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The Cell-Cycle Regulatory Protein P21cip1/Waf1 is Required for Cytolethal Distending Toxin (Cdt)-Induced Apoptosis

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The Cell-Cycle Regulatory Protein P21cip1/Waf1 is Required for Cytolethal Distending Toxin (Cdt)-Induced Apoptosis

Abstract

The Aggregatibacter actinomycetemcomitans cytolethal distending toxin (Cdt) induces lymphocytes to undergo cell-cycle arrest and apoptosis; toxicity is dependent upon the active Cdt subunit, CdtB. We now demonstrate that $p21^{CIP1/WAF1}$ is critical to Cdt-induced apoptosis. Cdt induces increases in the levels of $p21^{CIP1/WAF1}$ in lymphoid cell lines, Jurkat and MyLa, and in primary human lymphocytes. These increases were dependent upon CdtB's ability to function as a phosphatidylinositol (PI) 3,4,5-triphosphate (PIP3) phosphatase. It is noteworthy that Cdt-induced increases in the levels of $p21^{CIP1/WAF1}$ were accompanied by a significant decline in the levels of phosphorylated $p21^{CIP1/WAF1}$. The significance of Cdt-induced $p21^{CIP1/WAF1}$ increase was assessed by preventing these changes with a two-pronged approach; pre-incubation with the novel $p21^{CIP1/WAF1}$ inhibitor, UC2288, and development of a $p21^{CIP1/WAF1}$-deficient cell line ($Jurkat^{p21-}$) using clustered regularly interspaced short palindromic repeats (CRISPR)/cas9 gene editing. UC2288 blocked toxininduced increases in $p21^{CIP1/WAF1}$, and $Jurkat^{WT}$ cells treated with this inhibitor exhibited reduced suscepstibility to Cdt-induced apoptosis. Likewise, $Jurkat^{p21-}$ cells failed to undergo toxin-induced apoptosis. The linkage between Cdt, $p21^{CIP1/WAF1}$, and apoptosis was further established by demonstrating that Cdt-induced increases in levels of the pro-apoptotic proteins Bid, Bax, and Bak were dependent upon $p21^{CIP1/WAF1}$ as these changes were not observed in $Jurkat^{p21-}$ cells. Finally, we determined that the $p21^{CIP1/WAF1}$ increases were dependent upon toxin-induced increases in the level and activity of the chaperone heat shock protein (HSP) 90. We propose that $p21^{CIP1/WAF1}$ plays a key pro-apoptotic role in mediating Cdt-induced toxicity. © 2020 by the authors. Licensee MDPI, Basel, Switzerland.

Keywords

Aggregatibacter actinomycetemcomitans; Apoptosis; Cytolethal distending toxin; Lymphocytes; Virulence

Disciplines

Dentistry

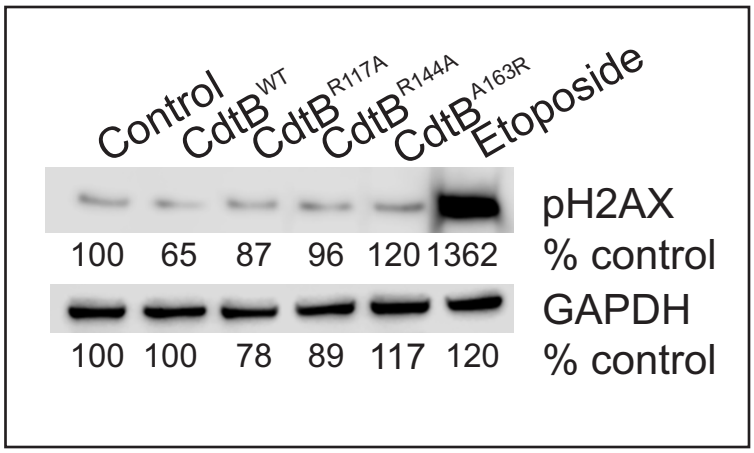

Figure S1: Effect of Cdt containing $CdtB^{WT}$ or one of the CdtB mutant proteins on phosphorylation of H2AX. $Jurkat^{WT}$ cells were incubated in the presence of 100 pg/ml Cdt containing either $CdtB^{WT}$ or a mutant CdtB protein for 4 hr. Cells were then fractionated and analyzed by Western blot for the presence of pH2AX. A representative blot of three experiments is shown. Numbers represent the amount of pH2AX expressed as a percentage observed in control cells incubated in medium alone. Cells treated with etoposide (25 uM) were included as a positive control for H2AX phosphorylation.

