

Chapter 8.2

Viral level: Do lymphotropic viruses fool dendritic cells?

Lymphotropic viruses target DC-SIGN⁺ DCs to mediate transmission

Lymphotropic viruses pathogenic to humans, such as HIV-1 and MV, have evolved mechanisms to reach their target cells in the lymphoid tissues. At present, transmission by DC-SIGN⁺ DCs is among the most likely scenarios to explain infection by lymphotropic viruses. The unique migration pathway of DCs to mediate antigen presentation to T cells connects the peripheral tissues with the lymphoid tissues. *In vitro* and *ex vivo* data support that lymphotropic viruses target DC-SIGN⁺ DCs to invade the body (Section 2-4). However, the *in vivo* evidence for a role of this DC subset in viral transmission from site of entry into the lymphoid tissues is still lacking.

Primary DC-SIGN⁺ DCs are difficult to isolate in high numbers, and in various labs, the culture of DC-SIGN⁺ monocyte-derived DCs (moDCs) is a standard and controlled method. Therefore, most of the times moDCs, and not primary DCs, have been used to unravel the interaction of viruses and DC-SIGN⁺ DCs. Primary subepithelial DCs in the cervix, foreskin and glans penis express DC-SIGN^{23,23,73}, but few studies have studied these transmission by these primary DC-SIGN⁺ DC subsets. DC-SIGN⁺ DCs are present throughout the whole rectal epithelium⁴⁸ and after isolation these cells mediate transmission to T cells via the receptor DC-SIGN⁴⁰. Moreover, dermal DC-SIGN⁺ DCs, which, based on their expression of surface markers, resemble the subepithelial DC-SIGN⁺ DCs, efficiently transmit HIV-1 *ex vivo*⁸⁶. Thus, DC-SIGN⁺-primary rectal and dermal DCs mediate HIV-1 transmission. However, more research is essential to investigate DC-SIGN⁺ DCs present in the other subepithelia and to determine the mechanisms that are involved in HIV-1 transmission by these primary cells. A further comparison of the phenotype of moDCs and primary DCs and their ability to transmit viruses will be an essential step towards understanding the *in vivo* situation. Transmission of a murine lymphotropic virus in a conditional DC knockout mouse^{9,50} might assess the role of DCs during transmission *in vivo*. However, the role of C-type lectins may not be adequately addressed in mice, since the expression and carbohydrate specificities of the murine DC-SIGN homologues are highly distinct^{60,61,98}.

Do Lymphotropic viruses infect Langerhans cells?

LCs are protected against MV and HIV-1 infection by the receptor Langerin. Furthermore, LCs protect against HIV-1 infection of other target cells, and might as such provide an innate antiviral barrier. However, LCs in human skin and vaginal explants *ex vivo* can be infected with CCR5-using viruses by high virus concentrations, resulting in transmission to CD4⁺ T cells (Table 8.2).

