Indirect Hemagglutinating Antibody Response to *Herpesvirus hominis* Types 1 and 2 in Immunized Laboratory Animals and in Natural Infections of Man

ARTHUR F. BACK and NATHALIE J. SCHMIDT

Viral and Rickettsial Disease Laboratory, California State Department of Health, Berkeley, California 94704

Received for publication 26 April 1974

Indirect hemagglutinating (IHA) antibody responses to *Herpesvirus hominis* types 1 and 2 (HVH-1 and HVH-2) were compared to complement-fixing and neutralizing antibody responses in immunized laboratory animals (rabbits, guinea pigs, and hamsters) and in natural infections of man. With the immunized animals, type specificity was seen only in the IHA test and only with antisera produced in hamsters and in the rabbits immunized with HVH-2. In human nongenital infections (considered to be caused predominately by HVH-1), IHA and neutralizing antibodies developed at about the same rate and reached approximately the same levels for HVH-1 and HVH-2. IHA titers tended to be higher than neutralizing antibody titers for both virus types. In genital infections (considered to be caused predominately by HVH-2), there was a rapid IHA antibody response to HVH-2, and the early HVH-2 antibody demonstrable by IHA, but not by neutralization tests, was found to be immunoglobulin M in nature. In genital infections, IHA titers for HVH-2 were markedly higher than neutralization titers, but there was no pronounced difference in neutralizing the IHA antibody titers for HVH-1. Several patients with genital infections failed to develop IHA antibody for HVH-1. The IHA test possessed no greater sensitivity than did complement fixation or neutralization tests for serodiagnosis of HVH infections. Despite the fact that a number of patients with genital infections produced IHA antibody only for HVH-2, the test was no more effective than the neutralization test in providing a type-specific serodiagnosis of infection, due largely to the fact that the rapid IHA antibody response to HVH-2 prevented demonstration of a further, significant antibody titer increase in a number of cases.

Although indirect hemagglutination (IHA) with *Herpesvirus hominis* was described by Scott et al. (16) as early as 1957, the test received little attention until 1970 when Fucillo et al. (4) adapted it to the microtiter system and showed, with a small series of patients, that IHA could be used for serodiagnosis of herpes simplex infections in man. Bernstein and Stewart (2) and Schneweis and Nahmias (15) have used inhibition of IHA for the type-specific identification of HVH antibodies or isolates. IHA tests have also been developed for assay of antibodies to human cytomegalovirus (1, 5), another member of the *Herpesvirus* group.

The present studies were undertaken for the purpose of better defining the IHA antibody response to HVH types 1 and 2 in immunized laboratory animals and in natural infections of man, and for determining the relative diagnostic value of the IHA test in human herpetic infections. IHA antibody responses were compared to complement-fixing (CF) and neutralizing antibody responses from the standpoint of temporal appearance of antibody, titers attained, type specificity, and ability of the tests to demonstrate diagnostically significant increases in antibody titer.

**MATERIALS AND METHODS**

Antisera to HVH types 1 and 2 produced in laboratory animals. Antisera to HVH were produced in rabbits, guinea pigs, and hamsters by using the MacIntyre strain of HVH type 1 (HVH-1) and the MS strain of HVH type 2 (HVH-2). Rabbits were immunized with virus propagated in primary rabbit kidney cells, guinea pigs with virus propagated in primary guinea pig embryo cell cultures, and hamsters with HVH-infected suckling hamster brain. All animals received a series of six weekly injections of antigen by the intraperitoneal route. Virus was inactivated with a 0.2% final concentration of beta-propiolactone for the first four inoculations and unoinactivated virus was used for the last two. The virus preparations (un diluted infected cell culture material or 10% brain suspension) were mixed with an equal volume of adjuvant (9 parts Standard mineral oil no. 3 and 1
indirect hemagglutination tests for HVH

part Arlacel A) for the first and third inoculations. For each inoculation, rabbits received 5 ml of antigen, guinea pigs 3 ml, and hamsters 1 ml. Samples of blood were collected at 4 and 6 weeks, and final bleedings were done 3 weeks after the last immunizing injection.

