MINIREVIEW

New pertussis vaccination approaches: en route to protect newborns?

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Abstract
Pertussis or whooping cough is a life-threatening childhood disease, particularly severe during the first months of life, although adolescent and adult pertussis is increasingly more noted. General vaccination has tremendously reduced its incidence but has failed to bring it completely under control. In fact, it remains one of the most poorly controlled vaccine-preventable diseases in the world. New vaccination strategies are thus being explored. These include vaccination of pregnant mothers to transmit protective antibodies to the offspring, a cocooning strategy to prevent the transmission of the disease from family members to the newborn and neonatal vaccination. All have their inherent limitations, and improved vaccines are urgently needed. Two types of pertussis vaccines are currently available, whole-cell, first-generation and second-generation, acellular vaccines, with an improved safety profile. Attempts have been made to discover additional protective antigens to the 1–5 currently included in the acellular vaccines or to include new adjuvants. Recently, a live attenuated nasal Bordetella pertussis vaccine has been developed and undergone first-in-man clinical trials. However, as promising as it may be, in order to protect infants against severe disease, a single approach may not be sufficient, and multiple strategies applied in a concerted fashion may ultimately be required.

Introduction
At the verge of the third millennium, pertussis or whooping cough continues to be a major global public health problem. In fact, it constitutes today one of the first infectious diseases whose incidence is increasing despite the widespread use of efficacious vaccines. As an example, after a dramatic drop to almost zero in the 1980s, the number of pertussis cases in California was again as high in 2010 as it was in 1947 (Kuehn, 2010). Despite a close to 85% worldwide vaccination coverage through the Expanded Program on Immunization of the World Health Organization (WHO, 2011), roughly 40 million pertussis cases and 300 000 pertussis-linked deaths are recorded annually. These facts illustrate the limits of current pertussis vaccination programmes and mark this disease as the most poorly controlled vaccine-preventable disease in the developed world.

Since the 1940s and 1950s, pertussis vaccines have been combined with diphtheria and tetanus toxoids in formulations called diphtheria-tetanus-pertussis (DTP) vaccines and are now sometimes combined in addition with hepatitis B, inactivated polio and/or Haemophilus influenzae b vaccines (Storsaeter et al., 2007). Primary infant vaccination against pertussis starts usually at 2 or 3 months of age and includes 2–3 immunizations, given at 1- or 2-month intervals, often followed by a booster vaccination during the second year of life (see Table 1 for pertussis vaccination schedules in Europe). However, deferred vaccination for more than 6 months is not uncommon (Luman et al., 2002). This implies that children are not optimally protected before the age of 6 months in the period when they are most vulnerable to the severe forms of the disease (Bisgard et al., 2005). Yet, the most important primary objective of infant vaccination strategies should be the protection against the severe
and deadly forms of the disease during the most vulnerable period. The scope of this review is to describe and discuss the different approaches currently proposed to reach this goal.

**Current pertussis vaccines**

**Whole-cell pertussis vaccines**

Shortly after the discovery of *Bordetella pertussis* as the aetiological agent of whooping cough, more than 100 years ago, first attempts to develop a vaccine were undertaken. The first vaccines based on whole, killed *B. pertussis* organisms were tested in children in Tunis and in Denmark (Howson et al., 1991). By the 1930s, these whole-cell pertussis vaccines (Pw) were produced and used in many countries and combined with the tetanus and diphtheria vaccines during the 1940s and 1950s. These DTPw combined vaccines are today still the most widely childhood formulations to protect simultaneously against diphtheria, tetanus and pertussis. There is little difference between production procedures of Pw vaccines. All contain whole B. pertussis bacteria that have been inactivated by heat, formaldehyde treatment or other chemical procedures.

The effectiveness of Pw vaccination to prevent pertussis in children is without question and has been documented through numerous efficacy trials, starting with those conducted by the British Medical Research Council in the 1940s (Medical Research Council, 1951), and long-term epidemiological studies (Storsaeter et al., 2007). However, not all Pw vaccines showed equal efficacy, leading to substantial differences in quality between the manufacturers (Fine & Clarkson, 1987).

Consequently, Pw vaccines have been administered to millions of children worldwide and have prevented hundreds of thousands of deaths. However, adverse events have resulted in decreased confidence in Pw vaccines during the 1970s and the 1980s. This, in conjunction with variability in vaccine quality, resulted in a drop of vaccination acceptance and in certain countries, such as Japan and Sweden, to a full vaccination stop. As an immediate consequence, pertussis epidemics increased rapidly in these countries (Storsaeter et al., 2007), thereby in fact illustrating the effectiveness of high coverage with Pw vaccines.

