Sphingolipid Antibodies in Sera of Animals and Patients with Central Nervous System Lesions.* (27789)

MITSUO YOKOYAMA,† EBERHARD G. TRAMS, AND ROSCOE O. BRADY
(Introduced by C. R. Treadwell)
Laboratory of Neurochemistry, National Institute of Neurological Diseases and Blindness, Bethesda, Md.

The presence of gangliosides, a class of complex sphingolipids, was originally demonstrated in normal brain tissue by Klenk(1). These compounds were later found to occur in larger amounts in the brains of patients with Tay-Sachs disease(2) and the accumulation of gangliosides in these brains is considered a significant pathognomonic feature. Interest in the possible role of lipids as antigens has recently been renewed by the demonstration by Rapport *et al.*(3,4) that a ceramide (N-acyl sphingosine) dihexoside called Cytolipin H has antigenic properties. These observations suggested that gangliosides which contain N-acetylgalactosamine and N-acetylneuraminic acid residues in addition to the sphingosine-, fatty acid-, glucose- and galactose-moieties of the dihexoside structure might also have antigenic properties. This supposition was proven to be correct by the recent demonstration that both gangliosides and asialogangliosides, materials from which the terminal N-acetylneuraminic acid residues had been removed, each caused the production of specific antibodies(5). There was little or no cross reaction between the antibodies to these materials.

The present communication describes the results of experiments in which the sera from animals and patients with various central nervous system lesions were examined for the presence of antibodies against gangliosides and asialogangliosides.

Materials and methods. Gangliosides were isolated and purified from beef and human brain tissue according to the method of Trams and Lauter(6). Asialogangliosides were prepared by mild acid hydrolysis of gangliosides (6). Blood group antisera were obtained from Ortho Pharmaceutical Corp., N. J. Sheep erythrocytes used in this investigation were prepared as described previously(5).

Hemagglutination test. The samples of serum were inactivated at 56°C for 30 minutes and heteroagglutinins were removed by absorption onto washed sheep erythrocytes immediately prior to use. The presence of anti-ganglioside or anti-asialoganglioside antibodies was examined by a hemagglutination test developed in this laboratory(5). Essentially, the technic consists of coating sheep erythrocytes with the antigen by incubating 1 volume of washed packed sheep erythrocytes with 9 volumes of a 0.1% solution of the appropriate lipopolysaccharide in 0.1 M sodium phosphate-sodium chloride buffer at pH 6.4 for 1 hour at 37°C. The coated erythrocytes were then washed twice with saline and 0.1 ml of a 1% suspension of the coated erythrocytes in saline was added to 0.1 ml of serum. The mixture was incubated in a 1.0 × 7.5 cm test tube for 1 hour at 23° C. A positive reaction is defined as one in which the erythrocytes were agglutinated in the form of a diffuse carpet over the bottom of the tube(7). A small circle of cells having a dark outer rim or a tight button of erythrocytes were scored as negative readings. The sera which showed a positive reaction were serially diluted with saline to determine the antibody titer.

Control tests were performed with sera of known anti-ganglioside and anti-asialogangli-

* The authors wish to express their gratitude to the following for their help in providing serum samples for this study: Dr. Anthony Morris, Division of Biologics Standards, Nat. Inst. Health; Dr. M. Kitaoka, Nat. Inst. Health of Japan; Dr. Marion Kies, Nat. Inst. of Mental Health; Dr. Bruno Volk, Jewish Chronic Disease Hospital, Brooklyn, N. Y.; Dr. Milton Shy, Nat. Inst. of Neurolog. Dis. and Blindness; Dr. Irwin Feinberg, St. Elizabeths Hospital, Washington, D.C.; Mr. Lewis Hartman, Multiple Sclerosis Clinic, George Washington Univ. Hospital, Washington, D.C.

† Present address: Dept. of Medicine, University of California School of Medicine, San Francisco.

TABLE I. Presence of Anti-Ganglioside and Anti-Asialoganglioside Antibodies in Sera.

Source	Condition	No. of sera examined	Sera containing antibodies against: Gangliosides No.	Gangliosides Titers	Asialogangliosides No.	Asialogangliosides Titers
Human	Normal	42	0		0	
Chimpanzee	Encephalitis without CNS damage	83	0		0	
Human	Japanese B encephalitis	14	0		6	(1:2–1:8)
"	Venezuelan "	1	0		1	(1:4)
"	Murray Valley "	1	0		1	(1:4)
Chimpanzee	Encephalitis with CNS damage	12	0		5	(1:2–1:4)
Human	Tay-Sachs disease	14	0		5	(1:1–1:4)
"	Schizophrenia	14	1	(1:8)	3	(1:1–1:4)
"	Multiple sclerosis	42	8	(1:1–1:4)	0	
"	Brain tumor	4	1	(1:8)	0	
"	Amyotrophic lateral sclerosis	3	1	(1:8)	0	
"	Kuru	3	0		0	
Guinea pig	Experimental allergic encephalitis	11	0		0	
" "	Injected with gangliosides	9	5	(1:8–1:32)	0	

oside antibody titers obtained from rabbits immunized against these materials(5). Negative controls were carried out with saline, normal human sera, non-immune animal sera, and non-coated sheep erythrocytes.

When samples of patients' blood were available, the erythrocytes were tested for the ABO, MN, Le^a, Rh (C, D, E, c, and e) blood type systems.

