The carbohydrate binding lectin Microvirin and its divalent form display potent anti-HIV-1 activity

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Background

The most effective approach to halt the HIV epidemic will be establishing effective prevention methods incorporating multiple types of intervention including microbicides.

Microvirin (MVN), a lectin isolated from the cyanobacterium *Microcystis aeruginosa* PCC7806 shows specificity for α(1,2)Man that are abundantly present on the envelope gp120 of HIV-1 **(Figure 1)**. MVN is a monomer of 14.3 kDa with 1 carbohydrate binding domain located in domain A of MVN. In addition, two new molecules were investigated: DA, a peptide that only consists of domain A of MVN and Di (stands for divalent), a protein wherein domain B was substituted with domain A, creating two possible carbohydrate binding domains **(Figure 2)**.

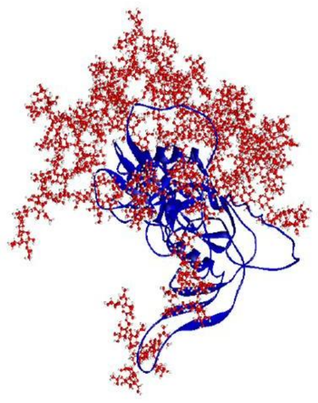

Figure 1 The envelope glycoprotein gp120 of HIV-1 is heavily glycosylated. Gp120 is shown in blue and the carbohydrates that form a shield around gp120 are shown in red. Carbohydrate binding agents have been proposed as innovative anti-HIV agents selectively targeting the glycans on gp120.

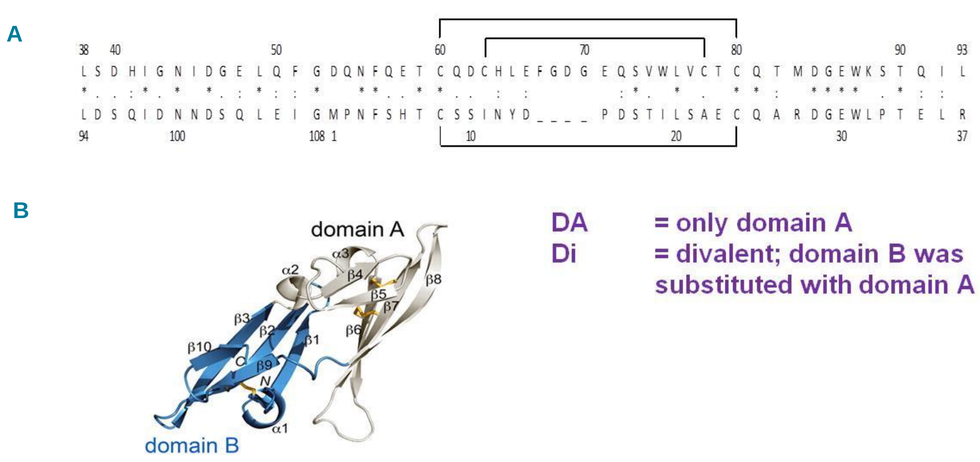

Figure 2 MVN, DA and Di. Internal amino acid sequence alignment of MVN **(A)** and ribbon diagram of the restrained minimized mean structure of MVN with the carbohydrate binding domain, domain A, colored *white* and domain B *blue* **(B)** **[1]**. DA is a peptide that only consists of domain A of MVN and Di stands for divalent and in this derivative of MVN domain B was substituted with domain A, creating two possible carbohydrate recognition sites.

Table 1 Anti-HIV activity of MVN and a few selected CBAs

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Agent[a]	HIV-1							
	lab strain		group M					group O
			A	B	C	D	A/E	
	NL4.3	BaL	UG273	US2	DJ259	UG270	ID12	BCF06
	(X4)	(R5)	(R5)	(R5)	(R5)	(X4)	(R5)	(X4)
MVN	0.12	0.32	0.13	0.03	2.39	0.69	0.06	>5
CV-N	0.42	0.24	1.4	0.18	0.23	0.32	0.051	0.15
HHA	0.12	6	29	5.4	>20	4.9	1.62	1.2
2G12 mAb	0.14	3.71	0.018	0.04	>50	>20	>50	>20

[a] 50 % inhibitory concentration (IC_{50}), or compound concentration in µg/ml required to inhibit viral p24 production with 50% in PBMCs.

