ORIGINAL REPORT

A STUDY ON TRANSPLANTATION IMMUNITY OF EHRLICH ASCITES TUMOR AND METHYLCHOLANTHRENE-INDUCED TUMOR IN MICE WITH SPECIAL REFERENCE TO LOCAL IMMUNITY OF LYMPH NODES DRAINING THE SITE OF SENSITIZATION

Morihisa KITANO and Yoshio OKADA

Department of Thoracic Surgery (Head: Prof. Chuzo NAGAISHI)
Chest Disease Research Institute, Kyoto University

(Received for publication July 22, 1967)

INTRODUCTION

In 1943, Gross[10] showed that methylcholanthrene-induced sarcomas of C3H mice can induce the resistance of its host to the transplantation of the same tumor in isologous mice.

It has been confirmed by Foley[8], Prehn[21] and by Révész[25], and analogous results have been obtained by Klein[15,16] on methylcholanthrene-induced sarcomas in autochthonous mice.

The experiments of Foley[8] showed resistance of C3H/He mice to the transplantation of MC sarcoma, by regression of the primary MC sarcoma, following the ligation method. Révész[25] showed that resistance can be established against the progressive growth of transplanted Ehrlich ascites tumors by pretreating the host with X ray-irradiated tumors. However, he reported that mice were not immunized against mammary carcinoma and lymphoma by similar method.

Some investigators also reported the successful induction of resistance to the growth of transplanted mouse tumors by pretreatment of mice with X ray-irradiated cells from the same kind of tumors[1,15,20,32,33].

According to these investigations it seems to be proved that the hosts acquire an immunological resistance to their own tumors and tumors of isologous origin. The authors attempted to confirm the transplantation immunity of Ehrlich ascites tumor and methylcholanthrene-induced sarcoma, to reveal difference of the immunological response between the regional lymph node and other parts.

MATERIALS AND METHODS

All the animals employed for this study were of the DDD- or C3H mice, weighing 20~25 gm, and 2~3 months of age. These strains were maintained by continuous, single-line, brother to sister mating at the Inbred Strain Animal Center, Kyoto University. All mice were kept on a standared pellet diet which, together with drinking water, was available ad libitum.

Two kinds of tumors, i. e. Ehrlich ascites carcinoma and methylcholanthrene-induced fibrosarcomas, were employed for this study. The former was obtained from the Research Institute, Microbial Diseases, Osaka University, and maintained in mice by weekly transfers. The transfers were carried out by intraperitoneal injection of ascitic fluid. The latter were induced by subcutaneous injection of 0.5~1.0 mg methylcholanthrene (L. Light & Co., Ltd., England), dissolved in 0.1 ml olive oil, into the dorsal skin of mice, and maintained in mice by subcutaneous transplantation at 10~14 day intervals. As the antigenicity of tumors may have changed by transfer over long periods, tumors transplanted over five generations were not employed in the present experiments.

One part of these tumors was inoculated subcutaneously into untreated isologous mice to maintain the tumor, while the rest was irradiated with a total dose of 15,000 R of ^{60}Co(30R/sec), mixed with an equal volume of physiological saline containing 100/I.U. penicillin, and homogenized with the Virtis blender at approximately 3000 r. p. m. for 5 minutes, so that the tumor homogenate was used as the antigen fluid. The homogenates were kept frozen at −20°C until use. Another fluid for control experiments was prepared on the same day from the pooled liver, kidney, and spleen tissues of an untreated isologous mouse.

When general immunization was intended, 0.15~0.2 ml tumor homogenate were injected into the subcutaneous tissue and peritoneal cavity. For local immunization, a total dose of 0.05~0.10 ml of tumor homogenate was injected into the thigh and the sole. Such treatments were performed once a week for four weeks. Tumor cells were inoculated one to two weeks after the last injection of antigen, then the growth rate of transplanted tumors was observed.

For B. C. G. sensitization, 0.5 mg of B. C. G. with the complete adjuvant was injected into the dorsal skin of DDD-mice, and one week later 0.5 mg B. C. G. was injected intravenously. Two weeks after intravenous injection, sensitization was confirmed by Mantoux reaction.

In the case of transplantation in the dorsal skin of mice, tumor tissue was inoculated subcutaneously without anesthesia. In the case of transplantation in the lymph node, tumor tissue was inoculated with a tuberculin syringe into the lymph node exposed by skin incision under general anesthesia accomplished by intraperitoneal injection of 1.5 ~ 2.0 mg/mouse of nembutal sodium.

Usually, on the 28 th day after the transplantation of tumors, both immunized and control animals were killed, examined for the presence of tumors, and any

tumors found were removed. The removed tumors were weighed and recorded at each inspection. After fixation of the removed tumors with 10% formalin, the tumors were sectioned in paraffin blocks, stained with hematoxylin and eosin, and then examined histologically.

RESULTS

A. Effects of General Immunization on the Transplanted Ehrlich Ascites Tumor.

1. Transplantation into subcutaneous tissue.

Ehrlich tumor cells (1×10^7) were transplanted into the dorsal subcutaneous tissue of mice. Figure 1 shows growth-rates of transplanted tumors in both immunized and non-immunized mice. Transplanted tumors in control group grow more rapidly than in immunized group. Average survival time after transplantation was six to seven weeks for immunized group, but three weeks for control group.

Average weight of tumors 4 weeks after transplantation was 2.17 gm in the immunized group, and 5.07 gm in control group as shown in Table 1.

In control group, as shown in Fig. 2, cells of growing tumor tissue are closely arranged, and the central parts of tumor tissue are usually necrotic due to rapid growth. Contrarily, in the immunized group, the arrangement of tumor cells is loose, and marked proliferation of fibrous tissue and infiltration of lymphocytes can be seen around the tumor tissue. Fig. 3 shows the most significant changes of the transplanted tumor tissues in the immunized animal. In this case, tumor cells are loosely arranged and surrounded by an aboundance of fibrous and lymphocyte-infiltration. Sometimes, gigantic tumor cells that seemed to be unusal mitosis can be found in the immunized group.

