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Immune Approaches for the Prevention of Breast Milk Transmission of HIV-1

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Introduction

Mother-to-child transmission (MTCT) of HIV-1 infection remains a significant cause of new HIV-1 infections, despite the increasing implementation of prevention strategies using antiretroviral therapy (ART) and the resulting decline in infections across the developing world. In 2009, the UNAIDS global report estimated 370,000 children under the age of 15 years were newly infected with HIV-1 (refer UNAIDS Report on the global AIDS epidemic, 2010 http://www.unaids.org/globalreport/Global_report.htm), most of whom acquired the infection from their mothers in low- and middle-income countries. Even with substantial progress, challenges remain for poor countries in providing comprehensive screening programs for pregnant women and implementing the full range of prevention services for those identified as HIV-1-infected. Although antiretroviral regimens and risk reduction counseling have been successfully used for pregnant women and their infants in many parts of the developing world, full implementation of these programs remains a challenge in many countries, especially where antenatal clinical attendance and HIV-1 screening is not yet widespread. In addition, potential toxicities of and development of drug resistance to ART in both mother and child are concerns. Therefore, the development of a safe effective immunoprophylaxis regimen begun at birth and continuing during breastfeeding, perhaps alongside neonatal chemoprophylaxis, remains an area of active research interest. An ideal pediatric vaccine for prevention of MTCT (PMTCT) would combine the immediacy of passive immunization designed to protect the infant during the first vulnerable weeks of life with the durability of active immunization to protect against the repeated low-dose homologous virus exposure delivered multiple times a day via breastfeeding.

In the absence of maternal ART, up to one half of all MTCT occurs through breast milk [1, 2]. In the first month of life, it can be challenging to define the route of infection as early breast milk transmission as opposed to late in utero or intrapartum transmission. After 1 month of age, the route of transmission is clearly breast milk and risk of transmission remains relatively constant throughout the duration of breastfeeding [2]. Increased risks of

breast milk transmission are associated with longer duration of breastfeeding [2] and high levels of HIV-1 in breast milk [3, 4].

Infants who acquire perinatal infection, particularly in developing countries, experience unusually rapid disease progression compared to that of adults, and mortality rates as high as 20–52% have been reported in the first 2 years of life [5, 6]. A key factor contributing to the rapid disease progression observed in infants may be the persistently high levels of HIV-1 viremia observed throughout the first year of life, with the “set-point” viral load (VL) rarely falling more than 1 log below the peak VL [7, 8]. However, there are some data to suggest that breast milk infection is associated with a better clinical outcome than in utero or intrapartum transmission. Both the peak VL and viral set-point are reported to be higher in babies infected at birth or in the first few weeks of life (early infection) than in those infected after 3 months of age (late infection) [7, 9]. These factors are associated with increased risk of disease progression and death in early infant HIV-1 infection [10]. Overall, after adjusting for length of infection, the risk of progression to AIDS and death is at least twofold higher for infants infected early compared with those infected through breast milk [11–13]. The most likely explanation for this is the relative immaturity of the infant immune system at birth, which is dominated by regulatory T-cell (Treg) [14, 15] and Th2 responses [16–18]. Following birth, there is a shift toward Th1 responses as the immune system switches from one predominantly adapted to tolerance of self and maternal antigens to a system better able to mount effective innate and acquired responses to external pathogens such as HIV-1 [19]. It is plausible that infants infected through breastfeeding may be more likely to become long-term survivors than those infected early, but there have been no natural history cohort studies assessing disease progression beyond 5 years to address this issue.

It is likely that co-infections acquired by babies in the developing world may play a role in rapid disease progression, such as human cytomegalovirus (CMV), which infects most West African children in the first year of life [20] and is associated with high VL in HIV-1 co-infected infants [21]. Co-infections may also contribute to the likelihood of transmission. Therefore, it is important to investigate and define the potential role of co-infections in breast milk transmission of HIV-1 so that these may be modified in preventive strategies.

