Chapter 1

Chapter 1: Introduction to the immune system, lentivectors, and transmission of HIV-1

Overview of thesis

In this thesis we explore the role of dendritic cells (DCs) in regulating the adaptive immune system and mediating the anti-viral immune response to HIV-1 derived lentiviral vectors (LVs). The study of immunology and LVs is important in gaining understanding of anti-viral immunity as well as HIV-1 pathogenesis. We seek to learn how DCs can be critical to the development of efficient anti-viral immune responses, but also be culprits in aiding viral dissemination.

The first chapter will provide background information on LVs and HIV-1 and the innate immune response in mediating immunity and infection to these viral vectors. It will also discuss the role of DCs in HIV-1 dissemination. In chapter 2, we will explore in depth how LVs activate DCs and result in priming of antigen-specific memory T cells. Our findings suggest that cellular DNA may act as a viral ligand to the STING pathway. In chapter 3 we continue to explore the role of DCs in HIV-1 infection. We find DCs that amplify HIV infection of T cells. This mode of transmission is relatively resistant to reverse transcriptase inhibitors compared to T cell infection in the absence of DCs. We consider the future directions and potential implications of this work in chapter 5, and discuss ongoing work to investigate innate immune signaling pathways associated with LV and HIV recognition, viral heterogeneity, and ways to address cell-to-cell infection.

Overview of Dendritic Cells

DCs are an important link between the innate and adaptive immune systems. They are derived from cells in the bone marrow and reside in tissues throughout the body, sampling the environment and processing this information for activation of the adaptive immune system. When DCs capture antigen and are activated, they will migrate to

nearby lymphoid tissues. The antigenic protein is presented on the surface of DCs on the major histocompatibility complex (MHC) to naïve T cells (Banchereau and Steinman, 1998). Activated DCs initiate the differentiation of naïve antigen-specific T cells into effector and memory T cells. Non-activated DCs can also present antigen to T cells, but without stimulation signals T cell tolerance is generated (Heath and Carbone, 2001). Thus, activation of DCs is important in regulating immune stimulation versus tolerance.

Overview of Innate Immune Recognition of Viral Pathogens

DC maturation leads to the downregulation of antigen-capture activity, the increased expression of surface MHC class II molecules and co-stimulatory molecules, cytokine secretion, and migration to the draining lymph node (Trombetta and Mellman, 2005). DCs contain several families of surface and intracellular pathogen recognition receptors (PRRs), which detect a variety of conserved structures on pathogens, termed pathogen-assoicated molecular patterns (PAMPs), and initiate appropriate intracellular cascading activation pathways. In certain instances, host factors can be recognized as “danger” signals, when they are presented in aberrant locations or abnormal molecular complexes as a consequence of infection, inflammation, or other types of cellular stress (Beg, 2002). PRRs that are thought to be important in sensing LVs and HIV-1 include Toll-like Receptors (TLRs), Rig-I-like Receptors (RLRs), and cytosolic nucleic acid sensors.

The TLRs are the most extensively studied class of PRRs. TLRs are composed of an extracellular or luminal ligand-binding domain containing leucine-rich repeat (LRR) motifs and a cytoplasmic signaling Toll/interleukin-1 (IL-1) receptor homology (TIR) domain (O'Neill and Bowie, 2007). Ligand binding to TLRs induces receptor oligomerization, which subsequently triggers intracellular signal cascades. To date, 10 human TLRs and 13 mouse TLRs have been discovered (Mogensen, 2009). TLRs 3, 7,

8, and 9 have been implicated in sensing viral pathogens. TLR 3 recognizes dsRNA during viral replication (Alexopoulou et al., 2001); TLR 7 and 8 sense viral ssRNA (Diebold et al., 2004, Heil et al., 2004); and TLR9 senses unmethylated CpG DNA present in the virus genomes (Hemmi et al., 2000). After ligand binding, TLRs recruit the TIR domain-containing adaptor molecules, which are critical to intracellular signal transduction. MyD88 is a key adaptor molecule that is involved in signaling triggered by all TLRs, including TLRs 7, 8, and 9. However, TLR 3 is dependent on signaling via the adaptor molecule TRIF. Thus, the TLR pathways are often divided into MyD88-dependent or MyD88-independent, TRIF-dependent. Both pathways are capable of activating NF-KB, but may differ in their ability to induce proinflammatory cytokines and the anti-viral type I IFN response(Mogensen, 2009).

