Published in final edited form as:

Nature. 2011 September 22; 477(7365): 466–470. doi:10.1038/nature10373.

Broad neutralization coverage of HIV by multiple highly potent antibodies

Laura M. Walker[1,*], **Michael Huber**[1,7,*], **Katie J. Doores**[1,7,*], **Emilia Falkowska**[1,7], **Robert Pejchal**[2], **Jean-Philippe Julien**[2], **Sheng-Kai Wang**[6], **Alejandra Ramos**[1], **Po-Ying Chan-Hui**[3], **Matthew Moyle**[3], **Jennifer L. Mitcham**[3], **Phillip W. Hammond**[3], **Ole A. Olsen**[3], **Pham Phung**[4], **Steven Fling**[5], **Chi-Huey Wong**[6], **Sanjay Phogat**[5], **Terri Wrin**[4], **Melissa D. Simek**[5], **Protocol G Principal Investigators**[†], **Wayne C. Koff**[5], **Ian A. Wilson**[2], **Dennis R. Burton**[1,7], and **Pascal Poignard**[1,5]

[1]Department of Immunology and Microbial Science and IAVI Neutralizing Antibody Center, The Scripps Research Institute, La Jolla, California 92037, USA

[2]Department of Molecular Biology, Skaggs Institute for Chemical Biology, and IAVI Neutralizing Antibody Center, The Scripps Research Institute, La Jolla, California 92037, USA

[3]Theraclone Sciences, Inc., Seattle, Washington 98104, USA

[4]Monogram Biosciences, Inc., South San Francisco, California 94080, USA

[5]International AIDS Vaccine Initiative, New York, New York 10038, USA

[6]Department of Chemistry, The Scripps Research Institute, La Jolla, California 92037, USA

[7]Ragon Institute of MGH, MIT, and Harvard, Cambridge, Massachusetts 02129, USA

Abstract

Broadly neutralizing antibodies (bnAbs) against highly variable viral pathogens are much sought-after to treat or protect against global circulating viruses. We have probed the neutralizing antibody repertoires of four HIV-infected donors with remarkably broad and potent neutralizing responses and rescued 17 new monoclonal antibodies (MAbs) that neutralize broadly across clades. Many of the new MAbs are almost 10-fold more potent than the recently described PG9, PG16, and VRC01 bnMAbs and 100-fold more potent than the original prototype HIV

Correspondence and requests for materials should be addressed to D.R.B. (burton@scripps.edu) or P.P. (poignard@scripps.edu).

*These authors contributed equally to this work.

†individuals and affiliations are listed in the supporting online material text as notes.

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Author Contributions Project planning was performed by L.M.W., M.H., K.J.D., E.F., P.P., P-Y.C-H., R.P., J-P. J., T.W., S.K.P, W.K., I.A.W. S.F., and D.R.B.; experimental work by L.M.W., A.R., M.H, K.J.D., E.F., R.P., J-P. J., P-Y.C-H., S-K.W., P.W.H., O.A.O., and T.W.; data analysis by M.H, L.M.W., K.J.D., E.F., P.P., T.W., P-Y.C-H., and D.R.B.; and manuscript composition and revision by L.M.W, M.H., K.J.D., R.P., J-P. J., E.F., W.K., I.A.W, D.R.B, and P.P.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Gene sequences of the reported antibodies and the primers used for antibody isolation have been deposited under GenBank accession numbers JN201894-JN201927. The authors declare competing financial interests: details accompany the paper on www.nature.com/nature. Readers are welcome to comment on the online version of this article at www.nature.com/nature.

bnMAbs[1–3]. The MAbs largely recapitulate the neutralization breadth found in the corresponding donor serum and many recognize novel epitopes on envelope (Env) glycoprotein gp120, illuminating new targets for vaccine design. Analysis of neutralization by the full complement of anti-HIV bnMAbs now available reveals that certain combinations of antibodies provide significantly more favorable coverage of the enormous diversity of global circulating viruses than others and these combinations might be sought in active or passive immunization regimes. Overall, the isolation of multiple HIV bnMAbs, from several donors, that, in aggregate, provide broad coverage at low concentrations is a highly positive indicator for the eventual design of an effective antibody-based HIV vaccine.

Most successful anti-viral vaccines elicit neutralizing antibodies as a correlate of protection[4,5]. For highly variable viruses, such as HIV, HCV and, to a lesser extent, influenza, vaccine design efforts have been hampered by the difficulties associated with eliciting neutralizing antibodies that are effective against the enormous diversity of global circulating isolates (i.e. bnAbs)[6,7]. However, for HIV for example, 10–30% of infected individuals do, in fact, develop broadly neutralizing sera, and protective bnMAbs have been isolated from infected donors[1,2,8–12]. It has been suggested that, given the appropriate immunogen, it should be possible to elicit these types of responses by vaccination[13] and understanding the properties of bnMAbs has become a major thrust in research on highly variable viruses.

