A Systematic Approach to HIV-1 Vaccine Immunogen Selection

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Abstract

A tremendous loss of financial and human resources from seven large-scale HIV vaccine efficacy trials suggest a need for a systematic approach to vaccine selection. We conducted a systematic analysis of three important envelope glycoprotein (Env) vaccine candidates: BG505 SOSIP.664, 1086.C gp140, and 1086.C gp120 to determine the most promising by comparing their structure and antigenicity. We found that the BG505 SOSIP trimer and 1086.C gp140 clearly outperformed the 1086.C gp120 monomer. BG505 SOSIP.664 bound the strongest to the most potent and broadest broadly neutralizing antibodies (bnAbs) PG9, PGT145, VRC01, and PGT121. Of interest, although BG505 SOSIP.664 did not bind to the CH58 mAb, 1086.C gp140 bound strongly to this mAb, which belongs to a class of non-neutralizing antibodies that may be protective based on correlates of protection studies of the RV144 HIV vaccine trial. The 1086.C gp120 monomer was the least antigenic of the three vaccine immunogens, binding the weakest to bnAbs and CH58 mAb. Taken together, the evidence provided here combined with previous preclinical immunogenicity and efficacy data strongly argue that the BG505 SOSIP.664 trimer and 1086.C gp140 are likely to be better vaccine immunogens than the monomeric 1086.C gp120, which was just recently tested and shown to be nonefficacious in a phase IIb/III trial. Thus, to best utilize our financial and valuable human resources, we propose a systematic approach by not only comparing structure and antigenicity, but also immunogenicity and efficacy of Env vaccine candidates in the preclinical phase to the selection of only the most promising vaccine candidates for clinical testing.

Keywords: HIV, vaccine, immunogen, trimer, preclinical trial

Introduction

HIV/AIDS STILL REMAINS one of the most devastating diseases affecting humans and a serious global public health threat. HIV currently afflicts ~36.9 million people and has cost 35.4 million lives since the start of the pandemic in the 1980s (UNAIDS 2018 Global HIV and AIDS Statistics). Despite the availability of effective treatment with antiretroviral therapy, a preventive vaccine is desperately needed to stop the spread of HIV infection. The development of vaccine strategies that can elicit either protective B cell or T cell responses or both are being pursued. However, accruing evidence from correlates of protection studies from the human Thai RV144 trial along with passive transfer studies in the nonhuman primate model has shift the focus of efforts more toward the development of HIV-1 envelope glycoprotein (Env) vaccines that will induce protective antibodies to prevent infection.[1–3]

Induction of broadly neutralizing antibodies (bnAbs) against HIV Envs is a viable vaccine strategy because passive administration of bnAbs can fully protect from infection in the nonhuman primate model of AIDS.[4] Moreover, some HIV patients develop potent bnAbs that can cross-neutralize a majority of global HIV isolates *in vitro*.[5,6] Thus far, the most promising Env vaccine for generating bnAbs is the soluble BG505 SOSIP trimer that resembles the native Env spike that expose many bnAb epitopes.[7] Initially created in 2013, the BG505 SOSIP trimer has undergone several iterations and with optimized antigen delivery, the trimer now has improved stability as well as antigenicity and immunogenicity.[8] Optimized BG505 SOSIP trimers have been shown to elicit neutralizing antibodies with increasing breadth inhibiting most Tier 1, autologous Tier 2, and some heterologous Tier 2 viruses.[8] Of importance, BG505 SOSIP trimer vaccine has been shown to protect against autologous Tier 2 SHIV challenge.[9] This finding is significant in that circulating Tier 2 viruses seem to be responsible for natural human infections and the epidemic.[10] Because of these promising preclinical data, the BG505 SOSIP trimer is now being tested by the

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International AIDS Vaccine Initiative (IAVI) in a phase I clinical trial (IAVI W001).

Developing a vaccine that elicits protective anti-V1/V2 loop antibodies could also be an effective strategy. The Thailand RV144 was the sole vaccine efficacy trial to indicate that it is possible to protect against HIV by vaccination, with a modest 31.2% protection. *Post hoc* analysis of the RV144 trial revealed that anti-V1/V2 loop apex functional antibody responses that were non-neutralizing, such as antibody-dependent cellular cytotoxicity (ADCC) correlated with protection.[3] The partial success of the RV144 trial has led to the exploration of the Pox vector prime/Env boost approach by a number of laboratories that includes Env from HIV strains such as transmitted/founder 1086.C Clade C virus. The 1086.C Env gp140 is a good candidate because it was highly immunogenic.[11,12] The Clade C 1086.C gp120 Env was selected in 2009 as a component of a bivalent vaccine to build on the RV144 results to address the HIV epidemic in sub-Saharan Africa where the majority of the population is infected by the Clade C virus.[13] The HIV-1 Clade C-based prime-boost vaccine regimen uses ALVAC-HIV (vCP2438) based on the ALVAC vector backbone (as in RV144) with Clade B (gp41, Gag, and Protease Lai strain) and Clade C (96ZM651 gp120) HIV-1 gene inserts and bivalent subtype C recombinant HIV Env gp120 (1086.C gp120 and TV1.C gp120). This vaccine regimen was just recently tested and found to be nonefficacious in a large phase IIb/III trial (HVTN 702) in Africa.

