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A Combination of Broadly Neutralizing HIV-1 Monoclonal Antibodies Targeting Distinct Epitopes Effectively Neutralizes Variants Found in Early Infection

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Neutralizing antibody protection against HIV-1 may require broad and potent antibodies targeting multiple epitopes. We tested 7 monoclonal antibodies (MAbs) against 45 viruses of diverse subtypes from early infection. The CD4 binding site MAb NIH45-46W was most broad and potent (91% coverage; geometric mean 50% inhibitory concentration [IC_{50}], 0.09 μg/ml). Combining NIH45-46W and a V3-specific MAb, PGT128, neutralized 96% of viruses, while PGT121, another V3-specific MAb, neutralized the remainder. Thus, 2 or 3 antibody specificities may prevent infection by most HIV-1 variants.

Studies in nonhuman primate models have demonstrated that passively infused neutralizing antibodies (NAbs) can protect against HIV-1 infection when present at the time of exposure (1, 3, 15, 18, 19, 21). However, an enormous challenge to preventing infection in naturally exposed populations is the requirement for NAb responses to recognize diverse circulating variants. Recently identified HIV-1 monoclonal antibodies (MAbs) capable of potently neutralizing diverse variants have spurred optimism for a NAb-based vaccine, as these MAbs may define key targets for protective NAb responses and may also be candidates for gene delivery (2, 16) and potentially for passive immunization to prevent or modify the course of infection (23). However, it is unclear how effective these MAbs are specifically against transmitted variants, which may comprise a unique subset of HIV variants (24) that have distinct characteristics compared to variants in chronic infection, such as shorter variable loop lengths and fewer potential N-linked glycosylation sites (PNGS) (7, 8, 25, 29) and, in some cases, different neutralization profiles compared to nontransmitted variants (8, 9, 29, 31).

We analyzed the neutralization profiles of 45 HIV-1 envelope variants of diverse subtypes (A, C, D), which were obtained soon after heterosexually acquired infection (median, 59 days postinfection) (4, 5, 17), against 7 recently identified broadly neutralizing MAbs targeting several distinct epitopes. These included the following: VRC01, which targets the CD4 binding site (CD4bs) (30); NIH45-46W (10), which also targets the CD4bs but is an engineered mutant that improves the neutralization breadth and potency of MAb NIH45-46, a clonal variant of VRC01 (26); PG9, PG16, and PGT145, which recognize a glycan-dependent quaternary epitope in V1/V2 and V3 (27, 28); and MAbs PGT121 and PGT128 (27), which form another class of antibodies targeted to glycan-dependent epitopes in V3. Serial dilutions of all MAbs were tested at a starting concentration of 1 μg/ml against envelope pseudoviruses in the TZM-bl assay as described previously (29). This starting MAb concentration was chosen due to the limited reagent availability and the reported breadth of the MAbs, even at low concentrations (10, 27, 28, 30).

The MAbs had differing neutralizing activities against the panel viruses, with 50% inhibitory concentration (IC_{50}) values ranging by more than 3 orders of magnitude from 0.0003 to >1 μg/ml (Fig. 1). The CD4bs MAb NIH45-46W neutralized 91% of variants with a geometric mean IC_{50} of 0.09 μg/ml, while VRC01, another CD4bs MAb, neutralized 71% of variants with a geometric mean IC_{50} of 0.36 μg/ml (Fig. 2). The glycan-dependent PG and PGT MAbs were less broad and potent than the CD4bs MAbs, neutralizing only 16% to 49% of variants with a geometric mean IC_{50} of 0.24 to 0.78 μg/ml.

Because the PG and PGT MAbs failed to neutralize a majority of variants, we investigated whether these variants lacked the PNGS required for neutralization by these MAbs (Fig. 1). In some cases, resistance to these MAbs could be explained by the absence of a key PNGS. For example, variants isolated from a number of patients, including Q769, QG984, QH209, and QH359, which were resistant to PGT121 and PGT128, lacked the N332 residue required for neutralization (22, 27). Two of the four PG9/16-resistant variants, isolated from subject QF495, did not have the full glycosylation sequon that is a target for these MAbs, despite having the N160 residue (20, 28). Similarly, one of the QD435 variants resistant to these MAbs did not have the N156 residue required for recognition by PG9 (20). However, for all other variants resistant to PG9/16, the absence of known PNGS targets could not account for resistance, as these variants possessed key residues required for neutralization (N156, N160) (18, 26). Moreover, the presence of positively charged residues at positions 168, 169, and 171, which have been reported to be important for recognition by PG9/16 (11), did not always predict sensitivity to these MAbs (data not shown).

