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Comparison of Serological Tests for Antibody to Hepatitis A Antigen, Using Coded Specimens from Individuals Infected with the MS-1 Strain of Hepatitis A Virus

JULES L. DIENSTAG,[1] SAUL KRUGMAN, DORIS C. WONG, AND ROBERT H. PURCELL*

Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014, and New York University School of Medicine, New York, New York 10016*

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To compare serological tests for antibody to hepatitis A antigen (anti-HA), we tested 15 paired serum specimens, submitted under code, from individuals infected with the MS-1 strain of hepatitis A virus. Immune electron microscopy (IEM), immune adherence hemagglutination (IAHA), and solid-phase radioimmunoassay (RIA) tests for anti-HA were performed with hepatitis A antigen (HA Ag) derived from human stool; results were also compared with previously reported titers determined by IAHA with HA Ag derived from marmoset liver. Antibody titers (IAHA and RIA) and ratings (IEM) determined with stool-derived HA Ag compared favorably, and a seroresponse to HA Ag was detected by all three methods for every serum pair tested. Differences in titers were noted between IAHA tests with liver-derived and with stool-derived HA Ag, but the discrepancies could be accounted for by differences in test technique. The agreement found in this study among the three techniques was quite good and confirms the specificity and sensitivity of tests for anti-HA that are done with stool-derived HA Ag.

Recently and in rapid succession, several in vitro serological tests have been developed to identify hepatitis A virus (HAV) infection. In 1973, using acute-phase stools from patients with type A hepatitis as a source of antigen, Feinstone et al. (6) described an immune electron microscopy (IEM) assay for antibody to hepatitis A antigen (HA Ag). Using HA Ag derived from the livers of marmosets with experimental HAV infection, Provost et al. (12) developed a complement fixation (CF) test and Miller et al. (10) developed an immune adherence hemagglutination (IAHA) test for antibody to HA Ag (anti-HA). Also described for the detection of HA Ag were solid-phase radioimmunoassays (RIAs) that could be modified for the detection of anti-HA (7, 13), and Moritsugu et al. (11) modified the IAHA test for use with HA Ag derived from acute-phase stools obtained from patients with type A hepatitis. The specificity of each of these tests for HAV infection as opposed to hepatitis B virus infection was demonstrated in the respective original reports; however, questions have been raised about the validity of any test that requires antigen purified from stool (1) and about attempts to quantitate antibody ratings by IEM (2). In the following study we compared three tests, IEM, IAHA, and RIA, in which human stool was the source of HA Ag, and assessed their specificity and sensitivity by testing under code paired serum samples from patients infected with a well-characterized and pedigreed strain of HAV, the MS-1 strain (9). Because the superiority of IAHA over CF has already been demonstrated (8), we did not include CF in this comparison.

MATERIALS AND METHODS

Serum samples. Preinoculation and convalescent serum samples (range, 2.5 months to 7 years after infection) were selected from 15 children who had type A hepatitis caused by the MS-1 strain of HAV (9). Serial serum specimens had been stored at −20°C for periods ranging between 5 and 10 years. All samples were randomized and coded in one laboratory and sent to the other investigators for testing. Before use, each sample was diluted 1:10 in Veronal-buffered saline supplemented with 0.1% bovine serum albumin. Each test was performed without knowledge of the results of the other two tests.

Preparation of HA Ag. For IEM, a pool of 2% stool filtrates from a patient with experimental HAV infection (Joliet Prison study) (2) was used and prepared as described (6). Approximately 25 to 50 HA Ag particles per electron microscope grid square were observed when this stool pool was used for IEM. For IAHA and RIA tests, HA Ag was purified by ultracentrifugation in cesium chloride and sucrose gradients (11) from acute-phase stools of

[1] Present address: Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA 02114.

humans involved in a foodborne outbreak of HAV (5). In an earlier study, the HA Ag samples from both the experimental and natural sources used here were shown to be immunologically indistinguishable (5).

