CANACELUNIN, A CANCER CELL AGGLUTININ FROM *STREPTOMYCES* SP.

Sir:

To improve the results of cancer chemotherapy, it is important to discover new anticancer agents with specific antitumor activity, and many biological studies have been undertaken using plant lectins such as concanavalin-A. On the other hand, microbial hemagglutinins were found *Streptomyces*.[1,2] On the assumption that there may be important lectins of microbial origin, we screened broth filtrates of *Streptomyces* sp., using ascites tumor cells, in an attempt to isolate a new compound with agglutination activity against tumor cells. From among the screened filtrates, we selected *Streptomyces* sp. No. 327 and isolated a new cancer cell agglutinin on which we now report.

Streptomyces sp. No. 327 was cultivated in a jar fermentor for 3 days at 27°C in a medium (pH 6.0)[1] containing 1% lactose, 0.5% peptone, 0.1% yeast extract, and 0.05% $MgSO_4 \cdot 7H_2O$. The agglutinating component was precipitated by adding 3 volumes of ethanol to the broth filtrate, and the precipitate (327-1, yield: 74 g from 15 liters broth filtrate) was subjected to Sephadex G-50 column chromatography. Agglutination activity was present in the fraction exhibiting UV absorption. The active component was isolated (327-1 A_1, yield: 112 mg from 3 g 327-1) and purified by DEAE Sephadex A-50 (phosphate type, pH 7.8) column chromatography with gradient elution, using a 0.25 mol NaCl solution. A single protein fraction with UV absorption exhibited agglutination activity; it was isolated and named canacelunin (yield: 5 mg from 400 mg 327-1 A_1 or 34.5 mg from 15-liter broth).

Canacelunin manifested UV absorption at 286 nm in 0.035 mol phosphate buffer solution (KH_2PO_4-Na_2HPO_4, pH 7.8) and gave a single band on polyacrylamide gel electrophoresis (PAGE). Its molecular weight was determined to be 62,000 by SDS-PAGE.

The amino acid components of canacelunin were: Lys, 2.11; His, 0.95; Arg, 0.95; Asp, 1.07; Thr, 1.03; Ser, 1.15; Glu, 1.08; Pro, 1.15; Gly, 1.06; Ala, 1.08; Val, 0.99; Ileu, 1.10; Leu, 1.06; Tyr, 1.13 and Phe, 1.10. The agglutination activity of canacelunin was approximately 1,000-fold higher than that of the ethanol precipitate (327-1). This agglutinin was heat-labile above 40°C, and no cell agglutination was observed in the absence of viable tumor cells. It did not react with cells at a pH below 5.7 or above 11; in the pH range from 6.0 to 7.5, the concentration of the phosphate buffer (0.05~0.8 mol) played no role in the agglutination activity. Strain-specificity of the agglutination activity of canacelunin was tested against several kinds of tumor cells such as S-180, EHRLICH carcinoma, L-1210, NTF sarcoma,[3~5] and CCM tumor,[6] and no difference was found, except that EHRLICH carcinoma was slightly resistant. Canacelunin agglutinated the viable cell suspension of solid S-180 and LEWIS lung carcinoma tumors. It did not have as high hemagglutination activity as the microbial hemagglutinins or plant lectins.

In general, agglutination by lectins is inhibited by the addition of special sugars.[7] Therefore, inhibition tests of sugars against canacelunin were carried out using ascites tumor cells. No inhibition was found in the tested sugars (10 m mol/ml), *i.e.* D-galactose, L-arabinose, D-galactosamine, N-acetyl-D-galactosamine, D-galacturonic acid, L-fucose, D-ribose, D-fructose, D-mannose, D-glucose, D-xylose, D-glucosamine, N-acetyl-D-glucosamine, D-glucuronic acid, L-rhamnose, L-sorbose, lactose, cellobiose, saccharose, maltose, trehalose, and dextran. Furthermore, the addition of amino acids, amines, or inorganic ions did not inhibit agglutination.

Canacelunin strongly induced lymphocyte blastoid transformation of mouse spleen cells, with ^{3}H-thymidine incorporation being used to measure the lymphocyte blastoid transformation of the spleen cells in the presence or absence of brain-associated anti-θ serum[8]. Results were compared with those obtained when *Phaseolus vulgaris* PHA or pokeweed mitogen were used. Our findings suggest canacelunin to be a T-cell mitogen.

Mice were subcutaneously inoculated in the right back foot pad with 10^7 S-180 ascites tumor cells. Starting with the 7th postinoculation day, every other day ($\times 5$), they received intratumor injections of 2 mg 327-A_1. After a 4-week period, complete tumor regression was noted in 4 of 6 mice tested. In the other 2 mice, the tumors were equal in size to those of controls.

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