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Cross-neutralizing activity of human anti-V3 monoclonal antibodies derived from non-B clade HIV-1 infected individuals

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ABSTRACT

One approach to the development of an HIV vaccine is to design a protein template which can present gp120 epitopes inducing cross-neutralizing antibodies. To select a V3 sequence for immunogen design, we compared the neutralizing activities of 18 anti-V3 monoclonal antibodies (mAbs) derived from Cameroonian and Indian individuals infected with clade AG and C, respectively. It was found that V3 mAbs from the Cameroonian patients were significantly more cross-neutralizing than those from India. Interestingly, superior neutralizing activity of Cameroonian mAbs was also observed among the nine VH5-51/VL lambda genes encoding V3 mAbs which mediate a similar mode of recognition. This correlated with higher relative binding affinity to a variety of gp120s and increased mutation rates in V3 mAbs from Cameroon. These results suggest that clade C V3 is probably weakly immunogenic and that the V3 sequence of CRF02_AG viruses can serve as a plausible template for vaccine immunogen design.

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Introduction

In light of the continuing worldwide spread of HIV-1 infection, it is accepted that a vaccine would be the most effective prophylaxis, although the genetic variation of the virus remains a major challenge. A certain optimism for designing an efficient vaccine was recently raised by the results from the RV144 clinical vaccine trial, which suggested that anti-HIV-1 antibodies (Abs) played a protective role (Rerks-Ngarm et al., 2009). Indeed, it was revealed that high titers of plasma anti-V1/V2 Abs correlated inversely with the rate of HIV-1 infection (Haynes et al., 2012). The neutralizing antibody response in the vaccine recipients was weak and did not correlate with the rate of infection but it was suggested that it can be partially protective against HIV-1 (Montefiori et al., 2012).

One concept for an HIV vaccine is to design a template with a set of immunogens representing various epitopes in the envelope. Such epitopes could include the V1/V2 regions as well as the V3 loop which is known to induce cross-clade neutralizing Abs (Corti et al., 2010; Hioe et al., 2010; Pantophlet et al., 2007; Scheid et al., 2009;2011). The contribution of anti-V3 Abs in protection against HIV-1 infection was documented by passive immunization experiments in animal models (Andrus et al., 1998; Emini et al., 1992; Watkins et al., 2011). Studies in vitro in several laboratories showed that the human V3 mAbs can neutralize the majority of tier 1 pseudoviruses (psVs) and some tier 2 viruses but their cross-clade neutralizing activity is relatively limited (Corti et al., 2010; Hioe et al., 2010; Pantophlet et al., 2007; Scheid et al., 2009;2011). Moreover, it was demonstrated in animal experiments that V3 immunogens can induce cross-clade neutralizing antibodies (Letvin et al., 2001; Zolla-Pazner et al., 2011).

The question still remains which V3 structure would be the most effective in inducing cross-neutralizing Abs. It was shown previously that anti-V3 mAbs derived from individuals infected with non-B clade viruses were more cross-reactive than clade B-derived V3 mAbs (Gorny et al., 2006). This appears to be due to the fact that the relatively conserved V3 motif GPGR in clade B viruses focuses the immune response on the positively charged Arg (R) and thus narrows the cross-reactivity of the responding

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V3 Abs, while the GPGQ motif in non-B clade viruses allows induction of Abs to other conserved elements of the V3 region (Jiang et al., 2010). The non-B clade viruses, with the GPGQ motif, are a very heterogeneous group, involved in ~85% of global HIV-1 infection with the most dominant being clade C, followed by clade A (Osmanov et al., 2002).

In this study we compared the neutralizing and binding activity of two panels of anti-V3 mAbs derived from subjects living in Cameroon and India and infected with non-B clade viruses containing the envelope (Env) sequence corresponding to clade A, mainly CRF02_AG, and clade C, respectively. The results showed that the mAbs from the Cameroonian patients were significantly more cross-neutralizing than those from India, suggesting that the V3 structure and sequence of CRF02_AG viruses would be better for immunogen design.

Results

Human anti-V3 mAbs

A panel of 18 anti-V3 mAbs derived from individuals infected with non-B clade HIV-1 includes five new anti-V3 mAbs, not yet described, generated from Cameroonian (mAbs 4210 and 4508) and Indian patients (mAbs 4139, 4487 and 4490) (Table 1). These five new mAbs belong to subclass IgG1 with four λ and one κ light chains (Table 1). The remaining 13 V3 mAbs have been previously described and characterized (Table 1).

Immunoglobulin gene usage

The variable fragment of the heavy (VH) and light (VL) chain genes were sequenced and analyzed using the IMGT system to determine the immunoglobulin (Ig) gene usage and percentage of mutations. A biased usage of VH genes was observed, as 9 of 18 (50%) anti-V3 mAbs were encoded by the VH5-51 gene segment: four mAbs developed from Cameroonian and five from Indian patients; however, these mAbs used different alleles, mainly *03 and *01, respectively (Table 2). Five other mAbs used VH1 family genes while three mAbs, 3074, 3881 and 4508 used one particular gene segment, VH4-59. The VH3 family genes, which are the most commonly used by Abs derived from healthy individuals (Tiller et al., 2007), were represented only by one V3 mAb which used the VH3-30 gene (Table 2).