**Human sera.** Acute- and convalescent-phase sera from 38 patients with good clinical evidence of herpetic infection were examined for neutralizing and IHA antibody to HVH types 1 and 2, and for CF antibody with an antigen prepared from a type 1 virus. Nineteen patients had nongenital infections, and 19 had genital infections. Of the patients with nongenital infections, eight had clinical manifestations of meningoencephalitis, and HVH-1 was isolated from the brain tissue of two. Three of the meningoencephalitis patients without virus isolation had no HVH antibody in their acute-phase serum specimens and high antibody titers in convalescent-phase specimens, suggesting an initial HVH infection. Ten of the patients with nongenital infections had gingivostomatitis, and HVH-1 was isolated from one; six others showed seroconversion, suggesting an initial HVH infection. One patient had a clinical diagnosis of herpetic dermatitis, and HVH-1 was isolated from a lesion specimen. HVH-2 was recovered from six of the 19 patients with genital tract infections, and eight additional patients in this group showed seroconversion or immunoglobulin M (IgM) antibody to HVH-2 in their acute-phase specimens. Thus, the clinical diagnosis of herpetic infection was strengthened in 10 of the 38 patients by virus isolation, in 17 others by seroconversion of IgM antibody, and in 11 by a fourfold or greater increase in HVH antibody by one or more serological tests. Twenty-six patients had acute-phase sera collected within 7 days of onset of illness, and convalescent-phase sera were collected 14 or more days after onset. The other 12 patients had serum pairs collected at different time intervals, but were confirmed to have HVH infections by one or more of the above criteria.

For the purposes of this study, patients with nongenital infections were considered to have probable type 1 infections, and those with genital infections were considered to have probable type 2 infections. However, it is recognized that type 2 virus can infect sites other than the genital tract and that genital infections are occasionally caused by type 1 virus.

**IHA test.** The IHA test for HVH types 1 and 2 was performed essentially as described by Bernstein and Stewart (1, 2) by using the MacIntyre strain of HVH-1 and the MS strain of HVH-2 for sensitizing antigens. These were propagated in the L-645 strain of human fetal diploid lung cells. Optimal dilutions of sensitizing antigen for use in antibody assays were determined by “box” titrations in which sheep erythrocytes sensitized with varying dilutions of antigen were tested against varying dilutions of known positive human and animal sera. The working dilution of antigen was the highest dilution which gave the highest antibody titers for the positive sera. IHA antibody titers were expressed in terms of the highest serum dilution which produced complete agglutination of the antigen-coated sheep erythrocytes.

**Neutralization test.** Neutralizing antibody assays for HVH types 1 and 2 were conducted by a micro method using the BS-C-1 line of grivet monkey kidney cells and the MacIntyre (HVH-1) and MS (HVH-2) virus strains. Tests were performed in sterile, disposable microtiter U plates (Linbro Chemical Co., New Haven, Conn.). The medium used for diluting serum, virus, and cells consisted of Medium 199 prepared in Earle balanced salt solution and supplemented with 5% fetal bovine serum (inactivated at 56 C for 30 min) and 1.5% of a 20% solution of glucose. Test sera were inactivated at 56 C for 30 min, and twofold dilutions were prepared in 0.05-ml volumes. To each serum dilution was added 0.05 ml of virus diluted to contain approximately 100 mean tissue culture infective doses. Serum-virus mixtures were incubated for 30 to 60 min at room temperature prior to addition of the cell suspension. Two thousand cells in a volume of 0.05 ml were added to serum-virus mixtures. Appropriate controls (12) were employed to determine the actual test dose of virus and to detect possible cytotoxicity of the test sera. Each cup received 0.08 ml of sterile mineral oil (viscosity 350), and the plates were then sealed with 3½-inch (ca. 8.3-cm) Paklon tape (Minnesota Mining and Manufacturing Co., St. Paul, Minn.). Tests were incubated at 35 to 36 C, and end points could be read either microscopically at 4 days or colorimetrically by pH change at 7 to 10 days.

