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Immunotypes of a Quaternary Site of HIV-1 Vulnerability and Their Recognition by Antibodies[▽]

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HIV-1 is neutralized by a class of antibodies that preferentially recognize a site formed on the assembled viral spike. Such quaternary structure-specific antibodies have diverse neutralization breadths, with antibodies PG16 and PG9 able to neutralize 70 to 80% of circulating HIV-1 isolates while antibody 2909 is specific for strain SF162. We show that alteration between a rare lysine and a common N-linked glycan at position 160 of HIV-1 gp120 is primarily responsible for toggling between 2909 and PG16/PG9 neutralization sensitivity. Quaternary structure-specific antibodies appear to target antigenic variants of the same epitope, with neutralization breadth determined by the prevalence of recognized variants among circulating isolates.

The extraordinary diversity of HIV-1 suggests that a vaccine serotype strategy, such as the approach used to provide broad protection against the various serotypes of poliovirus or human papillomavirus (1, 19), may have limited applicability to HIV-1. One potential alternative is to focus on conserved sites of HIV-1 vulnerability to antibody-mediated neutralization. If a limited number of immunological variants (immunotypes) exist for a given neutralization site, then a "site-of-vulnerability serotype" vaccine strategy may be possible. Several sites of HIV-1 vulnerability have been identified on the assembled envelope (Env) spike, which is composed of three gp120 exterior units and three gp41 transmembrane molecules. These sites include the initial site of viral attachment to the cellular receptor CD4 (4, 29–31), a glycan site recognized by the 2G12 antibody (22, 23), the V3 loop (6, 9, 10, 32), and the external region of gp41 proximal to the membrane (25, 33). Recently, an epitope composed of quaternary structure-dependent interactions of the V2 and V3 loops of gp120 was also identified (7, 8, 11, 21, 27, 28).

The first HIV-1 quaternary structure-specific antibody isolated was the human monoclonal antibody (MAb) 2909, which displays neutralization limited to strain SF162 (8, 11). In contrast, the quaternary structure-specific and clonally related human MAbs PG16 and PG9 neutralize 70 to 80% of circulating HIV-1 isolates (27). Interestingly, recognition by the PG antibodies was found to require an N-linked glycan at position 160 (based on HXB2 numbering) in the V2 region of gp120 (7, 27), and this glycan was shown previously to knock out 2909 recognition (8, 11). An asparagine at position 160 is conserved in over 90% of HIV-1 strains (http://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html). In contrast, SF162 contains a rare lysine at this position (11). The recent atomic-level structural analysis of antibodies PG16 and 2909 shows that both utilize protruding anionic 3rd complementarity-determining regions of the heavy chain (CDR H3) for recognition (5, 17, 18). The structural similarities of these antibodies, together with their distinct neutralization sensitivities associated with the alteration of a single residue in the V2 region of gp120, lead us to hypothesize that the PG and 2909 antibodies may target closely related variants of the same epitope on the HIV-1 viral spike. Specifically, we hypothesized that the presence or absence of the N-linked glycan in V2 at position 160 was a primary determinant of antibody recognition and that variation at this position defined two immunological variants (immunotypes) of this epitope.

To test the breadth of neutralization by MAbs 2909, PG16, and PG9, we selected 80 genetically defined HIV-1 envelopes (20 from clade A, 30 from clade B, and 30 from clade C) to use as DNA templates for the N160K point mutation (Fig. 1). In several cases (those of viruses 89.6, 6101.10, QH0692.42, and BR07), the natural residue at position 160 was neither N nor K and, hence, both 160N and 160K mutations were tested. For viruses that contained a natural 160K residue (SF162, BL01, and ZM214.15), the converse K160N mutation was tested. The full-length HIV-1 *rev-env* expression plasmids were described previously (2, 12–14, 20, 24, 29), and site-directed mutagenesis was performed using the QuikChange mutagenesis kit (Agilent Technologies, Santa Clara, CA). The individual wild-type and mutant HIV-1 *rev-env* expression plasmids were used to cotransfect 293T cells with an HIV-1 SG3Δ*env* plasmid to make Env pseudoviruses, and neutralization was measured using TZM-bl target cells as described previously (24, 29). Upon the introduction of N160K or other indicated mutations, some Env pseudoviruses displayed modestly reduced titers compared to the wild-type Env pseudoviruses; however, mu-

