Institut Pasteur
Microbes and Infection 16 (2014) 51–60
www.elsevier.com/locate/micinf

Original article

Protective role of adenylate cyclase in the context of a live pertussis vaccine candidate

Annabelle Lim [a,b], Jowin K.W. Ng [a,b], Camille Locht [c,d,e,f], Sylvie Alonso [a,b,*]

[a] *Department of Microbiology, National University of Singapore, CeLS Building #03-05, 28 Medical Drive, 115597 Singapore, Singapore*
[b] *Immunology Programme, National University of Singapore, CeLS Building #03-05, 28 Medical Drive, 115597 Singapore, Singapore*
[c] *Inserm, U1019, F-59019 Lille, France*
[d] *CNRS UMR8204, F-59019 Lille, France*
[e] *Univ Lille Nord de France, F-59000 Lille, France*
[f] *Institut Pasteur de Lille, F-59019 Lille, France*

Received 2 July 2013; accepted 3 October 2013
Available online 15 October 2013

Abstract

Despite high vaccination coverage, pertussis remains an important respiratory infectious disease and the least-controlled vaccine-preventable infectious disease in children. Natural infection with *Bordetella pertussis* is known to induce strong and long-lasting immunity that wanes later than vaccine-mediated immunity. Therefore, a live attenuated *B. pertussis* vaccine, named BPZE1, has been developed and has recently completed a phase I clinical trial in adult human volunteers. In this study, we investigated the contribution of adenylate cyclase (CyaA) in BPZE1-mediated protection against pertussis. A CyaA-deficient BPZE1 mutant was thus constructed. Absence of CyaA did not compromise the adherence properties of the bacteria onto mammalian cells. However, the CyaA-deficient mutant displayed a slight impairment in the ability to survive within macrophages compared to the parental BPZE1 strain. *In vivo*, whereas the protective efficacy of the CyaA-deficient mutant was comparable to the parental strain at a vaccine dose of 5×10^5 colony forming units (CFU), it was significantly impaired at a vaccine dose of 5×10^3 CFU. This impairment correlated with impaired lung colonization ability, and impaired IFN-γ production in the animal immunized with the CyaA-deficient BPZE1 mutant while the pertussis-specific antibody profile and Th17 response were comparable to those observed in BPZE1-immunized mice. Our findings thus support a role of CyaA in BPZE1-mediated protection through induction of cellular mediated immunity.

Keywords: *Bordetella pertussis*; Adenylate cyclase; BPZE1

1. Introduction

Despite high vaccination coverage, *Bordetella pertussis* infection remains endemic and reports of increasing incidence in Australia [1], Canada [2] and Europe [3] have been accumulating for the past twenty years. 2012 was the year with the highest whooping cough incidence in US and comparable outbreaks occurred also in the UK and Netherlands, in proportion of inhabitant numbers [4–6]. Adaptation of the circulating pertussis strains to the vaccines, as well as the waning and/or suboptimal efficacy of vaccine-mediated immunity during adolescence has been proposed to account for resurgence, with infected adolescent and adult populations being the major transmitters of disease in community representing a potential reservoir for disease transmission to young children who are yet to be fully vaccinated [7,8]. Furthermore, with the changing epidemiology, pertussis is increasingly becoming a real burden also in adults that experience long-lasting and very heavy cough periods of duration in weeks

* Corresponding author. National University of Singapore, Immunology Programme, CeLS Building #03-05, 28 Medical Drive, 117597 Singapore, Singapore. Tel.: +65 6516 3541; fax: +65 6778 2684.
E-mail address: micas@nus.edu.sg (S. Alonso).

1286-4579/$ - see front matter © 2013 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.micinf.2013.10.002

to months [9]. This underscores the need to pursue research efforts on this disease in order to provide suitable protection to the most vulnerable populations.

A highly attenuated *B. pertussis* strain, named BPZE1, has recently been described [10]. It produces enzymatically inactive pertussis toxin (PT), no dermonecrotic toxin and only trace amounts of tracheal cytotoxin. Markedly reduced lung pathology was observed in mice intranasally (i.n.) infected with BPZE1, compared to the virulent parent strain, while maintaining the ability to colonize and induce a strong protective immunity. In infant mice a single i.n. administration of BPZE1 induced stronger and longer-lasting protection than two administrations of current acellular pertussis vaccines [10,11]. In addition, the high genetic stability *in vitro* and *in vivo* [12], and a good safety profile in immunocompromised animals [13] have allowed BPZE1 to undergo the first-in-man clinical trial in adult healthy volunteers (http://www.child-innovac.org).

