The Difference in Immunological Properties between Lymph Node Metastatic and Non-Metastatic Cell Lines of MCA-Induced Fibrosarcoma of C4W Mice

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OGURI, H., SATO, Y., SUGAWARA, H., YOSHIDA, K., NISHIHIRA, T., MORI, S. and TACHIBANA, T. The Difference in Immunological Properties between Lymph Node Metastatic and Non-Metastatic Cell Lines of MCA-Induced Fibrosarcoma of C4W Mice. Tohoku J. Exp. Med., 1992, 168 (2), 409-412 — To pursue the process of lymph node metastasis, i.e., the preferential tumor growth in lymph node, we have established the non-metastatic M2B cell line which was derived from 3-methylcholanthrene-induced fibrosarcoma of C4W mouse and a metastatic cell line, M2BLN-M+ which was obtained from metastatic lymph nodes of irradiated C4W mouse which was subcutaneously implanted with cultured tumor cells, because implanted tumor cells were derived from the spontaneous metastatic lymph node of the parental M2B tumor, but regressed in naive C4W mouse. We examined the characteristics of both tumor cell lines in terms of the immunological cellular interactions. M2BLN-M+ showed unexpectedly to be more susceptible to cytotoxicity of immune effectors (NK cell, macrophage and cytotoxic T lymphocyte) than M2B did. When cultured both tumor cells with these effector cells, the growth inhibition of M2BLN-M+ was greater than that of M2B. The regional lymph node of tumor-bearer, however, showed no effective cytotoxic activity as reported by others. On the contrary, when cultured both tumor cells with non-immune lymph node cells, to be surprised, the proliferation of M2B was markedly suppressed, while that of M2BLN-M+ was slightly inhibited. The lymph node cells of M2B-bearing mice showed stronger cytostatic activity to M2B. The results suggest that the cytostatic activity of lymph node cells will be a pivotal factor, concerning the establishment of lymph node metastasis. — lymph node; metastasis; malignant tumor; cytotoxicity; cytostatic activity

Lymphatic metastasis is an important route in the spread of malignant tumors. In general, metastasis to the lymph node is considered to be the outcome of extensive local tumor invasion. But, the precise mechanisms of lymphatic metastasis are little known (Carr 1983). According to the knowledge of highly

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selective process of tumor dissemination, dependent on the interplay of host factors and tumor cell properties, lymph node metastasis alone will be no exception (Tachibana and Yoshida 1986). We have established a good experimental model of lymphatic metastasis; a metastatic M2BLN-M+(M+) cell line and a non-metastatic M2B cell line. The present study was concerned about the mechanism of the preferential growth of tumor cells in lymph node in the process of lymphatic metastasis.

**MATERIALS AND METHODS**

*Animals and tumors*

Male and female BALB/c-Fv-4W (C4W) mice (Odaka et al. 1981) were used at 6-12 weeks old for the experiments. A 3-methylcholanthrene-induced M2B fibrosarcoma cell line of C4W mouse origin was a non-metastatic. Several cultured cell lines were obtained from lymph nodes metastasized spontaneously in mice bearing M2B tumors, but all of them regressed in naive C4W mice. When implanted sc such a cell line in irradiated C4W mice, the cells formed local tumor and metastasized to the lymph nodes. A metastatic cell line, M2BLN-M+(M+) was easily established from the metastatic nodes.

*Immunization*

M2B and M+ tumor cell fractions for immunization were prepared from local tumor and metastatic nodes by digesting with the mixture of collagenase and DNase. Mice were immunized against each tumor by i.p. injections of a million irradiated tumors 3 times at 1-week interval. Seven days after the final immunization, mice were sc challenged with freshly excised tumor cells. Fourteen days later, the immune spleen cell suspension was prepared and used.

*Effector cells*

Cytotoxic T lymphocytes (CTLs) were obtained by in vitro sensitization of the immune spleen cells (5×10⁷) with irradiated tumor cells (10⁶, ⁶⁰Co 100 Gy) for 5 days at 37°C. As NK cells used were spleen cells from mice which were i.v. injected 0.01 mg poly (I: C) 3 days prior to use. Macrophages were collected from the peritoneal exudate cells of mice which received 0.1 mg OK432 i.v. 4 days prior to use. Cytotoxicity was determined by ⁵¹Cr-release assay for 4 hr (NK), 12 hr (CTL) and 18 hr (macrophage), and expressed as percent specific lysis.

*Proliferation assay*

Tumor cells were co-cultured with graded numbers of lymph node cells (30 Gy-irradiated or non-treated) for 72 hr at 37°C in 5% CO₂ in air. Uptake of [³H]TdR for the final 24 hr of culture was counted. The percent inhibition of tumor cell proliferation was calculated.

**RESULTS AND DISCUSSION**

*Metastatic (M+) and non-metastatic (M2B) cell lines*

M+ tumor cell line metastasized to axillary and/or inguinal lymph nodes naturally without exception when inoculated with more than 10⁶ cells sc on the back of C4W mice, while M2B did not proliferate in the lymph nodes.
Susceptibility of effector killing activities

M+ tumor cells showed to be more susceptible to every killing activity of both CTLs to M+ and M2B (Fig. 1A), to NK activity (Fig. 1B) and to cytocidal macrophage activity (Fig. 1C), compared to the susceptibility of M2B to every of three different effector cytotoxic activities. However, the regional lymph nodes of tumor-bearing 7 days after tumor implantation showed no measurable cytotoxic activity (data not shown), in agreement with the reports by others (Yoshida and Tachibana 1985). Thus, under the circumstances of tumor growing, the susceptibility of metastatic M+ tumor cells to effector cells seems to be no obstacle for the migrant tumor cells into the lymph node in this model.

Cytostatic activity of lymph node cells

Unexpectedly, non-immune lymph node cells markedly suppressed the prolif-
eration of M2B tumor cells, but slightly inhibited M+ tumor cells in vitro (Fig. 2). The lymph node cells of M2B-bearing mice showed stronger cytostatic activity to M2B, while those cells of M+ -bearing mice showed no remarkable change (data not shown). The results suggest that the cytostatic activity of non-immune lymph node cells will be a pivotal factor for the establishment of metastasis in the lymph node. The more precise study is now in progress.

References