R5 human immunodeficiency virus type 1 with efficient DC-SIGN use is not selected for early after birth in vertically infected children

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The binding of human immunodeficiency virus (HIV) to C-type lectin receptors may result in either enhanced trans-infected of T-cells or virus degradation. We have investigated the efficacy of HIV-1 utilization of DC-SIGN, a C-type lectin receptor, in the setting of intrauterine or intrapartum mother-to-child transmission (MTCT). Viruses isolated from HIV-1-infected mothers at delivery and from their vertically infected children both shortly after birth and later during the progression of the disease were analysed for their use of DC-SIGN, binding and ability to trans-infect. DC-SIGN use of a child’s earlier virus isolate tended to be reduced as compared with that of the corresponding maternal isolate. Furthermore, the children’s later isolate displayed enhanced DC-SIGN utilization compared with that of the corresponding earlier virus. These results were also supported in head-to-head competition assays and suggest that HIV-1 variants displaying efficient DC-SIGN use are not selected for during intrauterine or intrapartum MTCT. However, viruses with increased DC-SIGN use may evolve later in paediatric HIV-1 infections.
restricted, up to 15–20% of children born to infected mothers have been reported as becoming infected by this route (Lehman & Farquhar, 2007). Studies have, however, shown that several components of breast milk may inhibit DC-SIGN-mediated virus transmission (Groot et al., 2005; Hong et al., 2009; Naarding et al., 2005, 2006; Requena et al., 2008; Saeland et al., 2009; Yagi et al., 2010).

How DC-SIGN use by HIV-1 evolves over time, from early after infection to during disease progression, has not been clearly elucidated. However, studies by us and others suggest that DC-SIGN use by HIV-1 may vary within infected individuals (Borggren et al., 2008; Nabatov et al., 2006). In the present study we evaluated utilization of DC-SIGN by vertically transmitted HIV-1 to determine whether HIV-1 isolates with distinct ability to utilize DC-SIGN for trans-infection are selected for during MTCT, and we also analysed whether the utilization of DC-SIGN by the virus, evolving in the infected child, changes. For this purpose, six HIV-1-infected mothers and their six infected children were included in the present study. These individuals and their virus isolates have been characterized in previous studies (Casper et al., 2002a, 2002b; Contag et al., 1997; Navér et al., 1999). The mothers were asymptomatic and none of the mothers or children received antiretroviral therapy to reduce vertical transmission. All mothers were strongly advised not to breastfeed their children. The maternal isolates were sampled at or close to delivery. Virus isolates were obtained from the children just after birth, and also later on, when immune deficiency had developed (Table 1). However, there was a lack of a follow-up isolate from the child in mother–child pair 6.

In order to examine the efficacy of DC-SIGN trans-infection, virus-pulsed Ramos cells expressing DC-SIGN (Wu et al., 2004) were co-cultured with target PBMCs. The assay has been described previously in detail (Borggren et al., 2008; Wu et al., 2004). Briefly, irradiated Ramos/DC-SIGN cells (5 × 10⁵ cells per well) were pulsed with virus, corresponding to 75 pg of functional reverse transcriptase (RT), for 3 h at 37 °C. After virus pulsing, the Ramos/DC-SIGN cells were washed and co-cultured with 10⁵ PBMCs, and they were phytohaemagglutinin (PHA)-activated for 3 days. In parallel, conventional direct infections of 10⁵ PHA-activated PBMCs were set up. Seven days after infection, trans or direct, the p24 antigen content in culture supernatants was analysed by ELISA. The relative efficacy of DC-SIGN use was assessed to compensate for differences in PBMC infectivity of the isolates, by calculating the ratio of p24 release in DC-SIGN-mediated infections over p24 release in directly infected cultures. When comparing the maternal isolate obtained at delivery with the child’s earliest isolate, we found that in five out of the six mother–child pairs, DC-SIGN-mediated trans-infection of the transmitted virus was less efficient for the child’s isolate than it was for the corresponding maternal isolate (P < 0.17) (Fig. 1a). Furthermore, a comparison with the later isolates (sampled during disease progression) showed that these viral isolates had evolved with an increased DC-SIGN use in all five children, from whom follow-up virus isolates were available (P < 0.03) (Fig. 1b). In contrast, the efficacy of direct PBMC infections showed no such clear pattern (data not shown). Furthermore, the use of wild-type Ramos cells, lacking detectable DC-SIGN expression, did not result in virus transmission to PBMCs in the trans-infection assays (data not shown). Next, virus DC-SIGN use was analysed employing a DC-SIGN-binding assay, as described previously (Borggren et al., 2008). In brief, the percentage of virus (measured as p24 antigen) associated with DC-SIGN was determined. Virus was normalized according to RT activity (75 pg) and the p24 antigen levels were determined prior to pulsing of Ramos/DC-SIGN and Ramos/wild-type cells. Three hours after pulsing, cells were washed thoroughly, lysed and bound virus was determined by performing a p24 antigen ELISA. Results from the DC-SIGN-binding assay (Fig. 1c, d) correlated with those obtained in the DC-SIGN-mediated trans-infection assay (r = 0.86, P < 0.0001, according to Spearman Rank test). Thus, the first isolate of the child tended to bind DC-SIGN less efficiently compared with the maternal isolate and the follow-up viral isolate of the child. These results suggest that HIV-1 variants displaying efficient DC-SIGN use are not selected for early after birth in vertically infected children, whereas virus variants with an increased ability to utilize DC-SIGN may evolve over time.