Of the RLRs, RIG-I and melanoma differentiation-associated gene 5 (MDA5) are RNA helicases that sense cytoplasmic viral RNA and stimulate Type I IFN responses. The RLRs share highly conserved domain structures, including a central DExD/H-box helicase core composed of two helicase domains with a specific insertion within Hel-2 and a C-terminal domain that confers part of the ligand specificity (Kolakofsky et al., 2012). Although similar components are shared, the RIG-I and MDA5 have distinct viral sensing roles in certain cases. For example, RIG-I has been shown to be essential in detecting paramyxoviruses and influenza virus, whereas MDA5 seems to be critical for the response to picornavirus and norovirus (Kato et al., 2006). These differences may be due to length-dependent binding and recognition of 5’ triphophate ends on dsRNA (Kato et al., 2008). However, both sensors are important in the sensing of Dengue Virus and West Nile Virus (Loo et al., 2008). The intracellular signaling downstream of RIG-I and MDA5 depends on the adaptor protein IPS-1 (also known as MAVS, CARDIF, and

VISA). The loss of IPS-1 abolishes the induction of type I IFN and proinflammatory cytokines in response to RNA viruses.

Cytosolic sensors for DNA can signal through the adaptor protein, stimulator of IFN genes (STING), which is critical for inducing Type I IFN responses. Once activated by cytosolic DNA signaling, STING stimulates TBK1 and IRF3, resulting in type I IFN response. STING can also directly detect bacterial cyclic dinucleotides (Burdette et al., 2011). The definitive cytosolic DNA sensors that signals upstream to STING have not all been identified. Currently, there are various putative cytosolic DNA sensors, which include Cyclic GMP-AMP synthase (cGAS), IFI16, DDX41, DNA-dependent protein kinases, catalytic subunit (DNA-PKcs), MRE11, and DNA-dependent activator of IRFs (DAI) (Fig. 1) (Wu and Chen, 2014). It is to be determined whether cGAS functions only in specific cell types and whether other cytosolic DNA sensors signal in conjunction with cGAS.

Overview of cytosolic DNA sensors of the Innate Immune System

cGAS is activated by dsDNA through direct binding and catalyzes the production of cyclic GMP-AMP (cGAMP) from ATP and GTP. Structural analyses of cGAS show that the NTase and Mab21 domains and a unique zinc-binding motif are essential for B-form DNA binding (Civril et al., 2013). Upon direct binding of DNA, conformational changes of the NTase domain occur, allowing better access of GTP and ATP substrates required for the catalytic reactions to generate the second messenger cGAMP(Civril et al., 2013, Xiao and Fitzgerald, 2013). As a second messenger, cGAMP then activates the host STING signaling pathway. Knockdown of cGAS in mammalian cell lines abolishes Type I IFN production induced by either DNA transfection or DNA virus infection (Gao et al.,

2013). cGAS has also been shown to mediate the innate immune response to HIV and other retroviruses by detecting reverse-transcribed HIV-1 cDNA (Gao et al., 2013).

IFI16 is a DNA-binding AIM2 related protein that interacts with DNA via its two HIN domains on the C-terminus. It binds both dsDNA and ssDNA, which have both been shown to be stimulatory during lentiviral replication (Jakobsen et al., 2013). In addition, IFI16 immunopreciptates with STING in dsDNA-stimulated THP-1 cells (Unterholzner et al., 2010). Furthermore, knockdown of IFI16 leads to decreased antiviral Type I IFN response (Kis-Toth et al., 2011). However, there is no clear mechanism for how IFI16 binds to STING. IFI16 also has a PYRIN domain and PYRIN-containing proteins usually interact with one another. STING does not have a PYRIN domain; thus, it is unclear if other proteins are involved in the IFI16-STING interaction or if the PYRIN domain is even involved in STING signaling.

