CHAPTER 22

Virological Synapse for Cell-Cell Spread of Viruses

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Abstract

Cell-to-cell spread of retroviruses via virological synapse (VS) contributes to overall progression of disease. VS are specialized pathogen-induced cellular structures that facilitate cell-to-cell transfer of HIV-1 and HTLV-1. VS provide a mechanistic explanation for cell-associated retroviral replication. While VS share some common features with neurological or immunological synapses, they also exhibit important differences. The role of VS might not be limited to human retroviruses and the emerging role of a plant synapse suggests that VS might well be conserved structures for cell-cell spreading of both animal and plant viruses. Dissection of the VS is just at its beginning, but already offers ample information and fascinating insights into mechanisms of viral replication and cell-to-cell communication.

Neural, Immunological and Virological Synapse

The complex functioning of biological systems requires the capacity of cells to interact in a synchronized manner. The capacity of cells to come in close contact with one another enables rapid exchange of information through directed secretion. In complex systems such as the nervous and immune systems, characteristic rearrangements of plasma membrane proteins appear at the cell-cell junction, called synapse. A synapse is defined as "a stable adhesive junction across which information is relayed by directed secretion".1

The concept of the neural synapse (NS) was first introduced over a century ago and was depicted as a stable structure organized and specialized in intercellular signaling between neurons. Plasma membranes of the pre and post-synaptic neurons are contiguous and information is conveyed to the downstream cell via secretion of neurotransmitters. In order to generate a favorable microenvironment, stabilization of synapse by scaffolding proteins, mainly cadherins and other adhesion molecules, is required (reviewed in ref. 1).

In the immune system, interactions between T cells and antigen presenting cells (APC) are essential for an effective adaptive immune response. By analogy with the nervous system, these specialized interactions occur via an immunological synapse (IS). The concept of the IS has been extended to several types of cell-cell interactions within the context of the immune system (signaling via receptor engagement, lytic granules, directed secretion of cytokines) since its first description 20 years ago (reviewed in refs. 2,3). Although the IS shares many similarities with the NS, it also differs in two aspects. First, the panel of receptors and adhesion proteins recruited to the IS diverges from those in the neural synapse: integrins play the central role in stabilizing IS. Second, the establishment of an IS is a dynamic process between moving cells, whereas the neural synapse is long-lived. Therefore, in

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order to permit immune responses to take place, ISs need to be assembled and disassembled quickly. An example is CTL-mediated killing, where a single effector cell has been shown to contact sequentially target cells through several stable IS (for reviews see in refs. 1,5-7).

In recent years, the concept of the synapse has been further extended to cell-cell contacts during viral replication. To initiate an infection, viruses need to gain access to the replicative machinery of the host cell. In the cell-free virus model, viruses do so by crossing the plasma membrane of the target cell after binding to surface receptors. Nevertheless, some viruses use direct passage from cell-to-cell to spread within their host achieving, in the process, protection from neutralizing antibodies and complement as well as higher kinetics of replication (reviewed in ref 9). Recent articles have described virological synapses (VS) for two retroviruses, human T cell leukemia virus type 1 (HTLV-1) and human immunodeficiency virus type 1 (HIV-1) (reviewed in ref 16). VS, like their neural and immunological counterparts, suit the minimal criteria that define a synapse: both pre and post-synaptic cells implied in cell-cell contact remain discrete cells (no plasma membrane fusion), a stable adhesive connection is established between the two cells and directed transmission of information (viral genome) occurs from the infected cell (presynaptic cell) to the uninfected cell (post-synaptic).

**Virological Synapse during Retroviral Infection**

Although viral cell-to-cell transfer has been identified many years ago, we gained only recently some insight into the mechanisms of this mode of viral transmission. Cell-free HTLV-1 ineffectively infects T lymphocytes and spreads within and between individuals via cell-to-cell transfer. With the partial unraveling of the mechanisms involved in HTLV-1 dissemination from lymphocyte to lymphocyte via VS, puzzling questions, such as HTLV-1 cell tropism, regardless of the ubiquitous expression of its surface receptor, have found satisfying explanations.

