Effects of serum fractions obtained from cancer patients by double-filtration plasmapheresis on tumor growth and metastasis in mice.

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Abstract

We administered serum fractions obtained from cancer patients by double-filtration plasmapheresis (DFPP) to cancer-bearing mice to examine the effects on tumor growth and metastasis. Fraction 1 (whole plasma), fraction 2 (a plasma fraction containing substances with higher particle size), fraction 3 (a plasma fraction containing substances with smaller particle size) and saline were administered intravenously to cancer-bearing mice for 10 days following the inoculation of tumor cells. The tumor growth and metastasis in mice administered fraction 2 was far more rapid than that in the control mice. On the other hand, tumor growth in mice administered fraction 3 was significantly delayed compared with that in mice injected with fraction 2. These results suggest that factors in the higher particle-size fraction of cancer patients’ sera promote the growth and the metastasis of tumors in mice, and that DFPP, which remove these factors, is an effective therapy against cancer.

KEYWORDS: double-filtration plasmapheresis, immunosuppressive factors, cancer therapy

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Effects of Serum Fractions Obtained from Cancer Patients by Double-Filtration Plasmapheresis on Tumor Growth and Metastasis in Mice.

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We administered serum fractions obtained from cancer patients by double-filtration plasmapheresis (DFPP) to cancer-bearing mice to examine the effects on tumor growth and metastasis. Fraction 1 (whole plasma), fraction 2 (a plasma fraction containing substances with higher particle size), fraction 3 (a plasma fraction containing substances with smaller particle size) and saline were administered intravenously to cancer-bearing mice for 10 days following the inoculation of tumor cells. The tumor growth and metastasis in mice administered fraction 2 was far more rapid than that in the control mice. On the other hand, tumor growth in mice administered fraction 3 was significantly delayed compared with that in mice injected with fraction 2. These results suggest that factors in the higher particle-size fraction of cancer patients' sera promote the growth and the metastasis of tumors in mice, and that DFPP, which remove these factors, is an effective therapy against cancer.

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The effectiveness of plasma exchange in the treatment of cancer has been discussed since Browne (1) and Israel (2) first performed plasma exchange to treat cancer patients. This treatment was attempted to enhance the anti-tumor activity of the host by removing immunosuppressive factors from the patients' blood through plasma exchange. However, plasma exchange requires a lot of fresh frozen plasma (FFP) as a substitution fluid. For this reason, it has not come into wide use because there is a shortage of FFP. Therefore, new methods not requiring large volumes of substitution fluid have been tried (3). We have applied double-filtration plasmapheresis (DFPP), using a membrane separator, to patients with progressive cancer of the digestive tract and found that the removal of immunosuppressive factors was clinically effective as adjunctive therapy for malignant tumors (4). We also demonstrated that a suppressive effect of cancer patient's serum on the cellular immunity in vivo and in vitro could be reduced by DFPP (5).

In this study, we injected serum fractions obtained from cancer patients by DFPP into cancer-bearing mice to evaluate the effects of immunosuppressive factors on the growth and metastasis of tumors.

Materials and Methods

Mice. Male BALB/C and female C57BL/6 mice 6 weeks of age were obtained from the
Shizuoka Laboratory Animal Center (Shizuoka, Japan).

Tumors. Meth A is a methylcholanthrene-induced fibrosarcoma syngeneic to BALB/C mice. It was maintained in vivo by serial intraperitoneal (ip) transplantation into BALB/C mice. Lewis lung carcinoma, which is syngeneic to C57BL/6 mice and induces lung metastasis, was maintained in vivo by intramuscular (im) injection into the femoral region of C57BL/6 mice.

Fractionation of cancer patient's serum by DFPP. Serum fraction used in this experiment was obtained from cancer patients who underwent DFPP at the First Department of Surgery of Okayama University Medical School (5). Blood was drawn from the femoral vein and was circulated extracorporally at 100 ml/min. The blood was separated with the first filter (pore size: 0.2 μ) into a cellular component and a plasma component (the first filtrate). The first filtrate (fraction 1) was filtered through the second filter (pore size: 0.02 μ) and separated into fraction 2 containing substances with larger particle size and fraction 3 (the second filtrate) containing substances with smaller particle size including albumin. Fraction 3 together with the cellular component was infused back into the donor. Fraction 2 was discarded and replaced with substitution fluid such as FFP. Fraction 1, fraction 2 and fraction 3 were used in the following experiments (Fig. 1).

