

Induction of Cytotoxic Factor in Mice by Lignified Materials Combined with OK-432 (Picibanil)

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Abstract. Intravenous administration of pine cone lignin - related substance (Fr. VI) significantly stimulated OK-432 - elicited cytotoxic factor (CF) production in ICR mouse serum. The level of CF elicited after OK-432 administration peaked after 2 h and declined to basal level within 6 h. The CF producibility depended greatly on both dose and the interval between the administration of the Fr. VI and OK-432. Most natural and synthetic lignins, their degradation products, and polysaccharides, including pine cone hemicellulose fractions, had much weaker CF-inducing (priming) activity. When Fr. VI was treated with NaClO₂ to decompose the lignin portion, the priming activity was significantly reduced. The data suggest that the potent priming activity of Fr. VI might be a result of some conjugation between the lignin portion and other components including polysaccharides.

Several clinical trials were made for the treatment of cancer patients with exogenously prepared «tumor necrosis factor» (TNF), a cytokine or cytotoxic factor (CF) that is probably secreted as a self - defense mechanism against tumor cell growth. This type of cytokine treatment seems to be promising in itself, but results have not always been successful, due in part to severe side effects. Alternatively, various biological response modifiers (BRM) have been under consideration, some of which may induce endogenous TNF production (1, 2). We previously reported that some pine cone extracts (Frs. VI and VII) induced significantly higher levels of serum CF against mouse fibroblast L-929 cells, when the mice were administered OK-432 (Picibanil) which is regarded as a promising BRM for TNF production (3). We report here more detailed kinetics of CF production by these extracts

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and, in addition, their structurally related materials including phenolic natural products and polysaccharides. Structural requirement for this activity is also discussed.

Materials and Methods

Materials. Alkali-lignin and lignin sulfonate, humic acid, vanillin, gallic acid, 4-hydroxycinnamic acid (p-coumaric acid), and horseradish peroxidase were purchased from Tokyo Kasei Kogyo Co., Ltd, Tokyo. Tannic acid was purchased from Dainippon Pharmaceutical Co., Osaka. Sodium chlorite was obtained from Wako Pure Chemicals Co., Ltd, Osaka.

Pine cone of *Pinus parviflora* Sieb. et Zucc. were supplied by Mr. S. Matsuda. Seed shells of *Pinus parviflora* Sieb. et Zucc. were supplied by Mr. M. Yoshihara. Wood chips of slash pine (SP) (*Pinus ellioti* Engelm) and tallow wood (TW) (*Pentadesma* butyraceae Gutt.) were the gifts of Mr. H. Nakatsuka, Head of Seishi - Kogyo Shikengo, Shizuoka prefecture.

Carboxymethyl glucan of TAK (CM-TAK) (4) was kindly provided by Takeda Chemical Industries, Ltd., Osaka, Japan. Paramylon (5), a water - insoluble (1-3) - β- D-glucan with a molecular weight of 122 KD (DP756), was obtained from *Euglena gracilis*. PSK, prepared from the mycelium of a CM-101 strain of *Coriolus versicolor* (6), was kindly provided by Kureha Chem. Ind., Ltd., Tokyo. OK-432 (Lot No. A6J116, 50 KE/vial), a preparation of *Streptococcus pyogenes* (7) was supplied by Chugai Seiyaku Co., Tokyo.

Preparation of lignified materials. Lignified materials were prepared from various plant sources by successive extraction with hot water, 1% NaOH and 4% NaOH, after extensive alcohol washing. The details of the extraction procedure and the yield of each extract have been reported previously (8).

Fr. VI and Fr. VII were prepared by, respectively, acid - and ethanol - precipitation from NaOH extract of the pine cone (9).

RPMI 1640 medium was purchased from Grand Island Biological Co., Grand Island, New York, and fetal bovine serum (FBS) was obtained from Filtron, Pty. Ltd., Victoria, Australia.

Preparation of hemicellulose. Hemicellulose was prepared from pine cone as previously described (10). In brief, the pine cone of *Pinus parviflora* Sieb. et Zucc. was extracted with methanol, ethanol and hot water. The residue was ground, treated with NaClO₂ as previously described (11), and extracted with 1 N NaOH. The NaOH extract was dialyzed against distilled water and lyophilized.

Dehydrogenative polymerization of p-coumaric acid. The following solutions were used: Solution A: One gram of p-coumaric acid was neutralized with 1 N NaOH and diluted to 200 ml with 0.05 M Na-phosphate