The Role of Mannose Receptor on HIV-1 Entry into Human Spermatozoa

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Introduction

Since the beginning of the AIDS pandemic, sexual intercourse has been recognized as the most frequent mode of human immunodeficiency virus-1 (HIV-1) transmission. In most cases, the viral replication cycle begins after HIV-1 crosses the mucous barrier and infects its first target cells. Taking into consideration that M-tropic HIV strains are most frequently transmitted during primary infection, macrophages and dendritic cells are the first cells to become infected. It is known that entry of the virus into these cells is dependent on the expression of two proteins, CD4 and a chemokine receptor. In most cases the latter corresponds to the CCR5 molecule for M-tropic isolates, and to the CXCR4 molecule for T-tropic strains.

In this opinion article we consider the possibility that human spermatozoa have receptors for human immunodeficiency virus-1 (HIV-1). It is clear that sperm cells have the potential for transmitting HIV-1, but the mechanisms responsible for spreading or the virus by this vector are not known. In contrast to the traditional HIV-1 target cells, spermatozoa do not express CD4 receptors or the CCR5/CXCR4 co-receptors. Recent evidence indicates that astrocytes, which also do not express these molecules, can be infected with HIV-1 through the mannose receptor. Furthermore, a 160-kDa sperm receptor that interacts with the HIV gp 120 has been described. Therefore, we hypothesize that the mannose receptor, of 165–175 kDa, is the receptor that HIV-1 uses to invade spermatozoa, which could lead to both vertical and horizontal transmission of HIV-1.

Although the risk of becoming HIV-1 infected through sexual intercourse is relatively low, 0.1–1.0%, this route of transmission is the most frequent, with semen being the major source of virus. Semen is composed of spermatozoa, leukocytes and seminal plasma. It was initially considered that leukocytes were the cells responsible for HIV-1 transmission. However, it was subsequently shown that semen without leukocytes was also infectious. By using different techniques with high sensitivity such as atomic force microscopy, polymerase chain reaction (PCR) and in situ PCR, it was concluded that spermatozoa were also responsible for HIV-1 transmission. As these male germ cells do not express membrane CD4, CCR5 or CXCR4 molecules, the question can be raised as to what is the mechanism by which the infection of spermatozoa occurs?
Spermatozoa

The spermatozoa is composed of a head, a middle piece, and a tail. The head is elliptic, and contains a nucleus with highly condensed chromatin and an extensive region of secretory granules called the acrosome; this acrosome derives from the Golgi and provides the enzymes required for the spermatozoa to pass through the zona pellucida of the oocyte. The domains of the head region of the sperm surface in most mammals are the acrosomal cap and the equatorial segment overlying the acrosome and the post-acrosomal region respectively. The middle piece contains many mitochondria, which provide the energy for movement, and the tail consists of two single microtubules surrounded by nine microtubule doublets that provide the spermatozoa with motility.12

In the female reproductive tract, the spermatozoa undergoes a series of physiological and biochemical changes, called capacitation, that enables it to bind to and penetrate the extracellular matrix of the oocyte, the zona pellucida, and to release the acrosome content by the acrosomal reaction. The sperm binds them and fuses with the plasmatic membrane of the oocyte, which activates the cell cycle, and the embryo development begins. It is important to point out that the spermatozoa is transcriptionally inactive until it fuses with the oocyte and cell division occurs.12

Several proteins and glycoproteins have been proposed as possible sperm receptors for the zona pellucida such as the mannose receptor, the β-1,4 galactosyltransferase receptor, the receptor for progesterone, and the tyrosine-kinase receptor. The galactosyl-alkyl-acylglycerol (GalAAG) receptor is also expressed in spermatozoa. Although its function is still unknown, GalAAG was recently proposed as a candidate to allow HIV-1 entry into these cells.7

Mannose receptor

The mannose receptor, CD206, has a molecular size of 165–175 kDa, and is important in antigen recognition. It was originally described in macrophages, but is also expressed by other cell types such as immature dendritic cells, some epithelial cell subpopulations and spermatozoa.13–15 In sperm cells, this molecule participates in intergamete interaction and its topographical distribution is strongly correlated with the acrosome status. In acrosome-intact spermatozoa, mannose receptors are located over the entire head region13,16–19 and when the acrosomal reaction takes place, they are translocated to the equatorial region simultaneously with the release of the acrosomal

Fig. 1 Model of HIV infection of the fetus by spermatozoa. The spermatozoa and HIV virion; the spermatozoa is composed of a. head, b. middle piece, and c. tail (1); interaction between a 160kDa protein in spermatozoa (we propose that this protein corresponds to the mannose receptor) and HIV gp 120 protein; once inside the spermatozoa it is not clear where the virion is located (2); The infected spermatozoa might interact with the oocyte (3) resulting in infection of the embryo (vertical transmission from the father) (4).
content. This process allows fusion between the spermatozoa and the oocyte membranes.\textsuperscript{12,13,20} During the capacitation process, cholesterol is released due to the presence of cholesterol acceptor molecules. This efflux of cholesterol creates a thermodynamic status that favors the exit of mannose receptors from internal storage compartments to the plasma membrane.\textsuperscript{16} Therefore, the presence of these receptors in the sperm membrane is regulated by cholesterol levels.