Identical virus and antibody titers were noted by either reading method.

**CF test.** The CF technique employed was the standard microtiter method of this laboratory (7). Antigen produced from the MacIntyre strain of HVH-1 (13) was standardized in box titrations against sera from human type 1 and type 2 infections and used at a working dilution which detected antibody elicited by either type of infection.

**Indirect fluorescent-antibody tests.** HVH antisera produced in laboratory animals were examined by indirect fluorescent-antibody staining as described elsewhere (14).

**Isolation of HVH from clinical specimens.** Clinical materials suspected to contain HVH were inoculated into cultures of rhesus monkey kidney cells and human fetal diploid kidney cells by the standard procedures of this laboratory (12). Viral isolates were identified by direct fluorescent-antibody staining (14) using fluorescein-conjugated immune globulins produced in hamsters.

**RESULTS**

**Antibody responses in laboratory animals immunized with HVH.** Table 1 shows the antibody titers produced in rabbits, guinea pigs, and hamsters by a 6-week series of immunizations with HVH type 1 or 2. A diversity of antibody responses was seen between animal species and between individual animals of the same species. One of the two rabbits immunized with HVH-1 produced only low levels of IHA antibody, despite the fact that high levels of antibody were detected by the other tests. Both of the rabbits immunized with HVH-2 had low levels of neutralizing antibody but fairly high titers of antibody demonstrable by the other
tests. Guinea pigs immunized with either virus type showed little or no reactivity by IHA, and those immunized with HVH-2 had relatively low levels of neutralizing antibody. Considerable variation was seen between individual guinea pigs in antibody titers detected by the various tests.

Type specificity of the HVH antibody response was seen only in the IHA test and only with antisera produced in hamsters and those produced in rabbits immunized with type 2 virus. Sera collected from rabbits and hamsters at the trial bleedings on the 4th and 6th weeks showed no greater type specificity than did the final antisera. In general, antibody titers increased only two- to fourfold between the first trial and final bleedings.

**Temporal appearance and levels of antibody produced in human HVH infections.** Table 2 shows the geometric mean titers of CF, neutralizing, and IHA antibody in patients with nongenital and genital HVH infections relative

| Immunizing antigen | Host | Titer of immune serum by |
|--------------------|------|-------------------------|
|                    |      | CF                      |
|                    |      | Neutralization          |
|                    |      | Indirect FA*            |
|                    |      | IHA                     |
|                    |      | HVH-1                   |
|                    |      | HVH-2                   |
|                    |      | HVH-1                   |
|                    |      | HVH-2                   |
| HVH-1              | Rabbit 1 | ≥1,024                  |
|                    | 2      | ≥1,024                  |
|                    |        | 256                     |
|                    |        | 128                     |
|                    |        | 16                      |
|                    |        | 512                     |
|                    |        | 512<8                   |
| Guinea pig 1       | 2      | ≥1,024                  |
|                    |        | 256                     |
|                    |        | 128                     |
|                    |        | 16                      |
|                    |        | 512                     |
|                    |        | 512<8                   |
|                    |        | 256                     |
|                    |        | 128                     |
|                    |        | 16                      |
|                    |        | 512                     |
|                    |        | 512<8                   |
| Hamsters c          |        | 512                     |
| HVH-2              | Rabbit 1 | 512                     |
|                    | 2      | 16                      |
|                    |        | 32                      |
|                    |        | 16                      |
|                    |        | 512                     |
|                    |        | 512<8                   |
| Guinea pig 1       | 2      | 256                     |
|                    |        | 16                      |
|                    |        | 256                     |
|                    |        | 128                     |
|                    |        | 512                     |
|                    |        | 512<8                   |
|                    |        | 256                     |
|                    |        | 128                     |
|                    |        | 16                      |
|                    |        | 512                     |
|                    |        | 512<8                   |
| Hamsters c          |        | 128                     |
|                    |        | 128                     |
|                    |        | 256                     |
|                    |        | 1,024                   |
|                    |        | <8                      |

* FA, fluorescent antibody.  
* Reciprocal of antibody titer.  
* Pool of sera.