The most frequently observed adverse reactions are local pain, redness and mild to moderate swelling, as well as transient systemic effects, such as fever, drowsiness, irritability and anorexia (Heijbel et al., 1997). These adverse reactions are experienced by roughly 50% of the vaccinated children, and their incidence increases with subsequent doses. Other systemic adverse reactions, such as prolonged and unusual crying, hypotonic–hyporesponsive episodes and febrile convulsions, are much less common, and it is often difficult to establish a causal link with Pw vaccination, as some of them, such as febrile convulsions, are relatively common during early childhood (Waruiru & Appelton, 2004). More severe adverse events such as encephalopathy, permanent brain damage and infant death have been alleged to be associated with Pw vaccination, but are now generally considered to be Pw vaccination independent (Baker, 2003). Nevertheless, these allegations have resulted in a serious setback in childhood vaccination strategies. A strategy to reduce Pw reactogenicity by removing lipopolysaccharides present in the vaccine has been recently developed in Brazil and is currently under clinical evaluation (Zorzeto et al., 2009).

**Acellular pertussis vaccines**

The controversies around Pw vaccines have resulted in research efforts to develop new, safer vaccines against whooping cough, by trying to separate the protective antigens from toxic components of *B. pertussis* extracts. Initial attempts to identify protective antigens date back to the 1940s and 1950s. The antigen discovery strategy was based on the finding that in the British Medical Council trials (Medical Research Council, 1956), efficacy seemed to correlate with potency in a mouse model developed by Kendrick et al. (1947). This potency test was therefore used to identify protective antigens.

After the initial preparations of crude extracts, which showed potency in the Kendrick test, as well as the efficacy in clinical trials, procedures were developed to purify individual antigens, which are now part of the second-generation, acellular pertussis (Pa) vaccines. The first Pa vaccines were developed by Sato et al. (1984) in the 1970s and consisted of copurified *B. pertussis* antigens, essentially pertussis toxin (PT) and filamentous haemagglutinin (FHA). Subsequent Pa vaccines were derived from individually purified antigens, pooled to the final composition of 1–5 antigens. All currently marketed Pa vaccines contain at least detoxified forms of PT, most of them contain in addition FHA, and many further contain pertactin and sometimes fimbriae (Storsaeter et al., 2007). The amounts of the individual antigens per dose may vary between vaccines (Table 2).

Clinical trials have shown that Pa vaccines have a better safety profile than Pw vaccines and are efficacious (Ad Hoc Group, 1988; Greco et al., 1996). However, the level of Pa vaccine efficacy seemed to correlate with the number of protective antigens present in the formulation (Gustafsson et al., 1996), but this was not sustained over time (Gustafsson et al., 2006). Based on similar efficacy and improved safety over Pw vaccines, Pa vaccines progressively replace the latter in many countries. However,
Table 1. Pertussis vaccination schedules in Europe

<table>
<thead>
<tr>
<th>Country</th>
<th>2-6 months</th>
<th>10-24 months</th>
<th>3-17 years</th>
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<tr>
<td>Austria</td>
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Adapted from http://www.euvac.net

A, DTaP; a, Tdap; W, DTwP.
General pertussis vaccination has altered the epidemiology of the disease. In the prevaccination era, whooping cough was essentially a childhood disease, with the highest incidence in children between 6 months and 2 years, and only few cases were found in adults. In contrast, adolescent and adult pertussis is now becoming increasingly more frequent in countries with high vaccination coverage (Von König et al., 2002). Several, nonmutually exclusive reasons may explain this change, including B. pertussis strain adaptation (Mooi, 2010) and waning immunity postimmunization (Wendelboe et al., 2005). In the prevaccination era, virtually every child experienced B. pertussis infection, and because of continuous bacterial circulation, frequent booster exposures resulted in lasting immunity against disease. Neonates may therefore have been partly protected by maternal antibodies.

Adult or adolescent pertussis is usually not as severe as infant disease and is generally not life threatening. However, it may still cause significant morbidity, and the economic burden of adult pertussis is not negligible (Lee et al., 2004). In addition, B. pertussis-infected adolescents and adults may constitute an important reservoir for transmission to unvaccinated infants, which may be the reason for the rising incidence in unvaccinated or not completely vaccinated children (Lavine et al., 2010).

