HIV-1 CD4-binding site germline antibody–Env structures inform vaccine design

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BG24, a VRC01-class broadly neutralizing antibody (bNAb) against HIV-1 Env with relatively few somatic hypermutations (SHMs), represents a promising target for vaccine strategies to elicit CD4-binding site (CD4bs) bNAbs. To understand how SHMs correlate with BG24 neutralization of HIV-1, we report 4.1 Å and 3.4 Å single-particle cryo-EM structures of two inferred germline (iGL) BG24 precursors complexed with engineered Env-based immunogens lacking CD4bs N-glycans. Structures reveal critical Env contacts by BG24iGL and identify antibody light chain structural features that impede Env recognition. In addition, biochemical data and cryo-EM structures of BG24iGL variants bound to Env with CD4bs glycans present provide insights into N-glycan accommodation, including structural modes of light chain adaptations in the presence of the N276gp120 glycan. Together, these findings reveal Env regions critical for germline antibody recognition and potential sites to alter in immunogen design.

Current strategies to engineer a vaccine towards preventing HIV-1 infection involve designing Env-mimetic immunogens that can elicit broadly neutralizing antibodies (bNAbs). The CD4-binding site (CD4bs) epitope is a target of immunogen design as bNAbs in this class have been shown to be among the most potent and broad. Several studies have shown passive immunization using CD4bs bNAbs can confer protection from HIV-1 infection in animal models and human clinical trials, suggesting that immunization strategies to elicit these antibodies at effective concentrations would also be protective. This includes the VRC01-class of bNAbs that are derived from the VH1-2*02 variable heavy chain segment and are characterized by a short 5 amino acid complementary determining region 3 (CDR3) in the antibody (Ab) light chain and a shortened or flexible CDRL1. These characteristics are necessary for VRC01-class bNAbs to accommodate the heavily N-glycosylated landscape of the CD4bs of HIV-1 Env. Thus, VRC01-class bNAbs generally require high levels of somatic hypermutation (SHM), which is challenging to elicit through vaccination. Germline precursors of bNAbs do not generally show detectable binding to non-engineered, natively-glycosylated HIV-1 Env, and therefore, the germline-targeting approach to HIV-1 vaccine design involves efforts to engineer immunogens that can engage germline B-cell receptors (BCRs) and initiate bNAb development. Inferred germline (iGL) versions of mature bNAbs derived from predicted germline gene segment sequences represented in the human B-cell repertoire are used for the germline-targeting approach. Analysis of VRC01-class iGLs has shown that the human VH1-2*02 heavy chain gene segment encodes signature residues that are required for breadth and potency. Furthermore, germline VRC01-class precursors have been isolated from naïve individuals, and mature bNAbs have been identified from multiple HIV-1-infected human donors, suggesting that
raising this class of bNAbs is not uncommon in natural infection^{24,25}. Taken together, VRC01-class bNAbs are attractive targets for immunogen design.

The VRC01-class of bNAbs targets a particularly challenging epitope to elicit bNAbs against due to the presence of the CD4bs N-glycans that sterically obstruct interactions between Env and Ab CDRs^{32}. The glycans at positions N276gp120, N462gp120, N386gp120, and N197gp120 in the CD4bs^{3}. Two IgL versions of BG24 Fab constructs were made starting with the VH1-2*02 and VL2*01 heavy and light chain germline gene segment sequences: one containing the CDR3s from mature BG24 (BG24_{IGL-CDR3m}) and the other containing the IGL CDR3s (BG24_{IGL-CDR3IGL}). (Fig. 1a). Each BG24_{IGL} was structurally characterized in complex with GT1 and the V3 bNAbs 10-1074^{34}.

Cryo-EM structures of BG24_{IGL-CDR3igl} and BG24_{IGL-CDR3m} Fabs bound to GT1 were solved at 3.4 and 4.1 Å, respectively (Fig. 1b, c, Supplementary Fig. 1a–j, Supplementary Table 1). Both 3D cryo-EM reconstructions showed three BG24_{IGL} and three 10-1074 Fabs bound per Env trimer. However, for the BG24_{IGL-CDR3IGL}-GT1-10-1074 complex, a distinct 3D class contained two BG24_{IGL-CDR3IGL} Fabs bound to the GT1 Env (Supplementary Fig. 1e, f, Supplementary Table 1). We also solved a 1.4 Å crystal structure of unbound BG24_{IGL-CDR3igl} Fab (Supplementary Fig. 1k, Supplementary Table 2), which exhibited six disordered residues within CDR1, but otherwise superimposed with a 1.3 Å root mean square deviation (rmsd; calculated for 225 V_{H}-V_{L} atoms) with the Env-bound BG24_{IGL-CDR3m} Fab structure, suggesting no major structural differences upon Env binding.

