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Effect of sialic acid removal on the antibody response to the third variable domain of human immunodeficiency virus type-1 envelope glycoprotein

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Abstract

The gp160 envelope glycoprotein of human immunodeficiency virus type-1 (HIV-1) is an essential component of current vaccine trials. The glycans of gp160, part of which are highly sialylated, have been shown to influence gp160 immunogenicity. Here, using a panel of synthetic V3 peptides, we characterized the anti-V3 antibodies generated in rabbits immunized by desialylated recombinant $gp160_{LAI}$. Amino acid residues flanking the GPGR tip of V3 were necessary for the recognition by anti-V3 antibodies raised against either the native or desialylated gp160. Both types of antibodies reacted to V3 peptides of MN and SF2 strains and with a North American/European V3 consensus peptide, while anti-desialylated $gp160_{LAI}$ antibodies reacted in addition to the V3 of CDC4, WMJ2 and NY5 strains. Yet, the V3 peptides did not significantly differ in their secondary structure, as determined by circular dichroism. The titer and avidity for $V3_{MN}$ of anti-desialylated $gp160_{LAI}$ antibodies were significantly lower than those of anti-native $gp160_{LAI}$, which likely accounts for the inability of anti-desialylated $gp160_{LAI}$ sera to neutralize HIV-1_{MN}-induced syncytia. These results indicate that V3 immunogenicity may be influenced by subtle directed changes in the gp160 glycosylation pattern.

Key words: HIV; V3; Glycosylation; Antigenicity; Neutralizing antibody

1. Introduction

Much information has been gathered about the functions of N-linked glycans of human immunodeficiency virus type-1 (HIV-1) envelope glycoproteins and on their possible influence on immune recognition: glycans of mature gp120 and gp41 do not play a determinant role in virus infectivity (for reviews, see [1,2]) but they contribute to its antigenicity [3–7].

Envelope gp120 is composed of interspersed variable (V) and conserved (C) regions, some of which are disulphide-bonded [8]. The V4-C5 domains are considered to be involved in gp120 binding to CD4, the HIV receptor [9]. The V3 loop is not directly involved in the binding to CD4, but is likely to play a crucial role in subsequent post-binding events that result in virus/cell membrane fusion and virus entry into cells [9–11]. It is also a primary determinant of cell tropism [12]. About 35 amino acid (aa) residues long, V3 is the site of the principal neutralization determinant (PND) [11,13–15], and anti-V3 neutralizing antibodies (NA) appear to protect animals against a homologous HIV-1 challenge [16–19]. However, V3 variability [11,14] may represent a major obstacle to develop efficacious vaccines based on such responsiveness. Yet, most vaccine strategies are attempting to elicit strong and broadly reactive NA against V3 as presented by gp120 or gp160, based on the current knowledge of the key aa involved both in V3 biological activity and antigenicity [15,20–23].

An original approach to elicit broadly anti-V3 NA would be to modify gp120 overall carbohydrate padding in the hope of exposing hidden epitopes or to enhance V3 immunogenicity through conformational changes. This approach is supported by evidence that glycans may modulate the immunological properties of glycoproteins [24–26]. In most cases, N-linked glycans are not involved per se in the antibody response, but they act through their own conformation and/or by interacting with the peptide backbone, either by epitope masking or by conformational modifications [27]. The negative charges of sialic acids are known to play a prominent role in this respect [27–28].

It has recently been shown that the sialic acids presented by several glycans of gp120 and gp41 have important effects on their antigenicity: compared with native gp160, selective deglycosylation of gp160 increases the antigenicity of some of its regions, e.g. the V2 domain [5], and desialylation elicits antibodies in rabbits that recognize a conserved epitope downstream of the V2 loop of

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gp120 and that also cross-react with gp140 of HIV-2 [6,7].

