PERISCOPE: Road towards effective control of pertussis

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ABSTRACT

The resurgence and changing epidemiology of pertussis in high-income countries, the high infant mortality caused by pertussis in low-income countries and the increasing morbidity in all age groups worldwide call for a concerted effort to improve current and develop new vaccines and vaccination strategies against pertussis. In this Personal View, we identify several key obstacles that need to be overcome in order to develop a durable solution for global control of pertussis. In an effort to systematically address these obstacles, the PERISCOPE (Pertussis correlates of protection Europe) consortium was recently established. The objectives of this consortium are to increase our scientific understanding of vaccine- and infection-induced immunity to pertussis in humans, to identify biomarkers of protective immunity, and to generate technological tools and infrastructure that will enable the future development of improved pertussis vaccines. By paving the way towards the accelerated licensure and implementation of novel well-tolerated and effective pertussis vaccines, we hope to strengthen and stimulate further collaboration, cooperation and transparency between the key stakeholders, including the public, the scientific community, public health institutes, regulatory authorities and vaccine manufacturers.

INTRODUCTION

Whole cell pertussis (wP) vaccines have been a cornerstone of national immunisation programs (NIPs) since the 1940s-1950s. Today, most low- and middle-income countries (LMICs) continue using wP vaccines, while since the 1990s-2000s, acellular pertussis (aP) vaccines with a more favourable reactogenicity profile, have nearly completely replaced wP vaccines in high- and many middle-income countries. Unfortunately, despite nearly 70 years of universal childhood vaccination, it has proven to be difficult to control pertussis. The disease remains an important cause of infant mortality in low-income countries[1] and is associated with considerable morbidity in all age groups worldwide. Although combined diphtheria-tetanus-pertussis (DTP) vaccine coverage has improved significantly in many low-income countries following the inception of the Expanded Programme on Immunisation in 1974, large numbers of individuals still do not have access to vaccination[2], leaving vulnerable infants at risk of developing severe pertussis. Pertussis incidence has also been steadily rising in the last two decades in several countries with high vaccination coverage[1,3,4]. The pattern of disease resurgence is particularly obvious in school-aged children, adolescents and adults and is therefore thought to be related to waning of immunity with age[5-9]. Disease incidence is also increasing in infants too young to be protected by vaccination[10,11]. Although the effectiveness of current pertussis vaccines in infants is well established, there is a need to investigate the underlying causes of disease resurgence in other at-risk-populations, in particular concerning the differences between aP and wP vaccines in generating long-term protection. Defining immunological signatures linked to durable protection against pertussis disease, as well as identifying immunological correlates of protection (CoPs) against infection and transmission will be important to inform and expedite the design, development, and regulatory approval of new vaccines.

WHAT IS THE CAUSE OF THE RESURGENCE OF PERTUSSIS?

The epidemiology of pertussis is still not fully understood and many factors have likely contributed to the resurgence. Increased disease awareness and improved diagnostic tools such as PCR are known to have increased the number of reported pertussis cases[12], allowing identification of cases that previously remained undetected using traditional culture-based methods. Unfortunately, comparisons between countries are complicated by differences in surveillance systems, vaccination programs, vaccine composition and the use of molecular diagnostics in health care systems. Epidemiological data have yielded evidence of more persistent protection after primary vaccination with wP than with aP vaccines[7,8], suggesting that waning immunity contributes to the resurgence of pertussis in some countries with widespread use of aP-vaccination. Currently available data, while often incomplete and mostly based on clinical observations without laboratory confirmation, show no evidence that pertussis poses a major health problem in LMICs that still use wPs[13-15] and the current WHO recommendation is for these countries to continue using wPs[16]. Mathematical modelling of pertussis incidence and attack rates in the UK and USA suggests that asymptomatic transmission in vaccinated

populations may also contribute to resurgence[17,18]. The presence of high anti-pertussis toxin (PT) IgG has been used to indicate recent exposure in individuals vaccinated at least one year ago. In a large cross-sectional, population-based serosurveillance study in the Netherlands, a significant increase in high anti-PT IgG was found in individuals >9y, increasing from 4.0% in 1995-1996 to 9.3% in 2006-2007[19], supporting the hypothesis that there is significant circulation of pertussis, much of which remains undetected. It should be noted that pertussis resurgence is not universal and the incidence of pertussis already increased in some countries prior to the switch to aP vaccines[3,20]. Another factor potentially implicated in the resurgence of pertussis is genetic changes in *Bordetella pertussis*. Although there is still a lack of studies demonstrating a direct causal relationship between newly emerging lineages – such as the PtxP3 lineage - and vaccine effectiveness, the current hypothesis is that adaptation to vaccine-mediated selective pressure has resulted in strains with increased fitness[21,22]. A recent development is the emergence of *B. pertussis* strains that no longer express one or more of the vaccine antigens. Pertactin (Prn)-deficient strains in particular have been described to expand in several countries using aP vaccines, with prevalence reaching nearly 100% in some regions[23,24].