Because the greatest increase in pertussis incidence in high vaccine coverage countries is in teenagers, adolescent booster immunization is recommended in several countries, in order to limit transmission to nonvaccinated infants (Forsyth et al., 2007). However, in the Western world, the age-mixing patterns lead to little contact between teenagers and infants (Mossong et al., 2008). Thus, teenage booster immunization, although certainly of benefit for the teenage population, may have little

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Trade name</th>
<th>Manufacturer</th>
<th>Pertussis antigens</th>
<th>Targeted population</th>
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<tbody>
<tr>
<td>DTaP</td>
<td>Daptacel</td>
<td>Sanofi Pasteur</td>
<td>FHA (5 μg), glutaraldehyde-detoxified PT (10 μg), PRN (3 μg), Fim 2/3 (5 μg)</td>
<td>Infants and children 6 weeks to 6 years old</td>
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<tr>
<td></td>
<td>Infanrix</td>
<td>GlaxoSmithKline</td>
<td>FHA (25 μg), glutaraldehyde- and formaldehyde-detoxified PT (25 μg), PRN (8 μg)</td>
<td>Infants and children 6 weeks to 7 years old</td>
</tr>
<tr>
<td></td>
<td>Tripedia</td>
<td>Sanofi Pasteur</td>
<td>FHA (23.4 μg) and formaldehyde-inactivated PT (23.4 μg)</td>
<td>Infants and children 6 weeks to 7 years old</td>
</tr>
<tr>
<td>DTaP/Hib</td>
<td>TriHIBit</td>
<td>Sanofi Pasteur</td>
<td>FHA (23.4 μg) and formaldehyde-inactivated PT (23.4 μg)</td>
<td>Children 15–18 months old</td>
</tr>
<tr>
<td>DTaP/polio</td>
<td>Kinrix</td>
<td>GlaxoSmithKline</td>
<td>FHA (25 μg), glutaraldehyde- and formaldehyde-detoxified PT (25 μg), PRN (8 μg)</td>
<td>Children 4–6 years old</td>
</tr>
<tr>
<td>DTaP/polio/Hib</td>
<td>Pentacel</td>
<td>Sanofi Pasteur</td>
<td>FHA (20 μg), glutaraldehyde-detoxified PT (20 μg), PRN (3 μg), Fim 2/3 (5 μg)</td>
<td>Infants and children 6 weeks to 4 years old</td>
</tr>
<tr>
<td>DTaP/polio/hepatitis B</td>
<td>Pediarix</td>
<td>GlaxoSmithKline</td>
<td>FHA (25 μg), glutaraldehyde- and formaldehyde-detoxified PT (25 μg), PRN (8 μg)</td>
<td>Infants and children 6 weeks to 6 years old</td>
</tr>
<tr>
<td>Tdap</td>
<td>Adacel</td>
<td>Sanofi Pasteur</td>
<td>FHA (5 μg), glutaraldehyde-detoxified PT (2.5 μg), PRN (3 μg), Fim 2/3 (5 μg)</td>
<td>Adolescents and adults 11–64 years old</td>
</tr>
<tr>
<td></td>
<td>Boostrix</td>
<td>GlaxoSmithKline</td>
<td>FHA (8 μg), glutaraldehyde- and formaldehyde-detoxified PT (8 μg), PRN (2.5 μg)</td>
<td>Individuals 10 years of age and older</td>
</tr>
</tbody>
</table>

DTaP, diphtheria and tetanus toxoids, acellular pertussis adsorbed; DTaP/Hib, DTaP and Haemophilus influenzae type b; DTaP/polio, DTaP and inactivated poliovirus; DTaP/polio/Hib, DTaP, inactivated poliovirus and H. influenzae type b; DTaP/polio/hepatitis B, DTaP, inactivated poliovirus, and hepatitis B; Tdap, tetanus toxoid, reduced diphtheria toxoid, reduced acellular pertussis vaccine adsorbed.
New pertussis vaccines

New pertussis vaccines

Impact on protection against infant pertussis (Lavine et al., 2012). Booster vaccination appears to have a strong impact on herd protection within age groups, but not between age groups (Lavine et al., 2010, 2012). At the current stage of knowledge, it cannot be excluded that vaccinating teenagers further erodes the immunity in adults of child-bearing age and thereby increases the circulation of the disease in this age group (Lavine et al., 2012), which is the main source of severe pertussis in infants (Bisgard et al., 2004). The administration of frequent boosters to adults is likely also to be ineffective in reducing the pertussis burden in the infant population, as shown by using the epidemiological dynamics of pertussis in a mathematical model (Rohani et al., 2010).

