Role of DC-SIGN and L-SIGN receptors in HIV-1 vertical transmission

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A B S T R A C T

The innate immune system acts in the first line of host defense against pathogens. One of the mechanisms used involves the early recognition and uptake of microbes by host professional phagocytes, through pattern recognition receptors (PRRs). These PRRs bind to conserved microbial ligands expressed by pathogens and initiate both innate and adaptive immune responses. Some PRRs located on the surface of dendritic cells (DCs) and other cells seem to play an important role in human immunodeficiency virus type 1 (HIV-1) transmission. Dendritic cell–specific intercellular adhesion molecule–3 grabbing non-integrin, CD209 (DC-SIGN) and its homolog, DC-SIGN-related (DC-SIGNR or L-SIGN) receptors are PPRs able to bind the HIV-1 gp120 envelope protein and, because alterations in their expression patterns also occur, they might play a role in both horizontal and vertical transmission as well as in disseminating the virus within the host. This review aims to explore the involvement of the DC-SIGN and L-SIGN receptors in HIV-1 transmission from mother to child.

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1. Introduction

The Joint United Nations Program on HIV/AIDS estimates that 2.1 million children worldwide are infected with human immunodeficiency virus type 1 (HIV-1) [1] and that more than 430,000 children were newly infected in 2008. Mother-to-child transmission, also known as vertical transmission, accounts for more than 40% of all HIV-1 infections in children [2] and can occur in three ways [3–5]: during pregnancy through the placenta (transplacental or intrauterine transmission); during delivery (intrapartum transmission) through amniotic fluids, infected blood and cervical secretions; and during the process of breastfeeding. In addition, some independent factors have been associated with vertical transmission of HIV-1, such as high maternal viral load, low amount of CD4+ T cells, vaginal delivery and lower gestational age [4,5].

Global estimates show that, without specific medication, the rate of transmission of HIV-1 from mother to child is around 15–45% [6]; using antiretroviral therapies, this percentage has sharply dropped to 2%. However, although prophylactic antiretroviral therapy can reduce mother-to-child transmission to 2%, the limited access to timely diagnosis and drugs in many developing countries reduces the potential impact of this strategy [5]. Despite the sharp fall in the rate of viral transmission, a significant percentage of children are still being infected with HIV-1; the mechanisms used by the virus to escape the immune response and infect targets of cells of children born to infected mothers treated with antiretroviral therapies still remain to be clarified.

A better understanding of the immunologic mechanisms acting at the maternal–fetal interface and of host–pathogen interaction is essential for the development of alternative interventions aimed to prevent viral transmission. This review aims to explore the involvement of the dendritic cell–specific intercellular adhesion molecule–3 grabbing non-integrin, CD209 (DC-SIGN) and DC-SIGN-related C-type lectin domain family 4, member M (L-SIGN) receptors in HIV-1 transmission from mother to child.

2. DC-SIGN and L-SIGN receptors

The innate immune system is the first line of host defense against pathogens; it involves the early recognition and uptake of microbes by host professional phagocytes, such as dendritic cells (DCs) and macrophages, through germline-encoded receptors, known as pattern recognition receptors (PRRs) [7]. These proteins bind to conserved microbial ligands expressed by the pathogens, and initiate both innate and adaptive immune responses. PRRs are involved in phagocytosis, and antigen presentation could activate intracellular signaling and cytokine secretion. The efficiency of this initial pathogen recognition may have important consequences in the pathogenesis of infectious diseases [8].

Some PRRs located on the surface of DCs and other cells seem to play an important role in HIV-1 transmission. Of particular interest are the DC-SIGN and its close relative, L-SIGN (also known as DC-SIGN related [DC-SIGNR], CD209L or CLEC4M) receptors [9,10].
DC-SIGN and L-SIGN receptors, two C-type lectins, are long type II integral membrane proteins [11,12] that are involved in both innate and adaptive immunity [13–15]. They present strong dependence on calcium and act as cellular adhesion’s receptors and are involved in pathogen recognition [16,17]. As pathogen-recognition receptors, both these lectins recognize a wide range of microorganisms, some of which have a major impact on public health. For example, DC-SIGN captures viruses, such as Ebola virus [18], hepatitis C virus [19,20], Dengue virus [21], cytomegalovirus [22], and SARS coronavirus (SARS-CoV) [23,24], bacteria such as Mycobacterium tuberculosis [25] and Helicobacter pylori [26], and parasites such as Leishmania pifanoi [27]. L-SIGN is able to capture viruses such as Ebola virus [18], hepatitis C virus [19,20,28], and more recently SARS-CoV [23,29], as well as bacteria such as M tuberculosis [30], and Leishmania infantum [27]. Both DC-SIGN and L-SIGN can recognize and capture human immunodeficiency virus 1 by binding to the gp120 glycoprotein [31–35].

