Chapter 5

Binding of inferred germline precursors of broadly neutralizing HIV-1 antibodies to native-like envelope trimers

Kwinten Sliepen1, Max Medina-Ramírez1, Anila Yasmeen2, John P. Moore2, Per Johan Klasse2, Rogier W. Sanders1,2

1. Department of Medical Microbiology, Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands
2. Department of Microbiology and Immunology, Weill Medical College of Cornell University, New York, NY 10065, USA
* Co-first author

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Abstract
HIV-1 envelope glycoproteins (Env) and Env-based immunogens usually do not interact efficiently with the inferred germline precursors of known broadly neutralizing antibodies (bNAbs). This deficiency may be one reason why Env and Env-based immunogens are not efficient at inducing bNAbs. We evaluated the binding of 15 inferred germline precursors of bNAbs directed to different epitope clusters to three soluble native-like SOSIP.664 Env trimers. We found that native-like SOSIP.664 trimers bind to some inferred germline precursors of bNAbs, particularly ones involving the V1/V2 loops at the apex of the trimer. The data imply that native-like SOSIP.664 trimers will be an appropriate platform for structure-guided design improvements intended to create immunogens able to target the germline precursors of bNAbs.
Main text

To be effective, a HIV-1 vaccine should elicit bNAbs that target the trimeric Env spike on the virion surface [1]. No Env immunogen has been able to elicit bNAbs in animals or humans, but ~20% of HIV-1-infected patients do eventually develop these antibodies after ~2-3 years, and some exceptional patients develop bNAbs within a year [2]. Longitudinal analyses have shown that bNAbs generally emerge through a co-evolutionary process that is driven by iterative cycles of HIV-1 escape from more narrowly focused NABs, followed by renewed Ab affinity maturation [3,4].

To generate bNAbs by vaccination, it may be necessary to mimic such affinity maturation pathways [5]. Initiating any particular bNAb lineage requires activating the naïve B cells through their B cell receptor, i.e. the unmutated germline antibody [5]. For this to happen in a vaccine setting, the Env-based immunogen should, therefore, be capable of binding germline antibodies that have the potential to evolve into bNAbs. A complication is that most HIV-1 isolates appear incapable of interacting with the germline versions of bNAbs, which may be the outcome of how HIV-1 immune evasion strategies have evolved over time. In consequence, most recombinant Env proteins also cannot engage the inferred germline precursors of known bNAbs (gl-bNAbs) [6,7], either because they adopt non-native conformations or because they are derived from viruses that also lack the required reactivity. The problem is not universal, in that some Env proteins based on autologous founder virus sequences isolated from the patient from which a particular bNAb was isolated can sometimes bind the germline precursor of that bNAb [3,4,8]. Furthermore, Env immunogens can be specifically engineered to have such properties [9–12].

Recently, several soluble, recombinant SOSIP.664 Env trimers from clades A (isolate BG505), B (isolate B41) and C (isolates ZM197M and DU422) have been described [13–15] Electron microscopy imaging, glycan profiling and antigenicity studies show that these SOSIP.664 trimers mimic the virion-associated Env trimer [13–16]. In addition, the BG505 and B41 SOSIP.664 trimers have induced consistent NAb responses against the autologous tier 2 viruses, which has not been achieved by non-native Env immunogens [17].

Whether native-like trimers such as the above SOSIP.664 proteins can interact with gl-bNAbs is clearly relevant to strategies intended to induce neutralization breadth. There are reasons to believe that trimers that do so may be desirable. First, only native-like trimers consistently present several quaternary structure-dependent bNAb epitopes at the V1V2-apex or the gp120/gp41 interface [13,18,19]. Second, native-like trimers force the appropriate restrictions on the selection of Abs with the correct, trimer-compatible angles of approach, and thereby limit the exposure of immunodominant non-neutralizing epitopes that could interfere with the triggering of the desired bNAb germline [13,20,21]. We have, therefore, assessed whether the BG505, B41 and ZM197M SOSIP.664 trimers can interact with a set of 15 gl-bNAbs.

Epitope-tagged SOSIP.664-D7324 or SOSIP.664-His trimers, expressed in 293F cells, were purified by PGT145 bNAb-affinity chromatography [14]. We used ELISA and, in some cases, surface plasmon resonance (SPR) methods to assess trimer binding to 15 gl-bNAbs, targeting five distinct Env epitope clusters: the CD4 binding site (CD4bs) (VRC01, 3BNC60, 1NC9, CH103, CH31); the glycan-dependent V3 cluster (PGT121, PGT128);
the V1V2-apex (PG9, PG16, PGT145, VRC26.09, CH01) [4,22]; the gp120/gp41 interface (PGT151, 35O22) [18,19]; gp41 (3BC315) [23]. We did not test binding to gp120 monomers or uncleaved gp140 proteins, since the mature versions of PG9, PG16, PGT145, VRC26.09, PGT151, 35O22 and 3BC315 have been reported to bind these proteins very inefficiently [4,18,19,23–25].

