Langerhans cells and dendritic cells in innate defense against pathogens

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Chapter 1

General Introduction

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Introduction

Our body encounters millions of potential pathogens every day, such as bacteria, viruses and fungi that attempt to invade our body through our skin and mucosal tissues. To counteract invading pathogens, we are equipped with layers of mechanical protection: the stratum corneum of our skin serves as a physical barrier, while the mucosal tissues are lined with a mucus layer in which anti-microbial agents and mucus form a protective layer (1).

Nevertheless, pathogens often cross these barriers and at this point the immune system starts to play a crucial role. The immune system can be divided into innate and adaptive immunity. Innate immunity forms a strong, direct, non-specific line of defense toward pathogens. The innate immune system is constituted of natural killer cells, neutrophils, eosinophils, basophils, mastcells and macrophages. In contrast, the adaptive immune response is antigen- and pathogen-specific and induces immunological memory, which is mediated via T and B lymphocytes. Dendritic cells (DCs) bridge these two arms of the immune response (2). As professional antigen presenting cells, DCs reside in the peripheral tissues, sampling their environment for pathogens. Upon capture of pathogens or antigens, DCs undergo a series of phenotypical changes, also known as maturation, which is accompanied by increased expression of MHC-II and co-stimulatory molecules CD80, CD86, CD40, and acquisition of CD83 (2;3). Activation of DCs results in release of chemokines and cytokines, and up-regulation of CCR7, which in turn facilitates the migration of DCs toward the lymphatics. Subsequently, DCs migrate via the lymphatic system to peripheral lymph nodes. Antigens captured by DCs are processed and presented in MHC-I and -II complexes for the induction of antigen-specific naive T cells to initiate appropriate immune responses to eliminate pathogens (2). This process is tightly controlled and dysregulation can lead to tolerance, allergy or auto-immune diseases (4). Thus, DCs act as an important bridge between the innate and adaptive immune system; DCs recognize pathogens through innate recognition mechanisms and translate this information into induction of antigen-specific adaptive immunity.

1. Dendritic cell subsets

DCs were first described in 1868 by Paul Langerhans, who observed highly dendritic, non-pigmentary cells in the epidermis and named them Langerhans cells (LCs) (5). However, it was not until the 1970s that the lineage and function of DCs became clear(er) (6). DCs consist of two main categories: plasmacytoid DCs that circulate through the blood and play an important role in viral immunity by secreting high amounts of type I interferons upon activation and conventional DC, which are further subdivided into resident DCs and migratory DCs. Migratory DCs consist of DCs located in the dermis and sub-epithelium and LCs present in the epidermis and mucosal stratified squamous epithelium. By extending their long dendrites throughout the epidermis/epithelium and dermal/ sub-epithelial, DC subsets sample the surrounding environment for invading pathogens and antigens (7).

For a long time, LCs and sub-epithelial DCs were thought to exhibit similar immunological capacities. However, more and more differences between these two

cell types are being recognized. LCs in mucosal stratified squamous epithelium and skin epidermis and DCs in the sub-epithelium and dermis not only differ in their location, LCs are also phenotypically distinguishable from DCs by expression of the markers CD1a, the C-type lectin Langerin and E-cadherin, and presence of Birbeck granules (5;8;9), while DCs express other C-type lectins, such as mannose receptor and DC-SIGN (10).

It is thought that sub-epithelial DCs and LCs are derived from hematopoietic bone marrow progenitor cells, which differentiate via blood-circulating monocyte intermediates into immature cells and will then home to specific tissues (2;11;12). Transplantation studies and experiments in mice have provided additional insights in the ontogeny of DC subsets and suggest the presence of progenitor cells in the epidermis, which give rise to LCs (13-15). Although this remains to be further elucidated, it suggests that LCs and sub-epithelial DCs might have distinct progenitors (5).

