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Molecular mechanisms of HIV-1 mother-to-child transmission and infection in neonatal target cells

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Abstract

HIV-1 mother-to-child transmission (MTCT) occurs mainly at three stages, including prepartum, intrapartum and postpartum. Several maternal factors, including low CD4+ lymphocyte counts, high viral load, immune response, advanced disease status, smoking and abusing drugs have been implicated in an increased risk of HIV-1 MTCT. While use of antiretroviral therapy (ART) during pregnancy has significantly reduced the rate of MTCT, selective transmission of ART resistant mutants has been reported. Based on HIV-1 sequence comparison, the maternal HIV-1 minor genotypes with R5 phenotypes are predominantly transmitted to their infants and initially maintained in the infants with the same properties. Several HIV-1 structural, regulatory and accessory genes were highly conserved following MTCT. In addition, HIV-1 sequences from non-transmitting mothers are less heterogeneous compared with transmitting mothers, suggesting that a higher level of viral heterogeneity influences MTCT. Analysis of the immunologically relevant epitopes showed that variants evolved to escape the immune response that influenced HIV-1 MTCT. Several cytotoxic T lymphocyte (CTL) epitopes were identified in various HIV-1 genes that were conserved in HIV-1 mother-infant sequences, suggesting a role in MTCT. We have shown that HIV-1 replicates more efficiently in neonatal T-lymphocytes and monocytes/macrophages compared with adult cells, and this differential replication is influenced at the level of HIV-1 gene expression, which was due to differential expression of host factors, including transcriptional activators, signal transducers and cytokines in neonatal than adult cells. In addition, HIV-1 integration occurs in more actively transcribed genes in neonatal compared with adult cells, which may influence HIV-1 gene expression. The increased HIV-1 gene expression and replication in neonatal target cells contribute to a higher viral load and more rapid disease progression in neonates/infants than adults. These findings may identify targets, viral and host, for developing strategies for HIV-1 prevention and treatment.

Keywords

HIV-1 mother-to-child transmission; pediatric AIDS; HIV-1 genotypes and phenotypes; HIV-1 features associated with and lack of vertical transmission; viral load; HIV disease progression; HIV-1 replication in neonatal mononuclear cells; host factors

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Introduction

Mother-to-child transmission (MTCT) of HIV-1 occurs at a rate of 30% without any antiretroviral treatment and accounts for 90% of all HIV-1 infections in children (Ahmad, 2005; Ahmad, 2008a). While antiretroviral therapy in HIV-infected pregnant women has significantly reduced the rate of MTCT in developed countries, HIV-1 infection in children is still a major concern because approximately 500,000 new HIV-1 infected infants are born every year worldwide. In addition, more women in childbearing age group continue to be infected with HIV-1 worldwide increasing the risk of MTCT. More importantly, HIV-1 infected infants born to these infected mothers develop a higher viral load and progress to AIDS more rapidly than infected adults and their own infected mothers, including differences seen in clinical manifestations (Little et al., 2007). However, the molecular mechanisms of HIV-1 MTCT and differential infection in neonates/infants remain poorly understood. This article describes the characteristics of HIV-1 associated with and lack of MTCT and molecular mechanisms of differential HIV-1 infection in neonatal and adult target cells.

Overview of HIV-1 mother-to-child transmission

HIV-1 MTCT occurs mainly at three stages: prepartum (transplacental passage), intrapartum (exposure of infants skin and mucus membrane to maternal blood and vaginal secretions), and postpartum (breast milk) (Ahmad, 2005; Ahmad, 2008b). Several studies have demonstrated the infection of placentas or fetuses, including the capability of HIV-1 to pass through an intact placental barrier maintained *ex vivo* (Bawdon et al., 1994). The intrapartum transmission occurs in more than 50% of the cases due to the exposure of maternal blood to the child during labor and passage through birth canal (Kourtis et al., 2006). Postpartum HIV-1 MTCT mainly occurs through breastfeeding, with estimated rate of 14% to 29% and is a major route of MTCT in developing countries (Taha et al., 2009). While antibodies to HIV-1 in breast milk are not protective (Becquart et al., 2000), early development of T-helper cell responses to HIV-1 envelope proteins showed protection. Infants born to HIV-1 infected mothers are evaluated at regular follow-ups up to 3 years before they are declared uninfected based on Center for Disease Control (CDC) guidelines. Usually, a positive PCR on HIV-1 proviral DNA and virus culture in newborns' is considered to be indicative of HIV-1 infection.

While MTCT rate is around 30% without any antiretroviral treatment, viral and/or host factors protect the majority of children against HIV-1 infection. Several maternal parameters, including advanced clinical stages of the mother, low CD4+ lymphocyte counts, maternal immune response to HIV-1, recent infection, high level of circulating HIV-1, and maternal disease progression have been implicated in an increased risk of MTCT of HIV-1 (Ahmad, 2005; Ahmad, 2008b; Petropoulou, Stratigos, and Katsambas, 2006). Several studies indicate that elevated maternal viral load, plasma HIV-1 RNA levels, with different threshold may play an important role in MTCT (Garcia et al., 1999) However, the Ariel Project reported that the risk of transmission increased slightly with a higher viral load, but no threshold value of virus load was identified and that a high maternal viral load is insufficient to fully explain HIV-1 MTCT (Cao et al., 1997).

Several studies have demonstrated a direct association (Devash et al., 1990) or lack of correlation (Parekh et al., 1991) between the presence of maternal antibody against the V3 domain of the envelope protein and a lower rate of HIV-1 MTCT. More importantly, vertically transmitted HIV-1 variants were found to be neutralization resistant to their maternal plasma (Wu et al., 2006). Several other factors such as acute infection during pregnancy, the presence of other sexually transmitted diseases or other chronic infections,

disruption of placental integrity secondary to chorioamnionitis, and smoking have been shown to be associated with MTCT of HIV-1(Report, 1992).

The AIDS Clinical Trials Group recommended that women treated with zidovudine (ZDV) during pregnancy significantly reduces the risk of HIV-1 MTCT, including oral ZDV to the newborn for six weeks(Cooper et al., 1996). A short course of ZDV from 36 weeks' gestation and every 3-hour from onset of labor till delivery reduced MTCT in Thailand(Shaffer et al., 1999). The HIVNET study proved that oral administered of one dose of nevirapine to the mother and neonate could significantly reduce MTCT(Guay et al., 1999). Recently, highly active antiretroviral therapy (HAART) that includes at least three agents in combination of different classes of reverse transcriptase inhibitors and/or protease inhibitor has been found to reduce the MTCT rate to less than 2% (Taha et al., 2009). While use of antiretroviral therapy ART/HAART significantly reduces the risk of MTCT, MTCT of ART and multidrug-resistant HIV-1 has been reported (Johnson et al., 2001).

