Rational HIV Immunogen Design to Target Specific Germline B Cell Receptors
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Rational HIV Immunogen Design to Target Specific Germline B Cell Receptors


Vaccine development to induce broadly neutralizing antibodies (bNAbs) against HIV-1 is a global health priority. Potent VRC01-class bNAbs against the CD4 binding site of HIV gp120 have been isolated from HIV-1–infected individuals; however, such bNAbs have not been induced by vaccination. Wild-type gp120 proteins lack detectable affinity for predicted germline precursors of VRC01-class bNAbs, making them poor immunogens to prime a VRC01-class response. We employed computation-guided, in vitro screening to engineer a germline-targeting gp120 outer domain immunogen that binds to multiple VRC01-class bNAbs and germline precursors, and elucidated germline binding crystallographically. When multimerized on nanoparticles, this immunogen (eOD-G6) activates germline and mature VRC01-class B cells. Thus, eOD-G6 nanoparticles promise as a vaccine prime. In principle, germline-targeting strategies could be applied to other epitopes and pathogens.

Protection against disease by nearly all licensed vaccines is associated with induction of antibodies (1). Viruses with high antigenic diversity, such as HIV, influenza virus, and hepatitis C virus, pose major challenges for vaccine development (2). Most exposed surfaces on the Envelope glycoproteins (Env) of these viruses are hypervariable or shielded by glycans (3), and traditional vaccine approaches tend to induce neutralizing antibodies against only a small subset of viral strains (4–6). However, discoveries of bNAbs against each of these viruses have identified conserved epitopes as leads for vaccine design (2), and structural analysis has provided atomic definition for many of these epitopes (7, 8). Structure-based approaches are, therefore, needed to reverse-engineer vaccines capable of inducing bNAbs against these conserved epitopes (9).

High-potency VRC01-class bNAbs against the HIV gp120 CD4 binding site (CD4bs) have been isolated from several individuals infected with different strains of HIV-1 (10–12). VRC01-class bNAbs all derive from the human VH1-2*02 variable heavy gene but differ substantially in CDRL1 deletion in many VRC01-class bNAbs (Table 1 and supplementary materials). Therefore, wild-type Env constructs lacking the CD4bs on a minimal, engineered high-potency VRC01-class bNAbs are poor vaccine candidates to prime VRC01-class responses, because they are unlike-ly to reliably stimulate GL precursors to initiate antibody maturation.

Immunogen Design Strategy

To address the problem described above, we modified the CD4bs on a minimal, engineered outer domain (eOD) (17) to produce a germline-targeting vaccine prime (Fig. 1) with two important binding properties: (i) moderate affinity for multiple predicted VHL-2*02 GL-Ab to enhance the ability to activate VHL-2 GL B cells with appropriate light chains; (ii) high affinity for VRC01-class bNAbs to provide an affinity gradient to guide early somatic mutation toward

Vaccine design to induce VRC01-class bNAbs is attractive because VHL-2 genes are estimated to be present in ~2% of the human Ab repertoire (16) and, even considering restrictions on light chain usage, suitable precursors should be present in the naïve B cell repertoire of most individuals. However, predicted germline (GL) precursors for VRC01-class bNAbs exhibit no detectable affinity for wild-type Env (17, 18) (Table 1 and supplementary materials), a potential explanation for the rarity of VRC01-class bNAbs in HIV-1 infection (19). More important, wild-typeEnv constructs lacking GL affinity are poor vaccine candidates to prime VRC01-class responses, because they are unlikely to reliably stimulate GL precursors to initiate antibody maturation.

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the mature bNabs. Furthermore, we developed self-assembling nanoparticles presenting 60 copies of the germline-targeting eOD to enhance B cell activation and to improve trafficking to lymph nodes (Fig. 1).

**Engineering and Biophysical Analysis of Germline-Targeting Antigens**

Modifications to the VRC01 epitope included removing clashes and building new contacts between the CD4bs and the GL-Abs, as well as rigidifying the CD4bs in a conformation that is favorable for binding. Initially, we constructed a homology model of GL-VRC01 bound to gp120 and identified a likely clash between CDRL1 and the N276 glycan. Therefore, we evaluated the GL-VRC01 binding of an eOD (eOD-Base) that lacks glycans at 276 and on the nearby V5 loop owing to N276D and N463D mutations. The eOD-Base barely interacted with GL-VRC01 binding of an eOD (eOD-Base) that lacks glycans at 276 and on the nearby V5 loop owing to N276D and N463D mutations. The

