C-type lectins on dendritic cells: bittersweet interactions with viruses

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General Introduction

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Innate signaling in HIV-1 infection of dendritic cells.  
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Langerin functions as an antiviral receptor on Langerhans cells.  
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Inside the skin, dendritic cells (DCs) are professional antigen presenting cells (APCs) that constantly sample their surroundings for invading pathogens1. Immature DCs are specialized to capture pathogens using pattern recognition receptors (PRRs). PRRs recognize pathogen associated molecular patterns (PAMPs), which are conserved motives expressed by pathogens. Upon pathogen capture, PRR-signaling leads to maturation of DCs and up-regulation of chemokine receptor CCR7, which initiates migration towards the lymph nodes (LN) (Fig. 1). Besides CCR7 up-regulation, maturation of DCs involves several other functional and phenotypic changes, including up-regulation of Human leukocyte antigen (HLA) class II (HLA-II), up-regulation of co-stimulatory molecules CD86 and CD80 and production of immune-modulating cytokines2, required to combat the pathogen recognized. In the LN, DCs have fully evolved to APCs and present processed antigen to T cells that become activated, start to proliferate and migrate towards to the side of infection.

Simultaneous triggering of PPRs and cross-talk between PPR-signaling shapes the immune response induced by DCs. Depending on the array of PRRs triggered, DCs change the cytokines secreted to induce T helper (T_h) cell types, of which the best studied are the T_h1, T_h2 and T_h17 (ref. 1). Depending on the type of T_h cell, they activate macrophages and neutrophils to phagocytose extracellular pathogens. Moreover, DCs can activate CD8^+ T cells, which kill infected cells to clear intracellular infections. Hence, DCs play a crucial role in the induction of adaptive immune responses.

Langerhans cells

In this thesis we focused on two subsets of the DC family; the Langerhans cells (LCs) and DCs. LCs are located in the upper layers of the skin, the epidermis, and in mucosal tissue of the vagina and the glans penis3. With their dendrites, LCs form a dense network that functions as the first barrier against invading pathogens. Utilizing this network of dendrites, which penetrates through the tight junctions of the epithelium, LCs survey the mucosa/skin for pathogens and other danger signals4. Because the skin is in constant contact with the environment, LCs are most likely the first DC subset to encounter pathogens and commensal bacteria. However, LCs do not express the bacterial PAMP-recognizing PRRs Toll-like receptor (TLR) 4 and low levels of TLR2 and TLR5 (refs. 5, 6). This suggests
Figure 1. Adaptive immune responses are induced by DCs.

(a) Langerhans cells (LCs) and dendritic cells (DCs) screen for pathogens in the epidermis/mucosa and dermis/sub mucosa, respectively. Pathogens are recognized by PRRs that signal to activate and mature LCs and DCs. This co-insides with up regulation of lymph node homing receptor CCR7. In the lymph node, DCs and LCs present pathogen-derived antigens to T cells which induces clonal expansion of the T cell. (b) After having clonally expanded, T cells transform in effector cells. Effector T cells migrate towards the side of infection where they can activate macrophages and neutrophils to phagocyte pathogens, or they can kill infected cells to clear the infection.
that LCs are less sensitive to bacteria and this could prevent over-sensitivity to commensal bacteria present in the skin. However, it might reflect a more specialized function for LCs in virus infections as shown by their protective function in HIV-1 transmission. LCs interact with multiple pathogens including Herpes Simplex virus 2 (HSV-2) (ref. 7), HIV-1 (ref. 3) and several fungal species. Hence, LCs are APCs that can induce immune responses against pathogens, but their coverage might be limited due to the specific PRR expression profile.

**Dendritic cells**

Whereas LCs reside in the outer layers of our body, DCs are localized in the dermis and sub-mucosa. Here they detect pathogens, which have penetrated the epidermis or mucosa. DCs express TLR1 through TLR8 (ref. 5). This array of PPRs allows them to recognize fungi, viruses, helminthes and bacteria. DCs furthermore express several C-type lectin receptors (CLRs) that bind pathogens and can modulate immune responses. Hence, DCs are involved in inducing immune responses against several classes of pathogens. However, some pathogens have adapted to take advantage of the migratory capacity of DCs to facilitate their dissemination: DCs have been shown to increase transmission of Ebola, Hepatitis B virus, HIV-1 and Measles Virus.