Here, taking into account that viral gp160 and its recombinant counterpart produced in mammalians cells have a similar carbohydrate composition [8,29], we investigated whether enzymatic removal of sialic acid from recombinant gp160 obtained in mammalian cells modifies the antibody response to V3 of immunized rabbits.

2. Materials and methods

2.1. Immunization of the rabbits

Soluble recombinant gp160 of HIV-1_{LAI} produced by mammalian BHK21 cells was a gift from Pasteur Mérieux Sérums et Vaccins (Lyon, France).

Treatment of gp160 with neuraminidase from *Clostridium perfringens* (Sigma, St. Louis, MO) was performed under conditions that were previously shown to remove sialic acid without detectable side effects such as proteolysis or denaturation [6,30].

New Zealand White rabbits (2 rabbits/immunogen) were injected intradermally with 200 μg of native or neuraminidase-treated gp160 in complete Freund's adjuvant. This procedure was repeated with incomplete Freund's adjuvant on days 30, 60, 90 and 120, as described [6,7]. The anti-gp160 sera studied here were obtained one week after the last injection.

2.2. Enzyme-linked immunosorbent assay (ELISA)

V3 synthetic peptides, derived from the corresponding aa sequence of different HIV-1 strains (Los Alamos data base) were obtained from the Agence Nationale de Recherche sur le SIDA or purchased from Neosytem (Strasbourg, France).

Ninety-six-well microtiter plates (Nunc, Roskilde, Denmark) were coated for 2 h at 37°C with 500 ng/well of peptide in 50 μl of PBS, pH 7.4. After saturation and washing as described [6,7], 50 μl of diluted serum were added for 2 h at 37°C. Staining was performed with peroxydase-coupled swine anti-rabbit IgG (Dakopatts, Paris, France) as reported [6,7].

To assess antibody avidity, increasing amounts of peptides were preincubated for 1 h at 37°C with the sera before performing the ELISA.

2.3. Circular dichroism (CD) analysis

Low ultraviolet circular spectroscopies were recovered on a Jobin-Yvon spectrophotometer (Longjumeau, France). The instrument was calibrated with (+)-10-camphorsulfonic acid. Spectra were performed at 25°C by using a 0.5 mm path-length cell, with a 2-s time constant and a 0.5 nm/s scan rate. The peptide concentration was 0.1 mg/ml as determined by aa analysis. The spectra were cumulated 5-fold in water and 3-fold in 98% 2,2,2 trifluoroethanol (TFE). Values were automatically averaged.

2.4. Syncytia formation assay

Cells were cultured in RPMI 1640 supplemented with 5% fetal calf serum, 1% glutamine, 1% streptomycin-penicillin (Gibco, Irvine, Scotland). CEM or C8166 cells (a gift from H. Holmes, P. Tomlinson and A. Newberry, MRC-ADP, UK) were chronically infected with HIV-1_{LAI} or HIV-1_{MN}, respectively.

Infected cells (1×10^4 were incubated for 2 h with various serum dilutions in 96-well plates. Uninfected Molt-4 cells (4×10^4) were then added in 100 μl of culture medium. Syncytia were counted after 18 h at 37°C. To demonstrate that syncytium inhibition was essentially due to anti-V3 antibodies, sera were preincubated with 10 μg of the appropriate V3 peptide.

Preimmune sera were used as negative controls. Anti-Leu3a (Becton-Dickinson, Mountain View, CA, USA), a neutralizing anti-CD4 monoclonal antibody, was used at 2.5 μg/ml as positive control.

3. Results

3.1. Mapping of the V3 region recognized by the antibodies

We first determined the region of V3 to which anti-native (N1, N2) and anti-desialylated (D1, D2) gp160 antibodies reacted. We examined antibody binding to overlapping synthetic peptides encompassing $V3_{LAI}$ and to their $V3_{MN}$ counterparts, since the $V3_{MN}$ sequence appears as more closely related to that of most European and North American field isolates [14].

