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Broadly neutralizing antibodies targeting the HIV-1 envelope V2 apex confer robust protection against a clade C SHIV challenge

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Abstract

Neutralizing antibodies to the V2 apex antigenic region of the HIV-1 envelope (Env) trimer are among the most prevalent cross-reactive antibodies elicited by natural infection. Two recently

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Project planning was performed by D.H.B., D.R.B., J.R.M., R.A.K., L.M., and S.S.A.K.; the SHIV-325c was developed by L.J.T. and P.A.; antibodies were generated by A.P., P.L.M., D.S., X.C.; viral neutralization assays were performed and analyzed by M.K.L., R.T.B., A.P., D.S., S.D.S., N.A.D.-R., K.L.; env sequences were generated and analyzed by B.K.; plasma viral loads were measured by P.A.; PK-ELISA were performed by A.P., D.S., K.W.; synthesis and purification of CNE58-strandC-CAP256.SU SOSIP trimer for ELISA were performed by X.C., M.G.J., I.S.G., M.C., P.D.K.; PGDM1400 alanine scanning mutants were produced by D.S.; bNAbs coverage/breadth analysis was performed by M.S.S. and B.K.; and the manuscript was written by B.J., D.R.B., L.M., J.R.M. and D.H.B.

Competing interests

The authors declare that they have no competing financial interests.

described V2-specific antibodies, PGDM1400 and CAP256-VRC26.25, have demonstrated exquisite potency and neutralization breadth against HIV-1. However, little data exist on the protective efficacy of V2-specific neutralizing antibodies. We created a novel SHIV-325c viral stock that included a clade C HIV-1 envelope and was susceptible to neutralization by both of these antibodies. Rhesus macaques received a single infusion of either antibody at three different concentrations (2, 0.4, and 0.08 mg/kg) before challenge with SHIV-325c. PGDM1400 was fully protective at the 0.4 mg/kg dose, whereas CAP256-VRC26.25-LS was fully protective even at the 0.08 mg/kg dose, which correlated with its greater in vitro neutralization potency against the challenge virus. Serum antibody concentrations required for protection were <0.75 mg/ml for CAP256-VRC26.25-LS. These data demonstrate unprecedented potency and protective efficacy of V2-specific neutralizing antibodies in nonhuman primates and validate V2 as a potential target for the prevention of HIV-1 infection in passive immunization strategies in humans.

Introduction

The induction of broadly neutralizing antibodies (bNAbs) is a major goal of the HIV-1 vaccine field, but no HIV-1 Env immunogen to date has been able to elicit antibodies with broadly neutralizing activity (1). In contrast, many HIV-1 infected individuals produce neutralizing antibodies with some degree of breadth during the course of infection (2–4). Over the past few years, several antibodies targeting distinct epitopes of the HIV-1 Env trimer and with potent and broad activity against diverse clinical isolates have been identified (5–8). In particular, neutralizing antibodies directed towards the CD4 binding site and the V3 region have shown promise in preclinical studies, in which single intravenous doses of antibodies protected rhesus macaques against challenges with simian-human immunodeficiency virus (SHIV) (9–12). In the absence of a vaccine that can elicit such bNAb responses, passive immunization with bNAbs is being explored for HIV-1 prevention strategies.

While antibodies against several regions of the Env trimer have been described (6), neutralizing antibodies to the V2 apex antigenic region of the HIV-1 Env trimer are among the most prevalent cross-reactive antibodies elicited during infection (13–15). The V1V2 region, which harbors multiple glycans and is highly sequence diverse, is located at the Env apex and plays a vital role in the Env function by stabilizing the trimeric spike on the virion surface. It also shields V3 and the coreceptor binding sites in the prefusion state and exposes them upon CD4 binding (16). While these antibodies are common in HIV-1-infected individuals, we know very little about their ability to confer protection against infection. In the recent RV144 HIV-1 vaccine study, binding antibodies against the V1V2 region were associated with reduced risk of infection (17).

To date, V2-directed bNAbs have been isolated from several donors, including the IAVI protocol G donor 24 (PG9 and PG16) (18), the CHAVI donor 0219 (CH01–CH04) (19), the CAPRISA 256 donor (CAP256-VRC26.01–33) (20, 21), and the IAVI protocol G donor 84 (PGT141–145 and PGDM1400–1412) (5, 22). These antibodies bind to the intact trimer with a stoichiometry of one per trimer (20) and interact with glycans at N160 and to a lesser extent N156 (18). They also have a very long heavy chain complementarity-determining

region 3 (CDRH3), which enables them to effectively penetrate the glycan shield (21). For the present study, we selected two V2-specific mAbs, CAP256-VRC26.25 and PGDM1400, for their exquisite potency and neutralization breadth. CAP256-VRC26.25 neutralized 57% of global viral isolates and 70% of clade C isolates with a median 50% inhibitory concentration (IC₅₀) of 0.001 µg/mL against sensitive viruses (21, 23). Among the PGT145 antibody family, the somatic variant PGDM1400 had particularly broad and exceptionally potent neutralization activity with 83% global coverage at a median IC₅₀ of 0.003 µg/mL (22). These V2-specific antibodies have superior potency compared to the V3 glycan-dependent antibodies PGT121 and PGT128 (5), which are among the most potent bNAbs described to date. However, the protective efficacy of V2-specific bNAbs against pathogenic tier 2 SHIV challenges remains unexplored.

In this study, we evaluated the protective efficacy of these V2-specific bNAbs against SHIV challenge in nonhuman primates. We created a novel SHIV-325c stock that included a clade C Env and against which PGDM1400 and CAP256-VRC26.25 showed potent neutralization activity. Animals received a single infusion of PGDM1400 or CAP256-VRC26.25-LS (engineered with the Fc-LS mutation to increase *in vivo* half life) at three different concentrations and were challenged with SHIV-325c. 10 of 14 animals that received PGDM1400 and 12 of 12 animals that received CAP256-VRC26.25-LS were protected. The majority of breakthrough infections occurred in the lowest PGDM1400 dose group (0.08 mg/kg). Both antibodies protected all animals at the 0.4 mg/kg dose, demonstrating the unprecedented protective efficacy of these antibodies at exceptionally low doses as compared with previously tested bNAbs (9–11). These data validate V2 as a protective target against HIV-1.

