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Role of Glycosphingolipids in Dendritic Cell-Mediated HIV-1 *Trans*-infection

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Abstract

Glycosphingolipids (GSLs) are components of the cell membrane that comprise a membrane bound lipid, ceramide, coupled to an extracellular carbohydrate. GSLs impact numerous aspects of membrane biology, including membrane fluidity, curvature, and organization. The role of these molecules in both chronic inflammation and infectious disease and underlying pathogenic mechanisms are just starting to be recognized. As a component of the cell membrane, GSLs are also incorporated into lipid bilayers of diverse enveloped viruses as they bud out from the host cell and can go on to have a significant influence on viral pathogenesis. Dendritic cell (DC) subsets located in the peripheral mucosal tissues are proposed to be one of the earliest cell types that encounter transmitted viruses and help initiate adaptive immune responses against the invading pathogen by interacting with T cells. In turn, viruses, as obligatory intracellular parasites, rely on host cells for completing their replication cycle, and not surprisingly, HIV has evolved to exploit DC biology for the initial transmission event as well as for its dissemination and propagation within the infected host. In this review, we describe the mechanisms by which GSLs impact DC-mediated HIV *trans*-infection by either modulating virus infectivity, serving as a direct virus particle-associated host-derived ligand for specific interactions with DCs, or modulating the T cell membrane in such a way as to impact viral entry and thereby productive infection of $CD4^+$ T cells.

5.1 Introduction

Dendritic cells (DCs) play a sentinel role in the peripheral mucosal tissues, patrolling their immediate environment for foreign pathogens. Following pathogen uptake and sensing, DCs become activated, migrate to the draining lymph nodes, and process and present antigen in complex with major histocompatibility complex (MHC) Class I and II molecules to T cells that display cognate receptors specific for the antigen–MHC complexes displayed on the DC surface (Banchereau and Steinman 1998). Thus, DCs are specialized antigen-presenting cells that effectively link the innate and adaptive immune responses (Banchereau and Steinman 1998). Human DCs in the blood can be broadly divided into myeloid DCs (mDCs) or plasmacytoid dendritic cells (pDCs) (Geissmann et al. 2010), and both mDCs and pDCs specialize in the detection of virus infections and can function in innate and adaptive immunity. While pDCs are the primary type I interferon-producing cells upon viral infection (Liu 2005), mDCs have evolved primarily to prime and activate antiviral T cells (Banchereau and Steinman 1998). Because of the ease of availability either from human peripheral blood or generation in vitro from peripheral blood monocyte precursors (Sallusto and Lanzavecchia 1994), most of the work in the literature has involved study of pathogen

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interactions with mDCs or peripheral blood monocyte-derived DCs. Though peripheral blood monocytes are not the precursors to mDCs, monocytes can exit the blood and enter peripheral mucosal tissues upon pathogen infection, and give rise to inflammatory DCs that share many of the phenotypic and functional features of mDCs (Randolph et al. 1998). In this review, we focus our attentions primarily on HIV-1 interactions with peripheral blood monocyte-derived DCs.

DCs express a wide variety of innate receptors that recognize common pathogen-associated molecular patterns (PAMPs), a recognition that is essential for the induction of proinflammatory genes, DC migration and maturation, and activation of the adaptive immune response (Medzhitov and Janeway 1997). Since viruses are obligate human parasites that require host cell machinery to complete their life cycle, they have evolved diverse immune evasion strategies that interfere with these intrinsic effector mechanisms in DCs. To initiate infection, HIV must traverse the plasma membrane to gain access to the host cell cytoplasm. While HIV entry occurs predominantly at the plasma membrane upon sequential interactions with the receptor, CD4, and coreceptor, CCR5 or CXCR4 (Doms and Trono 2000), some recent studies have argued for receptor-dependent endocytosis of intact virus particles and fusion with endosomal membranes as an alternative entry mechanism (Miyauchi et al. 2009). Endocytosis offers invading virus particles a number of benefits, including a convenient and rapid transport across the plasma membrane and escape from cell-surface expressed pattern-recognition receptors such as the C-type lectin receptors (CLRs) (Figdor et al. 2002). However, the endocytic pathway is also fraught with danger for the incoming virion. Trafficking of virus-containing vesicular compartments to a low-pH late endosome/lysosome might lead to the degradation of viral capsids, exposure of viral nucleic acids, and initiation of IFN-dependent antiviral responses by the endosomal resident nucleic acid-sensing toll-like receptor (TLR) family members (Pichlmair and Reis e Sousa 2007). Alternatively, fusion of virus with DCs results in the delivery of virus core into the DC cytoplasm, a hostile environment equipped with a battery of intracellular viral nucleic acid-sensing RIG-I like receptors (RLRs), Nod-like receptors (NLRs), and, in the case of HIV, intrinsic cellular restriction factors that determine the course of viral pathogenesis in the infected host. How HIV navigates and exploits DCs for viral dissemination is a question under active study and of significant research interest. In this review, we discuss the role of both cell-associated glycosphingolipids (GSLs) and GSLs incorporated into HIV-1 particle membranes in mediating virus interactions with DCs and facilitating HIV evasion and dissemination via DC-mediated *trans*-infection pathways.

5.2 Glycosphingolipids

5.2.1 Glycosphingolipids Are an Integral Component of the Cellular Membrane

GSLs are part of a larger family of membrane-associated ceramide sphingolipids, defined simply as a sphingosine coupled to a fatty acid. The ceramide backbone is then modified to become sphingomyelin (SM), sulfatides, or glucosylceramide (GlcCer) (reviewed in (Zeidan and Hannun 2007; Rawat et al. 2005)). SM is one of the major membrane phospholipids that, with cholesterol, contributes to the structure and curvature of the membrane (Varki et al. 2008). GlcCer ultimately gives rise to the more complex GSLs, which are further broken down into gangliosides (defined as having a terminal sialic acid), asialogangliosides, and globosides. There are a large number of GSL variants, with gangliosides alone having over 40 known variants (Varki et al. 2008). Of the gangliosides, the monosialogangliosides GM3 and GM1 have been frequently studied, while the asialoganglioside, asialo-GM1 (the non-sialylated equivalent of GM1), and globoside globotriaosylceramide (Gb3) are frequently noted as examples of their respective subfamilies. Although GSLs only make up approximately 5% of the overall membrane lipid composition, they are highly enriched in membrane rafts (Varki et al. 2008; Bollinger et al. 2005). As HIV is known to bud from

membrane rafts (Nguyen and Hildreth 2000; Ono and Freed 2001), this results in an enrichment of GSLs within the HIV particle and can have significant consequences on viral function. Likewise, many of the cellular binding partners that have been described for HIV reside in membrane rafts and are therefore also subjected to the influence of GSLs enriched within these domains. GSLs are therefore poised to make significant contributions to HIV pathogenesis by impacting the virus particle directly, and by impacting the target cell.

5.2.2 Glycosphingolipid Expression Profiles and Variability in Membrane Distribution Within a Cell

There is a high degree of complexity and several nuances to the cellular distribution and expression profiles of individual GSLs (Zeidan and Hannun 2007; Alberts et al. 2002). For example, gangliosides are typically expressed at high levels in the brain while globosides are preferentially found on erythrocytes (Varki et al. 2008). Closer analyses of various disease states have elucidated further cellular associations. The ganglioside GM1 is abundant in intestinal epithelial cells, where it can be exploited by cholera toxin B (Alberts et al. 2002), while Gb3 is widely expressed on vascular endothelial cells and can be recognized by *Escherichia coli* verotoxin (Okuda et al. 2006). Burkitt's lymphoma cells have been found to express high levels of the globoside Gb3 (Nudelman et al. 1983) and a number of childhood neurodegenerative diseases are characterized by GSL abnormalities (reviewed in (Xu et al. 2010)). However, it is the cellular distribution of GSLs within leukocytes, or the "immune cell glycomes" (Haslam et al. 2008), that are particularly informative to our ongoing understanding of HIV pathogenesis. Gb3 can serve as a binding partner for HIV glycoprotein but is only found in macrophages and not T cells (Hammache et al. 1999; Ramegowda and Tesh 1996). Although both macrophages and activated $CD4^+$ T cells have high levels of GM3 (Hammache et al. 1999), it is found in higher levels within macrophages than within T cells (Chan et al. 2008). These variations in cellular distribution could potentially impact the tropism and mechanism of action of pathogens such as HIV. It is interesting to note that Gb3, enriched on macrophages, has a strong preference to bind CXCR4 using viruses, while GM3, enriched on T cells, preferentially binds CCR5 using variants (Nehete et al. 2002).

Dendritic cells also show differences in their glycome profile upon maturation. Maturation of DCs upregulates expression of varied glycosyltransferases, having broad effects on glycan structures, thereby impacting the profile of the DC-associated glycosphingolipidome (Haslam et al. 2008). Expression of ST3Gal1, a sialyltransferase, is upregulated upon DC maturation, resulting in increased expression of globosides and gangliosides. Similarly, bone marrow-derived murine DCs have been shown upon maturation to increase surface expression of globosides, while ganglioside levels are unchanged (Li et al. 2009). These differences in DC GSL composition are particularly interesting to note in light of the differences seen in how HIV interacts with an immature and mature DC (Izquierdo-Useros et al. 2010; Wu and KewalRamani 2006). Though maturation of DCs results in a global decrease in macropinocytosis and fluid-phase uptake (Austyn 1998), there is a dramatic enhancement of HIV-1 capture and enhanced transfer of captured virus particles to T cells, facilitated presumably by a maturation-dependent upregulation of co-stimulatory and adhesion molecules on the DC surface (Dong et al. 2007; Fahrbach et al. 2007; Hatch et al. 2009; Izquierdo-Useros et al. 2007, 2009; McDonald et al. 2003; Wang et al. 2007; Weissman et al. 1995). Similar to the effects observed with mature peripheral blood monocyte-derived DCs, HIV-1 binding and capture by activated Langerhans cells derived from cord blood $CD34^+$ stem cells (Fahrbach et al. 2007), vaginal epithelial sheets (Hladik et al. 2007), or human skin explants (de Jong et al. 2008) were also enhanced upon maturation. Whether differences in GSL composition upon DC maturation, and specifically

enhancement in cell surface expression of globosides and gangliosides, can impact the mechanism of HIV-1 capture and *trans*-infection by DCs remains to be determined.

