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Envelope glycoproteins of Human Immunodeficiency Virus type 1 variants issued from mother–infant pairs display a wide spectrum of biological properties

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ABSTRACT

Several studies have shown that the early virus population present in HIV-1 infected infants usually is homogeneous when compared to the highly diversified viral population present at delivery in their mothers. We explored the antigenic and functional properties of pseudotyped viruses expressing gp120 encoded by *env* clones issued from four mother–infant pairs infected by CRF01_AE viruses. We compared their sensitivity to neutralization and to entry inhibitors, their infectivity levels and the Env processing and incorporation levels. We found that both transmitted viruses present in infants and the variants present in their chronically infected mothers display a wide spectrum of biological properties that could not distinguish between them. In contrast, we found that all the transmitted viruses in the infants were more sensitive to neutralization by PG9 and PG16 than the maternal variants, an observation that may have implications for the development of prophylactic strategies to prevent mother-to-child transmission.

Introduction

Mother-to-child transmission (MTCT) is the leading source of human immunodeficiency virus (HIV) infection in children. In the absence of anti-retroviral prophylaxis, transmission can occur during pregnancy (*in utero*), during labor and delivery (intrapartum), or postnatally through breastfeeding (Scarlatti, 2004). Although infants have been found occasionally to be infected by a heterogeneous population of multiple maternal viral variants, molecular studies of MTCT have shown that, despite a heterogeneous viral population in the mother, homogeneous viral variants are generally transmitted to the infant, suggesting the selection of a limited number of maternal viral variants for establishment of a new infection in the infant (Ahmad et al., 1995; Dickover et al., 2001; Kishko et al., 2011; Lamers et al., 1994; Pasquier et al., 1998; Russell et al., 2011; Samleerat et al., 2008; Scarlatti et al., 1993b; Verhofstede et al., 2003; Wolinsky et al., 1992; Zhang et al., 2010b).

Maternal neutralizing antibodies (Nabs) are among the selective factors that are potentially responsible for this genetic bottleneck. Maternal antibodies of the IgG class cross the placenta into the fetal bloodstream, reaching high levels in the fetus at the end of pregnancy and protecting the infant against infection by numerous pathogens (Englund et al., 1998; Safrit et al., 2004). Therefore, MTCT of HIV-1 provides a model for studying the role of passively acquired antibodies present in the infant prior to virus exposure. However, reported studies have yielded conflicting results. Some studies have suggested a role of maternal Nabs in reducing MTCT, showing that non-transmitting mothers had more frequently detected or higher Nab responses than mothers who transmitted the virus to their infant (Barin et al., 2006; Bongertz et al., 2002; Lathey et al., 1999; Samleerat et al., 2009; Scarlatti et al., 1993a), or that viruses transmitted to infants are escape variants resistant to autologous maternal serum (Dickover et al., 2006; Wu et al., 2006; Zhang et al., 2010a). In contrast, others did not observe any difference neither in breadth or potency of neutralizing antibodies between sera from transmitting and non-transmitting mothers (Guevara et al., 2002; Hengel et al., 1998; Husson et al., 1995; Russell et al., 2011), nor in the sensitivity to neutralization between transmitted infant variants and maternal variants (Kishko et al., 2011; Russell et al., 2011). In addition, a recent study exploring the role of passively acquired HIV antibodies in exposed infants during

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breastfeeding suggested that the breadth and potency of the heterologous antibody response does not predict protection (Lynch et al., 2011).

Very few studies have been done focusing on the viral characteristics associated with MTCT, others than neutralization sensitivity. A correlation between HIV-1 transmission to infants and replicative fitness of transmitted viruses was suggested (Kong et al., 2008) but not confirmed (Kishko et al., 2011). Independently, several studies performed mainly on HIV-1 strains of subtypes A and C suggested that variants with shorter variable loops lengths and fewer potential N-linked glycosylation sites (PNGS) encoded by their *env* gene were selected during MTCT (Russell et al., 2011; Wu et al., 2006; Zhang et al., 2010b). In contrast, we and others did not observe these characteristics among *env* genes from mother–infant pairs infected with variants of B and CRF01_AE clades (Kishko et al., 2011; Samleerat et al., 2008). These discordant results may suggest that, similarly to what was observed during horizontal transmission, molecular properties linked to transmissibility could be subtype-specific (Chohan et al., 2005; Derdeyn et al., 2004; Frost et al., 2005). Nevertheless, in our study performed on CRF01-AE *env* variants of mother–infant pairs, we found that two PNGS, N301 in V3 and N384 in C3, were conserved in almost all infants' variants but were not uniformly present in variants from mothers. We hypothesized that these two PNGS may confer a selective advantage for transmission of the virus to the infants (Samleerat et al., 2008).

In the present study, we compared the biological properties of the virus conferred by the envelope of maternal and infant viral variants issued from four mother–infant pairs infected by HIV-1 of the CRF01_AE clade, in order to explore their association with the restrictive transmission of the virus. A better understanding of antigenic and functional properties of transmitted viral variants may help to the development of vaccines or improved prophylactic strategies to prevent MTCT.

