ON THE ROLE OF DENDRITIC CELLS IN HIV-1 INFECTION

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To my family
Abstract

Dendritic cells (DCs) are antigen-presenting cells with the capacity to initiate primary T cell responses against pathogens such as HIV-1. In addition, DCs express the receptors required for HIV-1 infection and as DCs are prevalent in the mucosa, they encounter the virus early after sexual transmission. DCs also express additional receptors able to bind and capture intact HIV-1 without becoming infected. Normally, DCs capture antigen in the periphery and migrate to draining lymph nodes to present the antigen to T cells to start immune responses. HIV-1 may utilize this process as, DCs can collect and carry HIV-1 and facilitate spread to CD4+ T cells. To optimize the T cell activation, DCs mature by upregulation of MHC and co-stimulatory molecules and induction of cytokine production. However, another consequence of efficient interaction between DCs and T cells is that it provides an optimal milieu for HIV-1 transmission and replication.

We studied the effects of HIV-1 infection on DC function. We found that monocyte-derived DCs (MDDCs) were productively infected by HIV-1 after in vitro exposure (as measured by intracellular production of HIV-1 p24). HIV-1 infected MDDCs upregulated co-stimulatory molecules in response to CD40ligand stimulation, comparable to uninfected MDDCs. However, intracellular cytokine staining revealed that while the HIV-1 infected DCs were able to produce TNFα, they failed to express IL-12 p70. This may impact the ability of DCs to induce optimal HIV-1 specific immune responses, as IL-12 is vital for the induction of cellular immune responses. Next, we expanded the studies by examining isolated primary myeloid DCs (MDCs) and plasmacytoid DCs (PDCs). MDCs and PDCs became productively infected by different HIV-1 isolates. The DC subsets displayed differential susceptibility to HIV-1. HIV-1 exposure induced some maturation in the DCs. However, TLR ligation induced full maturation and TNFα production in both uninfected and infected DCs. Productively infected MDCs and PDCs efficiently transferred HIV-1 to autologous CD4+ T cells, and antigen-reactivated T cells were more frequently infected than non-responding T cells. This suggests that induction of DC-dependent antigen-specific T cell responses, crucial to the immune defence, also can lead to preferential HIV-1 infection of responding T cell clones in infected individuals.

DCs can present antigens derived from apoptotic cells. We investigated whether apoptotic HIV-1 infected cells were capable of eliciting HIV-1 specific immune responses in vivo. Immunization of mice with apoptotic HIV-1/MuLV infected cells resulted in induction of HIV-1 specific T cell and antibody responses. Moreover, immunized mice handled challenge with live HIV-1/MuLV infected cells more effectively than nonimmunized mice. These data show that immunization of mice with apoptotic HIV-1 infected cells can induce high levels of HIV-1 specific systemic immunity and prime for mucosal immunity that could provide means for the mice to cope with challenge.

Collectively, our findings demonstrate that DCs are under certain conditions impaired by HIV-1 infection but that they efficiently transfer HIV-1 to CD4+ T cells. Taken together, an increased understanding of DC immunobiology may help us develop more effective HIV-1 therapy and/or an HIV-1 vaccine.

**Keywords:** dendritic cell, plasmacytoid, myeloid, HIV-1, cytokines, co-stimulation, transmission, antigen presentation, apoptotic cells, MuLV.

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Original papers

This thesis is based on the following original papers, which are referred to in the text by their Roman numerals:

I. Anna Smed Sörensen, Karin Loré, Lilian Walther-Jallow, Jan Andersson and Anna-Lena Spetz: HIV-1 infected dendritic cells upregulate cell surface markers but fail to produce IL-12 p70 in response to CD40ligand stimulation. Blood 2004; 104: 2810.

II. Anna Smed Sörensen, Karin Loré, Jayanand Vasudev an, Mark K. Louder, John R. Mascola, Jan Andersson, Anna-Lena Spetz and Richard A. Koup: Differential susceptibility to HIV-1 infection of myeloid and plasmacytoid dendritic cells. Submitted.


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Abbreviations

Acquired Immunodeficiency Syndrome  AIDS
Antiretroviral Treatment  ART
Azidothymidine  AZT
CC Chemokine Receptor  CCR
Cluster of Differentiation  CD
CD40ligand  CD40L
Carboxy-Fluorescein Diacetate Succinimidyl Ester  CFSE
C-type Lectin Receptor  CLR
Cytomegalovirus  CMV
Cytotoxic T Lymphocyte  CTL
CXC Chemokine Receptor  CXCR
Dendritic Cell  DC
DC-specific ICAM-3 Grabbing Nonintegrin  DC-SIGN
Dermal Dendritic Cell  DDC
Deoxyribonucleic Acid  DNA
Deoxynucleotide Triphosphate  dNTP
Epstein Barr Virus  EBV
Granulocyte/Macrophage-Colony Stimulating Factor  GM-CSF
Hepatitis C Virus  HCV
Human Immunodeficiency Virus  HIV
Human Leukocyte Antigen  HLA
Human T cell Leukemia Virus  HTLV
Intercellular Adhesion Molecule  ICAM
Interferon  IFN
Immunoglobulin  Ig
Interleukin  IL
Langerhans Cell  LC
Lipopolysaccharide  LPS
Myeloid Dendritic Cell  MDC
Monocyte-Derived Dendritic Cell  MDDC
Major Histocompatibility Complex  MHC
Mixed Lymphocyte Reaction  MLR
Mannose Receptor  MMR
Murine Leukemia Virus  MuLV
Pathogen-Associated Molecular Patterns  PAMP
Peripheral Blood Mononuclear Cell  PBMC
Plasmacytoid Dendritic Cell  PDC
Ribonucleic Acid  RNA
Reverse Transcriptase  RT
Staphylococcal Enterotoxin B  SEB
Simian Immunodeficiency Virus  SIV
Structured Treatment Interruption  STI
Transforming Growth Factor  TGF
Toll-Like Receptor  TLR
Tumor Necrosis Factor  TNF
Aims of this thesis

The general aim of this thesis was to study the effects of HIV-1 infection on DC function, as these effects are poorly understood but most likely central to our understanding of HIV-1 disease pathogenesis. Furthermore, we investigated the potential use of apoptotic HIV-1 infected cells as a vaccine. The specific objectives were:

To study the ability of HIV-1_{Bal} infected MDDCs to upregulate co-stimulatory molecules and produce cytokines in response to CD40L stimulation (paper I).

To assess the susceptibility of primary MDCs and PDCs to HIV-1_{Bal} and HIV-1_{IIIb} infection, and the capacity of these cells to upregulate co-stimulatory molecules and produce cytokines in response to TLR stimulation after HIV-1 infection (paper II).

To study the transfer of HIV-1_{Bal} and HIV-1_{IIIb} from infected primary MDCs and PDCs to autologous CD4+ T cells during antigen presentation, with focus on transmission efficacy and the nature of the T cells infected by DCs (paper III).

To determine whether apoptotic HIV-1 infected cells are capable of eliciting HIV-1 specific immune responses in vivo (paper IV).
Dendritic cells

Dendritic cells were first described in the human skin by the German medical student Paul Langerhans, who assumed that they were nerve cells based on their morphology, and published his observations on Langerhans cells (LCs) in 1868 (1). Later, the identification of cytoplasmic organelles termed Birbeck granules, which are unique for LCs, allowed further characterization of these cells (2). The function of Birbeck granules remains elusive but they are suggested to be involved in the endocytic pathway of LCs (3-5). Cells containing Birbeck granules were identified in the lymph, lymph nodes and thymus (6-8). It was further shown that these cells expressed major histocompatibility complex (MHC) class II on their cell surface (9-11), and were capable of antigen presentation (12) and lymphocyte activation (13-15). However, the discovery of a small population of “large stellate” cells named dendritic cells (DCs) in peripheral lymphoid organs of mice by Steinman and Cohn in 1973 (16-19), reinitiated modern DC research. It was established that LCs and DCs in the skin as well as in the thymus, are not nerve cells but originate from hematopoietic progenitors of the bone marrow (20-22).

Dendritic cell development

DCs are quite rare cells (1-3% of all skin cells and < 1% of peripheral blood mononuclear cells (PBMCs)), and laborious to isolate. Thus, the establishment of protocols to generate DCs in vitro from progenitors has had a considerable effect on the ability to study these cells. It was first shown that mouse blood and bone marrow contain progenitors that can develop into DCs when cultured in the presence of GM-CSF (23, 24). Also in the human setting, it is possible to generate large numbers of DC-like cells by culturing progenitors from bone marrow, cord blood and peripheral blood with specific cytokines (25-29). A lot of our current knowledge on the development and functions of human DCs comes from studies on monocyte-derived DCs (MDDCs), where peripheral blood monocytes are cultured in the presence of GM-CSF and IL-4 to give rise to DCs (26, 28) that resemble dermal DCs (30). The addition of TGFβ to progenitor cultures promotes development of cells that closely resemble LCs, with expression of Birbeck granules (31, 32).
Despite the central role of GM-CSF in in vitro culture systems, mice deficient in GM-CSF or its receptor still produce DCs (33). Increasing the levels of GM-CSF in mice results in only a small increase in the number of DCs (33), while another cytokine Flt3 ligand (a stimulus for growth and differentiation of early hematopoietic progenitors) substantially increases the number of circulating DCs, both in humans and mice (34-36). This indicates that some DC subsets found in vivo are less dependent on GM-CSF compared to MDDCs and also that other stimuli can induce DC differentiation. Data to support the use of MDDCs as an in vitro model was reported by Randolph and co-workers, who showed that human monocytes cultured with endothelium differentiated into DCs within 2 days without the addition of cytokines. Monocytes were shown to cross a layer of endothelial cells and enter a collagen matrix, mimicking the entry of monocytes into tissue from the blood stream. A proportion of these monocyte-derived cells migrated back across the endothelium, in parallel with DCs migrating from the tissue to the lymph. The cells that remained in the tissue-like matrix became macrophages, while those that migrated were identified as DCs. This phenomenon was enhanced if the cells actively phagocytosed material in the collagen matrix (37). These data provide the first direct evidence that human blood monocytes can be induced to become mature DCs in the absence of exogenous cytokines.

**Dendritic cell subsets**

DCs constitute a heterogeneous population of specialized bone-marrow derived leukocytes. Different subsets of DCs are distributed throughout the body and can be found in almost all tissues (38) (Figure 1). The majority of DC subsets described in various tissues is of myeloid origin and express myeloid markers like CD11c, CD11b and CD33 but lack expression of other lineage markers like CD3, CD19, CD20, CD14 and CD56 (39). In addition, a second lymphoid-related DC differentiation pathway was recently described, that give rise to plasmacytoid DCs (PDCs) (40). PDCs morphologically resemble antibody-secreting plasma cells, and secrete considerable levels of type I interferons in response to exposure of viruses or microbial components (41, 42). PDCs possess characteristics common to all DCs; high expression of MHC and co-stimulatory molecules upon activation and the capacity to induce T cell proliferation (43). However, PDCs do not express myeloid markers and are in humans often defined by their expression of CD123 (the $\alpha$ chain...
of the IL-3 receptor) and lack of CD11c. PDCs are sparsely distributed and only found in blood and lymphoid tissues, like thymus, tonsils and spleen (40, 44-47). Another DC subset also found in peripheral blood is myeloid DCs (MDCs). MDCs are widely distributed and are found throughout the body. LCs reside in the epidermis and dermal DCs (DDCs) are found in the dermis of the skin. Similar DC subsets of myeloid origin exist in the mucosal epithelia, and are then termed mucosal DCs and submucosal DCs, respectively. As described earlier, LCs are distinguished by Birbeck granules, and they also express langerin, CD1a and CD1c in addition to myeloid markers like CD11b and CD33 (48). DDCs share most of the phenotype of LCs but lack expression of langerin and Birbeck granules. In addition, DDCs express CD1b and in some cases the monocyte/macrophage marker CD14 (49-51).

