Immune Adherence Hemagglutination: Alternative to Complement-Fixation Serology

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Immune adherence hemagglutination (IAHA) was compared to complement fixation (CF), using standard procedures, for serological testing of human sera with a number of commercially available antigens. The antigens included herpes simplex, measles, cytomegalo-, and influenza (type B) viruses, as well as *Mycoplasma pneumoniae* and *Chlamydia psittaci* (*Chlamydia* group). The IAHA test was found to be as specific as the CF test, but 4 to 20 times as sensitive with all antigens tested. Antigen titers were also higher with the IAHA method, and the time required to complete the test was only 4 h for the IAHA method, compared with 20 h for the CF method. The increased sensitivity of the IAHA test should permit its use for determination of immunity, as well as for serodiagnosis of recent infections.

For the serodiagnosis of viral infections, complement-fixation (CF) tests are often used, as a number of antigens are commercially available, and only a few additional reagents are required for the standard procedure (6), regardless of the antigen tested. The principal shortcoming of the CF test as a diagnostic procedure is its relative insensitivity, compared with other serological techniques such as hemagglutination inhibition, passive hemagglutination, indirect immunofluorescence, etc. (5, 10). While the low sensitivity of the CF test has been found acceptable in the diagnosis of recent infections, the method is not suitable for the determination of immunity.

Immune adherence hemagglutination (IAHA) is a sensitive serological technique similar to CF in that both methods detect antibodies which react with the test antigen and activate complement to form immune complexes. IAHA tests detect immune complexes by the hemagglutination which results when the complexes bind to the C'3b receptors found on the surface of primate erythrocytes (9). CF tests detect immune complexes by their capacity to deplete (by activation) the complement required for the subsequent lysis of added antibody-coated erythrocytes. Various reports have shown IAHA tests to be sensitive for the detection of antibodies to varicella-zoster (2), hepatitis A (8), hepatitis B (7), and cytomegaloviruses, as well as viruses of other groups (1, 3).

We examined a number of commercially available CF antigens in IAHA tests to determine the feasibility of adopting this more sensitive tech-nique for routine serodiagnosis in place of CF tests. Sera submitted for routine viral serology were tested both by CF and IAHA procedures, and the results obtained by both methods were compared.

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MATERIALS AND METHODS

Human sera. Specimens of human serum were those received by the virology section of the clinical microbiology laboratory of Hahnemann Hospital for routine serodiagnosis of recent illnesses. Both single and paired (acute and convalescent) sera were submitted; occasional specimens were received with requests to determine (humoral) immunity to specific viruses.

Antigens. Antigens and control antigens for CF tests were obtained from Microbiological Associates, Bethesda, Md., and Flow Laboratories, Rockville, Md. Psittacosis antigen (*Chlamydia* group antigen) and control antigen were obtained from Burroughs-Wellcome, Inc., Research Triangle Park, N.C. Titers were determined by block titrations prior to use for both the CF and IAHA tests.

Complement. Complement (guinea pig) was obtained in lyophilized form from Flow Laboratories or Cordis Laboratories, Inc., Miami, Fla. The complement was stored at −80°C in small portions after it was reconstituted.

Diluents for the IAHA test. (i) Veronal-buffered saline (VBS) was prepared as for the CF test (6). (ii) Gelatin-Veronal buffer (GVB) was prepared by adding 0.125% autoclaved gelatin (Baker Chemical Co., Fisher Scientific Co., and Sigma Chemical Co.) to VBS.

(iii) Bovine serum albumin-Veronal buffer was prepared by adding 0.1% fraction V bovine serum albumin (Miles Laboratories, Sigma Chemical Co.) to VBS. (iv) Dithiothreitol-ethylenediaminetetraacetic acid-VBS was prepared by mixing 2 volumes of 0.1 M disodium ethylenediaminetetraacetic acid with 3 volumes of VBS and adding 3 mg of dithiothreitol per ml (Sigma Chemical Co., St. Louis). The isomer dithioerythritol (Sigma Chemical Co.) can be substituted.

Erythrocytes. Sheep blood in Alsever solution was obtained from Flow Laboratories, Inc. Sheep erythrocytes were prepared for use in the CF test as described (4). Samples of human blood were collected in 2 volumes of sterile Alsever solution from known type "O" donors and then stored at 4°C for up to 5 weeks. The erythrocytes from about one donor in three gave suitable results in the IAHA test. Immediately prior to use, the erythrocytes were washed at 5°C (twice in VBS, then once in GVB) and then resuspended to a concentration of 0.75% in GVB.

