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Histopathological Studies on Antitumor Effect of Sporamycin

Cell-mediated Immunity Against Allogeneic Tumor-bearing Mice

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Summary. *Mice that had received transplants of sarcoma-180 followed by treatment with sporamycin were examined histopathologically at periodic intervals. A marked degeneration of tumor cells was observed at an early stage after the administration of sporamycin, but the degeneration subsequently ceased and regrowth of the tumor was seen. Marked infiltration of lymphoid cells, granulation tissue, and fibrosis was seen in the stroma or surrounding tissue of the tumor at a late stage after the administration of sporamycin, and the regression of tumor cells became marked. With a few exceptions the mice were completely cured by about the 40th day.*

In the peripheral lymphoid tissues, a transitory decrease in the number of cells was observed after the administration of sporamycin, but this was followed by regeneration of the cells, followed by a marked increase in the B cell system. On the other hand, lymphoid cell depletion of the thymus had persisted.

Transplantation of intact sarcoma-180 to mice preliminarily inoculated with sporamycin-treated sarcoma-180 cells resulted in inhibition of tumor growth in most of the mice, and qualitatively the same tissue reactions as those in mice cured of sarcoma-180 by sporamycin were seen.

The results suggest that enhancement both of antigenicity of the tumor (cells) and of the subsequent immune response of the host by sporamycin may be involved in the cure of the experimental tumor.

Introduction

Sporamycin is an antitumor antibiotic obtained from the culture filtrate of *Streptosporangium pseudovulgare*, strain PO-357 [6, 12], and its antitumor effect has been confirmed in various experimental animal tumors [5]. When sarcoma-180 is transplanted to mice, marked inhibition of tumor growth follows treatment with sporamycin. The tumor showed marked regression from about 20 days after the treatment [3, 6].

In the present series of investigations, the correlation between regression of the tumor in response to sporamycin and the reaction of the host's lymphoid tissue was examined histopathologically in an attempt to elucidate the mechanism of sporamycin's suppressive effect on tumor growth.

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Materials and Methods

Animals

Four-week-old male ddY mice (Shizuoka Agricultural Cooperative for Experimental Animals, Hamamatsu) weighing 20–30 g were used.

Tumor

A solid sarcoma-180 tumor supplied by the Takeda Chemical Industries, Ltd., Osaka in 1961 and subsequently serially transplanted SC in dd mice in this laboratory was transplanted SC in the left axilla of each ddY mouse by means of a trocar.

Therapeutic Test

Three days after transplantation of sarcoma-180, 5.0 mg sporamycin/kg was administered into the tail vein in a single dose and growth of the tumor was observed periodically.

Histopathological Observation

Mice were killed from day 3 after sporamycin administration onward, at 3- to 4-day intervals. Tumor tissue, thymus, spleen, lymph node, bone marrow (thoracic vertebrae), and other main

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organs were extirpated and fixed in 10% neutral buffered formalin solution. After embedding in paraffin, 4-μm sections were prepared and stained with hematoxylin and eosin for microscopical observation.

