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Exosome release following activation of the dendritic cell immunoreceptor: A potential role in HIV-1 pathogenesis

Claude M. Mfunyi [1], Myriam Vaillancourt [1], Julien Vitry, Thy-René Nsimba Batomene, Alma Posvandzic, Alexandra A. Lambert, Caroline Gilbert *

Centre de Recherche du CHU de Québec, Faculty of Medicine, Department of Microbiology-Infectiology-Immunology, Université Laval, Québec, Canada

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ABSTRACT

Exosomes are extracellular vesicles (EVs) that play a role in intercellular communication. Stimulation of dendritic cells by the HIV-1 virus triggers their release. HIV-1 binds to dendritic cells via dendritic cell immunoreceptor (DCIR). This study shows that inhibiting the binding to DCIR significantly decreases exosome release by HIV-1-pulsed dendritic cells. In addition, exosome release from Raji-CD4 expressing DCIR cells stimulated by anti-DCIR or HIV-1 is decreased when the immunoreceptor tyrosine-based inhibition motif (ITIM) signaling motif of DCIR is mutated. Unlike the EVs released from Raji-CD4-DCIR cells after antibody stimulation, those released from HIV-1-infected cells contain the pro-apoptotic protein DAP-3. Furthermore, EVs from HIV-1 pulsed dendritic cells increase spontaneous apoptosis in uninfected CD4 T lymphocytes while they decrease it in neutrophils. This study describes for the first time that DCIR plays a role in the release of exosomes strengthening the importance of this receptor and EVs/exosomes in HIV-1 pathogenesis.

Introduction

The human immunodeficiency virus type 1 (HIV-1) is known to compromise immune defenses and lead to chronic diseases often resulting in death. The progression of HIV-1 infection towards AIDS is characterized by continuous elimination of infected CD4 T lymphocytes (CD4TL), insufficient renewal of circulating CD4TL as a result of thymic dysfunction, inflammatory damage to other lymphoid tissues (Douek et al., 1998; Elsasser et al., 2004), TGF-β-mediated fibrosis in lymph nodes (Banerjee et al., 2007; Schacker et al., 2002), and abnormal retention and hence reduced migration of effector-type T cells (Brenchley et al., 2004). This pathology is also associated with chronic systemic immune activation, including polyclonal activation of B cells (Moir and Fauci, 2009), dysregulation of neutrophil and macrophage functions (d'Ettorre et al., 2002; Roilides et al., 1990), increased T-cell activation (Hazenberg et al., 2000), and increased serum concentrations of pro-inflammatory cytokines and chemokines (Valdez and Lederman, 1997). HIV-infected individuals exhibit histological abnormalities in the gastrointestinal mucosa, malabsorption and lymphocyte depletion (Kotler et al., 1993). Several studies have shown that CD4TL are depleted first in the gastrointestinal tract during the acute phase of infection (Brenchley et al., 2004; Guadalupe et al., 2003). This is due to the virus itself (Arnoult et al., 2003), to cytotoxic activity of $CD8^+$ T cells (Sewell et al., 2000) and to cytopathic effects associated with accumulation of abortive HIV-1 reverse transcripts (Doitsh et al., 2010) or extracellular vesicles (EVs) including exosomes (Lenassi et al., 2010; Subra et al., 2011b).

EVs participate in intercellular communication by transmembrane signaling and vesicle-mediated cell-to-cell transfer of membrane receptors, proteins, mRNA, and miRNA (Nazarenko et al., 2013). Exosomes are EVs ranging from 30 to 100 nm in diameter, originating in endocytic compartments and following pathways of multivesicular bodies biosynthesis. They are produced primarily by hematopoietic, epithelial and tumor cells (Buzas et al., 2014; Robbins and Morelli, 2014). EVs or exosomes derived from B lymphocytes and dendritic cells are involved in antigen presentation to immune cells by MHC-II (Raposo et al., 1996; Thery et al., 2002a, 2002b, 1999). Those derived from dendritic cells are also

Abbreviations: DCIR, dendritic cell immunoreceptor; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; AChE, acetylcholinesterase; BK, Bradykinin; iMDDC, immature monocyte-derived dendritic cell; ITIM, immunoreceptor tyrosine-based inhibition motif; DCIR inhibitor, 1-methyl-4-[4-methyl p-NNO-azoxy] benzene; MTT, thiazolyl blue tetrazolium bromide; CD4TL, CD4 T lymphocyte

* Corresponding author. Fax: +1 418 654 2765.
E-mail address: caroline.gilbert@crchudequebec.ulaval.ca (C. Gilbert).
[1] Principal co-authors with equal contributions.