Figure 1. DC subset distribution. Immature Langerhans cells (LCs) reside in the epidermis of the skin and immature dermal DCs are found in the dermis. Similar DC subsets exist in the mucosa, termed mucosal DCs and submucosal DCs, respectively. Both immature myeloid DCs (MDCs) and plasmacytoid DCs (PDCs) exist in peripheral blood. Upon antigen encounter all DCs subsets mature and migrate to draining lymph nodes, where mature DCs present the antigen to T cells.
Several subsets of DCs have been described, but whether each subset has a particular function in terms of mounting immune responses against various pathogens is only beginning to be unravelled. The fact that the immune system is constituted by multiple DC subsets with distinct anatomical locations and expression of toll-like receptors (TLRs) used to recognize pathogens (described below), could suggest that the various DC populations specialize in handling certain types of pathogens.

**Recognition and presentation of antigens**

Immature DCs migrate via the blood into peripheral tissues where they encounter foreign antigen. To assist recognition, DCs express receptors that bind conserved structures commonly found on pathogens. Binding of foreign structures to these receptors facilitate maturation of DCs that present the antigens, which can help induce proper immune responses in order to combat the particular pathogen. DCs express TLRs that recognize conserved structures, known as pathogen-associated molecular patterns (PAMPs), that are unique to the microbial world and found on entire classes of pathogens (52, 53) (Figure 2).

**Figure 2.** Various toll-like receptors and their ligands. TLRs 4 and 5 expressed on the cell surface recognize bacterial LPS and flagellin, respectively. In contrast, TLRs 3, 7/8 and 9 recognize nucleic acids (CpG DNA, dsRNA, ssRNA) mainly originating from viral pathogens. These latter TLRs are therefore localized intracellularly and detect nucleic acids in compartments normally not accessible to the nucleic acids derived from the host.
When TLR ligands bind their receptors, an intracellular signaling cascade induces DC maturation as defined by upregulation of MHC class I and II, increased expression of co-stimulatory molecules and secretion of cytokines. Human DC subsets express distinct patterns of TLRs and may subsequently be suited to confront different pathogens and dictate immune responses accordingly (41, 42) (Figure 3). TLR3 recognizes double-stranded RNA and is expressed on MDCs (54), while TLR9, expressed by PDCs, interacts with unmethylated bacterial DNA with immunostimulatory CG motifs, known as CpG oligodeoxynucleotides (55, 56). MDCs also express low levels of TLR4 and TLR5 that bind LPS and flagellin, respectively (41, 42). Furthermore, MDCs and PDCs express TLR7 and TLR8 that both recognize single-stranded RNA and imidazoquinoline compounds, like imiquimod and R-848 (57-61).

In addition, DCs express C-type lectin receptors (CLRs). CLRs bind sugars in a calcium-dependent manner using highly conserved carbohydrate recognition domains (62).

**Figure 3. Expression of some TLRs and CLRs on different DC subsets.** *The expression of TLRs on LCs is poorly characterized. However, LCs respond to stimulation with bacterial peptidoglycan (TLR2), LPS (TLR4) and flagellin (TLR5) (63).*

Binding of antigens to CLRs can facilitate internalization of the antigens for degradation and presentation by DCs (64, 65). However, CLRs do not only function as antigen receptors, but also regulate migration of DCs and their interaction with T
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cells (66, 67). Different subsets of DCs express different CLRs, like the macrophage mannosreceptor (MMR/CD206) on MDDCs and DDCs, Langerin (CD207) on LCs, DC-SIGN (CD209) on MDDCs, blood MDCs and some DDCs (49, 64, 68, 69) (Figure 3). While TLRs bind structures mainly found on non-self antigens, CLRs bind sugar domains found frequently on self antigens, but also on pathogens. Although most CLRs bind mannosylated antigens, their specific self ligands are not completely defined. MMR is known to bind mannose and fucose and DC-SIGN binds mannan, ICAM-2 and ICAM-3, while any self ligand for Langerin has not been identified. A number of CLRs are known to bind gp120 on the human immunodeficiency virus type 1 (HIV-1), like DC-SIGN, MMR and Langerin (70, 71). DC-SIGN has also been described to bind several other pathogens, including simian immunodeficiency virus (SIV) (72), Ebola virus (73, 74), cytomegalovirus (CMV) (75), hepatitis C virus (HCV) (76-78), Mycobacterium tuberculosis (79, 80) and Candida albicans (81). New data indicate that there is a cross-talk between TLRs and CLRs that can affect the type of immune response induced by the DCs (82-85).

Upon recognition, DCs are able to take up antigens using several mechanisms, including receptor-mediated endocytosis, phagocytosis and macropinocytosis. They subsequently process the antigens to peptides that are loaded onto MHC class I and II molecules (86). The intracellular pathways for antigen processing and peptide loading on MHC class I and II molecules have been well characterized and are reviewed in detail elsewhere (86-89). In general, endogenous antigens are restricted to presentation on MHC class I and recognition by CD8+ T cells, while exogenous antigens are presented on MHC class II for the recognition by CD4+ T cells. However, in 1976 Michael Bevan demonstrated a phenomenon he termed cross-priming, priming of class I restricted CD8+ T cells as a consequence of presentation of exogenous antigens on MHC class I (90). The ability of DCs and other antigen-presenting cells to present internalized antigens on MHC class I molecules is referred to as cross-presentation (91).

Dendritic cell migration and maturation
As described above, DCs are distributed throughout the body. DCs are often defined as immature and mature, based on their function and expression of cell surface markers and cytokine production. This definition also reflects their location in vivo, as
the functions of immature and mature DCs are linked to their physical compartmentalization in the body (92) (Figure 4). Upon encounter with antigen and possibly signaling via TLRs, DCs migrate to draining lymph nodes. The migratory capacity of DCs was first demonstrated by staining the skin of mice with fluorescein isothiocyanate (FITC) and proving that FITC was concentrated to the DCs, which migrated to and remained in the draining lymph nodes. This demonstrated the relationship between skin DCs and lymph node DCs (93, 94).

![Diagram of immature and mature DCs](image)

**Figure 4. Some phenotypic and functional properties of immature and mature DCs.**

The expression of chemokine receptors is important for cells to migrate in response to chemokines secreted at sites of inflammation or in lymphoid compartments. The repertoire of chemokine receptors on DCs change throughout maturation, which affects their responsiveness to specific chemokines and facilitate migration (95, 96). Immature DCs express the chemokine receptors CCR1, CCR2, CCR5 and CXCR1 and respond to their respective ligands; MIP-1α, MCP-1, MIP-1β and IL-8, respectively, which are chemokines produced at inflammatory sites. Upon maturation, DCs downregulate CCR1, CCR5 and CXCR1 and instead express high levels of the homing receptor CCR7. One of the ligands of CCR7 is CCL19, which is
produced in lymphoid organs and is believed to facilitate DC migration to T cell areas of the lymphoid tissues (95, 96).

Not only the expression of chemokine receptors changes in response to antigen, but DCs undergo a series of maturational changes and become efficient antigen-presenting cells. Maturation of DCs and subsequent reduction of endocytic capacity, often call for additional stimuli like inflammatory cytokines or microbial products in addition to antigen uptake. A recent report shows, that the antigen capturing capacity of DCs is transiently increased immediately after TLR ligand stimulation and results in enhanced presentation of antigen on MHC class I and II (97).

Multiple receptors are described to be able to trigger DC maturation upon ligation, like TLRs, cytokine receptors, members of the TNF receptor family and Fc receptors (86). As described previously, DCs express TLRs that recognize various pathogenic compounds, like LPS (TLR4), CpG (TLR9) and double-stranded RNA (TLR7). These substances, or other TLR binding compounds like R-848 (TLR7/8), are frequently used in vitro to induce and study DC maturation (41, 43, 55, 59). DCs also sense infections indirectly by responding to inflammatory mediators like TNF, IL-1β and PGE2, which are often elevated in tissues in response to pathogens (98, 99).

Another potent inducer of DC maturation is signaling of CD40 on DCs by its ligand CD40ligand (CD40L or CD154). CD40L is expressed mainly on activated T cells (100). Also binding of other molecules present on T cells, like OX40 and FasL to their corresponding ligands on DCs, OX40L and Fas can induce DC maturation. In addition, DCs may also be activated through immunoglobulin Fc receptors by engagement of immune complexes or specific antibodies (101-103).

Upon signaling by any of the receptors mentioned, DCs upregulate the expression of a panel of molecules on their cell surface. MHC class II is upregulated, to facilitate good presentation of processed antigens (104). In addition, the expression of co-stimulatory molecules like CD80, CD86, CD83, CD40, OX40L and 4-1BBL increase to support adequate cross talk with T cells (28, 105-108) (Figure 5). Mature DCs also start to produce and secrete numerous pro-inflammatory cytokines, which are central in orchestrating the subsequent cellular immune response.
Dendritic cell mediated activation of T cells

DCs have been called “nature’s adjuvant” due to their ability to coordinate many protective immune functions in response to infections. DCs have the unique capacity to stimulate naïve T cells and are thereby essential for the induction of primary immune responses (Figure 5). The high capacity of DCs, compared to other antigen-presenting cells, to induce T cell proliferation was first demonstrated in primary mixed lymphocytes reactions (MLRs) (109-111). DCs were shown to be approximately 100 times more effective at inducing T cell proliferation than macrophages and B cells (109). Also, the removal of DCs dramatically reduced the MLR stimulatory capacity of splenocytes (112).

**Figure 5.** The close interaction between a DC and a CD4+ T cell. The T cell receptor on the CD4+ T cell recognizes the peptide-MHC class II complex presented by the DC. CD40-CD40L interaction conditions the DC to produce cytokines and upregulate the expression of the co-stimulatory molecules CD80/CD86. In turn, CD80 and CD86 bind to CD28 or CTLA-4 on the T cell. Binding of co-stimulatory molecules to CD28 provides a signal for T cell activation, while CTLA-4 engagement downregulates T cell activity.

DCs efficiently cluster with T cells (111, 113), which is probably a vital feature for efficient induction of T cell responses. In line with their higher expression of MHC and co-stimulatory molecules, mature DCs are superior to immature DCs at inducing T
cell proliferation (28). Furthermore, mature DCs can rapidly polarize immune responses with different effector functions, depending on the cytokines they secrete (114, 115). Cytokines are small proteins or glycoproteins that often act locally and regulate the immune system in a paracrine or autocrine fashion. If DCs secrete IL-12, IL-18 and RANTES the T cell response is skewed to a type 1 (Th1) response where the CD4+ T cells subsequently produce IL-2, IFN\(\gamma\) and TNF, and provide help for a cellular cytotoxic CD8+ T cell response. Th1 responses are also characterized by the induction of antibodies of certain isotypes, mainly IgG2a (116). IL-12 has proven to be a key cytokine for successful activation of cytotoxic T cell responses against viral infections (117-119). In addition to IL-12, the interaction between CD40L and CD40 is critical to bring DCs to a state of maturation to efficiently induce T cell killer responses (120-122). On the other hand, DCs can be triggered to induce a type 2 (Th2) response, where the CD4+ T cell produce IL-4, IL-5, IL-6 and IL-13 and provide help to induce a humoral antibody response (114). The presence of IgE is often a reflection of a Th2 immune response. DCs can also activate other innate protective cells like natural killer (NK) and NKT cells (123, 124). In addition to their central role in innate and adaptive immunity, DCs can also induce tolerance. While the immuno-stimulatory functions require mature DCs, an increasing literature show that DCs that are not fully mature induce T cell tolerance and can dampen the immune response and protect the host from autoreactivity (125).