Some viruses, such as those from QF495, QH343, and QA465, had key PNGS for PGT121 and PGT128 recognition (N301 and N332) (20) yet were resistant to one or both of these MAbs. For other viruses, such as those isolated from Q259, Q168, and

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Subject ID	Subtype	Year of infection	Days PI*	Virus	NIH45-46W	VRC01	PG9	PG16	PGT121	PGT128	PGT145	NIH45-46W + PGT128	N156	N160	N301	N332
							PG9, PG16				PGT145				PGT128	PGT121
Q769	A	1996	56	Q769b9	0.03	0.17	0.05	0.01	>1	>1	>1	0.04	.	.	.	x
		1996		Q769d22	0.16	0.23	0.14	>1	>1	>1	>1	0.20	.	.	.	x
		1996		Q769h5	0.31	0.41	0.03	0.10	>1	>1	>1	0.31	.	.	.	x
Q842	A	1994	49	Q842d12	0.03	0.13	0.11	0.03	0.004	0.007	>1	0.004	.	.	.	shift
		1994		Q842d14	0.09	0.37	0.01	0.003	0.004	0.02	>1	0.001	.	.	.	shift
		1994		Q842d16	0.08	0.52	0.46	0.07	0.06	0.11	>1	0.24	.	.	.	shift
Q259	A	1994	76	Q259d2.17	0.12	0.28	0.41	>1	>1	>1	>1	0.12	.	.	.	shift
		1994		Q259d2.26	0.04	0.43	>1	>1	>1	>1	>1	0.03	.	.	.	shift
		1994		Q259.w6	0.04	0.34	>1	>1	0.002	>1	>1	0.03	.	.	.	shift
Q461	A	1995	26	Q461c2	0.08	0.45	>1	>1	>1	>1	>1	0.04	.	.	.	shift
		1995		Q461d1	0.04	0.29	0.74	0.20	>1	>1	>1	0.02	.	.	.	shift
		1995		Q461e2	0.10	0.58	>1	>1	>1	>1	>1	0.23	.	.	.	shift
Q168	A	1995	23	Q168a2	0.05	0.42	0.31	0.05	>1	>1	>1	0.14	.	.	.	shift
		1995		Q168b23	0.04	0.17	0.04	0.004	>1	>1	>1	0.06	.	.	.	shift
Q23	A	1995	485	Q23ENV.17	0.37	0.36	0.004	0.0003	0.04	0.03	1.00	0.05	.	.	.	.
QB726	A	1996	70	QB726.70M.ENV.B3	0.09	0.72	0.30	0.57	0.77	0.08	0.35	0.04	.	.	.	.
QF495	A	2005	23	QF495.23M.ENV.A1	0.23	>1	>1	>1	>1	>1	>1	0.60	.	x	.	.
		2005		QF495.23M.ENV.A3	0.17	>1	>1	>1	>1	>1	>1	0.26	.	x	.	.
		2005		QF495.23M.ENV.B2	0.16	>1	>1	>1	>1	>1	>1	0.28	.	.	.	.
		2005		QF495.23M.ENV.D1	0.13	>1	>1	>1	>1	>1	>1	0.23	.	.	.	.