Table 8.2 Different models investigated HIV-1 transmission by Langerhans cells

| LC Infection model | Advantages | Disadvantages | Detection method | Virus concentration | Infection, transmission? | Reference |
|-----------------------------------------|--------------------------------|-------------------------------------------------------------|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|------------------------|
| <i>Ex vivo</i> skin | Primary LCs, untouched | No <i>in vivo</i> target cells, activation through surgery? | Infection LCs: p24 production, intracellular p24 Transmission to T cells: p24 production | 10 ⁴ -10 ⁵ TCID ₅₀ /sheet 50-200ng p24/sheet | Infection LCs and transmission to T cells | 51,86 |
| <i>Ex vivo</i> vagina, suction blisters | Primary target LCs | Activation through blisters? | Capture: GFP viruses, GFP facs Infection: GFP expression recombinant virus | 50-500 ng p24/vaginal sheet + spinoculation virus and tissue | Capture HIV-1 by LCs. Infection LCs absent or inefficient, in contrast to T cells. | 43 |
| <i>Ex vivo</i> cervix | Primary LCs, untouched | Activation through surgery? | UFISH HIV-1 gag-pol mRNA | 10 ⁴ TCID ₅₀ /biopsie | Infected T cells detected after 6 hours, LCs after 3 days (activation?) | 39 |
| MoLCs | Cellular interaction | LC-like cells (*) | Infection LCs: p24 production in time | 2x10 ⁷ TCID ₅₀ /100.000 LCs | Infection moLCs low compared to MoDCs | 53 |
| CD34 ⁺ LCs | Cellular | LC-like cells (*) | <i>Trans</i> -infection of T cells:luciferase activity | 10-60 ng p24/100.000 LCs | No <i>trans</i> -infection, only after activation with TNF α /LPS | 29 |
| SIV infection primates | <i>In vivo</i> , no activation | 100% infectivity chance (**) SIV instead of HIV | <i>In situ</i> PCR/ISH | 10 ³ -10 ⁵ TCID ₅₀ /ml 1 ml/animal | No infection epithelium, infection of T cells and DCs in lamina propria | 96,115 |
| SIV infection primates | <i>In vivo</i> , no activation | 100% infectivity chance (**) SIV instead of HIV | ISH | 10 ⁵ TCID ₅₀ /ml 1 ml/animal | Infection LCs | 45 |
| Isolated skin LCs | Primary cells, cellular | No <i>in vivo</i> target cells, activation through surgery? | Transmission of T cells, clearance by p24 production | Titration range: 10-2x10 ⁴ TCID ₅₀ (30pg-40ng p24)/100.000 cells | Only transmission with high viral loads. Protection via Langerin. No <i>trans</i> -infection | 23 Section 5 |

(*) MoLCs and CD34⁺ LCs express receptors that are normally not expressed on primary LCs, such as TLR-4 and sometimes DC-SIGN

(**) To minimize the groups of animals used for these experiments, infectivity is increased to high levels, not reflecting the *in vivo* situation.

Experiments in macaques have resulted in contradicting reports. Hu *et al.* observed SIV infection of LCs in the vaginal epithelium⁴⁵, whereas other reports observed only infection of non-LC target cells in the lamina propria^{52,96}. At first sight these different reports seem contradicting. However, a closer look reveals that experimental conditions are likely to have attributed to the different observations. Key determinants are the concentration of virus particles and activation status of LCs (8.2.), also important for transmission *in vivo*. Not much information is available on p24 concentrations in semen and cervicovaginal fluids, but they seem to be in the range of pg values/ml, similar to plasma values^{15,84,85,95}. This indicates that the concentrations we used in our experiments are in the higher range of the physiological levels in the relevant body fluids. Using these concentrations we observed protection against HIV-1 infection of LCs via Langerin, suggesting that the protective function of Langerin will not easily be breached *in vivo* due to saturation of the receptor. MV infects LCs *ex vivo*, which is enhanced after blocking Langerin (Chapter 5.3). We seldom observed infected DC subsets in the skin of MV-infected macaque and in human MV-infected epidermis *ex vivo*, suggesting that LCs are not target cells for MV at the site of entry and during established infection, which may be due to Langerin function and/or CD150 expression.

Lymphotropic viruses target DCs to enhance infection during chronic disease

In this thesis we have focussed on the role DC-SIGN⁺ DCs during HIV-1 transmission. However, this subset may also be involved in established HIV-1 infection. During established infection, DC-SIGN⁺ DCs in blood and lymphoid tissues might enhance infection of target cells by capture of cell-free virus from blood and lymph and transfer of the virus through the infectious synapse. Moreover, DC-SIGN⁺ DCs may serve a viral reservoir. Notably, a polymorphism in *DC-SIGN* has been associated with enhanced disease progression of HIV-1 patients⁵⁸, suggesting a role for DC-SIGN during established HIV-1 infection. Only small numbers of mature LCs are present in the lymphoid tissues, the site of replication of lymphotropic viruses. We therefore hypothesize that LCs and Langerin function have a minimal effect in enhancing or preventing established infection, but are important for inducing immune responses or immune suppression.

