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The rhesus macaque pediatric SIV infection model - a valuable tool in understanding infant HIV pathogenesis and for designing pediatric HIV prevention strategies

Kristina Abel

Introduction

The AIDS pandemic presents a major health problem with far-reaching socio-economic impact. Progress has been made in our understanding of the virus, how it evolved, and how it interacts with the immune system. Antiretroviral therapy has resulted in a significant increase of the life expectancy of HIV-1 infected patients and drastically reduced mother-to-child-transmission (MTCT). However, vaccine development has not proven successful so far. While the rate of HIV-1 infections has stabilized or been reduced in many developed countries, the pandemic continues in most resource-poor countries. In these countries, the young adult population, and therefore the primary work force, has been drastically reduced and MTCT threatens the future population. Thus, we need to understand not only the pathogenesis of adult HIV-1 infection, but also be able to recognize the specific interactions between the HIV-1 virus and the newborn. Several groups have devoted their work to the prevention of pediatric HIV-1 infection and recent reviews summarize their excellent work [1–10]. This review will focus on the rhesus macaque model of infant SIV infection and how it is uniquely able to address many open questions clinicians may ask. Overviews of this model have been provided and the reader is advised to consult these resources [11,12]. In particular, this review strives to address immunological problems and concepts of the developing immune system that need to be considered in the design of pediatric intervention strategies.

1. Pediatric HIV infections

Every minute a child becomes infected with HIV (UNAIDS). Women now represent the major group of newly HIV-1 infected people (UNAIDS). Many of these women are of childbearing age thereby increasing the risk of vertical transmission. Yearly, about 500,000 new children become infected with HIV-1. About 90% of all HIV-1 infected children live in Africa or other resource-limited geographic regions. In contrast, in highly industrialized countries, early detection methods, standard prenatal care and access to antiretroviral therapy (ART) have made mother-to-child-transmission (MTCT) a rare event. Infants that become infected with HIV-1 during birth or shortly thereafter tend to have a very rapid disease progression, ~25% of HIV-infected infants die within the first 2 years of life [13,14]. Most children survive longer, but they have significantly higher levels of virus replication than adults and virus levels only slowly decline to adult values [15–19].

While children may “only” represent a relatively small percentage of newly HIV- infected patients, the socio-economic effect of HIV infection in this age group is far reaching. Considering the persistent high incidence of HIV infections in infants in Africa and many resource-poor countries, their limited access to ART, the high risk of developing drug-resistant HIV-1 in those who do receive the most common prophylactic single ART regimen, nevirapine, and their rather rapid progression to AIDS, the testing of novel pediatric HIV-1 prevention

strategies should be a major research priority and an ethical responsibility towards this future generation.

2. Mother-to-child transmission

2.1. In utero MTCT

HIV-1 infection of pregnant women does not necessarily translate into HIV-1 infection of the fetus in-utero. It is estimated that only 20–30% of vertical transmission are due to in-utero infection [3,15,20]. In a recent study of pregnant women in Nairobi, the in-utero transmission rate was only 6.3% [21]. Risk factors of MTCT include, but are not limited to, maternal viral load, antiretroviral therapy of the mother, simultaneous infection with other pathogens, and direct HIV infection of placental cells [22,23]. Other confounding factors could include the gestational age at which the fetus is exposed to the virus, and whether and how the virus evolved under the maternal immune pressure. In addition, the immunosuppressive milieu that allows mother and fetus to coexist might limit virus replication, as only few activated target cells for the virus will be available and/or active immuno-suppressive mechanisms are at work. Still, the question remains why do not all HIV infected women transmit HIV? Are the majority of fetuses protected because (i) transfer of maternal immunity prevents transmission, (ii) the fetus mounts immune responses abrogating the infection, or (iii) are both, maternal and fetal immune responses, involved? Although there is some evidence of HIV-specific $CD8^+$T cell responses in the blood of HIV exposed, but uninfected infants [24–26], more work is needed to understand the mechanisms leading to HIV transmission from the mother to the child. An alternative explanation for cellular responses in seronegative infants would be an occult HIV infection. Studies to answer these questions will not be feasible in humans, but would be possible to perform in nonhuman primate hosts as discussed below. For example, occult infection has been postulated to occur in infant macaques after perinatal SF162P3 transmission [27,28].