QG984	A	2004	21	QG984.21M.ENV.A3	0.03	0.10	0.20	0.04	>1	>1	>1	0.03	.	.	.	shift
QH209	A	2005	13	QH209.14M.ENV.A2	0.02	0.17	>1	>1	>1	>1	0.07	0.04	.	.	.	shift
QH343	A	2005	21	QH343.21M.ENV.A10	>1	>1	>1	>1	>1	0.11	0.07	0.06	.	.	.	.
		2005		QH343.21M.ENV.B5	>1	>1	>1	>1	>1	0.10	0.06	0.06	.	.	.	.
QH359	A	2005	21	QH359.21M.ENV.C1	0.37	>1	>1	>1	>1	>1	0.80	0.80	.	.	.	shift
		2005		QH359.21M.ENV.D1	0.25	>!	>1	>1	>1	>1	>1	0.60	.	.	.	shift
QA255	A	1998	21	QA255.21P.ENV.A15	0.20	0.82	0.07	0.01	>1	0.04	>1	0.03	.	.	.	.
QA790	A/D	1996	204	QA790.204I.ENV.A4	0.03	0.06	>1	0.20	>1	>1	>1	0.05	.	.	.	T
		1996		QA790.204I.ENV.C1	0.001	0.05	0.21	0.01	>1	>1	>1	0.002	.	.	.	T
		1996		QA790.204I.ENV.C8	0.22	0.12	0.68	0.45	>1	>1	>1	0.07	.	.	.	T
		1996		QA790.204I.ENV.E2	0.01	0.11	>1	0.49	>1	>1	>1	0.02	.	.	.	T
QG393	A2/D	2004	60	QG393.60M.ENV.A1	0.01	0.04	0.16	>1	>1	>1	>1	0.01	.	.	.	shift
		2004		QG393.60M.ENV.B7	0.02	0.06	0.06	0.08	>1	>1	>1	0.02	.	.	.	shift
		2004		QG393.60M.ENV.B8	0.01	0.03	0.13	>1	>1	>1	>1	0.004	.	.	.	shift
QB099	C	1995	391	QB099.391M.ENV.B1	0.06	0.43	0.44	0.02	0.03	0.05	>1	0.02	.	.	.	.
		1995		QB099.391M.ENV.C8	0.20	0.15	0.63	0.06	0.03	0.06	>1	0.04	.	.	.	.
QC406	C	1997	70	QC406.70M.ENV.F3	0.13	>1	0.20	>1	0.01	0.007	>1	0.01	.	.	.	.
QA013	D	1995	70	QA013.70I.ENV.H1	0.11	0.81	>1	>1	>1	>1	>1	0.47	.	.	.	S
		1995		QA013.70I.ENV.M12	0.22	>1	>1	>1	>1	>1	>1	0.45	.	.	.	S
QA465	D	1993	59	QA465.59M.ENV.A1	>1	>1	>1	>1	0.03	>1	>1	>1	.	.	.	.
		1993		QA465.59M.ENV.D1	>1	>1	>1	>1	0.03	>1	>1	>1	.	.	.	.
QB857	D	1997	110	QB857.110I.ENV.B3	>1	>1	>1	0.20	>1	0.07	0.65	0.12	.	.	.	.
QD435	D	1994	100	QD435.100M.ENV.A4	0.17	0.35	>1	>1	>1	>1	0.20	0.13	.	.	.	shift
		1994		QD435.100M.ENV.B5	0.08	0.51	>1	>1	>1	>1	>1	0.08	D	.	.	shift
		1994		QD435.100M.ENV.E1	0.08	0.59	>1	>1	>1	>1	>1	0.26	.	.	.	shift

