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DISSERTATION

"ATP and its role in the leukotoxin-mediated killing of malignant lymphocytes"

by

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Molecular Biology, Genetics & Cancer Track

B.S., 2013, Montclair State University, Montclair, NJ
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10:00 A.M.
MSB C600

ABSTRACT

Leukotoxin (LtxA; Leukothera®) is a protein secreted from the oral pathogen Aggregatibacter actinomycetemcomitans (A.a) that targets and kills activated white blood cells (WBCs) by binding to lymphocyte function associated antigen-1 (LFA-1). Interaction between LtxA and Jurkat T-cells results in cell death and is characterized by increased intracellular Ca2+, activation of caspases, clustering of LtxA and LFA-1 within lipid rafts, and involvement of the Fas death receptor. Here, we show that LtxA is capable of killing malignant lymphocytes via apoptotic and necrotic forms of cell death. We show that LtxA causes activation of caspases and PARP, cleavage of pannexin-1 (Panx1) channels, and expulsion of ATP, ultimately leading to cell death via apoptosis and necrosis. CRISPR-Cas9 mediated knock-down of Panx1 in Jurkat cells prevented ATP expulsion and resulted in resistance to LtxA for both apoptotic and necrotic forms of death. Resistance to necrosis could only be overcome when supplementing LtxA treatments with endogenous ATP (bzATP). The combination of LtxA and bzATP promoted only necrosis, as no Panx1 K/O cells stained positive for phosphatidylserine (PS) exposure following the combined treatment. Inhibition of LtxA and bzATP induced necrosis was possible when pretreating Jurkat cells with oATP, a $P2X_7R$ antagonist. Similarly, blockage of $P2X_7Rs$ with oATP prevented the intracellular mobilization of Ca2+, an important early step in cell death. We show that LtxA is able to kill malignant lymphocytes through an apoptotic death pathway which is potentially linked to a Panx1/$P2X_7R$ mediated necrotic form of death. Thus, inhibition of ATP release appears to significantly delay the onset of LtxA induced apoptosis while completely disabling the necrotic death pathway in T-lymphocytes.