We have previously screened sera from approximately 1,800 HIV infected donors for neutralization breadth and potency, designating the top 1% as “elite neutralizers”, based on a score incorporating both breadth and potency[14]. In this study, we set out to isolate bnMAbs from the top four elite neutralizers (Supplementary Table 1) by screening antibody-containing memory B cell supernatants for broad neutralizing activity using a recently described high-throughput functional approach[2]. Antibody variable genes were rescued from B cell cultures that displayed cross-clade neutralizing activity and expressed as full-length IgGs. Analysis of the sequences revealed that all of the MAbs isolated from each individual donor belong to a distant, but clonally related cluster of antibodies (Supplementary Table 2). Since it has been proposed that antibodies from HIV infected patients are often polyreactive[15,16], we tested the new MAbs for binding to a panel of antigens and showed that they were not polyreactive (Supplementary Fig. 2).

The potency and breadth of the MAbs were next assessed on a 162-pseudovirus panel representing all major circulating HIV subtypes (Fig. 1, Supplementary Tables 3 and 4)[2]. All of the MAbs exhibited cross-clade neutralizing activity, but more strikingly, several displayed exceptional potency. The median IC_{50}s and IC_{90}s of PGT MAbs 121-123 and 125-128 were almost 10-fold lower (i.e. more potent) than the recently described PG9, PG16, VRC01, and PGV04 bnMAbs[1,2] (Falkowska et al., manuscript in preparation), and approximately 100-fold lower than other bnMAbs described earlier (Fig. 1). At concentrations less than 0.1 μg/ml, these MAbs still neutralized 27% to 50% of viruses in the panel (Fig. 1 and Fig. 4a). Although PGT MAbs 135, 136, and 137 displayed lesser neutralization breadth than the other MAbs, they all still potently neutralized over 30% of the clade C viruses on the panel (Supplementary Fig. 2 and Supplementary Table 3b). This

result is significant considering that HIV clade C predominates in sub-Saharan Africa and accounts for more than 50% of all HIV infections worldwide.

Interestingly, many of the clonally related MAbs exhibited differing degrees of overall neutralization potency. For example, the median IC_{50}s of PGT MAbs 131, 136, 137, and 144 were approximately 10- to 50-fold higher than those of their somatically related sister clones (Fig. 1). Also, in some cases, the somatically related MAbs exhibited similar neutralization potency, but differing degrees of neutralization breadth, against the panel of viruses tested (Fig. 1 and Supplementary Tables 3 and 4). For example, PGT 128 neutralized with comparable overall potency but significantly greater neutralization breadth than the clonally related PGT 125, 126, and 127 MAbs (Fig. 1 and Supplementary Tables 3 and 4). Overall, these observations suggest that serum neutralization breadth may develop from the successive selection of somatic variants that bind to a modified epitope or a slightly different envelope (Env) conformation expressed on virus escape variants. Comparison of the neutralization profiles of the MAbs isolated from a given donor with that from the corresponding serum revealed that the isolated MAbs could largely recapitulate the serum neutralization breadth and potency (Fig. 2 and Supplementary Fig. 3).

We next sought to gain information on the epitopes recognized by the newly isolated bnMAbs. ELISA binding assays indicated that PGT MAbs 121-123, 125-128, 130, 131, and 135-137, bound to monomeric gp120 (Supplementary Table 5). In contrast, the PGT 141-145 bnMAbs exhibited a strong preference for membrane-bound, trimeric HIV Env (Supplementary Fig. 4). Based on this result, we postulated that these bnMAbs bound to quaternary epitopes similar to those of the recently described PG9 and PG16 bnMAbs[2]. Indeed, this hypothesis was confirmed by competition studies, N160K sensitivity, and, for PGT MAbs 141-144, an inability to neutralize JR-CSF pseudoviruses expressing homogenous $Man_9GlcNAc_2$ glycans[17] (Supplementary Fig. 5).

To define the epitopes recognized by the remaining PGT antibodies, competition ELISA assays were carried out with a panel of well-characterized neutralizing and non-neutralizing antibodies (Fig. 3a). Unexpectedly, all of the remaining antibodies (PGT MAbs 121-123, 125-128, 130, 131, and 135-137) competed with the glycan-specific bnMAb 2G12. This result was surprising given that 2G12 had previously formed its own unique competition group. All of the MAbs, except for PGT MAbs 135, 136 and 137, also competed with a V3 loop-specific mAb and failed to bind to gp120 ΔV3, suggesting their epitopes were in proximity to or contiguous with V3 (Fig. 3a and Supplementary Table 5). Deglycosylation of gp120 with Endo H abolished binding by all the MAbs, indicating that certain oligomannose glycans were important for epitope recognition (Supplementary Table 5). Competition of these MAbs with 2G12 and lack of binding to deglycosylated gp120 prompted us to investigate whether these antibodies contacted glycans directly. Glycan array analysis revealed that PGT MAbs 125-128 and 130 bound specifically to both $Man_8GlcNAc_2$ and $Man_9GlcNAc_2$, whereas the remaining antibodies showed no detectable binding to high-mannose glycans (Fig. 3b). Interestingly, binding of PGT MAbs 125-128 and 130 to gp120 was competed by Man_9, but, unlike 2G12, was not competed by monomeric mannose or Man_4 (D1 arm of $Man_9GlcNAc_2$) (Fig. 3c,d), suggesting a different mode of glycan recognition. Furthermore, in contrast to 2G12, no evidence was found for

domain exchange and monomeric Fab fragments still exhibited potent neutralizing activity (Supplementary Fig. 7 and data not shown).

