EVALUATION OF PSK, AN ANTITUMOR PROTEIN-BOUND POLYSACCHARIDES, BY THYMOCYTE ELECTROPHORESIS

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It was clear that the proportion of high mobility cells in thymus tended to increase during tumor development by cell electrophoresis. Although the antitumor activity of PSK, protein-bound polysaccharide or mitomycin-C was similarly effective in sarcoma-180-bearing mice, the histogram pattern of thymocyte electrophoresis differed markedly from each other. Normally, the proportion of high mobility cells of thymocyte increased more with the administration of mitomycin-C than that of the untreated tumor-bearers, while PSK kept the thymic cell mobility histogram normal. Changes of thymocyte electrophoresis caused by the drugs also correlated with the depression of their antiinfectious activity. Using the fully automated cell electrophoretic instrument, the drug can be evaluated simply from the viewpoint of antiinfectious efficacy.

Keywords — thymocyte electrophoresis; tumor bearing; PSK; mitomycin-C; drug evaluation

INTRODUCTION

In the previous paper, it was reported that a high mobility peak appeared in the histogram of thymocyte electrophoresis in tumor bearing mice by using a fully automated analytical instrument.¹⁾ In addition, it was found that a high mobility peak of histogram pattern consisted of mainly $\text{Lyt-1}^+ 2^-$ mature cells, that might be the inducer of suppressor T cells.

The present study was undertaken to compare the histogram of thymocyte electrophoresis in the course of the administration of anti-cancer drug, PSK or mitomycin-C, to tumor bearing mice for drug evaluation using the automated cell electrophoretic instrument.

MATERIALS AND METHODS

Animal and Tumor — Female mice of ICR strain were obtained commercially from CLEA Japan Inc., Tokyo. Sarcoma-180 was maintained intraperitoneally in the mice.

Chemicals — Hanks' balanced salt solution

(HBSS) and Eagles, minimum essential medium (MEM Eagle) were purchased from Gibco, USA. Mitomycin-C (MMC) was commercially obtained from Kyowa Hakko Kogyo Co., Ltd., Tokyo. Other reagents used here were of analytical grade.

Administration of Drug — Sarcoma-180 (1×10^6) were subcutaneously injected into the right flanks of the mice. PSK (10 mg/kg) or MMC (0.5 mg/kg) was intraperitoneally injected every other day for 20 d from the day after implantation of sarcoma-180. At the day shown in Fig. 1, three mice were sacrificed and each thymus was used for cell electrophoretic measurement. At the same time, the weight of each tumor was measured after resection.

Preparation of Thymic Lymphocyte — The thymus was teased with fine forceps and cell suspension was prepared by passing the teased tissue through a stainless steel mesh. Erythrocytes were removed from these cells by hemolysis. After washed twice with HBSS and

once with MEM Eagle, the cells were resuspended in fresh MEM Eagle.

Cell Electrophoretic Measurement — The electrophoretic mobility of lymphocytes was determined with a fully automated cell electrophoretic apparatus (Parmoquant: produced by Kureha Chem. Ind. Co. Ltd., Tokyo). As the details of the apparatus and method of measurement were described previously.¹⁾ Only a brief description is given here. The cell suspension was admitted into the rectangular quartz chamber. Ten to twenty migrating cells in view were traced by image processing and the positions of each cells were stored instantaneously. After two hundred cells were measured, the data and conditions of electrophoresis were printed out. Cells were suspended in MEM Eagle at an approximate concentration of 5×10^5 cells/ml and placed in the chamber in 2 ml amounts. Electrophoresis was performed at 13 mA for 3 s at 24°C.

RESULTS

Changes in Tumor Size of Sarcoma-180-Bearing Mice by Treatment with PSK (an Immunomodulator) or MMC

Fourteen or 21 d after the subcutaneous implantation of the tumor, the tumor growth was evaluated by weighing the tumor mass after sacrificing the mice. As the tumor grew, the host in the untreated group died. In PSK- or MMC-treated group, the tumor began to regress after 21 d although the tumor mass existed in the host after 14 d (Fig. 1). Both drugs showed a similar antitumor action on this tumor system.

