Department of Microbiology, Tumor and Cell Biology

Characterization of dengue virus isolates from patients experiencing dengue fever, dengue hemorrhagic fever, and dengue shock syndrome

AKADEMISK AVHANDLING
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ABSTRACT

The four serotypes of dengue virus (DENV) belong to the genus flavivirus, and have a positive sense, single-stranded RNA genome of ~11 kb. The DENVs cause the most common arthropod-borne viral disease in man with ~100 million infections per year. The sole measure of control is limiting the mosquito vectors *Aedes aegypti* and *Ae. albopictus*, and there is an urgent need for an effective vaccine and potent anti-viral drugs.

DENV infection can be asymptomatic or a self-limited, acute febrile disease ranging in severity. The classical form, dengue fever (DF), is characterized by high fever, headache, stomach ache, rash, myalgia and arthralgia. Severe dengue, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are accompanied by thrombocytopenia, vascular leakage and hypotension. The fatal condition DSS is characterized by systemic shock.

Dengue research has been hampered by a lack of appropriate animal models of infection and disease. Furthermore, fundamental knowledge such as host cell tropism and virulence markers are still not established. This thesis focuses on the characterization of clinical DENV isolates from all four serotypes and clinical conditions (DF, DHF, and DSS) aimed at identifying viral features involved in pathogenesis.

Attempts to develop a strand-specific qRT-PCR to identify primary target cells for DENV replication, failed due to the self-priming phenomenon of the DENV genome. Self-priming was not restricted to any particular regions of the viral genome, nor to contaminating cellular nucleic acids, nor the lack of a poly(A)-tail at the 3' end. First-strand synthesis *in situ* of the DENV genome is believed to arise due to spontaneous loop-back structures functioning as transient primers for the reverse transcriptase.

In vitro studies in mammalian Vero cells revealed a decreased level of replication for all DENV isolates from DSS patients compared to DENV isolates from DF patients. The replication patterns of the DHF isolates resembled either the DF- or DSS-derived DENV isolates depending on serotype. The DSS isolates were further distinguished from milder case DENV isolates by induction of apoptosis in mosquito C6/36 cells.

Three DENV-1 isolates representing a DF, DHF, and a DSS case, were further characterized *in vivo* in BALB/c mice. Infection with the DF and DHF isolates peaked during the first week with viral RNA found primarily in lungs, liver, and to a certain extent in brain. In contrast, the DSS isolate was primarily neurotropic and persisted longer compared to the DF and DHF isolates.

Genomic sequencing revealed a preference for amino acid substitutions in the viral envelope protein and the non-structural (NS) protein NS1 and NS5. Thus, these viral proteins may influence pathogenesis either by immunomodulation, and/or host cells tropism and replication.

In conclusion, these results based on clinical DENV isolates indicate that DENVs within the same serotype and genotype may have both different phenotypes and genotypes. These intrinsic viral features could influence virus virulence and disease pathogenesis in humans.

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