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Tumor growth promoting activity of an immunosuppressive substance and its modulation by protein-bound polysaccharide PSK

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1. Summary

The role of an immunosuppressive substance (IS), which is increased in the serum of tumor-bearing animals, was examined in rats. IS isolated from cancerous ascites fluid of rats was administered to Walker 256 tumor-bearing rats to examine changes in the serum level of IS, tumor growth and survival rate. PSK, an immunomodulator, was also administered. Serum IS increased with tumor growth. The administration of IS to tumor-transplanted rats caused the tumor to grow and shortened the animals' survival time. The administration of PSK, however, inhibited the increase in serum level of IS, resulting in the suppression of tumor growth and a prolongation of survival time.

The findings suggested that IS is a useful parameter for predicting not only tumor growth but also the therapeutic effect of immunomodulators such as PSK.

2. Introduction

Cellular immunity is known to be reduced in the tumor-bearing body. The mechanism of such a reduction is not clear; it may be caused by substances produced or induced by cancer cells. Some studies [1, 2, 3] have demonstrated the presence in the serum of patients with advanced cancer of factors which non-specifically inhibit in vitro and in vivo lymphocyte function. Recently we have isolated an immunosuppressive glycoprotein (IS) from ascitic fluid of patients with advanced gastrointestinal cancer [4], and the serum levels of IS were found to be higher than those in normal serum [5, 6, 7]. Although the origin of IS has not been clarified, it was speculated that IS is produced or induced by tumor cells, and excreted into body fluid to influence host immunity. PSK, a protein-bound polysaccharide derived from Basidiomycetes, is an immunomodulator which is presently being used clinically in Japan. It has been demonstrated that PSK produces antitumor activity against some tumors by restoring the depressed delayed-type hypersensitivity reactions [8] or depressed antibody production in tumor bearing animals [9].

In this study we isolated IS from the ascitic fluid of tumor-bearing rats and examined its effect on the growth of tumors in rats. We also examined the effects of PSK on host immunity and tumor growth.

Key words: IS (immunosuppressive substance); PSK; Walker 256; Cellular immunity

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3. Materials and Methods

3.1. *Preparation of IS*

IS was prepared from the ascitic fluid of Donryu

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rats transplanted with Yoshida sarcoma as reported previously [4]. Briefly, ascitic fluid was saturated to 60% with ammonium sulfate at pH 7.0 and kept for 10 h at 4 °C. The supernatant was dialyzed against distilled water and lyophilized. Preparative isoelectric focusing was performed at pH 2.5 to 6.0. Fractions from pH 2.7 to 3.3 were collected and combined and the residual ampholyte was removed by dialysis and Sephadex G-50 filtration.

3.2. *Analysis of IS by electrophoresis*

SDS polyacrylamide electrophoresis was carried out according to the method of Weber and Osborm [10] on 10–20% gradient polyacrylamide slab gel. The electrophoresis was run at 60 mA constant current for 2 h at room temperature. Gels were stained with 0.025% Coomassie Brilliant Blue R-250.

3.3. *Detection of serum IS*

A specific anti-IS antibody was produced by the immunization of IS against rabbits. To check the specificity of the antibody, immunoelectrophoresis (at pH 8.6) was done by the method of Grabar and Williams [11]. IS in serum was quantified by the single radial immunodiffusion (SRID) method as described by Mancini et al. [12] using this anti-IS antiserum.

3.4. *Animals and tumors*

Eight-week-old Wistar-Imamichi rats weighing about 200 g were obtained from the Imamichi Institute for Animal Reproduction (Omiya, Japan) and kept for at least 10 days prior to the experiments. Walker 256 carcinoma 5-mm blocks were inoculated into the backs of experimental animals (10 animals/group).

3.5. *Treatment of IS and PSK*

IS (5 mg) was suspended in 0.5 ml of sterile phosphate buffer saline, and injected intravenously (i.v.) on days 1, 3 and 5 after inoculation. PSK was orally administered fourteen times every day at a dose of 1000 mg/kg from the day of the inoculation.

3.6. *Assay of suppressive activity*

The immunosuppressive activity of rat IS was evaluated by the amount of [^{3}H]thymidine incorporation in response to Con A stimulation of rat spleen cells. Spleens removed from rats were minced, filtered through a 200 stainless steel mesh and washed 3 times with RPMI 1640 medium. The cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum, L-glutamine and antibiotics. A 100-μl spleen cell suspension (1×10^5 cells) was placed in the wells of a 96-well Falcon microplate containing 100 μl of medium and 1 μg of Con A (type IV, Sigma Chemical Co.). All cultures were done in triplicate. The plates were incubated for 72 h and 1 μCi of [^{3}H]thymidine (Becton Dickinson Co.) was added to each well. The cells were further cultured for 6 h and harvested. The incorporation of [^{3}H]thymidine into DNA was measured by a liquid scintillation counter.