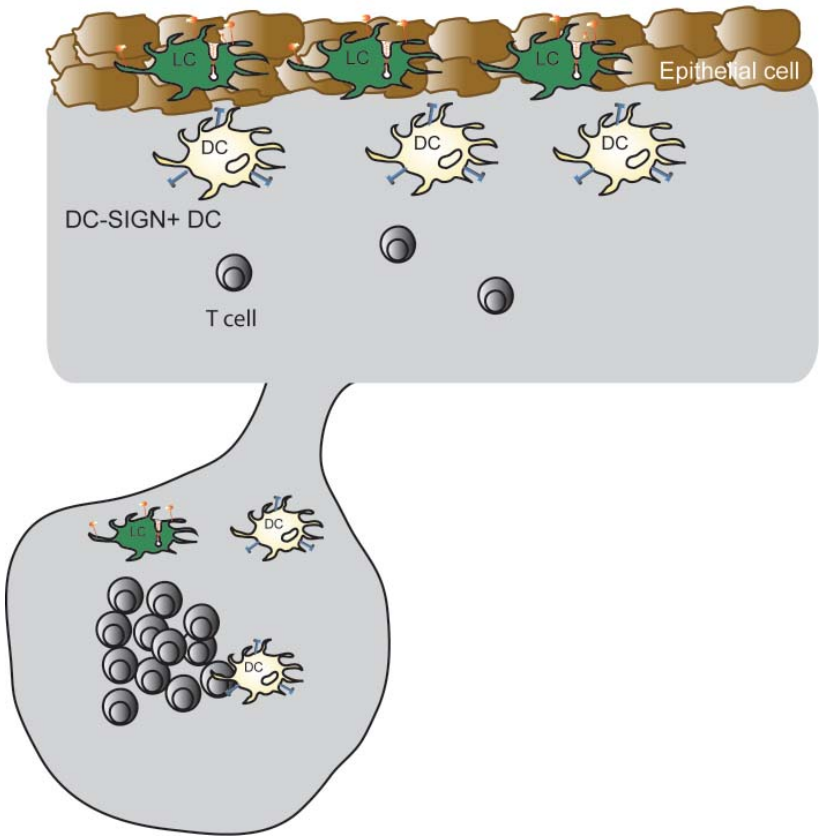

Figure 1. Schematic overview of LCs and DC-SIGN$^+$ DCs in mucosal tissues. LCs reside in the epithelium while DC-SIGN$^+$ DCs are present in the sub-epithelium. Upon activation LCs and DCs mature and migrate towards the lymphoid tissues. In the lymphnode specific T cells are activated and mediate adaptive immune responses.

2. Antigen presentation

DCs play an important role in the induction of adaptive immune responses. Depending on what type of antigen is encountered, a specific immune response is elicited. Exogenous antigens, such as bacteria and apoptotic cells, are internalized, processed and presented onto MHC-II for $CD4^+$ T cell activation while on the other hand, during DC infection with viruses and other pathogens, endogenous antigens are loaded onto MHC-I for $CD8^+$ T cell activation (2). Under certain circumstances, exogenous antigens are processed for $CD8^+$ T cell presentation via MHC-I, a process referred to as cross-presentation (16;17). These distinct pathways lead to an immune response tailored towards specific antigens.

$DC\text{-}SIGN^+$ DCs are able to process antigens for both $CD4^+$ and $CD8^+$ T cell activation and cross-presentation (2). However, little information is available about antigen presentation by human LCs. Mice studies demonstrated that LCs targeted with anti-Langerin antibodies present to both $CD4^+$ and $CD8^+$ T cells via MHC-II and MHC-I (18). On the other hand, a murine herpes simplex virus (HSV) model showed that LCs are not able to cross-present HSV. Instead it is thought that LCs transfer their antigens or apoptotic LC fragments to another DC subset (19). Whether this antigen transfer takes place in the epidermis or in the lymph nodes remains unclear (19;20). In addition, murine LCs migrate much slower from the epidermis to the lymph nodes compared to dermal DCs (21). Thus, several studies concluded that there is lack of involvement of LCs in the presentation of pathogens and together these data suggest that dermal DCs but not LCs play a pivotal role in early antigen presentation events during infection (19;22-26).

3. Pathogen recognition

Pathogens express evolutionary highly conserved sets of molecules also known as pathogen-associated molecular patterns (PAMPs). These are recognized by host pattern recognition receptors (PRR), which include Toll-like receptors (TLRs), C-type lectins, RIG-I like receptors, and (intra)cytoplasmic nucleotide oligomerization domain (NOD)-like receptors (NLRs) (4). Each of these receptors has a specialized function in the recognition of pathogens and the induction of immunity.

