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COMMENTARY

Early Life HIV-1 Immunization: Providing a Window for Protection Before Sexual Debut

Sallie Permar,[1,2] Ofer Levy,[3–5] Tobias R. Kollman,[6] Anjali Singh,[7] and Kristina De Paris[8]

Abstract

Limited success of current HIV-1 vaccines warrants new approaches. We discuss feasibility and potential benefits of early life HIV-1 immunization followed by vaccine boosts during childhood that may enable maturation of vaccine-induced broad anti-HIV-1 immunity over several years. By initiating this immunization approach in the very young, well before sexual debut, such a strategy may dramatically reduce the risk of HIV-1 infection.

Keywords: HIV prevention, vaccine, immune development

Introduction

Successful implementation of antiretroviral therapy (ART) has transformed HIV-1 infection from a fatal to a manageable chronic disease. The dependence of HIV-infected individuals upon lifelong ART, however, places an unsustainable burden on healthcare costs worldwide, underscoring the urgent need for a safe and effective HIV-1 vaccine.

HIV-1 vaccine research reached a crossroad between traditional approaches that can elicit non-neutralizing antibodies (Abs) and T cell responses and the development of novel vaccine candidates aiming to induce broadly neutralizing Abs (bnAbs). The three completed phase 3 HIV-1 vaccine trials failed to demonstrate generation of bnAbs that can neutralize the majority of HIV-1 variants.[1] In response, two new main vaccine strategies were developed to elicit mature Abs with high avidity and broad neutralization capacity. However, both approaches, the use of native-like trimeric HIV-1 envelope (Env) immunogens or B cell lineage design-based vaccines to guide the development of Env-specific B cell responses over a prolonged time, have yielded only incremental advancement. Thus, new strategies to direct vaccine-elicited, broad immune responses are needed.

During the 2016 HIVR4P Conference in Chicago (USA), the Division of AIDS (National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH]) sponsored a Satellite Session entitled *Vaccine-Elicited Immunity in the Pediatric Immune System* providing a forum to discuss the opportunities and challenges of pediatric immunization approaches, and for fostering collaborations to guide comprehensive HIV-1 prevention efforts. Herein, we consider key aspects for rational design of a pediatric HIV-1 vaccine by examining the unique features of the infant immune system and discussing novel tools focused on defining immune pathways that could be harnessed to elicit broad immunity to HIV-1 before sexual debut.

Rationale for HIV-1 Vaccine Implementation in Early Life

Young women (15–24 years) represent the highest risk group for new HIV-1 infections, and the number of young HIV-infected women has not declined in the past 5 years.[2,3] These transmission rates are unlikely to decline significantly in coming years without new interventions beyond the current antiretroviral programs. To protect these young women, a vaccine would have to provide protection against infection before sexual maturity is reached. Sexual maturity is reached within a wide span of ages, girls as young as 9 years can exhibit pubertal characteristics.[4] Therefore, as years of affinity maturation might be necessary to generate bnAbs, the concept of

[1]Department of Pediatrics, Duke University Medical School, Durham, North Carolina.
[2]Human Vaccine Institute, Duke University Medical School, Durham, North Carolina.
[3]Precision Vaccines Program, Division of Infectious Diseases, Boston Children's Hospital, Boston, Massachusetts.
[4]Harvard Medical School, Boston, Massachusetts.
[5]Broad Institute of MIT and Harvard University, Boston, Massachusetts.
[6]Division of Infectious Diseases, Department of Pediatrics, University of British Columbia, Vancouver, Canada.
[7]Vaccine Research Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland.
[8]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.

early life vaccination against HIV-1 followed by booster immunization in childhood/preadolescence to provide protection before the onset of sexual activity is attractive. However, thus far, most HIV-1 vaccines have been tested exclusively in adults, and utilized short-term immunization schedules. The underexplored approach of initiating HIV vaccine immunization in early life is appealing because (1) birth is a reliable point of healthcare contact worldwide, (2) pediatric vaccines have a strong track record in preventing infectious diseases in childhood and lifelong, (3) the substantial preadolescent window provides a unique opportunity to develop mature immune responses via booster immunization over several years, and (4) the introduction of vaccines against sexually transmitted disease in adolescence has proven difficult.[5,6]

