RESUMO SIMPLES - MICROBIOLOGIA E IMUNOLOGIA

HEMOCYTE MEDIATED EXTRACELLULAR DNA RELEASE IN STOMOXYS CALCITRANS: EVIDENCE OF ET-LIKE STRUCTURES AND NOVEL FILAMENTOUS PROJECTIONS

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Extracellular traps (ETs) have been extensively described as immune defense mechanism involving the release of extracellular DNA by neutrophils in mammals. However, in hematophagous insects, particularly Stomoxys calcitrans, this phenomenon remains underexplored, despite the biological and epidemiological relevance of this dipteran as a mechanical vector of diverse veterinary pathogens. Hemocytes, which are the central cellular components of insect innate immunity, are responsible for essential immune responses such as phagocytosis, encapsulation, nodulation, and humoral activation. Therefore, investigating the capacity of S. calcitrans hemocytes to release extracellular DNA, including ET-like structures, contributes to a better understanding of cellular defense mechanisms in insects. The aim of this study was to evaluate the release of extracellular DNA by S. calcitrans hemocytes after exposure to Serratia marcescens (Gram-negative bacterium frequently associated with insect microbiota) and Herpetomonas muscarum (a monoxenous

trypanosomatid known to naturally infect S. calcitrans). Adult flies (24–48 hours post-emergence and blood-fed) were used for hemolymph collection. Due to the very small volume obtained from each individual, 80–100 insects were pooled per assay. Hemocytes were concentrated by centrifugation and resuspended in Schneider's medium at a density of 10^5 cells. The cells were then incubated for 90 minutes with either 100 CFU of S. marcescens or 5×10^5 cells of H. muscarum. Supernatants were collected and analyzed for extracellular DNA (ET-DNA) quantification using PicoGreen® fluorescence assay. Fluorescence microscopy with Sytox Green staining was performed to qualitatively assess the presence of DNA-based extracellular structures. .Triplicate assays consistently demonstrated that hemocytes stimulated with S. marcescens released significantly higher concentrations of extracellular DNA compared to both the unstimulated control and the H. muscarum group. This result confirms that S. marcescens induces stronger cellular activation compatible with an ET-related immune response. The H. muscarum treated group exhibited slightly elevated DNA release compared to controls, but significantly lower than the S. marcescens group, suggesting that the protozoan may trigger a milder or potentially evasive immune response. Beyond the quantitative results, fluorescence microscopy revealed extracellular DNA filaments extending from the hemocytes after stimulation with S. marcescens, exhibiting morphological characteristics consistent with ET-like structures. These fluorescent filamentous projections emerged from the cellular body and formed extracellular extensions, although they did not always establish the classical three-dimensional net-like architecture typical of ETs in mammals. In contrast, these structures were absent or less frequent in control and H. muscarum samples, reinforcing the association between bacterial stimulus and ET formation. Notably, fluorescence and light microscopy also revealed the presence of fine, elongated filamentous structures produced by hemocytes after exposure to S.marcescens. These filaments are different from classical ETs because they lacked a reticular structure and appeared as thin projections extending outward from the hemocyte membrane. Their cellular origin, presence after bacterial exposure, and morphology suggests a previously undescribed defense mechanism, possibly representing an early stage of ET release or alternative filament-based immune response in dipteran hemocytes. The reduced occurrence of these filamentous structures in the H. muscarum group may indicate that this protozoan induces a different type of activation pathway or employs immune evasion strategies that prevent or modulate ET formation. Taken together, these findings support the hypothesis that S. calcitrans hemocytes are capable of

releasing extracellular DNA structures in response to bacterial stimulation, compatible with ET-like mechanisms, and further suggest the existence of additional filament-based immune process yet to be characterized. The combination of quantitative assays and microscopy provides robust evidence for ET-like activity in this species and highlights S. calcitrans as a valuable model for studying innate immune mechanisms in insects.

Palavras-chave: extracellular traps; s calcitrans; hemolymph; hemocytes; insect immunity.