BG24_{IGL} Fab structures recognize the modified CD4bs in GT1 Env

The GT1 complexes with BG24_{IGL} included density for CD4bs N-glycans attached to residues N234gp120, N363gp120, and N392gp120 (Fig. 2a, b). These N-glycans were also observed in the crystal structure of BG505 Env complexed with a mature BG24 Fab^{36} (BG24_{m}) (PDB 7UCF), which also included densities for N-glycans at N197gp120, N276gp120, and N386gp120 that are not present in GT1 (Fig. 2c). Despite additional glycans in BG505 compared with GT1, the CDR loops in the GT1-bound IGL Fabs showed similar orientations and positions as in the BG505-bound BG24mat Fab, except for CDR1, which is six residues longer in BG24igl than in BG24mat (Figs. 1a, 2d–f).

BG24_{igl} and BG24_{m} buried comparable surface areas on GT1 gp120 (953 and 951 Å², respectively) as compared with only slightly larger surface area (1056 Å²) buried on BG505 gp120 in the BG24mat-BG505 structure (PDB 7UCF) (Fig. 2g). We hypothesize that, although a germline precursor antibody presumably exhibits fewer contacts to an antigen than its counterpart somatically mutated bNAb, the overall interface BSA values for the gp120 peptide components of the BG24_{igl}-GT1 and BG24mat-BG505 structures were similar because the modifications in GT1 (both amino acid substitutions and removals of N-glycans) allowed increased contacts between BG24_{igl} and the GT1 gp120. However, differences in interactions between the BG24_{igl} and BG24mat-BG505 structures suggest that SHM substitutions enrich interactions in particular regions within the CD4bs (Fig. 2h, i).

For example, in the BG24mat-BG505 complex, BG24mat residue S100A{H}C hydrogen bonds with the gp120 inner domain residue K97gp120 (Fig. 2g–i). K97 is -90% conserved among HIV-1 Envs, making this a crucial interaction of broad and potent CD4bs bNAbs^{18}. Residue S100A{H}C is a germline-encoded residue, however, in both BG24_{igl}-GT1 structures, is not within the distance to form a hydrogen bond with K97gp120 (Fig. 2i). Compared to BG24_{igl}-GT1, BG24mat-BG505 also showed increased V_{H} buried surface area (BSA) in the gp120 exit loop (gp120 residues 472–476). Together, this analysis demonstrated differences in the distribution of BSA among BG24mat and BG24_{igl} in CD4bs regions.

BG24 somatic hypermutation and germline features play a role in CD4bs recognition

We next compared how differences in BG24_{igl} and BG24mat contribute to their recognition of GT1 and BG505, respectively. BG24_{igl} contains a
germline 11-residue CDRL1 that can recognize the mostly aglycosylated CD4bs in GT1, whereas the BG24mat CDRL1 is six residues shorter and includes a glycine to create a more flexible loop that can accommodate the N276gp120 glycan. In the BG24mat-BG505 structure, the five-residue BG24 CDRL1 is oriented adjacent to the N276gp120 glycan (Fig. 3a). The CDRL1 interface with GT1 in the BG24mat-BG505 structures showed the longer CDRL1s in the germline-overlaid with the germline-encoded residues at positions T57HC and S54HC, maintain interactions with Env also play a functional role by contributing to neutralization potency and breadth.