TLRs are important for DC function by recognizing PAMPs from bacterial, viral, fungal and parasitic origin. Recognition of a pathogen by TLRs results in DC maturation and the production of cytokines that induce a specific immune response tailored to the pathogen (27). Each TLR is specialized for a specific group of pathogens and the expression pattern of specific TLRs by DCs is indicative for their function. Gram positive bacteria and mycobacteria are mainly recognized by TLR-1, -2, and -6, whereas TLR-4 is specific for gram-negative bacteria and TLR-5 detects flagellated bacteria. TLR-3, -7, -8 and -9 are located in intracellular vesicles and recognize respectively dsRNA (TLR-3), ssRNA (TLR-7, -8) and unmethylated CpG oligonucleotides in bacterial DNA (TLR-9) (27).

$DC\text{-}SIGN^+$ DCs (both sub-epithelial DCs and monocyte-derived DCs) express TLR-1 through -9, while LCs express TLR-1, -2, -3, -6, and -7 but neither TLR-4, -5, and low or no TLR-8 and TLR-9 (3;28). This profile suggests that LCs preferentially

respond to viruses but not to gram-negative or flagellated bacteria. In general, LCs have low responsiveness to TLR ligands and secrete only low amounts of cytokines compared to keratinocytes or sup-epithelial DCs (4). Residing in the peripheral tissues LCs are almost continuously exposed to commensal bacteria and fungi and LCs should therefore not respond to every (commensal) microbe they encounter in order to prevent continuous inflammation. In contrast, once bacteria have breached the epidermal/epithelial barrier, indicative of severe damage, dermal/sub-epithelial DCs should recognize these bacteria and fungi and induce strong immune reactions.

4. C-type lectins

C-type lectins also play a role in pathogen recognition. C-type lectins are transmembrane proteins that bind carbohydrate structures in a calcium-dependent manner. C-type lectins are expressed by different antigen presenting cells and capture pathogens through binding of carbohydrate structures that are present on pathogens. C-type lectins function as endocytic receptors, and capture of a ligand leads to internalisation, degradation, processing and subsequently to antigen presentation (29).

LCs and sub-epithelial DCs express distinct C-type lectins: Langerin on LCs and DC-SIGN on DCs (Table 1). In human, Langerin is exclusively expressed by LCs in the epidermis and mucosal stratified epithelium, whereas DC-SIGN is expressed by DCs in dermis, mucosal sub-epithelium, rectal epithelium and some lymphoid tissues, but also by macrophages during inflammation (5;29). Both DC-SIGN and Langerin belong to the group I C-type lectin receptors (also known as mannose receptor family group) and are type II transmembrane proteins (30).

CD209 or **D**endritic **C**ell **S**pecific **I**ntercellular adhesion molecule-3-**G**rabbing **N**on-integrin (DC-SIGN) contains a CRD which includes two Ca^{2+} binding sites: one for conformation, one for coordination of carbohydrate structures, a neck region that is required for oligomerization, a transmembrane region, and a cytoplasmic domain, which is involved in internalization. DC-SIGN forms tetramers and this oligomerization of the lectin domain might be crucial for the affinity and specificity of carbohydrate recognition (29;31). DC-SIGN was first identified in 2000 and was shown to play a role in DC-T cell interaction and HIV-1 recognition (10;32). Since then, a wide range of functions have been described for DC-SIGN, such as cell-adhesion, migration, uptake of self- and non-self antigens for antigen presentation (29). It is now evident that DC-SIGN is an important PRR. DC-SIGN specifically interacts with mannose and fucose containing-carbohydrates expressed by pathogens, and binding of these carbohydrate structures in combination with TLR stimulation modulates TLR mediated immune responses such as cytokine secretion and T cell polarization (33). Thus, DC-SIGN plays an important role in the recognition of pathogenic structures and a plethora of viruses, bacteria, fungi and parasites that interact with DC-SIGN have been identified e.g. HIV-1, Measles Virus, Hepatitis C Virus*, Mycobacterium tuberculosis*, *Candida albicans*, and *Schistosoma mansoni* (32;34-39). The outcome of the interaction with these pathogens differs: e.g. interaction with DC-SIGN could lead to re-direction of the internalization route to non-lysosomal compartments for

protection and transmission to target cells or to escape immune surveillance by inhibition of the immunostimulatory function of DCs (29).

