Antiviral Research 83 (2009) 61–70

Contents lists available at ScienceDirect

Antiviral Research

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

Capture and transmission of HIV-1 by the C-type lectin L-SIGN (DC-SIGNR) is inhibited by carbohydrate-binding agents and polyanions

Joeri Auwerx[a], Katrien O. François[a], Els Vanstreels[a], Kristel Van Laethem[b], Dirk Daelemans[a], Dominique Schols[a], Jan Balzarini[a,*]

[a] Virology and Chemotherapy, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium
[b] Clinical and Epidemiological Virology, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 14 October 2008
Received in revised form 11 March 2009
Accepted 23 March 2009

Keywords:
L-SIGN
DC-SIGN
HIV
Virus transmission
Carbohydrate-binding agents (CBA)
Polyanions

ABSTRACT

It was recently shown that capture of HIV-1 by DC-SIGN-expressing cells and the subsequent transmission of HIV to $CD4^+$ T-lymphocytes can be prevented by carbohydrate-binding agents (CBAs), whereas polyanions were unable to block virus capture by DC-SIGN. In this study, we could show that a short pre-exposure of HIV-1 to both mannose- and *N*-acetylglucosamine (GlcNAc)-specific CBAs or polyanions dose-dependently prevented virus capture by L-SIGN-expressing 293T-REx/L-SIGN cells and subsequent syncytia formation in co-cultures of the drug-exposed HIV-1-captured 293T-REx/L-SIGN cells and uninfected C8166 $CD4^+$ T-lymphocytes. Additionally, the inhibitory potential of the compounds against L-SIGN-mediated HIV-1 capture and transmission was more pronounced than observed for DC-SIGN-expressing 293T-REx/DC-SIGN cells. The excess value of CBAs and polyanions to prevent HIV-1 capture and transmission by DC-SIGN and L-SIGN-expressing cells to susceptible T-lymphocytes could be of interest for the development of new drug leads targeting HIV entry/fusion.

1. Introduction

The human immunodeficiency virus (HIV) is one of the major human infectious pathogens with more than 33 million infected individuals. Since the first onset of the epidemic, some 25 million people have died of AIDS-related illnesses worldwide (UNAIDS, 2008). HIV establishes a persistent infection that is characterized by variable viremia and by an escape from the immune system through several strategies, e.g. continuous antigenic variation, downregulation of host MHC molecules and the destruction of infected and uninfected cells through apoptosis (Gougeon, 2003). Currently there is no curative treatment or vaccine available for HIV infection (Letvin, 2006).

The most common route for HIV-1 infection is through sexual transmission across the genital mucosa. Langerhans cells (LCs) that specifically express the C-type lectin langerin and dendritic cells (DCs) expressing the specific intercellular adhesion molecule-3 (ICAM-3)-grabbing nonintegrin (DC-SIGN or CD209) are part of the innate immune system and are present in mucosal tissues (de Witte et al., 2007). LCs reside in the epidermis of the skin and in most mucosal epithelia, whereas DC-SIGN-expressing DCs exist in the subepithelium layer (Geijtenbeek et al., 2000a; de Witte et al., 2007; Patterson et al., 2002). DCs in the mucosal tissues are thought to transmit HIV-1 to T-cells through capture of the virus particles by DC-SIGN (Geijtenbeek et al., 2000b; Turville et al., 2001, 2002).

DC-SIGN is a 44 kDa type II integral membrane protein with a short amino-terminal cytoplasmic domain and a carboxyl-terminal C-type (i.e. calcium-dependent) lectin domain (Geijtenbeek et al., 2000a). When the mucosal tissue is exposed to HIV-1, the viral surface glycoprotein gp120 will specifically bind to the DC-SIGN-expressing DCs that subsequently transport HIV to the draining lymph node, thereby facilitating infection of susceptible target cells *in trans* (Geijtenbeek et al., 2000b).

A significant proportion of the heavily glycosylated surface glycoprotein gp120 is highly (terminally) mannosylated (Geyer et al., 1988; Leonard et al., 1990). It is assumed that the variability of glycosylation of the HIV-1 gp120 envelope surface modulates the immunogenicity of gp120 as this glycoprotein is the main target for neutralising antibodies during HIV-1 infection. The continuous change in the glycan shield enables the virus to persist the presence of the evolving antibody repertoire (Wei et al., 2003).