Given their functional characteristics, DCs can play a role in disease progression, not only by presenting the pathogen to the immune system and orchestrating the T cell immune responses, but also by spreading the infection throughout the body.

**HIV-1**

In 1981 the first reports were published of an increased incidence of opportunistic infections like *Pneumocystis Carinii* and *Candida albicans* as well as an aggressive form of Karposi’s sarcoma among sexually active, young gay men in the United States (126, 127). Many of these patients had low numbers of CD4+ T cells, which was believed to account for the immunodeficiency symptoms they presented. The disease was named acquired immunodeficiency syndrome (AIDS). Two years later,
1983, a retrovirus was isolated from patients with AIDS and proposed to be the causative agent for the disease (128, 129). Dr. Robert C. Gallo, at the time working at the National Cancer Institute, Bethesda, MD, argued that the retrovirus was the known human T cell leukemia virus I (HTLV-I), while the group of Dr. Luc Montagnier at the Pasteur Institute in Paris, stated that this virus was a new HTLV. The following year Gallo and co-workers published four papers in Science describing a virus related to HTLV-I and HTLV-II as the causative agent for AIDS and named it HTLV-III (130-133). Later the virus was renamed HIV-1 (134). When some laboratory workers were tragically infected with HIV-1, and later developed disease, it provided the final evidence that HIV-1 is the causative agent for AIDS (135). The identification of HIV-1 as the cause for AIDS led to a long debate between the scientists involved in this discovery. Recently, Gallo and Montagnier, published their personal views on the now historical events concerning the discovery of HIV-1 (136, 137).

The origin of HIV-1

Strong evidence shows that HIV-1 was transferred to humans from chimpanzees, which harbor the related SIVcpz. In addition, phylogenetic data show that the related HIV-2 originates from SIVsm in sooty mangabeys (138-141). These monkeys live in Central Africa and coastal West Africa. In these areas, nonhuman primates live in close contact with humans as pets and wild animals are hunted and used as a source of food, which enables routes of cross-species transmission (142). Retrospectively, the earliest documented case of HIV-1 infection in humans was identified in a plasma sample from 1959, collected in former Leopoldville, Belgian Congo, now Kinshasa, Democratic Republic of Congo (143). Most likely, SIV was transmitted to humans by cutaneous or membrane exposure to infected animal blood (138). Based on phylogenetic analyses, it was established that these zoonotic transfers probably occurred repeatedly over time, no fewer than seven times and possibly more (138, 144-146). A broad range of factors have been suggested to explain the appearance of AIDS as an epidemic in the 20th century, including social disruption, urbanization, prostitution, population growth and additional changes in social behavior that are not yet fully understood (147, 148).
The HIV-1 structure and life cycle

HIV-1 is classified as a member of the Retroviridae family in the Lentivirus genus. The HIV-1 genome consists of two positive single-stranded RNA copies of approximately 9.2 kb and is composed of three major structural genes; \textit{gag}, \textit{pol} and \textit{env}. HIV-1 \textit{gag} codes for the nucleocapsid, capsid and matrix proteins (p6, p7, p17 and p24). HIV-1 \textit{pol} codes for reverse transcriptase (RT), integrase and protease, and HIV-1 \textit{env} codes for the envelope glycoproteins (gp120 and gp41). The HIV-1 provirus contains six additional open reading frames encoding the regulatory proteins Tat and Rev, and the accessory proteins Nef, Vif, Vpr and Vpu (149, 150).

HIV-1 infection is initiated when the virus binds via gp120 to the CD4 receptor of the target cell (151, 152) (Figure 6). After binding, the virus fuses with the membrane by interaction with gp41, gp120 and a co-receptor on the target cell, usually chemokine receptor CCR5 or CXCR4 (153-156).

HIV-1 isolates that utilize CCR5 to enter the target cell are referred to as R5-using isolates or R5 isolates, while isolates that use CXCR4 in short are called X4 isolates. After fusing, the virus enters the cell cytoplasm and the viral RNA genome is reverse transcribed by RT to obtain a double stranded DNA copy, which is transported into the cell nucleus (157). The proviral DNA is stably integrated in the target cell genome by the viral enzyme integrase. The virus can remain dormant in this stage for long periods of time, before it is activated and transcribed; replicates, assembles and
eventually buds from the cell membrane. There is an apparent preference for CCR5-
using isolates in the establishment of primary infection in most individuals (158-160),
although the tropism broadens as a result of mutations in the V3 loop of the virus
envelope protein, allowing the usage of a wider repertoire of co-receptors (Figure 7).
When the virus quasispecies (the population of virus variants that have evolved in the
host from the original infecting strain) change the overall usage of co-receptor from
CCR5 to CXCR4, there is usually a sharp decline in the number of CD4+ T cells as
well as increased viral load (161).

The course of HIV-1 infection
In general, HIV-1 infection follows three phases (Figure 7); the acute or primary
infection within the first weeks of infection can be asymptomatic or manifest as
mononucleosis-like illness. The level of virus in the plasma peaks and gradually
decreases to reach a steady state level, also called the viral set point.

![Figure 7. Diagram of the course of a typical HIV-1 infection.](image)

The drop in viral load coincides with the appearance of HIV-1 specific cytotoxic T
lymphocytes (CTLs, (162)). The chronic infection that follows can be clinically
asymptomatic for many years. However, ultimately the immune system fails to control
the infection, and as the disease progresses there is a decrease in CD4+ T cells and
a decline in the function of both innate and adaptive immune mechanisms (163, 164). This makes the patient susceptible to opportunistic infections and results in the third and final stage of HIV-1 infection: AIDS (165-167). WHO/UNAIDS estimates that 38 million people are currently infected with HIV-1. 4.8 million people were newly infected with HIV-1 in 2003 and 2.9 million died of AIDS during 2003 (168).

A hallmark of HIV-1 infection is the loss of CD4+ T cells. T cell dynamics are affected by HIV-1 infection, and the lifespan of both CD4+ and CD8+ T cells is shortened in HIV-1 infection to about one third of the lifespan of T cells in a healthy individual (169). HIV-1 replicates most efficiently in activated T cells (170), which could explain the qualitative loss of CD4+ T cell help, both to HIV-1 itself but also to other recall antigens (171). Also, HIV-1 preferentially infects HIV-1 specific CD4+ T cells (172). One feature of HIV-1 pathogenesis appears to be the failure to replace lost CD4+ T cells, which could be explained by thymic impairment (173). In addition, impaired DC function or generalized immune activation may also influence the loss of CD4+ T cell function and the course of HIV disease progression.

### Dendritic cells in HIV-1 infection

Since HIV-1 infection is characterized by loss of CD4+ T cells and failure to control the infection, eventually rendering the patient susceptible to opportunistic infections, one could argue that exclusively T cells are of interest to study in this disease. However, as efforts are being made to understand the pathogenesis of the disease, the number of factors that contribute to disease presentation increase. One contributing factor could be defects in DCs, impacting on the initiation of T cell activation and subsequent T cell responses ((174-179), paper I). DCs express the receptors required for HIV-1 infection; CD4 and the most commonly used co-receptors CCR5 and CXCR4 ((180-182), papers I-II). MDDCs and dermal DCs also express a C-type lectin called DC-SIGN that has been shown to facilitate transfer of HIV-1 from DCs to T cells in vitro (70). Also, in vivo DCs are located at sites where initial transmission of HIV-1 is most likely to occur, like mucosal surfaces and blood. Therefore, DCs could serve as one of the initial target cells of HIV-1 infection, and
contribute to the overall immunodeficiency observed in HIV-1 infected individuals. Hence, it is important to study DCs in the context of HIV-1 infection.

**HIV-1 infection of dendritic cells**

The question of whether DCs are infected with HIV has been a matter of debate for several years. It has been difficult to answer that question mainly for three reasons: i) DCs represent a relatively rare cell population in tissue; ii) DCs migrate from peripheral tissue upon contact with antigen. Conclusively, infected DCs remain in the periphery less than 2-3 days following mucosal inoculation of the virus (183, 184). Finally, iii) HIV-1 infection in DCs has been difficult to detect because viral replication in DCs occurs at much lower levels than in CD4+ T cells, that have been studied in detail with respect to HIV-1 infection. Despite the difficulties mentioned, the data obtained to date suggest that DCs are susceptible to HIV-1 infection and may represent a cellular reservoir for HIV-1 in infected individuals. In 1987, LCs from HIV-1 infected individuals were reported to contain HIV-1 as determined by staining for HIV proteins in explant cultures of skin biopsies (185, 186). Later both MDCs and PDCs have been identified as cellular reservoirs for HIV-1, in addition to LCs, albeit to a limited extent (187-190). However, some data state that blood DCs do not constitute an HIV-1 reservoir in vivo (191). Also spleen DCs are reservoirs for HIV-1 in infected individuals (192). In addition, a number of reports have confirmed the susceptibility of DCs to HIV-1 infection in vitro (35, 193-199).

Data suggest that once HIV-1 infection has established, the predominant source of new virus production and the main reservoir of HIV-1 is CD4+ T cells (183, 184). DCs may have a greater role in retaining the virus and spreading it to the more permissive CD4+ T cells, than producing large amounts of virus themselves.

In addition to DCs and CD4+ T cells, HIV-1 has been found to infect several cell types of the immune system (200), like macrophages (201, 202), CD8+ T cells (203), NKT cells (204, 205) as well as NK cells (206). However, it is not fully understood if and to what extent HIV-1 infection of these cells contributes to the pathogenesis of the disease.
Loss of dendritic cells during HIV-1 infection
Several studies have shown that the number of circulating MDCs and PDCs is reduced in HIV-1 infected individuals (176, 189, 207-213). Also, the loss of circulating DCs correlates with an increase in HIV-1 viral load (211, 212, 214). A recent report indicates a correlation between the recovery of circulating PDCs during ART and a lower viral load rebound after treatment interruption in patients with primary HIV-1 infection (215). It remains unclear why DCs are lost during HIV-1 infection. Several hypotheses have been proposed to explain this phenomenon; i) failure of DC precursors to differentiate into characteristic DCs, ii) death of DCs due to HIV-1 infection and iii) relocation of DCs to secondary lymphoid tissues, perhaps as a consequence of DC maturation induced by the virus or induced when DCs capture HIV-1 for antigen processing and presentation. During acute HIV-1 infection, an increased frequency of DCs is detected in lymph nodes, suggesting that migration from the periphery to these sites is increased (174). However, patients with AIDS have a reduced number of DCs in their lymph nodes, indicating that redistribution does not fully explain the loss of DCs in HIV-1 infection (174).

Functional impairment of DCs in HIV-1 infection
Viruses can suppress DC function in a number of ways, including induction of apoptosis, inhibition of maturation, impaired cytokine production, migratory deficiencies and inhibition of T cell activation. DCs isolated from HIV-1 infected individuals have a reduced capacity to stimulate resting T cells in vitro, compared to DCs isolated from HIV-1 seronegative persons (175-179). There is also a defect in IL-12 production in PBMCs isolated from AIDS patients (216, 217). The observed IL-12 impairment could be overcome by addition of soluble CD40L to monocyte cultures from HIV-infected patients (218). DCs isolated from HIV+ individuals produced similar amounts of IL-12 following stimulation with CD40L, as DC from HIV negative individuals (219). Both the nature of stimulus and the stage of disease determine the amount of IL-12 secreted from PBMCs isolated from HIV-1 infected individuals (220). In addition, there is an accumulation of DCs in the lymphoid tissue of HIV-1 infected patients during acute infection. These DCs have a reduced expression of the co-stimulatory molecules CD80 and CD86, which may limit their capacity to generate HIV-1 specific T cell responses (174). This reduced expression of co-stimulatory molecules could be a direct consequence of the virus or an indirect effect of the
infection, causing insufficient stimulation of DCs. An indirect impairment of DC function could be that DCs do not get appropriate stimulation and subsequently can not provide sufficient stimulation themselves to initiate proper immune responses. Increasing evidence suggests that HIV-1 has evolved mechanisms to suppress CD40L expression (221, 222). The lack of efficient immune responses against HIV-1 in infected individuals could therefore in part be explained by the lack of CD40L, as the interaction between CD40L and CD40 on DCs is crucial for maturing DCs in a way sufficient to trigger CTL responses (120-122). Furthermore, dysregulation of cytokine production in DCs could also contribute to incomplete CD4+ and CD8+ T cell activation. The failure of specific T cell help together with DC dysfunction might augment the disability of the host to respond to emerging virus quasispecies and to mount appropriate HIV-1 specific CTLs (223).

