

## Inhibitory Effect of Bacterial Infection on Tumor Growth

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This study is undertaken to ascertain whether concurrent infection in a tumor-bearing host exhibits an inhibition of tumor cell growth.

- 1) Infection of  $\beta$ -streptococcus origin has led to a reduction of transplanted methylcholantren (MC) tumor cell growth. Survival time after  $1 \times 10^6$  Ehrlich tumor cell transplantation was much more elongated with infection induced by  $\beta$ -streptococcus administration 5 days before tumor cell transplantation. As a route of infection to enhance the effects on an inhibition of tumor cell growth, the subcutaneous (SC) route is more prominent than the intraperitoneal (IP) one.
- 2) Phagocytic activity of the reticuloendothelial system is enhanced by an infection of  $\beta$ -streptococcus origin and also SC route for induction of infection is more prominent to evolve it than IP one.
- 3) Immunologic host response to the macrophage migration inhibition (MMI) and plaque forming cell (PFC) tests are apparently facilitated by  $\beta$ -streptococcus origin infection.

In conclusion, it has become clear that a preceding infection in tumor-bearing host effectively exhibits the antitumor activity.

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## INTRODUCTION

It is well known that microorganism at times plays an important role in reducing a malignant tumor sizes in concurrent tumor bearing host.<sup>1)</sup> Of interest is the fact that concomitant infection in tumor-bearing host may lead to inhibition of the tumor cell growth. The objective of this report is experimentally to clarify as to whether or not concurrent infection in a tumor-bearing host may cause the tumor regression.

## MATERIAL AND METHOD

Animals and cells: C<sub>3</sub>H mice, 6 weeks of age, were kindly supplied from Shionogi Research Laboratory. Ehrlich tumor cells supplied by Shionogi Research Laboratory were chosen as an inoculated tumor cell source.  $\beta$ -streptococcal microorganism was used for a concomitant infection origin. Tumor-bearing host: Ehrlich tumor cells were intraperitoneally inoculated in mice and  $1 \times 10^6$  Ehrlich tumor cells were used. Meanwhile,  $1 \times 1 \times 1$  cm Methylcholantren tumor (MC-tumor) was also used as a transplantable solid tumor. Inoculation of  $\beta$ -streptococcus: Microorganism of  $\beta$ -streptococcus were experimentally given in mice according to the following ways,  $1 \times 10^6$ ,  $3 \times 10^7$ ,  $6 \times 10^7$ ,  $1 \times 10^8$  and  $2 \times 10^8$   $\beta$ -streptococcus inoculations via intraperitoneal (IP) or subcutaneous (SC) routes on the same day with or on day 5 prior to tumor cell transplantation to assess a proper condition referable to the numbers of tumor cells and microbes in terms of survival time and growing tumor weight. Inoculation of BCG vaccine: 1mg BCG vaccine purified by Japan BCG production co. was used by dilution with saline in this study. Reticuloendothelial function test: 0.2ml of 1% Congo-red was injected from the vein in the tail of mouse and 0.1ml blood samples from the amputated extremities were taken 5 and 30min after giving congo-red dye. The color of the blood samples hemolysed with 0.83 NH<sub>4</sub>Cl was read in a spectrophotometer at 540m and corrected granuloplectic index ( $\alpha$ ) was calculated as the following equation.<sup>2)</sup>  $\alpha = \frac{W}{W_{L+S}} \cdot 3K$ , W: body weight, W<sub>L+S</sub>: liver and spleen weight, K: granuloplectic index. Macrophage migration inhibition test (MMI-test): The macrophage, which was assembled with a 20ml liquid paraffin intraperitoneally given prior to 4 days, was collected as a suspension with Eagle' solution.

MC-tumor antigen was prepared by homogenized tumor tissue in Eagle' solution.

MMI-test was carried out according to Revillard' method.<sup>3)</sup> The macrophage

migration area from the capillary tube was measured on the photopicture and calculated in a magnified ratio of a measured diameter of the capillary tube to an actual one.