The four sera strongly reacted against peptides (302–

peptides		amino acid sequences	serum ELISA reactivity (OD) N1	N2	D1	D2
V3(LAI)	302-334	C T R P N N N T R K S I R I Q R G P G R A F V T I G K I G N M R Q A	2.3	2.4	1.9	2.1
	302-324	T R P N N N T R K S I R I Q R G P G R A F V T	1.1	0.6	0.6	1.3
	307-327	C N T R K S I R I Q R G P G R A F V T I G K	2.4	1.9	1.6	1.9
	312-327	I R I Q R G P G R A F V T I G K	0.8	0.3	0.1	0.6
	306-320	N N T R K S I R I Q R G P G R	—	—	—	—
	308-323	T R K S I R I Q R G P G R A F V	—	—	—	—
	308-318	T R K S I R I Q R G P	—	—	—	—
	314-330	I Q R G P G R A F V T I G K I G N	0.1	—	—	—
	319-327	G R A F V T I G K	—	—	—	—
	321-334	A F V T I G K I G N M R Q A	—	—	—	—
V3(MN)	307-331	- K - - R - H - - - - - - - Y - T K N - I G T I C	2.0	1.7	1.0	0.9
	307-325	C - K - - R - H - - - - - - - Y - T K N	2.3	1.4	1.1	1.0
	307-320	- K - - R - H - - - - - - -	—	—	—	—
	311-325	R - H - - - - - - - Y - T K N	—	—	—	—
	319-331	- - Y - T K N - I G T I C	—	—	—	—

Fig. 1. Mapping of the regions of V3 recognized by sera raised against either native (N1, N2) or desialylated (D1, D2) $gp160_{LAI}$. Sera were diluted 1 : 100. The coated peptides were derived from either $V3_{LAI}$ or $V3_{MN}$. OD values were obtained at 492 : 620 nm (–) no reactivity: (OD < 0.1). Background OD was 0.03 (number of experiments, $n = 3$). C was added to the original sequence in order to potentially facilitate coupling to a carrier.

V3 peptides	amino acid sequences	%/Lai	serum ELISA reactivity (OD) N1	N2	D1	D2
Lai(307-327)	CNTRKSIRIQRGPGRAFVTIGK	100	2.4	2.4	1.4	2.4
HXB2	-----R-----------	95	1.8	1.5	1.4	1.4
SF2	-------Y- -------H-T-R	71	1.9	0.9	0.9	0.8
MN	--K--R-H- -------Y-TKN	57	2.3	1.4	1.1	0.9
Cons	-------H- -------Y-TGE	66	1.7	0.9	0.6	1.5
WMJ2	--V-R-LS- -------R-RE	55	-	-	1.2	1.2
NY5	---K-G-A- -----TLYARE-	48	-	-	0.9	0.8
CDC4	-H---RVTL -----VWY-T-E	42	-	-	0.9	0.9
SC	---TR--H- -------YAT-D	57	-	-	-	-
RF	--------TK -----VIYAT-Q	52	-	-	-	-
Z6	----Q-TP-GL-QALYTTRGRTK	29	-	-	-	-
ELI	----QRTP-GL-QSLYTTRSRS	24	-	-	-	-
Z2	--I-QRTS-GL-QALYTTKTRS	19	-	-	-	-

Fig. 2. Reactivity of sera raised against either native (N1, N2) or desialylated (D1, D2) $gp160_{LAI}$ to V3 peptides from different HIV-1 strains. Conditions are as in Fig. 1. %/Lai: % of homology between the LAI and the other PND sequences.

334) and (307–327) of $V3_{LAI}$, and also against (302–324) (Fig. 1). Weak or no reactivity was noted against peptide (312–327). Because the sera did not bind to peptides (306–320), (308–323) and (314–330), aa residues flanking the conserved GPGR sequence should be important for antibody recognition. This corresponds to what is already known regarding the PND region [11,20,22,31].

