A major research priority for HIV eradication is the elucidation of the events involved in HIV reservoir establishment and persistence. Cell-to-cell transmission of HIV represents an important area of study as it allows for the infection of cell types which are not easily infected by HIV, leading to the establishment of long-lived viral reservoirs. This phenomenon enables HIV to escape elimination by the immune system. This process may also enable HIV to escape suppressive effects of antiretroviral drugs. During cell-to-cell transmission of HIV, a dynamic series of events ensues at the virological synapse that promotes viral dissemination. Cell-to-cell transmission involves various types of cells of the immune system and this mode of transmission has been shown to have an important role in sexual and mother-to-child transmission of HIV and spread of HIV within the central nervous system and gut-associated lymphoid tissues. There is also evidence that cell-to-cell transmission of HIV occurs between thymocytes and renal tubular cells. Herein, following a brief review of the processes involved at the virological synapse, evidence supporting the role for cell-to-cell transmission of HIV in the maintenance of the HIV reservoir will be highlighted. Therapeutic considerations and future directions for this area of research will also be discussed.

Although the advent of highly active antiretroviral therapy (HAART) in the mid-1990s revolutionized the management and prognosis of human immunodeficiency virus (HIV)-infected individuals (van Sighem et al., 2010), it soon became apparent that discontinuation of HAART led to rapid rebound of HIV within peripheral blood (Chun et al., 2000; Davey et al., 1999). This viral rebound is due to release of HIV from reservoirs throughout the body (Blankson et al., 2002). Within these reservoirs, HIV is kept in a transcriptionally silent or latent state, but can reactivate to undergo HIV replication following appropriate stimulation (Bukrinsky et al., 1991; Chun et al., 1997) or cessation of antiretroviral pressures (Chun et al., 2010). Given that viral reservoirs are the main reason for our inability to eradicate HIV, there is strong interest in understanding the processes involved in the formation and persistence of these reservoirs.

CD4+ T-cells are well-characterized cellular reservoirs of HIV and become infected soon following HIV infection (Brenchley et al., 2004; Sáez-Cirión et al., 2013). Within these memory cells, HIV has been detected predominantly within central memory and transitional memory cell subsets (Chomont et al., 2009; Chun et al., 1997). Monocytes and macrophages (Redel et al., 2010) and dendritic cells (Keele et al., 2008) also play important roles as HIV reservoirs (Coleman & Wu, 2009; Zhu et al., 2002). Despite the fact that a very small percentage of monocytes (0.01–1%) harbour proviral HIV DNA (Zhu et al., 2002), they are of concern as they travel throughout the body and enter into anatomical compartments and tissues where antiretrovirals may not penetrate well and where immune pressures may enable their persistence. Furthermore, although macrophages are long-lived cells (Crowe et al., 1992, 1990), they can also proliferate in lymph nodes in the presence of HIV co-infection with Mycobacterium avium complex or Pneumocystis jiroveci (Orenstein et al., 1997). Reduced expression of drug transporters may limit HIV antiviral activity within monocytes and macrophages (Kim et al., 1998). Haematopoietic stem cells have also been shown to be cellular reservoirs of HIV (Slobod et al., 1996) in addition to some natural killer cells (Valentin et al., 2002) and astrocytes (Alexaki & Wiegahl, 2008). Meanwhile, with regard to anatomical reservoirs, the gut-associated lymphoid tissue (GALT) is the best characterized and harbours the majority of the body’s lymphocytes (Guadalupe et al., 2003; Mowat & Viney, 1997). Other recognized anatomical reservoirs include the central nervous system (CNS) (Canestri et al., 2010; Spudich et al., 2006), testes (Le Tortorec et al., 2008) and lungs (Costiniuk & Jenabian, 2014). These sites can harbour distinct viral variants compared with peripheral blood which, in turn, may differ in their propensity to infect cells (Blackard, 2012). Similarly, cellular factors, such as lineage and activation status can also affect the ability of cells to become infected (Del Portillo et al., 2011).
Cell-to-cell transmission of HIV allows infection of cell types not easily infected by HIV, leading to the establishment of long-lived viral reservoirs. An understanding of the mechanisms involved in cell-to-cell transmission of HIV is essential for rational development of therapeutic strategies targeted at minimizing or eliminating the HIV reservoir. After a brief review of the structures and processes involved at the virological synapse (VS), evidence supporting the role of cell-to-cell transmission of HIV in establishing and maintaining the HIV reservoir will be reviewed. Clinical implications of cell-to-cell transfer and future directions will also be discussed.