From the above mentioned findings, the mice generally sensitized by Ehrlich tumor cells show resistance to subcutaneous inoculation of the same tumor cells.

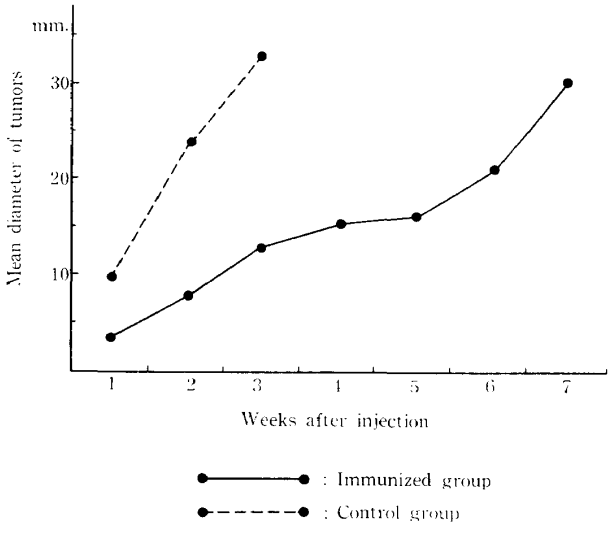


Fig. 1 Effect of general immunization on transplantation of Ehrlich carcinoma into the dorsal skin.

Table 1 Effect of general immunization on transplantation of Ehrlich carcinoma in the dorsal skin

	Case no.	Weight of tumors	Weight of the body	Weight of tumors / weight of the body
Immunized group	1	2.51gm	25.00gm	0.10gm
	2	2.33	23.92	0.09
	3	1.48	28.61	0.05
	Average	2.17gm	25.84gm	0.08gm
Control group	4	5.74gm	32.27gm	0.18gm
	5	8.78	31.42	0.28
	6	3.50	25.25	0.14
	7	5.00	31.20	0.16
	Average	4.07gm	30.03gm	0.19gm

Histologically, destruction of transplanted tumor cells and rejection-reaction to them can be seen in the sensitized mice.

2. Transplantation into lymph nodes.

Ehrlich tumor cells were inoculated into the dorsal skin and lymph nodes, and the growth-rates of transplanted tumor in mice sensitized by the same tumor and those of control mice were compared. Transplanted tumor cells in lymph node grew more rapidly than those in dorsal skin.

An amount of 1×10^3 cells of Ehrlich ascites tumor was injected into one inguinal lymph node of one side of DDD- mice, and these mice were sacrificed four weeks later. All transplanted Ehrlich tumor tissues were rejected in the immunized mice, but took 60% in control mice as shown in Table. 2.

Table 2 Effect of immunization on transplantation of Ehrlich ascites carcinoma into inguinal lymph nodes

Case no.	Immunized group		Control group	
	Occurrence of tumors	Weight of tumors	Occurrence of tumors	Weight of tumors
1	(−)		(+)	0.656gm
2	(−)		(+)	0.535
3	(−)		(+)	0.348
4	(−)		(+)	0.314
5	(−)		(+)	0.305
6	(−)		(+)	0.113
7	(−)		(−)	
8	(−)		(−)	
9			(−)	
10			(−)	
Rate of "take"	0/8		6/10	
Average weight	0		0.378gm	

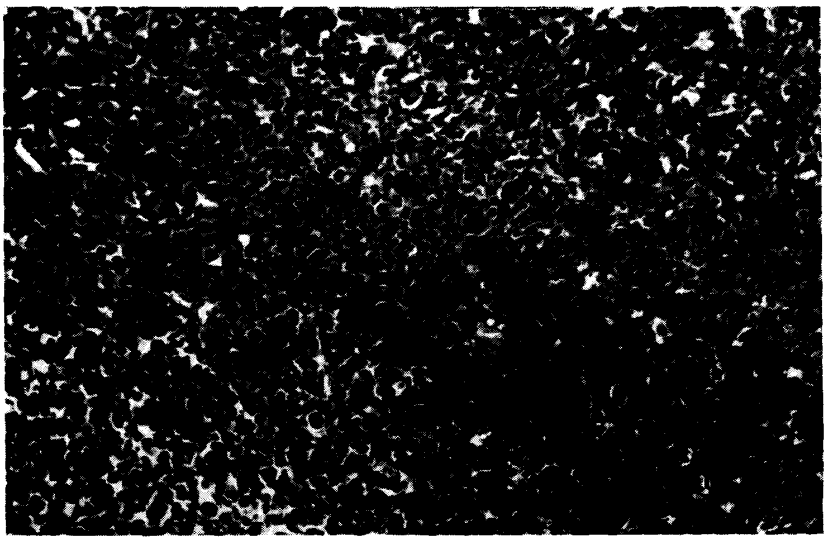


Fig. 2 Ehrlich tumor transplanted in the subcutaneous tissue of control mice. Tumor cells closely arranged like a stone-wall. (H.-E. staining)

