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CARBOHYDRATE-BINDING AGENTS, BUT NOT POLYANIONS EFFICIENTLY PREVENT HIV-1 CAPTURE BY DC-SIGN-EXPRESSING CELLS, AND SUBSEQUENT TRANSMISSION TO CD4+ T-LYMPHOCYTES

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BACKGROUND AND OBJECTIVES

Dendritic cells (DC) function as antigen-presenting cells that capture microorganisms that enter the peripheral mucosal tissues. It has been shown that DC-SIGN, expressed by DC, promotes efficient HIV infection in *trans* of cells that express CD4 and chemokine co-receptors CXCR4/CCR5 (Geijtenbeek et al., Cell 100, 587-597 (2000)). The design and development of candidate microbicide drugs have to be taken into account for this mechanism of dissemination of the incoming virus. It has also been shown that Raji/DC-SIGN transfected cells capture and transmit HIV at efficiencies comparable to those of monocyte-derived dendritic cells (Geijtenbeek et al., 2000, Cell 100, 575-585 (2000)). The outcome of this type of studies would be very helpful to guide the choice of potential candidate microbicide drugs.

MATERIALS AND METHODS

Test compounds. The mannose-specific plant lectins from *Galanthus nivalis* (GNA), *Hippeastrum* hybrid (HHA), *Narcissus pseudonarcissus* (NPA) and *Cymbidium* hybrid (CA) and the GlcNAc-specific plant lectin from *Urtica dioica* (UDA) were derived and purified from these plants and obtained from E. Van Damme (Ghent University, Belgium).

Cells. Human T-lymphocytic C8166 cells were obtained from the American Type Culture Collection (Manassas, VA). The Raji/DC-SIGN cells were constructed by Geijtenbeek et al. (Cell 100, 587-597, 2000) and were kindly provided by Dr. L. Burleigh (Institut Pasteur, Paris, France)).

Flow cytometry analysis. Cells were stained with anti-DC-SIGN (DCN46-FITC; BD Biosciences) and anti-CD4 (SK3-FITC; BD Biosciences) and were processed for flow cytometry as described previously (Vermeire et al., Mol. Pharmacol. 63, 203-210 (2003)).

Viruses. HIV-1(IIIB) was provided by Dr. R.C. Gallo (Institute of Human Virology, University of Maryland, Baltimore, MD).

Antiretrovirus assays. C8166 cells (5 x 105 cells per ml) were suspended in fresh culture medium and infected with HIV-1 at 100 CCID50 per ml of cell suspension. Then, 100 µl of the infected cell suspension were transferred to microplate wells, mixed with 100 µl of the appropriate dilutions of the test compounds, and further incubated at 37°C. After 3 to 4 days, C8166 giant cell formation was recorded microscopically (Condition A, Table 2).

Effect of continuous presence of test compounds on syncytia formation in co-cultures of virus-exposed Raji/DC-SIGN cells and uninfected C8166 cells. Raji/DC-SIGN cell cultures were exposed to HIV-1 for 30 min to allow virus capture and subsequently thoroughly washed with culture medium as described above. Then, virus-exposed Raji/DC-SIGN cells were seeded in 96-well microtiter plates in the presence of serial dilutions of the test compounds. Immediately after seeding, C8166 cells were added to each well. After 36 to 48 hrs, syncytia formation was recorded microscopically (condition B, Table 2).

Effect of short exposure of HIV-1 to test compounds on prevention of HIV-1-capture by Raji/DC-SIGN cells. High amounts of HIV-1 particles were exposed to serial dilutions of the test compounds for 30 min. Then, the drug-exposed virus suspensions were mixed with Raji/DC-SIGN cell suspensions for 60 min at 37°C after which the cells were thoroughly washed twice with 40 ml culture medium. The Raji/DC-SIGN cell cultures were then analysed for p24 content (data in Table 1, Fig. 1), mixed with 2 x 105 C8166 cells and further incubated in 48-well plates for 36-48 hrs at 37°C. Then, the syncytia formation in the cell cultures was recorded microscopically (Table 2, condition C).

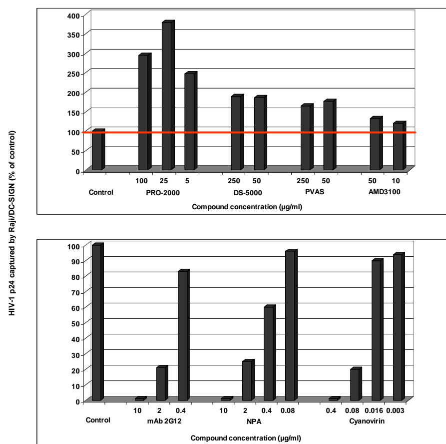

Fig. 1. Effect of test compounds on the capture of HIV-1 particles by Raji/DC-SIGN cells. HIV-1(IIIB) particles were exposed to various dilutions of the test compounds (30 min) prior to administration to Raji/DC-SIGN cells for 60 min. After removal of unbound virus by several washing (centrifugation) steps, cell-associated virus was qualified by p24 ELISA.