Characteristics of HIV-1 associated with and lack of mother-to-child transmission

Characterization of the molecular and biological properties of HIV-1 variants that are associated with and lack of MTCT has been performed by our and several other groups, with the idea that the strategies for prevention and treatment should be targeted at the properties of transmitted viruses. We and others have shown that a minor genotype, subtype or variant of maternal virus from a genetically heterogeneous virus population was transmitted to the infant based on HIV-1 envelope gp120 sequences analysis (Ahmad et al., 1995; Contag et al., 1997; Dickover et al., 2001; Mulder-Kampinga et al., 1995; Scarlatti et al., 1993; Wolinsky et al., 1992). The minor HIV-1 genotype predominates initially as a homogeneous population in the infant and then becomes diverse, as the infant grows older. However, transmission of a major or multiple (Dickover et al., 2001; Lamers et al., 1994) HIV-1 genotypes from mother-to-child has also been reported. In addition, selective transmission of minor SIV genotypes from mother-to-child was demonstrated in five macaque mother-child pairs following transplacental transmission (Amedee et al., 1995). Similar observations of selective transmission of minor HIV-1 genotypes have also been found in transmitter-recipient partners involving sexual transmission, including a homogeneous sequence population present initially in the recipients (Zhu et al., 1993).

Several studies have characterized the biological properties of HIV-1 associated with horizontal and vertical transmission and shown that macrophage-tropic and non-syncytium-inducing or R5 HIV-1 are transmitted during sexual (Zhu et al., 1993) or vertical transmission (Matala *et al*, 2001;. These maternal R5 viruses that are transmitted to infants utilize CCR5 chemokine receptor (Matala *et al*, 2001). In addition, the viral phenotype of SIV involved in MTCT was found to be R5 (Amedee et al., 1995). Furthermore, the role of CCR5 in HIV-1 MTCT was investigated and found that infants with two copies of 32 bp deletions in CCR5 were infectable by X4 viruses following vertical transmission. However, in a study of 552 mother-child pairs, no babies were found to be infected who were homozygous for 32 bp deletion for CCR5 (Philpott et al., 1999).

We have also analyzed the characteristics of HIV-1 from non-transmitting mothers (who failed to transmit HIV-1 in the absence of antiretroviral therapy) in the region of *env* V3 region, *gag* p17, *vif* and *vpr* and compared with transmitting mothers' isolates. We found that the coding potential of the envelope ORF, including several patient-specific amino acid motifs and earlier described molecular features across the V3 region were highly conserved in non-transmitting mothers (Matala et al., 2000). However, there was a low degree of viral heterogeneity within each non-transmitting mother's sequence as compared to transmitting

mothers' sequences. In addition, the estimates of genetic diversity of non-transmitting mothers' sequences were significantly lower compared to transmitting mothers' sequences (Matala et al., 2000). The *gag* p17 matrix sequences of HIV-1 were analyzed from three nontransmitting mothers, including multiple deliveries in case of one mother. There was a low degree of heterogeneity of gag p17 matrix sequences in nontransmitting mothers (Hahn and Ahmad, 2001) compared to our previously analyzed mother-infant pairs' sequences. Furthermore, *vif* and *vpr* sequences of non-transmitting mothers were less heterogeneous compared to transmitting mothers' sequences. Our results provide one reasonable explanation for the reduction of MTCT seen with ART/HAART. Since ART/HAART is known to negatively affect virus replication and lower viral load in infected patients, it reduces viral heterogeneity because of the lack of active virus replication. The decrease in viral heterogeneity due to initial ART/HAART therapy may result in reduced HIV-1 MTCT. These data support the notion that a limited genetic diversity of HIV-1 in infected mothers may reduce the risk of MTCT.

Characteristics of HIV-1 genes associated with and lack of mother-to-child transmission

We have characterized several HIV-1 genes, including the structural genes (*gag, pol* and *env*), regulatory (*tat* and *rev*) and accessory (*vif, vpr, vpu* and *nef*) genes from mother-child pairs. Since these genes are essential for viral replication and pathogenesis, molecular analysis should provide relevant information for developing strategies for prevention and treatment. The frequencies of the coding potential of the *env*, *gag* p17 and NC, *pol* RT, *env* (V3 region) and gp41, *tat, rev, vif, vpr, vpu* and *nef* genes from five to seven infected mother-infant pairs following MTCT were 86.2, 92.8, 87.2, 98.4, 84.17, 90.9, 93.9, 89.8, 92.17, 90.12 and 86.2%, respectively (Ahmad et al., 1995; Hahn et al., 1999; Hahn, Ramakrishnan, and Ahmad, 2003; Husain et al., 2001; Ramakrishnan et al., 2005; Ramakrishnan et al., 2006; Yedavalli, Chappey, and Ahmad, 1998; Yedavalli et al., 1998; Yedavalli et al., 2001). There was a low degree of sequence variability of HIV-1 sequences in the regions of *gag* p17 and NC, *pol* RT, *vif* and *vpr* compared to *env* V3 region, gp41, *tat, rev, vpu* and *nef* sequences. In addition, most of the mothers' HIV-1 sequences were more heterogeneous compared to infants' sequences, suggesting selective transmission. The data further suggested that HIV-1 sequences from epidemiologically linked mother-infant pairs were closer than those from epidemiologically unlinked individuals.

The functional domains required for the activity of HIV-1 genes, including Env V3 region and gp41, Gag p17and NC, Pol RT, Tat, Rev, Vif, Vpr, Vpu and Nef were analyzed by comparing mother-infant pairs' deduced amino acid sequences with previously published mutational analysis reports. In addition, the functional domains of HIV-1 *gag* p17, *vif* and *vpr* were analyzed from non-transmitting (NT) mothers, including a mother with multiple deliveries and compared with our previously analyzed transmitting mothers. The V3 loop of our five pairs mother-infant sequences were closer to the macrophage-tropic (R5) clone than lymphotropic (X4) clone (Matala et al., 2001) because the amino acids critical for R5 phenotype, including H at position 275, Y at 283, E or D at 287, T at 313 were mostly present in all our mother-infant V3 loop sequences (Ahmad et al., 1995). Comparison of the motif SIHIGPGRALYTTGEIIGDI from position 274 to 291, generally conserved in macrophage-tropic clones (Hwang et al., 1991; Shioda, Levy, and Cheng-Mayer, 1991), matched best with our mother-infant pair V3 region sequences. The functional domains and motifs required for the transmembrane envelope glycoprotein, Env gp41, activity in the deduced amino acid sequences, including hydrophobic fusion peptide (FP), HR1, HR2, precursor (gp160) cleavage, the four-glycosylation sites and cell signaling were mostly conserved in our mother-infant pairs sequences (Ramakrishnan et al., 2006). In addition, the

T20 target motifs showed a high degree of conservation, which was expected because these patients were never treated with T20 inhibitors. The susceptibility to T20 was influenced by CCR5 coreceptor usage (Reeves et al., 2002), suggesting that T20 might reduce MTCT because CCR5 utilizing R5 viruses are transmitted from mother to infant (Matala et al., 2001).