Plaque forming cell test :  $1.5 \times 10^8$  sheep red cells were injected to a mouse. On day 4, splenectomy was done and 0.05ml of 50% spleen cells were mixed with 0.4ml of  $1 \times 10^6$ /ml sheep red cells. These were incubated with a 0.05ml guinea pig complement at  $37^\circ\text{C}$  for 1 hour. Plaque forming cells were counted by using a light microscope according to Cunningham' method.<sup>4)</sup>

## RESULT

Survival periods of mice with a  $1 \times 10^6$  Ehrlich tumor cell IP transplantation were compared among various conditions of the timing and the dose of streptococcal infection as shown in Fig. 1.

The longest survival was obtained at the time when  $3 \times 10^7$   $\beta$ -streptococcal infection preceded at 5 days before tumor cell transplantation with an average of 24.4 days.

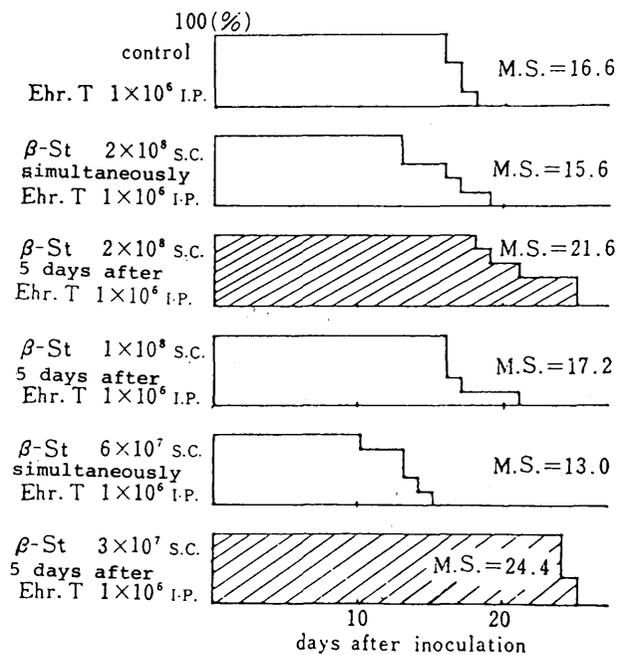


Fig. 1. Survival times after  $1 \times 10^6$  Ehrlich tumor cell transplantation with various conditions of additional streptococcal infection.

Next, the survival time of IP administration of  $2 \times 10^8$   $\beta$ -streptococcus followed with an average of 21.6 days. However, the shortest survival was gained from the mice receiving a  $6 \times 10^7$  IP administration of  $\beta$ -streptococcus with Erhlich tumor cell inoculation at the same time, averaging 13.0 days. It was shorter than that of the control.

Phagocytic activity in the reticuloendothelial system was tested after either  $2 \times 10^7$  IP  $1 \times 10^8$  SC administrations of  $\beta$ -streptococcus in mice. Fig. 2 showed that phagocytic activity of reticuloendothelial system was apparently enhanced on the 5th or 7th day following a subsequent infection of  $\beta$ -streptococcus administration. The SC administration of  $\beta$ -streptococcus was much more effective than IP one to promote the reticuloendothelial system function on phagocytosis against microorganism invasion and it lasted for 15 days, whereas in the IP administration it persisted for 5 to 7 days.

MC tumor transplantation were compared between with and without the pretreatment of BCG and/or  $\beta$ -streptococcus SC administrations 5 days prior to tumor transplantation by weighing on day 5, 10, 15 and 25 respectively as shown in Fig. 3. The rates of the tumor growth in non-pretreatment before tumor transplantation were manifest on day 10 and 15 as compared with those in the pretreatment with BCG or  $\beta$ -streptococcus. To make sure of immunological mechanism as to whether  $\beta$ -streptococcus administration inhibits tumor growth, MMI test was utilized. Fig. 4 revealed that macrophage migration of  $\beta$ -streptococcus SC administration was markedly inhibited on day 5 to 10 as compared with those of non-pretreatment and/or  $\beta$ -streptococcus IP administration although not so