These results demonstrate that SHMs in CDRH2 shown to interact with Env also play a functional role by contributing to neutralization potency and breadth. To understand the functional role of these CDRH2 SHMs, we tested the neutralization activity of BG24mat constructs in which individual SHMs were reverted to the corresponding germline residue (BG24 R53NHc, BG24 G54Nh, and BG24 V57Tic) and a combined construct with all three mutations reverted (BG24 CDRH2 GL3mut-a, CDRH2 GL3mut-b, CDRH2 GL3mut-c, CDRH2 GL3mut-d) against a 12-strain global panel of HIV-1 strains plus BG505 T332N35, and compared potencies and breadth against BG24mat (Fig. 3d). For BG24mat self-acting Env residue. (Fig. 3d)35 For BG24mat single mutations, we observed a greater than 5-fold decrease in potency against 6 of the 13 strains tested compared to BG24mat; BG24 G54Nh, most closely compared to BG24mat, showing a more than five-fold decrease in potency against only 3 strains. BG24 CDRH2 GL3mut-c was more strongly affected by the SHM reversions, showing more than a five-fold decrease in potency against almost all strains compared to BG24mat. These results demonstrate that SHMs in CDRL2 shown to interact favorably with Env also play a functional role by contributing to neutralization potency and breadth.

Signature residues encoded by the VH1-2*02 germline gene in VRC01-class bNAbs interact with conserved gp120 residues and are correlated with neutralization potency. These interactions have been structurally characterized in the context of VRC01-class IGLs bound to...
**Fig. 2 | Comparison of BG24_{iGL} and BG24_{mat} CD4bs epitopes.** Surface contacts made by BG24_{GL-CDR3iGL} VH (bright pink) and VL (light pink) on GT1 gp120 (light gray) (a), BG24_{GL-CDR3mat} VH (dark purple) and VL (light purple) on GT1 gp120 (light gray) (b), and BG24_{mat} VH (deep teal) and VL (light teal) on BG505 gp120 (light gray) (PDB 7UCF). (c) Surface representation of gp120 (light gray) with cartoon representations of BG24_{GL-CDR3iGL} (d), BG24_{GL-CDR3mat} (e), and BG24_{mat} (f) CDR loops. VH and VL CDR loops are colored the same as in a–c. g Summary table of gp120 buried surface area (BSA) (Å²) calculations for BG24_{GL-CDR3iGL}, BG24_{GL-CDR3mat}, and BG24_{mat} at the inner domain (inner), D loop, CD4bs loop, β20/21, β23, V5 loop, β24, and exit loop of the CD4bs. BSA calculations were conducted for gp120 peptide components and did not include glycan interactions. h Surface representation of gp120 (PDB 5T3Z) with CD4bs motifs colored and labeled (inner domain—purple, D loop—light pink, CD4bs loop—yellow, β20/21—bright pink, β23—orange, V5 loop—blue, β24—green, and exit loop—red). Distance measurements between K97{gp120} and S100A_{HC} for i BG24_{GL-CDR3iGL-GT1gp120}, j BG24_{GL-CDR3mat-GT1gp120}, and k BG24_{mat-BG505gp120} structures. The distances between atoms are represented by black dotted lines.
monomeric gp120s, but there are no known structures of VRC01-class iGLs bound to a trimeric Env, except when the iGL was chemically cross-linked to Env. To evaluate and verify VRC01-class VH1-2*02 germline-encoded interactions with an Env trimer, we compared these interactions in the BG24iGL-GT1 and BG24mat-BG505 structures (Supplementary Fig. 2c–f). Specifically, as previously described in structures involving gp120 monomers, germline-encoded R71HC in the BG24iGL-GT1 and BG24mat-BG505 structures formed a salt bridge with the conserved D368gp120 side chain, an Ab interaction that mimics the interaction of host receptor residue R59CD4 with D368 gp120 (Supplementary Fig. 2c). In the gp120 D loop, there were interactions between the backbone and side chain of N276gp120 with Y100DHC and germline-encoded W50HC side chains (Supplementary Fig. 2d). In the V5 loop, interactions between the conserved R456gp120 residue and germline-encoded N58HC are conserved in both structures (Supplementary Fig. 2e). In BG24iGL-CDR3mat-GT1, atoms within these Fab residues were separated by more than 5 Å from atoms within gp120 residues; thus, this is not defined as an interaction. In the light chain, E96LC interacted with the backbone of G459gp120 and the side chain of N280gp120 (Supplementary Fig. 2f).
GT1 CD4bs glycan modifications affect BG24 binding
To evaluate how glycan modifications in the GT1 immunogen contributed to BG24 binding, we evaluated the binding of BG24 constructs to GT1 with Env PNGSs either restored to or removed from the CD4bs (Fig. 4a). The BG24 constructs included BG24mat, BG24 with germline CDR1 (BG24CDR1GL) (Fig. 1a), BG24GLCDR3mat, BG24GLCDR3GL, and BG24 with an iGL light chain (BG24LCGL). PNGSs were individually restored at positions N197gp120, N386gp120, and N462gp120 and removed at N234gp120 to create five GT1 constructs with altered glycan landscapes (GT1N197gp120, GT1N234gp120, GT1N386gp120, GT1N462gp120, and GT1delN234gp120, respectively). BG505 and GT1 binding was evaluated by enzyme-linked immunosorbent assays (ELISAs). Restoring Env PNGSs at positions N197gp120, N386gp120, and N462gp120 and removing the PNGS at N234gp120 did not greatly affect the binding of BG24 IgG constructs (Fig. 4a).