Breast Milk Transmission of HIV-1: An Immunological Perspective

Mother-to-child transmission of HIV-1 is surprisingly inefficient: in the absence of treatment, 55–80% of HIV-1 exposed infants remain HIV-1 uninfected [22, 23]. This is striking when considering the large volumes (hundreds of liters) of HIV-1-contaminated milk ingested by infants breastfed by untreated HIV-1 uninfected mothers [24]. Why does such a large proportion of HIV-1-exposed uninfected children resist infection? Breast milk may confer protection from transmission through immunologic mechanisms. Oral exposure to HIV-1 has been reported to result in both systemic cellular and antibody immune responses detectable in ~13–28% of uninfected partners within HIV-1 discordant couple pairs [25, 26]. We have recently found that HIV-1 specific interferon-gamma (IFN- γ) secreting cells are present in the breast milk of the majority of HIV-1 infected mothers and confer a 70% reduction in the likelihood of early breast milk transmission, indicating that maternal HIV-1 specific immunity may protect against breast milk transmission (Lohman-Payne et al., manuscript submitted).

There are also some data that suggest that uninfected infants exposed to HIV in utero, at delivery or postnatally, can develop HIV-1-specific T-cell responses [27–30], which suggests that there has been sufficient exposure to replicating virus to prime such a response. Although the detection of an HIV-specific response in the absence of persistent infection

does not necessarily imply that T-cell immunity contributes to protection, resistance to postnatal HIV-1 transmission through breast milk was shown to correlate with the magnitude of the HIV-1-specific T-cell response in a prospective study in Nairobi, Kenya [24]. This finding provides some encouragement that enhancing the immune responses to HIV-1 through immunotherapeutic strategies in uninfected infants could confer protection against breast milk infection.

What are the consequences of later transmission for the infected infant? As previously mentioned, epidemiological studies suggest that the risk of disease progression to AIDS or death by the age of 12 months is halved in children infected later in infancy, compared to those who acquire infection before 1 month of age [6, 11–13]. This is consistent with the clinical experience of congenital infection with pathogens such as CMV, *Toxoplasma gondii*, and rubella, where disease is much more severe when infants are infected in utero.

During the first year of life, the infant immune system undergoes a transition from a predominantly regulated [14, 15], Th2 biased system [16–18], designed to maintain fetal life in the intrauterine environment, toward a more adult-like immune system, capable of mounting successful immune responses against a range of pathogens encountered in early life [19]. The precise timings of these changes in human infancy have not been well defined and much of the data come from studies comparing the phenotype and function of immune cells in cord blood with those in adult samples. Cord blood T cells have lower basal expression of CD3 and adhesion molecules, defects in cytokine production and CD8+ T-cell activity [31], while monocytes and dendritic cells (DC) express lower levels of costimulatory molecules, have altered differentiation pathways and reduced cytokine/chemokine production [32, 33]. Nevertheless, neonates generally develop immune responses to vaccines given at birth, including cell-mediated responses to several acute viral infections [34]. Bacilli Calmette-Guerin (BCG) vaccination, which is widely given at birth throughout the developing world as part of the Extended Program of Immunization (EPI), elicits a Th1-type response in newborns of similar magnitude to that achieved later in life [35]. Moreover, BCG itself can act as an adjuvant for other vaccines [36]. Pre- and postnatal exposure to environmental microbial products that activate innate immunity might accelerate this maturation, diminishing Th2 and/or enhancing Th1 cell polarization, and this could be incorporated into adjuvant design. Both natural and inducible CD4+CD25+ Treg numbers are increased in infancy and are thought to be required for maintaining peripheral T-cell tolerance through inhibition of Th1 cell immunity [37–39]. It is intriguing to note that the cord blood of HIV-1 exposed uninfected infants contains large populations of Treg cells and depletion of these cells ex vivo leads to an increased frequency of detection of IFN- γ secreting HIV-1 specific CD8+ T cells [38], consistent with Treg downregulation of antigen-specific cellular responses in cord blood. Exposure to foreign antigens, both allo-antigens [15] and antigens from *Plasmodium falciparum* present in placental malaria [40], promotes the development of regulatory T-cell populations at birth: this may mean that the infant exposed to HIV-1 and other pathogens in utero is less able to mount an efficient response to infections encountered in early life. Alternatively, perhaps depending on the timing of exposure, prenatal exposure might also promote Th1 responses in a similar manner to BCG given at birth [35].

