



Rapid Progression of HIV-1 Infection in Infants Associated with CCR-5 and not CXCR-4 Co-receptor Usage.

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ABSTRACT. The mean time from infection to first AIDS defining illness in adults is ten years but 20% of children infected through materno-fetal transmission present with AIDS or die within the first year of life. CXCR-4 co-receptor using viruses are associated with later stages of infection and more advanced disease in adults and children. Selection for CXCR-4 viruses by passage across trophoblasts has been reported *in vitro* (Lagaye *et al* 2001). We investigated co-receptor usage in children presenting with symptomatic HIV infection in the first year of life.

Methods. HIV-1 was isolated from cryopreserved PBMCs from 14 infants by co-cultivation with negatively selected PHA-activated CD4+ lymphocytes. HIV-1 co-receptor usage was determined by 4-5 days culture with U87-CD4 cells stably expressing the chemokine receptors CCR-1, CCR-3, CCR-5 or CXCR-4 with 128 – 1089 TCID of each primary isolate. Infection was detected by focal immunoassay. A 1.2 kb fragment of *env* was amplified from DNA extracted from PBMCs, before and after co-culture and sequenced. Assignment of the speculative biological phenotype was made according to the charge of amino acid residues at positions 11 and 25 in V3. **Results.** Co-receptor usage was identified for 11 of the 14 viral isolates. All children were infected with non-B clade viruses that utilized the CCR-5 co-receptor. Four of the children have died, the remainder are on combination anti-retroviral therapy.

Conclusions. These data are consistent with the view that the placenta is an efficient barrier to HIV-1 transmission. Transmission of maternal viruses, which are likely to use CCR-5, may occur when the barrier is breached or by-passed. The rapid advance of HIV infection in these infants was not due to CXCR-4 co-receptor usage. Should compounds that block CCR-5 co-receptors become clinically useful and not select for virus which uses CXCR-4, these data suggest that they might also be useful for the prevention of mother-infant transmission and for the treatment of infected infants.

Reference:
Lagaye S *et al*, *J Virol* 2001;75:4781-91

INTRODUCTION. Two viral phenotypes are described: Non-syncytium inducing (NSI) virus which utilises the CCR5 receptor (C-5) and is associated with earlier infection; and syncytium inducing (SI) virus which utilises the CXCR4 receptor (X-4) and which has is broadly associated with later stages of infection and more advanced disease. Whilst the mean time from infection to first AIDS defining illness (ADI) in adults is 10 years 20% of children infected in utero, at delivery or perhaps through breast-feeding present with an ADI and/or die in the first year of life. Presentation is usually at or after the age of three months. Selection for X4 virus by passage across trophoblasts has been demonstrated *in vitro* even when the predominant viral strain presented to the trophoblast cell layer is C5. We postulated that if this were the case *in vivo* X4 virus might be more commonly detected in children who present in early infancy following infection in utero or at delivery. Furthermore if X4 is indeed a more pathogenic virus strain this might partly explain the rapidity of progression in this patient subset. Third, one might consider whether viral rebound with a X4 virus should be more aggressively treated than if the predominant strain was a C5 virus. We therefore investigated the viral phenotype of infants presenting to our unit with symptomatic HIV infection.

| ID | TCID50 (/75ul) | TCID50 (ml) | CCR1 | CCR3 | CXCR4 | CCR5 | CoRx | Genotype |
|------|----------------|-------------|------|------|-------|------|-----------|----------|
| #1 | 280 | 3733 | NEG | NEG | NEG | ++ | R5 | NSI |
| #2 | 817 | 10893 | NEG | NEG | NEG | ++ | R5 | NO SEQ |
| #3 | 163 | 2173 | NEG | NEG | NEG | ++ | R5 | NSI |
| #4 | 163 | 2173 | NEG | NEG | NEG | + | R5 | SI |
| #5 | 96 | 1280 | NEG | NEG | NEG | +++ | R5 | NSI |
| #6 | 280 | 3733 | NEG | NEG | NEG | ++ | R5 | NSI |
| #7 | 280 | 3733 | NEG | NEG | NEG | +++ | R5 | NSI |
| #8 | 280 | 3733 | NEG | NEG | NEG | ++ | R5 | NSI |
| #9 | <10 | | NEG | NEG | NEG | + | R5 | NSI |
| #10 | 280 | 3733 | NEG | NEG | NEG | + | R5 | NSI |
| #11 | 163 | 2173 | NEG | NEG | NEG | ++ | R5 | NSI |
| 89.6 | POS CONTROL | | NEG | +++ | +++ | +++ | R5X4 (R3) | |

CONCLUSIONS Although rapid progression of HIV-1 infection in adults and children has been associated with a switch in co-receptor usage from CCR5 to CXCR4 this was not found in these 11 infants, who presented with ADI before 1 year of age. Whilst not excluding selection of CXCR4 using virus by trophoblasts *in vivo*, these data suggest that this may not be clinically relevant. Factors other than viral co-receptor usage were important in the rapid progression in these infants. These findings may have important implications for the future use of co-receptor blockers to prevent transmission and to treat infants with HIV-1 infection.

METHODS. Primary isolates were obtained by co-culture of PBMCs from HIV-1 infected infants with CD-8 depleted donor PBL. Each child presented with symptomatic HIV-1 infection during the first year of life.

Virus Co-receptor Utilization

A range of co-receptors CCR1, CCR3, CXCR4, and CCR5 expressed on the U87-CD4-CoRx cell line (a kind gift from Dr Dan Littman) were used to determine viral co-receptor usage. Each of the four U87-CD4-CoRx cell lines (10,000 cells/well) were seeded into duplicate wells and incubated for 24 hours to allow the cells to adhere before the addition of 100 µl of day 28 culture supernatant. The presence of HIV-1 induced syncytia was detected after 48 hours incubation and confirmed by an HIV-1 specific focal assay.

Genotyping. For each patient DNA was extracted from cell pellets collected before and after 28 days of co-culture. using in-house extraction buffers (TST, TE and TENT). HIV-1 *Env* was amplified by nested PCR using HMA primers, (ED3/ED14) for the first round, (ED5/ED12) for the second round, as described by Delwart *et al* 1994. The PCR product was cleaned with the Qiagen PCR purification kit, and sequencing was performed using the dRhodamine terminator cycle kit (Applied Biosystems, UK) and the forward primer ES7 (Delwart *et al* 1994). Both sequences (Day 0 and Day 28) were aligned using SeqEd 1.0.3(Applied Biosystem) and the consensus translated. Assignment of the speculative biological phenotype (non-syncytium-inducing, NSI, versus syncytium-inducing, SI) was made according to the charge of amino acid residues at positions 11 and 25 (numbering the first residue in the V3 as 1), according to Fouchier *et al* 1992 and 1995.

References:

- Fouchier RAM *et al*. *J Clin Microbiology* 1992;33:906-11,
- Fouchier RAM *et al*. *J Virol*. 1995;66:1381-7,
- Delwart EL *et al*. *J Virol*. 1994;68:6672-83