Immunological Relationship between
Herpes Simplex and Varicella-zoster Viruses Demonstrated
by Complement-fixation, Neutralization and
Fluorescent Antibody Tests

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SUMMARY

The antigenic relationship between the viruses of varicella-zoster and herpes simplex was studied by complement-fixation, fluorescent antibody staining and neutralization tests. Twenty-three of 75 patients with herpes simplex infections showed significant heterologous increases in complement-fixing antibody titre to varicella-zoster virus. These heterologous increases occurred in patients with serological evidence of a prior infection with varicella-zoster virus, and the greatest proportion occurred in patients in the younger age groups who had probably experienced the most recent varicella-zoster virus infections. Five of 42 patients with varicella-zoster infections showed heterologous complement-fixing antibody responses to herpes simplex virus; all were patients with serological evidence of a prior herpes simplex virus infection. The patients with herpes simplex infection who showed heterologous complement-fixing antibody responses to varicella-zoster virus also showed marked increases in neutralizing antibody and antibody demonstrable by immunofluorescent staining. However, none of the patients with varicella-zoster infection who showed heterologous increases in complement-fixing antibody titre to herpes simplex virus had significant increases in neutralizing antibody.

INTRODUCTION

The viruses of herpes simplex and varicella-zoster are similar morphologically (Almeida, Howatson & Williams, 1962) and the possibility that they might share common antigens was first suggested by Kapsenberg (1964), who noted that certain patients with herpes simplex infections showed increases in complement-fixing antibody titre to varicella-zoster antigens. Similar findings were also reported by Svedmyr (1965). Conversely, Ross, Subak-Sharpe & Ferry (1965) demonstrated heterologous increases in complement-fixing antibody titre to herpes simplex virus in certain patients with varicella or herpes zoster infections.

We have studied further the incidence of heterologous increases in complement-fixing antibody titre in herpes simplex and varicella-zoster virus infections, and have explored the possible effect of age or pre-existing antibody on heterologous complement-fixing antibody responses. Homologous and heterologous neutralizing and indirect fluorescent antibody responses were also studied to determine whether these antibody assays might be more specific than complement-fixing tests.
METHODS

Sera examined. These studies were made with acute- and convalescent-phase sera from patients with clinical illnesses attributed to infection with either herpes simplex or varicella-zoster virus, who showed fourfold or greater increases in complement-fixing antibody titre to herpes simplex or varicella-zoster antigen. Paired sera were examined from 75 patients with clinical diagnoses of herpetic disease of the skin or mucous membranes or with meningo-encephalitis compatible with infection by herpes simplex virus, and from 42 patients with clinical diagnoses of either chickenpox or herpes zoster.

Complement-fixation tests. Complement-fixing antigens for herpes simplex virus were prepared as described by Schmidt, Lennette & Shon (1960) from chick embryo cell cultures infected with the McINTYRE strain of virus. Complement-fixing antigens for varicella-zoster virus were prepared from human foetal diploid cell cultures infected with the BATSON strain of virus (Schmidt et al. 1964). Complement-fixing antibodies were titrated by our standard procedure adapted to use in the ‘Microtiter’ system (Lennette, 1964).

Fluorescent-antibody assays. Indirect immunofluorescent staining for detection of herpes simplex or varicella-zoster antibodies was performed by a technique described in detail by Schmidt et al. (1965).

Neutralizing-antibody assays. Microneutralization tests for herpes simplex virus were made in cups in disposable ‘Microtiter “U”’ plates (Cooke Engineering Co., Alexandria, Va, U.S.A.). To each cup was added 7500 human foetal diploid lung (HFDL) cells (of line no. 645, established by Dr J. H. Schieble of this laboratory) in 0.075 ml. of growth medium consisting of 10% foetal bovine serum and 90% Eagle’s minimum essential medium (MEM) prepared in Hanks’s balanced salt solution. The cups were sealed with 3-1/4 in. Paklon tape (Minnesota Mining and Manufacturing Co., St Paul, Minn., U.S.A.) and the plates were incubated at 36° for 24 to 48 hr, until confluent monolayers of cells had formed. The growth medium was aspirated from each cup before the addition of serum + virus mixtures.

Dilutions of serum (inactivated at 56° for 30 min.) and virus were prepared in maintenance medium consisting of 5% foetal bovine serum and 95% Eagle’s MEM prepared in Earle’s balanced salt solution. Serum dilutions were assayed against 32 to 100 TCD50 of virus, and serum + virus mixtures were incubated at 37° for 30 min. before inoculation of 0.05 ml. volumes on to the monolayers. Maintenance medium (0.025 ml.) was added to each cup. Plates were sealed with tape and the tests were incubated at 36° for 48 hr. Results were read microscopically, and the neutralizing antibody titres were expressed as the greatest serum dilution which completely prevented the cytopathic effect of the test dose of virus. Sera from five patients with varicella-zoster infections who showed significant increases in complement-fixing antibody titre to herpes simplex virus were also assayed for herpes simplex neutralizing antibody by the plaque reduction method described below for varicella-zoster virus. The overlay containing neutral red was added on the third day and tests were read on the fourth day.