For over three decades since the discovery of the HIV virus in 1983, there has been an overwhelming effort to develop a vaccine that will halt the HIV pandemic. Seven major efficacy trials (phase IIb/III) have been completed but none of the experimental vaccines tested have demonstrated significant efficacy for preventive measure. To date, >32,000 human volunteers have participated in the six completed efficacy trials.[14–19] An additional 8,000 volunteers were scheduled to enroll in the HVTN 702 (Uhambo) and HVTN 705 (Imbokodo) phase IIb/III efficacy trials. Unfortunately, the HVTN 702 efficacy trial just recently was stopped early because the vaccine failed to protect against HIV infection. In addition, many human volunteers have participated in the testing of vaccine candidates in numerous early phase I/IIa trials that were not advanced because of safety issues or lack of immunogenicity. The financial cost and human resources utilized to conduct these trials are tremendous. With many more vaccine immunogens being created and tested on a regular basis, it is imperative that we implement a systematic approach to vaccine candidate selection to better utilize limited financial and precious human resources.

In this study, we propose a systematic approach to candidate Env vaccine selection. To begin, we compared side-by-side the structure and antigenicity of the three aforementioned vaccine candidates being developed to induce protective antibody responses: BG505 SOSIP.664, 1086.C gp140, and 1086.C gp120. We found that overall the BG505 SOSIP.664 trimer outperformed both 1086.C Env immunogens with an ability to bind to the most potent bnAbs. However, interestingly, the 1086.C gp140 was strongly recognized by an anti-V1/V2 loop antibody that may possibly be protective based on correlates of HIV protection studies of the RV144 trial.[3] With these selection criteria (structure and antigenicity) along with historical data on the preclinical immunogenicity

of these Env vaccines, the BG505 SOSIP.664 trimer and 1086.C gp140 seem to be better vaccine candidates than the monomeric 1086.C gp120, which was recently evaluated to be noneffective in a human HIV vaccine efficacy trial. The profound structural and antigenicity differences among the three Env candidates that we observed in this study strongly argue for a more comprehensive and stringent criteria for Env immunogen selection for testing in human clinical trials.

Materials and Methods

Antibodies and Env proteins

The HIV-1 Env 1086.C D7gp120 and 1086.C gp140C Envs, CD4-IgG2, anti-HIV-1 envelope monoclonal antibodies 2G12, PGT121, 3869, CH58, PG09, PGT145, IgG1 b12, and VRCO1 were obtained from the NIH AIDS Reagents Program (Germantown, MD). The BG505 SOSIP.664 envelope trimer proteins were kindly provided by Dr. John P. Moore, Cornell University (Ithaca, NY).[7]

Polyacrylamide gel electrophoresis analysis of HIV-1 envelope protein

For reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis, HIV-1 envelope proteins were prepared in Laemli sample buffer containing 10% 2-mercaptoethanol and incubated at 95°C, 5 min. Samples were loaded onto 4%–20% polyacrylamide mini-protean TGX (Bio-Rad Laboratories, Hercules, CA) and electrophoresis was conducted at 90 V for 2 h using 1 × Tris/glycine/SDS buffer (Bio-Rad Laboratories). For western blot analysis, envelope proteins transferred on Invitrolon PVDF membranes (Novex, Carlsbad, CA) were incubated in blocking buffer (0.25% Tween-20, 1% nonfat dried milk, 5% fetal bovine serum in phosphate-buffered saline [PBS]) and detected with 1 μg/mL HIVIg (NIH AIDS Reagent Program) and a secondary antihuman IgG-HRP antibody (Millipore, Billerica, MA). Membranes were then stained using Super Signal West Pico (Thermo Scientific, Rockford, IL) and exposed on CL-XPosure film (Thermo Scientific).

For nondenaturing blue native-PAGE (BN-PAGE), the HIV envelope proteins were resuspended in native PAGE sample buffer and loaded into native PAGE 4%–16% Bis-Tris gel (Invitrogen, Carlsbad, CA). Electrophoresis was conducted at 120 V for 2 h in a light blue solution containing native PAGE running buffer and native PAGE cathode additive (Invitrogen). The HIV proteins were then stained with GelCode Blue Safe Protein Stain (Thermo Scientific) for 1–1.5 h and analyzed using the Innotech FluorchemQ Image III System. For reducing and nonreducing PAGE analysis, Precision Plus Protein ladder (Bio-Rad Laboratories) and NativeMark Protein Standard (Invitrogen) were used as a molecular weight marker, respectively.

Negative staining electron microscopy analysis

Negative staining electron microscopy (NS-EM) was performed at the Harvard Medical School Electron Microscopy Facility (Boston, MA). Carbon-coated meshes were glow discharged at 25 mA for 20 s. The meshes were floated over with 5 μL of sample for 20 s. After drying with filter paper, the meshes were washed for 30 s, dried, and stained with 7.5% m/v uranyl formate. Images were captured on a

Tecnai G^2 Spirit BioTWIN microscope with 68,000 × direct magnification at 80 kV using Homamatsu ORCA HR camera.

