Peripheral replication and latency reactivation kinetics of the non-neurovirulent herpes simplex virus type 1 variant 1716

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The terminal portion of the herpes simplex virus (HSV) genome long repeat region has been shown to contain a neurovirulence gene. Both HSV-1 and HSV-2 mutants deleted in this gene fail to cause central nervous system (CNS) disease in mice. The HSV-1 strain 17 variant 1716, which has a 759 bp deletion encompassing the gene, grows normally in tissue culture but fails to grow following intracerebral inoculation of mice. This paper demonstrates that 1716 is capable of peripheral replication in the footpads of mice. However, no acute replication of virus is detectable in dorsal root ganglia up to 10 days after footpad inoculation. These results imply that the replication defect in 1716 is not host-specific, but is tissue- and/or cell type-specific. Latency reactivation kinetics demonstrate that 1716 is capable of establishing a latent infection, but the kinetics of reactivation are significantly impaired compared to wild-type virus and are dose-dependent. Lack of acute ganglionic replication combined with impaired reactivation kinetics support the conclusion that a proportion of 1716 genomes initiate a lytic infection which then aborts, and a proportion enter the latent state. The results with 1716 imply that its inability to replicate in CNS and peripheral nervous system neurons is specific, and that the block in replication is beyond the stage of adsorption and entry. A prerequisite for any live attenuated HSV vaccine is an inability to initiate CNS involvement following peripheral inoculation. In this respect, 1716 has prototype vaccine potential with the proviso that a direct extrapolation is being made from mouse to man.

It is now agreed that the terminal 1 kb of the genome long repeat region (RL) of herpes simplex virus type 1 (HSV-1) strain F, HSV-1 strain 17 and HSV-2 strain HG52 contains a gene (Ackermann et al., 1986; Chou & Roizman, 1986, 1990; McGeoch et al., 1991; Dolan et al., 1992) which confers neurovirulence (Taha et al., 1989a, b; MacLean et al., 1991). Deletion or mutation of this gene results in variants which are incapable of replicating in central nervous system (CNS) neurons, and cause neither encephalitis nor subsequent death in mice (Taha et al., 1990). Both the HSV-1 strain 17 variant 1716, which has a 759 bp deletion in RL, and the HSV-2 strain HG52 variant 2604, with a 1488 bp deletion, grow as well as their wild-type (wt) parents in tissue culture, including mouse cells, but do not replicate in vivo when injected into the cerebral hemispheres of mouse brains. Wt viruses grow exponentially and all infected animals die within a few days of infection (MacLean et al., 1991). There is an approximately 106-fold difference in LD50 between wt viruses and variants in which the RL neurovirulence gene is deleted.

Knowing that deletion of the RL neurovirulence gene renders HSV incapable of destroying CNS tissue, it was important to establish whether these viruses are able to replicate in peripheral tissue. In addition, because the minor latency-associated transcript (LAT) of 8.3 kb (Mitchell et al., 1990) is antisense with respect to the RL neurovirulence gene, it was important to determine whether variants with deletions in this gene are capable of establishing and reactivating from latency, and whether infection of peripheral nervous system (PNS) neurons as opposed to CNS neurons is also abortive.

This paper reports the peripheral replication kinetics, the acute dorsal root ganglion (DRG) replication kinetics and the latency reactivation kinetics following DRG explant of the variant 1716 subsequent to peripheral infection of the footpads of mice.

To study peripheral replication, groups of 3-week-old BALB/c mice were injected in the right rear footpad with 10^5 p.f.u. of either HSV-1 strain 17 or the variant 1716 in 0.025 ml. From day 0 (day of infection) to day 10 post-infection (p.i.), two mice were killed daily, their right rear footpads were excised, homogenized and sonicated, and the virus in the resultant suspension was titrated on BHK21/C13 cells. It can be seen from Fig. 1(a) that the wt 17+ virus grows well, showing a 100-fold increase in growth between days 0 and 6 p.i. There were two peaks of growth, one on day 1 and the other on day 5.
Short communication