IC50 μg/ml
<0.03
0.03-0.08
0.09-0.23
0.24-1
>1

FIG 1 Summary of neutralization profiles of panel viruses against MAbs. Subject ID, virus subtype based on V1-V5 envelope sequence, and calendar year of infection are shown in the first 3 columns. Each row shows the virus name, IC_{50} for MAbs tested, and known core residues based on HXB2 numbering required for neutralization by MAbs shown. Viruses were obtained as described previously (4, 5, 17). For some patients, multiple viruses were obtained from the same time point to represent the diversity of the virus population at that time point as determined by phylogenetic analysis (5). Symbols: asterisk, estimated days postinfection at which envelope clone was obtained; dot, amino acid is present; x, amino acid is present but not in a glycosylation sequon; shift, amino acid is present but in position 334; D, T, S, amino acid substitutions. Darker shading indicates increasing MAb potency, as indicated by the key at the bottom, grouped by quartiles of IC_{50} values for all virus-MAb combinations. Gray shading indicates that 50% neutralization was not achieved at the highest concentration of MAb tested (1 μg/ml). The combination of NIH45-46W and PGT128 was tested at a starting concentration of 1 μg/ml of each MAb. IC_{50} values shown are averages from at least 2 independent experiments performed in duplicate.

QD435, the shift of PNGS at position 332 to position 334 may account for resistance to PGT121 and PGT128. However, this shift in PNGS did not always predict resistance to PGT121 and PGT128, as exemplified by Q842 variants, which had a shift of PNGS to position 334 yet were sensitive to these MAbs. Thus, the presence of known residues important for neutralization by the PG and PGT MAbs did not fully explain differing neutralization profiles among these early variants, suggesting that there may be other determinants of sensitivity to these MAbs. Indeed, the fact that some viruses, such as those from QF495 and QA465, had the expected epitope targets yet were resistant to most MAbs suggests that these viruses may have altered conformations that result in global neutralization resistance, as was observed for another early subtype A virus from heterosexual transmission (6) and for subtypes A and A/D vertically transmitted variants (14). Of note, variants that possessed the canonical epitopes for PG and/or PGT MAbs (QA013, QB857, QF495 QH343, and QH359 variants) were still not neutralized even when these MAbs were tested at a higher starting concentration of 10 μg/ml (data not shown). An alignment of V1-V3 sequences of all variants did not readily reveal signature sequences that would predict sensitivity to these MAbs (see Fig. S2 in the supplemental material), although QF495 variants had a large insertion in V2, including the addition of multiple PNGS, that could explain their resistance to most MAbs tested here.

NIH45-46W neutralized all but 5 viruses in the panel, including some viruses that were not neutralized by any other MAb (QF495, QH359, and QA013) (Fig. 1). Interestingly, although

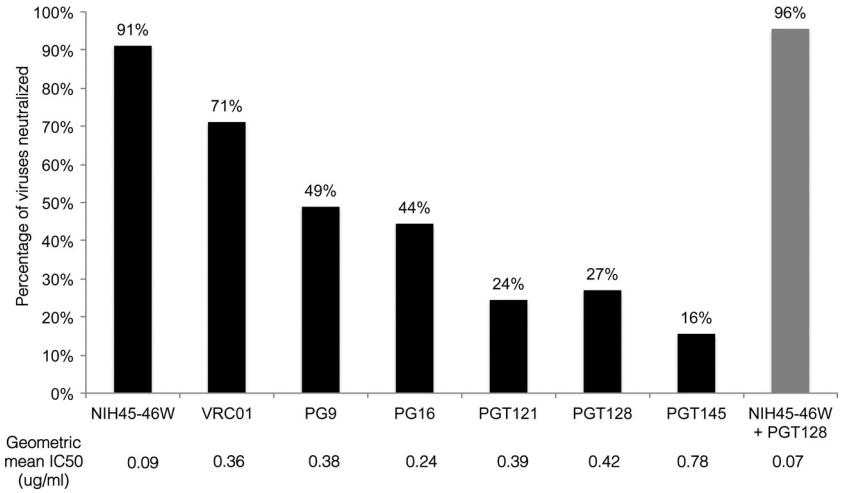

FIG 2 Summary of neutralization breadth and potency of MAbs against 45 viruses. Percentages of viruses neutralized for each MAb are indicated at the bottom of the graph. Geometric mean IC_{50} value for each MAb is indicated below the MAb name. IC_{50} values greater than the highest MAb concentration tested (1 μg/ml) were assigned a value of 1 in the geometric mean IC_{50} calculations.

MAbs PGT121, PGT128, and PGT145 displayed limited breadth at the highest concentration tested in these experiments (1 μg/ml), they potently neutralized variants that were resistant to all other MAbs, including NIH45-46W. (Fig. 1; see also Fig. S1 in the supplemental material). Specifically, QA465 and QH343 variants were only neutralized by PGT121, PGT128, and/or PGT145 ($IC_{50} \leq 0.11$ μg/ml). Hierarchical clustering analyses suggested that combining NIH45-46W and PGT128 would neutralize all but 2 variants, which were recognized by PGT121 (Fig. 3).

To test the hypothesis that NIH45-46W and PGT128 would complement rather than interfere with each other's neutralizing ability, we investigated the neutralization profiles of a subset of viruses against NIH45-46W and PGT128 alone or in a 1:1 combination. We chose viruses that were either (i) neutralized by one but not the other MAb (Q168.A2 and QH343.21 M.ENV.A10) or (ii) neutralized by both MAbs (QB099.391 M.ENV.B1 and QC406.70 M.ENV.F3). The presence of one MAb did not interfere with the activity of the other MAb regardless of whether the virus tested was sensitive to one (see Fig. S3A in the supplemental material) or both (see Fig. S3B) MAbs, with no increase in IC_{50} values for MAbs in a 1:1 combination compared to MAbs tested alone. Because NIH45-46W is an engineered antibody, we also confirmed that a naturally occurring broad and potent CD4bs MAb, VRC01, would not interfere with PGT128 neutralization (see Fig. S3). These results demonstrate that broad and potent MAbs targeting the CD4bs and V3 do not compete for neutralization.

