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Adaptive immune response to whole cell pertussis vaccine reflects vaccine quality: A possible complementation to the Pertussis Serological Potency test

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ABSTRACT

Whole cell *Bordetella pertussis* (wP) vaccines are still used in many countries to protect against the respiratory disease pertussis. The potency of whole-cell pertussis vaccine lots is determined by an intracerebral challenge test (the Kendrick test). This test is criticized due to lack of immunological relevance of the read-out after an intracerebral challenge with *B. pertussis*. The alternative *in vivo* test, which assesses specific antibody levels in serum after wP vaccination, is the Pertussis Serological Potency test (PSPT). Although the PSPT focuses on a parameter that contributes to protection, the protective immune mechanisms after wP vaccination includes more elements than specific antibody responses only. In this study, additional parameters were investigated, i.e. circulating pro-inflammatory cytokines, antibody specificity and T helper cell responses and it was evaluated whether they can be used as complementary readout parameters in the PSPT to assess wP lot quality. By deliberate manipulation of the vaccine preparation procedure, a panel of high, intermediate and low quality wP vaccines were made. The results revealed that these vaccines induced similar IL-6 and IP10 levels in serum 4 h after vaccination (innate responses) and similar antibody levels directed against the entire bacterium. In contrast, the induced antibody specificity to distinct wP antigens differed after vaccination with high, intermediate and low quality wP vaccines. In addition, the magnitude of wP-induced Th cell responses (Th17, Th1 and Th2) was reduced after vaccination with a wP vaccine of low quality. T cell responses and antibody specificity are therefore correlates of qualitative differences in the investigated vaccines, while the current parameter of the PSPT alone was not sensitive enough to distinguish between vaccines of different qualities. This study demonstrates that assessment of the magnitude of Th cell responses and the antigen specificity of antibodies induced by wP vaccination could form valuable complementary parameters to the PSPT.

1. Introduction

Whole cell pertussis (wP) vaccines are used since the '50 and are still widely applied as an effective strategy in Latin America, Africa and Asia [1] to induce protection against whooping cough, caused by the Gram-negative bacterium *Bordetella pertussis*. Due to occasional side effects associated with wP vaccination, wP vaccines have been replaced by safer acellular pertussis (aP) vaccines in the 1990's in most industrialized countries. Though both aP vaccines and wP vaccines are effective in pertussis prevention campaigns, recent studies indicate that wP vaccination provides superior protection and durability of immunity [2,3]. It is therefore likely that wP vaccines will stay the vaccine of choice in many regions of the world in the near future.

Vaccine production is a biological process and is therefore associated with inherent variability in vaccine lot potency and safety, characteristics that are measured by regulatory required animal tests. The relevance of many of these *in vivo* models is disputable because they are not always susceptible to the pathogen of interest

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[4] and they do not always accurately mimic the human immune system [5]. Since mice are normally not susceptible for respiratory *B. pertussis* infection, the protective properties of wP vaccines are assessed by an intracerebral challenge of vaccinated mice, with lethality as end parameter [4]. Because it is unlikely that the pathology and immunology of a cerebral infection accurately reflects the responses induced by a natural respiratory infection in humans, and because of animal welfare reasons, there is a urgent need for alternative *in vivo* models and *in vitro* models that can provide a more accurate reflection of clinically relevant aspects of wP vaccine quality.

One of the alternatives under development is the Pertussis Serological Potency test (PSPT) [6–8], a test based on the detection of wP vaccine-induced *B. pertussis*-specific antibodies in mouse serum, a parameter that contribute to protection. However, the protective immune mechanisms after wP vaccination also depend on the vaccine's capacity to induce appropriate activation of innate immune responses and subsequent vaccine-specific T- and B cell responses. It has been shown that in humans *B. pertussis* infection as well as wP vaccination induce the formation of *B. pertussis*-specific antibodies [9,10], T helper (Th) 1 cells and/or Th17 directed cellular responses [11–13]. Similar adaptive immune mechanisms contribute to clearance of respiratory *B. pertussis* infection in mice [14,15]. In this study, we investigated whether other immune parameters can complement the current readout parameter of the PSPT, being *B. pertussis*-specific antibody responses, using wP vaccines of various protective properties [16]. We studied the type of immune response these wP vaccines induce in two murine outbred strains (RIVM:NIH and CD1; strains used for wP potency testing) and evaluated whether the novel measured immune parameters reflect vaccine quality.