Lymphotropic viruses target DCs for immune suppression

The DC family has a crucial function in the initiation of adaptive immune responses against invading pathogens. Viruses are therefore thought to target DCs not only for transmission, but also for their survival in the host by suppressing DC function, thereby increasing survival in the host and maximizing the chance to spread further into the population. Indeed, to induce latency, chronic infection or immune suppression, most pathogenic viruses have evolved strategies to escape our immune system. *In vitro* studies have revealed different mechanisms, by which viruses target DCs for their escape, including interaction with both LCs and DC-SIGN⁺ DCs³. These mechanisms are often virus-specific and interfere with various levels of DC function, such as maturation, cytokine production and T cell polarisation³. Notably, interaction of pathogens with DC-SIGN in combination with TLR stimulation leads to a prolonged and increased production of IL-10 by signalling through Raf-1 kinase and the acetylation of NF-kappaB subunit p65. Cross-talk between DC-SIGN and TLRs appears to be pathogen specific⁴⁴. Strikingly, MV has been shown to interact with TLR2, which might normally be beneficial to the host. However, together with the MV interaction with DC-SIGN, this results in a strong a high production of IL-10³⁷, suggesting that MV uses the combination of both receptors to induce immune suppression. During clinical measles Th-1 cytokines are produced, resulting in an efficient CTL response. However after the

measles symptoms have faded, a cytokine imbalance is observed with a switch to Th-2 cytokines, including high levels IL-10 and reduced CTL responses^{6,36,80}. Thus, interaction of viruses with DC-SIGN may attribute to immune suppression; however, the pathways involved need to be further elucidated.

Measles virus versus HIV-1

Similarities. As discussed in the introduction, MV and HIV-1 are both lymphotropic immune-suppressive viruses and may therefore employ the same mechanisms to invade the host and to induce immune suppression. Indeed, DC-SIGN⁺ DCs mediate transmission and antigen presentation of both MV and HIV-1 to CD4⁺ T cells. Furthermore, DCs transmit MV to CD8⁺ T cells, suggesting that interaction of virus-carrying-DCs with both T cell subtypes results in the formation of an infectious synapse⁷⁵ and that expression of entry receptors restricts infection. The interaction of these viruses with DC-SIGN also induces similar signalling and this might contribute to immune suppression.

Differences. Two important differences between MV and HIV-1 pathogenesis are the infectivity and the effectivity of the immune response. Moreover, MV enters the body in the respiratory tract, whereas HIV-1 is transmitted through sexual or blood-blood contact. The high infectivity of MV compared to HIV-1 might implicate that this virus efficiently circumvents the protective LCs in the respiratory tract to reach other target cells, such as the DC-SIGN⁺ DCs. However other reasons such as higher stability of the virus or an easier access to targets cells in the respiratory tract might be involved. We did not evaluate antigen presentation of HIV-1, however other reports have demonstrated that LCs and DCs efficiently mediate antigen presentation of HIV-1^{23,78,79}, as we have seen for MV in the context of MHC class-II. This suggests that the potency of the immune response is not regulated at the level of C-type lectin binding. This could be an intrinsic factor in the virus, such as the high mutation rate of HIV-1 or the high level of glycosylation of HIV-1 gp120 that allow escape from immune responses. Further research will address whether differences and similarities of these viruses are generated at the level of DCs.