Liver-specific ICAM-3-grabbing nonintegrin (L-SIGN), DC-SIGN-related (DC-SIGNR) or CD209L has functional similarity in its interactions with ICAM-3 and HIV-1 (Bashirova et al., 2001; Geijtenbeek et al., 2002; Pöhlmann et al., 2001b). The sequences of DC-SIGN and DC-SIGNR are 88% identical, with 93% identity in the neck domains, 79% identity in the carbohydrate-recognition domains (CRDs), and 63% identity in the cytoplasmic tail and

* Corresponding author.
E-mail address: jan.balzarini@rega.kuleuven.be (J. Balzarini).

0166-3542/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2009.03.011

transmembrane domains. It is interesting that apparently minor sequence differences in each of these regions have important functional consequences, leading to differences in sugar-binding specificity and intracellular trafficking (Yu et al., 2009). Endothelial cells, but not DCs, of the liver and lymph nodes express high levels of L-SIGN. Similar to DC-SIGN, L-SIGN can bind to mannose residues of viral glycoproteins, for example HIV-1 gp120 and Hepatitis C virus (HCV) E2, through a C-terminal carbohydrate-binding domain (Feinberg et al., 2001; Gardner et al., 2003; Lozach et al., 2004, 2003; Pöhlmann et al., 2003). The oligomerisation of the extracellular domain of both the L-SIGN and DC-SIGN receptors was shown to be important for high affinity binding of these viral glycoproteins (Feinberg et al., 2001; Guo et al., 2004; Mitchell et al., 2001). Thus, L-SIGN and DC-SIGN function as capture receptors for HIV and HCV and may fulfil critical roles in viral pathogenesis and cell tropism. However, the real physiological functions of L-SIGN still remain unclear.

Carbohydrate-binding agents (CBAs) that directly interact with the intact viral envelope glycans compromise the efficient entry of the virus into its susceptible target cells. In addition CBAs could force the virus to delete parts of its glycan shield to escape CBA drug pressure, which might trigger an immune response against previously hidden uncovered immunogenic epitopes (Balzarini, 2005; Balzarini, 2007). Recently it was shown that the capture of HIV-1 by DC-SIGN and subsequent transmission to $CD4^+$ T-lymphocytes and macrophages was also efficiently prevented by CBAs (Balzarini et al., 2007a; Turville et al., 2005). Also, CBAs could block capture of HIV-1 particles by the macrophage mannose receptor (MMR) present on primary monocyte-derived macrophage cell cultures (Pollicita et al., 2007). In addition, CBAs were proven to efficiently block the entry of HCV into its target cells (Helle et al., 2006; Bertaux et al., 2007).

Given the different location and possibly also the different physiological function of L-SIGN *versus* DC-SIGN, we extended our studies on the inhibitory activity of CBAs against DC-SIGN-directed HIV-1 capture and transmission to L-SIGN. We showed that different classes of CBAs but also polyanions could prevent the capture and transmission of HIV-1 to T-lymphocytes by L-SIGN-expressing cells (293T-REx/L-SIGN). In addition, we confirmed that CBAs efficiently inhibit the capture and transmission of HIV-1 to T-lymphocytes by DC-SIGN-expressing cells (293T-REx/DC-SIGN). The significant inhibition of L-SIGN-mediated HIV-1 capture and transmission by polyanions show that its mode of capture and/or transmission must differ from the DC-SIGN-modulated transmission because polyanions did not show a prominent activity in DC-SIGN-directed virus capture and transmission. The potential of the CBAs to impair the L-SIGN receptor in its capacity to capture and to transmit HIV to T-lymphocytes could be an important property in the continuing search for new CBA lead compounds as members of a novel functional class of antivirals.

2. Materials and methods

2.1. Test compounds

The mannose-specific plant lectins from *Galanthus nivalis* (GNA), *Hippeastrum* hybrid (HHA), *Narcissus pseudonarcissus* (NPA) and *Cymbidium* hybrid (CHA) and the *N*-acetylglucosamine (GlcNAc)-specific plant lectin from *Urtica dioica* (UDA) were derived and purified from these plants as described previously (Van Damme et al., 1988a,b). Dextran sulfate (M_r 5000) and suramin were purchased from Sigma (St. Louis, MO). Cyanovirin (CV-N) was provided by Dr. J.B. Mc-Mahon (National Institutes of Health (NIH), Bethesda, MD) and Dr. C. Bewley (NIH, Bethesda, MD). The sulfonated polyvinyl alcohol (PVAS) was synthesized by Dr. S. Görög (Budapest, Hungary). Pradimicin A (PRM-A) was obtained from Prof. T. Oki and Prof. Y. Igarashi (Toyama, Japan).