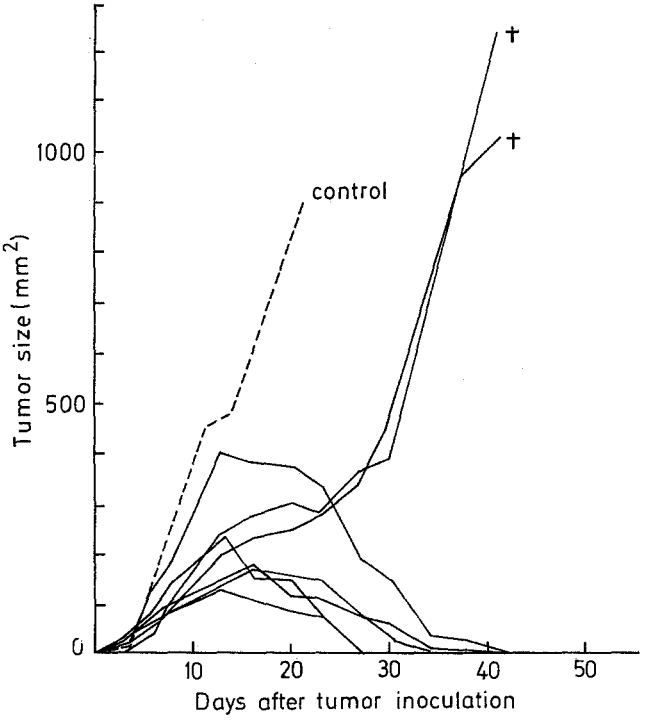

Fig. 1. Effect of sporamycin on growth of sarcoma-180

Preparation of Sarcoma-180 Tumor Cells and Presensitization with Sporamycin- or Mitomycin-treated Sarcoma-180 Cells

Sarcoma-180 tumor cells were separated into single cells by passing through a stainless steel mesh (80 mesh), washed several times with minimum essential medium (MEM), and prepared to give a suspension of 2×10^6 cells/ml in MEM. Sporamycin was added to this suspension at the rate of 100 μg/ml, and the mixture was incubated at 37° C for 90 min. The cells were then washed several times with MEM and used as the sporamycin-treated sarcoma-180 cells. As a control, 20 μg mitomycin C/ml was added to a suspension of 2×10^6 sarcoma-180 cells/ml, the mixture was allowed to stand for 2 h, and these cells were used as the mitomycin-treated sarcoma-180 cells.

The tumor cells thus obtained were transplanted SC in the left axilla of the mice at a dose of 10^6 cells/mouse, and intact sarcoma-180 cells were transplanted in a similar way in the opposite axilla 10 days later. Measurements of tumor size and histological examinations were carried out periodically.

Results

Effect of Sporamycin on Growth of Sarcoma-180

Sarcoma-180 transplanted to untreated control mice grew vigorously and killed all the mice within 30 days. In the sporamycin-treated group, the tumor showed gradual growth for 14–20 days after sporamycin administration; however, the degree of the growth rate was less than in untreated controls. After this period, consolidation and regression of the tumor became marked, and five of seven animals showed regression of the tumor growth by 40 days after sporamycin administration (Fig. 1).

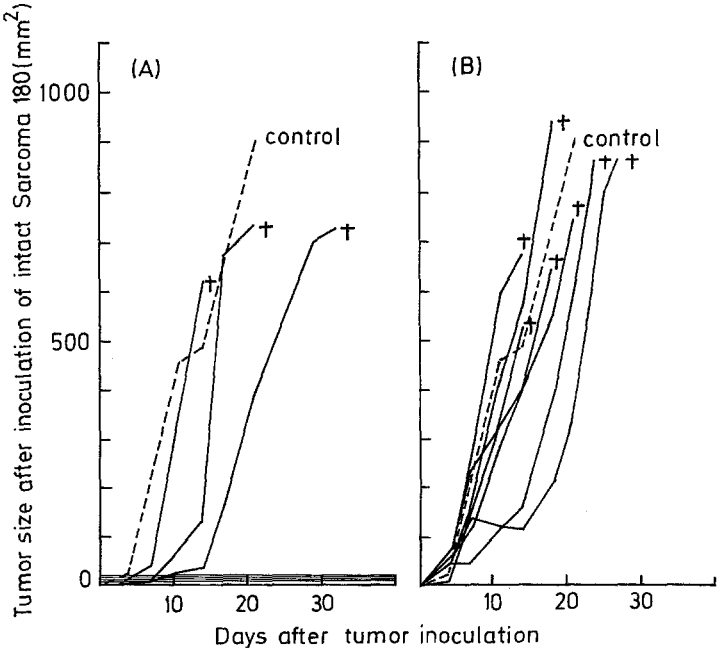

Fig. 2. Effect of inoculation with sporamycin- or mitomycin-treated S-180 cells. Mice were inoculated with sporamycin (A)- or mitomycin (B)-treated S-180 cells. After 10 days, intact S-180 cells were inoculated

Effect of Inoculation with Sporamycin- or Mitomycin-treated Sarcoma-180 Cells on Growth of Sarcoma-180

Sporamycin-treated sarcoma-180 cells were absorbed without growth in four of seven mice by about 17 days after transplantation. Intact sarcoma-180 cells did not show any growth in the mice in which sporamycin-treated sarcoma-180 cells had been absorbed (Fig. 2).

