FULL TITLE: A Glycomimetic Compound Inhibits DC-SIGN Mediated HIV Infection in Cellular and Cervical Explant Models

RUNNING TITLE: Glycomimetic Compound Inhibits HIV Infection

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ABSTRACT

Objective: DC-SIGN participates in the initial stages of sexually transmitted HIV-1 infection by recognizing highly mannosylated structures presented in multiple copies on HIV-1 gp120 and promoting virus dissemination. Inhibition of HIV interaction with DC-SIGN thus represents a potential therapeutic approach for viral entry inhibition at the mucosal level.

Design: Herein we evaluate the efficacy in inhibiting HIV-1 infection and the potential toxicity of a multimeric glycomimetic DC-SIGN ligand (Dendron **12**).

Methods: The ability of Dendron **12** to block HIV-1 infection was assessed in cellular and human cervical explant models. Selectivity of Dendron **12** towards DC-SIGN and Langerin was evaluated by Surface Plasmon Resonance studies. β chemokines production following stimulation with Dendron **12** was also analyzed. Toxicity of the compound was evaluated in cellular and tissue models.

Results: Dendron **12** averted HIV-1 *trans* infection of $CD4^+$ T lymphocytes in presence of elevated viral loads and prevented HIV-1 infection of human cervical tissues, under conditions mimicking compromised epithelial integrity, by multiple clades of R5 and X4 tropic viruses. Treatment with Dendron **12** did not interfere with the activity of Langerin and also significantly elicited the production of the β chemokines MIP-1α, MIP-1β and RANTES.

Conclusion: Dendron **12**, thus, inhibits HIV-1 infection by competition with binding of HIV to DC-SIGN and stimulation of β chemokines production. Dendron **12** represents a promising lead compound for the development of anti-HIV topical microbicides.

Introduction

HIV remains one of the leading causes of mortality and morbidity [1]. As the vast majority of HIV-1 infections occurs via sexual transmission through mucosal surfaces, the development of vaginal and rectal topical microbicides represents a promising approach to prevent sexually transmitted HIV-1 infection.

DC-SIGN (dendritic cell-specific ICAM-3 grabbing non-integrin) is involved in the initial step of HIV-1 sexually transmitted infection and it may be considered a promising therapeutic target [2,3].

Myeloid immature DCs located in mucosal tissue of vagina, cervix and rectum express DC-SIGN and are among the first cells to encounter sexually transmitted HIV [4-6]. Upon HIV-1 binding to DC-SIGN, the virus escapes (at least partially) degradation into lysosomes. Rather, it is internalised into endosomes and multivescicolar bodies, where it is protected from degradation and retained in a high infective state [7-9]. DCs transmit the virus in *trans* to adjacent $CD4^+$ T lymphocytes in genital mucosae or, after migration to lymphoid tissue, to $CD4^+$ T lymphocytes resident in lymphoid tissue, promoting HIV-1 dissemination [10-11]. DC-SIGN, facilitating HIV-1 interaction with CD4 and co-receptors, also enhances DCs infection in *cis*, that results in long term transmission of HIV [12,13].

In addition, binding of HIV-1 to DC-SIGN activates signaling pathways that modulate Toll Like Receptors (TRLs) signaling, inducing immunosuppressive responses and triggering HIV replication and transmission [14-17].

DC-SIGN specifically recognizes the high mannose glycan (Man_9), presented in multiple copies on HIV-1 gp120 [8-18]. Fragments of Man_9 terminated by a di- or a tri-mannoside bind to DC-SIGN almost as efficiently as the entire Man_9 [19]. Chemically synthesised analogues of $(Man)_9$ terminal di- and tri-saccharides, are more resistant to hydrolysis by glycosydases than the corresponding natural oligosaccharides and

interact efficiently with DC-SIGN [20]. These analogues can be linked to tetravalent (dendrons) scaffolds to obtain compounds endowed with stronger binding affinities to DC-SIGN [21, 22]. Such compounds can compete with binding of HIV gp120 to DC-SIGN and are suitable for the development of new anti-HIV microbicides.

