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IMINOSUGAR NB-DNJ DELAYS MORTALITY IN LETHAL MODEL OF DENGUE VIRUS INFECTION IN MICE

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The four serotypes of dengue virus (DV1-4) cause more human illness than any other arbovirus worldwide. DV infection results in dengue fever, an acute febrile illness, or the more severe, life-threatening dengue hemorrhagic fever/dengue shock. Immunity to one of the four DV serotypes can increase disease severity upon subsequent infection with another DV serotype via antibody-dependent enhancement (ADE) that facilitates entry of DV into Fcγ receptor-bearing cells. The DV genome consists of 3 structural (C, capsid; prM/M, membrane, and E, envelope) and 7 nonstructural (NS1-5) proteins, of which M, E, and NS1 are glycosylated. No antivirals or vaccines currently exist, and treatment is supportive in nature. We have developed a robust mouse model of DV infection and disease in mice lacking interferon α/β and γ receptors (AG129) that features many of the hallmarks of severe dengue in humans. Recently, we demonstrated ADE in these mice following passive transfer of anti-DV monoclonal antibodies or polyclonal immune sera. Iminosugars such as deoxynojirimycin (DNJ) and its N-alkylated derivatives are glucose analogues that inhibit host cell ER-resident glucosidases required for folding and maturation of glycoproteins. Iminosugars prevent interaction of many viral glycoproteins with ER chaperones, leading to incorrect glycoprotein folding, oligomerization and assembly of infectious virions. The N-linked glycosylation of the prM/M, E, and NS1 proteins are thought to play a role in viral morphogenesis and secretion. We tested the effect of *N*-butyl-DNJ (NB-DNJ) in our mouse model of ADE-induced lethal disease. Mice received 2ug of the anti-E 4G2 MAb intraperitoneally (ip) and were infected 24 hours later with $1x10^5$ DV2 D2S10 iv. Drug was administered daily or twice daily ip on the day of infection and for 7 days p.i.. NB-DNJ given at 0.2 mg/kg/day protected 3/5 (60%) mice from mortality, while PBS control mice all died by day 5 ($p=0.003$). Three of 5 mice receiving NB-DNJ at a much lower dose (0.0001 mg/kg/day) encapsulated in liposomes were protected from death vs. liposome alone, where all 5 mice died within 5 days ($p=0.003$). In addition, NB-DNJ treatment delayed morbidity in infected mice. In conclusion, glucosidase inhibitors reduce DV-induced disease and mortality *in vivo*, and we are currently evaluating other iminosugars with liposome delivery as promising antiviral therapies.

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DENGUE VIRUS PROTEASE NS2B/NS3 WITH A POTENTIAL INHIBITION OF THE TOLL-LIKE RECEPTOR 3 SIGNALING PATHWAY

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Dengue virus (DENV) is a mosquito-borne flavivirus of worldwide distribution that causes in humans, dengue fever (DF) an acute febrile illness or life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). The mechanisms of immune response against dengue virus are not completely understood. Toll-like receptors (TLR) are important in mediating inflammation and immune responses by recognizing pathogen specific ligands. TRL3 recognizes double-stranded RNA (dsRNA) activating the interferon (IFN) signaling pathway, a potent defense mechanism against viruses. However, viruses have developed strategies to avoid this response and the viral products that mediated this resistance are under study. After cytoplasmic nucleocapsid release, DENV RNA translates to a polyprotein that with the activity of host and virus peptidase results in the cleavage of three structural (C, E, prM) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins. Has been reported that DENV infected cells showed resistance to the antiviral action of IFN, for example, NS4B inhibit the α/β IFN signaling. In other flavivirus, Hepatitis C virus, has been described that the viral serine-protease NS3/4A cause a cleavage of TRIF (Toll-IL-1 receptor domain-containing adaptor inducing IFN-β) the adaptor protein linking TLR3. A sequence alignment of TRIF protein showed possible cleavage sites for DENV protease (NS2B/NS3). TLR3 induced activity of IRF3 was assessed using a luciferase reporter assay in the presence or absence of viral protease. We found that luciferase activity was decrease as effect of viral protease. All these events support our hypothesis that NS2B/NS3 DENV interrupts the TLR3 signaling pathway. This phenomenon could constitute an important mechanism of immune evasion in DENV.

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EFFECT OF FCγRII ISOFORMS ON ANTIBODY DEPENDENT ENHANCEMENT OF DENGUE VIRUS INFECTION

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The Antibody Dependent Enhancement (ADE) phenomenon is hypothesized to contribute to the pathogenesis of complicated dengue virus infection. Fc-γ receptor engagement by dengue immune complexes is linked with enhancement of infection. Our current work focuses on the role of FcγRs in ADE of dengue virus infection in primary human target cells. Our previous data showed that dendritic cells mainly express FcγRII; both FcγRIIa and FcγRIIb isoforms. We showed that in mature human dendritic cells, FcγRIIa largely mediates ADE. Therefore, we aimed to study the contributions of these two isoforms in ADE, especially in light of the inhibitory features of the FcγRIIb isoform and focused on downstream consequences. We generated FcγRIIa or FcγRIIb expressing cell lines by transfecting full-length cDNA of each isoform into non-Fc bearing cells, thereby allowing comparative study of cells including infection rates, viral output and infectivity of released virions. Our preliminary data, using binding and internalization studies, suggests that the FcγRIIa isoform mediates ADE more efficiently than FcγRIIb. We will report our current data set to advance the understanding of the involvement of FcγRII isoforms in ADE.

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COMPARISON OF NEUTRALIZING AND ENHANCING TITERS OF PATIENT AND VACCINEE SERA USING A HIGH-THROUGHPUT DENGUE REPORTER VIRUS DETECTION SYSTEM

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Despite the critical need for a safe and effective Dengue virus (DENV) vaccine, development has been hindered by the lack of reliable, high-throughput tools for measuring antibodies that may be protective or, equally important, infection-enhancing. Here we use a recently developed tool, the Dengue Reporter Virus (DRV), to rapidly monitor human serum for protective and pathogenic anti-DENV antibodies. DRVs have been developed by combining a subgenomic replicon encoding an optical reporter (GFP or luciferase) with structural components from defined strains of DENV. Infection is monitored by expression of the reporter gene, providing an objective output that can be quantified using standard optical detection platforms. DRVs retain the antigenic determinants