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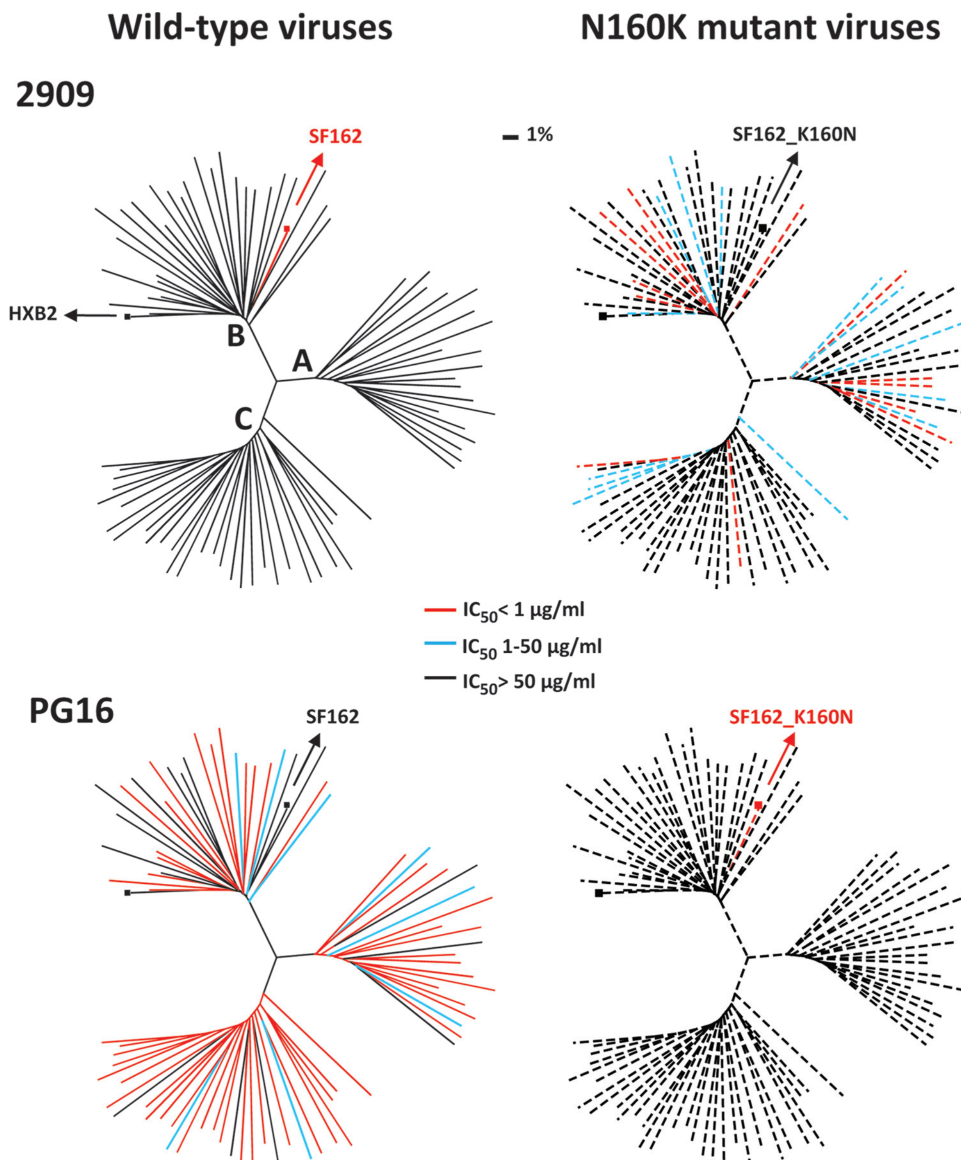


FIG. 1. Analysis of 2909 and PG16 neutralization of 80 wild-type (left; solid lines) and N160K mutant (right; dashed lines) HIV-1 Env pseudoviruses from clades A, B, and C. Dendrograms, made by the neighbor-joining method, show the wild-type envelope protein sequence distance. The clade B strain HXB2 was used to root the tree. The clades of HIV-1 are indicated, and the amino acid distance scale is indicated with a value of 1% distance as shown. The neutralization potencies of 2909 and PG16 are indicated by the color of the branch for each virus. The 160K mutant data for viruses 89.6, 6101.10, QH0692.42, and BR07 are shown in the N160K mutant dendrograms, although these isolates did not have an asparagine at position 160. Viruses SF162, BL01, and ZM214.15 each have a wild-type 160K residue, and they are included among the viruses in the wild-type dendrograms; the data for the converse K160N mutation are shown in the N160K mutant dendrograms. For JR-FL variants, the JR-FL_E168K data are shown in the wild-type dendrograms and the JR-FL_N160K_E168K data are shown in the N160K mutant dendrograms.

tant virus entry was sufficient for the neutralization assay, in which the virus entry levels were normalized to generate 20,000 to 200,000 relative light units in a luciferase assay (Promega, Madison, WI). One clade B Env pseudovirus, BG1168.1, lost entry with the N160K mutation; it was therefore removed from further analysis. All MAbs used in this study were produced by transient transfection using a mammalian expression system as described previously (5). Among the 80 wild-type Env pseudoviruses, MAb 2909 neutralized only SF162 (Tables 1, 2, and 3 and Fig. 1, top left) whereas MAb PG16 neutralized 62 pseudoviruses (78%), including 17 (85%) of 20 clade A, 18 (60%) of 30 clade B, and 27 (90%) of 30 clade C isolates (Tables 1 to 3 and Fig. 1, bottom left). Antibody PG9 displayed a neutralization breadth similar to that of PG16 (Tables 1 to 3). These results are consistent with the original reports on these antibodies (8, 11, 27). Thus, for Env pseudoviruses carrying the wild-type HIV-1 envelopes, MAb 2909 was SF162 specific and MAbs PG16 and PG9 were broadly neutralizing, with the greatest coverage against clades A and C and somewhat less coverage against clade B. Although the frequency of the 160N residue among clade B isolates (91%) is lower than that among clade A (97%) and C (94%) isolates, the more limited neu-

TABLE 1. Neutralization concentrations of MAbs 2909, PG16, and PG9 against 20 HIV-1 clade A Env pseudoviruses, either with wild-type Env or with the specific amino acid mutation indicated