4. Results

4.1. *Properties of IS*

IS showed a single band and its molecular weight was about 43000 (Fig. 1). The immunosuppressive effect of IS on [^{3}H]thymidine incorporation in response to Con A stimulation of rat spleen cells was determined in the presence of various concentrations of IS. As shown in Fig. 2, [^{3}H]thymidine incorporation decreased as the concentration of IS increased.

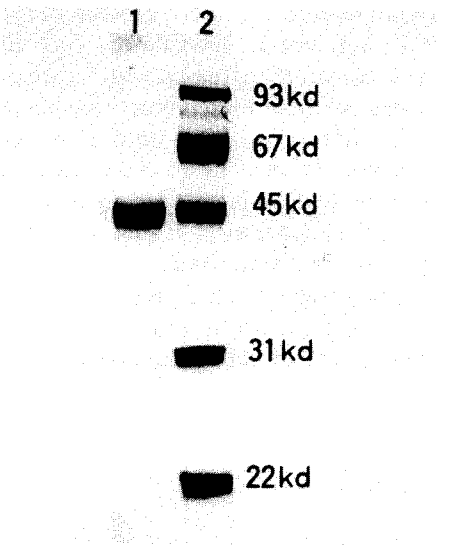

Fig. 1. Polyacrylamide gradient gel electrophoresis. (1) Rat IS; (2) marker proteins: phosphorylase b (92500); bovine serum albumin (66200); ovalbumin (45000); carbonic anhydrase (31000); soybean trypsin inhibitor (21500).

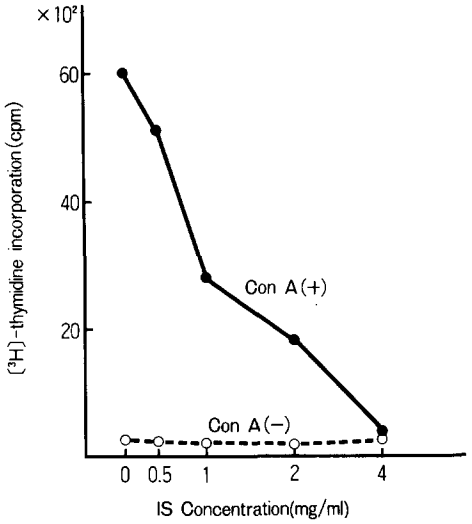

Fig. 2. Inhibitory effect of rat IS on spleen cell response to Con A. Data are presented as the mean cpm ($n = 3$).

The suppression was approximately 50% at 1 mg/ml and 100% at 4 mg/ml of IS.

4.2. *Serum levels of IS in normal and cancer-bearing rats*

Anti-IS antiserum from rabbit showed a single line of precipitation in immunoelectrophoresis versus the serum of Walker 256 tumor bearing rats (Fig. 3). With SRID using this anti-IS antiserum, the calibration curve for purified IS was almost linear at concentrations between 50 and 1500 μg/ml (data not shown).

Serum levels of IS in normal and Walker 256 tumor-transplanted rats are shown in Fig. 6. IS levels in normal rats (265 ± 74 μg/ml) were similar to those in tumor-bearing rats (271 ± 54 μg/ml) 4 days after tumor transplantation. On day 10 after transplantation, when the tumor became visible, the serum levels of IS in tumor-bearing rats had increased significantly ($P<0.01$), to 594 ± 113 μg/ml. The animals that survived until the 14th day had slightly higher serum levels of IS (747 ± 198 μg/ml).

4.3. *Effects of IS and PSK on tumor growth survival time*

Fig. 4 shows the size of tumors at various times after their transplantation into the backs of rats. Tumor sizes in the experimental group treated with IS and the control group began to show a difference on the 5th day after transplantation, and were significantly different ($P<0.01$) on the 15th day.