Results

Generation and evaluation of SHIV-325c

PGDM1400 and CAP256-VRC26.25 have been shown to neutralize HIV-1 broadly and with high potency (21, 22), but they did not neutralize several commonly used SHIVs (Table 1). We therefore generated a novel challenge stock SHIV-325c by cloning a clade C HIV-1 *env* sequence from an early HIV-1 infected individual from South Africa (24) into the SHIV KB9-AC backbone. Transfection of 293T cells yielded high SIV p27 levels (data not shown), and large-scale challenge stocks were generated by inoculation of the 293T transfection-derived supernatants into primary human peripheral blood mononuclear cells (PBMC), resulting in virus infectivity titers of 3.49×10^5 TCID₅₀/mL and viral loads ranging from $1.3\text{--}1.5 \times 10^9$ RNA copies/mL. We sequenced the *env* of the SHIV-325c challenge stock using single-genome amplification and compared it to the original HIV-1 *env* patient sequence (Fig. 1). The diversity of the viral stock was low (mean diversity 0.06%, excluding APOBEC mutated sequences). Among 25 SGA-derived sequences obtained from the SHIV-325c stock, 12 amplicons were 100% identical to the original HIV-1 *env* consensus sequences, and 11 amplicons showed one to three sporadic nucleotide differences (mean divergence 0.03%, max divergence 0.13%). Only one amplicon demonstrated several APOBEC mutated sequences. These data show that the *env* sequences in the SHIV-325c stock were very similar to the original HIV-1 *env* sequence.

We next evaluated the infectivity of the SHIV stock *in vivo*. 8 adult rhesus macaques were inoculated by the intrarectal route with 1 ml of the SHIV-325c challenge stock. All animals developed productive infection (Fig. 2A) and exhibited robust peak viral loads ranging from 4.7 to 6.7 log SIV RNA copies/ml after inoculation. These viral loads were comparable to those following SHIV-SF162P3 or SHIV-AD8 infection (25, 26). Viral loads declined and reached chronic setpoint levels by approximately week 10. Setpoint viral loads varied among animals (2.2 to 5.2 log SIV RNA copies/mL), and 75% (6 of 8) of infected animals exhibited chronic viremia at week 14, with mean setpoint viral loads of 3.9 log RNA copies/mL. During acute infection, mean CD4⁺ T cell numbers declined by 27% in SHIV-325c inoculated animals (pre-infection count: 1406 cells/mm³, post infection nadir: 1027 cells/mm³; $p < 0.02$) (Fig. 2B) but did not subsequently exhibit further decline.

When tested against a panel of 14 bNAbs, SHIV-325c exhibited sensitivity to the V2 antibodies PG9, CAP256-VRC26.25 and PGDM1400 but was resistant to several other bNAbs, including the CD4 binding site antibodies VRC01 and 3BNC117, the V3-glycan antibody PGT128, and several MPER and CD4i antibodies (Table 2). SHIV-325c sensitivity across the tested bNAbs was comparable to the frequently used tier 2 SHIV-SF162P3, which demonstrated resistance against 7 of the 14 tested bNAbs. In contrast, SHIV-325c was more resistant than the tier 1 SHIV-BaL, which was neutralized by 11 of the 14 bNAbs, indicating that SHIV-325c had a tier 2 phenotype. SHIV-325c neutralization sensitivity to PGDM1400 and CAP256-VRC26.25 (IC₈₀ 0.104 µg/mL and 0.006 µg/mL, respectively) was comparable to their median neutralization sensitivity of a multi-clade panel of 208 HIV pseudoviruses (IC₈₀ 0.04 µg/mL and 0.03 µg/mL, respectively) (Fig. 3A; Supplementary Table S1). PGDM1400 and CAP256-VRC26.25 also neutralized SHIV-325c at a similar potency to a panel of clade C pseudoviruses (IC₈₀ 0.02 µg/mL and 0.01 µg/mL, respectively) (Fig. 3B). No neutralization curve plateaus below 100% neutralization were observed with these three V2 antibodies against SHIV-325c (Fig. 4), unlike previously described for PG9 against SHIV-BaL (11).

These data indicate that SHIV-325c had a tier 2 neutralization phenotype and a representative neutralization profile for PGDM1400 and CAP256-VRC26.25 as compared with a large panel of clade C viruses, suggesting that it may be a useful challenge model for evaluating the protective activity of these antibodies.

***In vivo* protection against SHIV-325c challenge**

To assess the protective efficacy of PGDM1400 and CAP256-VRC26.25-LS against SHIV-325c challenge, we performed an antibody titration challenge study in rhesus macaques. CAP256-VRC26.25-LS is a variant of CAP256-VRC26.25 that has been engineered to include the LS mutation in its Fc region that increases *in vivo* half-life (27) from approximately 4.4 days to 8.8 days in rhesus macaques (Supplementary Fig. S1).

35 rhesus macaques were randomized to the following groups: two groups of 5 animals each received 2 mg/kg and 0.4 mg/kg of PGDM1400, and one group of 4 animals received 0.08 mg/kg of PGDM1400. Three additional groups of 4 animals each received 2 mg/kg, 0.4mg/kg and 0.08mg/kg of CAP256-VRC26.25-LS. A control group of 9 animals received saline. Antibodies were administered *i.v.* 24 h before the animals were challenged by the

intrarectal route with a single high dose of SHIV-325c (500 TCID₅₀). All animals in the control group became infected with detectable viremia between day 7 and 28, and median peak viremia was 5.8 log viral copies/ml (Fig. 5A), confirming the robust infectivity of the novel SHIV-325c challenge stock. In the high dose group (2 mg/kg), 1 out of 5 animals that received PGDM1400 became infected and showed plasma viremia on day 28 (Fig. 5B), while all of the CAP256-VRC26.25-LS pretreated animals at the same dose were protected (Fig. 5E). At 0.4 mg/kg, no infections occurred in either group (Fig. 5C and F). In the low dose group (0.08 mg/kg), 3 of 4 animals that received PGDM1400 were infected (Fig. 5D), while none of the animals that received CAP256-VRC26.25-LS were infected (Fig. 5G). These data demonstrate that the *in vitro* neutralization potency of these V2-specific antibodies translates into extraordinary protective efficacy *in vivo* at doses of 0.08–0.4 mg/kg.