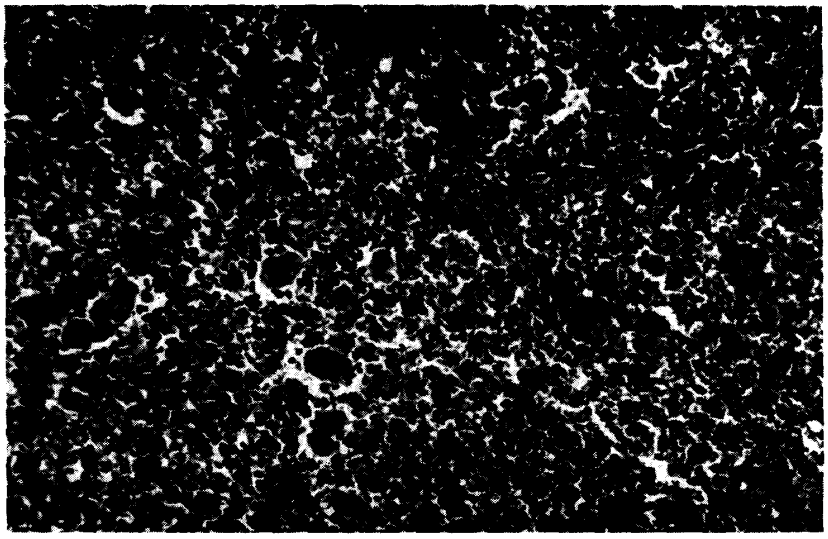
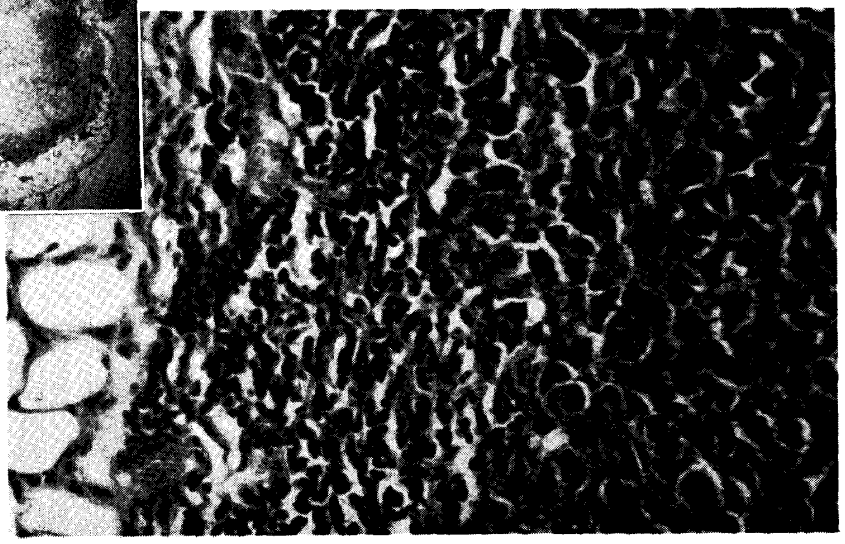


Fig. 3 Ehrlich tumor transplanted in the subcutaneous tissue of generally immunized mice. Tumor cells are loosely arranged and gigantic tumor cells and marked infiltration of lymphocytes can be found. (H.-E. staining)

(a)

Fig. 4 (a) A low power view of transplanted tumor in lymph nodes of generally immunized mice. The central portion of the tumor tissue is necrotic. (H.-E. staining)



(b) A high power of the same specimen. Lymphocytes invade in the tumor tissue. (H.-E. staining)

(b)

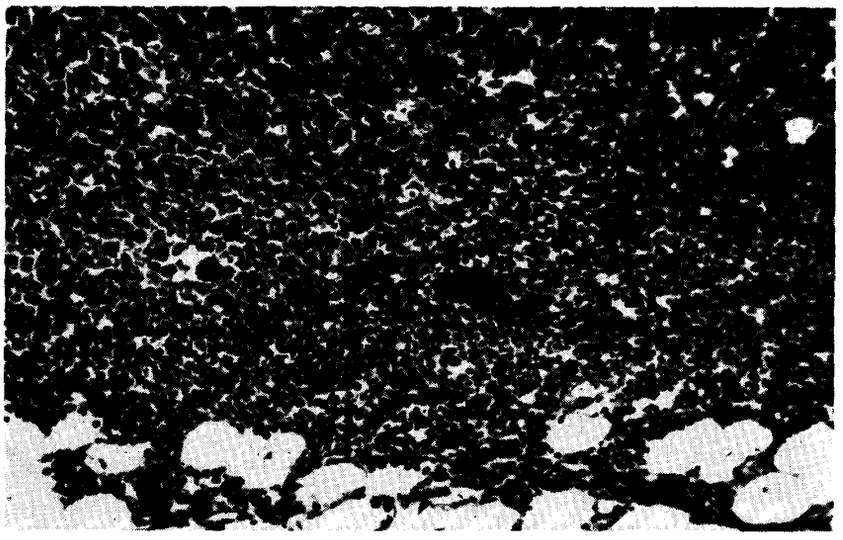


Fig. 5 Transplanted tumor in lymph nodes of generally immunized mice. Tumor cells with gigantic nucleus and marked lymphocyte-infiltration in tumor tissues can be seen. (H.-E. staining)