**Treatment strategies for HIV/AIDS**

At present there is no cure for HIV-1. The need to develop new therapies or a vaccine for HIV/AIDS is urgent, as the number of people infected with HIV-1 has grown to 38 million and continues to increase at an alarming rate in many developing countries. However, the existence of HIV-1 infected individuals that are able to control their infection as well as people that are repeatedly exposed to HIV-1 but not infected, provide hope for the possibility to find ways to prevent infection. Extensive efforts are made to gain a better understanding of the virus, the immune responses that contain HIV-1 infection, and the complex mechanisms used by the virus to evade those immune responses. Since HIV-1 targets, infects and compromises cells of the immune system, the interactions between this virus and the host are both complex and offer insights into mechanisms of immune defences.

**Current treatment of HIV-1 infection**

The introduction of triple antiretroviral treatment (ART) in 1996 dramatically reduced the rate of disease progression and death through control of viral replication and partial reconstitution of the immune system (224-226). However, ART alone does not seem to result in improved HIV-1 specific immune function (225, 227, 228). Most of
ON THE ROLE OF DENDRITIC CELLS IN HIV-1 INFECTION

the approved antiretroviral drugs currently in use are inhibitors of viral enzymes with no known direct human analogues; the viral RT and protease. These enzymes are essential for the virus replication and interference with them inhibits production of intact and infectious virus particles. In addition, one fusion inhibitor, T-20 is currently approved for clinical use in Sweden.

Initially, hopes were high that the virus could be controlled and eradicated, and therapy eventually stopped (229). However, in most chronically infected patients, attempts to discontinue ART have resulted in a rapid increase in plasma viral load and a decrease in CD4 counts (230, 231). Supervised or structured treatment interruptions (STI) has been considered as an approach to provide limited, but hopefully sufficient antigenic stimulus to the new naïve T cells that are generated during ART and to educate these cells to target HIV-1 (232). Indeed, STI in a limited number of patients with acute infection resulted in immune control (233), but unfortunately these data have been difficult to reproduce especially in patients with chronic HIV-1 infection (230). The probable cause of failure in STI, so far, is the persistence of a long-lived reservoir of infected cells, which consequently results in the need for lifelong treatment of HIV-1 infected patients (234).

Although the introduction of ART has changed the clinical course of HIV-1 and AIDS in a radical way, there are still drawbacks to consider. One of the main difficulties in a global perspective is poor access to antiretroviral drugs. The effective treatment is both costly and requires an infrastructure that makes it unavailable for most people in developing countries. The major problem in treated patients is compliance, where inconsistent intake of the drugs results in increased risk for development of drug resistant virus strains. Also, the therapy is accompanied by severe side effects like diarrhea, hyperlipidemia, muscular and neurological toxicity and increased risk of diabetes, hypertension and cardiac infarcts (235, 236). Therefore, the ideal solution to contain or even eradicate the virus and the AIDS epidemic would be to develop a vaccine. New and exciting strategies are being exploited to find prophylactic or therapeutic vaccines and improve antiretroviral treatment.
Introduction to vaccines

Vaccines against infectious diseases have had great impact on human health worldwide, as they prevent disease as well as disease symptoms of global infections. Two major categories of vaccination exist: passive and active immunization. In brief, passive immunization depends on the administration of purified antibodies or antibody-containing sera against a certain pathogen, to an individual at risk of contracting the particular infection. Passive immunization results in temporal protection against infectious agents, for example in newborns who receive maternal antibodies via breastfeeding. Active immunization, on the other hand, stimulates the immune system to induce effector molecules and/or effector cells against the immunogen. Edward Jenner noted that milkmaids who had contracted cowpox were immune to subsequent smallpox infection. Jenner confirmed this by injecting an eight-year-old boy with material from cowpox lesions and later deliberately infected the boy with smallpox. The boy was immune to smallpox and did not develop the disease (237). Later, Louis Pasteur observed that chickens inoculated with old cholera cultures caught the disease but recovered and developed immunity to subsequent cholera infection, while chickens inoculated with fresh cholera cultures died (238). Pasteur argued that the cholera had weakened by long-term culture and called the attenuated cholera strain a vaccine. In line with these findings, first-generation vaccines were live, attenuated pathogens, a strategy with obvious safety concerns. Next, chemically or physically inactivated pathogens were developed for immunization. Third-generation vaccines were purified or synthetic proteins derived from the pathogen and lately, a fourth-generation vaccine strategy is developed based on DNA and virus vectors coding for immunogenic proteins (239). Prophylactic vaccination prevents infection by inducing specific memory and effector immune responses that provide sterilizing immunity in non-infected individuals. Therapeutic vaccination, in stead prevent severe complications in a chronically infected individual by reinforcing and/or broadening specific immune responses in the host (240). The development a prophylactic HIV-1 vaccine, which could be administrated to people on a large scale, will hopefully be a reality. However, maybe it is more likely to first find a therapeutic vaccine that could improve the conditions for already infected individuals.
Requirements on an HIV-1 vaccine

In theory, to generate efficient protection against HIV-1, an ideal vaccine should stimulate four different components of the immune system. It should generate broadly cross-reactive neutralizing antibodies, induce high levels of antiviral T cells, stimulate mucosal immunity as well as provoke the innate immune system (164, 241). To obtain protective immunity, the major targets for an HIV-1 vaccine are most likely steps involved in virus entry and virus replication (242). Antibodies that block entry of the virus to the target cell could neutralize the virus and prevent infection. However, it has proven very difficult to generate antibodies that can neutralize primary isolates of HIV-1 (243). Also, the high error rate of the viral enzyme RT generates large antigenic variation in the viral proteins gp120 and gp41 involved in cell surface binding and cell entry (244). An effective vaccine should probably also induce cellular immune responses, which are mediated by T cells. Given the difficulty to raise neutralizing antibodies against HIV-1, most current vaccine strategies aim to induce HIV-1 specific T cell responses. Vaccines based on induction of HIV-1 specific T cell responses might not prevent infection, but can control virus replication. Still, HIV-1 specific cytotoxic T cells have been found in repeatedly HIV-1 exposed but uninfected individuals (245), providing hope for a protective capacity of T cell response induced by vaccination. CD8+ T cells clear infections by lysing infected cells (246), by release of antiviral cytokines (247-249) and chemokines (248, 250, 251). Induction of HIV-1 specific T cell helper responses is most certainly required for long-term control, since these cells are needed to maintain CTL function (252). However, there are still little data defining the magnitude, breadth and specificity of CD4+ helper or CD8+ CTL responses that results in true clinical benefit (253-255). The challenges in creating an effective HIV-1 vaccine are closely linked to the characteristics of the virus itself. In addition to variations within an individual patient there are at least 12 subtypes of the virus with broad geographical distributions (256). This results in difficulties in generating a worldwide applicable vaccine. Finally, since HIV-1 is a retrovirus, it integrates in the host genome and can hide from the immune system (257), evidently for long periods of time.

No single immune mechanism has been found to unaccompanied be prognostic for either protection against HIV-1 infection, viral clearance or disease progression. Historically, most vaccine candidates have entered clinical trials with limited
knowledge of their ability to stimulate immune responses and what immune responses result in protection against the pathogen (239). However, most effective vaccines available today are directed against acute virus infections like smallpox, polio, measles, rubella and influenza and protection is associated with generation of virus-specific neutralizing antibodies (258, 259). Cell-mediated immune responses are important in the control of established chronic infections like CMV and Epstein-Barr virus (EBV) (260, 261). In experimental SIV infection, vaccine-induced cellular immunity has been shown to control chronic disease (262-266). An increased knowledge in immunology allows us to obtain a more detailed understanding of the protective effects of vaccines and the correlates to protection.

**Dendritic cells in HIV-1 immunotherapy**

DCs are essential to the initiation of T cell responses against foreign antigens and probably play a role in HIV-1 pathogenesis. The administration of cytokines like IL-2 and GM-CSF (267-270) aim at modulating DCs and subsequently T cell responses in vivo. Other strategies considered are administration of factors that stimulate DC activity and mount stronger immune responses in HIV-1 infected individuals, like soluble CD40L or TLR ligands (271). As DCs express TLRs, and signaling via TLRs can affect the ability of DCs to orchestrate adaptive immunity, the effects of TLR ligation bridge innate and adaptive immunity (272, 273). TLRs could be useful drug targets, since they are prime sensors of microbial products. Thus, the interest in using compounds that bind to TLRs has intensified as these molecules could be used as vaccine adjuvants (43). In addition, therapeutic strategies are developed to generate or enhance HIV-1 specific cellular immunity by targeting DCs in vivo or using DCs loaded with antigens. Several studies have reported attempts to develop HIV-1 antigen containing vectors that target DCs (274-276). In animal models, immunization with DCs loaded with peptides or viral vectors that target DCs induces immune responses against viruses (277). In 2003, Lu and colleagues published the first evidence in an animal model that therapeutic immunization can augment immune control of SIV (278). Rhesus macaques with an established SIV infection were immunized five times with autologous DCs loaded with chemically inactivated SIV in two week intervals. This resulted in a marked reduction of viral load and an increase in CD4 counts, all in the absence of ART. The animals were immunized early in the disease progression, 56 days post infection. As mentioned earlier,
intervention by STI in acute infection has been promising also in humans, but less successful in chronic infection (230, 233). Thus, although the data from Lu and co-workers suggest that therapeutic vaccines work, they need to be repeated in animals with a chronic infection. Also, preparation of autologous DCs for in vitro loading and readministration is perhaps not feasible due to manufacturing requirements as an ideal HIV-1 vaccine.

**Dendritic cell immunotherapy using apoptotic cells**

There are several ways in which DCs can capture viral antigens, besides direct infection, and induce T cell responses. DCs can present nonreplicating virus, which are abundantly found in the plasma of HIV-1 infected individuals (279). DCs can also efficiently take up and present immune-complexed antigens (101, 280). In addition, DCs can present antigens derived from apoptotic cells. In vitro studies have shown that uptake of apoptotic virus-infected cells by DCs results in efficient presentation of viral epitopes on MHC class I (281-283).

