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Immune-Based Approaches to the Prevention of Mother-to-child-Transmission of HIV-1: Active and Passive Immunization

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Synopsis

Despite more than two decades of research, an effective vaccine that can prevent HIV-1 infection in populations exposed to the virus remains elusive. In the pursuit of an HIV-1 vaccine, does prevention of exposure to maternal HIV-1 *in utero*, at birth or in early life through breast-milk require special consideration? In this article we will review what is known about the immune mechanisms of susceptibility and resistance to mother-to-child transmission (MTCT) of HIV-1 and will summarise studies that have used passive or active immunisation strategies to interrupt - MTCT of HIV-1. We will also describe potentially modifiable infectious co-factors that may enhance transmission and/or disease progression (especially in the developing world). Ultimately an effective prophylactic vaccine against HIV-1 infection will need to be deployed as part of the Extended Programme of Immunisation (EPI) recommended by the World Health Organisation (WHO) for use in developing countries, so it is important to understand how the infant immune system responds to HIV-1 antigens, both in natural infection and presented by candidate vaccines.

Keywords

HIV-1; vaccine; infant; co-factor

Introduction

Mother to child transmission (MTCT) of HIV-1 infection remains an important cause of new HIV-1 infections worldwide, despite the increasing implementation of prevention strategies using anti-retroviral therapy (ART) across the developing world. In the year ending December 2008 an estimated 430, 000 children under the age of 15 years were newly infected with HIV-1 (UNAIDS Epidemic Update 2009), the great majority of whom acquired the infection from their mothers in low and middle-income countries. Despite clear evidence of significant progress, challenges remain for poor countries in providing comprehensive screening programmes for pregnant women and implementing the full range of prevention services for those identified as HIV-1-infected.

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For those children who acquire perinatal infection, disease progression appears to be unusually rapid compared to that of adults, particularly in developing countries, where mortality rates as high as 20–52% have been reported in the first two years of life^{1 2}. 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^{3 4}, despite the early appearance of HIV-1-specific T-cell responses⁵. It is likely that co-infections acquired in 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 life⁶ and is associated with high viral load in HIV-1 co-infected infants⁷. Co-infections, particularly malaria in pregnancy, may also contribute to the likelihood of transmission: therefore it is important to investigate and define the potential role of co-infections in MTCT so that these may be modified in preventive strategies.

Mother-to-child transmission of HIV-1: an immunological perspective

Vertical transmission of HIV-1 is not an inevitable consequence of exposure: in the absence of treatment 55–80% of HIV-1 exposed infants remain HIV-1 uninfected. This is striking when the large volumes of maternal blood containing HIV-1-infected cells circulating through the placenta throughout gestation are considered: in fact, HIV-1 can be detected with relative ease in the placentas of both transmitting and non-transmitting mothers⁸. Moreover, infants breastfed by untreated HIV-1 uninfected mothers will ingest hundreds of litres of HIV-1-contaminated milk but over 80% of them remain uninfected⁹. It is generally assumed that there are no serious long-term consequences of exposure to HIV-1 *in utero* or early life in children who escape infection. Nevertheless, uninfected babies born to HIV-1-infected mothers suffer from increased morbidity and mortality^{10, 11}. It has been difficult to distinguish the direct impact of HIV-1 exposure from the consequences of being born to a sick mother¹¹, but there are some suggestions that exposed uninfected babies may present with severe infections indicative of clinical immunodeficiency¹². With the widespread roll-out of PTMCT programmes in resource-poor settings, this represents an important area for future investigation.

When transmission does occur, *in utero* (transplacental) transmission accounts for an estimated 5–10% of infections, *peripartum* transmission (occurring during labor, delivery and early breastfeeding) contributes 10–15% of infections, whilst breastfeeding transmission accounts for 5–20% of MTCT (reviewed in¹³). In the developed world, the successful deployment of intervention strategies has reduced the overall transmission rate to substantially less than 5%. 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 widespread. In addition, the potential toxicities of and the development of drug resistance to anti-retroviral therapy (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.

The major risk factors for MTCT include factors associated with maternal viral load (duration and composition of ARV regimen and viral load at delivery) and infant exposure to infected fluids (duration of rupture of membranes, type of delivery, presence of other maternal sexually-transmitted infections (STI), and breastfeeding duration)^{14 15}. Breastfeeding considered alone is associated with a near doubling of the risk of MTCT of HIV¹⁶. Avoidance of breastfeeding and reduction of other maternal risk factors for MTCT

of HIV have led to remarkable declines in MTCT in settings with adequate resources. However, replacement feeding is only advisable when the “AFASS” criteria are met: namely, that formula feeding be culturally Acceptable, Feasible for the mother, Affordable, Sustainable and Safe - conditions rarely met in most areas of the world. Thus an ideal pediatric vaccine for 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.

Why does such a large proportion of HIV-1-exposed uninfected children resist infection? *In utero* transmission is thought to occur most commonly in the last trimester; it has been suggested that the placenta blocks the transmission of free HIV-1 virions¹⁷ (by mechanisms as yet unidentified) and infection depends either on breaks in the placental barrier or the transcytosis of infected cells¹⁸. There are some data that suggest that uninfected infants exposed to HIV *in utero*, at delivery or post-natally can develop HIV-1-specific T-cell responses^{19, 20 21 22}, 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 post-natal HIV-1 transmission was shown to correlate with the magnitude of the HIV-1-specific T-cell response in a prospective study in Nairobi, Kenya⁹. This finding provides some encouragement that enhancing the immune responses to HIV-1 through immunotherapeutic strategies in uninfected infants could confer protection against later infection.

Vaccine strategies to prevent mother-to-child-transmission of HIV-1

HIV-1 vaccine studies in adults

Three large-scale human efficacy studies have been completed to date. In the first of these, Vaxgen employed an envelope sub-unit protein construct with the aim of inducing a protective antibody response: however, a large phase III clinical trial showed no evidence of efficacy²³. The field moved towards testing T-cell-inducing vaccines and considerable hope was placed on 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 vaccine was tested in the phase 2b STEP trial (tested in high risk volunteers), which was terminated prematurely as study subjects were neither protected from HIV-1 infection nor experienced reduced viral load when infection occurred^{24 25}. This failure led to a great deal of introspection in the HIV vaccine field, as it called into question whether or not a vaccine designed to elicit a cytotoxic T-lymphocyte (CTL) response could indeed protect against HIV-1 infection, as well as casting doubt on the non-human primate models of HIV infection that did not accurately predict the outcome of the human trial. 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 setpoint viral loads as well as decreased AIDS-related mortality compared with control animals following challenge with a pathogenic SIV strain, SIVmac251²⁶. More recently, a novel approach using rhesus CMV constructs induced effector memory T-cells at mucosal sites that correlated with protection from infection in four of twelve macaques repeatedly challenged with the highly pathogenic SIVmac239 strain²⁷. Further encouragement to the HIV vaccine field has come 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 Thailand²⁸. Although the incidence of new infections was low in this study, meaning that significant efficacy was only seen in one of 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 cytotoxic T lymphocytes (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 simian immunodeficiency virus (SIV) and recombinant simian-human immunodeficiency virus (SHIV) infection in neonatal rhesus macaques are mucosal surfaces, similar tissues are likely to be exposed in MTCT of HIV-1^{29 30}. HIV-1 specific immunity present at these sites may be most relevant to preventing 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, dendritic cells, IgG, IgA and secretory IgA (SIgA) levels, and levels of cytokines such as TNF- α and IFN- γ ³¹.

Mucosal vaccines developed for PMTCT of HIV-1 could exploit the common mucosa-associated lymphoid tissue (MALT) 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^{32 33}. 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 vectors³¹. 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.