BG24mat was the only Ab that showed substantial binding to BG505, which unlike the GT1 Env, included all PNGSs. We conclude that BG24 constructs with a long, germline CDRL1 can accommodate the N276gp120 glycan on Envs that...
have been engineered to have a limited glycan landscape in the CD4bs. These results
contribute to existing studies of N276 glycan accommodation by germline CDRL1 (e.g., ref. 36).

To gain insight into BG24 CDRL1-iGL interactions with GT1 containing an N276 glycan, we solved a single-particle cryo-EM structure of BG24CDRL1-iGL bound to GT1 containing the restored N276 glycan. This structure allowed us to identify a unique 3D structure containing either one, two or three bound BG24 CDRL1 Fab structures (Supplementary Fig. 3a–h, Supplementary Table 1). Electron density in the Fab CDR1 was not optimal after cryo-EM processing; therefore, side chains were not modeled (Supplementary Fig. 3i).

The BG24CDRL1-iGL-GT1N276 complex structure showed that the Fab CDR1 main chain residues adopted a helix-like conformation to accommodate the N276 glycan (Fig. 4c). Available crystallographic and cryo-EM Env structures demonstrate that the N276 glycan is conformationally heterogeneous32-37,38 (Supplementary Fig. 3b). Indeed, the N276 glycan in the GT1 and BG505 Env structures exhibited different conformations (Fig. 4c). Thus, after superimposing the gp120 residues in the BG24CDRL1-iGL-GT1N276 and BG24CDRL1-iGL, it was evident that the N276 glycan in BG505 showed steric clashes with the CDR1-iGL in BG24CDRL1-iGL (Fig. 4c). Flexibility of the N276 glycan on BG505 may be more constrained than the counterpart glycan on GT1, as GT1 contains fewer N-glycans in the CD4bs, allowing for increased N276 glycan flexibility. This assumption is consistent with the ELISA results showing that BG24CDRL1-iGL bound to GT1, but not to BG505 Env trimers with an N276 glycan (Fig. 4c).

The only other known CD4bs-targeting bNAb with a helical CDRL1 is IOMA, another VH-2’02-derived bNAb29. IOMA contains features that distinguish it from VRC01-class bNAbs, including a normal length (8-residue) CDR3 and a 3-residue CDR1, which adopts a short α-helix to accommodate the glycan at N276. However, CLK31, an IOMA-like Ab isolated from naive human B cells using a VRC01 germline-targeting immunogen, did not include a helical CDRL122. Alignment of the LCs of BG24CDRL1-iGL, IOMA, and CLK31 showed that each CDR1 adopts a different conformation (Fig. 4d). These observations suggest CDR1 helical conformations are diverse and have only been observed when bound to gp120s that contain the glycan at N276.

**Discussion**

VRCo1-class bNAbs are promising targets for germline-targeting immunogen design as germline-encoded residues make signature contacts with gp120 that contribute to the impressive breadth and potency1,2,23,25. However, challenges in eliciting VRCo1-class bNAbs through a germline-targeting vaccine regimen include explicitly selecting for the VH-2’02 germline gene, overcoming CD4bs glycan barriers, and stimulating high levels of SHM40,41. Despite these challenges, progress has been made in developing a VRCo1-class bNAb germline-targeting approach22,25,46, which is initiated by engineering an immunogen that binds to the germline precursors of VRCo1 bNAbs. Priming immunogens are engineered to interact with specific germline-encoded residues and lack CD4bs glycans that obstruct germline recognition29,37,43. VRCo1-class priming immunogens include monomeric gp120 cores22,25,44,46, soluble CD4-based trimers37,44, and anti-idiotypic antibodies that recognize target BCRs with VH-2’02 gene segments44,46. Furthermore, selecting a particular strain of Env and a gp120+ trimeric Env-based platform to engineer priming characteristics that have proven to impact germline BCR selection in vivo1. Thus, identifying and developing the optimal priming immunogen for VRCo1-like bNAb elicitation will require a robust understanding of the structural and biophysical nature of Env recognition by germline precursors.