Biolayer interferometry analysis

Biolayer interferometry (BLI) was performed at the Harvard University Center for Macromolecular Interactions (Boston, MA). Antihuman Fc capture biosensors (Fortebio, Fremont, CA) were loaded with 10 μg/mL antibody solution for 2 min. Biosensors underwent a cycle of binding and dissociation as follows: basal signal for 2 min, binding step for 5 min, and dissociation for 10 min. For each antibody, protein dilutions in running buffer (0.1% bovine serum albumin in PBS pH 7.4) were assessed ranging from 3.9 to 250 nM. Data were processed using Fortebio software suite applying a global fit model. Binding measurement was obtained using a Fortebio Octet Red 384 system. Binding on-rate constant, K_{on} was expressed as $1/M \times s$ and off-rate constant, K_{off} as $1/s$. The affinity constant, K_D was calculated using the kinetic equation: $K_D = K_{off}/K_{on}$ and expressed in molarity.

Results

Expression and structural analysis of candidate Env vaccines

A number of studies have shown that soluble Env trimers are better than Env monomers at binding to bnAbs and are relatively more immunogenic.[7,8,20] We analyzed both the expression and structure of BG505 SOSIP.664, 1086.C gp140, and 1086.C gp120. Western blot analysis under reducing condition showed that monomeric BG505 SOSIP.664 and 1086.C gp120 Env proteins have a molecular weight of ~100 kDa and monomeric 1086.C Env gp140 has a molecular weight of ~150 kDa (Fig. 1A). Under native condition using BN-PAGE, the BG505 SOSIP.664 and 1086.C gp140 are trimers with an approximate molecular weight of 700 and 720 kDa, respectively, similar to previous studies (Fig. 1B).[12] The 1086.C gp120 migrated as a monomer, consistent with previous observation that this Env behaved as a stable monomer in solution (Fig. 1B).[13] NS-EM confirms the BG505
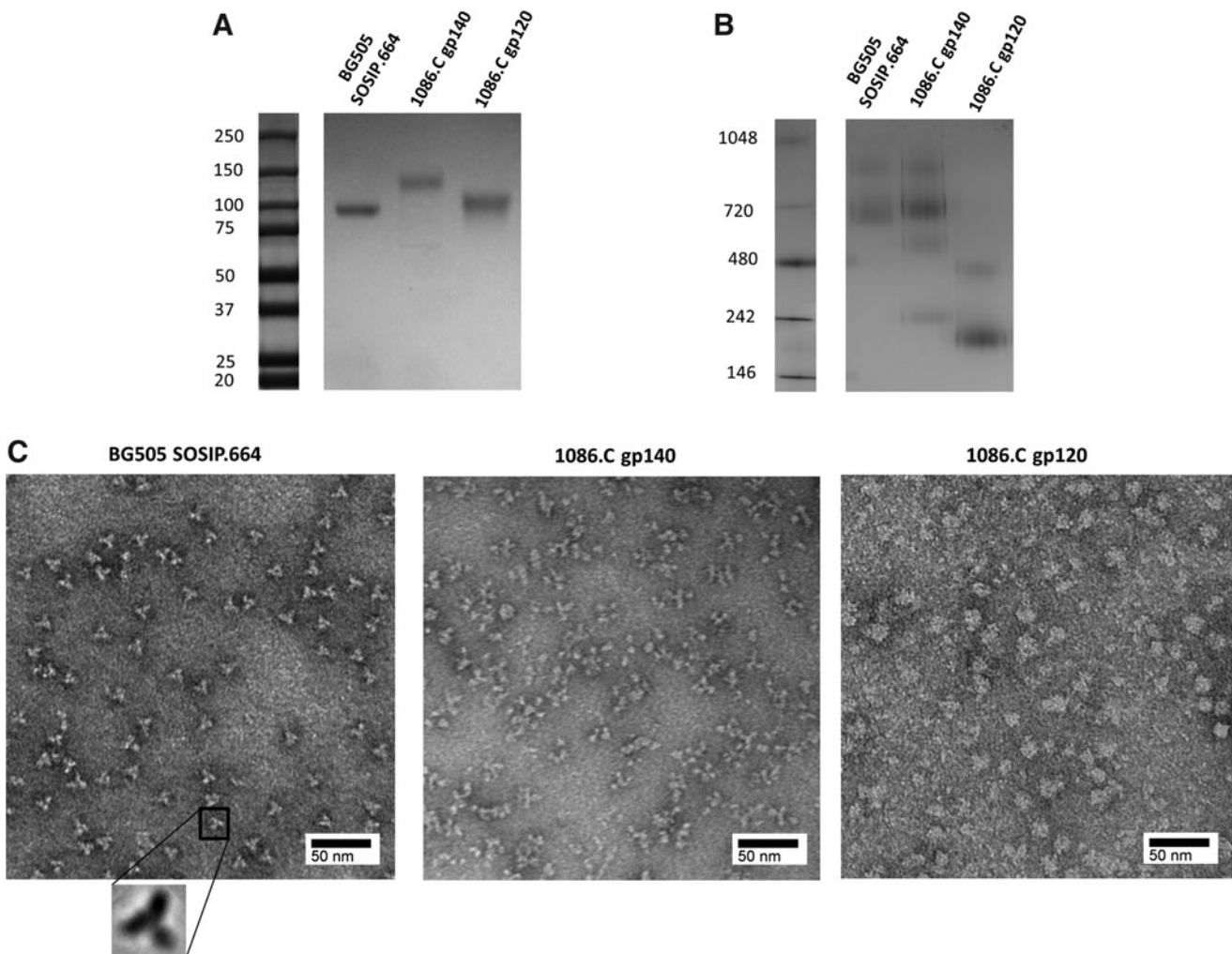

