Method for Typing Antisera to *Herpesvirus hominis* by Indirect Hemagglutination Inhibition

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Received for publication 23 December 1970

A test for typing antisera to *Herpesvirus hominis* that uses the method of indirect hemagglutination inhibition is described. The test, which is based upon the differential absorption of herpes antisera by preparations of type 1 and type 2 antigens, is rapidly and easily performed. The results permit some conclusions to be drawn regarding the antigenic relationships between the two virus types. Some of the practical limitations of the test are discussed.

The classification of *Herpesvirus hominis* (HVH) into two main antigenic types has important clinical correlations (2, 6). In particular, the presence of a type 2 herpes infection has a great deal of clinical significance since it may be transmitted to the newborn at the time of delivery and may produce a wide spectrum of disease ranging from mild central nervous system damage to fatal meningencephalitis (10). In addition, an association between type 2 herpes and carcinoma of the cervix has been found, and a causal relationship has been suggested (5).

For these reasons and because antiviral therapy requires early diagnosis, there has been a need for simpler and more rapid methods for typing herpes isolates and for detecting and typing the antibody produced in herpes infections. Large-scale typing by the microneutralization method is laborious, takes several days to complete, and may give equivocal results (8). Typing of virus by fluorescent-antibody techniques (7) requires supplies of specially prepared slides and reagents of considerable purity.

Recently, indirect hemagglutination (IHA) tests for antibody to HVH (3) and cytomegalovirus (CMV; references 1, 4) have been adapted for Microtiter systems. Inhibition of IHA by homologous virus in the CMV system (1) was shown to be a specific and sensitive method of identifying CMV antigen. A similar IHA inhibition test with type 1 and type 2 herpes antigens was performed and found to differentiate between the types of herpes antibody present in herpes antisera. This paper presents such a method and discusses the antigenic relationships demonstrated.

**Materials and Methods**

**Viral strains and antigens.** Type 1 HVH strain VR3 was obtained from Walter Dowdle, Virology Section, Laboratory Division, Center for Disease Control (CDC) (8). Type 2 HVH strain V229 was isolated from necropsy material of an infected infant and was typed by Andre Nahmias, Department of Pediatrics, Emory University School of Medicine, Atlanta, Ga. The viruses were grown in the RU-1 strain of diploid human fetal lung fibroblasts and were harvested, sonically treated, clarified, and stored as previously described for CMV (1).

**Antisera.** Monospecific rabbit sera to HVH type 1 (L2) and type 2 (US) were obtained from Walter Dowdle (8). Other antisera which were typed were CDC diagnostic specimens found to contain antibodies to HVH by complement fixation (CF) and IHA.

**IHA and IHA inhibition tests.** The IHA test was performed as described for CMV (1); the tanned sheep erythrocytes were sensitized with HVH type 1 (VR3) and with HVH type 2 (V229). Optimal sensitizing concentrations were determined for each antigen. The IHA titers for each serum to be typed were determined against cells sensitized with each type antigen. Portions of antiserum dilutions containing eight units of antibody to type 1-sensitized cells were mixed with equal volumes of each antigen type. (The antigen concentration for absorption was one previously shown to inhibit completely an IHA reaction produced by this amount of antibody.) The mixtures were allowed to react at room temperature for 30 min. The absorbed sera were then added in 0.05-ml amounts to Microtiter U-plates, and 0.05 ml of 0.5% type 1-sensitized cells was added to each well. The same procedure was followed with eight units of antibody to type 2-sensitized cells. Samples were absorbed with each antigen and tested against type 2-sensitized cells. Unabsorbed serum dilutions were included as controls. After sensitized cells were added, the plates were sealed with clear tape, shaken, and kept at room temperature until the cells settled (usually about 2 hr).

**Results**

**IHA with monospecific sera.** Rabbit antisera to type 1 and to type 2 HVH were titrated recip-
rocally against sheep cells sensitized with type 1 and type 2 antigens. Several diagnostic sera of unknown type were similarly titrated (Table 1).