To further explore our results on differences in DC-SIGN use of R5 HIV-1 isolates from mother–child pairs, we performed head-to-head competition assays to test the relative fitness of viruses in DC-SIGN mediated trans-infections. The competition assay has been described previously (Borggren et al., 2008). Briefly, virus isolates from mother–child pairs were mixed, i.e. the maternal isolate was mixed with the child’s first isolate, or the child’s first isolate was mixed with the child’s follow-up isolate at a 1:1 ratio based on RT activity. Virus mixtures were serially diluted and used in DC-SIGN-mediated trans-infections of PBMCs. After 7 days, supernatants were harvested and the p24 antigen content was analysed. Cells from p24 antigen-positive wells, where the virus was diluted to the limit, were lysed and the DNA was sequenced to identify the replicating viruses. The gp120 V1V2 region of the primary isolates from mother–child pairs 2 and 4 was sequenced prior to the competition assay to identify any unique signature sequences using a nested PCR approach, with E00 and ES8 (Trouplin et al., 2001), and E20 and 793seq4 (Mild et al., 2007; Trouplin et al., 2001) primer pairs (GenBank accession nos JX870008–JX870013). Competition between the maternal isolate and the child’s first isolate from mother–child pairs 2 and 4 revealed that the maternal isolate in both cases out-competed the respective child’s virus in the DC-SIGN-mediated trans-infection assay (Fig. 2a). The maternal isolate from mother–child pair 2 was detected exclusively in all trans-infections, whereas that from mother–child pair 4 was exclusively found in 75% of cultures. Next, intrapatient virus competitions were set up, mixing the
Table 1. Characteristics of HIV-1-infected mother–child pairs and virus isolates

Coreceptor use was determined by the infection of U87.CD4 and GHOST coreceptor indicator cell lines expressing CCR5 and CXCR4 (Casper et al., 2002a, b). Virus coreceptor use was R5 in all cases. NA, Not available.

<table>
<thead>
<tr>
<th>Mother–child pair</th>
<th>HIV-1 subtype</th>
<th>Isolate</th>
<th>Sampling time point*</th>
<th>CD4 count (cells mm(^{-3}))</th>
<th>CDC stage(\dagger)</th>
<th>Plasma HIV RNA load (copies ml(^{-1}))</th>
<th>Antiretroviral therapy</th>
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<tr>
<td>1</td>
<td>A</td>
<td>Maternal</td>
<td>1 month</td>
<td>140</td>
<td>N1</td>
<td>27 000</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Child first</td>
<td>1.5 months</td>
<td>3380</td>
<td>N1</td>
<td>48 000</td>
<td>None</td>
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<tr>
<td></td>
<td></td>
<td>Child follow-up</td>
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<td>170</td>
<td>B3</td>
<td>150 000</td>
<td>Zidovudine</td>
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<td>2</td>
<td>A</td>
<td>Maternal</td>
<td>Delivery</td>
<td>680</td>
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<td>18 000</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Child first</td>
<td>≤ 4 days</td>
<td>NA</td>
<td>N1</td>
<td>1 000</td>
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</tr>
<tr>
<td></td>
<td></td>
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<td>32 000</td>
<td>Zidovudine</td>
</tr>
<tr>
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<td>130 000</td>
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<td></td>
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<td>C3</td>
<td>1 900 000</td>
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<td>B</td>
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<tr>
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<td>Child follow-up</td>
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<td>6</td>
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<tr>
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<td>N1</td>
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</table>

*Sampling time point at or after delivery/birth.
†The Centers for Disease Control and Prevention classification was performed according to the 1994 classification for HIV in children less than 13 years of age (CDC, 1994).
child's first isolate with the follow-up isolate of the children in pairs 2 and 4. In pair 2, the follow-up isolate was found to completely out-compete the first isolate, while in pair 4 both types of viruses were detected (Fig. 2b). Thus, results from the competition assays support the conclusions that vertically transmitted R5 HIV-1 displays reduced DC-SIGN use as compared with that of viruses circulating in the mother from a similar time period. Furthermore, virus variants in the child seem to evolve with more efficient DC-SIGN use over time.