In a sequential immunization approach, boosting immunogens are introduced to shape the development of a germline precursor into a bNAb by stimulating favorable SHMs4. Example boosting immunogens reintroduce native Env glycans and heterogenous Env strains to develop bNAbs capable of overcoming steric glycan barriers and have heterologous-neutralizing activity36. The N276 glycan on HIV-1 Env
provides a particularly difficult roadblock, as VRC01-like germline CDRL1s must become shorter or more flexible through SHM to avoid steric clashes23,27,28,32,48. Several iterations of this approach have been tested in animal models; however, the elicitation of heterologous-neutralizing activity has not yet been accomplished40,49. BG24mat represents a VRC01-class bNAb that can be targeted for germline-targeting approaches30. BG24mat has a fraction of the SHMs found in VRC01 and other VRC01-class bNAbs and maintains notable breadth and potency. Together with previous studies of the VRC01-class PCIN63 lineage and construction of a minimally mutated VRC01, our studies of BG24 suggest that high levels of SHM are not absolutely required for the development of VRC01-class Abs. Our cryo-EM structures of the iGL precursors of BG24 bound to the priming immunogen GT1 contribute to understanding how VRC01-class bNAb precursors interact with immunogens. We found that VH1-2*02 germline-encoded residues make the predicted signature contacts with gp120, and the long germline CDRL1 is accommodated in the absence of the N276gp120 glycan in GT1, rationalizing removal of this glycan in a priming immunogen since modeling suggested the germline CDRL1 conformation would clash with the N276gp120 glycan. These observations validate the design of priming immunogens that nurture interactions with germline residues and remove the N276gp120 glycan from the CD4bs epitope. We further investigated how the glycan landscape of an immunogen affects germline binding, finding that...
BG24G-LC can evade clashes with the N276gp120 glycan when the BG24 HC includes bNab features and the CD4bs epitope is only minimally glycosylated. Based on these observations, we propose that boosting immunogens might first aim to target mature HC features, and then introduce the N276gp120 glycan in a limited CD4bs glycan landscape before moving to a fully glycosylated Env landscape.

We also characterized the binding of BG24CDRL1-iGL to the clade D 6405 Env, which suggested that some non-engineered HIV-1 Envs can accommodate some germline VRC01-class CDRL1s. In the case of BG24CDRL1-iGL, accommodation of the N276gp120 glycan occurred through a helix-like conformation in the iGL CDRL1. Furthermore, ELISA data suggested that other VH1-2*02-derived bNabs with iGL LCs can also bind to the 6405 Env. Taken together, we propose further investigation of the 6405 Env to identify properties of the CD4bs that led to the structural and biochemical observations reported here, which could be applicable to the design of CD4bs-targeting immunogens.

Methods

**BG24G constructs design**

Genes encoding the IGHV1-2*02 and IGLV2-11*02 germline sequences were reverted based on inferred sequences using IMGT/ V-QUEST. Mutations to BG24 IgG and Fab sequences were generated using QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent).

**Protein expression and purification**

Fabs and IgGs were expressed and purified as previously described. Briefly, Fabs were expressed by transient transfection using the Expi293 expression system (ThermoFisher). Fab expression vectors contained genes of LC and the C-terminal 6x-His-tagged HC. The Fab and IgG proteins were purified from cell supernatants by Ni²⁺-NTA (GE Healthcare) and protein A affinity chromatography (GE Healthcare), respectively, followed by size exclusion chromatography (SEC) using a Superdex 200 10/300 column (GE Healthcare).

**SOSIP.664 Env constructs contained the disulfide mutations S01C and 605C (SOS), I559P (IP), and the furin cleavage site mutated to six Superdex 200 10/300 column (GE Healthcare) and protein A affinity chromatography (GE Healthcare),** respectively, followed by size exclusion chromatography (SEC) using a Superdex 200 10/300 column (GE Healthcare).

