Immune Adherence Hemagglutination Test Applied to the Study of Herpes Simplex and Varicella-Zoster Virus Infections

ASMITA GILLANI AND LESLIE SPENCE*

Department of Microbiology, Toronto General Hospital, Toronto, Ontario, Canada M5G 1L7

Received for publication 27 September 1977

The immune adherence hemagglutination (IAHA) test has been used successfully to detect antibody to herpes simplex (HS) virus and varicella-zoster (V-Z) virus. Comparative studies between the complement-fixation (CF) test and the IAHA test revealed that, in most cases, the IAHA test was more sensitive than the CF test. Furthermore, diagnosis on the basis of a fourfold change in antibody titer was made more rapidly by the IAHA test. The IAHA test was found to be a very simple and practical technique requiring only a few hours for completion compared with the conventional CF test which required up to 24 h. In addition, both sera and cerebrospinal fluids could be tested in very low dilutions in the IAHA test, so that very low levels of antibody could be detected. Also, the IAHA test detected antibody to V-Z virus more frequently than did the CF test in adults with a history of varicella occurring 9 to 30 years prior to sampling. The level of cross-reaction between HS and V-Z viruses was examined by both the CF test and the IAHA test, and no major differences between the two techniques were found.

The technique of immune adherence hemagglutination (IAHA) is useful for the study of the immune response of the host to both viral and bacterial pathogens. It is simple to perform and relatively free of nonspecific reactions, and it can be used to detect either antigen or antibody (3-8). It has been adapted for the detection of varicella-zoster (V-Z) antibody in serum specimens (2), and more recently it has also been applied to the cytomegalovirus antigen-antibody system (1).

In 1966 Ito and Tagaya reported the potential of the IAHA technique in serodiagnosis of viral disease, and their preliminary findings showed that the IAHA test could be applied to herpes simplex (HS) virus (3). This report describes the application of the IAHA technique to antibody studies on sera and/or cerebrospinal fluids (CSF) received from patients with a clinical history of infection with either HS virus (type 1 and type 2) or V-Z virus.

MATERIALS AND METHODS

Buffers. Veronal buffer with 0.1% bovine serum albumin (VB-BSA) was used as diluent throughout the assay. A stock of 5× Veronal buffer was prepared as described by Gershon, Kalter, and Steinberg (2). One volume of the stock buffer was diluted as needed by adding 4 volumes of distilled water containing 0.125% bovine serum albumin. The final pH of the buffer was 7.4.

DTT-EDTA-VB. Ethylenediaminetetraacetic acid

(EDTA), 0.10 M, was prepared in distilled water and adjusted to pH 7.5. A solution containing 2 volumes of EDTA and 3 volumes of VB-BSA buffer was prepared, and to this a 3-mg/ml solution of the solid dithiothreitol (DTT; Sigma Chemical Co.) was added. This solution was also prepared freshly when needed.

Complement. Lyophilized guinea pig complement (GIBCO) was reconstituted with distilled water and stored in small volumes at -70°C. Dilutions of complement were made in VB-BSA buffer. Each batch was titrated to find the optimal dilution of complement which gave a 4+ agglutination of erythrocytes (RBC) with optimal dilutions of antigen and antibody. Usually, a 1:100 dilution was used.

Antigen. All antigens except HS antigen were obtained from a commercial source (Microbiological Associates). The HS antigen was obtained from the Laboratory Centre for Disease Control in Ottawa. Optimal titers were determined from standard checkerboard titrations (8).

RBC. Human group O cells obtained from Ortho Diagnostics were used in the HS and V-Z virus systems. The RBC were washed twice in normal saline and twice in the VB-BSA buffer.

Specimens. Twenty-five specimens (sera and CSF) were obtained from seven patients with clinical diagnoses of either HS or V-Z virus infection. Sera and CSF were inactivated by heating at 56°C for 0.5 h prior to testing.

CF test. A modification of the microtiter complement fixation (CF) procedure described by Sever (9) was used. The sheep cell concentration was reduced to a final concentration of 0.2%, which was a modification of the method suggested by Zissis and Clinet (11).