Mitomycin-treated sarcoma-180 cells were also absorbed in all the animals by about 10 days after transplantation; intact sarcoma-180 cells, however, showed marked proliferation, as in the untreated controls (Fig. 2).

Histopathological Observations

Tumor

Effect of Sporamycin on Growth of Sarcoma-180. In the untreated controls, the tumor showed marked growth with time after transplantation, and infiltration of a small number of histiocytic cells or lymphocytes was seen in the normal tissue around the tumor by 8–10 days. Thereafter, wide-spread necro-

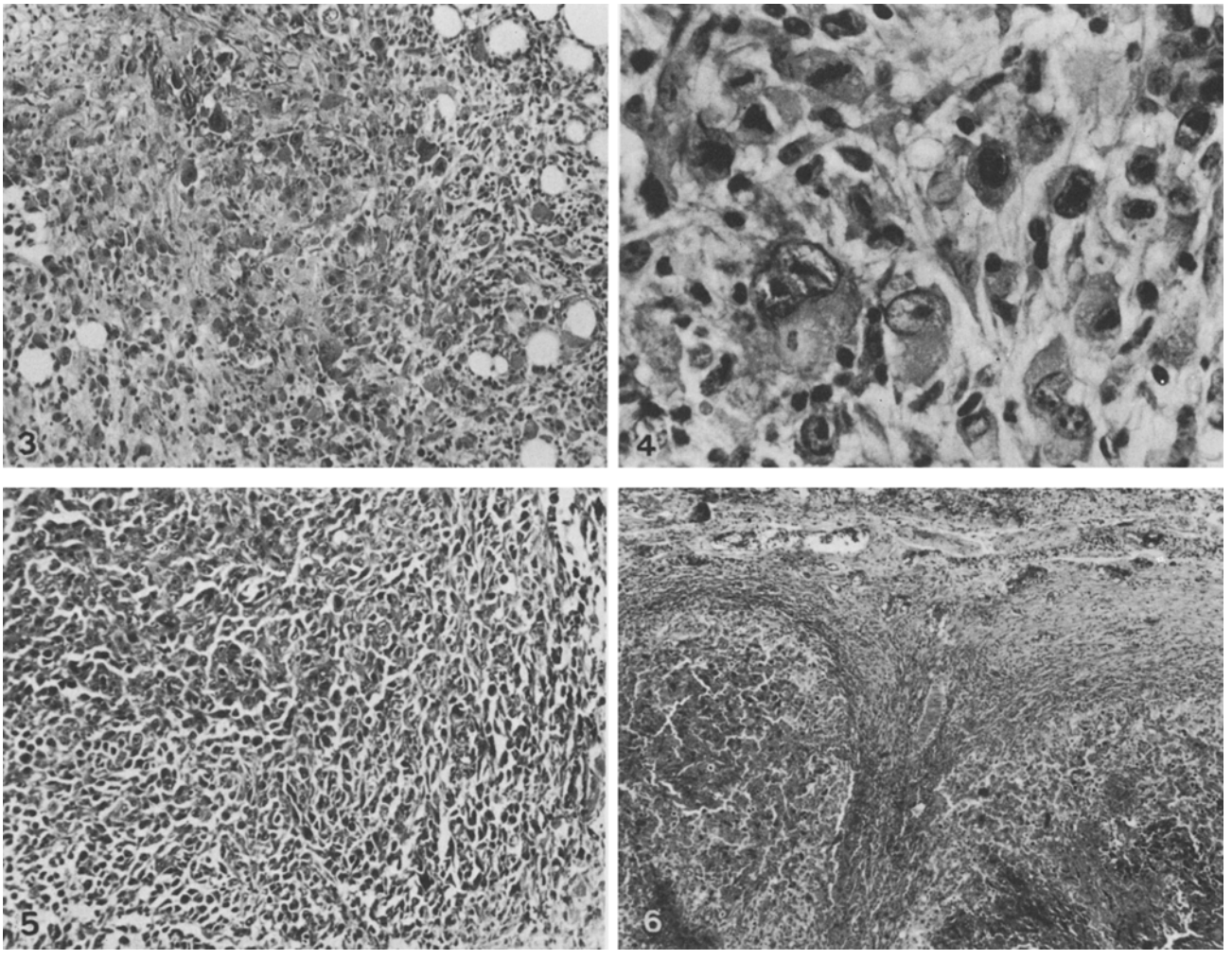

Fig. 3. Five days after treatment with 5.0 mg sporamycin/kg. Tumor cells show marked degeneration. (H and E, × 100)

