Radioimmunoassay of Herpes Simplex Virus Antibody: Correlation with Ganglionic Infection

By B. FORGHANI, TONI KLASSEN AND J. R. BARINGER
Viral and Rickettsial Disease Laboratory, California State Department of Health, Berkeley and Department of Neurology, Veterans Administration Hospital and University of California, San Francisco, California, U.S.A.

(Accepted 13 April 1977)

SUMMARY

Results of herpes simplex virus (HSV) isolation from a series of human post-mortem trigeminal, thoracic and sacral ganglia were correlated with the HSV antibody type(s) detected in the sera by radioimmunoassay (RIA). HSV type 1 was isolated from trigeminal ganglia of 44 out of 90 individuals, from thoracic ganglia of 1 out of 25, and from sacral ganglia of 1 out of 68 cases. HSV type 2 was recovered from sacral ganglia of 8 out of 68 individuals. In all cases in which an HSV was isolated from ganglia and serum was available for testing, homologous, type-specific antibody was demonstrable, and in a few instances antibody to the heterologous HSV was also detected. In those individuals in which HSV type 1 was isolated from trigeminal ganglia and HSV type 2 from sacral ganglia, antibody to both virus types was present in the sera, indicating that simultaneous latent infections with each of the two viruses can occur, and that antibody is produced to each virus independently. Antibody to HSV type 1, 2 or both types was demonstrated in 8 out of 10 cases in which virus isolation attempts were negative, suggesting either a higher sensitivity of RIA for detecting HSV infection, or the presence of latent HSV at some other site in the body which was not sampled.

INTRODUCTION

In recent years it has become increasingly appreciated that the two antigenically distinct types of herpes simplex virus (HSV) differ in a number of biological properties (Craig & Nahmias, 1973). In addition, they tend to produce lesions in different parts of the body; type 1 HSV is most frequently associated with oral or ocular lesions and the HSV encephalitis of children or adults, while type 2 HSV is more frequently responsible for genital infection, and is the cause of the majority of neonatal cases of HSV encephalitis and some cases of herpes meningitis in adults. A variety of lines of evidence have indicated that the virus can reside in sensory ganglia, and most probably in neurons within these ganglia. HSV can be recovered from the sensory ganglia of human cadavers when such ganglia are placed in explant culture and observed for the presence of a cytopathic effect (Bastián et al. 1972; Baringer & Swoveland, 1973; Baringer, 1974, 1975). Virus isolates from the trigeminal ganglia of humans have been of type 1, and those from the sacral ganglia have been of type 2.

This report correlates the results of virus isolation from a series of human post-mortem trigeminal, thoracic, and sacral ganglia with the HSV antibody type(s) detected in the sera by radioimmunoassay (Forghani, Schmidt & Lennette, 1975).
METHODS

Virus isolation from ganglionic tissue. Cadaver tissue was selected for study only on the basis of accessibility for removal within 24 h from the time of death. The patients ranged in age from 44 to 92 years and were all males from the general medical and surgical services of a metropolitan Veterans Administration Hospital.

Ganglia were obtained as previously described (Baringer & Swoveland, 1973; Baringer, 1974). After removal of the brain, the trigeminal ganglia were removed using sterile instruments. The thoracic and sacral areas of the cadaver were scrubbed with soap and rinsed with alcohol; using sterile instruments, dissection was carried out down to the spinal column and the laminae were removed. The ganglia were exposed, dissected free, and placed individually in sterile containers for transport to the laboratory.

Ganglia were minced finely and washed twice in Hanks' balanced salt solution. The trigeminal ganglia were divided into as many as four 25 cm² Falcon flasks, the S-2 ganglia into four flasks each, the S-3 ganglia into two to four flasks, and the S-4 ganglia into one or two flasks, depending upon the size of the individual ganglia. The thoracic ganglia were pooled and inoculated into one or two flasks. To the flasks was added a suspension of $1 \times 10^6$ freshly trypsinized human embryonic lung cells (Flow 2000) in Leibovitz (L-15) medium containing 10% foetal calf serum. The cell cultures were incubated at 36 °C and observed twice weekly for the appearance of a cytopathic effect. Medium was replenished weekly, and cultures were maintained for three months before being discarded as negative. The cytopathic effect (c.p.e.) was recognized initially as a focal rounding and detachment of cells from the surface of the flask; this was followed by aggregation of the cells into grape-like clusters. The virus was identified by neutralization of the c.p.e. by specific anti-HSV sera prepared in rabbits, or by fluorescent antibody staining. Typing of the virus was accomplished by radioimmunoassay using hamster antisera prepared against HSV types 1 or 2 (Back & Schmidt, 1974) as previously described (Forghani, Schmidt & Lennette, 1974).