The B-cell compartment is also affected in newborns, with responses to vaccines characterized by lower antibody levels, restricted diversity of antibody repertoire, and lower levels of IgG2 isotype as compared with responses induced in adults [41]. The implications of these deficiencies in pediatric vaccine development may include a requirement for enhanced stimulation of antigen-presenting cells with increased avidity of CD3/T-cell receptor (TCR) and co-stimulation molecule interactions to ensure immune activation.

There is a dearth of studies looking at the immune response and clinical course of infants infected through breast milk compared to those with early infection. We have shown that infants infected after the first month of life develop HIV-1-specific CD8⁺ T-cell IFN- γ responses more rapidly than infants infected early in life and the emergence of the response coincides with a decline in VL [42]; however, the overall magnitude of the response did not differ between early and late infection groups. Viral and host factors associated with the improved clinical outcome of breast milk infected infants have not been defined.

Co-infections and Breast Milk HIV-1 Transmission

HIV-1 infected infants in sub-Saharan Africa have poorer control over VLs and a higher risk of mortality compared to infants in Europe and the USA (~20–40 vs. ~10–20% mortality, respectively) [5, 7, 43–45]. Co-infections during infancy are thought to explain a large proportion of these cohort differences. In addition to causing significant morbidity as opportunistic infections, co-infections have the potential to increase both the risk of vertical HIV-1 transmission and infant HIV-1 progression. CMV [46–48], herpes simplex virus type-2 (HSV-2) [49, 50], and malaria [51, 52] may accelerate HIV-1 disease progression by causing transient or sustained elevations in HIV-1 VL. Since breast milk HIV-1 load is closely correlated with systemic VL, any infection that raises maternal HIV-1 VL could potentially affect the risk of breast milk transmission. Despite these associations, there have been few prospective studies examining the role co-infections play in breast milk transmission and downstream infant disease progression. CMV, which causes persistent asymptomatic infection in a large proportion of healthy adults, has been the most intensively studied co-infection in HIV-1. Longitudinal cohort studies conducted in West and East Africa suggest more than 80% of children may acquire CMV during their first year of life, regardless of HIV-1 status [20, 21]. Maternal CMV reactivation was associated with a fourfold increased risk of mortality in HIV-infected infants independent of maternal immunosuppression [53]. Among HIV-1 infected infants, co-infection with CMV is associated with higher peak CMV VLs, prolonged detection of CMV in the plasma [21], and a greater than twofold increased risk of mortality [47]. In co-infected women, there is a strong correlation between HIV-1 and CMV loads in both the blood and breast milk [21, 53, 54]. It is yet unclear whether a cause-and-effect relationship links these two pathogens; CMV could potentially act as a co-factor to increase HIV-1 replication directly (reviewed in ref. [55]) or indirectly by increasing cellular activation of breast milk lymphocytes. Further studies are needed to understand better the long-term effects of other highly prevalent persistent viral infections acquired during infancy, particularly those that are associated with the development of malignancies after years of HIV-1 infection, such as Epstein–Barr virus (EBV), human herpesvirus-8 (HHV-8), and hepatitis B virus.

