Complement Fixation Reactions between Adenovirus Antigens and Sera from Infectious Hepatitis Cases (33958)

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Several investigators (1–4) isolated adenoviruses of various serological types from patients with infectious hepatitis. Despite the repeated association of adenoviruses with hepatitis, no convincing evidence was obtained to support a direct etiological relationship between these viruses and the disease. To obtain further information about the association between adenoviruses and hepatitis, a study of the occurrence of antibody to adenovirus group complement fixing antigen was undertaken on sera from persons with infectious hepatitis and from persons not clinically ill with hepatitis.

Materials and Methods. Complement fixing antigens. Adenovirus group complement fixing antigen produced in KB cells from adenovirus type 4 (prototype strain, RI67) was obtained from the Biological Reagents Section, National Communicable Disease Center (NCDC). Complement fixing antigen was also prepared from San Carlos no. 8, an atypical adenovirus type 3 representative of a number of adenoviruses previously isolated from patients with infectious hepatitis (1, 3). For antigen production, this virus was grown in tubes of KB cells maintained on Eagle's minimal essential medium with 2% inactivated chicken serum. After 4+ cytopathic effect was observed, tubes were frozen and thawed five times, pooled, and clarified by centrifugation at 2000 rpm for 20 min (model PR-2 International centrifuge). The clarified material was used as antigen. Appropriate control antigens were obtained from the Biological Reagents Sections, NCDC, or prepared from uninoculated KB cells processed in parallel with the San Carlos no. 8 antigen.

Complement fixation method. The Laboratory Branch complement fixation method (macro modification) was used in this study (5) "Vacseal"[1] complement (Probio, Inc., Nyack, New York) and antisheep cell hemolysin, desiccated (Baltimore Biological Laboratory, Baltimore, Maryland), were employed throughout the experiments. Complement was titrated each day that tests were carried out; hemolysin was titrated each time that a different lot of sheep cells was used. Sheep cell suspension was standardized by the centrifugation method (5). All lots of adenovirus antigen were tested in a checkerboard titration against a reference human serum containing adenovirus group complement fixing antibody (obtained from Biological Reagents Section, NCDC) to determine the optimal dilution for use in the actual tests. Each serum included in the study was tested at the same time against both adenovirus group and appropriate control antigens. As a check on the overall validity of the tests, the reference human serum with adenovirus antibody was tested each day against the optimal dilution of antigen.

Human sera. Acute and convalescent sera were available from cases of infectious hepatitis in Cherokee, North Carolina; Portland, Maine; and Bristol, England. The Cherokee specimens were collected during an epidemic on the Cherokee Indian Reservation in 1963 (6). Specimens from Bristol were obtained in December, 1963, and January, 1964; cases at this time were occurring at a reduced rate compared to the high incidence observed in 1960 and 1961 (7; J. F. Skone, personal communication). The Portland sera were collected in December, 1963, and January, 1964, when numerous cases were present in the community (8). The diagnosis of infectious hepatitis was based on the presence of varying combi-

[1] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U. S. Department of Health, Education, and Welfare.

nations of the clinical signs and symptoms of the disease (jaundice, gray stools, dark urine, fever, vomiting, nausea, anorexia, and abdominal discomfort). All of the Cherokee and Bristol patients and 6 of the 9 Portland cases were jaundiced. The 3 anicteric patients from Portland were children, ages 9, 11, and 13 years; each had anorexia, nausea, vomiting, and abdominal discomfort. In addition to the clinical information, serum glutamic pyruvic transaminase levels were determined on the acute sera from all of the Cherokee cases and from 3 of the 9 Portland cases. In all instances, levels greater than 300 Karmen units were demonstrated. Acute sera were obtained within 7–10 days after onset of first symptoms. Convalescent sera were collected 1–2 months after onset of illness. Age ranges of the individuals tested were as follows: Cherokee, 4–18 years; Portland, 9–32 years; Bristol, 6–41 years. Median age for the hepatitis cases was 12 years. Twenty-four pairs of sera were tested with adenovirus type 4 antigen, 18 pairs with San Carlos no. 8 antigen. For comparison, sera provided by the NCDC serum bank from individuals without hepatitis in Los Angeles, California; Houston, Texas; Clearwater, Florida; Cincinnati, Ohio; and Seattle, Washington, were studied. None of these subjects were clinically ill when their sera were collected. However, no information is available about their previous history with regard to infectious hepatitis or adenoviral infections. The sera were collected in connection with the following programs: Los Angeles, Cincinnati and Seattle—measles vaccine studies, 1961–64; Houston—survey for antibodies to encephalitis viruses, 1965; Clearwater—survey for antibodies to encephalitis viruses, 1963. Prevaccination sera were used from the measles vaccine studies. Sera were selected from persons so that the age ranges corresponded approximately to those of the persons with infectious hepatitis. Age ranges were as follows: Los Angeles, 2–11 years; Cincinnati, 5–7 years; Seattle, 5–7 years; Houston, 15–35 years; Clearwater, 15–35 years. Median age for the individuals without hepatitis was 16 years. Forty-eight single sera were tested with adenovirus type

TABLE I. Complement Fixation Results with Paired Sera from Infectious Hepatitis Cases against Adenovirus Type 4 and San Carlos No. 8 Antigens.