Langerin is a C-type lectin expressed uniquely by human LCs and therefore often used as a marker for LCs (9). Langerin contains a calcium-dependent carbohydrate recognition domain (40) and a short cytoplasmic tail that contains a proline-rich motif, which might be involved in signaling (41). It is thought that Langerin contains a second carbohydrate recognition domain that acts in a calcium-independent manner (40), although whether this is important in its function is yet unclear. Langerin forms trimers on the cell-surface and has a carbohydrate specificity for mannose, fucose and N-acetyl-glucoseamine monosaccharides (GlcNAC) (42). Langerin is a pathogen receptor on human LCs that binds HIV-1 (43;44), as well as the mycobacterial cell-wall component ManLAM (45). Since mannose structures are abundantly expressed by pathogens, especially by viruses, it is to be expected that Langerin has a broad specificity for different pathogens including viruses.

Little is known about the function of Langerin as a pathogen receptor. Langerin-dependent presentation of *Mycobacterium*-derived structures has been documented (45). In addition, Langerin induces the formation of specific organelles called Birbeck granules (46). These Birbeck granules are part of the endosomal recycling pathway, which are exclusively present in LCs. They are tennis racket-like structures of 1 µm in diameter and approximately 50 nm thick (47). The formation of Birbeck granules occurs through cross-linking of either Langerin with a ligand on the cell-surface or a soluble ligand between Langerin molecules, resulting in “zipping” of the granules (41). Their small size and the lack of appropriate markers have complicated the research to understand the function of Birbeck granules and the recycling mechanism of Langerin. Data have shown that Birbeck granules are involved in degradation of viruses, since HIV-1 is internalized into these compartments by Langerin (44). Noteworthy, Langerin expression in mice is not restricted to epidermal tissues. Recently, a small population of Langerin^{+} CD103^{+} dermal DCs has been identified in mice (5;48). It remains to be determined whether this subset is also present in human, and whether Langerin has a similar function on these cells.

Table 1. Overview of the characteristics of C-type lectins Langerin and DC-SIGN in human.

	Langerin	**DC-SIGN**
Cell type	Langerhans cells	Dendritic cells, macrophages
Location	Epidermis, mucosal stratified squamous epithelium	Dermis, sub-epithelium, rectal mucosa and lymph node
Carbohydrate recognition	Mannose, Fucose, GlcNAc	Mannose, Fucose
Oligomerization	Trimers	Tetramers
Pathogen binding	HIV-1, mycobacteria	HIV-1, mycobacteria, *C. albicans*, HCV, *S. mansoni*, *H. pylori*, SARS and more
Characteristic	Induces Birbeck granules formation	Extensively studied in monocyte-derived dendritic cells

5. LCs and DC-SIGN$^+$ DCs in health and disease

Most of the times when a pathogen enters our body, the infection is cleared efficiently. However, along with the human immune system, pathogens have evolved as well and have developed different mechanisms to evade our immune system for their own benefit. This thesis describes the effect of different pathogenic agents on our immune system and in particular the roles that LCs and DC-SIGN$^+$ DCs play.

5.1 HIV-1

HIV-1, the causative agent of AIDS, has infected more than 33 million people world-wide, while another 1 to 3 million people acquire HIV-1 annually (49). The main target cells for HIV-1 are CD4$^+$ T cells, which express the entry receptor CD4, and co-receptors CCR5 (for R5-tropic viruses) and CXCR4 (for X4-tropic viruses). However, macrophages and DCs are infected as well. Upon entry, reverse transcription of viral RNA into DNA takes place, which is subsequently integrated into the host genome, thereby causing a life-long infection.

Sexual transmission is the major route of HIV-1 infection (50;51). In general, the transmission rate of HIV-1 is not very efficient: the male-to-female transmission probability through semen is 1 in 200 to 1 in 2000 per exposure (52). This might be due to the architecture of the genital tissues, which have been designed to form a protective barrier against invading pathogens. In order for HIV-1 to infect an individual via sexual transmission, HIV-1 has to circumvent a mucus layer, which traps pathogens to prevent further infection. In addition, the mucus layer acts as a primary microbicide since hydrogen peroxide- producing lactobacilli are present (53;54). Below this mucus layer, a stratified layer consisting of mainly epithelial cells is located. HIV-1 preferentially infects CD4$^+$ T cells but only few CD4$^+$ target cells are present at the site of HIV-1 entry in the genital tissue. However, they are abundant in lymphoid tissues. Therefore, HIV-1 needs to disseminate from the genital tissue to the lymphoid organs. LCs and DC-SIGN$^+$ DCs are thought to play a role in the dissemination of HIV-1. However, it is becoming clear that the interaction of HIV-1 with LCs and DC-SIGN$^+$ DCs has different effects.