Apoptosis, or programmed cell death, is a process of great importance during development and maintenance of tissue homeostasis. The process is characterized by caspase activation, DNA fragmentation and formation of apoptotic bodies (284). These apoptotic bodies are membrane-enclosed vesicles that contain both proteins and DNA from the cell of origin. The apoptotic bodies formed upon cell death are cleared through uptake by phagocytosing cells and do not induce immune responses, in contrast to another type of cell death; necrosis, which is usually the cause of inflammation and tissue damage (285). Still, in vitro studies have shown that uptake of virus-infected apoptotic cells by DCs result in presentation of viral antigens and induction of immune responses (274, 281-283, 286). Matzinger hypothesized that apoptosis of virus-infected or otherwise damaged cells may provide a signal that alerts the immune system (287, 288). Such signals could be inflammatory cytokines, necrotic cells, bacterial components or double-stranded RNA, which induce maturation of DC, a crucial step in the initiation of immune responses (285, 289-291). It has previously been demonstrated that EBV, HIV-1 and oncogenic DNA present in apoptotic cells can be transferred to antigen-presenting cells, analogous to the horizontal gene transfer frequently used as a mechanism to exchange genetic information in bacteria (292-294). In these studies, the transferred DNA was
subsequently expressed in the recipient antigen-presenting cell. The efficiency of horizontal DNA transfer correlated with the phagocytosing capacity of the recipient cells. We propose that horizontal gene transfer after uptake of apoptotic HIV-1 infected cells could be the hypothetical base for a novel HIV-1 vaccine strategy, where autologous apoptotic HIV-1 infected cells are administrated. This strategy has some theoretical advantages when considering the above-mentioned difficulties in constructing an HIV-1 vaccine. Using the patients’ own cells to generate apoptotic cells would include many or all of the virus variants present in the patient and potentially overcome the problem with high variability of HIV-1. Apoptotic cells contain both DNA and proteins, a combination shown to be a strong inducer of immune responses in vaccination trials (295, 296). Apoptotic HIV-1 infected cells also include the entire HIV-1 genome and given the high diversity of HIV-1 and concomitant escape from immune control (297), it is probably advantageous to include as many genes as possible to elicit a broad immune response.

Taken together, an increased understanding of the immunobiology of DCs in health and disease could help us exploit the features of DCs and use them to design effective immunotherapy.
Methods

The methods used in the studies included in this thesis are described in the original papers (papers I-IV). However, some of the central methods and experimental set-ups used in the different studies are outlined below.

Generation of monocyte-derived dendritic cells

Monocyte-derived DCs (MDDCs) were differentiated from PBMCs as described earlier (26, 298). PBMCs were separated from healthy blood donors using density gradient and monocytes were allowed to adhere for 1.5 hours at 37°C. Cells were carefully washed 3 times and adherent monocytes were cultured for 6 days in medium supplemented with 10% fetal bovine serum (FBS) and recombinant human IL-4 and GM-CSF, to obtain immature MDDCs (Figure 8). The frequency of MDDCs ranged from 75-90% in the cell cultures on day six. Immature MDDCs were CD14-CD1a+ with low expression of CD40, CD80, CD83 and CD86.

The frequency of CD14+ cells was 5-10% while the frequency of contaminating CD3+ cells ranged from 5-15% in the culture on day 6. Although monocytes can be enriched for based on their capacity to adhere to plastic, the cells are only loosely adherent and it is difficult to obtain cultures free of other cell populations. In some experiments, it was essential to obtain MDDCs absolutely free of contaminating cells and thus, CD14+ monocytes were enriched from PBMCs by negative selection. Monocytes were cultured in the presence of IL-4 and GM-CSF, as described. After 6 days 95% CD1a+ immature MDDCs were obtained with low to undetectable levels (<0.6%) of CD3+ T cells and CD14+ monocytes.
Isolation of circulating blood myeloid and plasmacytoid dendritic cells

Recently, we established a direct isolation procedure to purify relatively high numbers of primary human CD123+ PDCs and CD11c+ MDCs from blood (43). Briefly, PBMCs were collected from healthy HIV-1 seronegative blood donors by automated leukapheresis. Enriched populations of lymphocytes and monocytes were obtained by counterflow centrifugal elutriation. MDCs and PDCs were isolated from elutriated monocytes using magnetic beads followed by sequential separation on AutoMacs (Miltenyi Biotec), (Figure 9). The BDCA-4 and CD1c isolation kits were used for isolation of PDCs and MDCs, respectively.

![Figure 9. Isolation of human blood MDCs and PDCs. CD11c+ MDCs and CD123+ PDCs are isolated from elutriated monocytes, which are mainly large CD14+ cells. In the elutriated monocyte fraction, the DC subsets are characterized by high expression of HLA-DR and lack of expression of the lineage markers CD3, CD14, CD20 and CD56. MDCs are positively selected based in their expression of CD1c and PDCs are isolated based on their expression of BDCA-4 using magnetic beads.](image)

To maintain viability, the PDCs and MDCs were cultured in complete medium supplemented with 10% FBS and the recombinant human cytokines IL-3 or GM-CSF, respectively. On average, the recovery of isolated DCs was \(1.1 \times 10^6\) PDCs and \(2.2 \times 10^6\) MDCs per \(10^8\) elutriated monocytes. The subsets of DCs were highly enriched (>90%) as determined by lack of the lineage markers (CD3, CD20, CD14 and CD56), and expression of HLA-DR and CD123 or CD11c. The contaminating cells were CD14+ monocytes. Overnight culture in IL-3 or GM-CSF of freshly sorted PDCs and MDCs, respectively, lead to development of characteristic DC morphology (especially MDCs) and immature DC phenotype with low expression of CD40, CD80, CD83 and CD86.
Dendritic cell:CD4+ T cell co-cultures

DCs and CD4+ T cells were isolated from elutriated monocytes and lymphocytes, respectively, from healthy CMV seropositive donors with a detectable CD4+ T cell recall response against CMV. MDCs and PDCs were isolated as described above. Negatively isolated CD4+ T cells (memory and naïve) were obtained by depletion of CD1c+, CD8+, CD14+, CD15+, CD19+, CD56+ and BDCA-4+ cells using magnetic beads and separation on AutoMACS and frozen until further used. After overnight incubation, the MDCs and PDCs were infected with HIV-1BaL and HIV-1IIIB or mock and the cells were cultured for 72 hours. In some experiments, azidothymidine (AZT) was added to the cultures prior to addition of the virus isolate to prevent HIV-1 infection. The DCs were exposed to HIV-1 for 72 hours and in some cases stimulated with the TLR7/8-ligand R-848 during the final 24 hours, to induce DC maturation. Thereafter, HIV-1 exposed or unexposed DCs were thoroughly washed in pre-warmed media to minimize transfer of residual non-cell associated virus and then co-cultured with sorted autologous CFSE-labeled CD4+ T cells in a DC:T cell ratio of 1:10 (Figure 10). Most co-cultures were stimulated with whole CMV antigen lysate to activate CMV-specific memory T cells but in some experiments, the T cell receptor (TCR) superantigen SEB was used as an alternative antigen. The DC:T cell co-cultures were cultured for 1-8 days with addition of brefeldin A during the last 12 hours of co-culture. In some experiments, the protease inhibitor Indinavir was added to the cells at initiation of co-culture to prevent spreading and propagation of the virus among T cells, after the initial transfer of HIV-1 from DCs to T cells. After harvesting, the cells were surface stained with CD11c (for co-cultures including MDCs) or CD123 (for co-cultures including PDCs). Cells were then fixed and permeabilized for intracellular staining of HIV-1 p24 and cytokine expression (IFNγ, TNFα and IL-2). The cells were immediately analyzed to prevent leakage of CFSE dye from the cells. In addition, after 10 hours and 3.5 days of co-culture, four different fractions of T cells were sorted using a FACSVantage SE/DiVa (Becton Dickinson). Cells were gated on CD11c-/CD123- CFSE labeled T cells and subsequently 1) undivided cytokine producing, 2) dividing cytokine producing, 3) undivided cytokine non-producing and 4) dividing non-cytokine producing T cells were sorted. The T cells were subsequently analyzed for their content of HIV-1 ssDNA (an early product of replication) and full-length HIV-1 gag DNA copies per 10^5 cells using quantitative real time PCR.
Figure 10. Co-culture of HIV-1 exposed DCs and autologous CD4+ T cells. MDCs, PDCs and CD4+ T cells were isolated from elutriated monocytes and lymphocytes, respectively, from healthy CMV seropositive donors with a detectable CD4 recall CMV response. Total CD4+ T cells were isolated and frozen until further used. After overnight incubation, the DCs were infected with HIV-1 and cultured for 72 hrs. Thereafter, DCs were thoroughly washed to minimize transfer of residual non-cell associated virus and then co-cultured with sorted autologous CFSE-labeled CD4+ T cells in a DC:T cell ratio of 1:10 in the presence of antigen (CMV or SEB) and in some cases Indinavir. The DC:T cell co-cultures were cultured for 10 hours to 8 days. Thereafter the cells were harvested, stained and analyzed by flow cytometry.

Gating strategies in flow cytometric analyses of dendritic cell: T cell co-cultures
To obtain as much information as possible from the DC:T cell co-cultures, we set up a gating scheme to analyze both the DCs and the T cells in the same sample (Figure 11). First, DCs and T cells were separated based on cell size (FSC) and granularity (SSC), where DCs were found in the population of large and more granulated cells and T cells were less granulated and ranged in size from small (non-proliferating) to large (proliferating). Dead cells were excluded based on size as well as propidium iodide staining. DCs and T cells were further separated based on CFSE staining (as all T cells were dyed before initiation of co-culture) and CD11c (MDCs) or CD123 (PDCs). However, in the T cell gate, the highly CFSE expressing events, that had a brighter CFSE staining than the freshly stained T cells, were excluded as they most likely represent dead or dying cells and cellular conjugates (299). HIV-1 infection assessed by p24 expression as well as IFNγ, TNFα and IL-2 production could be
analyzed in both DCs and T cells. T cell proliferation could also be determined based on CFSE dilution.

**Figure 11.** Gating strategies for the DC:CD4+ T cell co-cultures. Unexposed (A) or HIV-1BaL exposed (B) MDCs were co-cultured with autologous CFSE-labeled CD4+ T cells for 3.5 days. The cells were subsequently harvested, stained and analyzed by flow cytometry. First, DCs and T cells were separated and dead cells were excluded based on cell size and granularity. DCs and T cells were further separated based on CFSE staining and CD11c (MDCs). The highly CFSE expressing events were excluded as they represent dead/dying cells or cellular conjugates. HIV-1 infection, cytokine production of the different cell populations and T cell proliferation could thereafter be determined.
Results and discussion

Below I discuss the results obtained in the studies included in this thesis in a more general context. For detailed information on and graphic illustrations of the data, please see the included papers (papers I-IV).

Dendritic cells are susceptible to HIV-1 infection

An increasing number of reports show that DCs are susceptible to HIV-1 infection, both in vivo and in vitro (35, 187-190, 192, 194-199) However, the somewhat conflicting reports on the level of susceptibility to HIV-1 infection and the capability of DCs to support productive infection could be due to the diversity of techniques used for isolation and culture of DCs. In addition, infection protocols optimized for infection of CD4+ T cells have sometimes been applied to DCs, which often include long culture periods and stimulation conditions that are not appropriate for DCs. The methods to determine HIV-1 infection have mainly been based on detection of secreted viral proteins in the cell culture supernatant and/or detection of viral RNA or DNA. These methods commonly estimate the overall infection in a culture and do not allow for further studies of the HIV-1 infected DCs per se.

In vivo, DCs in mucosa and blood, where initial transmission of HIV-1 occurs, express an immature phenotype (Figure 4). Immature DCs are therefore more relevant to examine with respect to HIV-1 susceptibility. We established an infection protocol that allowed us to study early HIV-1 infection of immature DCs in vitro using intracellular staining against the HIV-1 protein p24 and flow cytometric analysis. We showed that immature MDDCs (paper I) as well as isolated primary immature PDCs and MDCs (paper II) were susceptible to HIV-1 infection in vitro and that the virus replicated in these DC subsets. We exposed DCs to purified and highly concentrated HIV-1_{Bal} (R5) and HIV-1_{IIIB} (X4) isolates with high multiplicity of infection to generate high and fast infection without inducing cell death. In contrast to CD4+ T cells that need stimulation to induce significant viral replication, HIV-1 infection of DCs does not require prior activation of the cells (196, 300). We found that MDDCs (paper I), MDCs and PDCs (paper II) replicated HIV-1 and expressed significant amounts of p24 intracellularly without prior activation. This allowed us to study early HIV-1
infection in DCs, as viral DNA transcripts could be detected already 3 hours after exposure and HIV-1 p24+ cells appeared after 24 hours of viral exposure and increased over time. No viral replication was detected when the DCs were cultured in the presence of AZT. AZT is a nucleoside analogue reverse RT inhibitor, which inhibits viral replication by hampering RT activity through competition with the natural deoxynucleotide triphosphates (dNTPs) for the enzyme-binding site or by incorporation into the growing DNA chain resulting in chain termination. Addition of AZT to the cell culture thus prevents productive infection by inhibition of reverse transcription of the viral genome, integration and subsequent formation of viral proteins and virus particles.

