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FOUND IN TRANSLATION

Leveraging preclinical study designs to close gaps in vaccine development for perinatal pathogens

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Vaccines to perinatal pathogens are critical for both reducing the burden of endemic pathogens and preparing for the next pandemic. Although they are often at greater risk of severe disease from infection, pregnant people and children are routinely marginalized in the vaccine development process. We highlight several challenges in the vaccine development process and how three tools—translational animal models, human cohort studies of natural infection, and innovative data-use strategies—can speed vaccine development and ensure equity for pregnant people and children in the next pandemic.

Introduction

Vaccines are one of the most transformative public health tools of the last century. Congenital rubella syndrome, first described in 1941 and characterized by miscarriage, stillbirth, and congenital defects, affected up to four babies in every 1,000 live births prior to the introduction of the highly effective rubella vaccine in 1969 (Reef and Plotkin, 2013; World Health Organization, 2023). While the rubella vaccine is largely a success story, there are opportunities to improve the perinatal vaccine development process and to close persistent equity gaps.

For pathogens that are still circulating and for future pandemics, we must address both pathogen-specific challenges and equity issues. Pregnant people were completely left out of COVID-19 vaccine development and then had a slower uptake of COVID-19 vaccines compared to the general population despite an increased rate of severe COVID-19 disease in pregnancy (Galanis et al., 2022). The JYNNEOS vaccine was ready for use when monkeypox began spreading in the US; however, there was no clinical data on the JYNNEOS vaccine in pregnant people and children, leaving both providers and patients without data to make decisions about vaccine use (Centers for Disease Control and Prevention, 2023a, 2023b). More recently, a respiratory syncytial virus (RSV) vaccine was first approved for the elderly despite the major disease burden of RSV occurring in newborns. Approval of the novel vaccine for use in pregnancy is still pending, and it is unknown if it will be approved in enough time to make an impact on the RSV season this year, even after children's hospitals were overwhelmed with a significant viral surge of RSV last year.

Here, we use Zika virus (ZIKV), Ebola virus (EBOV), human cytomegalovirus

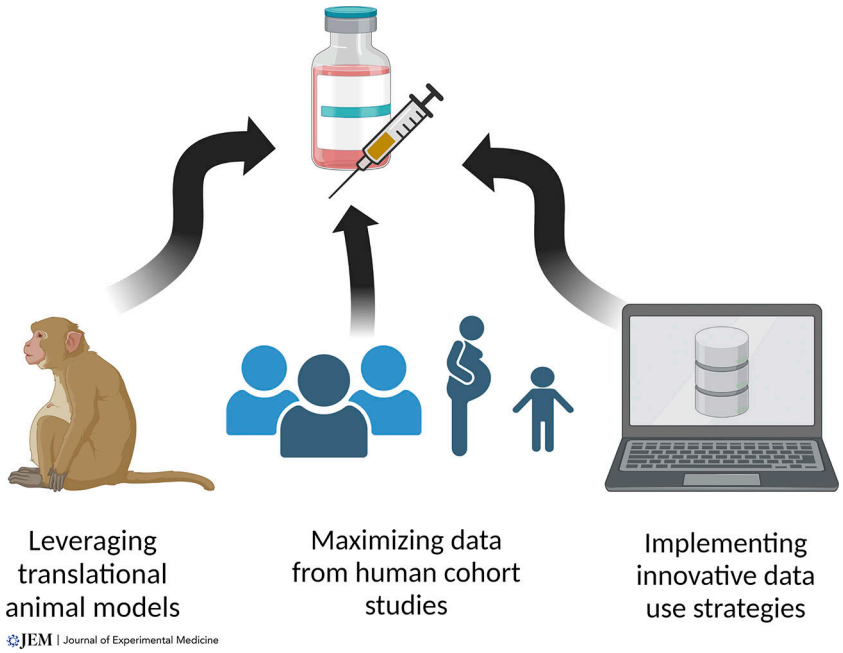

Figure 1. **Preclinical strategies to de-risk vaccine trials and ensure equity in vaccine development.** Figure generated using BioRender.