Pharmacokinetics of PGDM1400 and CAP256-VRC26.25-LS *in vivo*

Serum samples were obtained throughout the study, and PGDM1400 and CAP256-VRC26.25-LS levels were determined by ELISA. The results show that the animals that received PGDM1400 at 2 mg/kg, 0.4 mg/kg and 0.08 mg/kg had average serum antibody concentrations of 6.9 µg/mL, 2.5 µg/mL and 0.22 µg/mL at the time of challenge (Fig. 6). In contrast, serum concentrations of CAP256-VRC26.25-LS were 19.8 µg/mL, 3.3 µg/mL and 0.75 µg/mL for the animals that received 2 mg/kg, 0.4 mg/kg and 0.08 mg/kg of CAP256-VRC26.25-LS, respectively. Serum concentrations of CAP256-VRC26.25-LS were thus slightly higher than PGDM1400 ($P=0.02$ – 0.03 for all dose groups) at time of challenge, likely reflecting differences in distribution or elimination of these two antibodies. The half-life of PGDM1400 based on the level of serum antibody decay (two-phase exponential decay) was calculated to be 7.7 days for the 2 mg/kg dose group, 6.7 for the 0.4 mg/kg dose group and 6.3 for the 0.08 mg/kg group. The half-life of CAP256-VRC26.25-LS in this study was calculated to be 10.3 days for the 2 mg/kg dose group, 9.9 days for the 0.4 mg/kg dose group and 7.7 days for the 0.08 mg/kg dose group, consistent with the initial experiment (Supplementary Fig. S1). The higher serum concentrations and more potent neutralization activity of CAP256-VRC26.25-LS compared to PGDM1400 against SHIV-325c correlated with its higher protective efficacy.

Sequence analysis of breakthrough viruses

Although only 4 of 14 PGDM1400 pretreated animals developed plasma viremia, we were interested to examine potential escape/resistance pathways in SHIV-325c following PGDM1400 administration. We therefore generated single genome *env* sequences from plasma viruses at the time of peak viremia following breakthrough infection. Overall, there was very little sequence variation (mean divergence 0.08–0.19%) between the challenge strain and the plasma viruses. We then focused on the variable loop 1 and 2 region, and no specific pattern of sequence variation was noticeable comparing PGDM1400 and placebo control animals (Fig. 7). Position 167 in V2, a known resistance mutation for CAP256 lineage antibodies (18, 28, 29) showed D/N and rare D/E changes in several of the bNAbs pretreated but also in the placebo control animals, and the D/N variant was also found in the challenge stock. Mutations at this position (D/A) also confer resistance to PGDM1400 (Table 3), suggesting that the rare D/E variation seen in the PGDM1400 pretreated animal

could be an escape pathway. Similarly, one PGDM1400 pretreated animal developed a D/E mutation at position 165, which has been associated with escape from CAP256 lineage antibodies (30). While we do not see reduced neutralization susceptibility for PGDM1400 with an I/A mutation at this position (Table 3), the effect of I/E needs to be evaluated. In animal M97, which received 2 mg/kg of PGDM1400 but nevertheless developed high plasma viremia at day 28, no variant stood out in the V1/V2 region, but a fixed mutation (F210L) was observed in all 27 amplicons generated from this animal. This variant was absent in the challenge stock and was not detected in any other animal. Whether this mutation contributed to viral escape from PGDM1400 remains to be determined.

Complementary coverage of HIV-1 clade C viruses by V2- and V3-specific antibodies

PGDM1400 and CAP256-VRC26.25 both have potent neutralization activity against HIV-1 clade C viruses and exhibited 61–65% coverage of a large panel of 200 clade C *env* pseudoviruses (median IC₈₀ 1.17 µg/mL and 0.19 µg/mL for PGDM1400 and CAP256-VRC26.25, respectively) (Fig. 8A). A previous study reported that the combination of PGDM1400 with the V3 glycan-dependent antibody PGT121 provided 98% coverage of multi-clade viruses with a median IC₅₀ of 0.007 µg/mL (22). We therefore determined if the coverage of clade C viruses would improve if PGDM1400 or CAP256-VRC26.25 were combined with PGT121. Interestingly, PGT121, which covers 65% of the clade C virus panel at a median IC₈₀ of 1.85 µg/mL (23), targeted many of the “gaps” in viral coverage of PGDM1400 and CAP256-VRC26.25 (Figure 8A). PGT121 plus CAP256-VRC26.25 as well as PGT121 plus PGDM1400 showed robust coverage of >90% of clade C viruses and additive potency as compared to single bNAbs (IC₈₀ for combinations 0.017 µg/mL and 0.07 µg/mL respectively) (Fig. 8B).

Discussion

The V2 region of the HIV-1 Env trimer is a common target of bNAbs, and some bNAbs directed against this epitope have demonstrated exquisite neutralization potency and breadth. Our findings demonstrate the capacity of the V2-specific antibodies PGDM1400 and CAP256-VRC26.25-LS to protect rhesus macaques at extremely low titers against high-dose challenge with SHIV-325c. Protection was achieved at low serum antibody concentrations of <0.75 mg/ml for CAP256-VRC26.25-LS, consistent with the extraordinary neutralization potency of these bNAbs.

Protection at such low antibody concentrations has not previously been reported (31). Previous studies have tested the protective capacity of several bNAbs, including the CD4 binding site mAbs VRC01 and 3BNC117 as well as the V3-glycan dependant bNAbs PGT121 and 10-1074 against various SHIV strains (e.g. SHIV-AD8EO, SHIV-SF162P3, SHIV-BaL) (9–11). Average serum concentrations at the time of challenge necessary for protection were variable but higher than that reported here (31). The lowest serum antibody concentrations that conferred complete protection were with PGT121 at 15 and 22 µg/mL against SHIV-SF162P3 and SHIV-AD8EO challenges, respectively, and partial or no protection was seen at serum levels of 1.8 µg/mL (9, 10). VRC01 only protected 4 out of 10 animals against the neutralization-sensitive tier 1 virus SHIV-BaL at serum concentrations of

1.3 µg/mL, whereas 10E8 only protected 3 out of 6 animals against this virus at serum concentrations of 1.8 µg/mL (11). The low doses of PGDM1400 and CAP256-VRC26.25-LS required for protection suggest that they could be explored for subcutaneous administration (32). We did not measure anti-PGDM1400 or anti-CAP256-VRC26.25-LS antibodies in this study. Xenogeneic responses against human antibodies are commonly induced in macaques (27) but have not been observed to date in clinical studies with VRC01 and 3BNC117 (33, 34).

No previous studies have assessed the protective efficacy of V2-specific bNAbs against tier 2 SHIV challenges, and thus the present studies help validate V2 as a protective target for bNAbs. PG9 is the only other V2-specific bNAb that has been evaluated for protective efficacy to date in nonhuman primates. Partial protection was achieved at 5 mg/kg and no protection was observed at 0.3 mg/kg (serum antibody levels at day of challenge were 3.7 and 0.28 µg/ml, respectively) against the neutralization-sensitive tier 1 virus SHIV-BaL, despite a mean IC₅₀ of 0.06 µg/mL against SHIV-BaL (11). It remains to be determined if the inability of PG9 to achieve 100% neutralization of the challenge stock by TZM-bl assays *in vitro* (35) may have impacted its protective efficacy *in vivo*.

