The JH2604 deletion variant of herpes simplex virus type 2 (HG52) fails to produce necrotizing encephalitis following intracranial inoculation of mice

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Herpes simplex virus (HSV) is a neurotropic human herpesvirus responsible for a variety of conditions ranging from mild cutaneous lesions to a very rare fatal encephalitis. After acute infection at the surface of the body, the virus travels via axons of the sensory nerves to establish a latent infection in the dorsal root ganglia of the peripheral nervous system (Stevens & Cook, 1971). Following reactivation virus mainly travels to the periphery resulting in cutaneous lesions but transport to the central nervous system (CNS) can result in encephalitis. Encephalitis can also be a consequence of a primary infection (Nahmias et al., 1982). Acute necrotizing encephalitis is the most serious neurological disease caused by HSV (Finelli, 1975) and as direct study in humans is impossible, animal model systems have been employed. Intracranial inoculation of mice with HSV produces lesions similar to those seen in humans and we have therefore chosen the mouse as a suitable model in which to evaluate the role of specific regions of the HSV genome in neuropathogenesis.

A series of deletion variants of both HSV types 1 and 2 isolated in our laboratory provides the unique opportunity of exploring the roles of the deleted genes in the pathogenesis of the disease (Brown et al., 1984; Harland & Brown, 1985, 1989; Brown & Harland, 1987; MacLean & Brown, 1987a, b). One such variant (JH2604) of HSV-2 strain HG52 (Harland & Brown, 1985) has been shown to be completely avirulent following intracranial inoculation of mice with a LD50 of > 10^7 p.f.u./mouse compared to < 10^2 p.f.u./mouse for its parental wild-type (wt) virus (Taha et al., 1989a). Dideoxynucleotide sequencing analysis of JH2604 has shown that the genome has a deletion of 1488 bp within the 3 kb BamHI v fragment (0 to 0.02 and 0.81 to 0.83 map units) which encompasses one copy of the 17 bp direct repeat element of the 'a' sequence and terminates 522 bp upstream of the 5' end of the immediate early gene 1 (Taha et al., 1989b). It was concluded that sequences within the 1488 bp conferred neurovirulence on strain HG52.

In this study the morphological changes and spread of HSV antigen in the brains of mice inoculated intracranially with wt HG52 and JH2604 are described.

Three week old BALB/c mice (Bantin & Kingman) were anaesthetized and 0.025 ml of virus stock (10^5 p.f.u./mouse) was inoculated into the left cerebral hemisphere. HSV-2 strain HG52 (Timbury, 1971) and the variant JH2604 (Harland & Brown, 1985) were used. Control animals were either untreated or inoculated with phosphate-buffered saline containing 10% calf serum. At 0, 12, 24, 48, 72, 96, 120, 144 and 168 h post-infection, two surviving mice were sacrificed; their brains were removed rapidly and fixed in 10% formol-buffered saline. The left sides of the brains were marked with indelible ink for orientation; the forebrains were cut into
three equally spaced coronal slices; the hindbrains were cut transversely into two through the pons. The portions of brain were processed and embedded in paraffin wax. Multiple 7 μm-thick sections were cut, some of which were stained by haematoxylin and eosin and others processed for immunohistochemistry as described by Kennedy et al. (1985) using polyvalent rabbit antiserum.

On macroscopic examination it was possible to identify, in 70% of the specimens, intracerebral inoculation over the lateral aspect of the left cerebral hemisphere. There were no complications and, in particular, no evidence that the intracranial pressure had been high during life. On microscopic examination the brains of the control group of animals showed no evidence of inflammation, hydrocephalus or of internal herniation. By 24 h post-inoculation there was a mild degree of lymphocytic infiltration in the meninges of all animals whether injected with wt virus or JH2604. After 48 h, in wt virus-infected animals the infiltrate was confined to the basal meninges. In addition, focal infiltration of the ventricular system, especially the walls of the third ventricle, was seen in JH2604-infected animals. By 72 h in the wt-infected animals there were small foci of necrotizing encephalitis in the internal capsule and thalamus of the left cerebral hemisphere. Within a further 24 h the lesions were larger, bilateral and affected both grey and white matter. Within these areas there was focal necrosis of all neurons and glia, vacuolation of the white matter, swelling of related astrocytes and a mild infiltration by neutrophils and polymorphs (Fig. 1a). A proportion of the related blood vessels were lightly cuffed by macrophages and there was a moderate amount of lymphocytic infiltration of the basal meninges (Fig. 1b). In JH2604-infected animals examined 72 and 96 h post-inoculation, there was no evidence of necrotizing encephalitis even at the site of inoculation. There was, however, an encephalitis with mild cuffing of small vessels, largely by lymphocytes and the occasional
Table 1. Nature and severity of neurohistological changes in animals inoculated with wt and JH2604 virus