### Table 1. Putative gene usage and CDR3 sequences of the gl-bNAbs used in this study.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>VH-gene</th>
<th>HCDR3a</th>
<th>JH-gene</th>
<th>VL-gene</th>
<th>LCDR3a</th>
<th>JL-gene</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG9</td>
<td>V3-33*05</td>
<td>ARXGGPQDYKIDQYDDGQTYTYYMENY</td>
<td>J6</td>
<td>I1</td>
<td>T2</td>
<td>IMGT</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>PG16</td>
<td>V3-33*05</td>
<td>ARXGGPQDYKIDQYDDGQTYTYYMENY</td>
<td>J6</td>
<td>I1</td>
<td>T2</td>
<td>IMGT</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>PGT145</td>
<td>V1-8*01</td>
<td>G5XH9IID6YKIDQYDDGQTYTYYMENY</td>
<td>J6</td>
<td>I1</td>
<td>T2</td>
<td>IMGT</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>VRC26.09</td>
<td>V3-30*18</td>
<td>GADKEGGEENAEWDQIDISGYPRQ</td>
<td>J3</td>
<td>I1</td>
<td>50</td>
<td>IMGT</td>
<td>N.A.</td>
<td></td>
</tr>
</tbody>
</table>

The sequences of germline versions of PG9, PGT145, PGT151, 35O22, PGT128, 1NC9, 3BC315 were inferred using the IMGT/V QUEST online tools [26] (Table 1). We note that PG9 and PG16 are clonal relatives and originate from the same germline precursor [27]. However, it is difficult to infer an accurate germline sequence of the long heavy chain complementarity-determining regions 3 (HCDR3) of these antibodies. Therefore, we used two different germline precursors of the PG9/16 lineage: gl-PG16 was based on the previously published sequence [27] and gl-PG9 was inferred as described above. The gl-bNAb sequences were synthesized by Genscript, cloned into the pVRC8400 expression vector, transfected into 293F cells and then purified by a protein A/G agarose column (Thermo Scientific). The mature and germline versions of PG9, PG16 and PGT145 were expressed in the presence of exogenous tyrosylprotein sulfotransferase 1 (TPST1) to ensure they were tyrosine-sulfated [28]. The germline versions of 3BNC60, VRC01, CH31, CH103, PGT121, CH01 and VRC26 were kindly provided by colleagues (Table 1). The ELISA for measuring Ab-trimer binding was modified from a published method [13], as follows: 3 μg/ml of SOSIP.664-D7324 proteins were diluted in Tris-buffered saline pH 7.5 (TBS) containing 10% fetal calf serum (FCS), and captured on D7324 Ab-coated plates. Mature and gl-bNAbs were serially diluted in casein-blocking solution (Thermo Scientific). Half-maximal binding Ab concentrations (EC$_{50}$) were derived using Graphpad Prism (version 5.01). All 15 mature bNAbs bound to all three SOSIP.664-D7324 trimers, which is mostly consistent with the antigenicity profiles reported previously [13,14] (Julien et al. in press) (Table 2). We note that, here, the mature versions of PGT151 and 35O22 were reactive with the B41 SOSIP664 trimers in ELISA (Table 2), which was not seen in our previous study [14]. The difference may arise because we increased the assay sensitivity by using a higher input concentration of the B41 SOSIP664-D7324 trimer (i.e., 3 μg/ml vs. 0.3 μg/ml, previously) and we used a different blocking solution (i.e., casein blocking solution vs. 2% milk, previously).
Table 2. Summary of EC_{50} values (in ng/ml) derived from D7324-capture ELISA with different SOSIP664 trimers. For comparison, midpoint neutralization concentrations (IC_{50} in ng/ml) of the sequence-matched Env-pseudotyped viruses are also indicated.

<table>
<thead>
<tr>
<th>SOSIP664</th>
<th>BG505</th>
<th>B41</th>
<th>ZM197M</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pseudovirus</td>
<td>SOSIP664</td>
<td>Pseudovirus</td>
</tr>
<tr>
<td>PG9</td>
<td>&gt;25000</td>
<td>242</td>
<td>&gt;25000</td>
</tr>
<tr>
<td>PGT145</td>
<td>&gt;25000</td>
<td>65</td>
<td>&gt;25000</td>
</tr>
<tr>
<td>VRC01</td>
<td>&gt;25000</td>
<td>67</td>
<td>&gt;25000</td>
</tr>
<tr>
<td>CH01</td>
<td>&gt;25000</td>
<td>N.D.</td>
<td>&gt;25000</td>
</tr>
</tbody>
</table>

For comparison, midpoint neutralization concentrations (IC_{50} in ng/ml) of the sequence-matched Env-pseudotyped viruses are also indicated.