We recently demonstrated that a tetravalent dendron containing four copies of a linear pseudo-mannotrioside (Dendron **12**) was able to inhibit HIV-1 *trans* infection of $CD4^+$ T lymphocytes [23].

Experimental models based on infection with HIV of explants taken from human uterine cervix, albeit with some limitations, allow a better approximation of the conditions *in vivo* compared to cellular models [24-27]. Thus, in this study a cervical explant model was exploited to assess the efficacy in inhibiting HIV-1 infection and the toxicity of Dendron **12**, with the purpose of evaluating if the compound is a suitable candidate for the development as topical microbicide.

Material and Methods

Cell culture

B-THP1 and B-THP1/DC-SIGN cells (contributed by Drs Li Wu and Vinet N.KevalRamani) were cultured as described [23].

Virus

The following HIV-1 strains were used: BaL (contributed by Drs. S. Gartner, M. Popovic and R. Gallo, DU174 (Source: Dr L Morris), the R5 and X4 tropic primary isolates 8g and DPMVF (provided by Prof. Stefano Aquaro).

Inhibition of HIV infection in *trans*

Human $CD4^+$ T lymphocytes were purified from peripheral blood of volunteer healthy donors following written consent, and activated as described [23]. Dendron **12,** synthesized as described [23], were diluted to desired concentration into culture medium (RPMI 160 with 20% FBS, Penicillin and Streptomycin (PS) and L-Glutamine (LG), (all from Euroclone, Siziano, Italy). B-THP1/DC-SIGN or B-THP1 cells (10^6 cells/ml) were pre-incubated with Dendron **12** (250 μM), or culture medium alone for 30' prior to exposure to BaL (virus titer ranging from 5 $TCID_{50}$ to 80 $TCID_{50}$), in the continued presence of the inhibitor 3 h at 37 °C. After extensive washing, B-THP1/DC-SIGN cells were co-cultured with activated human $CD4^+$ T cells as previously described [23].

Human cervical explants infection

Cervical tissue was obtained, following written informed consent, from premenopausal women, HIV, HBV and HCV seronegative and without current genital infection, undergoing therapeutic hysterectomy at Unit of Obstetrics and Gynaecology of Sacco Hospital (Milan, Italy). The study was approved by the local ethic committee and conducted in compliance with international guidelines and local laws. Endocervical 3 mm x 3 mm explants biopsies comprised both epithelium and stromal tissue.

Within 1 hour after obtaining tissues the explants were extensively washed. Then explants were either immediately treated with the Dendron **12** and infected with R5 tropic strains or were pre-stimulated for 2 days in presence of IL-2 (R&D systems, Minneapolis, MN, USA) and PHA (Sigma-Aldrich, Saint Louis, Mo, USA) prior to X4 tropic DPMVF exposure.

Explants were pre-treated 30 minutes at 37°C with different concentrations of Dendron **12**. Afterwards explants were exposed to HIV-1 BaL, DU174 (both 2.6 $x10^4$ $TCID50_{50}$), 8g or DPMVF (both 10^4 $TCID50_{50}$) in the continued presence of the

compound 3h at 37°C. Subsequently explants were washed with RPMI. Unstimulated explants were cultured in RPMI medium supplemented with 20% FBS, PS,LG (Euroclone) and gentamycin (Sigma-Aldrich) at 37°C and 5% CO_2. Stimulated explants were cultured under the same conditions in presence of IL-2. Supernatants were collected 3 and 7 days post infection.

p24 ELISA

p24 concentration in the supernatants was assayed by Alliance HIV-1 p24 Antigen kit (Perkin Elmer, Whaltam, MA, USA). Plates were read using the IMark microplate reader equipped with Microplate Manager ® 6 software (Biorad, Segrate, Italy).