No. of pairs showing	Sera tested against adenovirus type 4 antigen				Sera tested against San Carlos no. 8 antigen			
	Bristol	Portland	Cherokee	Total	Bristol	Portland	Cherokee	Total
Twofold increase in titer	0	1	2	3	0	0	2	2 (3)[b]
Fourfold increase in titer	0	0	3	3	0	0	2	2 (2)
Decrease in titer[a]	2	3	1	6	0	3	1	4 (5)
Stable titer	1	3	1	5	1	3	1	5 (2)
No complement fixing antibody (<1:10)	4	2	1	7	2	2	1	5 (6)
Totals	7	9	8	24	3	8	7	18 (18)

[a] All decreases in titer observed were twofold.

[b] Values in parentheses are results on the same 18 pairs tested with adenovirus type 4 antigen.

TABLE II. Complement Fixation Titers of Hepatitis and Nonhepatitis Sera against Adenovirus Type 4 Antigen.

Complement fixation titers	Hepatitis sera				Nonhepatitis sera	
	Acute		Convalescent		Single	
	No.	(%)	No.	(%)	No.	(%)
<1:10	9	37	8	33	23	48
1:10	3	13	4	17	8	17
1:20	5	21	3	13	7	15
1:40	7	29	7	29	8	17
1:80 or greater	0	0	2	8	2	4
1:10 or greater	15	63	16	67	25	52
Geometric mean titer	1:14		1:16		1:12	

4 antigen, 18 with the San Carlos no. 8 antigen.

Results. The reactions obtained with adenovirus type 4 and San Carlos no. 8 antigens against paired sera from infectious hepatitis cases are summarized in Table I. Three of 24 pairs showed fourfold increases in titer with the adenovirus type 4 antigen. Two fold increases were observed with 3 additional pairs. Five of the 6 pairs showing an increase in titer, including all those with fourfold increases, were collected in Cherokee, North Carolina. With the San Carlos no. 8 antigen, fourfold increases in titer were observed with 2 of the 18 pairs of sera and twofold increases with 2 other pairs. All 4 of these pairs were again from Cherokee, North Carolina. Most of the paired sera studied showed decreases in titer, stable titers or no complement fixing antibody at the 1:10 dilution with both antigens. Sera from only 2 individuals showed conversions from <1:10 to 1:20 or higher.

A summary of the complement fixation titers against adenovirus type 4 antigen with the sera from both hepatitis cases and individuals not clinically ill with hepatitis is shown in Table II. Titers of 1:10 or greater were obtained on 63% of the acute sera, 67% of the convalescent sera, and 52% of the nonhepatitis sera. These percentages, however, are not significantly different (chi-square test with $p = .05$). In addition, the distributions of titers (and, therefore, the geometric mean titers) for the three groups are not significantly different (chi-square test).

The reactions of San Carlos no. 8 antigen with hepatitis and nonhepatitis sera are summarized in Table III. Titers of 1:10 or greater were found on 67% of the acute sera, 61% of the convalescent sera, and 56% of the nonhepatitis sera. As with the adenovirus type 4 antigen, these percentages are not significantly different (chi-square test with $p = .05$). Similarly, the distributions of titers among the three groups and their geometric mean titers are not significantly different (chi-square test).

Throughout the study there were no marked differences between the reactions observed with the adenovirus type 4 and San Carlos no. 8 antigens. With the 18 pairs of sera from hepatitis cases which were tested against both antigens, essentially the same number of pairs showed increases or decreases in titer (last column, Table I). Comparison of the reactions of the hepatitis and nonhepatitis sera tested against both antigens revealed no significant differences in the number of positives, the distributions of titers, or the geometric mean titers (Table III). Twenty-seven sera from the hepatitis cases and 12 sera from the nonhepatitis group gave exactly the same titers with the two antigens.

The complement fixation titer of the reference human antiserum included in each test was quite reproducible. Values of either 1:128 or 1:256 were obtained in all the tests with both adenovirus type 4 and San Carlos

TABLE. III. Complement Fixation Titers of Hepatitis and Nonhepatitis Sera against San Carlos No. 8 Antigen.

Complement fixation titers	Hepatitis sera				Nonhepatitis sera	
	Acute		Convalescent		Single	
	No.	(%)	No.	(%)	No.	(%)
<1:10	6 (7)[a]	33 (39)	7 (8)	39 (44)	8 (9)	44 (50)
1:10	3 (3)	17 (17)	1 (2)	6 (11)	2 (1)	11 (6)
1:20	4 (3)	22 (17)	3 (2)	17 (11)	2 (4)	11 (22)
1:40	4 (5)	22 (28)	5 (4)	28 (22)	5 (4)	27 (22)
1:80 or greater	1 (0)	6 (0)	2 (2)	11 (11)	1 (0)	6 (0)
1:10 or greater	12 (11)	67 (61)	11 (10)	61 (56)	10 (9)	56 (50)
Geometric mean titer	1:14 (1:13)		1:17 (1:14)		1:14 (1:12)	

[a] Values in parentheses show the reactions obtained with adenovirus type 4 antigen on the same 18 serum pairs and 18 single sera.

no. 8 antigens. Two pairs of sera from known adenoviral infections previously found to show a fourfold increase in titer to adenovirus group complement fixing antigen by another laboratory also showed similar fourfold increases in titer with our procedure. All human sera tested showed titers of <1:10 with appropriate control antigens.

