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Effect of GlcNAc-, Man- and Gal-Specific Lectins of the Cytosol, Nuclear Membrane, and Nuclear Matrix of Calf Brain Cortex on Proliferation Activity of Peripheral Blood Lymphocyte

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We studied the effect of GlcNAc-, Man-, and Gal-specific lectins isolated from cell cytosol, nuclear membrane, and nuclear matrix of calf brain cortex, lyophilized, and stored for 5 years on proliferation activity of human peripheral blood lymphocytes and on hemagglutination activity of trypsinized rabbit erythrocytes. Human peripheral blood lymphocytes treated lyophilized lectins demonstrated lower proliferation activity than lymphocytes treated with concanavalin A (positive control), but higher than control lymphocytes (incubated with saline). Lectins produced no effect on hemagglutination activity.

Key Words: *calf brain lectins; MTT assay; proliferation of human peripheral blood lymphocytes; hemagglutination activity*

Lectins are non-immune proteins reversibly [12] and selectively binding free and conjugated saccharides [9]. Some lectins induce lymphocyte proliferation and modulate various immune functions [10,11], which helps to study the mechanism of cell stimulation and proliferation. There are ample published data on the use of plant lectins, but little is known about animal lectins. We have previously demonstrated that freshly isolated GlcNAc-specific lectin from the nuclear membranes of rat brain cortex 2.5-fold increased lymphocyte proliferation [1].

Our aim was to find out whether GlcNAc-, Man-, and Gal-specific lectins isolated from cell cytosol, nuclear membrane, and nuclear matrix of calf brain cortex cells 5 years ago, lyophilized, and stored at 4°C stimulate proliferation of human peripheral blood lymphocytes and whether they retain their hemagglutination activity.

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MATERIALS AND METHODS

Brain specimens from 8-12-month-old calves were used in the study. From cell nuclei prepared as described elsewhere [8], protein fractions with lectin activity were isolated from the nuclear membrane [1,5]. Nuclear matrix was isolated as described previously [3,7]. Protein fractions with lectin activity from the nuclear matrix were obtained using a buffer solution [2] with high concentrations of Triton X-100 and NaCl [1,2,5,6] for extraction of these proteins from membrane structures.

GlcNAc-, Gal-, and Man-specific lectins were isolated from lectin-containing protein fractions of the cytosol, nuclear membranes, and nuclear matrix by affinity chromatography on affinity columns (1×4 cm) with immobilized GlcNAc, Gal, and Man. Lectin fractions were collected, dialyzed against PBS or distilled water; protein fractions were lyophilized and kept at 4°C. Lectin activity was evaluated by hemagglutination of trypsinized rabbit erythrocytes (microtitration) in U-bottom plates in a medium for agglutination [4]. Hemagglutination activity was defined by minimum protein concentration inducing agglutination. Protein

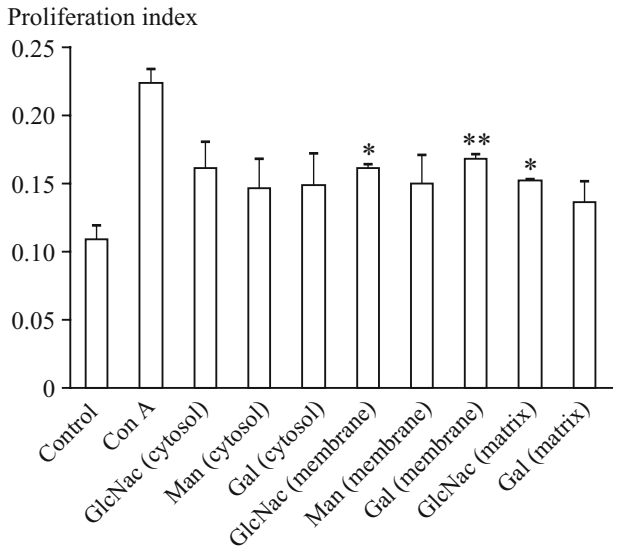

Fig. 1. Mitogenic stimulation of human PBL with lectins. The results of 4 independent experiments are presented. *$p<0.05$, **$p<0.01$ in comparison with the control.

content was measured by the method of Bradford.

Human peripheral blood lymphocytes (PBL) were isolated from the heparinized blood by standard density centrifugation on a Ficoll density gradient, resuspended to a concentration of 10^5/ml in complete RPMI-1640 medium (Sigma) supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. Then, 80 µl cells suspension were transferred to wells of a 96-well flat-bottom microplates and 2 µg mitogens (or 20 µl PBS in the control) were added. Concanavalin A (Sigma; 2µg in 20 µl PBS) was used as the positive control. The cells were then incubated for 72 h at 37°C and 5% CO_2 in a humidified incubator. On day 3, 10µl MTT (Sigma) was added to each well solution (5µg/ml in PBS) were added of the microplate. The cells were incubated at 37°C for 4 h, then 100 µl isopropanol (Sigma) were added to each well and the plate was shaken for 10 min to dissolve formazan crystals. Absorbance was measured on a Tecan Safire microplate reader (Magellan 6.5 software) at 562 nm (reference wavelength 620 nm).

The data were analyzed by with Microsoft Excel using two-sample Student's *t* test. The values were significant at $p<0.05$.

RESULTS

Activation of T cells can be induced by exogenous growth factors that interact with a specific receptor on the membrane of the target cell. Under physiological conditions, circulating PBL are resting cells and could be used for *in vitro* experiments, especially for studies of cell growth and development. Cells in resting phase need an antigen or a mitogen for expression of specific receptors for T or B cells. The latter activates growth factors and promoted proliferative response of lymphocytes [13]. For differentiation into a plasma cell, further activation steps and other lymphocytes are needed.

The studied lectins exhibited mitogenic activity, but showed no hemagglutination activity (Fig. 1).

It is well known that some lectins stimulate cell transformation from resting into blast-like cells and mitotic division of the latter, which attests to their participation in cell growth and development. In our experiments, GlcNAc-, Man- and Gal-specific lectins isolated from the cytosol, nuclear membrane, and nuclear matrix of calf brain cells less effectively stimulated proliferation of PBL than concanavalin A, but their effect was higher than that of PBS (control) after the loss of hemagglutination activity.

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