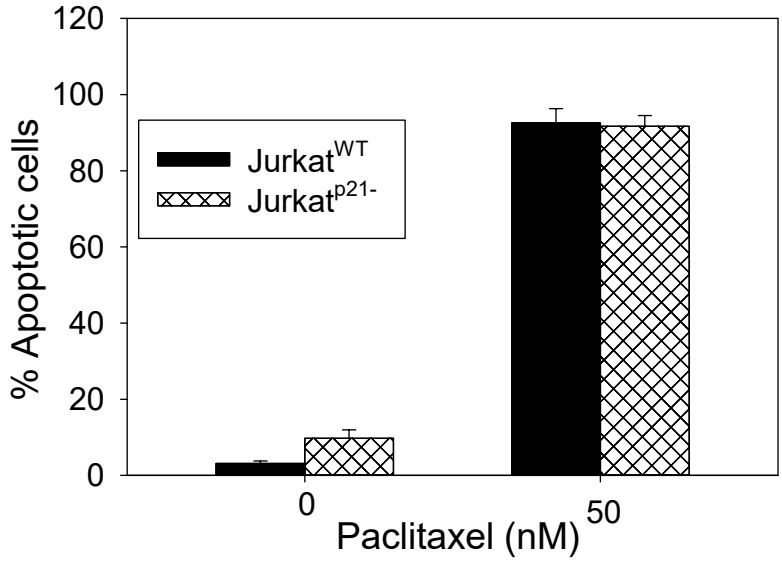

Figure S2: Susceptibility of Jurkat^{p21-} cells to paclitaxel. Jurkat^{WT} and Jurkat^{p21-} were incubated with 50 nM paclitaxel for 48 hr. The cells were then analyzed for apoptosis using the TUNEL assay and flow cytometric analysis. Data from three experiments are plotted as the percent of apoptotic cells (mean±SE).

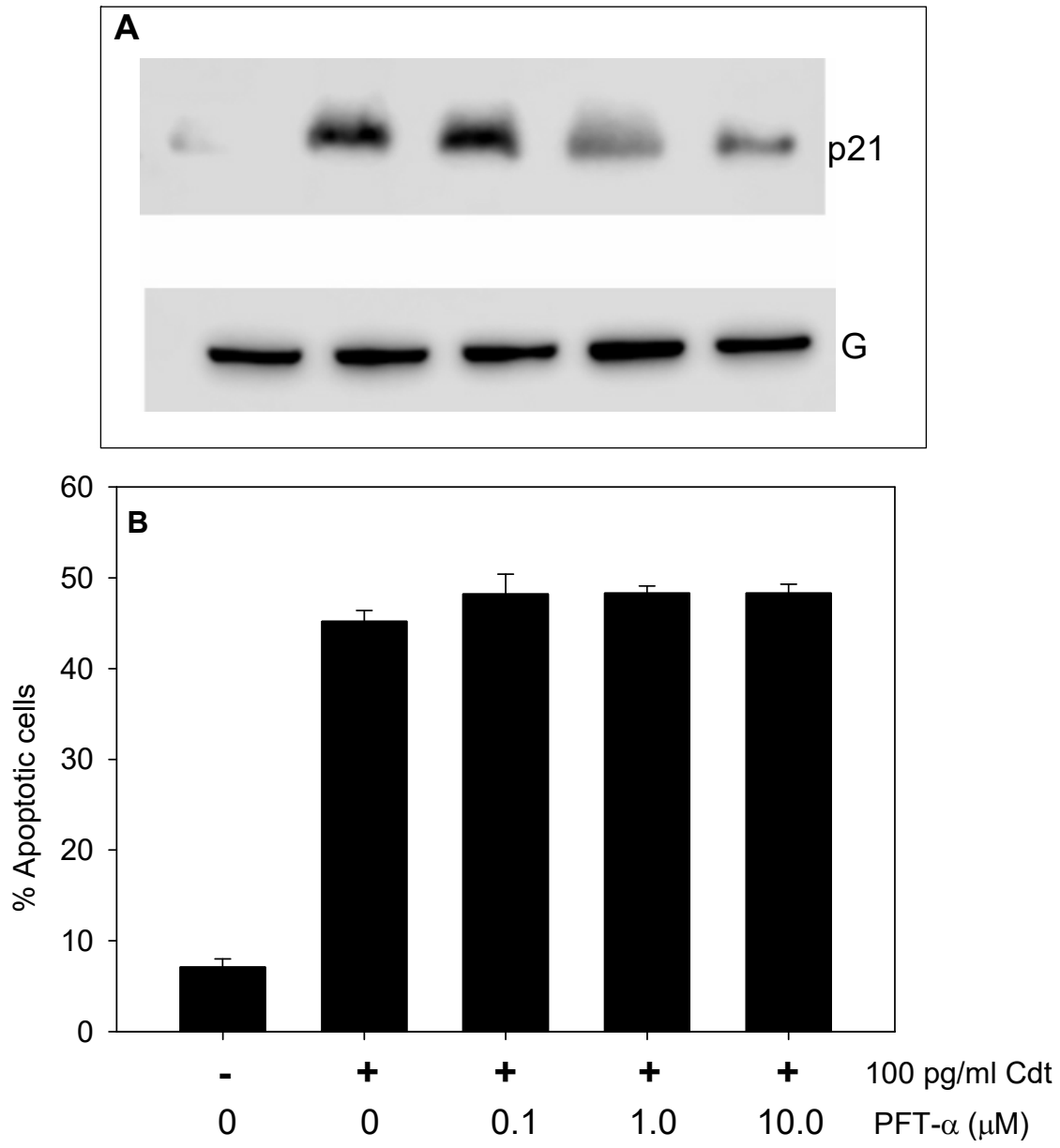

Figure S3: Effect of pifithrin-α (PFT) on Cdt-induced increases in $p21^{CIP1/WAF1}$ and apoptosis. $Jurkat^{WT}$ cells were pretreated with 0-10 μM PFT for 60 min followed by the addition of 100 pg/ml Cdt. Cells were analyzed for $p21^{CIP1/WAF1}$ levels by Western blot at 16 hr (panel A) and for apoptosis at 24 hr (panel B). The Western blot is representative of three experiments; bands showing $p21^{CIP1/WAF1}$ and GAPDH (G) are of (left to right) cells treated with medium alone, Cdt alone and Cdt in the presence of 0.1, 1.0 and 10.0 μM PFT-α. Apoptosis (TUNEL assay) results represent the mean±SD of three experiments.