The Avirulent A7 Strain of Semliki Forest Virus Has Reduced Cytopathogenicity for Neuroblastoma Cells Compared to the Virulent L10 Strain

By G. J. ATKINS

Department of Microbiology, Moyne Institute, Trinity College, Dublin 2, Ireland

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SUMMARY

Molecular and host range properties of the virulent L10 strain of Semliki Forest virus were compared to those of the avirulent A7 strain. No difference could be detected between the two strains in adsorption, nucleocapsid synthesis, protein synthesis, ratio of 42S:26S RNA, particle infectivity, interferon induction and susceptibility, or defective interfering particle production. A7 showed lower total RNA synthesis than L10 in BHK, G26-24 (oligodendroglioma) and C1300 (neuroblastoma) cells. Cytopathogenicity of A7 was reduced compared to L10 in C1300 cells but not in G26-24 cells. It is concluded that the avirulent A7 strain has similar host range and molecular properties to the neurovirulence mutants M9 and M136, and that demyelination may be produced by a similar mechanism.

Semliki Forest virus (SFV) is a neurotropic alphavirus which has been widely used as a model in studies of pathogenicity. Virulent strains of SFV produce a lethal encephalitis in weanling mice whereas avirulent strains produce a self-limiting encephalitis which involves demyelination. Several studies have compared virulent and avirulent strains in an attempt to relate virulence to properties of the virus. Woodward & Smith (1975) claimed to have detected defective interfering (DI) particles in the brains of mice infected with an avirulent strain but not a virulent strain, but this later proved to be due to an artefact (Woodward et al., 1978). The avirulent A7 strain of SFV multiplied more slowly in the brains of infected mice than the virulent strain (G. J. Atkins, unpublished results), possibly allowing immune defence. Woodward & Smith (1979) showed that the A7 strain was temperature-sensitive. However, the molecular basis of this defect was not determined, and the mechanism of demyelination by the A7 strain has not been fully elucidated (Suckling et al., 1978; Kelly et al., 1982).

Our approach to the analysis of SFV pathogenicity has been to isolate neurovirulence mutants of a highly virulent wild-type (wt) strain. Of four such mutants isolated, two (M9, M136) were found to enter the brain following intraperitoneal injection and produce demyelination, whereas the other two caused a viraemia but did not enter the brain. All four mutants allowed survival of the majority of mice infected with low doses, whereas the wt caused a lethal encephalitis in all cases (Barrett et al., 1980). We have shown that all four neurovirulence mutants are defective compared to the wt, and that the two mutants causing demyelination have separate defects in viral RNA synthesis (Atkins & Sheahan, 1982). These defects lead to a reduced capacity to multiply and cause a cytopathic effect (c.p.e.) in neuroblastoma cells, although the ability to cause a rapid c.p.e. in oligodendroglia cells is unaffected. This effect was also observed in the intact animal; the wt destroys both oligodendrocytes and neurons, whereas the mutants have lost the capacity to destroy neurons but not oligodendrocytes. We have concluded that destruction of neurons is lethal for the mouse and that destruction of oligodendrocytes is the trigger for demyelination (Sheahan et al., 1981, 1983; Atkins & Sheahan, 1982). The present study shows that the avirulent A7 strain also has reduced RNA synthesis compared to the virulent L10 strain and that its cytopathogenicity is reduced for neuroblastoma cells but not oligodendroglia cells.
The BHK cell line, the oligodendroglioma cell line G26-24 and the neuroblastoma cell line C1300 were grown as previously described (Atkins & Sheahan, 1982). The virulent L10 and avirulent A7 strains of SFV were grown from single plaque isolates and assayed as previously described (Atkins & Sheahan, 1982; Atkins et al., 1982). Their virulence was tested by injecting $10^4$ p.f.u. intraperitoneally into groups of six 40-day-old BALB/c mice; for L10 all six mice died within 7 days, whereas all six mice injected with A7 survived. The pathology of eight 40-day-old mice injected intraperitoneally with $10^4$ p.f.u. of A7 was also examined between 7 and 14 days after infection and mild demyelinating lesions were found in two of these mice.

