

Chapter 8.4

Level of the host: Host factors and HIV-1 transmission

Host factors

Different target cells, receptors, entry mechanisms and protective factors are involved in HIV-1 transmission. Epidemiological data indicate that these mechanisms and factors differ between individuals. This will be discussed in the light of our data in this thesis (Table 8.4).

Epithelial barrier

The epithelia are a crucial mechanical barrier against HIV-1 infection²³. We have demonstrated in chapter 6.2 that thickness of the vaginal and cervical epithelia are variable, which is influenced in females by androgens, suggesting a different susceptibility to acquire HIV-1. Although the epithelium protects against HIV-1 transmission, *in vitro* experiments suggest that HIV-1 infected monocytes or cell-free virus might be transported from the luminal surface towards the apical surface, referred to as transmigration and transcytosis respectively^{14,18,76}. Genital epithelial cell transcytosis depends on the attachment of the virus to syndecan-1 and -2¹². The rectal and endocervical epithelia are fragile, and therefore transcytosis and transmigration may be more efficient in these thinner epithelia, contributing to increased risk of HIV-1 acquisition during anal intercourse and of persons with cervical ectopy^{1,2,65,83,90}.

Table 8.4 Host factors affect HIV-1 transmission.

Host factor	Affected by	Effect
Breaches epithelium	Co-infection, thin epithelium, dry-sex, trauma	Less mechanical barrier, exposure sub-epithelial target cells
LC barrier	Langerin polymorphisms, decreased density LCs, factors in body fluids?	Decreased protection by Langerin
Increased exposure target cells	Increased target T cells; increase DC-SIGN ⁺ DCs	Increased infection and transmission
Increased susceptibility target cells	Upregulation chemokine receptors; LC activation	Trans-infection, enhanced replication, Increased entry
Decreased susceptibility target cells	CCR5 mutation, chemokine overexpression, auto-antibodies	Decreased viral infection.
High mannose structures in body fluid	???	Affect DC-SIGN/ Langerin function?
Anti-HIV-1 CTLs	???	Kill infected cells

LC barrier: Dr. Jekyll and Mr. Hyde?

Our results indicate that LCs both protect against- but also mediate HIV-1 transmission. This duality is strongly influenced by environmental triggers. High viral loads, HSV infection, and the pro-inflammatory cytokine TNF α increase transmission by LCs *in vitro* and *ex vivo* (**Section 5** and **6**). Transmission of HIV-1 under these conditions is dependent on infection of LCs via the entry receptors CD4 and CCR5. Furthermore, the TLR ligand Pam3CSK induces *trans*-infection by LCs, suggesting that other receptors are involved in transmission.

The opposite function of LC infection versus LC protection *in vivo* has to be determined. Circumstantial evidence indicates that LC infection is inefficient during steady-state conditions and that LCs protect against HIV-1 transmission as part of the epithelial barrier: i) The skin and oral mucosa do not or very rarely transmit HIV-1, while it is likely that LCs in these tissues incidentally encounter HIV-1. ii) Heterosexual transmission of HIV-1 is rare, while the chance that LCs encounter HIV-1 during intercourse with an HIV-1 infected person is high. iii) Female mucosal tissues have less and more variable densities of LCs compared to male tissues (**Section 5**) and the rate of HIV-1 transmission from male-to-female may be higher^{1,90}. iv) Transmission is highly increased during trauma, inflammation, and during practice of anal sex, exposing tissues that lack LCs to HIV-1. In contrast, the fact that primarily CCR5-using viruses are detected after transmission could implicate a role for LCs or activated LCs in transmission, since LCs express CCR5 and not CXCR4, while other target cells express both co-receptors⁵². However, other reports have proposed that selection of CCR5-using viruses occurs after primary infection¹⁰⁰.

Target cells

In **Section 2** we have extensively discussed the transmission of HIV-1 by DC-SIGN⁺ DCs. Furthermore, primary DC-SIGN⁺ DCs mediate transmission of HIV-1 *in vitro* and *ex vivo*^{28,40,46,86,101}. Interestingly, the amount of DC-SIGN⁺ DCs was not variable or different between vaginal, cervical and penile tissues (**Section 6**). However rectal epithelium lacks LCs and contains many DC-SIGN⁺ DCs, and thus the mode of sexual contact affects HIV-1 transmission via DCs. Moreover, genital herpes was correlated with increased densities of DC-SIGN⁺ DCs²³. Chapter 6.2 revealed that other target cells than LCs and DC-SIGN⁺ DCs are present in the mucosal tissues, including macrophages and CD4⁺ T cells. Interestingly, the amount of CD4⁺ T cells in the epithelium and subepithelium in female genital tissues was markedly higher than in male genital tissues and more variable between female individuals. This might be an important determinant for the susceptibility to acquire HIV-1. A role for CD4⁺ T cells is supported by different *ex vivo* and *in vivo* experiments^{39,43,96,114,115}. Furthermore, DCs might be important carriers of virus in this scenario, transporting T cell derived *de novo* virus into the lymphoid tissues.