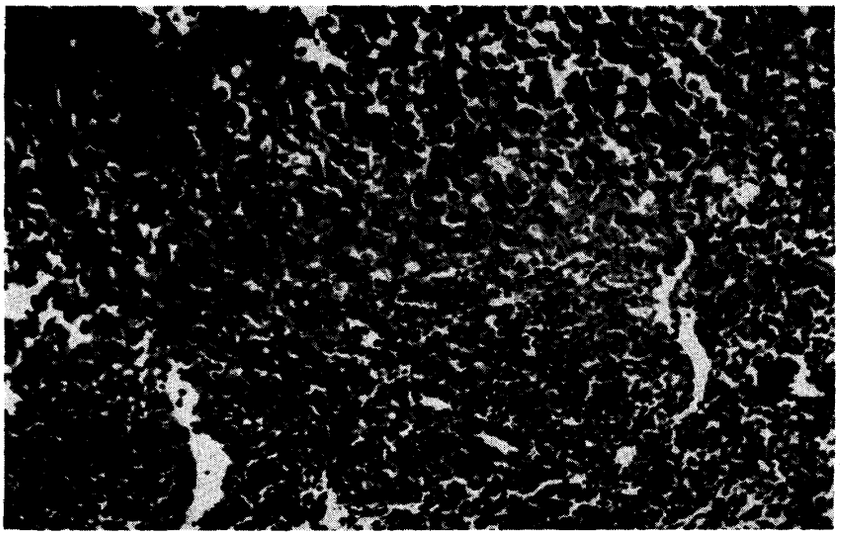
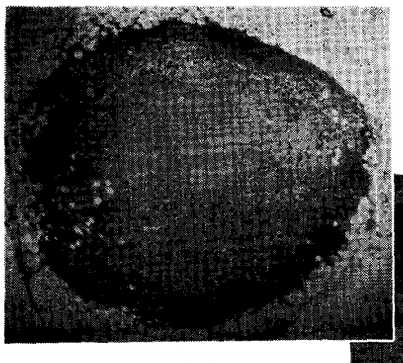
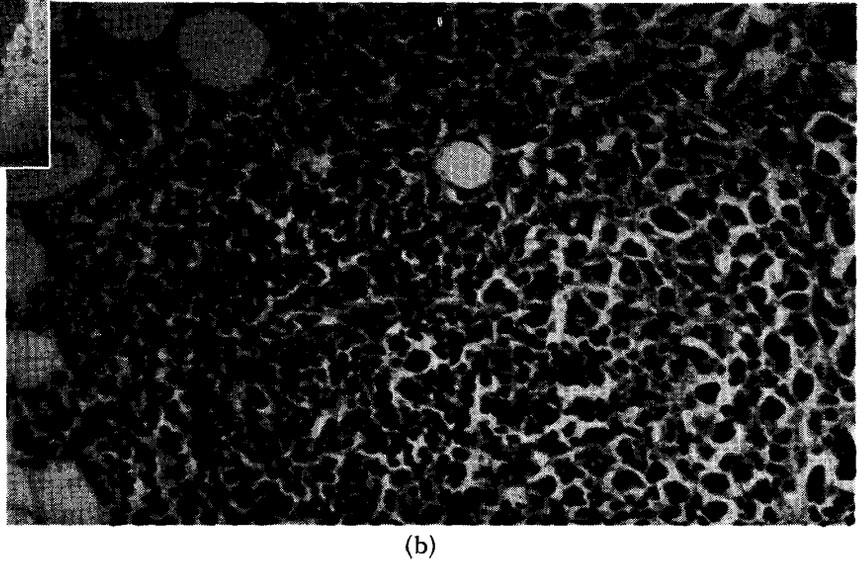


Fig. 6 Transplanted tumor in lymph nodes of non-immunized mice. (H.-E. staining)



(a)



(b)

Fig. 7 (a) A low power view of transplanted tumor in lymph nodes of generally immunized mice. Central-necrosis of tumor tissue. (H.-E. staining) (b) A high power view of the same specimen. marked lymphocyte-infiltration and giantic tumor cells are found. (H.-E. staining)

Table 3 Effect of general immunization on transplantation of Ehrlich ascites carcinoma into both inguinal lymph nodes

	Lymph nodes		Case number of mice 1	2	3	4	5	6	7	8	Rate of "take"	Average weight
Immunized group	left	Occurrence of tumors	(+)	(+)	(+)	(−)	(−)	(−)	(−)	(−)	4/16	0.360 gm
		Weight of tumors	0.476	0.282	0.214							
	right	Occurrence of tumors	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)		
		Weight of tumors				0.471						
Control group	left	Occurrence of tumors	(+)	(−)	(+)	(+)	(+)	(−)	(+)	(+)	12/16	0.750 gm
		Weight of tumors	0.647		0.932	0.083	0.518		0.663	1.20		
	right	Occurrence of tumors	(+)	(+)	(+)	(+)	(+)	(−)	(−)	(+)		
		Weight of tumors	0.677	0.574	0.605	0.950	0.657			1.50		

An analogous experiment of transplantation into the inguinal lymph nodes on both sides C3H-mice was performed. As shown in Table 3, transplanted Ehrlich tumor tissue was rejected in 75% of mice immunized by the same tumor tissues, while in 25% of control mice. Average weight of tumors grown was 0.36 gm in immunized mice and 0.75 gm in control mice.

Histologically, transplanted tumor cells in the central part of inguinal lymph node were observed to grow and invade the surrounding lymphoid tissue as shown in Fig. 4a. In the control group, most tumor cells in the peripheral zone of the tumor tissue seemed to be more vigorous than those in the central portion and they invaded into the surrounding lymphoid tissue as shown in Fig. 4b. In the peripheral zone of transplanted tumor that contacts lymphoid tissue of the immunized mice, the

Table 4 Effect of local immunization whith ^{60}Co irradiated Ehrlich ascites carcinoma on transplantation of the same tumor in regional lymph node

	Case no. of mice	1	2	3	4	Average weight
Immunized inguinal lymph nodes	Occurrence of tumors	(+)	(+)	(+)	(+)	2.93gm
	Weight of tumors	2.32	2.62	3.20	3.58	
non-immunized inguinal lymph nodes	Occurrence of tumors	(+)	(+)	(+)	(+)	2.25gm
	Weight of tumors	2.06	1.75	1.78	3.41	

arrangement of the tumor cells is loose and accompained by a marked lymphocyte-infiltration and proliferation of fibrous tissue as shown in Fig. 5. In an immunized case, gigantic tumor cells with large nuclei which seem to be caused by anomalous mitosis are found. On the contrary, in control mice, tumor cells are closely arranged, and degeneration of tumor tissue accompanied by lymphocyte-infiltration can not be found, as shown in Fig. 6.

From the above mentioned findings, it may be concluded that the immunological resistance to inoculation of Ehrlich tumor cells can be seen in the lymph nodes of the generally immunized mice by irradiated autogenous tumor cells.

B. Effects of Local Immunization on the Transplanted Ehrlich Ascites Tumor.

1. Transplantation into regional lymph nodes of mice after local immunization with Ehrlich tumor cells irradiated by ^{60}Co.

This experiment was designed to find the difference of transplantation immunity between the sensitization of regional lymph node and unsensitized lymph node. The homogenate of Ehrlich tumor was injected into the thigh and the sole of one side, and then 2×10^3 tumor cells were injected into inguinal lymph nodes on both sides. The rejection-reaction in the regional lymph nodes of sensitized mice differed little from that in the controls as shown in Table. 4.