Neonatal Immunity

In conjunction with the challenges of designing an effective vaccine to prevent transmission across mucosal surfaces, pediatric vaccinologists have the additional difficulty of working with a neonatal immune system in a state of extreme change as the newborn adjusts to life outside its intrauterine environment^{34, 35}. Although neonates generally develop immune responses upon immunization as well as cell-mediated responses to several acute viral infections, several deficiencies in the immunoregulatory pathways of T cells and antigen presenting cells have been documented in human cord blood with important implications for the development of immune-based therapies to prevent MTCT of HIV-1. Cord blood T cells have lower basal expression of CD3 and adhesion molecules, defects in cytokine production and CD8+ T-cell activity³⁶, while monocytes and dendritic cells express lower levels of costimulatory molecules, have altered differentiation pathways and reduced cytokine/

chemokine production^{37, 38}. Neonatal T cells have a bias against Th1 cell polarizing cytokines that leaves the newborn susceptible to microbial infection and may contribute to the impairment of neonatal immune responses^{39, 40}. An exception is bacilli Calmette-Guerin (BCG) vaccination: newborns are capable of mounting a Th1-type response of similar magnitude to that given later in life⁴¹. Moreover, BCG itself can act as an adjuvant for other vaccines⁴². Pre- and post-natal exposure to environmental microbial products that activate innate immunity might accelerate this maturation, diminishing the Th2 and/or enhancing Th1 cell polarization, and this could be incorporated into adjuvant design. Both natural and inducible CD4+CD25+ regulatory T-cell (Treg) numbers are increased in infancy and are speculated to be required for maintaining peripheral T cell tolerance through inhibition of Th1 cell immunity^{43–45}. It has been noted that exposure to foreign antigens, both allo-antigens⁴⁶ and antigens from *P. falciparum* present in placental malaria⁴⁷, promotes the development of regulatory T-cell populations at birth.

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 adults⁴⁸. 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/TCR and co-stimulation molecule interactions to ensure immune activation.

Vaccine strategies for PMTCT of HIV infection

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 hyperimmune serum (SIVIG/HIVIG) or neutralizing monoclonal antibodies at birth^{49–53}. The mechanism of protection from infection could include antibody binding alone, classic viral neutralization, or NK cell-mediated antibody dependent cellular cytotoxicity (ADCC). 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. A phase III randomized clinical trial 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 was completed in Uganda in 2006 (<http://www.mujhu.org/hiviglob2.html>). The study 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 age⁵⁴. 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 five days to mimic breast milk exposure. The immunization regimen was unable to prevent infection, however the 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 challenge⁵⁵. 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. Lastly, a topical DNA vaccine containing HIV-1 gag and env (DermaVir) represents an immunization strategy that targets lymph node dendritic cells (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 viremia⁵⁶.

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 vaccinees: ~40% of infants immunized with ALVAC vCP205 and 75% of infants immunized with ALVAC vCP1452^{57, 58}, McFarland, 2006 #1314. 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 is aiming to enrol 72 HIV-1 uninfected infants with 36 breastfeeding infants and 36 formula feeding infants by the end of 2010, with infants in follow-up for 18 months. Within each feeding group, 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 in the different feeding groups and the response to other national immunization program vaccines in the MVA.HIVA vaccinated or unvaccinated infants (Tomas Hanke, personal communication).

Co-infections have the potential to alter vertical HIV-1 transmission and vaccine efficacy

HSV-2

Genital co-infections play an important role in both vertical and horizontal HIV-1 transmission. A study of HIV-1 discordant couples in Uganda demonstrated that the presence of genital ulcers increased the probability of HIV-1 transmission per coital act 2.6-fold⁵⁹. Herpes simplex type-2 infection (HSV-2) is the primary cause of genital ulcer disease (GUD) in resource-poor settings. HSV-2 seroprevalence is high among HIV-1 infected populations globally, and is likely to have a significant population impact on HIV-1 transmission in endemic regions^{60, 61}; a recent meta-analysis of studies estimated HSV-2 infection confers a 3-fold increased risk of sexual HIV-1 acquisition⁶². Maternal HSV-2 seropositivity has also been associated with increased rates of peripartum HIV-1 transmission in some studies^{63, 64}, but not others^{65, 66}. Recurrent subclinical reactivation of HSV-2, characterized by HSV-2 shedding in the absence of ulcers, is common in HSV-2 infection⁶⁷. The presence of genital ulcers, or genital shedding of HSV-2 proximal to delivery has been associated with an increased risk of vertical transmission of HIV^{14, 66}. Several mechanisms may explain this association. First, ulcers may provide a physical

breach in the mucosa that increases infant exposure to HIV-1 virions or infected cells beneath the epithelium. HSV-2 ulcers contain high levels of HIV-1 RNA⁶⁸, and genital HIV-1 replication is a strong risk factor for transmission¹⁴. The concentration of HIV-1 in ulcers may result from the homing of activated CD4 T cells^{69, 70}, which are potential targets for HIV-1 infection. Even in the absence of ulcers, HSV-2 shedding is associated with increased HIV-1 shedding in the genital tract^{71, 72}. HSV-2 may also affect vertical transmission by more generalized effects on maternal systemic viral load; plasma HIV-1 viral load is increased during sub-clinical HSV-2 reactivation and declines with antiviral suppression of HSV-2 replication^{67, 73}. A randomized controlled trial (RCT) of valacyclovir versus placebo for 12 weeks demonstrated a 0.53 log₁₀ copies/ml decrease in HIV-1 plasma viral load, and a 0.29 log₁₀ decrease in HIV-1 RNA in the genital tract of HIV-1/HSV-2 co-infected women⁷⁴. The effect of HSV-2 co-infection on systemic HIV-1 replication may result from increased immune activation or direct interactions between viruses (reviewed in⁷⁵). Additionally, acyclovir has been shown to have an inhibitory effect on HIV-1 replication *in vitro*^{76, 77}. Together, these data suggested that HSV-2 suppression may be an effective tool to prevent HIV-1 transmission. However, results from clinical trials of sexual transmission have been disappointing; HSV-2 suppression with acyclovir did not prevent either HIV-1 transmission or acquisition^{78–80}. Importantly, acyclovir does not appear to affect egress of CD4 cells from lesion sites following healing; suggesting HIV-1 target cells remain concentrated in the genital mucosa⁸¹, and this may explain in part the failure of acyclovir to prevent HIV-1 transmission despite reductions in viral load. However, failure in the sexual transmission model may not exclude HSV-2 suppression as a strategy to reduce vertical transmission. A pharmacokinetic study showed acyclovir is actively transported to the amniotic fluid and breast milk⁸²; if HSV-2 suppression reduces HIV-1 viral load in these compartments, there is the potential for this approach to affect *in utero* or breast milk HIV-1 transmission. A randomized trial is currently underway in Kenya to address this question (NCT00530777).