2.2. Breast milk transmission

MTCT cannot only occur in-utero, but also intrapartum through the transmission of HIV by amniotic fluid, blood, and/or vaginal secretions, and by breast-milk transmission [4,5]. The number of neonatal/infant HIV-1 infection through breast-milk transmission is estimated to range from 25–50% of all MTCT cases [4,5]. In resource-limited countries, many women don't have a choice but to breast-feed their children for lack of clean water and to prevent malnutrition and disease. In fact, the cessation of breastfeeding at 6 months of age has been associated with a failure to thrive and increased morbidity as a result of non-HIV related infections common to geographical regions hardest hit by HIV infection [29].

The exact dose of HIV exposure during each feeding is very difficult to determine. The levels of virus in human breast milk are highest in the first few days after delivery [30,31]. However, HIV can be transmitted throughout the entire breast-feeding period and newer studies report equal risk factors for breast-milk transmission during the entire breast-feeding period [5]. The average virus load in breast milk lingers around 700 copies/ml, but can range from a few hundred to hundred of thousands of copies/ml breast milk. The total daily exposure of an infant could potentially result in more than a million copies per day [32]. Therefore, while the actual dose of virus might be low during each exposure, the number of exposures is exceedingly high and occurs consistently at short intervals for extended periods ranging from weeks to years.

Virological and immunological studies on MTCT by breast-feeding are difficult to perform due to ethical reasons and sample size and access. Thus, many questions remain to be answered: (i) What are the primary target cells of HIV in infants?, (ii) what are the primary replication site(s) of the virus?, (iii) How fast does the virus disseminate, (iv) can the infant immune system respond to the viral challenge and how?, (v) why does a subset of children not control HIV

replication and die before their 2nd birthday?, (vi) what are the differences between infant and adult immune responses to the virus in the early phases of infection and/or what are the differences between fast and normal progressing infants? These questions can be answered in a nonhuman primate model as we have the opportunity to control conditions of infection, e.g virus inoculum, route of infection, and age at infection, experimentally [12]. Pediatric nonhuman primate studies are sensitive in nature and are associated with very high costs. Further, the influence of genetic factors, for example the relationship between MHC and susceptibility to HIV-1 infection and disease progression, could not be easily studied. Still, the vast knowledge gained from nonhuman primate studies would likely contribute significantly to our understanding of vertical transmission of HIV and many other pathogens.

3. Lessons from the natural nonhuman primate hosts of SIV

In natural hosts of SIV, SIV transmission by infected mothers to their babies seems to be rare. In fact, SIVagm-positive babies born to SIV-infected African Green Monkey (AGM) have not been observed and seroconversion in AGM infected as neonates with SIVagm is delayed and progression to SIV-induced disease does not occur [20,33]. Similarly, a recent breast milk SIV transmission study in mandrills demonstrated that despite high virus levels in maternal milk, infection of infant animals was very rare [34]. The authors speculate that lower expression levels of the SIV (and HIV) coreceptor CCR 5 contributed to the lack of infection. However, the authors analyzed only peripheral blood and presented no data from tissues where the T cells with an activated/memory phenotype, the target cells for HIV/SIV, reside. Still, it is intriguing that natural hosts of SIV apparently don't transmit the virus vertically despite the high levels of virus replication in these animals. Thus, a comparative analysis of fetal immune responses in natural and experimental hosts might reveal important insights into the influence of viral and host factors on transmission.

4. The neonatal/infant rhesus macaque model of SIV infection

Non-human primates most closely resemble humans in their physiology and in their immune system development [35]. Rhesus macaques can be experimentally infected with simian immunodeficiency virus (SIV), a virus that is closely related to HIV and results in similar pathogenesis. Therefore, non-human primate studies of SIV infection in infants are of critical importance for understanding age-related differences in SIV/HIV pathogenesis between infants and adults. Further, an infant SIV infection model would be, and already has been proven (see below) to be extremely valuable for the testing of antiretroviral therapies and for the design and preclinical testing of pediatric HIV vaccines.