Challenges and Opportunities of Infant Immune Development

As with any approach, early life immunization includes challenges. While many have alluded to infant immunity as ''immature,'' the infant's immune system has in fact evolved to avoid persistent activation that would be detrimental to survival, because the newborn transitions from a low pathogen environment to balancing the establishment of normal flora with the simultaneous need to recognize pathogenic microbes.[7] These functional demands shape the infant immune system to be distinct from that of adults, but may provide possible leverage in the quest for anti-HIV bnAbs.

Adjuvants are agents that are added to enhance vaccine immunogenicity, with many working by enhancing the priming capacity of antigen-presenting cells (APCs).[8,9] Most current pediatric vaccines contain alum that promotes T helper cell 2 (Th2) responses.[8] Yet, an effective HIV-1 vaccine may require the induction of cytokines promoting Th1 responses associated with antiviral immunity. Infant APCs exhibit reduced IL-12 production to most stimuli,[10–13] and thus, adjuvants other than alum may be needed for protection against certain intracellular pathogens. Activation of pathogen recognition receptors (PRRs) such as Toll-like receptors (TLRs) can enhance immune responses. Indeed, multiple TLR agonists (TLRAs) demonstrate adjuvant activity *in vivo*, and several are now included in licensed vaccines, including monophosphoryl lipid A (MPL), a detoxified lipopolysaccharide TLR4A employed in certain human papilloma virus vaccines given to school-age children.[8]

As the establishment of the normal microbiome at mucosal surfaces is associated, at least transiently, with a more tolerogenic response to TLR2 and TLR4 agonists in infants after birth,[14] the use of TLR4-based adjuvants in early life vaccines to protect against mucosally transmitted HIV-1 remains to be conclusively determined. Growing evidence suggests that the host microbiome effects vaccine responses.[15] The importance of the microbiome in vaccine-mediated HIV-specific responses is further supported by the induction of polyreactive Abs to gp41 and antigens in the host flora in both adult and infant HIV-1 vaccine studies.[16] The induction of such cross-reactive Abs warrants consideration in Env immunogen design. Conversely, these findings imply that host flora antigens can imprint the B cell repertoire, a feature that could be exploited to generate targeted B cell responses by pediatric HIV-1 vaccines.

Much remains to be learned regarding age-specific effects of adjuvants. Of note, TLR7/8As, such as low molecular weight imidazoquinolines, can induce potent IL-12p70 production in infant APCs.[7] IL-12p70 is essential for Th1 differentiation, antiviral cellular immunity, and activation of NK cells that aid in clearing virally infected cells via direct killing or Ab-mediated cellular cytotoxicity. Indeed, inclusion of the TLR7/8-based adjuvant 3M-052 in the alum-adjuvanted pneumococcal vaccine (PCV), that is poorly immunogenic in human newborns, significantly accelerated and enhanced Ab responses in infant Rhesus macaques (RM) immunized at birth compared to PCV alone.[17]

Germinal center formation B cell development, an important factor in the development of effective Ab responses is critically dependent on T follicular helper (Tfh).[18,19] Mouse and nonhuman primate (NHP) studies suggest that infants have reduced Tfh frequencies and function.[20,21] In neonatal mice, limited Tfh cell expansion and therefore low GC B cell frequencies in response to an immunization with alum-adjuvanted tetanus toxoid could be overcome by the inclusion of a CpG adjuvant (TLR9A) into the neonatal vaccine formulation.[20]

More recently, 2′3′cGAMP, an agonist of the cytosolic PRR Stimulator of Interferon Genes (STING), demonstrated robust activation of neonatal APCs *in vitro* and marked adjuvanticity when formulated with alum *in vivo*.[22] Evidence suggests that combination adjuvantation systems may show age-specific interactions (e.g., additivity, synergy, or antagonism) further underscoring the importance of targeted age-specific vaccine development.[23] Thus, age-appropriate adjuvantation systems may be important for pediatric HIV-1 vaccine design, and novel adjuvants targeted for pediatric vaccines must be assessed for immunogenicity in the pediatric population.