Malaria

There is significant overlap between the malaria and HIV-1 epidemics (reviewed in⁸³). Malaria co-infection is associated with a transient, but significant increase (~0.25 log₁₀) in plasma HIV-1 RNA viral load^{84, 85}, but its role in transmission is unclear⁸⁶. Ayoub and colleagues reported higher rates of MTCT in Cameroon during the rainy season, and speculated that malaria may affect vertical HIV-1 transmission⁸⁷. A few studies have demonstrated a trend for increased vertical transmission in women with blood parasitemia during pregnancy, however this relationship disappears after adjusting for HIV-1 viral load^{88, 89} and other studies have shown no association^{90, 91}. Placental malaria is more commonly detected in HIV-1 infected women^{88, 89, 92, 93} and Malawian women with placental malaria were found to have a ~2.5 fold higher plasma HIV-1 RNA viral load⁹⁴. Some studies have demonstrated an association between placental malaria and increased rates of vertical transmission independent of HIV-1 viral load^{89, 92}; however other studies have shown no association⁹³. A study conducted in Western Kenya found HIV-1 transmission risk to be increased when placenta parasitemia was high (>10,000 parasites/ml) but reduced in cases of low parasitemia (<10,000 parasites/ml) compared to malaria-negative controls⁹⁰. The potential protective effect of placental malaria was confirmed in a second study conducted in Mozambique⁹¹. These conflicting findings may be attributable to different methods of detecting placental parasitemia, different regional epidemics (seasonal, holoendemic), unrecorded self-treatment of malaria, and including breast milk HIV-1 transmissions in the ascertainment of effect. Malaria may alter the risk of HIV-1 transmission *in utero* by causing inflammation in the placenta⁹⁵, increasing CC-chemokine production^{96, 97}, shifting cytokine production from Th2 to Th1-type responses⁹⁸ and increasing CCR5 expression on Hofbauer cells⁹⁹. Together these data suggest malaria has

the potential to alter the risk of vertical HIV-1 transmission, but further studies are needed to understand the interactions between immune responses to these two pathogens.

Independent of HIV-1 infection, malaria during pregnancy is associated with obstetrical problems and adverse birth outcomes, making malaria prevention, diagnosis and treatment an important component of antenatal care for all women in malaria endemic areas.

Bacterial Vaginosis (BV) and other vaginal infections

In addition to HSV-2 as discussed above, other sexually transmitted infections (STI) are also associated with an increased risk of horizontal HIV-1 transmission and acquisition (reviewed in¹⁰⁰). Ulcerative and non-ulcerative STIs, as well as vaginitis and cervicitis of unknown etiologies are associated with increased HIV-1 shedding in the female genital tract^{100–105}, and treatment of genital infections is associated with declines in HIV-1 shedding^{104, 106}. Genital infections are likely to increase HIV-1 transmission via recruitment of activated HIV-1 infected cells to sites of inflammation. Disruption of healthy vaginal flora due to bacterial vaginosis (BV)^{107–109}, or vaginal washing has also been associated with an increased risk of HIV-1 acquisition¹¹⁰. A recent prospective study conducted in Kenya reported a 3-fold increased risk of *in utero* HIV-1 transmission from women diagnosed with BV at 32 weeks gestation compared to women with normal vaginal flora¹¹¹. However, a multi-site RCT of metronidazole versus placebo in three African countries found no difference in rates of vertical transmission compared to placebo, despite a 16% reduction in BV¹¹². Further studies are needed to determine whether restoration of normal vaginal flora can reduce vertical HIV-1 transmission.

Helminth infection is associated with reduced vaccine responses

The greatest need for an HIV-1 vaccine is in Africa and Asia where helminths may infect 25–76% of the healthy adults and children^{113–116}. The first indication that helminths may compromise vaccine responses was the observation of reduced Bacillus Calmette-Guérin (BCG) efficacy in resource-poor countries^{117, 118}. Though helminths comprise a diverse group of organisms with heterogeneous routes and targets of infection, the most commonly encountered helminths (digenean flukes, cestodes, and nematodes) have similar effects on the host immune system; co-infection suppresses IFN- γ production and induces a Th2-type CD4 response in their hosts (reviewed in¹¹⁹). Following BCG vaccination, newborns whose mothers had schistosomiasis or bancroftian filariasis infection had weaker IFN- γ responses to purified protein derivative (PPD) and increased production of IL-5¹²⁰. This trend was also found at 14 months of age; PPD-specific CD4 responses in children exposed *in utero* to helminths had highly Th2-skewed responses, indicating that helminth exposure during priming of CD4 responses *in utero* affected the profile of memory cells later in life¹²⁰. De-worming prior to revaccination with BCG improves PPD-specific IFN- γ production and T cell proliferation in adults¹²¹. Similarly, IFN- γ and IL-2 responses to cholera vaccination were improved by de-worming of patients with *Ascaris lumbricoides* infection¹²².

Helminths may also affect HIV-1 disease progression and acquisition. Suppression of Th1-type responses may directly impair control over viral replication, and helminth-induced systemic immune activation^{123–125} may accelerate HIV-1 disease progression. The effect of helminths on the control over HIV-1 replication has been studied elegantly *in vivo* using primate models; following infection with SHIV clade C, *Schistosoma mansoni*-infected rhesus macaques had higher SHIV viral load compared with non-parasitized controls, and maintained higher IL-4 and IL-10 production¹²⁶. Introduction of *S. mansoni* into chronically SHIV-infected animals who were aviremic resulted in reactivation of SHIV replication and decline in CD4+CD29+ cells¹²⁷. These experiments also demonstrated that helminth infection may affect HIV-1 acquisition; *S. mansoni* co-infected animals became infected at

lower SHIV doses compared with non-parasitized control animals, and had higher peak viral loads and proviral loads in central memory CD4 T cells¹²⁸. One study in humans has found an increased risk of vertical HIV-1 transmission in the setting of maternal helminth infection⁸⁸. To date, two RCTs have evaluated treatment of helminthic infections as a strategy to reduce HIV-1 disease progression. Treatment with albendazole improved CD4 counts in HIV-1 infected individuals with *Ascaris lumbricoides* infection, but no effect was found in subjects infected with hookworm or *Trichuris trichiura*¹²⁹. Similarly, Kallestrup and colleagues found improved control over viral replication and increased CD4 counts in subjects treated for Schistosomiasis infection¹³⁰. In sub-Saharan Africa, periodic de-worming is now a recommended component of comprehensive HIV-1 care of women and children.

Taking into consideration the local helminth burden of target populations will enable the more strategic design and deployment of an HIV-1 vaccine. De-worming of vaccinees prior to or during immunization may improve the priming of a Th1-type response. In mouse models, elimination of *Schistosoma mansoni* with praziquantel prior to immunization with a HIV-clade C DNA vaccine resulted in much improved vaccine-specific IFN- γ responses¹³¹. Additionally, infection with vaccinia virus expressing HIV-1 gp¹⁶⁰ resulted in stronger CTL responses and more rapid viral clearance in nonparasitized mice compared to *S. mansoni* infected mice¹³². Though de-worming may improve immune responses to vaccination, re-infection rates are high, and this would be expected to reduce the benefit of de-worming in vaccines requiring multiple doses or boosting. Continual re-infection with helminths may similarly impair the efficacy of a non-sterilizing therapeutic HIV-1 vaccine. An alternate approach is the design of vaccines targeted to overcome the Th2 bias induced by helminths, by the strategic use of adjuvants^{133, 134}, cytokines¹³⁵, or Toll-like receptors (TLR) signalling^{136, 137}.

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.

There is general agreement that pediatric clinical trials are necessary for implementation of novel medical strategies for prevention or treatment of childhood illnesses. In the US, several regulatory agencies have committed to ensure ethical conduct in research involving children and development of pharmaceutical formulations specific for children (www.fda.gov/cder/pediatric/). Although government programs and assurances provide a level of support to including infants in clinical trials of vaccines designed for PMTCT, in countries with historic imbalances in political and medical power, community-based opinion affects the support of local testing. Infants and children are subject to cultural traditions as well, for example within the family, who is empowered to grant consent? Who makes medical decisions? What is the relative value of family members? Clearly there is need to include diverse populations in testing vaccines, and resources invested in community sensitization and education during all stages of the trial may help ensure continued involvement in subsequent trials.

A logistical concern for phase I/II studies in infants is the frequency and volume of blood collection, not only the physiological limit of body size, but the psychological limit of the caregiver's tolerance to blood draws from healthy infants. Many assays and procedures have been optimized for minimal blood volumes, including Elispot assays for the detection of antigen-specific responses¹³⁸, multicolor flow cytometry¹³⁹ and detection of HIV-1 DNA in dried blood spots for monitoring infection status¹⁴⁰. Continued development of assays that require minimal volumes of blood will benefit pediatric clinical trials.