Toxicity on PBMCs

PBMCs, purified from peripheral blood of healthy donors as described [23], were incubated with different concentrations of Dendron **12** for 3 or 7 days. The apoptosis was monitored evaluating the percentage of dead cells by staining with 7-AAD, (Beckman Coulter, Fullerton, CA, USA). Flow cytometric analyses were performed using a CYTOMICS FC-500 flow cytometer interfaced with CXP21 software (Beckman Coulter).

MTT assay

Toxicity of Dendron **12** was determined by a MTT based assay (Sigma-Aldrich). Viable explants reduce MTT to formazan crystals. Explants were cultured with increasing concentration of Dendron **12** diluted in medium culture for 3 and 7 days. The 50% of medium culture (containing the different concentration of Dendron **12**) was changed at day 3. After culturing, explants were washed and incubated in medium RPMI without phenol red plus 10% FBS and MTT. Formazan was dissolved by MTT solubilisation solution and formazan absorbance was measured at 595 nm.

Differentiation and treatment of MDDCs

$CD14^+$ monocytes were separated from PBMCs using the $CD14^+$ microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany) following manufacturer's instruction. Monocytes were differentiated into iDCs by culturing them in presence of IL-4 (20 ng/ml) and GM-CSF (20 ng/ml) (R&D Systems) for 6 days. DC-SIGN expression was checked by staining with anti human DC-SIGN-PE monoclonal antibody (clone AZND1, Beckman Coulter) and flow cytometric analysis. MDDCs were treated with Dendron **12**. Supernatants were harvested and RNA extracted from cells after 3, 24 and 72h. β chemokines concentration in culture supernatants was evaluated using DuoSet kits (R&D Systems).

RNA Extraction and Real time PCR

RNA was extracted using the acid guanidium thiocyanate–phenol–chloroform method and purified from genomic DNA with RNase-free DNase (RQ1 DNase, Promega, Madison, WI,USA). RNA was reverse transcribed using random examer primers and M-MLV reverse transcriptase (Clontech, Palo Alto, CA, USA). cDNA quantification for MIP1α, MIP1β, RANTES and GAPDH was performed by real-time PCR (DNA Engine Opticon 2; MJ Research, Ramsey, MN, USA). Reactions were performed using a SYBR Green PCR mix (Finnzymes, Espoo, Finland). Results were expressed as ΔΔCt and presented as ratios between the target gene and the GAPDH housekeeping mRNA.

Surface plasmon resonance analysis

Extracellular domain (ECD) of Langerin (residue 68-328) and DC-SIGN (residue 66-404) were overexpressed and purified as described [28,29]. SPR experiments were performed on a Biacore 3000 using functionalized CM4 sensor chips and the

corresponding reagents from Biacore. Two flow cells were activated as described [30]. Flow cell one was blocked with ethanolamine and used as a control surface. The second one was treated with BSA-Man**α**1-3[Man**α**1-6]Man (Man-BSA, Dextra) (60 µg/mL) in 10 mM acetate buffer, pH 4. Remaining activated groups were blocked with ethanolamine. The final density immobilized on the surface of the second flow cell was 5200 RU. The Man-BSA used to functionalize CM4 chip harbours 12 glycosylation sites. The affinities for DC-SIGN ECD and Langerin ECD of pseudo-mannotrioside and D-mannose were estimated by an inhibition assay, as described [22,31]. Each lectin was injected onto the Man-BSA surface, at 20 µM alone or in presence of an increasing concentration of compounds. Injections were performed at 5 µL/min using 25 mM Tris-HCl, pH 8, 150 mM NaCl, 4 mM $CaCl_2$, and 0.005% of P20 surfactant as running buffer. The surface was regenerated by 1' injection of 50 mM EDTA, pH 8. The IC_{50} values were determined as described [22,31].