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**Fig. 1. Development of a germline (GL)–targeted HIV immunogen. (A)** VRC01-class bNabs bind to gp120 primarily through paratope residues encoded by VH 1-2*02. gp120 is colored green, with the CD4 binding site highlighted in yellow. Glycans are represented as blue spheres with the critical N276 highlighted in magenta. VRC01 is shown as a secondary structure rendering and colored gray, with the VH1-2*02 region highlighted in red. (B) Steps in the engineering of a modified gp120-based nanoparticle capable of activating GL VRC01-class B cells. (C) This nanoparticle can be used in an HIV-1 vaccine GL-prime-boost strategy to bridge this initial recognition gap and initiate clonal expansion and start somatic hypermutation of VRC01-class bNAb precursors.
2.4 Å, respectively (Fig. 2 and table S4). The unliganded GL-VRC01 structure revealed that the gp120 contacting loops closely resemble those of VRC01 despite extensive affinity maturation of the latter (Fig. 2A and figs. S5 to S7). Unliganded eOD-GT6 showed a similar structure to the outer domains of unliganded and VRC01-bound gp120 core (1.2 Å Cα root mean square deviation (RMSD) in both instances) (Fig. 2B and fig. S8), suggesting good mimicry of the CD4bs. The structure of unliganded eOD-GT6 was also similar to eOD-GT6 bound to GL-VRC01 Fab (0.9 Å RMSD), with the largest differences occurring in the flexible loops and in the eOD exit loop (fig. S9). In addition, the structure of the eOD-GT6+GL-VRC01 complex indicated that the VH1-2-encoded domain of GL-VRC01 approaches eOD-GT6 at an angle nearly identical to that of VRC01 with gp120 (Fig. 2C) (4.2° angular difference when the complexes are superposed on the CD4 binding loop).

Overall, the buried surface area (BSA) of GL-VRC01 approaches eOD-GT6 nearly identical to that of VRC01 with gp120 (Fig. 2C), further demonstrating the high degree of similarity between the two structures (tables S5 and S6). Key hydrogen-bonding networks are preserved in the GL-VRC01+eOD-GT6 interaction, particularly in the CD4 binding loop (fig. S10). On the other hand, important differences in hydrogen bonds, BSA, and Cα positions are observed for interactions in loop D, V5, and the OD exit loop, which contribute to GL-VRC01 reactivity to eOD-GT6 (Fig. 2D and fig. S11).

**Mutation Analysis**

To understand the affinity contributions of individual mutations, we measured GL-VRC01 binding affinities for point reversions of each mutation (Table 2). Six mutations on eOD-GT6 conferred improved affinity for GL-Abs relative to the starting construct (eOD-Base) that lacked glycans at 276 and 463. The eOD-GT6+GL-VRC01 complex structure revealed that these mutations either are directly involved in the binding interface (T278R, I371F, and N460V) or stabilize loops involved in the interface (L260F, K357R, and G471S) (Fig. 2C). The two mutations with the largest effect on GL-VRC01 binding were G471S and I371F; reversion at these positions reduced GL-VRC01 affinity by factors of 39 and 10, respectively (Table 2). Ser471, together with Phe371 and Phe360, appear to play a role in altering the conformation of the OD exit loop to allow the GL-VRC01 CDRH2 to make H bonds with three additional gp120 residues (G472, G473, and D474) and bury an additional 120 Å² on gp120, resulting in improved binding (Fig. 2, C and D, right inset panel, tables S5 and S6, and fig. S9).

Also, the N460V mutation located in V5 improves packing with the antibody and appears to contribute to an altered V5 conformation and pattern of V5 H-bonding with VRC01, as compared with Clade A/E 93TH057 gp120 recognition of VRC01 (Fig. 2D and fig. S12). Reversion of the N460V mutation reduced GL-VRC01 binding by a factor of 2.5 (Table 2).

Removal of key glycosylation sites was necessary for GL affinity. Reintroduction of the N276 glycosylation site in eOD-GT6 (by a double reversion, D276N/R278T) reduced binding by a factor of 140, and the remaining binding was likely due to a small fraction of the eOD-GT6-D276N/R278T that underutilized the N276 glycosylation position (fig. S13). Reversion of R278T alone reduced affinity by a factor of only 3.6 (Table 2). Thus, removal of the 276 glycan appears to release a block on GL-VRC01 binding but does not confer appreciable eOD affinity; further interface modification was required to achieve high affinity. Indeed, the eOD-GT6+GL-VRC01 complex structure revealed that, in addition to removing a clash between the N276 glycan and CDRL1 (Fig. 2C, left inset panel), eOD-GT6 D276 and R278 make two additional H-bonds with GL-VRC01. eOD-GT6 also lacks glycans at positions 386 (I12) and 463 (V5). Restoration of these glycosylation sites reduced affinity for GL-VRC01 by a factor of 3 (table S7).