Usage of the light chain genes was also biased toward lambda genes which were used by 14 of 18 mAbs (Table 2) while in mAbs derived from healthy subjects there is dominance of κ over λ light chain genes (Tiller et al., 2007). Among lambda VL genes, the most frequently used was the VL3-1 gene in 8 of 14 mAbs (57%), which mainly paired with VH5-51 gene (6 of 9 pairs using VH5-51 gene).

The amino acid sequence of the complementarity-determining region 3 (CDR) of the heavy chain (H3) and light chain (L3) was unique for each mAb (Table S1).

Neutralization of pseudoviruses by anti-V3 mAbs derived from Cameroonian and Indian HIV-1 infected patients

A panel of 18 anti-V3 antibodies derived from the Cameroonian and Indian HIV-1 infected subjects and a control mAb 1418 (anti-parvovirus B19) were tested with 41 pseudotyped viruses from clade A, B, C and AG for their neutralizing activity. Twenty-one of 41 viruses tested were found to be neutralized by this panel of V3 mAbs with a 50% inhibitory concentration (IC_{50}) < 50 μg/ml (Table 3). The remaining 20 psVs were not neutralized at an IC_{50} below 50 μg/ml (data not shown); all but one of these were tier 2 viruses (HO29.12, HO30.7, HO35.18, HO61.14, WITO4160.33, SC42661.8, TRO.11, AC10.0.29, THRO4156.18, CAAN5342.A2, PVO.4, TRJO4551.58 [clade B], CAP45.2.00.G3, Du156.12, Du172.17, Du422.1, ZM53M.PB12, ZM135M.PL10, ZM214M.PL15, ZM249M.PL1 [clade C]).

Table 1
Human anti-V3 and control mAbs used for the study.

No.	mAbs[a]	Site	Isotype	Subtype of the infecting virus[b]	Country of origin	Reference
1	3869	V3	IgG1 λ	Nd	Cameroon	Gorny et al. (2009)
2	4210	V3	IgG1 λ	Nd	Cameroon	This study
3	3074	V3	IgG1 λ	CRF02_AG	Cameroon	Gorny et al. (2006)
4	3881	V3	IgG1 κ	CRF02_AG	Cameroon	Gorny et al. (2009)
5	4508	V3	IgG1 κ	CRF02_AG	Cameroon	This study
6	2557	V3	IgG1 λ	CRF02_AG	Cameroon	Gorny et al. (2004)
7	2558	V3	IgG1 λ	CRF02_AG	Cameroon	Gorny et al. (2004)
8	3019	V3	IgG1 λ	CRF02_AG	Cameroon	Gorny et al. (2006)
9	3694	V3	IgG1 λ	H	Cameroon	Gorny et al. (2009)
10	4139	V3	IgG1 λ	C	India	This study
11	3791	V3	IgG1 κ	C	India	Gorny et al. (2009)
12	4121	V3	IgG1 λ	C	India	Gorny et al. (2009)
13	3904	V3	IgG1 κ	C	India	Gorny et al. (2009)
14	3906	V3	IgG1 λ	C	India	Gorny et al. (2009)
15	4490	V3	IgG1 λ	Nd	India	This study
16	4022	V3	IgG1 λ	C	India	Gorny et al. (2009)
17	4025	V3	IgG1 λ	Nd	India	Gorny et al. (2009)
18	4487	V3	IgG1 λ	Nd	India	This study
	Controls:					
	447-52D	V3	IgG3 λ	B	USA	Gorny et al. (1993)
	1418[c]	B19	IgG1 κ	N/A	USA	Gigler et al. (1999)

Nd—Not determined.

[a] Each mAb is derived from one HIV-1 infected individual (except 1418 which was produced from a subject carrying anti-parvovirus B19 serum Abs) living in the depicted countries.

[b] The subtype of the infecting HIV-1 was determined in our laboratory by sequencing the envelope fragment (C2-V5) from the donor plasma virus.

[c] Human mAb 1418 is specific against parvovirus B19 and it serves here as negative control.

The V3 mAbs neutralized both sensitive tier 1 and the more resistant tier 2 psVs; however, the mAbs displayed different patterns of activity with these two categories of psVs. For example, most tier 1 viruses were neutralized at < 1 μg/ml, while most of the tier 2 viruses required > 10 μg/ml of the mAbs to reach 50% neutralization. In terms of frequency, 106 of 198 (53%) tier 1 psVs/mAb combinations showed neutralizing activity while only 30 of 180 (16%) tier 2 psVs/mAb combinations showed neutralization ($p < 0.001$) (Table 3).