SHIV-325c was neutralized by PGDM1400 and CAP256-VRC26.25 at IC₈₀ values that were comparable to the median IC₈₀ values against large global virus panels. In addition to their exquisite potency, both PGDM1400 and CAP256-VRC26.25 have 60–65% (IC₈₀) of 200 tested clade C viruses. The pattern of missed viruses were relatively similar for these two V2-specific bNAbs, suggesting that these antibodies could be combined with other bNAbs that target different epitopes to improve coverage. PGT121 has demonstrated potent antiviral activity in both therapeutic and protection studies in rhesus macaques (9, 36) and shows striking complementary coverage with both PGDM1400 and CAP256-VRC26.25-LS. This complementarity increases viral coverage of these antibody combinations to >90% of clade C viruses (23), suggesting that PGT121 may be a useful antibody to co-administer with either CAP256-VRC26.25-LS or PGDM1400 to prevent clade C HIV-1 infection in southern Africa.

V1V2 and V3 are in close spatial proximity (37, 38), and thus it has been suggested that co-administration of antibodies targeting these regions (e.g. PGT121 together with either PGDM1400 or CAP256-VRC26.25-LS) might impact each antibody's binding kinetics. Indeed, prior data has suggested a certain level of competition between PGT121 and the V2 specific antibody PG9 (5), but this was not observed for PGDM1400, which showed very little to no competition with PGT121 (22). Similarly, binding of CAP256-VRC26.08, which is from the same lineage as CAP256-VRC26.25, was only minimally reduced by PGT121 (20) in a standard binding competition assay. These data suggest that overall no significant reduction in antibody binding should be expected when these V2- and V3-specific bNAbs are co-administered, although studies with such bNAb combinations require future studies in nonhuman primates and humans.

In summary, our data demonstrate that potent protective efficacy can be achieved with bNAbs that target the HIV-1 *env* trimer apex at very low antibody doses. PGDM1400 and

CAP256-VRC26.25-LS, particularly when combined with a V3 glycan-dependent antibody such as PGT121, should therefore be explored in clinical trials for HIV-1 prevention.

Material and Methods

Animals and study design

SHIV-325c infection study—Eight Indian-origin, outbred, young adult, male and female, experimentally naïve rhesus macaques (*Macaca mulatta*) that did not express the class I alleles Mamu-A*01, Mamu-B*08 and Mamu-B*17 associated with spontaneous virological control were housed at Bioqual Inc, Rockville, MD. Animals were atraumatically challenged by the intrarectal route with 500 TCID₅₀ of SHIV-325c. All animals were monitored for viral loads and CD4+ T cell counts. The animal studies were approved by the appropriate Institutional Animal Care and Use Committees (IACUC). The study was carried out in strict accordance with the recommendations in the “Guide for the Care and Use of Laboratory Animals” of the National Institutes of Health.

CAP256-VRC26.25 pharmacokinetics study—All animal experiments were reviewed and approved by the Animal Care and Use Committee of the Vaccine Research Center, NIAID, NIH, and all animals were housed and cared for in accordance with local, state, federal, and institute policies in an American Association for Accreditation of Laboratory Animal Care-accredited facility at the NIH. Eight Indian-origin rhesus macaques were administered low-endotoxin antibody preparations (<1 EU/mg) intravenously at 10 mg/kg of body weight. Whole blood samples were collected prior to injection and at multiple time points till week 4 post-administration.

SHIV-325c protection study—Thirty-five Indian-origin, outbred, young adult, male and female, experimentally naïve rhesus macaques that did not express the class I alleles Mamu-A*01, Mamu-B*08 and Mamu-B*17 associated with spontaneous virological control were housed at Bioqual Inc, Rockville, MD and Alphagenesis Inc, Yemassee, SC. Animals were randomly allocated to the different antibody dose and saline control groups. The antibody or saline was administered i.v. 24 h before the animals were atraumatically challenged by the intrarectal route with 500 TCID₅₀ of SHIV-325c. Serum samples for antibody detection and viral load determination were obtained at week -1, day 0, 1, 3, 7, 14, 28, 42, and 56. The animal studies were approved by the appropriate Institutional Animal Care and Use Committees (IACUC). The study was carried out in strict accordance with the recommendations in the “Guide for the Care and Use of Laboratory Animals” of the National Institutes of Health.

Construction of SHIV-325c molecular clone

SHIV-325c was generated from an *env* sequence derived from an early HIV-1-infected individual CA325 (24, 39). The *env* sequence was synthesized by GeneArt (GeneArt, Invitrogen) and inserted into the KB9-AC plasmid as described (39, 40). The plasmid was sequenced and transfected into 293T cells using LipoD293 (SignaGen Laboratories). Cell culture supernatants were collected after 48 h and clarified through a 0.45 µm filter.

Generation of large-scale SHIV stocks

For the generation of the SHIV-325c virus stock, PBMC were isolated from 120 mL of human blood. Cell culture supernatants harvested from transiently transfected 293T cells were used to inoculate to ConA-stimulated PBMC in the presence of 20 U/mL human IL-2 (AIDS Research and Reference Reagent Program). The medium was replaced and collected every 3 days. Virus was quantified by SIV p27 enzyme-linked immunosorbent assay (ELISA; Zeptometrix), and the 50% tissue culture infectious dose (TCID₅₀) was determined in TZM-bl cells (39).

Antibody production

PGDM1400 was generated as previously described (5) and purified by Protein A affinity matrix (GE Healthcare). CAP256-VRC26.25-LS was purified after transient transfection of Expi293 cells (Invitrogen) with expression vectors encoding for its heavy and light chains. PBS was used as control in this study. All the monoclonal antibody preparations were endotoxin free. The CAP256-VRC26.25-LS antibody included the Fc-region mutation (LS) (27) that increased circulating half-life. The LS mutation did not affect the antibody combining site and thus neutralization potency was unaffected.

ELISA

PGDM1400 and CAP256-VRC26.25-LS antibody concentration in serum was determined by ELISA as described (41) using the BG505 SOSIP trimer or the novel CNE58-strandC-CAP256.SU SOSIP trimer, respectively. Briefly, microtiter plates were coated with 1 µg/mL SOSIP trimer and incubated overnight at 4°C. The plates were washed with PBS/0.05% Tween-20 and blocked with PBS/Casein (Pierce). After blocking, serial dilution of serum samples were added to the plate and incubated for 2 h at 37 °C. Binding was detected with a horseradish peroxidase (HRP)-conjugated goat anti-human IgG secondary antibody (Fisher Scientific) and visualized with SureBlue tetramethylbenzidine (TMB) microwell peroxidase (KPL Research Products).