Antiserum to the type 1 virus agglutinated the cells that were sensitized with either type 1 or type 2 antigens. Antiserum to the type 2 virus also agglutinated cells that were sensitized by either type antigen. Although all of the diagnostic sera agglutinated both types, several showed higher titers with one type of sensitized cell than with the other.

**IHA with absorbed sera.** Each monospecific rabbit antiserum was absorbed with each of the antigens, and the absorbed sera were then tested for hemagglutination activity against cells sensitized with type 1 antigen. The absorbed sera were similarly tested against cells sensitized with type 2 antigen. A mixture of type 1 and type 2 antisera was absorbed and tested in the same manner. The results are shown in Table 2.

IHA inhibition by only one of the two absorbing antigens indicated that the serum contained antibody homologous with the antigen producing inhibition. This was true when the absorbed sera were tested against cells sensitized with either antigen.

When mixtures of type 1 and type 2 antisera were prepared with varied proportions of the two components, and absorbed by either antigen, antibody activity could be demonstrated for both type 1 and type 2 virus. The one exception was in those mixtures where the proportion of one component was so small that antibody to only one type was demonstrated.

**Absorption of herpes antisera with non-herpes antigen.** Absorption of herpes antisera with uninfected RU-1 tissue culture antigen, CMV, and varicella-zoster CF antigens produced no IHA inhibition when the absorbed sera were tested against herpes-sensitized cells.

**IHA-inhibition patterns produced by sera of unknown type.** Several sera found to have CF and IHA antibody to HVH were tested for IHA activity after absorption as above (Table 3). Three IHA-inhibition patterns were produced; they corresponded to those produced by the rabbit sera of known specificity shown in Table 2. A fourth pattern was produced by serum no. 10; when this serum was retested (no. 11) at a higher concentration, it produced a pattern of the “mixed” type.

**IHA-inhibition patterns as a means of determining virus type.** The IHA-inhibition test was performed with known type 1 and type 2 sera (no. 13 and 3 in Tables 1 and 3, respectively), and coded HVH antigen preparations were used for absorption. Each antigen was used to absorb each serum, and the absorbed sera were tested against type 1 and type 2 sensitized cells. Antigens of known types 1 and 2 were included in the test (Table 4).

**DISCUSSION**

The IHA-inhibition method of typing antisera to HVH is rapid and easy to perform. It uses easily prepared reagents. Type 1, type 2, and mixed antisera each produced a different inhibition pattern when tested against both types of sensitized cells.

A few sera agglutinate cells sensitized with one of the antigens to a higher titer than they do cells sensitized with the other. Fuccillo (3) implies that such sera can be typed directly by IHA, without using differential absorption. However,
The antigen coating on the cells cannot be standardized in numbers of antigen units per cell; also, the proportions of antigen subclasses attaching to the cells cannot be regulated. Therefore, such a direct test may not permit correct interpretation (Table 1, antisera L2).

The IHA-inhibition test provides a means of studying the antigenic relationships between type 1 and type 2 HVH. Cross-neutralization testing shows that type 2 antisera will often neutralize both types of virus with equal efficiency, or will neutralize type 2 virus more efficiently than type 1 virus. On the other hand, type 1 antisera usually neutralize type 1 virus with considerably more efficiency than they do type 2 virus (6, 9).

A model is thus suggested where the two types share a common antigen, but type 2 has an additional, different antigen. The antigenic composition of type 1 virus would be denoted as "H" and that of type 2 virus as "H.2." An alternative model that would explain the neutralization results would postulate that the two types share a common antigen, but that each type has an additional antigen that the other lacks. In this model, type 1 virus would be denoted as "H.1" and type 2 virus as "H.2." Table 5 illustrates the neutralization results with each model. (The degree of neutralization in those situations denoted "+ to ++" would depend upon the relative amounts of the different antigens and corresponding antibodies present in each virus or antiserum, and their relative importance in infectivity.) Both models are compatible with previous experimental findings.