Microtest plates. V-type 96-well polystyrene microtest plates were used for the IAHA test; many lots of plates from different manufacturers were found to give poor results in the IAHA procedure. Plates produced in mold 14 from Linbro Scientific, Inc., and in molds 1 and 11 from Cooke Engineering, Inc., were satisfactory. Immediately before use, the plates were rinsed with GVB and then inverted and rapped dry against an absorbent surface. This step eliminated most nonspecific agglutination in the test.

Procedures. The CF tests were performed as described (4). The method used for the IAHA tests was a modification of that described by Gershon et al. (2). All reagents were added in drops of 0.025 ml. Test and control sera were diluted to twice the initial concentration desired in the test and then inactivated at 56°C for 30 min. Bovine serum albumin-Veronal buffer was added to every well of the microtest plates precoated with GVB as described above. The inactivated sera were then added to the plates, and serial twofold dilutions were made with microdilutors prewetted in GVB; several serum dilutions were tested against the control antigens, as prozones were encountered with high-titered sera. Antigen and control antigen, diluted in bovine serum albumin-Veronal buffer, were then added. The plates were vibrated briefly and then incubated (covered) at 37°C for 30 min. After adding complement, the plates were again vibrated and incubated at 37°C for 40 min. Dithiothreitol-ethylenediaminatetraacetic acid-VBS was added to stop the reaction, followed by the type O erythrocyte suspension. After a final mixing, the plates were left at room temperature for hemagglutination patterns to develop; these were often readable within 1 to 2 h. Agglutination of 3+ or greater was considered positive. Weak agglutination in control antigen wells was acceptable.

RESULTS

Complement titrations with the IAHA procedure showed that a complement dilution of 1:100 was satisfactory for all antigens tested; as test results were unaffected by a slight excess of complement, a uniform dilution of 1:90 was used

in all IAHA tests. Titration of complement for the CF procedure gave different titers with the different antigens, and complement dilutions of 1:37 or 1:42 (two units) were used in our work.

Antigen-block titrations, using the same reference sera for both the CF and IAHA tests, indicated that the commercial CF antigens available were suitable for use in the IAHA procedure and that a two- or fourfold increase in antigen titer was found with the IAHA procedure compared with the CF procedure. A comparison of optimal antigen titers obtained by both methods is shown in Table 1.

Serum antibody titers obtained by the CF and IAHA tests are compared in Fig. 1. Comparison tests were done with 22 sera for influenza (type B), 34 for measles, 31 for *Mycoplasma pneumoniae*, 28 for herpes simplex, 38 for cytomegalovirus, and 31 for *Chlamydia*. With few exceptions, antibody titers obtained by IAHA were 4 to 20 times greater than those found by CF test for all antigens tested. The specificities of the two methods appeared to correlate well, as

TABLE 1. *Comparison of optimal antigen titer obtained by IAHA and CF tests*

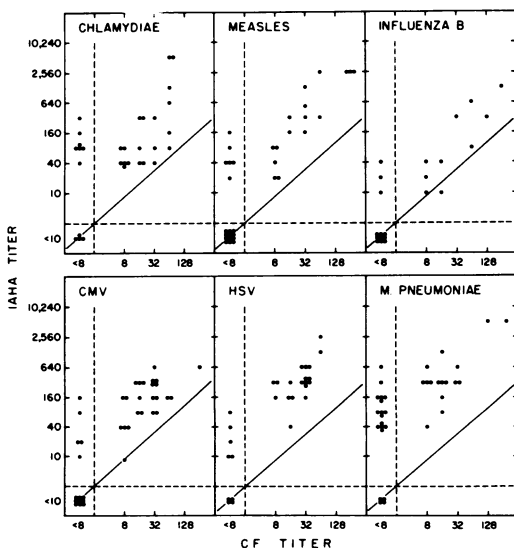| Antigen	CF titer	IAHA titer
Chlamydiae	1:16	1:32
M. pneumonia	1:16	1:64
Varicella	1:4	1:32
Cytomegalovirus	1;8	1:32
Herpes simplex	1:8	1:32
Measles	1:8	1:32
Influenza B	1:16	1:64

FIG. 1. *Comparison of IAHA and CF serum titers against various antigens.*

shown in Table 2. For all antigens, the number of sera positive by IAHA test exceeded that found by CF test. No sera found positive by CF test were found negative by IAHA test, and all sera negative by the IAHA test were also negative by CF test.

In Fig. 2, a comparison is shown of results obtained on serial sera of patients with chlamydial infections: six patients with psittacosis and one with lymphogranuloma venereum (patient 2). Earlier seroconversion was found by IAHA test for patient 3, and the increase in antibody titer was more rapid for patients 1, 4, 6, and 7.