Synthesis and purification of CNE58-strandC-CAP256.SU SOSIP trimer for ELISA

HIV-1 gp140 SOSIP-type molecule based on clade C strain CNE58 (42–44) including the ‘SOS’ mutations (A>501C, T605C), the isoleucine to proline mutation at residue 559 (I559P), mutation of the cleavage site to 6R (REKR to RRRRRR), and truncation of the C terminus to residue 664 (all HIV-1 Env numbering according to the HX nomenclature) was used in this study. The CNE58 SOSIP construct also utilized a chimera strategy by utilizing the gp41, N- and C-termini of BG>505 and part of the V1V2 C strand (residues 166–173) from the CAP256.SU strain (20, 28) and a C-terminal six amino acid glycine-serine linker, a HRV-3c cleavage site, His8 purification tag (GGSGGSGLEVLFGPGHHHHHHHH). The HIV-1 SOSIP molecule was expressed as previously described (45) by co-transfecting with furin in HEK 293F cells using HIV-1 SOSIP DNA and furin plasmid DNA. Transfection supernatants were harvested after 6 days, and the CNE58-strandC-CAP256.SU HIV-1 trimer supernatant was passed over a NiNTA affinity column. The eluate was concentrated and the peak corresponding to trimeric HIV-1 Env were identified, pooled, and concentrated.

Neutralization assays

Neutralization of diverse, replication-competent SHIV challenge stocks (including SHIV-325c) or Env-pseudoviruses was evaluated *in vitro* by using TZM-bl target cells and a luciferase reporter assay as described (11, 46, 47). Briefly, HIV-1 Env pseudoviruses were generated by transfection in 293T cells, and SHIV challenge stocks were produced by transfection of 293T cells with infectious molecular clone plasmids, followed by propagation in human or rhesus PBMC, as described above and as indicated in Table 1. The rhesus PBMC derived stock of SHIV-AD8 was kindly provided by Dr. Malcolm Martin (12) and SHIV-1157ipd3N4 was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH from Dr. Ruth Ruprecht. SHIV stocks or HIV-1 pseudoviruses were incubated with the antibody for 30 min at 37°C before TZM-bl cells were added. The protease inhibitor indinavir was added to assays with SHIV stocks to a final concentration of 1 µM to limit infection of target cells to a single round of viral replication. Luciferase expression was quantified 48 h after infection upon cell lysis and the addition of luciferin substrate (Promega).

Single genome amplification and analysis

Single genome amplification followed by direct sequencing of the Env gene was used to eliminate Taq induced errors and *in vitro* recombination as described (48). Briefly, viral RNA was isolated from plasma using a QIAamp viral RNA kit (Qiagen). Reverse transcription of RNA to single-stranded cDNA was performed using SuperScript III Reverse Transcriptase according to the manufacturer's recommendations (Invitrogen). In brief, a cDNA reaction of 1x RT buffer, 0.5mM of each dNTP, 5mM DTT, RNaseOUT [RNase (recombinant ribonuclease) inhibitor] (2U/mL), SuperScript III Reverse Transcriptase (10 U/mL), and 0.25 mM antisense primer EnvB3out 5'-TTG CTA CTT GTG ATT GCT CCA TGT-3'. RNA, primers and dNTPs were heated at 65°C for 5 min and then chilled on ice for 1 min then the entire reaction was incubated at >50°C for 60 min, followed by 55°C for additional 60 min. Finally, the reaction was heat-inactivated at 70°C for 15 min and then treated with 1 µl RNase H each at 37°C for 20 min. Then cDNA templates were serially dilute until only a fraction (~25%) of amplicons are PCR positive with the following PCR conditions: PCR amplification was carried out using the Platinum Taq (Invitrogen) with 1x buffer, 2mM MgCl₂, 0.2mM each deoxynucleoside triphosphate, 0.2 uM each primer, and 0.025 U/ul Platinum Taq polymerase. The primers for the first-round PCR were EnvB5out 5'-TAG AGC CCT GGA AGC ATC CAG GAAG-3' and EnvB3out 5'-TTG CTA CTT GTG ATT GCT CCA TGT-3'. The primers for the second-round PCR were EnvB5in 5'-CAC CTT AGG CAT CTC CTA TGG CAG GAA GAAG-3' and EnvB3in 5'-GTC TCG AGA TAC TGC TCC CAC CC-3'. The cycler parameters were 94°C for 2 min, followed by 35 cycles of 94°C for 15 sec, 55°C for 30 sec, and 68°C for 4 min, followed by a final extension of 68°C for 10 min. The product of the first-round PCR (1 µl) was subsequently used as a template in the second-round PCR under same conditions but with a total of 45 cycles. All PCR positive amplicons were directly sequenced using BigDye Terminator chemistry (Applied Biosystems). Any sequence with evidence of double peaks was excluded from further analysis.

Statistical analyses

Analyses of independent data were performed by two-tailed Mann–Whitney U-tests and Wilcoxon Rank Sum test. P values less than 0.05 were considered significant. Combination IC₈₀ titers were calculated using the Bliss-Hill model, which has been shown to provide accurate predictions of combination neutralization properties using those of individual bNAbs (23). Statistical analyses were performed using GraphPad Prism or the Stats module in Scipy (<http://www.scipy.org2015>).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Stephenson KE, D'Couto HT, Barouch DH. New concepts in HIV-1 vaccine development. *Current opinion in immunology*. 2016; 41:39–46. [PubMed: 27268856]
- Doria-Rose NA, Klein RM, Daniels MG, O'Dell S, Nason M, Lapedes A, et al. Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. *Journal of virology*. 2010; 84(3):1631–6. [PubMed: 19923174]
- Euler Z, van Gils MJ, Bunnik EM, Phung P, Schweighardt B, Wrin T, et al. Cross-reactive neutralizing humoral immunity does not protect from HIV type 1 disease progression. *The Journal of infectious diseases*. 2010; 201(7):1045–53. [PubMed: 20170371]
- Hraber P, Seaman MS, Bailer RT, Mascola JR, Montefiori DC, Korber BT. Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection. *Aids*. 2014; 28(2):163–9. [PubMed: 24361678]
- Walker LM, Huber M, Doores KJ, Falkowska E, Pejchal R, Julien JP, et al. Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature*. 2011; 477(7365):466–70. [PubMed: 21849977]
- Burton DR, Mascola JR. Antibody responses to envelope glycoproteins in HIV-1 infection. *Nature immunology*. 2015; 16(6):571–6. [PubMed: 25988889]
- Burton DR, Hangartner L. Broadly Neutralizing Antibodies to HIV and Their Role in Vaccine Design. *Annual review of immunology*. 2016; 34:635–59.
- Corti D, Lanzavecchia A. Broadly neutralizing antiviral antibodies. *Annual review of immunology*. 2013; 31:705–42.
- Moldt B, Rakasz EG, Schultz N, Chan-Hui PY, Swiderek K, Weisgrau KL, et al. Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109(46):18921–5. [PubMed: 23100539]
- Shingai M, Donau OK, Plishka RJ, Buckler-White A, Mascola JR, Nabel GJ, et al. Passive transfer of modest titers of potent and broadly neutralizing anti-HIV monoclonal antibodies block SHIV infection in macaques. *J Exp Med*. 2014; 211(10):2061–74. [PubMed: 25155019]