Vaccine Strategies to Prevent Breast Milk Transmission of HIV-1

HIV-1 Vaccine Studies in Adults

Three large-scale human efficacy studies have been completed to date and, although not designed to evaluate mother-to-child transmission, they provide an immunologic foundation for future studies to include breast milk transmission. The first trial, from Vaxgen, employed an envelope subunit protein construct with the aim of inducing a protective antibody response, but a large phase III clinical trial showed no evidence of efficacy [56]. A move toward testing T-cell-inducing vaccines led to the phase 2b STEP trial (tested in high-risk volunteers) of a replication-defective adenovirus type 5 (Ad5)-based recombinant vaccine produced by Merck that appeared to be the most immunogenic available construct in human studies. This study was terminated prematurely, as study subjects were neither protected from HIV-1 infection nor experienced reduced VL when infection occurred [57, 58]. However, progressive improvements in the constructs used to elicit T-cell responses have

provided some encouragement in the macaque model. For example, a heterologous rAd26 prime/rAd5 boost vaccine regimen expressing SIV Gag elicited broader and stronger cellular immune responses than had been seen with the homologous rAd5 regimen, which led to significantly lower set-point VLs as well as decreased AIDS-related mortality compared with control animals following challenge with a pathogenic simian immunodeficiency virus strain (SIVmac251) [59]. More recently, a novel approach using rhesus CMV constructs induced effector-memory T cells at mucosal sites that correlated with protection from infection in 4 of 12 macaques repeatedly challenged with the highly pathogenic SIVmac239 strain [60]. Further encouragement to the HIV vaccine field came from the results of the rv144 phase III efficacy trial of a combination of canary pox priming and HIV envelope protein boost tested in a low-risk population in Thailand [61]. Although the incidence of new infections was low in this study, meaning that significant efficacy was only seen in one of the three analyses (the modified intention-to-treat analysis), the vaccine appeared to confer 31% protection against infection, the first indication of efficacy in any human study. The mechanisms of protection are not yet known, but are unlikely to include either neutralizing antibodies or CTL, which are rarely induced by this vaccine approach.

Mucosal Vaccines

Mucosal immunization has been demonstrated to induce mucosal responses, and as the first sites of SIV and recombinant simian-human immunodeficiency virus (SHIV) infection in neonatal rhesus macaques are mucosal surfaces [62], similar tissues are likely to be exposed in MTCT of HIV-1 [63]. Both intrapartum and breast milk HIV-1 transmission are likely to be acquired through the oral route, and therefore HIV-1 specific immunity present at this site is most relevant to preventing breast milk transmission. Many cellular and cytokine properties of the neonatal oral and intestinal tissues are known to differ from the adult: these could either represent protective or susceptibility factors and include the levels of $\gamma\delta$ T cells, NK cells, macrophages, DC, IgG, IgA and secretory IgA levels, and levels of cytokines such as TNF- α and IFN- γ [64].

Mucosal vaccines developed for PMTCT of HIV-1 could exploit the common mucosa-associated lymphoid tissue to explore oral or nasal delivery. Oral delivery, although attractive for neonates, has challenges such as induction of tolerance, limitations in the choice of safe effective adjuvants, requirement for large doses of antigen, and the need for antigen stability in the gut. This last concern has been addressed through the development of lipid vesicles or polymeric nanoparticles that act as immunostimulants while preserving immunogens from intestinal enzymes. So far only a limited number of orally administered vaccines against HIV have been tested in humans. An attenuated canarypox vector (vCP 205) and Salmonella vaccine vector (CKS257) vaccine platforms were both reportedly well tolerated in humans but with less than expected mucosal immunogenicity [65, 66]. As opposed to oral vaccine delivery, the main advantage of nasally administered vaccine is the requirement for smaller doses of antigen. Several vaccine formulations have been tested in adults including peptides, DNA, and live bacterial and viral vectors [64]. Mucosal vaccine development against HIV-1 appears to require the deployment of stronger adjuvants, the use of which may be associated with safety concerns in young children.

In summary, a pediatric vaccine to prevent HIV infection would rapidly induce both antibody and cellular responses detectable at mucosal surfaces that would remain at effective levels during the duration of breastfeeding. A greater understanding of the most important inductive and effector sites in the newborn would guide research to address such questions as to whether systemic immunization can induce sufficient mucosal immunity, if oral or nasal vaccines can be effective, and if appropriate adjuvants can be developed for an effective vaccine development strategy for use in HIV-1-exposed infants.