Inhibitory effect of serum fractions on PHA-induced blastogenesis of normal lymphocytes. Lymphocytes were separated from the peripheral blood of healthy adults by the Ficoll-Conray density gradient centrifugation method. After washing with phosphate-buffered saline three times, they were suspended in RPMI 1640 (Nissui, Tokyo, Japan)-HEPES (Sigma Chemical Co., St. Louis, MO, U.S.A.) containing 10% fetal calf serum and were placed in 96-well microtiter plates (Corning Glass Works, Corning, NY, U.S.A.) with 1×10^4 cells/0.2 ml/well. Each fraction or serum obtained from normal human blood, AB type, (control) (0.2 ml) and phytohemagglutinin-P (PHA) (Difco Laboratories, Detroit, MI, U.S.A.) at a final concentration of 1% were added to each well. The mixtures were incubated at 37°C for 3 days. One μCi of ^3H-thymidine was added to each well 24 h before completion of the incubation. The uptake was measured by a liquid scintillation counter (Aloka, Tokyo, Japan). The counts per minute (cpm) were obtained as a mean of triplicate values. The inhibition rate was calculated as follows:

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\text{% inhibition} = \left(1 - \frac{\text{cpm of the culture with serum fraction}}{\text{cpm of the culture with control serum}} \right) \times 100
\]

Effects of serum fractions on tumor growth in cancer-bearing mice. To prepare cancer-bearing mice, 1×10^8 of Meth A cells were inoculated subcutaneously into the back of BALB/C mice. From the day when the cells of tumor were inoculated, the cancer-bearing mice were divided into five groups, and administered fraction 1, fraction

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Fig. 1 DFPP apparatus and fractions separated from cancer patients' sera by DFPP. Blood drawn from the patient was separated with the first filter into cellular component and the first filtrate (F1: fraction 1). The first filtrate was filtered through the second filter and separated into the discarded fluid (F2: fraction 2) and the second filtrate (F3: fraction 3). Fraction 3 was returned to the patient with the cellular component and fraction 2 was replaced with substitution fluid.
2, fraction 3, normal human serum, and saline, respectively. The serum fractions and other solutions (0.2 ml) were injected intravenously every day into each cancer-bearing mouse through the tail vein for 10 days. The diameter of the tumor was measured every day from the day when the tumor first became palpable.

**Effects of serum fractions on tumor metastasis in cancer-bearing mice.** A cell suspension (0.025 ml) containing 1×10⁴ Lewis lung carcinoma cells was injected into the footpads of C57BL/6 mice. The limbs with the tumor were amputated 10 days after the inoculation. From 11 days after the inoculation, the cancer-bearing mice were divided into five groups as described above, and 0.2 ml of the serum fraction or other solution was administered every day to each mouse through the tail vein for 10 days. Twenty-one days after the inoculation of tumor cells, lungs were extirpated after 1.2 ml of a 15% solution of India ink was given through the trachea. The lungs were bleached with Fekete's solution (6), and the lung metastases were counted. The metastases were easily countable because they formed white nodules on the surface of the lungs, while the parenchyma of the lungs turned black due to carbon particles from the ink.

Results

**Inhibitory effects of serum fractions on PHA-induced blastogenesis of normal lymphocytes.** Fraction 1 inhibited lymphocyte blastogenesis by 35.8% compared with control human serum, while fraction 2 remarkably inhibited it by 76.8%. The inhibition rate of fraction 3 was 18.4%, which was lower than those of the other two serum fractions (Fig. 2).

**Effects of serum fractions on tumor growth in cancer-bearing mice.** The diameter of the tumor 10 days after the inoculation was 4.9±2.5 cm in the control group administered saline, while that of the group administered fraction 2 was 7.6±2.5 cm, which was significantly greater than that of the control group (p < 0.05). In contrast, the diameter of the tumor in the group administered fraction 3 was 4.5±1.5 cm, which was approximately the same as that of the control group. There was a significant difference (p < 0.05) between the fraction 2 group and the fraction 3 group. The tumor grew in the group administered fraction 1 to the same degree as in the fraction 2 group (7.7±2.1 cm) (Fig. 3).