DC-SIGN$^+$ DCs are present in both genital and intestinal mucosa. In the cervix and vagina, DC-SIGN$^+$ DCs are located in the supepithelium underneath the squamous epithelial layer but in rectum and Peyer's patches in the intestine DC-SIGN$^+$ DCs are also abundantly present in the epithelium (55). In addition, DC-SIGN$^+$ DCs express low levels of CD4, CCR5 and CXCR4, and are therefore susceptible to HIV-1 infection. However, the level of HIV-1 infection and replication is low compared to CD4$^+$ T cells (56).

DC-SIGN on DCs promotes HIV-1 transmission by capturing HIV-1 and transmitting HIV-1 to T cells independently of infection, a process known as trans-infection (32;57). It was shown that DCs retain HIV-1 for a prolonged period of time after which DCs are still able to transmit virus to target cells (58). In addition, DC-SIGN binding to HIV-1 enhances HIV-1 infection by facilitating binding of HIV-1 to CD4 and co-receptors on the same cell, which is known as cis-infection (59). Activation of DC-SIGN$^+$ DCs enhances HIV-1 transmission to T cells; mature DCs,

however, have a reduced ability to support HIV-1 replication (60). Furthermore, animal studies have demonstrated that HIV-1 rapidly penetrates the vaginal mucosa and infects or associates with DC-SIGN$^+$ DCs, which subsequently transfer HIV-1 to target T cells (61;62). However, due to their location, not DC-SIGN$^+$ DCs but LCs are the first antigen presenting cells to encounter HIV-1.

LCs lining the mucosal stratified epithelium are among the first cells at the mucosal layer to encounter HIV-1. LCs express HIV-1 receptor CD4 and the co-receptor CCR5 (63;64). Therefore LCs are primarily infected with R5- but not with X4- tropic viruses. Since R5-tropic HIV-1 is predominantly present during the initial viremia after sexual transmission of HIV-1 (65), these data suggest that LCs are involved in HIV-1 transmission. *In vitro* and *in vivo* studies have demonstrated that LCs are refractory to HIV-1 infection since high virus concentrations are needed to infect LCs (44;66-69). These studies suggest that LCs form a barrier for HIV-1.

Langerin expressed by LCs has a high affinity for HIV-1. Capture of HIV-1 by Langerin prevents infection of LCs via CD4/CCR5 by rapid internalization into Birbeck granules, which leads to virus degradation. Inhibition of Langerin with antibodies or high virus concentrations allows LC infection, demonstrating that Langerin prevents LC infection but that high viral concentrations saturate its protective function (43;44). Therefore, Langerin forms a protective barrier against HIV-1. This protective barrier seems to be dependent on the activation status of LCs, since stimulation of LC-like cells results in trans-infection of target cells (70). Both *in vivo* and *ex vivo* data support a role for LC infection in HIV-1 transmission (66-69). Rhesus macaque infection with simian immunodeficiency virus (SIV) has demonstrated that LCs beneath the vaginal epithelium become infected within 24 hours of infection (68). Furthermore, several studies have used *ex vivo* skin explants models to demonstrate that LCs transmit HIV-1 to T cells (66;69;71).

Genital infections and/or inflammation of genital tissue lead to enhanced HIV-1 transmission (72-75). Genital infections have been linked to increased susceptibility to HIV-1 and include sexually transmitted infections (STIs), such as genital herpes, syphilis, gonorrhoea, chlamydia, and trichomoniasis (72;76), as well as yeast and bacterial vaginal infections (73;74). Pre-existing genital STIs are a risk factor for acquiring HIV-1, indicating that pathogens or host-responses to the pathogens create an environment that favours HIV-1 infection and dissemination (50).