Results

Dendron 12 inhibits *trans* infection in presence of HIV-1 elevated viral load

We firstly examined whether the Dendron **12** was able to inhibit the *trans* infection in the presence of elevated viral load. B-THP1/DC-SIGN cells were used as model to mimic HIV transmission to $CD4^+$ T cells, as previously described [6,23,32]. B-THP1/DC-SIGN cells were pre-incubated 30 minutes in the presence or in absence of Dendron **12** and then exposed to different inoculums of HIV-1 Bal. Then, B-THP1/DC-SIGN cells were washed and co-cultured with activated $CD4^+$ T cells. At lower viral concentration inhibition of BaL transmission to $CD4^+$ T cells was almost complete. Even at higher viral load (40 and 80 $TCID_{50}$) the Dendron **12** was able to counteract the transmission of the virus to $CD4^+$ T lymphocytes (more than 92% of inhibition) (Fig.1a).

Selectivity towards DC-SIGN

Both Langerin and DC-SIGN recognize Man_9 on gp120, but have distinct specificities towards complex oligosaccharides [33-36]. Specificity of pseudo-mannotrioside ligand for Langerin and DC-SIGN was tested by competition experiments using surface plasmon resonance (SPR), as previously described [37]. A CM4 sensorchip were functionalized with BSA-Mannotriose and a fixed amount of the extracellular domain of DC-SIGN and Langerin, were injected over the surface in the presence or absence of pseudo-mannotrioside or mannose (control). From the inhibition curves (Fig. 1b), an IC_{50} of the two ligands towards each lectins was evaluated (Fig. 1c). A limited difference in favour of DC-SIGN was observed for mannose. On the contrary, pseudo-mannotrioside is 20 times more potent toward DC-SIGN than against Langerin. Moreover, pseudo-mannotrioside is 14 times more potent than D-mannose towards DC-SIGN. Indeed, the use of pseudo-mannotrioside allows real improvement in affinity and in selectivity.

Inhibition of HIV-1 infection of human cervical tissue by Dendron 12

Endocervical tissue was obtained from premenopausal women, HIV, HBV and HCV seronegative, undergoing planned therapeutic hysterectomy. Explants were exposed to HIV-1 in a non-polarised manner, analogous to condition of compromised epithelium *in vivo*. As the laboratory adapted R5 strain HIV-1 BaL is able to infect resting tissue [25], explants were not activated to mimic physiological conditions.

Explants were pre-treated 30 minutes in absence or in presence of increasing concentration of Dendron **12** and then were exposed to BaL in the continued presence of compound. After washing to remove unbound Dendron **12** and virus, explants were maintained in culture up to 7 days. Data represent p24 levels and are presented as the mean of 5 independent experiments, using explants from separate

donors. Dendron **12** inhibited cervical explants BaL infection in dose dependent manner (Fig. 2). At the higher concentration tested, Dendron **12** reduced the infection by about 80%, at 3 and 7 days post infection.

The ability of Dendron **12** to avert explant infection mediated by primary HIV-1 isolates was also verified. R5 tropic isolate 8g was able to infect unstimulated tissue, but X4 tropic isolate DPMVF needed pre-activation to induce infection (not shown). Explants unstimulated or pre-activated 2 days with IL-2 and PHA were pre-treated with increasing concentration of Dendron **12** and infected respectively with 8g or DPMVF, as described before. Infection inhibition was dose dependent. At the concentration of 1mM, infection by both isolates was reduced by more than 85%. At 0.05 mM Dendron **12** decreases by 56% (day 3) and by 40% (day 7) the infection mediated by 8g (Fig. 3a and b), but the inhibitory effect against DPMVF was largely lost (Fig. 3c and d).

Furthermore we evaluated the capability of Dendron **12** to block explant infection by Clade C R5 tropic strain DU174. Unstimulated explants were pre-treated with the compound, exposed to DU174 and cultured as described. Dendron **12** reduced DU174 infection in dose dependent manner and by about 90% at 1 mM (Fig. 3e and f).

