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Role of Neutrophils in Dengue Patients: Clearance of Dengue Virus

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Abstract

The importance of neutrophils in acute inflammatory response, the activation, regulation and effector functions of innate and adaptive immune cells has been a central focus recently, with a primary role in clearance of extracellular pathogens. A unique type of cell death of neutrophil characterized by the active release of chromatin fibers, so-called neutrophil extracellular traps (NETs), which can trap and kill invading microbes extracellularly has been recognized. Neutropenia is one of the most salient clinical findings in dengue patients, suggesting that neutrophils have a crucial role in the pathogenesis of dengue virus infection. Platelets have been implicated in assisting the dissemination dengue virus in dengue patients. Viral tropism can activate platelets causing platelet dysfunctions and thrombocytopenia, a hallmark of severe dengue disease. Activated platelet-neutrophil interactions have been well established, and yet its role in the pathogenic cause of dengue remains poorly understood. Forty seven acute dengue confirmed patients were enrolled to study the platelet-neutrophil interactions. FACS, biochemical and immunochemical staining, and ELISA assays were utilized to evaluate and quantify the interactions, activation, and the relesates resulting from the engagements. The in vivo observation was further verified with in vitro experiments using neutrophils isolated from healthy individuals. Results revealed that about 15% (on average) of neutrophils were associated with dengue viral antigen positive platelets by FACS analysis. In addition, viral antigen positive platelets were often adhering, engaged and encapsulated, or closely associated with a NET structure in smear preps from peripheral blood of confirmed dengue patients. Quantitative assessment of the percentage of NETs revealed robust levels (~25%) in samples from dengue patients compared to healthy controls. Neutrophil elastase releasing from the activated platelet-neutrophil engagements were significantly higher in acute febrile samples compared to convalescent period. In contrast, antibody to histone was significantly higher in convalescent samples than in acute febrile stage. The NETs captured DV-infected platelets were substantiated with in vitro studies. Neutrophil elastase was significantly higher in the



supernatant of DVplatelet-neutrophil coculture than in LPS or platelet only. Results suggest that dengue viral antigen positive platelets associated with and encapsulated by the NETs, indicating that neutropenia in dengue patients may be a defense act by the host. The byproducts from neutrophil NET releasates may contribute to and promote dengue pathogenesis.