Cell-to-cell cross-talk

HIV’s ability to spread between cells is considered a major determinant of its virulence (Sowinski et al., 2008), with HIV cell-to-cell transfer being up to several thousand fold more efficient than infection associated with cell-free virions (Chen et al., 2007; Sourisseau et al., 2007). HIV takes advantage of a wide array of dynamic interactions between various cells of the immune system to spread throughout the body. T-cells travel many miles daily (Miller et al., 2004) and dendritic cells (DCs) contact thousands of T-cells hourly in search of appropriate antigenic patterns on major histocompatibility complex (MHC) molecules on T-cells (Bousso & Robey, 2003). Functional cross-talk between cells within the immune system occurs at immunological synapses (IS). Here, a T-cell receptor (TCR), CD4 and kinases come together at peptide MHCs on antigen-presenting cells (APCs) into a TCR microcluster (Burkhardt et al., 2008; Lehmann et al., 2011). This microcluster converges near the central supramolecular activation complex which is surrounded by adhesion molecules associated with talin and F-actin, which provide stability to the IS (Burkhardt et al., 2008; Lehmann et al., 2011). The microtubule-organizing centre becomes polarized towards the APC and T-cell activation ensues (Burkhardt et al., 2008; Lehmann et al., 2011). By hijacking components of the IS, HIV induces changes in cellular morphology and behaviour at the IS (Vasiliver-Shamis et al., 2008). A VS is formed when cells contact each other through stable adhesive junctions yet remain distinct cells that have not undergone fusion (Piguet & Sattentau, 2004). VSs are typically formed between T-cells and cells such as macrophages, DCs and other T-cells, which all bear HIV co-receptors (Felts et al., 2010; Lehmann et al., 2011; Piguet & Sattentau, 2004).

T-cell to T-cell and macrophage to T-cell transfer of HIV

Cell-to-cell transmission of HIV was initially described between HIV-infected Jurkat T-cell line and primary CD4 T-cells (Jolly et al., 2004). Within HIV-infected cells, Env and Gag proteins are recruited to sites of cell-to-cell contact (Jolly et al., 2004) and CD4, CCR5 and CXCR4 are recruited to these sites on target cells (Hübner et al., 2009). A major player in the process of reorganizing receptors on target cells is actin, which is also involved in the recruitment of the adhesion molecule, lymphocyte function-associated antigen (LFA)-1 (Dale et al., 2011; Feldmann & Schwartz, 2010; Lehman et al., 2008; Piguet & Sattentau, 2004). LFA-1 is organized into a supramolecular cluster at the site of cell-to-cell contact and contributes to junction stability (Feldmann & Schwartz, 2010; Lehman et al., 2008; Piguet & Sattentau, 2004). The formation of rosette-like structures is evidence of polysynapse formation, whereby there are multiple sites of cell-to-cell contact with VS formation (Rudnicka et al., 2009).

Over the span of approximately 3 h (Chen et al., 2007), HIV is transferred across the VS and undergoes budding followed by fusion with the target cell membrane (Piguet & Sattentau, 2004). HIV fusion requires that virions mature within endosomes of the acceptor cell (Miyachi et al., 2009). The process of HIV transmission between macrophages and T-cells is similar to that observed between T-cells and T-cells (Groot et al., 2008). Groot et al. (2008) showed that macrophages can endure for several weeks and may infect more than one T-cell every 6 h, underscoring the importance of macrophages in HIV persistence (Collman et al., 2003; Groot et al., 2008; Sharova et al., 2005).

