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The Effect of Xenogenic and Allogeneic Tumor Cells on Experimental Metastasis with Ehrlich Carcinoma

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Intravenous inoculation of viable Ehrlich carcinoma cells were given to ddY mice with or without xenogeneic Yoshida sarcoma or heat-killed allogeneic Ehrlich ascites carcinoma cells. There was no difference in the survival time of the mice due to metastasis among the group given Yoshida sarcoma or killed Ehrlich carcinoma cells and the control group. When these tumor cells were injected prior to the intravenous inoculation of the viable Ehrlich carcinoma cells, the survival of the host animals was remarkably prolonged. When Ehrlich tumor cells were given to mice successively two times, survival time of the hosts was dependent on the number of the total cells within 0 to 6 hours and on the number of the 1st inoculation when the interval was 24 hours.

Introduction

It has been known that the number of embolic tumor cells is an important factor in the process of experimental metastasis.[2] Most of the work on these problems indicates that its spread and growth are dependent largely on the morphological distribution of tumor cells and emboli. The quantitative relationship between the number of viable cells artificially introduced in the lung was also described.[3] Recently Nakamura and Suzuki[4] reported that the number of tumor cells neccessary to cause immediate death by pulmonary embolism was characteristically dependent on the time of injection and also on the tumor strain employed.

This communication describes the effects of xenogenic Yoshida sarcoma and heat-killed Ehrlich carcinoma on the metastasis in mouse produced by intravenous inoculation of viable Ehrlich carcinoma cells.

Experimental

The animals used in these experiments were mostly ddY male mice 4—5 weeks old. They were supplied from an animal farm in Shizuoka Prefecture and kept on standard diet CE-2 CLEA Japan Inc., in Tokyo with unlimited supply of water. The LP-12 subline[5] of Ehrlich ascites carcinoma cells which were maintained by serial intraperitoneal transplantation into ddY male mice, were employed for the experiments. Tumor cells harvested 7 days after transplantation were used. The preparation of killed Ehrlich cells was heated at 70° for 30 minutes in an incubator. Yoshida ascites sarcoma was serially maintained by intraperitoneal transplantation into Donryu female rat supplied from Nippon Rat Co., in Tokyo. They were fed in the same way as in the mice. Tumor cells obtained 3—4 days after transplantation were used.

1) Location: *a) Ukima 1-3-32, Kita-ku, Tokyo 115, Japan; b) Hongo 7-3-1, Bunkyo-ku, Tokyo, 113, Japan.*
2) I. Zeidman, M. McCutcheon and D.R. Coman, *Cancer Res.*, **10**, 357 (1950); D.R. Coman, R.P. deLong and M. McCutcheon, *Cancer Res.*, **11**, 648 (1951); I. Zeidman and J.M. Buss, *Cancer Res.*, **12**, 731 (1952); J.S. Wood, Jr., E.D. Holyoke, W.P.C. Clason, S.C. Sommers and J.S. Warren, *Cancer*, **7**, 437 (1954).
3) R. Baserga, P.B. Putong, S. Tyler and W.B. Wratman, *Brit. J. Cancer*, **14**, 173 (1960).
4) K. Nakamura and K. Suzuki, *GANN*, **60**, 483 (1969).
5) Y. Hasegawa, T. Irikura, M. Ishidate, Jr. and D. Mizuno, *GANN*, **61**, 73 (1970).

Result

Injection of Xenogeneic or Killed Allogeneic Tumor Cells

Table I shows the survival time of the hosts when Yoshida rat ascites sarcoma as xenogeneic tumor cells have been inoculated simultaneously with viable Ehrlich ascites carcinoma to ddY mice intravenously. All the mice inoculated with living Yoshida sarcoma cells alone survived more than 30 days. However, the tumor cells had microscopically invaded the lung tissues of the mice. When both inoculum sizes of viable Ehrlich and of Yoshida ascites cells were changed, survival time of the tumor-bearing mice was shown to dependent on the cell dose of the Ehrlich carcinoma but not on that of the Yoshida sarcoma cells. In the case of

Table I. Survival Time of the Hosts when Intravenous Inoculation of Viable Ehrlich and Yoshida Tumor Cells was given Simultaneously in to ddY Mice

Number of cells in inoculum		S/T[a]	Survival day Mean±S.D.
Ehrlich carcinoma	Yoshida sarcoma ($\times 10^6$)		
0	10	6/6	>30
1	0	3/6	24.7±6.3
1	5	3/6	25.0±6.4
1	10	4/6	26.7±5.5
3	0	0/6	11.0±1.6
3	3	0/6	11.0±1.5
3	10	0/6	11.0±2.4
6	0	0/6	8.7±0.9
6	3	0/6	9.2±0.9
6	10	0/4[b]	8.5±0.9

a) survivor for over 30 days per total treated
b) Many animals that died immediately after inoculation of cells are excluded from the table.