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 infections¹⁴¹. In newborn mice and humans *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) immunization induces a potent immune response and this response has been shown to alter immunity to unrelated vaccines¹⁴². Also, individual components of multivalent vaccines may induce responses that differ when given individually or in combination (reviewed in¹⁴³). 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.

Maternal antibodies have been clearly shown to interfere with the effectiveness of measles virus vaccination, although not affecting the immunogenicity of other vaccines administered in the presence of maternal antibodies¹⁴⁴. Passively transferred maternal antibodies may form antigen-antibody complexes with vaccine antigens thereby limiting vaccine exposure prior to the development of an infant specific immune response, although there is evidence for many vaccines of B-cell priming with development of protective antibody titers after boosting¹⁴⁵. Antigen-antibody complexes are efficiently taken up via Fc-mediated mechanism by professional antigen presenting cells, which may facilitate the development of antigen specific T- and B-cell responses. However in the setting of PMTCT of HIV, potential exists for co-circulation not only of maternal antibody and vaccine constructs aimed at priming immunity in the infant, but also maternal antibody and cell-free HIV-1 acquired from breastmilk. Thus, Fc-mediated uptake of antigen-antibody complexes by APCs could potentially lead to enhancement of HIV infection (reviewed in¹⁴⁶).

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), whilst the testing of adjuvant CRM₁₉₇ for use in multivalent pediatric vaccines has shown improve immunogenicity of certain vaccines¹⁴⁷. 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 infection¹⁴⁸.

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 immunisation 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 vertical 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 Extended Programme of Immunisation (EPI). Therefore a better understanding of infant immune responses to candidate vaccine antigens and adjuvants is an important area for future investigation.

References

1. Obimbo EM, Mbori-Ngacha DA, Ochieng JO, et al. Predictors of early mortality in a cohort of human immunodeficiency virus type 1-infected african children. *Pediatr Infect Dis J* Jun;2004 23(6):536–543. [PubMed: 15194835]
2. Newell ML, Coovadia H, Cortina-Borja M, et al. Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. *Lancet* Oct 2–8;2004 364(9441):1236–1243. [PubMed: 15464184]
3. Richardson BA, Mbori-Ngacha D, Lavreys L, et al. Comparison of human immunodeficiency virus type 1 viral loads in Kenyan women, men, and infants during primary and early infection. *J Virol* Jun;2003 77(12):7120–7123. [PubMed: 12768032]
4. Mphatswe W, Blanckenberg N, Tudor-Williams G, et al. High frequency of rapid immunological progression in African infants infected in the era of perinatal HIV prophylaxis. *Aids* Jun 19;2007 21(10):1253–1261. [PubMed: 17545701]
5. Lohman BL, Slyker JA, Richardson BA, et al. Longitudinal assessment of human immunodeficiency virus type 1 (HIV-1)-specific gamma interferon responses during the first year of life in HIV-1-infected infants. *J Virol* Jul;2005 79(13):8121–8130. [PubMed: 15956557]
6. Miles DJ, van der Sande M, Jeffries D, et al. Cytomegalovirus infection in Gambian infants leads to profound CD8 T-cell differentiation. *J Virol* Jun;2007 81(11):5766–5776. [PubMed: 17376923]
7. Slyker JA, Lohman-Payne BL, John-Stewart GC, et al. Acute cytomegalovirus infection in Kenyan HIV-infected infants. *AIDS* Oct 23;2009 23(16):2173–2181. [PubMed: 19617812]
8. Maury W, Potts BJ, Rabson AB. HIV-1 infection of first-trimester and term human placental tissue: a possible mode of maternal-fetal transmission. *J Infect Dis* Oct;1989 160(4):583–588. [PubMed: 2477467]
9. John-Stewart GC, Mbori-Ngacha D, Payne BL, et al. HIV-1-Specific Cytotoxic T Lymphocytes and Breast Milk HIV-1 Transmission. *J Infect Dis*. Feb 11;2009
10. Mussi-Pinhata MM, Freimanis L, Yamamoto AY, et al. Infectious disease morbidity among young HIV-1-exposed but uninfected infants in Latin American and Caribbean countries: the National Institute of Child Health and Human Development International Site Development Initiative Perinatal Study. *Pediatrics* Mar;2007 119(3):e694–704. [PubMed: 17296782]
11. Kuhn L, Kasonde P, Sinkala M, et al. Does severity of HIV disease in HIV-infected mothers affect mortality and morbidity among their uninfected infants? *Clin Infect Dis* Dec 1;2005 41(11):1654–1661. [PubMed: 16267740]
12. Slogrove AL, Cotton MF, Esser MM. Severe infections in HIV-exposed uninfected infants: clinical evidence of immunodeficiency. *J Trop Pediatr* Apr;2010 56(2):75–81. [PubMed: 19602487]
13. Lehman DA, Farquhar C. Biological mechanisms of vertical human immunodeficiency virus (HIV-1) transmission. *Rev Med Virol* Nov–Dec;2007 17(6):381–403. [PubMed: 17542053]
14. John GC, Nduati RW, Mbori-Ngacha DA, et al. Correlates of mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission: association with maternal plasma HIV-1 RNA load, genital HIV-1 DNA shedding, and breast infections. *J Infect Dis* Jan 15;2001 183(2): 206–212. [PubMed: 11120927]
15. Becquet R, Bland R, Leroy V, et al. Duration, pattern of breastfeeding and postnatal transmission of HIV: Pooled analysis of individual data from West and South African cohorts. *PLoS ONE* 2009;4:e3797.