DC-SIGN and L-SIGN receptors are organized into three structurally distinct regions (Fig. 1): an intracytoplasmatic tail domain responsible for internalization and signal transduction [36] that consists of N-terminal, LL, and YKSL motifs and a triacidic group (EEA), then a transmembrane domain and finally an extracellular domain, which is further divided into two structures, the neck repeat region and the carbohydrate recognition domain (CRD) [17].

The neck repeat region usually consists of 7 full and 1 incomplete tandem repeats of a sequence of 23 highly conserved amino acids, but the number of repeats can vary in the population: the neck repeat region of the L-SIGN receptor is highly polymorphic (4–10 repetitions are often found), while the one of DC-SIGN is less polymorphic (mainly seven repetitions) [12,17,37]. The neck repeats region plays a crucial role in tetramerization and supports carbohydrates’ recognition, thus directly influencing the receptor’s binding affinity to pathogens [8,17,36].

The number of tandem-neck repeats determines the multimerization status (Fig. 2). Feinberg et al. [38] showed that the lack of two repeats (five-repeat allele) results in partial dissociation of the final tetramer, whereas the lack of even more than five repeats causes a reduction in the overall stability of the molecule. By using a series of recombinant soluble receptors with different number of repeats, Synder et al. [39] showed that the binding affinity to HIV-1 gp120 glycoprotein was affected by the length of the repeats and multimerization status, with tetrameric forms presenting a higher affinity than shorter monomeric forms. The CRD, both in DC-SIGN and L-SIGN, is flexibly connected to the neck repeat region, allowing a departure from the membrane, which enables the binding of pathogens in a calcium-dependent manner [36].

DC-SIGN and L-SIGN belong to the CD209 gene family and probably originated following a gene duplication event [17]. The human genes encoding DC-SIGN and L-SIGN map on 19p13.2-3 and extend approximately for 13 kb [35,40]. In addition, they share the same introns and exons organization (consisting in seven exons and five introns) and the encoded proteins present a high similarity at the amino acid level (77% identity) [17].

The two receptors are characterized by different expression patterns. DC-SIGN is highly expressed in monocytes and CD34+ monocyte-derived DCs and in subsets of immature and mature DCs in various tissues such as dermis, mucosa, spleen, placenta (special-
Infection by HIV-1

Infection by HIV-1 can occur through two different paths: the trans- and the cis-infection (Fig. 3A, B). Although infection in trans occurs when DC-SIGN is expressed on a separate cell from the one that becomes infected, the infection in cis may occur when DC-SIGN is co-expressed with CD4 and chemokine receptor (e.g., CCR5) on a permissive cell type, such as macrophages [16,36,40]. Co-expression of CD4, CCR5, and CXCR4 receptors with DC-SIGN receptor has been blamed for the increase in the efficiency of HIV-1 infection, suggesting the involvement of the DC-SIGN receptor in viral transmission, besides increasing the efficiency of the infection in cis [51]. Both DC-SIGN and L-SIGN receptors are involved in HIV-1 infection in trans [34]. However, the HIV-1 infection in cis, has only been observed so far for DC-SIGN receptor.

The interaction of DC-SIGN and L-SIGN molecules with the HIV-1 occurs through the connection between their CRD with the gp120 viral envelope glycoprotein [16,17]. Binding of the gp120 to DC-SIGN and L-SIGN molecules may induce a conformational change in the gp120 itself that enables a more efficient interaction with CD4 and/or the chemokine receptor and subsequent membrane fusion with T cells. Alternatively, the binding of viral particles to the DCs may increase the probability that entry will occur after binding to the CD4 and co-receptor complex on target cells [10]. The ability of DC-SIGN and L-SIGN receptors to capture and transmit the HIV-1 to T cells may largely depend on their membrane organization in rafts or their capability to multimerize [10]. In addition, alternative splicing events can occur in DC-SIGN e L-SIGN, leading to the production of a vast repertoire of membrane-bound and soluble isoforms, which may also differently affect the process of HIV-1 transmission [5,17,52].