<table>
<thead>
<tr>
<th>Virus</th>
<th>Survival (h)</th>
<th>n</th>
<th>Lymphocytic meningitis</th>
<th>Encephalitis</th>
<th>Ependymitis</th>
<th>Hydrocephalus</th>
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<tbody>
<tr>
<td>Wt</td>
<td>0</td>
<td>2</td>
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<td>24</td>
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<td></td>
<td>48</td>
<td>2</td>
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<tr>
<td></td>
<td>72</td>
<td>3</td>
<td>+</td>
<td>+ (FN)†</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>3</td>
<td>+ +</td>
<td>+ (FN)</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>120</td>
<td>2</td>
<td>+</td>
<td>+ (FN)</td>
<td>+ (PC)†</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>144</td>
<td>1</td>
<td>+</td>
<td>+ (FN + PC)</td>
<td>+ (FN + PC)</td>
<td>+</td>
</tr>
<tr>
<td>JH2604</td>
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<td>+ (PC)</td>
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</table>

* Grade: +, mild; ++, moderate.
† FN, Focal necrosis; PC, perivascular cuffing by lymphocytes.

macrophage (Fig. 1c). The perivascular cuffing was limited to the site of inoculation and was associated with a mild basal lymphocytic meningitis and a marked focal infiltration of the ventricular walls, again by lymphocytes. In these animals there was also evidence of mild hydrocephalus. By 120 and 144 h post-inoculation, the wt-infected animals showed multiple foci of necrotizing encephalitis throughout the grey and white matter of both the cerebral hemispheres and hindbrain. There was considerable proliferation of microglia, macrophage formation and some neutrophil polymorphs; there was also hypertrophy and hyperplasia of astrocytes. Oligodendroglia were difficult to recognise and there was vacuolation of myelinated fibres plus a small amount of perivascular cuffing by lymphocytes. Marked periventricular inflammation by lymphocytes was seen and in some animals necrotic debris was seen within the ventricles (Fig. 1d). There was mild hydrocephalus and small numbers of lymphocytes were present in the basal meninges. Similar foci of necrosis were seen in the brain stem and cortex of the cerebellum. At these times, in the JH2604-infected animals the histological changes were completely different from those seen in wt-infected animals. There was widely distributed perivascular cuffing by lymphocytes plus a variable but moderate amount of lymphocytic infiltration of the basal meninges which extended from the meninges along the intracortical vessels. In this latter site there was also a glial and astrocytic response but no evidence of any neuronal involvement or necrosis. All of the animals had developed a moderate degree of hydrocephalus. By 168 h only JH2604-infected animals had survived and the only change observed was a quantitative one. A summary of the histological changes is seen in Table 1.

Turning to the immunohistochemistry observed on staining with anti-HSV polyclonal antibody, the control animals showed no positive staining in the various brain cell types, the meningeal covering or the ependymal lining of the ventricles (Fig. 2a). By 24 and 48 h after wt infection there was evidence of astrocytic staining along the line of inoculation and also in the ependymal cells of the third ventricle. In JH2604-infected animals positive staining was limited to the immediate vicinity of the site of inoculation and one or two ependymal cells in the walls of the third ventricle. By 96 h positive staining of astrocytes was present in and around the foci of necrotizing encephalitis in wt-infected animals (Fig. 2b). Apart from a few ependymal cells there was no positive staining elsewhere in the forebrain. There was, however, positive staining of both Purkinje cells and related reactive Bergmann glia in foci of early necrosis within the cortex of the cerebellum. A few positive ependymal cells were also seen, some of which had desquamated into the lumen of the ventricle. In JH2604-infected animals, the pattern was very different. Positive staining was limited to astrocytes in the immediate vicinity of the site of inoculation (Fig. 2c), a few ependymal cells in the wall of the third ventricle and related subependymal astrocytes. There was no staining of neurons within either the fore- or hindbrain. By 144 h, in wt-infected animals there was astrocytic and neuronal staining associated with necrotic foci in the fore- and hindbrains. In JH2604-infected animals, positive staining was limited to a few ependymal cells, some of which had desquamated into
Fig. 2. (a) Control section of normal white matter. (b) HG52 infection at 96 h. Reaction product is seen in both viable and necrotic nuclei (T) and extracellularly in the neuropile (A). (c) JH2604 infection at 96 h. Positive staining is limited to reactive astrocytes along the line of inoculation (T). Staining was with avidin, binding to biotinylated horseradish peroxidase-conjugated anti-HSV serum diluted 1:100. Bar marker represents 50 μm for all sections.

the lumen of the ventricle, associated subependymal astrocytes and astrocytes in the immediate vicinity of the site of inoculation. The remainder of the brain appeared normal and in particular there was no evidence of positive staining in neuronal populations of either the fore- or hindbrain.

