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Jaya Prakash

Washington University in St. Louis

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DO ENDOTOXINS DIRECTLY INJURE RED BLOOD CELLS?

Jaya Prakash

Mentor: Allan Doctor

This project examined the role of bacterial endotoxin (lipopolysaccharide, LPS), in the red blood cell (RBC) damage and abnormal oxygen delivery that is commonly observed in severe infection. Specifically, we are testing the hypothesis that LPS activates proteases involved in eryptosis (a special form of RBC death) and that these proteases damage proteins involved in energy metabolism. It has been demonstrated in the Doctor lab that in RBCs exposed to endotoxin, caspase (an enzyme involved in eryptosis initiation) is activated; however, the mechanism for this is not well understood. We predicted that the activation of caspase 3 and mu-calpain—after endotoxin exposure—would directly injure RBCs in lieu of operating through elements in plasma or white blood cells (WBCs). To test this, an endotoxin exposed human RBC model used whole blood from human volunteers and separated it via centrifugation into its components (washed RBCs, plasma, and WBCs), and samples from each subject were divided into three experimental groups: (1) unaltered whole blood, (2) washed RBCs, (3) washed RBCs + plasma. Each experimental group was incubated with LPS at varying concentrations. Samples from the three groups were evaluated with SDS PAGE and a western blot specific for procaspase 3, caspase 3, and mu-calpain to assess the injury caused by LPS exposure. After repeated trials, caspase 3 seemed to be activated most readily in whole blood where cleaved caspase 3 bands tripled the densitometry readings of those in washed RBCs. Similarly, mu-calpain was most readily activated in whole blood where the cleaved products had densitometry readings nearly 5 and 1.5 times those of the respective bands in washed RBCs. These differences in band densities between the experimental groups for mu-calpain and caspase 3 suggested that the mechanism of RBC injury through these enzymes might depend on signaling through the humoral immune system.