4. DC-SIGN and L-SIGN implications in vertical transmission of HIV-1

4.1. DC-SIGN and L-SIGN in trans-placental HIV-1 transmission

The human placenta is responsible for a close juxtaposition between fetal and maternal blood; however, this apparent barrier is permeable enough to display the HIV-1 from the mother, a fact that leads to fetal exposure to virus [53]. Thus, the placenta can play an important role in the transmission of HIV-1 infection. Most cases of vertical transmission via uterus occur through of the placenta (Fig. 4), especially during the third trimester of pregnancy [43]. It is estimated that the trans-placental or intrauterine HIV-1 transfer comprise 17–38% of cases of vertical transmission, but little is known about the mechanisms involved in the transmission of the virus [54]. Some cell types are pointed out as likely targets for the viral spread, such as macrophages present in the decidua and spe-

Fig. 3. Paths of infection by HIV-1. (A) Infection in trans using DC-SIGN receptor in DCs; this mechanism is very similar to the one used by L-SIGN receptor in endothelial cells. (B) Infection in cis using DC-SIGN in specialized macrophage.
cialized macrophages present in the placenta (Fig. 4), also known as Hofbauer cells [54]. Both decidual macrophages and Hofbauer cells play important roles in the placental physiology, the firsts promoting the development and control of blood flow and the latter acting in the defense against infectious agents (Hofbauer cells) [54].

Hofbauer cells, specialized macrophages from human placenta, support infection by HIV-1 both in vitro and in vivo [55]. This ability to support HIV-1 infection is probably associated with the expression of some cellular receptors related to the infection of T lymphocytes, such as CD4, CCR5, CXCR4, and DC-SIGN, also expressed in these cells [54,55]. During pregnancy, there is an increased expression of DC-SIGN by Hofbauer cells in the chorionic villi, and this expression has been correlated with increased rates of HIV-1 vertical transmission [54]. Thus, DC-SIGN can enhance the binding of HIV-1 on the surface of Hofbauer cells, providing an efficient mechanism by which the virus can be transmitted to other receivers permissible to HIV-1 in trans. Thus, one can wonder: How is the contact between native virus-bound infected cells with the fetal cells expressing receptors for HIV-1? The main physical barrier between fetal Hofbauer cells and maternal fluids is the wall of the trophoblast cells; however trophoblast cells can express receptors for HIV-1 entry, so they can be infected by the virus [55]. Moreover, breaches in the wall of the trophoblasts can originate from spontaneous processes or in consequence of infectious diseases (such as corioamnriotite) and behavioral habits such as smoking and drug use [55,56], allowing a direct contact between fetal Hofbauer cells DC-SIGN+CD4+CCR5+CXCR4+ and viral particles adsorbed to the maternal decidual macrophages or DCs expressing DC-SIGN, present in maternal blood [54,55,57]. Therefore, the contact between maternal and fetal cells through the wall of the trophoblasts, allows the efficient spreading of HIV-1 to fetal cells expressing receptors for viral binding and entry, allowing establishment of the infection [55].

A study has suggested that the mechanism of HIV-1 association with the cells, such as HIV-1 adsorbed to the DC-SIGN receptor, operates more efficiently in pregnancies where the viremia remains low because of the administration of antiretroviral therapies [54]. Why does this happen?

Some authors explain that binding of viral particles to DC-SIGN may focus or concentrate the virus particles at the surface of the DC and may thus increase the probability that entry will occur after binding to the CD4 and co-receptor complex on target cells [9]. In addition, the HIV-1 virus can remain viable for several days on the DC-SIGN–expressing cell, and then can be more efficiently transferred to T cells than to the transfer executed by free cells [10,16]. For the L-SIGN receptor, this fact is not observed. In addition, some authors propose three different mechanisms to explain how the HIV-1 virus is transmitted from mother to child, via the placenta. The first mechanism suggests that Hofbauer cells infected with HIV-1 or with the virus adsorbed to their cell membrane through
receptors, such as DC-SIGN, may enter the fetus through the umbilical vein \[54,55\]. The second mechanism is that Hofbauer cell infected by HIV-1 or carrying the virus adsorbed on the surface, remains \textit{in situ} in the chorionic villi, promoting HIV-1 antigen presentation and subsequent T lymphocytes infection. However, this mechanism seems unlikely, since T lymphocytes are inconspicuous in chorionic villi \[54,55\]. The third mechanism argues that Hofbauer cells may become infected by HIV-1 and may release infectious viral particles, which may become adsorbed to L-SIGN on the immediately adjacent placental capillary endothelium. The endothelium may, in turn, mediate infection of HIV-1 receptor-positive T-lymphocytes circulating in the blood. Infected T lymphocytes or Hofbauer cells either productively infected with HIV-1 or simply with the virus adsorbed to their surface may then travel between the placenta and the fetus in umbilical cord blood \[16,54,55\].

4.2. DC-SIGN and L-SIGN in post-partum HIV-1 transmission

In the intra- and post-partum vertical transmission of HIV-1, the virus is delivered and transmitted because of the contact with amniotic fluid, maternal blood, and cervical secretion (intra-partum) or with breast milk (post-partum) \[3,58\].