The duration of survival changed similarly in the control group: nearly half of animals survived until

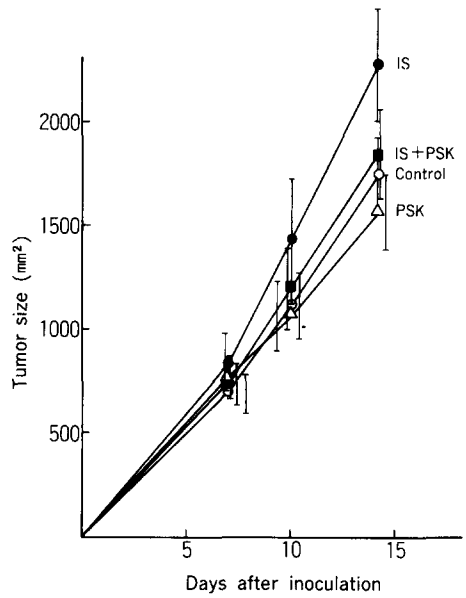

Fig. 4. Effect of IS and PSK on tumor growth in rats inoculated with Walker 256. Each group contained ten animals.

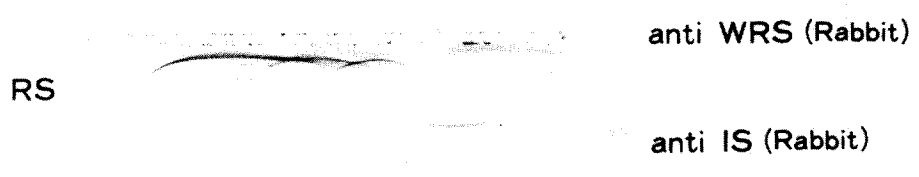

Fig. 3. Immunoelectrophoretic analysis of anti-IS antibody. Well contained the serum of Walker 256-bearing rat. Upper trough contained antiserum against rat whole serum, and lower trough contained antiserum against purified rat IS.

the 16th day but all died by 21st day, whereas in the experimental group about half the animals survived until the 11th day, but all died by the 17th day (Fig. 5).

The administration of PSK caused the mean tumor size to decrease slightly and the duration of survival to be prolonged in comparison with control group. In tumor-bearing animals treated with IS, the administration of PSK significantly decreased the tumor size and prolonged the survival rate.

4.4. *Serum levels of IS in tumor-bearing rats treated with IS and PSK*

Serum levels of IS in tumor bearing rats treated with IS and PSK were measured every 2nd day after transplantation. As shown in Fig. 6, in the control (untreated group), no change was observed in the IS level until the 7th day, whereas on the 8–10th days when the tumors began to grow, the level started increasing rapidly, being over 700 μg/ml on the 14th day. In the group treated with PSK, the serum level of IS increased slowly. It then reached a peak value of over 700 μg/ml by the 20th day, when death occurred. In the group intravenously treated with IS, the rate of increase in the level of IS was faster than that in the control group.

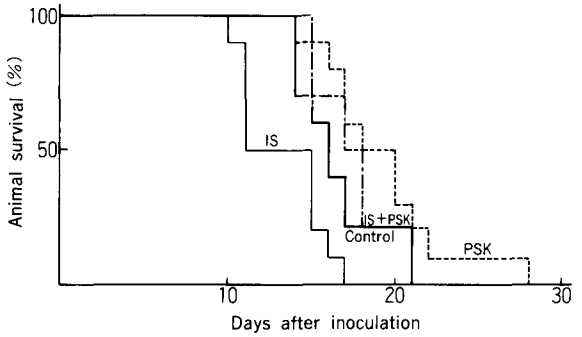

Fig. 5. Effect of IS and PSK on the survival of rats inoculated with Walker 256.

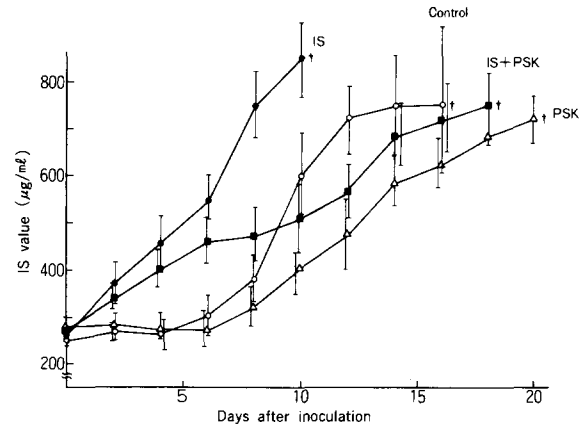

Fig. 6. Serum IS levels in Walker 256-transplanted rats treated with IS and PSK.

5. Discussion

Some studies [13, 14] have demonstrated the presence of immunosuppressive factors in serum and cell free ascitic fluid of tumor-bearing animals. We isolated an immunosuppressive glycoprotein from ascitic fluid of tumor bearing rats and found that the molecular weight of this glycoprotein was about 43000 and that it suppressed [^{3}H]thymidine incorporation into Con A-stimulated spleen cells. The molecular weight of this rat IS was different from the value (52000) for human IS obtained from ascitic fluid of cancer patients [4], but the level of immunosuppressive activity of rat IS was the same as that of human IS. Further studies on the physicochemical and biochemical analysis of rat IS are needed.

