

## Ganglioside and Monosaccharide Inhibition of Nonspecific Lymphocyte Mitogenicity by Group A Streptococcal Pyrogenic Exotoxins

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Group A streptococcal pyrogenic exotoxins (SPE) types A, B, and C are potent nonspecific lymphocyte mitogens. The mitogenicity of these exotoxins was inhibited by gangliosides and sialic acid, whereas concanavalin A was unaffected. The capacity of both concanavalin A and SPE-A to stimulate lymphocytes was suppressed by  $\alpha$ -methyl-D-mannopyranoside. Galactose reduced the activity of SPE-C. The sugars, glucose, *N*-acetylglucosamine,  $\alpha$ -methyl-D-glucopyranoside, and fucose, did not affect SPE mitogenicity.

Group A streptococcal pyrogenic exotoxins (SPEs) are low-molecular-weight proteins with a large number of biological properties. These include pyrogenicity (1, 11, 16, 21) and enhancement of host susceptibility to lethal shock (11, 16), myocardial damage (11, 17), and skin reactivity (14). Further, the three antigenically distinct toxins, A, B, and C, alter antibody production (5, 8, 9) and are potent nonspecific and specific lymphocyte mitogens; the mitogenicity is specific for T-cells (2, 15).

With such a large number of biological activities produced by low concentrations of SPEs, usually in nanograms or micrograms, it would be expected that the toxins interact with highly specific receptors on target tissues. This investigation was undertaken to study SPE-receptor interactions. The studies assessed the capacity of various monosaccharides and gangliosides to inhibit SPE-induced proliferation of normal rabbit lymphocytes.

All reagents and glassware used for toxin production and biological assays were maintained pyrogen free.

Group A streptococcal strains utilized for production of SPEs were NY-5 (M type 10/12) for A, B, and C toxins and T18P (M type 18) for C toxin. Stock cultures were maintained by lyophilizing in the presence of whole defibrinated fresh rabbit blood.

Streptococci were cultured in a modified beef-heart dialysate medium (11, 21). Highly purified preparations of SPEs were obtained from cell-free culture supernatant fluids after differential precipitation with ethanol and resolubilization in acetate-buffered saline (1, 11, 13), followed by preparative thin-layer isoelectric focusing (1, 13). Purified toxins were stored lyophilized in the presence of pyrogen-free streptococcal hyalu-

ronic acid (16). Hyaluronic acid did not affect the mitogenicity of the SPEs.

The capacity of SPEs to induce lymphocyte proliferation was assessed by incorporating [ $^3$ H]thymidine into deoxyribonucleic acid as a measure of mitogenesis (2). Optimal stimulatory doses of SPEs (1  $\mu$ g per well) and concanavalin A (ConA) (1  $\mu$ g per well) were used throughout the study. Inhibition of mitogenic activity by various monosaccharides was evaluated after incubation of lymphocytes with SPEs plus sugars. Sugar concentrations tested for inhibition of mitogenicity were 1, 10, and 50 mM unless stated otherwise. Gangliosides were a pool of ganglioside types and were obtained from P-L Biochemicals, Inc., Milwaukee, Wis. Ganglioside concentrations tested were 0.1, 1.0, and 5  $\mu$ g per well.

Data were reported as average counts per minute  $\pm$  1 standard deviation.

Lymphocyte proliferation can be induced nonspecifically by many lectins, including ConA (7, 12), phytohemagglutinin (3), and wheat germ mitogen (4). The mitogenicity of lectins is inhibited specifically by sugar residues (3, 4, 7, 12), suggesting that the monosaccharides are components of the host cell surface receptors.

Comparable to ConA, the nonspecific mitogenicity of SPE-A was inhibited by  $\alpha$ -methyl-D-mannopyranoside (Fig. 1). However, lymphocyte stimulation by SPE-B and -C was not affected significantly by the sugar.  $\alpha$ -Methyl-D-mannopyranoside at concentrations of 1 and 10 mM did not inhibit the activity of either SPE-A or ConA.

The nonspecific mitogenicity of SPE-C was reduced by 50 mM galactose (Fig. 2), but not at 1 and 10 mM concentrations. Complete inhibition of incorporation of [ $^3$ H]thymidine into DNA by galactose was not achieved by sugar concen-

trations up to 100 mM; galactose concentrations greater than 100 mM were not tested. The capacities of ConA and SPE-A and -B to induce lymphocyte proliferation were not affected by galactose at a concentration of 50 mM.