Body fluids

Body fluids, such as saliva, cervicovaginal fluid and semen, contain different anti-microbial agents, such as defensins and secretory leukocyte proteinase inhibitor^{20,31,54}. Notably, human breast milk, semen and a part of the cervicovaginal fluids contain ligands that interfere with DC-SIGN-mediated HIV-1 transmission^{49,81,82}. In breast milk, this component was shown to be bile-salt stimulated lipase that contains Lewis X type carbohydrate⁸¹. Since ligands for DC-SIGN potentially block Langerin function, we tested whether milk interacts with Langerin. Langerin did not interact with breast milk, in contrast to DC-SIGN (Figure 8.4). This can be explained by the difference in carbohydrate specificity for Lewis X between these two C-type lectins (Chapter 5.2). Subsequently we investigated the presence of ligands for DC-SIGN and Langerin in saliva from different glands. Similar to breast milk, saliva contains ligands

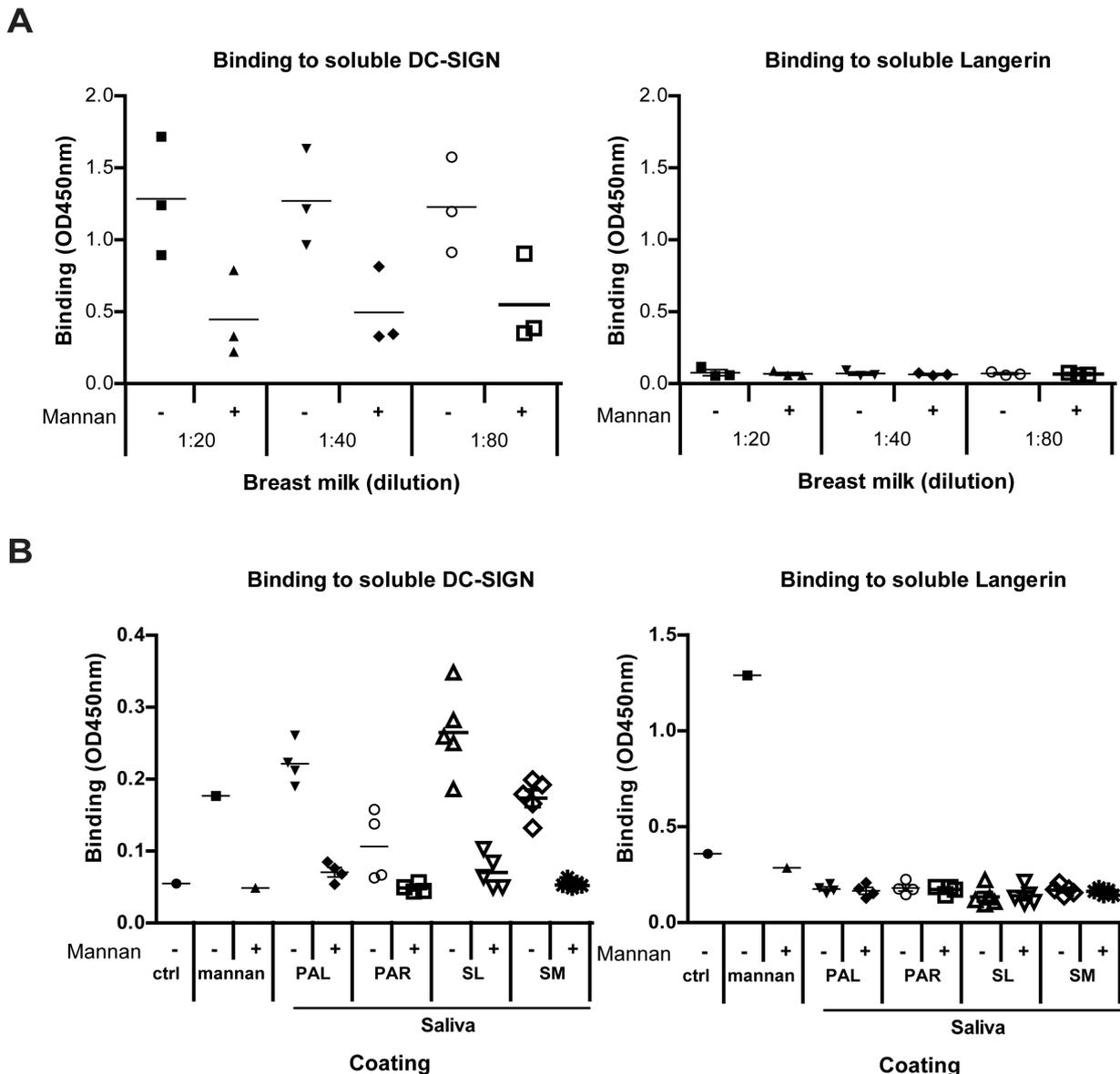


Figure 8.4 Breast milk and saliva contain ligands for DC-SIGN, but not Langerin.