IEM. Detection of anti-HA was performed by IEM as described by Feinstone et al. (6). Quantitation of antibody was based on the density of the antibody halo surrounding HA Ag particles and rated on a scale of 0 to 4+ as follows: 0, no antibody-coated particles observed; 1+, particles with distinct surfaces, lightly coated with antibody; 2+, particles with moderately blurred surfaces, moderately coated with antibody; 3+, particles with indistinct surfaces, heavily coated with antibody; 4+, particles with surfaces obscured by a very dense coating of antibody. Because the examination of serial dilutions for end-point titration would be too cumbersome by IEM, a single 1:10 dilution of each serum was evaluated. Studies in which serial dilutions of serum have been done indicate that a 10-fold dilution of serum yields an approximate decrement of 1+ in antibody rating (4, 5). A 1+ increment in rating between pre-illness and convalescent samples was considered a significant seroresponse, roughly equivalent to a 10-fold rise in antibody titer.

IAHA. Serum samples were tested for anti-HA by IAHA against 4 IAHA units of HA Ag according to the procedure described by Miller et al. (10) but modified by Moritsugu et al. (11) for use with antigen partially purified from acute-phase stools of humans with naturally acquired HAV (5). Each serum sample was diluted 1:10 in Veronal-buffered saline with 0.1% bovine serum albumin and heated in a 56°C water bath for 30 min to inactivate complement. All sera were screened initially at dilutions of 1:10, 1:100, and 1:1,000 to detect prozones. End-point titrations were performed with microtiter loops on samples positive at any dilution; prediluted samples were used for titration, and twofold dilution series did not exceed four dilutions. A hemagglutination pattern of 3 or 4 on a scale of 0 to 4+ was considered positive if the corresponding buffer control well was free of hemagglutination.

After the code was broken, antibody titers obtained with this IAHA test were compared with published titers found on the same sera with an IAHA test using marmoset liver-derived HA Ag as an antigen source (8). In the latter test, serum was diluted in serial twofold increments from 1:5 to 1:81,920.

RIA-B. A micro-solid-phase RIA for the detection of HA Ag was modified to permit detection of antibody by demonstrating blocking of the reaction of antigen with [^{125}I]anti-HA (13). Briefly, 5 RIA units of HA Ag (five times the minimum quantity sufficient to score as positive in a RIA test) was added to previously sensitized wells of a microtiter plate. After incubation, the wells were washed, serial 10-fold dilutions of the serum to be tested were added to respective wells, the plates were incubated overnight at 4°C, the wells were washed again, and radiolabeled anti-HA was added. After incubation and washing to remove unbound [^{125}I]anti-HA, the individual microtiter wells were cut apart and counted in a gamma spectrometer. Inhibition ("blocking") of binding of [^{125}I]anti-HA to the solid phase by a factor of 40% when compared with anti-HA-negative control serum was taken as evidence of anti-HA activity in the test serum. The RIA-blocking (RIA-B) titer was the highest dilution of a serum that exhibited such inhibition; exact RIA-B titers were extrapolated from the RIA-B curve for that serum.

RESULTS

Antibody titers determined with human stool-derived HA Ag by IAHA, RIA-B, and IEM are presented in Table 1 and compared with previously reported IAHA titers that had been determined with marmoset liver-derived HA Ag. Antibody titers and ratings determined with stool-derived HA Ag on 10-fold or greater dilutions of serum by the three methods compared favorably, and a seroresponse to HA Ag was detected by all three methods for every serum pair tested. Antibody titers determined by IAHA against liver-derived HA Ag were comparable or lower than IAHA titers against stool-derived HA Ag in 6 of the 15 serum pairs hairline; however, liver-derived HA Ag anti-HA titers were significantly higher in the other nine.

DISCUSSION

This study confirms the suitability of partially purified stool-derived HA Ag (11) as antigen in two recently described tests for anti-HA (11, 13). The preparation of HA Ag used in these tests had been obtained by isopycnic banding twice in cesium chloride followed by rate-zonal separation in a sucrose gradient and was sufficiently pure for both IAHA and RIA-B tests, without significant nonspecific reactivity. In one case (patient 4), however, preinoculation serum contained minimal blocking antibody; indeed, preinoculation serum samples varied in their ability to block the reaction between HA Ag and [^{125}I]anti-HA, although only patient 4 blocked such binding by a factor of 40% or more; such variation was observed previously (13). Most likely these low-level nonspecific reactions represent binding of antibody in these sera to stool components that contaminate the partially purified HA Ag used. Theoretically, such nonspecificity may be eliminated by one or both of the following: (i) using antigen that has been additionally purified or (ii) using ^{125}I-labeled immunoglobulin G purified from hyperimmune antiserum raised against liver-derived HA Ag. However, the HA Ag and [^{125}I]anti-HA reagents used in this study were sufficiently specific to distinguish under code between preinoculation and convalescent samples and to demonstrate a serological response in every serum pair.