Feasibility of Early Life Vaccines

Despite reduced costimulation by infant APCs, Th2 and Th17-prone $CD4^+$ T cell responses, and lower somatic hypermutation of Abs in infants,[7] early life vaccines are clearly feasible. In addition to polio vaccines (both live and inactivated) and Bacille Calmette-Guérin (BCG) vaccine, the more recent success of the alum-adjuvanted hepatitis B vaccine underscores the potential of inducing protective immunity in infants. In fact, a retrospective analysis of an early phase 1 pediatric HIV-1 immunization trial established that gp120 vaccine adjuvanted with MF59, an oil-in-water emulsion adjuvant containing squalene, elicited Ab responses of higher magnitude and persistence than that of an alum-adjuvanted vaccine.[24] Furthermore, MF59-induced responses were >20-fold higher than those elicited in the moderately protective adult HIV RV144 vaccine trial employing a poxviral vector prime/HIV Env boost vaccine regimen.[24]

The finding that HIV-infected infants develop bnAbs more rapidly than HIV-infected adults, despite limited hypermutation, further supports the idea of early life initiation of HIV immunizations.[25,26] Moreover, repeated boosts in the years leading up to adolescence could further promote affinity maturation, and enhance breadth and strength of bnAbs. Potentially, early life immunization could also facilitate the development of Abs with Fc-mediated effector function. In the RV144 trial, non-neutralizing plasma Abs that were associated with reduced risk of HIV-1 acquisition were predominantly of the IgG1 and IgG3 subtypes,[27] and IgG3 represents the predominant IgG subtype produced in infant plasma after maternal Ab waning in infant plasma.[28]

Similarly, when fetal RMs were exposed *in utero* to an attenuated simian immunodeficiency virus (SIV), they had more robust T cell responses following postnatal infection with pathogenic SIV, experienced less immune exhaustion, and demonstrated greater control of viral replication than unexposed infants.[29] Thus, early life exposure to SIV antigens without pathogenic infection, a scenario resembling vaccination, enhanced responses and controlled virus replication after infection.

Overall, these studies demonstrate the feasibility of early HIV vaccination, but also highlight our still limited understanding as to which immune responses would be optimal to induce protective immunity against HIV infection.

New Technologies to Guide HIV-1 Vaccine Design

Infant immune responses, as they transition from fetal to adult functional capacity, are tightly regulated by soluble and cell-associated molecules.[7] In this context, systems biology tools may provide key insights into unique age-specific features in HIV-1 vaccine responses. System serology approaches utilizing bead-based multiplex binding and functional assays provide a holistic look at complex Ab populations and their effector functions, while requiring only minimal volumes.[30,31] These polyclonal plasma responses can be integrated with assessment of the B cell repertoire through the genetic, antigen binding, and functional characterization of monoclonal Abs. Nanotechnologies have been applied to define HIV-specific $CD8^+$ T cell responses.[32]

Microfluidic single-cell analysis tools, for example, RNA-Seq transcriptome analysis, provide new windows into the molecular pathways controlling immune development and antigen-specific responses. Combinations of systems biology approaches, including proteomics and metabolomics of cells, plasma, and mucosal fluids, may identify factors predicting optimal HIV-specific immune responses.[33] These powerful systems biology tools have become increasingly available and cost effective, and we have started to adapt them to infant vaccinology.[34] The feasibility and validity of such systems approaches in early life was confirmed by the elucidation of multiple immune correlates of protection in the infant RTS,S malaria vaccine trial.[35,36]

Identification of molecular signatures, pathways, and hubs that correlate with broad antigen-specific immunity will enhance our mechanistic understanding of how vaccine-induced innate immune responses may shape effective memory responses that can confer protection upon HIV-1 exposure. Novel bioinformatic tools enable the mining of large data sets from multiple human and NHP vaccine studies to generate new hypotheses. These data will aid in advancing the rational design of age-specific vaccines that will leverage immune ontogeny to induce broad HIV-1 immunity in targeted populations.