Table II. Survival Time of the Hosts when Intravenous Inoculation of Viable and Killed Ehrlich Carcinoma Cells was given Simultaneously into ddY Mice

Number of cells in inoculum		S/T	Survival day Mean±S.D.
Viable	Killed ($\times 10^6$)		
0	1	6/6	>30
1	0	3/6	24.8±5.9
1	1	2/6	24.8±4.5
3	0	0/6	15.0±1.7
3	1	0/6	13.8±1.8
6	0	0/6	9.5±0.8
6	1	0/6	11.7±2.9

killed Ehrlich carcinoma cells injected to the mice, the results were similar to the case of xenogeneic Yoshida sarcoma cells as indicated in Table II. Of the mice injected with 16×10^6 cells in total (Table I) may died almost immediately. The mice also died immediately when 2×10^6 cells of killed Ehrlich carcinoma were injected intravenously in our preliminary findings. Nevertheless, the mice bearing Ehrlich carcinoma cells did not alter the survival time even after the additional treatment with these tumor cells. Consequently, the dose-response relationship between the number of viable Ehrlich cells inoculated and the incidence of sur-

vival time of the mice was observed regardless of the addition of killed Ehrlich carcinoma and of Yoshida sarcoma cells.

Survival times of ddY mice bearing viable Ehrlich cells were prolonged by a previous treatment either with the Yoshida sarcoma or with killed Ehrlich carcinoma cells as illustrated in Table III. The longest survival time was observed on an intravenous injection of Yoshida sarcoma cells. The data in Fig. 1 indicates that the percentage of mean survival time of the

Table III. Survival Time of the Hosts when Yoshida Sarcoma was given Intraperitoneally or Intravenously 2 Days prior to the Inoculation of 6×10^6 Ehrlich Carcinoma Cells into ddY Mice

Cells in inoculum	Route	S/T	Survival day Mean±S.D.	Percent (Treat./cont.)
Yoshida sarcoma				
Control		0/6	8.0±0.8	100
10^5	*i.p.*	0/6	9.0±1.1	113
10^6	*i.p.*	0/6	12.5±5.7	156
10^7	*i.p.*	0/6	13.7±4.2	171
10^5	*i.v.*	0/6	12.8±2.6	160
10^6	*i.v.*	0/6	14.2±3.3	178
10^7	*i.v.*	0/6	19.8±5.3	248
Killed Ehrlich carcinoma				
Control		0/6	10.3±0.5	100
10^6	*i.p.*	0/6	19.3±8.1	187
10^6	*i.v.*	0/6	12.5±2.0	121

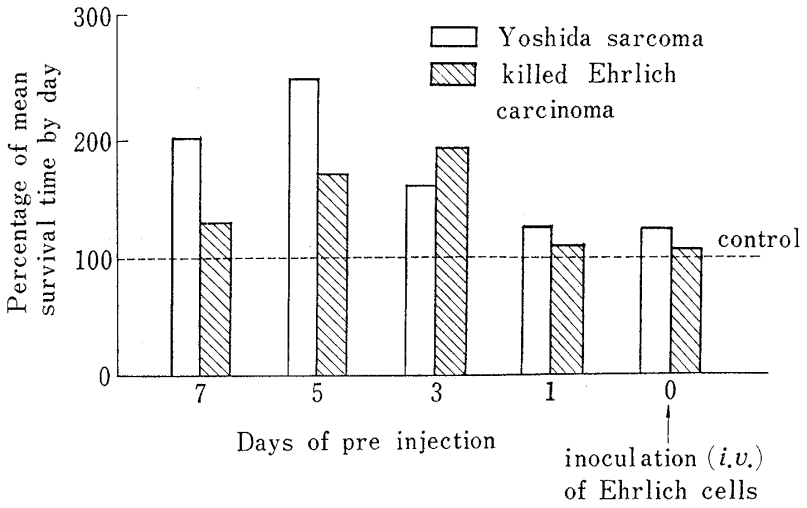

Fig. 1. Effect of Single Intraperitoneal Injection of Viable Yoshida Sarcoma or Killed Ehrlich Carcinoma Cells prior to the Intravenous Inoculation of Viable Ehrlich Carcinoma Cells

Killed Ehrlich (70° for 30 minutes) or viable Yoshida tumor cells (10^6) were given once at 0, 1, 3, 5 and 7 days prior to the inoculation of viable Ehrlich carcinoma cells (6×10^6). Survival animals more than 30 days were calculated as 30 days survivors. One group contained 6 mice.

hosts to which Yoshida sarcoma or killed Ehrlich carcinoma was injected intraperitoneally into the mice prior to the intravenous inoculation of viable Ehrlich carcinoma cells. The maximum survival time of the mice was obtained on the minus 5th day in the case of the Yoshida sarcoma cells and on the minus 3rd day in killed Ehrlich respectively.

