COMPARISON OF PRO-INFLAMMATORY AND PRO-COAGULANT EFFECTS OF NUCLEAR, MITOCHONDRIAL, AND BACTERIAL DNA

Bhagirath, Vinai1; Dwivedi, Dhruba1; Liaw, Patricia1
1Department of Medicine, McMaster University, Hamilton, Canada

Introduction: Sepsis is a syndrome in which infection triggers a systemic inflammatory and pro-coagulant response, with a prevalence of up to 3 cases per 1000 and a mortality rate of up to 40%. Cell-free DNA (cfDNA) is elevated in sepsis, and correlates with mortality. This DNA may come from nuclear, mitochondrial, or bacterial sources. CpG motifs on bacterial and mitochondrial DNA can stimulate inflammatory responses via TLR9, which is present on neutrophils, monocytes, and recently shown to be expressed on platelets. Nuclear cfDNA can activate coagulation via the intrinsic pathway. cfDNA may thus play an important pathogenic role in sepsis. This study elucidates the relative effects of nuclear, mitochondrial, and bacterial DNA on inflammatory and pro-coagulant pathways.

Objectives: To compare the pro-inflammatory and pro-coagulant properties of nuclear, mitochondrial, and bacterial DNA.

Methods: Nuclear and mitochondrial DNA concentrations were measured by PCR using plasma samples from septic patients. Nuclear and mitochondrial DNA were purified from human embryonic kidney 293 cells, and bacterial DNA was from E. coli. Neutrophils from healthy donors were cultured with purified nuclear, mitochondrial, or bacterial DNA at 1 or 15µg/mL. IL-6 levels in the supernatants were measured by ELISA at 16h, and neutrophil viability was measured at 20h by flow cytometry for annexin-V binding and propidium iodide exclusion. The three types of DNA were added at 1 or 10µg/mL to citrated human platelet-poor plasma, and continuous thrombin generation was measured (Technothrombin, Vienna, Austria). Washed platelets were treated with 1 or 10µg/mL of DNA and markers of platelet activation were measured by flow cytometry for P-selectin and activated integrin αIIbβ3. All reagents contained less than 0.06EU/mL of LPS by limulus amoebocyte lysate assay.

Results: Mitochondrial DNA as well as nuclear DNA are elevated in plasma from septic patients compared to healthy controls. Bacterial, but not mitochondrial or nuclear, DNA increased neutrophil IL-6 secretion. Both mitochondrial and bacterial DNA increased neutrophil viability. Nuclear, mitochondrial, and bacterial DNA increased thrombin generation in both platelet-poor plasma and platelet-rich plasma to a similar degree. This effect was reduced by addition of corn-trypsin inhibitor and in FXII-depleted plasma, and abolished in FXI-depleted plasma, indicating dependence on the intrinsic pathway of coagulation. The effect was not masked by incubation with TFAM, the major mitochondrial DNA-binding protein. Independently of coagulation, DNA from all three sources was capable of causing activation of platelet integrin αIIbβ3, but not surface expression of P-selectin or aggregation.

Conclusion: Both mitochondrial and nuclear DNA are elevated in sepsis. Bacterial DNA can stimulate neutrophil IL-6 release, while both mitochondrial and bacterial DNA, but not nuclear DNA, prolonged neutrophil viability. All three types of DNA can activate coagulation via the intrinsic pathway, and can also stimulate platelet activation. Thus, nuclear, mitochondrial, and bacterial DNA may play distinct roles in the pathogenesis of sepsis.

References: N/A