Dengue Virus NS1 Is a Viral Toxin That Activates Cells Via TLR4: Is Dengue a Case of "Aseptic" Shock?

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Dengue viruses are responsible for the most prevalent arboviral infection worldwide, affecting up to 400 million people annually. Life-threatening complications of infection include hemorrhage and shock, however the pathogenic mechanisms leading to these disease outcomes are not well understood. Factors that likely contribute include the activation of monocytes/macrophages and the subsequent release of vasoactive cytokines. The highly conserved dengue viral non-structural protein, NS1 is secreted from infected cells as a hexameric, lipid-associated species, and is found circulating in the blood of infected patients. Detection of high levels early during infection has been shown to correlate with disease severity. We hypothesized that NS1 might contribute to disease progression by triggering excessive innate immune responses leading to the production of vasoactive cytokines that promote vascular leak.

We found that physiologically relevant concentrations of the secreted form of NS1 activate mouse macrophages and human blood cells via toll-like receptor 4 (TLR4), inducing the release of a range of cytokines and chemokines. This activity was exhaustively verified as not being due to contaminating lipopolysaccharide (LPS). However, like the LPS response, induction of cytokines by sNS1 was completely prevented by incubation with an antagonistic form of LPS from Rhodobacter sphaeroides (LPS-RS). Thus, we have identified sNS1 as a dengue virus-encoded pathogen-associated molecular pattern (PAMP). In an *in vitro* model of vascular leak we further showed that human microvascular endothelial cells (HMEC-1) responded to both LPS and NS1, with production of IL- 6 and IL-8 and the disruption of HMEC-1 monolayer integrity. Both NS1-mediated activation of PBMCs and NS1-induced vascular leak *in vitro* were inhibited by a TLR4 antagonist and by anti-TLR4 antibody treatment. Finally, the importance of TLR4 activation *in vivo* was confirmed by the reduction of capillary leak by a TLR4 antagonist in a mouse model of dengue virus infection.

The striking similarities in cellular responses to LPS and NS1 via TLR4 points to NS1 being a viral toxin counterpart of bacterial endotoxin. Similar to the role of LPS in septic shock, NS1 may contribute to vascular leak in dengue patients with TLR4 antagonists an attractive therapeutic option for dengue disease.