RESULTS

Exposure of B-lymphocyte Raji/0 and Raji/DC-SIGN cell cultures to HIV-1(IIIB). Flow cytometry analysis revealed that the DC-SIGN-transfected Raji/DC-SIGN cells abundantly expressed DC-SIGN on their membrane, whereas the wild-type Raji/0 cells did not. When wild-type Raji/0 and stably DC-SIGN-expressing Raji/DC-SIGN cells were exposed to HIV-1(IIIB) particles (2.2 x 105 pg p24) for 60 min, after which the unadsorbed virus was carefully removed, no p24 Ag (< 10 pg p24 Ag/ml) (detection limit) was associated with the Raji/0 cells, whereas consistent p24 Ag was associated with Raji/DC-SIGN cells (HIV capture).

Inhibitory effect of test compounds on the ability of Raji/DC-SIGN to capture HIV-1 particles. The CBA dose-dependently inhibited capture of HIV-1 by the Raji/DC-SIGN cells. In contrast, none of the other classes of HIV entry inhibitors were markedly inhibitory at the concentrations tested. The polyanions did not inhibit HIV capture by the Raji cells. Instead, these compounds stimulated HIV capture by ~ 2- to 3.5-fold at the highest concentrations tested (Fig. 1, Table 1).

Co-cultivation of T-lymphocyte C8166 cells and HIV-exposed Raji/DC-SIGN cells. In co-cultures of uninfected C8166 cells and HIV-1-exposed Raji/DC-SIGN cells, an abundant amount of syncytia were formed at 36 hrs post-cultivation (Fig. 2). This assay sysem is useful to evaluate the inhibitory activity of the entry inhibitors on HIV transmission.

Inhibitory effect of entry inhibitors on syncytia formation in co-cultures of C8166 cells and virus-exposed Raji/DC-SIGN cells. In a first set of experiments, the entry inhibitors were administered to HIV-1-infected C8166 cells at the time of infection. Different antiviral potencies were observed depending the nature of the entry inhibitor (Table 2, first column).

In a second set of experiments, the test compounds were added to the co-cultures of uninfected C8166 cells and Raji/DC-SIGN cells that were pre-exposed to HIV and were kept present throughout the further cell culture incubation period (Table 2, second column). Most compounds had higher EC50 values for giant cell formation in these C8166 + HIV-1-exposed Raji/DC-SIGN co-cultures than in HIV-1-infected C8166 cell cultures.

In a third set of experiments, the entry inhibitors were only exposed to HIV-1 for a short time period (30 min) after which the drug-exposed virus was administered to the Raji/DC-SIGN cells for 60 min. Then, C8166 cells were added (Table 2, third column, condition C). Most CBAs showed a pronounced dose-dependent inhibitory potential. The differences in the potential of the various test compounds to prevent HIV transmission became evident if the ratios of EC50s (third column, condition C) to the EC50s (first column, condition A) in Table 2 were compared. (CBAs were endowed with EC50 ratios close to 1 (0.45 to 5.7); all other entry inhibitors including the polyanions showed much higher ratio numbers (> 24 to > 1428).

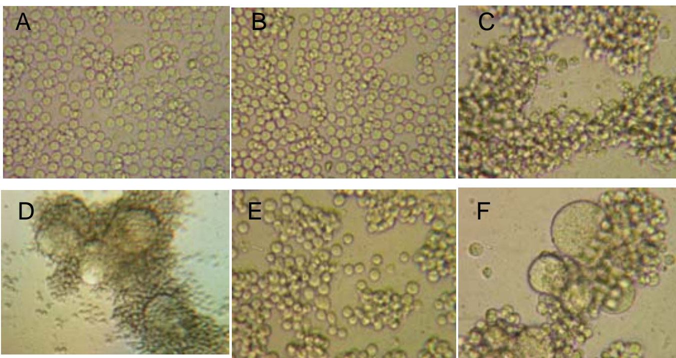

Fig. 2. Microscopic view of following cell cultures.
Panel A: Raji/DC-SIGN cells; Panel B: HIV-1-exposed Raji/DC-SIGN cells; Panel C: C8166 cells; Panel D: HIV-infected C8166 cells (3 days post infection with 100 CCID50 HIV-1(IIIB)); Panel E: Co-cultures of uninfected C8166 cells + Raji/DC-SIGN cells; Panel F: Co-cultures of uninfected C8166 cells + HIV-1-exposed Raji/DC-SIGN cells (the unadsorbed virus to the Raji/DC-SIGN cells was carefully removed after a 30-min incubation time prior to co-cultivation of the Raji/DC-SIGN cells with the uninfected C8166 cells).