FIG. 1. NS-EM and PAGE analysis of HIV-1 Env vaccine candidates. **(A)** Western blot analysis of BG505 SOSIP.664, 1086.C gp140, and 1086.C gp120 Envs under reducing condition. **(B)** BN-PAGE analysis of BG505 SOSIP.664, 1086.C gp140, and gp120 (2 μg of each protein). The molecular weight of marker (M) proteins are indicated **(B, C)**. **(C)** NS-EM analysis of BG505 SOSIP.664, 1086.C gp140, and 1086.C gp120 Envs stained with 7.5% uranyl formate. Electron microscopy was performed using Tecnai G^2 Spirit BioTWIN at 80 kV. Scale bar, 50 nm. BN-PAGE, blue native–polyacrylamide gel electrophoresis; Env, envelope glycoprotein; NS-EM, negative staining electron microscopy.

SOSIP.664 native-like trimeric structure as previously described (Fig. 1C).[7] Although the 1086.C gp140 showed a trimeric migration pattern on BN-PAGE gels, NS-EM analysis showed that 1086.C gp140 did not have the organized trimeric structure of the BG505 SOSIP.664 (Fig. 1C). The 1086.C gp140 formed complexes that were variable in shape and the 1086.C gp120 formed globular structures likely as a result of aggregation (Fig. 1C). As expected, these results demonstrate that only the BG505 SOSIP vaccine candidate and neither the 1086.C gp140 nor 1086.C gp120 was a native-like trimer.

Antigenicity and presence of bnAb epitopes on the candidate Env vaccines

The antigenicity profile assessment of the HIV-1 Env vaccine is critical to demonstrate the presence of epitopes recognized by bnAbs against HIV-1. The presence of the bnAb epitopes on Env vaccines allows for exposure and recognition by B cells for eliciting bnAb responses in the vaccinated host. We analyzed Env antigenicity and the presence of important bnAb epitopes including the high mannose patch/V3, CD4 binding site (CD4bs), and V1-V2 loops (apex) by determining the binding affinity of the candidate Env vaccine immunogens to important non-neutralizing antibodies and bnAbs that rec-ognize these vulnerable epitopes using BLI (Fig. 2A–C). The bnAbs selected have strong broadly neutralizing responses against HIV-1 (Fig. 2A–C).

