Vaccine: X 12 (2022) 100222

Contents lists available at ScienceDirect

Vaccine: X

journal homepage: www.elsevier.com/locate/jvacx

Immunization with HIV-1 trimeric SOSIP.664 BG505 or founder virus C (FVC_{Env}) covalently complexed to two-domain $CD4^{S60C}$ elicits cross-clade neutralizing antibodies in New Zealand white rabbits

Nancy L. Tumba *, Gavin R. Owen, Mark A. Killick, Maria A. Papathanasopoulos

HIV Pathogenesis Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

ARTICLE INFO

Article history:
Received 7 March 2022
Received in revised form 21 September 2022
Accepted 28 September 2022
Available online 30 September 2022

Keywords:
HIV-1 vaccine immunogens
Envelope glycoprotein trimers
Covalent complexes
Broadly neutralizing antibodies
New Zealand White rabbits
Immunogen design

ABSTRACT

Background: An ongoing challenge in HIV-1 vaccine research is finding a novel HIV-1 envelope glycoprotein (Env)-based immunogen that elicits broadly cross-neutralizing antibodies (bnAbs) without requiring complex sequential immunization regimens to drive the required antibody affinity maturation. Previous vaccination studies have shown monomeric Env and Env trimers which contain the GCN4 leucine zipper trimerization domain and are covalently bound to the first two domains of CD4 ($2dCD4^{S60C}$) generate potent bnAbs in small animals. Since SOSIP.664 trimers are considered the most accurate, conformationally intact representation of HIV-1 Env generated to date, this study further evaluated the immunogenicity of SOSIP.664 HIV Env trimers (the well characterized BG505 and FVC_{Env}) covalently complexed to $2dCD4^{S60C}$. **Methods:** Recombinant BG505 SOSIP.664 and FVC_{Env} SOSIP.664 were expressed in mammalian cells, purified, covalently coupled to $2dCD4^{S60C}$ and antigenically characterized for their interaction with HIV-1 bnAbs. The immunogenicity of BG505 SOSIP.664-$2dCD4^{S60C}$ and FVC_{Env} SOSIP.664-$2dCD4^{S60C}$ was investigated in New Zealand white rabbits and compared to unliganded FVC_{Env} and $2dCD4^{S60C}$. Rabbit sera were tested for the presence of neutralizing antibodies against a panel of 17 pseudoviruses. **Results:** Both BG505 SOSIP.664-$2dCD4^{S60C}$ and FVC_{Env} SOSIP.664-$2dCD4^{S60C}$ elicited a potent, HIV-specific response in rabbits with antibodies having considerable potency and breadth (70.5% and 76%, respectively) when tested against a global panel of 17 pseudoviruses mainly composed of harder-to-neutralize multiple clade tier-2 pseudoviruses. **Conclusion:** BG505 SOSIP.664-$2dCD4^{S60C}$ and FVC_{Env}SOSIP.664-$2dCD4^{S60C}$ are highly immunogenic and elicit potent, broadly neutralizing antibodies, the extent of which has never been reported previously for SOSIP.664 trimers. Adding to our previous results, the ability to consistently elicit these types of potent, cross-neutralizing antibody responses is dependent on novel epitopes exposed following the covalent binding of Env (independent of sequence and conformation) to $2dCD4^{S60C}$. These findings justify further investment into research exploring modified open, CD4-bound Env conformations as novel vaccine immunogens.

Introduction

The HIV/AIDS pandemic has now continued for four decades, with approximately 37.7 million individuals living with HIV globally, and 1.5 million new infections reported in 2020 [1]. While these numbers have vastly decreased from 1997 when the new infections peaked at 2.9 million [1], a decisive halt to this spread can only be achieved with an effective prophylactic vaccine. The broad diversity of HIV-1 has contributed to the difficulty in identifying an Env-based vaccine immunogen capable of inducing immunity against all circulating HIV-1 groups and clades. To address viral diversity, researchers have tried to design immunogens that direct the immune responses towards regions of the viral envelope glycoprotein (Env) that are highly conserved, such as the CD4 receptor binding site (CD4bs), the gp120-gp41 interface, the V1V2 trimer apex, and the membrane proximal external region (MPER) [2]. A number of broadly neutralizing antibodies (bnAbs) that target these conserved sites have been isolated from HIV-infected individuals thereby showing the possibility of their induction in humans. However, if HIV-infected individuals develop bnAbs, it is too late to impact on disease progression. By contrast,

* Corresponding author at: HIV Pathogenesis Research Unit, Faculty of Health Sciences, University of the Witwatersrand, 7th floor, Rm 7Q17, 7 York Road Parktown, 2193, South Africa.

E-mail addresses: Nancy.tumba@wits.ac.za (N.L. Tumba), gavin.owen@wits.ac.za (G.R. Owen), mark.killick@wits.ac.za (M.A. Killick), maria.papathanasopoulos@wits.ac.za (M.A. Papathanasopoulos).

https://doi.org/10.1016/j.jvacx.2022.100222
2590-1362/

their neutralizing potential, and their ability to protect against infection in animal challenge models has also been studied [3–5], providing evidence of the importance of preexisting humoral immune responses in preventing transmission.

In an effort to improve immunogens, the HIV-1 vaccine research field has made use of multiple techniques such as mutations, cleavage site disruption and the introduction of trimerization domains to create stabilized, recombinant HIV-1 Env trimers [6,7]. This gave rise to SOSIP trimers, the first Env-based immunogens with antigenic and topological structures that best represent the native HIV-1 virion trimeric spike [7–9]. When assessed in rabbits, the stabilized SOSIP trimers induce autologous neutralizing antibodies as well as neutralization of the more sensitive Tier-1 viruses [10]. Env trimers can be stabilized in the closed, prefusion conformation or the open, post-CD4 engaged state. These different Env conformations expose diverse epitopes and ultimately impact the elicitation of neutralizing antibodies, relevant for HIV-1 vaccine design [10,11]. Some of the broadest and most potent antibodies characterized from HIV-infected individuals target epitopes presented in the closed, prefusion conformation, when the trimer is in its quaternary fold. As a result, the majority of trimer and immunogen design efforts have been directed towards fastening the Env in the closed conformation, with studies on SOSIP trimers being the most advanced. However, not much research has been directed towards the use of SOSIP trimers in the CD4-bound, open conformation.

It is believed that sites of vulnerability of HIV-1 may be contextual and that the different Env conformations (i.e., prefusion, CD4-bound intermediates, pre-hairpin intermediate, and postfusion) structurally expose different epitopes for antibodies to target [2]. The CD4-bound conformation seems to contain less neutralization epitopes than the closed, prefusion conformation of Env, evidenced by the fewer number of antibodies identified from natural infection that specifically target this state [2]. The CD4-bound Env conformation exists in two distinct states- when a single gp120 monomer is engaged by CD4 (asymmetric trimer) or when all three gp120 monomers are bound to CD4 (open trimer) [12]. The transient nature of these CD4-bound conformations may contribute to the lack of antibodies to these epitopes in natural infection [13].

Transmitted/founder (TF) HIV-1 virions are defined as the viruses that establish productive infection following mucosal transmission [14,15]. They exhibit certain characteristics such as shorter variable loops, less putative *N*-linked glycans, enhanced interactions with dendritic cells, better infectivity as cell-free virions, and a preference for the chemokine receptor 5 (CCR5) [14]. Of interest, the shorter variable loops and reduced number of glycans have been linked to a modest increase in susceptibility to neutralizing antibodies [14,16]. Furthermore, TF viruses have displayed better interaction with the integrin pair α4β7 compared to chronic viruses [17]. $CD4^+$ T cells that express α4β7 are preferentially targeted in acute infection [18] and the binding of TF viruses to them could result in more efficient virus entry as well as cell-to-cell spread. The differences inherent in TF viruses compared to chronic ones highlight the significant role of TF viruses in HIV-1 infection and the need to develop immunogens that elicit antibodies able to recognize these 'fitter' viruses.