The Gag p17 matrix functional domains, including the targeting of Gag to the plasma membrane, virus assembly and release, envelope glycoprotein incorporation into virus particle, virus entry and localization of the virus preintegration complex to the nucleus of nondividing cells, were highly conserved in most of our mother-infant sequences (Hahn et al., 1999). Furthermore, KIEEEQN motif (amino acid position 103–109) at a major antibody binding site in Gag p17 were found to be significantly associated with MTCT (Narwa et al., 1996). In contrast, the polymerization site was less conserved and KIEEEQN motif was variable and the C-terminal 6-mer QVSQNY were conserved in nontransmitting mothers' (Hahn and Ahmad, 2001) sequences compared to transmitting mothers' sequences (Hahn et al., 1999). The other *gag* gene analyzed was the nucelocapisd (NC) gene, and the critical residues of the NC zinc fingers were highly conserved, while the basic residues throughout the NC protein displayed variability without overall loss of these basic amino acids (Wellenseil *et al*, 2003). The Gag p6 domains such as viral late domain (two prolines for Tsg101 binding), Vpr binding sites, and AIP1 binding site were all mostly conserved in mother-infant p6 sequences. We also analyzed the functional domains of RT enzyme's reverse transcriptase, DNA polymerase and RNAse H functions, including primer binding, template binding, positioning of template and primer (located in a-HelixH and a-Helix I), connexion subdomain critical for RNAseH activity and replication were mostly conserved in our mother-infant pairs' sequences (Sundaravaradan, Hahn, and Ahmad, 2005).

The functional domains of HIV-1 regulatory (Tat and Rev) were analyzed in our mother-infant pairs' deduced amino acid sequences. The first coding exon of Tat consists of 72 amino acid and contains five functional regions or domains, which are responsible for all major known functions, including binding of Tat to TAR region of nascent RNA, activation domains required for transactivation (amino-terminal, cysteine-rich, and core regions, amino acids 1–47) were conserved in six mother-infant pairs' sequences (Husain et al., 2001). Rev has two main functional domains; an arginine-rich sequence that serves both as the nuclear localization signal and the RNA binding domain and residues 75 to 93 that promote nuclear export through Rev-RRE interaction, were highly conserved in our mother-infant sequences (Ramakrishnan *et al*, 2006).

In HIV-1 accessory Vif, the two cysteines present at positions 114 and 133 are essential for viral infectivity, were present in most of the *vif* sequences. In addition, the domains for protein kinase C phosphorylation, N-myristoylation, cyclic AMP- and cyclic GMP-dependent protein kinase phosphorylation and SLQXLA (serine at position 144 required for phosphorylation) were conserved in mother-infant sequences (Yedavalli *et al,* 1998b). In contrast to a high functional conservation of *vif* genes in transmitting mothers' isolates, we found that there was a low degree of conservation of functional domains of these genes in non-transmitting mothers' (NT) isolates (Yedavalli and Ahmad, 2001). NT *vif* sequences contained stop codons, lack of initiation codons or a substitution of a highly conserved tyrosine to histidine at position 30.

Three domains have been classified in the Vpr protein, which are involved in virion incorporation, oligomerization, nuclear transport, cell cycle arrest and differentiation. These includes oligomerization domain, dipeptide (GC) and putative α-helix for virion incorporation, the HS/FRIG motif related with cytoskeleton function, leucine/isoleucine rich sequence (LR motif from amino acids 60–81) important for nuclear localization and highly

charged amino acids required for cell cycle arrest and differentiation were all highly conserved in our mother-infant sequences (Yedavalli, Chappey, and Ahmad, 1998). In non-transmitting mothers, *vpr* sequences contained either stop codons, lack of initiation codons, a substitution of serine in place of alanine at position 30, arginine in place of glycine at position 75 or sequences a deletion in the C-terminus that were absent in transmitting mothers' (Yedavalli and Ahmad, 2001) and are essential for Vpr function. In conclusion, a low degree of conservation of functional domains and heterogeneity of HIV-1 *vif* and *vpr* genes correlate with lack of MTCT.

Several studies have shown that Vpu can enhance the release of HIV-1 particles as well as degrade CD4 molecule in the endoplasmic reticulum to allow the transport of HIV-1 Env protein to cell surface were all conserved with little variability in our 162 mother-infant *vpu* sequences (Yedavalli et al., 2001). We found that the domains for Nef functions, including the myristoyl acceptor site (G^2), the patch of basic amino acids (residues 4–22), the residues W^{57}, L^{58} and E^{59} that comprise the direct CD4 binding site, the dileucine based endocytosis signal ($E/D^{160}xxxLL^{165}$) that can recruit APs to the cell membrane and enhance internalization of CD4 and MHC-I and motifs important for MHC-I downregulation were conserved in most of the mother-infant *nef* sequences (Hahn, Ramakrishnan, and Ahmad, 2003). In conclusion, these properties of the transmitted viruses should be targeted for HIV-1 MTCT preventive strategies.

Analysis of immunologically relevant epitopes associated with HIV-1 mother-to-child transmission

One of the major challenges in containing HIV-1 is the evasion of the host cytotoxic T-lymphocytes (CTL) response because of mutations in the key epitopes. While the immune responses against HIV-1 are generally effective, generation of HIV-1variants expose CTL to a large number of mutants that impairs the efficacy of the CTL. Escape mutants can arise early or late in HIV-1 infection and can also be transmitted (Goulder et al., 2001). Analysis of immunologically relevant mutations in HIV-1 genes, including Env gp41, Gag NC, Pol RT and Rev associated with MTCT that we have analyzed are summarized.

Two epitope clusters of Env gp41 (residues 770–780 and 835–843) have been described in HIV-1 infected patients over a period of 80 months that showed non-fixation of mutations. In the clones that were analyzed from five HIV-1 infected mother-infant pairs, Ile777 and Val778 were conserved across the board with some changes. These changes in the mother and infant clones suggest that these escape variants evolved to escape immune responses and influence transmission. CTL epitopes were identified within the NC and p6 proteins (http://www.hiv.lanl.gov/content/immunology/ctl_search), including CRAPRKKGC (amino acid (aa) positions 28–36), KEGHQMKDCTERQANF (aa 42–57, recognized by several HLA types), CTERQANFL (aa 50–56, recognized by HLA-B61) in NC protein and GNFLQSRPEPTAPPF (aa 70–84, recognized by several HLA types), KELYPLTSL (aa 105–114, recognized by HLA-B60 and is positioned within the $(LXX)_4$ Vpr binding), YPLTSLRSLF (aa 108–117 recognized by HLA-B7) in p6 protein were conserved in most of the mother-infant clones analyzed (Wellensiek et al., 2006).

