

In vitro platelet activation response induced by Dengue non structural protein 1

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Introduction: *In recent years, besides their important role in hemostasis and thrombosis, platelets have been recognized as mediators in immune response and host defense against several pathogens including viruses. Dengue virus (DENV) infection is an increasing health problem in tropical and subtropical areas, its clinical presentation goes from mild disease to severe and haemorrhagic fever. NS1 is a highly conserved DENV glycoprotein that can be detected in serum during the febrile phase infection. Recent results suggest a critical role for NS1 in the vascular leak and thrombocytopenia found in severe DENV infection, however, the mechanisms involved in DENV-induced platelet activation are not completely understood.*

Methods: *NS1 DENV2 was cloned into pHLSec plasmid. Competent E. coli cells were transformed and plasmid DNA was extracted to verify the presence of NS1 by agarose electrophoresis. HEK293 cells were transfected with plasmid DNA, the secreted protein was analyzed by silver staining and western blot while a liquid chromatography system was used to purify it. Platelet-rich plasma (PRP) was obtained from healthy donors by centrifugation of citrated blood at 800 rpm for 10 minutes; PRP was incubated with NS1 protein. After protein stimulation, platelets were stained with specific antibodies and cells fixed with paraformaldehyde solution. Platelets were detected by flow cytometry using anti-CD41-PC7 antibody. Expression of platelet activation markers P-selectin and GpIIb-IIIa was assessed using anti-CD62P-PE and PAC1-FITC antibodies, respectively.*

Results: *Flow cytometry analysis showed that DENV NS1 protein is able to induce expression of P-selectin and GpIIb-IIIa complex on platelet surface. Platelets activated with DENV NS1 have an activation response similar to that obtained when stimulated with collagen.*

Conclusions: *Our results suggest that DENV NS1 induces the expression of activation markers on platelet surface. These results may help to explain thrombocytopenia in dengue and emphasize the important role of DV NS1 in the pathogenesis. Further studies are needed to characterize platelets immune response against viruses.*