**Table 2. Mean titer of CF, neutralizing, and IHA antibody to HVH types 1 and 2 in patients with herpetic infections**

| Patient category | Test          | Mean titer to HVH-1 at day | Mean titer to HVH-2 at day* |
|------------------|---------------|---------------------------|-----------------------------|
|                  |               | 0-3 | 4-7 | 8-13 | 14-20 | ≥21 | 0-3 | 4-7 | 8-13 | 14-20 | ≥21 |
| Nongenital infections (19 patients) | CF Neutralization | 2   | 6   | 37   | 104   | 152 | 2   | 4   | 24   | 37   | 76   |
|                  | IHA           | 3   | 4   | 47   | 61   | 304 | 6   | 39  | 74   | 92   | 107  |
|                  |               | (7) | (7) | (5)  | (15) | (4) | (7) | (7) | (5)  | (15) | (4)  |
| Genital infections (19 patients) | CF Neutralization | 7   | 4   | 2    | 29   | 28  | 7   | 4   | 5    | 42   | 41   |
|                  | IHA           | 9   | 4   | 4    | 17   | 9   | 43  | 17  | 215  | 304  | 226  |
|                  |               | (7) | (8) | (4)  | (11) | (7) | (5) | (4) | (8)  | (11) |      |

* The CF test to determine mean titer to HVH-2 was not done.  
* Number of sera in each group is shown in parentheses.
to the time after onset of illness.

In patients with nongenital infections, CF titers against the type 1 antigen tended to be somewhat higher than those of patients with genital infections. Neutralizing antibody to virus types 1 and 2 developed at about the same rate, and comparable levels of antibody were produced to both virus types. IHA antibody to HVH-2 developed more rapidly than antibody to HVH-1 in a few of the patients with nongenital infections.

The patients with genital infections also developed neutralizing antibody to HVH-1 and HVH-2 at approximately the same rate, and levels of antibody were similar to both types. However, in genital infections neutralizing antibody titers tended to be lower than those elicited by nongenital infections. In genital infections, IHA antibody responses were more rapid to HVH-2 than to HVH-1, and markedly higher levels of antibody were attained. It is noteworthy that eight of the 19 patients with genital tract infections failed to develop IHA antibody for HVH-1; one of these also failed to show IHA antibody for HVH-2, but the rest had high levels of type 2 IHA antibody.

Table 3 summarizes a direct comparison of neutralizing and IHA antibody titers of each serum specimen. For patients with nongenital infections, IHA titers to HVH-1 tended to be two- to fourfold higher than neutralizing antibody titers, and IHA titers to HVH-2 tended to be four- to eightfold higher than neutralizing antibody titers. Ten sera from patients with genital infections had higher type 1 titers by neutralization, and 10 had higher titers by IHA. However, in genital infections IHA antibody titers for HVH-2 were markedly higher than neutralizing antibody titers.

**Nature of early IHA antibody to HVH-2.** Acute-phase sera from 11 patients (three with nongenital infections and eight with genital infections) showed IHA antibody to HVH-2, but not to HVH-1, and had no detectable HVH antibody in CF or neutralization tests. Five of the sera, with titers ranging from 1:32 to 1:512, were treated with 0.2 M 2-mercaptoethanol and dialyzed against 0.02 M iodoacetamide. The IHA activity for HVH-2 was completely abolished by 2-mercaptoethanol treatment, indicating that the early antibody was IgM in nature.

