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DC-SIGN plays a stronger role than DCIR in mediating HIV-1 capture and transfer

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ABSTRACT

The C-type lectin receptors (CLRs) expressed on dendritic cells (DCs), in particular DC-SIGN and DCIR, likely play an important role in HIV-1 early infection. Here, we systematically compared the capture and transfer capability of DC-SIGN and DCIR using a wide range of HIV-1 isolates. Our results indicated that DC-SIGN plays a stronger role than DCIR in DC-mediated HIV-1 capture and transfer. This was further strengthened by the data from transient and stable transfectants, showing that DC-SIGN had better capability, compared with DCIR in HIV-1 capture and transfer. Following constructing and analyzing a series of soluble DC-SIGN and DCIR truncates and chimeras, we demonstrated that the neck domain, but not the CRD, renders DC-SIGN higher binding affinity to gp120 likely via the formation of tetramerization. Our findings provide insights into CLR-mediated HIV-1 capture and transfer, highlighting potential targets for intervention strategies against gp120-CLR interactions.

Introduction

The majority of HIV-1 infections are acquired by mucosal exposure, with sexual transmission as the leading mode of HIV-1 infection worldwide (Haase, 2010; Royce et al., 1997). The anatomical distribution of dendritic cells (DCs) in the genital and anal mucosa (Edwards and Morris, 1985; Miller et al., 1992) together with the findings from *in vitro* studies (Masurier et al., 1998; Taruishi et al., 2004; Wiley and Gummuluru, 2006) inform that immature DCs are likely to be one of the first cell types targeted by HIV-1 (Harman et al., 2013; Hu et al., 2000; Wu and KewalRamani, 2006). Myeloid DCs are professional antigen presenting cells that can capture microorganisms in the peripheral mucosal tissues and then migrate to secondary lymphoid organs, where they present these in antigenic form to resting T cells and thus initiate adaptive immune responses (Banchereau et al., 2000; Banchereau and Steinman, 1998; Valitutti et al., 1995). Such process could be hijacked by HIV-1 to deliver itself from the mucosa to the secondary lymphoid organs, described as *in trans*-transmission or the "Trojan horse" model (Cameron et al., 1992; Izquierdo-Useros et al., 2010; Wiley and Gummuluru, 2006). In addition, DCs can also be directly infected by HIV-1 and transfer progeny viruses to $CD4^+$ T cells, named *in cis*-transmission (Nobile et al., 2005; Turville et al., 2004). Given that HIV-1/SIV-infected DCs are rarely detected *in vivo* (Spira et al., 1996; Steinman et al., 2003), extensive *in vitro* studies imply that *trans*-infection likely plays a more important role in HIV-1 early infection and dissemination (McDonald, 2010; Piguet and Steinman, 2007).

The receptor CD4 and co-receptors CCR5 and CXCR4 utilized by HIV-1 for infection are lowly expressed on DCs (Granelli-Piperno et al., 1996; Turville et al., 2001). In contrast, the expression of C-type lectin receptors (CLRs) on DCs is relatively high (Turville et al., 2003). CLRs share a common sequence motif indicative of similarly folded carbohydrate-recognition domain (CRD), which can recognize carbohydrates in a calcium-dependent manner (Feinberg et al., 2005; Zelensky and Gready, 2005). To date, a range of CLRs have been shown to bind HIV-1 gp120, the heavily glycosylated envelope protein with carbohydrates accounting for as much as 50% of its mass (Quinones-Kochs et al., 2002; Soilleux et al., 2002; Turville et al., 2003). Among the CLRs reported, DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) and DC immunoreceptor (DCIR), can bind gp120 and consequently enhance *trans*- and *cis*-infection of HIV-1 (Baribaud et al., 2001; Geijtenbeek et al., 2000; Lambert et al., 2008). DC-SIGN, originally cloned from a placental cDNA library as a gp120 binding protein with a greater affinity than CD4 (Curtis et al., 1992), binds preferentially to N-linked high mannose glycans on

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gp120, resulting in DC-mediated HIV-1 *in trans* and *in cis* infections by affecting viral internalization and immune evasion (Cambi et al., 2009; Pohlmann et al., 2001; van den Berg and Geijtenbeek, 2013). On the other hand, gp120 is also reported to be recognized by DCIR, a novel type II transmembrane molecule of the CLR family containing a consensus intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM) (Bates et al., 1999; Lambert et al., 2008). DCIR expression on $CD4^+$ T cells can be induced by HIV-1, and the ITIM-associated signal transduction pathway is necessary in DCIR-mediated enhancement of HIV-1 infection (Lambert et al., 2010, 2011). However, the carbohydrate structure on gp120 recognized by DCIR is unclear (Lambert et al., 2013). Furthermore, the importance of DC-SIGN in HIV-1 infection remains controversial (Boggiano et al., 2007; da Silva et al., 2011; Gummuluru et al., 2003). Given that DC-SIGN and DCIR both function in gp120 binding, we asked whether these two CLRs contribute equally or one over the other in DC-mediated HIV-1 capture and transfer.

Here we systematically compared the capability of DC-SIGN and DCIR in HIV-1 capture and transfer at cellular and biochemical levels. Our results indicate that both DC-SIGN and DCIR can capture and transfer HIV-1 independent of viral isolates, with DC-SIGN showing better capability than DCIR. Further study using DC-SIGN and DCIR truncates and chimeras reveal that the tetramerization structure likely plays an important role in the DC-SIGN-mediated enhancement of HIV-1 capture and transfer.