Other retroviruses, such as HIV-1 and SIV, also use VS to propagate within their respective hosts. Efficient HIV-1 infection requires permissive target cells to be located in close vicinity in order to initiate infection and subsequent spreading throughout different tissues. At least three modes of propagation have been described for HIV-1. Firstly, cell-free transmission of HIV-1 is well characterized. Cell-free HIV-1 binds surface receptors/coreceptors (CD4/CCR5 and CXCR4) of permissive cells before fusing with the plasma membrane of the target cell and following the subsequent steps of the viral replication cycle. Secondly, HIV is able to propagate through infection in trans. Cells such as dendritic cells (DC) capture virions through viral binding to cell-surface receptors such as C-type lectins. HIV-1 DCs, not necessarily infected themselves, then present the virus to target cells in trans via a VS or an Infectious Synapse. Thirdly, HIV-1-infected cells (also termed effector cells) are able to transmit the virus to uninfected target cells, without the previous requirement of virus budding in the extracellular milieu, illustrating direct cell-to-cell viral transmission through a VS. Until now, three types of VS have been described for HIV-1: the DC-T cell VS, also referred to as "Infectious Synapse", the T cell-T cell VS and the mononuclear cell-mucosal epithelial VS, implicated in HIV transcytosis through mucosal epithelia.

The use of VS for viral transmission is probably not limited to retroviruses and is exploited by other intracellular pathogens in order to disseminate through their host. Early in vitro experiments show a VS-like structure possibly contributing to SARS-coronavirus (SARS-CoV) dissemination from DCs to target cells.

As the concept of infectious or virological synapse is further applied to other organisms, such as plants, VS emerges as a general mechanism of cell-to-cell transmission for many pathogens and parasites.

**Virological Synapses during HIV Infection**

**Dendritic Cell-T Cell Infectious Synapse during HIV Sexual Transmission**

In model systems of sexual transmission, myeloid dermal DCs and Langerhans cells (LC) play a central role in the early steps of HIV-1 propagation (reviewed in refs. 36-40). DCs locate to the skin and mucosal tissues in an immature state (IDC) until coming across pathogen-derived antigens. DC
activation and differentiation into mature APCs results from contact with different stimuli such as bacterial products, TNF family ligands, double-stranded and single-stranded RNA. Migration of mature DCs (mDC) from the periphery to secondary lymphoid organs is strongly associated with maturation and allows DCs to encounter antigen-specific T cells in order to initiate adequate immune responses. Although HIV-1 infects CD4 T cells more effectively, LC and other DC types support low levels of viral replication, both in vivo and in vitro. DC are also able to capture HIV-1 in an infectious form and transfer such virions to target CD4 T cells without the need of virus replication within the effector cell (here the DC) (reviewed in refs. 37, 61). Recognitions of adhesion molecules inserted in the viral envelope or binding through lectin receptors, such as DC-SIGN, mannose receptor or langerin, allow DCs to bind HIV-1 efficiently. The C-type lectin DC-SIGN (CD209), strongly expressed in iDCs, plays a crucial role in capture and transfer of HIV-1 to T cells in trans. DC-SIGN was shown to mediate VS (or rather infectious synapse) formation in vitro between DCs and autologous resting T cells, favoring transfer of a CXCR4-using HIV-1. As a major attachment factor on DCs, DC-SIGN has been shown to bind many viruses such as HIV-1, HIV-2, simian immunodeficiency virus (SIV), Dengue virus, Cytomegalovirus (CMV), Ebola virus and SARS-CoV.