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(HCMV), HIV, and others as models to highlight some of the challenges associated with vaccine development and how preclinical tools can both speed the development of vaccines and ensure that pregnant people and children have access to safe and life-saving vaccines.

Barriers to perinatal vaccine development

Despite a significant reduction in cases since the 2015–2016 outbreak, ZIKV is well-positioned to re-emerge (Musso et al., 2019). However, we are unprepared to counter this threat because, despite the completion of Phase I and Phase II trials on several candidates in the 8 yr since the explosive outbreak in the Americas, we still do not have a licensed vaccine for ZIKV (Wang et al., 2022). As exemplified in the case of ZIKV countermeasure development, five key factors impede the development of safe and effective vaccines for emerging and re-emerging perinatal pathogens: complex immune dynamics, low case numbers, low-frequency adverse outcome during pregnancy, late inclusion of pregnant people in vaccine clinical trials, and reduced funding due to a perceived lack of urgency.

One of the most fundamental and often most challenging milestones in the development of a vaccine is the identification of a suitable vaccine target and the generation of an immune response against that target. For perinatal pathogens specifically, the bar for vaccine efficacy is high: vaccination must induce a response that is not only sufficient to protect the pregnant person from disease, but also prevent transmission of the pathogen to the fetus. Years of research have failed to identify the target(s) of neutralizing antibodies sufficient to protect against congenital infection with HCMV (Plotkin et al., 2020). In the case of ZIKV, researchers have identified the envelope protein as a target of potently neutralizing antibodies (Andrade and Harris, 2018). However, ZIKV interacts antigenically with the related dengue virus (DENV), and non-neutralizing antibodies to ZIKV may enhance, rather than protect against, subsequent infection with DENV, a phenomenon known as antibody-dependent enhancement (ADE). With the specter of the Dengvaxia rollout looming (Thomas and Yoon, 2019), in which vaccination with Dengvaxia in DENV-naive children led to ADE of subsequent DENV infections, many researchers are concerned about the potential for ZIKV vaccines to lead to ADE of infection in ZIKV and DENV endemic regions (Wang et al., 2022). For HIV, another virus with a high transmission rate in the perinatal period, a protective neutralizing response has been identified, but years of research have not yet identified a way to elicit this response through vaccination (Pegu et al., 2019; Nelson et al., 2022).

Low case frequency of emerging and re-emerging infections hampers progress toward vaccination. Since 2018, there have been, on average, 4,000 laboratory-confirmed cases of ZIKV in the Americas annually, compared to over 200,000 in 2016 alone (Pan American Health Organization, 2023). The reduction in cases necessitates recruiting many participants over a longer period to evaluate vaccines in clinical trials—extending the time and cost (Wilder-Smith et al., 2018; Vannice et al., 2019). In turn, this creates a high resource cost to test vaccines, reducing the likelihood of the development of a vaccine before the next pandemic. ZIKV is not the only virus to face this challenge: clinical trials for two EBOV vaccine candidates could not be completed due to a lack of cases of EBOV in West Africa in 2015 (Committee on Clinical Trials During the 2014–2015 Ebola Outbreak, 2017).

It is difficult to study viruses like ZIKV and HCMV that cause their most devastating effects in pregnancy: ethical concerns have long been associated with studies in pregnant people, and vaccine trials for these pathogens require screening a large number of participants to meet endpoints of the adverse pregnancy outcome. For example, a Phase III trial of HCMV-infected women required screening of more than 200,000 pregnancies over the course of 6 yr to test the therapeutic efficacy of a hyper-immune globulin therapy in nearly 400 women (Hughes et al., 2021). Government policy has historically disincentivized the inclusion of pregnant people in vaccine trials (Ren et al., 2021). Although there have been recent policy changes to support the inclusion of pregnant people in clinical trials, there is still little infrastructure to support their rapid inclusion in a pandemic scenario.