WHICH OBSTACLES DO WE NEED TO OVERCOME?

Vaccination continues to play a pivotal role in preventing pertussis-related morbidity and mortality. Nonetheless, recent indications that differences may exist between aP and wP vaccines in their ability to induce persistent immunity in humans and baboon studies that demonstrated that neither wP nor aP vaccines were able to prevent colonization and transmission have made it clear that there are significant gaps in our understanding of pertussis immunity. In order to find a more systematic and durable solution to the control of pertussis, several obstacles need to be overcome, as discussed below. We also include a glossary of definitions for various aspects related to clinical endpoints and CoPs (see Table 1), which may facilitate future discussions.

Limited resources, fragmentation of the field and research methods.

Following the large investment in the development of aP vaccines in the 1980s-1990s, aPs appeared to be effective and R&D expenditure was consequently reduced. Although precise numbers on local and global investments are difficult to obtain, there is a noticeable drop in the number of yearly publications on pertussis after the first DTaP licensure in 1996 and the subsequent implementation into childhood immunization programs (Figure 1). An unwanted consequence of discontinuous funding was fragmentation of pertussis research, with no clear integration of the epidemiological, microbiological, immunological, and clinical aspects. Targeted funding is thus required to ensure integrated research efforts involving all of the above areas can be undertaken. Additionally, recent advances in the field of vaccine research, in particular in immunology and systems biology, now offer new opportunities that will help us to better understand the mechanism(s) of protective immunity against *B. pertussis*.

Novel methodologies to evaluate vaccine efficacy and effectiveness.

Pertussis vaccines were licensed using clinical endpoints of protection against severe disease according to a specific case definition, as demonstrated from large, complex and expensive field trials conducted in the 1990s (reviewed in[25]), with different *B. pertussis* populations compared to now. Although durable protection against disease is an essential aspect of all pertussis vaccines, new insights gained from animal studies have raised the possibility of differences between individual vaccines with regards to their impact on other aspects of infection. For instance, studies in the baboon challenge model showed that while aP and wP vaccines prevent disease equally well, wP vaccines were more protective than aP vaccines against asymptomatic colonisation of the airways and concomitant transmission to a naïve animal[26]. Therefore, developing methodologies to evaluate protection against asymptomatic infection and prevention of transmission in humans will be useful in getting a more complete picture of the effectiveness of new vaccines against asymptomatic infection and controlling disease on a population basis.

Lack of validated CoPs

A major hurdle for the development and licensure of new pertussis vaccines is the lack of established immunological CoPs in humans. Most aP vaccine efficacy studies reported immunogenicity data based on specific antibody concentrations in blood, as quantified by ELISA. Evidence from clinical studies shows that high levels of anti-PT serum antibodies, and to a lesser extent Prn and fimbriae (Fim), correlate with protection against typical pertussis[27-29]. This is supported by observations from antenatal immunisation studies that demonstrate the efficacy of aP vaccine-induced antibodies in preventing death and severe morbidity from pertussis in newborns[30,31]. Although pertussis-antibody ELISA is currently the only immunoassay approved by regulatory authorities and used in licensing of pertussis vaccines, there are doubts about its predictive value for long-term effectiveness. There are many potential immunological parameters that could contribute to protection and are potential CoPs. For instance, assessment of functional antibody activity may paint a more relevant picture of the immunological responses induced by various pertussis vaccines, as reflected by reported differences in opsonizing bactericidal antibodies[32] and bacterial adherence inhibiting antibodies[33] between wP and aP vaccines. Similarly, work by Mills identified key differences in memory T-cells in mice, showing that aP and wP vaccines induce cellular immunity with a Th2/Th17 and a Th1/Th17 bias respectively[26,34]. Although studies in children by Buisman and coworkers demonstrated a similar polarization regarding Th1 and Th2 responses[35-37], the role of Th17 responses in humans has not yet been fully characterized[38]. Standardized methods to assess functional antibodies or pertussis-specific cell-mediated immunity are not yet available.

Lack of knowledge on waning of immunity.