In addition to cell type differences in GSLs, cell-intrinsic GSL expression levels can vary based on cell cycle and cell activation status (Hakomori 1990). For example, control of the cell surface expression level of gangliosides is a finely tuned process, and the Golgi-resident enzyme, GM3 synthase, also named ST3Gal-V or Sial-T1, plays a key regulatory role (Uemura et al. 2009). GM3 synthase catalyzes the transfer of a sialic acid residue to the terminal galactose of lactosylceramide, resulting in the synthesis of the ganglioside, GM3, the common precursor to nearly all of the cellular gangliosides (Kolter et al. 2002). In agreement with early observations that GM3 levels increase upon macrophage-like cell differentiation (Nojiri et al. 1986), the expression of GM3 synthase is dramatically upregulated upon monocyte differentiation into macrophages (Gracheva et al. 2007). TNF-α and other proinflammatory mediators are also associated with increased GM3 synthase gene transcription and expression levels (Tagami et al. 2002; Blander et al. 1999). GSLs are upregulated upon T cell activation, and Gb3 synthesis is induced in PHA/IL2-activated PBMCs (Lund et al. 2006). Interestingly, viral infection has also been demonstrated to impact cellular GSL levels. Both GM1 and asialo-GM1 are upregulated upon infection with respiratory syncytial virus (Moore et al. 2008), while peripheral blood mononuclear cells upregulate GM3 and Gb3 upon HIV infection (Fantini et al. 2000). The inducible modulation of GSL cell content, especially under proinflammatory conditions, has the potential to significantly impact how HIV interacts with the host cell while also influencing the composition of the cellular membrane from which de novo virions will bud.

In addition to intercellular heterogeneity in GSL expression, there is considerable heterogeneity in the intracellular localization of GSLs. Such subtleties in intracellular distribution can potentially impact the nature of the specific GSLs that are ultimately incorporated into a budding virion. Although there is a much higher concentration of GSLs at the plasma membrane than throughout the cell, intracellular membrane compartments do contain GSLs (Schwarz and Futerman 1997). Subcellular compartmentalization of sphingolipid biosynthetic enzymes along the *trans*-Golgi network and directional vesicular transport amongst organelle members of the endomembrane system can result in varied intracytoplasmic membrane concentrations of GSLs (D'Angelo et al. 2007, 2012; Yamaji et al. 2008). Within the cell, there are often higher levels of the ganglioside GM1 in the endoplasmic reticulum, while the *trans*-golgi network possesses higher levels of the ganglioside GM3 (D'Angelo et al. 2012; Yamaji et al. 2008). In some cases, such as motile T cells, distribution of GM1 and GM3 gangliosides can be polarized in response to chemotactic signals (Gomez-Mouton et al. 2001). While a stationary T cell shows relatively uniform distribution of the gangliosides GM1 and GM3, polarization of the cell results in a dramatic segregation of the two molecules, with GM3 redistributing to the leading edge, while GM1 becomes localized to the trailing uropod (Gomez-Mouton et al. 2001).

In the absence of such dramatic polarization, GSLs can also be found segregated into distinct membrane raft domains, sometimes at different perpendicular depths within an otherwise laterally equivalent region of membrane, although the precise nature of localization remains unclear. In Madin–Darby canine kidney epithelial cells, nanoscale topographic imaging was used to show that GM3 localizes to the peaks of microvillus-like membrane protrusions, while GM1 localizes to the valleys between the protrusions (Chen et al. 2008). However, confocal microscopy of similar membrane structures suggested the opposite distribution, with GM1 located in the microvilli and GM3 residing in the valleys (Janich and Corbeil 2007). Despite these conflicting observations, both lines of evidence demonstrate that GM1 and GM3 reside in separate membrane domains. Though assembly and budding from GSL-enriched lipid rafts is a feature that is well conserved amongst

diverse nonsegmented RNA virus families, including members of the orthomyxovirus and filovirus families (Suomalainen 2002), this segregation of unique GSL-containing plasma membrane microdomain sites may also help to explain the selective partitioning of virus assembly sites when a single cell is co-infected with two distinct “raftophillic” enveloped viruses, such as HIV-1 and Ebola (Leung et al. 2008) or HIV-1 and influenza (Khurana et al. 2007). In both instances, the HIV-1 glycoprotein is found to localize in membrane domains distinct from those containing Ebola GP or influenza HA glycoproteins.

5.3 The Role of Glycosphingolipids in Cellular Functions and Viral Pathogenesis

A growing body of research is steadily unveiling the requirement of GSLs in a breadth of cellular functions (reviewed in (Xu et al. 2010)). In broad terms, GSLs can act as cellular signaling molecules and contribute to membrane architecture, organization, fluidity, and rearrangements (Hakomori 1990; Hakomori et al. 1998). One mechanism by which GSLs exert these functions is through membrane rafts. Rafts are fluid membrane microdomains, typically 10–50 nm in diameter (Varki et al. 2008), that comprise cholesterol, sphingomyelin, and GSLs. These domains are believed to play a key role in membrane organization and are found to be enriched in specific types of molecules such as tetraspanins and GPI-anchored proteins (Simons and Gerl 2010). Membrane rafts have been shown to play a central role in receptor recruitment, rearrangement, and clustering (Nguyen et al. 2005), and raft-associated GSLs have been well established to act as cell surface signaling molecules that trigger membrane rearrangements (Nguyen et al. 2005; Hakomori and Handa 2002). This is highly relevant to HIV interactions with the target cell and is in part demonstrated by the role of GSLs in the recruitment and clustering of HIV receptors CD4 and CCR5 (Hug et al. 2000). While this review focuses on HIV, it is worth noting that several different viruses have been documented to exploit GSLs to also gain entry into the host cell. A range of viruses including members from orthomyxovirus (Suzuki et al. 1985), rhinovirus (Grassme et al. 2005), paramyxovirus (Cooling et al. 1995; Epand et al. 1995), and polyomavirus (Tsai et al. 2003; Haslam et al. 2008) virus families have all been described to use GSLs as attachment and entry factors.

5.3.1 HIV Buds from Lipid Rafts and Incorporates GM3

In addition to roles in virus entry, membrane rafts play a crucial role in HIV-1 assembly and release (Nguyen and Hildreth 2000; Ono and Freed 2001, 2005). Assembly of HIV-1 particles within lipid rafts involves multiple steps, all mediated by the viral Gag protein, which is sufficient for the assembly and release of virus particles (Adamson and Freed 2007; Ono and Freed 2001, 2005). Stable HIV-1 Gag lipid-raft membrane association is accomplished by a bipartite motif that includes a fatty acid myristate, added co-translationally to the N-terminus of Gag, and the first 31 amino acids of the HIV-1 matrix (MA) protein that form a highly basic patch on the surface of the protein (Spearman et al. 1994; Zhou et al. 1994; Lindwasser and Resh 2002; Ono et al. 2004; Ono and Freed 1999; Bryant and Ratner 1990), and allows for specific contacts with the inner leaflet of phosphatidylinositol (PI) 4,5-bisphosphate [PI(4,5)P_2]-enriched plasma membrane (Ono et al. 2004; Saad et al. 2006).

The general lipid composition of the HIV-1 membrane has been quantitatively analyzed by mass spectrometry and shown to comprise 45.1% (molar percentage) cholesterol, 8.8% phosphatidyl choline (PC), 4.4% Phosphatidylethanolamine (PE), 14.8% plasmalogen-PE (PI-PE), and 8.4% phosphatidylserine (PS) (Brugger et al. 2006; Chan et al. 2008). Although it is clear that HIV particles incorporate host-derived gangliosides, the precise nature and relative proportion of those gangliosides remain unclear, mainly because of lack of robust

quantitative mass spectrometry approaches to accurately determine GSL contents of virions. This may also be in part due to the inherent variability of GSL expression within the producer cell, and may further reflect true variations in viral GSL content during the course of natural infection that are acutely dependent upon immune activation status and the cellular source of progeny virions.

Multiple studies have suggested that HIV-1 particles incorporate both GM1 and GM3 gangliosides (Nguyen and Hildreth 2000; Chan et al. 2008; Wubbolts et al. 2003). However a recent report demonstrates that HIV-1 Gag assembly occurs at GM1-deficient lipid rafts, suggesting that GM1 is not incorporated into the virion (Lehmann et al. 2011). Results from our lab support this finding and demonstrate that direct staining of viral particles derived from the macrophage-like THP-1 cells is only able to detect appreciable levels of GM3, but not GM1 (Puryear et al. 2012). Interestingly, within the same cell, GM3 and GM1 seldom colocalize (Freund et al. 2010; Fujita et al. 2007). Thus matrix-dependent HIV-1 Gag targeting to unique plasma membrane microdomains might provide specificity in the types of GSLs incorporated into the budding virus particles. In further agreement for a role of viral incorporation of GM3 in HIV pathogenesis, a functional genomic screen found that knockdown of Gb3 and GM3 in the producer cell led to virions that were deficient in establishing infection (Brass et al. 2008). Interestingly, HIV-1 patients exhibit a significant overexpression of GM3 and Gb3 on their lymphocyte plasma membranes (Sorice et al. 1996) while also developing antibodies to both of these GSLs (Fantini et al. 1998). This correlative evidence lends further support to the idea that viral incorporation of GM3 is an important component of HIV-1 pathogenesis.

5.4 Mechanisms of HIV-1 Interactions with Dendritic Cells

It is well documented that the HIV-1 Envelope (Env) interacts with DCs via a number of attachment factors. The C-type lectin receptors such as DC-SIGN (expressed in the sub-epithelial rectal and lamina propria DCs) (Geijtenbeek et al. 2000; Jameson et al. 2002), mannose receptor (expressed on dermal DCs) (Turville et al. 2002), langerin (expressed on Langerhans cells in the skin and the genital epithelia (Hladik et al. 2007; Turville et al. 2002)), and DCIR (Lambert et al. 2008) are some of the best-described mechanisms of HIV capture by DCs. These receptors interact with virus particles by binding high-mannose oligosaccharides on the heavily glycosylated HIV Env (Feinberg et al. 2005; Guo et al. 2004; Lin et al. 2003). HIV Env can also bind to DCs by interacting with the charged residues of heparin sulfate proteoglycans (HSPG) (Mondor et al. 1998) or syndecans (Bobardt et al. 2003; de Witte et al. 2007a), as well as the GSL, galactosylceramide (Magerus-Chatinet et al. 2007). In addition, DCs have been shown to express HIV entry receptors, CD4 and CCR5 (Lee et al. 1999; Turville et al. 2002). Hence, the fate of the virus particle within DCs is dependent on the type of receptor(s) that HIV engages.