Results

HIV-1 mother–infant pairs (MIPs)

We selected HIV-1 CRF01_AE *env* sequences (V1 to V5 region of gp120) from four previously described MIPs [0377, 0858, 0978 and 1021; (Samleerat et al., 2008)]. Maternal *env* sequences were obtained from peripheral blood samples collected at delivery and infant *env* sequences from plasma samples obtained at the first time point at which the HIV-1 DNA PCR results was positive. One infant (0858) was positive at birth for HIV-1 DNA, indicating that he was infected *in utero* (Table 1). The three remaining infants (0377, 0978 and 1021) were HIV-1 DNA negative at birth but were found positive at 71, 55 and 67 days after birth, respectively. Because of these 3 infants were not breastfed, HIV-1 transmission occurred during delivery (intrapartum). Thirty-seven clones (9 from MIP 0377, 11 from MIP 0858, 12 from MIP 0978 and 5 from MIP 1021) were selected based on the fact that they were representative of the diversity of the variants present in the mothers and their babies, and that they possessed or not the PNGS at positions N301 and N384 (Fig. 1). Chimeric *env* genes were constructed by insertion of the V1 to V5 *env* fragment in a NL4.3 *env* backbone as previously described (Braibant et al., 2010), and the 37 corresponding Env-pseudotyped viruses were generated. Seventeen of them were infectious in TZM-bl cells: 6 from MIP 0377, 2 from MIP 0858, 6 from MIP 0978 and 3 from MIP 1021 (Table 2, Fig. 1).

Table 1
Characteristics of mother–infant pairs.

MIP	Subject	First positive	Transmission	Number clones selected	Number clones infectious
0377	Mother	–		8	5
	Infant	71 days	ip	1	1
0978	Mother	–		7	3
	Infant	55 days	ip	5	3
1021	Mother	–		2	1
	Infant	67 days	ip	3	2
0858	Mother	–		6	2
	Infant	at birth	iu	5	0

ip: intrapartum.
iu: *in utero*.

Sensitivity to neutralization by maternal plasma

We determined the sensitivity to neutralization of mother- and infant-derived Env-pseudotyped viruses with the maternal plasma collected during pregnancy just before the initiation of zidovudine (ZDV) prophylaxis, 4 to 11 weeks before delivery. Maternal variants issued from MIPs 0377, 0978 and 1021 were relatively resistant to autologous neutralization. Indeed, 0377 maternal clones presented a low sensitivity to neutralization (IC_{50} range: <20–103.1) and 0978 and 1021 maternal clones were particularly resistant to autologous antibodies, failing to reach 50% neutralization even using a 1:20 dilution of plasma, the highest plasma concentration tested (Table 2). Infant variants were also generally resistant to maternal plasma (IC_{50} range: <20–28.7), except a single clone from pair 0978, clone 0978-I2, which on contrary, displayed a high sensitivity to maternal plasma (IC_{50}: 883; Table 2). In contrast, the two maternal clones from MIP 0858, presented a high neutralization-sensitivity to maternal autologous plasma with IC_{50} values of 1197 and 1642. However, due to the lack of infectious infant clones for this pair, we were not able to compare their susceptibility to the transmitted variant(s). When the four pairs were considered together, we did not observe any difference in sensitivity to autologous plasmas between mother and infant variants ($P=0.38$, mixed model test; Fig. 2A).

Sensitivity to neutralization by heterologous sera

We investigated the sensitivity of maternal and infant variants to neutralization by a pool of ten heterologous broadly neutralizing sera selected from patients infected by CRF01_AE viruses in a previous study (Samleerat et al., 2009). The seventeen clones presented a broad and continuous range of sensitivity to heterologous antibodies (IC_{50} range: 123–5543; Table 2). Among MIP 0377, the infant clone presented a higher neutralization-sensitivity (IC_{50}: 1056) compared to the corresponding maternal clones (IC_{50} range: 123–339). On the contrary, maternal clones from pairs 0978 and 1021 presented similar sensitivity to heterologous neutralization (0978 IC_{50} range: 626–5543, 1021 IC_{50}: 990) compared to infants clones (0978 IC_{50} range: 2277–4150; 1021 IC_{50}: 585 and 746). When the four pairs were considered together, we did not observe any difference in sensitivity to heterologous plasmas between mother and infant variants ($P=0.97$, mixed model test; Fig. 2B).

Sensitivity to neutralization by monoclonal antibodies

We tested the sensitivity of our pseudotyped viruses to neutralization by the broadly neutralizing human monoclonal antibodies (mAbs) b12, PG9 and PG16. b12 is directed against an epitope overlapping the CD4-binding site (CD4BS) (Burton et al., 1994; Saphire et al., 2001), whereas PG9 and PG16 recognize a quaternary neutralizing epitope formed from conserved regions of V1/V2 and V3 variable loops (Pancera et al., 2010; Walker et al., 2009). All maternal and infant clones from MIPs 0377, 0978 and 1021, displayed a high level of resistance to neutralization by mAb b12 ($IC_{50}>50$ μg/mL), whereas the two maternal clones from pair 0858 were highly sensitive to b12 ($IC_{50}<0.1$ μg/mL; Table 2). On the contrary, we observed more heterogeneous results for PG9 and PG16 neutralization. Maternal clones issued from MIPs 0377, 0978 and 1021 displayed a broad and continuous range of sensitivity to both PG9 (IC_{50} range: 0.07 to