It has been reported that, in the absence of a prophylactic antiretroviral therapy, the breast milk of infected mothers is responsible for more than 40% of cases of children infected with HIV-1 via vertical transmission \[2-4,59\]. Some factors, such as the viral load in the plasma and breast milk may be relevant for vertical transmission of HIV-1 \[60\].

Transmission of the HIV-1 virus from mother to child via breast milk can occur by free virus particles and/or viral particles associated with cells \[4\]; in this case, the expression of cellular receptors for recognition and adhesion of pathogens is required. Among the cell types involved in the transmission of HIV-1 via breastfeeding, macrophages and mammary epithelial cells should be mentioned. Breast milk is the only bodily fluid that contains a large number of macrophages, comprising more than 80% of all cells present in colostrum \[61\]. Expressing CCR5, macrophages are prime targets of the HIV-1 virus, which uses the co-receptors CCR5 for viral entry \[4\]. In addition, macrophages, derived from peripheral blood monocytes (PBMo), are present in different concentrations throughout lactation, acting as immunoprotective in situ \[61\].

Moreover, it has been reported that macrophages also express DC-SIGN receptors \[61\]. In certain situations, the expression levels of DC-SIGN on macrophages can be quite high, especially when stimulated with interleukin (IL)-4, which also promotes reduction in the expression of CCR5 and CXCR4, suggesting the need for changes in local inflammatory Th2 dominance for an acceleration of HIV-1 transmission via breastfeeding \[59 – 61\]. Local production of IL-4 during infectious processes, as in mastitis, can over-regulate the expression of DC-SIGN on macrophages, suggesting the association of mastitis with high viral load in breast milk and high risk of vertical transmission of the virus \[61\].

Along with the macrophages, mammary epithelial cells may also be infected by HIV-1, through the co-receptor CXCR5 \[4\]. Some studies suggest the possibility of a HIV-1 compartmentalization between blood and milk, suggesting that the virus could be produced in and transmitted by the milk, through the mammary epithelial cells. In this sense, the viruses that derived from mammary gland, has been shown to inhibit DC-SIGN binding to CD4+ T cells, with monocytes and macrophages also acting as cellular reservoirs for HIV-1 \[3\]. The viral entry through the mucosa of the gastrointestinal tract can be mediated by the binding of the DC-SIGN receptor, expressed on DCs, to the viral gp120 protein \[2\]. This interaction appears to be more pronounced in the tonsils at the top of the esophagus and intestinal tract \[4\].

The infectivity of viruses associated with cells and captured by DC-SIGN is stable even in presence of the acidification process occurring in the gastrointestinal tract, suggesting that the virus bound to DCs through DC-SIGN is protected from the action of the gastric juice. The fact that the free viral particles loose their infectivity when exposed to acidic environments, suggest that the transmission of the virus to free cells in milk is hampered by gastric juice \[60\]. However, it is possible that free cells become infected in the oral mucosa and esophagus, where the acidity is not high. Studies with Raji cells expressing DC-SIGN, preincubated with PBS and with the HIV-1 virus, showed an efficient viral transfer. However, Raji cells expressing DC-SIGN, incubated with HIV-1 virus and uninfected human milk, showed a significant reduction of the binding of HIV-1 gp120 to DC-SIGN receptor. This suggests that in human milk some factors that could prevent the interaction between the gp120 and DC-SIGN receptor exist \[4\].

Similar tests were conducted for L-SIGN receptor, however, breast milk did not inhibit the interaction between the viral proteins and the receptor, suggesting that L-SIGN receptor can be used by the virus to increase its infectivity \[4\]. Some studies also report that exclusive feeding with uninfected mothers’ milk during the first months of life protects against a variety of infections, including HIV-1, and help fight morbidity and mortality, suggesting that there should be certain components in the human milk that may protect against the transmission of the virus \[59\]. So, what are these likely factors in breast milk, and how do they act in protection against the infection caused by HIV-1?