Productive or *cis*-infection of DCs, though feasible (Burleigh et al. 2006; Turville et al. 2004; Cameron et al. 2007), is ineffective for a myriad of reasons, including low CD4/CCR5 levels on the DC surface (Lee et al. 1999), as well as the presence of potent interferon-dependent (Granelli-Piperno et al. 1997; Neil et al. 2008, 2007; Pion et al. 2006, 2007; Van Damme et al. 2008) and interferon-independent antiviral restriction mechanisms (Granelli-Piperno et al. 1995, 1997). In vivo estimates of HIV-1-infected DCs is 10–100 times lower than that of HIV-1-infected $CD4^+$ T cells (Hosmalin et al. 1995; McIlroy et al. 1995), while in vitro, only 1–3% of peripheral blood mDCs can be productively infected (Smed-Sorensen et al. 2005). Recent studies have defined the molecular details of one such restriction mechanism that blocks HIV-1 infection of DCs at an early step in the viral life cycle (Hrecka et al. 2011; Laguette et al. 2011). SAMHD1, a protein that contains a sterile α-motif and an HD domain, is a deoxynucleotide triphosphohydrolase enzyme expressed in

monocytes, DCs, and macrophages that suppresses cellular dNTP levels to inhibit virus life cycle at the reverse transcription step (Goldstone et al. 2011; Lahouassa et al. 2012; Powell et al. 2011). These recent findings provide a mechanistic basis for the previous observations in the literature on the relative paucity of productively infected peripheral blood monocyte-derived and myeloid DCs (Cameron et al. 2007; Smed-Sorensen et al. 2005; Lore et al. 2005).

Interestingly, many primate lentiviruses, including SIV_{mac} and HIV-2, but not HIV-1 or SIV_{cpz}, express the accessory protein, Vpx, to target SAMHD1 for degradation and facilitate productive infection of DCs (Goujon et al. 2006, 2007; Lim et al. 2012). Forced expression of SIV_{mac} vpx gene in HIV-1-exposed DCs can rescue HIV-1 infection (Goujon et al. 2006, 2007), but the ensuing proviral transcription and translation result in the triggering of cryptic cytoplasmic sensors that detect newly synthesized HIV-1 proteins (Manel et al. 2010). Lack of SAMHD1-specific antagonizing function in HIV-1 has led to the provocative hypothesis that HIV-1 actively avoids the productive infection pathway in DCs to avoid activation of viral sensors and induction of antiviral signaling cascades (Manel et al. 2010).

In contrast to *cis*-infection, DCs have long been proposed to capture and internalize HIV-1 particles without initiating fusion. Interactions of HIV-1 with CLRs such as DC-SIGN have been implicated in targeting captured HIV-1 particles in immature DCs to intracellular endosomal compartments and subsequently to sites of DC–T cell infectious synapses (Arrighi et al. 2004a, b; Garcia et al. 2005). Such an HIV trafficking mechanism facilitated by DC-SIGN has been hypothesized to provide an escape mechanism for virus by targeting intra-cytoplasmic compartments that allow for viral persistence and evasion from lysosomal degradation pathways (Engering et al. 2002; Kwon et al. 2002). However, subsequent reports have questioned this hypothesis, and demonstrated DC-SIGN-dependent targeting of HIV to degradative pathways (Smith et al. 2007) and subsequent presentation of viral antigens via Class I MHC molecules (Moris et al. 2004, 2006). In contrast to DC-SIGN, the CLR, langerin, expressed on skin and vaginal epithelial $CD1a^+$ Langerhans cells (LCs), has been proposed to act as a “barrier” to HIV-1 infection. HIV-1 particles captured by $langerin^+$ LCs in vitro were endocytosed within Birbeck granules and targeted for degradation (de Witte et al. 2007b). Furthermore, endocytosis of model antigens by CLRs, such as the macrophage mannose receptor and Dectin-1, has been actively targeted to synergize with TLR-agonists for effective vaccine strategies (Bonifaz et al. 2002; Boscardin et al. 2006; Bozzacco et al. 2007). These studies hence question the prevailing hypothesis that CLRs are targeted by HIV to evade innate host defenses, and suggest that HIV-1 particles utilize alternative pathways to access compartments within DCs for persistence and evasion from host immune defenses.

In contrast to gp120–CLR-dependent interactions of HIV with DCs, our data argues for an existence of CLR-independent, GSL-dependent interactions of virus particles with DCs (Izquierdo-Useros et al. 2009; Hatch et al. 2009; Gummuluru et al. 2003). Interestingly, primary blood mDCs do not express DC-SIGN or other CLRs, and can capture and transfer HIV-1 particle to $CD4^+$ T cells (Izquierdo-Useros et al. 2007; Turville et al. 2001). Furthermore, HIV capture by DCs is dramatically enhanced upon maturation (Izquierdo-Useros et al. 2009). Interestingly, mature DCs downregulate surface DC-SIGN and CLR expression and enhancement of HIV-1 capture is largely independent of Env (Gummuluru et al. 2003; Hatch et al. 2009; Izquierdo-Useros et al. 2007, 2009; Puryear et al. 2012). HIV particles entirely lacking Env are still bound by mature DCs at levels similar to those of fully infectious Env-containing particles. This has been demonstrated with both Env-deficient infectious particles and Gag-EGFP-containing virus-like particles (Hatch et al. 2009; Izquierdo-Useros et al. 2009). Likewise, pretreatment of mature DCs with agents that block Env-dependent capture, such as mannan (to block CLRs), anti-CD4-neutralizing

antibodies (to block gp120–CD4 binding), or oligomeric soluble Env trimers (sgp140 to block all Env-mediated binding), also had minimal impact on viral capture (Gummuluru et al. 2003; Hatch et al. 2009). It is not surprising that HIV-1 gp120-independent attachment mechanisms can exist, considering that there are only 7–14 trimeric gp120 spikes irregularly clustered on the surface of an HIV-1 particle (Zhu et al. 2006), thus leaving open the possibility that host cell-derived determinants incorporated into the virus lipid bilayer can impact virus capture.

5.5 HIV Incorporation of the Host-Derived Ganglioside GM3 Allows the Virion to Bind DCs Independent of the HIV Glycoprotein

Alterations in HIV incorporation of GSLs do however lead to a dramatic decrease in virus capture by mature DCs (Hatch et al. 2009; Izquierdo-Useros et al. 2009). Virions derived from producer cells, rendered deficient in GSL levels, either by targeting GSL biosynthesis pathways by small molecule inhibitors or RNAi targeting GSL biosynthetic enzymes, show a dramatic decline in capture by mature DCs (Hatch et al. 2009; Izquierdo-Useros et al. 2009; Puryear et al. 2012). More specifically, capture of HIV-1 particles with or without Env by mature DCs is competitively inhibited by anti-GM3 antibodies and/or by GM3-containing liposomes while GM3 enrichment of HIV particles leads to enhanced DC capture (Puryear et al. 2012). GM3 enrichment can be artificially achieved by the addition of exogenous lipid to virus-producing cells; however changes in the producer cell can also result in concomitant changes in virion levels of GM3 incorporation. Upon stimulation with a synthetic TLR2/1 ligand Pam3CSK4 or Phorbol 12-myristate 13-acetate (PMA), moncytoid THP-1 cells can be induced to express high levels of surface GM3. HIV particles derived from unstimulated (monoycte-like) or stimulated (macrophage-like) THP-1 cells incorporate low or high levels of GM3, respectively. As compared to untreated THP-1 cells, virus produced from stimulated THP-1 cells displays an enhanced DC capture phenotype. This suggests that viruses derived from activated monocytes or T cells in vivo would also result in enhanced incorporation of GM3 within progeny virions with concomitant enhancements in DC capture and *trans*-infection.

Although the in vitro model of HIV-1 capture by DCs highlights the role of ganglioside GM3, it is possible that the related ganglioside GM1 could also play a similar role in vivo. Similar levels of increased DC capture are observed when virions are exogenously enriched with GM1 (Puryear et al. 2012). However, derivation of HIV-1 particles from Pam3CSK4-stimulated THP1 cells, that display a dramatic increase in cell surface GM1 expression, resulted in no associated increase in virion incorporation of GM1, and negligible enhancement in DC capture of HIV virions (Puryear et al. 2012). GSL binding interactions typically require a high avidity-binding event to overcome their inherently low-affinity interactions. It is therefore possible that even though GM1 is capable of mediating virion capture by DCs, the concentration of GM1 incorporated into the viral particles does not surpass a minimum threshold necessary to overcome the low-affinity binding interaction. This evidence suggests that in the in vitro model systems under study, HIV particles do not incorporate high enough levels of GM1 to mediate DC capture by the ganglioside-dependent mechanisms. It therefore follows that in vivo differences in cell type-specific GSL expression levels could tip the balance as to which ganglioside is incorporated into a de novo virion at high enough concentrations to mediate DC capture.

5.6 GSLs Impact *Trans*-infection by Influencing Membrane Fluidity on the Cells and on the Viron

DCs play a central role in viral dissemination through the process of *trans*-infection. DCs capture virus and traffic the particles to a DC–T cell junction. It remains unclear if the virus is maintained in an internal compartment (Frank et al. 2002; Garcia et al. 2005; Izquierdo-Useros et al. 2009; Wiley and Gummuluru 2006) or is surface associated (Cavrois et al. 2002; Yu et al. 2008) but regardless of the precise location, virus particles are maintained in an infectious form (Geijtenbeek et al. 2000) and ultimately transferred to $CD4^+$ T cell to establish productive infection by formation of "infectious synapses" between virus-containing DCs and T cells (McDonald et al. 2003; Pope et al. 1994; Frankel et al. 1996; Cameron et al. 1992). Furthermore, mDCs, pDCs, and langerhans cells all use similar mechanisms of HIV-1 *trans*-infection to T cells (Fahrbach et al. 2007; Lore et al. 2005).

A cardinal feature of HIV-1 infections is high level of chronic immune activation, which has been shown to be a strong predictor of disease progression in vivo (reviewed in (Douek et al. 2009)). DCs derived from peripheral blood of HIV-1-infected individuals have invariably been shown to be hyperresponsive to immune activation stimuli, such as TLR7/8 ligands (Sabado et al. 2010), thus triggering secretion of high levels of proinflammatory mediators and prolonged activation of T cells, a condition that is especially suitable for sustenance of high levels of virus replication in the lymph nodes. In fact, some estimates have placed the average daily production of HIV-1 at approximately 1×10^{10} virions, with much of the virus replication occurring in the paracortical regions of the peripheral lymphoid organs that are composed predominantly of DCs and $CD4^+$ T cells (Embretson et al. 1993; Finzi and Silliciano 1998; Pantaleo et al. 1993; Coffin 1996). Intriguingly, immune activation stimuli upregulate cell surface expression of GM3 in $CD4^+$ T cells and macrophages (Blander et al. 1999; Nojiri et al. 1986; Tagami et al. 2002; Gracheva et al. 2007), and hence, enhanced incorporation of GM3 on HIV-1 particles derived from these cells (Puryear et al. 2012). Furthermore, exposure to proinflammatory mediators enhances the ability of DCs to capture and disseminate HIV-1 particles (Izquierdo-Useros et al. 2007, 2009, 2010; Wang et al. 2007; Wu and KewalRamani 2006) in a GM3-dependent manner (Puryear et al. 2012). These findings highlight the nefarious nature of HIV to use DCs as vehicles for widespread dissemination within the host.