To further define the epitopes recognized by the MAbs, neutralizing activity against a large panel of HIV-1_{JR-CSF} variants incorporating single alanine substitutions was assessed using a single round of replication pseudovirus assay (Supplementary Table 6). In the panel of mutants, the N-linked glycans at positions 332 and/or 301 were important for neutralization by PGT MAbs 125-128, 130, and 131 suggesting their direct involvement in epitope formation. The apparent dependency on so few glycans suggests that, although these PGT MAbs contact $Man_{8-9}GlcNAc_2$ glycans directly, their arrangement in the context of gp120 is critical for high affinity glycan recognition and neutralization potency. This is further highlighted by the inability of the PGT MAbs to neutralize SIVmac239, HIV-2 or HCV, which display a high level of glycosylation (data not shown). Interestingly, although PGT MAbs 121-123 failed to exhibit detectable binding to high-mannose glycans and be competed by mannose sugars (Supplementary Fig. 6), the only substitutions that completely abolished neutralization by these MAbs were those that resulted in removal of the glycan at position 332. Although structural studies will be required to fully define the epitopes recognized by these antibodies, the above results suggest either that the PGT MAbs 121-123 bind to a protein epitope along the gp120 polypeptide backbone that is conformationally dependent on the N332 glycan or that the glycan contributes more strongly to binding in the context of the intact protein.

Vaccines against pathogens with low antigenic diversity, such as hepatitis B virus or measles, commonly achieve 90–95 % efficacy[18]. Similarly, the influenza vaccine achieves 85–90% efficacy in years when the vaccine and circulating seasonal strain are well-matched[19,20]. However, efficacy drops severely in years when there is a mismatch between the vaccine and circulating strain. In the case of HIV, the global diversity of circulating viruses is such that the match between the prophylactic antibodies and the circulating viruses, i.e. the antibody viral coverage, will be crucial for the degree of efficacy of active or passive prophylaxis approaches. To date, although the recent RV144 trial has led to speculation that some degree of protection against HIV may be achieved through extra-neutralizing activities of antibodies, such as antibody-dependent cell-mediated cytotoxicity or phagocytosis, the strongest evidence for protection is for neutralizing antibodies in non-human primate models using simian-human immunodeficiency virus (SHIV) challenge[21–25]. Passive administration of neutralizing antibodies in these models suggests that a serum antibody concentration of approximately or greater than 100 times the in vitro pseudovirus assay IC_{50} is required to achieve a meaningful level of protection[21–25]. Therefore, if a vaccine elicits a serum bNAb concentration on the order of 10 μg/ml[26] and if an IC_{50}: protective serum concentration ratio of 1:100 is assumed, then protection would be only achieved against viruses for which the bNAb IC_{50} is lower than 0.1 μg/ml. As a second more conservative scenario, for an IC_{50}: protective serum concentration ratio of 1:500, protection would be achieved against viruses for which the bNAb IC_{50} is lower than 0.02 μg/ml. As shown in Figure 4, although various bnMAbs display breadth at high concentrations, viral coverage often drops sharply at lower concentrations. Therefore, if elicited or delivered singly, only the most potent Abs, such as 121 and 128, would be able to achieve a meaningful level of viral coverage, in particular at concentrations corresponding to the more

conservative scenario given above. As bnMAbs display different and in some cases complementary breadth, we further looked at the coverage achieved by antibody combinations. For the two IC_{50}: protective serum concentration ratios above, a combination of PGV04 and VRC01, the two most potent CD4bs bnMAbs, would provide protection against 50% and 3% of viruses, respectively (Fig. 4b). In contrast, for a vaccine eliciting antibodies with high potency and favorable non-overlapping breadth, such as 128 and 145, coverage would be achieved against 70% and 40% of viruses for the two scenarios (Fig 4c). Several combinations of two bnMAbs, including those directed to overlapping epitopes, can yield this degree of coverage (Supplementary Fig. 8). In addition, a combination of all of the bnMAbs would cover 89% and 62% of viruses, correspondingly. Coverage against such a large proportion of viruses would likely have an important impact on the pandemic.

In summary, an effective vaccine against HIV will likely require the elicitation of a combination of complementary potent neutralizing antibodies. The demonstration that large numbers of potent and diverse bnMAbs can be isolated from several different individuals provides grounds for renewed optimism that an antibody-based vaccine may be achievable.

Methods Summary

Activated memory B cell supernatants were screened in a high throughput format for neutralization activity using a micro-neutralization assay, as described[2]. Heavy and light chain variable regions were isolated from B cell lysates of selected neutralizing hits by reverse transcription from RNA followed by multiplex PCR amplification using family-specific V-gene primer sets. For some antibodies, traditional cloning methods were used for antibody isolation, as described[2]. For other antibodies, amplicons from each lysate were uniquely tagged with multiplex identifier (MID) sequences and 454 sequencing regions (Roche). Single round of replication pseudovirus neutralization assays and cell surface binding assays were performed as described previously[2,27,28]. Glycan reactivities were profiled on a printed glycan microarray (version 5.0 from the Consortium for Functional Glycomics (CFG)) as described previously[29].