2. Transplantation into regional lymph nodes of mice after local immunization with living Ehrlich tumor cells.

As the titer of antibody seemed to rise more markedly in the cases immunized by living tumor cells than in the cases immunized by dead tumor cells, the analogous experiment of the foregoing paragraph was performed with living tumor cells. Living tumor cells (1×10^6) were transplanted into the right sole of DDD- mice. Ten days later, the right foot was removed after confirmation of a successful "take" of transplanted tumor. Three days later, tumor cells (1×10^3) were injected into inguinal lymph nodes on both sides. As shown in Table 5, the transplantability of tumor cells in the lymph node of the sensitized side was not inferior to that in the non-sensitized side. However, the average weight of tumor grown was 0.139 gm in the

Table 5 Effect of local immunization with living Ehrlich ascites carcinoma on transplantation of the same tumor in regional lymph node

Case no. of mice		1	2	3	4	5	6	7	8	Rate of "take"	Average weight
Immunized inguinal lymph nodes	Occurrence of tumors	(+)	(−)	(−)	(−)	(−)	(+)	(+)	(−)	3/8	0.139gm
	Weight of tumors	0.115					0.168	0.136			
Non-immunized inguinal lymph nodes	Occurrence of tumors	(+)	(−)	(+)	(−)	(−)	(−)	(+)	(−)	3/8	0.392gm
	Weight of tumors	0.502		0.242				0.434			

sensitized side, while 0.392 gm in the non-sensitized side. It is supposed, therefore, that the regional lymph nodes of the side of the tumor transplantation are more strongly immunized than those of the other side.

C. Effects of General Immunization on the Transplanted Methylcholanthrene-induced Tumors.

1. Transplantation into subcutaneous tissue.

A thick mixture containing $10^6 \sim 10^7$ of methylcholanthrene-induced tumor cells was transplanted into the dorsal skin of three groups of mice. The first group was immunized by subcutaneous and peritoneal injection of irradiated methylcholanthrene tumor homogenates; the second group was injected with physiological saline; and the third group was untreated. Transplanted MC tumor cells were rejected in 40 % with saline, and in 40 % untreated mice. Average weight of tumors grown was 0.26 gm in immunized mice, while 1.44 gm and 2.50 gm in each control grown as shown in Table 6.

The thick mixture of MC tumor cells was translpanted in another three groups, that is the first group immunized by the same tumor tissue, the second immunized by normal tissue (liver, spleen and kidney) and the third injected with physiological saline. As shown in Table 7, the average weight of removed tumors was 1.11 gm in the first group, 3.02 gm in the second and 3.38 gm in the third. From the results obtained, it was revealed that mice sensitized by the MC-induced sarcoma showed resistance to the transplantation of the same tumor.

2. Transplantation into lymph nodes.

As mentioned above, Ehrlich tumor transplanted into lymph node appeared to

Table 6 Effect of immunization on transplantation of MC-induced sarcomas in the dorsal skin

Case no. of mice	(I) Immunized group immunized with ^{60}Co irradiated MC tumor Occurrence of tumor and weight of tumors	(II) Control group treated with physiological saline Occurrence of tumor and weight of tumors	(III) Control group untreated Occurrence of tumor and weight of tumors
1	0.7	3.3	4.8
2	0.15	2.9	4.4
3	0.1	1.7	2.9
4	0.1	0.5	2.1
5	(−)	0.15	0.6
6	(−)	0.1	0.2
7	(−)	(−)	(−)
8	(−)	(−)	(−)
9	(−)	(−)	(−)
10	(−)	(−)	(−)
Rate of "take"	4/10	6/10	6/10
Average weight	0.26gm	1.44gm	2.50gm

Table 7 Effect of immunization on transplantation of MC-induced sarcoma in the dorsal skin

	(I) Immunized group immunized with ^{60}Co irradiated MC-tumor	(II) Control group immunized with ^{60}Co irradiated normal tissues	(III) Control group treated with physiological saline
Case no. of mice	Occurrence of tumor and weight of tumors	Occurrence of tumor and weight of tumors	Occurrence of tumor and weight of tumors
1	(−)	(−)	(−)
2	(−)	(−)	(−)
3	(−)	0.10	(−)
4	(−)	0.35	0.20
5	0.25	0.45	0.65
6	0.30	1.66	2.22
7	0.32	2.10	3.20
8	0.36	3.32	3.54
9	0.40	3.58	3.76
10	0.90	3.66	4.10
11	0.92	3.72	4.32
12	1.51	4.30	4.64
13	1.98	4.70	4.82
14	2.20	5.25	5.68
15	3.03	6.04	
Rate of "take"	11/15	13/15	11/14
Average weight	1.11gm	3.02gm	3.38gm

grow more rapidly than that transplanted into subcutaneous tissue. However, the growth of tumors transplanted in lymph nodes of immunized mice was inhibited as compared with non-immunized mice. This experiment was designed to inquire whether MC-induced tumors transplanted in lymph nodes of immunized mice could be inhibited or not.

The results obtained are shown in Table 8. Average weight of tumor grown was 1.41 gm in mice immunized by tumor tissue, while 3.62 gm in control mice.

MC sarcoma cells were transplanted in lymph nodes of three groups of mice. The first group was immunized by irradiated MC sarcoma, the second group was sensitized by B. C. G., and the third group was untreated. As shown in Table 9, transplanted MC sarcomas were rejected in 38% of mice immunized by tumor, while in 6% sensitized by B. C. G., and in 6% of untreated mice. Average weight of removed tumors was 0.63 gm in the first group, while 0.64 gm and 1.27 gm in the control groups.