Although both aP and wP vaccines are effective in preventing pertussis in infants[25,39], there are several epidemiological studies suggesting that aP-induced protection wanes more rapidly than wP-induced protection[7,8]. It should be noted that neither vaccination nor natural infection induces life-long protection[40,41]. Efforts to prolong protection by administering additional booster aP vaccine doses to children and adolescents have unfortunately not been as successful as expected[5,6]. Primary vaccination with wP or aP vaccines generates differences in the quality, quantity, longevity and 'boostability' of immunological memory[42]. This early imprinting of immunological memory by primary vaccination has been found to impact on the subsequent response to booster vaccination[37,43,44]. By understanding the underlying mechanisms of memory imprinting, it may be possible to confer more durable protection.

Protection of newborns against pertussis.

A key priority of pertussis control is to protect vulnerable newborns and young infants against severe pertussis from birth until they have been vaccinated. This is particularly important given the widespread circulation of *B. pertussis* amongst parents and older siblings[19]. Theoretically, this goal can be achieved by either giving pertussis vaccines to newborns or to pregnant women, which capitalizes on passive protection through transplacental transfer of maternal antibodies. Although aP vaccination studies in neonates showed that vaccination immediately after birth is safe and immunogenic, immunological interference against non-pertussis vaccine components was observed following subsequent immunisation at later time points[45-49]. Consequently, aP-vaccination of newborns has effectively been abandoned. Due to its success in the UK[30,31], the USA[50] and several other countries, antenatal pertussis vaccination is increasingly being implemented in high- and middle-income countries . Although the immediate benefits of such programs are evident, several questions remain to be answered concerning the potential impact of maternal antibodies on the infant's response to primary immunisation with wP or aP vaccines, or to other vaccines received in the first year of life.

THE PERISCOPE PROJECT

The resurgence and changing epidemiology of pertussis call for a concerted effort to improve current vaccines or develop new vaccines and vaccination strategies against pertussis. In an effort to systematically address these issues, the PERISCOPE (Pertussis correlates of protection Europe, http://www.periscope-project.eu) consortium was recently established as a public-private partnership, funded by the Innovative Medicines Initiative and the Bill & Melinda Gates Foundation. The objectives of the PERISCOPE consortium are discussed below.

ACCELERATION OF PERTUSSIS VACCINE DEVELOPMENT

The ultimate objective of PERISCOPE is to create a solid scientific basis for facilitating and accelerating the development of improved pertussis vaccines or vaccination strategies. PERISCOPE will approach this objective from multiple angles (Figure 2) through a series of preclinical and clinical studies. These studies will exploit existing know-how on pertussis biology and immunity[34,51,52] and build on solid experience of the partners with clinical and preclinical trials[37,42,43,53]. Together, these studies will help to *i.* gain a more thorough scientific understanding of the underlying mechanisms and biomarkers of protective immunity to *B. pertussis* in humans; *ii*. investigate differences between wP and aP vaccines in relation to immunological function and persistence; *iii.* investigate the impact of antenatal immunisation on infant responses to primary pertussis vaccination and *iv.* strengthen our technological means of testing novel vaccine candidates in animal and human models of disease and asymptomatic infection.

Clinical vaccine studies

Randomized multi-center clinical studies comparing aP versus wP-vaccination will be performed in infants in both Europe and Africa to identify differences in immunological memory. To understand the impact of antenatal vaccination, these trials will include an arm with infants born to mothers who received a booster dose of aP vaccine during pregnancy. Furthermore, vaccination trials will be performed in different age groups to study the effect primary vaccination on innate and adaptive responses to an aP-booster vaccine[37,42,43,53]. Data from these studies will serve as a reference for future studies with novel formulations.

Controlled human infections and natural infection studies

Another objective is to establish a safe and reproducible model of controlled *B. pertussis* infection in humans. For ethical reasons these studies are carried out in adults, most of whom will have been vaccinated against pertussis during infancy, which will influence their response to infection. This model can be used to address several key questions and offers a means to identify and eventually validate CoPs against asymptomatic infection. For instance, immune profiles can be compared between culture-positive and culture-negative individuals to help identify immune factors involved in protection. Challenging humans with *B. pertussis* will also provide important insights into the human pathobiology of, and the immune response to infection. Once this model has been established, future studies outside the scope of PERISCOPE can utilize it to evaluate novel vaccine formulations with *B. pertussis* colonisation as an endpoint. In addition to the controlled human challenge studies, we intend to establish and conduct immunological research in a cohort of naturally infected pertussis patients and their family members or contacts. This will allow analysis of potential correlates of protection in the context of natural exposure.