DISCUSSION

Our results indicate that the IAHA test compared favorably to the CF test for serodiagnosis of many viral infections and infections with *Chlamydia* and *M. pneumoniae* as well. The IAHA test was 4 to 20 times as sensitive as the CF test for all antigens tested and appeared to

TABLE 2. *Comparison of specificities of IAHA and CF tests in the detection of antibodies against various antigens*

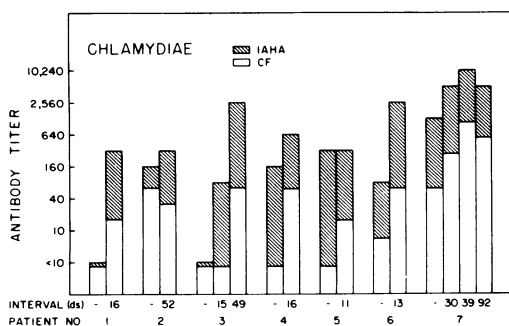| Antigen	CF		IAHA
	Positive	Negative	
Chlamydiae	20	7	Positive
Measles	16	6	
Influenza B	10	3	
Cytomegalovirus	24	5	
Herpes simplex	19	5	
M. pneumonia	16	15	
Chlamydiae	0	4	Negative
Measles	0	12	
Influenza B	0	9	
Cytomegalovirus	0	9	
Herpes simplex	0	4	
M. pneumonia	0	4	

FIG. 2. *Comparison of CF and IAHA titers of serial sera from seven patients with known chlamydial infections. Indicated intervals give days elapsed between collection of first and subsequent specimens.*

be equally specific. Consequently, seroconversions and increases in antibody titer were demonstrable by IAHA test earlier during various infections than by CF test. Antibodies persisted at a level detectable by the IAHA test long after they were no longer found by the CF test, as determined by tests of sera from people with no history of recent illness, but a previously diagnosed infection with an agent corresponding to one of our test antigens. In a study where IAHA and indirect immunofluorescent measurements of antibody to varicella-zoster virus were compared (2), the sensitivities of the two methods were found to be similar. Our results confirm earlier reports on the use of IAHA for serodiagnosis of various viral infections (1-3, 7, 8) and extend its use to the serodiagnosis of infections due to measles, influenza B, *Chlamydia*, and *M. pneumoniae.*

Results are usually obtained in 4 to 5 h with IAHA procedure, compared to the 20 or more hours required with the CF procedure. While the overnight incubation period of the standard CF test method may be shortened, a decrease in sensitivity of the test usually results (5). The IAHA test did not appear to gain any sensitivity when any of the incubation periods were lengthened. Fewer sera presented problems when tested by the IAHA method than with the CF method; most sera which were anti-complementary in CF tests gave normal agglutination patterns and results in the IAHA tests. Sera containing immune complexes will give positive reactions with control antigens in IAHA tests, but seldom to the point of causing difficulty in reading the test results; in addition, the suggested presence of immune complexes in the test serum may be of diagnostic interest.

For laboratories which use CF tests, use of the IAHA test is simpler and faster, and requires no equipment other than that already needed for the CF procedure. Many antigens commercially available for the CF techniques turned out to be equally satisfactory for the IAHA technique. The sensitivity of the latter is greater than that of the CF technique and appears comparable to that of immunofluorescence methods (2, 10; E. T. Lennette and D. A. Lennette, unpublished results).

The IAHA test also appears to have certain advantages when compared with the indirect immunofluorescence (IIF) tests. Antigens for IIF tests, fixed cells or tissues, are more difficult to handle than the fluid antigens used in IAHA tests and also require more effort to produce; relatively few IIF antigen systems are available commercially. The IAHA procedure is easily automated and useful for testing large numbers of sera; the IIF procedure is not as well adapted

to these conditions and is read microscopically, which becomes tedious when large numbers of sera are examined.

The IAHA technique should be applicable to the detection of antibodies to a wide variety of antigens—both soluble and particulate—and need not be confined to microbial antigens. Indeed, the IAHA technique has recently been found to be very sensitive and specific in the detection of heterophil antibodies in the sera of infectious mononucleosis patients, using soluble Paul-Bunnell-Davidsohn antigens (Lennette et al., in press).

Thus, the IAHA test appears to be a useful, simple, and economic alternative to both CF and IIF tests in the serodiagnosis and determination of immunity to infectious agents. It can also serve as a general-purpose serological technique in the detection of antibodies to a variety of antigens.

ACKNOWLEDGMENTS

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