Cell-free virus for use in varicella-zoster neutralization tests was prepared as described by Brunell (1967) by sonic oscillation of HFDL cells infected with the BATSON strain of virus. The infected cells from each 32 oz culture bottle were scraped into 2 ml. of growth medium and then treated at 20 kcyc./sec. for 20 sec. After centrifugation at
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1500 rev./min. for 15 min. (at 4°), the supernatant fluid was removed and sorbitol was added to give a final concentration of 10%. The cell-free virus preparations were stored at -70°.

Neutralizing antibody to the varicella-zoster virus was assayed by a plaque-reduction technique. Monolayers of HFDL cells were prepared in cups in Linbro Model FB-24 Disposo-trays (Linbro Chemical Co., Inc., New Haven, Conn., U.S.A.) by seeding each cup with 300,000 cells in 3 ml. of growth medium consisting of 10% foetal bovine serum and 90% Eagle's MEM in Hanks's solution. Linbro plastic covers were placed over the panels which were incubated in an atmosphere of 5% CO₂ in air at 36° for 2 days to produce confluent monolayers. The medium was aspirated from each cup just before inoculation with virus or serum + virus mixtures.

Dilutions of serum (inactivated at 56° for 30 min.) and virus were prepared in Eagle's MEM containing 2% foetal bovine serum. Each serum dilution was mixed with an equal volume of virus diluted to contain 80 to 100 p.f.u./0.1 ml. The serum + virus mixtures were incubated at 37° for 30 min. and then 0.2 ml. volumes were inoculated on to cell monolayers. The cultures were incubated at 36° for 1 hr to permit adsorption of virus, then the inocula were removed and each monolayer was washed with 2 ml. of medium. The cell sheets were then covered with 3 ml. of nutrient agar overlay consisting of 5% foetal bovine serum, 94.5% Eagle's MEM in Earle's saline (prepared without phenol red) and 0.5% Ionagar no. 2 (Consolidated Laboratories, Inc., Chicago Heights, Ill., U.S.A.). After the overlays had solidified the cultures were incubated in an atmosphere of 5% CO₂ in air at 36° for 6 days. Two ml. of nutrient agar overlay containing neutral red sufficient to give a final concentration of 1/50,000 was then added to each cup. Plaques were counted on the 8th day. Neutralizing-antibody titres were expressed as the highest dilution of serum producing a 50% or greater reduction in plaque count, compared with the count in virus controls containing either no serum or a known negative serum.

RESULTS

Frequency of heterologous complement-fixing antibody responses in patients with herpes simplex virus infections; relationship to age and presence of heterologous antibody in the acute-phase serum

No increases in heterologous antibody to varicella-zoster virus were seen in patients in the 1- to 10-year age-group (Table 1). Although chickenpox occurs fairly frequently in children from 3 to 10 years of age, only one patient in this particular group showed serological evidence of a prior infection with varicella-zoster virus, and he showed no significant increase in heterologous antibody titre. Fifteen of the 22 patients in the 11- to 20-year age-group showed a significant increase in heterologous titre to the varicella-zoster virus, and more than half the patients in this group had varicella-zoster antibody in their acute-phase serum specimens. A third of the patients in the 21- to 30-year age-group showed an increase in heterologous titre to the varicella-zoster antigen, but three-quarters of the patients in this group had varicella-zoster antibody in their acute-phase serum. None of the 13 patients 31 years of age or older showed an increase in heterologous complement-fixing antibody to varicella-zoster virus, although four of them had serological evidence of a previous infection with this virus.

Heterologous increases in complement-fixing antibody titre to varicella-zoster

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antigen may thus be related not only to a prior infection with varicella-zoster virus, but also to the length of time since the infection. The highest proportion of heterologous antibody responses was seen in individuals who had probably experienced the most recent infections with the varicella-zoster virus, i.e. those in the 11- to 20-year age-group. With increasing age the proportion of heterologous antibody responses decreased, despite the fact that fairly large numbers of the patients in the older age groups showed serological evidence of a prior infection with varicella-zoster virus.

