Virology 482 (2015) 189–201

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Exosomes in human semen restrict HIV-1 transmission by vaginal cells and block intravaginal replication of LP-BM5 murine AIDS virus complex

Marisa N. Madison [a], Philip H. Jones [a], Chioma M. Okeoma [a,b,*]

[a] Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242-1109, USA
[b] Interdisciplinary Program in Molecular and Cellular Biology, University of Iowa, Iowa City, IA 52242, USA

ARTICLE INFO

Article history:
Received 15 December 2014
Returned to author for revisions
20 January 2015
Accepted 23 March 2015

Keywords:
Exosome
Semen
Vaginal epithelia
HIV
Murine AIDS
mAIDS
LP-BM5 virus

ABSTRACT

Exosomes are membranous extracellular nanovesicles secreted by diverse cell types. Exosomes from healthy human semen have been shown to inhibit HIV-1 replication and to impair progeny virus infectivity. In this study, we examined the ability of healthy human semen exosomes to restrict HIV-1 and LP-BM5 murine AIDS virus transmission in three different model systems. We show that vaginal cells internalize exosomes with concomitant transfer of functional mRNA. Semen exosomes blocked the spread of HIV-1 from vaginal epithelial cells to target cells in our cell-to-cell infection model and suppressed transmission of HIV-1 across the vaginal epithelial barrier in our trans-well model. Our *in vivo* model shows that human semen exosomes restrict intravaginal transmission and propagation of murine AIDS virus. Our study highlights an antiretroviral role for semen exosomes that may be harnessed for the development of novel therapeutic strategies to combat HIV-1 transmission.

Introduction

Exosomes originate as a result of inward budding of endosomal membranes within the late endosomal compartment of many cell types (Gruenberg and Stenmark, 2004). Multivesicular bodies (MVB) are generated from this mechanism of inward budding with a large repertoire of exosomes within them (Gruenberg and Stenmark, 2004). Exosomes are endogenous carriers of genetic and proteinaceous cargo (Madison et al., 2014; Schorey and Bhatnagar, 2008; Simpson et al., 2008; Vojtech et al., 2014b, a) and they can deliver their cargo (Alvarez-Erviti et al., 2011) through endocytosis or fusion with target cells (Li et al., 2013; Nanbo et al., 2013; Newton et al., 2006; Orth et al., 2002; Skog et al., 2008; Sun et al., 2010) resulting in the conditioning of target cells. Thus, exosomes have been implicated in intercellular communication (Danzer et al., 2012; Demory Beckler et al., 2012), modulation of immune response (Kelly et al., 1991; Li et al., 2013), and regulation of microbial pathogenesis (Delorme-Axford et al., 2013; Khatua et al., 2009; Li et al., 2013; Madison et al., 2014). For example, exosomes derived from H9 cells, a T lymphocyte cell line, contain the antiviral factor, Apobec3G, which endows H9 exosomes the ability to partially protect recipient cells from HIV-1 infection (Khatua et al., 2009). Similar to H9 exosomes, semen exosomes (SE) from healthy human semen contain antiviral mRNA and was shown to inhibit infection of target cells by retroviruses, such as murine AIDS virus complex LP-BM5 and HIV-1 (Madison et al., 2014).

Subsequent to crossing the host genital mucosa entry portal, sexually transmitted Retroviridae including SIV, MLV, and HIV-1 utilize various mechanisms to gain a foothold and establish persistent infection in the host. Worldwide, HIV/AIDS is a pandemic with about 35.0 million people living with HIV in 2013. HIV-1 is predominantly sexually transmitted between discordant sexual partners and semen is the principal vector (Byrn et al., 1997; Clumeck et al., 1989; Royce et al., 1997; Vernazza et al., 1999). The genital tract may serve as a haven for HIV-1 to undergo selective pressures (Byrn et al., 1997; Delwart et al., 1998; Kroodsma et al., 1994; Taylor et al., 2003). Such pressure may facilitate the evolution of drug resistant HIV-1 variants, which are subsequently