Radioimmunoassay method for antibody detection. Blood from cadavers was obtained by cardiac puncture at the time of autopsy. Sera from children (age 6 months to 2 years) were obtained from unused but fresh samples submitted to the clinical laboratories at the University of California Hospital from the paediatric ward and clinic.

The McIntyre strain of HSV-1 and the MS strain of HSV-2 were used to produce infected cells as sources of antigen. Human foetal diploid lung (HFDL) cells were grown in one-dram glass vials in fortified Eagle's minimal essential medium supplemented with 10% foetal bovine serum. When the cells formed a complete monolayer in the bottom of the vial after two to three days, they were infected with HSV-1 at a ratio of approx. 0.3 infectious particle/cell, or with HSV-2 at a ratio of approx. 0.1 infectious particle/cell. The cultures showed a marked c.p.e. in 20 to 22 h and, as shown by direct fluorescent antibody staining, over 90% of the cells contained specific HSV antigen. The cells were fixed with acetone and stored at $-70^\circ$C. Uninfected cell cultures were prepared the same way. The complete details of these procedures have been described previously (Forghani et al. 1974, 1975).

Suspensions of uninfected HFDL cells and cells infected with type 1 or type 2 HSV were prepared and used as previously described (Forghani et al. 1975) for absorption of sera. Generally, one vol. of serum was mixed with ten vol. of packed cells for cross-absorption of antibodies. Residual antibody was detected in the absorbed sera by solid phase RIA as outlined below.

Anti-human or anti-hamster gamma globulin prepared in goats was purchased from Antibodies, Inc., Davis, California. The gamma globulin fraction was precipitated by
HSV antibody and ganglionic infection

Table 1. Recovery of HSV from sensory ganglia

<table>
<thead>
<tr>
<th>Ganglia examined</th>
<th>Number of cadavers</th>
<th>Number positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trigeminal</td>
<td>90</td>
<td>44*</td>
</tr>
<tr>
<td>Sacral</td>
<td>68</td>
<td>1</td>
</tr>
<tr>
<td>Thoracic</td>
<td>25</td>
<td>1</td>
</tr>
</tbody>
</table>

* In 28 cases HSV type 1 was recovered from both right and left ganglia.

1/3 saturation with ammonium sulphate and further purified by DEAE cellulose column chromatography. The purified IgG was labelled with $^{125}$I by the chloramine T method (Hunter & Greenwood, 1962; McConahey & Dixon, 1966). Assuming 100% recovery of labelled IgG, the specific radioactivity was approx. 0.5 μCi/μg of protein.

The complete radioimmunoassay (RIA) procedures for typing HSV isolates and typing HSV antibody in human sera have been described in detail (Forghani et al. 1974, 1975). Briefly, cross-absorbed human sera or hamster typing sera were reacted with (1) uninfected, (2) HSV type 1-infected and (3) HSV type 2-infected cells in the glass vials, the excess antibody was washed off, and the presence of type-specific HSV antibody combined with antigen in the cells was detected by addition of the $^{125}$I-labelled anti-species globulin. After incubation and washing, the residual radioactivity was counted directly in the vials using a gamma counter. Binding ratios for HSV type 1 and HSV type 2 were calculated by dividing the counts per minute obtained with the absorbed serum against virus-infected cells by that obtained against uninfected cells. A binding ratio of 2.1 or greater was considered indicative of the presence of antibody for the test HSV type. Patterns of binding ratios for sera containing antibody to HSV type 1, HSV type 2, or both types of antibody are shown elsewhere (Forghani et al. 1975). In the present studies, positive binding ratios for absorbed sera ranged from 2.1 to 10.3, and negative binding ratios from 1.0 to 1.6.

RESULTS

Virus isolation

Results of virus isolation attempts are summarized in Table 1. Trigeminal ganglia were examined from 90 cadavers. Of these, HSV was recovered from 44 (49%) and in 28 of the 44 positive cases (64%) the virus was recovered from both right and left ganglia. The c.p.e. was evident in from two to six weeks after co-cultivation of the ganglia. In each case the virus was identified as HSV type 1. In 10 ganglia from these individuals, the virus was recovered in all four flasks; more often, the virus was recovered from one or two of the four flasks prepared. From the sacral ganglia of 68 cadavers, HSV was recovered from 8 at intervals from 2 to 13 weeks after co-cultivation. Virus was recovered only from second and third sacral ganglia. In seven of these instances the virus was identified as type 2. In one instance the virus was identified as type 1 on the basis of fluorescent antibody staining, radioimmunoassay and neutralization by specific antisera, each performed in a different laboratory. Of the thoracic gangia from 25 cadavers, one culture yielded a type 1 virus. A simultaneous recovery of HSV from the trigeminal and sacral ganglia was accomplished in three patients: in these, the virus from the trigeminal was type 1 and that from the sacral ganglia was type 2.
Antibody typing