Methods

Antibodies and Antigens

The following antibodies and reagents were procured by the IAVI Neutralizing Antibody Consortium: antibody 2G12 (Polymun Scientific, Vienna, Austria), antibody F425/b4E8 (provided by Lisa Cavacini, Beth Israel Deaconess Medical Center, Boston, MA), soluble CD4 (Progenics, Tarrytown, NY), HxB2 gp120, SF162 gp120, BaL gp120, JR-FL gp120, JR-CSF gp120 and YU2 gp120 (provided by Guillaume Stewart-Jones, Oxford University). Purified ADA gp120 was produced in the laboratory of Robert Doms, University of Pennsylvania. Fab X5 was expressed in *E.coli* and purified using an anti-human Fab specific affinity column. Deglycosylated gp120 JRFL was expressed in HEK 293S $GnTI^{-/-}$ cells and treated with Endo H (Roche).

Donors

The donors identified for this study were selected from the IAVI sponsored study, Protocol G[14]. Eligibility for enrolment into Protocol G was defined as: male or female at least 18 years of age with documented HIV infection for a least three years, clinically asymptomatic at the time of enrolment, and not currently receiving antiretroviral therapy. Selection of individuals for monoclonal antibody generation was based on a rank-order high throughput screening and analytical algorithm[14]. Volunteers were identified as elite neutralizers based on broad and potent neutralizing activity against a cross-clade pseudovirus panel[14].

Isolation of MAbs

The method for isolating human MAbs from memory B cells in circulation has previously been described[2]. Surface IgG^+ B cells seeded at near clonal density in 384-well microplates were activated in short-term culture. Supernatants were screened for neutralization activity against 2-4 pseudotyped viruses for which neutralization activity was detected at high titers in the donor serum. Heavy and light chain variable regions were isolated from B cell lysates of selected neutralizing hits by reverse transcription from RNA followed by multiplex PCR amplification using family-specific V-gene primer sets. Amplicons from each lysate were uniquely tagged with multiplex identifier (MID) sequences and 454 sequencing regions (Roche, Indianapolis, IN). A normalized pooling of gamma, kappa and lambda chains was performed based on agarose gel image quantitation and the pool was analysed by 454 TitaniumR sequencing. Consensus sequences of the V_H and V_L chains were generated using the Amplicon Variant Analyzer (Roche) and assigned to specific B cell culture wells by decoding the MID tags. Selected V_H and V_L chains were synthesized and cloned in expression vectors with the appropriate IgG1, Igκ or Igλ constant domain. Monoclonal antibodies were reconstituted by transient transfection in HEK293 cells followed by purification from serum-free culture supernatants.

PGT antibody expression and purification

Antibody genes were cloned into an expression vector and transiently expressed with the FreeStyle 293 Expression System (Invitrogen, Carlsbad, CA). Antibodies were purified using affinity chromatography (Protein A Sepharose Fast Flow, GE Healthcare, UK) and purity and integrity checked with SDS-PAGE.

Neutralization assays

Neutralization by monoclonal antibodies and donor sera was performed by Monogram Biosciences using a single round of replication pseudovirus assay as previously described[31]. Briefly, pseudoviruses capable of a single round of infection were produced by co-transfection of HEK293 cells with a subgenomic plasmid, pHIV-1lucu3, that incorporates a firefly luciferase indicator gene and a second plasmid, pCXAS that expressed HIV-1 Env libraries or clones. Following transfection, pseudoviruses were harvested and used to infect U87 cell lines expressing co-receptors CCR5 or CXCR4. Pseudovirus neutralization assays using $HIV\text{-}1_{JR\text{-}CSF}$ alanine mutants are fully described elsewhere[2]. Neutralization activity of MAbs against $HIV\text{-}1_{JR\text{-}CSF}$ alanine mutants was measured using a TZM-BL assay, as described[2]. Kifunensine-treated pseudoviruses were produced by treating 293T cells with 25

μM kifunensine on the day of transfection. Memory B cell supernatants were screened in a micro-neutralization assay against a cross-clade panel of HIV-1 isolates and SIV_{mac239} (negative control). This assay was based on the 96- well pseudotyped HIV-1 neutralization assay (Monogram Biosciences) and was modified for screening 15 μl of B cell culture supernatants in a 384-well format.

Cell surface binding assays

Titrating amounts of antibodies were added to HIV-1 Env transfected 293T cells, incubated for 1 hr at 37°C, washed with FACS buffer, and stained with goat anti- human IgG $F(ab')_2$ conjugated to phycoerythin (Jackson ImmunoResearch, West Grove, PA). Binding was analyzed using flow cytometry, and binding curves were generated by plotting the mean fluorescence intensity of antigen binding as a function of antibody concentration. For competition assays, titrating amounts of competitor antibodies were added to the cells 30 min prior to adding biotinylated PGT MAbs at a concentration required to give EC_{50}.