Discussion. The results showed no significant differences in the incidence of adenovirus group complement fixing antibody or the geometric mean complement fixation titers with sera from cases of infectious hepatitis and sera from persons not clinically ill with hepatitis. The sera from the individuals without hepatitis, however, did not represent optimal controls for the hepatitis sera tested. Ideally, control sera should have been obtained concurrently from persons with no clinical or biochemical evidence of current hepatitis and with no history of hepatitis. These persons should have been from the same general populations and from the same age groups as those which yielded the hepatitis sera. Unfortunately, such control sera were not available.

Other reports have indicated the incidence and titers of adenovirus group complement fixing antibody found in sera from individuals not suffering from hepatitis (9–12). In certain United States military personnel and their dependents, the incidence of antibody varied from 21% in a 1-year-old group to 74% in an 18–25-year-old group composed mainly of soldiers recently completing basic training (10). Children in Sheffield, England, showed an incidence of antibody varying from 29% at 5–11 months of age to 50% at 8 years of age (12). Geometric mean titers of 1:2 to 1:12 were found in groups of new military recruits (9, 11). Although our data and the results from these studies cannot be directly compared, both the incidence of antibody and the geometric mean titers observed by us are similar to those reported previously in individuals without hepatitis.

Four hepatitis cases, all from Cherokee, North Carolina, showed fourfold increases in adenovirus group complement fixing titer from acute to convalescent sera; in two of these, conversions from <1:10 to 1:20 or higher were observed. The remainder of the cases fell into one of three categories: (i) those showing twofold increases or decreases in titer; (ii) those showing stable titers; or (iii) those with no complement fixing antibody detectable at 1:10 dilution. Since a twofold fluctuation in titer is not considered significant, the results on paired sera as a whole fail to support the involvement of adenoviruses in the etiology of infectious hepatitis by the usual virological criteria. Anamnestic responses or antibody persisting from previous adenoviral infections could account for most, if not all, of the complement fixation reactions obtained. Similar conclusions were reached about neutralization test results with San Carlos no. 8 virus against paired sera

from hepatitis cases (3). The reasons for the occurrence of fourfold increases in complement fixation titer only in the cases from Cherokee, North Carolina, are not known. However, since all four of these cases were in children (ages 4, 6, 11, and 12 years) in the fall and early winter, intercurrent adenoviral infections were possible.

Several factors could have influenced the complement fixation reactions observed. For example, although group complement fixing antibody is not usually detectable in typical adenoviral infections until about 7 days after onset of illness (13), antibody might have already developed in some of the hepatitis cases by the time the acute sera were collected, and thus might have prevented demonstration of significant increases in titer. Also, the group complement fixation test was less sensitive than either the neutralization or the hemagglutination–inhibition test in the serodiagnosis of adenoviral infection (14, 15). Because of this lack of sensitivity, some significant increases in antibody titer in the hepatitis cases might have gone undetected. Lastly, to conserve serum, the lowest dilution used in the tests was 1:10. Consequently, some low complement fixing titers might have been overlooked.

In general, the results obtained fail to relate adenoviruses to the etiology of infectious hepatitis. Throughout the study, no significant differences were evident between the reactions with the San Carlos no. 8 and adenovirus type 4 antigens, despite the fact that the San Carlos no. 8 virus was originally isolated from a case of infectious hepatitis (1, 3).

Summary. Complement fixation tests were performed on paired sera from infectious hepatitis cases and on single sera from persons not clinically ill with hepatitis. Antigens for the tests were prepared from prototype adenovirus 4 and from San Carlos no. 8, an atypical adenovirus type 3 isolated from a case of infectious hepatitis. Incidence of antibody varying from 61 to 67% was observed with the two antigens against acute and convalescent hepatitis sera. Geometric mean titers ranged from 1:14 to 1:17. However, only 4 fourfold increases in titer were demonstrated; twofold increases or decreases in titer and stable titers occurred most frequently. Incidence of antibody in control sera collected in various other geographic areas was 52 and 56% against adenovirus type 4 and San Carlos no. 8 antigens, respectively, with geometric mean titers of 1:12 and 1:14. These findings were not significantly different from those with the hepatitis case sera. No significant differences were observed between the reactions with prototype adenovirus 4 and San Carlos no. 8 antigens, despite the fact that the latter virus was isolated from a case of hepatitis. No clear evidence was obtained to indicate direct involvement of adenoviruses in the etiology of infectious hepatitis.

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