The IHA-inhibition test provides a means of determining which of these two models will more

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**Table 3. Typing of herpes antisera by indirect hemagglutination (IHA)-inhibition**

| Serum no. | Clinical history | IHA-inhibition results with type 1-sensitized cells after serum absorption with<sup>a</sup> | IHA-inhibition results with type 2-sensitized cells after serum absorption with | Virus type |
|-----------|-----------------|-----------------------------------------------|-----------------------------------------------|------------|
| L2        | Rabbit immune serum | Type 1 | Type 2 | No absorption | Type 1 | Type 2 | No absorption |
| US        | Rabbit immune serum | 0 + + + | 0 0 + + | 0 0 + + | 1 |
| US and L2 | Rabbit immune serum | 0 + + + | 0 0 + + | 0 0 + + | 2 |
| 1         | Mother of microcephalic | 0 + + + | 0 0 + + | 0 0 + + | Mixed |
| 2         | Newborn with jaundice and hepatosplenomegaly | 0 0 + + | + 0 0 + | 1 2 |
| 3         | Mother of no. 2 | 0 0 + + | + 0 0 + | 0 + + | 2 |
| 4         | Microcephalic newborn | 0 + + + | 0 0 + + | 0 0 + + | 1 |
| 5         | Mother of baby with congenital CMV infection | 0 0 + + | + 0 0 + | 0 + + | 2 |
| 6         | Microcephalic newborn | 0 + + + | 0 0 + + | 0 0 + + | 1 |
| 7         | 5-year-old microcephalic | 0 + + + | 0 0 + + | 0 0 + + | 1 |
| 8         | Newborn with seizures | 0 0 + + | + 0 0 + | 0 + + | 2 |
| 9         | Mother of no. 8 | 0 + + + | 0 0 + + | 0 0 + + | Mixed |
| 10        | 42-year-old woman with liver disease | 0 0 + + | + 0 0 + | 0 + + | Unknown |
| 11        | Serum no. 10, concentrated eightfold | 0 + + + | + 0 0 + | 0 0 + | Mixed |
| 12        | Mother of premature infant | 0 + + + | 0 0 + + | 0 0 + | 1 |
| 13        | Mother of infant with intracranial bleeding and seizures | 0 + + + | 0 0 + + | 0 0 + | 1 |
| 14        | Mother of baby with congenital CMV infection | 0 + + + | 0 0 + + | 0 0 + | 1 |
| 15        | Adult with recurrent oral herpes | 0 + + + | 0 0 + + | 0 0 + | 1 |

<sup>a</sup> + = Agglutination; 0 = inhibition.
accurately explain the antigenic relationship between the strains. As shown in Tables 6 and 7, the two models predict different results for the same IHA-inhibition experiment. A comparison of the two predictions with the actual experimental findings shown in Table 2 enables one to reject the "H-H.1" model as inconsistent with the experimental evidence.

If the "H.1-H.2" model is valid, we might draw some further conclusions regarding the immune response to type 1 and type 2 herpes. In the present study, the virus preparation used for absorbing sera and for sensitizing cells was unpurified tissue culture material containing multiple antigens, both group and type-specific. The sera that were absorbed by these antigens contained mixtures of antibodies against both the type-specific antigens (1 or 2) and the common group antigen(s) (H) in different proportions and combinations. It has been shown in Tables 2 and 5 how these absorbed sera produce results in an IHA test that differentiate pure type 1 from pure type 2 and either of these from mixtures of both types of antisera.

The unexpected results produced by serum no. 10 (Table 3), however, have not yet been discussed in terms of the antigenic model. After samples of this serum were absorbed by each antigen and tested against cells coated with each antigen, complete IHA inhibition was produced. This "untypable" pattern could result if the serum contained primarily IHA antibody activity against the common group antigen(s) and little activity against the type-specific antigens (1 and 2) at the serum dilutions used in the test.