New pertussis vaccination strategies

Maternal vaccination

The primary objective of pertussis vaccination strategies should be to protect infants against severe disease and death, particularly threatening the very young (<3 months of age). Given the possibility of maternal antibodies to transfer protection to the newborns (Van Rie et al., 2005), it would intuitively seem reasonable to propose immunization during pregnancy (Mooi & De Greeff, 2007). It has indeed been shown that administration of Pw (Kendrick et al., 1945) or Pa (Lewis, 2011) vaccines late in pregnancy is safe for the mother and the infant and that it results in high levels of B. pertussis-specific antibodies in infants. The levels of these antibodies may be high enough to protect the infants through the highest risk period. However, placently transferred maternal antibodies are unlikely to persist at high enough concentrations to sustain protection. In addition, they may potentially interfere with actively induced immune responses upon vaccination of the infants, and innovative formulations may thus have to be used for the neonates in order to overcome the vaccine interference by maternal antibodies (Polewicz et al., 2011). Finally, the protective effect may be very limited for pre-term infants, where the risk of pertussis-linked mortality is particularly high (Haberling et al., 2009).

A cocoon strategy

In some countries, such as France, the United States and Australia, a ‘cocoon’ strategy has been proposed to prevent pertussis in newborns (De la Rocque et al., 2007; DeMaria & Lett, 2010). This strategy consists of immunizing the parents and other household contacts immediately after the birth of the new child to prevent the transmission of the disease to the newborn, according to the assumption that most of the neonatal pertussis is acquired from infected parents, grandparents or siblings. A successful cocoon programme implies very high numbers of contacts to be vaccinated in order to reach a significant impact on severe infant pertussis, hospitalization and death. In low-incidence countries, the numbers would be too high and resource intensive (Skowronski et al., 2012). Notwithstanding cost-effectiveness, cocooning is difficult to implement. It requires intense parental education, a prolonged window of opportunity to immunize, which is logistically challenging and costly (Healy et al., 2011). Finally, cocooning may be limited in its ability to prevent infant pertussis in the first weeks of life, because the disease may already be circulating within the household at the time of delivery and may therefore be transmitted to the newborn before vaccine-induced immunity develops in the contacts.

New pertussis vaccines

New pertussis vaccines

Neonatal vaccination

Considering the limits of maternal, adolescent and cocooning immunization, neonatal vaccination appears thus as an attractive option. Immunization at birth with DTPw vaccines has been tempted more than 50 years ago. However, it was found to result in ‘immune tolerance’, whereby antibody responses to B. pertussis antigens upon both primary and booster vaccinations were reduced compared to infants vaccinated later in life (Provenzano et al., 1965). Similar observations were made in mouse models (Roduit et al., 2002). Nevertheless, despite reduced antibody levels, neonatal vaccination with Pw or Pa vaccines induced protection levels in 1-week-old mice similar to those induced in 3-week-old mice. Administration of a three-component Pa vaccine given to infants at birth and at 3, 5 and 11 months induced a poor antibody response after the first dose, but resulted in efficient priming, as evidenced by a comparison of their antibody responses at 5 months with those of infants who were not vaccinated at birth (Belloni et al., 2003). However, at 12 months, the anti-PT antibody levels were lower in the first group than in the second group.

A more recent study showed significantly lower antibody responses to B. pertussis antigens upon immunization at birth with a DTPa vaccine, followed by a dose at 2, 4, 6 and 17 months, compared to those observed in infants who were not immunized at birth (Halasa et al., 2008). This difference was maintained up to 18 months. In contrast, when a stand-alone three-component Pa vaccine was used for immunization of newborns, no interference with subsequent vaccinations was observed. Instead, it accelerated the antibody responses to the B. pertussis antigens, although at 7 months, the antibody responses were similar to those observed in the absence of early Pa
vaccination (Knuf et al., 2008). The administration of a second dose of a stand-alone Pa vaccine at 1 month of age in addition to vaccination at birth significantly increased the antibody responses to *B. pertussis* antigens and had no negative impact on antibody titres upon subsequent DTPa vaccinations (Wood et al., 2010).

Neonatal immunization with current Pa vaccines is generally well tolerated and may thus accelerate antibody responses to *B. pertussis* antigens. However, even with an accelerated antibody response, infants in the first 2 months of life, the most vulnerable time window for severe and life-threatening pertussis (Haberling et al., 2009), remain still unprotected. Furthermore, early Pa vaccination may interfere with antibody responses to other recommended vaccines, such as the hepatitis B, *H. influenzae* type b and diphtheria vaccines (Knuf et al., 2008, 2010). In addition, up till now most studies have focused on the antibody responses upon at-birth immunization. Little information on T-cell responses after early immunization is currently available, although protection against pertussis is at least partially conferred by cell-mediated immunity of the Th1 type (Mills et al., 1998; Feunou et al., 2010a), and the limited information available suggests a strong Th2 polarization of the cellular immune memory to neonatal Pa vaccination (White et al., 2010).