11. Pegu A, Yang ZY, Boyington JC, Wu L, Ko SY, Schmidt SD, et al. Neutralizing antibodies to HIV-1 envelope protect more effectively in vivo than those to the CD4 receptor. *Science translational medicine*. 2014; 6(243):243ra88.
12. Gautam R, Nishimura Y, Pegu A, Nason MC, Klein F, Gazumyan A, et al. A single injection of anti-HIV-1 antibodies protects against repeated SHIV challenges. *Nature*. 2016; 533(7601):105–9. [PubMed: 27120156]
13. Walker LM, Simek MD, Priddy F, Gach JS, Wagner D, Zwick MB, et al. A limited number of antibody specificities mediate broad and potent serum neutralization in selected HIV-1 infected individuals. *PLoS pathogens*. 2010; 6(8):e1001028. [PubMed: 20700449]
14. Georgiev IS, Doria-Rose NA, Zhou T, Kwon YD, Staupe RP, Moquin S, et al. Delineating antibody recognition in polyclonal sera from patterns of HIV-1 isolate neutralization. *Science*. 2013; 340(6133):751–6. [PubMed: 23661761]
15. Landais E, Huang X, Havenar-Daughton C, Murrell B, Price MA, Wickramasinghe L, et al. Broadly Neutralizing Antibody Responses in a Large Longitudinal Sub-Saharan HIV Primary Infection Cohort. *PLoS pathogens*. 2016; 12(1):e1005369. [PubMed: 26766578]
16. Pan R, Gorny MK, Zolla-Pazner S, Kong XP. The V1V2 Region of HIV-1 gp120 Forms a Five-Stranded Beta Barrel. *Journal of virology*. 2015; 89(15):8003–10. [PubMed: 26018158]
17. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *The New England journal of medicine*. 2012; 366(14):1275–86. [PubMed: 22475592]
18. Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, Goss JL, et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science*. 2009; 326(5950):285–9. [PubMed: 19729618]
19. Bonsignori M, Hwang KK, Chen X, Tsao CY, Morris L, Gray E, et al. Analysis of a clonal lineage of HIV-1 envelope V2/V3 conformational epitope-specific broadly neutralizing antibodies and their inferred unmutated common ancestors. *Journal of virology*. 2011; 85(19):9998–10009. [PubMed: 21795340]
20. Doria-Rose NA, Schramm CA, Gorman J, Moore PL, Bhiman JN, DeKosky BJ, et al. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. *Nature*. 2014; 509(7498):55–62. [PubMed: 24590074]
21. Doria-Rose NA, Bhiman JN, Roark RS, Schramm CA, Gorman J, Chuang GY, et al. New Member of the V1V2-Directed CAP256-VRC26 Lineage That Shows Increased Breadth and Exceptional Potency. *Journal of virology*. 2016; 90(1):76–91.
22. Sok D, van Gils MJ, Pauthner M, Julien JP, Saye-Francisco KL, Hsueh J, et al. Recombinant HIV envelope trimer selects for quaternary-dependent antibodies targeting the trimer apex. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111(49):17624–9. [PubMed: 25422458]
23. Wagh K, Bhattacharya T, Williamson C, Robles A, Bayne M, Garrity J, et al. Optimal Combinations of Broadly Neutralizing Antibodies for Prevention and Treatment of HIV-1 Clade C Infection. *PLoS pathogens*. 2016; 12(3):e1005520. [PubMed: 27028935]
24. Gray GE, Allen M, Moodie Z, Churchyard G, Bekker LG, Nchabeleng M, et al. Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. *The Lancet Infectious diseases*. 2011; 11(7):507–15. [PubMed: 21570355]
25. Harouse JM, Gettie A, Eshetu T, Tan RC, Bohm R, Blanchard J, et al. Mucosal transmission and induction of simian AIDS by CCR5-specific simian/human immunodeficiency virus SHIV(SF162P3). *Journal of virology*. 2001; 75(4):1990–5. [PubMed: 11160699]
26. Gautam R, Nishimura Y, Lee WR, Donau O, Buckler-White A, Shingai M, et al. Pathogenicity and mucosal transmissibility of the R5-tropic simian/human immunodeficiency virus SHIV(AD8) in rhesus macaques: implications for use in vaccine studies. *Journal of virology*. 2012; 86(16):8516–26. [PubMed: 22647691]
27. Ko SY, Pegu A, Rudicell RS, Yang ZY, Joyce MG, Chen X, et al. Enhanced neonatal Fc receptor function improves protection against primate SHIV infection. *Nature*. 2014; 514(7524):642–5. [PubMed: 25119033]

