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Cell-to-cell spread of viruses *via* virological synapse

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A b s t r a c t

Virological synapse (VS) formation is an excellent example of an strategy, which viruses (mainly retroviruses) exploit for immune cell-cell spreading. VS may be defined as a virus-induced, specialized area of cell-to-cell contact that promotes the directed transmission of the virus between cells, what protect them from effector mechanisms of the host immune response. Such cell–cell viral dissemination appears to function by triggering existing cellular pathways involved in antigen presentation and T-cell communication. In this minireview, we summarize the mechanisms of VS formation in the context of human immunodeficiency virus type 1 (HIV-1) and human T-lymphotropic virus-1 (HTLV-1) infection. Despite the essential differences between VS and immunological synapse (IS), those synapses have some common features, which are also discussed.

K e y W o r d s: virological synapse, immunological synapse, HIV-1, HTLV-1

Introduction

Viruses have developed many strategies to spread from cell to cell, including actin tails formation (Boratyńska *et al.*, 2010; Doceul *et al.*, 2010), budding into the extracellular environment (Chazal and Gerlier, 2003), cells fusion and syncytium formation (Watkins *et al.*, 1997) and assembly of the virological synapse (VS) (Igakura *et al.*, 2003; Jolly and Sattentau, 2005). First detailed description of a VS was reported for the transmission of human T cell leukemia virus type 1 (HTLV-1) (Igakura *et al.*, 2003). VS is a virus induced specialized area of cell-cell contact that promotes directed transmission of the virus between cells. VS is actively induced by contact between an infected cell and another cell, which can be mobile, such as leukocytes. Formation of VS limits the exposure of the virus to hosts defense mechanisms, that's way maximizes the efficiency of transmission (Nejmeddine and Bangham, 2010).

Definition and features of VS

The VS in comprised of a stable adhesive junction that forms at the physical contact between virally infected (effector) and permissive, uninfected (target) immune cells, and contains viral antigens and cellular receptors colocalised at the conjugate interface. The great number of investigations indicates engagement of the VS assembly during HIV-1 (human immunodeficiency virus type 1), and HTLV-1 (human T-lymphotropic virus-1) infection (Jolly and Sattentau, 2004; Nejmeddine *et al.*, 2007), however, some preliminary evidence suggests, that the spread of the SARS (severe acute respiratory syndrom) coronavirus (SARS-CoV) might be due to VS formation, since a SARS-CoV–pseudotyped lentiviral vector was shown to be transferred from DCs to target cells through a structure analogous to the HIV-1 VS (Yang *et al.*, 2004).

Two types of VSs have been described in the case of HIV-1 infection: DC–T cell (McDonald *et al.*, 2003) and T cell–T cell (Jolly *et al.*, 2004). Using green fluorescent protein (GFP)-tagged HIV-1 virions, it was observed (McDonald *et al.*, 2003), that in response to contact between an HIV-1-pulsed (effector) DC and a (target) T-cell, the DC rapidly recruited virus to the conjugate interface. During antigen-independent interactions between DCs and T cells, CD4, LFA-1, CXCR4 and CCR5 were partially polarised to the interface on the T-cell and spread of HIV-1 to target cell took place across the synapse. Recruitment of HIV-1 to the synapse in the DC was resistant to protease treatment, suggesting that these viral particles resided in an internal compartment which was trafficked to the synapse. This intracellular

compartment containing HIV-1 has not been formally identified, but recent work suggests that the virus localises within multivesicular bodies (MVB) for translocation to the synapse and subsequent egress from cells *via* the exosome pathway (Jolly and Sattentau, 2004). Therefore, virus release from both the plasma membrane and MVB compartments can occur directionally into VSs (Morita and Sundquist, 2004). Recently it has been shown (Deneka *et al.*, 2007), that in macrophages virus assembled directly into compartment rich in tetraspanins CD81/CD9/CD53, and similarities between the viral compartments of DCs and macrophages suggest that the latter compartment may also be involved in virus transfer to target cells *via* virological synapse.

In T cell–T cell VS, HIV-1-infected T cells rapidly polarize viral receptors toward uninfected T cells following cell-cell contact. It has been shown (Jolly *et al.*, 2004), that the binding of CXCR4-using HIV-1–infected effector T cells to primary $CD4^+/CXCR4^+$ target T cells resulted in rapid recruitment to the cell-cell contact surface of CD4, CXC chemokine receptor 4 (CXCR4), talin, and LFA1-1 on the target cell. This was concomitant with recruitment of Env and Gag to the site of cell contact in the effector cell. This interaction of the HIV-1 Env protein on an effector cell, with CD4 and CXCR4 expressed on naive T cells, is necessary to activate actin-dependent recruitment of the viral receptors and LFA-1 into a supramolecular cluster at the site of cell-cell contact (Piguet and Sattentau, 2004). Therefore, in the case of HIV-1 there is a cytoskeleton-dependent recruitment of viral receptors and adhesion molecules into the target cell-side of the synapse. In the effector cell, the core proteins (Gag) are focussed at the synapse and antigen rapidly transfers across the synaptic junction into the target cell in a microtubule-dependent manner (Jolly and Sattentau, 2004). Directed HIV-1 budding into the synaptic junction in the T-cell-T-cell VS, what facilitated transfer of HIV-1 into the previously uninfected target cell, probably followed by fusion of virions with the target cell plasma membrane. Interestingly, only limited cell-cell fusion of infected and uninfected cells has been observed during retroviral transfer (Martin *et al.*, 2010). Polarised budding of HIV-1 in cell–cell junctions has been previously observed using electron microscopy, and implies a mechanism for targeting virus to the site of cell contact (Pearce-Pratt *et al.*, 1994; Piguet and Sattentau, 2004). Synaptic transfer between T cells may be a strategy of HIV-1 to spread effectively within secondary lymphoid organs.