Interestingly, we found a difference in susceptibility of primary PDCs and MDCs to HIV-1\textsubscript{BaL} and HIV-1\textsubscript{IIIB} (paper II). We found that MDCs were more effectively infected by HIV-1\textsubscript{BaL} than PDCs. MDCs were less susceptible to infection with HIV-1\textsubscript{IIIB} than HIV-1\textsubscript{BaL}. However, the differences in susceptibility of the DC subsets could not be correlated to the expression level (MFI) of the co-receptors CCR5 and CXCR4 on MDCs and PDCs prior to infection (paper II). Still MDCs express higher levels of CCR5 than PDCs, in analogy with their higher susceptibility to the R5-using HIV-1\textsubscript{BaL}. Early after transmission, HIV-1 isolated from infected individuals is almost always CCR5-using (158-160). This could be due to the fact that CD4\textsuperscript{+} T cells and DCs in the mucosa and submucosa at the local site of infection express much more CCR5 than CXCR4 (301-303). MDCs, which express high levels of CCR5, are more prevalent in mucosal tissues, while PDCs normally are found only in blood and lymph nodes. Thus, MDCs may encounter HIV-1 in the periphery early after initial infection and contribute to the selection of virus strains that are transported to the lymph nodes. In contrast, PDCs may primarily be exposed to virus that reaches lymphatic tissues and may play a role in DC mediated transfer of HIV-1 to T cells during antigen presentation. One could thus speculate that the difference in susceptibility to HIV-1 infection between the DC subsets may play a role for the initial selection of virus at transmission as well as the virus spread to T cells.

Functional properties of HIV-1 infected dendritic cells
The effects of HIV-1 on DC function are poorly understood but most likely important to our understanding of the pathogenesis of the disease. The presence of detectable
p24+ DCs allowed us to extend prior findings with detailed studies of the effects of HIV-1 infection on DC maturation and cytokine production (papers I-II).

**Phenotypic maturation**

We found that in vitro HIV-1 exposure of MDDCs did not lead to detectable phenotypic maturation overall in the culture as determined by increased expression (MFI) of CD80, CD86, CD83 and HLA-DR compared to the unexposed donor-matched MDDCs (paper I), in agreement with previous reports (219, 304, 305). However, when we studied the expression of CD86 on the MDDCs that were not only exposed but HIV-1 infected (p24+), the average CD86 expression tended to be higher than on the uninfected p24- MDDCs in the same culture (paper I). HIV-1 exposure of primary MDCs and PDCs, using an identical infection protocol and virus dose as for MDDCs, resulted in a moderate increase in the expression of co-stimulatory molecules overall on MDCs (CD86) and PDCs (CD86, CD40 and CD83) after virus exposure compared to the medium control (paper II). It is therefore possible that i) HIV-1 infected DCs are more mature than uninfected DCs and ii) that primary DCs are more sensitive to HIV-1 exposure than in vitro-derived DCs. In vitro HIV-1 exposure of PDCs has previously been shown to induce significant upregulation of co-stimulatory molecules (199, 306). Although HIV-1 exposure alone did not induce maturation in MDCs, they matured as bystander cells to exposed PDCs (306). In our studies, regardless of prior differentiation caused by the virus, HIV-1 exposure and/or infection did not inhibit further maturation by CD40L or TLR ligation in any of the DC subsets. Furthermore, full maturation did not increase HIV-1 replication in infected DCs, which is a major concern when designing immunomodulatory therapies that target DCs. Taken together, regardless if HIV-1 exposure causes some differentiation or not, it at least appears that HIV-1 does not impair the ability of DCs to respond to stimuli and upregulate expression of essential co-stimulatory molecules. However, the presence of these receptors on the cell surface does not exclude that HIV-1 infected DCs could have an impaired antigen-presenting function.

As mentioned in the introduction, one theory as to why the frequency of circulating DCs decreases in HIV-1 infected individuals is that DCs become infected and die. We did not see an increased number of dead cells in either the MDDCs or the
primary DCs after 72 hours of HIV-1 exposure as compared to the medium control (papers I-II). However, at later time points (> 4 days) increased cell death was evident in the HIV-1 exposed DC cultures (papers I-II). Overall, PDCs were found to be more sensitive to long-term culture than MDCs regardless of virus exposure. HIV-1 did not induce cell death in MDCs and PDCs as an instant effect of in vitro infection. However, one could of course question how this corresponds to the in vivo situation. Another theory to the loss of DCs in blood during HIV-1 infection is that DCs become activated by HIV-1, mature and migrate and are redistributed in the body. Our data may indirectly support this theory, as we detected an increased expression of co-stimulatory molecules CD86 (on MDCs and PDCs) as well as of CD40 and CD83 (on PDCs) after HIV-1 exposure (paper II). However, we did not observe upregulation of the homing receptor for migration to lymph nodes, CCR7, on MDCs and PDCs after HIV-1 exposure alone, as reported by others (306).

Cytokine profile

Signaling via CD40 induces DCs to upregulate of co-stimulatory molecules and secrete pro-inflammatory cytokines like IL-12 (307-309). A hypothesis in the HIV-1 field has been that DCs from HIV-1 infected individuals are defective in their ability to produce IL-12 and thereby fail to provide the help required for cytotoxic T cell responses. To address this, we studied the cytokine profile of HIV-1\textsubscript{Bal} exposed MDDCs in response to CD40L stimulation. Analyses of the cell culture supernatants did not reveal any differences in the amounts of TNF\textsubscript{α} and IL-12 p70 secreted from HIV-1\textsubscript{Bal} exposed or unexposed MDDCs stimulated with CD40L (paper I). However, intracellular cytokine stainings revealed that while TNF\textsubscript{α} was produced by both p24- and p24+ DCs after stimulation with CD40L, IL-12 p70 was exclusively produced by the p24- DCs. This emphasizes the difference between studying effects of CD40L on cytokine production in the total culture (where no differences were found between HIV-1 exposed and unexposed DCs) and the cytokine profile in individual cells. This is especially important in HIV-1 infection where a relatively small fraction of the DCs is infected. Thus, by applying intracellular staining to study the expression of cytokines and HIV-1 p24 at the single cell level, we were able to determine that while HIV-1\textsubscript{Bal} infection did not alter TNF\textsubscript{α} production in response to CD40L stimulation it did interfere with IL-12 p70 production (paper I). Our data therefore indicate that HIV-
1 infected DCs are impaired in their ability to produce IL-12. Downmodulation of IL-12 may be a common strategy by viruses to evade the host immune system. Suppression of IL-12 has been documented in other viral infections like herpes virus, CMV and measles infection (310-314). It has been shown that PBMCs isolated from AIDS patients have a severe defect in their IL-12 production (216, 217). This impairment could be overcome by addition of soluble CD40L to monocyte cultures from HIV-1 infected patients, indicating that this defect may be due to insufficient signalling from bystander CD40L+ T cells and not due to impairment of the IL-12 producing cells themselves (218). However, our data suggest that reconstitution of CD40L in HIV-1 infected individuals may not fully restore the function of HIV-1 infected DCs in terms of IL-12 p70 production.

Next, we extended these findings by showing that HIV-1 infected primary MDCs and PDCs also produce TNFα upon TLR ligation with R-848 (paper II). R-848 is a low molecular weight imidazoquino-like compound that signals through TLR7 and 8. Human MDCs and PDCs both express TLR7 and have been shown to upregulate cell surface markers and secrete cytokines upon R-848 stimulation (43). However, we failed to detect intracellular IL-12 p70 in R-848 stimulated MDCs and PDCs. Still, by ELISA we found that R-848 stimulation induced IL-12 p70 secretion from MDCs, irrespective of HIV-1 exposure (43 and data not shown). Primary MDCs produce significantly less IL-12 p70 than MDDCs (315), which could explain why we detected IL-12 p70 intracellularly in MDDCs but not in MDCs and PDCs. Therefore, while we found that HIV-1 infected MDDCs are impaired in the ability to produce IL-12 p70 we cannot conclude that such a defect is present also in primary HIV-1 infected DCs. Primary DCs seem to retain functional capabilities despite HIV-1 infection. Indeed, in our studies using autologous DC:CD4+ T cell co-cultures we did not see an impaired ability of HIV-1 exposed/infected primary MDCs and PDCs to induce recall T cell responses (paper III). However, it is possible that the function of HIV-1 infected DCs, as well as their potential impairment in cytokine production, is more critical for induction of primary T cell responses than for memory responses. Although we established productive HIV-1 infection in primary MDCs and PDCs, only 1-7% of the DCs were HIV-1 infected as determined by intracellular p24 staining. Dysfunctions in HIV-1 infected DCs may be difficult to detect, since the majority of the DCs in the cultures were uninfected (p24-).
We found that HIV-1 exposure induced production and secretion of IFNα from PDCs (paper II). It is well established that PDCs produce IFNα in response to exposure to different viruses like herpes simplex virus (HSV), influenza virus, Sendai virus as well as HIV-1 (199, 306, 316-319). It appears that exposure rather than infection is required for induction of IFNα production, as shown by the production of IFNα by PDCs that had been cultured in the presence of AZT, which inhibits productive infection. IFNα has a documented anti-viral effect (320-323). However, somewhat at odds with the antiviral effect of IFNα, we saw a positive correlation between the frequencies of HIV-1 infected p24+ PDCs and the levels of IFNα detected in the cell culture supernatant after 72 hours of exposure to HIV-1BaL. Perhaps higher concentration of viral antigen in the cell culture (as a consequence of higher infection rate) resulted in induction of more IFNα. Intracellular IFNα stainings of HIV-1 infected PDCs were not performed in our study but one could speculate that the PDCs that are HIV-1 infected do not produce IFNα and vice versa PDCs that produce IFNα would not become infected.

The function of MDDCs vs. primary DCs after HIV-1 infection

Although MDDCs resemble primary MDCs with respect to cell surface markers and their response to stimuli, they are phenotypically and functionally distinct DC populations in a number of ways. While both subsets express myeloid markers like CD11c, MDDCs are often characterized by their expression of CD1a, which is not expressed by MDCs. On the other hand MDCs express CD1d, which MDDCs lack (315). The two DC populations express overlapping but not identical TLRs; MDDCs express high levels of TLR4 but no TLR7, while MDCs express TLR7 and lower levels of TLR4 (41, 42, 55, 59, 272, 324). MDCs and MDDCs are different also with respect to their migratory capacity, ability to secrete cytokines like IL-6, IL-10 and IL-12 p70 in response to various stimuli as well as their capacity to induce cytokine production in T cells (315, 325, 326). We found several marked differences between the MDDCs and primary MDCs, although we did not compare the DC subsets side by side. While MDDCs produce high levels of IL-12 p70 in response to CD40L (paper I), the levels of IL-12 p70 produced by MDCs in response to R-848 stimulation was much lower and not detectable by intracellular cytokine staining and flow cytometry (paper II). However, CD40L and R-848 stimulation are two different stimuli, reported
to induce different levels of IL-12 p70 in MDDCs (327). Comparing MDCs and MDDCs from the same donor, MDCs were found to produce less IL-12 p70 in response to different stimuli than MDDCs (315). Still, our intracellular analyses of cytokine production in MDDCs after HIV-1 infection and the observed inability of HIV-1 infected p24+ MDDCs to produce IL-12 p70 illustrates a possible mechanism to explain the inadequate T cell responses observed in vivo.