Table 1. *Heterologous complement-fixing antibody responses to varicella-zoster virus (V-Z) in patients with herpes simplex virus infections*

<table>
<thead>
<tr>
<th>Age-group (years)</th>
<th>No. of patients</th>
<th>No. of patients with fourfold or greater increase in titre to CF antibody to V-Z</th>
<th>No. of patients with fourfold or greater increase in titre to both herpes simplex and V-Z</th>
<th>No. of patients with CF antibody to V-Z in acute-phase serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 10</td>
<td>16</td>
<td>16</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>11 to 20</td>
<td>22</td>
<td>7</td>
<td>15 (68%)</td>
<td>13</td>
</tr>
<tr>
<td>21 to 30</td>
<td>24</td>
<td>16</td>
<td>8 (33%)</td>
<td>18</td>
</tr>
<tr>
<td>31 +</td>
<td>13</td>
<td>13</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Totals</td>
<td>75</td>
<td>52</td>
<td>23 (31%)</td>
<td>36</td>
</tr>
</tbody>
</table>

Table 2. *Relationship between presence or absence of heterologous complement-fixing antibody in acute-phase sera and the type of heterologous complement-fixing antibody response seen in patients with herpes simplex or varicella-zoster virus infection*

<table>
<thead>
<tr>
<th>Group of patients</th>
<th>Heterologous complement-fixing antibody in acute-phase serum</th>
<th>No. of patients</th>
<th>No change or decrease in titre</th>
<th>Two-fold increase in titre</th>
<th>Four-fold or greater increase in titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes simplex</td>
<td>V-Z antibody absent*</td>
<td>39</td>
<td>31</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>V-Z antibody present</td>
<td>36</td>
<td>8</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Varicella-zoster</td>
<td>Herpes simplex antibody absent*</td>
<td>28</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Herpes simplex antibody present</td>
<td>14</td>
<td>6</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

* Titre < 1/8.

The occurrence of heterologous complement-fixing antibody responses is further related to serological evidence of previous infection with varicella-zoster virus in Table 2. Of the 39 herpes simplex patients with no heterologous complement-fixing antibody to varicella-zoster virus in their acute-phase sera, eight subsequently showed a serological response to the varicella-zoster antigen. However, some patients without demonstrable complement-fixing antibody in their acute-phase sera did have low concentrations of varicella-zoster antibody demonstrable by neutralization or indirect fluorescent antibody tests (cf. Table 4). Fifteen of the 36 patients with complement-fixing antibody to varicella-zoster virus in the acute-phase serum had fourfold or greater increases in titre, and 13 showed increases in titre of twofold. Thus, about
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three-quarters of the patients with serological evidence of prior infection with varicella-
zoster virus responded to herpes simplex virus infections with an increase in heterologous complement-fixing antibody to varicella-zoster virus.

Frequency of heterologous complement-fixing antibody responses in patients with varicella-
zoster virus infections; relationship to age and presence of heterologous antibody in the
acute-phase serum

None of the varicella-zoster patients in the 1- to 10-year age-group showed increases
in heterologous complement-fixing antibody titre to herpes simplex antigen, and none
showed serological evidence of prior infection with herpes simplex virus (Table 3).
Sera from only a few patients between 11 and 30 years of age were available for study,
and none of these showed increases in heterologous titre or evidence of previous
herpes simplex infection. Four of the six patients in the 31- to 40-year age-group had
antibody to herpes simplex virus in their acute-phase sera, but none showed increases
in heterologous complement-fixing antibody titre. Ten of the 12 patients 41 years of
age or older had complement-fixing antibody to herpes simplex virus in their acute-
phase serum; five of them had significant increases in heterologous complement-fixing
antibody titre to the herpes simplex antigen.

Table 3. Heterologous complement-fixing antibody responses to herpes simplex virus in
patients with varicella-zoster infections

<table>
<thead>
<tr>
<th>Age-group (years)</th>
<th>No. of patients</th>
<th>No. of patients with four-fold or greater increase in titre to V-Z only</th>
<th>No. of patients with four-fold or greater increase in titre to both V-Z and herpes simplex</th>
<th>No. of patients with CF antibody to herpes simplex in acute-phase serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 10</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11 to 20</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21 to 30</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>31 to 40</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>41 &gt;</td>
<td>12</td>
<td>7</td>
<td>5 (42%)</td>
<td>10</td>
</tr>
<tr>
<td>Totals</td>
<td>42</td>
<td>37</td>
<td>5 (12%)</td>
<td>14</td>
</tr>
</tbody>
</table>

None of the varicella-zoster patients without demonstrable complement-fixing anti-
body to herpes simplex virus at the onset of illness showed heterologous antibody
responses, but five of the 14 patients who had heterologous antibody in the acute-
phase serum specimen had fourfold increases in titre and three had twofold increases
(Table 2).

As with herpes simplex infections, increases in heterologous complement-fixing
antibody titre seem to occur only in varicella-zoster patients who have previously
experienced an infection with the heterologous virus.