Interestingly, nine mAbs from the Cameroonian patients neutralized 21 psVs significantly more potently than the nine mAbs from the Indian patients by comparing their IC_{50} values ($p = 0.001$). The significantly more potent neutralizing activity of the anti-V3 mAbs from the Cameroonian patients was observed separately for (a) tier 1 ($n = 11$ psVs, $p < 0.01$), (b) tier 2 ($n = 10$ psVs, $p < 0.0001$), (c) clade B ($n = 12$ psVs, $p = 0.002$), and (d) non-B clade viruses ($n = 9$ psVs, $p = 0.01$). Statistical significance was determined using the Chi-Squared test.

Table 2
Analysis of immunoglobulin gene usage and percent mutations in the variable fragment of human anti-V3 mAbs.

mAb[a]	IGHV[b]	IGHJ[b]	mut (%)[c]	IGLV[b]	IGLJ[b]	mut (%)[c]
3869	1-f*01	4*02	8.3	L3-1*01	2*01/3*01	8.6
4210	1-f*01	3*02	5.6	L3-1*01	3*02	4.3
3074	4–59*01	3*01	5.9	L1-51*02	2*01/3*01	5.3
3881	4–59*01	3*02	11.2	K3-15*01	1*01	5.4
4508	4–59*01	4*02	4.6	K4-1*01	3*01	4.7
2557	5–51*01	3*01	14.2	L3-1*01	3*02	14.0
2558	5–51*03	4*02	7.3	L3-1*01	2*01/3*01	5.7
3019	5–51*03	3*01	5.9	L3-1*01	2*01/3*01	9.7
3694	5–51*03	1*01	18.1	L3-1*01	2*01/3*01	11.5
4139	1–8*01	4*02	6.6	L2-14*01	2*01/3*01	6.6
3791	1–18*01	4*02	12.2	K1-3301	4*01	4.3
4121	1–18*01	4*02	5.2	L1-44*01	1*01	1.4
3904	3–30*03	4*03	5.9	K1-39*01	3*01	5.0
3906	5–51*01	3*02	7.3	L3-1*01	2*01/3*01	9.7
4490	5–51*01	4*02	4.5	L3-1*01	2*01/3*01	5.0
4022	5–51*01	5*02	11.8	L3-10*01	3*02	7.9
4025	5–51*01	6*02	3.8	L3-10*01	1*01	4.7
4487	5–51*03	4*02	8.3	L1-51*02	2*01/3*01	5.6

[a] The first nine mAbs are derived from Cameroon while the second nine mAbs are from India HIV-1 infected subjects, separated by a space.
[b] Immunoglobulin genes and alleles (*) were determined using the nucleotide sequence of the variable fragments of mAbs analyzed by IMGT system.
[c] mut — percent of mutation in IGHV and IGLV indicate the difference in nucleotide sequence between the tested mAbs and corresponding germline.

In terms of frequency, which also reflects cross-neutralization, 83 of 189 psVs/mAb combinations (44%) of the mAbs from the Cameroonian patients displayed neutralization compared to 53 of 189 psVs/mAb combinations (28%) displayed by the mAbs from the Indian patients (Table 3).

To understand the difference in the neutralizing activity we analyzed (a) Ig gene usage, (b) percentage of mutations, (c) relative binding affinity and (d) the epitopes of the mAbs derived from the Cameroonian and Indian patients.

Neutralization of VH5-51- and non-VH5-51-derived anti-V3 mAbs

Nine of 18 V3 mAbs, including four from Cameroonian and five from Indian patients, used the VH5-51 gene paired with VL lambda genes; six VL3-1, two VL3-10 and one VL1-51 gene (Table 2). Using the neutralization data from Table 3, statistical analysis revealed that the VH5-51-derived V3 mAbs from the Cameroonian patients were significantly more potent than the corresponding mAbs from the Indian patients ($p=0.006$, Table S2). Similarly, we analyzed the remaining mAbs encoded by non-VH5-51 genes including the VH1 family, the VH4-59 and VH3-30 genes. It was also found that the mAbs from the Cameroonian compared to Indian patients were significantly more potent in neutralization of psVs ($p=0.01$, Table S3) as determined using the Chi-Squared test, suggesting that the neutralizing efficiency of V3 mAbs from the Cameroonian patients may be independent of their VH gene usage.

Percentage of mutations in the heavy and light chain genes of anti-V3 mAbs

The percentage of point mutations in the nucleotide sequences of the variable (*V*) region of the heavy and light chain genes was determined by alignment with the corresponding germline genes using the IMGT system. The percentage of mutations varied for the VH genes from 3.8% to 18.1%, with a mean of 8.1%, while for VL genes varied from 1.4% to 14.0% with a mean of 6.6% (Table 2). We compared the percentage of mutations in the heavy and light chain genes combined together using the Student's *t*-test; the mean percentage for mAbs derived from the Cameroonian patients was higher than for those from the Indian patients, 16.7% versus 12.8%, but the difference was not significant ($p=0.20$) (Table S4).