The monosaccharides fucose, glucose,  $\alpha$ -methyl-D-glucopyranoside, and N-acetylglucosamine did not inhibit SPE-induced lymphocyte mitogenicity (Table 1).

Gangliosides effectively inhibited the mitogenic activity of all SPE types (Fig. 3), whereas the activity of ConA was unaltered. It is not known which type of ganglioside contributed to the suppression since the gangliosides used may have contained many types. The inhibition of

SPE mitogenicity was specific since comparable doses of gangliosides failed to suppress ConA-induced lymphocyte proliferation. Studies are underway to identify the ganglioside or related compound responsible for the inhibition of SPE activity.

A major component of gangliosides, sialic acid, also inhibited lymphocyte proliferation induced by all SPE types (Fig. 4). The higher dose of sialic acid (50 mM) inhibited both SPE and ConA stimulation of lymphocyte proliferation (data not shown). This inhibition of activity probably represented a nonspecific effect (20).

Other protein toxins have either ganglioside or sialic acid as components of host cell surface

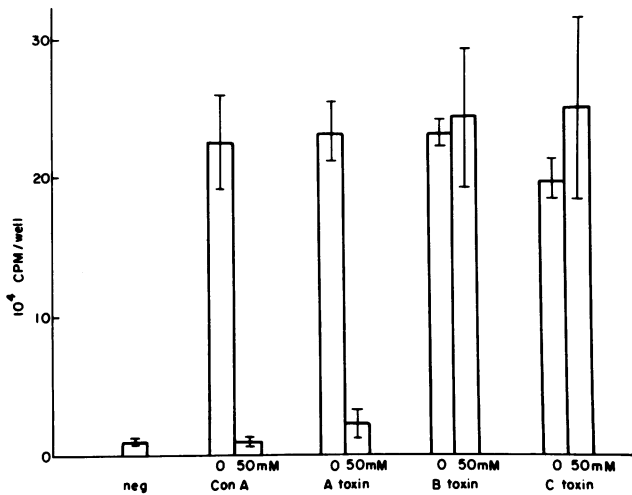


FIG. 1. Effect of  $\alpha$ -methyl-D-mannopyranoside on SPE-induced proliferation of spleen cells from normal rabbits. ConA and SPE concentrations were 1  $\mu$ g per well. Bars,  $\pm 1$  standard deviation.

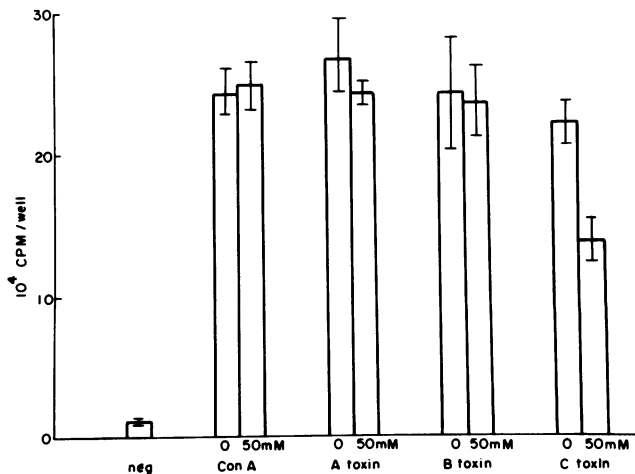


FIG. 2. Effect of D-galactose on SPE-induced proliferation of spleen cells from normal rabbits. ConA and SPE concentrations were 1  $\mu$ g per well. Bars,  $\pm 1$  standard deviation.

TABLE 1. Effect of several monosaccharides on SPE stimulation of spleen cells from normal rabbits

Monosaccharide (mM)	SPE-A <sup>a</sup>	SPE-B <sup>a</sup>	SPE-C <sup>a</sup>
Fucose			
0	240,000 ± 18,000	122,905 ± 4,080	340,000 ± 40,000
50	220,000 ± 36,000	141,370 ± 10,313	320,000 ± 38,000
Glucose			
0	95,000 ± 4,000	94,000 ± 16,000	105,000 ± 4,000
50	93,000 ± 16,000	90,000 ± 11,000	105,000 ± 20,000
$\alpha$ -Methyl-D-glucopyranoside			
0	170,000 ± 15,000	200,000 ± 15,000	270,000 ± 11,000
50	175,000 ± 14,000	180,000 ± 25,000	250,000 ± 15,000
N-acetylglucosamine			
0	170,000 ± 20,000	140,000 ± 20,000	238,000 ± 15,000
50	140,000 ± 25,000	150,000 ± 15,000	230,000 ± 15,000

<sup>a</sup> Counts per minute  $\pm$  1 standard deviation.

