Figures and figure supplements

Structural basis for germline antibody recognition of HIV-1 immunogens

Louise Scharf et al
Figure 1. Sequence Alignments of Inferred Germline and Mature Forms of 3BNC60 and NIH45-46. Alignments of (A) VH and (B) VL sequences of inferred germline progenitors (3BNC60<sub>GL</sub> and NIH45-46<sub>GL</sub>), mature 3BNC60 (3BNC60<sub>MAT</sub>), mature NIH45-46 (NIH45-46<sub>MAT</sub>), and the predicted germline V gene segments from which they were derived. Antibody framework regions (FWR) and CDR loops (CDR) are marked and CDR loops are colored blue (CDR1), green (CDR2), and red (CDR3). CDR3 sequences for germline Fabs were taken from mature antibody sequences as done in previous studies (Dosenovic et al., 2015; Hoot et al., 2013; McGuire et al., 2013; Scharf et al., 2013).

DOI: 10.7554/eLife.13783.003
Figure 2. SPR binding assays. Representative sensograms (red), fits (black, where applicable), residuals, and $K_D$, $k_a$, and $k_d$ values (mean ± standard deviation from 3 independent experiments) for binding of germline and mature Abs to gp120 cores. NIH45-46$_{GL}$, 3BNC60$_{GL}$, NIH45-46$_{MAT}$ and 3BNC60$_{MAT}$ IgG were captured on a protein A biosensor chip, and 426c.TM1ΔV1-3, 426c.TM4ΔV1-3 and 93TH057 gp120 cores were flowed over the chip as a 2-fold dilution series with top concentrations of 16 mM and 400 nM for germline and mature Abs, respectively.

DOI: 10.7554/eLife.13783.004
Figure 3. Overview of Bound and Unbound Structures of Germline and Mature Forms of NIH45-46 and 3BNC60. Superposition of unbound (grey) and bound (colored) Fab structures of (A) NIH45-46GL (blue), (B) NIH45-46MAT (orange), (C) 3BNC60GL (green), (D) 3BNC60MAT (purple), and (E) VRC01GL (teal). The crystal structures were superimposed on their VH VL domains and are shown as wire representations with CDR loops colored blue (CDR1), green (CDR2), and red (CDR3). Panels (F–I) show detailed areas of interest for the corresponding structure comparisons shown in panels (A–D). Protein backbones are shown as wire diagrams, side chains are shown as stick representations (red, oxygen; blue, nitrogen). (J) Superposition of unbound (grey) and bound (red) structures of 426c.TM4ΔV1-3 shown as wire diagrams. (K) Table summarizing Ca rmsds for the indicated Ab and gp120 pairs. Since no unbound crystal structure of 3BNC117MAT was available, the structure of the close clonal relative, 3BNC60MAT (93%HC/96% LC sequence identity) was substituted. Similarly, no unbound structure of VRC01MAT was available, so that of NIH45-46MAT (88%HC/96% LC sequence identity) was substituted, omitting its four-residue insertion in CDRH3 from the rmsd calculation.

DOI: 10.7554/eLife.13783.006
**Figure 4.** Comparison of Signature VRC01-class and CD4-mimicry Contacts in Germline Ab-gp120 Immunogen Complexes. Top panels show (A) CD4 binding loop, (C) HC, (E) LC contacts in superimposed NIH45-46GL/426c.TM1.V1-3 and NIH45-46MAT/93TH057 (PDB 3U7Y) complexes. Bottom panels show (B) CD4-binding loop, (D) HC, (F) LC contacts in superimposed 3BNC60GL/426c.TM4.V1-3 and 3BNC117MAT/93TH057 (PDB 4JPV) complexes. Protein backbones are shown as wire diagrams, interacting residues are shown as stick representations (red, oxygen; blue, nitrogen). Yellow dashed lines indicate putative hydrogen bonds (distance < 3.5 Å, A-H–D angle > 90°). Ab coloring: blue, NIH45-46GL HC; light blue, NIH45-46GL LC; orange, NIH45-46MAT HC; yellow, NIH45-46MAT LC; green, 3BNC60GL HC; light green, 3BNC60GL LC; purple, 3BNC66MAT HC; light pink 3BNC60MAT LC. gp120 coloring: blue, CD4-binding loop, green, loop D; teal, loop VS. (E, F) Interacting residues of the Cγ strand of Fab HCs and the CD4-binding loop of gp120 (grey) are shown as sticks. The Fab residue numbers are indicated since the amino acid sequence differs at some positions between germline and mature Abs.