Previous work using an Env immunogen composed of the consensus sequence obtained from over 1,800 subtype C TF envelopes [19]; namely, the Founder Virus C Env (FVC_{ENV}), covalently complexed to the first two domains of CD4 (2dCD4) via cysteine linkage through targeted intermolecular binding of gp120 to CD4 by introduction of a serine to cysteine substitution in CD4 [20] ($2dCD4^{S60C}$) gave rise to exceptional immunogens [21]. The former study made use of various Env conformations, from monomeric to leucine-zipper linked gp140 trimers, with the same outcome: all consistently elicited potent, cross-neutralizing antibody activity against clinically-relevant HIV-1 isolates in New Zealand white rabbits [21] and rhesus macaques (Pereira *et al.* unpublished data).

Here we describe the SOSIP trimerization of FVC_{ENV} as compared to the well characterized BG505. SOSIP trimers constitute the best antigenic mimics of HIV-1 in its native state and, therefore, provide an excellent immunogen model. Moreover, by locking the BG505 and FVC_{ENV} SOSIP.664 trimer in the CD4-bound, open state by covalently complexing our SOSIP Env trimer to $2dCD4^{S60C}$, we interrogated whether the CD4-bound conformation of SOSIP trimers is antigenically compromised or would replicate and possibly improve on results seen with other SOSIP.664 and Env-$2dCD4^{S60C}$ conformations. Consequently, the immunogenicity of BG505 SOSIP.664-$2dCD4^{S60C}$ and FVC_{ENV} SOSIP.664-$2dCD4^{S60C}$ was evaluated in New Zealand white rabbits and the antisera response characterized and compared for neutralization potential.

Materials and methods

Ethics statement. Twenty female New Zealand white rabbits were maintained at the Wits Research Animal Facility (WRAF) in a pathogen-free environment, where all animal work was conducted in accordance with the Animal Research Ethics Committee (AREC) directives and National/International recommendations. Animal housing, care and the immunization and bleed protocols were all conducted in accordance with the institutional requirements set by AREC and WRAF. All AREC comply with the South African National Standard on the care and use of animals for scientific research. Clearance was obtained from the Animal Ethics Screening Committee, University of the Witwatersrand (certification #: 2018/02/10B).

Recombinant plasmids description. The BG505 SOSIP.664 clone in the VRC3831 plasmid was kindly provided by Dr. Peter Kwong. The subtype C derived FVC *env* gene constructed from the consensus sequence of TF viruses [19] was used to incorporate the SOSIP.664 mutations: I559P, A501C, T605C, ^{508}REKR^{511} to R6, and MPER truncation at residue 664 [7,8]. The codon-optimized FVC_{ENV} SOSIP.664 clone was synthesized by GeneArt (Life Technologies, Regensburg, Germany) and cloned into the pCDNA3.3 expression vector (Invitrogen, Grand Island, NY). The plasmid containing the coding-sequence of the furin protease, i.e., pcDNA3.1-Furin, was obtained from Dr. J.P. Moore [8]. Two-domain CD4 (2dCD4) plasmids- pET15b-$2dCD4^{(WT/S60C)}$ were used for the expression of wild-type (WT) and S60C mutant versions of 2dCD4. The pET15b (Novagen, Germany) plasmids contain a 6 × polyhistidine (His) tag at the 3′ terminus in frame with the 2dCD4 sequence to allow for Nickel affinity purification.

Mammalian cell lines and antibodies. The CD4- and CCR5-positive TZM-bl cells were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (ARRRP), (Contribution of Dr. John C. Kappes, Dr. Xiaoyun Wu, and Tranzyme Inc.). The HEK293T cells were obtained from the American Type Culture Collection, also through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH. TZM-bl and 293 T cells were cultured in Dulbecco's minimal essential medium (DMEM; Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% v/v heat-inactivated fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 2 mM Glutamax (Gibco/Invitrogen, Waltham, MA, USA), and 100 U/mL penicillin–streptomycin (Gibco/Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) to prepare the complete growth medium. Cells were incubated at 37°C in 5% CO_2 and 80% relative humidity. Purified mAbs PG9 (Dr. D. Burton), VRC01 & VRC03 (Xueling Wu, Zhi-Yong Yang, Yuxing Li, Gary Nabel, John Mascola) [22], HJ16 (Dr. Antonio Lanzavecchia), and 10E8 (Dr. Mark Connors) were obtained from ARRRP.

Expression plasmids encoding heavy- and light-chain sequences of the monoclonal antibodies 17b and PGT145 were a generous gift from Dr. Pascal Poignard.

Subcloning of FVC_{Env} into the VRC3831 backbone. To improve the expression of FVC_{ENV} recombinant protein, the FVC_{ENV} coding region from the pCDNA3.3 plasmid was subcloned into the VRC3831 plasmid backbone using the Gibson Assembly cloning method (New England Biolabs, USA), according to manufacturer's instructions. The integrity of the resultant clones was verified by sequencing along the 5′- and 3′-end portions of the inserted gene using vector-specific primers (SeqinF: 5′TGTGATCAGATATCGCGĠ3 and SeqinR: 5′AAGGCACTGGGGAGGGGĆ3) with the ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) using an automated ABI 3100 genetic analyzer. Chemically competent *E. coli* DH5α bacterial cells were transformed with the final DNA constructs as previously described [23]. Transformants were selected on Luria Bertani agar plates supplemented with 50 μg/mL kanamycin. Plasmid DNA was isolated and purified using QIAGEN™ plasmid purification kits (Qiagen, Maryland, USA) according to the manufacturer's instructions.

Expression and purification of Env SOSIP.664 trimers. HIV-1 Env were expressed in HEK293T cells following transient co-transfection with the Env plasmids and pcDNA3.1-Furin as described previously [22]. Supernatants containing the secreted Env recombinant proteins were clarified by centrifugation at low speed and filtration through a 0.22 μm filter. Purification of the SOSIP.664 trimers from the supernatant was performed using agarose beads-conjugated *Galanthus nivalis* lectin (Sigma-Aldrich) column chromatography with affinity for mannose glycans, as previously described [24]. A second purification by gel filtration chromatography (HiPrep Sephacryl S-300; GE Healthcare, Piscataway, NJ) with an AKTA FPLC (GE Healthcare) was performed to isolate the trimers from aggregates, dimers, and monomers, followed by concentration using centrifugal filtration with a 50 kDa MWCO Amicon Ultra filter (Millipore, Billerica, MA, USA). The final protein concentration was determined by bicinchoninic acid protein assay (Pierce, Rockford, IL). The purity of expressed proteins was evaluated by reducing and non-reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and by Western blotting to confirm size and identity, as well as by blue native (BN)-PAGE to confirm the trimeric state.

Expression and purification of 2dCD4. Competent BL21* *E. coli* (DE3; Invitrogen) cells (New England Biolabs, Ipswitch, MA, USA) were transformed with pET15b-$2dCD4^{WT/S60C}$ plasmids using a standard protocol (Novagen, USA). Bacterial cells were propagated at 37°C under ampicillin selection pressure in LB medium (1% w/v tryptone, 0.5% w/v yeast, 1% w/v NaCl, pH 7.3). Recombinant $2dCD4^{WT/S60C}$ proteins were expressed and purified as described previously [20]. Briefly, the 2dCD4 insoluble fraction was purified by denaturing the proteins using chaotropic agents, capturing the his-tagged 2dCD4 using Nickel affinity, and refolding by a gradual removal of chaotropes in buffer-exchange dialysis. Following refolding of the expressed 2dCD4 proteins using a series of dialysis steps, the proteins were concentrated with an Amicon Ultra filter 10 kDa MWCO (Millipore, Billerica, MA, USA) and stored at −80°C.

Env:CD4 immunogen complex formation and purification. The purified, recombinant Env proteins (BG505 SOSIP.664 or FVC_{ENV} SOSIP.664) were complexed to the purified 2dCD4 proteins ($2dCD4^{WT}$ or $2dCD4^{S60C}$) by overnight incubation at 4°C using a 3-fold molar excess of the soluble 2dCD4 proteins. Formation of the intermolecular disulphide bridge in the complexes with the mutant $2dCD4^{S60C}$ was facilitated by addition of 1 mM β-mercaptoethanol as a reducing agent. The complexes were centrifuged at 3,500 × g for 5 min to remove precipitates. The complexes were purified from unbound 2dCD4 by size exclusion chromatography using a HiPrep 16/60 Sephacryl S-300 HR column (GE Healthcare), followed by concentration with a 50 kDa MWCO Amicon centrifugal filter.