Results of HSV antibody typing on cross-absorbed sera by RIA on 27 cases from whom virus was recovered from either trigeminal or sacral ganglia, or both, are shown in Table 2. In addition, serum was available in ten cases in which co-cultivation studies were carried out but no virus was recovered. When only type 1 virus was isolated, the sera generally contained antibodies only to that type, but occasionally to both type 1 and type 2 viruses. In the three cases in which only type 2 virus was recovered, antibody was detected either to type 2 or to types 1 and 2. In the three cases of dual virus isolation, specific antibody to both virus types was present in the serum. In ten cases where no virus was recovered, antibody to type 1 was present in five, to type 2 in one, to types 1 and 2 in two, and no antibody was detected in two. In RIA of serum specimens from young children, no antibody was detected in any of the 11 specimens tested (binding ratios of 1.0 to 1.6).

**DISCUSSION**

Applying co-cultivation techniques to sensory ganglia, we recovered type 1 HSV from approx. 50% of an unselected elderly male autopsy population. Attempts to establish a correlation of the presence of virus in the trigeminal ganglia with a history of recurrent HSV infection on the lip or mouth by review of charts or by interview of surviving relatives were unsuccessful. In the majority of cases there was no known history of HSV infection, or the character of possible ulcerative lesions was so incompletely described that it could not be established whether the lesions were herpetic. In 28 of the 44 positive cases, HSV was recovered from right and left ganglia, suggesting a high frequency of bilateral involvement. Virus was recovered from sacral ganglia of 8 out of 68 patients, and in all except one the virus was type 2. In the single exception, the virus was independently identified as type 1 by neutralization, fluorescent antibody test and RIA. The trigeminal ganglia was not removed from this patient, excluding the possibility of contamination from that site. RIA of the serum revealed antibody only to type 1 virus in the patient.

Despite the fact that the sacral ganglia 2, 3 and 4 were all placed in culture, virus was recovered only from the third and fourth sacral ganglia, and never from the second. Of interest was the fact that recovery of HSV type 2 from the sacral ganglia frequently required a longer time of in vitro cultivation than that required for type 1. Although all ganglionic cultures were maintained for three months, recoveries of type 1 virus from trigeminal ganglia all took place within the second to sixth week after co-cultivation. While most of the isolations of type 2 virus from sacral ganglia were accomplished in a similar period, one isolate was recovered at six weeks, three at seven weeks, two at 10 weeks, and two at 13 weeks after explantation. The reasons for this slower appearance of type 2 virus are not evident.

In one patient, type 1 virus was recovered from the pooled thoracic ganglia at seven weeks after explantation. The possibility of contamination cannot be completely excluded, but
seems unlikely on the basis of persistently negative results from cultivation of thoracic ganglia in the other 24 cases. We were unable to ascertain whether this patient had any herpetic eruption during life. No evidence of a cytopathic effect consistent with varicella-zoster virus was seen in any of the cultured thoracic ganglia.

In three individuals, simultaneous latent infections with type 1 virus in the trigeminal ganglia and type 2 virus in the sacral ganglia were identified; in each of these individuals, antibody to both types of virus was present in the serum. These results indicate that it is possible to have independent and simultaneous latent infections by each of the two viruses, and that antibody to each is produced independently.

In 10 cases from which ganglionic cultures maintained for three months did not reveal virus, RIA revealed antibody to type 1 virus in five, to type 2 virus in one, and to both types in two. These results might reflect a difference in sensitivity in the test methods, technical difficulties in isolating HSV, or the presence of latent virus at some other site in the body which was not sampled. The specificity of the RIA was indicated by the observation that coded sera from children did not contain antibody.

The degree of correspondence between the isolation of virus from the trigeminal and sacral ganglia with the detection of type-specific antibody by RIA suggests that virus infection at these sites may be responsible for persistence of type-specific antibody for the lifetime of the individual. The use of RIA provides a highly sensitive and specific tool for detection of type-specific antibody in sera, and results appear to correlate well with the presence of latent ganglionic infection by HSV.

This work was supported in part by Public Health Research Grant AI-01475 from the National Institute of Allergy and Infectious Diseases and by Grant RG-895-B-2 from the Multiple Sclerosis Society.

The authors are indebted to Dr Nathalie J. Schmidt for consultation during the course of these studies and for reviewing this manuscript.

REFERENCES


(Received 27 January 1977)