Overall, the combination of NIH45-46W and PGT128 neutralized 96% of variants with a geometric mean IC_{50} of 0.07 μg/ml (Fig. 1 and 2). The remaining 2 variants not neutralized by NIH45-46W and PGT128 alone or in combination were potently neutralized by PGT121 (Fig. 3). Because PGT121 and PGT128 have previously been shown to compete for binding (27), we investigated whether these MAbs would interfere with each other's neutralizing capacity against these 2 viruses. The combination of PGT121 and PGT128, with or without NIH45-46W present, neutralized both viruses, which were sensitive to PGT121 but resistant to PGT128, to a similar extent as PGT121 alone (see Fig. S4 in the supplemental material). These results demonstrate that the presence of PGT128 does not interfere with PGT121 neutralization against these variants. It is possible that these observations reflect an absence of binding of PGT128 to the viruses tested rather than a lack of competition between the MAbs.

We observed generally similar neutralization breadth for the individual MAbs NIH45-46W and VRC01 and only slightly lower breadth for PG9 and PG16 compared to previous reports (10, 28, 30). However, the neutralization breadth of PGT121, PGT128, and PGT145 (16 to 27%) was 2- to 3-fold lower than that observed previously (27). In the prior study by Walker et al. (27), these MAbs were tested against a panel of viruses weighted toward variants from chronic infection, potentially suggesting differences in efficacy of the MAbs against variants in early versus chronic infection. However, differences in assays used and the subtypes of viruses examined could also be relevant and studies that directly compare these variables will be needed to understand differences in efficacy against different virus panels.

Prior studies of a subset of MAbs tested here, including CD4bs and V1/V2 MAbs, suggested that the combination of PG9 and VRC01 provided almost universal coverage of the viruses tested, which included subtype B viruses (13) and viruses from diverse subtypes (12). When PG9 and VRC01 were tested at 1 μg/ml, 26% of viruses in our panel were resistant to both MAbs (Fig. 1), which is more than what was reported in the prior cross-clade study (~10%) (12). This difference could again reflect differences in the virus panel, which included viruses from both acute and chronic infection from a study by Doria-Rose et al. (12). Additionally, differences in the calendar period from which viruses were isolated could also influence sensitivity to these MAbs (13), but our sample size and distribution over the sampling period (Fig. 1) were not adequate to rigorously address this issue. Finally, the study by Doria-Rose et al. used higher MAb concentrations (50 μg/ml) and a larger dilution range, and at this higher concentration, we have observed a tendency to obtain lower IC_{50} values than those obtained with the lower starting concentration (1 μg/ml) and tighter dilution range used here for viruses that were potently neutralized ($IC_{50} < 0.1$ μg/ml). However, this would not have