DC to T-cell transfer of HIV. Immature DCs can capture HIV virions through DC-SIGN, a C-lectin that is the major mediator of attachment of HIV to DCs (Arrighi et al., 2004; Geijtenbeek et al., 2000). Meanwhile, Langerhans cells, a DC subtype that are found within mucosa and skin, can capture HIV through mannose receptors and langerin (Turville et al., 2008). Other adhesion molecules, such as ICAM-1, are also capable of binding to ligands on the HIV membrane during budding (Hioe et al., 1998). Like HIV transfer between T-cells, HIV transfer from DCs to T-cells occurs at contact areas within the VS, where HIV receptors, HIV co-receptors, tetraspanins and actin are concentrated. HIV is transferred from immature DCs to T-cells on membrane extensions (Lehmann et al., 2011). Immature DCs are mainly present in mucosal tissues and skin and they mature during their migration to peripheral and secondary lymphoid organs (Martin-Fontecha et al., 2009). The VS is formed between mature DCs and T-cells when large membrane sheets or extensions of the cell membrane, emanating from DCs, form an envelope around T-cells (Lehmann et al., 2011) as depicted in Fig. 1. As DCs are unable to fully break down HIV, some virions remain in endosomal compartments but are not degraded and are accessible to the cell surface (Bosch et al., 2008). Although there are similarities between VSs formed between DCs and T-cells and those established between T-cells and other T-cells, there are also important differences between these interactions. For example, bonds between mature DCs and T-cells may be sufficient enough to induce synapse formation, but additional adhesive forces, such as those provided by Env, may be required between two T-cells (Felts et al., 2010).

Evidence for the contribution of cell-to-cell spread of HIV in the establishment of HIV reservoirs

The notion that cell-to-cell transfer could be playing a role in HIV dissemination throughout the body was first
entertained when it was noted that in vitro shaking of lymphocyte cultures resulted in reduced HIV replication compared with cultures that were not shaken (Sourisseau et al., 2007). In chronic HIV infection, a large proportion of HIV replication occurs within lymphoid tissue. Lymphoid tissue consists predominantly of tightly packed CD4 T-cells (Feldmann & Schwartz, 2010; Haase, 1999). This tight packing impedes cellular movement and facilitates intercellular contact (Feldmann & Schwartz, 2010; Sourisseau et al., 2007). Observations that T-cells within lymph nodes frequently display polarized morphologies support the notion that cell-to-cell transmission of HIV is common within these tissues (Llewellyn et al., 2010). Similarly, the clustering of cells in lymphoid tissues suggests that polysynapses, whereby one infected cell forms synapses with several target cells (Rudnicka et al., 2009), may be present. These findings all account for the rapid escalation of HIV in acute infection (Llewellyn et al., 2010). Furthermore, it has been shown that the mean number of integrated HIV proviruses in the spleen is 3.2 per cell (Jung et al., 2002). This high number of integrated proviruses suggests that more than one virion is transferred per synapse to the target cells (Del Portillo et al., 2011). Alternatively there may be polysynapse formation, which enables the transfer of more than one virion to a cell (Feldmann & Schwartz, 2010). Furthermore, distinct quasispecies within separate germinal centres of a spleen have been reported (Cheynier et al., 1994; Feldmann & Schwartz, 2010; Ince et al., 2009), which suggests that cell-to-cell transmission may be involved in HIV spread within the spleen (Feldmann & Schwartz, 2010).

It has been suggested that close cell-to-cell contact may provide sufficient ‘stimuli’ to activate neighbouring cells (Carr et al., 1999). In peripheral blood, over 90% of CD4 T-cells do not show evidence of activation (Carr et al., 1999). In contrast, a much greater proportion of CD4 T-cells would be expected to show evidence of activation in areas such as densely packed lymphoid tissues. The finding that cell-to-cell contact may serve as a stimulus to activate cells is significant given that activated cells are more permissive to HIV infection (Carr et al., 1999).