During VS formation between HTLV-1 infected T lymphocytes and autologous cells, polarization of viral proteins Gag, Env and Tax in the region of contact formed with the target cell have been observed in numerous investigations (Igakura *et al.*, 2003; Nejmeddine *et al.*, 2005). HTLV-1 Tax protein acts in synergy with crosslinking of ICAM-1 on the infected cell surface, to cause polarization of the infected cell's microtubule cytoskeleton to the VS. For that reason Tax protein may have an enhancing role in VS assembly. The formation of the HTLV-1 VS between T cells is accompanied by the polarization of the MTOC in the infected cell toward the cell contact formed with the target cell, what reveals the distinction between VS and immunological synapse (IS). MTOC polarization depends on the integrity of both actin and microtubule components of the cytoskeleton and requires the activity of the small GTPases Rac1 and Cdc42 (Nejmeddine and Bangham, 2010). This pathway of polarization is distinct from that activated by TCR engagement (Nejmeddine *et al.*, 2009). Three dimensional ultrastructure of HTLV-1 VS using electron tomography revealed, that VS contains more than one synaptic cleft, spatially distinct and separated by areas of close membrane-membrane apposition. This contrasts with the IS where secretory lysosomes are secreted into a single synaptic pocket in the cytotoxic synapse (Nejmeddine and Bangham, 2010). Packets in the membrane contain enveloped viral particle, and are site of virus budding (Majorovits *et al.*, 2008). Therefore, virions may be transferred to the target cell across this clefts or/and at the periphery of the contact zone between cells (Nejmeddine and Bangham, 2010).

VS and IS similarities

Virological synapse has several common features with immunological synapse. IS forms between antigen presenting cell (APC) and effector cell (e.g. T cell, NK cell) and it is a mulitmolecular platform characterized by a central region of antigen receptors surrounded by a ring of integrin family adhesion molecules (Szulc and Niemiałtowski, 2007). Similarly to IS, at the cell-cell contact junction in the VS formed between T $CD4^+$ T cells, interaction between an adhesion molecule LFA-1 (lymphocyte function-associated antigen 1) and ICAM (intercellular adhesion molecule) occurs during HIV-1 infection (Jolly *et al.*, 2007). Those interaction stabilize the virological synapse and facilitate the transmission of HIV-1. Moreover, it has been also shown, that $CD4^+$ T cells deficient in LFA-1 or with modified LFA-1 function were less able to support VS assembly and cell-cell transfer of HIV-1. This emphasize important role of adhesion molecules in formation of the VS.

Another similarity between IS and VS is that recruitment of receptor and viral antigen to virological synapses is actin and tubulin dependent. The cell membrane, where immunological synapse is formed, consists of lipid raft membrane domains that are stabilized by actin scaffold linked to the inner leaflet of the membrane *via* adaptor proteins. Actin filaments are important for recruitment of receptors and signaling molecules to the IS and are necessary for assembly of signaling complexes, because disruption of actin filaments blocks T cell activation immediately (Dustin and Cooper, 2000). Additionally, microtubules interact with cortical actin *via* adaptors and may influence the spatial organization of actin-lipid raft complexes during cell adhesion, migration and IS formation. Meanwhile, it has been shown (Jolly *et al.*, 2007), that in HIV-1 infected T cells polarized viral assembly platforms were enriched in viral proteins Gag, Env, and ganglioside GM1 and were disrupted by interference with either the actin or tubulin cytoskeleton. Inhibition of actin and tubulin remodeling in HIV-1 infected cells interfered with cell-cell spread across a VS and reduced new viral DNA synthesis. It is therefore proposed, that HIV-1 may follow a similar microtubule-directed trajectory to gain access to the T-cell plasma membrane, from which it can then disseminate *via* cell-free or cell-cell spread (Jolly *et al.*, 2007).

Architecture of virological synapse formed between HTLV-1-infected T cell and another T cell shows analogous to the immunological synapse. It has been detected **(**Igakura *et al.*, 2003), that HTLV-I Gag protein accumulated in the cenral zone of cell-cell junction. Additionally, viral Gag proteins were surrounded by talin, the LFA1-actin linker molecule. Similar location of talin is observed in IS, where it is found in the periphery region of IS and surrounds central region of IS (Dustin and Cooper, 2000). Viral Gag protein in complex with the HTLV-I genome, appeared to be transported to the MTOC (microtubule organizing center) by a microtubule-dependent process. MTOC polarization was associated with HTLV-I infection of the T cell and was not triggered by antigen recognition. However, the MTOC polarization occurred within the HTLV-I-infected cell, not toward the infected cell. MTOC (and Golgi) orientation toward the synapse is an indication of directed secretion in the IS and may explain how viral Gag moves to the intercellular junction in the VS (Igakura *et al.*, 2003). The accumulation of mitochondria near the HTLV-1 VS also resembles the immunological synapse (Majorovits *et al.*, 2008; Stinchcombe *et al.*, 2006).

Conclusions

Virological synapse formation is an excellent strategy which allows viruses to spread from cell to cell and minimize exposure to environmental factors and effector mechanisms of immune response. Although this phenomenon has so far been described only for two retroviruses (HIV-1 and HTLV-1), we cannot exclude, that other pathogenic viruses that infect cells of the immune system use related ways of spread. Despite important differences between VS and IS, those synapses exhibit some common features. Mechanisms responsible

for assembly of VS must be investigated in details so as intervention in VS formation could function as a novel therapeutic target.

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