With respect to $V3_{MN}$, the sera reacted also against peptides (307–331) and (307–325), but not against peptides (307–320), 311–325) and (319–331).

3.2. Reactivity of the sera against V3 peptides from different HIV-1 isolates

To examine whether desialylation of $gp160_{LAI}$ modified the immunogenicity of its V3 domain, we compared the reactivity of anti-native and anti-desialylated $gp160_{LAI}$ antibodies against peptides mimicking the core of V3 (corresponding to the 307–327 aa sequence of $V3_{LAI}$) of clone HXB2 and of different HIV-1 strains known to be more or less homologous to $HIV\text{-}1_{LAI}$: namely MN, SF2, WMJ2, CDC4, NY5, SC, RF, Z2, Z6, ELI. We also used a peptide derived from the North American/European consensus (Cons) sequence [14]. Beside the expected reactivity to $V3_{HXB2}$, three reactivity patterns could also be distinguished (Fig. 2).

(i) Peptides derived from MN, Cons and SF2 V3 sequences, the homology of which with $V3_{LAI}$ is about 60–70%, were efficiently recognized by all four sera.

(ii) Only sera D1 and D2 reacted in addition with V3 peptides derived from WMJ2, NY5 and CDC4, which present 42–55% homology with $V3_{LAI}$.

(iii) The third group of peptides (SC, RF, Z2, Z6 and ELI) did not react with any of the sera, even though SC and RF were, respectively, 57% and 52% homologous to $V3_{LAI}$ while the level of homology to $V3_{LAI}$ of the three other peptides was 19–29%.

As controls, it was verified that the four immune sera strongly reacted against a peptide corresponding to gp41 conserved immunodominant epitope and that the preimmune sera did not react to V3 nor to the gp41 peptide (data not shown; [7]).

3.3. CD analysis of the V3 peptides

We then examined whether the pattern of antibody reactivity observed could be accounted for by the degree of homology of the peptides' secondary structure, as determined by CD analysis. The intensive negative band around 200 nm noted for all peptides in aqueous solution (Fig. 3A, and data not shown) indicated the predominance of nonperiodic (unordered) conformer populations [32]. When spectra were obtained in TFE, a membrane-like environment, all peptides exhibited a modified shape indicating that they tended to adopt a helix conformation characterized by typical double minima near 208 nm and 222 nm and increase of positive ellipticity below 200 nm (Fig. 3B, and data not shown). An intense negative band was shown by the peptides from Z2, Z6, and ELI (data not shown).

3.4. Inhibition of syncytium formation by the sera

We have previously shown that rabbit antisera to both native (N1, N2) or desialylated (D1, D2) $gp160_{LAI}$ neutralize $HIV\text{-}1_{LAI}$-induced syncytia and infection to the same extent [6]. Because these sera reacted with $V3_{MN}$ as well as with $V3_{LAI}$ in ELISA, we tested whether they could also inhibit $HIV\text{-}1_{MN}$-induced syncytia.