GSLs contribute to this process in a number of ways that have previously been reviewed (Lingwood and Branch 2011; Waheed and Freed 2010), and additional mechanisms are sure to be uncovered as our understanding of intracellular viral trafficking continues to expand. The formation of the infectious synapse between DCs and T cell is likely under significant influence from the membrane composition of GSLs both on the DC and the T cell. Synapse formation requires a large degree of membrane rearrangement in order for synaptic junction molecules to assemble at the site of contact. GSLs have an established ability to trigger membrane rearrangements (Hakomori and Handa 2002; Nguyen et al. 2005) and have been shown to play an important role in the recruitment and clustering of HIV receptors CD4 and CCR5 (Hug et al. 2000). Productive infection of the partnering T cell can be influenced by GSL content, not only for the GSL contribution to receptor rearrangement, but also for GSL contributions to membrane fusion that are required for viral entry. GSL depletion has been shown to inhibit viral fusion (Hug et al. 2000; Puri et al. 1998, 2004) and Gb3 and GM3 in particular can impact HIV-1 fusion with primary T cells (Brass et al. 2008). This inhibition is likely attributed to either an impairment of lateral receptor mobility or impairment of fusion pore formation. Reconstitution of the GSL-depleted membrane with Gb3 or GM3 restores the ability of HIV to establish infection (Hug et al. 2000; Puri et al. 1998, 1999). Similarly, the enrichment of target cells with Gb3 or GM3 can enhance viral infection (Hammache et al. 1999; Hug et al. 2000; Nehete et al. 2002). Recent reports further suggest

that GSL content, particularly GM3, can impact $CD4^+$ T cell receptor signaling and activation, which could further impact the formation of the immunological synapse (Zhu et al. 2011; Nagafuku et al. 2012).

The GSL composition of the HIV-1 particle itself can also impact how efficiently the virus is transferred from DCs to $CD4^+$ T cells. Our recent work demonstrating a role for gangliosides in DC capture also found that GSL content impacts DC-mediated *trans*-infection. HIV particles produced from cells where GSL synthesis was inhibited produced virions with impaired transfer (Hatch et al. 2009). In contrast, HIV particles that were enriched for GM3 incorporation show enhanced T cell *trans*-infection (Puryear et al. 2012). Upon contact of the viral particle with the T cell, it remains unclear how many Env spikes are required for productive infection to occur (Yang et al. 2005; Magnus et al. 2009). Some studies suggest that two or more spikes may be required (Magnus et al. 2009) and the spatial requirements of binding may require the Env spikes to relocate within the viral membrane. The ability of the glycoprotein cytoplasmic tail, gp41, to move laterally within the virion is influenced by both cholesterol and sphingomyelin, and impairment of this mobility can lead to a potential decrease in the ability of the virus to fuse with the target cell (Saez-Cirion et al. 2002). Since gangliosides are known to impact membrane fluidity it is also within reason that differences in ganglioside composition of the virus also have a similar effect. This is supported by the evidence from a functional genomic screen showing that virions produced from Gb3- or GM3-deficient cells go on to be impaired for establishing productive infections (Brass et al. 2008).

5.7 Conclusions and Future Directions

Mounting evidence shows that GSLs impact DC capture and *trans*-infection of HIV on multiple levels; however several key questions still remain. One particularly fascinating area of inquiry warranting further investigation is that of immune evasion strategies of HIV in DCs and the ways in which GSLs may contribute to HIV's ability to hijack the DC for efficient dissemination. The ability of HIV to be captured by DCs and to remain in an infectious form that can subsequently be delivered to $CD4^+$ T cells to establish productive infection remains an enigma. Given the data discussed in this review, future efforts would be well spent to address the contribution of cellular GSLs in trafficking the virus through the DC to a non-lysosomal compartment of the cell.

The ability of HIV to interact directly with DCs via GSLs is particularly intriguing in this regard. It is possible that by binding to a GSL-recognizing receptor, the virus triggers a signaling event that results in membrane rearrangements and provides a mechanism by which the virus can efficiently "surf" the DC membrane. Such interactions may permit the virus to access trafficking pathways within the DC that are separate from the endosomal pathways typical of gp120–CLR binding, or the predominantly dead-end productive infection pathway resulting from gp120 engagement of CD4 and coreceptor. It will be interesting to discern the relative contribution of the various DC binding mechanisms to overall pathogenesis and to better understand how each pathway contributes to the fate of the virion in vivo (Fig. 5.1). Future research should address the proportional frequency of each type of binding event and determine which pathway is preferentially utilized in natural infection and how differences in immune activation or disease state affect preferential receptor engagement. Likewise, given the role of GSLs in DC capture and viral infectivity and given the variability in cellular GSL expression based on cell type and activation status, the contribution of GSLs to overall viral fitness is an additional area of research that warrants further exploration. Viral particles that are otherwise genetically identical could show dramatic differences in viral fitness based simply on the GSL composition that is incorporated into their viral envelopes.

The contribution of virally incorporated gangliosides to DC capture and *trans*-infection is, we believe, a particularly provocative one. The identification of the DC receptor that captures HIV-1 particles in a GM3-dependent manner might provide a potentially novel target for early intervention strategies such as microbicides. To date, all such efforts have been directed against the HIV Env. This has been a particularly challenging target due to the inherent variability and rapid evolution of the viral protein. If further studies verify that a substantial proportion of the early interaction between HIV and the sentinel mucosal DCs is actually mediated by a host-derived ganglioside that is incorporated into the virus membrane, this provides a much less fickle target and one that is potentially much more difficult for the virus to mutate around. Rigorous studies would clearly need to be pursued to ensure that targeting a host ganglioside on HIV does not have detrimental effects on normal functions within the host.

Several studies have shown that exosomes and HIV-1 particles have shared physical properties, including approximate size, composition, and GM3 enrichment (Chan et al. 2008; Wubbolts et al. 2003). Interestingly, DC trafficking characteristics are also very similar between exosomes and HIV. The two types of particles compete for DC capture (Izquierdo-Useros et al. 2009), traffic to a tetraspanin-rich region of the DC to evade lysosomal degradation (Wiley and Gummuluru 2006; Gould et al. 2003), and ultimately arrive at the immunological synapse (Hladik and McElrath 2008; Izquierdo-Useros et al. 2010). This is particularly intriguing given the ways in which the HIV particle mirrors an exosome's GSL composition, and may represent a novel form of molecular mimicry that mediates viral immune evasion.

For the purpose of navigating through the DC, avoiding degradation, and retaining infectivity and competency for transfer at the DC–T cell infectious synapse, exosome mimicry is a fortuitous one for HIV, in that the virus particle presumably appears to the immune system as a "self-antigen." This propensity to hide in plain sight would provide an efficient means of escaping recognition and degradation by the DC. In addition to immune evasion, exosome mimicry provides an efficient means of DC-mediated viral dissemination. Exosomes are part of an intrinsic DC *trans*-dissemination pathway that have been postulated to help sustain an elevated immune response by providing a means to gather and store antigen (Izquierdo-Useros et al. 2009, 2010) while controlling endosomal acidification (Savina et al. 2006). The antigen-containing exosomes avoid fusion (Gould et al. 2003; Izquierdo-Useros et al. 2009) and go on to cross-present antigen to T cells (Savina et al. 2006). Exosomes also serve as a means of intercellular communication, again avoiding fusion while transiting through DCs and providing a mechanism to deliver membrane proteins, signaling proteins, mRNA, and miRNA (Record et al. 2011). Hence, the process of HIV *trans*-infection draws many parallels to the exosome process of *trans*-dissemination, in that upon arrival at the DC–T cell infectious synapse, the virus is then free to infect $CD4^+$ T cells through conventional interactions between HIV Env and CD4/coreceptor.

The burgeoning of our understanding as to how GSLs impact numerous areas of cellular biology and immune function and how these mechanisms impact viral pathogenesis is an exciting one. This is sure to be an active area of research in the coming years and will undoubtedly yield intriguing new insights for both HIV and immune function. It is our hope that such insights will also yield promising new treatment directions as these novel pathways are further defined.

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References

Adamson CS, Freed EO. Human immunodeficiency virus type 1 assembly, release, and maturation. Adv Pharmacol. 2007; 55:347–387. [PubMed: 17586320]

Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. Molecular biology of the cell. 4. Garland Science; New York: 2002.

Arrighi JF, Pion M, Garcia E, Escola JM, van Kooyk Y, Geijtenbeek TB, Piguet V. DC-SIGN-mediated infectious synapse formation enhances X4 HIV-1 transmission from dendritic cells to T cells. J Exp Med. 2004a; 200(10):1279–1288. [PubMed: 15545354]

Arrighi JF, Pion M, Wiznerowicz M, Geijtenbeek TB, Garcia E, Abraham S, Leuba F, Dutoit V, Ducrey-Rundquist O, van Kooyk Y, Trono D, Piguet V. Lentivirus-mediated RNA interference of DC-SIGN expression inhibits human immunodeficiency virus transmission from dendritic cells to T cells. J Virol. 2004b; 78(20):10848–10855. [PubMed: 15452205]

Austyn JM. Dendritic cells. Curr Opin Hematol. 1998; 5(1):3–15. [PubMed: 9515196]

Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998; 392(6673): 245–252. [PubMed: 9521319]

Blander JM, Visintin I, Janeway CA Jr, Medzhitov R. Alpha(1,3)-fucosyltransferase VII and alpha(2,3)-sialyltransferase IV are up-regulated in activated CD4 T cells and maintained after their differentiation into Th1 and migration into inflammatory sites. J Immunol. 1999; 163(7):3746–3752. [PubMed: 10490970]

Bobardt MD, Saphire AC, Hung HC, Yu X, Van der Schueren B, Zhang Z, David G, Gallay PA. Syndecan captures, protects, and transmits HIV to T lymphocytes. Immunity. 2003; 18(1):27–39. [PubMed: 12530973]

Bollinger CR, Teichgraber V, Gulbins E. Ceramide-enriched membrane domains. Biochim Biophys Acta. 2005; 1746(3):284–294. [PubMed: 16226325]

Bonifaz L, Bonnyay D, Mahnke K, Rivera M, Nussenzweig MC, Steinman RM. Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. J Exp Med. 2002; 196(12):1627–1638. [PubMed: 12486105]

Boscardin SB, Hafalla JC, Masilamani RF, Kamphorst AO, Zebroski HA, Rai U, Morrot A, Zavala F, Steinman RM, Nussenzweig RS, Nussenzweig MC. Antigen targeting to dendritic cells elicits long-lived T cell help for antibody responses. J Exp Med. 2006; 203(3):599–606. [PubMed: 16505139]