Results

Knockdown of DC-SIGN expression on iMDDCs significantly decreases HIV-1 capture and transfer

To address the proportional contribution of DC-SIGN and DCIR in DC-mediated HIV-1 capture and transfer, we used immature monocyte derived dendritic cells (iMDDCs) as a model. iMDDCs have been widely used in studies to mimic DCs and may also have *in vivo* relevance. For instance, monocytes were observed to develop into iMDDCs at sites of inflammation as a second recruitment of antigen-presenting cells (Randolph et al., 1999). After the differentiation of monocytes into iMDDCs, we analyzed the expression levels of HIV-1 related receptors by FACS. Consistent with results from previous studies by others (Turville et al., 2001), the expression of CD4 and CCR5 on iMDDCs was very low, while the levels of C-type lectin receptors (CLRs), including DC-SIGN and DCIR, were relatively high (Fig. 1A). To investigate the contribution of DC-SIGN and DCIR in HIV-1 capture and transfer, DC-SIGN or DCIR specific siRNA was used to knockdown the expression of corresponding CLRs. After specific siRNA treatment, the surface expression of DC-SIGN and DCIR decreased to a similar percentage level, with approximately 40% of expression by mean fluorescence intensity (MFI) or 35% of expression by relative value to β-actin compared to control groups (Fig. 1B–D). Results from HIV-1 capture assay showed that iMDDCs with DC-SIGN or DCIR knockdown both demonstrated decreased capability in capturing HIV-1 BaL, showing ~60% and ~40% reduction in mediating HIV-1 capture, respectively (Fig. 1E). Although results slightly differed among different donors, the trends were similar, showing that iMDDCs with DC-SIGN knockdown had less capability in HIV-1 capture than those with DCIR knockdown (Fig. S1). Similar results were observed in HIV-1 transfer experiments (Fig. 1F).

DC-SIGN demonstrates stronger capability than DCIR in HIV-1 capture and transfer

To investigate the capability of DC-SIGN and DCIR in mediating HIV-1 capture and transfer, 293T and QT6 cells transiently expressing DC-SIGN or DCIR were initially used for HIV-1 capture and transfer experiments. DC-SIGN and DCIR expressions on 293T and QT6 cells at the time of assay were determined by FACS, showing that the surface expression levels of the two CLRs were similar (Fig. 2A). Considering that antibody used for DCIR detection may have a different affinity to that used for DC-SIGN detection, we further confirmed the expression of DC-SIGN and DCIR by engineering flag labeled constructs. Flag was introduced to the N terminal of DC-SIGN and DCIR, and the expression was determined by western blotting using anti-flag antibody. Consistent with the results from FACS, western blotting data showed that the two proteins were expressed at similar levels on all the tested cell lines (Fig. S2). Following expression determination, 293T and QT6 cells expressing DC-SIGN or DCIR were used to examine their capability in HIV-1 capture and transfer. As shown in Fig. 2B and C, both DC-SIGN and DCIR, regardless of cell types, demonstrated the capability in capturing and transferring HIV-1 BaL. The effect could be blocked by mannan (Fig. 2) while kifunensine (Eggink et al., 2010) enhanced the effect of DC-SIGN but not DCIR in HIV-1 capture (data not shown), indicating a specific interaction between CLR and the glycans of HIV-1 Env. Moreover, DC-SIGN possessed considerably stronger ability than DCIR in both capture and transfer experiments. Further assessment showed that this capability of HIV-1 capture by CLR-expressing cells was dose dependent and a specific interaction between CLRs and HIV-1 Env was necessary (Fig. S3).

We next confirmed the results using DC-SIGN or DCIR stably expressing Raji cells. Raji cell line is a CD4 and lectin-negative human B-cell line, and Raji-DC-SIGN has been widely used in previous studies (Wu et al., 2004). In the current study, we generated DCIR-expressing stable cell line. DC-SIGN and DCIR were expressed at comparable levels on stable Raji cells as assessed by FACS (Fig. 2A). The results in HIV-1 capture and transfer assays were consistent with those obtained from 293T and QT6 cells, revealing that both DC-SIGN and DCIR possessed the ability in capturing and transferring HIV-1 BaL, with the capability of DC-SIGN higher than that of DCIR (Fig. 2D).

DC-SIGN is more efficient than DCIR in capturing a wide range of HIV-1 isolates

After demonstrating that DC-SIGN and DCIR are both capable of capturing and transferring HIV-1 BaL, with DC-SIGN possessing better capability than DCIR, we further conducted capture experiments using a wide range of HIV-1 isolates including laboratory-adapted strains, primary isolated as well as transmitted/founder (T/F) Env-pseudotyped and infectious viruses, specified as follows: IIIB, a laboratory-adapted X4 clone; MWS2 *env*, cloned from semen of a subject known to have infected women by vaginal intercourse; CH811 *env*, cloned from a blood sample isolated from a Chinese patient; and T/F HIV-1 clones, isolated very early after transmission and thought to be more physiologically relevant than laboratory-adapted strains (Keele et al., 2008). As shown in Fig. 3, in general, both DC-SIGN and DCIR expressing Raji cells were capable of capturing the tested HIV-1 isolates, with DC-SIGN demonstrating much stronger capability than DCIR, although slight differences were observed when different viruses were tested. Moreover, DC-SIGN, but not DCIR, seemed to have better capability in capturing HIV-1 infectious viruses than Env-pseudotyped viruses (Fig. 3B). Of note, DCIR-expressing cells failed to show significant increase in capturing several T/F strains (Fig. 3C), compared to the parental Raji cells. In addition, iMDDCs with DCIR knock-down showed large (BaL), moderate (CH042.C) and small (REJO.D12.1972) reduction in HIV-1 capture. In contrast, DC-SIGN knock-down severely impaired the capture of all the three tested viruses by iMDDCs (Fig. S4). Taken together, results