**Novel pertussis vaccines**

**New vaccine antigens**

Currently available pertussis vaccines have thus clearly revealed their limits, and new vaccines are needed for better control of this disease. Since the advent of Pa vaccines, relatively little effort has been devoted to the development of new pertussis vaccines. As current Pa vaccines contain up to five antigens, detoxified PT, FHA, Prn, serotype 2 and serotype 3 fimbriae, one line of research aims at identifying additional protective antigens. The first one to focus considerable attention was the adenylate cyclase toxin. It has been known for some time that immunization with adenylate cyclase toxin can protect mice against intranasal challenge with *B. pertussis* (Guiso et al., 1991). In addition, this antigen expresses adjuvant activities (MacDonald-Fyall et al., 2004). Repeated coadministration of a genetically inactivated adenylate cyclase toxin with Pa vaccine enhanced the protective effect of Pa vaccine in a murine nasal challenge model, probably due to an augmentation of the Th1 and Th2 responses to the other *B. pertussis* antigens (Cheung et al., 2006).

Based on the important role of the autotransporter BrkA in *B. pertussis* virulence in mouse models and on the observation that antibodies to BrkA augment serum killing of *B. pertussis*, this protein was also assessed as a potential vaccine antigen (Marr et al., 2008). However, the administration of BrkA did not induce significant protection in mice against nasal challenge with *B. pertussis*, although the addition of BrkA to FHA and PT improved the protection over FHA and PT alone. On the other hand, no further improvement of protection was noted when BrkA was added to a trivalent Pa vaccine, containing PT, FHA and Prn, or to a Pw vaccine. Interestingly, whereas Prn-deficient and even PT-deficient clinical isolates have been described (Bouchez et al., 2009), so far, all tested strains of *B. pertussis* produce similar levels of BrkA with essentially identical sequences, suggesting that BrkA-containing vaccines may be broadly protective, even against strains that lack Prn or PT.

Recently, an antigen induced by iron starvation, named IRP1-3, has been described to be strongly recognized by antibodies from *B. pertussis*-infected human subjects and to be protective in mouse challenge models (Alvarez Hayes et al., 2011). Again, there is little, if any, sequence variation in this protein amongst *Bordetella* strains. Anti-IRP1-3 antibodies can induce opsonization and neutrophil uptake of iron-starved *B. pertussis*, and vaccination with this antigen induced slight but significant protection against nasal challenge with iron-starved *B. pertussis*. Whether the addition of IRP1-3 to the currently available Pa or Pw vaccines improves their protective potential has not been assessed so far.

**New vaccine formulations**

In addition to the search for new protective antigens, some effort has been devoted to the development of new vaccine formulations. Based on the observations that outer membrane vesicles (OMV) of Gram-negative organisms can induce potent protective immunity, this technology has also been applied to pertussis vaccine development (Asensio et al., 2011). *Bordetella pertussis* OMVs contain a large array of potentially protective antigens in addition to those already present in the current Pa vaccines, including immunostimulatory molecules, such as lipooligosaccharide.

All commercial vaccines contain alum as an adjuvant. Alum-adjuvanted vaccines most often favour a strong Th2-type immune response, at the expense of the Th1 response. There is now overwhelming evidence indicating that strong Th1 responses are required in addition to antibodies in order to achieve effective clearance of *B. pertussis* both in animal models and in humans (Mills et al., 1998; Feunou et al., 2010a). Therefore, Th1-inducing adjuvants have been explored for their use in future pertussis vaccines. CpG motif-containing oligodeoxynucleotides are potent inducers of Th1 responses, and their addition to
alum-adjuvanted DTP vaccines shifts the anti-PT IgG isotype profile towards IgG2a in mice, indicative of a Th1 response (Sugai et al., 2005). Furthermore, vaccine formulations containing CpG oligodeoxynucleotides can overcome the interference of maternal antibodies on the vaccine take of the neonates in animal models (Polewicz et al., 2011). Combining CpG oligodeoxynucleotides with additional immunostimulatory molecules, such as polyphosphazenes and cationic innate defence regulator peptides, further increases B. pertussis-specific IgG2a responses, both in adult and in neonatal mice (Gracia et al., 2011). In addition, this adjuvant formulation resulted in earlier and longer-lasting immune responses than vaccines with each of the single adjuvants. Encapsulation of these formulations within microparticles further enhanced the immune responses and induced strong protection in mice, even after a single immunization (Garlapati et al., 2011). These microparticles were found to induce strong Th1 responses in addition to the strong Th1 responses.

However, none of the new antigens or formulations has undergone further product or clinical development so far.