**Implications of cell-to-cell transfer for HIV transmission and persistence**

Cell-to-cell transmission of HIV in vivo has several important implications. As there is less diffusion, there is a greater probability that a virion will attach to and infect a cell. Zhong et al. (2013) noted that cell-to-cell spread of HIV resulted in greater amounts of proviral DNA within target cells than observed with infection of target cells by cell-free HIV particles. Another implication of cell-to-cell spread of HIV is the risk that endogenous restriction factors could become saturated by the entry of many virions into cells simultaneously (Del Portillo et al., 2011). When proviral DNA is reactivated upon stimulation of quiescent T-cells, these cells may become re-infected with another HIV virion, which sets the stage for recombinatorial events (Tang et al., 1995). Inheritance of multiple copies of HIV-1, referred to as multiploid inheritance,
enables virus in relatively low titres and with low infection frequency to infect cells in multiple copies (Del Portillo et al., 2011). As discussed by Del Portillo et al., point mutations can be spontaneously inherited with non-mutant virus and virus can accumulate recessive mutations, enabling less fit mutants to survive (Del Portillo et al., 2011; Gelderblom et al., 2008).

Cell-to-cell spread of HIV may enable virus to escape the effect of antiretrovirals in the extracellular space (Sigal et al., 2011). Using an in vitro model, tenofovir was shown to decrease cell-free infection of peripheral blood mononuclear cells by approximately 30-fold, whereas tenofovir was associated with a less than twofold decrease in infection arising through cell-to-cell transmission of HIV (Sigal et al., 2011). However, a recent study by Agosto et al. (2014) revealed different findings when the efficacy of a panel of antiretrovirals was tested in the prevention of cell-to-cell and cell-free HIV spread. Although several nucleoside reverse transcriptase inhibitors (NRTIs) displayed reduced activity during cell-to-cell HIV transmission, non-nucleoside reverse transcriptase inhibitors, entry inhibitors and protease inhibitors remained effective in preventing cell-to-cell transmission. Moreover, although single NRTIs were not able to prevent cell-to-cell transmission of HIV, a combination of NRTIs was able to inhibit HIV cell-to-cell transmission and cell-free infection equally well (Agosto et al., 2014). These authors indicate that their findings support the observations that combinations of agents contribute to the clinical effectiveness of HAART (Agosto et al., 2014). Moreover, HIV antigens may be presented to the immune system differently when expressed on infected cells compared with when these antigens are detected as part of free virions (Ferrari et al., 2011). This has implications for cytotoxicity mediated by CD8 T-cells and cytotoxicity that is antibody-dependent, given that HIV may escape recognition and neutralization by antibodies and complement (Garcea et al., 2005; Kremensov et al., 2009). When co-cultures of HIV-infected T-cells and primary lymphocytes were studied, only a portion of broadly neutralizing antibodies (bNAbs) that targeted the CD4 binding site or glycan/V3 loop were able to block HIV cell-to-cell transmission (Koppensteiner et al., 2012; Malbec et al., 2013). Furthermore, although some bNAbs can block HIV infection by cell-to-cell transmission with the same efficiency as with cell-free infection, other bNAbs are required in higher concentrations to inhibit cell-to-cell, compared with cell-free, HIV infection (Sagar et al., 2012; Schiffner et al., 2013). This has been attributed, in part, to steric hindrance whereby bNAbs may not be able to easily access the VS (Koppensteiner et al., 2012; Schiffner et al., 2013). Furthermore, it has been demonstrated that neutralization efficiency may also be influenced by the time at which antibodies are added to co-cultures (Martin et al., 2010; Schiffner et al., 2013). Another implication of HIV cell-to-cell transmission relates to microbicide development, although microbicides that inhibit free virus may not provide protection against virus acquired from cell-associated virus.