DOI: 10.7554/eLife.13783.007
Figure 5. Accommodation of Asn276_{gp120} Glycan by Germline Ab Light Chains. Superposition of gp120 (grey) complexes with germline and mature Abs. (A) CDRL1 from 45-46m2_{MAT} (yellow), NIH45-46_{GL} (blue) and 3BNC60_{GL} (green) is positioned near the Asn276_{gp120} glycan. Two- and four-residue insertions in NIH45-46_{GL} and 3BNC60_{GL}, respectively, result in a widening of the tip of CDRL1 rather than a more extended loop, which would clash with gp120 protein residues and/or the Asn276_{gp120} glycan. gp120 (grey) complexes with (B) 3BNC117_{MAT} (pink) and 45-46m2_{MAT} (yellow) (C) NIH45-46_{GL} (blue) and 45-46m2_{MAT} (yellow), (D) 3BNC60_{GL} (green) and 45-46m2_{MAT} (yellow). Protein backbones are shown as wire diagrams and the Asn276_{gp120} glycan from the 45-46m2_{MAT}/93TH057 gp120 complex (PDB code 4JKP) is shown as sticks (yellow, carbon; red, oxygen; blue, nitrogen). The positions of CDRL1 and FWR3_{LC} are indicated. (E) CDRL1 from VRC-CH31_{MAT} (magenta), NIH45-46_{GL} (blue) and 3BNC60_{GL} (green) is positioned near the Asn276_{gp120} glycan. The CDRL1 loop of VRC-CH31_{MAT} is of the same length as that of 3BNC60_{GL}, and uses increased backbone conformational flexibility due to somatically mutated glycine residues to avoid clashes with gp120 protein residues and/or the Asn276_{gp120} glycan.

DOI: 10.7554/eLife.13783.008
Figure 6. Comparison of the Binding Interfaces in gp120 Complexes with Germline and Mature Abs. (A) gp120 residues contacted by Fabs and (B) Fab residues contacted by gp120s are shown as surfaces over ribbon diagrams. gp120 domains are colored as in Figure 3. Ab coloring: blue, NIH45-46GL HC; light blue, NIH45-46GL LC; purple, NIH45-46CHIM HC; light purple, NIH45-46CHIM LC; orange, NIH45-46MAT HC; yellow, NIH45-46MAT LC; green, 3BNC60GL HC; light green, 3BNC60GL LC; pink, 3BNC60MAT HC; light pink, 3BNC60MAT LC. (C) Quantitation of buried surface areas ($\text{Å}^2$) depicted in (A) and (B). The columns labeled total are the sums of areas for outer domain, bridging sheet and inner domain for gp120, and of heavy chain and light chain for Abs. Surface areas buried due to complex formation were calculated using a 1.4 Å probe. DOI: 10.7554/eLife.13783.009
Figure 7. Comparisons of Binding Mode in Germline Ab-gp120 Immunogen Complexes. (A) Superpositions of Fab-gp120 complexes depicted as wire diagrams. The following Fab/gp120 complexes were compared by alignment of their gp120s: 3BNC117MAT/93TH057 (PDB code 4JPV), 3BNC117MAT/C1086 (PDB code 4LSV), 3BNC117MAT/93TH057 (PDB code 4JPV), 3BNC60GL/426c.TM4ΔV1-3, NIH45-46MAT/93TH057 (PDB code 3U7Y), VRC01MAT/ KER.2018.11 (PDB code 4LSS), NIH45-46GL/426c.TM1ΔV1-3. (B) Rotation angle and translation distance of VH domains of mature, chimeric and germline Fabs in complex with gp120s relative to VRC01MAT in complex with 93TH057 gp120 (PDB code 3NGB). Data points for complexes of mature Fabs bound to non-immunogen gp120s are shown as blue diamonds, complexes of germline Fabs bound to immunogen candidates are shown as red squares (TM1 = 426c.TM1ΔV1-3, TM4 = 426c.TM4ΔV1-3, eOD = eOD-GT6), the complex between the half mature, half germline NIH45-46CHIM and to a non-immunogen gp120 is shown as a purple diamond, and 3BNC55MAT bound to 426c.TM4ΔV1-3 is shown as a green square. When two complexes were found in the crystallographic asymmetric unit, rotation and translation parameters are shown for both complexes (denoted as #1 and #2). Standard deviations for the translation distance and rotation angle for mature VRC01-class bNAb-gp120 complexes shown as vertical and horizontal lines, respectively. (C) Alignment of the 3BNC60GL/426c.TM4ΔV1-3 (VH,VL shown in red) and VRC01MAT Fab/gp120 (PDB code 3NGB) (VH,VL shown in blue) structures onto the gp120 region of a native-like Env trimer structure (BG505 SOSIP.664; PDB code 5CEZ) (gray). Modeled structures are shown looking down the trimer three-fold axis (left panel) and from the side (right panel). Figure 7 continued on next page.
Figure 7 continued