**Relative value of the IHA test for serodiagnosis of HVH infections.** Table 4 compares the IHA test to CF and neutralization tests in detecting diagnostically significant HVH antibody titer increases.

For patients with nongenital infections, the CF and neutralization tests detected significant antibody titer rises in 18 and 19 cases, respectively, whereas the IHA test diagnosed 16 cases. Most of the patients in this group had fourfold or greater neutralizing and IHA antibody titer rises to both virus types, but five showed significant neutralizing antibody increases and four showed significant IHA antibody increases only to HVH-1. One patient showed a significant neutralizing antibody titer rise only to HVH-2. The three patients who failed to show significant IHA titer rises had stationary antibody levels to both virus types.

All three of the tests were less sensitive for serodiagnosis of HVH genital infections than for diagnosis of nongenital infections. The neutralization test provided a diagnosis for 15 patients, 10 of whom showed a significant titer rise to both virus types, three to HVH-1 only and two to HVH-2 only. Only 12 of the 19 patients showed a significant titer rise in the CF test; of

**Table 3. Differences between neutralizing and IHA antibody levels to HVH-1 and HVH-2 in patients with herpetic infections**

| Patient category                  | No. of sera | HVH-1 |          |           |          |          |          |          |          |          |          |          |
|-----------------------------------|-------------|-------|----------|-----------|----------|----------|----------|----------|----------|----------|----------|----------|
|                                   |             |   | Neutral- | Neutralization |     | Neutraliz | Neutraliz |     | Neutraliz | Neutraliz |     | Neutraliz | Neutraliz |     | Neutraliz | Neutraliz |
|                                   |             |   | ization |   titer higher |     | and IHA |   titer higher |     | and IHA |   titer higher |     | and IHA |   titer higher |     | and IHA |   titer higher |     |
|                                   |             |   | 2x      | 4x        | ±8x      | 2x      | 4x        | ±8x      | 2x      | 4x        | ±8x      | 2x      | 4x        | ±8x      | 2x      | 4x        | ±8x      | 2x      | 4x        | ±8x      |
| Nongenital infections (19 patients, 38 sera) |             |   |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|                                   |             |   | 14*     | 5         | 11       | 4        | 4         | 9*       | 3        | 2         | 5        | 9        | 10       |          |          |          |          |          |          |
| Genital infections (19 patients, 38 sera)   |             |   | 18*     | 3         | 6        | 1        | 7         | 2        | 1        | 4*       | 1        | 4        | 6        | 23       |          |          |          |          |          |          |

* Ten titers < 1:8 in both tests.
* Five titers < 1:8 in both tests.
* Ten titers < 1:8 in both tests.
* All titers < 1:8 in both tests.
the seven failing to do so, five had moderately high stationary CF antibody titers and two had no demonstrable antibody at a 1:8 dilution of convalescent-phase serum. Thus, an accelerated CF antibody response, rather than lack of response, occurred in most of the genital infections in which the CF test failed to give a positive diagnosis. The IHA test also gave a positive serodiagnosis for only 12 of the 19 patients with genital infections, and, again, this was a reflection of the presence of high levels of IHA antibody, particularly to type 2 virus, in the acute-phase sera and lack of a further, significant increase in antibody titer. Six of the patients with genital infections showed significant IHA antibody increases only to HVH-2, and one showed a significant titer rise only to HVH-1.

**Type specificity of neutralizing and IHA antibody responses in human herpetic infections.** Table 5 summarizes the difference in neutralizing and IHA antibody titers to type 1 and type 2 viruses by patient category. In nongenital infections differences in neutralizing antibody titers were rarely significant ($\geq 4$-fold), and, whereas three sera from this patient group had $\geq 4$-fold higher titers to HVH-1, two had significantly higher titers to HVH-2. In the IHA test six sera from nongenital infections had significantly higher titers to HVH-1, but 10 sera had significantly higher titers to HVH-2.