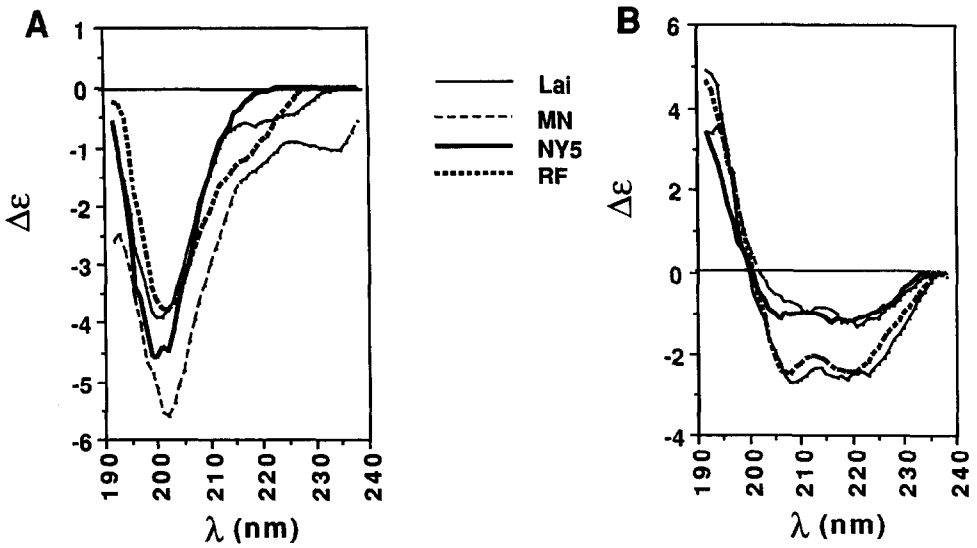

Fig. 3. Circular dichroism spectra of V3 peptides in water (A) and in 98% TFE (B). $\Delta\varepsilon$ corresponds to the variation of molar aa residue absorption coefficient expressed in $M^{-1} \cdot cm^{-1}$.

As reported, all sera neutralized syncytia induced by HIV-1_{LAI} [6] but only N1 and N2, and not D1 and D2, inhibited HIV-1_{MN}-mediated syncytia in a dose-dependent manner (Fig. 4). Preincubation of N1 (and N2; data not shown) with $V3_{MN}$ peptide (307–325) abolished the serum capability to inhibit HIV-1_{MN} syncytia. Therefore, this inhibitory effect was mainly due to anti-V3 antibodies.

As controls, it was shown that anti-Leu3a antibodies neutralized syncytia and that preimmune sera had no effect.

Sample	serum dilution		
	1/10	1/30	1/100
N1	–	x	xx
N2	x	xx	xxx
D1	xxx	xxxx	xxxx
D2	xxxx	xxxx	xxxx
N1+V3MN	xxx	xxxx	xxxx
Anti-Leu3a	–		
Preimmune	xxxx	xxxx	xxxx

Fig. 4. Ability of sera raised against either native (N1, N2) or desialylated (D1, D2) $gp160_{LAI}$ to inhibit HIV-1_{LAI}- or HIV-1_{MN}-mediated syncytia. HIV-1_{MN} infected C8166 cells (1×10^4 cells) were incubated in microtiter plates with different serum dilutions for 2 h before the addition of Molt-4 cells (4×10^4 cells). Syncytia were scored after 18 h. Syncytium inhibition relative to the control (preimmune sera or no serum) was scored as follows: – = 98% reduction of syncytia; + = 90–98% reduction; ++ = 60–90% reduction; +++ = 20–60% reduction; ++++ = < 20% reduction. Preincubation of $V3_{MN}$ peptide (10 μg) with N1 (N1 + V3MN) before adding C8166 cells and co-incubation of Leu3a monoclonal antibody (Leu3a) with infected C8166 cells and Molt-4 cells were also performed.

3.5. *Characterization of the cross reactive anti-V3 antibodies*

Inhibition of HIV-1_{MN}-mediated syncytia by N1 and N2 correlated with their ability to bind to $V3_{MN}$ in ELISA but discrepant results were obtained in this respect with D1 and D2, which were unable to block HIV-1_{MN}-induced fusion though they bound also to $V3_{MN}$ peptides. This different behaviour may be due either to differences in the amounts of $V3_{MN}$ cross-reacting antibodies or to differences in the affinity of the antibodies for the peptides.

ELISA titration experiments (Fig. 5A) showed that the four sera recognized $V3_{MN}$ (307–325) in a dose-dependent manner, but with different titers. For example, if the titer is defined as the serum dilution which results in a 1 OD value, titers were significantly higher for N1 and N2 than for D1 and D2 sera (1×10^{-3} and 3×10^{-4} vs. 1×10^{-2}), respectively.