The present study demonstrated that serum level of rat IS increased with tumor growth and that its intravenous administration caused the transplanted tumor to develop rapidly, resulting in a remarkably shortened survival time in tumor-bearing animals. These findings suggest that IS is produced by cancer or other cells, and that its increase in the body fluid decreases the host's immunological competence, thus allowing tumor cells to proliferate. The origin of IS remains to be clarified. Recently Hamada et al. [15] reported the presence of IS produced by the leukocytes of cancer patients. As to the role of IS, it has been shown to inhibit natural killer cell activity and interferon production [16]. IS may influence cellular immunity by means of macrophages; by acting on macrophages, it may cause PGE to be produced, thus leading to a decrease in lymphocyte activity and suppression of IFN production.

Some studies [17–20] demonstrated that PSK enhanced cellular immunity and inhibited the suppres-

sion of IFN production in tumor-bearing mice. Thus, it is possible that PSK exerts its anticancer effect by normalizing the host's decreased cellular immunity resulting from tumor-bearing. The present study suggests that PSK inhibited the production of IS, which is increased in tumor-bearing animals, thus controlling tumor growth and leading to a prolongation of survival time.

Further studies are required to clarify the origin of IS and its function in the body. For that purpose, the experimental model used in the present study is useful. Serum levels of IS may serve as a parameter to monitor the efficacy of immunotherapy.

References

[1] Glasgow, A. H., Nimberg, R. B., Menzojan, J. O., Saporoschetz, I., Cooperband, S. R., Schmid, K. and Mannick, J. A. (1974) N. Engl. J. Med. 291, 1263.

[2] Urushizaki, I., Ishitani, K., Nagai, T., Gocho, Y. and Koyama, R. (1977) Gann 68, 413.

[3] Hanna, H., Ovadia, J. and Nelken, D. (1978) Immunology 43, 1007.

[4] Fujii, M., Takahashi, N., Hayashi, H., Matsunaga, K. and Yoshikumi, C. (1987) Clin. Biochem. 20, in press.

[5] Mannami, T., Fuchimoto, S., Amamiya, S., Morioka, S., Tanaka, S., Tanaka, K., Onaga, E. and Orita, K. (1982) Jpn. J. Cancer Chemother. 9, 2104.

[6] Noguchi, H., Shiozawa, I., Sakai, Y., Ishi, J., Suzuki, A. and Tsukahara, Y. (1983) Acta Obst. Gynaec. Jpn. 35, 1931.

[7] Yamashita, K., Hayashi, H., Mure, K., Ishikawa, M. and Shimizu, T. (1986) Cancer 57, 69.

[8] Nomoto, K. (1981) Jpn. J. Clin. Med. 39, 1863.

[9] Nomoto, K., Yoshikumi, C., Matsunaga, K., Fujii, T. and Takeya, K. (1975) Gann 66, 365.

[10] Weber, K. and Osborm, M. (1969) J. Biol. Chem. 244, 4406.

[11] Grabar, P. and Williams, C. A. (1953) Biochim. Biophys. Acta 10, 193.

[12] Mancini, G., Carbonara, A. O. and Heremans, J. F. (1965) Immunochemistry 2, 235.

[13] Yamazaki, K. and Umezawa, H. (1973) Gann 64, 83.

[14] Badger, A. M., Cooperband, S. R. and Mannick, J. A. (1974) Fed. Proc. 33, 749.

[15] Hamada, S., Fuchimoto, S., Onishi, K., Kamiya, S., Naramoto, Y., Houra, R., Yonehana, T. and Orita, K. (1984) Proc. Jpn. Cancer Assoc., 109.

[16] Hamada, S., Fuchimoto, S., Itano, S., Kimura, T., Kamiya, S., Naramoto, Y., Akura, Y., Tanaka, K. and Orita, K. (1985) Proc. Jpn. Cancer Association, 105.

[17] Tsukagoshi, S., Hashimoto, Y., Fujii, G., Kobayashi, H., Nomoto, K. and Orita, K. (1984) Cancer Treat. Reviews 11, 13.

[18] Tsuru, S. and Nomoto, K. (1983) J. Clin. Lab. Immunol. 4, 215.

[19] Kitani, H., Tsuru, S., Oguchi, M., Watanabe, M. and Zinnaka, Y. (1984) J. Clin. Lab. Immunol. 5, 211.

[20] Ebina, T. (1981) Tampakushitsu Kakusan Koso (suppl.), 81.