Bozzacco L, Trumpfheller C, Siegal FP, Mehandru S, Markowitz M, Carrington M, Nussenzweig MC, Piperno AG, Steinman RM. DEC-205 receptor on dendritic cells mediates presentation of HIV gag protein to CD8+ T cells in a spectrum of human MHC I haplotypes. Proc Natl Acad Sci U S A. 2007; 104(4):1289–1294. [PubMed: 17229838]

Brass AL, Dykxhoorn DM, Benita Y, Yan N, Engelman A, Xavier RJ, Lieberman J, Elledge SJ. Identification of host proteins required for HIV infection through a functional genomic screen. Science. 2008; 319(5865):921–926. [PubMed: 18187620]

Brugger B, Glass B, Haberkant P, Leibrecht I, Wieland FT, Krausslich HG. The HIV lipi-dome: a raft with an unusual composition. Proc Natl Acad Sci U S A. 2006; 103(8):2641–2646. [PubMed: 16481622]

Bryant M, Ratner L. Myristoylation-dependent replication and assembly of human immunodeficiency virus 1. Proc Natl Acad Sci U S A. 1990; 87(2):523–527. [PubMed: 2405382]

Burleigh L, Lozach PY, Schiffer C, Staropoli I, Pezo V, Porrot F, Canque B, Virelizier JL, Arenzana-Seisdedos F, Amara A. Infection of dendritic cells (DCs), not DC-SIGN-mediated internalization of human immunodeficiency virus, is required for long-term transfer of virus to T cells. J Virol. 2006; 80(6):2949–2957. [PubMed: 16501104]

Cameron PU, Freudenthal PS, Barker JM, Gezelter S, Inaba K, Steinman RM. Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells. Science. 1992; 257(5068):383–387. [PubMed: 1352913]

Cameron PU, Handley AJ, Baylis DC, Solomon AE, Bernard N, Purcell DF, Lewin SR. Preferential infection of dendritic cells during human immunodeficiency virus type 1 infection of blood leukocytes. J Virol. 2007; 81(5):2297–2306. [PubMed: 17166903]

Cavrois M, De Noronha C, Greene WC. A sensitive and specific enzyme-based assay detecting HIV-1 virion fusion in primary T lymphocytes. Nat Biotechnol. 2002; 20(11):1151–1154. [PubMed: 12355096]

Chan R, Uchil PD, Jin J, Shui G, Ott DE, Mothes W, Wenk MR. Retroviruses HIV and MLV are enriched in phosphoinositides. J Virol. 2008; 82(22):11228–11238. [PubMed: 18799574]

Chen Y, Qin J, Chen ZW. Fluorescence-topographic NSOM directly visualizes peak-valley polarities of GM1/GM3 rafts in cell membrane fluctuations. J Lipid Res. 2008; 49(10):2268–2275. [PubMed: 18603643]

Coffin JM. HIV viral dynamics. AIDS. 1996; 10(Suppl 3):S75–S84. [PubMed: 8970715]

Cooling LL, Koerner TA, Naides SJ. Multiple glycosphingolipids determine the tissue tropism of parvovirus B19. J Infect Dis. 1995; 172(5):1198–1205. [PubMed: 7594654]

D'Angelo G, Polishchuk E, Di Tullio G, Santoro M, Di Campli A, Godi A, West G, Bielawski J, Chuang CC, van der Spoel AC, Platt FM, Hannun YA, Polishchuk R, Mattjus P, De Matteis MA. Glycosphingolipid synthesis requires FAPP2 transfer of glucosylceramide. Nature. 2007; 449(7158):62–67. [PubMed: 17687330]

D'Angelo G, Rega LR, De Matteis MA. Connecting vesicular transport with lipid synthesis: FAPP2. Biochim Biophys Acta. 2012; 1821(8):1089–1095. [PubMed: 22266015]

de Jong MA, de Witte L, Oudhoff MJ, Gringhuis SI, Gallay P, Geijtenbeek TB. TNF-alpha and TLR agonists increase susceptibility to HIV-1 transmission by human Langerhans cells ex vivo. J Clin Invest. 2008; 118(10):3440–3452. [PubMed: 18776939]

de Witte L, Bobardt M, Chatterji U, Degeest G, David G, Geijtenbeek TB, Gallay P. Syndecan-3 is a dendritic cell-specific attachment receptor for HIV-1. Proc Natl Acad Sci U S A. 2007a; 104(49): 19464–19469. [PubMed: 18040049]

de Witte L, Nabatov A, Pion M, Fluitsma D, de Jong MA, de Gruijl T, Piguet V, van Kooyk Y, Geijtenbeek TB. Langerin is a natural barrier to HIV-1 transmission by Langerhans cells. Nat Med. 2007b; 13(3):367–371. [PubMed: 17334373]

Doms RW, Trono D. The plasma membrane as a combat zone in the HIV battlefield. Genes Dev. 2000; 14(21):2677–2688. [PubMed: 11069884]

Dong C, Janas AM, Wang JH, Olson WJ, Wu L. Characterization of human immunodeficiency virus type 1 replication in immature and mature dendritic cells reveals dissociable cis- and trans-infection. J Virol. 2007; 81(20):11352–11362. [PubMed: 17686876]

Douek DC, Roederer M, Koup RA. Emerging concepts in the immunopathogenesis of AIDS. Annu Rev Med. 2009; 60:471–484. [PubMed: 18947296]

Embretson J, Zupancic M, Ribas JL, Burke A, Racz P, Tenner-Racz K, Haase AT. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS [see comments]. Nature. 1993; 362(6418):359–362. [PubMed: 8096068]

Engering A, Geijtenbeek TB, van Kooyk Y. Immune escape through C-type lectins on dendritic cells. Trends Immunol. 2002; 23(10):480–485. [PubMed: 12297419]

Epand RM, Nir S, Parolin M, Flanagan TD. The role of the ganglioside GD1a as a receptor for Sendai virus. Biochemistry. 1995; 34(3):1084–1089. [PubMed: 7827024]

Fahrbach KM, Barry SM, Ayehunie S, Lamore S, Klausner M, Hope TJ. Activated CD34-derived Langerhans cells mediate transinfection with human immunodeficiency virus. J Virol. 2007; 81(13):6858–6868. [PubMed: 17442711]

Fantini J, Tamalet C, Hammache D, Tourrès C, Duclos N, Yahi N. HIV-1-induced perturbations of glycosphingolipid metabolism are cell-specific and can be detected at early stages of HIV-1 infection. J Acquir Immune Defic Syndr Hum Retrovirol. 1998; 19(3):221–229. [PubMed: 9803963]

Fantini J, Hammache D, Pieroni G, Yahi N. Role of glycosphingolipid microdomains in CD4-dependent HIV-1 fusion. Glycoconj J. 2000; 17(3–4):199–204. [PubMed: 11201791]

Feinberg H, Guo Y, Mitchell DA, Drickamer K, Weis WI. Extended neck regions stabilize tetramers of the receptors DC-SIGN and DC-SIGNR. J Biol Chem. 2005; 280(2):1327–1335. [PubMed: 15509576]

Figdor CG, van Kooyk Y, Adema GJ. C-type lectin receptors on dendritic cells and Langerhans cells. Nat Rev Immunol. 2002; 2(2):77–84. [PubMed: 11910898]

Finzi D, Silliciano RF. Viral dynamics in HIV-1 infection. Cell. 1998; 93(5):665–671. [PubMed: 9630210]

Frank I, Piatak M Jr, Stoessel H, Romani N, Bonnyay D, Lifson JD, Pope M. Infectious and whole inactivated simian immunodeficiency viruses interact similarly with primate dendritic cells (DCs): differential intracellular fate of virions in mature and immature DCs. J Virol. 2002; 76(6):2936–2951. [PubMed: 11861860]

Frankel SS, Wenig BM, Burke AP, Mannan P, Thompson LD, Abbondanzo SL, Nelson AM, Pope M, Steinman RM. Replication of HIV-1 in dendritic cell-derived syncytia at the mucosal surface of the adenoid. Science. 1996; 272(5258):115–117. [PubMed: 8600520]

Freund D, Fonseca AV, Janich P, Bornhauser M, Corbeil D. Differential expression of bio-functional GM1 and GM3 gangliosides within the plastic-adherent multipotent mesenchymal stromal cell population. Cytotherapy. 2010; 12(2):131–142. [PubMed: 20196693]

Fujita A, Cheng J, Hirakawa M, Furukawa K, Kusunoki S, Fujimoto T. Gangliosides GM1 and GM3 in the living cell membrane form clusters susceptible to cholesterol depletion and chilling. Mol Biol Cell. 2007; 18(6):2112–2122. [PubMed: 17392511]

Garcia E, Pion M, Pelchen-Matthews A, Collinson L, Arrighi JF, Blot G, Leuba F, Escola JM, Demaurex N, Marsh M, Piguet V. HIV-1 trafficking to the dendritic cell-T-cell infectious synapse uses a pathway of tetraspanin sorting to the immunological synapse. Traffic. 2005; 6(6):488–501. [PubMed: 15882445]

Geijtenbeek TB, Torensma R, van Vliet SJ, van Duijnhoven GC, Adema GJ, van Kooyk Y, Figdor CG. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell. 2000; 100(5):575–585. [PubMed: 10721994]

Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science. 2010; 327(5966):656–661. [PubMed: 20133564]

Goldstone DC, Ennis-Adeniran V, Hedden JJ, Groom HC, Rice GI, Christodoulou E, Walker PA, Kelly G, Haire LF, Yap MW, de Carvalho LP, Stoye JP, Crow YJ, Taylor IA, Webb M. HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. Nature. 2011; 480(7377):379–382. [PubMed: 22056990]

Gomez-Mouton C, Abad JL, Mira E, Lacalle RA, Gallardo E, Jimenez-Baranda S, Illa I, Bernad A, Manes S, Martinez AC. Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization. Proc Natl Acad Sci U S A. 2001; 98(17):9642–9647. [PubMed: 11493690]

Goujon C, Jarrosson-Wuilleme L, Bernaud J, Rigal D, Darlix JL, Cimarelli A. With a little help from a friend: increasing HIV transduction of monocyte-derived dendritic cells with virion-like particles of SIV(MAC). Gene Ther. 2006; 13(12):991–994. [PubMed: 16525481]

Goujon C, Riviere L, Jarrosson-Wuilleme L, Bernaud J, Rigal D, Darlix JL, Cimarelli A. SIVSM/HIV-2 Vpx proteins promote retroviral escape from a proteasome-dependent restriction pathway present in human dendritic cells. Retrovirology. 2007; 4:2. [PubMed: 17212817]

