Molecular characteristics of the HIV-1 envelope glycoproteins of CRF01_AE variants transmitted from mother to child

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Background

Mother-to-child transmission (MTCT) of HIV

- The mother to child transmission is the predominant mode of pediatric HIV infection.

- MTCT is when an infected mother passes the virus to her baby. This can occur during:
  - pregnancy especially in the last trimester (in utero)
  - labour and delivery (intrapartum)
  - after birth through breastfeeding
Background

- Heterogeneous virus population with highly diverse envelopes is usually detected in the infected mothers, compared with a more homogeneous virus population in the infant (Wolinsky et al. 1992, Dickover et al. 2001, Verhofstede et al. 2003).

- This indicates the presence of selective pressure in which a limited number of maternal variants are selected to establish a new infection in the infants.

- In MTCT setting, maternal antibody could play a role in limiting transmission of neutralization-sensitive variants. The infant viral isolates are resistant to neutralization by maternal plasma (Kliks et al. 1994, Dickover et al. 2006).

→ **What are the molecular characteristics of the viral envelope that might be associated with a selective advantage allowing transmission to the infant?**
Background

Molecular characteristics of HIV-1 envelope

- Most extensive molecular studies were performed in populations infected by subtypes B (Dickover et al. 2006) or A (Verhofstede et al. 2003), or A, C and recombinant forms (Wu et al. 2006).

  ➔ A population infected with CRF01_AE isolates in Thailand

- Only a few studies analyzed the entire gp120 gene (others analyzed V3 region because identified earlier as the main target for neutralizing antibody).

  ➔ Entire gp120 (V1-V5)

- Some studies have suggested that timing of transmission (in utero or intrapartum) might influence the selection of the transmitted variants, however, it remains unclear.

  ➔ The timing of transmission (in utero or intrapartum) was known.
  ➔ The study was performed blinded to the timing of transmission status of the mother-child pairs.
Population

- Perinatal HIV Prevention Trial: PHPT-1 study – a clinical trial assessing various ZDV treatment durations for the prevention of MTCT in Thailand (Lallemand et al., 2000). The infants were not breastfed.

- 17 mother-child pairs: 6 infants infected in utero, 11 infants infected intrapartum

- Maternal PBMC at the time of delivery and infant’s plasma at the first-positive-time-point for HIV-1 DNA PCR were used.
  - Proviral DNA from the mothers
    - to obtain more complete information concerning the viral population that evolved during their infection.
    - HIV RNA was difficult to amplify from maternal samples collected at delivery due to ZDV prophylaxis (low viral load).
  - Viral RNA from the infants
    - to obtain information concerning the viral variants that were successfully transmitted.
Methods

Sequences present internal stop codons were removed.

353 sequences from 17 mother-child pairs
175 from mothers (mean: 10.3; 4-15)
178 from infants (mean: 10.5; 6-15)

Nested-PCR using specific primers for CRF01_AE
Cloning

10-18 clones per sample

Sequencing

- Sequences present internal stop codons were removed.
- 353 sequences from 17 mother-child pairs
  - 175 from mothers (mean: 10.3; 4-15)
  - 178 from infants (mean: 10.5; 6-15)
Unrooted neighbor-joining tree of 353 HIV-1 env gp120 nucleotide sequences for 17 mother-child pairs

- All were infected with CRF01_AE strains.

- Env nucleotide sequences of the epidemiologically linked mother-child pairs were closer than those of epidemiologically unlinked individuals.

Inter-pair genetic distance median: 14.5%

Intra-pair genetic distance median: 3.1% (P<0.01)
14 trees support the transmission of a single maternal variant

3 trees support the transmission of multiple maternal variants

P2

mo = 3.6
in = 0.6

P3

mo = 2.4
in = 0.6

P14

mo = 4.8
in = 0.8

P7

mo = 3.0
in = 2.9

P1

mo = 0.8
in = 0.7

P17

mo = 0.9
in = 0.8

infant sequence

mother sequence
Comparing genetic diversity in V1-V5 sequence between mothers and infants

- The lower diversity of infant’ sequences compared to maternal sequences indicates the transmission of limited maternal viral variants.
- No significant difference between mothers or infants’ sequences depending of timing of transmission, in utero or intrapartum.
Comparing V1-V5 length and number of potential N-linked glycosylation sites (PNGS)

Several studies of heterosexually transmitted viruses collected early after infection suggested that they have fewer PNGS and shorter variable loop sequences than later isolates, depending of subtype (Derdeyn et al. 2004, Chohan et al. 2005, Frost et al. 2005, Li et al. 2006).

Are these properties also associated with a selective advantage in MTCT context?
Comparing V1-V5 length and number of potential N-linked glycosylation sites (PNGS)

No significant difference in V1-V5 length between:

• mothers and infants
• the timing of transmission

No significant difference when each variable or constant region of gp120 were analyzed separately.
We performed amino acids alignment and numbering according to the CRF01_AE consensus sequence obtained from the HIV database.

We noted each position where a PNGS was found at least in a single individual.

We calculated the frequency of PNGS at each position in each subject.

**Blue** for highly conserve PNGS position (≥80%)

**Yellow** for 79 - 50%

**White** for ≤49%
PNGS positions at position N301 in V3 was found in all maternally transmitted CRF01_AE virus that were sequenced in an independent study in Thailand (Sutthent et al., 1998).

In *in vitro* studies, N301 was also associated with both reduced neutralization sensitivity of HIV-1 to CD4BS antibodies and modification of the interaction of the envelope with CD4 and chemokine receptors (Malenbaum et al., 2000, Kiszaka et al., 2002, Koch et al., 2003).
Evidence for selective transmission of a recombinant virus in pair P9
Conclusion (1)

- We have analyzed the gp120 env sequences (V1-V5) in a homogeneous population of 17 mother-child pairs infected by a single HIV-1 clade, CRF01_AE.

- Our results confirm the transmission of a genetically-restricted viral population in the setting of MTCT, but without any difference in timing of transmission, either in utero or intrapartum.

- We observed no difference between maternal and infant viruses both in length of V1-V5 and number of PNGS (even when focusing on each region).
Conclusion (2)

- PNGS at position N241, N301, N354, N384 were found in almost all the sequences from infant’s viruses, but less frequency in the maternal viruses.

- This could suggest that these PNGS particularly N301 might be associated with a selective advantage, at least for CRF01_AE viruses.

- One infant was infected with a single viral population issued from recombination between maternal variants.
Conclusion (3)

- Although our findings need to be confirmed by functional studies with pseudotyped viruses (in progress), they provide additional evidences for the role of the “glycan shield” in the biology of HIV to escape immunity.

- Dissecting the characteristics of viruses transmitted to infant in the setting of MTCT where exposure to the virus occurs in the presence of passively transferred antibodies could help find ways to prevent HIV infection.
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