

Hepatitis A Virus Hemagglutination and a Test for Hemagglutination Inhibition Antibodies

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Received 22 December 1988/Accepted 16 February 1989

Like enteroviruses, hepatitis A virus (HAV) hemagglutinated various species of erythrocytes under similar conditions. HAV-specific antibodies in both acute- and convalescent-phase sera were found to inhibit hemagglutination. The HAV hemagglutination inhibition test can be used for diagnosis, epidemiological surveillance, and vaccine assessment.

On the basis of its biophysical and biochemical characteristics, hepatitis A virus (HAV) has been classified as a picornavirus in the *Enterovirus* genus (4). A number of enteroviruses agglutinate erythrocytes, and this property of hemagglutination (HA) has been used to test for specific antibodies in the HA inhibition (HAI) test (5). The source of the hemagglutinin is usually untreated, infectious cell culture harvests that are free of serum. In the current study, the HM175 strain of HAV, adapted to MRC-5 cells, was found to hemagglutinate various species of erythrocytes under conditions similar to those under which other enteroviruses do so.

HAV (HM175 strain) was grown in MRC-5 cells as previously described (1). Clarified HAV-infected cell culture fluids were concentrated by centrifugation at $63,000 \times g$ for 2 h. The virus pellets were suspended in 6.7 mM phosphate-buffered saline (pH 7.4) to 1:100 of the original volume. The HAV concentrate was stored at 4°C until it was used for HA tests. A control antigen preparation of uninfected MRC-5 cell culture fluids was prepared identically.

Factors known to influence HA by enteroviruses include temperature, pH, and the species of erythrocyte used in the test. For HAV, the optimal incubation temperature for HA appeared to be 4°C for tests done with human O cells (Table 1). The optimal pH range varied for the species of erythrocyte used in the test. With human O cells, the pH optimum was limited to 5.4 to 5.6; for goose erythrocytes, this range extended to pH 6.0. HAV also had a broad range of susceptible cells that were agglutinable, including human, guinea pig, and goose erythrocytes. Additionally, human A cells were susceptible to HA by HAV (data not shown). MRC-5 control cell fluids, concentrated identically to the HAV-infected fluids, did not hemagglutinate any of the erythrocyte species.

Inhibition of HAV-specific HA can be used to measure antibody responses to HAV infection. These assays were performed by a microdilution modification of methods described by Clarke and Casals (2) and Melnick et al. (5). All sera used in the tests were adsorbed with kaolin (25%) at room temperature for 30 min to remove nonspecific inhibitors of HA. The sera were also adsorbed with human O cells to remove natural agglutinins prior to testing. Various sera from humans (obtained from the Hepatitis Serology Laboratory, Department of Virus Diseases, Walter Reed Army Institute of Research) and a chimp known to have been infected with HAV (National Institutes of Health reference

reagents, catalog numbers V811-801-573 and V811-501-573) contained HAI antibodies to HAV (Table 2 and Fig. 1). A commercially available human immune serum globulin preparation (Gammar, lot no. C11905; Armour Pharmaceutical Co.) was also tested and found to be positive. Both acute- and convalescent-phase sera had significant titers of HAV-specific antibodies (range, 640 to 5,120). A human acute-phase serum specimen (JV271) was obtained from a patient with hepatitis less than 1 month after the onset of disease; convalescent-phase sera were routine clinical specimens obtained from healthy individuals. The same sera were tested in commercial enzyme-linked immunosorbent assays used for HAV diagnosis (Connaught Laboratories, Inc., Toronto, Ontario, Canada). The results are shown in Table 2 for both total immunoglobulins and immunoglobulin M (IgM) antibodies. The acute-phase human serum, which contained IgM, had a high HAI titer. This result indicates that the HAI test may be useful in detecting early serum responses to HAV infection and so would be useful for diagnosis.

Although HA has been described for other enteroviruses, this is the first report of this phenomenon for HAV. Since the first description of cell culture adaptation and growth of HAV in monkey diploid cells (6), interest has flourished in characterizing this virus and attempting to understand more about the disease and how to prevent it. The source of the hemagglutinin used in this study was MRC-5 cell-adapted HAV, which can be grown to titers of approximately 10^7 infectious units per ml of cell culture lysates. On the basis of electron microscopy studies of similar HAV preparations, such viral harvests contain approximately 10^9 HAV particles (J. Ticehurst, personal communication). In our studies, HAV harvests without concentration were just below the detectable level of HA. Other strains of HAV which may replicate to higher titers in cultures or the use of techniques to increase the concentration of HAV in cell culture fluids may result in more readily available preparations of hemagglutinin for various studies.