Sexual transmission and cell-to-cell transfer of HIV. As sexual transmission is the most common route of HIV transmission worldwide (Beyrer, 2007), cell-to-cell transmission of HIV within the genital tract has great interest. HIV transmission from HIV-infected monocytes to inner foreskin mononuclear cells has been observed in vitro with a co-culture time as brief as 1 h (Cameron et al., 1996; Ganor et al., 2010; Zhou et al., 2011). However, infection of inner foreskin mononuclear cells was not observed with exposure to cell-free HIV virions (Ganor et al., 2010). VSs were identified between HIV-infected cells and apical foreskin keratinocytes, which resulted in polarized budding of HIV then internalization by inner foreskin Langerhans cells (Ganor et al., 2010). Virus was transmitted from Langherans cells to T-cells at the epidermis–dermis barrier, culminating in HIV transmission (Ganor et al., 2010). In contrast, urogenital cells have been shown to behave somewhat differently from foreskin epithelial cells as primary cervical and prostate cells do not become productively infected with either cell-free HIV or cell-associated HIV (Dezzutti et al., 2001). Although not productively infected with HIV, these cells were nonetheless able to sequester HIV and transfer HIV to immune cells (Dezzutti et al., 2001). With regard to vaginal transmission, Sallé et al. (2010) induced systemic SIV infection by macaque vaginal exposure to SIV-infected macaque spleen cells. SIV was found in proximal and distal lymphoid tissues within 1–2 days post-exposure. Iba et al. (1997) reported that murine peritoneal lymphoid cells placed into the mouse vagina resulted in invasion into mucosa and iliac lymph nodes within 24 h. In another animal model study, chimpanzees became infected after inoculation of 300 infectious peripheral blood mononuclear cells into the endocervical canal (Girard, 1992). Moreover, in an in vitro study examining human cervical submucosa, Maher et al. (2005) found that the cervical submucosa can become infected following exposure to HIV-infected seminal cells within 3–4 days. Meanwhile, another study demonstrated that semen promotes lymphocyte adherence to stratified epithelium of the ectocervix, facilitating cell-to-cell transmission of HIV (Pearce-Pratt & Phillips, 1993).

With regard to rectal transmission of HIV, Kolodkin-Gal et al. (2013) studied the ability of cell-associated and cell-free HIV to infect mucosa. Using a human colonic mucosal explant system, in addition to in vivo challenge with SIV in the distal colan of rhesus macaques, they showed that exposure to cell-associated virus was much more efficient at producing infection than exposure to cell-free virus. They found that exposure of the colon explant system to cell-associated HIV produced infection with low doses of HIV (7 TCID50 per well) while an inoculum of 50 000 peripheral blood mononuclear cells with up to $2.4 \times 10^4$ DNA copies per inoculum was sufficient to simulate exposure to sexually transmitted HIV.

Cell-to-cell transmission of HIV within GALT. In the aforementioned study by Kolodkin-Gal et al. (2013), it was
shown that CD4 cells can migrate through intestinal tissue and spread HIV to other CD4 cells via cell-to-cell spread. However, compared with the genito-urinary tract, cell-to-cell transmission of HIV has been much less studied in gastrointestinal tissues. Nonetheless, GALT has multiple features that make it ideal for cell-to-cell spread of HIV. It contains the majority of lymphocytes within the body (Guadalupe et al., 2003; Mowat & Viney, 1997), represents one of the most active regions of HIV replication and is a well-documented HIV anatomical reservoir (Chun et al., 2008, 2010). The dense concentration of lymphoid cells in close proximity provides a good setting for cell-to-cell spread of HIV. The gut homing receptor α4β7 is able to bind HIV envelope protein gp120 and is threefold larger than the CD4 receptor, allowing it to capture HIV very efficiently (Cicala et al., 2010). These interactions result in the activation of LFA-1, thereby facilitating the formation of a VS (Cicala et al., 2010). Furthermore, the presence of drug efflux transporters along the gastrointestinal tract (Paine et al., 2006; Zhang et al., 1999) may reduce the concentration of antiretrovirals within gut cells, enabling HIV replication to occur unchekced.

Breaches in the integrity of the gastrointestinal mucosa due to direct effects of HIV facilitate translocation of Gram-negative bacteria and result in high plasma lipopolysaccharide levels (Brenchley et al., 2006). It has been suggested that elevated lipopolysaccharide levels may potentially drive HIV dissemination throughout the body (Wang et al., 2007). The gut mucosa is also continuously exposed to antigenic stimulation from food and external antigens (Mehandru, 2012). This, in turn, puts cells in an activated state, increasing their susceptibility to HIV infection and, in turn, contributing to HIV persistence.