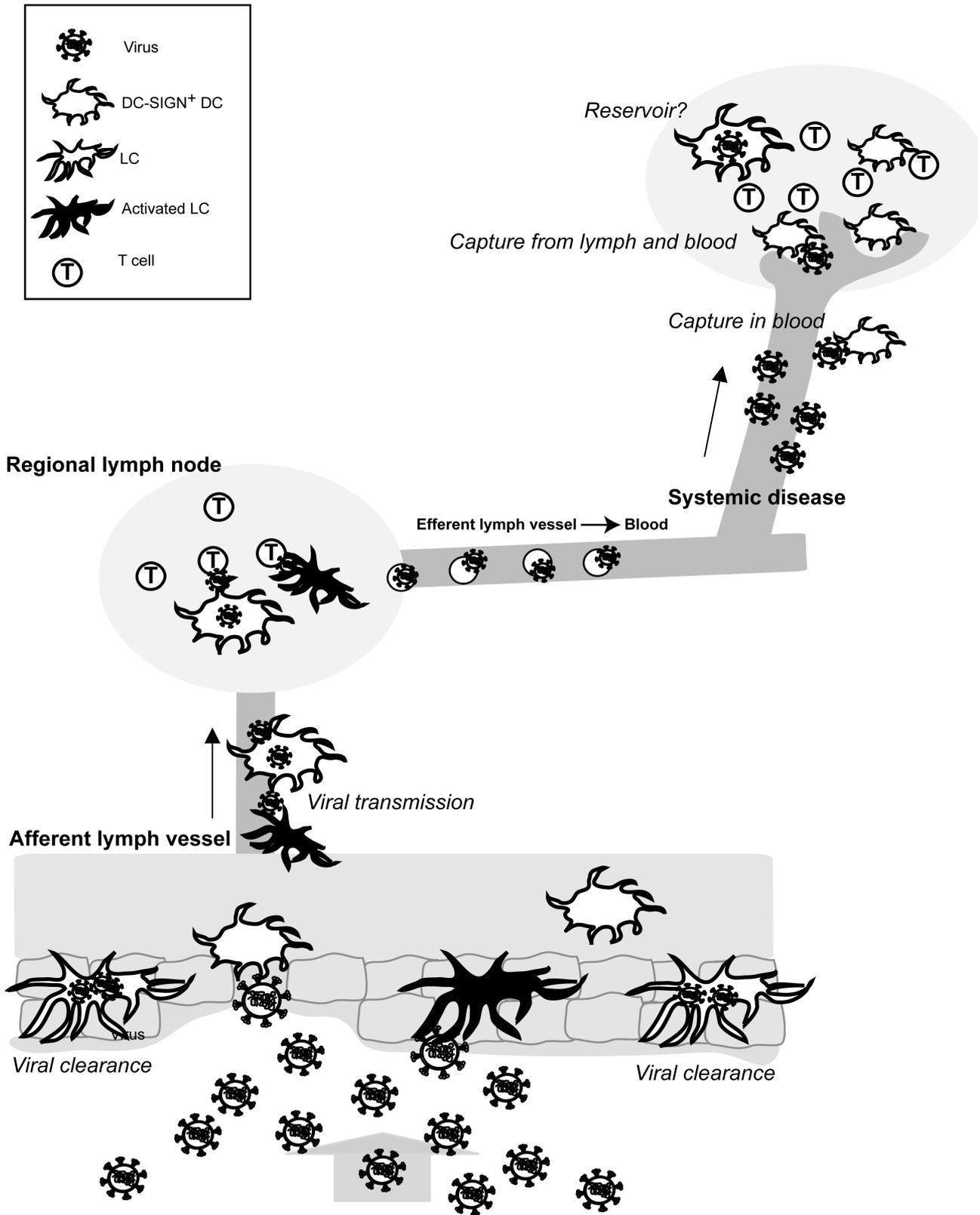


Figure 8.2 Viruses target DCs at the site of entry and during established disease.

At the site of entry immature LCs clear invading virus particles via the receptor Langerin. However, activation of LCs or rupture of the epithelial barrier resulting in contact of virus with DC-SIGN⁺ DCs, allows DC-mediated transmission of the virus to the lymphoid tissues. During systemic infection, virus-infected cells and viruses are present in the blood and lymph and these might be captured by DC-SIGN⁺ DCs in blood, lymph node or spleen, to either mediate viral transmission or antigen presentation.