Successive Inoculation of Ehrlich Carcinoma within a Short Period

The Ehrlich carcinoma cells ($1\times$, $3\times$ or 6×10^6) were inoculated intravenously into the tail vein of mice and the 2nd challenge was performed 24 hours later by the same route. As shown in Table IV, the survival time of the mice bearing Ehrlich carcinoma cells depended

Table IV. Survival Time of the Hosts when Successive Inoculation of Ehrlich Carcinoma Cells was given Intravenously into ddY Mice

Number of cells in inoculum			S/T	Survival day Mean±S.D.
1st	2nd[a)]	Total ($\times10^6$)		
1	0	1	3/6	25.0±5.2
1	5	6	1/6	18.5±6.2
3	0	3	0/6	11.5±3.0
3	3	6	0/6	11.2±4.4
6	0	6	0/6	9.5±1.4
6	3	9	0/6	8.8±0.7

a) 24 hours after the 1st inoculation

on the size of the 1st inoculation of the cells but not on that of the 2nd inoculation and not different from that of the control group. However, in the case of comparatively small size (10^6 cells) of the 1st inoculation, survival time was dependent on the 2nd inocualtion. The time of the 2 nd inoculation was changed successively 0 (simultaneous time), 1, 3, 6 or 24 hours after the 1st inoculation in Table V. These was an increase in the incidence of meta-

Table V. Relations between Time of 2nd Inoculation of Ehrlich Carcinoma Cells and Survival Time of ddY Mice

Time of 2nd inoculation (hours)	*S/T*	Survival day Mean±S.D.
Control	0/6	13.0±3.1
0	0/6	8.6±1.3
1	0/6	8.3±1.1
3	0/6	8.2±0.9
6	0/6	8.8±1.6
24	0/6	12.2±2.8

3×10^6 cells of Ehrlich carcinoma were intravenously inoculated both 1st and 2nd times. Control group was not given at the 2nd time.

stasis and high mortality in those groups between 0 and 6 hours. When the 2nd inoculation was performed 24 hours after the 1st, the survival time was not different from the case of a single injection at the 1st.

Discussion

Present studies were attempted to determine whether the effects of xenogeneic tumor cells on blood-borne metastasis were ascribed to mechanical embolism or not. From the experiments (Table I) there can be no doubt that metastasis is not dependent on the number of the xenogeneic tumor cells, though the size of Yoshida ascites sarcoma is smaller than that of Ehrlich ascites carcinoma. There was no difference of the survival time of Ehrlich tumor-bearing mice between the group injected with Yoshida sarcoma or killed Ehrlich carcinoma cells and the control group (Table I and II). The animals, however, died almost immediately

following the inoculation of extraordinary number of these tumor cells, suggesting that this size of inoculation causing emboli did not correlate with the metastasis. These results suggest that the additional dosage of xenogeneic or killed tumor cells does not correlate with the metastasis of the viable carcinoma cells.

The injection of Yoshida sarcoma or killed Ehrlich carcinoma cells prior to the inoculation of viable Ehrlich carcinoma cells exert a significant effect on the survival of the Ehrlich tumor-bearing mice (Table III and Fig. 1). It is uncertain whether or not the prolongation of survival of the host animals is due to the production of cell-mediated immunity in a broader sense. The immunotherapy against murine tumor cells by an injection of rat ascites hepatoma AH-39 was discussed by Akiyama.[6]

Baserga, *et al.*[3] have reported that the incidence of lung metastasis is about twice when mice are injected, 80 days apart, with two similar doses of viable Ehrlich tumor cells. They suggested that a previous treatment with viable tumor cells dose not alter the response of the host to the successive 2nd injection of viable tumor cells. The simultaneous injection of killed and viable Ehrlich ascites cells significantly reduced the minimal number required for the production of pulmonary metastasis. As shown in Tables IV and V, survival time of the tumor-bearing mice with successive inoculation of the same cells was not different from that of the control with a single inoculation. The mechanism by which these tumor cells are correlate with each other is not clear in some parts, but these results suggest that immunity of the host animals may have been concerned.[6,7]

6) T. Akiyama, *Minophagen Med. Rev.*, **9**, 165 (1964); *idem*, *ibid.*, **10**, 18 (1965).
7) T. Yoshida, "Yoshida Nikushu," Nara Shobo, Tokyo, 1964; P.S. Russell and A.P. Monako, "The Biology of Tissue Transplantation," Little, Brown and Company, Boston, 1965; G.H. Fairley, *Brit. Med. J.*, **2**, 467 (1969).