**Cell-to-cell spread of HIV in the CNS.** Despite the ability of HAART to suppress peripheral blood viraemia, HIV-infected individuals continue to suffer from HIV-associated neurocognitive disorders (HANDs) (Heaton et al., 2011). There is evidence for ongoing HIV replication and evolution in the CNS (Canestri et al., 2010; Spudich et al., 2006) and CNS immune activation despite HAART (Yilmaz et al., 2008). Antiretroviral penetration into the CNS may not be ideal (Best et al., 2009, 2012), which may facilitate ongoing HIV replication within the CNS.

HIV may enter the CNS through different mechanisms, although the ‘Trojan horse’ hypothesis is the most favoured theory (Albright et al., 2003; Kolson et al., 1998). This theory describes the migration of HIV-infected cells across the blood–brain barrier by infiltration of immune cells such as monocytes and lymphocytes (Gartner, 2000). The processes that establish and maintain the HIV reservoir within the CNS have been difficult to study due partly to the relative difficulty of obtaining fluids and tissues from this compartment, and the concern that in vitro models do not accurately reflect the features of in vivo systems.

Although very plausible, currently it has not yet been demonstrated whether cell-to-cell transmission of HIV within the CNS contributes to the persistence of the CNS HIV reservoir. Within the CNS are vast networks of complex intercellular interactions, and a relatively slow rate of cerebrospinal fluid movement, which can facilitate intercellular interactions. Interestingly, HIV can infect various types of cells that do not bear CD4 receptors, such as neurons and astrocytes (Harouse et al., 1989; Liu et al., 2004). Therefore, in addition to determining whether cell-to-cell spread actually does occur in the CNS, future studies into the mechanisms by which HIV is able to infect cells that do not bear CD4 receptors would be worthwhile.

**Mother-to-child transmission of HIV.** Vertical transmission of HIV may ensue either by in utero exposure to HIV across the placenta or after birth via exposure of the infant’s oral cavity to HIV-infected breast milk. In vitro studies show that placental cells are more likely to become infected by HIV when co-cultured with infected T-cell blasts and infected HIV placental cells than when simply exposed to cell-free virus (Arias et al., 2003). HIV-infected placental cells express increased adhesion molecule ICAM-1, which facilitates attachment of leukocytes to placenta (Arias et al., 2003). With regard to breast-feeding, within 1 day of exposure of oral mucosa to SIV, primates become systemically infected with the virus (Milush et al., 2004, 2007). Oral keratinocyte cell lines (OKF6/TERT-2; TERT-2) harbour HIV-1 and can transmit the virus to permissive cells (Vacharaksa et al., 2008). Furthermore, mammary epithelial cells (MECs), which are involved in milk secretion, are thought to be HIV reservoirs. HIV DNA from paired breast-milk and peripheral blood samples from HIV-infected women that was subjected to phylogenetic analyses showed that HIV from these two compartments differs genetically (Bequhart et al., 2002; Permar et al., 2010; Shapiro et al., 2005). HIV-infected MECs can transfer virus to susceptible target cells (Dorosko & Connor, 2010). Even if not directly infected themselves with integrated proviral DNA, MECs can pass along HIV from its endosomal compartment to CD4 cells trafficking through the mammary epithelial cell layer (Dorosko & Connor, 2010). Furthermore, studies of breast-milk from HIV-infected women on HAART have shown negligible impact on cell-associated or HIV proviral DNA levels, in contrast with a rapid decline in cell-free HIV RNA in breast-milk (Lehman et al., 2008; Shapiro et al., 2005).

