Short Communication: Man α 1-2Man-Binding Anti-HIV Lectins Enhance the Exposure of V2i and V3 Crown Neutralization Epitopes on the V1/V2 and V3 Hypervariable Loops of HIV-1 Envelope

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Abstract

This study aimed to explore the contribution of high-mannose glycans in the masking of conserved V3 crown (GPG) and V2i epitopes on the hypervariable loops of most exposed distal surface of HIV-1 Env. Using lectins specific to Man α 1-2Man residue containing Man₆₋₉GlcNAc₂ glycans extensively decorating HIV-1 Env, we found that Man α 1-2Man-binding lectins enhance the exposure of these partially and transiently exposed epitopes and consequentially increase the neutralization strength of antibodies against these epitopes.

Keywords: *N*-linked glycans, HIV-1, envelope, hypervariable loop epitopes, anti-HIV lectins

THE HIV-1 ENV is one of the known proteins with the highest genetic and antigenic variability. It is one of the most highly *N*-glycosylated proteins found in nature, extensively covered with ~25 potential *N*-linked glycosylation sites contributing to ~50% of the Env molecular mass.¹ The coating of the Env's exposed and antigenically vulnerable surface, with poorly antigenic V1-V5 hypervariable loops together with coating by extraordinary dense glycans, which further shield the underlying antigenic surface, plays a pivotal role in the determination of Env structure, epitope exposure, and consequently affects antigenicity, immunogenicity, antibody neutralization, infectivity, and receptor binding.^{2,3}

Despite being hypervariable, the V3 and V1/V2 loops present certain conserved features to perform certain well-defined functions in the virus, such as CD4 engagement, coreceptor interaction, viral tropism determination, and infectivity.⁴ A well-defined neutralizing domain characterized within the tip of V3 and V1/V2 loops is the target of antibodies that are made by essentially all HIV-infected individuals and are easily induced by most candidate HIV vaccines.⁵ These epitopes display conformational heterogeneity and are known to be dependent upon the native conformation of Env and remain occluded within the unliganded trimeric Env spike.⁶

Recent structural data suggest that these epitopes remain extensively covered with *N*-linked glycans on and adjacent to V1/V2 and V3 loops on the native Env, and contribute to the con-

formational heterogeneity of these epitopes. The predominant type, ~98% of glycans on native Env, is immature unprocessed Man₅₋₉GlcNAc₂ type *N*-glycans with fully processed mature GlcNAc₂Man₃GlcNAc₂ and the GlcNAcMan₅GlcNAc₂-derived *N*-glycans representing only minor population. It has also been shown that Man α 1-2Man terminating glycans (Man₆₋₉GlcNAc₂) are threefold more abundant on native Env than the monomeric gp120.^{1,6} Taking these facts into consideration, we designed a study to elucidate the biological significance of the masking of V3 crown and V2i epitopes by high-mannose glycans using Man α 1-2Man-binding lectins.^{7,8}

We generated pseudoviruses (PsVs) by cotransfecting the HEK 293T cells with a viral plasmid containing *env* gene and Δenv backbone plasmids pNL4-3 Δenv or pSG3 Δenv . The PsVs were used to infect TZM-bl reporter cells at 100 to 200 tissue culture infectious dose (TCID₅₀) in the presence of di-ethyl amino-ethyl (DEAE)-dextran (10 μ g/ml) for 48 h. The lectins were added to the viruses at a fixed concentration (IC₅₀) and incubated for 30 min at 37°C. For neutralization experiments, serial dilutions of mAbs were added to the virus-lectin mixture and incubated for 1 h at 37°C followed by adding the TZM-bl cells along with DEAE-dextran. After 48 h, the virus neutralization was measured using a β -galactosidase-based assay.

We tested three anti-HIV lectins: griffithsin (GRFT), cyanovirin-N (CV-N), and scytovirin (SV-N), which recognize

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TABLE 1. HIGH-MANNOSE-BINDING LECTINS WITH OLIGOSACCHARIDE RECOGNITION SPECIFICITY AND IC₅₀ VALUES IN TZM-BL CELLS

Lectin	Specificity	Binding on HIV Env	Bal.01 (IC ₅₀)	SF162.LS (IC ₅₀)	Bal.ec1 (IC ₅₀)	Median (IC ₅₀)
<i>Griffithsia</i> sp. (GRFT)	-Man α (1-2)Man	D1, D2 or D3 arm of Man ₆₋₉	0.001 $\pm$ 0.001	0.003 $\pm$ 0.001	0.008 $\pm$ 0.002	0.003
Cyanovirin-N (CV-N)	-Man α (1-2)Man- α (1-2)Man -Man α (1-2)Man	D1 or D3 arm of Man ₆₋₉	0.039 $\pm$ 0.001	0.034 $\pm$ 0.002	0.138 $\pm$ 0.039	0.039
<i>Scytonema varium</i> (SV-N)	-Man α (1-2)Man- α (1-6)Man- α (1-6)	D3 arm of Man ₉	0.356 $\pm$ 0.02	0.294 $\pm$ 0.01	1.868 $\pm$ 0.09	0.356