Binding to high mannose patch/V3 bnAbs. The 2G12, PGT121, and 3869 bnAbs recognize the high mannose patch/V3 (Fig. 2A–C).[21–23] PGT121 was previously shown to neutralize roughly 70% of HIV-1 with high potency (Fig. 2C).[24,25] BLI analysis revealed that 2G12 bound BG505 SOSIP.664 the strongest ($<1 \times 10^{-3}$ nM K_D), followed by 1086.C gp140 (3.3 nM K_D) (Fig. 3A–C). 2G12 did not bind the 1086.C gp120 immunogen (Fig. 3A–C). PGT121 bound BG505 SOSIP.664 with the highest affinity (0.153 nM K_D), followed by 1086.C gp140 (1.36 nM K_D) (Fig. 3A–C). Relatively weaker binding of PGT121 to the 1086.C gp120 (25.8 nM K_D) was observed. With the 3869 bnAb, binding to BG505 SOSIP.664 and 1086.C gp140 were strong (2×10^{-3} nM K_D and 1.0×10^{-3} nM K_D, respectively) (Fig. 3A–C), whereas binding affinity to 1086.C gp120 was significantly lower (0.52 nM K_D) (Fig. 3A–C). These results show that the high mannose patch/V3 bnAbs bind much more strongly to the BG505 SOSIP trimer followed by the 1086.C gp140. The 1086.C gp120 was poorly antigenic to this class of bnAbs.
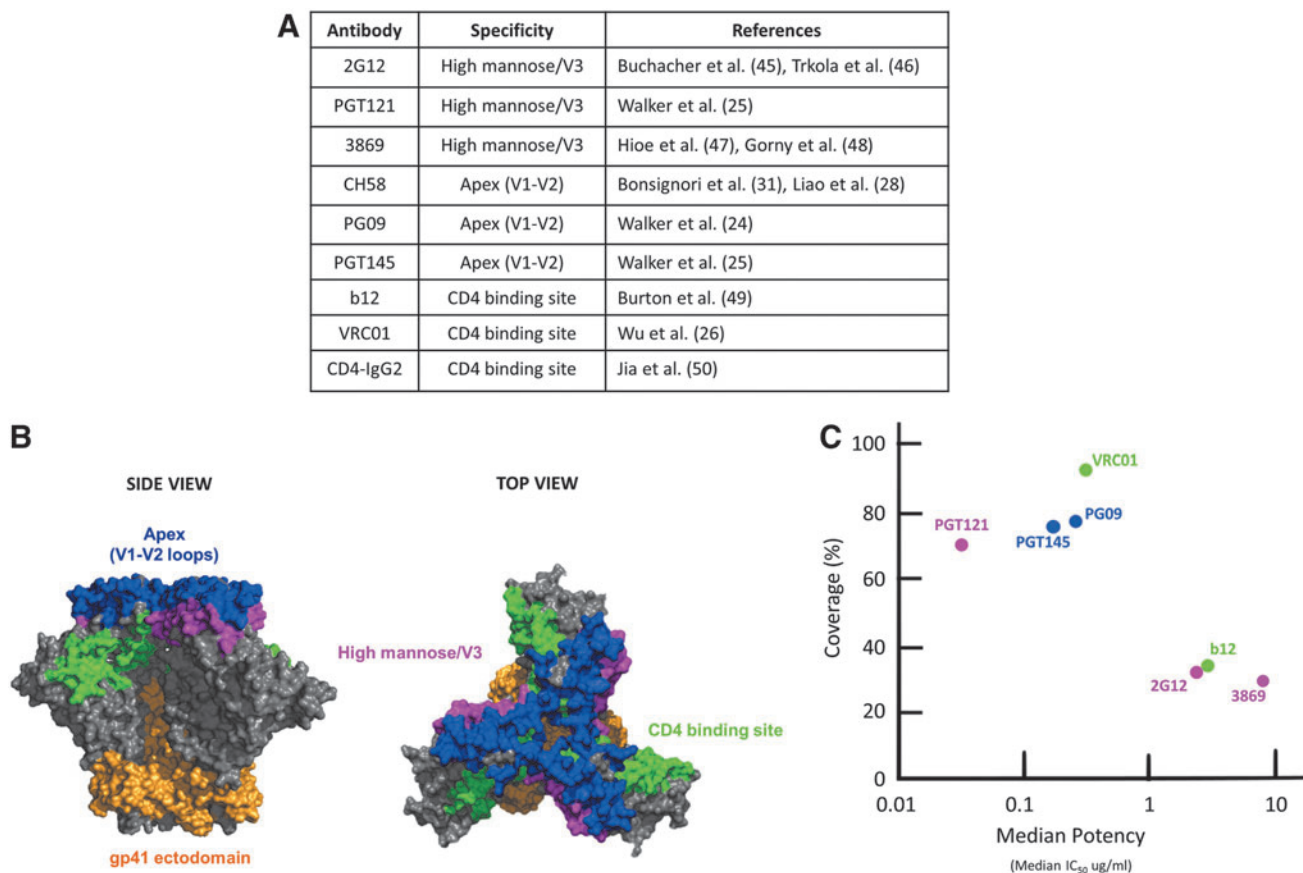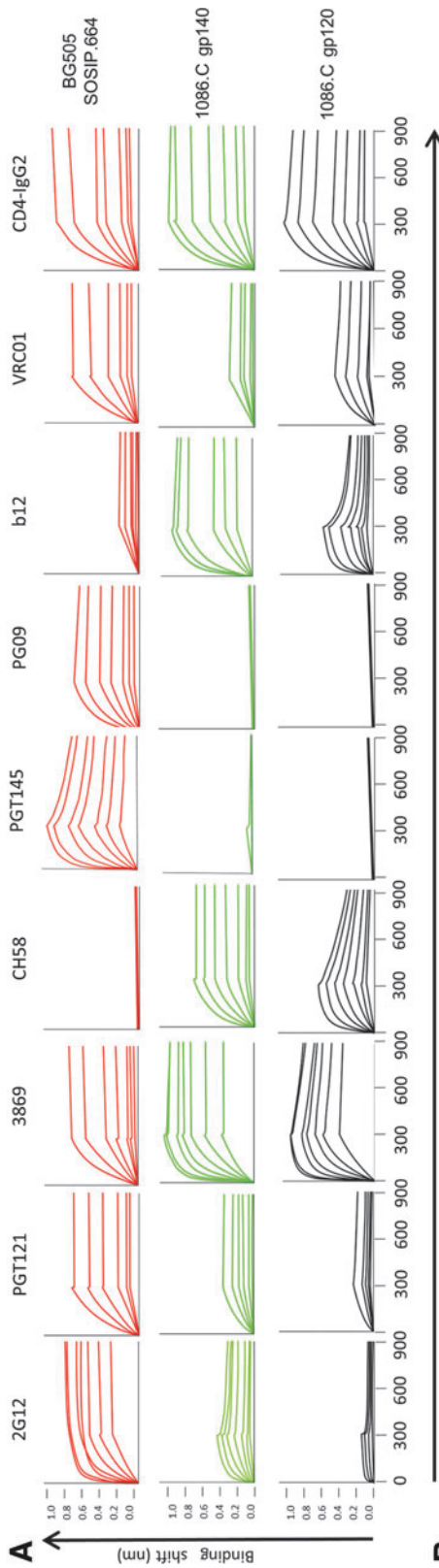
Antibody	Specificity	References
2G12	High mannose/V3	Buchacher et al. (45), Trkola et al. (46)
PGT121	High mannose/V3	Walker et al. (25)
3869	High mannose/V3	Hioe et al. (47), Gorny et al. (48)
CH58	Apex (V1-V2)	Bonsignori et al. (31), Liao et al. (28)
PG09	Apex (V1-V2)	Walker et al. (24)
PGT145	Apex (V1-V2)	Walker et al. (25)
b12	CD4 binding site	Burton et al. (49)
VRC01	CD4 binding site	Wu et al. (26)
CD4-IgG2	CD4 binding site	Jia et al. (50)