In addition to the logistic and scientific challenges associated with reduced cases, research funding for emerging threats tends to be reactive rather than proactive. There are no records of grant awards for "Zika" from the National Institutes of Health prior to 2016 when the ZIKV outbreak in the Americas occurred, highlighting a lack of preparedness for a pathogen that began causing outbreaks in 2007. Emergency funding for ZIKV was stalled in Congress for months, and funds that were still being used to respond to the West African EBOV outbreak had to be repurposed for ZIKV in the absence of new funds (McNeil, 2016). The ebb and flow of funding to specific pathogens over time prevents sustained research on pathogens needed to prepare for the next pandemic.

De-risking vaccine trials by leveraging preclinical tools

Addressing these challenges to develop vaccines that prevent low-frequency adverse outcomes of infection in pregnancy will require efforts from entities across the public health system. Here, we propose three solutions specifically in the preclinical space to de-risk large and costly human clinical trials: (1) better leveraging translational animal models of perinatal pathogens, (2) maximizing the utility of large human cohorts to assess immune correlates of natural infection, and (3) innovative data-use strategies (Fig. 1).

Employment of translational animal models of perinatal pathogens

Translational animal models fill critical gaps in the development of vaccines for both pathogens and populations that are difficult to study in humans. In the absence of high caseloads, research on pathogenesis, immunology, and interventions for pathogens can continue in animal models to inform and complement human trials. The ZIKV vaccines that were developed following the outbreak in the Americas drew upon previous work in a variety of models to develop vaccines for other related viruses such as West Nile virus. Studies in mice and non-human primates (NHP) preceded the only Phase II/IIb clinical trial of a ZIKV vaccine for the National Institute of Allergy and Infectious Diseases VRC5283 vaccine (Dowd et al., 2016; Wang et al., 2022). In the case of COVID-19 vaccination, the rapid pace at which vaccines were licensed and developed was due in large part to the work that was conducted on SARS-CoV and MERS-CoV in

various animal models, including NHP, in the previous 20 yr (Wellcome Trust, 2022).

For perinatal pathogens such as ZIKV and HCMV that cause some of their most severe outcomes in the fetus, NHP models are particularly relevant since their reproductive physiology and placentation more closely mimics humans than other animal models. The models do, however, have limitations: ethical and financial constraints often limit the number of animals in NHP studies, and NHP models do not always recapitulate the spectrum of human disease. However, efforts to develop high-risk models for congenital ZIKV (Rosinski et al., 2022) and HCMV (Bialas et al., 2015) have been successful at generating reproducible phenotypes that enable testing the efficacy of vaccines and therapeutics, allowing a pathway for de-risking vaccine trials that may include a pregnant population.

Animal models can provide safety data and dosing information for pregnant people and children in pandemic situations while Phase I trials in adults are ongoing. In the development of the COVID-19 vaccines, studies in infant NHP recapitulated the reduced dose required for reaching levels of protective immunity in children (Milligan et al., 2023; Padmanabhan et al., 2022). Including this type of study early in clinical testing of pandemic vaccines could speed the approval of vaccines for children and would provide needed data for pregnant people and parents considering whether they want to get a vaccine during a pandemic. Policymakers should consider how animal study data could speed emergency use authorization of vaccines for pregnant people and children in response to future threats.

Maximize human cohort studies of natural infection to define immune correlates

Large human cohort studies provide an opportunity to further de-risk costly and time-intensive clinical trials of vaccine efficacy. Intentional design and ready-made funding pathways of prospective human cohorts in areas of disease transmission can maximize the amount of data we are able to generate from a single study and can provide the infrastructure to identify and respond to new threats. A pediatric cohort of children in Nicaragua has existed for over 20 yr and has been used to primarily study dengue transmission, although samples have also enabled studies of influenza and chikungunya. Samples from this cohort and a separate cohort study of HCMV seroprevalence were able to be re-purposed to define fundamental knowledge about the ZIKV epidemic and its antigenic interactions with DENV when ZIKV emerged in the Americas (Katzelnick et al., 2020; Barbosa et al., 2018; Coutinho et al., 2021).