The development of a MTCT transmission model of SIV infection to study pediatric HIV infection started soon after the discovery of SIV. Direct inoculation of amniotic fluid with pathogenic SIV could result in effective in utero SIV transmission to the fetus [36–39]. Further, experiments demonstrated that pregnant rhesus monkeys could transmit SIV to the fetus during different stages of gestation [40]. However, the very late seroconversion observed in some infants born to perinatally infected dams suggested that these infants did not become infected in-utero, but more likely during the breast-feeding period [37,38]. In fact, Amadee et al. showed that, similar to HIV in humans, SIV can be transmitted throughout the entire lactation period [41,42]. Thus, SIV transmission from SIV-infected mothers to infants can occur by the same routes and with similar frequencies as observed in humans, validating the use of this model. However, the testing of antibodies directed against the envelope protein of the virus or strategies aimed at interfering with virus binding to the host cells would not be possible because of significant difference in the envelope proteins of SIV and HIV. To allow the more direct study of HIV intervention and vaccine strategies in nonhuman primates, a model of MTCT was developed in pigtailed macaques using the CCR5 tropic virus SHIV SF162P3 [27].

However, the propagation of SHIV SF162P3 is difficult and often virus replication is not sustained after infection. The majority of MTCT studies have been performed in infant rhesus macaques.

In contrast to SIV infection in adult rhesus macaques, most infant macaques infected with pathogenic SIVmac251 progress to simian AIDS (SAIDS) within 6 months, therefore being most representative of the subset of HIV-infected infants that progress rapidly to AIDS [43–45]. Studies to define the age-related differences in immune responses resulting in the different pathogenic outcome have been rare. We observed that the relatively higher prevalence of regulatory T cells (Treg) in infant compared to adult macaques might interfere with SIV-specific $CD4^+$T cell responses, which in turn could impair the development of effective SIV-specific $CD8^+$T cell and antibody responses, contributing to accelerated disease progression [46]. However, caution should be used in targeting Treg in vaccine design as the suppression of Treg could promote general immune activation and thereby immunopathology. A much more comprehensive knowledge of the mechanisms of HIV-1 transmission and local host immune responses is needed to design new immune interventions aimed at preventing pediatric HIV infections and to slow progression to AIDS.

5. Breast milk SIV transmission model in infant rhesus macaques

In utero and intrapartum SIV transmission occur only infrequently. The large animal numbers required to obtain scientifically meaningful data are associated with extremely high costs. Unfortunately, this resulted in a lack of mechanistical studies looking at in utero MTCT transmission of SIV. The most practical approach to study SIV infection in infant macaques appears to be the oral SIV transmission model. Although the oral SIV infection model best reflects breast milk transmission, certain cases of intrapartum HIV-1 transmission are likely to occur by the oral route as well. Direct breast-milk SIV transmission studies from pregnant dams to their infants have been performed by Dr. Amadee's laboratory [41,42]. Dr. Marthas and her colleagues have developed a model to mimic human breast milk transmission of HIV by-1 exposing infant macaques three times a day for five days with SIV by bottle-feeding [47]. While each individual SIV dose of 2×10^4/ml given in those studies [47] is relatively high, SIV levels can reach up to 2×10^3–4×10^5 copies per ml of breast milk when mothers are acutely infected [42]. One should consider that, due to the high costs and the sensitive nature of infant SIV studies, an infection regimen had to be established that would result in the infection of the majority of infant macaques. Importantly though, the active feeding of virus at multiple times during the day reflects the active drinking of HIV-infected breast milk by human babies. This multiple oral SIV feeding model in neonatal/infant macaques enables us to study the very early virus host interactions in specific anatomic compartments and will provide detailed insight and understanding of virus dissemination in relation to host immune responses in the developing infant.