16. John-Stewart G, Mbori-Ngacha D, Ekpini R, et al. Breast-feeding and Transmission of HIV-1. *J Acquir Immune Defic Syndr* Feb 1;2004 35(2):196–202. [PubMed: 14722454]
17. Dolcini G, Derrien M, Chaouat G, Barre-Sinoussi F, Menu E. Cell-free HIV type 1 infection is restricted in the human trophoblast choriocarcinoma BeWo cell line, even with expression of CD4, CXCR4 and CCR5. *AIDS Res Hum Retroviruses* Oct;2003 19(10):857–864. [PubMed: 14585217]
18. Lagaye S, Derrien M, Menu E, et al. Cell-to-cell contact results in a selective translocation of maternal human immunodeficiency virus type 1 quasispecies across a trophoblastic barrier by both transcytosis and infection. *J Virol* May;2001 75(10):4780–4791. [PubMed: 11312350]
19. Cheyner R, Langlade-Demoyen P, Marescot M-R, et al. Cytotoxic T lymphocyte responses in the peripheral blood of children born to HIV-1-infected mothers. *Eur. J. Immunol* 1992;22:2211–2217. [PubMed: 1381309]
20. Rowland-Jones SL, Nixon DF, Aldhous MC, et al. HIV-specific CTL activity in an HIV-exposed but uninfected infant. *Lancet* 1993;341:860–861. [PubMed: 8096564]
21. Aldhous MC, Watret KC, Mok JY, Bird AG, Froebel KS. Cytotoxic T lymphocyte activity and CD8 subpopulations in children at risk of HIV infection. *Clin Exp Immunol* 1994;97(1):61–67. [PubMed: 8033422]
22. Kuhn L, Coutoudis A, Moodley D, et al. T-helper cell responses to HIV envelope peptides in cord blood: protection against intrapartum and breast-feeding transmission. *AIDS* Jan 5;2001 15(1):1–9. [PubMed: 11192849]
23. Pitisuttithum P, Gilbert P, Gurwith M, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J Infect Dis* Dec 15;2006 194(12):1661–1671. [PubMed: 17109337]
24. Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* Nov 29;2008 372(9653):1881–1893. [PubMed: 19012954]
25. McElrath MJ, De Rosa SC, Moodie Z, et al. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *Lancet* Nov 29;2008 372(9653):1894–1905. [PubMed: 19012957]
26. Liu J, O'Brien KL, Lynch DM, et al. Immune control of an SIV challenge by a T-cell-based vaccine in rhesus monkeys. *Nature* Jan 1;2009 457(7225):87–91. [PubMed: 18997770]
27. Hansen SG, Vieville C, Whizin N, et al. Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. *Nat Med* Mar;2009 15(3):293–299. [PubMed: 19219024]
28. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* Dec 3;2009 361(23):2209–2220. [PubMed: 19843557]
29. Abel K, Pahar B, Van Rompay KKA, et al. Rapid virus dissemination in infant macaques after oral simian immunodeficiency virus exposure in the presence of local innate immune responses. *J. Virol* 2006;80:6357–6367. [PubMed: 16775324]
30. Kumar RB, Maher DM, Herzberg MC, Southern PJ. Expression of HIV receptors, alternate receptors and co-receptors on tonsillar epithelium: implications for HIV binding and primary oral infection. *Virol. J* 2006;3:25–38. [PubMed: 16600047]
31. Azizi A, Ghunaim H, Diaz-Mitoma F, Mestecky J. Mucosal HIV vaccines: A holy grail or a dud? *Vaccine* 2010;28:4015–4026. [PubMed: 20412879]
32. Wright PF, Mestecky J, McElrath MJ, et al. Comparison of systemic and mucosal delivery of 2 canarypox virus vaccines expressing either HIV-1 genes or the gene for rabies virus G protein. *J. Infect. Dis* 2004;189:1221–1231. [PubMed: 15031791]
33. Kotton CN, Lankowski AJ, Scott N, et al. Safety and immunogenicity of attenuated *Salmonella enterica* serovar *Typhimurium* delivering an HIV-1 gag antigen via the *Salmonella* Type III secretion system. *Vaccine* 2006;24:6216–6224. [PubMed: 16824652]
34. Jaspan HB, Lawn SD, Safrit JT, Bekker L-G. The maturing immune system: implications for development and testing HIV-1 vaccines for children and adolescents. *AIDS* 2006;20:483–494. [PubMed: 16470112]

35. Marchant A, Newport M. Prevention of infectious diseases by neonatal and early infantile immunization: prospects for the new millennium. *Curr. Opin. Infect. Dis* 2000;13:241–246. [PubMed: 11964793]
36. Adkins B, Leclerc C, Marshall-Clarke S. Neonatal adaptive immunity comes of age. *Nature Reviews* 2004;4:553–564.
37. Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nature Rev. Immunol* 2007;7:379–389. [PubMed: 17457344]
38. Velilla PA, Rugeles MT, Chouhnet CA. Defective antigen-presenting cell function in human neonates. *Clin. Immunol* 2006;121:251–259. [PubMed: 17010668]
39. Chheda S, Palkowetz KH, Garofalo R, Rassin DK, Goldman AS. Decreased interleukin-10 production by neonatal monocytes and T cells: relationship to decreased production and expression of tumor necrosis factor - [alpha] and its receptors. *Pediatr. Res* 1996;40:475–483. [PubMed: 8865287]
40. Lilic D, Cant AJ, Abinun M, Calvert JE, Spickett GP. Cytokine production differs in children and adults. *Pediatr. Res* 1997;42:237–240. [PubMed: 9262229]
41. Marchant A, Goetghebuer T, Ota MO, et al. Newborns develop a Th1-type immune response to *Mycobacterium bovis* bacillus Calmette-Guerin vaccination. *J. Immunol* 1999;163:2249–2255. [PubMed: 10438968]
42. Ota MO, Vekemans J, Schlegel-Haueter SE, et al. Influence of *Mycobacterium bovis* bacillus Calmette-Guerin on antibody and cytokine responses to human neonatal vaccination. *J Immunol* Jan 15;2002 168(2):919–925. [PubMed: 11777990]
43. Godfrey WR, Spoden DJ, Ge YG, et al. Cord blood CD4+CD25+-derived T regulatory cell lines express FoxP3 protein and manifest potent suppressor function. *Blood* 2005;105:750–758. [PubMed: 15374887]
44. Legrand FA, Nixon DF, Loo CP, et al. Strong HIV-1-specific T cell responses in HIV-1-exposed uninfected infants and neonates revealed after regulatory T cell removal. *PLoS One* 2006;1:e102. [PubMed: 17183635]
45. Hartigan-O'Connor DJ, Abel K, McCune JM. Suppression of SIV-specific CD4+ T cells by infant but not adult macaque regulatory T cells: implications for SIV disease progression. *J. Exp. Med* 2007;204:2679–2692. [PubMed: 17954571]
46. Mold JE, Michaelsson J, Burt TD, et al. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. *Science* Dec 5;2008 322(5907):1562–1565. [PubMed: 19056990]
47. Flanagan KL, Halliday A, Burl S, et al. The effect of placental malaria infection on cord blood and maternal immunoregulatory responses at birth. *Eur J Immunol* Apr;2010 40(4):1062–1072. [PubMed: 20039298]
48. Gans H, Yasukawa L, Rinki M, et al. Immune responses to measles and mumps vaccination of infants at 6, 9, and 12 months. *J. Infect. Dis* 2001;184:817–826. [PubMed: 11528592]
49. Van Rompay KKA, Otsyula MG, Tarara RP, et al. Vaccination of pregnant macaques protects newborns against mucosal simian immunodeficiency virus infection. *J. Infect. Dis* 1996;173:1327–1335. [PubMed: 8648204]
50. Van Rompay KKA, Berardi CJ, Dillard-Telm S, et al. Passive immunization of newborn rhesus macaques prevents oral simian immunodeficiency virus infection. *J. Infect. Dis* 1998;177:1247–1259. [PubMed: 9593009]
51. Hofmann-Lehmann R, Vlasak J, Rasmussen RA, et al. Postnatal passive immunization of neonatal macaques with a triple combination of human monoclonal antibodies against oral simian-human immunodeficiency virus challenge. *J. Virol* 2001;75:7470–7480. [PubMed: 11462019]
52. Ferrantelli F, Rasmussen RA, Buckley KA, et al. Complete protection of neonatal rhesus macaques against oral exposure to pathogenic simian-human immunodeficiency virus by human anti-HIV monoclonal antibodies. *J. Infect. Dis* 2004;189:2149–2153. [PubMed: 15181560]
53. Ferrantelli F, Buckley KA, Rasmussen RA, et al. Time dependence of protective post-exposure prophylaxis with human monoclonal antibodies against pathogenic SHIV challenge in newborn macaques. *Virology* 2007;358:69–78. [PubMed: 16996554]