**Mucosal administrations**

Whooping cough is a strictly respiratory disease, and B. pertussis infection is limited to the upper respiratory tract in humans. With very few exceptions (Troseid et al., 2006), there is no dissemination of B. pertussis to other organs. It is therefore likely that mucosal immunity may play a role in protection against this disease, although the contribution of mucosal immunity in protection against pertussis has only attracted limited attention (Hellwig et al., 2001), and the current vaccines are all administered parenterally.

Nevertheless, mucosal immunization against pertussis has been tempted. Oral administration of a Pw vaccine to newborn babies on days 2, 3, 4 and 5 after birth and at 6 weeks elicited salivary anti-B. pertussis IgA, as well as serum IgG and cellular immune responses (Baumann et al., 1985). In addition, the immune responses occurred earlier than after parenteral immunization, and oral vaccination provided significant protection against pertussis morbidity in infants. A single dose of a Pw vaccine delivered nasally to adults induced B. pertussis-specific antibodies in nasal secretions, but not in the serum, whereas the reverse was seen after intramuscular vaccination (Thomas, 1975). It was therefore concluded that this mode of administration of Pw vaccines is not recommended for use in infants. A more recent study confirmed the induction of mucosal anti-B. pertussis antibodies after nasal spraying of a nonadjuvanted Pw vaccine (Berstad et al., 2000a). They were predominantly detected in nasal secretions and much less in the saliva. In this study, serum anti-B. pertussis IgG and IgA were also detected. However, no antibodies specific for PT were found, neither in the serum nor in the mucosa, and only modest anti-FHA antibodies were found in the nasal secretions.

In addition to antibodies, nasal administration of the Pw vaccine also induced T-cell responses, as evidenced by T-cell proliferation upon stimulation with B. pertussis antigens (Berstad et al., 2000b). FHA-specific T cells were detected in those vaccinees who also responded with anti-FHA IgA in their nasal secretions. The antigen-specific T-cell proliferation persisted for at least 9 weeks after nasal immunization.

Pa vaccines have not yet been applied intranasally to human volunteers. As Pa vaccines are based on only a handful of purified antigens, they may be less immunogenic that Pw vaccines when administered mucosally and therefore may need the help of powerful mucosal adjuvants. In mice, a mixture of FHA and PT in combination with chitosan administered nasally induced systemic and mucosal antibody responses to these two antigens, which were much lower in the absence of chitosan (Jabbal-Gill et al., 1998). Similar results were found when pertussis vaccines were administered intranasally together with polyphosphazene as adjuvant (Shim et al., 2010). This formulation elicited a mixed Th1/Th2 response and strong protection against respiratory infection with B. pertussis.

By far the strongest mucosal adjuvants are cholera toxin and the related Escherichia coli heat-labile enterotoxin (LT) and their nontoxic derivatives. Nasal vaccination of neonatal mice with Pa or Pw vaccines together with genetically detoxified LT resulted in significant protection against B. pertussis challenge (Hale et al., 2004). Similarly, a chimeric protein composed of the S1 subunit of PT fused to cholera toxin induced mucosal and serum antibodies to PT and some level of protection against B. pertussis when delivered nasally (Lee et al., 2003). However, nasal administration of detoxified LT or cholera toxin as adjuvants may result in unacceptable adverse events in humans, as illustrated by the significant increased risk of Bell’s palsy upon intranasal immunization with LT-adjuvanted inactivated influenza vaccine (Mutch et al., 2004).

**Live attenuated vaccines**

One of the first suggestions for the use of attenuated live vaccines to protect against pertussis comes from nonhuman primate studies. The effect of repeated nasal infections of macaque monkeys by virulent B. pertussis has led to the conclusion that ‘ultimate protection against
whooping cough probably best follows a live *B. pertussis* inoculation’ (Huang et al., 1962). Infection induces long-lasting protection. Initially estimated to be nearly lifelong (Gordon & Hood, 1951), it is now well established that natural immunity can wear off with time. Mathematical modelling using epidemiological signatures from the well-documented incidence data for England and Wales indicates that the average duration of infection-induced immunity lasts for approximately 30 years (Wearing & Rohani, 2009). However, it is inherently variable, and some individuals may lose immunity quite rapidly after infection. Nevertheless, vaccine-induced immunity appears to wane faster than infection-induced immunity (Hallander et al., 2011; Lavine et al., 2012), and second episodes of pertussis are usually milder than the first infections, are often atypical and are therefore difficult to diagnose. In addition, despite the assumed poor capacity of neonates to induce strong Th1 responses, natural infection by *B. pertussis* leads to strong and long-lasting Th1 responses, even in very young infants (Mascart et al., 2003).