Results

MVN is able to inhibit infection by a wide variety of HIV-1 laboratory-adapted strains and various clinical isolates of different tropisms and subtypes in peripheral blood mononuclear cells (PBMCs) **(Table 1 and Fig. 3 panel A)** [2]. MVN also inhibits syncytium formation between persistently HIV-1-infected T cells and uninfected CD4⁺ T cells **(Fig. 3 panel B and Fig. 4)**. It also inhibits DC-SIGN-mediated HIV-1 binding **(Fig. 3 panel C)** and subsequent transmission to CD4⁺ T cells **(Fig. 3 panel D)**. DA that consists of domain A of MVN lost approximately 100-fold in antiviral activity when tested in MT-4 cells against HIV-1 NL4.3. In contrast, Di, the divalent molecule, became 10-fold more active **(Table 2 column A)**. Comparable results were observed in the giant cell formation assay were DA could not inhibit the syncytia formation while Di was more potent than MVN in inhibiting the syncytia formation between persistently infected cells and uninfected CD4⁺ T cells **(Table 2 column B)**.

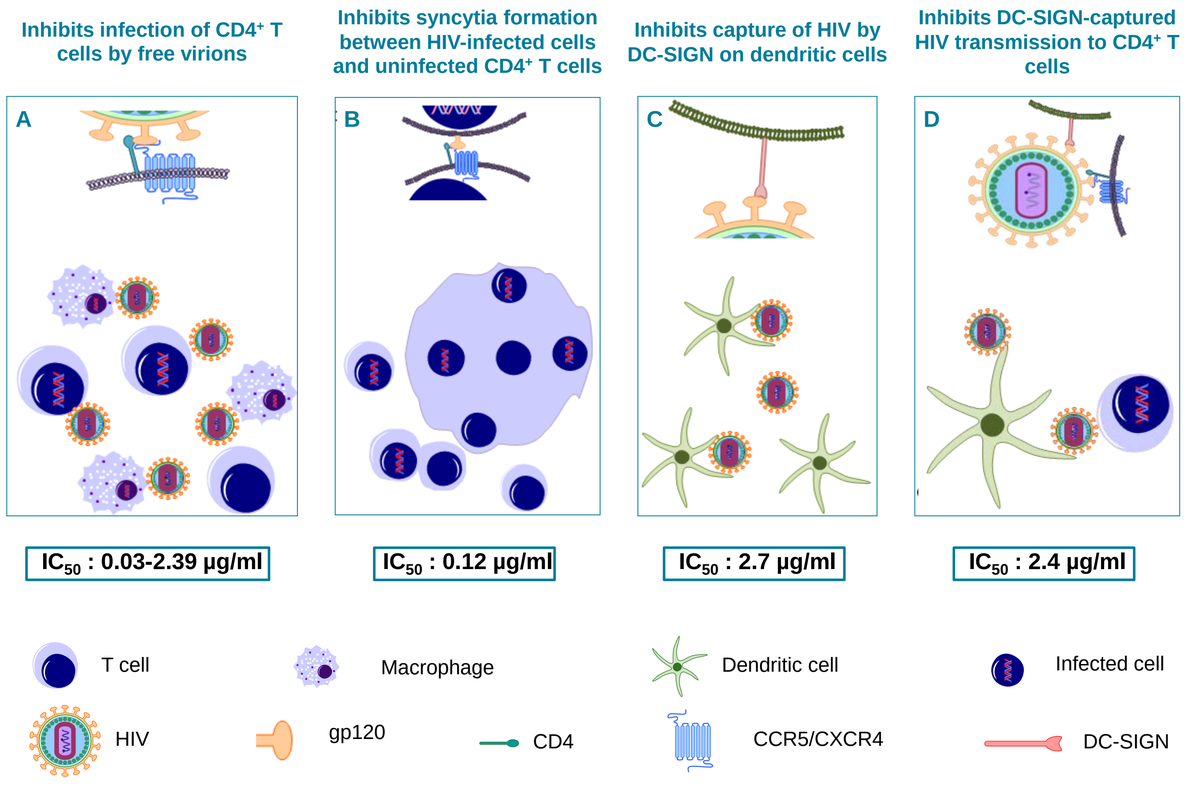

Figure 3 MVN inhibits the different transmission pathways of HIV-1. Infection of free virions. PBMCs were cultured in the presence of MVN and HIV-1 strains or clinical isolates and viral replication was determined by measuring p24 Ag production **(A)**. Infection of CD4⁺ T cells by HIV-infected cells. Persistently HIV-1(IIIB)-infected HUT-78 cells were cocultured with uninfected CD4⁺ SupT1 cells in the presence of MVN and syncytium (giant cell) formation was determined **(B)**. Capture of virus to the DC-SIGN receptor. Initially, the HIV-1 strain HE was pre-exposed to MVN before the virus was administered to DC-SIGN⁺ Raji cells. After 1 h, free virus and MVN were removed, and the amount of captured virus was determined by measurement of the p24 Ag content of the cells **(C)**. Transmission of DC-SIGN-captured HIV to CD4⁺ T cells. HIV-1 strain HE was given the opportunity to be captured by DC-SIGN⁺ Raji cells. Then the HIV-1-captured Raji/DC-SIGN cells were co-cultured with C8166 cells in the presence of MVN and giant cell formation was determined **(D)**.