For the patients with genital infections, the neutralization and IHA tests differed markedly in type specificity. The neutralization test detected only two sera with significantly higher titers to HVH-2, whereas in the IHA test 12 acute-phase and 16 convalescent-phase sera showed significantly higher titer for HVH-2.

Figure 1 compares the IHA antibody titers to HVH-1 and HVH-2 in greater detail. Four of the patients with nongenital infections showed an accelerated antibody response to HVH-2 in their acute-phase sera in the absence of antibody to HVH-1, but all patients showed anti-

---

**Table 4. Comparison of diagnostically significant HVH antibody increases detected by neutralization and IHA tests in human herpetic infections**

| Patient category | No. of patients | Test         | No. of $\geq 4$-fold antibody titer rises to: |
|------------------|----------------|--------------|---------------------------------------------|
|                  |                |              | Both virus types | HVH-1 only | HVH-2 only | Neither virus type |
| Nongenital infections | 19*          | Neutralization | 13 | 5 | 1 | 3* |
|                   |                | IHA          | 12 | 4 | 1 | 3* |
| Genital infections   | 19*         | Neutralization | 10 | 3 | 2 | 4* |
|                   |                | IHA          | 5  | 1 | 6 | 7* |

*Eighteen patients had significant CF antibody titer rises.

*Twelve patients had significant CF antibody titer rises.

*Five patients had stationary antibody levels to both virus types; two had stationary antibody only to HVH-2.

---

**Table 5. Comparison of the type specificity of neutralizing and IHA antibody in human herpetic infections**

| Patient category | No. of patients | Test         | Serum         | No. of patients showing |
|------------------|----------------|--------------|---------------|-------------------------|
|                  |                |              | No significant difference in titer to HVH-1 and HVH-2$^a$ | $\geq 4 \times$ higher titer to |
|                  |                |              |               | HVH-1 | HVH-2 |
| Nongenital infections | 19          | Neutralization | Acute         | 17 | 1 | 1 |
|                   |                | IHA          | Convalescent  | 16 | 2 | 1 |
|                   |                |              | Acute         | 12 | 1 | 6 |
|                   |                |              | Convalescent  | 10 | 5 | 4 |
| Genital infections  | 19          | Neutralization | Acute         | 19 | 0 | 0 |
|                   |                | IHA          | Convalescent  | 17 | 0 | 2 |
|                   |                |              | Acute         | 7  | 0 | 12 |
|                   |                |              | Convalescent  | 3  | 0 | 16 |

*Includes sera showing twofold differences in titer to HVH-1 and HVH-2.
body to both virus types in their convalescent-phase sera. On the other hand, eight of the 19 patients with genital infections showed moderate to high levels of IHA antibody to HVH-2 in acute-phase serum specimens, in the absence of antibody to HVH-1, and seven of these patients had no antibody to HVH-1 in their convalescent-phase sera. Both acute- and convalescent-phase sera of patients with genital infections showed markedly higher titers to HVH-2 than to HVH-1.

DISCUSSION

In addition to studying the IHA antibody response to HVH, the various laboratory animals were immunized with HVH-1 and HVH-2 in an effort to determine the most suitable animal host for production of potent and type-specific herpesvirus antisera. The outstanding findings were that guinea pigs generally failed to produce antibodies reactive in the IHA test, and that type specificity was seen only in the IHA test, and only with antisera produced in hamsters and with the type 2 antisera produced in rabbits.

