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Japanese encephalitis virus: Biological clones from a clinical isolate quasispecies show differing neurovirulence *in vitro* and in a mouse model

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Abstract

The Japanese encephalitis virus (JEV), a leading cause of encephalitis, exists as quasispecies in clinical isolates. Using a limiting dilution method combined with immunohistochemistry to detect viral antigens, 10 biological clones were isolated and purified from a clinical JEV isolate (CNS138/9) derived from an autopsy brain. These biological clones were tested for neurovirulence in SK-N-MC and NIE-115 neuronal cells, and a 2-week-old, footpad-infected, JE mouse model. Nine clones were found to be neurovirulent; one clone neuroattenuated. Although further studies are needed to determine genotypic differences, if any, in these clones, the limiting dilution purification and neurovirulence testing methods described herein should be useful for phenotypic studies of quasispecies of neurotropic viruses in general, and JEV and other flaviviruses in particular.

Keywords: Japanese encephalitis virus, quasispecies, biological clones, limiting dilution

INTRODUCTION

Japanese encephalitis virus (JEV) is an enveloped, single-stranded positive-sense RNA arbovirus belonging to the family *Flaviviridae* and genus *Flavivirus*. The approximately 11 kb genome comprises capsid, pre-membrane and envelope (E) genes that encode for 3 corresponding structural proteins, respectively, and 7 non-structural genes (NS1, NS2A and B, NS3, NS4A and B, and NS5). The open reading frame is flanked by 5' and 3' untranslated regions.[1-3]

JEV is one of the leading causes of mosquito-borne viral encephalitides with an annual global estimate of 68,000 Japanese encephalitis (JE) cases, and 13,600 to 20,400 deaths in affected areas.[4] In endemic areas, JE largely involves children, but in non-endemic areas, all age groups are at risk of infection.[1] With a 1:25 to 1:1000 symptomatic to asymptomatic ratio[1,5], JE fatality rate ranges from 25% to 50%, and more than 50% of the survivors suffer permanent neurological sequelae.[6]

Similar to other flaviviruses like dengue virus (DENV) and West Nile virus (WNV), JEV replicates in the absence of proof-reading and repair of newly synthesized viral RNAs. Hence, routinely isolated viruses exist as a quasispecies which is a mixture of viral strains or biological clones with closely-similar genomes.[7,8] These biological clones may result in phenotypic differences including alterations in cell tropism, virulence, host range, and resistance to antiviral agents and host immune responses.[7,9] Isolation of biological clones from a flavivirus quasispecies population is possible, as had been demonstrated in WNV.[10-12] Since JEV is very difficult to isolate because of low and transient viraemia in humans, relatively little work has been done on its quasispecies.

One method that is commonly used for virus purification is the viral plaque assay. It uses serial dilutions of viruses to infect susceptible cell monolayers, and the application of a semi-solid nutrient medium overlay to prevent virus from spreading to nearby uninfected cells, resulting in a distinct plaque that can be easily visualized after staining. The method is useful for viruses that are able to produce good visible cytopathic effects (CPE).[13] Unfortunately, JEV infection does not produce good CPE and plaque formation, so the plaque assay is unsuitable for its purification.

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