Fig. 4. Higher magnification of Fig. 1. Swelling and lysis of the tumor cells. (H and E, × 400)

Fig. 5. Twelve days after treatment with 5.0 mg sporamycin/kg. Tumor cells show marked regrowth. (H and E, × 100)

Fig. 6. Twenty days after treatment with 5.0 mg sporamycin/kg. Tumor mass is encapsulated by a thick layer of newly formed granulation tissues with round cell infiltration. (H and E, × 100)

Table 1. Histopathological findings in sarcoma-180 tumor-bearing mice after treatment with sporamycin

	Days after treatment							
	3	7	12	15	18	20	30	40
Degeneration of tumor cells	3	2	0	0	1	2	2	C[a]
Round cell infiltration with formation of granulation tissue around the tumor	0	0	0	1	2	3	3	F[b]
Cell proliferation in the thymus	0	0	0	1	0	0	0	0
Cell depletion in the thymus	3	2	2	1	0	0	0	0
Cell proliferation in the spleen and lymph node[c]	0	0	1	2	3	3	3	2
Cell depletion in the spleen and lymph node[c]	3	2	0	0	0	0	0	0

Grade: All lesions graded for severity as follows: 0, absent; 1, mild; 2, moderate; 3, severe
[a] Cured
[b] Fibrosis
[c] Mesenteric lymph node

sis was noted in the central part of the tumor, and infiltrative growth of the tumor to the surrounding tissue became obvious, although there was no infiltration of inflammatory cells into the tumor tissue.

In the sporamycin-treated animals, swelling and lysis of the cytoplasm or swelling of the nucleus and nucleoli of tumor cells became marked about 5 days after sporamycin administration (Figs. 3 and 4). Some tumor cells formed binuclear or multinuclear giant cells. Seven days after sporamycin administration, tumor cells showing strong degeneration were seen in the marginal zone of markedly proliferating tumor tissue. After this period the degenerative process of the tumor cells was entirely absent, and regrowth became marked (Fig. 5).

About 18–20 days after sporamycin administration, infiltration of macrophages or lymphocytes along the border of the tumor and in its vicinity and tumor cell degeneration were seen again. Subsequently, fibrosis around the tumor progressed gradually, resulting in the encapsulation of tumor tissue by a band of dense fibrosis (Fig. 6). At the same time, lymphoid cell infiltration in the interstitial tissue became marked, and attachment of lymphocytes to the tumor cells was observed. These tumor cells became swollen and the cytoplasm appeared clear, with the presence of eosinophilic granules, but no striking change was seen in the nucleus (Fig. 7). Then tumor tissue formed a large necrotic focus and was infiltrated and demarcated by granulation tissue, with concomitant marked infiltration of monocytic cells. The tumor-bearing mice were cured by about 40 days after sporamycin administration (Table 1).

Effect of Inoculation with Sporamycin-treated Sarcoma-180. The animals that received transplants of sporamycin-treated sarcoma-180 showed the same histological features as seen in those treated with sporamycin alone (Fig. 8), although in the case of the former the time to regression of the tumor was shorter.

Lymphoid Tissue

In the animals that received sporamycin, atrophy of the thymus with lymphocyte depletion in the cortex became marked until 7 days after the administration. Depletion of lymphocytes was observed in the thymus-dependent area of the spleen and lymph node, but the lymph follicles were approximately normal. Regeneration of cortical lymphocytes was observed in the thymus at 15 days after the administration of sporamycin, and enlargement of the lymph follicles became marked in the spleen and lymph nodes (Fig. 9), with a concomitant increase in size of germinal centers. Proliferation of plasma cells became marked around the central arterioles of the spleen (Fig. 10). These changes in the spleen and lymph nodes became especially marked around 20 days after sporamycin administration, when regression of the tumor started (Table 1).