**Recognition of Pathogens**

DCs need to discriminate between different classes of pathogens to induce proper adaptive immune responses. Discrimination of pathogens depends on the array of PRRs expressed on the DC. Several classes of PPRs are expressed by DCs, including TLRs, NOD-like receptors, RIG-I like receptors and CLRs. These PRRs induce signaling events that activate immune responses and tailor the responses to be specific for the type of pathogen detected. Therefore the array of PRRs that recognizes a pathogen determines the type of adaptive immune response that is induced by DCs. TLRs and CLRs recognize PAMPs at the cell surface or after uptake in endosomes/lysosomes, and are therefore able to sense pathogens prior to infection of the cell. Once infected, the PAMPs released into the cytosol are recognized by sensors such as NOD- and RIG-I-like receptors.
The C-type lectin DC-SIGN is highly expressed by DCs and interacts with both mannose- and fucose-expressing pathogens, which include bacteria, viruses and helminthes. Recent reports show that DC-SIGN is crucial in shaping adaptive immune responses to specific pathogens\textsuperscript{15,16} (Fig. 2). DC-SIGN triggering alone does neither result in DCs maturation nor in transcription of inflammatory genes. Nevertheless, DC-SIGN signaling shapes immune responses by modifying signaling cascades activated by other PRRs. Therefore DC-SIGN either enhances or suppresses specific pro-inflammatory cytokines\textsuperscript{16}. Notably, signaling induced by DC-SIGN discriminates between mannose- and fucose-containing pathogens\textsuperscript{16}. Mannose-carrying pathogens such as HIV-1 trigger signaling via a signalosome that is pre-assembled at the cytosolic domain of DC-SIGN. This signalosome contains KSR, CNK and Raf-1, that link to the cytoplasmic domain of DC-SIGN through the adaptor protein LSP1. The signalosome is required for the constitutive recruitment of Raf-1 to DC-SIGN. Upon binding of mannose-containing
pathogens by DC-SIGN, Raf-1 becomes activated by recruitment of the upstream effectors LARG and RhoA to the DC-SIGN signalosome. Signaling downstream of Raf-1 induces phosphorylation of the NF-κB subunit p65 at serine 276, which allows subsequent acetylation of p65 at different lysine residues\textsuperscript{15}. Acetylation of p65 prolongs its activity and also enhances its transcription rate at genes such as \textit{IL12A}, \textit{IL12B} and \textit{IL6} (ref. 15). Thus, p65 acetylation enhances expression of pro-inflammatory cytokines that are required for T\textsubscript{h}1 differentiation\textsuperscript{17}. Because Raf-1 activation alone does not induce NF-κB activation, prior triggering of other PRRs such as TLRs is required to activate NF-κB subunit p65. In contrast, fucose-expressing ligands actively disassociate KSR, CNK and Raf-1 from DC-SIGN, which results in activation of a signaling cascade that suppresses pro-inflammatory cytokines\textsuperscript{16}. It has become clear that binding of DC-SIGN to pathogens such as HIV-1 induces signals that shape adaptive immunity and/or promote HIV-1 infection and transmission. HIV-1 triggering of DC-SIGN activates Raf-1, which enhances TLR-induced pro-inflammatory cytokines IL-12p70 and IL-6, as well as the suppressive cytokine IL-10 (refs. 15, 16). These data suggest that HIV-1 can use this modulatory signaling pathway to affect adaptive immune responses in HIV-1-infected individuals. Currently, it is unclear whether the modulation is beneficial to the host or virus.