HIV-1 exposure of MDCs, on the other hand, resulted in upregulation of the co-stimulatory molecule CD86 but not CD40 and CD83 (paper II). In contrast, the bulk culture of MDDCs did not mature after HIV-1 exposure alone, but a slight upregulation of co-stimulatory molecules was only seen on the fraction of MDDCs that were HIV-1 infected (paper I). However, stimulation resulted in full maturation as defined by a marked increase in the expression of these cell surface markers; both in HIV-1 exposed unexposed MDDCs and MDCs (papers I-II). MDCs appear to be more sensitive to HIV-1 exposure than MDDCs, at least in terms of upregulation of cell surface markers (219, 304, 305). Also, MDCs are more efficient than MDDCs in polarizing proliferating T cells to a Th1 type response (325), despite that they produce less IL-12 compared to MDDCs. In addition, different protocols to generate MDDCs most likely result in different DC-like cells, depending on the source of serum, concentration and source of recombinant cytokines, cell density and length of culture, which complicate matters even more. These differences between in vitro and primary in vivo generated DCs are important to consider when interpreting data and consequently when choosing what type of DCs to use for potential clinical applications.

Taken together, our findings suggest HIV-1 infection has multiple effects on the functional properties of DCs, which could help provide insight in the full complexity of the contribution of DCs to the pathogenesis of the disease.

**The role of dendritic cells in spreading HIV-1 infection**

The migratory nature of DCs and their susceptibility to bind to and become infected by HIV-1, along with their ability to interact with numerous T cells in the lymphoid tissue, identifies them as strong candidates for a central role in spreading HIV-1 in the host (328). After sexual transmission, HIV-1 crosses the mucosal epithelium and eventually ends up in the lymphoid tissue where a permanent infection is established.
ON THE ROLE OF DENDRITIC CELLS IN HIV-1 INFECTION

Thus, DCs are proposed to play a central role in the early events of HIV-1 transmission by transporting the virus from the periphery to the lymphoid compartment. As DCs and T cells interact to generate immune responses, this interaction also serve as a ideal microenvironment for HIV-1 replication and transfer between cells (113). In experimental transmission of SIV across the vaginal epithelium of rhesus macaques, intraepithelial DCs become productively infected at a low level within 18 hours after inoculation of the virus (330). The DCs subsequently migrate to draining lymph nodes where they infect resident CD4+ T cells (330). Naïve T cells require specific activation signals from antigen-presenting DCs in order to initiate their expansion and differentiation into memory and effector T cells and DCs possess the ability to cluster with numerous T cells to facilitate clonal expansion during immune activation. It is also known that complexes of T cells and DCs dramatically increase HIV-1 production by the T cells, as activated T cells become highly susceptible to HIV-1 and enhance viral replication (113, 183, 184, 304, 331, 332). A study using highly enriched HIV-1 infected MDDCs showed that these cells were poor stimulators of allogeneic T cell proliferation and that HIV-1 infected DCs indeed may contribute to impaired T cell mediated immune responses (300). In our study, we analyzed recall CD4+ T cell responses against CMV and SEB (paper III). It would have been interesting to study transfer of HIV-1 to HIV-1 specific CD4+ T cells. However, this would have required the use of DCs and T cells isolated from HIV-1 infected individuals or the induction of primary HIV-1 specific immune responses. To mimic the HIV-1 transfer taking place in vivo between infected DCs and naïve T cells using a cell culture system, would require establishment of primary HIV-1 infection and expansion of HIV-1 specific T cells in vitro, which would be technically challenging.

As described earlier, we showed that both MDCs and PDCs are susceptible to productive infection with both R5-using HIV-1_{Bal} and X4-using HIV-1_{IIIB} and that there is a difference in susceptibility to HIV-1 infection between the two DC subsets (paper II). Next, we could show that both MDCs and PDCs were able to transfer the two HIV-1 isolates to autologous CD4+ T cells (paper III). To study the transfer of HIV-1 from infected DCs to T cells and not the following spread of virus between T cells and subsequent propagation of virus, we added Indinavir at the initiation of the co-culture. Indinavir is a protease inhibitor that binds to the active site of the viral protease and
thereby inhibits the proteolytic cleavage of the Gag and Gag-Pol precursors resulting in only non-infectious particles being produced. Consequently, addition of Indinavir to the co-cultures of HIV-1 infected DCs with autologous CD4+ T cells allowed transfer of the virus produced in the DCs prior to co-culture but no further spread of the virus. Addition of Indinavir substantially reduced the frequency of p24+ T cells, compared to the frequencies of p24+ T cells detected in the absence of Indinavir in the DC-T cell co-cultures. This shows that under normal conditions, i.e. in the absence of antiretroviral drugs, HIV-1 is mainly propagated by T cell to T cell spread after the initial transfer from DCs. With Indinavir present in the co-culture, significantly higher frequencies of p24+ T cells were found after co-culture with HIV-1_{Bal} exposed MDCs as compared to HIV-1_{IIIB} exposed MDCs. In contrast, HIV-1_{IIIB} exposed PDCs were again able to infect higher numbers of T cells as compared to HIV-1_{Bal} exposed PDCs. HIV-1_{IIIB} was transferred equally well by PDCs and MDCs. In contrast, HIV-1_{Bal} was more efficiently transferred to T cells by MDCs than PDCs. Taken together, these data indicate that there is a differential susceptibility and subsequent transfer of HIV-1_{Bal} and HIV-1_{IIIB} by the different DC subsets. This may play an important role in both the initial selection of HIV-1 strains after transmission as well as subsequent dissemination of virus in the infected host.

The role of DC-SIGN and productive infection of DCs in spreading HIV to T cells

HIV-1 interacts with DCs using several different pathways. In addition to the conventional CD4 and co-receptor mediated infection, HIV-1 can also bind to various CLRs on DCs without infecting the cells. The receptor studied in most detail in this respect is DC-SIGN, which is highly expressed on MDDCs. MDDCs can capture HIV-1 via gp120 binding to DC-SIGN expressed on the cell surface. The virus particle is then internalized into an early endosome and preserved intact and infectious. Upon contact with CD4+ T cells, DC-SIGN and virus is reexposed on the cell surface of the DCs and T cells may become infected (70, 333). DC-SIGN-bound virus markedly enhances infection of T cells in the presence of extremely small doses of virus; doses that normally would not infect T cells. Different subsets of DCs have been shown to express distinct arrays of CLRs that are capable of binding HIV-1 gp120 (49). Turville and co-workers investigated the expression of CLRs on LCs and DDCs, which are similar, but not identical, to the DC populations found in the squamous epithelia of the vaginal and anogenital tract. DC-SIGN was found only on CD14+ CD1a^{low} DDCs.
and not on CD1a^{high} dermal DCs and LCs. LCs transfer HIV-1 to T cells in a CD4- and CCR5-dependent manner (334). However, LCs express langerin and DDCs express the mannose receptor, both known to bind gp120 (49). Therefore, if LCs, or the equivalent mucosal DCs, are the first cell to capture the virus, as they reside in the outer epithelium, other receptors than DC-SIGN must be involved. Once the virus reaches the submucosa, via LC transmission or through a damaged mucosal surface, DC-SIGN may play a role in spreading the virus. Interestingly, DDCs that migrate out of skin explants downregulated their expression of CLRs and gp120 binding becomes predominantly CD4 dependent (49). Although MDCs and PDCs express very low or undetectable levels of DC-SIGN (data not shown), other CLRs with a similar capacity to bind HIV-1 may be utilized. In order to examine the ability of MDCs and PDCs to transfer HIV-1 to CD4+ T cells without being productively infected themselves, we supplemented the cultures with AZT during the 72 hours of HIV-1 exposure to prevent productive infection of DCs (paper III). AZT was thereafter washed out together with residual virus before the DCs were co-cultured with autologous CD4+ T cells for 3.5 days. We found that transmission of virus to T cells by PDCs and MDCs required productive infection by the DCs. T cells co-culture with previously AZT treated DCs did not show any p24 expression. This was shown in T cells co-cultured with either PDCs or MDCs exposed to either HIV-1_{BaL} or HIV-1_{IIIB}. These data show that DC-mediated transfer of HIV-1 to T cells require productive HIV-1 infection of immature PDCs and MDCs and that no or undetectable levels of HIV-1 are transferred by other mechanisms in our co-culture system. The controversy of whether DCs must be productively infected to support transfer of virus to T cells may be dependent on the DC subset. It may also be a matter of time of viral exposure, as viral transfer from MDDCs to T cells is dependent on DC-SIGN early after HIV-1 exposure of the MDDCs (335). However, at later time points productive infection of MDDCs is required for transfer to T cells to occur (335). Here, we exposed the DCs to virus for 72 hours that resulted in productive infection of at least a fraction of the DCs, which could lessen the usage of DC-SIGN in this system. In addition, primary blood DCs may not the most relevant DC subset to study to determine the relevance of DC-SIGN mediated transfer of HIV-1 to T cells, since these DCs express very low or undetectable levels of DC-SIGN (paper III). Still, the relative contribution of these different pathways in vivo remains to be determined.
The nature of HIV-1 infected T cells after viral transfer from dendritic cells

DCs efficiently transfer HIV-1 to T cells. However, the exact nature of the T cells that become infected is still not known. It has been reported that HIV-1 preferentially infects CD4+ T cells specific for HIV-1 antigens as compared to T cells with other antigen specificities in infected individuals (172). Early studies showed that when DCs were pulsed with HIV-1 and presented an additional antigen (superantigen, alloantigen or tetanus toxoid); antigen-responding T cells appeared to be preferentially infected (170, 328, 336). Although HIV-1 preferentially infects antigen-specific T cells, other T cells are also targets for HIV-1 infection, possibly by transfer from DCs, and may function as viral reservoirs in infected individuals (184, 337, 338). We examined the CD4+ T cells that became infected by transfer from HIV-1 infected MDCs and PDCs during antigen presentation using a co-culture and flow cytometry model system that allowed detailed characterization of the DCs and T cells (paper III). As described above, we found that MDCs and PDCs efficiently transferred both an R5 and an X4 HIV-1 isolate to autologous CFSE-labeled CD4+ T cells. HIV-1 infected MDCs and PDCs presenting CMV antigens were also able to activate CMV-specific T cells to a similar extent as uninfected donor-matched DCs as measured by production of effector cytokines; IL-2, TNFα and IFNγ and proliferation. The responding antigen-specific T cells became HIV-1 infected more frequently as compared to non-responding T cells, i.e. T cells that did not divide and did not produce cytokines. In conclusion, both PDCs and MDCs preferentially transmitted HIV-1 to antigen-specific CD4+ T cells during DC-mediated activation of the T cells. Our results suggest that most DC-mediated HIV-1 infection of CD4+ T cells does not occur randomly. HIV-1 infected MDCs and PDCs presenting CMV antigens activated pre-existing autologous CMV-specific memory CD4+ T cells and preferentially transferred the virus to them. This pattern was also seen when using SEB and subsequent cross-linking of TCRs as an alternative to CMV antigens to activate T cells. This may help explain why HIV-1 specific T cells in infected individuals more frequently contain HIV-1 than T cells with other TCR-specificities (172).

Generation of HIV-1 specific immune responses after immunization with
HIV-1 infected apoptotic cells

DCs present viral antigens to T cells after uptake of apoptotic cells derived from virus infected cells in vitro (274, 281-283, 339-342). However, it is unclear whether
apoptotic virus infected cells are capable of generating immune responses in vivo. To address the question whether HIV-1 infected apoptotic cells are capable of inducing HIV-1 specific immune responses in vivo, we used a pseudotype virus composed of the envelope of an amphotropic retrovirus murine leukemia virus (MuLV) and the HIV-1\textsubscript{LAI} genome, to overcome the cellular tropism of HIV-1 and be able to use a mouse model to study HIV-1 immune responses (paper IV, (343)) (Figure 12). The pseudovirus was constructed by super-infection with HIV-1\textsubscript{LAI} of a cell line persistently infected with MuLV (344, 345).