6. The primary site of SIV replication after oral SIV infection of infant rhesus macaques

6.1. The intestine

In humans, the intestinal tissue has been identified as a major site of early virus replication resulting in a significant loss of $CD4^+$T cells that cannot be restored [48–51]. The impact on the intestinal tissue appears to be independent on the route of virus entry and becomes evident in the acute phase of infection. Consistent with these findings, Veazey et al. have recently shown that infant macaques show rapid $CD4^+$T cell loss in the intestinal mucosa after intravenous SIV infection [52]. Further, these authors confirmed that the major target cells for the virus are activated $CD4^+$T cells with a memory phenotype. In *oral* SIV infection studies

of infant macaques, the tonsil, and the oral, eosophagal and intestinal mucosa were identified as primary replication sites [53,54]. The intestinal tissue represents the most likely primary entry site if the virus inoculum is swallowed. An interesting question is how the possible immediate exposure to HIV-1 could be influenced by the establishment of the normal flora at mucosal surfaces after birth and vice versa [55-58].

6.2. The tonsil

In addition to pathogen transmission from infected mothers to their infants by breast-feeding, the oral uptake of antigens represents a very common route in infants due to their natural habit of exploring new things by putting them into their mouth. The central location of the tonsil makes it a primary target organ for the uptake of these antigens. The tonsil can be considered a lymphoid organ, but is also part of the mucosa-associated lymphoid tissue (MALT) [59]. In contrast to lymph nodes, tonsils do not have afferent lymphoid vessels [59]. Therefore, the exposure to antigens relies on the contact of antigens with immune cells across the epithelium covering tonsils. The epithelial layer that surrounds the tonsil contains M cells that are specialized in antigen uptake [60]. Thus, the tonsil is predisposed to efficient antigen uptake and transport of these antigens through the mucosa to the underlying effector cells, including dendritic cells (DC) and T cells. In the case of HIV/SIV infection, this mechanism may contribute to the rapid dissemination of the virus.

Consistent with the frequent exposure to antigens, the cells in the tonsil are characterized by a more activated phenotype compared to cells present in lymph nodes of the same animal. Thus, in neonates/infants, the early entry organs of HIV-1 are rich in activated T cells expressing a memory phenotype, cells identified as the preferred target cells of HIV/SIV in adults [61,62]. In fact, we have data demonstrating that there is a rapid $CD4^+$T cell loss in the tonsil that is intermediate to the $CD4^+$T cell loss observed in lymph nodes and the intestine during the acute stage of infection (Abel, unpublished data).

In summary, the (primary) target organ(s) of orally transmitted HIV/SIV could be the oral buccal mucosa, the tonsil, the esophageal mucosa that is lined with many lymph nodes, and/or the intestinal tissue. Where the virus enters depends on mucosal breaches, the duration of breast milk exposure in the oral cavity and/or the immediate swallowing of the milk without any prolonged exposure in the infant's mouth. Common to all entry sites is the mucosal nature of the tissue. The primary target cells of HIV/SIV have not been identified after oral exposure and likely vary depending on the primary entry site of the virus. In vitro experiments show that epithelial cells are susceptible to HIV-1 infection. In vivo studies of oral SIV infection demonstrated infection of T cells and monocytes in the first few days of infection [54].

7. Early events in infant SIV pathogenesis

Pathogenesis studies have shown that SIV disseminates within one week after oral SIV exposure [53,63]. Therefore, the window for preventing dissemination and containing and/or abrogating infection at the site of local entry is extremely small. Importantly, as is observed in adults, profound and irreversible $CD4^+$T cell depletion occurs rapidly in intestinal tissue of infant macaques independent of the route of infection. Adaptive response could not be detected in local entry sites until after systemic infection has already been established. In addition, we observed that the relatively higher prevalence of regulatory T cells in infant compared to adult macaques might interfere with SIV-specific $CD4^+$T cell responses, which in turn impairs the development of effective SIV-specific $CD8^+$T cell and antibody responses, contributing to accelerated disease progression [46]. The delay of adaptive HIV/SIV-specific responses could be due to a lack of efficient priming. Thus, infant dendritic cells may not provide the proper signals for adequate induction of HIV/SIV-specific T and B cell responses. We have previously demonstrated that oral SIV infection in infant macaques rapidly induces innate inflammatory