Breast milk is provided with a series of antimicrobial compounds, such as lactoferrin, lysozyme, secretory leukocyte protease inhibitor, lactodifucotetrase, lacto-N-fucopentose I, II, and III, and monofucosilacto-N-hexose II, among others, which are associated with a reduced rate of HIV-1 transmission \[2,59\]. Some studies attribute this reduction in viral transmission to certain antigens, such as Lewis structures, which compete with the gp120 for a binding site in the DC-SIGN receptor, inhibiting the viral transfer to CD4+ T cells \[2\]. Inhibition of the binding between gp120 and DC-SIGN receptor is probably due to the size of the compound, which contains many Lewis structures that mask the interaction sites \[4\]. Compounds containing Lewis structures and present in breast milk were shown to interact with DC-SIGN, blocking the response of Th1 cells and resulting in an increased responsiveness of Th2 cells, suggesting that these compounds may influence the immune response by acting as immunomodulatory factors \[4\]. A constituent of human milk, bile salt-stimulated lipase, a Lewis X (Le-)–containing glycoprotein secreted by the pancreas as well as by mammary gland, has been shown to inhibit DC-SIGN binding to HIV and DC-SIGN-mediated transfer of HIV-1 to CD4+ lymphocytes, by competing with the virus for the binding to DC-SIGN \[62\]. The binding of bile salt-stimulated lipase to DC-SIGN can be prevented using an antibody against Le–, thus demonstrating the importance of the Le \[10\] epitope.

Others constituents of human milk, such as human milk oligosaccharides and MUC1 (epithelial mucin), have shown promising results and could be used to develop drugs that inhibit the HIV-1 binding to DC-SIGN \[2,59\]. Hong et al. \[2\] found a reduction of more than 60% in the interaction between gp120 and the receptor protein DC-SIGN when using human milk oligosaccharides at a concentration of 0.5 g/l, the one usually present in breast milk. Additionally,
5. Polymorphisms in DC-SIGN and L-SIGN genes and vertical transmission of HIV-1

For some genes, susceptibility and/or resistance to certain (infectious but not only) diseases has been associated with gene expression levels and with the presence of gene variations/mutations. Can this happen also for DC-SIGN and L-SIGN? Can variations in the gene encoding DC-SIGN and L-SIGN be associated with vertical transmission of HIV-1?

To date, except one study regarding the L-SIGN gene, no other genetic studies trying to associate mutations in the genes encoding for DC-SIGN and L-SIGN with the vertical transmission of the HIV-1 have been performed. Boily-Larouche et al. [5] performed an association study in a well-characterized cohort of 197 HIV-1–infected mothers and their children from Zimbabwe, and found that children with two copies of H1 and/or H3 haplotype of L-SIGN were about 3.6 times more at risk for intrauterine transmission of HIV-1 and 5.7 times at risk for intrapartum transmission. The H1 and H3 haplotypes are characterized by two single nucleotide polymorphisms in the promoter region (p-198A) and the intron 2 (int2-180A) that associate with a reduction of the transcriptional activity. The same study also showed that infants homozygous for the H1 haplotype showed a more than fourfold decrease in the level of placental L-SIGN transcripts, and in particular of the membrane linked isoforms [5].

A reduced expression of L-SIGN (especially of the membrane isoforms) in the endothelial cells of capillaries in the placenta may facilitate the binding of HIV-1 to viral entry receptors of endothelial cells, such as CCR5, which can facilitate the migration of maternal HIV-1 across the placental barrier, resulting in intrauterine transmission of HIV-1 [5]. The membrane bound L-SIGN receptors are responsible for catching the virus. After capture, the virus adhered to L-SIGN may undergo degradation processes or be presented as antigens. Thus, these receptors act to protect the infant against infection by HIV-1 [5]. Boily-Larouche et al. [5] explain these discoveries with the hypothesis that when the levels of placental L-SIGN-bound membrane are reduced, virus fails to bind to L-SIGN and binds preferentially to CCR5 receptors on endothelial cells of capillaries, resulting in loss of integrity of the placental barrier and increase the passage of cells infected by HIV-1 in fetal circulation, leading to vertical transmission.

6. Concluding remarks and future directions

In view of what has been discussed, much evidence exist that the DC-SIGN and L-SIGN receptors are involved in the transmission of HIV-1 from mother to child. Therefore, the DC-SIGN and L-SIGN receptors should be likely targets for the development of new drugs and antiretroviral therapies, to challenge the spread of viral transmission.

In addition to this, given that only a few genetic studies have been performed to investigate the possible involvement of DC-SIGN and L-SIGN receptors in the genetic mechanisms correlated with vertical transmission of the HIV-1 virus, we believe that more detailed studies aiming to elucidate the role of genetic variants from different worldwide populations in susceptibility and/or resistance to HIV-1 infection are needed.

Acknowledgments

We thank the Laboratory of Immunopathology Keizo Asami, the Department of Genetics, Federal University of Pernambuco, the Graduate Program in Genetics and Molecular Biology for supporting physical and scientific, as well as FACEPE and CNPq, for financial support. S.L. is recipient of a fellowship grant (APQ-0020-4.01/08) from FACEPE.