(a,b) The soluble DC-SIGN and Langerin binding assays were performed as described in chapter 5.3 and 6.4. Different dilutions of human breast milk and saliva from different glands were coated onto Maxisorp plates (NUNC, Denmark) for 1 hour at 37°C. After blocking the plate with 5% BSA in TSM (TSA) for 30 minutes at 37°C, Jurkat-Langerin lysate (1×10^8 cells/ml; 1:2 lysate:TSA) or DC-SIGN-Fc ($1 \mu\text{g/ml}$ in TSA) was added and incubated for 2 hours at room temperature. To detect soluble DC-SIGN binding, DC-SIGN-Fc ($1 \mu\text{g/ml}$) was added for 2 hours at room temperature. DC-SIGN-Fc binding was determined using a peroxidase-conjugated goat anti-human Fc antibody. To detect Langerin binding the plate was incubated with the specific non-blocking anti-Langerin antibody DCGM4 ($10 \mu\text{g/ml}$). Subsequently, the plate was incubated with a goat anti-mouse peroxidase conjugate and binding was detected using ELISA. To determine specificities for the binding sites of Langerin and DC-SIGN, the lysate or DC-SIGN Fc construct was pre-incubated with mannan for 1 hour at 37°C. The icons represent different donors. (b) Human whole saliva was collected by drooling in ice-chilled vials and cleared by centrifugation in a bench top centrifuge at 10,000g for 2 min. Glandular salivas from the parotid (Par), submandibullary (SM), sublingual (SL), and palate (Pal) glands were collected using custom-fitted devices as previously described¹⁰⁶. Cleared glandular salivas were obtained from 6 healthy laboratory workers and frozen until use. All subjects gave their informed consent (protocol OWBR010, approved by the Institutional Ethical Board of the hospital). As positive control for Langerin and DC-SIGN binding mannan was coated.

for DC-SIGN but not Langerin (Figure 8.4). Thus body fluids contain compounds that specifically block DC-SIGN, but not Langerin function, indicating that body fluids preserve the protective function of LCs and block DC-mediated transmission by DC-SIGN. In seminal plasma the component binding to DC-SIGN is thought to contain high mannose structures, and might thus possibly interfere with Langerin function during intercourse. This should be further investigated. Other factors in body fluids such as the pH, can also influence HIV-1 transmission.

Currently, experimental research on HIV-1 transmission is mostly carried out with cell-free virus lab-produced HIV-1, but it is evident that factors in body fluids affect HIV-1 transmission. Moreover, both cell-free and cell-bound virus are present in these body fluids and this might further influence HIV-1 transmission. To mimic as closely as possible the *in vivo* situation, infected semen or cervicovaginal fluids could be used in transmission models. Sperm derived from infected SIV/SHIV monkeys might be a more physiological method for *in vivo* primate infections. However, experimental problems including low rates of infection may hamper these experiments.

Other protective host factors

Studies on highly exposed persistently seronegative individuals, such as uninfected prostitutes in areas with high HIV-1 prevalence and uninfected partners of HIV-1 positive individuals, revealed different host factors that attributed to HIV-1 transmission⁶³. The most well-known factor is the homozygous CCR5-delta32 mutation that highly protects against HIV-1 infection^{47,67}. However, also polymorphisms in the chemokine SDF-1 (ligand for CXCR4) and upregulation of chemokine production might protect against HIV-1 infection⁶³. Furthermore, auto-antibodies, such as antibodies against CD4 or CCR5^{16,68}, high levels of cytotoxic T cells directed to HIV-1 proteins^{11,35,64} and high levels of soluble suppressor factors, such as defensins, have been associated with resistance to HIV-1²⁰. Last but not least, safe-sex is a key determinant in HIV-1 transmission.

Acknowledgements

I would like to thank Dr. E.C. Veerman and Dr. J.G. Bolscher (Department of Oral Biochemistry, Academic Centre for Dentistry Amsterdam, Vrije Universiteit and Universiteit van Amsterdam, 1081 BT Amsterdam, The Netherlands) for providing us with the saliva samples and E. Saeland for providing us with the breast milk samples.