The percentage of mutations was also analyzed separately for VH5-51-encoded V3 mAbs and was higher (although not significantly) for mAbs derived from the Cameroonian patients compared to those from the Indian patients, 21.6% versus 13.7% ($p=0.13$), respectively. The non-VH5-51 mAbs, which used the VH1 family, VH4-59 and VH3-30 genes, had only a slightly higher percentage of mutations amongst the mAbs from Cameroon vs. those from India, 12.7% and 11.8% ($p=0.71$) (Table S4). These results indicate that the percentage of mutations is higher among the V3 mAbs from Cameroon compared to those from India, especially in VH5-51-derived mAbs, but statistically comparable.

We analyzed the possible association between the percentage of mutations in the 18 V3 mAbs and the number of neutralized pseudoviruses and we found only a trend but not a significant correlation ($p=0.2668$ for all psVs and $p=0.1927$ for tier 2 psVs; Fig. S1). This study has some limitations because the CDR3 sequence of the heavy chain (CDR H3) is not included in the analysis of mutation rate due to lack of the corresponding germline sequence.

Binding of anti-V3 mAbs to recombinant gp120s

The lower neutralizing activity of the V3 mAbs from the Indian patients compared to those from the Cameroonian patients could be related to lower binding affinity. To test this hypothesis, all 18 anti-V3 mAbs were tested for their reactivity to 18 recombinant gp120s with sequences from one clade A, seven clade C and 10 clade B viruses (Table 4). Twelve gp120s corresponded to the respective psVs tested in the neutralization assay, but only four of these psVs (SF162.LS, ZM197M.PB7, JRFL.JB and REJO4541.67) were neutralized, as shown in Table 3. Although recombinant gp120 cannot mimic the native trimeric structure of the envelope which is targeted by the mAb in order to neutralize, binding indicates the presence of the epitope in the virus envelope. A representative binding pattern of mAbs to gp120s of clade C, A and B supported these data (Fig. S2A–D).

The binding experiments showed that the anti-V3 mAbs were able to bind all the clade C and one clade A gp120s and the only difference between the mAbs from Cameroon and India was observed in binding to clade B gp120s (Table 4). Particularly, none of the mAbs from the Indian panel was able to bind two of the clade B gp120s (CDC451 and 11.B11.1550) while four mAbs from the Cameroonian panel showed reactivity (Table 4, Fig. S2D).

The relative affinities measured by 50% of maximal binding were higher for Cameroonian versus Indian V3 mAbs; for binding to clade C and A gp120s the difference was not significant ($p=0.529$) while for binding to clade B gp120s the difference was highly significant ($p=0.0002$, Chi-Squared test) (Table 4).

Study of the V3 epitopes based on mAbs binding to gp120s

The binding experiments revealed that clade C and clade A gp120s were recognized by all V3 mAbs in contrast to clade B gp120s that was reactive with majority of mAbs. Three mAbs, 4508, 3791 and 3904 (derived from one Cameroonian and from two Indian patients) did not bind to any of the clade B gp120s which differ from clade C and A gp120s by residue Arg (R) at position 315 in the V3 region (Tables 4 and 5) suggesting that the epitope of these mAbs include the tip of the V3 loop. The remaining 15 mAbs were tolerant for changes in R/Q/K residues at position 315, suggesting that this residue is not an important component of the epitope. Furthermore, the mAbs

Table 3
Neutralization of pseudoviruses by anti-V3 mAbs derived from Cameroonian and Indian HIV-1 infected individuals[a].