Enzyme-Linked Immunosorbent Assay (ELISA). The monoclonal antibodies 17b and PGT145 were expressed and purified as described previously [25,26]. To determine the structural integrity and conformational antigenicity of the recombinant BFG505 SOSIP.664 and FVC_{ENV} SOSIP.664 trimers and the complexes, the reactivity of the Env to human-derived HIV-1 specific mAbs was evaluated in ELISAs as previously described [9]. Immuno MaxiSorp 96-well plates (Nunc) were coated with Env or Env-$2dCD4^{S60C}$ complexes used in the vaccinations at 2 μg/ml. The following HIV-1 specific mAbs were added in a 3-fold serial dilution starting at 10 μg/ml: PG9, 10–1074, IgG1b12, VRC01, A32, 35O22, PGT145, 10E8, and 17b. The presence of bound antibodies was detected with a horseradish peroxidase-conjugated anti-human IgG antibody (GE Healthcare).

Immunizations and serum collection. New Zealand white rabbits were divided into four groups with five animals in each group. All experimental animals weighed between 3 and 5 kg at the commencement of the study. The rabbits were given intramuscular injections of 50 μg total protein/animal of either FVC_{ENV} SOSIP.664 (group A), $2dCD4^{S60C}$ (group B), BG505 SOSIP.664-$2dCD4^{S60C}$ complex (group C), or FVC_{ENV}-$2dCD4^{S60C}$ complex (group D). Each injection was adjuvanted with Adjuplex Adjuvant (Advanced BioAdjuvants, Omaha, Nebraska) comprising of detergent-free lecithin and the carbomer homopolymer. The immunization schedule consisted of multiple doses with injections on days 0, 28, 56, and 84 with bleeds on days 0 (prior to immunization), 14, 42, 70, and 98.

Pseudovirion production. The plasmid encoding the HIV-1 *env* gene of strains QH0692.42, 398_F1, 246F3, TRO.11, X2278, CAP210.2.00.E8, Du422.12, 246F, 25710, CE0217, ZM53.12, CNE8, CNE55, CH119.10, BJOX2000, CE1176, X1632, and the *env* gene of the vesicular stomatitis virus glycoprotein (VSV-G; control) were obtained from the ARRRP and used in the production of pseudovirions. Recombinant pseudoviruses were produced in HEK293T cells as previously described [27]. The $TCID_{50}$ of each pseudovirus batch was determined, as described previously [28].

TZM-bl neutralization assay. Neutralization of pseudovirions was measured with the TZM-bl cells-based luciferase assay as previously described [29]. Neutralization was determined as the difference in RLU between the test wells and the cells-only control wells divided by the difference in RLU values between the virus-only control and cells-only control wells [30]. Each serum was tested against the VSV-G pseudoviruses to confirm the HIV-1 Env specificity of neutralization. Positive controls included human monoclonal antibodies (mAbs) PG9 and VRC03 at concentrations ranging between 0.005 and 10 μg/mL. Neutralization was reported as the 50% inhibitory dilution (ID_{50}) for sera, or 50% inhibitory concentration (IC_{50}) for mAbs which represent the dilution of serum (or mAbs) resulting in a 50% reduction in RLU.

Results

FVC_{ENV} SOSIP.664 amino acid sequence comparison to BG505 and other Env sequences

The BG505 SOSIP.664 modifications [9] were introduced in the original FVC_{ENV} sequence [19] to produce the FVC_{ENV} SOSIP.664 gp140 protein. The changes included the A501C and T605C mutations to create the interprotomer SOS disulphide link, the I559P mutation to stabilize the trimer, the replacement of the R517, E518, K519, R520 to to 6×Arg (R517-R522) for the furin cleavage site, and finally the sequence truncation after the Asp at position

664 to delete the MPER region of gp41 and to enhance the solubility of the protein (Supplementary Fig. S1). An Asn at position 332 and a Ser at position 334, the potential *N*-linked glycosylation site on which certain V3-directed antibodies are highly dependent, was already present in the FVC_{ENV} sequence. Comparatively, the FVC_{ENV} sequence, which is a consensus envelope of subtype C founder viruses [19], contained shorter V1V2 and V4 loops (Supplementary Fig. S1). The FVC_{ENV} SOSIP.664 sequence contains 17 *N*-linked glycosylation sequons (Supplementary Fig. S1).

FVC_{ENV} SOSIP.664 and BG505 SOSIP.664 express native-like trimers and form protein complexes with 2dCD4^{S60C}

The FVC_{ENV} SOSIP.664 and BG505 SOSIP.664 trimers were successfully expressed in HEK293T cells and purified via lectin-affinity chromatography followed by size-exclusion chromatography (*SEC*) (Fig. 1). While the trimer fraction constituted the largest peak, there was a proportion of dimers and monomers formed for both SOSIP.664 Envs (Fig. 1A). Reducing and non-reducing SDS-PAGE confirmed successful cleavage of the FVC_{ENV} and BG505 trimers as observed by the transition of gp140 to gp120 in the presence of DTT (Fig. 1B). This indicates that the optimal 6-arginine cleavage site and co-transfection with furin provided efficient cleavage. Purified FVC_{ENV} and BG505 SOSIP.664 trimers were complexed to 2dCD4^{S60C} in the presence of a low concentration of reducing agent, to facilitate reduction of the native disulphide bond and subsequent formation of the intermolecular covalent bond between Env and 2dCD4 locking the FVC_{ENV} and BG505 SOSIP.664 Envs in the open, CD4-bound conformation. *SEC* purification of the FVC_{ENV}-2dCD4^{S60C} and BG505-2dCD4^{S60C} complexes showed elution of the higher molecular weight complexes (Fig. 1A) whose increased molecular mass was confirmed by BN-PAGE (Fig. 1C).

FVC_{ENV} SOSIP.664 produces native-like trimers with preserved antigenic qualities, similar to BG505 SOSIP.664

We used ELISAs to quantify the binding of various HIV-1 neutralizing antibodies to the SOSIP.664 gp140 trimers in the unliganded and 2dCD4^{S60C} complexed forms. The SEC-purified SOSIP.664 trimers (FVC_{ENV} and BG505) were immobilized onto ELISA plates and we monitored the binding of serially-diluted mAbs that recognize various regions of the Env, namely, the quaternary epitope V1V2 apex (PG9 & PGT145), a V3 glycan epitope (10–1074), the CD4 binding site (VRC01 & IgG1b12), the first and second constant region C1-C2 (A32), the gp120/gp41 interface (35O22), and the MPER (10E8) (Fig. 2A). Antibodies whose epitopes are only formed when the trimer is in the quaternary native fold-such as PG9, PG16 and PGT145- are used to confirm trimers have adopted the correctly folded, quaternary conformation. All three of these antibodies recognize the V1V2 crown of the trimer when the protomers are in close proximity to each other in the closed, prefusion trimeric conformation (Fig. 2B). This epitope is compromised in the open, CD4-bound conformation although the PG9 and PG16 somatically-related antibodies indiscriminately bind to the monomeric versions of Env albeit with lower affinity [31]. These antibodies are also dependent on N160 or N156 glycans which are present in the FVC_{ENV} and BG505 trimers based on the amino acid sequence analysis (Supplementary Fig. S1). We observed strong binding of the VRC01, IgG1b12, 10–1074 and PG9 antibodies and weaker binding of the A32, 35O22 and PGT45 antibodies for the FVC_{ENV} SOSIP.664 trimer (Fig. 2A). Similarly, VRC01, 10–1074 and PG9 bound well to the BG505 SOSIP.664 trimer, however,