Virus designation[a]	Residue[b] at position:				IC_{50} (μg/ml) of:			IC_{80} (μg/ml) of:		
	156	160	165	168	2909	PG16	PG9	2909	PG16	PG9
Q259.17	N	N	L	K	>50	0.030	0.041	>50	0.488	0.166
Q259.17_N160K	N	K	L	K	0.006	>50	>50	1.2	>50	>50
Q23.17	N	N	L	K	>50	0.001	0.002	>50	0.003	0.005
Q23.17_N160K	N	K	L	K	0.047	>50	>50	1.1	>50	>50
KNH1209.18	N	N	L	K	>50	0.283	0.167	>50	>50	19.1
KNH1209.18_N160K	N	K	L	K	0.151	>50	>50	2.0	>50	>50
Q842.d12	N	N	L	K	>50	0.009	0.019	>50	0.031	0.070
Q842.d12_N160K	N	K	L	K	0.289	>50	>50	6.3	>50	>50
DJ263.8	N	N	L	K	>50	8.2	0.218	>50	>50	2.6
DJ263.8_N160K	N	K	L	K	0.620	>50	>50	6.3	>50	>50
BS208.B1	N	N	L	K	>50	0.003	0.005	>50	0.008	0.049
BS208.B1_N160K	N	K	L	K	1.1	>50	>50	44.5	>50	>50
UG037.8	N	N	L	K	>50	0.003	0.011	>50	0.011	0.044
UG037.8_N160K	N	K	L	K	8.4	>50	>50	>50	>50	>50
Q168.a2	N	N	L	K	>50	0.019	0.045	>50	0.078	0.173
Q168.a2_N160K	N	K	L	K	12.8	>50	>50	>50	>50	>50
KER2018.11	N	N	L	K	>50	0.004	0.010	>50	0.011	0.033
KER2018.11_N160K	N	K	L	K	25.4	>50	>50	>50	>50	>50
KER2008.12	N	N	L	K	>50	0.008	0.017	>50	0.051	0.068
KER2008.12_N160K	N	K	L	K	27.4	>50	>50	>50	>50	>50
0330.v4.c3	N	N	L	R	>50	0.001	0.004	>50	0.007	0.019
0330.v4.c3_N160K	N	K	L	R	>50	>50	>50	>50	>50	>50
Q769.h5	N	N	L	K	>50	0.009	0.009	>50	0.067	0.033
Q769.h5_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
3718.v3.c11	N	N	L	K	>50	0.012	0.088	>50	0.136	0.328
3718.v3.c11_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
3415.v1.c1	N	N	Q	K	>50	0.017	0.116	>50	0.358	0.814
3415.v1.c1_N160K	N	K	Q	K	>50	>50	>50	>50	>50	>50
RW020.2	N	N	L	K	>50	0.037	0.052	>50	0.385	0.269
RW020.2_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
Q461.e2	N	N	L	K	>50	2.7	1.5	>50	>50	10.9
Q461.e2_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
0260.v5.c1	D	N	L	K	>50	3.2	1.4	>50	>50	31.9
0260.v5.c1_N160K	D	K	L	K	>50	>50	>50	>50	>50	>50
QH209.14 M.A2	N	N	V	R	>50	>50	>50	>50	>50	>50
QH209.14 M.A2_N160K	N	K	V	R	>50	>50	>50	>50	>50	>50
398-F1-F6-20	N	N	L	R	>50	>50	>50	>50	>50	>50
398-F1-F6-20_N160K	N	K	L	R	>50	>50	>50	>50	>50	>50
0439.v5.c1	N	N	L	K	>50	>50	>50	>50	>50	>50
0439.v5.c1_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
Neutralization breadth[c] for:										
Wild-type viruses (n = 20)					0 (0%)	17 (85%)	17 (85%)	0 (0%)	13 (65%)	17 (85%)
Mutant viruses (n = 20)					10 (50%)	0 (0%)	0 (0%)	6 (30%)	0 (0%)	0 (0%)

[a] The viruses are ordered first by the 2909 IC_{50}, then by the PG16 IC_{50}, and finally by the PG9 IC_{50}. Mutant viruses are designated by the corresponding wild-type strain name followed by the relevant amino acid mutation.
[b] Residues at positions 156, 160, 165, and 168 (based on HXB2 numbering) with potential importance in the PG and 2909 epitope are shown.
[c] Neutralization breadth was calculated as the number (percentage) of viruses neutralized with an antibody IC_{50} or IC_{80} of ≤50 μg/ml.

tralization coverage of clade B by PG16/PG9 did not appear to be due to the inclusion of the few non-160N-containing strains because the 160N versions of these viruses were also tested and generally remained resistant to PG16/PG9 neutralization (Table 2).

Among the Env pseudoviruses containing a wild-type or mutant 160K residue, MAb 2909 neutralized 10 (50%) of 20 clade A, 11 (37%) of 30 clade B, and 6 (20%) of 30 clade C viruses (Fig. 1, top right, and Tables 1 to 3). In total, 27 (34%) of 80 160K-containing Env pseudoviruses were sensitive to 2909 neutralization. Remarkably, PG16 and PG9 neutralized none of the N160K mutant viruses (Fig. 1, bottom right, and Tables 1 to 3). Because wild-type SF162 had a lysine at position 160, we mutated this residue to asparagine, thereby restoring the N-linked glycan site. As described previously, the K160N mutation resulted in complete resistance to MAb 2909 while the PG MAbs were now able to neutralize the glycan-containing mutant of SF162 (21, 27). In total, these data indicate that a single glycan-specific mutation at position 160 in V2 can flip the overall neutralization landscape of antibodies 2909 and PG16/PG9 (Fig. 1 and Tables 1 to 3). Additionally, these neutralization profiles were mutually exclusive; there was no case of a virus that was sensitive to both 2909 and PG16/PG9 neutralization (Fig. 1 and Tables 1 to 3).