DDX41 was found in a systematic RNAi screen of a family of DExD/H helicases required for induction of type I IFNs by cytosolic DNA in mouse and human cell lines (Zhang et al., 2012). The knockdown of DDX41 led to decreased type I IFN production in response to cytosolic DNA, HSV-1 and bacterial cyclic nucleotides (Zhang et al., 2012, Parvatiyar et al., 2012). In response to cytosolic DNA, DDX41 colocalizes with STING suggesting a connection with the STING signaling pathway (Parvatiyar et al., 2012). DDX41 has a DEAD domain, which is responsible for binding to DNA, but a clearly defined signaling domain that is distinct from its nucleic acid–binding domain has not been identified. Therefore, it is unclear how DDX41 recruits signaling adaptors such as STING for downstream signaling.

DNA-PKcs is an enzyme belonging to the phosphatidylinositol 3-kinase-related kinase (PI3K) protein family, which is required for the non-homologous end joining (NHEJ) pathway of DNA repair, which rejoins dsDNA breaks. This complex was identified in affinity pull-down experiments as being directly bound to dsDNA and DNA-PKcs-deficient mouse embryonic fibroblasts (MEFs) or mice showed decreased Type I IFN response to DNA virus infection (Ferguson et al., 2012).

The protein MRE11A was also found to be involved in dsDNA repair and is involved in nonhomologous joining of DNA ends. MRE11 has also been found to be a cytosolic dsDNA sensor, which signals through STING, but was not important to HSV-1 or *L. monocytogenes* anti-microbial responses (Kondo et al., 2013).

DAI was the first putative cytosolic DNA sensor found to be important to DNA-mediated induction of type I IFN response in non-immune cell lines (Takaoka et al., 2007). However, later studies found that MEFs, dendritic cells, and macrophages deficient in DAI responded normally to DNA transfection or DNA virus infection. Furthermore, DAI knockout mice showed normal immune responses to DNA vaccination (Ishii et al., 2008).

Although great progress has been made in identifying putative cytosolic DNA sensors, the exact mechanism of cytosolic DNA innate immune sensing remains unclear. It also remains to be seen how mechanisms involved in DNA damage repair overlap with viral DNA sensing pathways.

Overview of DC vaccines including LVs

Since DCs are critical to eliciting innate and adaptive immune responses, vaccination strategies involving DCs are being investigated. In particular, DC vaccines are currently

being developed to stimulate tumor-specific effector and memory T cells that can clear and suppress tumor growth. In order to achieve successful DC vaccination, tumor antigens have to be processed by the DCs and maturation of DCs has to occur. For example, the ex vivo DC vaccine derives DCs from a patient, cultures them ex vivo, loads them with tumor antigens, matures them with an adjuvant, and then injects them back into the patient with the goal of eliciting tumor-specific effector T cells that induce tumor regression (Tacken et al., 2007). However, this method is labor and resource-intensive and only appropriate for a limited number of patients. In vivo targeting vaccines directly target DCs to take up antigen and become activated in vivo. This can be achieved by LVs, which are HIV-1-derived replication-defective vectors that effectively transduce dividing and non-dividing cells such as DCs. Thus, LVs are advantageous in their efficiency, manufacturability, and accessibility to a wide number of patients.

To produce LVs, a reporter gene or gene of interest is cloned into a vector sequence between the LTRs. This ensures that only the gene of interest becomes integrated into the target cell genome. The viral genes required to generate the viral capsid, matrix, envelope, and enzymes are encoded on separate vectors (Fig. 2). These vectors are on separate plasmids, to ensure the LV is replication-deficient. After the plasmids are transfected into cells, LVs can be collected in the cell supernatant.

The LV vaccine capitalizes on the ability of the vector to integrate and generate long-term gene expression. Interestingly, the persistent expression of antigen does not lead to immunotolerance, but immunostimulation without evidence of exhaustion (Karwacz et al., 2009, Obst et al., 2007). This pattern of antigen persistence leading to effective memory responses instead of immune tolerance has also been seen with other viral infections (Zammit et al., 2006, Turner et al., 2007).

Since LV vaccines are being used clinically, it is critically important to understand how they stimulate innate and adaptive immunity. The identification of the mechanisms that bestow LVs with their inherent ability to activate innate and adaptive will have great scientific and public health relevance, because it will deepen our understanding of host responses to viruses and ultimately develop effective vaccines against pathogens and cancer.