The avidity for $V3_{MN}$ of the corresponding antibodies was also examined (Fig. 5B). The concentration of soluble $V3_{MN}$ peptide that inhibited by 50% ($K_{0.5}$) N1 and N2 antibody binding to the same coated peptide was in the 3×10^{-7} M range, while it was about 1×10^{-5} M for D1 and D2.

Comparatively, the four sera recognized $V3_{LAI}$ to the same extent, 1 OD values being noted with sera diluted 1×10^{-3} to 3×10^{-4}, and antibodies in these sera had a similarity high avidity for the peptide ($K_{0.5}$ from 5×10^{-7} to 1×10^{-6} M; data not shown).

Therefore, both the titer and the avidity of D1 and D2 antibodies for $V3_{MN}$ may be considered as lower than those of N1 and N2 antibodies.

These characteristics were also determined with V3 peptides of WMJ2, NY5 and CDC4, which were recognized only by D1 and D2. The titers of D1 and D2 (data

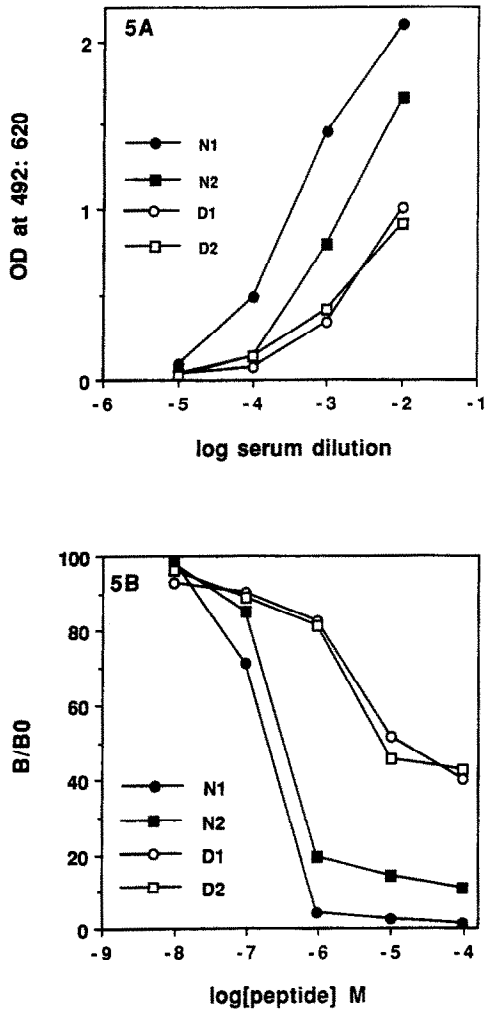

Fig. 5. Determination of the titer (A) and avidity (B) to $V3_{MN}$ peptide (307–325) of sera raised against either native (N1, N2) or desialylated (D1, D2) $gp160_{LAI}$. The diluted sera were added to $V3_{MN}$-coated plates (A). Different amounts of $V3_{MN}$ peptide were preincubated with the sera before addition to the plates (B). B/B0, binding obtained in the presence of competitor/binding in the absence of competitor.

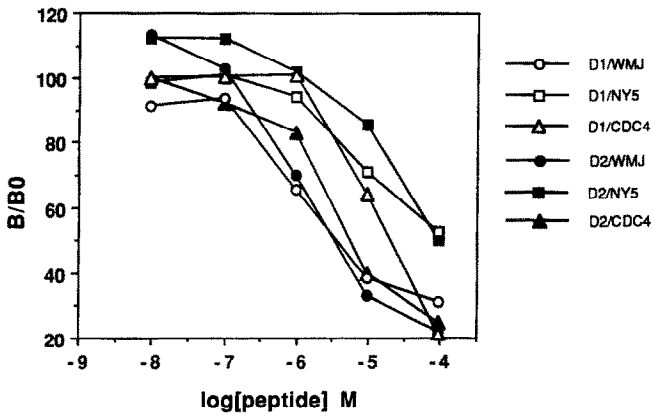

Fig. 6. Determination of the avidity to V3 peptides from WMJ2, NY5 and CDC4 isolates of sera raised against desialylated (D1, D2) $gp160_{LAI}$. Conditions are as in Fig. 5.

not shown), and the corresponding avidity ($K_{0.5}$ from 1×10^{-4} to 1×10^{-5} M: Fig. 6) were as low as to $V3_{MN}$.