FIG. 2. Non-neutralizing antibodies and bnAbs to HIV-1 Env vaccine candidates. **(A)** Non-neutralizing and bnAbs used in this study with their specificities are listed in the table. **(B)** The crystal structure of BG505 SOSIP.664 trimer in a *side view* (*left*) and *top view* (*right*), and the location of bnAb epitopes: apex, V1-V2 loops (*blue*), CD4 binding site (*green*), high mannose patch/V3 (*pink*), and the gp41 ectodomain (*orange*). **(C)** Neutralization breadth as presented by neutralization coverage (%) (y-axis) of large panels of global isolates and potency (median IC$_{50}$, in μg/mL) (x-axis) of bnAbs as determined previously.[5,25] bnAbs, broadly neutralizing antibodies. Color images are available online.
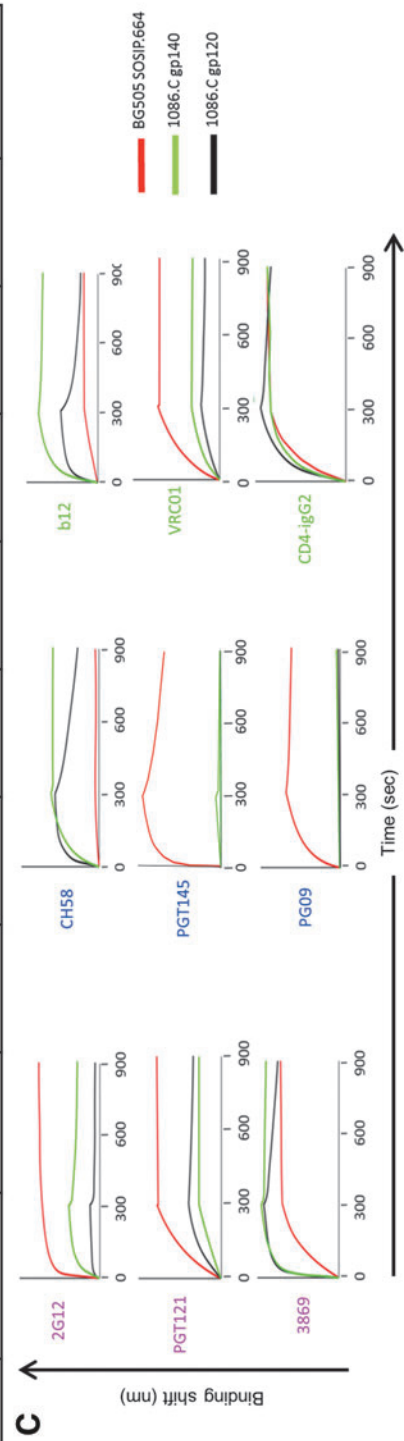
FIG. 3. Comparison of non-neutralizing antibodies and bnAb binding to HIV-1 Env vaccine candidates. **(A)** BLI curves. Anti-HIV-1 Env bnAbs (2G12, PGT121, 3869, PGT145, PG09, IgG1 b12, and VRC01), the non-neutralizing antibody CH58 and CD4-IgG2 were immobilized on anti-hIgG Fc biosensors. In the association step, each sensor was incubated with Env proteins ranging from 3.9 to 250 nM. Binding curves of BG505 SOSIP.664 (*red*), 1086.C gp140 (*green*), and 1086.C gp120 (*black*) are shown. **(B)** The table indicates binding on-rate (K_{on}) and off-rate (K_{off}) constants and affinity (K_D) of anti-HIV-1 Env antibodies to the HIV-1 Env glycoproteins. HIV Env with PB and NB are indicated. **(C)** BLI curves of BG505 SOSIP.664 (*red*), 1086.C gp140 (*green*), and 1086.C gp120 (*black*) binding to bnAbs (2G12, PGT121, 3869, PG09, PGT145, IgG1 b12, and VRC01), the non-neutralizing antibody CH58 and CD4-IgG2. BLI, biolayer interferometry; NB, no binding; PB, poor binding. Color images are available online.