Gould SJ, Booth AM, Hildreth JE. The Trojan exosome hypothesis. Proc Natl Acad Sci U S A. 2003; 100(19):10592–10597. [PubMed: 12947040]

Gracheva EV, Samovilova NN, Golovanova NK, Andreeva ER, Andrianova IV, Tararak EM, Prokazova NV. Activation of ganglioside GM3 biosynthesis in human monocyte/macrophages during culturing in vitro. Biochemistry (Mosc). 2007; 72(7):772–777. [PubMed: 17680770]

Granelli-Piperno A, Pope M, Inaba K, Steinman RM. Coexpression of NF-kappa B/Rel and Sp1 transcription factors in human immunodeficiency virus 1-induced, dendritic cell-T-cell syncytia. Proc Natl Acad Sci U S A. 1995; 92(24):10944–10948. [PubMed: 7479915]

Granelli-Piperno A, Chen D, Moser B, Steinman RM. The HIV-1 life cycle is blocked at two different points in mature dendritic cells. Adv Exp Med Biol. 1997; 417:415–419. [PubMed: 9286396]

Grassme H, Riehle A, Wilker B, Gulbins E. Rhinoviruses infect human epithelial cells via ceramide-enriched membrane platforms. J Biol Chem. 2005; 280(28):26256–26262. [PubMed: 15888438]

Gummuluru S, Rogel M, Stamatatos L, Emerman M. Binding of human immunodeficiency virus type 1 to immature dendritic cells can occur independently of DC-SIGN and mannose binding C-type lectin receptors via a cholesterol-dependent pathway. J Virol. 2003; 77(23):12865–12874. [PubMed: 14610207]

Guo Y, Feinberg H, Conroy E, Mitchell DA, Alvarez R, Blixt O, Taylor ME, Weis WI, Drickamer K. Structural basis for distinct ligand-binding and targeting properties of the receptors DC-SIGN and DC-SIGNR. Nat Struct Mol Biol. 2004; 11(7):591–598. [PubMed: 15195147]

Hakomori S. Bifunctional role of glycosphingolipids. Modulators for transmembrane signaling and mediators for cellular interactions. J Biol Chem. 1990; 265(31):18713–18716. [PubMed: 2229037]

Hakomori S, Handa K. Glycosphingolipid-dependent cross-talk between glycosynapses interfacing tumor cells with their host cells: essential basis to define tumor malignancy. FEBS Lett. 2002; 531(1):88–92. [PubMed: 12401209]

Hakomori S, Yamamura S, Handa AK. Signal transduction through glyco(sphingo)lipids. Introduction and recent studies on glyco(sphingo)lipid-enriched microdomains. Ann N Y Acad Sci. 1998; 845:1–10. [PubMed: 9668338]

Hammache D, Yahi N, Maresca M, Pieroni G, Fantini J. Human erythrocyte glycosphingo-lipids as alternative cofactors for human immunodeficiency virus type 1 (HIV-1) entry: evidence for CD4-induced interactions between HIV-1 gp120 and reconstituted membrane microdomains of glycosphingolipids (Gb3 and GM3). J Virol. 1999; 73(6):5244–5248. [PubMed: 10233996]

Haslam SM, Julien S, Burchell JM, Monk CR, Ceroni A, Garden OA, Dell A. Characterizing the glycome of the mammalian immune system. Immunol Cell Biol. 2008; 86(7):564–573. [PubMed: 18725885]

Hatch SC, Archer J, Gummuluru S. Glycosphingolipid composition of human immunodeficiency virus type 1 (HIV-1) particles is a crucial determinant for dendritic cell-mediated HIV-1 trans-infection. J Virol. 2009; 83(8):3496–3506. [PubMed: 19193785]

Hladik F, McElrath MJ. Setting the stage: host invasion by HIV. Nat Rev Immunol. 2008; 8(6):447–457. [PubMed: 18469831]

Hladik F, Sakchalathorn P, Ballweber L, Lentz G, Fialkow M, Eschenbach D, McElrath MJ. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. Immunity. 2007; 26(2):257–270. [PubMed: 17306567]

Hosmalin A, McIlroy D, Cheynier R, Clauvel JP, Oksenhendler E, Wain-Hobson S, Debre P, Autran B. Splenic interdigitating dendritic cells in humans: characterization and HIV infection frequency in vivo. Adv Exp Med Biol. 1995; 378:439–441. [PubMed: 8526113]

Hrecka K, Hao C, Gierszewska M, Swanson SK, Kesik-Brodacka M, Srivastava S, Florens L, Washburn MP, Skowronski J. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature. 2011; 474(7353):658–661. [PubMed: 21720370]

Hug P, Lin HM, Korte T, Xiao X, Dimitrov DS, Wang JM, Puri A, Blumenthal R. Glycosphingolipids promote entry of a broad range of human immunodeficiency virus type 1 isolates into cell lines expressing CD4, CXCR4, and/or CCR5. J Virol. 2000; 74(14):6377–6385. [PubMed: 10864648]

Izquierdo-Useros N, Blanco J, Erkizia I, Fernandez-Figueras MT, Borras FE, Naranjo-Gomez M, Bofill M, Ruiz L, Clotet B, Martinez-Picado J. Maturation of blood-derived dendritic cells enhances human immunodeficiency virus type 1 capture and transmission. J Virol. 2007; 81(14): 7559–7570. [PubMed: 17475656]

Izquierdo-Useros N, Naranjo-Gomez M, Archer J, Hatch SC, Erkizia I, Blanco J, Borras FE, Puertas MC, Connor JH, Fernandez-Figueras MT, Moore L, Clotet B, Gummuluru S, Martinez-Picado J. Capture and transfer of HIV-1 particles by mature dendritic cells converges with the exosome-dissemination pathway. Blood. 2009; 113(12):2732–2741. [PubMed: 18945959]

Izquierdo-Useros N, Naranjo-Gomez M, Erkizia I, Puertas MC, Borras FE, Blanco J, Martinez-Picado J. HIV and mature dendritic cells: Trojan exosomes riding the Trojan horse? PLoS Pathog. 2010; 6(3):e1000740. [PubMed: 20360840]

Jameson B, Baribaud F, Pohlmann S, Ghavimi D, Mortari F, Doms RW, Iwasaki A. Expression of DC-SIGN by dendritic cells of intestinal and genital mucosae in humans and rhesus macaques. J Virol. 2002; 76(4):1866–1875. [PubMed: 11799181]

Janich P, Corbeil D. GM1 and GM3 gangliosides highlight distinct lipid microdomains within the apical domain of epithelial cells. FEBS Lett. 2007; 581(9):1783–1787. [PubMed: 17428477]

Khurana S, Krementsov DN, de Parseval A, Elder JH, Foti M, Thali M. Human immunodeficiency virus type 1 and influenza virus exit via different membrane microdomains. J Virol. 2007; 81(22): 12630–12640. [PubMed: 17855546]

Kolter T, Proia RL, Sandhoff K. Combinatorial ganglioside biosynthesis. J Biol Chem. 2002; 277(29): 25859–25862. [PubMed: 12011101]

Kwon DS, Gregorio G, Bitton N, Hendrickson WA, Littman DR. DC-SIGN-mediated internalization of HIV is required for trans-enhancement of T cell infection. Immunity. 2002; 16(1):135–144. [PubMed: 11825572]

Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, Segeral E, Yatim A, Emiliani S, Schwartz O, Benkirane M. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature. 2011; 474(7353):654–657. [PubMed: 21613998]

Lahouassa H, Daddacha W, Hofmann H, Ayinde D, Logue EC, Dragin L, Bloch N, Maudet C, Bertrand M, Gramberg T, Pancino G, Priet S, Canard B, Laguette N, Benkirane M, Transy C, Landau NR, Kim B, Margottin-Goguet F. SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. Nat Immunol. 2012; 13(3):223–228. [PubMed: 22327569]

Lambert AA, Gilbert C, Richard M, Beaulieu AD, Tremblay MJ. The C-type lectin surface receptor DCIR acts as a new attachment factor for HIV-1 in dendritic cells and contributes to trans- and cis-infection pathways. Blood. 2008; 112(4):1299–1307. [PubMed: 18541725]

Lee B, Sharron M, Montaner LJ, Weissman D, Doms RW. Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages. Proc Natl Acad Sci U S A. 1999; 96(9):5215–5220. [PubMed: 10220446]

Lehmann M, Rocha S, Mangeat B, Blanchet F, Uji IH, Hofkens J, Piguet V. Quantitative multicolor super-resolution microscopy reveals tetherin HIV-1 interaction. PLoS Pathog. 2011; 7(12):e1002456. [PubMed: 22194693]

Leung K, Kim JO, Ganesh L, Kabat J, Schwartz O, Nabel GJ. HIV-1 assembly: viral glycoproteins segregate quantally to lipid rafts that associate individually with HIV-1 capsids and virions. Cell Host Microbe. 2008; 3(5):285–292. [PubMed: 18474355]

Li Y, Thapa P, Hawke D, Kondo Y, Furukawa K, Hsu FF, Adlercreutz D, Weadge J, Palcic MM, Wang PG, Levery SB, Zhou D. Immunologic glycosphingolipidomics and NKT cell development in mouse thymus. J Proteome Res. 2009; 8(6):2740–2751. [PubMed: 19284783]

Lim ES, Fregoso OI, McCoy CO, Matsen FA, Malik HS, Emerman M. The ability of primate lentiviruses to degrade the monocyte restriction factor SAMHD1 preceded the birth of the viral accessory protein Vpx. Cell Host Microbe. 2012; 11(2):194–204. [PubMed: 22284954]

Lin G, Simmons G, Pohlmann S, Baribaud F, Ni H, Leslie GJ, Haggarty BS, Bates P, Weissman D, Hoxie JA, Doms RW. Differential N-linked glycosylation of human immunodeficiency virus and Ebola virus envelope glycoproteins modulates interactions with DC-SIGN and DC-SIGNR. J Virol. 2003; 77(2):1337–1346. [PubMed: 12502850]

Lindwasser OW, Resh MD. Myristoylation as a target for inhibiting HIV assembly: unsaturated fatty acids block viral budding. Proc Natl Acad Sci U S A. 2002; 99(20):13037–13042. [PubMed: 12244217]

Lingwood CA, Branch DR. The role of glycosphingolipids in HIV/AIDS. Discov Med. 2011; 11(59): 303–313. [PubMed: 21524384]

Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol. 2005; 23:275–306. [PubMed: 15771572]

Lore K, Smed-Sorensen A, Vasudevan J, Mascola JR, Koup RA. Myeloid and plasmacytoid dendritic cells transfer HIV-1 preferentially to antigen-specific CD4+ T cells. J Exp Med. 2005; 201(12): 2023–2033. [PubMed: 15967828]