Results. Antibody against gangliosides and asialogangliosides was not detected by the hemagglutination method used in the present experiments in 42 samples of normal human serum or in 83 sera of chimpanzees inoculated with Japanese encephalitis virus whose sera showed complement fixing antibody titers of 1:4 to 1:32 and hemagglutination inhibiting antibody titers of 1:10 to 1:5120 but without histological evidence of brain damage (Table I). In 8 out of 16 cases of human encephalitis, anti-asialoganglioside antibodies were present in the sera. Similar antibodies were observed in 5 of 12 sera from encephalitic chimpanzees with brain lesions. Of the 14 sera obtained from patients with Tay-Sachs disease, 5 showed the presence of anti-asialoganglioside antibody. Anti-ganglioside antibodies were not detected in these sera.

In 3 of the sera of 14 schizophrenic patients, anti-asialoganglioside antibodies were found, and in another, the presence of anti-ganglioside antibody was also detected. Anti-ganglioside antibodies were present in the sera of 8 of 42 cases of multiple sclerosis. The serum obtained from one of 4 patients with brain tumors and 1 of 3 sera obtained from patients with amyotrophic lateral sclerosis showed anti-ganglioside antibodies. No antibodies against gangliosides or asialogangliosides were detected in 3 cases of kuru. Similar negative results were obtained in 11 sera of guinea pigs with experimental allergic encephalomyelitis produced by the injection of a partially purified spinal cord protein(8). When a solution of gangliosides was injected into guinea pigs along with a basic brain protein fraction(9), sera from 5 of the 9 animals injected showed the presence of anti-ganglioside antibodies. No anti-asialoganglioside antibodies were detected in these animals.

The results of the blood group tests showed that all of the patients with multiple sclerosis whose sera showed the presence of anti-ganglioside antibodies were homozygous with respect to the hr'(c) blood group antigen. However, not all of the sera of the multiple sclerosis patients with the hr'(c) antigen contained anti-ganglioside antibodies.

Discussion. The possibility that autoimmune phenomena contribute to the pathogenesis of a number of disease states has been actively investigated(10-12). The demonstration by Rapport *et al.* of the antigenicity of a ceramide dihexoside suggested that the sera of patients and experimental animals with lesions of the central nervous system should be examined for the presence of sphingolipid antibodies. The present data

indicate that in a number of instances, brain damage is attended by the presence of antibodies in the serum against gangliosides and asialogangliosides. The formation of these antibodies appears to be specific since no cross reactions were observed in the present series. This finding is consistent with the production of specific antibodies when either gangliosides or asialogangliosides were injected into experimental animals(5).

Only anti-asialoganglioside antibodies were present in human and chimpanzee sera from encephalitis cases with evidence of brain damage. It is perhaps significant in this regard that the commonly accepted mechanism of penetration of influenza virus into cells is thought to occur with the concomitant liberation of neuraminic acid(13). Thus, through the action of the receptor destroying enzyme, one might expect the production of asialoganglioside as an antigenic residue on a portion of the surface of the penetrated cell.

It was somewhat surprising to find anti-ganglioside antibodies in the sera of patients with multiple sclerosis in view of the fact that gangliosides apparently are not characteristic components of the myelin sheath. It may be that the integrity of the myelin sheath depends upon the functional state of the neuron and these observations reflect some derangement of the neuronal cell.

Conversely, it is difficult to explain satisfactorily the finding of anti-asialoganglioside antibodies in sera of patients with Tay-Sachs disease, a condition in which an excess of gangliosides accumulates in the neurones. Such conflicting observations render premature attempts to assess the magnitude of the role of the auto-immune phenomena in these disease states, particularly in cases such as multiple sclerosis where it appears that the observations in the present study may have revealed only one of a number of possible antigenic agents.

The observation that only patients homozygous for hr'(c) blood group antigen showed anti-ganglioside antibodies in the serum suggests that genetic factors may play a role in this response. The role of such a hereditary factor and whether a similar relationship exists in other cases of multiple sclerosis with perhaps other antigenic materials as yet undetected require further investigation.

Summary. Anti-asialoganglioside or anti-ganglioside antibodies were not detected in 42 samples of normal human serum. Sera obtained from animals and patients with viral encephalitis having clinical evidence of brain damage contained antibodies against asialogangliosides. The presence of these antibodies was also observed in 5 of the sera from 14 patients with Tay-Sachs disease and in 3 of 14 cases of schizophrenic patients. Anti-ganglioside antibodies were found in the sera of 8 of 42 patients with multiple sclerosis. The formation of this antibody appeared to be linked to the presence of homozygous hr'(c) blood group antigen in these patients. The participation of auto-immunizing phenomena in the pathogenesis of this disease state is considered.

1. Klenk, E., *Z. Physiol. Chem.,* 1935, v235, 24.
2. ———, *A.M.A. J. Dis. Children,* 1959, v97, 711.
3. Rapport, M. M., Graf, L., Skipski, V. P., Alonzo, N. F., *Nature,* 1958, v181, 1803.
4. ———, *Cancer,* 1959, v12, 438.
5. Yokoyama, M., Trams, E. G., Brady, R. O., *J. Immunology,* in press.
6. Trams, E. G., Lauter, C. J., *Biochim. Biophys. Acta,* 1962, v60, 350.
7. Stavitsky, A. B., *J. Immunol.,* 1954, v72, 360.
8. Roboz, E., Henderson, N., *Allergic Encephalomyelitis,* Charles C Thomas, Springfield, Ill., 1959, 281.
9. Kies, M. W., Murphy, J. B., Alvord, E. C., Jr., *Chemical Pathology of the Nervous System,* Pergamon Press, New York, 1961, 197.
10. Steiner, J. W., Volpe, R., *Canad. Med. Assn. J.,* 1961, v84, 1165.
11. ———, *ibid.,* 1961, v84, 1227.
12. ———, *ibid.,* 1961, v84, 1297.
13. Klenk, E., Uhlenbruck, G., *Hoppe-Seyl. Z. Physiol. Chem.,* 1958, v311, 227.

Received July 18, 1962. P.S.E.B.M., 1962, v111.