Fig. 1. (a) In vivo growth kinetics of HSV-1 strain 17 (○) and the deletion variant 1716 (●) in the footpads of BALB/c mice. Three-week-old mice were injected in the right rear footpad with 0.025 ml of virus. From day 0 (day of infection) to day 10 p.i., two mice were killed daily, their infected footpads were removed, homogenized and sonicated, and the resultant suspension was titrated on BHK21/C13 cells. (b) In vivo growth kinetics of HSV-1 strain 17 (○) and the deletion variant 1716 (●) in the DRG of mice. Mice were infected in the right rear footpad with 0.025 ml of virus. From day 0 to day 10 p.i., two mice were killed daily and nine DRG (six lumbar, two thoracic and one sacral) explanted from the right side of the spinal cord. The nine ganglia from each mouse were homogenized and sonicated, and the virus in the resultant suspension was titrated on BHK21/C13 cells.

To determine the amount of acute replication in DRG following footpad inoculation, the same animals used to study footpad replication had nine DRG (two thoracic, six lumbar and one sacral) removed from the right-hand side of the spinal cord of two animals daily between days 0 and 10. The DRG were pooled, homogenized as for the footpad tissue and virus was titrated on BHK21/C13 cells. It can be seen in Fig. 1(b) that in 17+-infected animals, infectious virus was first detectable by day 2 and reached a peak of 1.2 × 10^5 p.f.u./ml by day 5, after which the titre declined until, by day 8, it was below the threshold level of detection (< 1 p.f.u.). In 1716-infected animals there was no detectable infectious virus (< 10^5 p.f.u./ml) released from the DRG on any day between days 0 and 10.

To determine whether variant 1716 was capable of establishing and reactivating from a latent infection, groups of four 3-week-old BALB/c mice were infected via the right rear footpad with different doses of HSV-1 strain 17 (10^4 and 10^5 p.f.u./animal) and variant 1716 (10^5, 10^6 and 10^7 p.f.u./animal) in 25 μl volumes. Six weeks p.i. the mice were killed and the same nine DRG used to study acute replication were explanted separately into microtitre plate wells. Every second day, the supernatant from the wells was transferred to BHK21/C13 cells, which were subsequently stained and scored for c.p.e. The results shown in Fig. 2 illustrate that by day 3 post-explantation virus from 55% of the DRG from mice infected with 17+ at a dose of 10^5 p.f.u./animal had reactivated. At a dose of 10^4 p.f.u., virus first appeared 7 days post-explantation and, by day 15, 30% of the DRG had released virus. DRG from animals infected with a dose of 10^5 p.f.u. of 1716 did not release virus until day 14 post-explantation and by day 16 only 8% had shed virus. The rate and frequency of reactivation increased when the input dose of 1716 was increased to 10^6 p.f.u., virus appearing first on day 12 and 10% of DRG being positive for virus release by day 16. By increasing the dose to 10^7 p.f.u., virus was released first on day 9, and by day 15 29% of DRG had shed virus. The
rate and frequency of reactivation were therefore dose-dependent.

Three fundamental prerequisites for the development of a live attenuated HSV vaccine are: (i) the virus must be incapable of causing CNS disease, (ii) the virus must be able to replicate at the periphery and thereby induce an immunological response; (iii) the virus should be unable to establish latent infection, or, if this is not achievable its ability to reactivate should be abolished.