Live attenuated pertussis vaccines have a number of advantages over the current vaccines. Their nasal administration is needle-free and avoids the use of invasive procedures. Compliance is therefore higher than for parenteral vaccination, as nasal vaccination overcomes the fear of injections (Davis, 2001). In comparison with injectable vaccines, needle-free vaccine administration is better suited for mass vaccination and may thereby help to achieve universal vaccine coverage, even in resource-poor settings, as it is simple, safe and cost-effective (Levine, 2003). This is well illustrated by the promising effects of measles aerosol vaccines on the reduction in the overall burden of this disease and its high potential for the global elimination of measles (Higginson et al., 2011). Needle-free vaccination eliminates the need of trained personnel, reduces the risk of contamination and of needle-stick injury.

Similar to natural infection, nasally applied live attenuated *B. pertussis* may induce both mucosal and systemic immune responses, which may result in faster and broader immunity, compared to parenteral vaccine administration. In contrast to current pertussis vaccines, *B. pertussis* infection also leads to protection against other *Bordetella* species, such as *Bordetella parapertussis* (Watanabe & Nagai, 2001), the agent of a milder form of pertussis, whose incidence has been increasing over the last decades and against which no vaccine is currently available.

The first attempts to develop live attenuated *B. pertussis* vaccines date back to the 1980s, when Roberts et al. (1990) developed an *aroA* mutant of *B. pertussis*. This mutant strain was highly attenuated, but it failed to efficiently colonize the respiratory tract of mice. Therefore, repeated nasal administrations of high doses were required to obtain significant protective effects using this strain.

More recently, we have developed an attenuated vaccine strain, based on the knowledge of the specific molecular mechanisms of pertussis pathogenesis. This vaccine strain, named BPZE1, contains genetic alterations that eliminate or inactivate three different *B. pertussis* toxins (Mielcarek et al., 2006). In BPZE1, the derrnecrotic toxin gene was deleted, and the PT gene was genetically modified by altering two different codons, so that the enzymatic ADP-ribosyltransferase activity of the toxin was abolished, yielding a fully inactive protein. Finally, the *B. pertussis ampG* gene was replaced by *E. coli ampG*, which resulted in virtual absence of the tracheal cytotoxin. This strain was found to be nonpathogenic in mouse models and caused no pulmonary inflammation, yet it is able to colonize the mouse respiratory tract nearly as long as the virulent parent strain. A single nasal administration of BPZE1 induces full protection against challenge with virulent *B. pertussis* in mice. BPZE1-induced protection is mediated by both antibodies and CD4+ T cells (Feunou et al., 2010a). Protection against challenge is dose dependent (Mielcarek et al., 2010) and is directly related to the ability of the vaccine strain to colonize the respiratory tract and to induce both anti-*B. pertussis* antibodies and IFN-γ-secreting cells.

In preclinical mouse studies, BPZE1 induced long-lasting protection (Feunou et al., 2010b; Skerry & Mahon, 2011). For up to at least 1 year, a single nasal administration of BPZE1 to adult or infant mice provided total protection against nasal challenge with virulent *B. pertussis*, whereas immunity induced by two administrations of Pa vaccine started to wane at 6 months after the last immunization. Interestingly, 1 year after vaccination, protection could still be transferred by either antibodies or T cells from BPZE1-immunized mice, whereas the protection could not be transferred by antibodies or T cells from Pa-vaccinated animals. Furthermore, BPZE1 vaccination induced rapid protection, which can be detected as early as a few days after immunization (Debrie et al., in preparation). This is reminiscent of the observations made on a live attenuated *Bordetella bronchiseptica* vaccine shown to provide protection against kennel cough in dogs as early as 48 h after vaccination (Bey et al., 1981).

In addition to *B. pertussis*, BPZE1 vaccination also protects mice against *B. parapertussis* (Mielcarek et al., 2006; Feunou et al., 2010a) and *B. bronchiseptica* infection (Kammoun et al., in preparation). However, unlike protection against *B. pertussis*, protection against *B. parapertussis* could only be transferred by BPZE1-induced T cells and not by antisera from BPZE1-vaccinated mice.
(Feunou et al., 2010a). This finding is consistent with the fact that Pa vaccines only poorly protect against B. parapertussis, as their effector mechanisms rely essentially on antibody production.