Induction of β1 chemokines production by Dendron 12

We wonder if, in addition to competitive inhibition of DC-SIGN, other mechanisms account for the antiviral effect of the compound **12**. So we investigate if Dendron **12** stimulates the production of factors interfering with HIV infection. Due to difficulty of isolating sufficient amounts of primary mucosal DCs, immature monocytes-derived dendritic cells (iMDDCs), that share with mucosal DCs similar features and DC-SIGN expression, were used as a model [38]. iMDDCs were treated with Dendron **12** for 3, 24 and 72 hours. Expression and production of β chemokines MIP-1α, MIP-1β, and

RANTES following stimulation were analyzed by quantitative real time PCR and ELISA. The treatment increased expression level of mRNA specific for MIP-1α, MIP-1β and RANTES (Fig. 4a-c) after 3 hours. Also MIP-1α, MIP-1β and RANTES production increased after 24 and 72 hours of stimulation (Fig. 4d-f).

Evaluation of Dendron 12 toxicity

Cytotoxicity against PBMCs was evaluated by labelling with 7-Amino-Actinomycin D (7-AAD), that identify non-viable cells, after Dendron **12** treatment. Compound exposure for 3 and 7 days did not alter significantly the viability of PBMCs (Fig. 5a and b).

To assess toxicity of Dendron **12** towards cervical tissue, effect of non-polarized exposure of the compound on explants viability was monitored (Fig. 5c). After 3 and 7 days treatment in absence or in presence of different concentration of Dendron **12**, viability was evaluated by a MTT based assay. Viability of the compound treated explants was compared to viability of untreated control. No significant difference between control and treated explants was observed up to a concentration of 1mM (the higher concentration tested in assessment of compound efficacy against HIV-1 infection).

Discussion

Three decades after HIV discovery HIV-AIDS pandemic continues and millions of people are infected every year. Thus, the development of effective, non-toxic and low cost topical microbicides represents a valid alternative approach to prevent the sexual transmission of HIV [3]. However, so far almost all compounds failed to prevent HIV transmission in efficacy trials. A recent exception was a vaginal gel formulation of tenofovir, that reduced HIV infection by 50% [39].

Tenofovir and other topical microbicides can prevent localized infection of target cells in genital mucosae. However DCs DC-SIGN+ internalize and transport HIV to secondary lymphoid organs, rendering the virus inaccessible to inhibitory effect of the microbicides. Therefore inhibition of DC-SIGN is essential to block HIV-1 uptake and dissemination from migratory DCs.

We have previously reported that the tetravalent Dendron **12**, was able to block almost completely the HIV-1 *trans* infection of $CD4^+$ T cells at micromolar range [23]. This compound exerts its activity by competitive inhibition of HIV-1 gp120 binding to DC-SIGN. In our initial experiments we demonstrated that Dendron **12** (at the same concentration previously assayed) even in presence of higher viral loads retains its ability to inhibit HIV-1 *trans* infection. This potent inhibitory activity is due both to elevate affinity for DC-SIGN of Dendron **12** pseudo-trisaccharide units and to high avidity of binding, guaranteed by the tetravalent presentation on the compound scaffold.

Different DC-SIGN inhibitors have been described so far. Dendrons displaying complex oligomannoses in high density inhibited binding of gp120 to DC-SIGN with IC_{50} in nanomolar range [40]. Nevertheless the complexity of the oligosaccharides used limits the possibility of a therapeutic application. Gold nanoparticles displaying mannosyl oligosaccharides are potent inhibitors of DC-SIGN mediated HIV-1 *trans* infection of human PBMCs [41], but may have toxic effects because of gold accumulation.

Unlike DC-SIGN, the C-Type lectin Langerin, expressed on Langerhans cells (LCs) appears to play a protective role against HIV infection. LCs are located in the stratified mucosal epithelia of female and male genital tissue. Both DC-SIGN and Langerin recognise high mannose glycans on gp120 through their CRD. Recent data demonstrate that Langerin prevents HIV transmission by LCs, at least in the presence of low concentration of the virus, promoting rapid degradation and

clearance of HIV-1 [32-33]. Using a biosensor with a SPR detection method we showed that the Dendron **12** is much more selective for DC-SIGN than for Langerin. Considering the similarity between the CRD of DC-SIGN and Langerin, the fact that the Dendron **12** does not interfere with Langerin function is a remarkable advantage in view of developing new microbicides.