28. Moore PL, Sheward D, Nonyane M, Ranchobe N, Hermanus T, Gray ES, et al. Multiple pathways of escape from HIV broadly cross-neutralizing V2-dependent antibodies. *Journal of virology*. 2013; 87(9):4882–94. [PubMed: 23408621]
29. Bhiman JN, Anthony C, Doria-Rose NA, Karimanzira O, Schramm CA, Khoza T, et al. Viral variants that initiate and drive maturation of V1V2-directed HIV-1 broadly neutralizing antibodies. *Nature medicine*. 2015; 21(11):1332–6.
30. Moore PL, Gray ES, Sheward D, Madiga M, Ranchobe N, Lai Z, et al. Potent and broad neutralization of HIV-1 subtype C by plasma antibodies targeting a quaternary epitope including residues in the V2 loop. *Journal of virology*. 2011; 85(7):3128–41. [PubMed: 21270156]
31. Pegu A, Hessel AJ, Mascola JR, Haigwood NL. Use of broadly neutralizing antibodies for HIV-1 prevention. *Immunol Rev*. 2017; 275(1):296–312. [PubMed: 28133803]
32. Ledgerwood JE, Coates EE, Yamshchikov G, Saunders JG, Holman L, Enama ME, et al. Safety, pharmacokinetics and neutralization of the broadly neutralizing HIV-1 human monoclonal antibody VRC01 in healthy adults. *Clin Exp Immunol*. 2015; 182(3):289–301. [PubMed: 26332605]
33. Bar KJ, Sneller MC, Harrison LJ, Justement JS, Overton ET, Petrone ME, et al. Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption. *The New England journal of medicine*. 2016; 375(21):2037–50. [PubMed: 27959728]
34. Scheid JF, Horwitz JA, Bar-On Y, Kreider EF, Lu CL, Lorenzi JC, et al. HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. *Nature*. 2016; 535(7613):556–60. [PubMed: 27338952]
35. McCoy LE, Falkowska E, Doores KJ, Le K, Sok D, van Gils MJ, et al. Incomplete Neutralization and Deviation from Sigmoidal Neutralization Curves for HIV Broadly Neutralizing Monoclonal Antibodies. *PLoS pathogens*. 2015; 11(8):e1005110. [PubMed: 26267277]
36. Barouch DH, Whitney JB, Moldt B, Klein F, Oliveira TY, Liu J, et al. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. *Nature*. 2013; 503(7475):224–8. [PubMed: 24172905]
37. Mao Y, Wang L, Gu C, Herschhorn A, Xiang SH, Haim H, et al. Subunit organization of the membrane-bound HIV-1 envelope glycoprotein trimer. *Nature structural & molecular biology*. 2012; 19(9):893–9.
38. Rusert P, Krarup A, Magnus C, Brandenberg OF, Weber J, Ehlert AK, et al. Interaction of the gp120 V1V2 loop with a neighboring gp120 unit shields the HIV envelope trimer against cross-neutralizing antibodies. *The Journal of experimental medicine*. 2011; 208(7):1419–33. [PubMed: 21646396]
39. Chang HW, Tartaglia LJ, Whitney JB, Lim SY, Sanisetty S, Lavine CL, et al. Generation and evaluation of clade C simian-human immunodeficiency virus challenge stocks. *Journal of virology*. 2015; 89(4):1965–74. [PubMed: 25473043]
40. Tartaglia LJ, Chang HW, Lee BC, Abbink P, Ng'ang'a D, Boyd M, et al. Production of Mucosally Transmissible SHIV Challenge Stocks from HIV-1 Circulating Recombinant Form 01_AE env Sequences. *PLoS pathogens*. 2016; 12(2):e1005431. [PubMed: 26849216]
41. Nkolola JP, Peng H, Settembre EC, Freeman M, Grandpre LE, Devoy C, et al. Breadth of neutralizing antibodies elicited by stable, homogeneous clade A and clade C HIV-1 gp140 envelope trimers in guinea pigs. *Journal of virology*. 2010; 84(7):3270–9. [PubMed: 20053749]
42. Julien JP, Cupo A, Sok D, Stanfield RL, Lyumkis D, Deller MC, et al. Crystal structure of a soluble cleaved HIV-1 envelope trimer. *Science*. 2013; 342(6165):1477–83. [PubMed: 24179159]
43. Sanders RW, Derking R, Cupo A, Julien JP, Yasmeen A, de Val N, et al. A next-generation cleaved, soluble HIV-1 Env trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. *PLoS pathogens*. 2013; 9(9):e1003618. [PubMed: 24068931]
44. Shang H, Han X, Shi X, Zuo T, Goldin M, Chen D, et al. Genetic and neutralization sensitivity of diverse HIV-1 env clones from chronically infected patients in China. *The Journal of biological chemistry*. 2011; 286(16):14531–41. [PubMed: 21325278]

45. Pancera M, Zhou T, Druz A, Georgiev IS, Soto C, Gorman J, et al. Structure and immune recognition of trimeric pre-fusion HIV-1 Env. *Nature*. 2014; 514(7523):455–61. [PubMed: 25296255]
46. Li M, Gao F, Mascola JR, Stamatatos L, Polonis VR, Koutsoukos M, et al. Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. *Journal of virology*. 2005; 79(16):10108–25. [PubMed: 16051804]
47. Montefiori DC. Measuring HIV neutralization in a luciferase reporter gene assay. *Methods Mol Biol*. 2009; 485:395–405. [PubMed: 19020839]
48. Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105(21): 7552–7. [PubMed: 18490657]

Summary

Neutralizing antibodies to the HIV-1 envelope V2 apex protect macaques against SHIV challenge at unprecedented low serum concentrations, suggesting that these antibodies should be considered for HIV-1 prevention.

Figure 1. Highlighter amino acid sequence alignment of *env* derived from the SHIV-325c stock and the parental HIV-1 *env*
Amino acid substitutions that differ from the parental HIV-1 CA325 *env* sequence are indicated in color. Dashes indicate amino acid sequences identical to the parental sequence. A total of 25 sequences from SHIV-325c were generated by SGA.

Figure 2. Plasma viral loads and CD4+ T-cell counts in rhesus macaques challenged with SHIV-325c

(A) All animals were infected after challenge with SHIV-325c and 6 out of 8 animals established chronic infection with mean viral loads set-point of 3.9 log RNA copies/mL. The red line represents the mean log RNA copies/mL. (B) During acute infection mean CD4+ T cell numbers declined by 27%.

Figure 3. Neutralization profiles of PGDM1400 and CAP256-VRC26.25 against a multi-clade panels of 208 pseudoviruses (A) and highlighting specifically the activity of both bNAbS against clade C pseudoviruses (B)

(A) Each bar represents the IC_{80} ($\mu\text{g/mL}$) of PGDM1400 (upper graph) or CAP256-VRC26.25 (lower graph) against a single virus. Viruses are ranked according to increasing IC_{80} values. Bars reaching the dotted line represent IC_{80} values $>50 \mu\text{g/mL}$. The red bars highlight the IC_{80} values for PGDM1400 (upper graph) or CAP256-VRC26.25 (lower graph) against SHIV-325c. (B) IC_{80} values for PGDM1400 and CAP256-VRC26.25 against SHIV-325c (red dot) and against the clade C pseudoviruses included in panel (A). The horizontal bars represent the mean of IC_{80} values of neutralizable pseudoviruses.

Figure 4. Neutralization of SHIV-325c by CAP256-VRC26.25, PGDM1400 and PG9
Neutralization was measured using replication competent challenge stock SHIV-325c infection of TZM-bl cells. All 3 antibodies showed 100% neutralization of SHIV-325c with no evidence of a plateau effect below 100% neutralization.