No.	Virus ID	Tier	Clade	Cameroon mAbs						India mAbs											
				2558 5-51	**2557 5-51**	**3019 5-51**	**3694 5-51**	3869 1-f	4210 1-f	3074 4-59	4508 4-59	3881 4-59	**3906 5-51**	**4022 5-51**	**4487 5-51**	**4490 5-51**	**4025 5-51**	3791 1-18	4121 1-18	4139 1-8	3904 3-30	1418
1	MW965.26	1A	C	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	–
2	271-11	1B	AG	2	3.6	5.5	19.5	0.9	1.2	< 0.4	< 0.4	–	0.4	3.2	3.1	–	3.9	< 0.4	17.9	1.3	3.4	–
3	DJ263.8	1B	A	1.9	–	–	4.4	2.2	–	< 0.4	< 0.4	< 0.4	3.1	0.7	–	9.1	0.8	< 0.4	6.2	0.6	1.7	–
4	25710-2.43	1B	C	–	–	–	–	–	–	11.7	14.5	46	–	–	–	–	–	43	–	–	–	–
5	242-14	1B	AG	–	–	–	–	–	–	49.9	–	–	–	–	–	–	–	–	–	–	–	–
6	SF162.LS	1A	B	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	–	–	18.4	< 0.4	3.7	3.2	< 0.4	–	12	25.8	–	–
7	Bx08.16	1B	B	0.8	0.4	0.8	6.9	1.1	3.9	3.3	–	–	–	10.6	2.8	2.8	–	–	32.3	23.7	–	–
8	BaL.26	1B	B	0.4	< 0.4	0.7	1.4	1.4	3.7	5.2	–	–	–	–	4.7	4	–	–	37.8	–	–	–
9	SS1196.1	1B	B	2.6	2.6	5.2	25.5	1	10.7	7.5	–	–	–	7.8	14.4	21.8	–	–	–	–	–	–
10	BZ167.12	1B	B	0.5	–	–	2.5	–	30.2	–	–	–	0.4	–	–	–	–	–	4	–	–	–
11	HXB2.DG	1B	B	45.4	–	–	–	–	–	–	–	–	9.4	–	–	–	–	–	–	–	–	–
12	ZM109F.PB4	2	C	24.9	25.3	–	–	14.5	15.7	6.6	2.5	–	5.1	26.6	–	–	49.1	8.7	–	–	–	–
13	ZM233M.PB6	2	C	–	–	–	–	44.3	–	49.7	–	–	37.8	–	–	–	–	–	–	–	–	–
14	ZM197M.PB7	2	C	–	–	–	–	–	–	–	–	–	–	–	16.7	–	–	–	–	–	–	–
15	CAP210.2.00	2	C	–	–	–	–	–	–	39.5	–	–	–	–	–	–	–	–	–	–	–	–
16	6535.3	2	B	32.6	2.9	–	–	1.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	QH0692.42	2	B	46.1	41.8	–	–	9.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–
18	HO31.7	2	B	13.9	5.9	13.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19	JRFL.JB	2	B	30.4	21.2	47.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
20	REJO4541.67	2	B	–	14.1	–	–	49.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–
21	RHPA4259.7	2	B	–	–	–	–	26.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] All 18 mAbs were tested against 41 pseudotyped viruses using the TZM-bl cell assay; only 21 viruses, reactive with at least one mAb are shown in the table. Nine mAbs from each group (Cameroon and India) are shown with sample IDs (4 digit numerical) at the top of each column and immunoglobulin VH gene usage in the next row below. Monoclonal Abs encoded by VH5-51 gene are bold. The space separates tier 1 from tier 2 pseudotyped viruses. The IC_{50} values which indicate the amount of mAbs (μg/ml) needed for 50% neutralization are shown in each cell; they were estimated from the titration curves. The IC_{50} > 50 ug/ml (–) have been omitted. Monoclonal Ab 1418 is specific to parvovirus B19 and is used here as a negative control.

Table 4
Relative affinity binding of mAbs to recombinant gp120s[a].

gp120	Clade	Cameroon mAbs						India mAbs												
		2557 5-51	**2558 5-51**	**3694 5-51**	**3019 5-51**	3869 1-f	4210 1-f	3074 4-59	3881 4-59	4508 4-59	**4022 5-51**	**4490 5-51**	**4487 5-51**	**3906 5-51**	**4025 5-51**	4121 1-18	4139 1-8	3791 1-18	3904 3-30	p^b
Du151	C	0.026	0.010	0.042	0.020	0.013	0.064	0.028	0.010	0.024	0.003	0.012	0.024	0.025	0.010	0.075	0.045	0.024	0.020	
Du156.12	C	0.010	0.005	0.005	0.001	0.027	0.025	0.019	0.005	0.011	0.002	0.010	0.019	0.017	0.008	0.009	0.027	0.013	0.002	
Du422.1	C	0.006	0.003	0.007	0.002	0.019	0.010	0.018	0.004	0.006	0.001	0.007	0.017	0.008	0.006	0.011	0.022	0.007	0.003	
ZM53M.PB12	C	0.006	0.006	0.003	0.006	0.008	0.009	0.013	0.002	0.006	0.001	0.008	0.011	0.012	0.004	0.006	0.007	0.002	0.002	0.529
Du172.17	C	0.017	0.009	0.018	0.001	0.024	0.021	0.024	0.009	0.017	0.003	0.021	0.028	0.021	0.014	0.045	0.024	0.009	0.007	
C.ZA.1197MB	C	0.009	0.004	0.007	0.001	0.020	0.014	0.015	0.004	0.015	0.001	0.014	0.016	0.068	0.005	0.018	0.023	0.009	0.002	
ZM197M.PB7	C	0.017	0.006	0.007	0.005	0.019	0.012	0.017	0.004	0.030	0.003	0.038	0.030	0.012	0.021	0.040	0.027	0.003	0.005	
92 RW020	A	0.030	0.016	0.006	0.001	0.033	0.029	0.033	0.008	0.021	0.002	0.008	0.018	0.029	0.005	0.009	0.023	0.010	0.003	
SF162	B	0.058	0.011	0.009	0.004	0.031	0.025	0.019	0.160	–	0.001	0.008	0.011	0.020	0.004	0.014	0.023	–	–	
WITO4160	B	0.027	0.004	0.012	0.007	0.008	0.018	0.017	0.434	–	0.006	0.014	1.934	0.198	0.798	0.050	0.020	–	–	
TRO	B	0.077	0.010	0.021	0.022	0.006	0.029	0.022	0.949	–	0.074	0.046	–	–	–	0.097	0.096	–	–	
JRFL	B	0.018	0.008	0.008	0.002	0.019	0.016	0.072	–	–	0.053	0.027	0.029	–	–	0.174	–	–	–	
PVO	B	0.019	0.007	0.018	0.008	0.008	0.024	0.094	–	–	0.003	0.032	0.032	0.023	0.008	–	0.072	–	–	**0.0002**
REJO4541	B	0.020	0.009	0.026	0.009	0.009	0.631	0.022	–	–	0.004	0.029	1.697	–	0.074	–	–	–	–	
SC422661.8	B	0.022	0.011	0.015	0.010	0.010	0.016	1.473	–	–	0.024	0.009	0.013	0.024	0.076	–	–	–	–	
MN	B	0.055	0.026	0.026	0.005	0.029	0.037	–	–	–	0.016	0.027	0.023	0.090	–	0.122	–	–	–	
CDC451	B	0.011	0.259	0.008	–	0.050	–	–	–	–	–	–	–	–	–	–	–	–	–	
11.B11.1550	B	0.137	0.296	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