and antiviral cytokine responses in lymphoid tissues, but no or only low antiviral IFN-alpha responses in inflamed mucosal tissues [53] (Abel, unpublished observations). Therefore, we need to identify the necessary interactions between innate and adaptive effector cells that would result in protective immunity. Further, we need to determine (i) whether there are differences in the cell populations present in different tissues, (ii) what the main cell populations responding to SIV challenge with cytokine production are and what their distribution pattern in mucosal versus lymphoid tissues is, (iii) whether there are tissue-specific factors that influence virus-host interactions and result in a particular cytokine milieu in a tissue, (iv) how the recruitment of cells is regulated in various tissues and (v) how local factors affect cell migration after virus challenge. Understanding the mechanisms of oral HIV-1 transmission and local host immune responses will be critical in developing pediatric HIV-1 vaccines and immune interventions to prevent pediatric HIV-1 infections and to slow progression to AIDS.

8. The challenges of the infant immune system

In the first few months of life, the relative frequencies, activation, and functional capacity of cell populations undergo continuous changes. These changes are likely to be major factors resulting in reduced immune responses in infants. Thus, at a time when infants are constantly exposed to multiple and new pathogens, their immune responses appear to be characterized by inefficient priming, limited effector function, and even active suppression by regulatory T cells. The understanding of developmental changes in the immune system and their underlying mechanisms, their correlation to disease outcome and differences in pathogenesis between infants and adults will be critical for the design of intervention therapies and vaccines that can be safely administered to infants and provide long-term protection [64–66].

8.1. Infant dendritic cell function

In contrast to adult human dendritic cells (DC), infant DC express lower levels of MHC and adhesion molecules [67,68], their ability to produce IL-12 is reduced due to stricter regulation of the expression of the IL-12p35 unit [69,70], and responses to Toll-like receptor ligation are reduced due to limited expression of adapter proteins and/or the limited activation of downstream signaling molecules [71–76].

Most studies focused on human myeloid DC's as they represent the main antigen-presenting cell type. However, there is evidence demonstrating reduced responsiveness of human infant PDC as well [77]. Our own unpublished observations in the infant SIV infection model show a rapid response of PDC in local lymph nodes draining the oral entry site of SIV suggesting that they are functionally competent. Clearly, more functional studies on infant DC in healthy and SIV infected hosts are needed to assess their role in SIV immunity and how to exploit their function in vaccine design. It has been demonstrated in adults that plasmacytoid and myeloid DC decline in frequency in adult peripheral blood with progression to AIDS, but these studies have not been thoroughly undertaken in pediatric patients. A single study in infected children (mean age 11 years) showed that pDC numbers and function decreased in pediatric patients as well over time of infection with HIV [78].

As a result of decreased functional activity of myeloid infant DC, the priming of the T cell response to infectious pathogens in infants is compromised. However, the fact that IL-12 addition during the priming phase of naïve infant human PBMC results in the production of IFN-g and IL-4 compared to IFN-g only in adult PBMC [79] suggests infant T cell function may also be compromised.

8.2. Infant T cell function

8.2.1. The central role of HIV-specific CD4$^+$T cell responses—Lymphocyte populations in blood and several lymphoid nodes early after birth consist primarily of T cells and only few B cells [53,80]. Among T cells, CD4$^+$T cells can represent up to 80% of all T cells. However, as most of them express a naïve phenotype, the high number of CD4$^+$T cells cannot explain the higher levels of HIV/SIV replication observed in infants [53,80]. The preponderance of activated cells, the preferred targets of HIV/SIV, in mucosal tissues explains the very rapid and often irreversible loss of CD4$^+$T cells at these sites after HIV-1 infection. Thus, the preservation of central memory T cells at mucosal sites seems to be critical in slowing progression to AIDS. Therefore, a thorough analysis of oral and intestinal tissues is absolutely essential to test the efficacy of novel strategies aimed at preventing HIV transmission or at reducing virus replication and preserving immune function in pediatric patients. The important role of CD4$^+$T cells in pediatric HIV-1 pathogenesis has been underlined by a recent report showing that long-term non-progressor (LNTP) children had sustained HIV-1 specific CD4$^+$T cell responses compared to children with lower survival [24].