BPZE1-induced protective immune responses were found to involve both antibodies and CD4+ T cells. Further analyses into the protective CD4+ T cells revealed IFN-γ-producing and IL-17-producing subsets, indicating that BPZE1 induces both Th1 and Th17 CD4+ T cells in mice [14]. To further study the mechanisms involved in BPZE1-mediated protection and in particular the contribution of some virulence factors in the protective efficacy, we investigated the role of adenylate cyclase (CyaA) which is produced at wild type levels in BPZE1. CyaA is a dual activity toxin composed of two functional domains; the N-terminal cell invasive adenylyl cyclase enzyme, which elevates cAMP levels in phagocytes, and the C-terminal pore-forming 'hemolysin-toxin' activity (Hly) [15]. *In vivo* studies have suggested that CyaA exerts immunosuppressive effects on both the innate and adaptive immune system, specifically targeting CD11b/CD18 expressing-myeloid cells such as macrophages, neutrophils and dendritic cells [16–18]. The CyaA Hly domain mediates the binding to CD11b/CD18 host receptor and internalization of the enzymatic moiety into the host cells [19]. Upon entry into the cell, the AC enzymatic moiety is activated by host calmodulin and subverts cellular signaling by unregulated conversion of ATP to cAMP. Intracellular cAMP accumulation yields to suppression of bactericidal activities of phagocytes. In particular, it was shown that CyaA primarily and very sensitively blocks complement-mediated opsonophagocytic killing of bacteria by neutrophils [20]. Other functions such as chemotaxis, FcR-mediated phagocytosis and superoxide production were also found impaired by CyaA [20–23] which eventually leads to apoptosis [24]. The role of the Hly activity of CyaA in Bordetella virulence remains unclear, but its pore-forming activity may potentially be important in harnessing the pro-inflammatory response during bacterial colonization, yielding IL-1 induction and promoting Th17 response that appears required for bacterial clearance [25]. CyaA was previously shown to be involved in bacterial colonization of the airways and displays important immunomodulatory properties [26,27]. Infection with CyaA deficient *B. pertussis* strains results in reduced pathology and inability to cause lethality in infant mice [16,28–30]. On the other hand, CyaA has been reported to play a protective role against pertussis [25,31] and also displays adjuvant properties [32]. In this study, we constructed a CyaA-deficient BPZE1 mutant and analyzed its lung colonization profile, immunogenicity, and protective efficacy in an infant mouse model of *B. pertussis* infection.

2. Materials and methods

2.1. Ethic statement

All the animal experiments were carried out under the guidelines of the National Advisory Committee for Laboratory Animal Research (NACLAR) in the AAALAC-accredited NUS animal facilities (http://nus.edu.sg/iacuc/). NUS has obtained a license (#VR008) from the governing body Agri-Food & Veterinary Authority of Singapore (AVA) to operate an Animal Research Facility. The animal experiments described in this work were approved by the IACUC from National University of Singapore under protocol number 118/09. Non-terminal procedures were performed under anesthesia, and all efforts were made to minimize suffering.

2.2. Bacterial growth conditions

BPSM is a streptomycin-resistant Tohama I-derived B. pertussis strain [33]. BPAL10 was derived from *B. pertussis* BPZE1, a BPSM derivative strain producing inactivated pertussis toxin (PTX), no dermonecrotic toxin (DNT) and background levels of tracheal cytotoxin (TCT) [10]. *B. pertussis* strains were cultivated at 37 °C for 72 h on Bordet-Gengou (BG) agar (Difco, Detroit, Mich.) supplemented with 1% glycerol, 10% defibrinated sheep blood and 100 μg/ml streptomycin (Sigma Chemical CO., St Louis, Mo.). Liquid cultures were performed as described previously [33] in Stainer–Scholte (SS) medium containing 1 g/L heptakis(2,6-di-o-methyl) β-cyclodextrin (Sigma).

2.3. Cell lines

The human pulmonary epithelial cell line A549 (ATCC CCL-185) and the mouse macrophage cell line J774A.1 (ATCC TIB-67) were cultured according to the ATCC guidelines.

2.4. Construction of the CyaA-deficient BPZE1 strain

The CyaA-deficient BPZE1 strain, named BPAL10, was obtained by double homologous recombination strategy as described before [34]. A 999-bp PCR1 DNA fragment encompassing the 989-bp sequence upstream the first nucleotide of the *cyaA* ORF and the first 10-bp of *cyaA* was first PCR amplified from purified BPZE1 chromosomal DNA using the primers, 5′-TTTCTAGAGGCGGTGCCCCGGCCTCG-3′and 5′-TTGAG CTCTCGCACCGACGCAACCGGTG-3′ (*Xba*I site underlined). Similarly, a 996-bp PCR2 fragment including the 986-bp sequence downstream the STOP codon of *cyaA* ORF and the last 10-bp of *cyaA*, was PCR-amplified using the primers 5′-