Several hypotheses have been described to explain the increased susceptibility to HIV-1 infection in the presence of STIs and these are not mutually exclusive. Some STIs, such as genital herpes and syphilis, cause ulceration, which leads to breaching of the epithelium and influx of CD4$^+$ T cells (77). This might allow HIV-1 to circumvent LCs in the epithelium. Consequently, HIV-1 can directly interact with either CD4$^+$ T cells residing beneath the basal membrane or sub-epithelial DC-SIGN$^+$ DCs, which are efficient in transmitting HIV-1 to CD4$^+$ T cells. In addition, ulceration and inflammation caused by STIs could lead to the production of pro-inflammatory cytokines and chemokines, which results in the recruitment of susceptible inflammatory cells to the genital tissue. These susceptible inflammatory cells persist for a prolonged time even after the lesions have healed, thereby forming

a potential target for HIV-1 infection (78;79). However, little is known about the role of STIs and vaginal flora on LC function.

In **chapter 3** of this thesis, the effect of STIs on the susceptibility of LCs to HIV-1 is described. Both pro-inflammatory cytokine TNF-α and bacterial and fungal TLR ligands enhance HIV-1 transmission from LCs to target cells through distinct mechanisms, transforming LCs from a protective barrier against HIV-1 infection into cells that facilitatie HIV-1 transmission. Whereas TNF-α enhances HIV-1 replication in LCs, TLR-2 ligand Pam3CSK4 enhances trans-infection through cell-surface bound HIV-1. Furthermore, **chapter 4** describes the response of LCs to HSV-2 and viral TLR ligands and subsequent consequences for HIV-1 transmission. LCs are productively infected with HSV-2, the most common viral STI, and interaction of LCs with HSV-2 is mediated via Langerin. In addition, both viral TLR ligands and HSV-2 induces activation of LCs, which results in down-regulation of Langerin from the cell-surface. Subsequently, LCs are more susceptible to HIV-1 infection.

To date, no curative treatments or protective vaccines against HIV-1 are available, while the HIV-1 pandemic is still expanding world-wide. Therefore, alternative protective measures, such as topically applied microbicides in the form of genital gels, creams, or rings, are needed to prevent HIV-1 transmission (49). Novel microbicides and mucosal vaccines should maintain Langerin function. However, since availability of primary LCs is limited, and primary LCs are difficult to isolate and vary between donors, it is not possible to perform large screening for the development of microbicides using primary LCs. In **chapter 5**, we have investigated whether LCs derived from a human $CD34^+$ acute myeloid leukaemia cell-line Mutz-3 (Mu-LCs) provide a valuable tool to investigate the role of LCs in HIV-1 transmission and microbicide screening. Mu-LCs resemble human LCs and Mu-LCs may be useful as an *in vitro* model to study the effect of microbicides on Langerin function.

Microbicides could be either naturally occurring or synthetically produced compounds. Both semen and breast milk contain several compounds that have been shown to modulate HIV-1 transmission (80;81). Mother-to-child transmission of HIV-1 frequently occurs via breast milk (82). However, it has been demonstrated that exclusive breast feeding during early months of life protects against HIV-1 infection (83-85). As described in **chapter 6,** MUC1, an epithelial mucin abundantly present in human milk interacts specifically with DC-SIGN on DC-$SIGN^+$ DCs, thereby inhibiting HIV-1 transmission from DC-$SIGN^+$ DCs to target cells.

Potential microbicides can also be derived from natural, non-human organisms. Mermaid is a calcium dependent mannose specific C-type lectin that is secreted by the marine nematode *Laxus oneistus*. The carbohydrate recognition domain is structurally and functionally similar to the one of DC-SIGN (86;87). **Chapter 7** describes the potential role of mermaid in HIV-1 transmission since mermaid blocks DC-mediated HIV-1 transmission by preventing DC-SIGN binding to HIV-1.