Therefore, the identification of a neurovirulence gene (Ackermann et al., 1986; Chou & Roizman, 1986, 1990; McGeoch et al., 1991; Dolan et al., 1992) and the isolation of variants in which this gene is deleted (Taha et al., 1989a, b; MacLean et al., 1991) provides the starting material for a prototype vaccine strain. Variant 1716 is totally avirulent for BALB/c mice and the virus is incapable of replication in brain tissue (MacLean et al., 1991). Therefore it was important to establish whether 1716 replicated in mouse tissues other than the CNS. The results presented here demonstrate that wt 17+ replicates efficiently in cells of the footpad, and the two peaks of replication possibly represent reinitiation of cells by virus travelling back from the DRG after the first burst of replication. The amount of replication seen in 1716-infected footpads by day 1 p.i. is comparable to that in the wt infection, but no second burst of replication takes place; the titre declines by day 3 and by day 6 no infectious virus is detectable. The difference in titres at day 0 probably reflects the logistics of the experiment, in that there was a longer time between infection and dissection of the footpad in 1716-infected animals than in 17+-infected animals. Consequently, input 1716 had already been disassembled by the time of footpad removal, whereas in 17+-infected animals some residual input virus was still present. The results clearly show that 1716 is capable of productive replication in mouse footpad tissue, and that the defect in CNS replication is tissue- and/or cell-specific and not species-specific. Therefore it could be assumed that 1716 should elicit a good immunological response; a cell-mediated response in mice (B. Rouse, personal communication) and an antibody response in guinea-pigs (M. Slaoui, personal communication) have been demonstrated.

As expected, 17+ demonstrated efficient ganglionic replication with a peak titre on day 5 p.i. and virus still being detectable on day 8. This is comparable with previous results on 17+ replication in the trigeminal ganglia after intraocular infection of mice (Steiner et al., 1989). However, ganglia from 1716-infected animals released no detectable infectious virus (< 10 p.f.u.) up to 10 days after footpad inoculation (low levels of replication below the detectable threshold level cannot be entirely ruled out). These results strongly indicate that 1716 is incapable of replication in neurons of the PNS and support our conclusion that the replication defect in 1716 is tissue- and/or cell type-specific.

The latency reactivation experiments demonstrated that >50% of ganglia from 17+-infected animals released virus post-explantation. This figure varies from experiment to experiment, and it is known from previous studies that as many as 80 to 90% of the DRG harbour latent HSV-1 genomes. In animals infected with a comparable dose of 1716, only 8% of ganglia released infectious virus and this was delayed 10 days in comparison to that of wt virus. The rate and frequency of reactivation improved on increasing the input dose, but even with a 10-fold greater concentration of inoculum, only 29% of the ganglia were positive for virus release, with a 6 day delay compared to wt virus. Therefore it appears that although 1716 is capable of establishing and reactivating from latent infection, and by analogy is capable of entering PNS neurons, the acute replication data would suggest that the latent virus is derived entirely from the input inoculum. The lower frequency and slower rate of reactivation reflects either fewer neurons with latent genomes or fewer latent genomes per neuron, or a combination of both.

It is of course already known that replication is not a requirement for the establishment of latency. The HSV-1 variant in 1814, with a mutation in Vmw65, although incapable of replication in vivo establishes an efficient latent infection and reactivates at the same frequency and rate as wt virus (Steiner et al., 1990). It is assumed with Vmw65 mutants that there is no competition between the establishment of latency and the lytic cycle, and hence all virus reaching the neurons becomes latent. As the stage at which replication is blocked in 1716 is not yet known, the virus may be capable of entering a lytic cycle which is then rapidly aborted, and hence only a proportion of the genomes reaching the ganglionic neurons will establish latency. Therefore the rate and frequency of reactivation would be expected to be lower than those of wt virus. The fact that dose increases improve the reactivation parameters in 1716 infections would support this conclusion. The effect of the 1716 deletion on the expression of the minor and major LATs is at present under study.

With the proviso that a direct extrapolation is being made from results in mice to man, it is clear that HSV-1 strain 17 variant 1716 provides a prototype for a live attenuated vaccine because (i) it is incapable of inducing CNS disease and, as the mutation is a deletion, the question of reversion does not arise; (ii) it replicates efficiently at the periphery and induces a good immune response; (iii) it is unlikely to spread from the PNS to the CNS following the natural route of peripheral infection as it does not replicate in sensory ganglia; (iv) although capable of establishing a latent infection it is severely
impair in its capacity to reactivate. We have previously isolated a mutation in the LAT promoter that also severely impairs the reactivation kinetics of the virus (Steiner et al., 1989). A variant with the LAT promoter deletion in conjunction with the 759 bp 1716 deletion has been constructed and its in vitro and in vivo characteristics are currently under study.

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