We next used contingency tables to categorize the 80 viral strains based on their neutralization sensitivities to PG16 and

TABLE 2. Neutralization concentrations of MAbs 2909, PG16, and PG9 against 30 HIV-1 clade B Env pseudoviruses, either with wild-type Env or with the specific amino acid mutation(s) indicated

Virus designation[a]	Residue[b] at position:				IC_{50} (μg/ml) of:			IC_{80} (μg/ml) of:		
	156	160	165	168	2909	PG16	PG9	2909	PG16	PG9
SF162	N	K	I	K	0.015	>50	>50	0.804	>50	>50
SF162_K160N	N	N	I	K	>50	0.002	0.003	>50	0.016	0.012
AC10.29	N	N	M	K	>50	0.009	0.012	>50	0.038	0.073
AC10.29_N160K	N	K	M	K	0.001	>50	>50	0.004	>50	>50
JR-FL	N	N	I	E	>50	>50	>50	>50	>50	>50
JR-FL_N160K	N	K	I	E	>50	>50	>50	>50	>50	>50
JR-FL_E168K	N	N	I	K	>50	0.003	0.008	>50	0.015	0.055
JR-FL_N160K_E168K	N	K	I	K	0.001	>50	>50	0.005	>50	>50
7165.18	N	N	I	K	>50	0.426	>50	>50	>50	>50
7165.18_N160K	N	K	I	K	0.017	>50	>50	0.077	>50	>50
WITO.33	N	N	I	K	>50	0.002	0.005	>50	0.006	0.009
WITO.33_N160K	N	K	I	K	0.105	>50	>50	0.411	>50	>50
HT593.1	N	N	I	K	>50	0.056	0.214	>50	2.5	2.1
HT593.1_N160K	N	K	I	K	0.109	>50	>50	0.793	>50	>50
YU2	N	N	I	K	>50	0.258	3.86	>50	>50	>50
YU2_N160K	N	K	I	K	0.492	>50	>50	2.3	>50	>50
ADA	N	N	I	K	>50	0.012	0.128	>50	0.060	5.2
ADA_N160K	N	K	I	K	1.24	>50	>50	50.0	>50	>50
SC422.8	N	N	I	K	>50	>50	0.238	>50	>50	>50
SC422.8_N160K	N	K	I	K	4.5	>50	>50	>50	>50	>50
TRO.11	N	N	I	K	>50	0.077	29.4	>50	>50	>50
TRO.11_N160K	N	K	I	K	5.0	>50	>50	50.0	>50	>50
REJO.67	N	N	P	K	>50	0.005	0.003	>50	0.024	0.015
REJO.67_N160K	N	K	P	K	6.7	>50	>50	>50	>50	>50
JR-CSF	N	N	I	K	>50	0.001	0.002	>50	0.008	0.008
JR-CSF_N160K	N	K	I	K	>50	>50	>50	>50	>50	>50
3988.25	N	N	I	K	>50	0.005	0.016	>50	0.022	0.062
3988.25_N160K	N	K	I	K	>50	>50	>50	>50	>50	>50
5768.4	N	N	L	K	>50	0.008	0.031	>50	0.580	1.3
5768.4_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
SS1196.1	K	N	I	K	>50	0.020	0.074	>50	0.179	0.695
SS1196.1_N160K	K	K	I	K	>50	>50	>50	>50	>50	>50
RHPA.7	N	N	I	K	>50	0.334	10.0	>50	3.5	>50
RHPA.7_N160K	N	K	I	K	>50	>50	>50	>50	>50	>50
THRO.18	N	N	V	K	>50	0.498	13.2	>50	50.0	>50
THRO.18_N160K	N	K	V	K	>50	>50	>50	>50	>50	>50
BaL.01	N	N	I	K	>50	0.993	0.033	>50	>50	1.1
BaL.01_N160K	N	K	I	K	>50	>50	>50	>50	>50	>50
TRJO.58	N	N	T	K	>50	2.7	1.9	>50	>50	42.7
TRJO.58_N160K	N	K	T	K	>50	>50	>50	>50	>50	>50
PVO.4	N	N	I	R	>50	6.6	4.3	>50	>50	34.5
PVO.4_N160K	N	K	I	R	>50	>50	>50	>50	>50	>50
CAAN.A2	N	N	M	K	>50	25.0	14.4	>50	>50	>50
CAAN.A2_N160K	N	K	M	K	>50	>50	>50	>50	>50	>50
6535.3	N	N	R	K	>50	>50	0.056	>50	>50	0.411
6535.3_N160K	N	K	R	K	>50	>50	>50	>50	>50	>50
89.6	N	Y	I	K	>50	>50	>50	>50	>50	>50
89.6_Y160N	N	N	I	K	>50	>50	0.140	>50	>50	>50
89.6_Y160K	N	K	I	K	>50	>50	>50	>50	>50	>50
R2	N	N	I	K	>50	>50	>50	>50	>50	>50
R2_N160K	N	K	I	K	>50	>50	>50	>50	>50	>50
R2_A162T	N	N	I	K	>50	>50	10.3	>50	>50	>50
R2_N160K_A162T	N	K	I	K	>50	>50	>50	>50	>50	>50
HXB2	N	N	I	K	>50	>50	>50	>50	>50	>50
HXB2_N160K	N	K	I	K	>50	>50	>50	>50	>50	>50
6101.10	N	D	I	K	>50	>50	>50	>50	>50	>50
6101.10_D160N	N	N	I	K	>50	>50	>50	>50	>50	>50
6101.10_D160K	N	K	I	K	>50	>50	>50	>50	>50	>50
QH0692.42	N	S	I	K	>50	>50	>50	>50	>50	>50
QH0692.42_S160N_P162T	N	N	I	K	>50	>50	>50	>50	>50	>50
QH0692.42_S160K_P162T	N	K	I	K	>50	>50	>50	>50	>50	>50
BL01	N	K	L	K	>50	>50	>50	>50	>50	>50
BL01_K160N	N	N	L	K	>50	>50	>50	>50	>50	>50
BR07	N	H	R	K	>50	>50	>50	>50	>50	>50
BR07_H160N	N	N	R	K	>50	>50	>50	>50	>50	>50
BR07_H160K	N	K	R	K	>50	>50	>50	>50	>50	>50
QH0515.1	N	N	I	R	>50	>50	>50	>50	>50	>50
QH0515.1_N160K	N	K	I	R	>50	>50	>50	>50	>50	>50
Neutralization breadth[c] for:										
Wild-type viruses (n = 30)					1 (3%)	18 (60%)	20 (67%)	1 (3%)	11 (37%)	13 (43%)
Mutant viruses (n = 30)					10 (33%)	2 (7%)	3 (10%)	8 (27%)	2 (7%)	2 (7%)

