SUMMARY

Dissemination of herpes simplex virus (HSV) in nude mice after intracutaneous inoculation in the midflank, and the effect of passively administered antibody on the course of infection were investigated. In untreated infected mice the skin lesions developed rapidly and HSV could first be recovered from the homogenate of the dorsal root ganglia on day 3 after infection, from the spinal cord on day 7 and from the brain on day 11. HSV could not be recovered from the blood, spleen or liver. In mice passively immunized with human gamma globulin, development of the skin lesions was rather slow and HSV could not be recovered from the homogenate of the dorsal root ganglia until day 16. From the results of explant culture of the ganglia, HSV was found to have reached the ganglia as early as 48 h after infection, even in mice administered human gamma globulin. The protective action of antibody seems to originate from the inhibition of virus growth not only at the inoculation site but also in the dorsal root ganglia.

There have been several reports on the dissemination of herpes simplex virus (HSV) in experimental animals (Johnson, 1963; Wildy, 1967; Cook & Stevens, 1973; Knotts et al., 1974; Oakes, 1975a; Renis et al., 1976; Davis et al., 1979). Although the site of inoculation varied from experiment to experiment and haematogenous spread operated in some systems, the results of these experiments suggest that HSV can spread to the central nervous system (CNS) by way of peripheral nerves and regional sensory ganglia.

In addition to the examination of virus dissemination, analysis of the immune mechanism responsible for protection against or recovery from HSV infection has been reported from several laboratories. In general, cell-mediated immunity seems to play the most important role in the recovery from HSV infection (Oakes, 1975b; Rager-Zisman & Allison, 1976). Humoral immunity also appears to be involved in the mechanisms of resistance to HSV infection (Worthington et al., 1980a, b; Oakes & Lausch, 1981). We have also previously investigated possible resistance mechanisms, using the nude mouse system (Nagafuchi et al., 1979; Hayashida et al., 1982) in which nude mice are inoculated intracutaneously with HSV in the midflank according to the method of Sydiskis & Schultz (1965). This system has the advantage that skin lesions are readily visible and can be evaluated semiquantitatively. Furthermore, because of the complete deletion of the mature T cell population in athymic nude mice, humoral and cell-mediated immunity can be evaluated separately in transfer experiments. In this system, mice challenged with HSV die at about 14 days after infection. The recipient mice acquire complete resistance by adoptive transfer of immune spleen cells. In the group given hyperimmune mouse serum, the mean survival time is prolonged significantly although the mice die eventually.

In the present study, we have compared virus dissemination from the site of inoculation to the CNS in the nude mouse system with or without antibody treatment in order to determine the organ in which the antibody acts.

Vero cells grown in Eagle’s minimal essential medium (MEM) supplemented with 5% calf serum were used for virus propagation and titration. HSV type 1, strain Hayashida, which was originally isolated by Dr H. Oda from a vesicular skin lesion of herpes labialis and passaged five times in Vero cells, was used for inoculation. Six-week-old nude Balb/c mice (supplied by Central Laboratory of Experimental Animals Ltd., Tokyo, Japan) were used throughout the study. The mice were inoculated intracutaneously in the midflank with $2.5 \times 10^4$ p.f.u./0.05 ml...
Fig. 1. Amount of HSV present in various organs and tissues after intracutaneous inoculation with or without human gamma globulin treatment. Six-week-old nude mice were inoculated with $2.5 \times 10^4$ p.f.u. HSV-1 intracutaneously in the midflank. Inoculated mice were divided into two groups: one group (a) received PBS only and served as a control; the other group (b) was given 1 ml human gamma globulin intraperitoneally 3 h after infection. On the day of sampling, six mice showing a similar degree of lesion development were killed and the various organs and tissues assayed for virus. Lesion scores of mice are as follows: 0, no lesion; 1+, local vesicle; 2+, local erosion; 3+, scattered zosteriform lesion; 4+, confluent zosteriform lesion. Homogenates of organs and tissues were assayed on Vero cell monolayers. Each point represents the geometric mean titre of virus from three to six mice ± the standard deviation. Lines A and B represent levels of sensitivity for assay in the brain (A), and the ganglia and spinal cord (B) respectively. ●, Skin; ○, dorsal root ganglia; △, spinal cord; ▲, brain.