5.2 Herpes Simplex Virus

HSV is a double stranded DNA virus that belongs to the family of the *Alphaherpesviridae*. HSV is a common human pathogen that causes painful, but mild infections of the skin and mucosa, resulting in cold sores and blisters. Two subtypes of HSV have been described: HSV-1, which mostly causes oral-facial lesions and HSV-2, which is associated with genital herpes. HSV is transmitted by direct body contact with infected lesions or body fluids and enters the body at the mucosal tissues or lesions of the skin. The primary target cells for HSV are epithelial cells and keratinocytes. During primary infection, HSV enters cutaneous sensory neurons and travels to the dorsal root ganglia where it establishes the characteristic lifelong latent infection. At times when the immune system is suboptimal, such as during stress, trauma, UV-light, fatigue or a common cold, the virus escapes immune surveillance and migrates via the peripheral nerve towards the epithelium or skin, where it causes recurrent herpes simplex lesions (88;89). In the dermis and sub-epithelium DC-SIGN$^+$ DCs encounter HSV. DC-SIGN$^+$ DCs express HSV entry receptors HVEM (Hve-A) and nectin-2 (Hve-B), and heperan sulfates that mediate attachment and DCs are productively infected with HSV, which results in apoptosis (89).

HSV has developed several mechanisms to escape immune surveillance by DCs; HSV-1 infection strongly affects DC function by interfering with DC maturation, including down-regulation of co-stimulatory molecules as well as CD83, CD1 molecules and MHC-I (90-92). HSV-1 infection also decreases IL-12 production and lowers the allostimulatory capacity of DCs (93;94). In **chapter 8**, the interaction of DC-SIGN with HSV is investigated. Both HSV-1 and -2 interact with DC-SIGN via glycoproteins gB and gC, while binding of HSV to DCs is mediated by heparan sulfate proteoglycans (HSPGs) and DC-SIGN. DC-SIGN not only increases DC infection by HSV-1 but also captures HSV-1 for transmission to permissive target cells. Therefore, DC-SIGN is an attachment receptor for HSV-1 that contributes to the infectivity and transmission of HSV. In addition, in **chapter 4** the ability of HSV-2 to infect LCs and the effect of HSV-2 on the anti-HIV-1 function of LCs is described.

5.3 Hepatitis B Virus

More than 350 million people are chronically infected with Hepatitis B Virus (HBV) worldwide (95). Similar to HIV-1, HBV is transmitted via sexual intercourse and infects bodily fluids; HBV is, however, one hundred times more contagious than HIV-1. HBV is a DNA virus, consisting of a core particle enveloped by small (S), middle (M) and large (L) surface antigens, generally referred to as HBsAg (96). HBV primarily infects hepatocytes. Upon infection, most people will develop antibodies and clear the infection. However, when HBV is not eliminated efficiently, a chronic infection will occur, which can lead to liver cirrhosis or hepatocellular carcinoma (96).Despite the high incidence of infection, a cellular receptor for HBV entry is still unknown. Several proposed candidates include human serum albumin (97), asialoglycoprotein receptor (98), heparin (99) and mannose binding lectin (100), but their exact role in HBV attachment and uptake remains unclear (101). HBV research

has been hampered because of the lack of a viral culture system. Several reports have shown the presence of HBV on or within DCs (102-105). Whereas DCs express DC-SIGN, its homologue L-SIGN is expressed on sinusoidal endothelial cells in liver and lymph nodes (106). These receptors could be involved in HBV binding. However, **chapter 9** describes the lack of interaction between HBV and DC-SIGN or L-SIGN. This interaction is hampered because of the glycosylation pattern of HBV. This mechanism provides a potential escape mechanism for HBV.

5.4 *Borrelia burgdorferi*

Borrelia burgdorferi is a spirochete bacteria and is the causative agent of Lyme disease. *Ixodes scapularis*, also known as the 'deer tick', is the main arthropod vector for the transmission of *B. burgdorferi* (107). *Ixodes scapularis* feeds on birds, small rodents and large mammals, including humans and this is when transmission of *B. burgdorferi* takes place. During the course of the blood meal, *I. scapularis* penetrates the skin and introduces saliva and *B. burgdorferi* into the host. Tick saliva itself contains a wide range of components, which interfere with host processes and interaction with pathogens (108). Local immune suppression of the host by tick molecules assists *B. burgdorferi* in establishing an infection. Salp15, a 15-kDa salivary gland protein, is a major immunomodulatory protein in *I. scapularis* saliva (109). Salp15 has been shown to impair $CD4^+$ T cell activation and proliferation (110;111). Under normal circumstances there are very few $CD4^+$ T cells located at the site of the tick-bite, whereas DCs are abundantly present. **Chapter 10** describes the effect of Salp15 on DC function in the presence of *B. burgdorferi*. We demonstrate that local interaction of Salp15 with DCs in the presence of *B. burgdorferi* will lead to immunosuppression, which prevents proper immune activation and might therefore play an important role in the pathogenesis of Lyme disease.