ELISA assays

For antigen-binding ELISAs, serial dilutions of MAbs were added to antigen-coated wells and binding was probed with alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG) $F(ab')_2$ Ab (Pierce, Rockford, IL). For competition ELISAs, titrating amounts of competitor MAbs were added to gp120-coated ELISA wells and incubated for 30 min prior to adding biotinylated PGT MAbs at a concentration required to give IC_{70}. Biotinylated PGT MAbs were detected using alkaline phosphatase conjugated streptavidin (Pierce) and visualized using p-nitrophenol phosphate substrate (Sigma, St. Louis, MO).

Glycan microarray analysis

Monoclonal antibodies were screened on a printed glycan microarray version 5.0 from the Consortium for Functional Glycomics (CFG) as described previously[29]. Antibodies were used at a concentration of 30 μg/ml and were precomplexed with 15 μg/ml secondary antibody (goat-anti-human-Fc-rPE, Jackson Immunoresearch) before addition to the slide. Complete glycan array data sets for all antibodies may be found at www.functionalglycomics.org in the CFG data archive under “cfg_rRequest_2250”.

Oligomannose Dendron synthesis

The oligomannose dendrons (Man_4D and Man_9D) were synthesized by Cu(I) catalyzed alkyne-azide cycloaddition between azido oligomannose and the second generation of AB_3 type alkynyl dendron. Detailed procedures and characterization were previously reported[30].

Fabrication of gp120 microarray

NHS-activated glass slides (Nexterion slide H, Schott North American) were printed with robotic pin (Arrayit 946) to deposit gp120 JRFL at concentrations of 750 or 250 μg/ml in printing buffer (120 mM phosphate, pH 8.5; containing 5% glycerol and 0.01 % Tween 20). 12 replicates were used for each concentration. The printed slides were incubated in relative humidity 75% chamber overnight and treated with blocking solution (superblock blocking

buffer in PBS, Thermo) at room temperature for 1 h. The slides were then rinsed with PBS-T (0.05% Tween 20) and PBS buffer, and centrifuged at 200 *g* to remove residual solution from slide surface.

Oligomannose dendron-gp120 competition assay with MAbs

Serial diluted oligomannose dendrons were mixed with MAb (40 μg/ml) in PBS-BT buffer (1% BSA and 0.05 % Tween 20 in PBS). The mixtures were applied directly to each sub-array on slide. After incubation in a humidified chamber for 1 h at RT, the slides were rinsed sequentially with PBS-T and PBS buffer, and then centrifuged at 200 *g*. Each sub-array was then stained with Cy3 labeled goat anti-human Fc IgG (7.5 μg/ml in PBSBT) for 1 h in a humidified chamber. The slides were then rinsed sequentially with PBS-T and demonized water and centrifuged at 200 *g*. The fluorescence of the final arrays was imaged at 10 μm resolution (Ex: 540 nm; Em: 595 nm) with an ArrayWorx microarray reader (Applied Precision).

Sequence analysis

Germ line genes were predicted using the immunoglobulin sequence alignment tools IMGT/V-QUEST[32] and SoDA2[33]. Clonally related sequences were identified by common germ line V-genes and long stretches of identical N-nucleotides.

Statistics

Statistical analyses were done with Prism 5.0 for Mac (GraphPad, La Jolla, CA). Viruses that are not neutralized at an IC_{50} or IC_{90} < 50 μg/ml were given a value of 50 μg/ml for median calculations. For combinations of antibodies, a virus was counted as covered if at least one of the MAbs was neutralized depending on individual concentrations (IC_{50}). This approach does not take additivity into account and therefore underestimates the neutralization potency of antibody combinations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank N. Schultz, K. Le, and V. Thaney for technical assistance at TSRI. We also thank C. Corbaci, S. M. Eagol and C. Williams for assistance with Figure 3. We would also like to thank the research staff members of the Theraclone I-STAR Discovery and Antibody Purification Team, and K. Limoli, S. Jauregui, K. Tran, P. Sitou, J. Perea, B. Huang, and P. Guinto at Monogram BioSciences. We thank R. McBride for assistance with the glycan array and are grateful to W. Olson for providing purified KNH1144 SOSIP trimer, R. Wyatt for providing the YU2 Foldon trimer, and J. Mascola for providing MAb VRC01. In addition, we would like to thank all of the IAVI Protocol G project, clinical and site team members and all of the Protocol G clinical investigators, specifically, G. Miiro, A. Pozniak, D. McPhee, O. Manigart, E. Karita, A. Inwoley, W. Jaoko, J. DeHovitz, L.-G. Bekker, P. Pitisuttithum, R. Paris, J. Serwanga, and S. Allen. This work was supported by the International AIDS Vaccine Initiative (IAVI), NIAID, the Ragon Institute, and the United States for International Development (USAID). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government.