SIV and SHIV Models for Vaccine Strategies for PMTCT of HIV Infection—Active and Passive Immunization

Animal models, especially the rhesus macaque/SIV–SHIV model, have been used to address areas of uncertainty in pediatric vaccine design, including the most appropriate vaccine, the timing of immunizations, and the duration of vaccine elicited responses. The SIV models for vertical transmission of HIV-1 and for neonatal vaccine development were validated in the 1990s and many important proof-of-concepts have been demonstrated including protection of newborn macaques from oral SIV/SHIV challenge through maternal vaccination and through administration of passive hyper-immune serum (SIVIG/HIVIG) or neutralizing monoclonal antibodies at birth [67–71]. The mechanism of protection from infection could include antibody binding alone, classic viral neutralization, or NK cell-mediated antibody dependent cellular cytotoxicity. But will passive immunization to prevent MTCT of HIV-1 work? There are many challenges to this approach, particularly the composition of the antibody cocktail, which would need to be effective against more than one HIV clade and circulating recombinant forms, and the scale-up delivery logistics are daunting. Alternatively, boosting of maternal antibody levels by vaccination represents an important strategy to augment passive immunity during the infant's early months of life, until the infant can be actively immunized. However, the role of maternal antibody in reducing the effectiveness of immune responses in the newborn, especially T-cell responses, is a concern. This may be addressed in the results of a phase III randomized clinical trial that was completed in Uganda in 2006 (<http://www.mujhu.org/hiviglob2.html>). The trial was designed to compare the standard single-dose mother/infant nevirapine regimen for PMTCT with the addition of HIV immune globulin (HIVIGLOB) or a second arm of extended infant NVP dosing compared with the standard single-dose NVP regimen alone without HIVIGLOB and enrolled 722 mothers with 204 in the HIVIGLOB arm. The data from the HIVIGLOB arm were pooled with other data from Johns Hopkins trials in Ethiopia and India, and results are not yet available.

Live attenuated vaccines have the advantage of prolonged antigen delivery and stimulation of both innate and adaptive immunity. Modified vaccinia virus Ankara (MVA) and canarypox viral vectors (ALVAC) have been tested in the rhesus macaque/SIV–SHIV models in advance of human trials. MVA expressing SIV gag, pol, and env or expressing SIVmac1A11 was used to immunize infant macaques at birth and at 3 weeks of age [72]. The infants were challenged at 4 weeks of age with uncloned SIVmac251, using a multiple low-dose challenge model to deliver virus three times a day over 5 days to mimic breast milk exposure. Although the immunization regimen was unable to prevent infection, immunized infants mounted antibody responses and had improved clinical outcome compared to controls. An attenuated recombinant canary pox vector expressing SIV gag, pol, and env (ALVAC-SIV) was used to immunize infant macaques at birth, 2 and 3 weeks of age, followed by repeated oral low-dose challenge [73]. In this experiment, significantly fewer immunized infants were infected (6/16) compared to the unimmunized controls (14/16), demonstrating that neonatal immunization provided partial protection from infection. Finally, a topical DNA vaccine containing HIV-1 gag and env (DermaVir) represents an immunization strategy that targets lymph node DC. Rhesus macaques immunized with DermaVir generated HIV-1 specific Th1 and Th2 cytokines and antigen-specific memory T cells, while serum antibody levels were boosted after p27/gp140 protein boosting. Following mucosal challenge, none of the animals were protected from infection; however 4/5 immunized monkeys had reduced peak and set-point viremia [74].