IAHA test for antibody detection. Serial dilutions (1:2 to 1:256) of serum and CSF were made in VB-BSA (0.025 ml/well) in microplates. Tissue culture grade V plates (Linbro) were used. Two sets of dilutions were made for each test, one to which the antigen was added and the other as a control. Antigen at the predetermined dilution was added in 0.025-ml amounts to all test wells except the controls, to which 0.025 ml of diluent was added. The plates were shaken for 10 s on a micromixer and were incubated in a water bath at 37°C for 1 h. Complement at the appropriate dilution was added to each well (0.025 ml/well). The plates were shaken again for 10 s and allowed to incubate for a further 40 min at 37°C.

DDT-EDTA-VB was added next (0.025 ml/well), and the plates were shaken for 10 s. Finally, a 1% suspension of RBC in VB-BSA was added (0.025 ml/well), and the plates were shaken for 60 s and left at room temperature for the hemagglutination to develop. Usually within 30 min a pattern formed, but final readings were not made until 1 h later. The pattern did not change significantly upon further standing for 2 to 3 h or overnight. Hemagglutination ranged from 0 to 4+, and a reading of 3+ and 4+ was taken as positive, provided the controls did not show nonspecific hemagglutination. Antigen, complement, and RBC controls, as well as the laboratory reference antisera, were always included in each test.

RESULTS

Comparison of IAHA titers and CF titers. The specimens were tested for antibody to HS and V-Z by both CF and IAHA techniques (Table 1). The antibody titers were found to be higher by the IAHA test than by the CF test. The convalescent antibody levels were between 2 and 32 times higher by the IAHA test.

When daily serum specimens were obtained from a patient with fatal herpes encephalitis (case no. 6, Table 1) it was found that the IAHA antibody titer increased more rapidly than did the CF antibody titer. Similarly, serial CSF specimens from another patient with nonfatal herpes encephalitis (case no. 7, Table 1) also demonstrated the same phenomenon; that is, the IAHA antibody titer in the CSF increased more rapidly than did the CF antibody titers. This same trend also occurred in a patient who had aseptic meningitis (case no. 5, Table 1) associated with a herpes group virus infection. In this patient the titer of IAHA antibody to both HS and V-Z had increased faster than the corresponding CF antibody titer.

Determining the immune status to V-Z. Six members of our laboratory staff who had histories of varicella 9 to 30 years prior to testing were tested for V-Z antibody by both CF and IAHA techniques. All six had detectable V-Z antibody by IAHA, with titers ranging from 1:4 to 1:512. Three were negative by CF, and of the three that had CF antibody the levels were

lower, ranging from 1:8 to 1:64.

Cross-reactions between V-Z and HS antigens. Some of the sera were tested to determine whether a rise in V-Z antibody titer, demonstrable by IAHA and CF, occurred during infection with the HS virus or whether a rise in HS antibody titer occurred during infection with V-Z virus. Our findings (Table 2) showed that, when a change in antibody titer to the heterologous virus occurred in the CF test, it also occurred in the IAHA test. One of the patients in whom the diagnosis was based entirely on serology showed increases in antibody titers to both HS and V-Z by both techniques (case no. 5, Table 1) so that neither the IAHA nor the CF test could demonstrate conclusively the causal agent of the illness. Both tests showed the antibody titer to V-Z to be rising faster than the antibody to HS, although the IAHA antibody titers were much higher than the CF titers. In other instances, when the CF titers of the heterologous antibody remained unchanged the IAHA titer did not change.

DISCUSSION

The IAHA test consists of the reaction between antigen and antibody, the reaction of the antigen-antibody complex with complement resulting in the activation of the C3 component of complement, and, finally, the interaction of the receptor sites located on human erythrocytes and the C3 component, resulting in IAHA. This reaction, which was described by Nelson (8) in 1953, is being used more frequently to detect viral antigens and antibody. There has been a great deal of interest in the technique among workers in the field of viral hepatitis, and it has been used to detect antigens and antibodies associated with hepatitis A virus (4, 6, 7), hepatitis B surface antigen (5), and hepatitis B core antigen and antibody (10).