2.2. Cells

Human T-lymphocyte C8166 cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA) and were cultivated in RPMI-1640 medium (Invitrogen, Merelbeke, Belgium) supplemented with 10% fetal calf serum (FCS) (Cambrex Bio Science Verviers, Verviers, Belgium), 1% penicillin/streptavidin, 2 mM L-glutamine and 75 mM $NaHCO_3$. The human embryonic kidney cells 293T were purchased from the ATCC and cultivated in DMEM supplemented with 10% FCS, 1% penicillin/streptavidin and 75 mM $NaHCO_3$. U87/$CD4^+$/$CXCR4^+$/$CCR5^+$ cells were cultivated in DMEM containing 10% FCS supplemented with 0.4% geneticin and 1% puromycin for selection of CD4, CCR5 and CXCR4, respectively (Princen et al., 2004). 293T-REx/DC-SIGN and 293T-REx/L-SIGN cells were a kind gift from Dr. A. Marzi (Erlangen, Germany). The T-REx cell lines had been constructed by a stable transfection of 293T cells with DC-SIGN or L-SIGN Tet-On plasmids, making the respective nonintegrin expressions inducible with doxycycline (Sigma) (Simmons et al., 2003). The cells were maintained in 293T medium, as described above, supplemented with zeocine (50 μg/ml) and blasticidine (2.5 μg/ml).

2.3. Viruses

The pNL4.3-EGFPΔenv construct that was used for production of wild-type NL4.3 virus through homologous recombination with the *env* gene, was provided by M. Quiñones-Mateu of The Cleveland Clinic Foundation (Cleveland, OH) (Weber et al., 2006). All rights, title, and interest in these materials are owned by The Cleveland Clinic Foundation. After a standard calcium dichloride based transfection of pNL4.3-EGFPΔenv and the wild-type *env* gene into 293T cells, the produced HIV-NL4.3-EGFP virus was transferred to U87/$CD4^+$/$CXCR4^+$/$CCR5^+$ cells for higher virus production. The produced virus in the cell culture supernatants was harvested after 3 days post-infection and 1 ml aliquots were frozen at −80 °C until use. HIV-1_{IIIB} and HIV-1_{BaL} were provided by R.C. Gallo and M. Popovic (at that time at the National Cancer Institute (NCI), National Institutes of Health, Bethesda, MD). HIV-1_{HE} is a clinical isolate derived from a Belgian AIDS patient in 1987 and later propagated in MT-4 cells.

Fluorescent virions were produced by transient co-transfection of pNL4-3 and pGFP-Vpr, a plasmid expressing the GFP-Vpr fusion protein (a kind gift from Dr G.N. Pavlakis, NCI, Frederick, MD). Supernatants holding GFP-Vpr containing HIV-1 were collected 60 h after transfection. Cell debris were removed from the supernatant by centrifugation (450 × *g* for 10 min) and the supernatant was used for infection or stored at −80 °C.

2.4. Flow cytometric analysis

293T, 293T-REx/DC-SIGN and 293T-REx/L-SIGN cells were stained with the monoclonal anti-human DC-SIGN-phycoerythrin antibody and the L-SIGN specific monoclonal anti-human DC-SIGN2-fluorescein antibody (R&D System, UDA) and were processed by flow cytometry. In a 48-well plate 40,000 293T cells and 50,000 293T-REx/DC-SIGN and 293T-REx/L-SIGN cells were seeded on day 0. On day 1, the cells were stimulated with doxycycline or mock-treated. On day 2, the supernatant was removed and replaced by 200 μl of fresh medium supplemented with one of the monoclonal antibodies. After incubation for 30 min at 37 °C, the cells were washed three times with phosphate buffered saline (PBS). Finally, the cell pellet was resuspended in 500 μl 2% paraformaldehyde