Professional APCs play a central role in antigen processing. As the archetypal APC, DCs are rich in degradative compartments. Nevertheless, efficient digestion of HIV-1 occurs in DCs, but a small fraction DC-SIGN-internalized virus remains infectious for extended periods of time and can be transferred in trans to target cells. The characteristic DC lysosomal degradative functions are activated upon DC maturation. Several studies suggest that HIV-1-induced maturation is only partial and might fail to induce a full activation of the lysosomal system. HIV-loaded DCs retains a population of infectious virus within an intracellular compartment that, until recently, was poorly described. Surprisingly, dissection of non replicating (CXCR4-using) HIV-1 trafficking pathways in monocyte-derived DCs revealed that, virus does not accumulate in lysosomes after capture but in a novel mildly acidic nonconventional compartment distinct from the classical late endosome/multivesicular body (MVB). This novel endosome targeted by HIV after capture by DCs is enriched in specific tetraspanins (CD81 and CD9) but contains only little CD63 (marker of MVB) and virtually no LAMP-1 (marker of lysosomes). This tetraspanin rich compartment targeted by HIV in DCs resembles the structures where HIV assembles in macrophages, where HIV assembles in macrophages, the location and mechanisms of HIV-1 replication and budding within DCs remain to be characterized.

Importantly, both HIV-infected and HIV-pulsed DCs are able to transmit a strong infection to T cells in trans. The recent depiction of a VS formed between uninfected T lymphocytes and DCs pulsed with fluorescently tagged HIV-1 has shed some light on the molecular processes at play. The DC-T cell VS has also been termed "Infectious Synapse". In the DC-T cell situation the dendritic cell is not necessarily replicating virus and is transferring HIV to a target cell in trans, whereas in the T cell-T cells VS both cells (pre and postsynaptic) are productively infected. For the purpose of clarity in this review we will use the term VS also in the case of the DC-T cell Infectious Synapse. In DC-T cells conjugates, virions polarize to the contact surface between the adjacent cells. Simultaneously, HIV-1 receptors (CD4) and coreceptors (CXCR4/CCR5) seem to be at least partially enriched on the T cell side of the junction with the DC (EG and VP, unpublished observations). VS formation is possibly initiated by normal cellular interactions in which T cells "scan" DC in an antigen-independent fashion, searching for the cognate peptide presented by the APC. Upon contact with T cells, internalized HIV-1 relocates rapidly to the VS in which the tetraspanins CD81 and CD9 are also redistributed. Given the apparent role of CD81 as an element of the IS (reviewed in see refs. 6, 88), HIV-1 subverts a pathway involved in IS formation and T cell activation to spread from DCs to uninfected CD4 T cells. On the T cell side of the synapse, engagement of the CD81 receptor might also play a role in increasing viral gene expression.
The dissection of the DC-T cell VS is still ongoing and many questions remain to be answered. Is VS formation relevant in the context of sexual transmission of HIV-1? Shown to facilitate nonreplicative HIV-1/SIV transfer in DC-T cell conjugates, DC-T cell VS usage by HIV-1 has to be confirmed with replicative CCR5-using strains. What is the relationship between the DC-T cell immunological synapse and the DC-T cell VS? The molecular basis of DC-T cell VS assembly remains poorly understood. Interference studies using receptor-blocking antibodies, inhibitors of cellular processes involved in cytoskeletal rearrangements and signaling, and RNA interference of surface receptor expression are ongoing in order to address this issue.

**HIV-1 T Cell-T Cell Virological Synapse**

Upon cell-to-cell contact, HIV-1-infected T cells are able to induce rapid clustering of viral receptors on uninfected T cells. The molecular interactions behind this process were recently detailed and led to the description of an HIV-1 induced VS between T cells. Interactions between HIV-1 Env protein on the effector cell with CD4 and CXC chemokine receptor 4 (CXCR4) on the naïve T cell are essential to induce a fast actin-dependent recruitment of viral receptors and lymphocyte-associated antigen 1 (LFA-1) to the VS. F-actin disassembly/reassembly is central to the mobilization of all players within the T cell VS, as demonstrated by inhibitors for both processes. Indeed, stable antigen-independent clusters between CD4+ T cells seldom occur when compared with antigen-dependent DC-T cell clusters. Therefore, stabilization of T cell-T cell contacts must be triggered by a specific signal. In the case of HIV-1 VS, Env seems to function as the triggering signal. Blocking antibodies and chemical inhibitors preventing Env binding to CD4 and CXCR4 on the naïve T cell reduce T cell VS formation as well as T cell-T cell conjugates.