**eOD-GT6 Nanoparticle Generation**

To enable eOD-GT6 to activate GL B cells via cross-linking of B cell receptors, and to develop a multivalent platform for eOD-GT6 that mimics the size, shape, multivalency, and symmetric surface geometry of many viruses for improved immunogenicity (21), we sought to fuse eOD-GT6 to a self-assembling virus-like nanoparticle. From a search of large homomeric particles in the Protein Data Bank (PDB), we prioritized 60-mer molecular modules for human VH1-2 (Fig. 3A) (22, 23), with a known VH gene (24). Chimeric GL-Ab constructs were produced in which the GL VH genes from mice or macaques were paired with GL VH genes from mice or macaques (Fig. 3B and figs. S14 and S15). The GL-VRC01 antibody was expressed from the germline sequence in an Escherichia coli-based expression system with a polyhistidine tag at the C-terminus (Fig. 3C). Both IgM and IgG B cell lines were generated for GL 12A12, and we observed no significant differences in activation magnitude or kinetics between the two antibody isotypes (fig. S16).

**In Vitro B Cell Activation**

The ability of eOD-GT6 nanoparticles to activate GL B cells expressing GL and mature VRC01 [immunoglobulin M (IgM)] (23, 24) and VRC01 [immunoglobulin G (IgG)] (24) was tested in Ca²⁺-dependent activation assays. The nanoparticles potently activated both GL and mature B cells with 1 μM outer domain (16 nm particle) and modestly activated all three cell lines at 1000-fold lower concentrations (Fig. 3C and fig. S16). In contrast, monomeric eOD-GT6 was nonstimulatory, probably due to an inability to cross-link B cell receptors (28). Trimeric eOD-GT6 activated both GL and mature B cells, but less potently and rapidly than the 60-mer nanoparticles, and a soluble gp140 trimmer from HIV-1 strain YU2 (25) showed no activation of GL B cells but did activate the mature counterparts (Fig. 3C). Both IgM and IgG B cell lines were generated for GL 12A12, and we observed no significant differences in activation magnitude or kinetics between the two antibody isotypes (fig. S16).

**Animal Models for Human VH1-2**

**Germline Targeting**

We then assessed whether eOD-GT6 might interact with related GL-Abs in animal models. Analysis of VH genes from rabbit (fig. S7) (26), mouse (figs. S18 and S19) (27), and macaque (fig. S20) revealed that none of these commonly used model organisms have a known VH gene containing all of the critical residues for GL binding (15). To measure binding experimentally, chimeric GL-Abs were produced in which the human VH1-2*02 gene from GL-VRC01 was replaced with GL VH genes from mice or macaques containing the essential Arg167 and as many other

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**Table 1. Binding of GL and mature (Mat) antibodies to gp120 and eOD variants.** Values represent K₅₀ in nM measured by surface plasmon resonance (SPR). Detectable binding to GL antibodies is in boldface.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>VRC01</th>
<th>12A12</th>
<th>3BNC60</th>
<th>NH45-46</th>
<th>PGV04</th>
<th>PGV19</th>
<th>PGV20</th>
<th>VRC-CH31</th>
</tr>
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<tbody>
<tr>
<td>Wild-type</td>
<td>Core.HXB2</td>
<td>GL&gt;10⁰</td>
<td>5 &gt;10⁰</td>
<td>6 &gt;10⁰</td>
<td>36&gt;10⁰</td>
<td>35&gt;10⁰</td>
<td>48&gt;10⁰</td>
<td>20&gt;10⁰</td>
<td>19&gt;10⁰</td>
</tr>
<tr>
<td>Germline-targeted</td>
<td>eOD-Base</td>
<td>N276D</td>
<td>&gt;10⁰</td>
<td>5 &gt;10⁰</td>
<td>380 &gt;10⁰</td>
<td>4100&gt;10⁰</td>
<td>14&gt;10⁰</td>
<td>110&gt;10⁰</td>
<td>3100&gt;10⁰</td>
</tr>
<tr>
<td>Core.BaLG1</td>
<td>1800</td>
<td>0.5</td>
<td>3200</td>
<td>1</td>
<td>14,600</td>
<td>4 &gt;10⁰</td>
<td>0.6&gt;10⁰</td>
<td>170</td>
<td>25</td>
</tr>
<tr>
<td>eOD-GT1</td>
<td>44,000</td>
<td>1</td>
<td>&gt;10⁰</td>
<td>2300</td>
<td>83 &gt;10⁰</td>
<td>3 &gt;10⁰</td>
<td>4 &gt;10⁰</td>
<td>7800</td>
<td>10000</td>
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<td>44</td>
<td>2</td>
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<td>14,000</td>
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<td>410</td>
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Critical residues as possible. Chimeric GL-Abs with mouse VH genes had no detectable binding to eOD-GT6. Chimeric Abs derived from two of the three rhesus VH genes bound weakly to eOD-GT6, with $K_D$ so &~30 mM and ~40 mM (table S8). The rhesus chimeric GL-Ab most similar to human GL-VRC01 contained only 10 mutations in the VH gene (95.5% identity over the antibody Fv region) but showed no detectable binding to eOD-GT6. Annotation of the rhesus macaque antibody repertoire and analysis of gene usage frequencies will be useful to construct bona fide macaque GL VH1-2 Abs. These analyses illustrate the potential difficulty for using animal models to produce VRC01-class bNAbs and suggest that immunization of humans or mice engineered to produce human Abs may be essential for testing and iteratively optimizing such immunogens.