The efficacy of the HIV/SIV-specific CD4$^+$T cell response depends on adequate immune priming by antigen-presenting cells (APC), as discussed above (7.1), the functional capacity of infant CD4$^+$T cells and the possible suppression of CD4$^+$T cell responses by external factors. Generally, infant CD4$^+$T helper (Th) cell responses are characterized by lower frequencies of antigen-specific T cells, a reduced magnitude of the response, and often a Th2 bias [81–83]. The observed Th2 bias could be a reflection of a different threshold needed for Th cell activation, similar to the high/low dose tolerance phenomena. In fact, it has been documented that lower doses of antigen can elicit effective Th1 responses [83–85]. Alternatively, factors intrinsic to infant CD4$^+$T cells, most importantly the hypermethylation of the interferon gamma (IFN-γ) promoter could increase the activation threshold for infant CD4$^+$T cells [86] and result in reduced Th1 cytokine production. Indeed, pediatric HIV-infected patients show very low IFN-γ and IL-2 responses in the first few months of life [87]. Consistent with the human data, we have shown that rhesus infant peripheral blood CD4$^+$T cells have a reduced capacity to produce IFN-γ after stimulation with polyclonal stimuli compared to adult CD4$^+$T cells [46]. We have further demonstrated that infant SIV-specific CD4$^+$T cell responses are rarely detectable [46], because they are actively suppressed by regulatory T cells (Treg). Thus, multiple factors contribute to the altered T cell response profile observed in infants compared to adults. The role of the newly described CD4$^+$T cells producing IL-17 in infant infection has not been explored.

Thus, reduced functional capacity of infant CD4$^+$T cells seems to be one factor responsible for the altered virological outcome between infant and adult patients. Future studies need to be expanded from peripheral blood to a more thorough tissue analysis. T cell responses in tissues might differ due to the anatomic micromilieu and the activation status of T cells present in tissues.

8.2.2. CD8$^+$T cell immunity in infants—The number of CD8$^+$T cells, as pointed out above, increases in the first few weeks of life. Consistent with relatively poor CD4$^+$T cell responses in HIV-1 infected children, HIV-specific CD8$^+$T cell responses show a clear age-dependence with regard to the frequency, magnitude and quality in pediatric patients [87–91]. Lack of effective CD8$^+$T cell function in HIV-1 infected children is associated with a worse prognosis [92]. Some human studies suggest that CD8$^+$T cells in infants expand less well than adult CD8$^+$T cells, presumably due to the lack of sufficient CD4$^+$T cell help, and therefore only a limited number of effector and memory T cells is generated [90,93]. The sparse data in infant SIV infection models seem to be consistent with these observations. Only low numbers of

memory T cells are generated, and those are often not multifunctional (unpublished observation).

In summary, understanding the influence of T cell function on pathogenesis in the context of immune system development will require the analysis of T cells in different phases of the infection, and/or in distinct anatomic sites. Many of the human studies on infant immune function are predominantly descriptive in nature and it appears imperative to define the functional differences between infant and adult T cells further on the molecular level to design effective strategies aimed at enhancing infant T cell function.

8.3. B cell immunity

Human antibody responses are not fully developed in the first six months of life. In fact, histological analysis of lymph nodes at birth shows that no germinal centers have formed yet. Maternal antibodies that have been passed through the placenta provide protection to the newborn during this time. Therefore, babies born to HIV-1 infected mothers are likely to have circulating HIV-1 specific antibodies [65,94]. The efficacy of these antibodies in preventing transmission is questionable as some babies become infected in the presence of maternal antibodies It is possible that the virus has evolved under the maternal immune pressure and therefore, antibodies are no longer effective against the predominant virus. In addition, only IgG antibodies can pass the placenta, but HIV-1 infection occurs predominantly through mucosal sites. HIV-specific IgG and IgA antibodies are detectable in breast-milk and can be transferred to the nursing infant [95], a finding that has been confirmed in the SIV infection model [96,97]. The question whether the quantity and specificity of IgA can prevent HIV/SIV transmission needs further clarification.