DISCUSSION

Only the CBA were able to efficiently prevent virus transmission from virus-captured Raji/DC-SIGN cells to uninfected C8166 cells. Although CV-N looks most promising, it should be mentioned that CV-N shows pronounced toxicity at antivirally active concentrations, a phenomenon not observed for the plant lectins (Balzarini et al., J. Virol. 80, 8411-8421 (2006)) (see also poster of D. Huskens et al., no. Y-145). None of the other entry inhibitors proved very effective in interrupting the HIV transmission process. It was even observed that the polyanions reliably stimulated HIV-1 capture by Raji/DC-SIGN cells (Fig. 1). However, several of its members, PRO-2000 (and also cellulose sulfate (CS), Usher gel) are currently subject of clinical microbicide trials. The stimulatory properties of the polyanions on HIV capture and subsequent transmission found in our assay system – if physiologically relevant – is not recommended to occur in the presence of a potential microbicide candidate drug. Very recently (31 January 2007), a microbicide trial with CS has been put on hold, due to the indication of a higher HIV infection rate in the placebo group (http://www.medicalnewstoday.com). It would be relevant to reveal whether increased DC-SIGN-mediated virus capture by polyanions, as observed in our cell culture studies, is related to the observation made in the clinical trial with CS.

Table 1. Effect of test compounds on the ability of Raji/DC-SIGN cells to capture HIV-1(III_B) particles

Test compound	IC_{50}[a] (µg/ml)
HHA	8.5
GNA	18
NPA	0.7
CA	2.4
CV-N	0.035
UDA	10
mAb 2G12	0.80
T-20	> 25
AMD3100	> 50
DS-5000	> 250
PVAS	> 250
PRO-2000	> 25

[a]50% inhibitory concentration or compound concentration required to prevent HIV-1 capture (adsorption) by Raji/DC-SIGN cells as measured by HIV-1 p24 ELISA.

Table 2. Inhibitory activity of test compounds against HIV-1-infected C8166 cultures or HIV-1-exposed Raji/DC-SIGN cells cocultivated with uninfected C8166 cells

Test compound	Condition [A] EC_{50}[a] (µg/ml) (HIV-1-infected C8166 cells)	Condition [B] EC_{50}[b] (µg/ml) (continuous presence of test compound in the Raji/DC-SIGN + C8166 co-culture after virus exposure to Raji/DC-SIGN cells)	Condition [C] EC_{50}[c] (µg/ml) (virus pre-incubated with test compound prior to exposure to Raji/DC-SIGN)
HHA	13 ± 6.5	> 20	32 ± 7.5
GNA	20 ± 0.0	> 20	42 ± 2.5
NPA	0.90 ± 0.1	3.1 ± 0.9	0.9 ± 0.3
CA	0.85 ± 0.05	7.0 ± 3.0	1.5 ± 0.5
CV-N	0.007 ± 0.003	0.35 ± 0.15	0.04 ± 0.0
UDA	25 ± 15	45 ± 5.0	30 ± 10
mAb 2G12	2.0 ± 0.0	22 ± 2.5	0.9 ± 0.1
T-20	0.75 ± 0.05	2.7 ± 1.2	> 25
AMD3100	0.035 ± 0.015	0.85 ± 0.65	> 50
DS-5000	0.8 ± 0.0	4.8 ± 3.2	> 250
PVAS	1.4 ± 0.6	2.2 ± 0.25	≥ 250
PRO-2000	0.3 ± 0.1	6.0 ± 4.0	≥ 25

[a]50% effective concentration, required to prevent syncytia formation in HIV-1(III_B)-infected C8166 cell cultures at day 3 post infection.
[b]50% effective concentration, required to prevent syncytia formation in HIV-1(III_B)-exposed Raji/DC-SIGN cell cultures that were cocultivated with uninfected C8166 cells in the continuing presence of different concentrations of the test compounds.
[c]50% effective concentration, required to prevent syncytia formation in test compound-pre-exposed HIV-1(III_B)-infected Raji/DC-SIGN cell cultures, co-cultivated with uninfected C8166 cells in the absence of the test compounds

CONCLUSION

CBAs, but not other type of HIV entry inhibitors can efficiently interrupt and prevent HIV particle capture by DC-SIGN-expressing cells. Although a variety of different (viral and non-viral) properties are important to be taken into account in the eventual choice to proceed with new microbicide drug leads, the potential to interrupt the HIV transmission pathway by such drugs in the described assay model should not be neglected, but, instead, seriously be taken into account, when making a choice on potential candidate microbicide drugs.

Data published in Balzarini, J., Van Herrewege, Y., Vermeire, K., Vanham, G. & Schols, D. Carbohydrate-binding agents efficiently prevent dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN)-directed HIV-1 transmission to T-lymphocytes. Mol. Pharmacol., 71: 3-11 (2007).