The use of live attenuated vaccines raises the important issue of their biosafety, especially if they are destined to general vaccination. It is important to illustrate that the genetic attenuation is stable and that reversion to virulence does not occur. Because no natural horizontal gene transfer mechanism exists between B. pertussis cells, reversion of large deletions, such as those for the dermonecrotic and ampG genes, is thus impossible. PT was inactivated in BPZE1 by two independent mutations affecting two amino acid residues that are both critical for its ADP-ribosyltransferase activity, one being essential for substrate binding and the other for catalysis. Reversion of both to active amino acid residues is required to regain enzyme activity, which again is virtually impossible. The genetic stability of BPZE1 has been established upon continuous serial passages both in vitro and in vivo in mice for up to 1 year (Feunou et al., 2008).

In addition to the genetic stability, live attenuated vaccines must also be safe as such, including in immunocompromised subjects, such as those infected with HIV. Attenuated B. pertussis derivatives appear to be especially interesting in that regard, as even infection with fully virulent, wild-type B. pertussis is not more frequent in patients with AIDS than in non-HIV-infected individuals (Cohn et al., 1993). Although B. pertussis generally causes a strictly upper respiratory tract infection without bacteremia, in severely immunocompromised individuals, the organism can occasionally be isolated from blood (Troseid et al., 2006). This atypical disseminated infection can be mimicked in IFN-γ receptor-deficient mice (Mahon et al., 1997). Whereas virulent B. pertussis disseminates to the liver of these mice upon aerosol infection, BPZE1 does not (Skerry et al., 2009). Similar conclusions can also been drawn from experiments with SCID mice. Furthermore, although infection with virulent B. pertussis may kill neonatal mice to a significant extent, no mortality was observed in neonatal immunocompetent or immunodeficient mice upon the administration of BPZE1.

Further preclinical safety data also indicated that BPZE1 does not exacerbate airway pathology associated with allergen sensitization, although virulent B. pertussis infection exacerbates inflammation in that model (Kavangh et al., 2010). Instead, BPZE1 administration actually protects against experimentally induced allergic pulmonary pathology and reduces allergen-driven IL-4, IL-5 and IL-13 production. The anti-inflammatory properties of BPZE1 have also been evidenced in a heterologous respiratory infection model. In a mouse model of severe influenza virus–induced pneumonitis, nasal BPZE1 administration prior to virus challenge provided significant and sustained protection (Li et al., 2010). Protection was not paralleled by a reduction in viral load, but was attributable to the dampening of the cytokine storm induced by the viral challenge, reduced lung inflammation and tissue damage, as well as decreased neutrophil infiltration.

The excellent safety profile of BPZE1 has allowed it to be declassified from a safety level 2 to a safety level 1 organism in several countries. Together with its immunoprotective properties in preclinical models, this has now allowed BPZE1 to be tested in phase I clinical safety trials in human volunteers. A placebo-controlled, double-blind, dose-escalating safety trial was initiated at the end of 2010 (Clinicaltrials.gov ID: NCT01188512). The volunteers were followed up for safety and immunogenicity for 6 months, and a full trial report is expected in the months to come.

In addition to providing protection against pertussis and other Bordetella infections, as well as to its anti-inflammatory properties, BPZE1 may also constitute an interesting platform for the production of heterologous antigens, in order to develop vaccines that can protect simultaneously to several infections upon a single nasal administration (Mielcarek et al., 2001).

**Conclusion and outlook**

Despite the wide vaccination coverage, pertussis is not under control, highlighting the shortcomings of current vaccination strategies. Neither severe and deadly pertussis in infants nor pertussis in general, including in the older populations, has been controlled satisfactorily by current vaccination strategies, and the incidence of the disease is rising even in countries with very high vaccination coverage. Several approaches to solve this problem have been tempted and met with limited success. These include maternal immunization, neonatal vaccination and cocooning strategies. Each one has its limits, owing to biological and immunological constrains and/or difficulties to implement such measures in an effective way. Since the advent of Pa vaccines, little effort has been devoted to the development of new pertussis vaccines until recently. New antigens, adjuvants and immunization routes have been explored in animal models, but only few have reached clinical tests in humans. Nevertheless, the development of improved parenterally administered pertussis vaccines for neonates remains an option. A promising recent line of research has led to the development of a live attenuated pertussis vaccine, currently in clinical phase I trials. Similar to the Bacille Calmette-Guérin, the only available vaccine against tuberculosis, the target
population of this vaccine is the newborns in order to provide early protection against the most severe forms of the disease and to prime anti-pertussis immunity to be boosted later in life by Pa vaccines (Locht, 2008).

It is likely that pertussis in general will not be controlled by a single approach. Perhaps by combining maternal immunization with cocooning strategies and innovative neonatal immunization, at least severe and life-threatening pertussis in the infant population may be controlled in the next generations.

References


New pertussis vaccines