Abbreviations: HIV-1, human immunodeficiency virus type 1; AIDS, acquired immunodeficiency syndrome; mAIDS, murine acquired immunodeficiency syndrome; SIV, simian immunodeficiency virus; SE, semen-derived exosomes; PBMC, peripheral blood mononuclear cells; IVag, intravaginal; FRT, female reproductive tract; RT, reverse transcriptase; A3, APOBEC3, apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3; PBL, peripheral blood leukocytes; BE, blood exosomes; LIPO, liposomes

* Correspondence to: Department of Microbiology, Carver College of Medicine, University of Iowa, 51 N Road, Iowa City, IA 52242-1109, USA. Office #Tel.: +1 319 335 7906; Lab Tel.: +1 319 335 7612; fax: +1 319 335 9006.
E-mail address: chioma-okeoma@uiowa.edu (C.M. Okeoma).

http://dx.doi.org/10.1016/j.virol.2015.03.040
0042-6822/

sexually transmitted (Boden et al., 1999; Hecht et al., 1998; Little et al., 1999). It has been shown that HIV-1 patients whose viremia is persistently suppressed by antiretroviral therapy shed viral RNA into the genital tract (Cu-Uvin and Caliendo, 2011; Cu-Uvin et al., 2010), suggesting that shedding of HIV-1 in semen can occur in the absence of detectable viremia. However, sexual transmission of HIV-1 requires a high number of exposures (O'Brien et al., 1994; Varghese et al., 2002), an indication that not all viral particles in semen retain infectivity or that some anti-HIV-1 activity is operative in the mucosa or semen.

Despite decades of research, the component of semen that enhances or decreases efficacy of sexual transmission of HIV-1 is not fully understood. Previously, we showed that exosomes purified from the semen of healthy human donors inhibit replication of HIV-1 and LP-BM5 murine AIDS virus complex (mAIDS), but do not have an effect on propagation of Herpes simplex virus types -1 and -2 (HSV-1 and HSV-2) (Madison et al., 2014), suggesting that the antiviral effect of SE may be retroviral specific.

In this study, we report the role of SE on retroviral transmission using *in vitro* and *in vivo* models. We show that SE are internalized by human vaginal epithelial cells *ex vivo* via endocytosis and by murine vaginal cells *in vivo*. In addition, SE is internalized by HIV susceptible T (SUPT1) and monocytic (U937) cell lines. Functionally, SE blocked *trans* infection and cell-to-cell spread of HIV-1 and potently protected mice from intravaginal infection and propagation of mAIDS. Furthermore, our data show that SE-mediated inhibition of retroviral propagation involves impairment of viral RNA reverse transcription process necessary for synthesis of nascent viral copy DNA required for establishing persistent infection. Thus, our data identify SE as a critical factor that may reduce efficacy of sexually transmitted retroviruses, suggesting new opportunities for the development of therapeutics against such viruses.

Results

Human vaginal epithelial cells internalize semen-derived exosomes

To examine whether cells of the female reproductive tract (FRT) internalize SE, E6/E7 transformed human vaginal epithelial cells (V428) (Peterson et al., 2005) were exposed to PKH67Green-labeled SE for 3 h followed by confocal microscopy (Fig. 1A). V428 cells predominantly exhibited a punctate SE staining pattern, but some diffuse staining was also observed (Fig. 1A). PKH67Green was not transferred to V428 cells co-incubated with vehicle control (Fig. 1B). To examine whether differences exist in the ability of transformed and primary vaginal epithelial cells to internalize SE, we labeled primary human vaginal epithelial cells V428 with PKH26Red and co-incubated the cells with PKH67Green-labeled SE. At 3 h post-exposure, confocal microscopy indicated that PKH26Red-labeled V428 cells internalized PKH67Green-labeled SE (supplemental Fig. 1A) and exhibited more diffuse SE staining and less punctate SE staining pattern. Similar to transformed V428 cells, PKH67Green was not transferred to V428 cells co-incubated with vehicle control (supplemental Fig. 1B), signifying that exosome labeling and internalization was specific. These results show that both fusion between SE and the V428 plasma membrane as well as V428 cellular uptake of intact SE occur in primary and transformed vaginal epithelial cells with some minor differences. Since both primary and transformed V428 cells take up SE, transformed V428 cells were utilized for the remainder of the study due to ease of culturing/availability.