**Cryo-EM data collection and processing**

Data for single-particle cryo-EM were collected on either a Talos Arctica (BG24G-LC-CDR3mat-GT1-10-1074, BG24G-LC-CDR3GCL-GT1N276gp120-10-1074, BG24G-LC-CDR3mat-GT1-10-1074) or a Titan Krios (BG24G-LC-CDR3mat-GT1-10-1074) transmission electron microscope, operating at 200 and 300 kV, respectively. Movies were collected with beam-image shift over a single exposure per hole in a 3-by-3-pattern of 2 μm holes. For datasets collected on the Talos Arctica, movies were recorded in super-resolution mode on a Falcon III camera (Thermo Fisher) at 1.436 Å/pixel or a K3 camera (Gatan) at 0.4345 Å/pixel. Movies obtained from samples on the Titan Krios were collected in super-resolution mode on a K3 camera (Gatan) equipped with an Bio-Quantum energy filter (Gatan) with a 20 eV slit width at 0.4327 Å/pixel. The defocus range was set from 1.0 to 3.0 μm for each dataset.

The data processing workflow described below was performed similarly for all datasets using RELION. Movies were motion-corrected using MotionCor2 after binning. GCTF was used to estimate CTF, and micrograph power spectra that showed poor CTF fits or bad ice were removed. A subset of particles was manually picked and used for reference-free 2D classification. Classes representing the defined complex were used as references for RELION AutoPicking to generate 2D classes. Subsequent 2D classes were inspected, and 2D classes representing a defined complex were selected for 3D classification. An ab initio model was generated using cryoSPARC using a subset of particles for each dataset and used as a reference in 3D classification, which assumed C1 symmetry. 3D classes representing a defined complex were selected for auto-refinement and post-processing in Relion. Particles used in 3D refinement were then extracted and un-binned. Particles were then subjected to 3D classification with the map generated with un-binned particles used as a reference. Distinct classes representing a particular defined complex (C1 or C3 symmetric) were selected for 3D auto-refinement after masking out Fab CβC1 domains. Iterative rounds of particle CTF refinement, particle polishing, 3D auto-refinement, and post-processing were used for each class to generate final maps. To improve the resolution of Fab Cβ CDRL1s, a soft mask surrounding the Fab VH-VL-gp120 interface was created in chimera and used for local refinements in cryoSPARC to improve density in this region and allow for CDRL1 fitting and refinement. Resolutions were calculated in RELION using the gold-standard FSC 0.143 criterion. FSCs were generated by the 3DFSC program.

**Cryo-EM model building and refinement**

Model coordinates were generated by fitting reference gp120 (PDB 5T32), gp41(PDB 5T32), 10-1074 (PDB 5T32), and BG24-derivative B-factor refinement in Phenix, followed by several cycles of iterative manual building in Coot and real-space refinement with TLS groups in Phenix.

Assembly of protein complexes and cryo-EM sample preparation

Protein complexes for cryo-EM were generated by incubating a purified BG24 Fab and the 10-1074 Fab with an Env trimer in a 3:1 Fab/trimer molar ratio and incubating at 4 °C overnight. The complex was then SEC purified over a Superdex 200 1/150 column (GE Healthcare). The peak corresponding to the complex was pooled and concentrated to 1.0 mg/mL. Quantifoil R2/2 400 mesh cryo-EM grids (Ted Pella) were prepared by glow-discharging for 1 min at 20 mA using a PELCO easiGLOW (Ted Pella). Fab-Env complexes (3 μL) were then applied to grids and blotted with Whatman No. 1 filter paper for 3–4 s at 100% humidity at room temperature. The grids were vitrified by plunge-freezing in liquid ethane using a Mark IV Vitrobot (ThermoFisher).
Fabs (this study) chains into cryo-EM density with UCSF Chimera\textsuperscript{67}. Initial models were refined using the Phenix command \texttt{phenix.real_space_refine}\textsuperscript{67,69}. Sequence updates to the model and further manual refinement was conducted with Coot\textsuperscript{66}. Iterative rounds of Phenix auto-refinement and manual refinements were done to generate the final models (Supplementary Table 1).