Overview of cell-to-cell transmission of HIV-1

DCs also play an important role in HIV-1 infection involving activation and coordination of innate and adaptive immune responses to the virus. In addition, DCs may also play a role in the viral reservoir and dissemination via DC-mediated transmission of HIV-1 to CD4+ T cells. DCs, in particular activated ones, are able to capture HIV-1 on their surface without being infected. Virus particles concentrated at or near the surface of DCs are then transmitted to uninfected target CD4+ T cells via close contact in a process termed *trans*-infection (Fig. 3) (Turner et al., 2007, Coleman et al., 2013). In contrast, HIV-1 can infect DCs, productively replicate and release viral particles to new target cells in a process called *cis*-infection. However, productive infection of DCs is rare.

Host cellular molecules have been demonstrated to affect DC-mediated transmission of HIV-1 to $CD4^+$ T cells, including dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), CD4, and intercellular adhesion molecules (ICAMs). Viral factors can also affect DC-mediated transmission of HIV-1 to $CD4^+$ T cells, largely by directly interacting with and/or altering the expression of the host cell molecules. As

such, the effects of viral factors on DC-mediated transmission of HIV-1 to $CD4^+$ T cells will be described in the context of their effects on the host cell.

There are host cellular molecules and viral factors that have been demonstrated to affect DC-mediated transmission of HIV-1 to $CD4^+$ T cells. The DC-SIGN is a surface adhesion molecule highly expressed on DCs and is involved in formation of the virologic synapse and binds to HIV Env to capture the virus (Geijtenbeek et al., 2000). The blocking of DC-SIGN inhibits and the overexpression ehances DC-mediated HIV transmission. However, DC-SIGN is mainly important for HIV transmission by immature DCs (Wang et al., 2007). Another surface adhesion molecule, ICAM-1, binds to Leukocyte function-associated mocleule-1 (LFA-1) expressed on the surface of CD4+ T cells. The interaction of these two molecules is important to stabilization of the virologic synapse, and thus for DC-to-T cell HIV transmission (Sanders et al., 2002). The viral factors found to be important to DC-mediated viral tranmission include the composition of glycosolation of the HIV Env (van Montfort et al., 2011) and Nef protein (Sol-Foulon et al., 2002).

Further exploration is required to better understand the mechanism of how the virologic synapse forms and mediates HIV transmission between cells. DC-mediated HIV-1 transmission is likely a potent mode of infection, but the implication of how this relates to drug efficacy has yet to be explored. This area needs to be further studied to develop strategies to inhibit persistent infection.

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Receptor	Cellular localization	Microbial component(s)	Origin(s)
TLRs			
TLR1/TLR2	Cell surface	Triacyl lipopeptides	Bacteria
TLR2/TLR6	Cell surface	Diacyl lipopeptides	*Mycoplasma*
		Lipoteichoic acid	Gram-positive bacteria
TLR2	Cell surface	Lipoproteins	Various pathogens
		Peptidoglycan	Gram-positive and -negative bacteria
		Lipoarabinomannan	Mycobacteria
		Porins	*Neisseria*
		Envelope glycoproteins	Viruses (e.g., measles virus, HSV, cytomegalovirus)
		GPI-mucin	Protozoa
		Phospholipomannan	*Candida*
		Zymosan	Fungi
		β-Glycan	Fungi
TLR3	Cell surface/endosomes	dsRNA	Viruses
TLR4	Cell surface	LPS	Gram-negative bacteria
		Envelope glycoproteins	Viruses (e.g., RSV)
		Glycoinositolphospholipids	Protozoa
		Mannan	*Candida*
		HSP70	Host
TLR5	Cell surface	Flagellin	Flagellated bacteria
TLR7/8	Endosome	ssRNA	RNA viruses
TLR9	Endosome	CpG DNA	Viruses, bacteria, protozoa

Table 1. List and description of human TLRs.