Histologically, transplanted tumor tissues were seen in the central part of lymph node invading surrounding lymphoid tissue as shown in Fig. 7a. In the central portion of transplanted tumor tissues, cells frequently were found to be degenerated, while in the peripheral zone of the tumor tissue, most cells were vigorous and infiltrating the adjacent lymphoid tissue as shown in Fig. 7b. In immunized animals,

Table 8 Effect of general immunization on transplantation of MC-induced sarcomas into both inguinal nodes

Case no. of mice	Immunized group		Control group	
	r.-inguinal lymph node	l.-inguinal lymph node	r.-inguinal lymph node	l.-inguinal lymph node
1	2.5 gm	2.0 gm	6.7 gm	4.1 gm
2	1.8	3.1	4.4	3.9
3	1.6	1.8	3.3	5.5
4	0.6	3.9	2.3	5.4
5	0.5	1.7	2.6	3.1
6	0.8	1.3	2.3	3.1
7	0.2	1.3	1.8	2.3
8	0.5	0.8		
9	0.6	0.4		
Average weight	1.41 gm		3.62 gm	

peripheral parts of the tumor tissue were seen to be devided into small groups by lymphocyte-infiltration as shown in Fig. 8.

From these facts, it is concluded that immunological resistance to the transplantation of MC sarcoma can be seen in the subcutaneous tissue and lymph node of mice generally sensized by the seme tumor tissue.

D. Effects of Immunization on the Transplanted Methylocholanthrene-Induced Tumors.

According to the experiment with Ehrlich ascites tumor, it is supposed that the antibody related to transplantation immunity of tumor tissue is not equally distributed through the whole body, but concentrated in the regional lymph node of the side of the tumor transplantation.

After local immunization by MC sarcoma tissue, 0.0125 ml of tissue mixture of the same tumor was injected both into the immunized regional lymph nodes and

Table 9 Effect of general immunization on transplantation of MC-induced sarcomas into both inguinal nodes

	Lymph nodes	Case no. of mice									Rate of "take"	Average weights
		1	2	3	4	5	6	7	8	9		
I	right	(−)	(−)	(−)	0.65	0.60	0.65	0.45	1.10		10/16	0.630 gm
	left	(−)	(−)	0.55	(−)	0.35	0.60	0.65	0.70		(62.5%)	
II	right	(−)	0.15	0.20	0.20	0.60	0.65	0.65	1.35	1.90	17/18	0.647 gm
	left	0.75	0.25	0.45	0.45	0.55	0.55	0.75	0.95	0.60	(94.4%)	
III	right	1.40	0.50	0.80	1.30	1.40	1.55	1.40	2.20		15/16	1.279 gm
	left	(−)	0.45	1.45	1.25	0.90	1.10	1.35	2.00		(93.7%)	

I: immunized with ^{60}Co irradiated MC-tumors
II: sensitized with B.C.G.
III: treated with physiological saline

Fig. 8 Transplanted tumor in lymph nodes of generally immunized mice. Marked lymphocyte-infiltration in tumor tissues can be seen. (H.-E. staining)

Table 10 Effect of local immunization with ^{60}Co irradiated MC-induced sarcomas on transplantation of the same tumor in regional lymph node

	Case no. of mice	1	2	3	4	5	6	7	8	9	10	Rate of "take"	Average weight
Immunized inguinal lymph nodes	Occurrence of tumors	(−)	(−)	(−)	(−)	(+)	(+)	(+)	(+)	(+)	(+)	6/10	1.60 gm
	Weight of tumors					0.75	1.0	1.2	1.4	1.9	3.35		
Non-immunized inguinal lymph nodes	Occurrence of tumors	(−)	(−)	(−)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	7/10	2.68 gm
	Weight of tumors				0.95	1.6	2.3	2.8	3.2	3.8	4.1		

into the non-immunized lymph nodes, and the rate of "take" and growth of transplanted tumors were observed for four weeks. No significant difference was seen in the rate of successful "take" of transplanted tumors between local immunization and control (Table 10).

However, average weight of tumors grown was 1.60 gm in the inguinal lymph node of immunized side, and 2.68 gm in control non-immunized side.

It can be concluded that, in the experiment on MC-tumor also, the immunological resistance to the growth of the tumor is proved to be in the lymph node draining the site of sensitization by autogenous tumor cells.

DISCUSSION

When the host resistance to the transplanted tumor is weak, the mechanism of immunological resistance may easily be masked by the rapid proliferation of the tumor tissue. The efficiency of the host response can be increased, however, by use of a very small number of tumor cells for transplantation or by pretreatment with heavily irradiated tumor cells.

The immunized-method experiment reported in this paper was carried out under pretreatment with tumor cells irradiated with 15,000 R of ^{60}Co. The data presented here demonstrated that Ehrlich ascites tumors and MC-induced sarcomas were capable of producing an immunity against subsequent inoculation with the same tumor in mice of the inbred strain.

Transplantation Immunity against Ehrlich Ascites Tumor:

Ever since Ehrlich (1906) made his classical observation that an animal host became resistant or "immune" to reimplanted grafts of the same tumor after a tumor graft failed to "take," a widespread interest was shown in the theoretical and practical implications of this phenomenon.

Haaland[12] (1909), Contamin[6] (1910), Mottran and Russ[18] (1917), Chamber and Scott[5] (1922) and Goldfeder[9] (1954) have shown that host resistance to growth of tumors can be developed by pretreatment with X ray-irradiated tumor cells, followed by a challenge with a small viable inoculum of the same tumor.

To study the effect of X ray-irradiated cells on the growth of the Ehrlich tumor, Révész[24,25] (1955, '60) has observed the growth of Ehrlich tumor after subcutaneous inoculation of mixtures of viable and irradiated Ehrlich carcinoma cells. Tumor cells damaged by irradiation of 5,000 R exerted either an inhibiting or an enhancing effect upon the growth of admixed viable cells depending on quantitative proportion. The irradiated cells inhibited the growth of a small population of viable cells inoculated simultaneously. This finding was interpreted as being due to an immunization of the host to the dead cells. On the other hand, the irradiated cells appeared to exert an enhancing influence on the growth of a large population of viable cells. This observation suggested either that dead cells might release substance capable of stimulating tumor growth or that surviving cells which recovered from the effects of irradiation might later have contributed to the total number of growing tumor cells.