Preclinical vaccine evaluation capacity

Additional preclinical evaluation capacity is needed to support the screening of novel vaccines, particularly in the baboon model that was established at the US Food and Drug Administration (FDA) by the Merkel group[54-56]. The baboon model is the only animal model to date that allows evaluation of *B. pertussis* infection, disease[54] and transmission[26,55]. One of our objectives is therefore to make this model more accessible by expanding its use in Europe. By harmonizing study designs across baboon and human studies, it will be possible to compare immune response profiles and link these to long-term protection against both *B. pertussis* transmission *and* disease. The selective use of mouse models, including knock-out mice[34,52], will be essential to complement studies in humans and baboons and decipher the mechanisms of protective immunity and the biological role of putative biomarkers.

Standardized immuno-assays

A crucial step forward for the field is to develop a number of standardized immunological assays to characterise the spectrum of immune responses to pertussis in humans and to identify biomarkers and potential CoPs that could help expedite the development process of novel vaccines. We anticipate that antibody assays that measure functional activity of vaccine-induced antibodies will more likely yield biomarkers and CoPs than antibody assays that solely measure antigen binding capacity. We will focus on antibody-mediated inhibition of bacterial attachment to respiratory epithelial cells (early colonisation), bactericidal activity (early to late infection), opsonophagocytosis and killing (early to late infection) and neutralization of PT (disease).

Although cellular immunity likely plays an important role in protection against *B. pertussis*, T-cell responses against *B. pertussis* have not been extensively studied in humans, largely due to the lack of well-established and fully standardized assays to analyze *B. pertussis*-specific T-cells in a clinical trial setting. It is therefore imperative to develop a standardized pertussis T-cell assay, which will allow a more thorough investigation of T-cell responses against *B. pertussis* and enable cross-study comparisons. Although memory B-cells have been more extensively studied in humans[37,43,53], it is also important to establish a standardized assay to quantify *B. pertussis* antigen-specific plasma and memory B-cells.

To minimize the risk that putative CoPs are solely directed against a single strain or antigen, a representative panel of *B. pertussis* strains will be used for testing in relevant immunoassays.

Systems biology approach to pertussis research

Using systems biology approaches[57,58], discrete immune signatures may be uncovered that provide important clues on how immunological memory is (re)programmed following aP and wP-vaccination respectively, and how this differs from infection-induced immunity. An important deliverable of PERISCOPE is the establishment of a TranSmart database that will facilitate an integrated data

analysis. This will allow us to analyse patterns of the early immune response and link these to adaptive immune responses or clinical endpoints.

In-depth analysis of T- and B-cell memory

Studies will be performed to investigate the development and maintenance of B- and T-cell immunological memory following vaccination and infection. For instance, a comprehensive investigation of how the antigen-specific B-cell response develops over time can provide important clues towards the role of antibodies in protection. This will also provide an opportunity to analyse the B-cell receptor repertoire and combine it with functional antibody readouts, an approach that has proven to be incredibly useful for influenza vaccine research[59]. A similar analysis of T-cell immunity is warranted, as a better understanding of the functional plasticity of *B. pertussis*-specific T-cells could help guide the design and use of novel pertussis booster vaccines.

Remaining knowledge gaps

It should be mentioned that PERISCOPE is a human-centric project with a strong focus on the identification of putative immunological correlates of protection. There are several areas of interest that PERISCOPE will not be able to address, even though they might bring important insights. These include genetic changes in *B. pertussis*, potential new vaccine formulations, novel vaccine antigens and vaccination beyond one pregnancy, among others.

REBUILDING THE SCIENTIFIC ECOSYSTEM FOR PERTUSSIS RESEARCH

Through PERISCOPE we aim to foster scientific innovation and rebuild the ecosystem and technical infrastructure that is needed to evaluate novel pertussis vaccines. Ultimately the potential modification of current vaccine formulations, immunisation schedules and R&D of novel vaccine formulations will be impacted by the availability of reliable preclinical and clinical models and a robust immunological toolbox to be deployed in clinical studies. The legacy of the PERISCOPE consortium will be an increase in the ability of academic researchers and biotechnology and pharmaceutical organizations all over the world to evaluate pertussis vaccines and select and evaluate the most promising ones for further clinical development.