Lund N, Branch DR, Mylvaganam M, Chark D, Ma XZ, Sakac D, Binnington B, Fantini J, Puri A, Blumenthal R, Lingwood CA. A novel soluble mimic of the glycolipid, globotriaosyl ceramide inhibits HIV infection. AIDS. 2006; 20(3):333–343. [PubMed: 16439866]

Magerus-Chatinet A, Yu H, Garcia S, Ducloux E, Terris B, Bomsel M. Galactosyl ceramide expressed on dendritic cells can mediate HIV-1 transfer from monocyte derived dendritic cells to autologous T cells. Virology. 2007; 362(1):67–74. [PubMed: 17234232]

Magnus C, Rusert P, Bonhoeffer S, Trkola A, Regoes RR. Estimating the stoichiometry of human immunodeficiency virus entry. J Virol. 2009; 83(3):1523–1531. [PubMed: 19019953]

Manel N, Hogstad B, Wang Y, Levy DE, Unutmaz D, Littman DR. A cryptic sensor for HIV-1 activates antiviral innate immunity in dendritic cells. Nature. 2010; 467(7312):214–217. [PubMed: 20829794]

McDonald D, Wu L, Bohks SM, KewalRamani VN, Unutmaz D, Hope TJ. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. Science. 2003; 300(5623):1295–1297. [PubMed: 12730499]

McIlroy D, Autran B, Cheynier R, Wain-Hobson S, Clauvel JP, Oksenhendler E, Debre P, Hosmalin A. Infection frequency of dendritic cells and CD4+ T lymphocytes in spleens of human immunodeficiency virus-positive patients. J Virol. 1995; 69(8):4737–4745. [PubMed: 7609039]

Medzhitov R, Janeway CA Jr. Innate immunity: the virtues of a nonclonal system of recognition. Cell. 1997; 91(3):295–298. [PubMed: 9363937]

Miyauchi K, Kim Y, Latinovic O, Morozov V, Melikyan GB. HIV enters cells via endocytosis and dynamin-dependent fusion with endosomes. Cell. 2009; 137(3):433–444. [PubMed: 19410541]

Mondor I, Ugolini S, Sattentau QJ. Human immunodeficiency virus type 1 attachment to HeLa CD4 cells is CD4 independent and gp120 dependent and requires cell surface heparans. J Virol. 1998; 72(5):3623–3634. [PubMed: 9557643]

Moore ML, Chi MH, Goleniewska K, Durbin JE, Peebles RS Jr. Differential regulation of GM1 and asialo-GM1 expression by T cells and natural killer (NK) cells in respiratory syncytial virus infection. Viral Immunol. 2008; 21(3):327–339. [PubMed: 18788941]

Moris A, Nobile C, Buseyne F, Porrot F, Abastado JP, Schwartz O. DC-SIGN promotes exogenous MHC-I-restricted HIV-1 antigen presentation. Blood. 2004; 103(7):2648–2654. [PubMed: 14576049]

Moris A, Pajot A, Blanchet F, Guivel-Benhassine F, Salcedo M, Schwartz O. Dendritic cells and HIV-specific CD4+ T cells: HIV antigen presentation, T-cell activation, and viral transfer. Blood. 2006; 108(5):1643–1651. [PubMed: 16675708]

Nagafuku M, Okuyama K, Onimaru Y, Suzuki A, Odagiri Y, Yamashita T, Iwasaki K, Fujiwara M, Takayanagi M, Ohno I, Inokuchi J. CD4 and CD8 T cells require different membrane gangliosides for activation. Proc Natl Acad Sci U S A. 2012; 109(6):E336–E342. [PubMed: 22308377]

Nehete PN, Vela EM, Hossain MM, Sarkar AK, Yahi N, Fantini J, Sastry KJ. A post-CD4-binding step involving interaction of the V3 region of viral gp120 with host cell surface glycosphingolipids is common to entry and infection by diverse HIV-1 strains. Antiviral Res. 2002; 56(3):233–251. [PubMed: 12406507]

Neil SJ, Sandrin V, Sundquist WI, Bieniasz PD. An interferon-alpha-induced tethering mechanism inhibits HIV-1 and Ebola virus particle release but is counteracted by the HIV-1 Vpu protein. Cell Host Microbe. 2007; 2(3):193–203. [PubMed: 18005734]

Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature. 2008; 451(7177):425–430. [PubMed: 18200009]

Nguyen DH, Hildreth JE. Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts. J Virol. 2000; 74(7):3264–3272. [PubMed: 10708443]

Nguyen DH, Giri B, Collins G, Taub DD. Dynamic reorganization of chemokine receptors, cholesterol, lipid rafts, and adhesion molecules to sites of CD4 engagement. Exp Cell Res. 2005; 304(2):559–569. [PubMed: 15748900]

Nojiri H, Takaku F, Terui Y, Miura Y, Saito M. Ganglioside GM3: an acidic membrane component that increases during macrophage-like cell differentiation can induce monocytic differentiation of

human myeloid and monocytoid leukemic cell lines HL-60 and U937. Proc Natl Acad Sci U S A. 1986; 83(3):782–786. [PubMed: 3456169]

Nudelman E, Kannagi R, Hakomori S, Parsons M, Lipinski M, Wiels J, Fellous M, Tursz T. A glycolipid antigen associated with Burkitt lymphoma defined by a monoclonal antibody. Science. 1983; 220(4596):509–511. [PubMed: 6836295]

Okuda T, Tokuda N, Numata S, Ito M, Ohta M, Kawamura K, Wiels J, Urano T, Tajima O, Furukawa K, Furukawa K. Targeted disruption of Gb3/CD77 synthase gene resulted in the complete deletion of globo-series glycosphingolipids and loss of sensitivity to verotoxins. J Biol Chem. 2006; 281(15):10230–10235. [PubMed: 16476743]

Ono A, Freed EO. Binding of human immunodeficiency virus type 1 Gag to membrane: role of the matrix amino terminus. J Virol. 1999; 73(5):4136–4144. [PubMed: 10196310]

Ono A, Freed EO. Plasma membrane rafts play a critical role in HIV-1 assembly and release. Proc Natl Acad Sci U S A. 2001; 98(24):13925–13930. [PubMed: 11717449]

Ono A, Freed EO. Role of lipid rafts in virus replication. Adv Virus Res. 2005; 64:311–358. [PubMed: 16139599]

Ono A, Ablan SD, Lockett SJ, Nagashima K, Freed EO. Phosphatidylinositol (4,5) bisphosphate regulates HIV-1 Gag targeting to the plasma membrane. Proc Natl Acad Sci U S A. 2004; 101(41):14889–14894. [PubMed: 15465916]

Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, Fox CH, Orenstein JM, Kotler DP, Fauci AS. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease [see comments]. Nature. 1993; 362(6418):355–358. [PubMed: 8455722]

Pichlmair A, Reis e Sousa C. Innate recognition of viruses. Immunity. 2007; 27(3):370–383. [PubMed: 17892846]

Pion M, Granelli-Piperno A, Mangeat B, Stalder R, Correa R, Steinman RM, Piguet V. APOBEC3G/3F mediates intrinsic resistance of monocyte-derived dendritic cells to HIV-1 infection. J Exp Med. 2006; 203(13):2887–2893. [PubMed: 17145955]

Pion M, Stalder R, Correa R, Mangeat B, Towers GJ, Piguet V. Identification of an arsenic-sensitive block to primate lentiviral infection of human dendritic cells. J Virol. 2007; 81(21):12086–12090. [PubMed: 17728230]

Pope M, Betjes MG, Romani N, Hirmand H, Cameron PU, Hoffman L, Gezelter S, Schuler G, Steinman RM. Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. Cell. 1994; 78(3):389–398. [PubMed: 7914836]

Powell RD, Holland PJ, Hollis T, Perrino FW. Aicardi-Goutieres syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase. J Biol Chem. 2011; 286(51):43596–43600. [PubMed: 22069334]

Puri A, Hug P, Jernigan K, Barchi J, Kim HY, Hamilton J, Wiels J, Murray GJ, Brady RO, Blumenthal R. The neutral glycosphingolipid globotriaosylceramide promotes fusion mediated by a CD4-dependent CXCR4-utilizing HIV type 1 envelope glycoprotein. Proc Natl Acad Sci U S A. 1998; 95(24):14435–14440. [PubMed: 9826718]

Puri A, Hug P, Jernigan K, Rose P, Blumenthal R. Role of glycosphingolipids in HIV-1 entry: requirement of globotriosylceramide (Gb3) in CD4/CXCR4-dependent fusion. Biosci Rep. 1999; 19(4):317–325. [PubMed: 10589997]

Puri A, Rawat SS, Lin HM, Finnegan CM, Mikovits J, Ruscetti FW, Blumenthal R. An inhibitor of glycosphingolipid metabolism blocks HIV-1 infection of primary T-cells. AIDS. 2004; 18(6):849–858. [PubMed: 15060432]

Puryear WB, Yu X, Ramirez NP, Reinhard BM, Gummuluru S. HIV-1 incorporation of host-cell-derived glycosphingolipid GM3 allows for capture by mature dendritic cells. Proc Natl Acad Sci U S A. 2012; 109(19):7475–7480. [PubMed: 22529395]

Ramegowda B, Tesh VL. Differentiation-associated toxin receptor modulation, cytokine production, and sensitivity to Shiga-like toxins in human monocytes and monocytic cell lines. Infect Immun. 1996; 64(4):1173–1180. [PubMed: 8606075]

Randolph GJ, Beaulieu S, Lebecque S, Steinman RM, Muller WA. Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. Science. 1998; 282(5388):480–483. [PubMed: 9774276]

Rawat SS, Johnson BT, Puri A. Sphingolipids: modulators of HIV-1 infection and pathogenesis. Biosci Rep. 2005; 25(5–6):329–343. [PubMed: 16307380]

Record M, Subra C, Silvente-Poirot S, Poirot M. Exosomes as intercellular signalosomes and pharmacological effectors. Biochem Pharmacol. 2011; 81(10):1171–1182. [PubMed: 21371441]

Saad JS, Miller J, Tai J, Kim A, Ghanam RH, Summers MF. Structural basis for targeting HIV-1 Gag proteins to the plasma membrane for virus assembly. Proc Natl Acad Sci U S A. 2006; 103(30): 11364–11369. [PubMed: 16840558]

Sabado RL, O'Brien M, Subedi A, Qin L, Hu N, Taylor E, Dibben O, Stacey A, Fellay J, Shianna KV, Siegal F, Shodell M, Shah K, Larsson M, Lifson J, Nadas A, Marmor M, Hutt R, Margolis D, Garmon D, Markowitz M, Valentine F, Borrow P, Bhardwaj N. Evidence of dysregulation of dendritic cells in primary HIV infection. Blood. 2010; 116(19):3839–3852. [PubMed: 20693428]

