

Rift Valley fever virus NSm protein inhibits virus-induced apoptotic cell death

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Rift Valley fever virus (RVFV) (genus *Phlebovirus*, family *Bunyaviridae*) causes severe epidemics among ruminants and causes fever, myalgia, a hemorrhagic syndrome, ocular disease, and encephalitis in humans. Most recently, in 2006-2007 an outbreak of RVFV occurred in Kenya with a high case-fatality ratio. RVFV M segment encodes two major structural envelope proteins Gn and Gc, a minor structural protein 78-kDa protein, and a nonstructural protein NSm. The viral M segment-derived mRNA has five in-frame translational initiation codons upstream of the region encoding Gn and Gc proteins (the pre-Gn region). NSm protein and 78-kDa protein were translated from the first AUG and second AUG, respectively. The biological functions of the NSm and 78-kDa proteins are unknown, while both proteins are dispensable for viral replication in cell culture. To find biological functions of NSm and 78-kDa proteins, we generated a mutant RVFV (arMP-12-del21/384) carrying a large deletion in the pre-Gn region by using a reverse genetics system of an attenuated vaccine candidate of RVFV, MP-12. Neither NSm nor 78-kDa proteins were synthesized in arMP-12-del21/384-infected cells. arMP-12-del21/384 and its parental arMP-12 showed similar virus growth kinetics, viral RNA synthesis and viral protein accumulation in infected Vero E6 and 293 cells, yet the former produced larger plaques than the latter in Vero E6 cells. MTT based cell-viability assay showed that arMP-12-del21/384 replication in Vero E6 cells and 293 cells induced rapid and more extensive cell death than the parental arMP-12. Annexin V binding and flow cytometry analysis further revealed that RVFV infection induced apoptosis and arMP-12-del21/384 replication triggered apoptosis early in infection as compared with arMP-12 replication. Consistent with the annexin V binding assay, the activation of caspase-3, which is a hallmark of apoptosis, and the cleavage of its downstream substrate, poly-ADP-ribose polymerase, occurred much earlier in arMP-12-del21/384-infected cells than in arMP-12-infected cells. We further demonstrated that the kinetics and severity of caspase-3 activation was suppressed by expression of NSm protein in arMP-12-del21/384-infected 293 cells. These results demonstrated that the NSm protein delayed or inhibited virus-induced apoptotic cell death, suggesting that NSm protein may be involved in viral survival and pathogenesis in infected hosts.