IC₅₀ values (in μ g/ml) are shown for the three viruses tested, the inhibition assay was performed in duplicate, and data are shown as the averages of all data points from two experiments. Data are represented as mean $\pm$ SD.

the Man α 1-2Man-containing residues present on the terminals of D1, D2, and D3 arms of the Man₆₋₉GlcNAc₂ (Table 1). All the lectins inhibited the viruses, Figure 1a, GRFT being most potent followed by CV-N and SV-N, with median IC₅₀ values 0.003, 0.039, and 0.356 μ g/ml, respectively. The inhibitory potential of lectins correlated with their avidity, that is, number of glycan motifs recognized on the high-mannose structure. Subsequently, we estimated the neutralization sensitivity of the viruses to two representative antibodies against V2i (LDI/V) and V3(GPG) epitopes. We found that all viruses were more sensitive to V3 mAb2557 with median IC₅₀ of 1.25 μ g/ml, whereas V2i mAb1393A was relatively less efficient in neutralizing the viruses, median IC₅₀ 24.86 μ g/ml. These findings are in agreement with the published literature that V3 antibodies display more potency and breadth than V2i antibodies, Figure 1b.^{9,10}

While determining the effect of Man α 1-2Man-binding lectins on the neutralization sensitivity of viruses to antibodies,¹¹ it was observed that in the presence of lectins, the neutralization capacity of all the antibodies displayed a highly significant increase, which was many fold higher than the predicted additive values (Fig. 1c, d). Such results were obtained only when viruses were incubated with lectins before the addition of mAbs. No enhancement in the neutralization potency was observed when viruses were incubated with mAbs followed by addition of lectins (Fig. 1c).

The fold change difference in the neutralization potency of 2557 against Bal.ec1 was highly significant in the presence of GRFT ($p < .0001$) and CV-N ($p < .0001$) followed by SV-N ($p < .001$). Similar results were obtained with SF162 and Bal.01 with GRFT being most potent in its enhancement of neutralization potency of 2557 followed by CV-N and SV-N. The fold change differences observed with 1393A against

Bal.ec1 and other viruses displayed a similar pattern. The most peculiar observations were that experimental “observed values” obtained when mAb and lectins were used together were highly significant than the predicted additive values (Table 2 and 3). In addition, the enhancement in neutralization potency correlated positively with the avidity of the lectin binding to high-mannose glycans.

Since it is well established that V3 and V1/V2 epitopes displaying conformational heterogeneity with Man₆₋₉GlcNAc₂ types of glycans abundantly present on the HIV-1 Env with terminal D1, D2, and D3 branches ending with Man α 1-2Man remain occluded and display partial and transient exposure,¹² we attempted to explore whether Man α 1-2Man-binding lectins, which recognize Man₆₋₉GlcNAc₂, affect the neutralization sensitivity of viruses to V3 crown and V2i antibodies. Only tier 1 viruses were chosen for this study as V2i antibodies efficiently neutralize tier 1 viruses exclusively. All the viruses were strongly inhibited by GRFT followed by CV-N and SV-N, suggesting the relative contribution of Man₆₋₉GlcNAc₂ glycans on the virus Env.

The higher IC₅₀ values for GRFT followed by CV-N and SV-N can be explained by avidity of these lectins for the Man₆₋₉GlcNAc₂ glycan with GRFT recognizing all the three arms D1–D3, followed by CV-N with two binding sites and SV-N recognizing only D3 arm. The strongly enhanced neutralization sensitivity of the viruses to 2557 and 1393A mAb in the presence of Man α 1-2Man-binding lectins can be explained because of increased exposure of V3 crown epitope, which lies beneath V1/V2 loop with its epitope-bearing crown pointing toward the inner trimer axis, away from the trimer surface. Furthermore, N-glycans, although not part of V2i epitope, have been shown to point away from V2i in the crystal structure of scaffolded V1/V2, influencing the

FIG. 1. Inhibitory properties of Man α 1-2Man-binding lectins and exposure of glycan-shielded loops. **(a)** Inhibitory properties of three high-mannose-binding lectins against HIV-1 viruses tested in TZM-bl reporter cells. In parallel to all lectin-mediated inhibition experiments, cell viability was performed and only lectin concentrations were chosen to give 100% cell viability. **(b)** The three viruses were neutralized using a V3 antibody 2557 and a LDV/I-specific V2i antibody 1393A. The V3 crown antibody compared with V2i strongly neutralized all the viruses. We do not find any inhibition with 1418 a B19 protein-specific parvovirus antibody. **(c)** Effect of time of addition of lectins on the neutralization potency of lectins. Bal.01 viruses were first incubated with viruses before addition of mAbs, or viruses were first incubated with mAb followed by addition of lectins. **(d)** Effect of Man α 1-2Man-binding lectins on the enhancement of neutralization strength of the V3 crown and V2i antibodies was measured. Lectins at IC₅₀ were added to all viruses and incubated with the serial dilution of V3 and V2i antibodies. The results shown are of Bal.ec1, the other viruses were similarly inhibited (data not shown). 1418 mAb is a parvovirus B19 protein-specific antibody used as control. All experiments were performed in duplicate, and data are shown as the average of all data points from two experiments, error bars represent SD.