**Figure 12.** A mouse model for HIV-1 infection. A human T cell line (CEM-1B) with an integrated MuLV, was super-infected with HIV-1\textsubscript{LAI}. Upon infection and replication, the CEM cells produced a mixture of HIV-1, MuLV and HIV-1/MuLV chimeric virions. HIV-1/MuLV pseudotype virus particles with the MuLV envelope infected activated murine splenocytes and the HIV-1 p24 content of infected cells was determined. The syngeneic HIV-1/MuLV infected splenocytes were injected in the peritoneal cavity of mice. After injection of live infected cells it was possible to detect infectious particles in the peritoneal cavity and spleen 8-14 days after inoculation, thereafter the cells are naturally cleared by the host. The level of HIV-1 in the collected tissues was determined by culture on HIV-1 permissive cells and measurement of secreted p24. Modified from (346).
The supernatant collected contained a mixture of virus particles; MuLV, HIV-1, particles with the MuLV envelope containing the HIV-1 genome and vice versa and mosaic virus particles with HIV-1/MuLV envelope with either HIV-1 or MuLV genome. This supernatant was used to infect primary murine splenocytes from naïve mice. Only particles with the MuLV envelope were able to infect the splenocytes. Infected cells were assayed for the presence of HIV-1 by p24 ELISA (347). Batches of HIV/MuLV infected cells were frozen and used as source of apoptotic cells. Since the infection efficiency varied between different batches and in repeated experiments, the dose of 840±15 pg p24 per immunization was kept constant by varying the number of infected cells administrated (1-2x10^6 cells). Apoptosis was induced in infected splenocytes by γ-irradiation (348).

C57BL/6 mice were immunized intra-peritoneally (i.p.) according to the schedule in Figure 13, with apoptotic HIV-1/MuLV-infected or noninfected syngeneic splenocytes and subsequently challenged i.p. with live HIV-1/MuLV-infected splenocytes. We were not able to isolate replicating virus from the mice immunized with apoptotic HIV-1/MuLV infected cells, i.e. there was no release of infectious HIV-1 in the peritoneal cavity from the infected apoptotic cells.

![Figure 13. Immunization(s) with apoptotic HIV-1/MuLV infected or noninfected cells and challenge with live HIV-1/MuLV infected cells.](image)

The i.p. immunization route was chosen to target antigens to the spleen. The spleen is a central immunological organ (349) and i.p. immunization potentially allows the study of antigen response in both B and T cells. The i.p. route can be questioned should this vaccination strategy be considered in human trials, but it is possible to
administrate vaccines i.p. also in humans (350). Still, additional studies in mice or non-human primates on alternative administration routes like intra-venously, intra-muscularly or intra-dermally, would be beneficial to improve this vaccine strategy.

We could show that immunizing mice with apoptotic HIV-1/MuLV infected cells induced HIV-1-specific immunity. Immunization with apoptotic HIV-1/MuLV-infected syngeneic splenocytes resulted in strong Nef-specific CD8+ T cell proliferation and p24 induced CD4+ and CD8+ T cell proliferation as well as IFNγ production. In addition, systemic HIV-1 specific IgG and IgA as well as mucosa-associated IgA responses were generated. Moreover, mice vaccinated with apoptotic HIV-1/MuLV cells cleared the challenge using live HIV-1/MuLV-infected cells, as no live HIV-1/MuLV-infected cells could be isolated from the peritoneal cavity of the mice 10 days after challenge. On the other hand, it was possible to isolate live HIV-1/MuLV-infected cells that could infect activated human T cells, from mice vaccinated with apoptotic noninfected (8/10) or MuLV-infected (3/4) splenocytes 10 days after challenge with live HIV-1/MuLV infected cells. This shows that immunization with apoptotic HIV-1/MuLV infected cells shifted the kinetics for clearing the infection in the mice as compared to mice immunized with apoptotic noninfected or MuLV infected cells.

The fact that we were able to induce HIV-1 specific immune responses using apoptotic HIV-1/MuLV infected cells without the use of an adjuvant implies that the apoptotic cells per se provided the necessary signals for immune activation. We hypothesize, although this remains to be established, that phagocytes in the peritoneal cavity, like macrophages or DCs, take up the apoptotic cells. The antigen presenting cells then mature and migrate to a nearby lymphoid site and present the antigen to T cells and generate the immune responses detected in the immunized mice.

It has been very difficult to generate antibodies that can neutralize primary isolates of HIV-1 (243). The generation of a strong humoral response with neutralizing antibodies is perhaps the most challenging part of generating an effective HIV-1 vaccine. We are encouraged by the broad systemic and mucosal-associated humoral HIV-1 specific immune response and the presence of cross-clade reactive antibodies.
against the gp41 clade B/LAI peptide containing the ELDKWASLWN epitope, which is a part of a highly conserved broad HIV-1 subtype neutralizing epitope (351), after immunization with apoptotic HIV-1/MuLV infected cells. However, the potential HIV-1 neutralizing capacity of these antibodies remains to be determined.

After challenge with live HIV-1/MuLV infected cells in naïve mice it is possible to recover HIV-1/MuLV infected cells that can infect activated human T cells (as measured by p24 ELISA), from the peritoneal cavity and the spleen 8-14 days post inoculation, thereafter the infection is naturally cleared by the host (344, 345, 352). To study the potential long-term protective capacity of vaccination with apoptotic HIV-1/MuLV infected cells, these questions need to be addressed in a truly pathogenic animal model since HIV-1/MuLV has a limited replication capacity in mice as compared to SIV or SHIV in macaques (353). However, our data show that i.p. immunization with apoptotic HIV-1 infected cells induced HIV-1 specific systemic immune responses, primed for mucosal immune responses, and accelerated the clearance of challenge with live HIV-1 infected cells in mice.

These findings may have implications for the development of therapeutic and prophylactic HIV-1 vaccines. A therapeutic vaccine using this concept would include isolation of the circulating quasispecies of HIV-1 in the patient and subsequent superinfection of autologous cells with the patient’s own virus, which would hopefully overcome the problem with the high variability of HIV-1. However, this strategy requires modern laboratory facilities and trained personnel, which put demands on infrastructure. A prophylactic vaccine candidate based on this concept would perhaps use a cell line transfected with multiple truncated HIV-1 genes of different clades, coding for the known immunodominant epitopes of the various genes to obtain a broad response. This strategy excludes the potential benefit of using the patient’s own viral repertoire. On the other hand, such transfected cells could be obtained in large quantities and would be easier to distribute on a more extensive scale.
Concluding remarks

DCs are important in the generation of T cell responses. Still, it is unknown if DCs are as central to the pathogenesis of HIV-1 infection as they are for generating effective adaptive HIV-1 immune responses. Numerous parameters are involved in HIV-1 pathogenesis and contribute to disease progression in the infected host. The frequency of circulating DCs is reduced in HIV-1 infection and the reduction of DCs correlates with disease progression (176, 189, 207-213). Some data suggest that DCs are redistributed in the body and accumulate in lymphoid compartments in HIV-1 infected individuals (174). In addition, DCs isolated from HIV-1 seropositive persons have demonstrated a number of functional defects, like reduced capacity to induce T cell proliferation and decreased cytokine production as compared to DCs isolated from healthy donors (175-179). Based on these findings, we considered it important to study DCs in the context of HIV-1.

Here, we present data demonstrating an impaired ability of HIV-1 infected DCs to produce IL-12, an important cytokine in the initiation of cellular immune responses (paper I). In addition, we found that primary MDCs and PDCs were differentially susceptible to two different isolates of HIV-1 (paper II) and that productively infected DCs transferred HIV-1 primarily to antigen-reactivated CD4+ T cells (paper III). Furthermore, we showed that it was possible to induce HIV-1 specific immune responses in mice after immunization with apoptotic syngeneic HIV-1/MuLV infected cells and that these responses appeared to accelerate the ability of the mice to clear a challenge with live HIV-1/MuLV infected cells as compared to control animals (paper IV). Therefore, our data contribute both to an increased understanding of how DCs are affected by HIV-1 infection and subsequently provide insight as to how development of new strategies to combat HIV-1/AIDS could be developed.

Still many questions remain unanswered as to what the key elements in HIV-1 pathogenesis are and how we should address them. One strategy to obtain more knowledge on this subject is to continue the studies on the interaction between DCs and T cells during HIV-1 infection. It is important to understand how to obtain a balance between optimal induction of HIV-1 specific immune responses and reduced
risk of viral transfer and replication. In a continuation of the studies presented here, it would be beneficial to develop a procedure to isolate and examine pure populations of viable HIV-1 infected DCs. Pure populations of HIV-1 infected DCs would allow for a detailed study of how any impairments of HIV-1 infected DCs affect the induction of T cell responses. However, it is ultimately important to address the relative contribution of HIV-1 infected DCs to HIV-1 pathogenesis in vivo. One way to do that could be to study the transfer of HIV-1 from infected DCs to autologous HIV-1 specific T cells using blood or cells isolated from lymphoid compartments of HIV-1 infected individuals. Another way could be to take advantage of the SIV macaque models available. The use of apoptotic HIV-1 infected cells as a potential vaccine needs to be evaluated in an animal model where it is possible to establish an infection, like the SIV or SHIV infection in macaques. It is necessary to address if it is possible to administrate apoptotic infected cells without infecting and/or induce viral replication in the vaccinee. In addition, the immune responses elicited after immunization with apoptotic HIV-1/MuLV infected cells require more detailed study. Such efforts are currently made in the laboratory and the outcome of these experiments will be very interesting to follow.

An increased understanding of how DCs function under pathological and normal conditions is crucial to our understanding of immune defects in various diseases and I hope to continue my research on this topic in the future.
Populärvetenskaplig sammanfattning

I detta avhandlingsarbete har vi försökt förstå hur HIV påverkar dendritiska celler (DC). Vi har även undersökt möjligheten att använda döda HIV-infekterade celler som ett HIV-vaccin.


Vi har undersökt hur DC påverkas av HIV-infection. DC är en typ av vita blodkroppar som har till uppgift att ta upp olika ämnen i kroppen, t.ex. virus och bakterier och visa upp dem för resten av immunsystemet. På så sätt kan kroppens immunförsvar känna igen och bekämpa sjukdomsalstrande organismer. DC kan bli infekterade av HIV, men de kan även bära med sig viruset när de förflyttar sig i kroppen, utan att själva bli infekterade. På så vis kan HIV snabbt och effektivt spridas i kroppen och komma i kontakt med ett stort antal celler som går att infektera. Dessutom kan HIV påverka DCs förmåga att på ett korrekt sätt visa övriga immunförsvaret att kroppen är infekterad med HIV och på så sätt hindra eller påverka immunförsvarets möjlighet att bekämpa infektionen.

Vi fann att HIV-infekterade DC är defekta i sin produktion av cytokinen IL-12, vilket är en substans som behövs för att generera s.k. cytotoxiska T-celler, en slags immunceller som känner igen och dödar virusinfekterade celler i kroppen. Att DC inte gör IL-12 när de är HIV-infekterade skulle kunna bidra till att färre cytotoxiska T-celler genereras och att kroppen därför har svårt att eliminera virusinfekterade celler. Vi fann även att när HIV-infekterade DC interagerar med T-celler, vilket är nödvändigt


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References

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in vitro from CD34(+) hematopoietic progenitor cells is primarily determined by their maturation stage. Blood 93:3866.


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