TABLE 1. *Anti-HA in paired serum samples from individuals infected with the MS-1 strain of hepatitis A virus: comparison of tests*

Patient	Pre- or post-hepatitis A	Anti-HA as measured by indicated method			
		IAHA[a]	IAHA[a]	RIA-B[b]	IEM[c]
1	Pre	<5	<10	0	0
1	Post (6 months)	2560	16,000	174	3+
2	Pre	<5	<10	0	0
2	Post (2.5 months)	≥81,920	≥64,000	3055	2+
3	Pre	<5	<10	0	0
3	Post (4 years)	40,960	4,000	1413	2+
4	Pre	<5	<10	13	0
4	Post (7.5 months)	40,960	16,000	49	2-3+
5	Pre	<5	<10	0	0
5	Post (9 months)	20,480	8,000	4169	1-2+
6	Pre	<5	<10	0	0
6	Post (4 months)	20,480	4,000	18	2+
7	Pre	<5	<10	0	0
7	Post (10 months)	10,240	8,000	169	1-2+
8	Pre	<5	<10	0	0
8	Post (10 months)	≥81,920	2,000	10	1+
9	Pre	<5	<10	0	0
9	Post (1 year)	≥81,920	8,000	347	1-2+
10	Pre	<5	<10	0	0
10	Post (8 years)	1,280	2,000	173	2-3+
11	Pre	<5	<10	0	0
11	Post (11 months)	≥81,920	16,000	1462	2-3+
12	Pre	<5	<10	0	0
12	Post (6 years)	5,120	4,000	1884	2+
13	Pre	<5	<10	0	0
13	Post (7 years)	20,480	4,000	1429	3-4+
14	Pre	<5	<10	0	0
14	Post (7 years)	2,560	2,000	813	3+
15	Pre	<5	<10	0	0
15	Post (3 months)	20,480	8,000	140	1+

[a] HA Ag was extracted from the livers of *Saguinus mystax* marmosets; these results were published previously (8).

[b] HA Ag was partially purified from stools of patients who were acutely infected with HAV (4, 11).

[c] HA Ag was derived from 2% filtrates of stools from patients with experimental HAV infection (3, 6).

When we compared IAHA anti-HA titers obtained with liver-derived and stool-derived HA Ag, a tendency toward significantly higher titers was observed with liver-derived antigen. Since in other published comparisons (5a, 13) the two IAHA antigens have been shown to be comparable, the differences noted here may derive from a difference in test technique. In tests with liver-derived antigen, a 1:2.5 dilution of serum was serially diluted 15 times with a microdiluter, a practice dictated by the scarcity of liver-derived HA Ag at that time but one that, because of the viscosity of serum, introduces an additive error yielding higher titers. Tests in which stool-derived antigen were used employed serum prediluted to within three to four dilutions of the end point before subsequent microdilution.

By subjecting these antibody tests to a coded study of carefully pedigreed paired sera, we have demonstrated their validity; each of the tests can be used reliably for a diagnosis of HAV. Although agreement among the tests using stool-derived HA Ag was good, several differences emerged. As observed earlier by Purcell et al. (13), RIA-B antibody titers tended to be lower than IAHA antibody titers, and IEM ratings did not correspond linearly with IAHA titers; however, reasonably good correlation was observed, a finding in agreement with previous observations (4). In other comparative studies with the new tests for anti-HA, the kinetics of antibody development were different when measured by CF and IAHA (8, 10) or by IEM and IAHA (4). Considering, then, that each technique may be measuring different antibodies to HA Ag, the agreement found in this study was quite good. However, based on specificity, sensitivity, and ease of application to large numbers of serum samples, the IAHA

technique was the most useful for serological evaluation of type A hepatitis, regardless of the source of antigen.

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