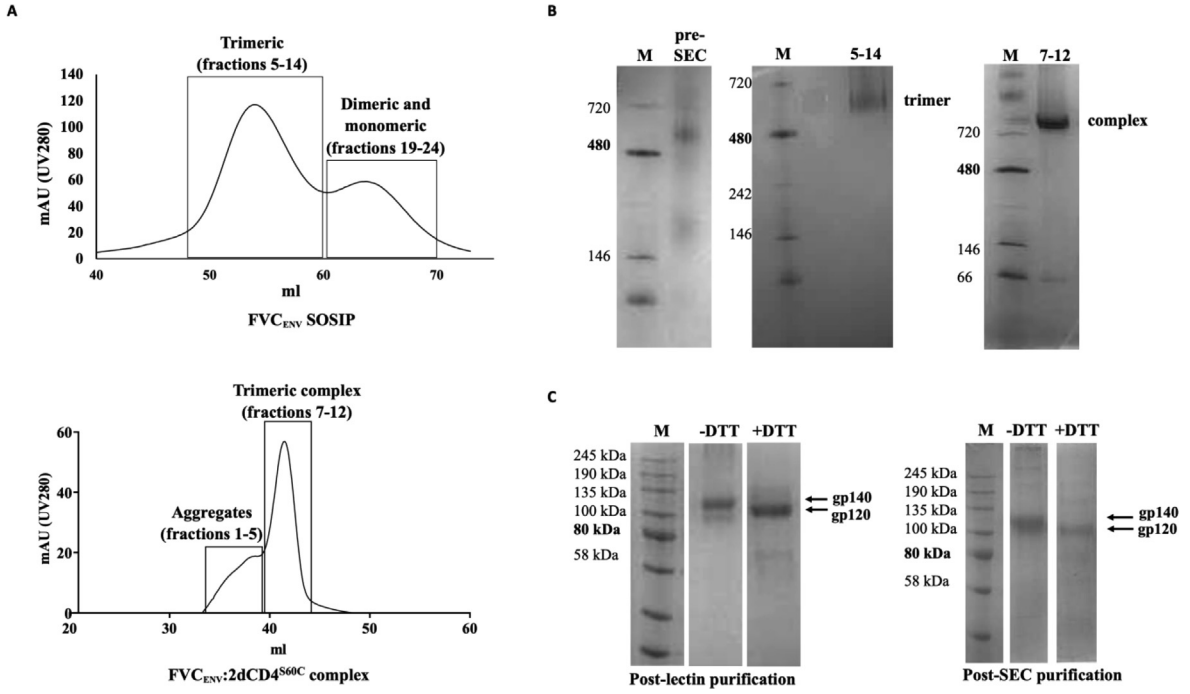

Fig. 1. Biochemical characterization of FVC_{ENV} and BG505 SOSIP.664 trimers covalently complexed to 2dCD4^{S60C}. FVC_{ENV} and BG505 SOSIP.664 trimers complexed to 2dCD4^{S60C} were purified using size-exclusion chromatography and analysed by SDS-PAGE and BN-PAGE. (A) HiPrep Sephacryl S-300 HR *SEC* profile of lectin-purified FVC_{ENV} SOSIP.664 and BG505 SOSIP.664 gp140 expressed in HEK293T cells before and after complexing to 2dCD4^{S60C} with peaks correlating to aggregates, trimers and monomers annotated. (B) Reducing (+DTT) and non-reducing (-DTT) SDS-PAGE analysis with 10% Tris-Glycine gels of lectin-purified FVC_{ENV} SOSIP.664 gp140 and BG505 SOSIP.664, following Coomassie blue staining. Subsequent SEC-purified FVC_{ENV} SOSIP.664 gp140 also shown. The shift of the gp140 band to gp120 under reducing conditions indicates furin-mediated cleavage to allow gp120-gp41 dissociation. The molecular weight marker (M), indicated on the left, is the broad-range, color prestained protein standard (NEB). (C) Coomassie-stained BN-PAGE analysis of FVC_{ENV} and BG505 pre- and post-*SEC* purification as well as the FVC_{ENV}:2dCD4^{S60C} complex post- *SEC* pooled fractions. The molecular weight marker (M), indicated on the left, is the NativeMark™ protein standard (Thermo Fisher Scientific).

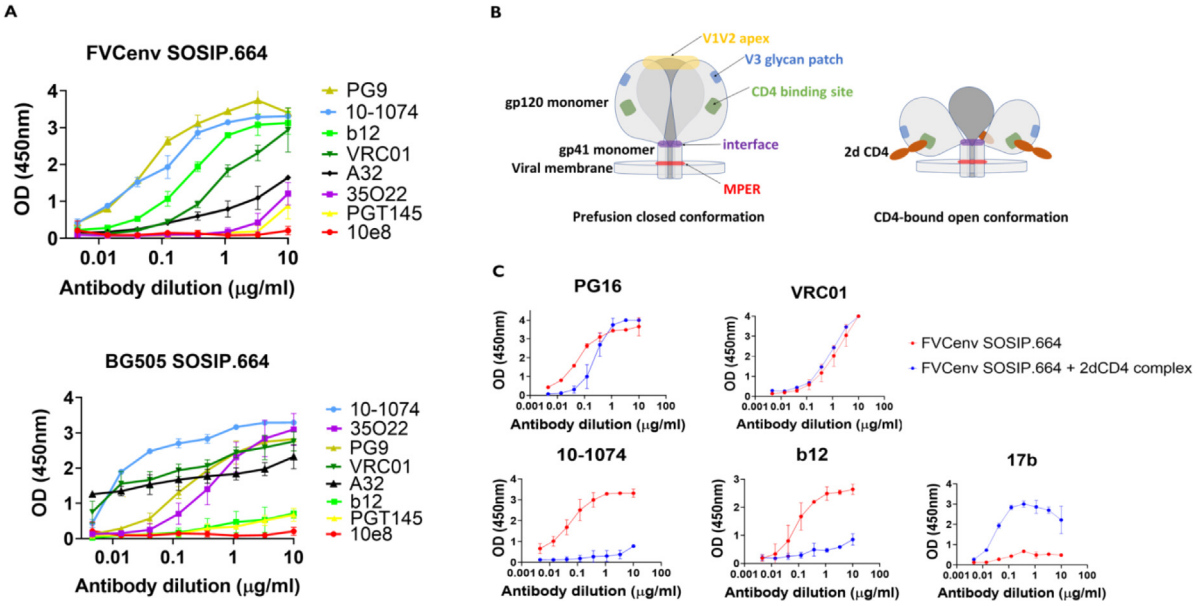

Fig. 2. Antigenicity of SOSIP.664 trimers (FVC_{ENV} and BG505) and complexes. (A) Recognition of FVC_{ENV} and BG505 trimers by a selection of antibodies targeting the V1V2 apex (yellow curves- PG9 & PGT145), the CD4 binding site (green curves- IgG1b12 & VRC01), the V3 region (blue curve- 10–1074), the gp120/gp41 interface (purple curve- 35O22), the C1-C2 region (black curve- A32), and an MPER epitope (red curve- 10E8) used as a negative control. (B) Model of unliganded SOSIP.664 trimer in the closed, prefusion conformation (left) and the open, CD4-bound conformation (right). The trimer spike is represented with two of the three protomers shown in light grey and the third protomer shown in dark grey with broadly neutralizing antibodies epitopes indicated with arrows and colored in orange for the V1V2 apex, blue for the V3 glycan patch, green for the CD4 binding site, purple for the gp120/gp41 interface, and red for the MPER region. (C) Effect of complexing FVC_{ENV} to $2dCD4^{S60C}$ on the high-binding antibodies PG16, VRC01, IgG1b12, and 10–1074 as well as the CD4-induced mAb 17b. Red curves represent FVC_{ENV} SOSIP.664 trimers and blue curves the FVC_{ENV} SOSIP.664 trimers complexed to $2dCD4^{S60C}$.

the binding of IgG1b12 - while detectable - was significantly lower than its recognition of FVC_{ENV} (Fig. 2A). In contrast, antibodies A32 and 35O22 displayed more pronounced binding to the BG505 SOSIP.664 Env compared to the FVC_{ENV} SOSIP.664 trimer (Fig. 2A). 10E8 was included as a negative control as its epitope is absent in the SOSIP.664 trimers and it showed no binding to the FVC_{ENV} SOSIP.664 trimer (Fig. 2A).