Thus, our results suggest that intrapatient evolution of R5 HIV-1 isolates, from birth to the onset of disease, leads to an increased efficiency in DC-SIGN utilization for transmission. We also highlight findings that imply that efficient DC-SIGN use may not be a requirement for HIV-1 MTCT.

The involvement of DC-SIGN in MTCT has been implicated (da Silva et al., 2011), but knowledge of the role of DC-SIGN-mediated trans-infection and the effects are clearly limited. It is well recognized that the viral population in the new host after vertical transmission is genetically quite homogeneous (Scarlati et al., 1993; Wolinsky et al., 1992). It is thus possible that DC-SIGN use could be such a bottleneck for transmission. DC-SIGN is expressed on cells in close proximity to both sides of the trophoblast cells in the placenta (Soilleux et al., 2001). However, unexpectedly, we noted that virus variants with a reduced ability to utilize DC-SIGN for trans-infection tended to emerge in children early after birth, suggesting that efficient DC-SIGN use may not be needed for vertical transmission, or may even be a disadvantage. This may seem inconsistent but could possibly be explained by the finding of an association between a polymorphism resulting in reduced expression of placental DC-SIGN, a DC-SIGN homologue, and an increased risk of HIV-1 vertical transmission (Boily-Larouche et al., 2009). DC-SIGN has been shown to support HIV trans-infection (Pöhlmann et al., 2001b), but also functions as an HIV-1 antigen-capture receptor (Snyder et al., 2005). Thus, Boily-Larouche et al. (2012) hypothesized that placental DC-SIGN may protect against HIV-1 infections by capturing and degrading the virus, instead of mediating HIV-1 trans-infection. It should, however, be noted that another recent study links polymorphisms in the DC-SIGN promoter region with reduced DC-SIGN expression and higher virus affinity (Boily-Larouche et al., 2012). In fact, similar to DC-SIGNR, dual functions of DC-SIGN have also been reported, i.e. as a receptor for virus trans-infection and virus internalization and processing (Tsegaye & Pöhlmann, 2010). In the placental structure, DC-SIGNR and DC-SIGN are expressed in close proximity to each other, but on separate fetal cells located adjacent to the trophoblasts, on capillary endothelium and Hofbauer cells, respectively (da Silva et al., 2011). Perhaps minor virus variants with reduced affinity for DC-SIGN may be selected for during vertical transmission, while virus variants displaying efficient DC-SIGN use, dominating in the mother, may be selected against during transmission.

We, and others, have previously reported on intrahost virus variation related to the efficacy of DC-SIGN use in
HIV-1-infected adults (Borggren et al., 2008; Nabatov et al., 2006). By studying R5 HIV-1 isolates sequentially obtained during disease progression, we noted that virus isolates from the chronic phase of the disease were more efficient in their ability to utilize DC-SIGN than corresponding isolates obtained from the severe immunodeficient stage (Borggren et al., 2008). We speculated that efficient DC-SIGN use could be an important viral feature selected for during the immune-competent phase, since there are reports associating DC-SIGN interactions with immune evasion from neutralizing antibodies (Marzi et al., 2012). Naturally-occurring genetic variants in human DC-SIGN increase HIV-1 capture, cell-transfer and risk of mother-to-child transmission. (Lancet, 1991), virus evolution in the children may not include the same magnitude of changes in DC-SIGN use as in adults, in whom the infection has been ongoing for many years. It is also plausible that if more samples had been analysed from the children during the immune-competent phase, virus variants with even more efficient DC-SIGN use may have been identified before the onset of severe immune suppression, similar to our findings in the late stage of the disease in adults.

We believe that further studies on receptors involved in vertical HIV-1 transmission and the understanding of intrapatient virus evolution may be of importance in the development of preventive and therapeutic interventions against HIV.

**References**


**Fig. 2. In vitro selection of mother–child R5 HIV isolates in head-to-head competitions.** DC-SIGN-mediated trans-infection competition assays were set up, mixing the maternal isolate obtained at delivery with the first isolate of the child (a), and the first isolate with the follow-up isolate of the child (b). Replicating viruses were identified by sequencing the V1V2 region in three to six parallel cultures.


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