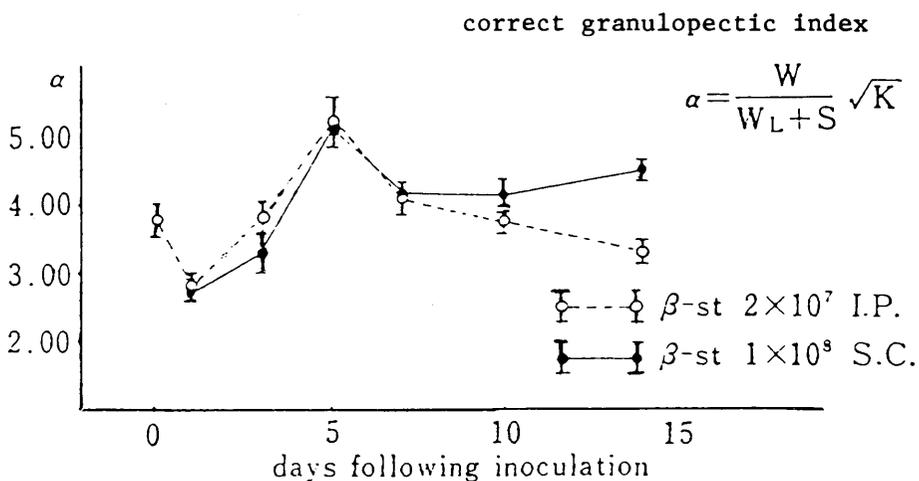


Fig. 2. Phagocytic activity of the reticuloendothelial system following  $2 \times 10^7$  IP or  $1 \times 10^8$  SC streptococcal inoculations.

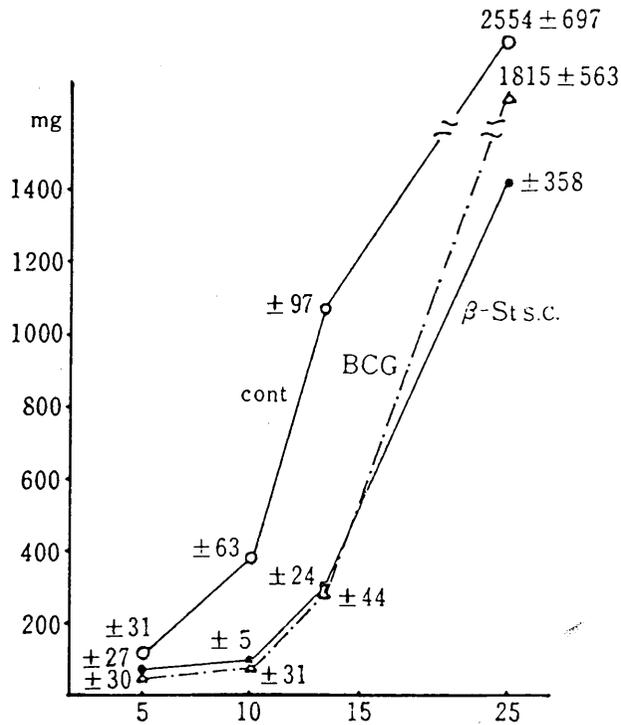


Fig. 3. Comparison in the growing transplanted tumor weight of  $1 \times 1 \times 1$  cm MC-tumor between BCG inoculation and  $\beta$ -streptococcal administration.

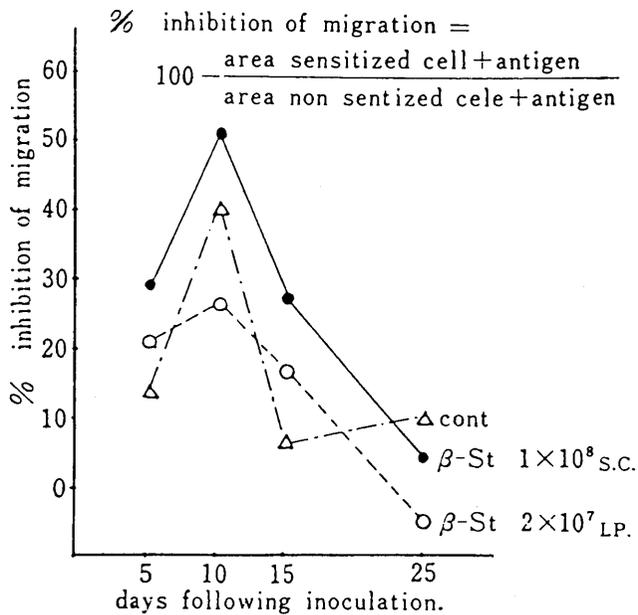


Fig. 4. Changes in percent inhibition of macrophage migration in comparison between  $\beta$ -streptococcal SC and IP administrations.