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involved in the development of immune tolerance (Peche et al., 2003; Segura et al., 2005a, 2005b), and via co-stimulatory molecules present on them, in activation of CD4TL (Thery et al., 2002a, 2002b). In some cases, they appear to contribute to the events leading from HIV-1 infection to AIDS (Lenassi et al., 2010; Subra et al., 2011b).

Early events in HIV-1 infection are major determinants of the irreversible damage inflicted on the immune system (Haase, 2010, 2011) and of the progression of infection towards AIDS. Cell-free virions do not penetrate genital epithelial cells efficiently, using instead primarily dendritic cells to cross it (Steinman et al., 2003). Dendritic cells internalize the virus and migrate to secondary lymphoid organs where they communicate with cells of the innate and adaptive immune systems (Cameron et al., 1992; Tsunetsugu-Yokota et al., 1997). HIV-1 and dendritic cells interact during primary infection via viral external envelope glycoprotein gp120 oligosaccharides and cell-bound mannose receptor (CD206), langerin (CD207), DC-SIGN (Geijtenbeek et al., 2000; Turville et al., 2003) or dendritic cell immunoreceptor (DCIR) (Bloem et al., 2014). Via their lectin receptors, dendritic cells internalize the virus in late endosomes.

The DCIR is a membrane-bound glycoprotein, member of the C-type lectin family. It is expressed on the surface of cells of the myeloid lineage, including dendritic cells, neutrophils, monocytes and macrophages, on B lymphocytes (Bates et al., 1999), CD4TL from HIV-1 patients (Lambert et al., 2010) and on CD4 and CD8 T lymphocytes of arthritic patients (Eklow et al., 2008). Previous studies have shown that dendritic cells or apoptotic CD4TL bind HIV-1 via DCIR and that this leads to both *trans* and *cis* infection of CD4TL (Lambert et al., 2008, 2010). In addition, a series of DCIR inhibitors of small molecular mass have been identified and shown to inhibit HIV-1 interactions with dendritic and CD4TL cells (Lambert et al., 2013). The DCIR structure includes an extracellular ligand-binding domain consisting of a carbohydrate recognition domain (CRD), EPS motif (Glu-Pro-Ser), neck domain, and an immunoreceptor tyrosine-based inhibition motif (ITIM) involved in intracellular signaling and internalization (Drickamer, 1993; Kanazawa et al., 2004; Richard et al., 2003). DCIR ITIM motif is involved in the modulation of cellular responses (Kanazawa, 2007) and plays a role in transducing signals that inhibit leukocyte activation mediated by tyrosine phosphatases SHP-1 and SHP-2 (Richard et al., 2006). Activation of DCIR by specific antibodies promotes its internalization in endosomes, leading to inhibition of pro-inflammatory cytokine synthesis (IL-12, TNF and IFN-α) induced by TLR9 and TLR8 activation (Meyer-Wentrup et al., 2008, 2009).

Many physical and biochemical similarities between exosomes and HIV-1 have been reported, as has the coincidence of exosome dissemination with the capture and transfer of HIV-1 particles by dendritic cells (Izquierdo-Useros et al., 2009). HIV-1 biogenesis and release through the normal cell-encoded exosome or vesicle biogenesis pathway was first suspected several years ago (Nguyen et al., 2003; Pelchen-Matthews et al., 2004). In dendritic cells and macrophages, exosomes are formed by reverse budding inside endosomal compartments called multivesicular bodies and released into the extracellular environment by fusion of the latter with the cell membrane (Pelchen-Matthews et al., 2004; Thery et al., 2002a, 2002b). The similarity between exosomes and viral particle budding (Izquierdo-Useros et al., 2011; Ostrowski et al., 2010) suggests that some sharing of the cellular machinery is involved. Some observations indicate that budding takes place mainly at CD81-, CD9- and CD63-rich invaginated micro-domains in the plasma membrane, which are similar to endosomal domains. Exosomes can contain viral material, miRNA of TAR (Narayanan et al., 2013), Gag p17 (Columba Cabezas and Federico, 2013) or accessory proteins (Lenassi et al., 2010). Presence of Nef in exosomes has been shown to cause activation-induced death of resting peripheral blood lymphocytes in vitro (Lenassi et al., 2010). It has also been shown that HIV-1-loaded dendritic cells release exosomes that induce apoptosis in CD4TL (Subra et al., 2011b).