Our data confirm the preliminary findings of Ito and Tagaya (3) and show that the IAHA test could be of great value for the diagnosis of HS and V-Z infections. This becomes particularly important in cases of herpes encephalitis, in which recovery of the virus from the CSF is seldom achieved and serology may become the only noninvasive method for diagnosis. The use of the IAHA test to detect antibody in daily blood specimens, or in one or more CSF specimens from encephalitis patients, may provide the diagnosis and avoid the need for a brain biopsy. In one fatal case of herpes encephalitis seen in this hospital, the CF test, which was then the routine serological test done in this laboratory, showed a change in antibody titer to HS from <1:8 to 1:8 between the first specimen and

TABLE 1. *Comparison of IAHA and CF titers in patients with HS and V-Z virus infection*

Case no.	Clinical diagnosis	Date of onset	Date of specimen	Specimen	Anti-gen	Serum titers		Remarks
						IAHA	CF	
1	Herpes zoster	Not known; vesicle fluid received on 11 June 1976 for virus isolation	11 June 1976	Serum	V-Z	1:128	1:8	V-Z isolated
			18 June 1976	Serum	V-Z	1:4,096	1:256	
2	Herpes zoster	1 July 1976	5 July 1976	Serum	V-Z	1:8,192	1:256	V-Z isolated
3	Varicella	27 Dec. 1975	29 Dec. 1975	Serum	V-Z	Negative	<1:8	
4	Genital herpes	13 Feb. 1976	7 Jan. 1976	Serum	V-Z	1:1,024	1:256	HS isolated
			19 Feb. 1976	Serum	HS	Negative	<1:8	
5	? Varicella meningitis ? Herpes meningitis	28 July 1976	4 Mar. 1976	Serum	HS	1:2,048	1:512	
			1 Aug. 1976	Serum	HS	1:8	<1:8	
			11 Aug. 1976	Serum	HS	1:32	<1:8	
			10 Sept. 1976	Serum	HS	1:256	1:128	
			1 Aug. 1976	Serum	V-Z	1:64	1:16	
			11 Aug. 1976	Serum	V-Z	1:256	1:64	
			10 Sept. 1976	Serum	V-Z	>1:8,192	1:256	
			30 June 1976	Serum	HS	1:8	<1:8	
6	Herpes encephalitis	25 June 1976	2 July 1976	Serum	HS	1:8	<1:8	HS virus was demonstrated by immunofluorescence in the brain biopsy performed on 5 July 1976
			5 July 1976	Serum	HS	1:32	1:8	
			6 July 1976	Serum	HS	1:128	1:16	
			7 July 1976	Serum	HS	1:256	1:64	
			8 July 1976	Serum	HS	1:512	1:128	
			9 July 1976	Serum	HS	1:1,024	1:128	
			13 Oct. 1976	CSF	HS	1:1	Negative	
			14 Oct. 1976	CSF	HS	1:2	1:1	
7	Herpes encephalitis	1-7 Sept. 1976	14 Oct. 1976	CSF	HS	1:2	1:1	
			15 Oct. 1976	CSF	HS	1:4	1:2	
			18 Oct. 1976	CSF	HS	1:16	1:8	
			20 Oct. 1976	CSF	HS	1:32	1:16	
			22 Oct. 1976	CSF	HS	1:64	1:32	
			29 Oct. 1976	CSF	HS	1:128	1:32	

TABLE 2. *Cross-reactions between the V-Z and HS antigens*

Causal agent	Specimen	Date of specimen	Heterologous antibody titers		Remarks
			CF	IAHA	
V-Z virus	Serum	11 June 1976	HS: <1:8	HS: Negative	V-Z isolated
	Serum	18 June 1976	HS: <1:8	HS: Negative	
HS virus	Serum	19 Feb. 1976	V-Z: 1:8	V-Z: 1:64	HS isolated
		4 Mar. 1976	V-Z: 1:512	V-Z: 1:4,096	
HS virus (fatal)	Serum	30 June 1976	V-Z: <1:8	V-Z: 1:16	HS isolated from brain
	Serum	2 July 1976	V-Z: <1:8	V-Z: 1:16	
	Serum	5 July 1976	V-Z: <1:8	V-Z: 1:16	
	Serum	6 July 1976	V-Z: <1:8	V-Z: 1:16	
	Serum	7 July 1976	V-Z: <1:8	V-Z: 1:16	
	Serum	8 July 1976	V-Z: 1:8	V-Z: 1:32	
HS virus	CSF	All specimens	V-Z: Negative	V-Z: Negative	See case 7, Table 1

the third specimen (case no. 6, Table 1). This was not convincing in establishing HS virus as the causal agent, and a brain biopsy had to be performed. HS virus was demonstrated in the brain by immunofluorescence and electron microscopy. The IAHA test done later showed a change in antibody titer to HS from 1:8 to 1:32 (Table 1), which was clearly more convincing.