To confirm that SE are incorporated and retained within cells rather than at the cellular surface, V428 cells exposed to increasing concentrations of PKH67Green-labeled SE for 24 h were trypsinized. FACS analysis was used to enumerate the level of SE uptake by trypsinized V428 cells post exposure to SE (Fig. 1C). To confirm that vaginal epithelial cells take up SE, we utilized VK2 cells, another immortalized human vaginal epithelial cell line. FACS analysis show that, similar to V428 cells, VK2 cells incorporate PKH67Green-labeled SE but not PK67Green-labeled vehicle in a time (1 h, 3 h, and 6 h post exposure) dependent manner (Fig. 1D).

To compare V428 cell uptake efficiency of SE with other types of vesicles, we incubated V428 cells with 25 μg/ml of PKH67Green-labeled blood exosomes (BE), liposomes (LIPO), or SE (Fig. 1E). FACS analysis after 24 h incubation reveal that SE was most efficiently taken up (MFI 119), compared to LIPO (MFI 85.2) and BE (MFI 4.4) in that order (Fig. 1E). A similar pattern of uptake efficiency was observed with VK2 vaginal epithelial cells (Fig. 1F). Increases in MFI across all samples in VK2 cells (Fig. 1F) compared to V428 cells (Fig. 1E) may indicate that VK2 cells more efficiently incorporate vesicles than V428 cells.

To further demonstrate that SE are internalized by vaginal epithelial cells, we pre-treated V428 cells with inhibitors of endocytosis (Dynasore) or macropinocytosis (EIPA). Treatment with Dynasore but not EIPA inhibited internalization of PK67Green-labeled SE (Fig. 1G), suggesting that the majority of SE uptake by V428 cells may occur via endocytosis. In all experiments, internalization of SE by vaginal epithelial cells was shown to be specific as PKH67Green-labeled vehicle was not taken up (Fig. 1B–G and supplemental Fig. 1B).

Semen-derived exosomes inhibit cell-to-cell spread of HIV-1

During mucosal HIV-1 infection, it is likely that epithelial cells in the FRT are the first cells to come into contact with HIV-1. It is still debatable whether or not these cell types support active HIV-1 infection (Asin et al., 2004). Although epithelial cells of the FRT may or may not support active HIV-1 infection, they have been shown to transmit infectious virus to target cells (Stoddard et al., 2007).

To evaluate the role of SE on direct cell-to-cell spread of HIV-1 from vaginal V428 cells to target cells of infection, as a way to model infection during sexual transmission, we first sought to determine the properties of the vaginal V428 cells with respect to HIV-1 primary receptor CD4 and co-receptors CCR5 or CXCR4. RT-qPCR analysis show that in comparison to CD4+CCR5+ monocytic cells (U937) or CD4+CXCR4+ T lymphocytes (SupT1), V428 cells have little or no CD4 (Fig. 2A), CCR5 (Fig. 2B), and CXCR4 (Fig. 2C) encoding mRNA.

Since V428 cells internalize SE, we evaluated the ability of HIV-1 target monocytic (U937) and lymphocytic (SUPT1) cells to incorporate and retain SE. Thus, U937 and SupT1 cells were exposed to PKH67Green-labeled PBS or SE for 3 h or 24 h. Following trypsinization and PBS washing, FACS analysis confirms that SE are incorporated and retained within U937 (Fig. 2D) and SUPT1 (Fig. 2E) cells. Although both cell lines internalized SE, differences exist in level of SE incorporation, which was more efficient in U937 cells and increased with time from 3 h (MFI 7.79) to 24 h (MFI 10.8) post exposure (Fig. 2D). In contrast, SUPT1 cells did not internalize SE efficiently and there were no differences in incorporation rate at 3 h (MFI 6.23) and 24 h (MFI 6.39) post exposure (Fig. 2E). These data are consistent with a previous report showing that T lymphocytes are inefficient at incorporation of exosomes (Chivero et al., 2014). Given the differences in SE internalization between U937 and SUPT1 cells, we assessed whether such differences exist in internalization of other vesicles including BE and LIPO (used as positive control). FACS analysis reveal that U937 (supplemental Fig. 2C) and SUPT1 (supplemental Fig. 2D) cells incorporated BE and LIPO more efficiently than SE at 24 h post exposure. Similar to SE, SUPT1 cells incorporated lower BE (MFI 39.3)