Changes of Thymocyte Population in Sarcoma-180-Bearing Mice Following Treatment with PSK or MMC

The border line between fast and slow mobility cells was tentatively placed at a mobility of $0.85 \mu\text{m/s/volt/cm}$ in the histogram (fast cells $\geq 0.85 \mu\text{m/s/volt/cm}$). Following the tumor growth, the proportion of fast cells had a tendency to increase, which became obvious 21 d after tumor implantation (Fig. 2). In the PSK-treated group, the percentage of the fast cells was hardly altered, while it was increased remarkably in the

mitomycin-C-treated group. Mitomycin-C seemed to give more harm to thymocytes of the tumor-bearing mice.

Electrophoretic Mobility Pattern of Thymocytes in Drug-Treated Mice 21 d after the Tumor Implantation

As shown in Fig. 3, in the tumor-bearing mice, a new peak appeared in the histogram

FIG. 1. Changes in Tumor Size of Sarcoma 180-Bearing mice by Treatment with Mitomycin-C or Immunomodulator, PSK

●—● control, ○- -○ PSK-treated (10 mg/kg), □- -□ MMC-treated (0.5 mg/kg).

FIG. 2. Changes in Percentage of Fast Cells of Thymocytes in Sarcoma 180-Bearing Mice by Treatment with Mitomycin-C or Immunomodulator, PSK

●—● control, ○- -○ PSK-treated (10 mg/kg), □- -□ MMC-treated (0.5 mg/kg).

around $1.0 \mu\text{m/s/volt/cm}$ during tumor growth. In the MMC-treated group, the proportion of fast cells seems to increase as a result of the marked decrease in the slow cells. However, the electrophoretic mobility pattern of thymocytes remained normal in the PSK-treated group. In other word, PSK brought about recovery in the host by keeping the thymus harmless, from the thymocyte electrophoretic mobility pattern.

DISCUSSION

PSK, a protein-bound polysaccharide obtained from *Coriolus versicolor* (FR.) Quél. which belongs to the Basidiomycetes, was found to exhibit host-mediated antitumor activity against several murine tumors.²⁻⁴⁾ In mice bearing sarcoma-180, PSK as well as mitomycin-C showed a marked antitumor activity followed by complete recovery.

In the previous paper, it was reported that the proportion of high mobility cells ($1.0 \mu\text{m/s/}$

volt/cm) to low mobility cells ($0.7 \mu\text{m/s/}$ volt/cm) increased in thymus as the tumor grew in the mice.¹⁾ In this communication, we examined changes of the histogram after drug administration to the tumor bearers, and found the proportion of the fast cells in the thymus increased more remarkably in MMC-treated group than in untreated tumor bearers (Fig. 3). In the tumor bearers treated with PSK, an immunomodular, however, the thymocyte mobility histogram was similar to that of normal thymocyte.

The different effect of these drugs on thymus seems to be caused by different antitumor actions. Namely, MMC has a direct action to cancer cells by the inhibition of desoxyribonucleic acid (DNA) synthesis and depressed the immunity of the host as well. However, the mechanism of antitumor activity of PSK is thought to be the enhancement of immune responses specific for tumor, by the general recovery of suppressed immunity caused by tumor burden. Tsuru *et al.*^{5,6)} reported that in tumor-bearing mice, the size, weight, cell number and area of the cortex of the thymus were reduced, and the scatter profile of thymus cells analyzed with a fluorescence activated cell sorter changed. In addition, he found intraperitoneal or oral administration of PSK prevented such modulation in the thymus of tumor-bearing mice. According to North,⁷⁾ it is said that the thymus gland is important to establish a thymus-derived lymphocyte-mononuclear phagocytic immunity in infections. For example, congenitally athymic mice defecting in the cell-mediated immunity is known to be more sensitive to bacterial and viral infections than original mice with thymus.⁸⁾ Therefore, although the antitumor activity of these drugs can be similar as shown in our experiment (Fig. 1), PSK, and immunomodulator, is thought to be better than MMC, from the viewpoint of antiinfectious activity. In fact, Nomoto *et al.* reported PSK restored antiinfectious activity in tumor-bearing host, although mitomycin-C depressed it further under the same circumstances. Namely, PSK prolonged life span, while mitomycin-C short-

FIG. 3. Electrophoretic Mobility Pattern of Thymocytes in Drug-Treated Mice 21 d after Tumor Implantation

ened it in the tumor-bearing mice with *Pseudomonas aeruginosa* infection.

From these facts, it can be said that changes in thymocyte electrophoresis predicted the effect of the drug on antiinfectious activity, and a pattern analysis of the thymocyte electrophoretic mobility histogram will be a simple method to evaluate the drug efficacy, if using fully automated instrumentation.

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