In newborn macaques, numerous studies demonstrated that the passive transfer of SIV-specific antibodies could prevent SIV infection [9,98–104]. Recently, it has been suggested that ADCC is one of the protective mechanisms involved [105]. Studies demonstrating the protective efficacy of a neutralizing antibody cocktail against a broad spectrum of HIV-1 clades are very encouraging and underline the important role antibodies play in preventing HIV-1 transmission [102,106]. A caveat of all these studies is the very high amount of antibody needed to achieve protective efficacy. Although the large-scale production of these antibodies might be difficult and expensive, antibody treatment for a limited time should not be ruled out. Such treatment may allow the immune system to develop up to a critical, yet still undefined, threshold at which a pediatric vaccine or booster vaccine would be more efficacious.

Although the data collected so far are limited, they support the notion that the pediatric SIV infection model can be used to thoroughly characterize the infant immune response in blood and tissues. Importantly, immune responses can be analyzed in different anatomic compartments and correlated to virus dissemination and replication, and in the context of normal changes due to immune system development. The knowledge on how differentiation and maturation of various immune effector cells in relation to age, infection, and organ development alter the overall immune response in an individual may have far reaching applications to multiple pediatric diseases in addition to HIV infection.

9. Pediatric HIV-1 vaccine studies

9.1. Pediatric SIV vaccine studies

The knowledge gained in pathogenesis studies should form the basis for a more rationale vaccine design and for the testing of new intervention therapies. Nonhuman primate infant vaccine studies have demonstrated that passive antibody transfer is effective at preventing oral SIV transmission [103,104]. Similarly, infection of neonatal macaques with live attenuated SIVmac1A11 given orally at birth provides partial protection against oral challenge with

pathogenic SIV in infants [107,108]. The broad and continuous induction of innate and adaptive responses is likely one of the main reasons why live attenuated vaccines, e.g. SIVmac1A11, are superior to other vaccine candidates in providing protective efficacy [107,108]. Several poxvirus vaccine strategies (ALVAC-SIV and MVA-SIV) could not prevent oral SIV infection, but increased the survival time of SIV-infected infants [45,47]. Many of the data obtained in these preclinical trials have since then translated into, or at the least supported the moving forward process of clinical trials in human infants.

9.2. Pediatric HIV vaccine trials

Despite the fact that AIDS is now considered the leading cause of deaths in children under the age of 5 years, support for pediatric HIV vaccine trials has been met with reluctance. Several reasons account for this: (i) an effective HIV vaccine for adults would eventually eliminate MTCT, (ii) ART of HIV-1 infected mothers during pregnancy and administration of nevirapine to babies born to these mothers at birth has significantly reduced MTCT, and (iii) the safety and ethical challenges inherent to these trials.

In the US, two Phase I and one Phase I/II pediatric vaccine studies have been conducted. The Phase I trials PACTG 218 and PACTG 230 tested the safety and immunogenicity of subunit vaccines consisting of the HIV-1 envelope protein gp120. The only Phase I/II trials (PACTG 326) evaluated a prime-boost ALVAC-HIV/gp120 regimen in infants that were born to HIV-1 infected mothers. The evaluation of the data obtained in these trials showed that (i) an accelerated vaccination regimen in infants effectively induces anti-HIV immune responses, and that (ii) a prime-boost regimen induces better T and B cell responses than a subunit vaccine alone [109]. As a result of these observations, based on the safety of the ALVAC-HIV vaccine in adults and preclinical studies in nonhuman primates, the first Phase I clinical trial in newborns of HIV-infected women in Africa (Uganda, HPTN 027) was initiated and closed enrollment in May 2007. These infants received 4 injections of ALVAC-HIV vCP1521 within the first 3 months of life. Similarly, and also based on preclinical studies in nonhuman primates [99,102,104], two clinical trials are now under way in Africa to test the efficacy of (1) HIV antibodies in African children to pregnant mothers in late gestation and to newborns (HIVIGLOB- Uganda), and of (2) monoclonal antibodies to prevent postpartum MTCT (South Africa) [110].