Larger numbers of CTL escape variants are found in transmitting mothers as compared to non-transmitting mothers that may influence HIV-1 MTCT (Wilson et al., 1999). Several regions in the RT gene have been shown to elicit strong CTL responses. (http://www.hiv.lanl.gov/content/immunology/ctl_search). These eptitopes include TVLDVGDAY (amino acid [aa] positions 107–115, higly conserved), TAFTIPSI (aa 128–135, an HLA-B51 restricted epitope), AIFQSSMTK (aa 158–166, recognized by several HLA types) and YPGIKVRQL (aa 271–279) that are conserved among known HIV-1

isolates and some epitopes believed to be associated with MTCT (Wilson et al., 1999) were mostly conserved in our mother infant sequences (Sundaravaradan, Hahn, and Ahmad, 2005). In Rev there are three high epitope motif dense regions, namely RTVRLIKLLY (region 14–23 of *rev* exon 1, the multimerization domain), ISERILSTY (region 55–63 of *rev* exon 2, the multimerization domain) and SAEPVPLQLP (region 67–76, the NES). Interestingly, we observed variability within these three CTL epitope clusters, which were mostly patient specific (Ramakrishnan et al., 2005). The possibility exists that these changes give rise to CTL escape variants without compromising on Rev function but influencing MTCT. The CTL epitopes for Nef protein comprise of two immunodominant regions, amino acid positions 66–100 and 118–149 that included SH3 motif, were highly conserved in our mother-infant Nef sequences (Hahn, Ramakrishnan, and Ahmad, 2003). However, a second CTL epitope that is recognized in most HLA backgrounds and includes, in addition to the SH3 binding motif, the downstream α-helix A, displayed variability at positions 83–87 in 5 of the seven mother infant pairs. The B8-restricted epitope (FLKEKGGL) at positions 90–97 that was described to initiate a strong CTL response early in infection, the epitope YFPDWQNYT, positions 120–128, was described in a mother-infant setting (Wilson et al., 1999) were conserved in Nef sequences of mothers and infants (Hahn, Ramakrishnan, and Ahmad, 2003). In addition, Nef-specific CTL was found to be negatively correlated with age, with poor Nef-specific CD8 T cell response in HIV-1 infected children compared with adults (Buseyne et al., 2006).

Mechanisms of HIV-1 infection in neonatal target cells

Neonates and infants infected with HIV-1 have a higher viral load and progress to symptomatic AIDS more rapidly than infected adults and their own infected mothers, including differences seen in clinical manifestations (Little et al., 2007). HIV-1 infected children commonly experiment recurrent bacterial infections, otitis media, sinusitis, viral respiratory infections, bacterial pneumonia, meningitis, lymphocyte interstitial pneumonitis, encephalopathy, neurological, and physical growth deficits. However, the common opportunistic infection in both children and adults is Pneumocystis carnii or *jiroveci* pneumonia. In addition, HIV-1 infected infants have more central nervous system (CNS) disorders than infected adults. In neonates, HIV-1 replication is probably supported in the thymus and the thymic injury from HIV-1 infection may have a profound impact on development of the immune system and populating the immune system with T-cells. The immunologic abnormalities observed in HIV-1 infected infants include a decreased percentage of thymic $CD4^+$ cells, drastic reduction in cortical CD4/CD8 double positive cells, and an increased percentage of $CD8^+$ cells (Koup and Wilson, 1993). In addition, the stromal cells, which support a thymocyte development, are damaged. In the periphery, an inverted ratio of CD4/CD8, an increased quantitative Ig, a decreased *in vitro* response to mitogens/antigens, a decreased CTL response, and a decreased phagocytosis have been reported (Koup and Wilson, 1993). In addition, poor antibody response to vaccination with T-dependent and T-independent antigens, increased production of IL-1β, IL-2, IL-6, and interferon-γ in lymph nodes, and decreased production of IL-2, IL-4, and interferon-γ by CD4 T-cells have been observed in HIV-1 infected children (Koup and Wilson, 1993).

Since the infants' immune system is immature and developing, the immune responses generated against HIV-1 cannot contain the virus (Tiemessen and Kuhn, 2006). Contrary to HIV-1-infected adults where strong CTL responses are associated with reductions in viremia, HIV-1-infected neonates generate HIV-1-specific $CD8^+$-T-cell responses early in life that are not clearly associated with reduction in viremia and improved clinical outcomes (Lohman et al., 2005). In contrast to X4 viruses associated with AIDS progression in adults, rapidly progressing HIV-1-infected infants generally harbor viruses of R5 phenotype that is associated with high viral load (Cao et al., 1997). Since R5 viruses have been shown to be

involved in MTCT (Matala et al., 2001), interaction of R5 viruses with monocytes/macrophages and $CD4^+/CCR5^+$ T-cells may play an important role in pathogenesis of HIV disease. The majority of HIV-1 infected $CD4^+$ T-cells in the blood of most infected infants and children have the memory ($CD45RO^+$) phenotype, despite the relative scarcity of these cells (Sleasman et al., 1996). Infection of naïve ($CD45RA^+$) $CD4^+$ T-cells is associated with a rapid decline in $CD4^+$ T-cells count in infants and children (Zaitseva et al., 1998) and may correlate with deaths as seen in adults. Therefore, elucidation of the molecular mechanisms of HIV-1 infection in neonatal target cells may provide some insights into immunopathogenesis of HIV disease in infants.

We have compared HIV-1 replication kinetics between neonatal and adult blood mononuclear cells and determined the mechanisms of HIV-1 replication in these cell types. We have used cord blood in place of neonatal blood because, like neonatal blood, it has more $CD45RA^+$ T-cells and less $CD45RO^+$ T-cells and is immature compared with adult blood (Mo et al., 1998), and is also available in a larger volume than neonatal blood. We have shown that HIV-1 replicates more efficiently in cord blood MDM and T- lymphocytes compared with adult blood cells isolated from 7 different donors (Sundaravaradan et al., 2006). There was no significant difference in the cell proliferative capabilities, levels of HIV-1 receptor (CD4) and coreceptors (CXCR4 and CCR5) for virus entry, and in the levels of post entry events (reverse transcription and translocation of preintegration complex into the nucleus) of cord vs. adult mononuclear cells (Sundaravaradan et al., 2006). However, there was a significant upregulation in HIV-1 gene expression in cord monocytes/macrophages and T-lymphocytes compared with adult cells, suggesting that the differential HIV-1 replication in cord and adult target cells is regulated at the level of HIV-1 gene expression (Sundaravaradan et al., 2006).

HIV-1 has more target cells available in neonates and infants than adults because neonates and infants have more circulating T-lymphocytes than adults. Furthermore, a higher level of HIV-1 replication in neonatal compared with adult blood T-lymphocytes (Sundaravaradan et al., 2006) would provide a higher viral load in neonates and infants than adults, which may result in a more rapid disease progression in neonates and infants than adults. There are two possibilities to explain the differential HIV-1 replication in neonatal compared to adult blood T-lymphocytes (Sundaravaradan et al., 2006). One such possibility is the ratio of subsets of T-lymphocytes, namely naïve to memory found in cord (9:1) and adult (1:1) blood cells and the other is that there might be different levels of inherent cellular factors in cord vs. adult cells that regulate HIV-1 gene expression and increase HIV-1 replication. We, therefore, compared the replication kinetics of HIV-1 in neonatal (cord) and adult blood naïve ($CD45RA^+$) and memory ($CD45RO^+$) T-lymphocytes from five different donors. We have recently found that an increased HIV-1 replication in cord compared with adult blood T-lymphocytes is not influenced by the variable ratios of naïve to memory T-lymphocytes but regulated at the level of HIV-1 gene expression (Mehta and Ahmad, 2010, unpublished observation).

