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Academic Background: Date of Entry: 9/2004
Degrees Expected: MD/PhD
Expected Year of Graduation/Program Completion: 2015
Major or Field of Study: Pathobiology
Academic Level: MD/PhD Candidate

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Department of Surgery, Rhode Island Hospital, Providence, Rhode Island
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Title: "An Extracellular Matrix-based Mechanism of Rapid Neutrophil Extracellular Trap Formation in Response to *C. albicans*"

Category: Microbiology, Immunology, Genetics, or Molecular Biology

Research Sponsor(s): *This work was supported by the National Institute of Health Grant (GM066194) (JSR); A.S. Byrd is supported by UNCF/Merck Graduate Science Research Dissertation Fellowship*

Statement of the Problem/Background:
Polymorphonuclear leukocytes (PMNs, neutrophils) are innate immune cells responsible for host defense against opportunistic fungal infections. Patients who have a reduction in the number of peripheral blood PMNs are at risk for acquiring invasive candidiasis, a nosocomial infection that is associated with 40-80% mortality and causes approximately 10,000 deaths per year in the US. The armament of neutrophil-mediated host defense against pathogens includes the extrusion of a lattice of DNA and microbicidal enzymes known as Neutrophil Extracellular Traps (NETs).

Research Question/Hypothesis:
We hypothesize that there is a novel and significant regulatory role for the ubiquitous matrix component fibronectin (Fn) in NET release and that this response is receptor mediated.

Research Design/Methods Used in the Investigation:
Global tyrosine phosphoproteomic analysis mitigated the quantitative analysis of phosphorylated sites of neutrophils adherent to immobilized Fn ± β-glucan and was validated via Western blot analysis. Light, confocal and transmission electron microscopy was implemented to observe NET formation after addition of Sytox Green, indicating a breach in membrane integrity.

Results/Summary of the Investigation:
We report that recognition of purified fungal PAMP β-glucan by human neutrophils causes rapid (≤ 30 mins) homotypic aggregation and NET release by a mechanism that requires Fn. Alone, immobilized β-glucan induces reactive oxygen species (ROS) production but not NET release, whereas in the context of Fn, ROS production is suppressed and NETs are extruded. NET release to Fn+ β-glucan is robust, accounting for 17.2 ± 3.4% of the total DNA in the cell population. Aggregation and NET release are dependent on β-glucan recognition by CR3 (CD11b/CD18), ERK MAPK, but not Dectin-1, or ROS. The process of NET release included filling of intracellular vesicles with nuclear material that was eventually extruded. NET formation to C. albicans hyphae was also found to depend on β-glucan recognition by CR3, require Fn and ERK but not ROS and result in hyphal destruction.

Interpretation/Conclusion of the Investigation:
We show promotion of homotypic aggregation and NET formation to ligand combinations suggesting a regulatory role for CR3 in mediating the host response to a fungal PAMP. We have found that this phenomenon depends on ERK, but is independent of the respiratory burst allowing NET production in the absence of ROS, which in turn may minimize collateral tissue damage. We have successfully shown a correlation between PMN defense mechanisms within a reductionist model and responses to a more physiologically relevant stimulus, C. albicans hyphae. Further work will recapitulate an in vivo representation, mitigating how diverse mechanisms converge, optimizing the host defense against pathological fungal infections.