4. Discussion

The glycans of gp160, which are almost equally divided into oligomannosidic and highly sialylated complex species [8] may influence its antigenicity. For example, antibodies elicited by gp160 whose mannose moieties are cleaved fail to neutralize HIV-1 infection in contrast to antibodies raised against native or desialylated gp160 [6,7]. Interestingly, the latter antibodies recognize an otherwise silent conserved epitope downstram of gp120 V2 loop, and they cross-react with HIV-2 gp140.

It was then of interest to investigate whether sialic acid removal from $gp160_{LAI}$ modifies the way antibodies recognize V3, by examining their cross-reactivity to V3 synthetic peptides from different HIV-1 strains and their possible cross-neutralizing capacity for a relatively divergent strain such as $HIV\text{-}1_{MN}$.

The top of the V3 loop comprises a conserved GPGR sequence shared by most isolates, which is flanked by variable aa residues. Several epitopes may be recognized by V3-specific NA, the binding of which requires either or both of GPGR flanking regions [11,20,22,31].

Here, overlapping synthetic peptides were used to map the regions of V3 that were recognized by antibodies elicited against either desialylated or native gp160. All sera reacted with $V3_{LAI}$ (aa 307–327) and $V3_{MN}$ (307–325) central region known to include the PND, which confirms its immunodominance. Peptides lacking aa residues flanking either or both sides of the GPGR tip did not attach antibodies, indicating that the corresponding aa are necessary for antibody recognition.

When assayed against V3 peptides of different strains representative of American and European dominant isolates [14] all the sera also cross-reacted with the peptides derived from MN and SF2, which display about 65% homology with $V3_{LAI}$, but not with those from more divergent SC, RF, or ELI, Z2 and Z6 African strains. Interestingly, antibodies against desialylated $gp160_{LAI}$ reacted in addition with the V3 of WMJ2, CDC4 and NY5, which present about 50% homology with $V3_{LAI}$. This finding could be explained by the occurrence of a broadly reactive antibody population elicited by sialic acid removal from $gp160_{LAI}$. One possibility is that desialylation induces conformational changes of gp160 that reveal structures that are common on the V3 of LAI, WMJ2, CDC4 and NY5.

Such broad antibody binding ability might then be explained by the existence of epitopes within V3 that share homologies either in their primary or secondary structures. While the GPGR crown and the overall

length and charge are generally conserved among these peptides, their sequences present an important variability of the flanking aa residues. This presumably accounts for the different reactivity of the two sets of sera. Therefore, we analyzed these peptides by CD to investigate whether the observed reactivity pattern was actually related to a common secondary structure. In line with what was previously reported for $V3_{MN}$ and $V3_{IIIB}$ [33], all peptides in aqueous solution showed similar spectra which were characteristic of predominantly random conformations [32]. In TFE, all peptides tended to form α-helices. Thus, there is no obvious correlation between the antibody reactivity pattern and the secondary structure of the peptides defined by CD. These results suggest that even though mutations, deletions and insertions may occur within V3, they do not significantly modify their flexibility.

However, such analysis (% of homology, CD) does not address all the effects of individual aa changes. Indeed, antibody-antigen interactions depend on the mobility, packing density, shape, surface exposure and negative electrostatic potential of the epitope [34]. Thus, some aa contained within a sequence present a greater contribution than others to the reactive epitope, and change of a single aa can be sufficient to completely abolish antibody recognition. This has been verified for V3 [15,21–23,35]. For example, change of P^{318} to Q almost completely abolishes the binding and neutralizing activity of monoclonal antibody 0.5β [21] and change of FR^{322} to L increases resistance to neutralization by soluble CD4 [23].