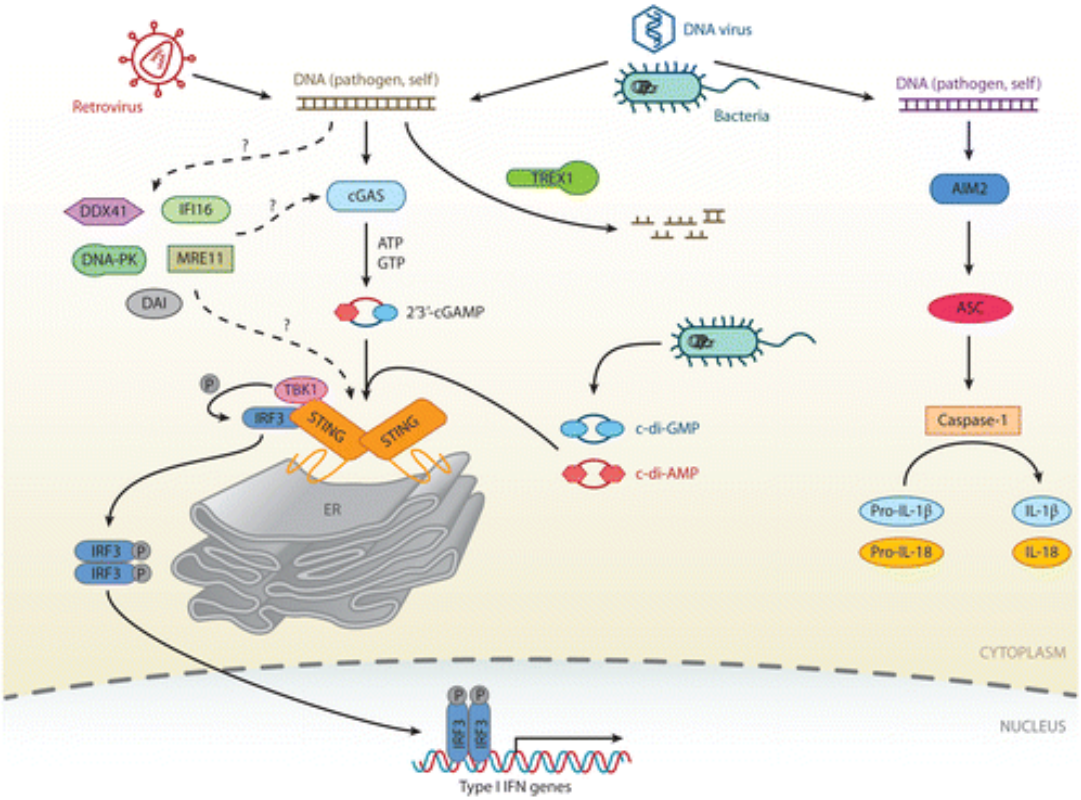

Figure 1. Putative cytosolic DNA sensors.

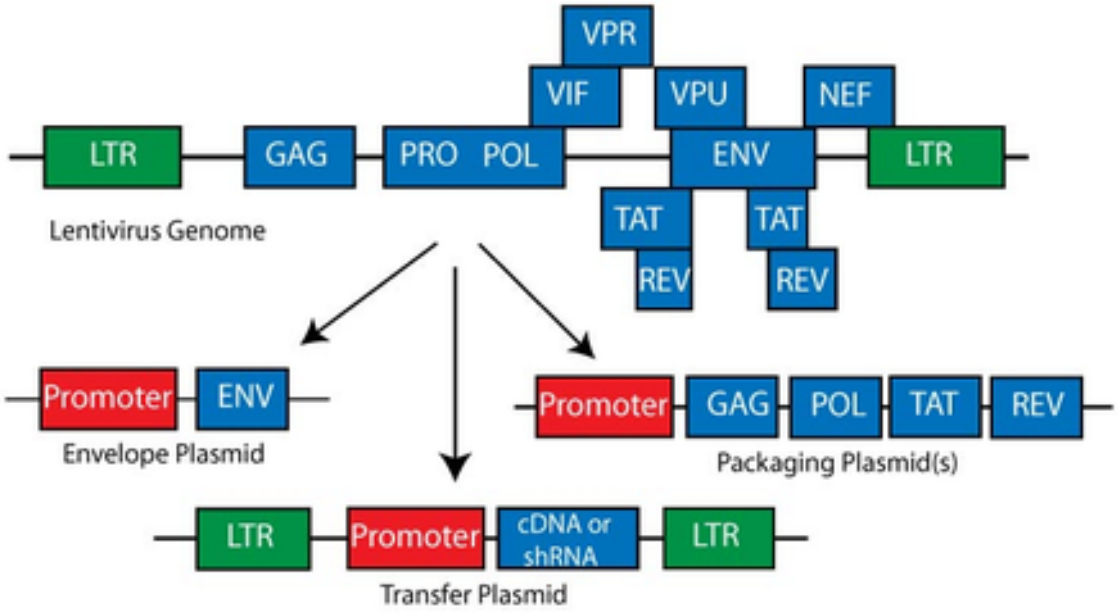

Figure 2. Schematic of plasmids required to generate lentivector (LV).