Such "untypable" sera could be the result of consecutive infection with different virus types, in which a boost in titer primarily to the common antigen(s) occurred and the antibody to the type-specific antigens remained at low levels that were not detectable under the conditions of the test.

| Absorbing antigen | IHA results a | Virus type |
|-------------------|--------------|------------|
|                   | Absorbed type 1 serum tested against cells sensitized with | Absorbed type 2 serum tested against cells sensitized with |
|                   | Type 1 | Type 2 | Type 1 | Type 2 |
| Controls          |        |        |        |        |
| VR3 (type 1)      | 0      | 0      | 0      | ++     |
| VR29 (type 2)     | +      | 0      | 0      | 0      |
| Unknowns          |        |        |        |        |
| US                | +      | 0      | 0      | 2      |
| MCH-A             | 0      | 0      | 0      | +      |
| MCH-B             | 0      | 0      | 0      | +      |
| MS                | +      | 0      | 0      | 2      |
| DI                | 0      | 0      | 0      | +      |
| No absorption     | +      | +      | +      | +      |

a + = Agglutination; 0 = inhibition.

| Antiserum | H-H.2 model a | H.1-H.2 model |
|-----------|---------------|---------------|
|           | Virus (Type 1) H | Virus (Type 2) H.2 | Antiserum | Virus (Type 1) H.1 | Virus (Type 2) H.2 |
| Anti-H    | ++           | + to ++        | Anti-H.1 | ++           | + to ++        |
| Anti-H.1  | ++           | ++             | Anti-H.2 | + to ++        | ++             |

a ++ = Good neutralization; + = partial neutralization.

| Initial serum specificity | Absorbed with | Postabsorption effective specificity | IHA results against cells sensitized with a |
|--------------------------|---------------|-------------------------------------|-------------------------------------------|
|                          |               |                                     | (Type 1) H | (Type 2) H.2 |
| Anti-H (anti-type 1)     | H (type 1)    | No antibody                         | 0           | 0           |
| Anti-H                   | H.2 (type 2)  | No antibody                         | 0           | 0           |
| Anti-H.2 (anti-type 2)   | H             | Anti-2                              | 0           | +           |
| Anti-H.2                 | H.2           | No antibody                         | 0           | 0           |

a + = Agglutination; 0 = inhibition.
The IHA end point of the type-specific antibody present may be considerably lower than that of the group-specific antibody used to determine the serum dilution employed in the IHA-inhibition test.) It should be possible to demonstrate the type-specific antibody in these sera by using the sera in the IHA-inhibition test at a greater concentration. The sera must be absorbed with enough antigen to remove all group-specific antibody but leave detectable levels of type-specific antibody. This is, in fact, indicated by serum no. 11 (Table 3), a concentrated preparation of serum no. 10.

When a serum tested by IHA-inhibition is said to contain only type 1 or type 2 antibody, it is likewise possible that small amounts of the heterologous type-specific antibody are also present but at levels too low to detect in the serum dilution tested. Sera could be tested over a range of dilutions, but this would increase the complexity of the test and decrease one of its advantages over other typing methods. The clinical usefulness of the IHA-inhibition test might be best evaluated by a large-scale comparative study in which sera are typed by both the currently accepted micro-neutralization test and by IHA inhibition. Ultimately, if pure type-specific 1 and 2 antigens free of any group antigens can be prepared, unequivocal typing of antiserum by simple IHA will then be possible. The inhibition test would remain a useful method for typing virus isolates and would prove a useful tool for studying antigenic relationships between virus strains.

**ACKNOWLEDGMENTS**

We thank Robert Kissling and Walter Dowdle for their encouragement and advice and Janet Habas for her technical assistance.

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