Pediatric Clinical Trials of HIV Vaccines

The most recently reported clinical trials of live attenuated vaccine constructs tested as PACTG 326 part 1 and part 2 include the ALVAC constructs vCP205 and ALVAC-HIV

vCP1452. Immunization of neonates was well tolerated and induced lymphoproliferative and/or cytotoxic T-cell responses in vaccines: ~40% of infants immunized with ALVAC vCP205 and 75% of infants immunized with ALVAC vCP1452 [75]. An MVA-vectored vaccine is also currently under evaluation in an open randomized phase I/II study evaluating safety and immunogenicity of a candidate HIV-1 vaccine, MVA. HIVA, administered to healthy infants born to HIV-1 infected mothers in Nairobi, Kenya. This active study aims to enroll 72 HIV-1 uninfected infants by the end of 2010, with infants in follow-up for 18 months. Infants will be randomized to receive MVA.HIVA or to remain unvaccinated. The design of the study will allow for multiple secondary aims for comparison of immunogenicity to other national immunization program vaccines in the MVA.HIVA vaccinated or unvaccinated infants (Tomas Hanke, personal communication).

Concerns Regarding Infant Vaccine Development

Many key questions regarding the development of a successful adult HIV-1 vaccine are equally valid for a neonatal immune-based intervention for PMTCT. However, pediatric vaccine development also faces a series of unique concerns. These include, but are not limited to, regulatory/ethical issues applicable to vulnerable populations, physiologic constraints of blood volumes that may limit the degree of safety and immunogenicity testing, existing immunization schedules and potential vaccine interference, and simultaneous exposure to both vaccine and pathogen in the presence of maternal antibodies and the developing neonatal immune system.

Currently, infants are immunized worldwide against an array of infections delivered over the first 2 years of life, including BCG, polio, hepatitis B, diphtheria, pertussis, tetanus, pneumococcus, and *Hemophilus influenza* b (Hib). Investigations into the sequence of exposure to murine viruses have demonstrated that the magnitude and specificity of the immune response elicited by the most recent infection are modified by the host's history of previous infections [76]. In newborn mice and humans, *Mycobacterium bovis* BCG immunization induces a potent immune response and this response has been shown to alter immunity to unrelated vaccines [77]. Also, individual components of multivalent vaccines may induce responses that differ when given individually or in combination (reviewed in ref. [78]). Therefore, the timing of introduction of new vaccines into the existing Expanded Program of Immunizations has the potential to modify responses to both previous and subsequent vaccines.

Vaccine constructs and adjuvants may also react differently in infants, although thus far, recombinant HIV-1 gp120 delivered either in alum or MF59, and recombinant canarypox vectored vaccines for HIV have proven safe in pediatric populations (PACTG 230, 326, and HPTN 027), while the testing of adjuvant CRM₁₉₇ for use in multivalent pediatric vaccines has shown to improve immunogenicity of certain vaccines [79]. Live attenuated SIV vaccines tested in neonatal macaques have also proved safe, with the rare exception of a multiply deleted SIVmac239 that when administered to neonates, showed unexpected pathogenicity not initially observed in adults. Pathogenesis in adults was later documented in ~25% of vaccinated adults after a median of ~3 years of infection [80].

These concerns can be addressed readily through investment in the use of animal models, of continued testing in adults, and of basic research into underlying mechanisms of neonatal immune regulation, maternal antibody interference, and vaccine interference.

Conclusions and Future Directions

Although the HIV vaccine field still has some way to go before an effective vaccine to prevent infection becomes available, the special issues of mother-to-child transmission and

infant immunization deserve further study. We have highlighted the approaches tested to date, as well as highlighting the potentially modifiable infectious co-factors that can facilitate transmission of HIV-1 from mother to child in the developing world and commenting on some of the issues that will need to be considered in the development of a pediatric HIV vaccine. Even if deployment of strategies to prevent mother-to-child transmission of HIV-1 becomes universal, a scenario that currently seems some distance away in resource-poor settings, it is very likely that a prophylactic HIV vaccine will ultimately need to be given as part of the EPI. Therefore, a better understanding of infant immune responses to candidate vaccine antigens and adjuvants is an important area for future investigation.

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