SUMMARY (English)

The first part of this PhD. thesis deals with molecular mechanism of action of the adenylate cyclase toxin (CyaA), a key virulence factor of the whooping cough agent *Bordetella pertussis*. CyaA belongs to the family of RTX (Repeat-in-ToXin) proteins secreted by Gram-negative bacteria and primarily targets myeloid phagocytes, expressing the CD11b/CD18 integrin receptor (also known as $\alpha_M\beta_2$, CR3 or Mac-1). Upon binding, CyaA permeabilizes cell membranes by forming small cation-selective pores, and subverts cellular signaling by delivering into host cells an adenylate cyclase (AC) enzyme that converts ATP to cAMP. Elevation of the cytosolic cAMP levels by CyaA then knocks down bactericidal functions of host innate immunity.

CyaA is unique among other enzymatically active toxins in its capacity to penetrate cells directly from cell surface across the cytoplasmic membrane, without the need for endocytosis. Penetrating activity of CyaA depends on plasma membrane potential and on an intact, acylated and calcium-loaded RTX cytolysin moiety. By examining a set of 18 CyaA constructs that bear overlapping deletions within AC domain and a $CD8^+$ OVA T-cell epitope tag, we showed that the first 371 amino-terminal residues are dispensable for the CyaA capacity to deliver a passenger OVA epitope into cytosol of dendritic cells, as determined *in vitro* by stimulation of OVA-specific $CD8^+$ T cells. This observation suggested a passive passenger role of the AC domain during its membrane penetration.

In addition, we demonstrated that CyaA suppresses the bactericidal activities of macrophages by provoking futile membrane ruffling and showed for the first time that cAMP signaling of the CyaA toxin causes a rapid and complete inhibition of CR3-mediated phagocytosis. We further reported that the molecular mechanism of the repeatedly documented capacity of CyaA to undermine bactericidal activities of macrophages may well rely on RhoA inactivation, as a result of cAMP signaling. Besides that, by flow cytometry analysis and ELISA assays, we characterized the ability of CyaA to modulate maturation of dendritic cells (DCs), and showed that CyaA suppresses LPS-induced CD40 and CD54 molecule expression, and enhances IL-10 cytokine production. Moreover, we demonstrated that CyaA-treated DCs have a reduced capacity to prime proliferation of antigen-specific $CD4^+$ as well as $CD8^+$ T cells, and we unraveled the prominent subversive role of cAMP-activated PKA in these processes. Collectively, these findings corroborate the previous observations that CyaA subverts host immune responses.

The second part of the PhD. thesis is focused on the use of adenylate cyclase toxoids for antigen delivery. The penetration of recombinant CyaA/AC$^-$ toxoids to cell cytosol could be previously exploited for delivery of passenger CD8$^+$ epitopes to the major histocompatibility complex (MHC) class I presentation pathway, and induction of cytotoxic CD8$^+$ T lymphocyte (CTL) responses. An efficient therapeutic antitumor immunity in mice, and a full prophylactic protection against lethal lymphocytic choriomeningitis virus challenge was, indeed, conferred upon immunization with CyaA/AC$^-$ toxoids that bear appropriate CD8$^+$ T-cell epitopes. We tested here the capacity of the CyaA-CSP toxoids, containing an epitope of the circumsporozoite protein of the rodent malaria parasite *Plasmodium berghei,* to induce protective anti-malaria immunity in mice. Immunization of mice with CyaA-CSP toxoid induced high numbers of CSP-specific IFN-γ-secreting CD8$^+$ T cells, while no protective immunity against challenge with *P. berghei* sporozoites was achieved. However, when the anti-CTLA-4 was administered during boost immunization, or when CyaA-CSP toxoid was employed in a heterologous prime/boost vaccination regimen, using live recombinant *Salmonella* delivering the CSP epitope through type III secretion system as a primary vaccination strategy, we observed significant enhancement of the CSP-specific CD8$^+$ T cells and induction of protective immunity. Taken together, these results document the potential of CyaA to confer protection against a parasitic infection and to boost efficacy of vaccines in heterologous prime/boost immunizations.