[a] The viruses are ordered first by the 2909 IC_{50}, then by the PG16 IC_{50}, and finally by the PG9 IC_{50}.

[b] Residues at positions 156, 160, 165, and 168 (based on HXB2 numbering) with potential importance in the PG and 2909 epitope are shown.

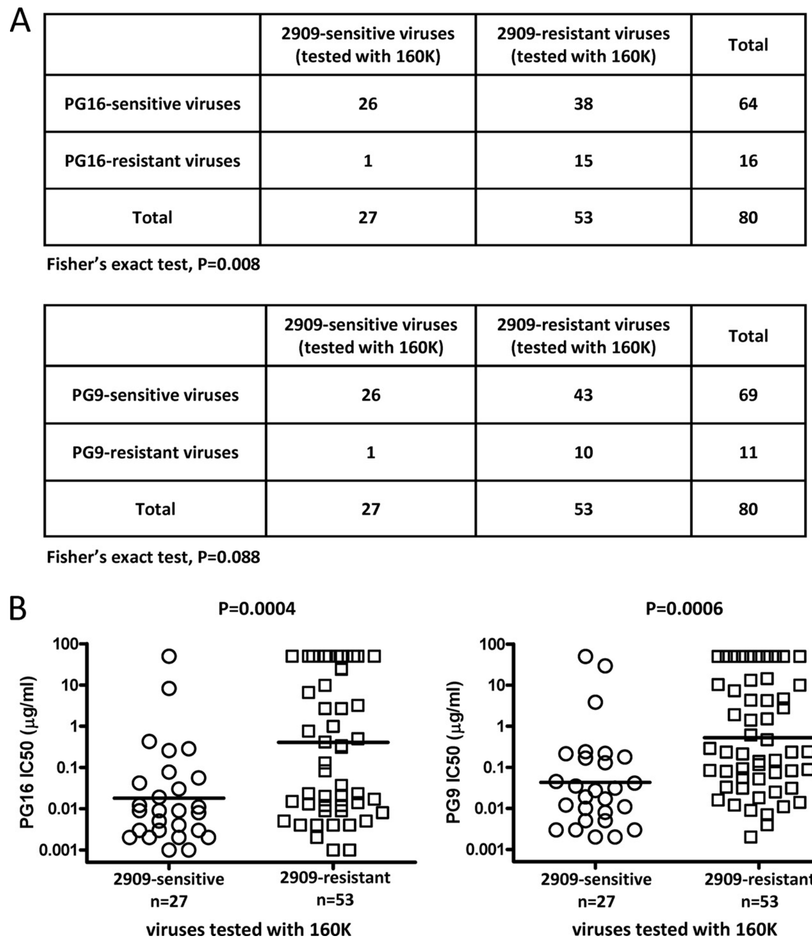
[c] Neutralization breadth was calculated as the number (percentage) of viruses neutralized with an antibody IC_{50} or IC_{80} of $\leq$50 μg/ml.