Chemical treatment of HAV with Formalin results in a loss of infectivity but a retention of antibody-binding activity as well as immunogenicity (1). Formalin-inactivated HAV vaccine has been used successfully to immunize various laboratory animals and, more recently, humans (7). However, this procedure destroys HA activity (data not shown). HA activity is most likely a surface phenomenon that is dependent on one or more capsid proteins. Analysis of the HAV virion surface with specific monoclonal antibodies will be important for understanding the HA phenomenon.

Complete inactivation of infectious HAV with the reten-

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TABLE 1. Effect of erythrocyte species, pH, and temperature on the HAV HA test

Erythrocyte species	pH	Temp (°C)	HA titer
Human O	5.4	4	64
	5.5		64
	5.6		64
	5.7		16
	6.0		<2
Guinea pig	5.4	4	128 ^a
	5.5		128 ^a
	5.6		128 ^a
	5.7		128
	6.0		<2
Goose	5.4	4	128
	5.5		128
	5.6		128
	5.7		128
	6.0		128
	6.2		<2

^a Partial hemolysis.

tion of HA activity is desirable prior to general use of the HA or HAI test in diagnostic laboratories. HA activity is significantly reduced when HAV is treated with 0.05% Formalin or heated at 100°C for 5 min. Other inactivation procedures that preserve HAV HA are being assessed.

A practical application of HAV HA is to use it to test various sera for HAV-specific antibodies that would inhibit the HA reaction. The HAI test is a simple, inexpensive, and rapid technique for the serological identification of HAV infections. In selected sera, there was a complete correlation with the results of commercially available assays for both acute- and convalescent-phase sera. The appearance of IgM

TABLE 2. HAI test for HAV-specific antibodies

Serum	HAI titer ^a	Total immunoglobulins ^b	IgM ^c
Human 258 (normal)	<10	—	—
Human 274 (normal)	<10	—	—
Human 270 (HAV convalescent phase)	1,280	+	—
Human 335 (HAV convalescent phase)	640	+	—
Human JV271 (HAV acute phase)	2,560	+	+
Human ISG ^d	5,120	+	+
Chimp (pre-HAV)	<10	—	—
Chimp (post-HAV)	640	+	+

^a Reciprocal of the dilution that inhibited 8 units of HAV hemagglutinin.

^b The test serum was considered positive for anti-HAV antibody if absorbance values were less than or equal to the cutoff value. The cutoff value was determined by dividing the sum of the negative control and positive control mean absorbance values by 2.

^c The test serum was considered positive for IgM antibody if absorbance values were equal to or greater than the cutoff value. The cutoff value was determined by adding 0.10 to the mean absorbance value of the negative control.

^d ISG, Immune serum globulin.

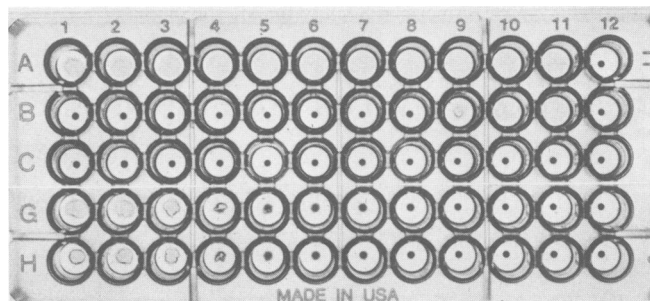


FIG. 1. HAI test. Row A, Chimp pre-HAV serum; row B, chimp post-HAV serum; row C, human immune serum globulin. All sera were diluted twofold starting at a 1:10 dilution in column 1. Column 12 is a cell control containing a 1:10 dilution of test serum and human O cells. Rows G and H, Titration of HAV HA antigen starting with a 1:2 dilution in column 1.

after HAV infection is used as a confirmation of infection. The HAI test may therefore be used as a substitute for an enzyme-linked immunosorbent assay or a radioimmunoassay for the detection of HAV-specific IgM. The HAI test could be modified to detect specific immunoglobulins such as IgM. One technique described involves the capture of IgM from sera onto a solid phase with anti-IgM followed by a HAI test (8). Immune adherence HA is another serological test for HAV antibodies that is sensitive and quantitative (3). However, this test does not detect early antibodies and often does not become positive until 4 to 6 weeks after the illness.

We thank M. Sjogren for providing the human sera. The technical assistance of R. Timchak, D. Barvir, N. Bristol, and B. Martinez is gratefully acknowledged.

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