54. Van Rompay KKA, Greenier JL, Cole KS, et al. Immunization of newborn rhesus macaques with simian immunodeficiency virus (SIV) vaccines prolongs survival after oral challenge with virulent SIVmac251. *J. Virol* 2003;77:179–190. [PubMed: 12477823]
55. Van Rompay KKA, Abel K, Lawson JR, et al. Attenuated poxvirus-based simian immunodeficiency virus (SIV) vaccines given in infancy partially protect infant and juvenile macaques against repeated oral challenge with virulent SIV. *Journal of acquired immune deficiency syndromes* (1999) 2005;38:124–134. [PubMed: 15671796]
56. Cristillo AD, Lisiewicz J, He L, et al. HIV-1 prophylactic vaccine comprised of topical DermaVir prime and protein boost elicits cellular immune responses and controls pathogenic R5 SHIV162P3. *Virology* 2007;366:197–211. [PubMed: 17499328]
57. McFarland EJ, Johnson DC, Muresan P, et al. HIV-1 vaccine induced immune responses in newborns of HIV-1 infected mothers. *AIDS* Jul 13;2006 20(11):1481–1489. [PubMed: 16847402]
58. Johnson DC, McFarland EJ, Muresan P, et al. Safety and immunogenicity of an HIV-1 recombinant canarypox vaccine in newborns and infants of HIV-1-infected women. *J. Infect. Dis* 2005;192:2129–2133. [PubMed: 16288378]
59. Gray RH, Wawer MJ, Brookmeyer R, et al. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *Lancet* Apr 14;2001 357(9263):1149–1153. [PubMed: 11323041]
60. Corey L, Wald A, Celum CL, Quinn TC. The effects of herpes simplex virus-2 on HIV-1 acquisition and transmission: a review of two overlapping epidemics. *J Acquir Immune Defic Syndr* Apr 15;2004 35(5):435–445. [PubMed: 15021308]
61. Hitti J, Watts DH, Burchett SK, et al. Herpes simplex virus seropositivity and reactivation at delivery among pregnant women infected with human immunodeficiency virus-1. *Am J Obstet Gynecol* Aug;1997 177(2):450–454. [PubMed: 9290467]
62. Freeman EE, Weiss HA, Glynn JR, et al. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. *AIDS* Jan 2;2006 20(1):73–83. [PubMed: 16327322]
63. Cowan FM, Humphrey JH, Ntozini R, et al. Maternal Herpes simplex virus type 2 infection, syphilis and risk of intra-partum transmission of HIV-1: results of a case control study. *AIDS* Jan 11;2008 22(2):193–201. [PubMed: 18097221]
64. Bollen LJ, Whitehead SJ, Mock PA, et al. Maternal herpes simplex virus type 2 coinfection increases the risk of perinatal HIV transmission: possibility to further decrease transmission? *AIDS* Jun 19;2008 22(10):1169–1176. [PubMed: 18525263]
65. Chen KT, Tuomala RE, Chu C, et al. No association between antepartum serologic and genital tract evidence of herpes simplex virus-2 coinfection and perinatal HIV-1 transmission. *Am J Obstet Gynecol* Apr;2008 198(4):399, e391–395. [PubMed: 18177832]
66. Drake AL, John-Stewart GC, Wald A, et al. Herpes simplex virus type 2 and risk of intrapartum human immunodeficiency virus transmission. *Obstet Gynecol* Feb;2007 109(2 Pt 1):403–409. [PubMed: 17267842]
67. Schacker T, Zeh J, Hu H, Shaughnessy M, Corey L. Changes in plasma human immunodeficiency virus type 1 RNA associated with herpes simplex virus reactivation and suppression. *J Infect Dis* Dec 15;2002 186(12):1718–1725. [PubMed: 12447756]
68. Schacker T, Ryncarz AJ, Goddard J, et al. Frequent recovery of HIV-1 from genital herpes simplex virus lesions in HIV-1-infected men. *JAMA* Jul 1;1998 280(1):61–66. [PubMed: 9660365]
69. Cunningham AL, Turner RR, Miller AC, Para MF, Merigan TC. Evolution of recurrent herpes simplex lesions. An immunohistologic study. *J Clin Invest* Jan;1985 75(1):226–233. [PubMed: 3880773]
70. Koelle DM, Abbo H, Peck A, Ziegweid K, Corey L. Direct recovery of herpes simplex virus (HSV)-specific T lymphocyte clones from recurrent genital HSV-2 lesions. *J Infect Dis* May;1994 169(5):956–961. [PubMed: 8169426]
71. Mbopi-Keou FX, Gresenguet G, Mayaud P, et al. Interactions between herpes simplex virus type 2 and human immunodeficiency virus type 1 infection in African women: opportunities for intervention. *J Infect Dis* Oct;2000 182(4):1090–1096. [PubMed: 10979904]

72. McClelland RS, Wang CC, Overbaugh J, et al. Association between cervical shedding of herpes simplex virus and HIV-1. *AIDS* Dec 6;2002 16(18):2425–2430. [PubMed: 12461416]
73. Mole L, Ripich S, Margolis D, Holodniy M. The impact of active herpes simplex virus infection on human immunodeficiency virus load. *J Infect Dis* Sep;1997 176(3):766–770. [PubMed: 9291329]
74. Nagot N, Ouedraogo A, Foulongne V, et al. Reduction of HIV-1 RNA levels with therapy to suppress herpes simplex virus. *N Engl J Med* Feb 22;2007 356(8):790–799. [PubMed: 17314338]
75. Van de Perre P, Segondy M, Foulongne V, et al. Herpes simplex virus and HIV-1: deciphering viral synergy. *Lancet Infect Dis* Aug;2008 8(8):490–497. [PubMed: 18652995]
76. McMahon MA, Siliciano JD, Lai J, et al. The antiherpetic drug acyclovir inhibits HIV replication and selects the V75I reverse transcriptase multidrug resistance mutation. *J Biol Chem* Nov 14;2008 283(46):31289–31293. [PubMed: 18818198]
77. Lisco A, Vanpouille C, Tchesnokov EP, et al. Acyclovir is activated into a HIV-1 reverse transcriptase inhibitor in herpesvirus-infected human tissues. *Cell Host Microbe* Sep 11;2008 4(3):260–270. [PubMed: 18779052]
78. Celum C, Wald A, Hughes J, et al. Effect of aciclovir on HIV-1 acquisition in herpes simplex virus 2 seropositive women and men who have sex with men: a randomised, double-blind, placebo-controlled trial. *Lancet* Jun 21;2008 371(9630):2109–2119. [PubMed: 18572080]
79. Celum C, Wald A, Lingappa JR, et al. Acyclovir and transmission of HIV-1 from persons infected with HIV-1 and HSV-2. *N Engl J Med* Feb 4;2010 362(5):427–439. [PubMed: 20089951]
80. Watson-Jones D, Weiss HA, Rusizoka M, et al. Effect of herpes simplex suppression on incidence of HIV among women in Tanzania. *N Engl J Med* Apr 10;2008 358(15):1560–1571. [PubMed: 18337596]
81. Zhu J, Hladik F, Woodward A, et al. Persistence of HIV-1 receptor-positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. *Nat Med* Aug;2009 15(8):886–892. [PubMed: 19648930]
82. Kimberlin DF, Weller S, Whitley RJ, et al. Pharmacokinetics of oral valacyclovir and acyclovir in late pregnancy. *Am J Obstet Gynecol* Oct;1998 179(4):846–851. [PubMed: 9790357]
83. Rowland-Jones SL, Lohman B. Interactions between malaria and HIV infection-an emerging public health problem? *Microbes Infect* Oct;2002 4(12):1265–1270. [PubMed: 12467769]
84. Kublin JG, Patnaik P, Jere CS, et al. Effect of Plasmodium falciparum malaria on concentration of HIV-1-RNA in the blood of adults in rural Malawi: a prospective cohort study. *Lancet* Jan 15–21;2005 365(9455):233–240. [PubMed: 15652606]
85. Hoffman IF, Jere CS, Taylor TE, et al. The effect of Plasmodium falciparum malaria on HIV-1 RNA blood plasma concentration. *AIDS* Mar 11;1999 13(4):487–494. [PubMed: 10197377]
86. Whitworth JA, Hewitt KA. Effect of malaria on HIV-1 progression and transmission. *Lancet* Jan 15–21;2005 365(9455):196–197. [PubMed: 15652587]
87. Ayoub A, Nerrienet E, Menu E, et al. Mother-to-child transmission of human immunodeficiency virus type 1 in relation to the season in Yaounde, Cameroon. *Am J Trop Med Hyg* Oct;2003 69(4):447–449. [PubMed: 14640507]
88. Gallagher M, Malhotra I, Mungai PL, et al. The effects of maternal helminth and malaria infections on mother-to-child HIV transmission. *AIDS* Nov 4;2005 19(16):1849–1855. [PubMed: 16227793]
89. Brahmbhatt H, Sullivan D, Kigozi G, et al. Association of HIV and malaria with mother-to-child transmission, birth outcomes, and child mortality. *J Acquir Immune Defic Syndr* Apr 1;2008 47(4):472–476. [PubMed: 18332766]
90. Ayisi JG, van Eijk AM, Newman RD, et al. Maternal malaria and perinatal HIV transmission, western Kenya. *Emerg Infect Dis* Apr;2004 10(4):643–652. [PubMed: 15200854]
91. Naniche D, Lahuerta M, Bardaji A, et al. Mother-to-child transmission of HIV-1: association with malaria prevention, anaemia and placental malaria. *HIV Med* Oct;2008 9(9):757–764. [PubMed: 18651857]
92. Brahmbhatt H, Kigozi G, Wabwire-Mangen F, et al. The effects of placental malaria on mother-to-child HIV transmission in Rakai, Uganda. *AIDS* Nov 21;2003 17(17):2539–2541. [PubMed: 14600529]