TABLE 3. Neutralization concentrations of MAbs 2909, PG16, and PG9 against 30 HIV-1 clade C Env pseudoviruses, either with wild-type Env or with the specific amino acid mutation(s) indicated

Virus designation[a]	Residue[b] at position:				IC_{50} (μg/ml) of:			IC_{80} (μg/ml) of:		
	156	160	165	168	2909	PG16	PG9	2909	PG16	PG9
ZM233.6	I	N	L	K	>50	0.001	0.002	>50	0.002	0.007
ZM233.6_N160K	I	K	L	K	0.004	>50	>50	2.2	>50	>50
Du422.1	N	N	L	K	>50	0.042	0.178	>50	0.924	2.0
Du422.1_N160K	N	K	L	K	0.843	>50	>50	12.1	>50	>50
CAP45.G3	N	N	L	K	>50	0.002	0.003	>50	0.007	0.014
CAP45.G3_N160K	N	K	L	K	2.17	>50	>50	21.7	>50	>50
ZM249.1	N	N	L	K	>50	0.009	0.031	>50	0.192	0.162
ZM249.1_N160K	N	K	L	K	3.73	>50	>50	22.7	>50	>50
Du156.12	N	N	L	K	>50	0.002	0.035	>50	0.019	0.109
Du156.12_N160K	N	K	L	K	7.28	>50	>50	>50	>50	>50
Du123.6	N	N	I	K	>50	0.011	0.027	>50	0.050	0.138
Du123.6_N160K	N	K	I	K	8.8	>50	>50	>50	>50	>50
ZM176.66	N	N	L	K	>50	0.002	0.011	>50	0.006	0.036
ZM176.66_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
Du151.2	N	N	I	R	>50	0.004	0.012	>50	0.016	0.054
Du151.2_N160K	N	K	I	R	>50	>50	>50	>50	>50	>50
25710-2.43	N	N	L	K	>50	0.004	0.014	>50	0.098	0.080
25710-2.43_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
BR025.9	N	N	V	K	>50	0.004	0.018	>50	0.019	0.089
BR025.9_N160K	N	K	V	K	>50	>50	>50	>50	>50	>50
SO18.18	N	N	I	K	>50	0.004	0.031	>50	0.057	0.106
SO18.18_N160K	N	K	I	K	>50	>50	>50	>50	>50	>50
TV1.29	N	N	L	K	>50	0.005	0.007	>50	0.147	0.036
TV1.29_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
ZM53.12	N	N	L	K	>50	0.009	0.092	>50	0.031	0.330
ZM53.12_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
286.36	N	N	L	K	>50	0.012	0.084	>50	0.043	0.390
286.36_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
TZBD.02	N	N	L	K	>50	0.013	0.211	>50	0.101	1.1
TZBD.02_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
CAP244.D3	N	N	L	K	>50	0.014	0.082	>50	0.048	0.341
CAP244.D3_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
25711-2.4	N	N	L	K	>50	0.015	0.468	>50	0.101	4.7
25711-2.4_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
CAP210.E8	I	N	L	K	>50	0.021	0.080	>50	0.159	0.438
CAP210.E8_N160K	I	K	L	K	>50	>50	>50	>50	>50	>50
TZA125.17	N	N	I	K	>50	0.023	0.149	>50	0.367	0.721
TZA125.17_N160K	N	K	I	K	>50	>50	>50	>50	>50	>50
Du172.17	N	N	I	K	>50	0.023	0.240	>50	0.147	0.952
Du172.17_N160K	N	K	I	K	>50	>50	>50	>50	>50	>50
288.38	N	N	V	K	>50	0.083	0.610	>50	22.5	>50
288.38_N160K	N	K	V	K	>50	>50	>50	>50	>50	>50
ZM106.9	N	N	I	K	>50	0.129	0.234	>50	>50	3.6
ZM106.9_N160K	N	K	I	K	>50	>50	>50	>50	>50	>50
ZM55.4a	N	N	L	I	>50	0.308	4.2	>50	14.5	41.2
ZM55.4a_N160K	N	K	L	I	>50	>50	>50	>50	>50	>50
ZA012.29	N	N	I	K	>50	0.414	4.6	>50	>50	>50
ZA012.29_N160K	N	K	I	K	>50	>50	>50	>50	>50	>50
ZM197.7	D	N	V	R	>50	0.765	0.287	>50	>50	2.5
ZM197.7_N160K	D	K	V	R	>50	>50	>50	>50	>50	>50
ZM109.4	H	N	V	R	>50	9.8	0.235	>50	>50	3.7
ZM109.4_N160K	H	K	V	R	>50	>50	>50	>50	>50	>50
16845-2.22	N	N	L	K	>50	23.9	7.3	>50	>50	>50
16845-2.22_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
ZM215.8	N	N	V	K	>50	>50	0.025	>50	>50	0.437
ZM215.8_N160K	N	K	V	K	>50	>50	>50	>50	>50	>50
ZM135.10a	N	N	L	K	>50	>50	2.8	>50	>50	>50
ZM135.10a_N160K	N	K	L	K	>50	>50	>50	>50	>50	>50
ZM214.15	N	K	L	K	>50	>50	>50	>50	>50	>50
ZM214.15_K160N	N	N	L	K	>50	>50	>50	>50	>50	>50
ZM214.15_N162T	N	K	L	K	>50	>50	>50	>50	>50	>50
ZM214.15_K160N_N162T	N	N	L	K	>50	>50	>50	>50	>50	>50
Neutralization breadth[c] for:										
Wild-type viruses (n = 30)					0 (0%)	27 (90%)	29 (97%)	0 (0%)	22 (73%)	25 (83%)
Mutant viruses (n = 30)					6 (20%)	0 (0%)	0 (0%)	4 (13%)	0 (0%)	0 (0%)

[a] The viruses are ordered first by the 2909 IC_{50}, then by the PG16 IC_{50}, and finally by the PG9 IC_{50}.
[b] Residues at positions 156, 160, 165, and 168 (based on HXB2 numbering) with potential importance in the PG and 2909 epitope are shown.
[c] Neutralization breadth was calculated as the number (percentage) of viruses neutralized with an antibody IC_{50} or IC_{80} of ≤50 μg/ml.