Saez-Cirion A, Nir S, Lorizate M, Agirre A, Cruz A, Perez-Gil J, Nieva JL. Sphingomyelin and cholesterol promote HIV-1 gp41 pretransmembrane sequence surface aggregation and membrane restructuring. J Biol Chem. 2002; 277(24):21776–21785. [PubMed: 11929877]

Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus inter-leukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med. 1994; 179(4):1109–1118. [PubMed: 8145033]

Savina A, Jancic C, Hugues S, Guermonprez P, Vargas P, Moura IC, Lennon-Duménil A-M, Seabra MC, Raposo G, Amigorena S. NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. Cell. 2006; 126(1):205–218. [PubMed: 16839887]

Schwarz A, Futerman AH. Determination of the localization of gangliosides using anti-ganglioside antibodies: comparison of fixation methods. J Histochem Cytochem. 1997; 45(4):611–618. [PubMed: 9111239]

Simons K, Gerl MJ. Revitalizing membrane rafts: new tools and insights. Nat Rev Mol Cell Biol. 2010; 11(10):688–699. [PubMed: 20861879]

Smed-Sorensen A, Lore K, Vasudevan J, Louder MK, Andersson J, Mascola JR, Spetz AL, Koup RA. Differential susceptibility to human immunodeficiency virus type 1 infection of myeloid and plasmacytoid dendritic cells. J Virol. 2005; 79(14):8861–8869. [PubMed: 15994779]

Smith AL, Ganesh L, Leung K, Jongstra-Bilen J, Jongstra J, Nabel GJ. Leukocyte-specific protein 1 interacts with DC-SIGN and mediates transport of HIV to the proteasome in dendritic cells. J Exp Med. 2007; 204(2):421–430. [PubMed: 17296787]

Sorice M, Garofalo T, Sansolini T, Griggi T, Circella A, Massetti A, Tai T, Pavan A. Overexpression of monosialoganglioside GM3 on lymphocyte plasma membrane in patients with HIV infection. J Acquir Immune Defic Syndr Hum Retrovirol. 1996; 12(2):112–119. [PubMed: 8680881]

Spearman P, Wang JJ, Vander Heyden N, Ratner L. Identification of human immunodeficiency virus type 1 Gag protein domains essential to membrane binding and particle assembly. J Virol. 1994; 68(5):3232–3242. [PubMed: 8151785]

Suomalainen M. Lipid rafts and assembly of enveloped viruses. Traffic. 2002; 3(10):705–709. [PubMed: 12230468]

Suzuki Y, Matsunaga M, Matsumoto M. N-Acetylneuraminyllactosylceramide, GM3-NeuAc, a new influenza A virus receptor which mediates the adsorption-fusion process of viral infection. Binding specificity of influenza virus A/Aichi/2/68 (H3N2) to membrane-associated GM3 with different molecular species of sialic acid. J Biol Chem. 1985; 260(3):1362–1365. [PubMed: 3838173]

Tagami S, Inokuchi JiJ, Kabayama K, Yoshimura H, Kitamura F, Uemura S, Ogawa C, Ishii A, Saito M, Ohtsuka Y, Sakaue S, Igarashi Y. Ganglioside GM3 participates in the pathological conditions of insulin resistance. J Biol Chem. 2002; 277(5):3085–3092. [PubMed: 11707432]

Tsai B, Gilbert JM, Stehle T, Lencer W, Benjamin TL, Rapoport TA. Gangliosides are receptors for murine polyoma virus and SV40. EMBO J. 2003; 22(17):4346–4355. [PubMed: 12941687]

Turville SG, Arthos J, MacDonald K, Lynch G, Naif H, Clark G, Hart D, Cunningham AL. HIV gp120 receptors on human dendritic cells. Blood. 2001; 98(8):2482–2488. [PubMed: 11588046]

Turville SG, Cameron PU, Handley A, Lin G, Pohlmann S, Doms RW, Cunningham AL. Diversity of receptors binding HIV on dendritic cell subsets. Nat Immunol. 2002; 3(10):975–983. [PubMed: 12352970]

Turville SG, Santos JJ, Frank I, Cameron PU, Wilkinson J, Miranda-Saksena M, Dable J, Stossel H, Romani N, Piatak M Jr, Lifson JD, Pope M, Cunningham AL. Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. Blood. 2004; 103(6):2170–2179. [PubMed: 14630806]

Uemura S, Yoshida S, Shishido F, Inokuchi J. The cytoplasmic tail of GM3 synthase defines its subcellular localization, stability, and in vivo activity. Mol Biol Cell. 2009; 20(13):3088–3100. [PubMed: 19420140]

Van Damme N, Goff D, Katsura C, Jorgenson RL, Mitchell R, Johnson MC, Stephens EB, Guatelli J. The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. Cell Host Microbe. 2008; 3(4):245–252. [PubMed: 18342597]

Varki, A.; Cummings, R.; Esko, J.; Freeze, H.; Stanley, P.; Bertozzi, C.; Hart, G.; Etzler, M. Essentials of glycobiology. 2. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 2008.

Waheed AA, Freed EO. The role of lipids in retrovirus replication. Viruses. 2010; 2(5):1146–1180. [PubMed: 20740061]

Wang JH, Janas AM, Olson WJ, Wu L. Functionally distinct transmission of human immunodeficiency virus type 1 mediated by immature and mature dendritic cells. J Virol. 2007; 81(17):8933–8943. [PubMed: 17567699]

Weissman D, Li Y, Orenstein JM, Fauci AS. Both a precursor and a mature population of dendritic cells can bind HIV. However, only the mature population that expresses CD80 can pass infection to unstimulated CD4+ T cells. J Immunol. 1995; 155(8):4111–4117. [PubMed: 7561124]

Wiley RD, Gummuluru S. Immature dendritic cell-derived exosomes can mediate HIV-1 trans infection. Proc Natl Acad Sci U S A. 2006; 103(3):738–743. [PubMed: 16407131]

Wu L, KewalRamani VN. Dendritic-cell interactions with HIV: infection and viral dissemination. Nat Rev Immunol. 2006; 6(11):859–868. [PubMed: 17063186]

Wubbolts R, Leckie RS, Veenhuizen PT, Schwarzmann G, Mobius W, Hoernschemeyer J, Slot JW, Geuze HJ, Stoorvogel W. Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. J Biol Chem. 2003; 278(13):10963–10972. [PubMed: 12519789]

Xu Y-H, Barnes S, Sun Y, Grabowski GA. Multi-system disorders of glycosphingolipid and ganglioside metabolism. J Lipid Res. 2010; 51(7):1643–1675. [PubMed: 20211931]

Yamaji T, Kumagai K, Tomishige N, Hanada K. Two sphingolipid transfer proteins, CERT and FAPP2: their roles in sphingolipid metabolism. IUBMB Life. 2008; 60(8):511–518. [PubMed: 18459163]

Yang X, Kurteva S, Ren X, Lee S, Sodroski J. Stoichiometry of envelope glycoprotein trimers in the entry of human immunodeficiency virus type 1. J Virol. 2005; 79(19):12132–12147. [PubMed: 16160141]

Yu HJ, Reuter MA, McDonald D. HIV traffics through a specialized, surface-accessible intracellular compartment during trans-infection of T cells by mature dendritic cells. PLoS Pathog. 2008; 4(8):e1000134. [PubMed: 18725936]

Zeidan YH, Hannun YA. Translational aspects of sphingolipid metabolism. Trends Mol Med. 2007; 13(8):327–336. [PubMed: 17588815]

Zhou W, Parent LJ, Wills JW, Resh MD. Identification of a membrane-binding domain within the amino-terminal region of human immunodeficiency virus type 1 Gag protein which interacts with acidic phospholipids. J Virol. 1994; 68(4):2556–2569. [PubMed: 8139035]

Zhu P, Liu J, Bess J Jr, Chertova E, Lifson JD, Grise H, Ofek GA, Taylor KA, Roux KH. Distribution and three-dimensional structure of AIDS virus envelope spikes. Nature. 2006; 441(7095):847–852. [PubMed: 16728975]

Zhu Y, Gumlaw N, Karman J, Zhao H, Zhang J, Jiang J, Maniatis P, Edling A, Chuang W-L, Siegel C, Shayman JA, Kaplan J, Jiang C, Cheng SH. Lowering glycosphingolipid levels in CD4+ T cells attenuates T cell receptor signaling, cytokine production, and differentiation to the Th17 lineage. J Biol Chem. 2011; 286(17):14787–14794. [PubMed: 21402703]

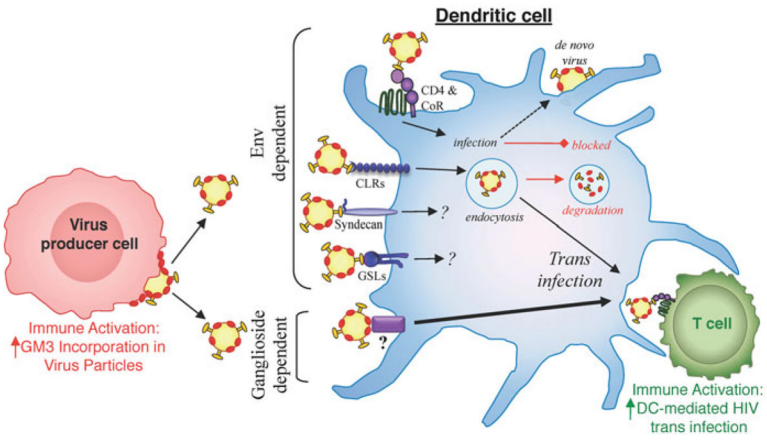

Fig. 5.1.

Model of HIV-1 interactions with dendritic cells. HIV-1 gp120-independent virus capture mechanism by DCs is dependent on the ganglioside GM3. Recognition of virus particle-associated GM3 by a yet-to-be identified DC receptor results in trafficking of virus particles to $CD81^+$ compartments, and upon initiation of DC–T cell contact, re-localization of the virus particles to the DC–T cell infectious synapse. Exposure of virus producer cells to proinflammatory mediators, such as microbial TLR ligands, results in enhanced expression of GM3 in the virus producer cell, and hence, enhanced incorporation of GM3 in the budding virus particles. Immune activation and maturation of DCs result in enhanced capture of HIV-1 particles by DCs, establishment of long-lived contacts with $CD4^+$ T cells, and enhanced *trans*-infection of $CD4^+$ T cells. We posit that recognition of HIV-1 gp120 by pathogen recognition receptors such as CLRs is an integral part of the host innate response to the invading virus, while GSL-dependent interactions of HIV with DCs are part of the virus evasion response