Relevant Preclinical Models

Vaccine development relies on predictive preclinical models. A combination of approaches may greatly enhance early life vaccine development including (1) human *in vitro* modeling employing age-specific humoral (i.e., blood plasma) and cellular (e.g., leukocytes) immune system components,[37] and (2) preclinical testing of novel candidates in relevant newborn and infant animal models. Animal models must overcome the species-specific nature of HIV-1 via study of a related virus, for example, SIV, or humanizing of immune cells, such as in humanized mice. The time required for humanization and differences in immune development exclude the humanized mouse model for studies of early life immunity. In contrast, immune ontogeny in humans and RM is very similar, and the RM SIV infection model can recapitulate all human HIV-1 transmission modes and many aspects of pathogenesis.[38] As the majority of HIV-1 infections occur in sub-Saharan Africa where clade C is prevalent, molecular design of novel chimeric simian-human immunodeficiency virus (SHIVs) with clade C Env and Env optimized for binding to Rhesus CD4, enables development of clinically relevant pediatric SHIV infection models. RM reaches sexual maturity by age 3–4 years. Thus, the hypothesis that vaccination at birth, followed by repeated boosts, will increase breadth and strength of HIV-1 immune responses to provide protection against HIV-1 infection during adolescence can be directly tested in a compressed time frame in this model.[39]

The infant RM oral SIV/SHIV infection model made significant contributions to our understanding of pediatric HIV-1 infection, including (1) safety and efficacy testing of tenofovir in infant macaques before human pediatric use,[40] (2) proof of effective pre-exposure prophylaxis in infant macaques by passive transfer of polyclonal Abs,[41] and (3) prevention of infection and reduced viral reservoir seeding by administration of bnAbs before or up to 1 day post-oral SHIV162P3 challenge.[42] Although still limited by small sample volume, the pediatric model allows invasive biopsy sampling and tissue collection, providing a major advantage over human infant studies. Importantly, the RM B cell repertoire and a number of true human homologues evolve in parallel to that in humans after HIV-1 vaccination.[43] Therefore, while small animal models of early life immunization can contribute to screening of vaccine candidates, the NHP model is the preferred model to investigate the complexities of the developing immune system and elicitation of broad HIV-1 immunity.

Concluding Remarks

The idea of initiating HIV-1 immunization in early life to promote time-dependent maturation of broad anti-HIV-1 immune responses and protect high risk adolescents, is paradigm shifting. Studies of immune ontogeny and development of age-specific adjuvantation systems support approach and inform research directed toward early life HIV-1 vaccine development.

Advantages of early life HIV-1 vaccination

- Birth is a reliable point of healthcare contact, and early life HIV-1 immunizations could be incorporated into infant vaccine schedules in both resource poor (e.g., Expanded Immunization Program) and resource rich settings.
- There is strong precedent for preventing infection via early life immunization.
- Knowledge of immune ontogeny enables age-appropriate vaccine adjuvantation and targeted Ab development.
- Multidisciplinary systems biology and data integration approaches can identify potential mechanisms of in-

duction of broad immunity in infants and correlates of protection.
- Early immunization will allow for time to mature vaccine-induced immune responses to protect high-risk group of young adults, and thereby also reduce pediatric HIV-1 infections.

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Address correspondence to:
Kristina De Paris, PhD
Department of Microbiology and Immunology
University of North Carolina at Chapel Hill
Burnett-Womack Building, Room 9029
160 Dental Circle, CB No.7292
Chapel Hill, North Carolina 27599-7292

E-mail: abelk@med.unc.edu