Several factors, viral and host, could influence this increased viral gene expression, including the site of HIV-1 integration within infected host cells. Integration of the HIV-1 genome into the host DNA is a critical event in the viral lifecycle and could have a profound effect on viral transcription. Recent studies suggest that HIV-1 integrates within active host genes, which would place the host transcription machinery in close proximity to the viral genome resulting in increased transcription, virus production and subsequently a high viremia in infected hosts. Since neonatal immune cells are under immune activation, HIV-1 may integrate into highly expressed genes in neonatal cells compared with adult cells, resulting in increased HIV-1 gene expression and replication in neonatal than adult cells. Moreover, differential expression levels of these factors between neonates and adults may

affect the efficiency and location of HIV-1 integration. Recently, we have characterized 468 HIV-1 integration sites within cord and adult blood T-lymphocytes and monocytes/ macrophages from five donors(Wellensiek et al., 2009). Several functional classes of genes were identified by gene ontology to be over represented, including genes for cellular components, maintenance of intracellular environment, enzyme regulation, cellular metabolism, catalytic activity and cation transport. Numerous potential transcription factor binding sites at the sites of integration were identified. Furthermore, the genes at the site of integration, transcription factors which potentially bind upstream of the HIV-1 promoter and factors that assist HIV-1 integration were found to be expressed at higher levels in cord than adult cells(Wellensiek et al., 2009). Taken together, these results suggest HIV-1 integration occurred in a more actively transcribed genes in neonatal cells compared with adult cells (Wellensiek et al., 2009), which may help explain a higher level of HIV-1 gene expression and replication in neonatal compared with adult cells (Sundaravaradan et al., 2006).

During infection, HIV-1 drives a vast program of host cell factors that redirect cellular machinery and interfere with cellular pathways that are not conducive to viral survival (Copeland, 2005). However, the global effects of HIV-1 infection on host cell gene expression have not been comprehensively addressed and understanding the biochemical changes that occur in HIV-1 infection is important for continuing efforts aimed at countering its effects. We have previously shown a higher level of HIV-1 replication and gene expression in neonatal (cord) blood mononuclear cells (CBMC) compared with adult blood cells (PBMC) (Sundaravaradan et al., 2006), which could be due to differential expression of host factors. We performed the gene expression profile of CBMC and PBMC and found that 8013 genes were expressed at higher levels in CBMC than PBMC and 8028 genes in PBMC than CBMC, including 1181 and 1414 genes upregulated after HIV-1 infection in CBMC and PBMC, respectively (Sundaravaradan et al., 2010). Several transcription factors (NF-κB, E2F, TFIIE, Cdk9, Cyclin T1), signal transducers (STAT3, STAT5A) and cytokines (IL-1β, IL-6, IL-10) were upregulated in CBMC than PBMC, which are known to influence HIV-1 replication. In addition, a repressor of HIV-1 transcription, YY1, was down regulated in CBMC than PBMC and several matrix metalloproteinase (MMP-7, 12, 14) were significantly upregulated in HIV-1 infected CBMC than PBMC (Sundaravaradan et al.). Furthermore, we show that CBMC nuclear extracts interacted more efficiently with HIV-1 LTR cis-acting sequences, including NF-κB, NFAT, AP1 and NF-IL6 compared with PBMC nuclear extracts and retroviral based short hairpin RNA (shRNA) for STAT3 and IL-6 down regulated their own and HIV-1 gene expression, signifying that these factors influenced differential HIV-1 gene expression in CBMC than PBMC (Sundaravaradan et al., ; Sundaravaradan et al., 2010)

HIV-1 infected infants have a higher level of viremia and progress more rapidly to AIDS compared with infected adults, including their own infected mothers. However, the mechanisms behind this differential disease progression remain unclear. An explanation, or at least a partial one, has been that an infant's (neonates) immune system is immature and simply is unable to contain the virus. The other aspect may be the differential interaction of HIV-1 with neonatal and adult immune cells, which was explored in our previous study which showed that HIV-1 replicated more efficiently in cord target cells compared with adult cells, and was influenced at the level of HIV-1 gene expression. This may result in a high viral load and rapid disease progression in neonates and infants. The data presented in our current study shows HIV-1 integration into more highly expressed host genes within cord compared to adult cells may contribute to this increased HIV-1 gene expression and replication in neonatal than adult cells. These results provide novel insights into the mechanisms of differential HIV-1 gene expression, replication and disease progression in neonates and infants compared with adults.

While HIV-1 infected infants have a high viral load and progress to symptomatic AIDS more rapidly than adults, the mechanisms of the differential HIV disease are not known. We have provided some evidence before showing that HIV-1 replicates more efficiently in neonatal than adult cells. We have also presented data that HIV-1 integration into more highly expressed host genes within cord compared to adult cells may contribute to this increased HIV-1 gene expression and replication in neonatal than adult cells (Wellensiek et al., 2009). In addition, we provide additional data that support the notion that differential levels of host factors and their interactions with HIV-1 contribute to higher levels of HIV-1 gene expression and replication in neonatal vs. adult cells (Sundaravaradan et al.). While HIV disease is multifactorial in nature, the new information on the role of host factors provided in this study likely contributes to a better understanding of differential HIV-1 pathogenesis and disease progression in neonates and adults.

Drugs of abuse and HIV-1 mother-to-child transmission and neonatal infection

Drugs of abuse, including cocaine, heroin, opiates, methamphetamine, and others taken during pregnancy increases the risk of HIV-1 MTCT (Bulterys et al., 1997; Rodriguez et al., 1996). These studies have suggested that drugs of aduse modulated immunity and increased incidence of preterm births and therefore increased the risk of HIV-1 MTCT. Several studies have shown that drugs of abuse, including cocaine, heroin, methamphetamine and others enhance HIV-1 replication, modulate immune functions and increase CCR5 expression (Cabral, 2006; Dhillon et al., 2007; Nair et al., 2000). Increased HIV-1 replication and/or increased CCR5 expression due to drugs of abuse likely increase the viral load in infected mothers and influence HIV-1 MTCT. In addition, drugs of absue induced increased level of CCR5 expression would favor R5 HIV-1 replication in infected mothers and influence MTCT, as shown before in our and others studies that R5 HIV-1 is predominantly transmitted from mother to child (Huestis and Choo, 2002; Matala et al., 2001; Wolinsky et al., 1992). Furthermore, exposure of drugs of abuse in utero can have a severe impact on the development of the fetus as well as development of the child (Huestis and Choo, 2002). These drugs of abuse can modulate the developing and immature immune sytem and enhance HIV-1 replication and disease progression in neonates and infants. However, more research should be directed to understand the role of drugs of abuse in HIV-1 MTCT and pathogenesis of HIV-1 infection in neonates and infants.