[a] Nine mAbs from each group (Cameroon and India) were tested by ELISA against 18 recombinant gp120s coated onto plastic plate at 1 μg/ml. All mAbs were titrated at a concentration ranging from 10 to 0.00001 μg/ml (12 dilutions). At the top of each column the ID of the mAb is shown and immunoglobulin gene VH usage in the next row below; VH5-51-derived mAbs from each panel are bold. The space separates gp120 clade C and A from clade B. The binding activity of V3 mAbs to gp120 is shown as 50% of maximal binding and represents the relative affinity. Human anti-parvovirus B19 mAb 1418 was used as a negative control and was not reactive (data not shown). Each experiment was performed at least two independent times. —no reactivity (> 10.0).

[b] p — p Value was determined using the Chi-Squared longrank test; it indicates significantly higher relative affinities of Cameroonian compared to Indian mAbs for binding to clade B gp120s.

were sensitive to the presence of two conserved residues, I309 and F317, suggesting that these two highly conserved residues on the hydrophobic face of V3 (Gorny et al., 2011; Jiang et al., 2010) are involved in the epitopes of 15 mAbs. This conclusion is supported by the fact that mutations at these two positions, in the gp120s of CDC451 and 11.B11.1550 (Table 5) abrogated or substantially reduced the binding of the majority of the mAbs (Table 4).

Discussion

Analysis of human mAbs that display cross-neutralizing activity to diverse HIV-1 subtypes is critical for gaining insight into the design of a prophylactic vaccine. We previously showed that non-B clade anti-V3 mAbs are more potent and cross-neutralizing than clade B derived mAbs (Gorny et al., 2006). Notably, in the present study we found that the neutralizing activity of mAbs derived from non-B clade-infected individuals can be significantly different depending on whether the donor was infected by strains circulating in Cameroon, mainly CRF02_AG or with strains circulating in India, mainly clade C HIV-1. The greater neutralizing activity of the Cameroon-derived V3 mAbs was unexpected given the close resemblance of the consensus V3 sequences between CRF02_AG and clade C viruses (only one residue Ile is replaced by Val in the entire V3 sequence; …RKSI/VRIGPGQ…) and the conservation of the GPGQ motif in the V3 crown of both clades.

Similarly, the Cameroon-derived mAbs were more potent than the Indian-derived mAbs even when the mAbs were encoded by the same VH5-51 and VL lambda genes. We have already observed that human anti-V3 mAbs display biased usage of Ig genes, preferentially using the pair of VH5-51 and VL lambda genes which is most likely related to an antigen requirement (Gorny et al., 2011;2009). The superior neutralizing activities of Cameroonian versus Indian V3

Table 5
V3 sequences of recombinant gp120s tested in the binding assay.

gp120	Clade	V3 sequence[a]
Consensus	C	**C**TRPNNNT**RKSIRIGPGQTFY**ATGDI**I**GDIRQAH**C**
		296 315 323a 331
Du156.12	C	----------V----------
Du172.17	C	-----S-------V---------F----------
Du422.1	C	----------V----------E-------E---
Du151	C	----------E---N--E---
ZM53M.PB12	C	----G----------A-F--TN---------Y-
C.ZA.1197MB	C	----------A-----NE---------Y-
ZM197M.PB7	C	-V---------V---------F---E----------
92 RW020	A	----------GV-------A-----G----------
PVO	B	----------S-----**R**A----------
SF162	B	----------T-----**R**A----------
TRO	B	---------R--H-----**R**A----------
REJO4541	B	----------H-A--**R**A-----E-------K-Y-
WITO4160	B	-----G-----R--N-----**R**A-----A-------K---
MN	B	-----Y-K--R-H-----**R**A--T-KN-K-T------
SC422661.8	B	----------G-T-----**R**V--*--E-V-----V--
JRFL	B	----------H-----**R**A--T--E----------
CDC451	B	-------H---RVT**L**---**R**V**W**-T--E-L-N------
11.B11.1550	B	-I---------R-T**M**---**K**V**Y**-T--Q----------