Subsequently, the IAHA technique was used to diagnose another case of herpes encephalitis by demonstrating rising antibody levels to HS in the CSF (case no. 7, Table 1). This was confirmed by the CF test also.

The real advantage of the IAHA test is the speed with which the results can be obtained and the ease with which it can be performed. It

takes approximately 4 h for completion, whereas the CF test usually takes 24 h. Furthermore, the IAHA test is more sensitive than the CF test and is also more economical (1) as the antigens can be used at higher dilutions. In our system, the V-Z antigen which was used at a dilution of 1:4 in the CF test was used at a dilution of 1:16 in the IAHA test.

The usefulness of this technique in determining the immune status of an individual to V-Z has already been reported by Gershon, Kalter, and Steinberg (2). Our findings show similarly that persons with a history of varicella up to 30 years prior to testing still had demonstrable antibody to V-Z by IAHA. The person who had a V-Z antibody titer of 1:512 by IAHA had a titer of 1:64 by CF. This relatively high level of antibody to V-Z in this person was thought to be due to an infection of HS type 1 at the time of testing, which may have enhanced the V-Z antibody titer.

The IAHA test demonstrated cross-reactions between HS and V-Z viruses in much the same way as the CF test did.

The availability of human O cells from a commercial source greatly facilitates this technique. Not all human O cells are suitable for all viral systems but, for the HS and V-Z systems, the commercially obtained cells, which were from at least two donors, always gave good results.

ACKNOWLEDGMENT

We thank John Polley of the Laboratory Centre for Disease Control, Ottawa, for providing the HS antigen.

LITERATURE CITED

1. Dienstag, J. L., W. L. Cline, and R. H. Purcell. 1976. Detection of cytomegalic virus antibody by immune adherence haemagglutination. *Proc. Soc. Exp. Biol. Med.* **153**:543-548.
2. Gershon, A. A., Z. G. Kalter, and S. Steinberg. 1976. Detection of antibody to varicella-zoster virus by immune adherence haemagglutination. *Proc. Soc. Exp. Biol. Med.* **151**:762-765.
3. Ito, M., and I. Tagaya. 1966. Immune adherence haemagglutination test as a new sensitive method for titration of animal virus antigens and antibodies. *Jpn. J. Med. Sci. Biol.* **19**:109-126.
4. Krugman, S., H. Friedman, and C. Lattimer. 1975. Viral hepatitis A identified by complement fixation and immune adherence. *N. Engl. J. Med.* **292**:1141-1143.
5. Mayumi, M., K. Okochi, and K. Nishioka. 1971. Detection of Australia antigen by means of immune adherence haemagglutination test. *Vox Sang.* **20**:178-181.
6. Miller, W. J., P. J. Provost, W. J. McAlcer, O. L. Ittensohn, V. M. Villarejos, and M. R. Hilleman. 1975. Immune adherence assay specific for hepatitis A antibody. Application to diagnostic and epidemiological investigations. *Proc. Soc. Exp. Biol. Med.* **149**:254-261.
7. Moritsugu, Y., J. L. Dienstag, J. Valdesuso, D. C. Wong, J. Wagner, J. A. Routenberg, and R. H. Purcell. 1976. Purification of hepatitis A antigen from feces and detection of antigen and antibody by immune adherence haemagglutination. *Infect. Immun.* **13**:898-908.
8. Nelson, R. A., Jr. 1953. The immune adherence phenomenon. *Science* **118**:733.
9. Sever, J. L. 1962. Application of microtechnique to viral serological investigations. *J. Immunol.* **77**:320.
10. Tsuda, F., T. Takahashi, K. Takahashi, Y. Miyakawa, and M. Mayumi. 1975. Determination of antibody to hepatitis B core antigen by means of immune adherence haemagglutination. *J. Immunol.* **115**:834.
11. Zissis, G., and G. Clinet. 1974. Viral antibody detection by a more sensitive complement fixation reaction. *Lancet* **1**:754.