References

1. Wu X, et al. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science. 2010; 329:856–861. [PubMed: 20616233]
2. Walker LM, et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science. 2009; 326:285–289. [PubMed: 19729618]
3. Binley JM, et al. Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies. J Virol. 2004; 78:13232–13252. [PubMed: 15542675]
4. Amanna IJ, Messaoudi I, Slifka MK. Protective immunity following vaccination: how is it defined? Hum Vaccin. 2008; 4:316–319. [PubMed: 18398296]
5. Plotkin SA. Immunologic correlates of protection induced by vaccination. Pediatr Infect Dis J. 2001; 20:63–75. [PubMed: 11176570]
6. Barouch DH. Challenges in the development of an HIV-1 vaccine. Nature. 2008; 455:613–619. [PubMed: 18833271]
7. Karlsson Hedestam GB, et al. The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus. Nat Rev Microbiol. 2008; 6:143–155. [PubMed: 18197170]
8. Stamatatos L, Morris L, Burton DR, Mascola JR. Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? Nat Med. 2009; 15:866–870. [PubMed: 19525964]
9. Trkola A, et al. Cross-clade neutralization of primary isolates of human immunodeficiency virus type 1 by human monoclonal antibodies and tetrameric CD4-IgG. J Virol. 1995; 69:6609–6617. [PubMed: 7474069]
10. Stiegler G, et al. A potent cross-clade neutralizing human monoclonal antibody against a novel epitope on gp41 of human immunodeficiency virus type 1. AIDS Res Hum Retroviruses. 2001; 17:1757–1765. [PubMed: 11788027]
11. Burton DR, et al. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. Science. 1994; 266:1024–1027. [PubMed: 7973652]
12. Kwong PD, Wilson IA. HIV-1 and influenza antibodies: seeing antigens in new ways. Nat Immunol. 2009; 10:573–578. [PubMed: 19448659]
13. Schief WR, Ban YE, Stamatatos L. Challenges for structure-based HIV vaccine design. Curr Opin HIV AIDS. 2009; 4:431–440. [PubMed: 20048708]
14. Simek MD, et al. Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. J Virol. 2009; 83:7337–7348. [PubMed: 19439467]
15. Haynes BF, et al. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science. 2005; 308:1906–1908. [PubMed: 15860590]
16. Mouquet H, et al. Polyreactivity increases the apparent affinity of anti-HIV antibodies by heteroligation. Nature. 2010; 467:591–595. [PubMed: 20882016]
17. Walker LM, et al. A limited number of antibody specificities mediate broad and potent serum neutralization in selected HIV-1 infected individuals. PLoS Pathog. 2010; 6
18. Plotkin. Vaccines. Elsevier Health Sciences; Philadelphia: 2008.
19. Bridges CB, et al. Effectiveness and cost-benefit of influenza vaccination of healthy working adults: A randomized controlled trial. JAMA. 2000; 284:1655–1663. [PubMed: 11015795]
20. Herrera GA, et al. Influenza vaccine effectiveness among 50–64-year-old persons during a season of poor antigenic match between vaccine and circulating influenza virus strains: Colorado, United States, 2003–2004. Vaccine. 2007; 25:154–160. [PubMed: 17064823]
21. Parren PW, et al. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. J Virol. 2001; 75:8340–8347. [PubMed: 11483779]
22. Nishimura Y, et al. Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. J Virol. 2002; 76:2123–2130. [PubMed: 11836389]

23. Hessell AJ, et al. Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. Nat Med. 2009; 15:951–954. [PubMed: 19525965]
24. Hessell AJ, et al. Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. PLoS Pathog. 2009; 5:e1000433. [PubMed: 19436712]
25. Willey R, Nason MC, Nishimura Y, Follmann DA, Martin MA. Neutralizing antibody titers conferring protection to macaques from a simian/human immunodeficiency virus challenge using the TZM-bl assay. AIDS Res Hum Retroviruses. 2010; 26:89–98. [PubMed: 20059398]
26. Martell BA, et al. Cocaine vaccine for the treatment of cocaine dependence in methadone-maintained patients: a randomized, double-blind, placebo-controlled efficacy trial. Arch Gen Psychiatry. 2009; 66:1116–1123. [PubMed: 19805702]
27. Pantophlet R, et al. Fine mapping of the interaction of neutralizing and nonneutralizing monoclonal antibodies with the CD4 binding site of human immunodeficiency virus type 1 gp120. J Virol. 2003; 77:642–658. [PubMed: 12477867]
28. Li 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. J Virol. 2005; 79:10108–10125. [PubMed: 16051804]
29. Blixt O, et al. Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. Proc Natl Acad Sci U S A. 2004; 101:17033–17038. [PubMed: 15563589]
30. Wang SK, et al. Targeting the carbohydrates on HIV-1: Interaction of oligomannose dendrons with human monoclonal antibody 2G12 and DC-SIGN. Proc Natl Acad Sci U S A. 2008; 105:3690–3695. [PubMed: 18310320]
31. Richman DD, Wrin T, Little SJ, Petropoulos CJ. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc Natl Acad Sci U S A. 2003; 100:4144–4149. [PubMed: 12644702]
32. Brochet X, Lefranc MP, Giudicelli V. IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis. Nucleic Acids Res. 2008; 36:W503–508. [PubMed: 18503082]
33. Munshaw S, Kepler TB. SoDA2: a Hidden Markov Model approach for identification of immunoglobulin rearrangements. Bioinformatics. 2010; 26:867–872. [PubMed: 20147303]

a

	Median IC_{50}
PGT121	0.03
PGT122	0.05
PGT123	0.03
PGT125	0.04
PGT126	0.04
PGT127	0.08
PGT128	0.02
PGT130	0.16
PGT131	0.52
PGT135	0.17
PGT136	7.81
PGT137	3.46
PGT141	0.35
PGT142	0.21
PGT143	0.31
PGT144	2.06
PGT145	0.29
PG9	0.23
VRC01	0.32
PGV04	0.20
b12	2.82
2G12	2.38
4E10	3.41