The type specificity of HVH antisera produced in laboratory animals has varied in different investigations. Some workers using CF (10), neutralization (3, 10), and indirect fluorescent-antibody staining (9) have indicated that antisera to HVH-1 had significantly higher titers to HVH-1 than to HVH-2, whereas antisera to HVH-2 had similar titers to both virus types. With the exception of the hamster serum

Fig. 1. Comparison of IHA antibody titers to HVH-1 and HVH-2 in patients with herpetic infections.
in the IHA test, type specificity was not pronounced with our type 1 antisera. Other investigators using the IHA test (2) have reported that antisera produced in rabbits showed marked heterotypic reactivity between HVH-1 and HVH-2, but our HVH-2 antisera produced in rabbits showed remarkable type specificity in the IHA test. It has been noted that the specificity of HVH antisera produced in laboratory animals may be influenced to some extent by the size of the immunizing virus dose (11) and by the length of time after infection at which serum is collected (6). In our animals, however, we observed no change in type specificity over the course of immunization. Our studies showed that variation in antibody response occurred between animal species and even between individual animals of the same species, and it appears that the major factors influencing the magnitude and type specificity of HVH antibody responses in immunized animals remain to be determined.

As concerns HVH antibody responses in human herpetic infections, CF, neutralizing, and IHA antibody developed at about the same rate in patients with nongenital (presumably type 1) infections. In contrast, a number of patients with genital (presumably type 2) infections had HVH-2 antibody present during the first week after onset of illness, and this was most frequently detected by the IHA test.

It is of interest that the early type 2 antibody detectable by IHA, but not by neutralization tests, was found to be IgM in nature. The IHA test has been shown to detect mercaptoethanol-sensitive antibody to bacteriophage in rabbits early after immunization, before antibody was demonstrable by conventional neutralization tests (17). Also, Lerner et al. (8) have reported IHA tests to be highly sensitive for detection of IgM antibody to HVH-2 in spinal fluids of neonatal patients with HVH encephalitis. Further evaluation of the sensitivity of the IHA test for the detection of IgM antibody to HVH would be of interest and possibly of diagnostic significance.

The accelerated IHA antibody response to HVH-2 tended to limit the value of the test for serodiagnosis of HVH genital infections as compared with the neutralization test, since a fairly high proportion of the patients had moderate to high levels of IHA antibody in their acute-phase serum specimens and failed to show a further, significant increase in antibody titer.

On the assumption that nongenital infections were caused primarily by HVH-1, and genital infections by HVH-2, the IHA test did not possess the value for type-specific diagnosis suggested by Fucillo et al. (4) on the basis of their study on predominately type 1 infections. Bernstein and Stewart (2) have also shown that differences in IHA titer to HVH-1 and HVH-2 are not a reliable indication of the antibody type demonstrable by IHA inhibition. Of our 19 patients with nongenital infections, only four showed a significant IHA antibody titer rise to HVH-1 only, and six of the 19 patients with genital infections showed a significant IHA titer rise only to HVH-2. Although the IHA antibody response in genital infections was more specific for HVH-2 than the neutralizing antibody response, and more specific than the IHA response to HVH-1 in patients with nongenital infections, the possible diagnostic value of type specificity was offset by the rapid appearance of IHA antibody to HVH-2, making it impossible to demonstrate a significant antibody increase in a number of cases.

It is recognized that the amount of specific HVH antigen which coats erythrocytes is highly variable when crude preparations are used as sensitizing antigens, and it is possible that this contributed in part to the heterotypic IHA activity seen in this study and in those reported by others (2). A recent study conducted in this laboratory (A. F. Back and N. J. Schmidt, Infect. Immunity, in press) indicated that the type specificity and diagnostic value of the IHA test for HVH was not improved through the use of partially purified envelope, capsid, or soluble antigens for sensitization of erythrocytes, and that development of antigens for direct type-specific identification of HVH antibodies will require further separation of the specific type 1 and type 2 antigens from common antigens.

ACKNOWLEDGMENTS
We are indebted to Juanita Dennis and Helen H. Ho for assistance in these studies. This study was supported by Public Health Service grant AI-01475 from the National Institute of Allergy and Infectious Diseases to the State of California Department of Health and by Public Health Service General Research grant 5-S01-RR-05441 and General Purpose Traineeship grant 5 A03 AH00505-07 to the School of Public Health, University of California, Berkeley.