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Binding to CD4bs bnAbs and CD4-IgG2. The bnAbs IgG1 b12 (b12), VRCO1 and CD4-IgG2 recognize and bind to the CD4bs of the HIV-1 envelope trimer (Fig. 2A–C).[26,27] BLI binding analysis showed that CD4-IgG2 bound 1086.C gp140 ($<1\times10^{-3}$ nM K_D) the strongest, followed by BG505 SOSIP.664 (1.6×10^{-3} nM K_D), and 1086.C gp120 the weakest (1.8 nM K_D) (Fig. 3A–C). With regard to b12 binding, the 1086.C gp140 bound strongly (0.33 nM K_D), 1086.C gp120 also bound but with less affinity (6.0 nM K_D) (Fig. 3A–C). In contrast, BG505 showed no binding to b12. VRCO1 is one of the most potent bnAbs observed capable of neutralizing $\sim$91% of HIV-1 viruses (Fig. 2A–C).[26] VRC01 bound BG505 with strong affinity (2.08×10^{-3} nM K_D) and 1086.C gp140 and gp120 with markedly less affinity (17.7 nM K_D and 11.7 nM K_D, respectively) (Fig. 3A–C). Taken together, these results show that BG505 trimer contains a bnAb neutralization epitope, recognized strongly by one of the most potent bnAbs, VRC01. Of interest, the CD4bs on 1086.C gp140 is preferentially recognized by b12 and CD4-IgG2 over the BG505 SOSIP.664 (Fig. 3A–C). The 1086.C gp120 showed the weakest binding to the CD4bs bnAb class overall compared with the BG505 SOSIP trimer and 1086.C gp140 Env vaccines (Fig. 3A–C).

Binding to V1-V2 loops (apex). Anti-apex bnAbs are found to be highly potent capable of neutralizing the majority of HIV-1 viruses. bnAbs to the trimer apex include PG09, which can neutralize roughly 84% of HIV-1 viruses (Fig. 2A–C).[28,29] PG09 bound well to BG505 SOSIP.664 trimer (2.1 nM K_D) but binding to 1086.C gp140 and gp120 immunogens were undetectable (Fig. 3A–C). The PGT145 binds to the quaternary structure of the V2 apex and is a highly potent bnAb, neutralizing $\sim$80% of HIV-1 isolates (Fig. 2A–C).[25] PGT145 only binds specifically to the HIV-1 Env in trimeric configuration.[30] PGT145 bound strongly to BG505 SOSIP.664 trimer (2.57 nM K_D) but binding to 1086.C gp140 and gp120 immunogens were not observed (Fig. 3A–C).

Another class of anti-V2 apex antibodies that includes CH58 are nonbroadly neutralizing and are associated with vaccine-induced protection in the RV144 HIV vaccine trial.[28,31] CH58 bound 1086.C gp140 with the highest affinity (0.22 nM K_D) and 1086.C gp120 with lower affinity (9.31 nM K_D) (Fig. 3A–C). Of interest, CH58 did not bind to the BG505 SOSIP.664 trimer (Fig. 3A–C). Taken together, these results show that the BG505 SOSIP.664 trimer is better than the nontrimer 1086.C gp140 and gp120 at binding to PG09 and trimer-specific PGT145 bnAb. However, the 1086.C gp140 clearly is a better immunogen at binding to the RV144 patient CH58 mAb.

Discussion

For almost four decades since the diagnosis of the first case of HIV/AIDS, seven preventive HIV-1 vaccine efficacy trials[14–19] and numerous early-phase human trials have failed, amounting to a significant loss of financial and most importantly, human resources. A major lesson learnt from these trials is to establish a more systematic approach to selecting only the most promising vaccine strategies for testing in human clinical trials. To this end, we began to perform a comparative study on three HIV envelope proteins: 1086.C gp120, 1086.C gp140, and BG505 SOSIP that are considered promising HIV vaccine candidates.[7,11–13,32] Each of these Env immunogens were selected for vaccine development based on one or more of the following criteria: structure, antigenicity, preclinical immunogenicity, and efficacy trials in small laboratory animals and nonhuman primates. The BG505 SOSIP trimer is currently being tested for immunogenicity and safety in a human phase I clinical trial (IAVI W001). The 1086.C gp120 is a component of a bivalent vaccine that was recently tested and found to be ineffective in preventing HIV infection in a large human vaccine efficacy trial (HVTN 702).[33,34]

Exterior HIV Envs are in a trimeric configuration on the virion surface. We confirmed in this study that the BG505 SOSIP.664 is a native-like trimer by NS-EM analysis and binding to the trimer-specific PGT145 bnAb. In contrast, the 1086.C gp120 and gp140 Envs were found to exist in monomeric and oligomeric or aggregate forms. HIV-1 gp120 and gp140 glycoproteins have the propensity to form aggregates because of the presence of noncanonical disulfide bonds not seen in BG505 SOSIP.664 trimers.[35–37] These noncanonical disulfide bonds often result in aberrant intermolecular disulfide bonds that cause the crosslinking of Env glycoproteins into dimers and aggregates.[35–37]