**HIV–spermatozoa interaction**

It has been established that HIV-1 can enter spermatozoa, which in turn, facilitates the spread of the virus to cervicovaginal cells.\textsuperscript{21} However, the mechanism by which HIV-1 enters these cells is still controversial. Spermatozoa were recently reported to have a 160 kDa receptor that binds the HIV-1 glycoprotein of 120 kDa.\textsuperscript{7} The binding of HIV-1 is not inhibited by \textit{in vitro} pre-incubation of spermatozoa with anti-CD4 antibodies.\textsuperscript{7} This result suggests that binding and internalization of the virus into sperm cells is independent of the CD4 molecule. Additionally, the fraction obtained by extraction of sperm proteins using organic solvents did not show binding with gp120, excluding the possibility that the glycolipid GalAAG is the receptor that allows HIV-1 to enter these cells. Consistent with this finding are reports of alternative receptors for HIV-1 entry that allow infection of different cells in the organism, even without CD4 molecules in their membranes.\textsuperscript{22–24} Inhibition assays using mannan and anti-human mannose receptor antibody suggested that human astrocytes are infected with HIV-1 through the mannose receptor.\textsuperscript{23} Studies using the same cells demonstrated that infection through this receptor induces a signal that leads to the production of metalloproteinase-2 and dysfunction of astrocytes, which could be involved in pathogenesis of AIDS dementia.\textsuperscript{24} Furthermore, the mannose receptor was found to have an important role in HIV-1 binding and infection of macrophages.\textsuperscript{22}

The HIV-1 gp 120 envelope protein has 24 glycosylation sites, 50% of which are mannosylated.\textsuperscript{25} Several studies have shown that some molecules, such as lectins that bind mannan and concavalin A, inhibit HIV-1 infection suggesting an important role of the mannosylated ends of gp 120 during infection.\textsuperscript{22} Similarly, some soluble mannosylated molecules such as mannan and mannosyl can block HIV-1 infection of macrophages and astrocytes \textit{in vitro}.\textsuperscript{23,26}

**HIV and reproductive health**

The high incidence of HIV-1-infected individuals who are at reproductive age raises a challenge in reproductive health, as serodiscordant couples continue to procreate. When the man is the infected partner, isolation of the sperm motile fraction has been used, considering that spermatozoa were not the major reservoir of HIV-1. However, insemination procedures using spermatozoa from HIV-1 positive individuals have resulted in infection of recipient women. It has been reported that 0.3–0.4% of women inseminated with spermatozoa from HIV-1-positive males seroconverted.\textsuperscript{27,28} Reports are contradictory regarding the safety of methods currently used to isolate uninfected spermatozoa from HIV-1-positive individuals. A study using reverse transcription-polymerase chain reaction (RT-PCR), reported detectable levels of HIV-1 RNA in the isolated fraction of spermatozoa in 5.6% of the procedures.\textsuperscript{28} Approximately 12% of sperm motile fractions isolated from HIV-1-positive individuals who were negative by RT-PCR were positive for viral DNA using nested PCR.\textsuperscript{30} These results indicate that commonly used techniques to discard the presence of the virus in sperm fractions were not adequately discriminatory to guarantee safe insemination procedures. Therefore, the potential for transmitting HIV-1 infection when spermatozoa from HIV-1-positive individuals are used during insemination procedures still exists.

A reason for not finding viral particles in washed semen samples could be that the isolation techniques currently used permit recovery of motile spermatozoa with specific structural characteristics only, such as normal morphology, intact membrane and normal packing of DNA.\textsuperscript{31} One study used atomic force microscopy to demonstrate that spermatozoa from patients who have received antiretroviral therapy exhibited the virus bound and fused with the sperm membrane, altering the morphology and affecting the middle piece and the tail.\textsuperscript{6} In contrast, HIV-1-positive patients who have not received antiretrovirals exhibited fewer morphological alterations, although in higher numbers than the uninfected control group.\textsuperscript{5,12}

**Possible implications of HIV-1 infection of spermatozoa**

Once HIV-1 infection of spermatozoa have been demonstrated, the question becomes, does this
infection affect sperm viability or does it have some implication during the fertilization process? It was shown that human oocytes are not infected with HIV-1 in vitro, possibly because these cells lack CD4, CCR5, CXCR4 and other molecules used as alternative receptors. It has been further demonstrated that human oocytes can be fertilized in vitro with purified spermatozoa isolated from HIV-1-positive individuals. These spermatozoa underwent the normal process of acrosomal reaction and could penetrate the oocyte, bringing with them viral particles that were later detected in the blastomeres by electronic microscopy. These results can establish a model in which the spermatozoa could not only infect the mother (horizontal transmission) but also infect the newborn in a new form of ‘vertical transmission from the father’ (Fig. 1). The concept of ‘vertical transmission from the father’ may be possible, as previous evidence demonstrated that animal retroviruses such avian leucosis virus might be vertically transmitted not only through the passage of virions to the egg but also when the germ cells, oocyte and spermatozoa contain the provirus (genetic transmission).

Hypothesis

Based on the finding that (i) HIV-1 can infect astrocytes and macrophages through the mannose receptor (a protein of 160kDa) in a CD4-independent manner, (ii) spermatozoa do not express CD4, CCR5 or CXCR4 molecules, and (iii) a 160 kDa receptor present in spermatozoa can interact with HIV-1 gp 120 independent of the CD4 molecule, we propose that this receptor resembles the mannose receptor and the molecule present in the spermatozoa that interacts with HIV gp 120 correspond to the same molecule. According to this, the HIV enters the spermatozoa through the mannose receptor, resulting in HIV-1 infection of the mother and fetus/neonate (Fig. 1).

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References


