Distribution of Antibodies to Type 4 Adeno-Associated Satellite Virus In Simian and Human Sera.* (32553)

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High hemagglutinating activity (HA) is characteristic of all the small rodent DNA-containing viruses (picodnaviruses) which are capable of autonomous replication in tissue culture(1,2,3). We have reported recently that high HA activity is also associated with the type 4 adeno-associated satellite virus isolated from cultures of simian adenovirus SV15 in our laboratory(4,5). The HA titer was demonstrable with a wide spectrum of cells, was highest with human erythrocytes (type O) at 4°C, and was capable of elution from red cells at 37°C without destruction of receptor sites. A hemagglutination inhibition (HI) test has been described(4,5), and when reacted with adenovirus and 'picodnavirus' X14, HA was established as a specific property of the type 4 satellite. Such an HI test is valuable for detection of naturally occurring antibodies against satellite viruses in man and animals. This paper presents data obtained from examination of simian and human sera

Materials and methods. Viruses and antisera. The strain of type 4 adeno-associated satellite virus ASV4 isolated from simian adenovirus SV15 in our laboratory(6,7) was used. Satellite-free adenovirus SV15(O) was used as helper and virus stocks were grown in either primary green monkey kidney (GMK) cells or the GMK continuous cell line BSC-1 as previously described(7). Antisera against density gradient purified ASV4 and SV15(O) were prepared in guinea pigs as reported previously(7,8). Normal sera from African green and rhesus monkeys were obtained from animals in the laboratory colony through the kindness of Mr. Ira Wimberly. Normal human sera were made available by Dr. Wade Parks.

* This work was supported in part by Public Health Service grants CA-04600 from the National Cancer Institute, and AI 05382 and 5 T1 AI 74 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Hemagglutination titrations. Our pattern test using 0.4% human erythrocytes (type O) has been reported(5). Endpoints were read as the highest dilutions of virus agglutinating 50% of the cells. Titers were expressed as the reciprocal of the dilution causing this degree of hemagglutination.

Assay of antisera by hemagglutination inhibition (HI). 0.05 ml of two-fold dilutions of antiserum were mixed with an equal volume containing 8 HA units of virus in a Linbro plastic tray. After overnight incubation at 4°C, the same concentration of human erythrocytes was added (0.1 ml of a 0.4% suspension) and the mixtures allowed to stand for 2-3 hours at 4°C. The HI titer of antiserum was expressed as the reciprocal of the highest dilution which prevented partial (50%) agglutination of the cells.

Results. Specificity of hemagglutinating activity of type 4 satellite virus. As reported previously(5) antiserum against type 4 satellite inhibited the HA reaction of simian adenovirus contaminated with satellite SV15(4) strongly, but did not inhibit adenovirus HA present in SV15(O) which is free of satellite. The HI reaction of satellite antiserum is type-specific for type 4 satellite virus.

Application of the hemagglutination-inhibition test to study antibody distribution against type 4 adeno-associated satellite virus. Trials were carried out to check the HI antibody distribution against type 4 satellite and SV15(O) in normal human and African green monkey sera. No significant level of satellite antibody was detectable in 40 normal human sera tested. In the case of purified type 4 satellite antigen, a non-specific inhibition of HA was frequently observed at the lower dilutions of sera (1:160 or less). This inhibition was still present after application of simple procedures in routine use for elimination of non-specific inhibitors (heating for 20 minutes at 60°C and adsorption with kaolin). Therefore, an HI reaction was only judged

to be positive when the testing serum had an HI titer of greater than 1:160. In the case of normal green monkeys, high titers of antibody against type 4 satellite were found in 13 out of a total of 14 monkey sera tested (Table I). In some of them the HI titer was as high (5120) as those of some antisera prepared by specific immunization with purified satellite virions. On the other hand, only 3 of them contained HI antibody against adenovirus SV15(O). Two rhesus monkey sera were also tested and found positive for both type 4 satellite and the simian adenovirus (Table II). The frequency of HI-antibody distribution to the satellite in green monkey sera suggests that the type 4 satellite is more prevalent in monkeys than in humans.

TABLE I. Antibody Levels in Green Monkeys.

Virus‡	No. of green monkeys containing positive or negative HI antibody against type 4 satellite or adenovirus SV15	
	+*	—†
Type 4 satellite§	13	1
SV15(O) adenovirus	3	10

* HI titer more than 320.
† HI titer less than 160.
‡ Eight HA units of virus were used for HI titration.
§ Purified type 4 satellite by banding at 1.42 g/cm³.

TABLE II. Hemagglutination-Inhibition (HI) Antibody Titers in Normal Simian Sera.