The efficacy and the safety of the Dendron **12** were evaluated in a human cervical explants model. Endocervix is more susceptible to HIV-1, being lined by a single layer of columnar epithelium, and contain DCs DC-SIGN in the subepithelial region [4,5,24]. Explants were exposed to HIV-1 to mimic a condition of compromised epithelium *in vivo*; condition that highly increases the risk of HIV infection.

R5-tropic viruses strains predominate during HIV-1 transmission *in vivo* and cause the majority of new infections [42]. The results obtained showed that Dendron **12** strongly reduced the infection of cervical explants by different HIV-1 R5 tropic strains, such as BaL, the primary isolate 8g and the Clade C DU174. This may have a great impact, considering that Clade C is the most abundant subtype in all countries of Southern Africa and in some countries of eastern Africa and Asia, areas where the majority of HIV-1 infected people resides [43,44].

Rare cases of HIV-1 infection by X4-tropic strains were observed in CCR5Δ32 homozygotes and X4 tropic strain can infect immune activate human cervical tissue [25,42]. Dendron **12** was able to inhibit DPMVF primary X4 tropic strain infection at higher concentration assayed, but this effect was in part lost at lower concentrations of the compound.

Data obtained suggest that Dendron **12** might have additional mechanisms of action besides competitive inhibition of HIV-1 binding to DC-SIGN. Flow cytometric studies indicate that treatment with Dendron **12** reduces DC-SIGN expression on B-THP1/DC-SIGN [23]. This effect may be due to increased internalisation of the receptor after binding of the compound to DC-SIGN.

Furthermore Dendron **12** induced an augmentation of the production of β chemokines, such as MIP-1α, MIP-1β and RANTES, by iMDDCs. These β chemokines, natural ligands of CCR5, suppress HIV-1 R5 tropic strain replication, competing with the binding of the virus to CCR5 or inducing receptor internalisation [45-47]. The Dendron **12**, enhancing β chemokines production, could interfere, at least partially, with the direct infection of $CCR5^+$ $CD4^+$ T lymphocytes and macrophages located in genital mucosae.

The Dendron **12** do not reduce the vitality of PBMCs and of explants. However additional experiments, such as Rabbit Vaginal Irritation Assay [48], would be needed for a more accurate evaluation of potential toxic effects.

Vaginal epithelium has limited permeability to particles greater than 30 nm [2]. However the diameter of Dendron **12** is certainly below that threshold, so the compound could enter and diffuse into intact mucosal tissue. Furthermore the Dendron **12** scaffold can be easily modified to improve absorption of the compound, without decreasing affinity to DC-SIGN.

The tetravalent Dendron **12** prevents HIV *trans* infection of $CD4^+$ T lymphocytes at micromolar range, even in presence of elevated viral load, displays high solubility in physiological media, a neglectable toxicity and a long lasting effect. Moreover it inhibits in dose dependent manner HIV-1 infection of human cervical explants. These features make the Dendron **12** a good candidate as a lead compound to develop new microbicide drugs.

However the Dendron **12** inhibits 80-90% of cervical explants HIV-1 infection, but cannot block it completely. To overcome these limitations, structure of this compound can be improved in both the scaffold and the active pseudo-saccharide ligand to develop of new ligands of DC-SIGN more effective and easier to synthesize. Furthermore, Dendron **12** (or its derivatives) can be used in combination with other molecules directed against different HIV targets. In particular multivalent structures,

presenting on the same scaffold multiple copies of DC-SIGN and HIV co-receptors inhibitors, simultaneously blocking different HIV targets, could protect against different routes of HIV transmission.