2. Materials and methods

2.1. Preparation of wP vaccines

In this study, experimental wP vaccines were prepared as described in detail elsewhere [16]. Briefly, vaccines were generated from the *B. pertussis* strain 509 (Intravacc) cultured in a 3L bioreactor (2L working volume). The bioreactor was equipped with a six-bladed Rushton stirrer (Applikon, Schiedam, The Netherlands). After a pre-culture of the bacterium, the reactor was seeded at 5% (v/v), grown at 35 °C and after 3–5 reactor volumes steady state was assumed. Subsequently, deliberate downregulation of virulence genes ($t = 0$) was induced by addition of medium containing 50 mM $MgSO_4$. Meanwhile, $MgSO_4$ was added directly to the culture to instantly change the concentration to 50 mM $MgSO_4$. Samples were taken just before addition of $MgSO_4$ (0 h) and after 6 and 24 h. To generate vaccines, the samples were inactivated by adding formaldehyde (16 mM) followed by heating at 56 °C (for 10 min). Three individual runs were performed and corresponding vaccine preparations were pooled and referred to as vaccine A ($t = 0$), vaccine C ($t = 6$) and vaccine E ($t = 24$). The vaccine were diluted to 8 IOU/mL and 0.5 IOU/mL based on the OD_{590} of the vaccines ($1 * OD_{590} = 20$ IOU). The *in vivo* challenge test [16] revealed that the potency of vaccine A was the highest (7.0 IU/mL), the potency of vaccine C was intermediate (4.8 IU/mL) and the potency of vaccine E was the lowest (0.8 IU/mL) (Table 1). Vaccine A and C fulfilled the regulatory requirements with respect to potency, while vaccine E did not. In contrast to the behaviour of the vaccines in the intracerebral challenge test, differences in protective properties were not consistently found in respiratory infection experiments (data not shown). This discrepancy indicates that the intracerebral infection model has a higher sensitivity for subtle difference in wP vaccine quality, confirmed by earlier studies using similar vaccines [17,18]. If a respiratory challenge model would be used to detect qualitative differences between wP vaccines, adaptation and optimisation of this *in vivo* method will be required. In contrast, *in vitro* and immunochemical evaluation [19] showed that hTLR4 and moDC activating as well as the virulence protein content corresponds well with *in vivo* potencies (Table 1).

2.2. Ethical statement and immunisation of mice

This study was approved by the local Committee on Animal Experimentation of PD-Alt under permit numbers 201300053 and 201400128. Groups of adult (20–24 g) mice (n = 6 mice/group) of the outbred RIVM:NIH strain were vaccinated once i.p. with wP vaccine A, C or E at a dose of 0.25 IOU/mouse or 4 IOU/mouse, or were not vaccinated. Adult (20–24 g) mice of the outbred CD1 strain (Harlan, the Netherlands) were injected once i.p. with wP vaccine A, C or E at a dose of 4 IOU/mouse wP vaccine ($n = 12$ mice/group) or PBS ($n = 8$ mice). Similar to the conventional PSPT, all groups consisted of an equal number of females and males. CD1 and NIH mice both originate from Swiss mice, but their lineages were separated in 1930 [20]. These mouse strains are used for quality control testing of wP vaccines, i.e. for the Kendrick test [21] and the PSPT [7].

From the CD1 mice, blood samples were taken by orbital puncture 4 h after vaccination. 28 days after vaccination, CD1 and RIVM:NIH mice were bled under anaesthesia (isoflurane in O_2) and sacrificed (cervical dislocation) and sera and spleens were isolated. Whole blood was collected in blood collection tubes (Z Serum Sep GOLD, Greiner Bio-One), centrifuged (15 min, 3500 r.p.m.) and serum was stored at −80 °C until further use.

2.3. Analysis of B. pertussis-specific antibodies

IgG1 and IgG2A antibodies were measured using ELISA plates coated with inactivated whole *B. pertussis* bacteria (Kh96/01 or BP18323 as indicated). Sera were diluted 1:100 followed by a dilution series of 1:3. *B. pertussis*-specific antibodies were detected by HRP-conjugated goat-anti-mouse IgG1 and IgG2A antibodies (Southern Biotech) and subsequent incubation with peroxidase substrate for 10 min, and 2 M H_2SO_4. The presented values are the detected ODs at 450 nm (ELISA reader, Bio-Tek) × dilution of the serum. The limit of detection for both antibody subclasses was at

Table 1
Properties of vaccines used within this study.

	Vaccine A	Vaccine C	Vaccine E	Reference
Virulence proteins content	High	Intermediate	Low	Hoonakker et al. (submitted)
Protective properties	7.0[a]	4.8[a]	0.8[a]	Metz et al. [16]
Activation of moDC	High	High/intermediate	Low	Hoonakker et al. [19] Biologicals
Activation of hTLR4	High	Intermediate	Low	Hoonakker et al. (submitted)
Activation of mTLR4	High	High	High	Hoonakker et al. (submitted)

[a] Sufficient is a potency of at least 4 IU/mL and 95% interval with a lower limit of 2 IU/mL.