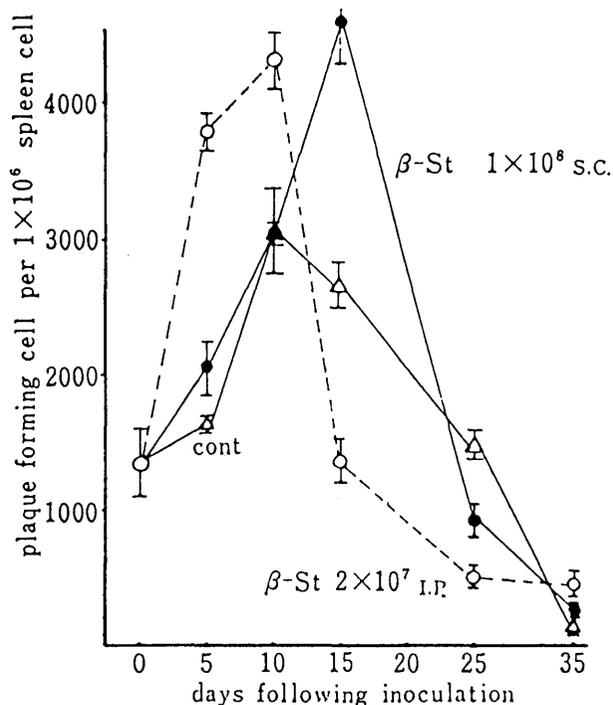


Fig. 5. Changes in the number of plaque forming cells in comparison between  $\beta$ -streptococcal infection via SC and IP routes.

on day 15.

PFC test against  $1 \times 10^6$  spleen cells reported by Jerne was also used for evaluation of a host response to tumor growth at  $1 \times 10^8$  SC and  $2 \times 10^7$  IP administrations of  $\beta$ -streptococcus 5 days prior to a  $3 \times 3 \times 3$  mm methylcholantren tumor transplantation.

Fig. 5 indicated that the plaque forming cells increased on day 5 to 10 after  $\beta$ -streptococcus IP administration although it continued up to 20 days after SC administration. It was a reflection of the different routes of infection induced by  $\beta$ -streptococcus.

## DISCUSSION

It is now evident that concomitant infection in tumor-bearing host occasionally inhibits the tumor cell growth. We experimentally attempted to elucidate the fact that an infection by  $\beta$ -streptococcus origin is effectively inhibit the transplanted methylcholantren and Ehrlich tumor cell growth or enhance the immunologic response to a host. HAVAS<sup>5)</sup> explained that the mechanism is based on a modified Schwartzman reaction.

The tumor-growth inhibitory effect in Ehrlich-tumor-bearing mice was presented as being a maximum in case of preexisting  $\beta$ -streptococcal infection. This result means that infection exerts on facilitation of a host-mediated immunologic response and associates with the existing duration and/or its severity.

This also is in excellent agreement with promotion of phagocytic activity in the reticuloendothelia system. It, therefore, is clear that the host-mediated tumor cell growth inhibition is due partly to phagocytic activation of the reticuloendothelial system.  $\beta$ -streptococcal infection via SC rather than IP administration potentiates a clearance activity of the endothelial system against a foreign body of Congo-red dye.

A  $3 \times 3 \times 3$ mm MC tumor implanted to a mouse grows with time. Of interest is the fact that concurrent infection induced by SC administration of  $1 \times 10^8$   $\beta$ -streptococcus 5 days prior to MC tumor transplantation exhibits a striking inhibition of tumor cell growth during a period of 10 to 15 days. The average weight of the tumor was reduced by 25% on day 10 and 27% on day 15. These experimental results indicate that simultaneous infection in tumor-bearing host benefits from tumor growth reduction. Furthermore, the route of  $\beta$ -streptococcus administration is closely associated with the effect on tumor-growth inhibition. It is apparent that SC route offers the greater effect rather than IP route.

It is our conviction on the basis of a result in the present study that coexisting infection in tumor bearing host helps a reduction of tumor cell growth. However, the severity and duration of infection are more likely to affect a host response to an inhibition of tumor cell growth. Our hypothesis is that mild and long standing infection is essential to depress a tumor growth.

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