LITERATURE CITED
1. Bernstein, M. T., and J. A. Stewart. 1971. Indirect hemagglutination test for detection of antibodies to cytomegalovirus. Appl. Microbiol. 21:84-89.
2. Bernstein, M. T., and J. A. Stewart. 1971. Method for typing antisera to Herpesvirus hominis by indirect hemagglutination inhibition. Appl. Microbiol. 21:680-684.
3. Dowdle, W. R., A. J. Nahmias, R. W. Harwell, and F. P.
Pauls. 1967. Association of antigenic types of *Herpesvirus hominis* with site of viral recovery. J. Immunol. 99:974–980.

4. Fuccillo, D. A., F. L. Moder, L. W. Catalano, Jr., M. M. Vincent, and J. L. Sever. 1970. *Herpesvirus hominis* types I and II: a specific microindirect hemagglutination test. Proc. Soc. Exp. Biol. Med. 133:735–739.

5. Fuccillo, D. A., F. L. Moder, R. G. Traub, S. Hensen, and J. L. Sever. 1971. Micro indirect hemagglutination test for cytomegalovirus. Appl. Microbiol. 21:104–107.

6. Leinikki, P. 1971. Immunofluorescent assay of herpesvirus type 1 and type 2 antibodies in rabbit and human sera. Arch. Gesamte Virusforsch. 35:549–555.

7. Lennette, E. H. 1969. General principles underlying laboratory diagnosis of viral and rickettsial infections. In E. H. Lennette and N. J. Schmidt (ed.), *Diagnostic procedures for virus and rickettsial infections*, 4th ed. American Public Health Association, Inc., New York.

8. Lerner, A. M., C. B. Lauter, D. C. Nolan, and M. J. Shippey. 1972. Passive hemagglutinating antibodies in cerebrospinal fluids in herpesvirus hominis encephalitis. Proc. Soc. Exp. Biol. Med. 140:1460–1466.

9. Nahmias, A. J., W. T. Chiang, I. del Buono, and A. Duffey. 1969. Typing of *Herpesvirus hominis* strains by a direct immunofluorescent technique. Proc. Soc. Exp. Biol. Med. 132:386–390.

10. Nahmias, A. J., W. R. Dowelle, and Z. M. Naib. 1968. Genital infections with *Herpesvirus hominis* types 1 and 2 in children. Pediatrics 42:659–666.

11. Feuherer, J. F. 1970. The specificity of rabbit antisera to *Herpesvirus hominis* and its dependence on the dose of virus inoculated. J. Med. Microbiol. 2:267–272.

12. Schmidt, N. J. 1969. Tissue culture technics for diagnostic virology. In E. H. Lennette and N. J. Schmidt (ed.), *Diagnostic procedures for virus and rickettsial infections*, 4th ed. American Public Health Association, Inc., New York.

13. Schmidt, N. J., and E. H. Lennette. 1971. Comparison of various methods for preparation of viral serological antigens from infected cell cultures. Appl. Microbiol. 21:217–228.

14. Schmidt, N. J., E. H. Lennette, J. D. Woodie, and H. H. Ho. 1965. Immunofluorescent staining in the laboratory diagnosis of varicella-zoster virus infections. J. Lab. Clin. Med. 66:403–412.

15. Schneweis, K. E., and A. J. Nahmias. 1957. Antigens of herpes simplex virus type 1 and 2—immunodiffusion and inhibition passive hemagglutination studies. Z. Immunforsch. 140:471–487.

16. Scott, L. V., F. G. Felton, and J. A. Barney. 1957. Hemagglutination with herpes simplex virus. J. Immunol. 78:211–213.

17. Walker, W. S., M. Fishman, and F. L. Adler. 1971. Detection of anti-phage antibody by passive hemagglutination. II. Early antibodies produced in *vivo* or in *vitro*. J. Immunol. 107:363–395.