**Virological Synapse and HIV-1 Transcytosis across Mucosal Epithelia**

Mucosal epithelia are the first line of defense of the human body against sexual transmission of HIV-1. The virus needs to circumvent this obstacle in order to gain a foothold within a new individual. In addition to capture by DCs or Dendritic Cells residing in mucosal epithelia, transcytosis of infectious virions across epithelial cells at mucosal sites of exposure may well be a strategy used by HIV-1. Early studies showed convincingly that transcytosis with cell-associated HIV-1 was much more efficient than transcytosis of cell-free virions through epithelial cell layers. Virological synapses, in which HIV-1-infected blood mononuclear cells establish contacts with mucosal epithelial cells, were recently described, providing a likely explanation for this cell-to-cell viral transmission. In this context, HIV-1 buds locally from the effector cell, followed by endocytosis and transcytosis without fusion from the apical to the serosal pole of epithelial cells. Infection grants HIV-1-loaded cells the ability to interact with epithelial cells by upregulating the expression of surface adhesion molecules and by the presence of the viral envelope proteins gp120 and gp41. Epithelial cells also take part in VS formation and stabilization as well as in proper initiation of HIV-1 transcytosis. The heparan sulfate proteoglycan (HSPG) agrin, present in the scaffolding complexes of neural and immunological synapses, serves as an HIV-1 attachment receptor through gp41-binding, reinforcing virion interactions with its previously described endocytic receptor galactosyl ceramid. Nevertheless, this is not sufficient to initiate HIV-1 transcytosis and additional signals supplied by the synaptic scaffold are crucial. Stable interactions between epithelial cells and HIV-1-infected PBMCs result partially from epithelial expression of the RGD-dependant Beta-1 integrin. Contacts between RGD-containing molecules, either at the surface of HIV-1-infected PBMCs or released as soluble factors, with Beta-1 integrins potentially initiate the signaling pathways leading to an efficient HIV-1 transcytosis and its subsequent spread throughout the host.

These three examples of HIV-1 VS demonstrate that VS play a central role in HIV cell-to-cell transmission. The benefit of VS for HIV spread is observed so far in vitro, but suggests an important function for VS in vivo.

**Virological Synapse for HTLV-1 Replication**

HTLV-1 is an oncogenic retrovirus spreading from infected T lymphocytes to uninfected T lymphocytes through VS, with little if any contribution from cell-free virions. Upon cell-to-cell
Figure 1. DC-T cell HIV-1 Virological Synapse. Left) Immature Dendritic Cells (DC) were incubated with HIV-1 for 24 hrs at 37°C. HIV-1 accumulates in an intracellular “viral endosome”. Right) Lipopolysaccharide-matured Dendritic Cells (DC) were incubated with HIV-1 for 2 hrs at 37°C. Upon encountering Jurkat CD4* T cells, HIV-1 is redistributed from this intracellular compartment to the zone of contact (infectious synapse) between the DC and the CD4* T cell (D center and right). Immunological synapse marker MHC-II (HLA-DR) does not appear enriched in the infectious synapse. (Green: Immunostaining of HIV-1 p24^*^; Red: HLA-DR; Blue: Lamp-1)