HSV according to the method of Sydiskis & Schultz (1965). The skin lesions were scored every day.

A 1 ml amount of human gamma globulin prepared from pooled human plasma and having a neutralizing titre of 1:64 was injected intraperitoneally into mice 3 h after inoculation. The neutralizing activity of human gamma globulin was measured using the microplate method described previously by Nagafuchi et al. (1979), where the neutralizing antibody titre was expressed as the highest dilution which showed 80% plaque reduction. Mice which received only phosphate-buffered saline (PBS) served as controls.

At various times after inoculation, blood samples were taken from each mouse along with tissue samples which included the sites of skin lesions (the minimum area encompassing the whole lesion), dorsal root ganglia from L (lumbar)-1 to L-5, the spinal cord, brain, liver and spleen (three to six mice were used for each). Heparinized whole blood and pooled dorsal root ganglia were assayed for virus soon after removal. Dorsal root ganglia were homogenized using a mortar and pestle in 1 ml MEM supplemented with 2% calf serum, and 10-fold serial dilutions were made from the homogenate. The other organs and tissues were kept in a deep freeze at −70 °C until required. Before titration, tissue samples were homogenized with a Teflon homogenizer, and the skin was separately homogenized using quartz sand and a mortar and pestle. After centrifugation at 2000 rev/min for 10 min, serial 10-fold dilutions were made from supernatant fluids. Each dilution was inoculated on to a Vero cell monolayer in Falcon plastic dishes and allowed to adsorb for 1 h before the cells were overlaid with MEM containing 2% calf serum and 2% methyl cellulose. The medium was removed 3 days later, the cells stained with 0.5% crystal violet, and the plaques counted. The titres of virus recovered from the various homogenates were expressed as p.f.u./organ or tissue.

In some experiments, dorsal root ganglia were examined for the presence of virus after explant culture. Pooled dorsal root ganglia from L-1 to L-5 were removed 24, 48 and 72 h after infection and placed in MEM supplemented with 2% calf serum. Immediately after removal of the ganglia, a suspension of Vero cells in MEM supplemented with 2% calf serum was added, cultured for a further 10 days, and then examined for virus-induced cytopathic effect.

The distribution of HSV and the lesion score of mice sampled on various days after infection.
are shown in Fig. 1. The typical course of lesion development in mice with and without human gamma globulin is as follows. In the control mice which had not received gamma globulin, small vesicles appeared on day 3 after infection and developed into local erosion by day 5. The lesion began to spread after day 7 and eventually developed into a zosteriform skin lesion after day 11, and almost all mice died within 14 days after infection. In the mice which had received human gamma globulin 3 h after infection, the skin lesion developed rather more slowly. In this group, the local lesions appeared on the same day as in the control group; however, later development of skin lesions was suppressed. The lesions remained at the site of inoculation for up to 13 days and developed into zosteriform lesions by day 19 (see also Hayashida et al., 1982).

In order to determine the organ in which the administered antibody acts, we examined the route of virus spread in nude mice with or without gamma globulin treatment. As shown in Fig. 1(a), the organs from which the virus could be recovered before death were the skin, pooled dorsal root ganglia from L-1 to L-5, the spinal cord and brain. No virus could be recovered from the liver or spleen throughout the experiment, neither could virus be isolated from the peripheral blood. The titre of the virus in the skin remained at a low level as long as the lesion remained localized at the site of inoculation. Virus titre rose, however, as the zosteriform skin lesions began to develop and the highest titre in the skin was recorded just before death. The virus could be recovered from the homogenates of the dorsal root ganglia as early as day 3, and from the spinal cord homogenates by day 7 after infection. The titre of virus in the dorsal root ganglia rose on day 7, at which time zosteriform skin lesions began to appear, and reached a plateau. Similarly, the virus titre in the spinal cord rose on day 7 and stayed at a high level until death of the mice. The virus could be recovered from the brain only in the case of moribund mice.