CONCLUSION

The epidemiology of pertussis has changed significantly since the introduction of universal pertussis childhood vaccination programs. Although several research groups are now actively developing novel pertussis vaccines, major technical and scientific hurdles remain that need to be overcome to enable this effort. In this Personal View, the PERISCOPE Consortium outlines essential steps that may help to expedite the development of novel pertussis vaccines and to reduce the risks of late-stage vaccine candidate failure. We acknowledge the huge collaborative effort required and hope that working

together with partners in all parts of the world along this initial roadmap will strengthen and stimulate further collaboration, cooperation and transparency between the key stakeholders and increase the chance of achieving the ultimate goal of bringing pertussis under control.

Contributors

DAD, PLH and RdG developed the concept and drafted the text. DAD conceptualized and produced the figures. UH conceptualized Table 1. All authors have been involved in the design of the PERISCOPE project and have critically reviewed and edited the manuscript.

Declaration of interests

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338 **Table 1. Definition of terms***

Term	Definition
B. pertussis	Gram negative pathogenic coccobacillus of the genus *Bordetella*
Transmission	Transmission of *B. pertussis* is defined as the successful transfer of *B. pertussis* between individuals or by experimental inoculation, i.e. demonstration of the presence of *B. pertussis* in the nasopharynx (or lower airways) **by PCR** with primers specific for *B. pertussis* at a given time point in the exposed individual. Transmission of *B. pertussis* may lead to colonisation within a reasonable period of time of exposure (<4 weeks) or not.
Colonisation	*B. pertussis* colonisation is defined as demonstration of the presence of viable *B. pertussis* in the nasopharynx (or lower airways, e.g. on autopsy) **by culture** (=replicating bacteria) at a given time point. Colonisation may or may not lead to infection.
Carriage	*B. pertussis* carriage is defined as demonstration of colonisation at least at two different time points in the absence of infection, with a maximum interval <4 weeks. The period of carriage is defined by the first and last demonstration of *B. pertussis* colonisation.
Infection	*B. pertussis* infection is defined by the presence of *B. pertussis* in the nasopharynx (or lower airways on autopsy) as demonstrated **by culture or PCR** with primers specific for *B. pertussis* or serological criteria as defined by EU Reference laboratories[60] which causes damage and induces an immune response of the host. If damage induced by *B. pertussis* infection is significant, this will lead to disease (=pertussis). Otherwise the infection may remain asymptomatic.
Pertussis (=disease)	Pertussis (or whooping cough) is a disease defined by cough with or without paroxysms, whooping or vomiting with convincing evidence of causation by *B. pertussis* infection rather than any other explanation.
Correlate(s) of protection	This is defined as a measurable profile of immune response(s) in an individual who resolved proven *B. pertussis* infection or disease or who was immunised against pertussis, which correlates with a specified state of protection when subsequently exposed to *B. pertussis*. Ideally, the profile is measured before exposure, or after pertussis, or after the last dose of immunisation.
Immune profile	A measurable immunological indicator, or a combination of measurable indicators, that define the potential for protection against *B. pertussis* colonisation, carriage or infection in an individual who does not currently have clinically apparent pertussis or is colonised by *B. pertussis*. Indicators may include specific cells, molecules, genes or gene products, including antibodies, cytokines, metabolites, etc. Indicators may be transiently measurable, such as gene transcription shortly after vaccination, or may be measurable over a longer duration of time, like serum antibodies.
Correlate of protection against transmission	Immune profile that correlates with **prevention of *B. pertussis* transmission** when exposed to *B. pertussis*.
Correlate of protection against colonisation and carriage	Immune profile that correlates with **prevention of *B. pertussis* colonisation** when exposed to *B. pertussis*. If colonisation cannot be prevented but on further testing within a short period of time (few days) *B. pertussis* colonisation is not detected anymore, at least **carriage** was prevented.
Correlate of protection against infection	Immune profile that correlates with **prevention of *B. pertussis* infection** when exposed to *B. pertussis.*
Correlate of protection against pertussis (= disease)	Immune profile that correlates with **prevention of pertussis or reduction of severity of pertussis** when exposed to *B. pertussis*.

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340 ***Result of consensus finding amongst PERISCOPE investigators based on relevant publications[27-29,60-62]

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FIGURE LEGENDS

Figure 1. Number of publications with 'pertussis' in the title or abstract in the period of 1945-2017.

Figure 2. Overview of the clinical and preclinical studies in PERISCOPE. The outer circle shows the general study design of the various (pre)clinical studies and the measurements and endpoints in PERISCOPE. The middle circle shows the comparative analyses that can be made between the immunological measurements and the endpoints for each study. The inner circle shows the biological insights and biomarkers that each study is anticipated to deliver.

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