		ASV antigen		SV15(O) antigen
		Crude	Purified	
Green monkey	G-1	640	1280	320
	G-2	5120	5120	<160
	G-3	640	1280	640
	G-4	10,240	20,480	<160
	G-5	<160	<160	<160
	G-6	1280	5120	<160
	G-7	5120	10,240	<160
	G-8	5120	20,480	<160
	G-9	1280	2560	<160
	G-10	640	5120	<160
	G-11	2560	10,240	<160
	G-12	1280	5120	<160
	G-13	640	2560	<160
	G-14	2560	5120	320
Rhesus	R-1	1280	2560	5120
	R-2	640	1280	2560
Hyperimmune guinea pig	Anti ASV4	10,240	20,480	<160
	Anti SV15(O)	<160	<160	5120

Discussion. The satellite HA reaction is inhibited by specific antiserum. This means that a hemagglutination inhibition reaction provides a simple and practical tool for detection and measurement of specific antibodies against type 4 satellite. Our results on the HI antibody distribution indicate that there is a high level of this antibody in normal green monkey sera in high frequency (Tables I and II).

Atchison *et al*(9) reported that an adeno-associated satellite virus isolated from SV15 stock did not agglutinate rhesus monkey, rat or guinea pig red cells. This strain of satellite virus has been identified subsequently as type 1 which differs from type 4 both in its antigenicity and its density(10). Atchison *et al*(9) also found CF antibody against type 1 satellite in approximately 20% of the normal monkey and human sera tested.

The presence of low levels of non-specific inhibitors, which resisted routine physical procedures for removal, has restricted us to limiting positive scoring of sera in the HI test to titers of 160 or greater. Our inability to detect evidence of type 4 satellite antibody in human sera is not inconsistent with the existence of low levels which cannot be detected by the HI test at this time. It is possible that further study of human sera using complement fixation techniques, immunofluorescence(7), or neutralizing antibody titrations, may reveal low levels of type 4 satellite antibodies.

Summary. The HI antibody study presented suggests that the type 4 adeno-associated satellite virus is of simian rather than human origin.

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Received September 7, 1967. P.S.E.B.M., 1967, v126

Ceramide and Ganglioside Accumulation in Farber's Lipogranulomatosis.* (32554)

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Farber's lipogranulomatosis is a rare disorder of infants and children with unique clinical and pathological features. Most of the available information about this disease has been reviewed recently(1). Eight cases have been reported; the fact that two of the patients were sibs suggests that the disorder is genetically determined. The pathological findings include striking granulomata of periarticular and subcutaneous tissues and a neuronal storage process. The granulomata contain a variable number of foam cells. Histochemical studies suggest that both the foam cells and the involved neurons contain glycolipids, although one group of investigators concludes that there was polysaccharide accumulation on the basis of a study of formalin-fixed paraffin-embedded sections(2). Uzman *et al* performed biochemical analyses and reported the accumulation of a lipoglycoprotein(3).

The pathogenesis of Farber's lipogranulomatosis is unresolved. Crocker *et al*(1) have emphasized that there appear to be two seemingly unrelated elements: "a proliferative infiltrative process . . . in the skin, subcutaneous tissues, tendons and viscera, and also an apparently intrinsic nervous system handicap." The first element would tend to place the disorder in the category of the histiocytoses, while the neuronal storage process suggests an inborn error of metabolism, as in the lipidoses. Resolution of this problem requires a decision as to whether lipid accumulation is the cause of the granulomata or is secondary to some other factor which causes tissue proliferation and breakdown.

We will present biochemical analyses of post-mortem tissues from a patient with Farber's lipogranulomatosis. We found a marked excess of ceramide both within granulomatous lesions and in visceral organs in which there was little or no granuloma formation. There was also a moderate accumulation of ganglioside. These findings suggest that lipid accumulation in Farber's lipogranulomatosis is a primary event and that the syndrome should be classified as one of the lipidoses.

Material and methods. A patient (MGH Case #132-24-00) with the typical clinical and pathological features of Farber's lipogranulomatosis died when she was 11 months old.† Tissues from this patient and suitable controls were taken at the time of the autopsy and stored at —50°C. The following control samples were available: liver, 7 samples from patients varying in age from 4 months to 9 years; kidney, 2 and 3 years; lymph node, 5 samples from patients varying in age from 5 days to 4 years; lung, 10 weeks, 6 years.

The tissues were extracted, partitioned, the gangliosides concentrated and the neuraminic acid (NANA) estimated by the methods described by Suzuki(5). Lipid hexose was estimated by a modification of the orcinol method of Sørensen and Haugaard(6), and chloroform:methanol extractable protein was determined by the method of Lowry *et al*(7).

To measure liver mucopolysaccharide con-

* This investigation was supported in part by USPHS Grant NB-02672, and a grant from the National Multiple Sclerosis Society.

† The ocular changes in this patient have already been published(4); a report of the clinical and pathological features is being prepared.