Sphingolipid Antibodies. II. Specificity of Antibody in Patients with Demyelinating Diseases.* (28673)

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Yokoyama, Trams and Brady(1) recently demonstrated antiganglioside activity in sera from some patients with certain neurological diseases, especially multiple sclerosis. Although the titers were low and the percentage of patients with multiple sclerosis exhibiting this reaction was modest (20%), the finding is of particular interest for several reasons. It represents the first *in vitro* detection of a specific chemically-defined antigen-antibody reaction in this disorder. The pattern of the disorders and frequencies in which it was detected is reminiscent of the tissue culture demyelinating activity of various sera as described by Bornstein(2). Additionally, gangliosides have not been implicated in the structure of the myelin sheath. Since ganglioside preparations isolated from various sources can be shown on thin layer chromatography to consist of 4 to 8 fractions(3-5) believed to represent at least mono-, di-, and trisialoganglioside fractions, an attempt was made to determine if the human antiganglioside activity were specific for one or another of the various species of gangliosides.

Materials and methods. Monosialoganglioside was obtained by fractionation of crude Sigma ganglioside batch No. 81B-623 by silicic acid column chromatography according to Svennerholm(6). The criteria used for designating this as monosialoganglioside consisted of the following: resistance to extensive treatment with neuraminidase; N-acetyl neuraminic acid content of 0.8 μmoles/mg, assayed as described by Trams and Lauter (7) and a single spot on thin layer chromatography.

Polysialogangliosides were prepared from beef brain acetone powder by a technique combining the methods of Booth(8) and that of Trams and Lauter(7). This material was found to have a NANA content of 1.2 μmoles/mg and therefore assumed to have an appreciable amount of ganglioside homologues containing more than 1 molecule of NANA.

The hemagglutination reaction for antiganglioside activity developed by Yokoyama, Trams and Brady(9) was used for assay and determination of titers of 5 human sera exhibiting antiganglioside activity. Four were from patients with multiple sclerosis and one from a patient with amyotrophic lateral sclerosis.

Hemagglutination inhibition was carried out as described by Kabat(10). Rabbit antiganglioside serum was produced by the method of Yokoyama *et al.*(9), utilizing the unfractionated Sigma ganglioside preparation #81B-623 as antigen.

Sheep erythrocytes were coated with the various ganglioside preparations in a concentration of 1 mg per ml in 0.01 M phosphate buffered saline (pH 6.4) using 20 volumes of solution to 1 volume of washed packed cells.

Considerable variation in the susceptibility of sheep cells to the coating procedure was found. It was also noted that coating was best with cells used on the 3rd to 5th day after bleeding. Insofar as possible, hemagglutination and inhibition reactions were carried out simultaneously on several sera utilizing one batch of sheep cells, and confirmed using other batches of sheep cells. Positive controls for coating were simultaneously car-

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TABLE I. Demonstration of Specificity of Anti-ganglioside Antibodies.

Source of serum	Reaction with cells coated with: Monosialo-ganglioside	Polysialo-ganglioside	Tay-Sachs' ganglioside	Inhibition with: ‡ Monosialo-ganglioside	Polysialo-ganglioside	Tay-Sachs' ganglioside
	Titer					
Patient No. 4	1:1	0	0	*	*	*
5	1:1	0	0	*	*	*
10†	1:2	0	0	+	0	*
37	1:8	0	*	+	0	*
67	1:8	0	*	+	0	*
Immunized rabbit antiserum	1:8	1:8	0	+	+	0

+ = Inhibition of hemagglutination. 0 = No inhibition of hemagglutination.

* Not determined.

† This serum was obtained from a patient with amyotrophic lateral sclerosis.

‡ Tests on human sera carried out with monosialoganglioside-coated erythrocytes. Tests on rabbit sera carried out both with erythrocytes coated with monosialo- and polysialogangliosides except that the inhibition by Tay-Sachs' ganglioside was studied only against cells coated with monosialoganglioside.

ried out with rabbit antiganglioside sera and negative controls were carried out using saline, normal human sera and uncoated cells.