Key (μg/ml): < 0.2; 0.2 - 2; 2 - 20

b

	Viruses neutralized		
	IC_{50} < 50 μg/ml	IC_{50} < 1 μg/ml	IC_{50} < 0.1 μg/ml
PGT121	70	57	44
PGT122	65	48	36
PGT123	67	54	40
PGT125	52	40	32
PGT126	60	50	40
PGT127	50	37	27
PGT128	72	60	50
PGT130	52	35	23
PGT131	40	23	13
PGT135	33	23	13
PGT136	16	6	3
PGT137	22	8	4
PGT141	56	36	15
PGT142	57	40	23
PGT143	56	37	17
PGT144	38	16	3
PGT145	78	52	27
PG9	77	54	29
VRC01	93	74	20
PGV04	88	65	25
b12	34	10	2
2G12	32	11	1
4E10	96	13	1

Key (%): > 90; 60 - 90; 30 - 60; 1 - 30

Figure 1. Neutralization activity of the newly identified PGT antibodies

a, Median neutralization potency against viruses neutralized with an IC_{50} < 50 μg/ml. The color-coding is as follows: yellow, 2 - 20 μg/ml; orange, 0.2 - 2 μg/ml; red, < 0.2 μg/ml. **b**, Neutralization breadth at different IC_{50} cut-offs. The color-coding is as follows: green, 1% to 30%; yellow, 30% to 60%; orange, 60% to 90%; red, > 90%.

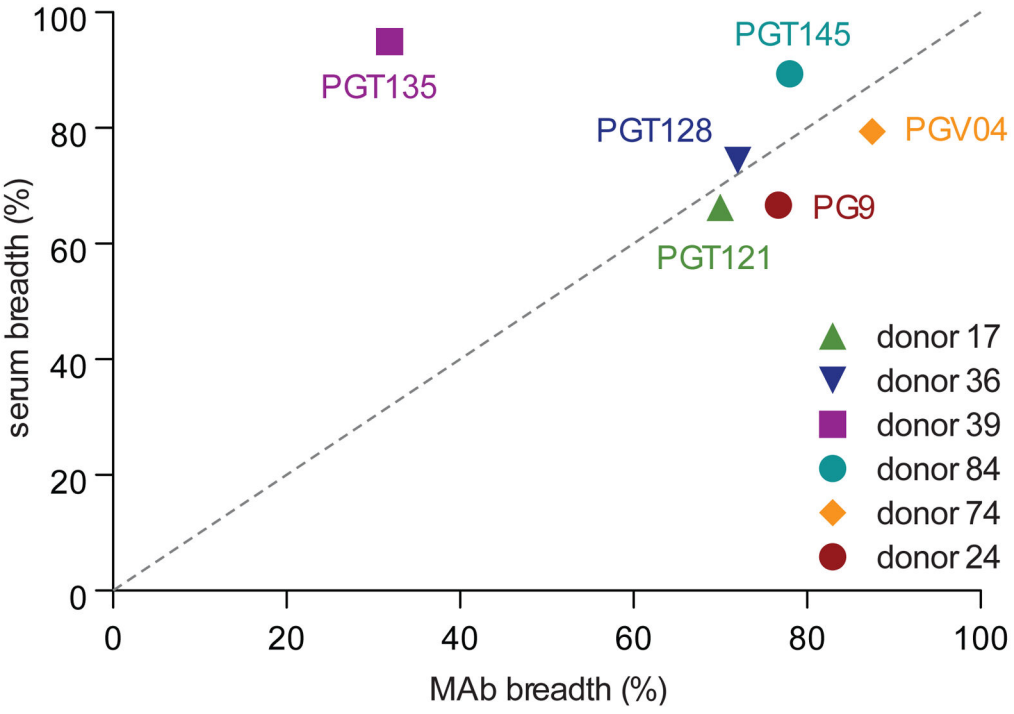

Figure 2. Key MAbs fully recapitulate serum neutralization by the corresponding donor serum

Serum breadth was corelated with the breadth of the broadest MAb for each donor (% viruses neutralized at NT_{50} > 100 or IC_{50} < 50 μg/ml, respectively). Of note, MAbs isolated from donor 39 could not completely recapitulate the serum neutralization breadth.