93. Inion I, Mwanyumba F, Gaillard P, et al. Placental malaria and perinatal transmission of human immunodeficiency virus type 1. *J Infect Dis* Dec 1;2003 188(11):1675–1678. [PubMed: 14639538]
94. Mwapasa V, Rogerson SJ, Molyneux ME, et al. The effect of *Plasmodium falciparum* malaria on peripheral and placental HIV-1 RNA concentrations in pregnant Malawian women. *AIDS* Apr 30;2004 18(7):1051–1059. [PubMed: 15096809]
95. Moormann AM, Sullivan AD, Rochford RA, et al. Malaria and pregnancy: placental cytokine expression and its relationship to intrauterine growth retardation. *J Infect Dis* Dec;1999 180(6):1987–1993. [PubMed: 10558956]
96. Abrams ET, Brown H, Chensue SW, et al. Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated beta chemokine expression. *J Immunol* Mar 1;2003 170(5):2759–2764. [PubMed: 12594307]
97. Chaisavaneeyakorn S, Moore JM, Mirel L, et al. Levels of macrophage inflammatory protein 1 alpha (MIP-1 alpha) and MIP-1 beta in intervillous blood plasma samples from women with placental malaria and human immunodeficiency virus infection. *Clin Diagn Lab Immunol* Jul;2003 10(4):631–636. [PubMed: 12853396]
98. Fried M, Muga RO, Misore AO, Duffy PE. Malaria elicits type 1 cytokines in the human placenta: IFN-gamma and TNF-alpha associated with pregnancy outcomes. *J Immunol* Mar 1;1998 160(5):2523–2530. [PubMed: 9498798]
99. Tkachuk AN, Moormann AM, Poore JA, et al. Malaria enhances expression of CC chemokine receptor 5 on placental macrophages. *J Infect Dis* Mar 15;2001 183(6):967–972. [PubMed: 11237815]
100. Fleming DT, Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sex Transm Infect* Feb;1999 75(1):3–17. [PubMed: 10448335]
101. Wolday D, Gebremariam Z, Mohammed Z, et al. The impact of syndromic treatment of sexually transmitted diseases on genital shedding of HIV-1. *AIDS* Mar 26;2004 18(5):781–785. [PubMed: 15075513]
102. Kreiss J, Willerford DM, Hensel M, et al. Association between cervical inflammation and cervical shedding of human immunodeficiency virus DNA. *J Infect Dis* Dec;1994 170(6):1597–1601. [PubMed: 7996003]
103. John GC, Nduati RW, Mbori-Ngacha D, et al. Genital shedding of human immunodeficiency virus type 1 DNA during pregnancy: association with immunosuppression, abnormal cervical or vaginal discharge, and severe vitamin A deficiency. *J Infect Dis* Jan;1997 175(1):57–62. [PubMed: 8985196]
104. Ghys PD, Fransen K, Diallo MO, et al. The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Cote d'Ivoire. *AIDS* Oct;1997 11(12):F85–93. [PubMed: 9342059]
105. Mostad SB, Overbaugh J, DeVange DM, et al. Hormonal contraception, vitamin A deficiency, and other risk factors for shedding of HIV-1 infected cells from the cervix and vagina. *Lancet* Sep 27;1997 350(9082):922–927. [PubMed: 9314871]
106. McClelland RS, Wang CC, Mandalia K, et al. Treatment of cervicitis is associated with decreased cervical shedding of HIV-1. *AIDS* Jan 5;2001 15(1):105–110. [PubMed: 11192850]
107. Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS. Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. *AIDS* Jul 31;2008 22(12):1493–1501. [PubMed: 18614873]
108. Taha TE, Hoover DR, Dallabetta GA, et al. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. *AIDS* Sep 10;1998 12(13):1699–1706. [PubMed: 9764791]
109. van de Wijgert JH, Morrison CS, Cornelisse PG, et al. Bacterial vaginosis and vaginal yeast, but not vaginal cleansing, increase HIV-1 acquisition in African women. *J Acquir Immune Defic Syndr* Jun 1;2008 48(2):203–210. [PubMed: 18520679]

110. McClelland RS, Lavreys L, Hassan WM, et al. Vaginal washing and increased risk of HIV-1 acquisition among African women: a 10-year prospective study. *AIDS* Jan 9;2006 20(2):269–273. [PubMed: 16511421]
111. Farquhar C, Mbori-Ngacha D, Overbaugh J, et al. Illness during pregnancy and bacterial vaginosis are associated with in-utero HIV-1 transmission. *AIDS* Jan 2;2010 24(1):153–155. [PubMed: 19952542]
112. Taha TE, Brown ER, Hoffman IF, et al. A phase III clinical trial of antibiotics to reduce chorioamnionitis-related perinatal HIV-1 transmission. *AIDS* Jun 12;2006 20(9):1313–1321. [PubMed: 16816561]
113. Belyhun Y, Medhin G, Amberbir A, et al. Prevalence and risk factors for soil-transmitted helminth infection in mothers and their infants in Butajira, Ethiopia: a population based study. *BMC Public Health* 2010;10:21. [PubMed: 20085635]
114. Naish S, McCarthy J, Williams GM. Prevalence, intensity and risk factors for soil-transmitted helminth infection in a South Indian fishing village. *Acta Trop* Jul;2004 91(2):177–187. [PubMed: 15234667]
115. Yatch NJ, Yi J, Agbenyega T, et al. Malaria and intestinal helminth co-infection among pregnant women in Ghana: prevalence and risk factors. *Am J Trop Med Hyg* Jun;2009 80(6):896–901. [PubMed: 19478245]
116. Nguyen PH, Nguyen KC, Nguyen TD, et al. Intestinal helminth infections among reproductive age women in Vietnam: prevalence, co-infection and risk factors. *Southeast Asian J Trop Med Public Health* Sep;2006 37(5):865–874. [PubMed: 17333727]
117. Ponnighaus JM, Fine PE, Sterne JA, et al. Efficacy of BCG vaccine against leprosy and tuberculosis in northern Malawi. *Lancet* Mar 14;1992 339(8794):636–639. [PubMed: 1347338]
118. Fine PE. Variation in protection by BCG: implications of and for heterologous immunity. *Lancet* Nov 18;1995 346(8986):1339–1345. [PubMed: 7475776]
119. van Riet E, Hartgers FC, Yazdanbakhsh M. Chronic helminth infections induce immunomodulation: consequences and mechanisms. *Immunobiology* 2007;212(6):475–490. [PubMed: 17544832]
120. Malhotra I, Mungai P, Wamachi A, et al. Helminth- and Bacillus Calmette-Guerin-induced immunity in children sensitized in utero to filariasis and schistosomiasis. *J Immunol* Jun 1;1999 162(11):6843–6848. [PubMed: 10352306]
121. Elias D, Wolday D, Akuffo H, et al. Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guerin (BCG) vaccination. *Clin Exp Immunol* Feb;2001 123(2):219–225. [PubMed: 11207651]
122. Cooper PJ, Chico M, Sandoval C, et al. Human infection with *Ascaris lumbricoides* is associated with suppression of the interleukin-2 response to recombinant cholera toxin B subunit following vaccination with the live oral cholera vaccine CVD 103-HgR. *Infect Immun* Mar;2001 69(3):1574–1580. [PubMed: 11179329]
123. Weisman Z, Kalinkovich A, Borkow G, et al. Infection by different HIV-1 subtypes (B and C) results in a similar immune activation profile despite distinct immune backgrounds. *J Acquir Immune Defic Syndr* Jun 1;1999 21(2):157–163. [PubMed: 10360808]
124. Bentwich Z, Weisman Z, Moroz C, Bar-Yehuda S, Kalinkovich A. Immune dysregulation in Ethiopian immigrants in Israel: relevance to helminth infections? *Clin Exp Immunol* Feb;1996 103(2):239–243. [PubMed: 8565306]
125. Kalinkovich A, Weisman Z, Greenberg Z, et al. Decreased CD4 and increased CD8 counts with T cell activation is associated with chronic helminth infection. *Clin Exp Immunol* Dec;1998 114(3):414–421. [PubMed: 9844052]
126. Chenine AL, Buckley KA, Li PL, et al. *Schistosoma mansoni* infection promotes SHIV clade C replication in rhesus macaques. *AIDS* Nov 4;2005 19(16):1793–1797. [PubMed: 16227786]
127. Ayash-Rashkovsky M, Chenine AL, Steele LN, et al. Coinfection with *Schistosoma mansoni* reactivates viremia in rhesus macaques with chronic simian-human immunodeficiency virus clade C infection. *Infect Immun* Apr;2007 75(4):1751–1756. [PubMed: 17283092]

