

Abstract

The adenylate cyclase toxin (CyaA) is a key virulence factor of the whooping cough agent *Bordetella pertussis*. CyaA primarily penetrates CR3-expressing myeloid phagocytes and subverts cellular signaling by a rapid conversion of ATP to cAMP. In parallel, CyaA can form cation-selective pores within cellular membrane, provoking massive potassium efflux from cell cytosol. An enzymatically inactive adenylate cyclase toxoid (CyaA-AC⁻) has then been abundantly used as an efficient antigen delivery tool over the past 20 years.

This work focused mainly on the mechanism of action of CyaA toxin and of its toxoid on dendritic cells. We studied the potency of the CyaA toxoid to act as adjuvant, its penetration capacity and its potential use in delivery of influenza epitopes. We show that the pore-forming activity and the activation of MAP kinases JNK and p38 were crucial for the adjuvant effects of the CyaA-AC⁻, which provokes maturation of dendritic cells (DC) independently of Toll-like receptor (TLR) or inflammasome signaling. Furthermore, such CyaA-AC⁻-stimulated DC acquired the ability to induce CD8⁺ and CD4⁺ T cells responses, as was determined both *in vitro* and *in vivo*. We further showed that the first 371 amino acids are dispensable for the capacity of CyaA to deliver its AC domain with inserted epitopes into cytosol of DC, implicating that the role of the AC domain polypeptide in the process of translocation across the cytoplasmic membrane of cells is rather passive. Our CyaA toxoid construct with an inserted antigen from the HA2 subunit of the hemagglutinin of influenza A viruses then induced both humoral and cellular immune responses in mice without the need for any added adjuvant and the responses protected mice against challenge with both homologous and heterologous influenza A viruses.

We further examined the role of the CyaA toxin in virulence of *B. pertussis*. We analysed the modulatory effects of CyaA action on TLR-activated murine and human DC. CyaA enhanced TLR-induced dissolution of cell adhesive contacts and chemotactic migration of DC *in vitro*, while it decreased the capacity of DC to present protein antigens and induce proliferation of antigen-specific CD4⁺ and CD8⁺ T cells. Manipulation of mouse DC by CyaA *in vitro* was shown to depend solely on the cAMP signaling and not on the pore-forming activity of the toxin. We further demonstrated in the mouse respiratory challenge model that the pore-forming activity of CyaA was not required for bacterial colonization. It, however, provoked neutrophil infiltration and the pore-forming activity importantly contributed to the overall pathology of lungs infected by *B. pertussis*.