Host cell killing mechanism of *Vibrio vulnificus* RtxA1 Toxin: Programmed necrotic cell death through calcium-dependent mitochondrial dysfunction

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Vibrio vulnificus, a halophilic estuarine bacterium which causes fatal septicemia and necrotic wound infection, is highly cytotoxic to eukaryotic cells. We recently reported that RtxA1, an RTX toxin predicted to be 501 kDa in size, kills host cells only after contact between the bacterial and host cells and plays an essential role in the pathogenesis of *V. vulnificus* infection. RTX toxins are virulence-associated multifunctional exotoxins with classical C-terminal GD-rich repeats. The cell death phenomena caused by the RtxA1 toxin is reproducible and proceeds in a programmed manner. This study was performed to elucidate the mechanism how the RtxA1 toxin mediates the programmed necrotic cell death in HeLa cells. In the present study, we show that the RtxA1 toxin causes programmed necrotic cell death through mitochondrial dysfunction. We found that the 501 kDa RtxA1 toxin was processed into two fragments by confocal microscopy and immunoblotting analysis of infected cells. The larger, N-terminal fragment (RtxA1-N) remained associated with the host cell membrane, while the smaller, C-terminal fragment (RtxA1-C, ~130 kDa) was internalized into the host cell cytoplasm. RtxA1-C, which contains GD-repeats and calcium-binding domains, specifically interacted with mitochondrial voltage-dependent anion channels (VDACs), and a VDAC inhibitor, 4,4'-diisothiocyanatostibene-2,2'-disulfonate (DIDS), inhibited RtxA1-induced host cell necrosis in a dose-dependent manner. The cell death mechanism also employed calcium-dependent mitochondrial pathways, causing irreversible mitochondrial membrane dysfunction and ATP depletion, which were later accompanied by plasma membrane integrity disruption. Based on our findings, for the first time, we disclose the molecular mechanism of RtxA1-mediated programmed necrotic host cell death. We also propose a new cell death model whereby a bacterial toxin is processed upon host cell contact and one fragment is internalized to specifically interact with mitochondrial VDAC1, which consequently result in an irreversible mitochondrial dysfunction though calcium sequestration.