

# Induction of Antimicrobial Activity by Antitumor Substances from Pine Cone Extract of *Pinus Parviflora* Sieb. et Zucc.

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**Abstract.** Pretreatment with two distinct antitumor substances extracted from pine cone of *Pinus parviflora* Sieb. et Zucc. protected mice from the lethal effects of *E. coli* infection. Intraperitoneal administration of these fractions transiently induced differentiation-inducing factor (DIF) with a peak at 1-2 hr. The rapid decay of DIF activity from the peritoneal cavity was followed by polymorphonuclear cell (PMN) accumulation and enhancement of superoxide generation (assayed with luminol-dependent chemiluminescence (LDCL)) by peritoneal exudate cells. The superoxide generation by adherent cells was similarly enhanced by pretreatment, but was only 10-20% of that of the peritoneal exudate cells. Fractions that showed comparable antitumor/antimicrobial activity were also obtained from seed shells and cones of other pine trees of Japanese and foreign origin. On the other hand, a neutral polysaccharide fraction from *Pinus parviflora* Sieb. et Zucc. that lacked any of these activities did not induce PMN accumulation, DIF activity or LDCL generation. The results suggest a significant role in PMN activation for the expression of antimicrobial activity induced by pine cone extracts.

We have reported previously that the pine cone extract of *Pinus parviflora* Sieb. et Zucc., which has been used as a Japanese traditional home medicine for gastric cancer, contains differentiation-inducing substance(s) against human leukemic cell lines (1), and antitumor substance(s) against transplanted tumor cells (2). Partial purification of 10 different polysaccharide fractions revealed that the 10 kD Fr. VI and 70-200 kD Fr. VII of NaOH extract had the most potent antitumor activity (2). However, there was a question whether or not similar antitumor substances exist in other kinds of pine trees grown in Japan or other countries. We thus investigated

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the geographical distribution of origins of antitumor substances extracted from cones and seed shells of various pine trees.

We have previously reported the immunopotentiating activities of these fractions which induced production of differentiation-inducing factor(s) by macrophages (2) and modulated the function of polymorphonuclear cells (PMN) (3). It has been reported that the bactericidal and fungicidal activity of macrophages and PMNs was activated by various cytokines (4, 5). We therefore also investigated the possibility of induction of antimicrobial activity in mice by treatment with these fractions.

## Materials and Methods

The cones of various pine trees were supplied by Mr. S. Matsuda, Mr. M. Matsuda, Mr. S. Sato (*Pinus parviflora* Sieb. et Zucc. and *Pinus thunbergii* Parl.), Mr. T. Kitajima (*Pinus densiflora* Sieb. et Zucc.), Mr. T. Colliander (*Pinus sylvestris* L.), Mr. S. Okabe and Mr. K. Yokoyama (*Pinus elliotii* var. *elliottii*, *Pinus taeda* L. *Pinus carbaea* var. *Hondurensis*). The seed shells of *Pinus parviflora* Sieb. et Zucc. and *Pinus armandii* Franch. were supplied by Mr. M. Yoshihara.

PSK, a protein-bound polysaccharide prepared from the mycelium of a CM-101 strain of *Coriolus versicolor* belonging to the *Basidiomycetes* (6), was kindly provided by Kureha Chem. Ind. Co., Ltd., Tokyo, Japan. Lentinan, an antitumor polysaccharide purified from *Lentinus edodes* (Berk.) Sing. (7), was purchased from Yamanouchi Co., Ltd., Tokyo, Japan. TAK, a glucan purified from *Alcaligenes faecalis* var. *myxogenes*, IFO 13140 (8), and the carboxymethylglucan of TAK (CM-TAK) (9), were kindly provided by Takeda Chem. Ind. Ltd., Osaka, Japan.

**Mice.** Female ICR mice (5 weeks old, 23-25 g) and ddY mice (5 weeks old, 24-26 g) were obtained from Sankyo Labo. Service Co. The mice were used for experiments at 6-7 weeks of age.

**Tumors.** Sarcoma-180 was maintained serially in *ascites* form by weekly *in vivo* transfer in ICR mice.

**Cell culture.** Human monoblastic leukemic U-937 cells (10) were cultured in RPMI1640 medium (GIBCO) supplemented with 10% heat-inactivated fetal bovine serum (FBS)(GIBCO), as described previously (1).

**Isolation of antitumor substances.** Various antitumor substances of pine cones were fractionated as described previously (2). In brief, cones and seed shells of various pine trees were extracted with boiling water after