**Other HIV reservoirs: thymocytes and renal tubular cells.** Other cells such as thymocytes and renal tubular cells have been studied in the context of cell-to-cell transmission of HIV. Within the thymus, DCs are spaced closely together and are able to become infected by HIV (Cameron et al., 1996). Plasmacytoid DCs have been shown to transfer HIV to mature and immature thymocytes (Evans et al., 2011). Although the thymus has not received any significant attention for its role as an HIV reservoir, there may be clinical implications as HIV persistence within the thymus may contribute to impaired T-cell production in immunological non-responders to HAART (Evans et al., 2011).
With regard to renal tubular cells, interaction of HIV with proximal renal tubular cell receptor DEC-205 leads to internalization of HIV (Mikulak et al., 2009). Although HIV is typically degraded within lysosomes and productive infection is not established, some virions will not be degraded and may be transferred to T-cells, thus supporting the role of renal tubular cells as HIV reservoirs (Mikulak et al., 2009). Furthermore, while renal tubular epithelial cells do not bear CD4 receptors, it has been shown that co-culture of HIV-infected T-cells and renal tubular epithelial cells results in HIV uptake and gene expression in renal tubular epithelial cells, with larger amounts of HIV being transferred to renal epithelial cells from HIV-infected T-cells than from cell-free HIV (Chen et al., 2007). Stable adhesions that required neither CD4 nor Env have been observed (Chen et al., 2007).

### Future directions

While there is evidence to date supporting a potentially important role for cell-to-cell transmission of HIV and its role in the establishment of the HIV reservoir, there are still several questions remaining. Some of these questions are rather fundamental, such as the direction of HIV transfer, as this may have implications with regard to the functional capacities of the cells (Jolly et al., 2011). More research into the role of cell-to-cell spread of HIV within the CNS is also merited, given the very rich networks of intercellular communication and the rapidity of spread of HIV within the CNS (Kadiu & Gendelman, 2011). More investigations into cell-to-cell spread of HIV within the lungs and testes are also merited given the potential roles of these organs as anatomical reservoirs of HIV (Costiniuk & Jenabian, 2014; Le Tortorec et al., 2008). Understanding the possible effect of cell-to-cell transmission of HIV in the liver would also be of interest, in addition to the effect of co-infections such as HIV–hepatitis C virus (HCV) given the complex interaction between these two viruses (Chen et al., 2014). Furthermore, it is important to gain a better understanding of the ways in which HIV infects cells that are not easily infected by HIV, such as those that do not bear CD4 receptors (Harouse et al., 1989; Liu et al., 2004). This may reveal additional insights into the role of cell-to-cell spread of HIV and its contribution to building and maintaining HIV reservoirs.

Although cells have been observed to form clusters in mucosal and lymphoid tissue of primates with SIV infection, the actual transfer of HIV through VS and the functioning of filopodia and nanotubules have not yet been witnessed in vivo (Feldmann & Schwartz, 2010). It has been suggested that visualizing cell-to-cell spread of HIV in animals may yield insight into the mechanisms by which HIV infection is established during acute infection and unfolds during chronic infection (Feldmann & Schwartz, 2010). Furthermore, more HIV/SIV challenge studies with animals should examine cell-associated virus in addition to cell-free virus.

With regard to therapeutics, strategies to inhibit cell-to-cell transfer of HIV should include the use of HIV entry inhibitors, as the use of these agents in preventing cell-to-cell transmission is currently not well-characterized (Durham et al., 2012). Furthermore, exploration of compounds that can inhibit structures and interactions necessary for cell-to-cell spread of HIV (such as actin inhibitors etc.) should be undertaken (Wang et al., 2007). As discussed by Wang et al. (2007), efforts to develop such therapies will likely also encounter challenges whereby there is widespread disruption of normal, healthy cell-to-cell interactions. Therefore, selectivity for cell-to-cell interactions between HIV-infected and non-infected cells will be critically important. In the context of vaccine development, although numerous broadly neutralizing antibodies and peptide inhibitors have been examined for their potential to block cell-to-cell transfer of HIV and VS-mediated cell infection, thus far only antibodies preventing Env–CD4 interaction appear capable of these roles (Durham et al., 2012). Therefore much more work remains to be done with regard to overcoming the challenges of cell-to-cell transmission of HIV and vaccine development. Finally, given the many strategies HIV employs to evade immune recognition and susceptibility to antivirals, a functional cure is a more feasible goal than a sterilizing one. A functional cure would suppress HIV to such low levels that the host’s immune system would be able to control the infection without the need for antiretroviral therapy, harm would not be inflicted upon the host and HIV would not be transmitted to other individuals.

### References


HIV-associated neurocognitive disorders before and authors (2011).