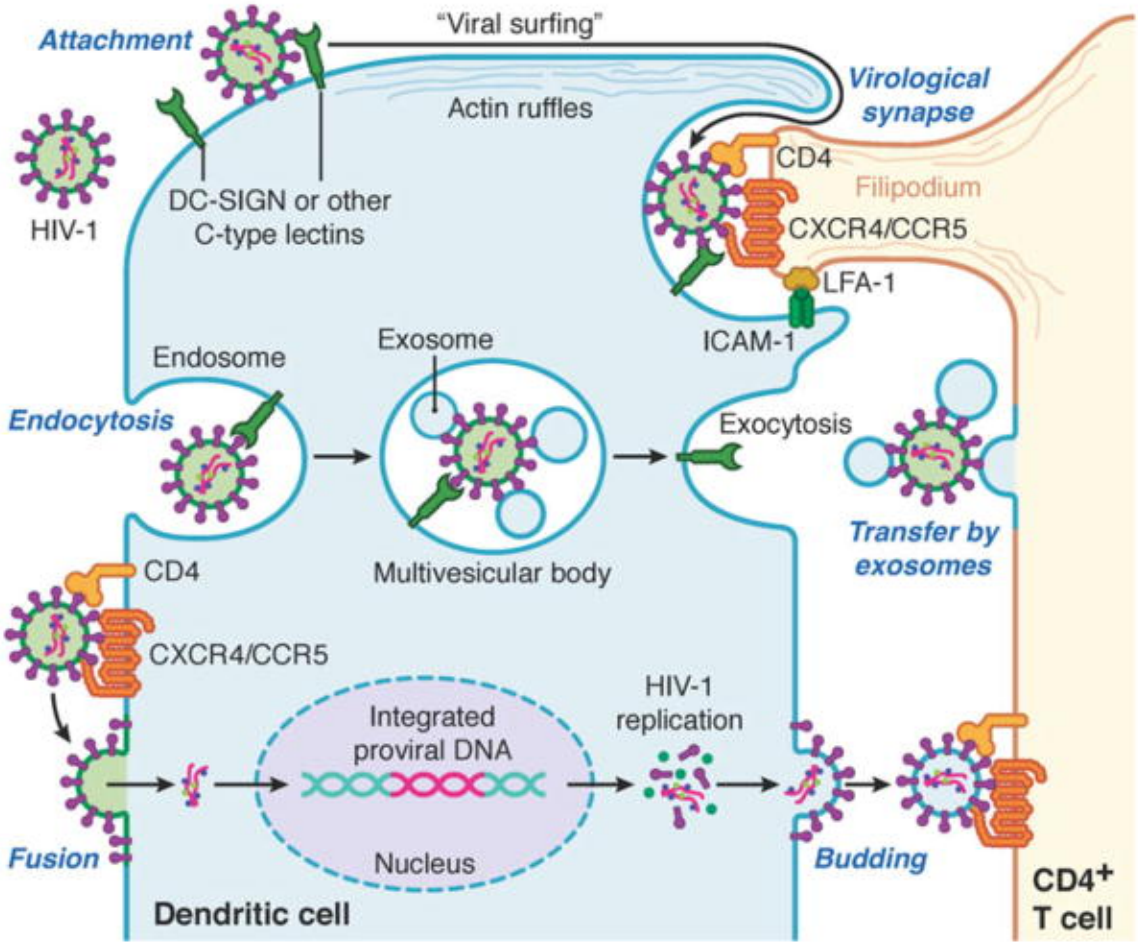

Figure 3. Mechanisms of DC-mediated HIV-1 transmission to $CD4^+$ T cells.