Fusion of endosomal compartments with lysosomes allows degradation of their content or, alternatively, biogenesis of intraluminal vesicles, which later merge with the plasma membrane to release exosomes and/or virions. The latter process appears to lead to *trans* infection of CD4TL (Izquierdo-Useros et al., 2010). At least one recent study indicates that HIV-1 infection increases exosome secretion by dendritic cells (Subra et al., 2011b). However, it is not yet known if increased exosomes amount by HIV-1-infected cells is DCIR-dependent. The aim of the present study was to examine the involvement of DCIR and its signaling pathways in the release of exosomes from HIV-1-infected cells into the extracellular environment. Our results suggest significant roles for DCIR and the ITIM motif in exosome production and show that EVs and exosomes released by HIV-1-infected cells can intervene in the apoptosis pathway, with opposite effects on CD4TL and neutrophils.

Results

Impact of the DCIR-directed inhibitor on exosome release by iMDDCs

Several inhibitors of binding to DCIR have been identified using the 3-D structure of the receptor (Lambert et al., 2013). Among these, we chose 1-methyl-4-[4-methyl p-NNO-azoxy] benzene (DCIR inhibitor), known to bind to the EPS motif. We studied the impact of this DCIR inhibitor on HIV-1 infection of iMDDCs. Panel A in Fig. 1 shows that pre-incubating iMDDCs with 1, 5, 10 and 50 μM of inhibitor decreases viral binding by about, respectively, 20%, 60%, 70% and 80%. The impact of the DCIR inhibitor at a concentration of 10 μM is significant until 10 days post-infection (Fig. 1, panel B), without affecting cellular viability as previously described (Lambert et al., 2013) and illustrated in Supplementary Fig. 1.

We demonstrated in our previous studies that the exosome release from iMDDCs is increased following HIV-1 cell pulsing and that these exosomes have a deleterious effect on neighboring CD4TL (Subra et al., 2011a, 2011b). However, the role of DCIR in exosome release from HIV-1-pulsed-dendritic cells remains unknown. Based on measurements of hydrodynamic size of nanoparticles using the Zetasizer Nano-ZS, we confirmed the presence of homogenous vesicles with an average diameter of 100 nm, which match the known size range of exosomes (see Supplementary data for a typical example, Fig. 2). We quantified exosomes by measuring AChE activity of velocity gradient fractions as described previously by us (Cantin et al., 2008) (Fig. 2). We show that NL4-3Bal*env* increases exosome release from iMDDCs, while pre-incubating them with the DCIR inhibitor decreases this response. In order to validate the effect of the DCIR inhibitor, we performed the test using a different R5-tropic virus, NLAD8 (Supplementary data, Fig. 3). In conclusion, we confirm that the exosome release by dendritic cells is increased in the presence of HIV-1 and is decreased when the cells are pre-treated with the DCIR inhibitor.

In order to provide additional support for the link between HIV-1, exosome release, DCIR and its signaling motif, exosomes were collected from the extracellular medium after 5 days of culture of Raji-CD4-DCIR cells exposed to NL4-3 virions. (Zetasizer Nano-ZS analysis of our preparation quality is provided in Supplementary Fig. 2). Based on results obtained by AChE assay, we show that the contact with HIV-1 increases exosome release, while the inhibitor suppresses this response (Fig. 3A). To determine whether or not the ITIM signaling motif plays a role in HIV-1 binding, Raji-CD4-DCIR with either normal ITIM motif or its mutant form (tyrosine substituted for phenylalanine; cell-line designated as