**Figure 3. Structure of Langerin**

The crystal structure mannose bound to the CRD of Langerin (PDB ID: 3P7G, ribbon generated with Cn3D 4.3). Mannose is bound by the primary calcium-dependent (gray sphere) carbohydrate binding site. The secondary carbohydrate binding site is separated from the primary site by the long loop region. Alpha-helices are colored green, beta-sheets are colored orange.
Langerin

LCs express the C-type lectin Langerin that has a carbohydrate specificity with similarities to that of DC-SIGN. Although Langerin is solely expressed by LCs in humans\textsuperscript{18}, Langerin-positive dermal DCs have been identified in mice\textsuperscript{19-21}. Because of the similarities between the carbohydrate recognition domain (CRD) of DC-SIGN and Langerin, it is not surprising that Langerin has a similar pathogen recognition profile as DC-SIGN. Indeed, Langerin and DC-SIGN both bind to HIV-1\textsuperscript{3,22} and \textit{M. tuberculosis}\textsuperscript{23,24}, and both C-type lectins are able to internalize antigens and process them for antigen presentation\textsuperscript{18,25}. However, their function in HIV-1 transmission is completely opposite; whereas DC-SIGN promotes HIV-1 transmission to T cells, Langerin chelates HIV-1 and prevents its transmission\textsuperscript{3}. This specialized function of Langerin might reflect an antiviral function of LCs.

Upon discovery of Langerin\textsuperscript{26}, sequence analysis of its CRD revealed high similarity to the Mannose Receptor and Mannose Binding Lectin, which are other mannose binding lectins. The CRD of Langerin contains an EPN (Glu-Pro-Asn) motive, which is predictive for mannose specificity\textsuperscript{27}. Langerin was shown to bind to mannose, fucose, N-acetylglucosamine (GlcNAc) structures\textsuperscript{28}, sulfated carbohydrates\textsuperscript{29} and β-glucans\textsuperscript{8}. Recent studies using crystallography confirmed these \textit{in silico} data, demonstrating that the Langerin CRD has high structural...
similarities to that of other typical C-type lectins\textsuperscript{30}. Two anti-parallel beta-sheets divide the CRD into two lobes (Fig. 3). The upper lobe contains the long loop region with an extra alpha helix compared to other CRD and forms the primary calcium-dependent carbohydrate binding site. Co-crystallization of Langerin with maltose or mannose revealed a second, calcium-independent binding site. This additional binding site is formed by an extra two stranded beta-sheet, which is, compared to the primary binding site, located on the other side of the long loop region. Modeling high-mannose structures in a crystal structure suggested the presence of a third binding site for high-mannose\textsuperscript{31}. Although it is not clear whether these additional binding sites are functional without the primary binding site, it suggests that these sites strengthen the interaction of ligands with the primary binding site of Langerin.

![Figure 5. Langerhans cells are a barrier against HIV-1 infection.](image)

(a) Immature Langerhans cells (LCs) capture and degrade HIV-1 through Langerin in healthy mucosa preventing infection and spreading of the virus. (b) However, in presence of coinfections (such as \textit{C. albicans}) or inflammation, LC are rendered susceptible to HIV-1 infection. Infected LC migrate towards the lymph nodes and transmit the virus to T cells. (c) When the LC are removed by abrasions or bypassed by micro-cuts, HIV-1 has direct access to the mucosal DC-SIGN\textsuperscript{+} dendritic cells. These efficiently capture and transmit HIV-1 to T cells in the lymph node and initiate the viral dissemination.
Sequence analysis of Langerin in several populations revealed single nucleotide polymorphisms (SNPs) that alter the amino acid sequence of Langerin. These SNPs can affect Langerin stability and affinity for certain carbohydrates. The most common SNP with an allele frequency of 48% (rs741326), results in an alanine substitution for a valine at position 278, which neither influences carbohydrate binding nor protein stability. However, a rarer SNP resulting in an asparagine (Asn, N) substituted for an aspartic acid (Asp, D) at position 288 (rs13383830), causes an at least ten-fold lower affinity for mannose-BSA. It is currently unknown whether these SNPs also affect Langerin binding to pathogens, and LC function.

Viral infections

HIV-1 and Measles virus (MV) are two lymphotropic and myelotropic viruses used in this thesis to study the interaction of viruses with DCs and LCs. HIV-1 is a RNA virus which has two genomic single stranded RNA strands that need to be reverse transcribed into DNA before integration into the genome of the host. The genome of MV contains RNA strands that, in contrast to the HIV-1 genome, do not become integrated into the host DNA. Instead, it replicates in the cytosol of the infected cell. Both viruses are most prevalent in sub-Saharan Africa, where they are a major cause of mortality and morbidity. Following infection, severe immune suppression causes opportunistic pathogens to infect patients, which are the major cause of HIV-1-related mortality and increase the mortality of MV.