We then tested the effect of complexing FVC_{ENV} SOSIP.664 to $2dCD4^{S60C}$ on the antibodies which bound well to the trimer. We observed that the binding of the CD4bs antibody IgG1b12 was substantial for the FVC_{ENV} SOSIP.664 trimer but was significantly reduced in FVC_{ENV} SOSIP.664-$2dCD4^{S60C}$ (Fig. 2C). The binding of the other CD4bs mAb VRC01 remained unchanged between FVC_{ENV} SOSIP.664 and FVC_{ENV} SOSIP.664-$2dCD4^{S60C}$ (Fig. 2C). Recognition of the V1V2 apex mAb PG16 was also similar for the unliganded FVC_{ENV} SOSIP.664 and the $2dCD4^{S60C}$-complexed trimer (Fig. 2C). Another monoclonal antibody that lost most of its binding capacity in FVC_{ENV} SOSIP.664-$2dCD4^{S60C}$ was the V3 glycan binding mAb 10–1074 (Fig. 2C). In addition, we were able to confirm CD4 binding through preferential recognition of the CD4-induced epitope by the 17b mAb in the FVC_{ENV} SOSIP.664-$2dCD4^{S60C}$ complex (Fig. 2C).

Immunization with BG505 SOSIP.664 and FVC_{ENV} SOSIP.664-$2dCD4^{S60C}$ elicits potent, cross-neutralizing antibodies in rabbits.

To evaluate the ability of the covalently complexed BG505 SOSIP.664-$2dCD4^{S60C}$ and FVC_{ENV} SOSIP.664-$2dCD4^{S60C}$ to induce humoral immune responses, we conducted immunogenicity studies with the purified recombinant proteins (expressed in mammalian cells with natural glycosylation preserved) using rabbits as the pre-clinical hosts. Each group of immunized animals was given either the $2dCD4^{S60C}$ protein, unliganded FVC_{ENV} SOSIP.664, BG505 SOSIP.664-$2dCD4^{S60C}$ or FVC_{ENV} SOSIP.664-$2dCD4^{S60C}$, as outlined in the immunization schedule in Fig. 3A.

The presence of neutralizing antibodies in the immunized rabbit sera was determined using the standard TZM-bl neutralization assay against 17 Tier 2 pseudoviruses comprising clades A, AC, B, C, AE, BC, E, and G (Fig. 3B). These Tier 2 pseudoviruses are considered as excellent determinants of antibodies neutralizing potential as they provide an exceptional representation of biologically relevant strains that have Env conformations of most circulating viruses [32]. We used sera collected two weeks after the last immunization as the antibody responses are shown to be at the highest titres following the fourth longitudinal immunization and neutralizing antibodies PG9 and VRC03 were used as controls (Fig. 3B). Results demonstrate that the covalently complexed Env SOSIP trimers (both FVC_{ENV} and BG505) were able to elicit antibodies that display robust neutralization of 12 (BG505 complex) and 13 (FVC_{ENV} complex) of the seventeen pseudoviruses tested (Fig. 3B). Comparatively, sera from the rabbits immunized with the unliganded FVC_{ENV} (Env control) were only able to weakly neutralize two of the pseudoviruses (398_F1 and CH119.10) and the sera from animals who received the $2dCD4^{S60C}$ show negligible neutralization with only two rabbits able to neutralize one pseudovirus (QH0692.42) at titers below ID_{50} values of 240 (Fig. 3B). Of interest, all five animals who received FVC_{ENV} SOSIP.664-$2dCD4^{S60C}$ generated a cross-neutralizing antibody response against twelve of the thirteen pseudoviruses neutralized with two antisera - from rabbits 0289 and 0330 - failing to neutralize the Du422.12 pseudovirus (Fig. 3B). Similarly, all the animals who received BG505 SOSIP.664-$2dCD4^{S60C}$ elicited cross-neutralizing antibodies against ten of the twelve neutralized pseudoviruses, with two rabbits (0300 and 0329) whose sera could not neutralize two of the clade C pseudoviruses (CAP210.2.00.E8 and Du422.12) (Fig. 3B). Overall, the cross-neutralizing response was consistently elicited in all the rabbits who received the covalent complexes with a few exceptions for one or two pseudoviruses. As expected, PG9 and VRC03 displayed different neutralization profiles against the panel of 17 pseudoviruses.