Results. Titers of the 5 patients' sera and combined immunized rabbit antisera against the ganglioside preparations are given in Table I. The reaction with monosialoganglioside-coated erythrocytes could be completely blocked by preincubation of these sera with this ganglioside preparation. The human sera did not react with the polysialoganglioside on any occasion although coating of the test cells with this ganglioside was adequate and comparable to that with the monosialoganglioside as determined by immune rabbit antisera controls. Pre-treatment of sera with the polysialoganglioside failed to inhibit the reaction with the monosialoganglioside-coated cells. In contrast with the specificity shown by the human antiganglioside antibodies, the rabbit antiganglioside antibodies, produced by administration of a crude mixture of Sigma gangliosides, reacts equally well both directly and by inhibition with mono- and polysialogangliosides. In addition, no reaction was obtained when 3 of the patients' sera and the immune rabbit sera were run against cells coated with Tay-Sachs' ganglioside. Pre-treatment of rabbit immune serum with Tay-Sachs' ganglioside did not prevent the agglutination of cells coated with monosialoganglioside.

Yokoyama, Trams and Brady noted that the human antiganglioside activity in patients with multiple sclerosis was associated with a homozygous state for hr′ (c) blood group substance. Gangliosides have been shown to possess some blood group activity (9). To ascertain whether the observations with patients' sera represented true antiganglioside activity or reflected the presence of anti-C antibody, the positive sera were thoroughly absorbed with human cells homozygous for the C antigen. This treatment did not remove or reduce the antiganglioside activity.

Discussion. The present studies indicate that the antiganglioside antibody present in sera of 4 patients with multiple sclerosis and one patient with amyotrophic lateral sclerosis is highly specific for monosialoganglioside. The antigenic determinants can therefore be stated with some degree of certainty. The presence of 1 and only 1 molecule of N-acetyl neuraminic acid (NANA) is required. The presence of at least 2 molecules of galactose is obligatory since the only demonstrable difference noted so far between Tay-Sachs' ganglioside and monosialoganglioside is the absence of one molecule of galactose in the Tay-Sachs' ganglioside. Previous studies have demonstrated the resistance of the monosialoganglioside to enzymatic hydrolysis by neuraminidase(4,7,11) and a difference in the nature of the linkage of this molecule of NANA to the glycolipid residue compared with the other molecules of NANA in the di- and trisialogangliosides(12). These findings

suggest that there may be some relative difficulty in the catabolism of the monosialoganglioside which may then persist in the blood stream as a glycolipid hapten.

Summary. Five human patients' sera containing antiganglioside activity were examined with various ganglioside preparations utilizing hemagglutination and hemagglutination inhibition techniques. The results indicate that the activity in these cases is specific for monosialoganglioside.

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Effect of Cholestyramine,* A Bile Acid Binding Polymer on Plasma Cholesterol and Fecal Bile Acid Excretion in the Rat. (28674)

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The hypercholesteremic state has received considerable clinical and laboratory attention in recent years because of its possible importance in atherogenesis. Siperstein *et al.*(1) have suggested that lowering plasma cholesterol through the binding of bile acids in the intestine may serve as a means of controlling arteriosclerosis. These authors found that feeding ferric chloride to cholesterol-fed cockerels inhibited the rise in plasma cholesterol and retarded the accompanied atheromata. They associated this effect with decreased cholesterol absorption due to precipitation of the bile acids in the intestinal tract.

More recently(2) it has been shown that feeding of cholestyramine, a bile acid binding polymeric organic base, inhibited the plasma cholesterol rise and aortic plaque formation in cholesterol-fed cockerels and depressed plasma cholesterol levels in normocholesteremic cockerels and dogs. Bergen *et al.*(3) have described the lowering of serum cholesterol levels by cholestyramine in patients suffering from coronary artery disease. The present report describes the effect of cholestyramine on plasma cholesterol level, fecal bile acid excretion and cholesterol synthesis rate in the rat.

Methods and materials. In vivo *experiments.* Holtzman male albino rats were obtained at 130-140 g in weight and maintained on Purina Laboratory Chow for one week. The rats were then randomized according to weight into groups of 10 rats each, with 20 rats in the control group, and placed on the experimental diets. The normocholesteremic diet consisted of Purina Laboratory Chow to

* Generic name for a quaternary ammonium anion exchange resin in which the basic groups are attached to a styrene-divinyl benzene copolymer skeleton by carbon-to-carbon bonds. Its equivalent weight is about 230. The material used in these experiments is in the chloride form. It is essentially dry as prepared, but picks up moisture upon exposure to air. The dietary con'ents reported in the tables represent the dry weight of the resin incorporated.