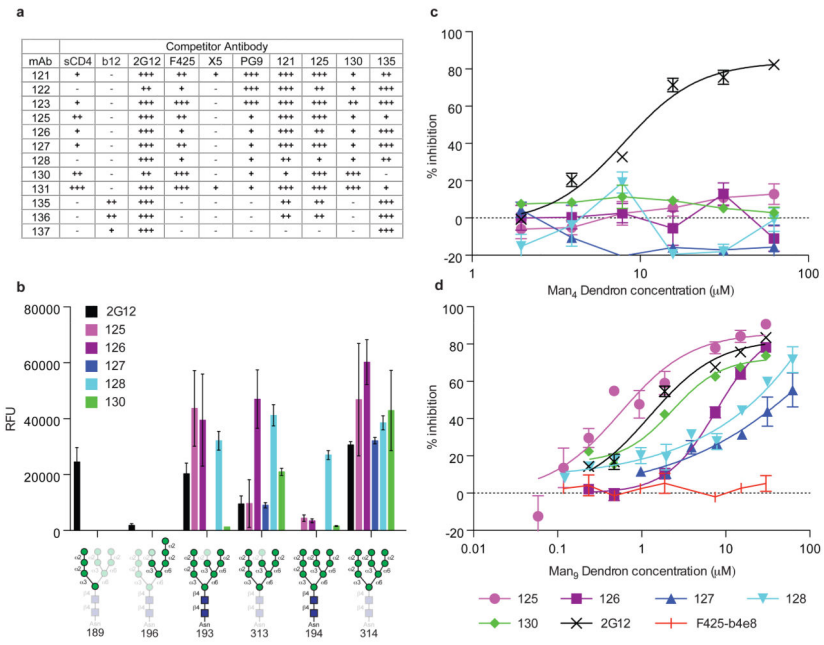

Figure 3. Epitope mapping of PGT antibodies

a, Competition of PGT MAbs with sCD4 (soluble CD4), b12 (anti-CD4bs), 2G12 (anti-glycan), F425/b4e8 (anti-V3), X5 (CD4i), PG9 (anti-V1/V2 and V3, quaternary) and each other. Competition assays were performed by ELISA using $gp120_{Bal}$ or $gp120_{JR\text{-}FL}$, except for the PG9 competition assay, which was performed on the surface of $JR\text{-}FL_{E168K}$ or JR-CSF transfected cells. Boxes are color coded as follows: red, 75–100% competition; orange, 50–75% competition; yellow, 25–50% competition; gray, <25% competition. Experiments were performed in duplicate, and data represent an average of at least two independent experiments. **b,** Glycan microarray analysis (Consortium for Functional Glycomics, CFG, v 5.0) reveals that PGT MAbs 125, 126, 127, 128, and 130 contact Man_8 (313), $Man_8GlcNAc_2$ (193), Man_9 (314) and $Man_9GlcNAc_2$ (194) glycans directly. Only glycans structures with RFU (relative fluorescent units) > 3000 are shown. PGT-131 showed no detectable binding to the CFG glycan array but bound to Man_9-oligodendrons[30] (data not shown). Error bars represent standard deviation. **c, d,** Binding of PGT MAbs 125, 126, 127, 128 and 130 to gp120 is competed by Man_9 oligodendrons but not Man_4 oligodendrons. Binding of 131 to immobilized gp120 was too low to measure any competition.

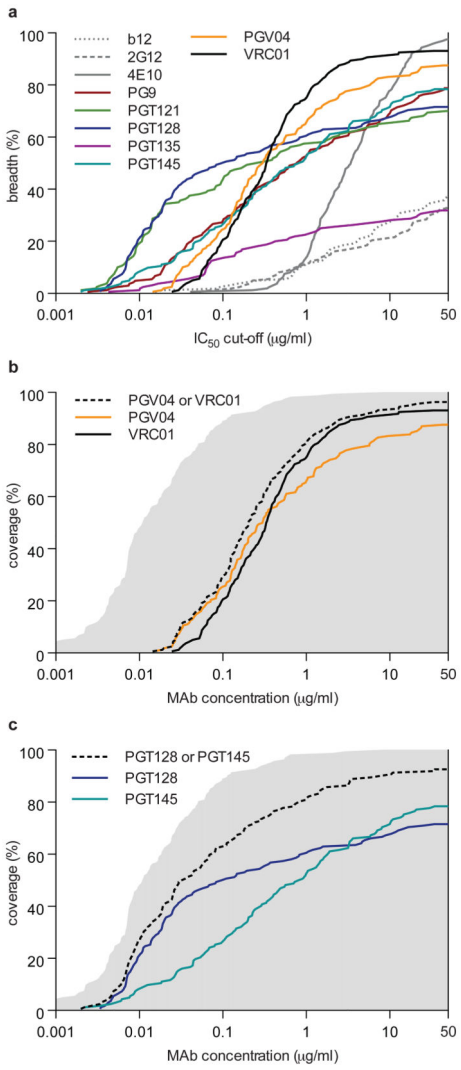

Figure 4. Certain antibodies or antibody combinations are able to cover a broad range of HIV isolates at low, vaccine achievable, concentrations

a, Cumulative frequency distribution of IC_{50} values of broadly neutralizing MAbs tested against a 162-virus panel. The y-axis shows the cumulative frequency of IC_{50} values up to the concentration shown on the x-axis and can therefore also be interpreted as the breadth at a specific IC_{50} cut-off. **b, c,** Percent viruses covered by single MAbs (solid lines) or by at least one of the MAbs in dual combinations (dashed black lines) dependent on individual concentrations. The grey area in both panels is the coverage of 26 MAbs tested on the 162-virus panel (PGT121-123, PGT125-128, PGT130-131, PGT135-137, PGT141-145, PG9, PG16, PGC14, VRC01, PGV04, b12, 2G12, 4E10, 2F5) and depicts the theoretical maximal achievable coverage known to date.