Conclusion and future prospective

While the use of ART during pregnancy has been significantly reduced the risk of HIV-1 MTCT in developed countries, this treatment is not readily available in developing countries where most of the HIV-1 MTCT cases occur. In addition, MTCT of ART and multi-drug resistant HIV-1 has been reported. There is also a major concern regarding the long-term effect of ART/HHART on the development of uninfected children born to these mothers. Several factors, including drugs of abuse may influence HIV-1 MTCT even in the presence of ART/HAART. Therefore, there is a need to develop better, effective and new antivirals and a preventive vaccine that could prevent HIV-1 MTCT and infection in children. This is only possible if we understand the molecular mechanisms of HIV-1 MTCT and infection in infants, including the properties of transmitted viruses and the role of viral and host factors in transmission and pathogenesis. Several studies have shown a selective transmission of HIV-1 from mother to child, including the biological properties of the transmitted viruses to be R5 utilizing CCR5 coreceptor. We have also characterized several molecular features of HIV-1 in structural, regulatory and accessory genes that may be associated with MTCT. In addition, we have shown that HIV-1 sequences from non-transmitting mothers are less heterogeneous than transmitting mothers, suggesting that viral heterogeneity influences

MTCT. After MTCT of HIV-1, there is a higher viral load and a more rapid disease progression in neonates and infants compared with their own infected mothers and infected adults. We have shown that HIV-1 replicates more efficiently in neonatal T-lymphocytes and MDM compared with adult cells and this differential replication is significantly influenced at the level of HIV-1 gene expression. The increased HIV-1 gene expression in neonatal cells is regulated by the differential levels of host factors and integration of HIV-1 in more actively transcribed genes in neonatal than adult target cells. However, abusing drugs during pregnancy could have different outcomes both for MTCT and disease progression in infected mothers and their infected infants. Research should also focus on the role of drugs of abuse on HIV-1 MTCT and pathogenesis of HIV-1. Furthermore, the molecular and biological properties of HIV-1 associated with MTCT and role of host factors in differential HIV-1 gene expression and replication should be targeted for developing new strategies for prevention and treatment of HIV-1 infection.

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References

Ahmad N. The vertical transmission of human immunodeficiency virus type 1: molecular and biological properties of the virus. Critical Review s in Clinical Laboratory Sciences. 2005; 42(1):1–34.

Ahmad N. Molecular mechanisms of HIV-1 vertical transmission and pathogenesis in infants. Advances in Pharmacology. 2008a; 56:453–508. [PubMed: 18086421]

Ahmad N. Molecular mechansims of HIV-1 vertical transmission and pathogenesis in infants. Advances in Pharmacology. 2008b; 56:453–508. [PubMed: 18086421]

Ahmad N, Baroudy BM, Baker RC, Chappey C. Genetic analysis of human immunodeficiency virus type 1 envelope V3 region isolates from mothers and infants after perinatal transmission. Journal of Virology. 1995; 69(2):1001–1012. [PubMed: 7815476]

Amedee AM, Lacour N, Gierman JL, Martin LN, Clements JE, Bohm R Jr, Harrison RM, Murphey-Corb M. Genotypic selection of simian immunodeficiency virus in macaque infants infected transplacentally. Journal of Virology. 1995; 69(12):7982–90. [PubMed: 7494311]

Bawdon RE, Gravell M, Hamilton R, Sever J, Miller R, Gibbs CJ. Studies on the placental transfer of cell-free human immunodeficiency virus and p24 antigen in an ex vivo human placental model. Journal of the Society for Gynecologic Investigation. 1994; 1(1):45–8. [PubMed: 9419745]

Becquart P, Hocini H, Levy M, Sepou A, Kazatchkine MD, Belec L. Secretory anti-human immunodeficiency virus (HIV) antibodies in colostrum and breast milk are not a major determinant of the protection of early postnatal transmission of HIV. J Infect Dis. 2000; 181(2):532–539. [PubMed: 10669336]

Bulterys M, Landesman S, Burns DN, Rubinstein A, Goedert JJ. Sexual behavior and injection drug use during pregnancy and vertical transmission of HIV-1. Journal of Acquired Immune Deficiency Syndrome and Human Retrovirology. 1997; 15(1):76–82.

Buseyne F, Scott-Algara D, Corre B, Porrot F, Monchatre E, Bellal N, Burgard M, Rouzioux C, Blanche S, Riviere Y. Poor recognition of HIV-1 Nef protein by CD8 T cells from HIV-1-infected children: impact of age. Virology. 2006; 354(2):271–9. [PubMed: 16904156]

Cabral GA. Drugs of abuse, immune modulation, and AIDS. Journal of Neuroimmune Pharmacology. 2006; 1(3):280–295. [PubMed: 18040805]

Cao Y, Krogstad P, Korber BT, Koup RA, Muldoon M, Macken C, Song JL, Jin Z, Zhao JQ, Clapp S, Chen IS, Ho DD, Ammann AJ. Maternal HIV-1 viral load and vertical transmission of infection: the Ariel Project for the prevention of HIV transmission from mother to infant. Nature Medicine. 1997; 3(5):549–552.

Contag CH, Ehrnst A, Duda J, Bohlin AB, Lindgren S, Learn GH, Mullins JI. Mother-to-infant transmission of human immunodeficiency virus type 1 involving five envelope sequence subtypes. Journal of Virology. 1997; 71(2):1292–1300. [PubMed: 8995653]

Cooper ER, Nugent RP, Diaz C, Pitt J, Hanson C, Kalish LA, Mendez H, Zorrilla C, Hershow R, Moye J, Smeriglio V, Fowler MG. After AIDS clinical trial 076: the changing pattern of zidovudine use during pregnancy, and the subsequent reduction in the vertical transmission of human immunodeficiency virus in a cohort of infected women and their infants. Women and Infants Transmission Study Group. Journal of Infectious Disease. 1996; 174(6):1207–11.

Copeland KF. Modulation of HIV-1 transcription by cytokines and chemokines. Mini-Reviews in Medicinal Chemistry. 2005; 5(12):1093–1101. [PubMed: 16375755]

Devash Y, Calvelli TA, Wood DG, Reagan KJ, Rubinstein A. Vertical transmission of human immunodeficiency virus is correlated with the absence of high-affinity/avidity maternal antibodies to the gp120 principal neutralizing domain. Proceedings of the National Academy of Sciences U S A. 1990; 87(9):3445–3449.

Dhillon NK, Williams R, Peng F, Tsai YJ, Dhillon S, Nicolay B, Gadgil M, Kumar A, Buch SJ. Cocaine-mediated enhancement of virus replication in macrophages: implications for human immunodeficiency virus-associated dementia. Journal of Neurovirology. 2007; 13(6):483–495. [PubMed: 18097880]

Dickover RE, Garratty EM, Plaeger S, Bryson YJ. Perinatal transmission of major, minor, and multiple maternal human immunodeficiency virus type 1 variants in utero and intrapartum. Journal of Virology. 2001; 75(5):2194–2203. [PubMed: 11160723]

Garcia PM, Kalish LA, Pitt J, Minkoff H, Quinn TC, Burchett SK, Kornegay J, Jackson B, Moye J, Hanson C, Zorrilla C, Lew JF. Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. Women and Infants Transmission Study Group. The New England Journal of Medicine. 1999; 341(6):394–402. [PubMed: 10432324]

Goulder PJ, Brander C, Tang Y, Tremblay C, Colbert RA, Addo MM, Rosenberg ES, Nguyen T, Allen R, Trocha A, Altfeld M, He S, Bunce M, Funkhouser R, Pelton SI, Burchett SK, McIntosh K, Korber BT, Walker BD. Evolution and transmission of stable CTL escape mutations in HIV infection. Nature. 2001; 412(6844):334–338. [PubMed: 11460164]