**Figure 1.** Representative binding curves of a panel of mature and gl-bNAbs tested in the same ELISA format ("mock": the wells contained only 10% FCS in TBS), except gl-CH103, for which the background was considerably higher ("gl-CH103 mock").

The BG505 SOSIP.664-D7324 trimer bound to gl-PG9 and its somatic relative gl-PG16 (EC_{50}: 1.0 and 15 μg/ml, respectively), its ZM197M counterpart bound to gl-PG9 (EC_{50}: 6.8 μg/ml) but not gl-PG16, while the B41 trimer bound neither gl-PG9 nor glPG16 (Fig. 1 and Table 2). Compared to gl-PG16, gl-PG9 contains more tyrosines that are potentially sulfated, which could increase affinity for the cationic groove at trimer apex. This might explain the increased reactivity of gl-PG9 with BG505 and ZM197M SOSIP.664 trimers. The BG505 and B41 trimers also bound to gl-CH01 (EC_{50}: 0.64 and 1.0 μg/ml respectively), gl-VRC26 and gl-PG15, which bind to a similar epitope as PG9 and PG16, but have entirely different HCDR3 loops and are derived from different germline genes, did not bind to any of the SOSIP.664 trimers. We observed a remarkably high affinity interaction between gl-3BC315 and the BG505 and ZM197M trimers (EC_{50}: 0.27; 0.54 μg/ml respectively) (Fig. 1 and Table 2). We note, however, that the HCDR3 of the germline- and mature versions of 3BC315 were identical, since it was not possible to reliably infer the germline HCDR3 sequence from the mature version. As the HCDR3 of 3BC315 is known to make important hydrophobic contacts with the gp41/ gp120 interface [23], this interaction may contribute to the high affinity the gl-3BC315 antibody has for the BG505 and ZM197M trimers. However, as gl-3BC315 did not bind to the B41 trimers, there are complexities to the Ab-trimer binding events that remain to be understood, such as the involvement of topologically proximal
glycans in either the formation or the occlusion of the epitope [23].

None of the three trimers bound the gl-bNabs targeting the glycan-dependent V3 epitopes (PGT121 and PGT128). They also did not interact detectably with the VRC01, 3BNC60, 1NC9 or CH31 gl-bNabs against the CD4bs, perhaps because of the shielding effects of various trimer glycans. An example is the clash between the N276 glycan and the gl-VRC01 light chain [9]. While gl-CH103 was prone to generating a high level of non-specific background signals in ELISA (Fig. 1, “gl-CH103 mock”), we did detect some specific binding of this antibody to the ZM197M trimers (EC$_{50}$: 18.1 μg/ml) although not to their BG505 and B41 counterparts (Fig. 1 and Table 2).

To corroborate the binding of gl-PG16 to the BG505 SOSIP.664-D7324 trimers, we performed SPR analyses using the His-tagged version of the same trimers. The data were fitted with a bivalent model, and binding parameters for the monovalent component in the bivalent model are given [24]. We confirmed that gl-PG16 bound to BG505 SOSIP.664-His trimers, although with a lower affinity (dissociation constant, $K_d = 320$ nM) than mature PG16 ($K_d = 24$ nM) (Fig. 2A). The reduced affinity arises because $k_{on}$ (on-rate constant) and $k_{off}$ (off-rate constant) were ~5-fold lower and ~3-fold higher, respectively, for gl-PG16 (Fig. 2B). As the binding stoichiometry was also lower for gl-PG16 ($S_m = 0.4$) than for mature PG16 ($S_m = 1.3$) only a subset of the trimers can bind gl-PG16, perhaps because of variation in the presence or composition of particular glycan sites (Fig. 2B). The difference in binding to BG505 SOSIP.664 between gl-PG16 and mature PG16 were more substantial in ELISA (EC$_{50}$ values = 13.1 μg/ml and 0.031 μg/ml, respectively) than in SPR ($K_d = 320$ nM and 24 nM, respectively). This can probably be explained by the high off-rate, which results in loss of binding signal in ELISA during washing steps. We note that the BG505 trimers are based on a transmitter/founder virus sequence that is similar in the relevant regions to viruses isolated from the donor of PG9 and PG16 [13,29]. This sequence homology may help explain why gl-PG9 and gl-PG16 bind to the BG505 trimers. In contrast, but consistent with the ELISA data, gl-VRC01 did not bind to BG505 SOSIP.664-His trimers in the SPR assay (Fig. 2A).

Gl-bNab sequences are based on in silico predictions. Whether these inferred gl-bNabs truly represent the in vivo naïve B cell receptors remains to be determined. Nevertheless, this exploratory study indicates that various SOSIP.664 trimers can bind to several gl-bNabs, mainly ones that are directed to the V1/V2 loops. Hence these trimers are good starting points for engineering immunogens that more efficiently activate naïve
B cell receptors and thereby initiate pathways that lead to the eventual emergence of bNAbs.

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**References**