Consistent with many studies demonstrating that HIV Env native-like trimer immunogens are better at binding and eliciting bnAbs than nontrimer and monomeric gp120 Env immunogens,[20,29,38] we showed in this study that BG505 SOSIP.664 trimer for the most part outperformed both 1086.C gp140 and gp120 immunogens. The BG505 SOSIP trimer showed markedly the strongest affinity to PG09, VRC01, PGT145, and PGT121 that are considered among the most potent and broadest bnAbs.[5,6] The BG505 SOSIP trimer strongly bound the bnAbs recognizing the high mannose patch/V3 region (2G12, PGT121, and 3869), the CD4bs bnAb VRC01, and the apex-specific PG09 and the trimer-specific PGT145 bnAbs. However, there was no detectible binding to IgG1 b12 bnAb. The binding of 2G12 to BG505 SOSIP trimer was stronger than previously observed by surface plasmon resonance (SPR) binding analysis probably because of differences between BLI and SPR technologies in dissociation constants measurements especially for some high-affinity antibodies.[39] The 1086.C gp140 bound all the antibodies but with less affinity than the BG505 SOSIP trimer. The 1086.C gp140 did not bind to PG09 and PGT145, but it strongly bound to IgG1 b12 bnAb. The 1086.C gp120 monomer showed some affinity to the bnAbs PGT121, 3869, IgG1 b12, and VRCO1 and no binding to 2G12, PG09, and PGT145. However, the 1086.C gp120 monomer bound to these antibodies much weaker than either BG505 SOSIP.664 trimer or 1086.C gp140 immunogen.

Several studies have shown that the presence of bnAb epitopes on Env immunogens including trimers does not translate to immunogenicity in that these Env immunogens often do not elicit broadly neutralizing responses at a reasonable titer in vaccinated animals.[40] However, despite the lack of direct correlation between HIV Env antigenicity and immunogenicity, the presence of bnAb epitopes on Env immunogens, especially native-like trimers such as BG505 SOSIP.664, is important for the recognition and induction of B cells capable of producing bnAbs. It must be noted that the structural and antigenicity studies conducted here are only the initial steps for selecting the most promising immunogens

in the systematic evaluation of HIV Env vaccine candidates. Testing only Env immunogens that contain bnAb epitopes for further evaluation of immunogenicity in vaccinated animals is warranted.

The next critical steps for vaccine selection are immunogenicity and efficacy studies in laboratory animals. As these studies were not conducted here, we rely on historical immunogenicity and efficacy assessments of the three Env vaccine immunogens that also demonstrate that the BG505 SOSIP trimer is the most promising for possibly inducing bnAb responses.[41] BG505 SOSIP.664 and a few other native-like trimer immunogens have been shown to elicit autologous and some heterologous Tier 2 virus neutralization.[42] Furthermore, the BG505 SOSIP.664 trimer vaccine was shown to elicit protection against homologous Tier 2 SHIV infection.[9] On the contrary, the monomeric envelope immunogens including 1086.C gp120 have only been found to elicit neutralization of Tier 1 viruses[43] and protection against Tier 2 SHIV infection *in vivo* has not been observed.

Of interest, in a human correlate study, host IgG1 binding of the BG505 SOSIP trimer was the best predictor of HIV-1 neutralization breadth in the plasma of HIV-infected individuals,[44] which suggests that in humans it might be possible to induce broadly neutralizing responses by immunization with the BG505 SOSIP trimer vaccine. Altogether, there is substantial evidence indicating that the BG505 SOSIP trimer is so far the most viable immunogen for eliciting bnAb responses. However, enthusiasm with the BG505 trimer vaccine is dampened by studies showing that the bnAb responses in vaccinated animals lacked significant potency and breadth against heterologous Tier 2 global virus isolates.[40] It will be important to improve trimer immunogens and vaccination strategies to elicit more bnAb responses.

The RV144 trial was the only efficacy trial that showed some protection (31.2%) that was correlated with non-neutralizing antibodies binding to the V1-V2 apex and mediate ADCC.[3] Antibodies that bind residue K169 in the V1-V2 region of the HIV-1 envelope correlated with reduced risk of infection. Here we tested Env vaccine binding to CH58 mAb that was one of RV144 vaccine-derived V1-V2 monoclonal antibodies isolated.[28] The CH58 mAb recognizes residue K169, which underscores the importance of the CH58 epitope in vaccine-induced protection.[28] We found that the 1086.C gp140 is better than the BG505 trimer in that it bound with high affinity to CH58, whereas BG505 trimer did not bind detectibly. Moreover, the 1086.C gp140 bound to CH58 markedly stronger than the 1086.C gp120. This suggests that the 1086.C gp140 would be a better choice than the 1086.C gp120 to improve the effectiveness of RV144 pox prime subunit Env vaccine regimen. It would be important to assess whether the 1086.C gp140 immunogen is capable of eliciting protective CH58-like ADCC responses.

The purpose of this study was to conduct a systematic approach to selecting which Env immunogens should be developed and advanced for clinical testing. Based on the results of our comparative structural and antigenicity studies combined with historical data on the preclinical immunogenicity and efficacy studies in nonhuman primates of the Env vaccines studied here, both the BG505 SOSIP.664 and 1086.C gp140 have outperformed the 1086.C gp120 vaccine immunogen. It is therefore not surprising that just recently, the HIV-1 Clade C gp120 bivalent vaccine regimen, which included the 1086.C gp120 immunogen, failed to protect against HIV infection in the large HVTN 702 vaccine efficacy trial. For financial but most importantly for ethical reasons, we propose in the future a collaborative effort among laboratories to conduct side-by-side comparative structural, antigenicity, immunogenicity, and efficacy of Env vaccine candidates in the preclinical phase to best select and prioritize only the most viable vaccine candidate(s) for human testing.

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