contact, HTLV-1 Env and Gag proteins polarize in the effector cell (presynaptic cell). On the post-synaptic side, talin polarizes as well at the site of cell-cell interaction and within minutes of synapse formation. Subsequently, HTLV-1 Gag protein transfer through VS is closely followed by HTLV-1 RNA genome transmission to the post-synaptic cell.10 Interestingly, HTLV-1 T cell VS shares a common feature with the CTL-mediated IS: in both cases, the microtubules organizing center (MTOC) polarizes toward the cell-cell junction within the effector cell. Recognition of the cognate peptide and engagement of the TCR are responsible for MTOC movement in the CTL-mediated IS, while in the HTLV-1-induced VS polarization occurs regardless of the potential antigen presented.10 The molecular basis underlying HTLV-1 T cell VS formation have partially been revealed. Using an antibody-coated bead-cell assay used previously to analyze T cell activation followed by interfering experiments, engagement of the intercellular adhesion molecule-1 (ICAM-1) on the effector cell (presynaptic cell) by lymphocyte function-associated antigen-1 (LFA-1) (on the postsynaptic side) was shown to be a crucial signal causing microtubules to polarize to the VS.21 VS formation is also facilitated by viral encoded proteins such as HTLV-1 transcriptional activator protein (Tax).22 Tax resides in the nucleus of unconjugated HTLV-1-infected T cells.20,23 Upon contact with naïve T cells, Tax is found at the site of contact between cells and around the MTOC, in association with the cis-Golgi apparatus.22 Transient transfection of Jurkat cells with Tax demonstrated a facilitating role for Tax in cell-cell contact-induced MTOC polarization, suggesting that Tax synergizes with ICAM-1 engagement to cause microtubule reorientation during VS formation.22 Finally, the recent identification of HTLV-1 receptor, glucose transport protein 1 (GLUT-1),104 will certainly lead to further understanding of the mechanisms involved in HTLV-1 T cell VS formation.

Emerging Role for a Plant Virological Synapse

Passage of intracellular pathogens, such as viruses, bacteria and parasites, between animal cells has been an area of intense scrutiny (reviewed in refs. 9,105,106). Thus it is likely that the concept of virological synapse or rather infectious synapse might be extended beyond animal viruses described above. Recently, the concept of synapse, including the VS has been extended to plants.35 Plant viruses are known to take advantage of plasmodesmata to gain access to the next cell.
Plasmodesmata are cytoplasmic channels formed and maintained between neighboring plant cells\(^{107,108}\) that selectively allow passage of macromolecules as well as viral particles. In a physiological context, plant synapses share limited similarities with the mammalian neuronal as well as immunological synapse, allowing plants to deal with pathogen attacks, as well as establishing symbiotic interactions, by polarizing the endocytic and secretory machineries towards the intruding organisms (reviewed in ref 35). The use of a VS-like structure in plants, implicating genetic transfer from one discrete cell to another has been recently demonstrated in the case of Tobacco Mosaic Virus (TMV), supporting the concept of VS in plants.\(^{109}\) Unlike HIV-1 DC-T cell VS that originates in tetraspanin rich multivesicular endosomes (MVB),\(^{15}\) TMV replication originates in the endoplasmic reticulum, before cell-to-cell propagation across plasmodesmata.\(^{109}\) There are significant differences between the VS of mammalian viruses when compared to VS-like structures in plants. Plasmodesmata are membrane linked pores in plant cell walls that provide continuity between adjacent cells, whereas in the immune system contacts between cells are transient and do not necessitate the formation of a pore. Nevertheless, cell-to-cell propagation of TMV through a plant VS-like structure is very reminiscent of the VS of mammalian retroviruses.

**Conclusions**

The identification and characterization of the virological synapse provides a satisfying explanation for cell-cell spread of retroviruses within the immune system. VS contribute to stealthy retroviral replication as these viruses hop from cell-to-cell across VS without possibility of neutralization by the immune system. Plant viruses use a plant VS-like structure, indicating that VS are conserved evolutionary structures facilitating replication of animal as well as plant viruses. For each virus and cellular context VS present themselves differently. Only in-depth study of VS in its various forms will provide us with a useful knowledge that may potentially allow us to interrupt cell-cell viral spread.

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