In the mice that received sporamycin-treated sarcoma-180 cells by injection the spleen and lymph node(s) showed the same proliferative reaction of the cells as in the animals that received sporamycin, but the degree of this reaction was slight. There was no marked change in the tyhmus.

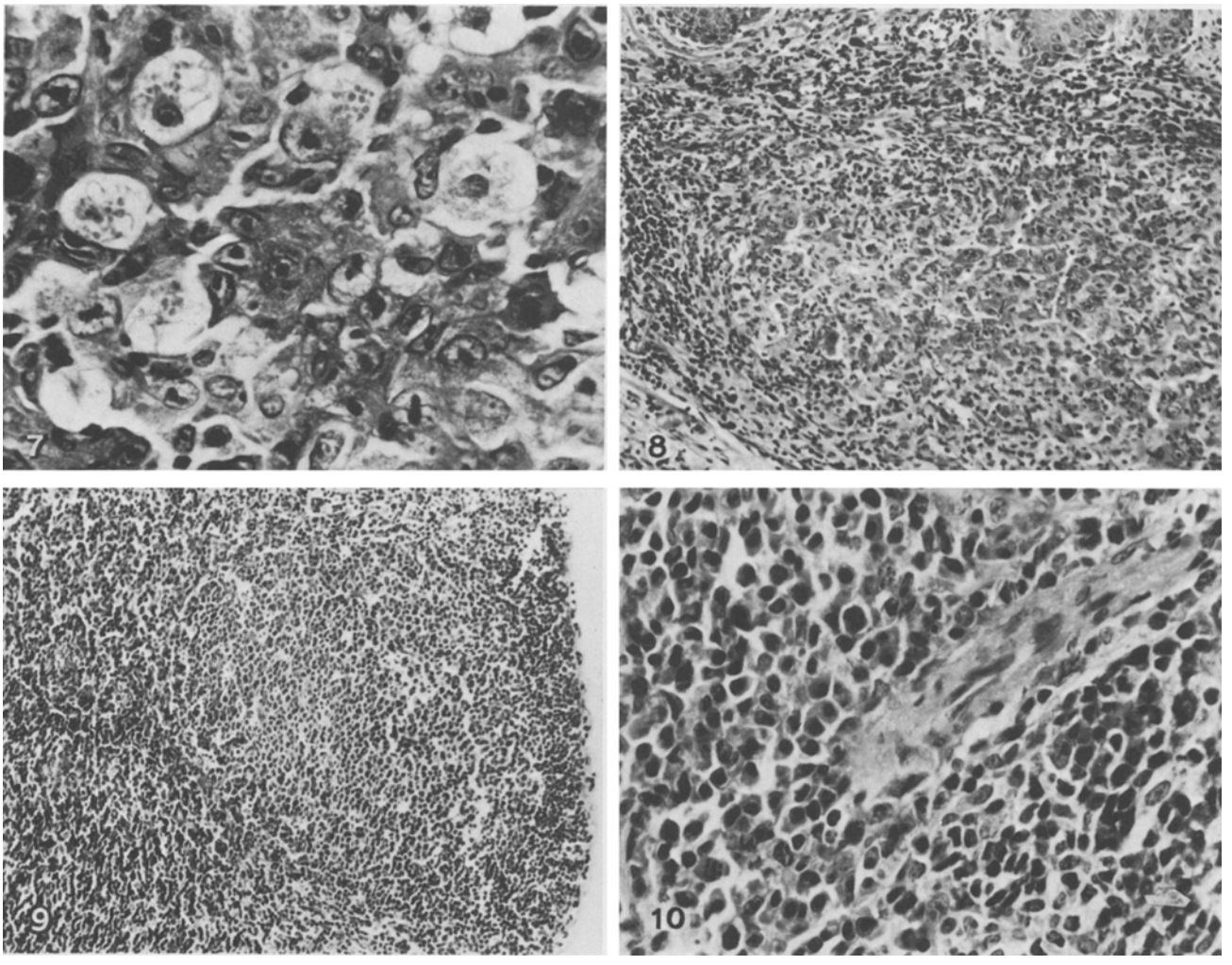

Fig. 7. Twenty days after treatment with 5.0 mg sporamycin/kg. Adsorption of lymphocytes to the tumor cells. (H and E, × 400)