**Structural analyses**
Structure figures were made using PyMol (Schrödinger LLC), UCSF Chimera\textsuperscript{66}, and UCSF ChimeraX\textsuperscript{66,69}. PyMol was used to calculate r.m.s.d. values after the pairwise alignment of Cα atoms. PDBePISA\textsuperscript{70} was used to calculate buried surface areas using a 1.4 Å probe. Calculations for gp120 BSA were for peptide components of gp120 and did not include glycan interactions. Defined interactions were assigned tentatively due to the low resolution of complexes using the following criteria: hydrogen bonds were assigned pairwise interactions that were less than 4.0 Å and with an A-D-H angle >90°, and van der Waals interactions were assigned as distances between atoms that were less than 4.0 Å.

**TZM.bl neutralization assay**
The neutralizing activities of BG24 CDRH2 mutant IgGs were measured using a luciferase-based TZM.bl assay according to standard protocols\textsuperscript{71}. Each assay was performed in duplicate. Data were analyzed using Antibody Database (v2.0)\textsuperscript{72}. Five-parameter curve fitting was used to determine 50% inhibitory concentrations (IC\textsubscript{50}s), and non-specific activity was detected by testing against murine leukemia virus (MuLV).

**Enzyme-linked immunosorbent assay**
SOSIP trimers were randomly biotinylated following the manufacturer’s guidelines using the EZ-Link NHS-PEG4-Biotin kit (Thermo Fisher Scientific). The Pierce Biotin kit (Thermo Fisher Scientific) was used to quantify biotin molecules per SOSIP protomer: biotin estimations ranged from 1 to 10 biotin molecules per SOSIP protomers. Streptavidin-coated 96-well plates (Thermo Fisher Scientific) were coated with 5 µg/mL of randomly biotinylated SOSIPs diluted in 3% BSA in TBS-T (20 mM Tris, 150 mM NaCl, 0.1% Tween20) and incubated at room temperature (RT) for 2 h. Plates were washed to remove unbound SOSIPs. Serial dilutions of IgGs were made in 3% BSA in TBS-T and applied to the plates. After a 2 h incubation at RT, plates were washed twice in TBS-T. Goat anti-human IgG Fc conjugated to horse-radish peroxidase (Southern BioTech) was added at 1:8000 dilution for 30 min, followed by three washes with TBS-T. 1-Step Ultra TMB-ELISA Substrate Solution (ThermoFisher Scientific) was added for colorimetric detection, and color development was quenched with 1 N HCl. Absorbance was measured at 450 nm. Two independent, biological replicates (n = 2) were performed.

**Reporting summary**
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
The atomic model generated for the X-ray crystallography structure of the BG24\textsubscript{GGL-CDR3}Fab in this study has been deposited in the Protein Data Bank (PDB) under accession code 7UGM. The cryo-EM maps and atomic structures have been deposited in the PDB and/or Electron Microscopy Data Bank (EMDB) under accession codes 7UGN and EMDB-26490 for BG24\textsubscript{GGL-CDR3}GTL1-10-1074 Class I, EMD-26491 for BG24\textsubscript{GGL-CDR3}GTL1-10-1074 Class II, 7UGD and EMD-26492 for BG24\textsubscript{GGL-CDR3}MAc- GTI1-10-1074, 7UGP and EMD-26493 for BG24\textsubscript{GGL-CDR3}GTL1N276Gp120-10-1074 Class I, EMD-26494 for BG24\textsubscript{GGL-CDR3}GTL1N276Gp120-10-1074 Class II, EMD-26495 for BG24\textsubscript{GGL-CDR3}GTL1N276Gp120-10-1074 Class III, and 7UGQ and EMD-26496 for BG24\textsubscript{CDR3}Fab-6405-10-1074. Local refinement maps used to model CDRL1s of BG24-derivatives have been deposited with PDB and EMDB accession codes for each respective structure. The ELISA data generated in this study are provided in the Supplementary Information and Source Data File. Source data are provided with this paper.

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K.A.D., C.O.B., H.B.G., T.S., M.C.N., and P.J.B. designed the research. K.A.D. performed protein purification, structural studies, and ELISA experiments. P.N.P.G. performed in vitro neutralization assays. K.A.D., C.O.B., and H.B.G. analyzed results. K.A.D. and P.J.B. wrote the manuscript with input from co-authors.

Competing interests

The authors declare no competing interests.

Additional information

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