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MC, ABernardi, DT conceived the study; ABerzi, MC wrote the paper, ABerzi, MB performed the experiments and analyzed the data; JoRe, RO synthesized the pseudo-mannotrioside; JaRo, MSN synthesized the Dendron scaffold; FF, IS performed SPR experiments and analyzed the data; EC expressed and purified DC-SIGN and Langerin. IC, PA obtained cervical explants.

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FIGURE LEGENDS

Fig. 1 Evaluation of Dendron 12 ability to inhibit HIV-1 Bal *Trans* infection in presence of increasing amounts of the virus (a) and selectivity to DC-SIGN (b,c). (a) After pre-treatment with Dendron **12** (250 µm) or medium culture, B-THP-1/DC-SIGN cells were pulsed with BaL 3 hours. After washing cells were co-cultured 3 days with $CD4^+$ T lymphocytes from healthy donors. Levels of infection were quantified by measuring p24 in the supernatants of co-cultures by ELISA. Data were obtained from 3 different healthy donors. Each donor was tested in duplicate. Values are mean ± SD. (b, c) SPR experiment results of DC-SIGN ECD and Langerin ECD binding to Man-BSA/dextran surface inhibition by pseudo-mannotrioside (psTri) and D-mannose. (b), inhibition curves, and (c) lectin selectivity histograms.

Fig. 2. Inhibition of endocervical tissue infection mediated by HIV-1 Bal. After a 30 minutes pre-treatment with the Dendron **12** or medium culture, endocervical explants were incubated 3 hours with BaL in the continued presence of indicated concentrations of Dendron **12**. Then explants were washed and cultured for 7 days. Infection was monitored by ELISA measurement of p24 in explant culture supernatants at day 3 (a) and 7 (b) post infection. Values represent the mean ±SD of 5 independent experiments. ***p values < 0.001, **p values<0.01, *p values<0.05 (Student T Test).

Fig. 3. Inhibition of endocervical infection induced by HIV-1 primary isolates (8g and DPMVF) and HIV-1clade C DU174. (a,b,e,f) Unstimulated explants were pre-treated 30’ with Dendron **12** or medium culture and challenged with 8g or

DU174. (c,d) Explants immuno-stimulated were pre-treated 30' with Dendron **12** or medium culture and infected with DPMVF.

After washing explants were put in culture. Infection was monitored by ELISA measurement of p24 in explant culture supernatants at day 3 and 7 post infection. Values represent the mean ±SD of 3 independent experiments. *p values<0.05 (Student T Test)

Fig. 4. β chemokines induction upon Dendron **12** (250µM) stimulation of human iMDDCs from healthy donors. (a-c) β chemokines expression following 3 h Dendron **12** (250 µM) treatment. mRNA levels of MIP1α (a), MIP1β (b) and RANTES (c) were assessed by quantitative real time PCR. Expression is normalized to GAPDH and shown as fold changes expression from the unstimulated sample, set as 1. (d-f) β chemokines protein production after stimulation with Dendron **12** (250 µM). The concentration of MIP1α (d), MIP1β (e) and RANTES (f) in the culture supernatants was assayed by ELISA at 24 and 72h. (a-f). Values represent the mean ±SD.

Fig. 5. Toxicity of compound 12. (a and b) Percentage of 7-AAD positive (non viable) PBMCs after 3 or 7 days of incubation with different concentrations of Dendron **12** (0.25 mM, 0.5 mM and 0.1 mM), or in the absence of the inhibitor (Medium). Percentage of 7-AAD was determined by flow cytometry. Experiments were performed on PBMCs from 3 healthy donors. Values are mean ± SD.

Effect of Dendron **12** on cervical explants viability (c). Endocervical explants, derived from the same donors, were exposed in non polarised manner to Dendron **12** or culture medium (control) for 3 and 7 days. Effect of Dendron **12** on tissue viability was determined by the MTT assay. Explants were weighted to normalize optical density of formazan yielded. Viability was expressed as percentage. Experiments

were performed on explants from 3 donors. Data are reported as the average percent viability (± SD).