Human cohort studies overcome one of the key limitations of NHP studies, in that scientists can study a much larger sample set as compared to most animal studies and capture the natural variation in human populations. However, natural variability is a double-edged sword and can lead to difficulties in drawing generalizable conclusions. These types of cohorts also take a huge investment; additional policies such as the National Institutes of Health (NIH)'s recent establishment of Centers for Research in Emerging Infectious Diseases serve as a model for expanding this type of collaborative, multidisciplinary translational research.

Design of in silico modeling for defining biomarkers and immune thresholds of disease

As discussed, both animal models and human cohort studies have inherent limitations. Here we suggest using innovative data-use strategies to mitigate these limitations and amplify the utility of both NHP and human cohort studies. Comparative analysis of challenge experiments conducted in multiple NHP species may reveal biomarkers and protective mechanisms that are consistent across species, and hence more likely to translate to the human context. Similarly, identifying commonalities in natural history and vaccine studies across different viral species from the same family may identify high-value targets for vaccine development. Bayesian models that can use historical data to construct informative prior distributions for parameters of interest can be particularly useful for the analysis of NHP studies, which typically have small group sizes. When new data coincides with the prior, this approach increases precision; when new data diverge from the prior, this approach suggests that we should update our scientific model.

Systems biology and machine learning approaches can identify patterns and potential mechanisms of protection from high-dimensional pathogen and immune assays (Loos et al., 2023). Mathematical models of pathogen dynamics and the contribution of vaccine-elicited immune effector functions to control pathogen replication can be used to evaluate vaccine development strategies in silico and suggest the in vitro or in vivo experiments that have the highest predicted payoffs (Byrne et al., 2022; Padmanabhan et al., 2022). These mathematical models can also generate vaccination outcomes in simulated populations—with a range of vaccine efficacies or at different trimesters—and provide information to guide vaccine distribution policies.

Recently, artificial intelligence (AI) methods based on large language models (LLMs) have been in the news due to the impressive emergent properties of programs like GPT-4. One direct application of these AI models is as a virtual research assistant, able to keep up with the exponential increase in publications and summarize the state of research interactively and near instantaneously. However, the risk of "hallucination" where LLMs assert knowledge that is false needs to be addressed before they can safely used (Hou and Ji, 2023 *Preprint*). Protein language models, a variant of LLMs trained on billions of protein sequences, are another AI technology that may revolutionize vaccine research. These models can predict protein structures and protein–protein interactions, potentially including antibody–antigen interactions (Olsen et al., 2022; Hie et al., 2023), and massively increase our ability to search for promising vaccine targets. For example, protein structure–based design informed the development of the RSV vaccine shown to be 82% effective at preventing neonatal RSV in the first 90 d of life when administered during pregnancy (McLellan, 2015; Kampmann et al., 2023). Using predictive models could speed up this type of design by predicting the protein structures and protein–antibody interactions most likely to elicit a protective immune response.

The development of robust in silico models for infectious diseases relies on accessible data sharing. There are several platforms that currently facilitate this, and the new NIH data sharing policy provides a catalyst to expand these kinds of platforms. For example, the ClinicalTrials.gov infrastructure could be expanded to include NHP studies, which would increase the rigor and reproducibility of these studies and reduce duplicative studies. That said, institutions

should provide support and easy-to-use platforms to improve data sharing so that the onus of responsible data sharing does not fall solely on individual investigators.

Conclusion

Vaccines are critical tools for combatting inevitable future outbreaks and reducing the mortality from endemic communicable diseases for vulnerable populations. Here we have proposed three strategies—leveraging translational animal models, maximizing the utility of human cohort studies, and innovative data-use strategies—to de-risk future clinical trials and ensure vaccine equity before the next pandemic.

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