2909 (Fig. 2A, upper panel) and to PG9 and 2909 (Fig. 2A, lower panel). As noted above, 27 of 80 Env pseudoviruses became sensitive to 2909 in the setting of the appropriate 160K sequence. Among these 27 160N-containing (mostly wild-type) viruses, all were sensitive to either PG16 or PG9. There was only one 160N wild-type virus (SC422.8) that was resistant to PG16 and one (7165.18) that was resistant to PG9. Conversely, among the 10 viruses resistant to both PG9 and PG16, none became 2909 sensitive upon 160K mutation. These data suggest that the 2909 epitope is generally not present or exposed



A

	2909-sensitive viruses (tested with 160K)	2909-resistant viruses (tested with 160K)	Total
PG16-sensitive viruses	26	38	64
PG16-resistant viruses	1	15	16
Total	27	53	80

Fisher's exact test, P=0.008

	2909-sensitive viruses (tested with 160K)	2909-resistant viruses (tested with 160K)	Total
PG9-sensitive viruses	26	43	69
PG9-resistant viruses	1	10	11
Total	27	53	80

Fisher's exact test, P=0.088

FIG. 2. Analysis of correlations between 2909 and PG16 or PG9 neutralization sensitivities among 80 HIV-1 Env pseudoviruses. (A) Contingency tables show the relationships between viral sensitivities to 2909 and PG16 (upper panel) and to 2909 and PG9 (lower panel). The viruses were tested with 160N for PG16 and PG9 sensitivity and with 160K for 2909 sensitivity. A virus with a 50% inhibitory concentration (IC_{50}) of ≤50 μg/ml was considered to be sensitive. Fisher's exact test was used to determine the significance of the correlations. (B) Neutralization IC_{50} values of PG16 (left) and PG9 (right) are plotted for 2909-sensitive ($n = 23$) and 2909-resistant ($n = 57$) strains. The geometric mean for each group is indicated with a horizontal bar. The unpaired Student *t* test was used to determine the significance of the difference between the geometric means for the 2909-sensitive and 2909-resistant strains.

properly among strains resistant to PG16 and PG9. Among the 64 PG16-sensitive viruses, 26 (41%) became 2909 sensitive in the setting of the appropriate 160K sequence, which was statistically significant ($P = 0.008$; Fisher's exact test). Likewise, 26 (38%) of 69 PG9-sensitive viruses became 2909 sensitive ($P = 0.088$; Fisher's exact test).

To further assess the relationship between the PG and 2909 epitopes, we categorized the 160K versions of the 80 Env pseudoviruses as either 2909 sensitive or 2909 resistant. We then compared the neutralization sensitivities of these two categories of viruses to PG9 and PG16. The 160N versions of the 2909-sensitive viruses displayed significantly greater neutralization sensitivity to PG16 (Fig. 2B, left) and to PG9 (Fig. 2B, right) than the 160N versions of the 2909-resistant viruses. Taken together, the ability of a single mutation to alter neutralization sensitivity between PG and 2909 MAbs and the greater PG neutralization sensitivity among 2909-sensitive 160K mutants suggest that the 2909 and PG MAbs target antigenic variants of the same quaternary structure-specific epitope.

In addition to the residues at position 160, we reviewed naturally occurring sequence variations at positions 156, 165, and 168 in or adjacent to V2 that were described previously to be important for PG or 2909 binding (Tables 1 to 3). Prior studies showed that mutating the well-conserved N-glycan at position 156 to alanine in virus strain JR-CSF causes a decrease in neutralization potency for PG9 and PG16 (7, 27). However, we found that this glycan is not required for PG or 2909 antibody recognition because six isolates that did not contain the N-glycan at position 156 were sensitive to the PG antibodies and one of them (ZM233.6) was sensitive to 2909 (in the context of the N160K mutation). An analysis of residues at position 165 suggested that an isoleucine might be important for MAb 2909 but not PG9 or PG16 recognition, as an I165A mutation rendered SF162 resistant to 2909 but did not alter the PG antibody neutralization of JR-CSF (28). However, we observed two viruses (AC10.29 and REJO.67) with residues at position 165 differing from the consensus isoleucine or leucine that were neutralized by 2909 (in the setting of the appropriate N160K mutation). Thus, antibody 2909 appears to tolerate

TABLE 4. IC_{50} values of six rhesus monkey quaternary structure-specific MAbs against a selected panel of 14 HIV-1 clade A, B, and C Env pseudoviruses sensitive to MAb 2909, either with wild-type Env or with the N160K or K160N (for SF162) mutation

