from cancer patients on autochthonous tumor cell. *Acta Med. Okayama* **27**, 133-140, 1973.
29. Wolberg, W. H.: Inhibition of migration of human autogenous and allogenic leucocytes by extracts of patients cancer. *Cancer Res.* **31**, 798-802, 1971.
30. Anderson, V., Bendixen, G. and Schiødt, T.: An *in vitro* demonstration of cellular immunity against autologous mammary carcinoma in man. *Acta Med. Scand.* **186**, 101-103, 1969.
31. Segall, A., Weiler, O., Genin, J., Lacour, J. and Lacour, F.: *In vitro* study of cellular immunity against autochthonous human cancer. *Int. J. Cancer* **9**, 417-425, 1972.