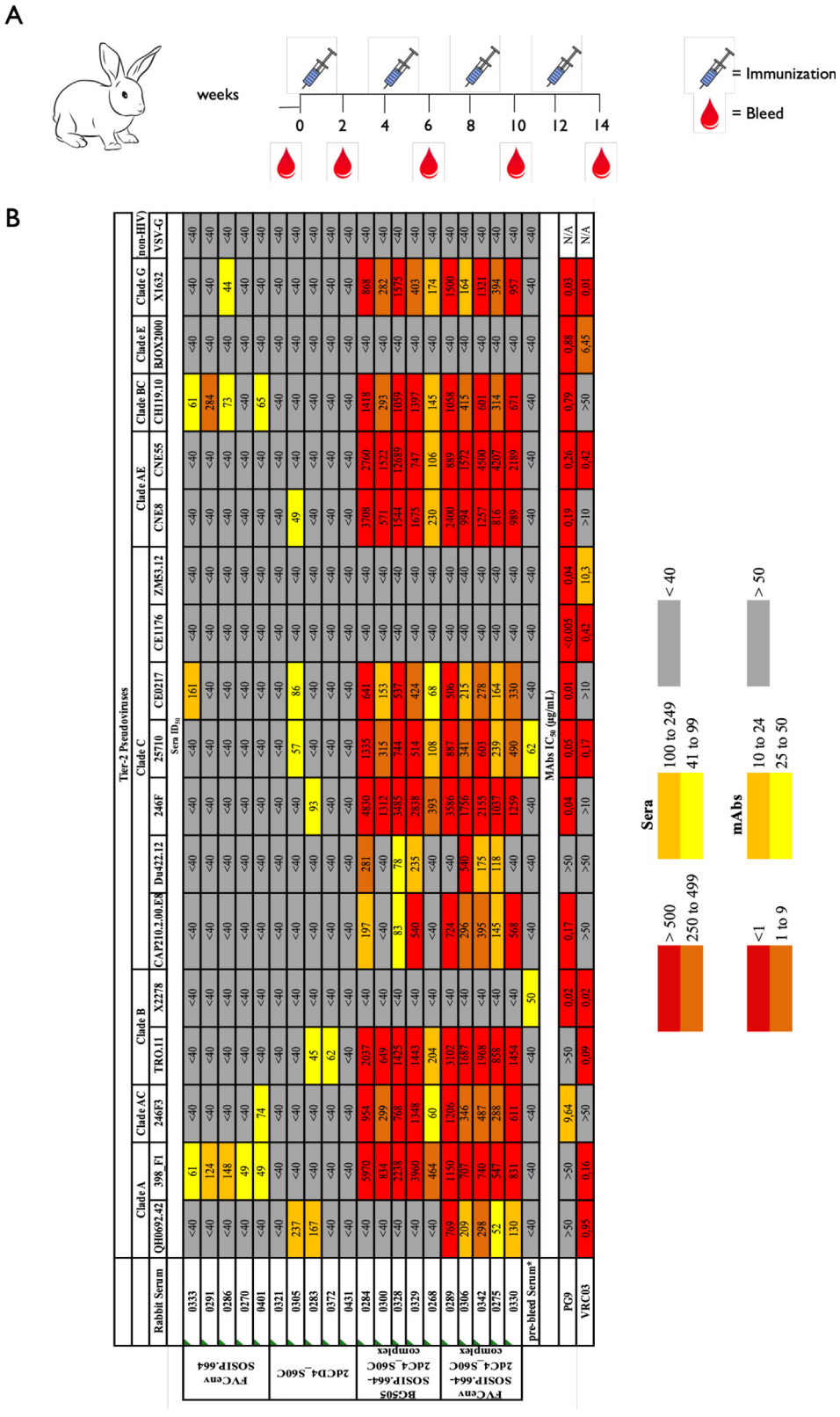

Fig. 3. Rabbit sera neutralization of a Tier 2 pseudovirus panel. (A) Schematic of rabbit immunization schedule with FVC_{ENV} SOSIP.664, $2dCD4^{S60C}$, BG505 SOSIP.664-$2dCD4^{S60C}$ and FVC_{ENV} SOSIP.664-$2dCD4^{S60C}$. The rabbits were immunized four times, monthly, with 50 μg of the soluble proteins adjuvanted with Adjuplex. Two weeks prior to the first immunization and two weeks following each immunization, blood samples were collected. Immunizations and bleeds time-points are indicated in weeks. (B) Neutralization titers of the rabbit sera in each of the four immunization groups. Sera obtained prior to immunizations (pre-bleed) and at week 14, following the final immunization, were tested in a TZM-bl reporter cell assay against the VSV-G specificity control pseudovirus and HIV-1 pseudoviruses QH0692.42, 398_F1, 246F3, TRO.11, X2278, CAP210.2.00.E8, Du422.12, 246F, 25710, CE0217, ZM53.12, CNE8, CNE55, CH119.10, BJOX2000, CE1176, and X1632. Monoclonal antibodies (mAbs) PG9 and VRC03 were included as neutralization controls for each pseudovirus except the non-HIV VSV-G pseudovirus. Numerical values represent ID_{50} (for sera) and IC_{50} (for mAbs) neutralization titers. ID_{50} < 40 and IC_{50} > 10 indicate no detectable neutralization for the rabbit sera or mAbs, respectively.

Discussion

An important milestone in HIV- vaccine development is the ability to elicit potent, bNAbs following vaccination. Our results demonstrate the ability to induce antibodies that neutralize heterologous HIV-1 tier 2 pseudoviruses in rabbits, with a single immunogen complex composed of trimeric SOSIP.664 envelopes covalently coupled to 2dCD4^{S60C}. Sera from rabbits immunized with the FVC$_{ENV}$ SOSIP.664-2dCD4^{S60C} displayed neutralization against 13/17 (76,5%) of tier 2 HIV-1 isolates from clades A, AC, B, C, AE, BC, and E, at notably high titers. Similarly, sera from rabbits immunized with the BG505 SOSIP.664-2dCD4^{S60C} complex were also able to neutralize the same pseudoviruses, with the exception of the clade A QHO692.42 pseudovirus, with comparatively high titers. The foundation of this work was accomplished in a previous study where various HIV-1 Env conformations (monomeric gp120 and GCN4 trimeric Env) covalently complexed to 2dCD4^{S60C} elicited potent, broadly neutralizing antibodies against 100% of twelve subtype B and C isolates made up of tier-1, tier-2 and a single tier-3 pseudoviruses [21]. This study further investigated the impact of SOSIP.664 (the archetype HIV-1 trimer mimic) on the immunogenicity of the covalently bound complexes locked in an open, 2dCD4-bound conformation in small animal models, comparing the well characterized BG505 sequence to FVC$_{Env}$.

Overall, sera from rabbits immunized with BG505 SOSIP.664-2dCD4^{S60C} and FVC$_{ENV}$ SOSIP.664-2dCD4^{S60C} neutralized over 70% of the Tier-2 pseudoviruses tested, at exceptionally high potencies. While these results are significant, the spectrum of neutralization cross-reactivity of the SOSIP.664-2dCD4^{S60C} complexes is narrower compared to the Envs in the previously reported study. This could be attributed to the difference in Env conformation or to the inclusion of pseudoviruses from clades A, AC, AE, BC, E and G which were not tested in the previous study [21]. Of note, one clade B (X2272) and two clade C (CE1176 and ZM53.12) pseudoviruses were resistant to neutralization in this study while all clade B and C pseudoviruses tested were neutralized in the Killick *et al.* study [21]. However, no direct comparison can be made as the pseudovirus panels used in the antibody neutralization assay varied between the two studies.

As expected, the rabbit sera from the groups immunized with either FVC$_{ENV}$ SOSIP.664 or 2dCD4^{S60C} exhibited low potency, antibody neutralization potential, with narrow breadth. A limitation of this study is that we did not include immunization with unliganded BG505 SOSIP.664 as a control. However, multiple groups have previously tested unliganded BG505 SOSIP.664 in rabbit immunizations, and shown its immunogenicity is limited to eliciting antibodies capable of neutralizing the autologous, immunogen-matched pseudovirus with the occasional cross-neutralization but of very limited breadth and potency [11,33]. Thus, in an effort to reduce the number of animals used in experimentation, the ethical decision was taken not to include an unliganded BG505 SOSIP.664 group, particularly since an unliganded FVC$_{Env}$SOSIP.664 group was included. In summary, this is the first report of covalently bound SOSIP.664-2dCD4^{S60C} complexes capable of eliciting potent, broadly neutralizing antibodies in small animals, and further expands our knowledge on SOSIP.664 immunogenicity.

The observation that neither the Env sequence (BG505 or FVC$_{ENV}$), nor the Env conformation (SOSIP trimer in this study versus monomeric or GCN4-linked Env trimers described in Killick *et al.*, 2015) used influences the ability to elicit the potent, heterologous neutralizing response is suggestive that covalently complexing the Env with 2dCD4^{S60C} creates the auspicious conformational change responsible for the elicitation of the potent bnAbs displayed here.

The CD4-bound conformation has not been widely explored, particularly in terms of immunogen development, as prefusion (ligand-free) states are known to favour neutralizing antibodies epitopes [34,35], including conformational ones [9,36] while non-neutralizing antibodies sometimes target epitopes exposed upon CD4 binding such as the CD4-induced (CD4i) and the crown of the third hypervariable (V3) loop [37,38] epitopes. As such, immunogen development strategies have sought to maximize prevention of immunodominant (neutralization-hypersensitive) regions, targets of non-neutralizing antibodies, by engineering Envs with increasing mutations for structural stability of the trimer in its native state with much reduced CD4 affinity [39,40]. While it cannot be argued that the preferential antigenic properties of these improved trimers have been finely mapped and their improved immunogenicity in preclinical studies well demonstrated [11,41], the limitations of focusing on the closed, ligand-free trimer as an immunogen have been highlighted here and in other studies [21].

The CD4-bound conformation of Env has been shown to exhibit some conformational plasticity [42] as intermediate transitional conformations occur when the various loops engage with CD4 [43]. We have shown that the CD4-bound FVC$_{ENV}$ displays the open trimer conformation as evidenced by the improved binding of CD4-induced mAb 17b. We have also observed the loss of some bnAb epitopes in the CD4-bound conformation, most of which could be explained by either steric hindrance or epitope loss due to the conformational changes involved in binding to CD4 (as may have been the case for PGT145; however, the ELISA binding was not substantial enough to perform a comparative assessment between CD4-liganded and -free conformations). In the first instance, we observed the contrast between two CD4 binding site antibodies whereby IgG1b12 seemed to compete with the bound CD4 molecule evidenced by the reduced binding of the mAb in the CD4-bound conformation and VRC01 whose binding remained unaffected in the CD4-liganded and -unliganded conformations. This is in accord with other studies that have shown VRC01 to have less steric hindrance than IgG1b12 in the way they approach their cognate epitopes, with IgG1b12 having a loop-proximal angle (similar to soluble CD4) and being more sensitive to loop packing, whereas VRC01 has a different angle of approach and its interaction with the functional virus spike is amenable to some alteration of the spike configuration [44]. The loss of recognition of the V3-targetting antibody 10–1074 was unexpected as the V3 loop exposure should be heightened post-CD4 attachment with the removal of the V2 glycan (N197) which normally occludes V3 antibodies from accessing their epitopes [9]; however, a study looking at cell surface expressed Env conformational states with increasing CD4 engagement has shown that antibodies targeting the V3 high mannose patch can drastically differ in their reactivity to unbound- and CD4 induced triggered conformations [45]. Specifically, of the four V3-directed bNAbs they tested, PGT128 and PGT135 bound better to the CD4 engaged, open trimer compared to the closed trimer, the binding of 2G12 was largely unchanged for all Env conformations, while PGT121 preferentially bound to the ligand-free, closed trimer with a noticeable decrease in binding potential for the CD4-bound Env [45]- similar to our result for 10–1074. Future studies to validate the different structural conformations between our FVC$_{ENV}$ SOSIP.664-2dCD4$^{WT/S60C}$ complexes can be conducted using cryoelectron microscopy.