To assess whether the ability of sera to cross-react with divergent V3 peptides correlates with their capacity to neutralize the corresponding isolates, we studied their ability to inhibit syncytia induced by prototype HIV-1_{MN}. While all sera inhibited syncytia mediated by HIV-1_{LAI} [6] only sera raised against native, but not those raised against desialylated $gp160_{LAI}$, impaired HIV-1_{MN}-induced syncytia. Because soluble V3 peptide efficiently abrogated the syncytium inhibitory effect of these sera, this indicates that we specifically adressed the anti-V3 response in our assay.

Because both types of sera recognized the same $V3_{MN}$ sequence, the discrepancy between the ability of D1 and D2 to additionally react to divergent V3 peptides but not to neutralize HIV-1_{MN}-mediated syncytia, cannot be explained by differences due to antibodies recognizing different epitopes. This may rather be related to the fact that their antibody titeres and avidity for $V3_{MN}$ were 10- and 100-fold lower, respectively, relative to N1 and N2. For example, when considering anti-V3 neutralizing human monoclonal antibodies 'the higher the affinity for $V3_{MN}$ the less monoclonal antibody is needed to neutralize the virus', and only antibodies with high affinities ($< 1 \times 10^{-6}$ M) are able to neutralize in vitro [15,31] or to apparently prevent HIV-1 vertical transmission [36]. This may well apply to D1 and D2, whose $K_{0.5}$ was about 1×10^{-5} M. Also, the avidity of these two sera for WMJ2, CDC4 and NY5 V3 peptides was low ($K_{0.5} > 10^{-5}$ M), which would indicate that they should fail to neutralize infection by these isolates in the same manner as they do for HIV_{MN}.

Nevertheless, these results indicate that sialic acid significantly influence V3 antigenicity. This may be surprising if one considers the limited glycosylation of V3 as compared with other gp120 domains, all the more so that the only glycan in the loop has been determined as being of the high-mannose or hybrid type, and the former do not present sialic acids [8]. However, it is possible that the three closely located or other more distant complex- or hybrid-type glycans affect V3 immunological properties. There is also other evidence for the direct role of glycans on V3 biological and immunological properties: (i) when considering vertical HIV transmission, 'a conserved N-linked glycosylation site within the V3 region, present in each mother's sequence set, was absent in all of the infant's sequence set' [37]; (ii) antibodies elicited by V3 peptide (301–315) failed to recognize $gp120_{SF2}$ but not its unglycosylated counterpart [4]; (iii) resistance of HIV-1 to neutralization was achieved by adding glycan in the V3 loop adjacent to the NA binding site [35].

Sialic acids have been reported to influence the conformation of glycoproteins in solution, especially through their negative charge that attracts electropositive basic aa to maintain the carbohydrate umbrella conformation [27–28], and this may prevent immune recognition by steric hindrance [38–39]. As to positively charged V3, it is possible that sialic acid removal from close or distant glycans restores the glycan branch mobility, giving thus access to new epitopes. Such an effect has been suspected for CAEV [38], oversialylation of the envelope glycoproteins of which may possible be responsible for the limited capacity of the virus to induce NA, whereas closely related visna virus whose envelope is less sialylated can elicit efficient NA.

Glycosylation, especially sialylation, may therefore play a role in modulating the protective immune response against HIV. As noted here, desialylation improves the ability of sera to cross-react with V3 peptides of relatively divergent viral strains, but at the cost of avidity. Future studies should then aim at designing immunization methods with abnormally glycosylated *env* productes in order to improve both the production and affinity of the resulting antibodies, in the hope to enhance their neutralizing capacity.

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