**Concluding Remarks**

The events that led to GL VH1-2*02 B cell activation in the HIV-infected individuals from which VRC01-class bNAbs were isolated remain unclear. Our finding that a small number of rare or previously undocumented Env mutations confers high affinity GL binding suggests that Env variants might have acquired one or more such mutations stochastically during infection and thereby gained the ability to prime GL VRC01-class B cells. Vaccines to induce VRC01-class responses will need to activate such B cells dependably and drive appropriate somatic mutation to produce high-affinity bNAbs (28). We propose the eOD-GT6 nanoparticle as a promising candidate for a vaccine prime based on its ability to bind diverse VH1-2*02 GL Abs, activate VRC01, 12A12, and NIH45-46 B cell lines in vitro, and provide an affinity gradient for early
somatic mutation. We further propose that ultimate elicitation of mature VRC01-class bNAbs will require, at minimum, boosting with different immunogens that present a less engineered, more native CD4bs, including the glycans around the CD4bs.

### References and Notes

Lorentz Meets Fano in Spectral Line Shapes: A Universal Phase and Its Laser Control

Christian Ott, Andreas Kaldun, Philipp Raith, Kristina Meyer, Martin Laux, Jörg Evers, Christoph H. Keitel, Chris H. Greene, Thomas Pfeifer

Symmetric Lorentzian and asymmetric Fano line shapes are fundamental spectroscopic signatures that quantify the structural and dynamical properties of nuclei, atoms, molecules, and solids. This study introduces a universal temporal-phase formalism, mapping the Fano asymmetry parameter $\varphi$ to a phase $\varphi$ of the time-dependent dipole response function. The formalism is confirmed experimentally by laser-transforming Fano absorption lines of autoionizing helium into Lorentzian lines after attosecond-pulsed excitation. We also demonstrate the inverse, the mapping of symmetric Lorentzian and asymmetric Fano line shapes, which is the superposition of a continuous spectrum and continuum, respectively. The exponential dipole response is shifted in phase with respect to the Lorentzian response, which is the origin of the asymmetric line shape of the Fano resonance. By a mathematical transformation [supplementary text (12)], we mapped this phase shift $\varphi$ in the time domain into the parameter $q$, which was introduced by Ugo Fano ($I$, $2$) and thereafter used to characterize and quantify the asymmetric Fano line shape. The cross section at photon energy $E = \hbar \omega$ is given in terms of $q$ by

$$\sigma_{\text{Fano}}(E) = \sigma_0 \left( \frac{q + \epsilon}{1 + e^{-\epsilon}} \right)$$

where $\epsilon$ denotes the reduced energy containing $E_0$ and $\Gamma$ as the position and width of the resonance, respectively, $\hbar$ denotes the reduced Planck constant, and $\sigma_0$ is the cross section far away from the resonance.

In general, the absorption cross section $\sigma_{\text{abs}}$ is proportional to the imaginary part of the index of refraction, which in turn is directly related to the polarizability ($\nu$) and thus to the frequency-dependent dipole response function $d(E)$:

$$\sigma_{\text{abs}}(E) \propto \text{Im}[d(E)]$$

Via the Fourier transform, $d(E)$ is connected to the time-dependent linear response $d(t)$ of the medium after a deltalike excitation pulse. For a Lorentzian spectral line shape of width $\Gamma$, $d_{\text{Lorentz}}(t)$ is an exponentially decaying function.