Notably, the conformational difference between closed and open states could expose additional epitopes and may explain the potent cross-neutralizing response seen here and similar studies [21]. Depletion of the antisera response in the Killick *et al.* study showed that the neutralizing response could be broadly mapped to the CD4 portion of the immunogen [21]. Exposure of previously shielded residues could explain the improved immunogenicity

seen with Env covalently complexed to 2dCD4^{S60C} as the epitopes may be presented in the Env and CD4 components of the complex. The potential concern in the vaccine field with using CD4-bound HIV-1 Env immunogens is that following vaccination, they could induce "self" antibodies that target the host CD4$^+$ *T*-lymphocytes. This concern was fueled by research conducted in the 1990's showing the presence of CD4-targeting autoantibodies in HIV-1 infected individuals which presumably arise from the presence of naturally occurring gp120-CD4 complexes during disease progression [46–55]. In a variety of clinical settings, anti-CD4 antibodies result in a decrease in CD4 expressing T cells [56–58]. However, in HIV-1 preclinical studies, anti-CD4 autoantibodies are rarely elicited by vaccination as evidenced by immunotoxicologic studies conducted in immunized cynomolgus macaques [59].

Of interest, antibodies which target host receptors have been discovered and engineered- Ibalizumab is an example of such a monoclonal antibody, which targets host CD4, and has shown efficiency in reducing viral load when administered as an antiviral therapeutic [60,61]. Furthermore, Ibalizumab has been FDA approved for use in combination antiretroviral treatment against multi-drug resistant HIV-1 in treatment experienced patients [62,63]. While Ibalizumab is a humanized monoclonal antibody, its approved use in a clinical setting sets a precedent for looking at modifying the current Env-2dCD4^{S60C} immunogen using rational design to ensure we only elicit Ibalizumab-like antibodies. Such an immunogen would be capable of consistently eliciting potent, broadly neutralizing antibodies against a range of clinically relevant HIV-1 strains, without the requirement of complex, sequential Env immunization regimens currently being tested for driving appropriate neutralizing antibody affinity maturation.

Funding

This study was supported in part by grants from the department of Molecular Medicine and Haematology, HIV Pathogenesis Research Unit (HPRU) and the South African National Research Foundation (Thuthuka 113998 & 129859).

CRediT authorship contribution statement

Nancy L. Tumba: Conceptualization, Funding acquisition, Validation, Writing – original draft. **Gavin R. Owen:** Conceptualization, Supervision, Validation. **Mark A. Killick:** Conceptualization, Supervision, Validation. **Maria A. Papathanasopoulos:** Conceptualization, Funding acquisition, Supervision, Validation.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvacx.2022.100222.

References

[1] UNAIDS. Fact sheet - Global HIV & AIDS statistics - 2021 fact sheet. 2021.
[2] Pancera M, Changela A, Kwong PD. How HIV-1 entry mechanism and broadly neutralizing antibodies guide structure-based vaccine design. Curr Opin HIV AIDS 2017;12:229–40.
[3] 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;503:224–8.
[4] Zwick MB, Labrijn AF, Wang M, Spenlehauer C, Saphire EO, Binley JM, et al. Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. J Virol 2001;75:10892–905.
[5] Walker LM, Sok D, Nishimura Y, Donau O, Sadjadpour R, Gautam R, et al. Rapid development of glycan-specific, broad, and potent anti-HIV-1 gp120 neutralizing antibodies in an R5 SIV/HIV chimeric virus infected macaque. Proc Natl Acad Sci U S A 2011;108:20125–9.
[6] Leaman DP, Zwick MB. Increased functional stability and homogeneity of viral envelope spikes through directed evolution. PLoS Pathog 2013;9:e1003184.
[7] Sanders RW, Vesanen M, Schuelke N, Master A, Schiffner L, Kalyanaraman R, et al. Stabilization of the soluble, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. J Virol 2002;76:8875–89.
[8] Binley JM, Sanders RW, Clas B, Schuelke N, Master A, Guo Y, et al. A recombinant human immunodeficiency virus type 1 envelope glycoprotein complex stabilized by an intermolecular disulfide bond between the gp120 and gp41 subunits is an antigenic mimic of the trimeric virion-associated structure. J Virol 2000;74:627–43.
[9] 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 Pathog. 2013;9:e1003618.
[10] Sanders RW, van Gils MJ, Derking R, Sok D, Ketas TJ, Burger JA, et al. HIV-1 VACCINES. HIV-1 neutralizing antibodies induced by native-like envelope trimers. Science. 2015;349:aac4223.
[11] de Taeye SW, Ozorowski G, Torrents de la Pena A, Guttman M, Julien JP, van den Kerkhof TL, et al. Immunogenicity of Stabilized HIV-1 Envelope Trimers with Reduced Exposure of Non-neutralizing Epitopes. Cell 2015;163:1702–15.
[12] Ma X, Lu M, Gorman J, Terry DS, Hong X, Zhou Z, et al. HIV-1 Env trimer opens through an asymmetric intermediate in which individual protomers adopt distinct conformations. Elife 2018;7.
[13] Munro JB, Gorman J, Ma X, Zhou Z, Arthos J, Burton DR, et al. Conformational dynamics of single HIV-1 envelope trimers on the surface of native virions. Science 2014;346:759–63.
[14] Parrish NF, Gao F, Li H, Giorgi EE, Barbian HJ, Parrish EH, et al. Phenotypic properties of transmitted founder HIV-1. Proc Natl Acad Sci U S A 2013;110:6626–33.
[15] Shaw GM, Hunter E. HIV transmission. Cold Spring Harb Perspect Med 2012;2.
[16] Derdeyn CA, Decker JM, Bibollet-Ruche F, Mokili JL, Muldoon M, Denham SA, et al. Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. Science 2004;303:2019–22.
[17] Nawaz F, Cicala C, Van Ryk D, Block KE, Jelicic K, McNally JP, et al. The genotype of early-transmitting HIV gp120s promotes α (4) β(7)-reactivity, revealing α (4) β(7) +/CD4+ T cells as key targets in mucosal transmission. PLoS Pathog 2011;7:e1001301.
[18] Tokarev A, McKinnon LR, Pagliuzza A, Sivro A, Omole TE, Kroon E, et al. Preferential Infection of α4β7+ Memory CD4+ T Cells During Early Acute Human Immunodeficiency Virus Type 1 Infection. Clin Infect Dis 2020;71:e735–43.
[19] Killick M, Capovilla A, Papathanasopoulos MA. Generation and characterization of an HIV-1 subtype C transmitted and early founder virus consensus sequence. AIDS Res Hum Retroviruses 2014;30:1001–5.
[20] Cerutti N, Mendelow BV, Napier GB, Papathanasopoulos MA, Killick M, Khati M, et al. Stabilization of HIV-1 gp120-CD4 receptor complex through targeted interchain disulfide exchange. J Biol Chem 2010;285:25743–52.
[21] Killick MA, Grant ML, Cerutti NM, Capovilla A, Papathanasopoulos MA. Env-2dCD4 S60C complexes act as super immunogens and elicit potent, broadly neutralizing antibodies against clinically relevant human immunodeficiency virus type 1 (HIV-1). Vaccine 2015;33:6298–306.
[22] Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, Seaman MS, et al. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science 2010;329:856–61.
[23] Hanahan D. Studies on transformation of Escherichia coli with plasmids. J Mol Biol 1983;166:557–80.
[24] Guenaga J, Dubrovskaya V, de Val N, Sharma SK, Carrette B, Ward AB, et al. Structure-Guided Redesign Increases the Propensity of HIV Env To Generate Highly Stable Soluble Trimers. J Virol 2015;90:2806–17.
[25] Scharf L, Scheid JF, Lee JH, West Jr AP, Chen C, Gao H, et al. Antibody 8ANC195 reveals a site of broad vulnerability on the HIV-1 envelope spike. Cell Rep 2014;7:785–95.
[26] Diskin R, Scheid JF, Marcovecchio PM, West AP, Klein F, Gao H, et al. Increasing the potency and breadth of an HIV antibody by using structure-based rational design. Science 2011;334:1289–93.