Neutralizing and fluorescent antibody responses of patients with increases in
heterologous complement-fixing antibody titre

Paired sera from 12 patients with herpes simplex virus infections and five patients
with varicella-zoster infections who showed increases in heterologous complement-
fixing antibody were examined for homologous and heterologous antibody by neutrali-
zation and indirect fluorescent antibody tests (Table 4). Heterologous responses to varicella-zoster virus were demonstrable for the herpes simplex patients not only by complement-fixing, but also by indirect fluorescent antibody and neutralization tests; some patients attained concentrations of heterologous antibody similar to those of homologous antibody. However, the complement-fixing test detected more heterologous varicella-zoster antibody responses in the herpes simplex patients than did the fluorescent antibody test or the neutralization test (Table 5). Of the five patients with
varicella-zoster infections with significant increases in herpes simplex complement-fixing antibody, only one showed a significantly increased titre in the fluorescent antibody test, and none showed even a fourfold increase in neutralizing antibody to herpes simplex virus.

Table 5. Increase in heterologous antibody in herpes simplex or varicella-zoster virus infections detected by neutralization tests (Neut.), fluorescent antibody staining (FA) or complement-fixation tests (CF)

<table>
<thead>
<tr>
<th>Group of patients</th>
<th>No. of patients</th>
<th>Fourfold or greater increase in titre to herpes simplex</th>
<th>Fourfold or greater increase in titre to V-Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes simplex infections</td>
<td>12</td>
<td>12 12 12</td>
<td>8 10 12</td>
</tr>
<tr>
<td>Varicella-zoster infections</td>
<td>5</td>
<td>0 1 5</td>
<td>4 3 5</td>
</tr>
</tbody>
</table>

DISCUSSION

Twenty-three of the 75 patients with herpes simplex infection in this study showed increases in heterologous complement-fixing antibody to varicella-zoster virus. Kapsenberg (1964) demonstrated heterologous complement-fixing antibody responses in 24% of her patients, and Svedmyr (1965) reported the phenomenon in nine of 14 patients. A number of the increases in heterologous complement-fixing antibody described by Kapsenberg occurred in patients with herpes simplex infection who were under 10 years of age but, in contrast to our study group of the same age, most of the patients had serological evidence of a prior varicella-zoster virus infection. All nine of the patients in Svedmyr's study who showed heterologous complement-fixing antibody responses were between 12 and 33 years of age, and seven of them showed serological evidence of a prior varicella-zoster virus infection.

Only 12% of the varicella-zoster patients in our study showed increases in heterologous complement-fixing antibody to herpes simplex virus, which is less than the 48% of varicella patients and 26% of zoster patients reported by Ross et al. (1965). However, relatively few of our varicella-zoster patients had serological evidence of a previous herpes simplex virus infection, and all except one of the increases in heterologous antibody described by Ross et al. occurred in patients with herpes simplex antibody in their acute-phase serum specimens.

A previous antigenic stimulus with the varicella-zoster or herpes simplex virus thus appears to be essential in eliciting a heterologous antibody response. Also, Svedmyr (1965) reported that animals hyperimmunized with herpes simplex virus fail to show complement-fixing antibodies to the varicella-zoster virus. The occurrence of heterologous antibody responses also seem to be related to the length of time elapsing since infection with the heterologous virus. This is suggested by our demonstration that in patients with herpes simplex infection a higher proportion of increases in heterologous complement-fixing antibody to varicella-zoster virus occurred in individuals in younger age-groups, and also by the findings of Ross et al. (1965) that younger patients with varicella showed a higher proportion of heterologous antibody responses to herpes simplex virus than did older patients with herpes zoster.

Ross et al. (1965) assayed neutralizing antibody for herpes simplex virus in the sera
of patients with varicella-zoster infection and found that some of those with increases in heterologous complement-fixing antibody also had slight increases in neutralizing capacity, but the increases were less than fourfold. Similarly, the few varicella-zoster patients in our study who showed an increase in heterologous complement-fixing antibody to herpes simplex virus failed to show significant increases in neutralizing antibody, either by the microneutralization test or by plaque reduction.

However, a high proportion of the herpes simplex patients with increases in heterologous complement-fixing antibody to varicella-zoster virus also showed marked increases in neutralizing antibody and antibody demonstrable by immunofluorescent staining. This is further evidence that the two viruses share common antigens. The fact that concentrations of varicella-zoster neutralizing antibody were boosted by infection with herpes simplex virus raises the question as to whether immunity to varicella-zoster virus might be reinforced by infections with herpes simplex virus.

The authors are indebted to Mrs Florence W. Jensen for performing the complement-fixation tests and to Mr James D. Woodie for performing the fluorescent-antibody assays.

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REFERENCES


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