Although using the intracranial and not peripheral route of inoculation is criticized for being artificial, it has the clear advantage of bypassing many of the steps that affect HSV pathogenesis following peripheral inoculation, such as replication at the peripheral site of inoculation, neural entry, spread within the nervous system and escape from immunological control. As the primary purpose of the present experiments was not to investigate the pathogenesis of HSV encephalitis but rather to determine the distribution of virus antigen and describe the cellular response to the presence of the parental HG52 and its avirulent variant JH2604, it was decided to use the well established model of direct intracerebral inoculation. The procedure was standardized as far as possible to deliver microinjections of HSV into one thalamus rather than to either the hypothalamus or cerebellum as has been used previously (McFarland & Hotchin, 1987).

Up to 48 h the changes were few and essentially limited to a mild lymphocytic meningitis. Thereafter the nature and severity of the changes were markedly different following inoculation of the two viruses. On HG52 infection, the resulting encephalitis was characterized by foci of necrosis not only along the length of the needle track but also widely distributed throughout the brains. Because of the necrotizing nature of the process, identification of cell types was difficult but comparison with the contralateral unaffected side indicated that both neurons and glia were affected by the process. Apart from the occasional polymorphonuclear cell, there was a remarkable paucity of inflammatory cells and it was not until between 96 and 144 h that small numbers of macrophages and lymphocytes became evident.

The microscopic appearance of the brains of the animals inoculated with the variant JH2604 was strikingly different with a complete absence of necrotizing encephalitis even at the site of inoculation. By 72 h and to a certain extent increasingly with time, the lesion was characterized by perivascular cuffing of small vessels by a combination of lymphocytes and macrophages, limited to the site of inoculation or the ependymal lining of the ventricles. There was therefore a fundamental difference in the histological appearance in response to each virus, the wt infection being characterized by disseminated foci of necrosis with a minimal associated inflammatory cell response and the JH2604 infection by localized non-necrotizing encephalitis characterized by a marked mononuclear cell response. A probable consequence of the ependymitis that characterized infection by the variant was the development of hydrocephalus, a feature readily seen in the JH2604-infected animals compared to wt. The hydrocephalus is probably due to a narrowing of
cerebrospinal fluid pathways due to a combination of desquamating debris and the marked inflammatory cell infiltrate, features that have been described previously in HSV-1-induced hydrocephalus in mice (Hayashi et al., 1986). In the present series of experiments, distribution of virus was determined by immunohistochemistry. In both the wt- and JH2604-infected animals surviving up to 48 h, positive staining was limited to astrocytes close to the inoculation site and to some of the ependymal cells lining the ventricular system. Thereafter, however, in wt-infected animals, virus-containing cells were identified very near the necrotizing foci of encephalitis in the form of both intranuclear and intracytoplasmic staining of astrocytes and neurons. In some animals, there was also positive staining in the Purkinje cells of the cerebellum. Positive staining for virus in the JH2604-infected animals was limited to astrocytes in the immediate vicinity of the site of inoculation with a few ependymal cells in the walls of the ventricular system also staining; there was no distinct pattern of spread (selective vulnerability). The distribution of virus antigen closely reflected the histological identification of the encephalitic process in the brains of the inoculated animals.

It appears therefore that the HG52 variant JH2604 is unable to replicate efficiently within the cells of the brains of mice and that the pathological response to the virus is confined to an inflammatory infiltrate. The dose of virus used for wt and JH2604 inoculations was 10^5 p.f.u. and it is possible that with higher doses there would be an indication of virus spread and cell necrosis. However, we have shown that the LD_{50} of JH2604 is > 10^7 p.f.u./mouse compared to < 10^2 p.f.u./mouse for HG52 and that there was no in vivo replication as measured by titration of infectious virus recovered from brains at different times post-inoculation (Taha et al., 1989a). It seems unlikely therefore that increasing the infectious dose would alter the response within the brain.

We conclude therefore that JH2604 is avirulent due to an inability to replicate within neurons of the CNS of mice and that this phenotype is a direct result of the loss of information from within the 1488 bp deleted from the TR_L and IR_L regions of this virus. The nature of the replication defect is under study.

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References


Taha, M. Y., Clements, G. B. & Brown, S. M. (1989a). A variant of herpes simplex virus type 2 strain HG52 with a 1-5 kb deletion in Rs between 0 to 0.02 and 0.81 to 0.83 map units is non-neuroviral for mice. Journal of General Virology 70, 705-716.


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