DCs capture both viruses through DC-SIGN and increase transmission to T cells. In strong contrast, immature LCs have been shown to protect against HIV infection via the C-type lectin Langerin. The consequence of pathogen recognition by Langerin is for most pathogens unknown. Recent data strongly suggest that Langerin has an antiviral function by capturing HIV-1 for degradation and thereby preventing HIV-1 infection of LC (Fig. 4a). Langerin interacts with HIV-1 (ref. 35) by binding to high-mannose structures present on the envelope glycoprotein gp120 (refs. 36, 37). After capture by Langerin, HIV-1 is internalized into Birbeck granules, an organelle only found in LCs. Comparison between cell-lines expressing Langerin and DC-SIGN shows that Langerin capture of HIV-1 leads to rapid degradation of the virus. Thus, Langerin capture...
Figure 6. Antigen presentation pathways in DCs.

DCs need to process antigens to be able to present them to T cells. (a) Misfolded and old endogenous proteins are degraded by the proteasome and transported back into the endoplasmic reticulum (ER) by TAP. HLA-I is loaded with peptides provided by TAP, and the loaded complex is transported through the golgi to the cell membrane. On the cell membrane peptide loaded HLA-I is presented to CD8+ T cells. (b) Exogenous antigens are taken up by DCs and routed into lysosomes and processed for presentation. Newly synthesized HLA-II is transported to the lysosomal compartment, where it is loaded with antigen-derived peptides. Loaded complexes are presented on the cell membrane to activate CD4+ T cells. (c) Exogenous antigens can also be presented in HLA-I, a process called cross-presentation. The exact mechanism is elusive, but antigens are taken up and are loaded in HLA-I. Cross-presentation allows DCs to activate CD8+ T cells to pathogens and cancers that do not affect them. Figure adapted from Villadangos & Schnorrer51.
of HIV-1 and subsequent targeting to the Birbeck granules\textsuperscript{18} protects both LCs and surrounding cells from HIV-1 infection\textsuperscript{1}. Indeed, LCs expressing functional Langerin are resistant to HIV-1 infection and do not transmit HIV-1 to T cells. Moreover, co-cultures with Langerin-expressing cells show reduced infection of bystander T cells, supporting a protective role for Langerin and LCs in HIV-1 infection\textsuperscript{1}.

HIV-1 infection of LCs occurs only when Langerin function is inhibited or saturated by high viral titers\textsuperscript{3,38-42}. Inhibition of Langerin allows HIV-1 infection of LCs and subsequent transmission to T cells (Fig. 4b). Studies using vaginal explants\textsuperscript{41,43} and reconstructed vaginal mucosa\textsuperscript{44} show that LCs can be infected with HIV. However, these experiments were done with relative high viral titers, saturating Langerin, hence blocking its function. Epidemiological studies show that coinfections increase the risk of acquiring HIV-1 (refs. 45-47). Notably, activation of LCs by TNF or TLR2 agonist PAM3CSK4 increases HIV-1 infection of LCs and enhances HIV-1 transmission by LCs to T cells\textsuperscript{38,39,42}. TNF increases HIV-1 replication in LCs, whereas the TLR2 agonist enhanced HIV-1 capture by LCs through an as yet unknown mechanism\textsuperscript{38}. Moreover, activation of LCs results in down-regulation of Langerin and therefore less efficient capture of HIV-1. Thus, immature LCs protect against HIV-1 infection as long as Langerin is functional. However, activated LCs are as efficient in transmitting the virus to T cells as DCs (Fig. 5). Moreover, micro-cuts that breach the epithelial/mucosal barrier provide direct access to DCs resulting in dissemination of the virus\textsuperscript{48,49}. As described above, SNP influence binding of mannose-BSA to Langerin. Because binding is essential for the protective function of Langerin, SNPs in \textit{langerin} might increase susceptibility to HIV-1 infection.