Guay LA, Musoke P, Fleming T, Bagenda D, Allen M, Nakabiito C, Sherman J, Bakaki P, Ducar C, Deseyve M, Emel L, Mirochnick M, Fowler MG, Mofenson L, Miotti P, Dransfield K, Bray D, Mmiro F, Jackson JB. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet. 1999; 354(9181):795–802. [PubMed: 10485720]

Hahn T, Ahmad N. Genetic characterization of HIV type 1 gag p17 matrix genes in isolates from infected mothers lacking perinatal transmission. AIDS Research and Human Retroviruses. 2001; 17(17):1673–80. [PubMed: 11779356]

Hahn T, Matala E, Chappey C, Ahmad N. Characterization of mother-infant HIV type 1 gag p17 sequences associated with perinatal transmission. AIDS Research and Human Retroviruses. 1999; 15(10):875–888. [PubMed: 10408724]

Hahn T, Ramakrishnan R, Ahmad N. Evaluation of genetic diversity of human immunodeficiency virus type 1 NEF gene associated with vertical transmission. Journal of Biomedical Sciences. 2003; 10(4):436–50.

Huestis MA, Choo RE. Drug abuse's smallest victims: in utero drug exposure. Forensic Sci Int. 2002; 128(1–2):20–30. [PubMed: 12208017]

Husain M, Hahn T, Yedavalli VR, Ahmad N. Characterization of HIV type 1 tat sequences associated with perinatal transmission. AIDS Research and Human Retroviruses. 2001; 17(8):765–773. [PubMed: 11429117]

Hwang SS, Boyle TJ, Lyerly HK, Cullen BR. Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. Science. 1991; 253(5015):71–74. [PubMed: 1905842]

Johnson VA, Petropoulos CJ, Woods CR, Hazelwood JD, Parkin NT, Hamilton CD, Fiscus SA. Vertical transmission of multidrug-resistant human immunodeficiency virus type 1 (HIV-1) and continued evolution of drug resistance in an HIV-1-infected infant. Journal of Infectious Disease. 2001; 183(11):1688–1693.

Koup, RA.; Wilson, CB. Clinical immunology of HIV-infected children. In: Pizzo, PA.; Wilfert, CM., editors. Pediatric AIDS: The challenge of HIV infection in infants, children, and adolescents. Williams and Wilkins; 1993. p. 158-165.

Kourtis AP, Lee FK, Abrams EJ, Jamieson DJ, Bulterys M. Mother-to-child transmission of HIV-1: timing and implications for prevention. The Lancet Infectious Diseases. 2002; 6(11):726–732. [PubMed: 17067921]

Lamers SL, Sleasman JW, She JX, Barrie KA, Pomeroy SM, Barrett DJ, Goodenow MM. Persistence of multiple maternal genotypes of human immunodeficiency virus type I in infants infected by vertical transmission. Journal of Clinical Investigation. 1994; 93(1):380–390. [PubMed: 8282808]

Little K, Thorne C, Luo C, Bunders M, Ngongo N, McDermott P, Newell ML. Disease progression in children with vertically-acquired HIV infection in sub-Saharan Africa: reviewing the need for HIV treatment. Current HIV Research. 2007; 5(2):139–153. [PubMed: 17346131]

Lohman BL, Slyker JA, Richardson BA, Farquhar C, Mabuka JM, Crudder C, Dong T, Obimbo E, Mbori-Ngacha D, Overbaugh J, Rowland-Jones S, John-Stewart G. 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. Journal of Virology. 2005; 79(13):8121–8130. [PubMed: 15956557]

Matala E, Crandall KA, Baker RC, Ahmad N. Limited heterogeneity of HIV type 1 in infected mothers correlates with lack of vertical transmission. AIDS Research and Human Retroviruses. 2000; 16(15):1481–1489. [PubMed: 11054261]

Matala E, Hahn T, Yedavalli VR, Ahmad N. Biological characterization of HIV type 1 envelope V3 regions from mothers and infants associated with perinatal transmission. AIDS Research and Human Retroviruses. 2001; 17(18):1725–1735. [PubMed: 11788024]

Mo H, Monard S, Pollack H, Ip J, Rochford G, Wu L, Hoxie J, Borkowsky W, Ho DD, Moore JP. Expression patterns of the HIV type 1 coreceptors CCR5 and CXCR4 on CD4+ T cells and monocytes from cord and adult blood. AIDS Research and Human Retroviruses. 1998; 14(7):607–617. [PubMed: 9591715]

Mulder-Kampinga GA, Simonon A, Kuiken CL, Dekker J, Scherpbier HJ, van de Perre P, Boer K, Goudsmit J. Similarity in env and gag genes between genomic RNAs of human immunodeficiency virus type 1 (HIV-1) from mother and infant is unrelated to time of HIV-1 RNA positivity in the child. Journal of Virology. 1995; 69(4):2285–2296. [PubMed: 7884875]

Nair MP, Chadha KC, Hewitt RG, Mahajan S, Sweet A, Schwartz SA. Cocaine differentially modulates chemokine production by mononuclear cells from normal donors and human immunodeficiency virus type 1-infected patients. Clincal and Diagnostic Laboratory Immunology. 2000; 7(1):96–100.

Narwa R, Roques P, Courpotin C, Parnet-Mathieu F, Boussin F, Roane A, Marce D, Lasfargues G, Dormont D. Characterization of human immunodeficiency virus type 1 p17 matrix protein motifs associated with mother-to-child transmission. Journal of Virology. 1996; 70(7):4474–4483. [PubMed: 8676472]

Parekh BS, Shaffer N, Pau CP, Abrams E, Thomas P, Pollack H, Bamji M, Kaul A, Schochetman G, Rogers M, George M. NYC, Collaborative Group. Lack of correlation between maternal antibodies to V3 loop peptides of gp120 and perinatal HIV-1 transmission. The NYC Perinatal HIV Transmission Collaborative Study. AIDS. 1991; 5(10):1179–1184. [PubMed: 1786145]

Petropoulou H, Stratigos AJ, Katsambas AD. Human immunodeficiency virus infection and pregnancy. Clincal Dermatology. 2006; 24(6):536–542.

Philpott S, Burger H, Charbonneau T, Grimson R, Vermund SH, Visosky A, Nachman S, Kovacs A, Tropper P, Frey H, Weiser B. CCR5 genotype and resistance to vertical transmission of HIV-1. Journal of Acquiried Immune Deficiency Syndrome and Human Retrovirology. 1999; 21(3):189–193.