Donaldson and Mitchell[7] (1959) have reported that survival time of the immunized animals injected with irradiated Ehrlich ascites tumor cells could be prolonged by retransplantation of the same tumors, and such effects were not significant in post-immunization but in pre-immunization.

McKee et al[17]. (1959) have found C57 BL mice, after pretreatment by intraperitoneal injection of Ehrlich ascites carcinoma cells which were attenuated by irradiation with 2,000 ~ 8,000 R X-ray, were able to produce a resistance to the simultaneous reimplantation of viable Ehrlich carcinoma cells. Furthermore, they showed that it is necessary to inject X-ray attenuated tumor cells more than five times in order to produce extremely high resistance to the retransplantation of the same tumor. There is partial recovery of tumor cells irradiated with 2,000~8,000 R X-ray, indicated by their ability to kill mice, and the resistance factor being the presence of an antibody in sensitized animals against tumor tissue was revealed by complement fixation test.

These authors[13] have analysed the immunological resistance to replantation of Ehrlich ascites tumor in the dorsal skin and the lymph node of mice generally or locally immunized by the homogenates of ^{60}Co irradiated tumor cells. The immunological resistance to the inoculation of tumor cells was clearly observed in the subcutaneous tissue of sensitized mice. For local immunity, the resistance of the growth of the transplanted Ehrlich tumor cells in the lymph node was more significant in the sensitized side than in the opposite side.

Transplantation Immunity against Methylcholanthrene-induced Sarcoma:

For many years investigators have been trying to induce transplantation immunity to tumors. But in the past such experiments employed hybrid animal as host. Therefore, even with the antigenicity of tumor being sufficient to produce successful transplantation immunity, the participation of genetic deviations between host and tumor-bearing animal and of genetic incompatibility due to oft-transplanted tumors, must be considered. On the other hand, it has become necessary to study tumors in inbred animals without genetic deviations between tumor and host according to recent advances in immunogenetics. In order to study transplantation immunity of tumors, then, animals of inbred strains whose histocompatibility system is certified, and tumors induced in these inbred lines must be employed.

Initial studies were reported by Gross[10,11] (1943, '45) and Foley[8] (1953), who took the concept of histocompatibility system in the immunological aspect of cancer and demonstrated cancer-specific antigen in mice by the method of cancer transplantation. Later, similar facts were confirmed by Prehn[21,22] (1957, '61), Klein[15,16] (1960, '66), Old[19] (1962), Ushubuchi[30,31] (1962, '65), Weiss[32] (1964), Alexander[1,2] (1964, '66) and Takeda[27~29] (1964, '66, '67).

Gross[10] found that intracutaneous inoculation of small doses of methylcholanthrene-induced sarcomas which arose in C3H/He mice regularly induced a state of immunity which prevented growth of the same tumors when fragments were later implanted. This observation may be explained by assuming that immunity acquired against tumors is directed specifically against the tumor, and that tumor immunity in these animals is not caused by genetic differences between the cells of the host and those of the animal in which the tumor originated.

Foley[8] tried to confirm the findings of Gross, using ligation method. He studied transplantation immunity of spontaneous mammary carcinoma and sarcomas induced by methylcholanthrene in C3H/He mice, and found that these two types of tumors differ from each other in their capacity to decrease the susceptibility of mice to reimplantation.

Prehn[21] demonstrated that dibenzanthracene-induced sarcoma is as capable as MC-induced fibrosacoma in producing an immunity against subsequent inoculation of the same tumor within mice of the inbred strain, and that the antigen was peculiar to and specific for its own tumor tissue. Further he[22] mentioned that immunization against some types of carcinogenesis may be feasible by use of tumor

antigen.

Klein et al.[15] removed the tumor bearing foot after tumors were produced by intracutaneous injection of methylcholanthrene into one foot of the mice, and stored resected tumors for the antigen. After that, the autochthonous mice of which tumor had been operatively removed, together with groups of isologous animals, were repeatedly pretreated with irradiated sarcoma cells and subsequently inoculated with viable tumor cells of the same origin. Untreated isologous mice were also inoculated. Thus, an increased resistance to transplanted tumors could be demonstrated in the autochthonous and in the isologous hosts, treated by the irradiated tumor cells, but not in untreated isologous groups. As isologous mice pretreated with irradiated normal tissues and subsequently inoculated with viable sarcoma cells showed almost no resistance to a given sarcoma, they interpreted such resistance to retransplantation as an immune phenomenon depending on the tumor-specific antigen.

Weiss et al.[32,33] (1964) revealed the existence of a resistance to transplanted mammary carcinoma in the original host. Further, they described that the lymph nodes draining the sites of tumor implantation showed lymphoid hyperplasia in original animals.

Old, Boyse, et al.[19] (1962) as well as Prehn and Main have observed that the subcutaneous tumors in inbred strains of mice induced by methylcholanthrene or dibenzpyrene have the capacity to immunize the isogenic host against the same tumor. Furthermore, they[20] (1962, '64) summarized the immunological aspects of experimental tumors.

In this experiment, the authors[14] examined the immunological resistance against tumor transplantation which might be shown by restraint of the growth of MC-induced sarcomas after pretreatment with ^{60}Co irradiated isogenous tumor cells.

The results obtained indicated that mice generally immunized with ^{60}Co irradiated MC sarcoma cells showed specific resistance to the retransplantation of MC-induced sarcoma. These findings agreed with the reports by Klein, Old and Takeda that the MC-induced sarcomas contained a specific antigen for its own tissue.

The problem whether this immune type of resistance can be detected equally over the whole body or conspicuously in special sites is essential in the research on transplantation immunity.