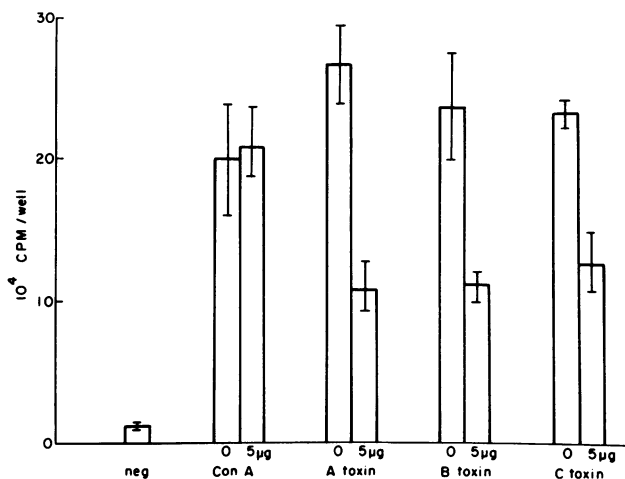


FIG. 3. Inhibition of SPE-induced proliferation of spleen cells from normal rabbits by gangliosides. ConA and SPE concentrations were 1  $\mu$ g per well. Bars,  $\pm$ 1 standard deviation.

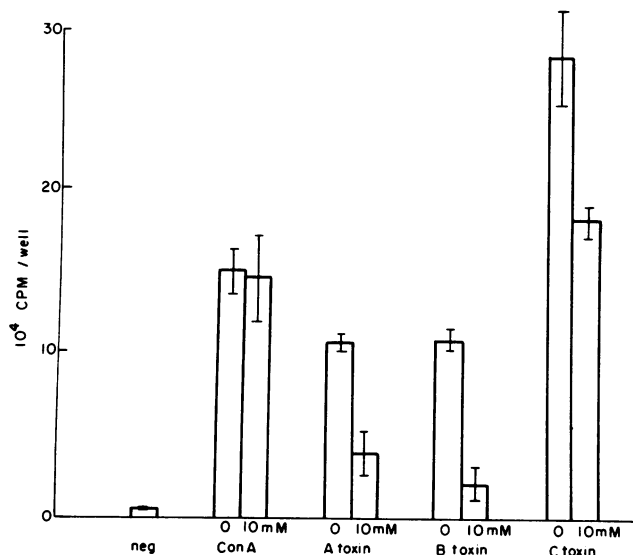


FIG. 4. Inhibition of SPE-induced proliferation of spleen cells from normal rabbits by sialic acid. ConA and SPE concentrations were 1  $\mu$ g/well. Bars,  $\pm$ 1 standard deviation.

receptors. Tetanus toxin, which is a potent neurotoxin, binds two sialic acid residues of ganglioside (18, 20). Cholera and heat-labile *Escherichia coli* enterotoxins bind GM<sub>1</sub> ganglioside of intestinal tissues or cultured adrenal cells (6, 10, 19). The diversity in biological activities observed for these exotoxins and SPEs is probably caused by differences in arrangements of sugar residues within the gangliosides and by the content of specific ganglioside types on various host cells.

It is interesting that the mitogenicity of SPE-A and -C was reduced by two sugar residues: SPE-A by  $\alpha$ -methyl-D-mannopyranoside and SPE-C by galactose. Sialic acid inhibited all three types. The inhibition of SPE-A and -C by two different sugars indicates that the toxins may bind residues on cell types that do not contain the specific ganglioside. The possibility exists, therefore, that some activities of the SPEs are regulated by a ganglioside (or related compound) binding property, whereas others may be controlled by toxin binding to other sugars.

Studies are presently in progress to investigate this hypothesis and to examine the kinetics of binding of SPE to various cell types expected to have receptors for the toxins.

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