Figure 5. Protective efficacy of PGDM1400 and CAP256-VRC26.25-LS against SHIV-325c in rhesus macaques

Plasma viral RNA (log RNA copies/mL) are shown for animals that received placebo control (A), 2 mg/kg PGDM1400 (B), 0.4 mg/kg PGDM1400 (C), 0.08 mg/kg PGDM1400 (D), 2 mg/kg CAP256-VRC26.25-LS (E), 0.4 mg/kg CAP256-VRC26.25-LS (F), or 0.08 mg/kg CAP256-VRC26.25-LS (G). All placebo controls became infected. Protection was observed in all animals that received CAP256-VRC26.25-LS at all doses. 1/5 animals in the PGDM1400 2 mg/kg dose group and 3/4 animals in the 0.08 mg/kg PGDM1400 dose group were infected. The assay sensitivity limit was >50 RNA copies/mL.

Figure 6. Serum concentration of CAP256-VRC26.25-LS and PGDM1400 in antibody treated animals

bNAb concentrations were determined by ELISA. The results show average serum concentrations of 19.8 µg/ml, 3.3 µg/ml and 0.75 µg/mL on the day of challenge in the CAP256-VRC26.25-LS 2 mg/kg, 0.4 mg/kg and 0.08 mg/kg groups, respectively, and 6.9 µg/mL, 2.5 µg/mL and 0.22 µg/mL on the day of challenge in the PGDM1400 2 mg/kg, 0.4 mg/kg and 0.08 mg/kg groups, respectively.

Single genome envelope amplification was performed with plasma viral RNA on day 28. For one placebo control animal, H515, viral amplification failed and therefore is not shown here. The *env* sequence at the top presents the SHIV-325c molecular clone inoculum. The column on the right reports the frequency of distinct amplicons (based on unique sequence variations in V1/V2) per total *env* amplicons obtained from a given animal. Most sequence variation is observed in residues 165–175 in the V2 region. Mutations at position 167 are associated with escape to CAP256 V2-lineage antibodies and but are present in the challenge stock, PGDM1400 and placebo treated animals. Interestingly, animal M97, which developed infection in the highest PGDM1400 dose group had a fixed F210L mutation in all 27 amplicons (marked with ●), that was not present in the challenge molecular clone or developed in any other animal.

Figure 8. Potency and breadth profiles of single and combination bNAbs against 200 clade C HIV-1 Env pseudoviruses

(A) Heatmaps of IC_{80} values for single bNAbs and bNAb combinations respectively. Rows represent Env pseudoviruses, and columns represent single and combination bNAbs. Darker hues of red indicate more potent neutralization and grey cells indicate IC_{80} above threshold. (B) Potency-breadth curves for single bNAbs and bNAb combinations are shown. IC_{80} scores for combinations and single bNAbs were compared using Wilcoxon rank sum test. Antibody a, PGDM1400; Antibody b, CAP256-VRC26.25; Antibody c, PGT121.

Table 1
Neutralization profiles of PGDM1400, CAP256-VRC26.25 and PG9 against SHIV challenge stocks

IC₅₀ and IC₈₀ values are in µg/mL. Values between 0.001–0.01 µg/mL are highlighted in red, values between 0.01–0.1 µg/mL are highlighted in orange, values between 0.1–1 µg/mL are highlighted in yellow, values between 1–10 µg/mL are highlighted in light green, and values between 10–50 µg/mL are highlighted in dark green. Virus suffix indicates PBMC cell type used for production: .Rh, rhesus PBMCs; .Hu, human PBMCs.

Virus	Env	clade	PGDM1400				CAP256-VRC26.25				PG9	
			IC50	IC80	IC50	IC80	IC50	IC80	IC50	IC80	IC50	IC80
SF162P3.Rh	B		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
BaLP4.Rh	B		>50	>50	>50	>50	>50	>50	>50	>50	0.552	>50
327c.Rh	C		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
327c.Hu	C		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
325c.Hu	C		0.037	0.104	0.003	0.006	0.976	2.33				
AD8EO.Rh	B		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
1157ipd3N4.Rh	C		0.197	12.4	>50	>50	>50	>50	0.527	>50	>50	>50

Table 2

Neutralization properties of three different SHIV viruses in TZM-bl assays against a panel of bNAbs targeting distinct epitopes

IC₅₀ and IC₈₀ values are in µg/mL. Values between 0.001–0.01 µg/mL are highlighted in red, values between 0.01–0.1 µg/mL are highlighted in orange, values between 0.1–1 µg/mL are highlighted in yellow, values between 1–10 µg/mL are highlighted in light green, and values between 10–50 µg/mL are highlighted in dark green.

Epitope region	Antibody	SHIV 325c		SHIV SF162P3		SHIV BaLP4	
		IC ₅₀	IC ₈₀	IC ₅₀	IC ₈₀	IC ₅₀	IC ₈₀
CD4 binding site, VRC01 class	VRC01	>50	>50	2.43	6.75	0.067	0.208
	3BNC117	>50	>50	>50	>50	0.027	0.058
	VRC07-523	7.53	21.4	0.284	0.629	0.015	0.039
	N6	11.3	26.7	0.421	0.800	0.023	0.051
CD4 binding site, other	F105	>50	>50	>50	>50	11.1	>50
	CH103	>50	>50	4.68	12.7	1.57	>50
	CD4-Ig	>50	>50	3.69	11.8	0.055	0.118
V1/V2	PG9	0.976	2.33	>50	>50	0.552	>50
	CAP256-VRC26.25	0.003	0.006	>50	>50	>50	>50
	PGDM1400	0.037	0.104	>50	>50	>50	>50
Glycan V3	PGT128	>50	>50	0.082	0.197	0.488	>50
MPER	2F5	>50	>50	>50	>50	14.2	>50
	10E8	29.2	>50	9.23	35.6	0.475	2.39
CD4i	17b	>50	>50	>50	>50	>50	>50

Table 3
Neutralization of JR-CSF alanine variants by PGDM1400 or PG9

A panel of HIV_{JRCSF} Env pseudovirus alanine scanning mutants was developed to examine the neutralization potency of PGDM1400 compared with PG9. A single mutation in V2 of JR-CSF, such as D167A resulted in resistance to neutralization by PGDM1400 but not PG9. In contrast, single mutations in 165 (I/A), a site previously associated with escape for CAP256 antibodies (30) showed no resistance to neutralization by either antibody.

JR-CSF	IC ₅₀ (µg/mL)	
	PGDM1400	PG9
WT	0.003	0.003
V127A	0.009	0.005
N134A	0.032	0.001
N160K	>50	>50
T162A	>50	>50
I165A	0.003	0.001
R166A	0.073	0.004
D167A	>50	0.001
K168A	0.002	0.002
E172A	0.001	0.001
Y173A	0.002	0.002
Y177A	0.019	0.006
L179A	0.002	0.001