The period from tumor inoculation to the day when the tumor became palpable was 6.1±0.9 days in the control group, while it was 5.2±0.9 days in the fraction 2 group, which was significantly shorter than in the control group (p < 0.05). The latent periods in the fraction 1 group and in the fraction 3 group were 5.3±0.8 days and 6.2±1.0 days, respectively (Table 1).
Effects of serum fractions on tumor metastasis in cancer-bearing mice. The number of lung metastases was 27.8 ± 6.2 in the control group, while it was 41.6 ± 8.0 in the fraction 2 group, which was significantly greater than in the control group (p < 0.01). It was 31.2 ± 14.5 in the fraction 1 group which was greater than in the control group, but there was no significant difference between them. On the other hand, it was 23.0 ± 7.7 in the fraction 3 group, which was significantly less than that in the fraction 2 group. The number of lung metastases in the normal human serum group was about the same as that in the control group (28.2 ± 7.9) (Fig. 4).

Discussion

From this study, it is suggested that substances with higher particle size in cancer patients' sera are immunosuppressive and promote tumor growth in mice, and these substances can be eliminated from cancer patients' sera by DFPP.

Various specific and non-specific immunosuppressive factors existing in the sera of cancer patients have been reported (7-12). Tumor-specific antigens, antibodies, and immune complexes are known as specific immunosuppressive factors. Non-specific
Possess the ability to depress immunological functions of the host, including lymphocyte blastogenesis stimulated by mitogens, specific lymphocyte cytotoxicity, and natural killer cell cytotoxicity (13, 14). Therefore, the elimination of immunosuppressive factors seems to be important in enhancing antitumor activity of the host and increasing the effectiveness of immunochemotherapy. For these reasons, plasma exchange has been studied in cancer patients. In 1977, Israel et al. applied plasma exchange to 56 cancer patients and reported that there was more than 50% tumor regression in 14 out of the 45 patients who responded to plasma exchange (15). Thereafter, many investigators have reported that plasma exchange is an effective treatment for malignant tumors (16, 17). However, plasma exchange has not been widely employed because it requires a lot of FFP as a substitution fluid.

For the above reason, methods which do not require a lot of substitution fluid, such as DFPP, cryofiltration, and plasmapheresis, have replaced plasma exchange (3, 18, 19). We have clinically applied DFPP to cancer patients as adjunctive therapy and have reported that immunological function was recovered by removing immunosuppressive factors in vitro (4, 5), but we had no in vivo data to evaluate the effects of DFPP on tumor growth.

In the present study, we separated cancer patients’ sera by DFPP and examined the inhibitory effects of the serum fractions on PHA-induced blastogenesis of normal lymphocytes. We previously reported that the inhibitory effect of the first filtrate (fraction 1), the discarded fluid (fraction 2), and the second filtrate (fraction 3) obtained from 11 cancer patients on PHA-induced blastogenesis of normal lymphocytes was 35.3 ± 18.0%, 76.8 ± 19.5%, and 24.4 ± 13.9%, respectively (5). In this study, the percent inhibition to normal human serum was 35.8%
in fraction 1, 76.8% in fraction 2, and 18.4% in fraction 3. These results were comparable with our previous data. These serum fractions were administered to cancer-bearing mice, and their effect on tumors was examined. Tumor growth in the group administered fraction 2, which markedly inhibited PHA induced blastogenesis of lymphocytes, was more rapid than that in the control group. On the other hand, tumor growth in the group administered fraction 3, which had a lower inhibitory effect on PHA induced blastogenesis, was delayed significantly more than in the group administered fraction 2. The number of lung metastases in the group administered fraction 2 clearly increased more than in the control group and the fraction 3 group. These results suggest that fraction 2, which is discarded by DFPP, contains substances which promote tumor growth, or substances which reduce the antitumor activity of the host. As a result, DFPP which eliminates these serum factors is considered to be an effective procedure for the treatment of cancer even if it is primary, recurrent or metastatic.

DFPP in cancer patients is still an experimental procedure. However, another animal model showed that the effectiveness of immunochemotherapy in cancer-bearing rats was augmented by removal of the immunosuppressive factors by plasma exchange (20). We have also examined adsorbents which were combined with DFPP to remove immunosuppressive factors with molecular weights lower than that of albumin (21). These procedures combined with chemotherapy are expected to be effective as adjunctive therapy for malignant tumors.

References


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