The recent failure of adenovirus-based HIV-1 vaccine trial in adults creates urgency for the development of a pediatric HIV-1 vaccine because another adult HIV-1 vaccine candidate to prevent HIV-1 in women of child-bearing age will not be available for several years.

9.3. Challenges of a pediatric HIV-1 vaccine to prevent breast-milk transmission

Considering that neonatal HIV exposure to breast milk starts shortly after birth and continues for many months to years, a pediatric vaccine would need to be administered early after birth. Maternal antibodies could interfere with the efficacy of a vaccine given within the first few weeks/months of age. The results of the PACTG 326 study, however, demonstrated that anti-HIV antibodies could be induced in infants despite the presence of maternal antibodies.

Further, a pediatric HIV/SIV vaccine must elicit immune responses in tissues of the oral and intestinal mucosa to prevent or reduce viral replication after oral HIV-1 exposure. Thus, for a vaccine to be effective, large numbers of effective T and B cells would have to be present in local mucosal tissues to prevent the early focal virus replication in the first few days after infection and to limit dissemination. This implies, that a pediatric HIV-1 vaccine would have to be administered with an accelerated vaccination regimen. While an accelerated vaccination regimen has been demonstrated to elicit immune responses (see above), it has not been determined how such a regimen affects the quality and quantity of the HIV-1 vaccine-induced

memory responses. Could a booster vaccination too early after the first vaccination in human infants result in increased death of primed cells as shown in mouse models [111–113]? It is encouraging that accelerated Hepatitis A and B virus (HAV and HBV) vaccine schedules (doses given at 1, 4 or 6–8 week intervals) can provide rapid seroprotective immunity as well as memory virus antigen-specific antibody responses in both infants and adults that are non-inferior to antibodies elicited by longer dosing schedules [114–120]. However, few data are available regarding the impact of different immunization intervals on T cell immunity for approved human viral vaccines in children or adults. Thus, it is essential that the relationship between vaccination intervals, memory T cell development and survival will be established in primate infant samples. Maybe, the efficacy of combination strategies, like the simultaneous administration of (i) passive antibodies or (ii) antiretroviral therapy and a vaccine, would be worthwhile testing [121,122]. The passive antibodies and/or ART [7] would be given for several weeks to suppress virus replication until another vaccine boost would be effective in increasing memory anti-HIV responses. In fact, the efficacy of PMPA against oral SIV challenge in infant rhesus macaques has been well established [123]. The observations from the nonhuman primate studies were successfully translated into human treatment and ART now belongs to the routine treatment options in newborns [124].

Alternatively, strategies need to be developed that are aimed at the optimization of DC priming. This could be achieved through the testing of various adjuvants, similar to studies performed by Seder [125,126]. One study actually describes the superiority of a TLR8 agonist in activating pediatric DC [127]. The efficacy of the BCG vaccine, one of the most potent pediatric vaccines in inducing cellular immune responses, has been demonstrated to depend on part on the activation of TLR9 [128,129]. Thus, the inclusion of innate response mechanisms into vaccine design should be tested more thoroughly.

Outlook

This review attempted to provide an overview about our current knowledge about the early immune responses to SIV infection. By no means, has this review been encompassing of every publication or every aspect of the disease. Instead, it tried to raise questions that might be important in our understanding of immune mechanisms unique to infants and how they might influence pediatric HIV-1 pathogenesis. Furthermore, inefficient infant T cell function T has also been described in other infections of infancy and childhood, e.g. RSV and CMV, supporting the idea that immune system development has to be considered in the design of pediatric antiviral intervention strategies [130,131]. Thus, there are unique characteristics to pediatric HIV-1 infection, but we should keep an open mind and look for similarities in other pediatric infectious diseases. Lastly, it should be pointed out that the infant immune system is capable of mounting effective immune responses, but we need to define how to stimulate it in an optimal manner [64]. While the nonhuman primate model certainly has its limitation, this review hopes to show the great value the nonhuman primate model of SIV infection could have for pediatricians involved in HIV-1 research and treatment in the understanding of basic mechanisms, virus-host interactions in multiple anatomic compartments and for the testing of novel concepts and strategies.

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