DOI: 10.7554/eLife.13783.010

The following source data is available for figure 7:

**Source data 1.** Rotation angle and translation distance data of VH domains of mature, chimeric and germline Fabs in complex with gp120s relative to VRC01MAT in complex with 93TH057 gp120.

DOI: 10.7554/eLife.13783.011
Figure 8. Comparison of Electrostatic Surface Characteristics of Fab-gp120 Complexes. The binding surfaces of Fabs (left panels) and gp120s (right panels) are shown. Each binding partner is shown in an orientation looking into the binding interface; the corresponding complex would be obtained by rotating each binding partner by ~90° about the vertical axis. The top panel shows the locations of landmarks on surface representations of Fabs (dark grey, V\textsubscript{H}; light grey, V\textsubscript{L}) and gp120s (yellow, outer domain; grey, inner domain; orange, bridging sheet; blue, CD4-binding loop; green, loop D; teal, loop V5; ordered residues of the Asn\textsubscript{276\text{gp120}} glycan shown as yellow sticks (normally a complex-type N-glycan, but a high mannose N-glycan in crystal structures); approximate locations of Figure 8 continued on next page
Figure 8 continued

Asn460_{gp120} and Asn463_{gp120} shown as light pink and magenta dots, respectively. The lower panels show electrostatic potentials on surface representations of Fabs (left panels) and gp120s (right panels) colored blue (positive electrostatic potential) to red (negative electrostatic potential). The binding interfaces are outlined with a dotted black line. The approximate footprints of the complex-type Asn276_{gp120} glycan on Fab surfaces are indicated with a black triangle (the Asn463_{gp120} glycan, also complex-type, is not resolved in any Env structures, thus its footprint on Fab surfaces cannot be shown).

DOI: 10.7554/eLife.13783.012
Figure 8—figure supplement 1. The combining sites of germline (left panels) and mature Fabs (right panels) are shown as surface representations and electrostatic potentials are indicated using blue for positive electrostatic potential and red for negative electrostatic potential. Abs shown are CH58GL and CH58MAT (PDB codes 4RIR and 4HPO), 4E10GL and 4E10MAT (PDB codes 4ODX and 2FX7), 10-1074GL and 10-1074MAT (PDB codes 4FQQ and 4FQ2) (shown in two orientations to better illustrate electrostatic changes), and CH103GL and CH103MAT (PDB codes 4QHK and 4JAM). The antibody paratopes are outlined with a dotted black line. The approximate footprint of Asn137gp120, Asn156gp120, and Asn332gp120 glycans on 10-1074GL and 10-1074MAT Fab surfaces are indicated with black triangles.

Figure 8—figure supplement 1 continued on next page
Figure 8—figure supplement 1 continued
DOI: 10.7554/eLife.13783.013