Recently, Prince et al.[23] have reported that so-called partial resistance was found in DAB mice with transplantable adenocarcinoma which had originated in the same strain.

The method of transplanting tumor cells into lymph nodes was designed by us for the study of immunity, especially of local immunity, to cancer. According to our investigation resistance to growth of transplanted tumors appears to be higher in regional lymph nodes draining the sites of immunization than in other body areas.

Old et al.[20] (1964) showed that B.C.G. infection markedly activated the reticulo-endothelial function in Swiss mice and greatly inhibited the growth of sarcoma-180 in the host. Our experiment showed that the infection with B. C. G. stimulated resistance in mice to MC-induced sarcoma.

Method of Transplantation in Lymph Nodes:

Observing host responses to a transplantable "ascites" tumor, Siegler and Koprowska[26] found that there was a striking resistance to tumor growth in lymph nodes, spleen, thymus, and bone marrow, in spite of the fact that the tumor cells grew extensively in the peritoneal cavity and invaded the blood stream.

Recently Berg[3] studied sinus histoicytosis of the axillary lymph nodes in breast cancer patients, and concluded that patients who showed more sinus histiocytosis had a better prognosis than those who showed less sinus histiocytosis. Thus, he emphasized that sinus histiocytosis of the regional lymph node was the significant prognostic factor in cancer patients.

Afterwards, Black and Speer[4] observed the sinus histiocytosis of lymph nodes removed from patients with and without cancer. They reported that it may be termed an expression of host resistance, since sinus histiocytosis is a reactive change in a tissue of the host and is associated with an increased survival.

The authors[13] designed a method for transplanting tumor cells into the inguinal lymph nodes of mice for purpose of testing the local immunity in regional lymph node draining the site of the inoculation of the tumor. By this method, the immunological resitance to the growth of the tumor was proved to be stronger in the regional lymph node of the sensitization site than in other lymph nodes.

SUMMARY

The possible influence of the immunological mechanism in transplantable tumors of mice was investigated by noting the tumor inhibitory effects in mice generally or locally sensitized by Ehrlich ascites carcinoma and methylcholanthrene-induced sarcoma.

The animals were pretreated with tumor cells irradiated with ^{60}Co (15,000 R). They showed a marked immune resistance to subsequent transplantation of the same tumor in the subcutaneous tissue, whereas no resistance was observed in animals similarly pretreated with normal mouse tissue.

The authors designed a method for transplanting tumor cells into the inguinal lymph nodes of mice for the purpose of testing the local immunity in the regional lymph node draining the site of inoculation of the tumor.

REFERENCES

1) Alexander, P., and Delorme, E. J.: Lancet, 18: 117, 1964.
2) Alexander, P., Mikulska, Z. B., and Smith, C.: J. Nat. Cancer Inst., 36: 29, 1966.
3) Berg, J. W.: Cancer N. Y., 9: 935, 1956.

4) Black, M. M., and Speer, F. D.: Surg. Gyn. Obst., 106: 163, 1958.
5) Chamber, H., Scott, G. M., and Russ, S.: Lancet, 1: 212, 1922.
6) Contamin, A.: C. R. Acad. Sci., 150: 128, 1910.
7) Donaldson, D. M., and Mitchell, J. R.: Proc. Soc. Exper. Biol. and Med., 101: 204, 1959.
8) Foley, E. J.: Cancer Res., 13: 835, 1953.
9) Goldfeder, A.: Brit. J. Cancer, 8: 320, 1954.
10) Gross, L:. Cancer Res., 3: 326, 1943.
11) Gross, L.: J. Immunol., 50: 91, 1945.
12) Haaland, M.: Proc. Roy. Soc., 82: 293, 1909.
13) Kitano, M. *et. al.*: Proc. Jap. Cancer Assoc., 24: 73, 1965.
14) Kitano, M. *et. al.*: Jap. Soc. Cancer Ther., 1: 44, 1966.
15) Klein, G., Sjögren, H. O., Klein, E., and Hellström, K. E.: Cancer Res., 20: 1561, 1960.
16) Klein, G.: Internat. Cancer Congr., 9: 289, 1966.
17) Mckee, R. W., Garcia, E., Troeh, M. R., and Schultz, W.: Acta Unio Internat. contra cancrum, 15: 955, 1959.
18) Mottran, J. C., and Russ, S.: Proc. Roy. Soc., 90: 1, 1917.
19) Old, L. J., Boyse, E. A., Clarke, D. A., and Carswell, E. A.: Ann. N. Y. Acad. Sci., 101: 80, 1962.
20) Old, L. J., and Boyse, E. A.: Ann. Rev. Med., 15: 167, 1964.
21) Prehn, R. T., and J. M. Main; J. Nat. Cancer Inst., 18: 769, 1957.
22) Prehn, R. T.: Ann. N. Y. Acad. Sci., 94: 107, 1961.
23) Prince, J. E., Fardon, J. C., Nutini, L. G., and Sperti, G. S.: Cancer Res., 17: 312, 1957.
24) Révész, L.: J. Nat. Cancer Inst., 15: 1691, 1955.
25) Révész, L.: Cancer Res., 20: 443, 1960.
26) Siegler, R., and Koprowsk, I.: Cancer Res., 22: 1278, 1962.
27) Takeda, K. *et. al.*: SAISHIN-IGAKU, (Japan) 19: 474, 1964.
28) Takeda, K. *et. al.*: Internat. Cancer Congr., 9: 293, 1966.
29) Takeda, K.: Gen. Ass. Jap. Med. Congr., 17: 22, 1967.
30) Ushubuchi, I.: Jap. J. Cancer Clin., 8: 615, 1962.
31) Ushubuchi, I. *et. al.*: J. Cancer Immunopathology, (Japan) 1: 71, 1965.
32) Weiss, D.E., Faulkin, L. T., and De Ome, K. B.: Cancer Res., 24: 732, 1964.
33) Weiss, D. E., Attia, M. A., and De Ome, K. B.: Cancer Res., 25: 451, 1965.