Fig. 8. Four days after transplantation of intact sarcoma-180 cells, i.e., 14 experimental days after immunization with sporamycin-treated sarcoma-180. Lymphoid cell infiltration is prominent around the tumor cells. (H and E, × 100)

Fig. 9. Fifteen days after treatment with 5.0 mg sporamycin/kg. Enlargement of the germinal center is clearly seen in the lymph node. (H and E, × 100)

Fig. 10. Fifteen days after treatment with 5.0 mg sporamycin/kg. Proliferation of plasma cells is noticeable around the white pulp artery of the spleen. (H and E, × 400)

Discussion

Sporamycin causes inhibition of DNA synthesis in tumor and normal cells [6], and the antibiotic was found to disappear rapidly from the body on measurement of its levels in mouse organs [4]. Consequently, the direct action of sporamycin in the tumor is considered to cease in a relatively short time after its administration.

About 1 week after the administration of sporamycin, sarcoma-180 cells showed marked degeneration of the nucleus and cytoplasm. Thereafter, the tumor tended to proliferate. The tumor cells, however, became degenerative again about 18 days after the administration of sporamycin, accompanying a mesenchymal reaction inside the tumor and its surrounding area. Degenerative change in this period was characterized by ballooning of the cytoplasm and hardly any change in the nucleus.

Degeneration of sarcoma-180 cells appeared at a late stage of the experimental period, and the process of cure in response to this antibiotic is presumed to be

mediated by a factor other than cytotoxicity of sporamycin.

Inoculation of sporamycin-treated sarcoma-180 cells to mice prevented the growth of intact sarcoma-180 cells. This fact indicates that sporamycin may have some action modifying the quality of tumor cells in addition to its direct cytocidal action on tumor cells, like macromomycin and another high-molecular antibiotic [2].

On the other hand, peripheral lymphoid tissue of the animals showed transient atrophy in the early period after the administration of sporamycin, followed by regeneration of this tissue. The early transient atrophy of the lymphoid tissues was caused by cell depletion only in the thymus-dependent areas, but the other areas of lymphoid tissue had remained unaffected by sporamycin. Thereafter, the spleen and lymph nodes showed enlargement accompanied by plasma cell proliferation around the central arterioles in the spleen and paracortical areas or meduallary cords of the lymph node. Marked cell proliferation of the peripheral lymphoid tissue was noted in the sporamycin-treated animals, but only weak cell proliferation was found in the animals inoculated with sporamycin-treated sarcoma-180 cells. This difference between the two groups could be caused by the presence vs absence of a direct effect of sporamycin on the lymphoid tissue. This is thought to be characteristic of sporamycin as against other antitumor agents, most of which finally induce almost complete destruction of the whole hematopoietic system. Therefore, sporamycin seems to act to enhance the immune response of the host.

Following the combined use of sporamycin with BCG, as an adjuvant that is considered to enhance the immune response and especially cell-mediated cytotoxicity when inoculated IV [8], a marked proliferative cell reaction was observed in the lymph nodes or spleen, suggesting a synergistic action of these two agents resulting in a more marked antitumor effect [3]. It is worthy of note that remarkable atrophy of the thymus delayed cell regeneration and there was marked cell proliferation in the peripheral lymphoid tissue in the sporamycin-treated mice. These phenomena are quite similar to the proliferation of the B cells in the absence of suppressor T cells in man or experimental animals [1, 9–11]. These findings suggest that the cell-mediated immune response could be induced by treatment with sporamycin. In fact, we observed that the neutralization activity (Winn's method) against syngeneic tumor cells was more marked following pretreatment with sporamycin than in the nontreated group [7].

The results suggest the presence of an antitumor effect involving an immunological mechanism besides sporamycin's direct action on the tumor.

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