[a] The V3 loop sequences of gp120s were retrieved from the Los Alamos HIV-1 database and were aligned with consensus C V3 sequence (shown at the top) using the seqpublish program (http://www.hiv.lanl.gov). The numbering of the V3 consensus C sequence corresponds to HXB2 gp160 position numbers 296-331 with a two residue deletion (310=*Q* and 311=*R*) and one residue insertion (323a=*I*); this was determined using the HIV Sequence Locator program (http://www.hiv.lanl.gov). To emphasize the numbering of V3 consensus C, the four residues are underlined and numbered below: two cysteines (C) at positions 296 and 331, a glutamine (Q) at 315 and isoleucine (I) at 323a. The V3 crown is comprised of approximately 13 amino acids (bold), to which all the mAbs bind. The residues Arg (R) and Lys (K) both at position 315 are present in the clade B viruses and are bold and underlined. The hydrophobic residues I309 and F317 are prerequisite for binding most of the mAbs tested; mutations in these positions, bold and underlined, result in limited reactivity of gp120s with V3 mAbs.

mAbs coded for by VH5-51/VL lambda genes suggest that this is related to higher binding affinity rather than different epitope recognition since these mAbs have similar shape of the antigen-binding site and bind the same V3 epitopes (Gorny et al., 2011; Jiang et al., 2010). The binding studies confirmed that relative affinities of Cameroonian compared to Indian V3 mAbs were significantly higher for binding to clade B gp120s and comparable with certain trends for higher binding to clade C and A gp120s. Thus the mAbs from India possibly did not mature as efficiently, resulting in their lower affinity and lower neutralizing activities. Lastly, it cannot be excluded that host genetics contributed differently in regulating the virus-specific antibody response in the two study populations.

Studies of the mutation rates are consistent with this hypothesis, as the antibodies from India compared to those from Cameroon had a lower percentage of mutations in the variable fragment of the heavy and light chain genes, particularly in mAbs encoded by the same VH5-51/VL lambda genes (though the difference did not reach statistical significance). Thus, it has been suggested that the percentage of mutations in VH and VL may reflect affinity maturation and length of antigenic stimulation. For example, it was observed that human anti-gp120 mAbs produced from B cells derived from recipients of a gp120 HIV-1 vaccine had 3.8% (range 0 to 8.2%) of V(H) somatic mutations after six months of immunization (Moody et al., 2012). In contrast, broadly neutralizing mAbs, which usually appear after 2–3 years of HIV-1 infection, have 20%–35% of mutations (Walker et al., 2011;2009; Wu et al., 2010). Another explanation may be the greater range of V3 sequences that are found in AG-infected individuals compared to clade C-infected individuals in which the V3 region is quite constant (Patel et al., 2008). Thus, antibody maturation could reflect both length of antigenic stimulation and the diversity of the antigen driving the immune response.

Given the similar recognition mode of the majority of the mAbs described here, the question arose why mAbs from India, even using the same VH5-51/VL lambda genes, have significantly lower neutralizing potency and relative affinity compared to those from Cameroon. The data suggest that V3 of clade C compared to clade AG viruses have lower immunogenicity resulting from different V3 structures which impact the maturation of anti-V3 antibodies.

This hypothesis is supported by two studies that analyzed the conformation of the V3 loop in clade C viruses. It was shown that intrinsic differences in V3 conformation exist between clade B and C which are related to the sequence variability in the stem and turn regions, but not in the base of the V3 loop (Patel et al., 2008). Different V3 conformations may have consequences on the antibody response, with less cross-reactivity and limited evolution of the V3 region in the clade C viruses towards evolving X4-tropic variant loops (Patel et al., 2008). Indeed, clade C envelopes carry a relatively conserved V3 sequence, and X4 variants of clade C evolve less frequently compared with clade B (Morris et al., 2001; Peeters et al., 1999).

Another study visualized the dynamic 3D structure of the V3 crown by ab initio folding and suggested a rigid, not beta-hairpin-like, structure of the V3 loop crown in the consensus cladeC V3 sequence compared to the flexible beta-hairpin structure in consensus clade B V3 (Almond et al., 2012). This conformational alteration may impart immune evasion to the virus but, conversely, antibodies specific to consensus clade C V3 would be less effective against other, more beta-hairpin-like strains.

In conclusion, it was found that human anti-V3 mAbs derived from Cameroonian patients infected mainly with clade AG displayed higher relative binding affinity to various recombinant gp120s and significantly higher neutralizing activities than V3 mAbs from Indian patients infected mostly by clade C HIV-1. Particularly interesting is the fact that among anti-V3 mAbs encoded by the VH5-51/VL lambda genes, those mAbs from Cameroon have a higher percentage of mutations and displayed significantly more efficient neutralization than those from India. These results suggest the clade C V3 region may have lower immunogenicity compared to clade AG V3 loop from Cameroon and its sequence can serve as a plausible template for vaccine immunogen design.

