

Induction of Antiparasite Activity by Pine Cone Lignin-Related Substances

MASAFUMI ABE¹, KENICHI OKAMOTO¹, KUNIO KONNO² and HIROSHI SAKAGAMI²

¹Department of Medical Biology and ²First Department of Biochemistry, School of Medicine, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan

Abstract. Pretreatment with two distinct lignin-related antitumor substances extracted from pine cone of *Pinus parviflora* Sieb. et Zucc. protected infant mice from *Hymenolepis nana* (Cestoda) infection. Subcutaneous administration of these fractions (10 mg/kg) to 1 week old mice evoked strong protective immunity against oral infection by *Hymenolepis nana* eggs. Significant antiparasite effects were also induced in 4 week old mice by intraperitoneal or oral administration of these fractions. These fractions had more potent antiparasite activity than pine cone extracts with lower antitumor and antiviral activity, and various polysaccharides derived from plants and bacteria.

Oral intake of a water soluble extract from the cones of the pine tree, *Pinus parviflora* Sieb. et Zucc., is a popular practice on the Kyushu island of Japan, because of its legendary antitumor potential described in folklore. Partial purification of 10 different fractions of pine cone extracts revealed that the 10 kD Fr. VI and the 70-200 kD Fr. VII of NaOH extract induced the most potent antitumor activity in mice (1). We previously reported that these fractions stimulated the production of differentiation-inducing factor(s) by macrophages (1), granulocytic cell iodination (2) and splenocyte proliferation (3). These fractions also induced antimicrobial activity in mice (4), and inhibited the proliferation of various viruses including herpes simplex virus (5, 6), influenza virus (7), and human immunodeficiency virus (8). We have tentatively concluded that the active principle of Fr. VI or Fr. VII might be complex(es) of lignin-like polyphenolic skeleton and hydrophilic components such as sugars or polysaccharides (9).

Correspondence to: Dr. Masafumi Abe, Department of Medical Biology, School of Medicine, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan.

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There are several reports of effects of antitumor agents on experimental parasitic infection (10-12). In contrast to the investigation of the antiproliferative activity of agents, such as Acivicin (13), against *Crithidia fasciculata* (Protozoa), there has been no detailed study of the antiparasite activity of the antitumor polysaccharides against other parasitic infection. Therefore, we investigated possible antiparasite activity of the pine cone antitumor substances (Frs. VI and VII) against *Hymenolepis nana*. We report here that pretreatment with these fractions effectively inhibited the growth of larvae of this parasite (Cestoda).

Materials and Methods

Materials. PSK, a protein-bound polysaccharide prepared from the mycelium of a CM-101 strain of *Coriolus versicolor* (14), was kindly provided by Kureha Chem. Ind. Co. Ltd., Tokyo, Japan. Lower molecular weight Schizophyllan (15) was kindly provided by Dr. N. Komatsu. TAK, a glucan purified from *Alcaligenes faecalis* var. *myxogenes* IFO 13140 (16), and the carboxymethylglucan of TAK (CM-TAK)(17), were kindly provided by Takeda Chem. Ind. Ltd., Osaka, Japan.

Mice. ICR mice (1 and 4 weeks old) were obtained from Nippon Bio-Supp. Center Co. Ltd., Tokyo Japan.

Isolation of antitumor substances. Antitumor substances (Frs. VI (10 kD) and VII (70-200 kD)) were obtained by acid- and ethanol precipitation from NaOH extract of the pine cone of *Pinus parviflora* Sieb. et Zucc. after extensive washing with ethanol and hot-water as described previously (1).

Assay for antiparasite activity. Mice (6 mice per group) were pretreated once with saline (control) or one of the test samples (10 or 50 mg/kg) 1 week before oral administration of shell- removed eggs of *Hymenolepis nana* (Cestoda). Selection of the 10 mg/kg dose was based on prior data from investigations into antitumor activity (1). The antiparasite effect of *Hymenolepis nana* infection was then determined by counting, under light microscopy, the number of cysticercoids (larvae) grown in the intestinal villi 4 days after infection. The antiparasite activity of each test sample was determined by the following formula:

$$\% \text{ inhibition} = \left(1 - \frac{\text{No. of cysticercoids (tested)}}{\text{No. of cysticercoids (control)}} \right) \times 100 (\%)$$