TABLE 2. HIGH-MANNOSE-BINDING LECTINS ENHANCE THE EXPOSURE OF V3 (GPG) EPIOTOPE AND DISPLAY INCREASED VIRAL NEUTRALIZATION BY ANTI-V3 ANTIBODY

	2557	GRFT +2557		CV-N + 2557		SV-N + 2557		1418
		Additive (calculated)	Experimental (observed)	Additive (calculated)	Experimental (observed)	Additive (calculated)	Experimental (observed)	
Bal.01	0.62 ± 0.19	0.62 ± 0.19	0.0004 ± 0.0003	0.71 ± 0.09	0.02 ± 0.01	1.28 ± 0.46	0.008 ± 0.001	>50
SF162.LS	0.62 ± 0.36	0.13 ± 0.03	<0.0001	0.53 ± 0.08	0.007 ± 0.0002	0.83 ± 0.12	0.01 ± 0.01	>50
Bal.ec1	1.04 ± 0.24	0.09 ± 0.01	<0.0001	0.53 ± 0.12	<0.001	0.86 ± 0.002	<0.001	>50

■ <0.0001; ■ 0.0001–0.001 ■ 0.001–0.01 ■ 0.01–0.1 ■ 0.1–2 ■ >50.

Data represent IC₅₀ values (μg/mL) shown for the V3 mAb 2557, the inhibition assay was performed in duplicate repeated twice, and data are shown as the averages of all data points from two experiments. Data are represented as mean ± SD.

TABLE 3. HIGH-MANNOSE-BINDING LECTINS ENHANCE THE EXPOSURE OF V2i (LDI/V) EPIOTOPE AND DISPLAY INCREASED VIRAL NEUTRALIZATION BY ANTI-V2i ANTIBODY

	1393A	GRFT +1393A		CV-N + 1393A		SV-N + 1393A		1418
		Additive (calculated)	Experimental (observed)	Additive (calculated)	Experimental (observed)	Additive (calculated)	Experimental (observed)	
Bal.01	37.23 ± 5.05	24.02 ± 1.52	8.39 ± 0.09	14.00 ± 0.60	6.73 ± 0.30	20.45 ± 0.58	3.50 ± 0.41	>100
SF162.LS	23.36 ± 2.34	14.62 ± 0.73	1.66 ± 0.23	18.94 ± 1.48	10.56 ± 1.76	29.94 ± 0.11	3.73 ± 0.74	>100
Bal.ec1	29.77 ± 3.90	7.81 ± 1.97	1.50 ± 0.72	13.38 ± 0.67	1.30 ± 0.54	8.01 ± 0.16	1.68 ± 0.05	>100

■ <5 ■ 5–10 ■ 10–15 ■ 15–20 ■ 20–40 ■ >100

Data represent IC₅₀ values (μg/mL) shown for the V2 mAb 1393A, the inhibition assay was performed in duplicate repeated twice, and data are shown as the averages of all data points from two experiments. Data are represented as mean ± SD.

antibody recognition and function mainly to induce and maintain proper folding of the V1/V2 domain.^{13,14}

Recent structural data have shown that *N*-linked glycans cover the entire surface of Env and are important players in the control of antigenicity and immunogenicity of V3 epitopes. Furthermore, the removal of glycans at positions N187 and N197 in the V2 loop and at position N301 on the V3 base has been shown to dramatically increase the exposure of V3 crown epitopes, which are wedged between N301 to N157 glycans. With an increase in the number of V2 glycosylation sites demonstrated in V2i antibody resistance viruses compared with sensitive sites, the additional glycosylation sites being included were mainly in the hypervariable fragments of V2 domain.⁹

Lectins in contrast do not interact with the protein component of Env but are exclusively specific to the glycan residues, which, in turn, affect the packing and stabilization of highly flexible crown and stem of V3 and V2 loops, thus affecting their exposure by limiting the mobility of these highly flexible regions.¹⁰ However, we found this effect only in tier 1 viruses but not in tier 2 and tier 3 viruses, possibly other conformational constraints restrict the accessibility of antibodies to these epitopes.

This study reveals the masking of V3 crown and V2i epitopes by glycan clusters as has been reported previously. Using Manz1-2Man-binding lectins, which stabilize the highly flexible loops on the most proximal surface of HIV-1 Env and allow access of antibodies to these epitopes, resulted in the enhancement of their neutralization potency.

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Author Disclosure Statement

No competing financial interests exist.

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