Materials and Methods

Monoclonal antibodies

Eighteen human anti-V3 mAbs developed from non-B clade infected individuals living in Cameroon and India were used in this study (Gorny et al., 2004;2009;2006). All donors were chronically HIV-1 infected of unknown duration. Five of these non-B clade V3 mAbs, 4139, 4210, 4487, 4490 and 4508 (Table 1), were recently developed using cellular techniques as previously described with some modifications (Gorny et al., 1991). Briefly, peripheral blood mononuclear cells (PBMC) were transformed with Epstein-Barr virus (EBV) and cultured with a polyclonal B cell activator, CpG (Traggiai et al., 2004) which enhances EBV infection and B cell transformation. The culture supernatants were screened for binding activity to V3-cholera toxin B (CTB) fusion protein (Totrov et al., 2010) containing consensus V3 sequences from clade B and C. The reactive cells were fused with the heteromyeloma cell line SHM-D33 (Teng et al., 1983) and the resulting hybridoma cells that continued to make functional V3 Abs were repeatedly cloned until monoclonality was achieved.

In addition, one anti-V3 mAb 447-52D, generated from a clade B infected individual (Gorny et al., 1993), and one control mAb, 1418, specific to parvovirus B19 (Gigler et al., 1999) were also tested as controls (Table 1).

Envelope proteins

Seventeen recombinant gp120s representing sequences of primary HIV-1 isolates from clade A, B and C (produced in 293 cells) were purchased from Immune Technology Corp. (http://www.immune-tech.com); $gp120_{MN}$ (clade B) was purchased from Immunodiagnostics, Inc. (Woburn, MA).

RT-PCR amplification of the Ig variable region of the heavy and light chain genes

The nucleotide sequence of the Ig variable genes and gene usage by the newly developed human anti-V3 mAbs was determined as previously described (Gorny et al., 2011;2009). Briefly, messenger RNA was extracted from the hybridoma cell lines producing anti-V3 mAbs and reverse transcribed into cDNA using oligo dT primer. The variable domain of the heavy and lambda light chain genes (VH and VL) was amplified from poly-C tailed cDNA by PCR using deoxyinosine-containing anchor primer as the forward primer (Invitrogen) and gene specific primer as the reverse primer located in the constant region of VH family genes, κ and/or λ genes. PCR amplification was performed using a cycling program and ethidium bromide-stained 0.8% agarose gels were used to visualize the PCR products. The bands of appropriate size were excised, purified and then cloned into the 2.1-TOPO TA cloning vector (Invitrogen). For each chain, 6 to 12 independent clones were screened. The plasmids with the appropriate inserts were sequenced in both directions using the M13 primers. All sequencing reactions were performed at the Macrogen, Rockville, MD. The sequence data were analyzed using Pregap4, BioEdit softwares and the International ImMunoGene Tics (IMGT) information system (http://imgt.cines.fr).

Binding assay

The binding activity of the anti-V3 mAbs against the gp120s was tested by ELISA as described (Gorny et al., 1997). Briefly, ELISA plates were coated overnight with gp120s at 1.0 μg/ml, blocked with 2% bovine serum albumin in PBS, and then incubated with mAbs at a concentration ranging from 10 to 0.00001 μg/ml. The bound mAbs were detected by incubation with alkaline phosphatase-conjugated goat anti-human IgG (γ specific) (SouthernBiotech, Birmingham, AL) followed by adding substrate to develop color and the plates were read at 410 nm. The relative affinities of mAb binding to gp120s were determined by measuring the concentration of mAbs required for 50% maximal binding defined when the binding curve reached the saturation level as described (Gorny et al., 2000).

TZM-bl neutralization assay

The anti-V3 mAbs were tested for neutralizing activities against 41 psVs using the TZM-bl cell line as target cells, as described (Li et al., 2005; Seaman et al., 2010). Pseudoviruses expressing single cloned envelopes (Env) derived from tier 1 and tier 2 viruses were classified as neutralization-sensitive and -resistant, respectively. Briefly, 2-fold serial dilutions of mAbs, starting from 50 μg/ml, were pre-incubated with the psVs and the mAb/virus mixtures were incubated 48 h with TZM-bl cells expressing CD4, CXCR4 and CCR5. The virus infectivity was determined by measuring the luciferase activity in the cell lysates. The 50% inhibitory concentration of mAb (IC_{50}) was determined at which relative luminescence units were reduced 50% compared to virus control wells.

Statistical analysis

A Chi-Squared test was used for analysis of the binding data and the neutralizing IC_{50} values of two panels of anti-V3 mAbs, including the separate analysis of tier 1, tier 2, clade B, non-clade B psVs, as well as VH5-51 and non-VH5-51 encoded V3 mAbs. Because some of the mAb/psV or mAb/gp120 combinations failed to react, the neutralizing IC_{50} and relative affinity data was treated as censored data and therefore the Chi-Squared longrank test was used. The Student's *t*-test was used for analysis of mutation rates and relative affinity of V3 mAbs binding to individual gp120s.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.virol.2012.12.010.

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