128. Chenine AL, Shai-Kobiler E, Steele LN, et al. Acute *Schistosoma mansoni* infection increases susceptibility to systemic SHIV clade C infection in rhesus macaques after mucosal virus exposure. *PLoS Negl Trop Dis* 2008;2(7):e265. [PubMed: 18648516]
129. Walson JL, Otieno PA, Mbuchi M, et al. Albendazole treatment of HIV-1 and helminth co-infection: a randomized, double-blind, placebo-controlled trial. *AIDS* Aug 20;2008 22(13):1601–1609. [PubMed: 18670219]
130. Kallestrup P, Zinyama R, Gomo E, et al. Schistosomiasis and HIV-1 infection in rural Zimbabwe: effect of treatment of schistosomiasis on CD4 cell count and plasma HIV-1 RNA load. *J Infect Dis* Dec 1;2005 192(11):1956–1961. [PubMed: 16267767]
131. Da'dara AA, Harn DA. Elimination of helminth infection restores HIV-1C vaccine-specific T cell responses independent of helminth-induced IL-10. *Vaccine* Feb 3;2010 28(5):1310–1317. [PubMed: 19941988]
132. Actor JK, Shirai M, Kullberg MC, et al. Helminth infection results in decreased virus-specific CD8+ cytotoxic T-cell and Th1 cytokine responses as well as delayed virus clearance. *Proc Natl Acad Sci U S A* Feb 1;1993 90(3):948–952. [PubMed: 8094248]
133. Ayash-Rashkovsky M, Weisman Z, Diveley J, et al. Generation of Th1 immune responses to inactivated, gp120-depleted HIV-1 in mice with a dominant Th2 biased immune profile via immunostimulatory [correction of imunostimulatory] oligonucleotides--relevance to AIDS vaccines in developing countries. *Vaccine* Jun 21;2002 20(21–22):2684–2692. [PubMed: 12034094]
134. Ayash-Rashkovsky M, Weisman Z, Zlotnikov S, et al. Induction of antigen-specific Th1-biased immune responses by plasmid DNA in schistosoma-infected mice with a preexistent dominant Th2 immune profile. *Biochem Biophys Res Commun* Apr 20;2001 282(5):1169–1176. [PubMed: 11302738]
135. Kusakabe K, Xin KQ, Katoh H, et al. The timing of GM-CSF expression plasmid administration influences the Th1/Th2 response induced by an HIV-1-specific DNA vaccine. *J Immunol* Mar 15;2000 164(6):3102–3111. [PubMed: 10706700]
136. Wille-Reece U, Flynn BJ, Lore K, et al. HIV Gag protein conjugated to a Toll-like receptor 7/8 agonist improves the magnitude and quality of Th1 and CD8+ T cell responses in nonhuman primates. *Proc Natl Acad Sci U S A* Oct 18;2005 102(42):15190–15194. [PubMed: 16219698]
137. Sieling PA, Chung W, Duong BT, Godowski PJ, Modlin RL. Toll-like receptor 2 ligands as adjuvants for human Th1 responses. *J Immunol* Jan 1;2003 170(1):194–200. [PubMed: 12496400]
138. Lalvani A, Brookes R, Wilkinson RJ, et al. Human cytolytic and interferon gamma-secreting CD8+ T lymphocytes specific for *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A* 1998;95(1):270–275. [PubMed: 9419365]
139. Maino VC, Maecker HT. Cytokine flow cytometry: a multiparametric approach for assessing cellular immune responses to viral antigens. *Clin. Immunol* 2003;110:222–231. [PubMed: 15047200]
140. DeVange Panteleeff D, John GC, Nduati R, et al. Rapid method for screening dried blood samples on filter paper for human immunodeficiency virus type 1 DNA. *J. Clin. Microbiol* 1999;37:350–353. [PubMed: 9889216]
141. Selin LK, Lin M-L, Kraemer KA, et al. Attrition of T cell memory: Selective loss of LCMV epitope-specific memory CD8 T cells following infections with heterologous viruses. *Immunity* 1999;11:733–742. [PubMed: 10626895]
142. Ota MO, Vekemans J, Schlegel-Haueter SE, et al. Influence of *mycobacterium bovis* bacillus Calmette-Guerin on antibody and cytokine responses to human neonatal vaccine. *J. Immunol* 2002;168:919–925. [PubMed: 11777990]
143. Vidor E. The nature and consequences of intra- and inter-vaccine interference. *J. Comp. Path* 2007;137(suppl 1):S62–S66. [PubMed: 17560593]
144. Gans HA, Arvin AM, Galinus J, et al. Deficiency of the humoral immune response to measles vaccine in infants immunized at 6 months of age. *J. Amer. Med. Assoc* 1998;280:527–532.
145. Siegrist C-A, Cordova M, Brandt C, et al. Determinants of infant responses to vaccines in presence of maternal antibodies. *Vaccine* 1998;16:1409–1414. [PubMed: 9711780]

146. Stoiber H. Complement, Fc receptors and antibodies: a Trojan horse in HIV infection? *Curr. Opin. HIV AIDS* 2009;4:394–399. [PubMed: 20048703]
147. Shinefield HR. Overview of the development and current use of CRM197 conjugate vaccines for pediatric use. *Vaccine* 2010;28:4335–4339. [PubMed: 20452430]
148. Baba TW, Liska V, Khimani AH, et al. Live attenuated, multiply deleted simian immunodeficiency virus causes AIDS in infant and adult macaques. *Nature Med* 1999;5:194–203. [PubMed: 9930868]