Contribution of Dengue Virus Nonstructural Protein 1 to Platelet Activation and Thrombocytopenia in Hemorrhagic Mice Model

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Dengue is a global-threatening arthropod-borne viral infection disease, which causes approximately 390 million people and causes 500,000 hospitalized infections every year. Most of these cases are mild self-limited dengue fever, known as dengue without warning sign, but some cases develop potentially severe dengue disease, which is characterized with plasma leaking, fluid accumulation, severe bleeding, and organ impairment. There is much research trying to investigate the underlying mechanisms of how mild dengue progresses into severe dengue and find the biomarkers for clinical prediction. Thrombocytopenia, a common feature observed in both mild and severe dengue disease, is more correlated with progression to severity than other factors and has been considered as a predicted biomarker for severe dengue. In addition, platelet activation (elevated surface P-selectin) and apoptosis (increased phosphatidylserine, PS expression) are correlated to the thrombocytopenia occurred in dengue patients and dengue virus (DENV) could induce platelet activation and apoptosis in vitro. Trugilho et al. have also revealed the underlying mechanism of DENV-induced platelet activation through the proteomic analysis of dengue patient platelets. However, whether and which viral component is participated in platelet activation and apoptosis is still
Dengue nonstructural protein 1 (NS1) is a 48 kDa glycoprotein that can be expressed on the DENV-infected cell surface as a dimer and is the only viral protein secreted into the blood circulation, as a hexamer, in dengue patients. The concentration of NS1 in the sera of DHF/DSS patients is range from 0.01-50 μg/ml and correlated with disease severity. Recently, more and more studies have shown that NS1 plays a critical role in dengue pathogenesis both in vitro and in vivo, which includes enhancing DENV replication/infection, directly inducing vascular leakage, and cytokine release from immune cells. Modhiran et al. have further demonstrated that the majority of a receptor of NS1 in immune cells is toll-like receptor 4 (TLR4), a well-known receptor of lipopolysaccharide (LPS). It is known that LPS could induce platelets activation and potentiates platelet aggregation via TLR4/MyD88 signaling transduction. Since both NS1 and LPS can activate immune cells through TLR4, we propose and test the hypothesis that NS1 could induce platelet activation and enhance aggregation through TLR4, which leads to the over-destruction of platelets during dengue infection in this study.

**Specific aim**

1. To investigate whether NS1 contributes to DENV-induced platelets activation
   
   1.1 To investigate whether NS1 could induce platelet activation
   
   1.2 To confirm whether NS1 induces platelet activation through TLR4

2. To investigate what is the fate of NS1-activated platelets
   
   2.1 To investigate whether NS1-activated platelets have stronger interaction to endothelial cell.
   
   2.2 To investigate whether NS1-activated platelets are more susceptible to phagocytosis by macrophage.
3. To investigate whether NS1 contributes to DENV-induce hemorrhage, bleeding time prolong and thrombocytopenia in mice model.

**Experimental design**

We used an *in vitro* model of isolated platelets stimulation with DENV NS1 to investigate whether NS1 could trigger platelet activation and result in the enhancement of platelet aggregation, the interaction between platelets and endothelial cell even make platelets being more susceptible to phagocytosis. We also used NS1-depleted DENV to evaluate the pathogenic role of NS1 during DENV infection by using hemorrhagic mice model. To understand the underlying mechanism, we further used various TLR4 inhibitors and TLR4 knock-out mice to investigate whether DENV NS1 activates platelets through TLR4.

**Result**

In this study, we demonstrate that DENV NS1-treated platelets exhibit classic features of dengue patient platelets that include increased surface P-selectin expression and phosphatidylserine exposure as compared to control. Consistent with previous studies, we also found that DENV NS1 induces platelets activation through TLR4 signaling transduction. Moreover, the activation of platelets induced by DENV NS1 leads the increase of platelets aggregation, adhesion to endothelial cells, and phagocytosis by macrophages. Finally, we also prove that depletion of NS1 from DENV supernatant could rescue the DENV-induced thrombocytopenia and hemorrhage in mice model.