Ramakrishnan R, Hussain M, Holzer A, Mehta R, Sundaravaradan V, Ahmad N. Evaluations of HIV type 1 rev gene diversity and functional domains following perinatal transmission. AIDS Research and Human Retroviruses. 2005; 21(12):1035–1045. [PubMed: 16379607]

Ramakrishnan R, Mehta R, Sundaravaradan V, Davis T, Ahmad N. Characterization of HIV-1 envelope gp41 genetic diversity and functional domains following perinatal transmission. Retrovirology. 2006; 3:42. [PubMed: 16820061]

Reeves JD, Gallo SA, Ahmad N, Miamidian JL, Harvey PE, Sharron M, Pohlmann S, Sfakianos JN, Derdeyn CA, Blumenthal R, Hunter E, Doms RW. Sensitivity of HIV-1 to entry inhibitors correlates with envelope/coreceptor affinity, receptor density, and fusion kinetics. Proc Natl Acad Sci U S A. 2002; 99(25):16249–54. [PubMed: 12444251]

Report. Report of a Consensus Workshop, Siena, Italy, January 17–18, 1992. Maternal factors involved in mother-to-child transmission of HIV-1. J Acquir Immune Defic Syndr. 1992; 5(10): 1019–29. [PubMed: 1453317]

Rodriguez EM, Mofenson LM, Chang BH, Rich KC, Fowler MG, Smeriglio V, Landesman S, Fox HE, Diaz C, Green K, Hanson IC. Association of maternal drug use during pregnancy with maternal HIV culture positivity and perinatal HIV transmission. AIDS. 1996; 10(3):273–282. [PubMed: 8882667]

Scarlatti G, Leitner T, Halapi E, Wahlberg J, Marchisio P, Clerici-Schoeller MA, Wigzell H, Fenyo EM, Albert J, Uhlen M, Rossi P. Comparison of variable region 3 sequences of human immunodeficiency virus type 1 from infected children with the RNA and DNA sequences of the virus populations of their mothers. Proceedings of the National Academcy of Sciencs, U S A. 1993; 90(5):1721–1725.

Shaffer N, Chuachoowong R, Mock PA, Bhadrakom C, Siriwasin W, Young NL, Chotpitayasunondh T, Chearskul S, Roongpisuthipong A, Chinayon P, Karon J, Mastro TD, Simonds RJ. Short-course zidovudine for perinatal HIV-1 transmission in Bangkok, Thailand: a randomised controlled trial. Bangkok Collaborative Perinatal HIV Transmission Study Group. Lancet. 1999; 353(9155):773–780. [PubMed: 10459957]

Shioda T, Levy JA, Cheng-Mayer C. Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene. Nature. 1991; 349(6305):167–169. [PubMed: 1986308]

Sleasman JW, Aleixo LF, Morton A, Skoda-Smith S, Goodenow MM. CD4+ memory T cells are the predominant population of HIV-1-infected lymphocytes in neonates and children. AIDS. 1996; 10(13):1477–1484. [PubMed: 8931781]

Sundaravaradan V, Hahn T, Ahmad N. Conservation of functional domains and limited heterogeneity of HIV-1 reverse transcriptase gene following vertical transmission. Retrovirology. 2005; 2(36)

Sundaravaradan V, Mehta R, Harris DT, Zack JA, Ahmad N. Differential expression and interaction of host factors augment HIV-1 gene expression in neonatal mononuclear cells. Virology. 2010; 400(1):32–43. [PubMed: 20138641]

Sundaravaradan V, Saxena SK, Ramakrishnan R, Yedavalli VRK, Harris DT, Ahmad N. Differential HIV-1 replication in neonatal and adult blood mononuclear cells is influenced at the level of HIV-1 gene expression. Proceedings of the National Academy of Sciences, U S A. 2006; 103(31): 11701–11706.

Taha TE, Kumwenda J, Cole SR, Hoover DR, Kafulafula G, Fowler MG, Thigpen MC, Li Q, Kumwenda NI, Mofenson L. Postnatal HIV-1 transmission after cessation of infant extended antiretroviral prophylaxis and effect of maternal highly active antiretroviral therapy. Journal of Infectious Disease. 2009; 200(10):1490–7.

Tiemessen CT, Kuhn L. Immune pathogenesis of pediatric HIV-1 infection. Current HIV/AIDS Reports. 2006; 3(1):13–9. [PubMed: 16522254]

Wellensiek BP, Ramakrishnan R, Sundaravaradan V, Mehta R, Harris DT, Ahmad N. Differential HIV-1 integration targets more actively transcribed host genes in neonatal than adult blood mononuclear cells. Virology. 2009; 385(1):28–38. [PubMed: 19100594]

Wellensiek BP, Sundaravaradan V, Ramakrishnan R, Ahmad N. Molecular characterization of the HIV-1 gag nucleocapsid gene associated with vertical transmission. Retrovirology. 2006; 3(21)

Wilson CC, Brown RC, Korber BT, Wilkes BM, Ruhl DJ, Sakamoto D, Kunstman K, Luzuriaga K, Hanson IC, Widmayer SM, Wiznia A, Clapp S, Ammann AJ, Koup RA, Wolinsky SM, Walker BD. Frequent detection of escape from cytotoxic T-lymphocyte recognition in perinatal human immunodeficiency virus (HIV) type 1 transmission: the ariel project for the prevention of transmission of HIV from mother to infant. Journal of Virology. 1999; 73(5):3975–85. [PubMed: 10196293]

Wolinsky SM, Wike CM, Korber BT, Hutto C, Parks WP, Rosenblum LL, Kunstman KJ, Furtado MR, Munoz JL. Selective transmission of human immunodeficiency virus type-1 variants from mothers to infants. Science. 1992; 255(5048):1134–1137. [PubMed: 1546316]

Wu X, Parast AB, Richardson BA, Nduati R, John-Stewart G, Mbori-Ngacha D, Rainwater SM, Overbaugh J. Neutralization escape variants of human immunodeficiency virus type 1 are transmitted from mother to infant. Journal of Virology. 2006; 80(2):835–44. [PubMed: 16378985]

Yedavalli VR, Ahmad N. Low conservation of functional domains of HIV type 1 vif and vpr genes in infected mothers correlates with lack of vertical transmission. AIDS Research and Human Retroviruses. 2001; 17(10):911–923. [PubMed: 11461677]

Yedavalli VR, Chappey C, Ahmad N. Maintenance of an intact human immunodeficiency virus type 1 vpr gene following mother-to-infant transmission. Journal of Virology. 1998; 72(8):6937–6943. [PubMed: 9658150]

Yedavalli VR, Chappey C, Matala E, Ahmad N. Conservation of an intact vif gene of human immunodeficiency virus type 1 during maternal-fetal transmission. Journal of Virology. 1998; 72(2):1092–1102. [PubMed: 9445004]

Yedavalli VR, Husain M, Horodner A, Ahmad N. Molecular characterization of HIV type 1 vpu genes from mothers and infants after perinatal transmission. AIDS Research and Human Retroviruses. 2001; 17(11):1089–98. [PubMed: 11485627]

Zaitseva MB, Lee S, Rabin RL, Tiffany HL, Farber JM, Peden KW, Murphy PM, Golding H. CXCR4 and CCR5 on human thymocytes: biological function and role in HIV-1 infection. Journal of Immunology. 1998; 161(6):3103–3113.

Zhu T, Mo H, Wang N, Nam DS, Cao Y, Koup RA, Ho DD. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science. 1993; 261(5125):1179–1181. [PubMed: 8356453]