[27] deCamp A, Hraber P, Bailer RT, Seaman MS, Ochsenbauer C, Kappes J, et al. Global panel of HIV-1 Env reference strains for standardized assessments of vaccine-elicited neutralizing antibodies. J Virol 2014;88:2489–507.
[28] 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. J Virol 2005;79:10108–25.
[29] Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, et al. Antibody neutralization and escape by HIV-1. Nature 2003;422:307–12.
[30] Sarzotti-Kelsoe M, Bailer RT, Turk E, Lin CL, Bilska M, Greene KM, et al. Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1. J Immunol Methods 2014;409:131–46.
[31] 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;326:285–9.
[32] Montefiori DC, Roederer M, Morris L, Seaman MS. Neutralization tiers of HIV-1. Curr Opin HIV AIDS 2018;13:128–36.
[33] Klasse PJ, LaBranche CC, Ketas TJ, Ozorowski G, Cupo A, Pugach P, et al. Sequential and Simultaneous Immunization of Rabbits with HIV-1 Envelope Glycoprotein SOSIP.664 Trimers from Clades A, B and C. PLoS Pathog. 2016;12: e1005864.
[34] Ringe RP, Ozorowski G, Yasmeen A, Cupo A, Cruz Portillo VM, Pugach P, et al. Improving the Expression and Purification of Soluble, Recombinant Native-Like HIV-1 Envelope Glycoprotein Trimers by Targeted Sequence Changes. J Virol 2017;91.
[35] Yasmeen A, Ringe R, Derking R, Cupo A, Julien JP, Burton DR, et al. Differential binding of neutralizing and non-neutralizing antibodies to native-like soluble HIV-1 Env trimers, uncleaved Env proteins, and monomeric subunits. Retrovirology 2014;11:41.
[36] 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. Proc Natl Acad Sci U S A 2014;111:17624–9.
[37] Thali M, Moore JP, Furman C, Charles M, Ho DD, Robinson J, et al. Characterization of conserved human immunodeficiency virus type 1 gp120 neutralization epitopes exposed upon gp120-CD4 binding. J Virol 1993;67:3978–88.
[38] Sattentau QJ, Moore JP. Conformational changes induced in the human immunodeficiency virus envelope glycoprotein by soluble CD4 binding. J Exp Med 1991;174:407–15.
[39] Hauser A, Carnell G, Held K, Sulbaran G, Tischbierek N, Rogers L, et al. Stepwise Conformational Stabilization of a HIV-1 Clade C Consensus Envelope Trimer Immunogen Impacts the Profile of Vaccine-Induced Antibody Responses. Vaccines (Basel) 2021;9.
[40] Henderson R, Lu M, Zhou Y, Mu Z, Parks R, Han Q, et al. Disruption of the HIV-1 Envelope allosteric network blocks CD4-induced rearrangements. Nat Commun 2020;11:520.
[41] Chuang GY, Geng H, Pancera M, Xu K, Cheng C, Acharya P, et al. Structure-Based Design of a Soluble Prefusion-Closed HIV-1 Env Trimer with Reduced CD4 Affinity and Improved Immunogenicity. J Virol 2017;91.
[42] Wang H, Barnes CO, Yang Z, Nussenzweig MC, Bjorkman PJ. Partially Open HIV-1 Envelope Structures Exhibit Conformational Changes Relevant for Coreceptor Binding and Fusion. Cell Host Microbe. 2018;24: 579-92.e4.
[43] Wang Q, Finzi A, Sodroski J. The Conformational States of the HIV-1 Envelope Glycoproteins. Trends Microbiol 2020.
[44] Li Y, O'Dell S, Walker LM, Wu X, Guenaga J, Feng Y, et al. Mechanism of neutralization by the broadly neutralizing HIV-1 monoclonal antibody VRC01. J Virol 2011;85:8954–67.
[45] Ivan B, Sun Z, Subbaraman H, Friedrich N, Trkola A. CD4 occupancy triggers sequential pre-fusion conformational states of the HIV-1 envelope trimer with relevance for broadly neutralizing antibody activity. PLoS Biol 2019;17: e3000114.
[46] Keay S, Tacket CO, Murphy JR, Handwerger BS. Anti-CD4 anti-idiotype antibodies in volunteers immunized with rgp160 of HIV-1 or infected with HIV-1. AIDS Res Hum Retroviruses 1992;8:1091–8.
[47] Keiser P, Keay S, Wasserman S, Wecksler W. Anti-CD4 antibodies are associated with HIV-1 seroconversion and may be detectable before anti-HIV-1 antibodies. The Multicenter AIDS Cohort Study. AIDS Res Hum Retroviruses 1992;8:1919–27.
[48] Chams V, Idziorek T, Klatzmann D. Biological properties of anti-CD4 autoantibodies purified from HIV-infected patients. AIDS 1991;5:565–9.
[49] Wilks D, Walker LC, Habeshaw JA, Youle M, Gazzard B, Dalgleish AG. Anti-CD4 autoantibodies and screening for anti-idiotypic antibodies to anti-CD4 monoclonal antibodies in HIV-seropositive people. AIDS 1990;4:113–8.
[50] Callahan LN, Roderiquez G, Mallinson M, Norcross MA. Analysis of HIV-induced autoantibodies to cryptic epitopes on human CD4. J Immunol 1992;149:2194–202.
[51] Martin L, Idziorek T, Lehen A, Gluckman JC, Klatzmann D. Anti-CD4 autoantibodies in HIV-infected individuals: detection by two immunoenzymatic techniques. AIDS 1994;8:389–90.
[52] Müller C, Kukel S, Bauer R. Antibodies against CD4+ lymphocytes in plasma of HIV-infected patients are related to CD4 cell depletion in vivo. Immunol Lett 1994;41:163–7.
[53] Burastero SE, Gaffi D, Lopalco L, Tambussi G, Borgonovo B, De Santis C, et al. Autoantibodies to CD4 in HIV type 1-exposed seronegative individuals. AIDS Res Hum Retroviruses 1996;12:273–80.
[54] Beretta A, Furci L, Burastero S, Cosma A, Dinelli ME, Lopalco L, et al. HIV-1-specific immunity in persistently seronegative individuals at high risk for HIV infection. Immunol Lett 1996;51:39–43.
[55] Lopalco L, Magnani Z, Confetti C, Brianza M, Saracco A, Ferraris G, et al. Anti-CD4 antibodies in exposed seronegative adults and in newborns of HIV type 1-seropositive mothers: a follow-up study. AIDS Res Hum Retroviruses 1999;15:1079–85.
[56] Larché M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. J Allergy Clin Immunol 2003;111:450–63. quiz 64.
[57] Mason U, Aldrich J, Breedveld F, Davis CB, Elliott M, Jackson M, et al. CD4 coating, but not CD4 depletion, is a predictor of efficacy with primatized monoclonal anti-CD4 treatment of active rheumatoid arthritis. J Rheumatol 2002;29:220–9.
[58] Wee SL, Stroka DM, Preffer FI, Jolliffe LK, Colvin RB, Cosimi AB. The effects of OKT4A monoclonal antibody on cellular immunity of nonhuman primate renal allograft recipients. Transplantation 1992;53:501–7.
[59] Schwartz JA, Prado I, Misamore J, Weiss D, Francis J, Pal R, et al. An HIV gp120-CD4 Immunogen Does Not Elicit Autoimmune Antibody Responses in Cynomolgus Macaques. Clin Vaccine Immunol 2016;23:618–27.
[60] Emu B, Fessel J, Schrader S, Kumar P, Richmond G, Win S, et al. Phase 3 Study of Ibalizumab for Multidrug-Resistant HIV-1. N Engl J Med 2018;379:645–54.
[61] Bruno CJ, Jacobson JM. Ibalizumab: an anti-CD4 monoclonal antibody for the treatment of HIV-1 infection. J Antimicrob Chemother 2010;65:1839–41.
[62] Ibalizumab MA. First Global Approval. Drugs 2018;78:781–5.
[63] Markham A. Correction to: Ibalizumab: First Global Approval. Drugs 2018;78:859.