HSV seems to spread to the CNS only by the neurotropic route in nude mice inoculated intracutaneously in the midflank and not haematogenously. Intra-axonal transport of HSV has been proposed as a neurotropic route by Hill et al. (1972) and Cook & Stevens (1973). Using mice inoculated in the footpad, Cook & Stevens (1973) found that maximal quantities of virus were regularly present in the ganglia earlier than in the sciatic nerves and from these findings, they suggested retrogressive infection of the nerves by the virus travelling centrifugally in axons from the infected neurons. Likewise, the zosteriform lesions found in our study seem to be formed by retrogressive infection of the skin by the virus which had once grown in neurons in the dorsal root ganglia. Similarly, infection of the spinal cord seems to be an ascending infection by the virus travelling from the infected neurons, and the cause of death in the present experimental system is considered to be encephalomyelitis.

The profile of virus recovery from gamma globulin-treated mice (Fig. 1b) was quite different from that of the control mice. The titres of virus recovered from the skin lesion remained constant or declined slowly during the first 7 days and thereafter rose gradually until day 13. From day 13 titres rose rapidly along with the development of the zosteriform skin lesions. As with the control mice, the maximal virus titre in the skin lesion was detected just before death.

In nude mice infected with HSV and treated with gamma globulin, no virus could be detected in homogenates of the dorsal root ganglia until the zosteriform skin lesions began to appear (day 13). However, there is a possibility that the assay technique was not sensitive enough to detect the virus before this time. Surgical removal of the inoculation site was reported to be ineffective in altering the disease in footpad challenge (McKendall et al., 1979). In order to test this possibility, explant cultures of the ganglia were prepared. As shown in Table 1, no virus could be recovered 24 h after infection. However, the virus could be recovered from some of the cultures
Table 1. Recovery of HSV from dorsal root ganglia after midflank intracutaneous inoculation in nude mice with or without human gamma globulin treatment*

<table>
<thead>
<tr>
<th>Time after infection (h)</th>
<th>GG†</th>
<th>Control‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>48</td>
<td>2/5</td>
<td>2/5</td>
</tr>
<tr>
<td>72</td>
<td>4/5</td>
<td>3/5</td>
</tr>
</tbody>
</table>

* The dorsal root ganglia ipsilateral to the inoculation site were removed and explanted into MEM supplemented with 2% calf serum. A Vero cell suspension was added to the explants and observations for a virus-induced cytopathic effect were made for 10 days.
† Received 1 ml human gamma globulin 3 h after infection.
‡ Control group received only PBS.

478

of dorsal root ganglia taken from the mice in both control and gamma globulin-treated groups 48 h after infection, and at 72 h after infection, the virus was present in cultures from the majority of each group. Cytopathic effects were observed within 7 days after explant in both control and gamma globulin-treated groups. From these results it is evident that HSV had reached the dorsal root ganglia as early as 48 h after infection from the midflank, but its multiplication in the dorsal root ganglia was suppressed as long as a sufficient level of gamma globulin was maintained. A similar phenomenon was reported by Cook & Stevens (1973) using footpad inoculation. Despite the arrival of HSV at the dorsal root ganglia in the early stage of infection, virus multiplication here is considered to be suppressed by antibody administration.

Our data indicate that the inhibitory effect of antibody on the course of infection originates from the suppression of virus growth not only at the inoculated skin site but also in the dorsal root ganglia, and following retrogressive and ascending infection.

This investigation was supported by a grant from Ministry of Education, Science and Culture of Japan. We thank Dr Mary Louise Robbins for reviewing the manuscript.

Department of Microbiology
School of Medicine
Kyushu University
Fukuoka 812, Japan

Yoichiro Kino†
Yoshinobu Hayashi
Isao Hayashida
Ryoichi Mori*

† Present address: The Chemo-Sero-Therapeutic Research Institute, Shimizumachi, Kumamoto 860, Japan.

REFERENCES


(Received 26 April 1982)