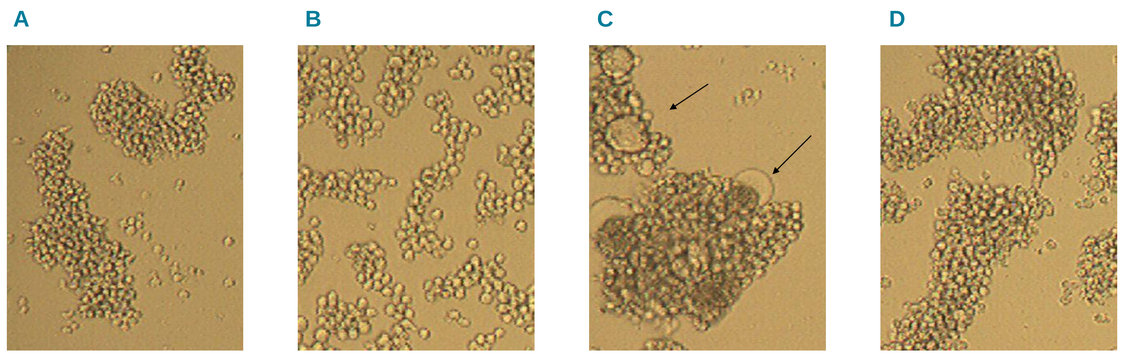

Figure 4 MVN inhibits giant cell formation. Light microscopic pictures of the following cell cultures: SupT1 cells **(A)**; HUT-78 cells persistently infected with HIV-1 IIIB **(B)**; Co-culture of SupT1 cells and HUT-78/HIV-1 IIIB cells (several giant cells are indicated with arrows) **(C)**; Co-culture of SupT1 cells and HUT-78/HIV-1 IIIB cells in the presence of 2 µg/ml MVN **(D)**.

Table 2 Anti-HIV activity of MVN, DA and Di

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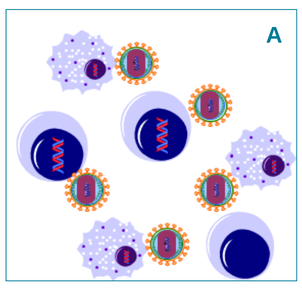
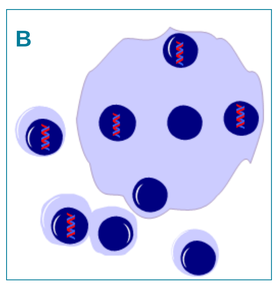


	IC_{50} (µg/ml)[a]	
	A	B
	MT-4 HIV-1 NL4.3	HUT-78 HIV-1 IIIB/SupT-1 giant cell formation
MVN	0.02	0.12
DA	3.1	>5
Di	0.003	0.04



[a] 50 % inhibitory concentration (IC_{50}), or compound concentration in µg/ml required to inhibit virus replication (A) or syncytia formation (B) with 50%.

Conclusion

Our data demonstrate that MVN may qualify as a useful lectin for potential microbicidal use based on its broad and potent antiviral activity and its potential to inhibit the different transmission pathways of HIV-1. The DA molecule that consists of domain A of MVN had almost no anti-HIV-1 activity. In contrast, the divalent molecule that has two instead of one carbohydrate binding site was more potent than MVN.

References

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2. Huskens, D., Férir, G., Vermeire, K., Kehr, J.-C., Balzarini, J., Dittmann, E., Schols, D. (2010) *J. Biol. Chem.* 285: 24845-24854.

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