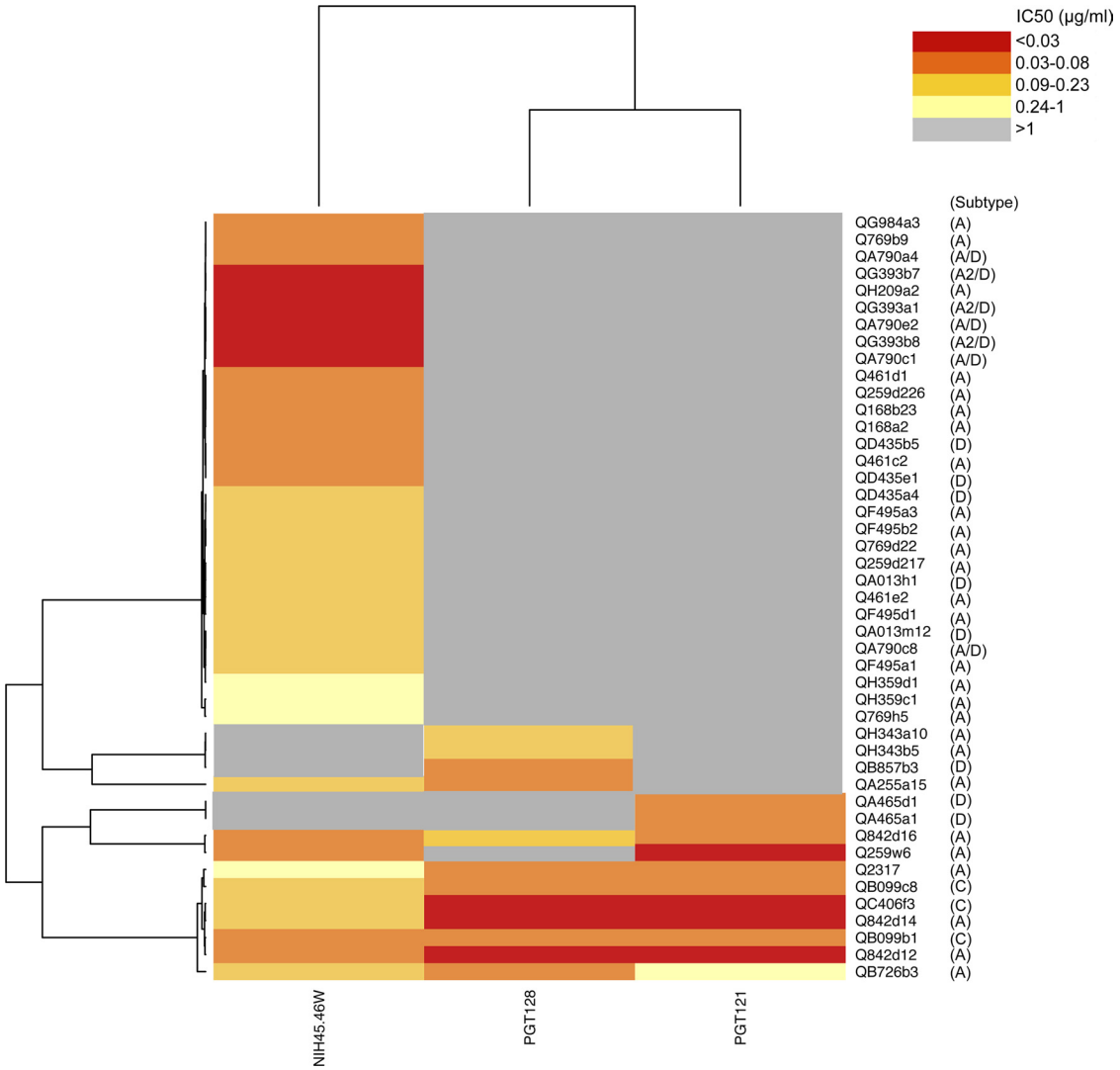

FIG 3 Hierarchical clustering of MAbs NIH45-46W, PGT128, and PGT121 (bottom) and panel viruses (right). A heatmap of IC_{50} values for each virus-MAb combination is shown, with darker shading indicating increasing potency, as indicated by the key. Gray shading indicates that 50% neutralization was not achieved at the highest concentration of MAb tested (1 μg/ml).

altered our overall results, which focused on whether MAbs could neutralize variants at1 μg/ml, although it could lead to small differences in the geometric mean IC_{50}. Notably, at the lower MAb concentration used here, only 4% of viruses in our panel were resistant to NIH45-46W and PGT128, two MAbs that were not included in previous studies, implying that these MAbs may be among the most effective against variants found early in infection. Although 7/45 viruses were obtained later in infection (Q23, QA790, and QB099), and thus may not be representative of recently transmitted variants, removing these viruses from the analysis did not significantly affect the results of our study. For example, even by excluding these viruses, NIH45-46W was still the most broad and potent MAb, while PGT121, PGT128, and PGT145 were the least broad and potent MAbs against our panel.

In summary, we demonstrated that recently identified broadly neutralizing HIV-1 MAbs have variable activity against variants found early in infection. NIH45-46W, an engineered mutant of NIH45-46 that targets the hydrophobic CD4 binding cavity in gp120 (10), displayed remarkable breadth and potency against these viruses. However, this MAb was unable to neutralize ~10% of the viruses in the panel, which were potently neutralized by glycan-dependent MAbs PGT121, PGT128, and/or PGT145. PGT128 and NIH45-46W displayed no competition for neutralization, and a combination of these MAbs neutralized 96% of variants, with PGT121 neutralizing the only 2 viruses not neutralized by this combination. Our results suggest that optimal neutralization coverage of transmitted variants may be achieved by combining a potent CD4bs NAb with one or more NAbs directed to glycan-dependent epitopes in V3. It is currently unclear whether this particular combination of broad and potent NAbs can develop within a patient during natural infection, and it is likely that eliciting such responses will be challenging. However, the results presented here provide motivation to focus on these epitopes, given that the antibody combination against them can neutralize viruses representing recently transmitted variants.

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