5.5 *Candida* and *Cryptococcus* species

Fungal species are ubiquitous residents of human skin and gut flora and many cause invasive infections especially in immune compromised individuals. Since the epidermis provides a protective barrier against invading pathogens, LCs are the first antigen presenting cells to encounter cutaneous fungi. Few pathogens have been identified that interact with Langerin, however based on the carbohydrate recognition specificity for mannose, fucose and GlcNAc, it is likely that Langerin has a broader specificity for pathogens than has been identified thus far (42). Fungi ubiquitously express β-glucans and mannosylated carbohydrate structures (112;113). **Chapter 2** demonstrates that Langerin not only recognizes mannose, fucose and GlcNAc structures, but also β-glucan structures. In addition, Langerin is a receptor for *Candida* species, *Saccharomyces cerevisiae*, and *Malassezia furfur*, but only weakly interacts with *Cryptococcus gattii* and *Cryptococcus neoformans*. This could explain the pathogenicity of different fungi. Table 2 shows an overview of the pathogens discussed in this thesis.

Table 2. Overview of pathogens discussed in this thesis.

Pathogen	Type	Natural route of transmission	Pathogenicity	Target cell for infection
HIV-1	RNA-virus (lenti-virus)	Sexual transmission/ mother-to-child transmission via blood and body fluids	AIDS	Activated $CD4^+$ T cells, macrophages, DCs, LCs
HSV-1/-2	DNA-virus	Infected lesions, body fluids	Oral-facial lesions ('cold sore)/ genital herpes	Epithelial cells, keratinocytes, LCs, DCs
HBV	DNA-virus	Blood, body fluids	Liver cirrhosis/ hepatocellular carcinoma	Hepatocytes, DCs?
Borrelia burgdorferi	Bacteria (extracellular spirochete)	Tick bite by *Ixodes scapularis*	Lyme disease	N/A
Candida albicans	Fungus	Opportunistic, commensal in oral mucosa and gut	(vaginal) candidiasis	N/A
Cryptococcus neoformans	Fungus	opportunistic	Fungal meningitis (AIDS patients)	N/A

6. Thesis outline

The aim of this thesis is to increase our understanding of the role of LCs and DCs in innate defense against pathogens. Since fungal species are ubiquitously present on human skin and mucosa, the interaction of LCs with different fungal species was investigated and these data demonstrate that Langerin is an important fungal receptor on LCs (**chapter 2**). Fungi, bacteria and viruses are all causative agents of sexually transmitted infections (STIs) and epidemiological studies show that STIs increase HIV-1 susceptibility. Our experiments showed that STIs increase HIV-1 transmission by LCs, but that distinct mechanisms are involved (**chapter 3 and 4**). Furthermore, we investigated whether Mutz-3 derived LCs could be a valuable tool to screen potential microbicides for their ability to prevent LC-mediated HIV-1 acquisition (**chapter 5**). This model could overcome the low availability of human tissues and the difficulty of isolating primary LCs.

DCs located in the sub-epithelium and the gastro-intestinal tract also play an important role in HIV-1 transmission. We have tried to identify novel potential microbicides that prevent transmission and we have demonstrated that human milk protein MUC1 (**chapter 6**) and marine nematode *Laxus oneistus* C-type lectin mermaid (**chapter 7**) both interfere with DC-SIGN-mediated HIV-1 transmission by DCs. DC-SIGN on DCs not only facilitates HIV-1 transmission, but is also involved in the dissemination of other viruses. As described in **chapter 8**, DC-SIGN mediates infection and transmission of HSV-1 by DCs, while in contrast HBV does not interact with DC-SIGN (**chapter 9**). It is becoming evident that DC-SIGN is important in the induction of adaptive immunity to different pathogens. We have shown that the tick protein Salp15 impairs immune activation by DCs through its interaction with DC-SIGN, and this immunosuppression might play a role in Lyme disease (**chapter 10**). These studies are placed in a broader perspective in the general discussion (**chapter 11**).

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