Our approach was to compare molecular properties of A7 and L10 using the strategy and techniques described earlier (Atkins & Sheahan, 1982). No significant difference could be detected between A7 and L10 in adsorption to BHK, C1300 or G26-24 cells, in nucleocapsid synthesis in BHK cells, in protein synthesis in the three cell lines, in interferon induction or susceptibility to interferon in C1300 and G26-24 cells or in particle : infectivity ratio for BHK cells. Since it has been shown that the administration of SFV DI particles can reduce virulence (Crouch et al., 1982), the ability of A7 and L10 to produce DI particles in BHK cells was measured. Confluent monolayers of BHK cells in 90 mm diam. plastic dishes were infected with 20 p.f.u./cell of A7 or L10, and the virus produced after 16 h was used to infect four fresh monolayers. This was continued for 10 passages without dilution. In early passages a multiplicity of infection of 20 to 50 was maintained for both A7 and L10. At each passage, one plate received 0.1 p.f.u./cell of added unpassaged standard virus, one received 1 μCi/ml $[^3]$H]uridine, the yield from one plate was plaque-assayed and the yield from the fourth plate was used for further passage. Labelled virus was used to measure particle : infectivity ratio as previously described (Atkins & Sheahan, 1982) and the yields produced from the standard virus alone compared to standard virus in the presence of passaged virus gave a measure of the interfering capacity of the preparation. For A7, a drop in yield of log$_{10}$ p.f.u. occurred at passage 7, and this was associated with an increase in particle : infectivity ratio and interfering capacity. For L10 this occurred at passage 6. Therefore, the lack of virulence of A7 could not be associated with faster production of DI particles, at least in BHK cells. These experiments could not be repeated in mouse cells because of the low yields of virus produced.

Like L10 and its neurovirulence mutants, A7 produced a rapid c.p.e. in G26-24 cells which was detectable at 4 h after infection and complete at 8 h. This c.p.e. was not associated with inhibition of host protein synthesis. In contrast, C1300 cells showed a c.p.e. at 24 h after infection with L10, but the c.p.e. for A7 was slight and similar to that obtained for neurovirulence mutants. This effect was analysed further by measuring virus yields and RNA synthesis in these three cell lines (Fig. 1). Unlike the neurovirulence mutants M9 and M136, A7 produced a greater yield of infectious virus in BHK cells, although viral RNA synthesis was diminished compared to L10. Thus, A7 has an enhanced ability to incorporate RNA into virions in BHK cells compared to L10. There was also no difference in the ratio of 42S : 26S RNA in BHK cells infected with L10 compared to A7. For G26-24 cells, A7 also produced a greater yield than L10, but viral RNA synthesis was diminished. For C1300 cells, viral RNA synthesis was undetectable for A7, although it could be detected for L10. Unlike the result obtained for BHK and G26-24 cells, infectious virus production was higher for L10 than A7 in C1300 cells.

Thus, it can be concluded that strain A7 is similar to mutants M136 and M9 in that its cytopathogenicity in neuroblastoma cells is reduced compared to L10, but cytopathogenicity for oligodendrogloma cells is similar to L10. For the mutant M9 we have shown that virus can be detected in oligodendrocytes in the intact animal, and that virus-induced changes can be detected in these cells early in the disease. We have suggested that infection of oligodendrocytes and subsequent release of myelin antigens results in immune-mediated demyelination in a manner similar to experimental allergic encephalomyelitis (Atkins & Sheahan, 1982; Sheahan et al., 1983). For A7, these early changes have not been reported, but immune-mediated demyelination has been described (Suckling et al., 1978; Kelly et al., 1982). This may be related to the less severe demyelination produced by A7 in about 25% of mice (Suckling et al., 1978) compared to the severe demyelination produced in 94% of surviving mice by M9 (Atkins & Sheahan, 1982), making early events more difficult to detect for A7. The molecular and host...
range properties of A7 compared to M9 and M136 are consistent with the suggestion that these viruses produce demyelination by a similar mechanism.

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REFERENCES


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