

Abstract

The adenylate cyclase toxin (CyaA) plays a key role in the virulence of *Bordetella pertussis*. CyaA penetrates CR3-expressing phagocytes and catalyzes the uncontrolled conversion of cytosolic ATP to the key second messenger molecule cAMP. This paralyzes the capacity of neutrophils and macrophages to kill bacteria by oxidative burst and opsonophagocytic mechanisms. Here we show that CyaA suppresses the production of bactericidal reactive oxygen and nitrogen species in neutrophils and macrophages, respectively.

The inhibition of reactive oxygen species (ROS) production is most-likely achieved by the combined PKA-dependent inhibition of PLC and Epac-dependent dysregulation of NADPH oxidase assembly. Activation of PKA or Epac interfered with fMLP-induced ROS production and the inhibition of PKA partially reversed the CyaA-mediated inhibition of ROS production. CyaA/cAMP signaling then inhibited DAG formation, while the PIP3 formation was not influenced. These results suggest that cAMP produced by CyaA influences the composition of target membranes.

We further show here that cAMP signaling through the PKA pathway activates the tyrosine phosphatase SHP-1 and suppresses the production of reactive nitrogen species (RNS) in macrophages. Selective activation of PKA interfered with LPS-induced iNOS expression in macrophages, while the inhibition of PKA largely restored the production of iNOS in CyaA-treated murine macrophages. CyaA/cAMP signaling induced SHP phosphatase-dependent dephosphorylation of the c-Fos subunit of the transcription factor AP-1 and thereby inhibited the TLR4-triggered induction of iNOS gene expression. Selective siRNA knockdown of the phosphatase SHP-1 then rescued the production of TLR-inducible RNS in toxin-treated cells. Finally, the inhibition of SHP phosphatase abrogated *B. pertussis* survival inside murine macrophages. These results reveal that an as yet unknown cAMP-activated signaling pathway controls SHP-1 phosphatase activity and may regulate numerous receptor signaling pathways in leukocytes. The hijacking of SHP-1 by CyaA action then enables *B. pertussis* to evade RNS-mediated killing inside macrophages.

In conclusion, we propose a model of CyaA-provoked signaling which allows *Bordetella pertussis* to evade killing by the sentinel cells of the host immune system.