Virus clade (no. of wild-type viruses) and designation	IC_{50} (μg/ml) of:							
	2909	PG16	2.2G	2.3E	2.5B	1.8E	LW10E	1.6F
Clade B (5)								
SF162	0.015	>50	<0.003	0.029	<0.003	<0.003	<0.003	<0.003
SF162_K160N	>50	0.002	>50	>50	>50	>50	>50	>50
AC10.29	>50	0.009	>50	>50	>50	>50	>50	>50
AC10.29_N160K	0.001	>50	0.009	0.272	>50	5.4	>50	0.022
7165.18	>50	0.426	>50	>50	>50	>50	>50	>50
7165.18_N160K	0.017	>50	>50	>50	>50	>50	>50	>50
WITO.33	>50	0.002	>50	>50	>50	>50	>50	>50
WITO.33_N160K	0.105	>50	>50	>50	>50	>50	>50	>50
YU2	>50	0.258	>50	>50	>50	>50	>50	>50
YU2_N160K	0.492	>50	>50	>50	>50	>50	>50	>50
Clade A (5)								
Q259.17	>50	0.030	>50	>50	>50	>50	>50	>50
Q259.17_N160K	0.006	>50	>50	>50	>50	>50	>50	>50
Q23.17	>50	0.001	>50	>50	>50	>50	>50	>50
Q23.17_N160K	0.047	>50	>50	>50	>50	>50	>50	>50
KNH1209.18	>50	0.283	>50	>50	>50	>50	>50	>50
KNH1209.18_N160K	0.151	>50	>50	>50	>50	>50	>50	>50
DJ263.8	>50	8.2	>50	>50	>50	>50	>50	>50
DJ263.8_N160K	0.620	>50	>50	>50	>50	>50	>50	>50
BS208.B1	>50	0.003	>50	>50	>50	>50	>50	>50
BS208.B1_N160K	1.1	>50	>50	>50	>50	>50	>50	>50
Clade C (4)								
ZM233.6	>50	0.001	>50	>50	>50	>50	>50	>50
ZM233.6_N160K	0.004	>50	>50	>50	>50	>50	>50	>50
Du422.1	>50	0.042	>50	>50	>50	>50	>50	>50
Du422.1_N160K	0.843	>50	>50	>50	>50	>50	>50	>50
CAP45.G3	>50	0.002	>50	>50	>50	>50	>50	>50
CAP45.G3_N160K	2.17	>50	>50	>50	>50	>50	>50	>50
ZM249.1	>50	0.009	>50	>50	>50	>50	>50	>50
ZM249.1_N160K	3.73	>50	>50	>50	>50	>50	>50	>50

some amino acid variation at this site. Finally, the virus JR-FL contains an unusual glutamic acid at position 168 rather than the more common lysine. As described previously (7, 27), this 168E residue accounts for the JR-FL resistance to the PG MAbs. As expected, we found that the E168K mutant version of JR-FL was sensitive to the PG antibodies and that the subsequent N160K mutation altered viral sensitivity from the PG antibodies to antibody 2909 (Table 2). In total, these data suggest that the contact sites for antibodies PG16/PG9 and 2909 share some common features among these residues in or adjacent to V2. Specifically, both the PG and 2909 MAbs do not require an asparagine at position 156 for neutralization, both the PG and 2909 antibodies tolerate amino acid variation at position 165, and neither the PG nor the 2909 MAb could tolerate a glutamic acid at position 168.

Other MAbs dependent on the quaternary interaction of V2 and V3 have been isolated from rhesus monkeys infected with the chimeric simian-human immunodeficiency virus $SHIV_{SF162p4}$ (21). Like MAb 2909, these rhesus quaternary structure-specific MAbs neutralized SF162 potently but could not neutralize other HIV-1 wild-type isolates. To examine if these MAbs target the same epitope as MAb 2909, we selected SF162 and 13 N160K mutant strains with high-level sensitivity to 2909 (Table 4). Of the 13 viruses, only 1 (AC10.29_N160K) was neutralized by some of the rhesus monkey quaternary structure-specific MAbs (Table 4). These data are consistent with the recent finding that these rhesus monkey MAbs recognize an epitope partially overlapping the 2909 epitope but display different amino acid and glycosylation pattern requirements for recognition of the Env trimer (21).

The analysis of antibody specificities in sera from HIV-1-infected donors shows that approximately 20% of highly selected, broadly neutralizing sera contain quaternary structure-specific antibodies sensitive to the N160K mutation (16, 28). Additionally, other quaternary structure-specific neutralizing antibodies have recently been reported (3). The observations that quaternary structure-specific antibodies may be generated with reasonable frequency during natural HIV-1 infection and that such antibodies can be broadly reactive with diverse HIV-1 isolates have focused attention on position 160 as a site of HIV-1 vulnerability (15, 26). While the atomic-level structures of PG16 and 2909 Fabs have recently been published (5, 17, 18), the glycan and quaternary requirements for binding to the Env trimer make liganded structures of these antibodies difficult to attain. Hence, nonstructural studies that provide an improved understanding of the antigenic characteristics of this epitope may foster improvements in the design of Env-based vaccine immunogens. Our data strongly suggest that the epitopes defined by 2909 and the PG MAbs are antigenic variants of the same region of the Env trimer. Hence, these

antibodies define alternative variants of an immunogenic region in the quaternary structure formed by the V2 and V3 loops. Ongoing efforts seek to find forms of gp120 or full-length HIV-1 Env that can be used as vaccine immunogens to elicit antibodies to this region of vulnerability on the HIV-1 Env. The data reported herein regarding the antigenic properties of the PG and 2909 epitope will help focus design efforts on immunogens that can better elicit the more broadly reactive forms of these quaternary structure-specific antibodies.

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