**Antigen presentation**

DCs efficiently capture and process pathogens for induction of adaptive immune responses. Antigen presentation requires that intracellular and extracellular antigens are processed and routed to HLA class-I (HLA-I) or HLA-II. DCs take up antigen from their surroundings and process them for presentation in HLA-II. Furthermore, DCs are able to present intracellular peptides and extracellular antigens via a process called cross-presentation in HLA-I.
Endogenously synthesized proteins are sampled from the cytosol and transported into the endoplasmic reticulum (ER) by the transporter associated with antigen presentation (TAP). There the peptides are loaded into HLA-I for presentation via the classical HLA-I pathway (Fig. 6a). The profile of peptides expressed in HLA-I on the cell membrane reflects the proteins produced inside the cell. Hence, virus-infected cells present virus-derived peptides in HLA-I on the cell surface to allow recognition of activated CD8+ cytotoxic T cells (CTL) to clear the infection. Whereas all nucleated cells are able to present intracellular antigens in HLA-I, only DCs are able to present intracellular pathogen-derived peptides to induce a CD8+ T cell-response. DCs detect intracellular pathogens by PPRs and subsequently up-regulate co-stimulatory molecules. Naive CD8+ T cells that recognize the peptides presented on DCs in combination with co-stimulatory molecules, start to proliferate and transform into CTL. Activated CTL can kill infected cells via recognition of the pathogen-derived peptides in HLA-I of the infected cell.

Extracellular antigens that are taken-up by DCs are routed to lysosomal compartments to be processed and presented in HLA-II (Fig. 6b). New HLA-II molecules are synthesized in the endoplasmic reticulum and routed to the lysosomal compartments where they are loaded with the pathogen-derived peptides. Antigen-HLA-II complexes are transported to the cell membrane where they are presented to CD4+ T cells. Depending on the cytokines expressed by DCs, these CD4+ T cells differentiate into different T helper subsets, of which Th1, Th2 and Th17 cells are the most studied subtypes.

Besides presentation of endogenous antigens in HLA-I, DCs are able to cross-present exogenous antigens in context of HLA-I (Fig. 6c). Cross-presentation is a process in which exogenous antigens are taken up and routed to be presented in HLA-I, as if they were endogenous to the DC. This allows activation of CD8+ T cells specific for viruses that do not infect DCs. The mechanism of cross-presentation remains elusive, although several hypothesis have been proposed (reviewed in ref. 50). It is becoming clear that the different DC subsets have different cross-presenting capacities, at least in mice. In this thesis we have assessed the capacity of human DCs and LCs to cross-present viral antigens.
**Thesis Outline**

In this thesis we aimed to better understand the role of C-type lectins, DCs and LCs in immunity. C-type lectins play a crucial role in innate immunity and shaping immune responses. DCs express DC-SIGN that facilitates transmission of HIV-1 to T cells. We investigated whether DC-SIGN could not only shape adaptive immunity, but also affect HIV-1 replication in DCs. We have demonstrated that DC-SIGN signaling is required for HIV-1 replication (Chapter 2). HIV-1 activates NF-κB through TLR8, however DC-SIGN signaling is required for continuation of transcription to produce full length transcripts. In contrast to DC-SIGN, Langerin prevents HIV-1 infection. Langerin is expressed by LCs and degrades the virus upon binding. Interfering with Langerin increases susceptibility of LCs to HIV-1 infection. Therefore we investigated the effect of a SNP in the *langerin* gene on HIV-1 transmission in cohorts. We found that variant rs13383830 D288 in *langerin* associates with HIV-1 infection in women, but not in men (Chapter 3) which probably reflects the presence of Langerin in vaginal mucosa while it is absent in the rectum. Besides viral transmission, C-type lectins also capture antigens for presentation to T cells. We assessed the capacity of DCs and LCs to present MV-derived antigens to T cells. We found that DCs capture MV through CD150 and DC-SIGN (Chapter 4). Subsequently, MV-antigens are presentation to CD4+ T cells, which is predominantly mediated by DC-SIGN. Langerin on LCs captures MV for presentation to CD4+ T cells as well. However, whereas DCs are able to cross-present MV-antigens, LCs cannot cross-present MV-antigens from cell-free MV or MV-infected apoptotic cells (Chapter 5). The findings presented in this thesis demonstrate that, although DCs and LCs both belong to the DC family, they have very different characteristics, most likely reflecting their distinct localizations and functions.
Reference List


