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Antibodies from rabbits immunized with HIV-1 clade B SOSIP trimers can neutralize multiple clade B viruses by destabilizing the envelope glycoprotein

MM van Haaren[1], LE McCoy[2,3], JL Torres[4], W Lee[4], CA Cottrell[4], JL Copps[4], P van der Woude[1], A Yasmeen[5], SW de Taeye[1], A Torrents de la Peña[1], JP Moore[5], DR Burton[2,6,7], PJ Klasse[5], AB Ward[4,6,7], RW Sanders[1,5¥], MJ van Gils[1¥]

[1]Department of Medical Microbiology, Amsterdam Infection & Immunity Institute, Amsterdam UMC, location AMC, University of Amsterdam, Amsterdam, 1105AZ, The Netherlands.

[2]Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, California, 92037, USA.

[3]Division of Infection and Immunity, University College London, London, WC1E 6BT, UK.

[4]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, California, 92037, USA.

[5]Department of Microbiology and Immunology, Weill Medical College of Cornell University, New York, New York, 10065, USA.

[6]International AIDS Vaccine Initiative - Neutralizing Antibody Center (IAVI-NAC), The Scripps Research Institute, La Jolla, CA 92037, USA.

[7]Center for HIV/AIDS Vaccine Development (CHAVD), The Scripps Research Institute, La Jolla, CA 92037, USA.

[¥] **Corresponding authors:**

Rogier W. Sanders, PhD; R.W.Sanders@amsterdamumc.nl

Marit J. van Gils, PhD; M.J.vangils@amsterdamumc.nl

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ABSTRACT

The high HIV-1 viral diversity is a formidable hurdle for the development of an HIV-1 vaccine. Elicitation of broadly neutralizing antibodies (bNAbs) would offer a solution, but so far immunization strategies have failed to elicit bNAbs efficiently. To overcome the obstacles, it is important to understand the immune responses elicited by current HIV-1 envelope glycoprotein (Env) immunogens. To gain more insight, we characterized monoclonal antibodies (mAbs) isolated from rabbits immunized with Env SOSIP trimers based on the clade B isolate AMC008. Four rabbits that were immunized three times with AMC008 trimer developed robust autologous and sporadic low-titer heterologous neutralizing responses. Seventeen AMC008 trimer-reactive mAbs were isolated using antigen-specific single B cell sorting. Four of these mAbs neutralized the autologous AMC008 virus and several other clade B viruses. When visualized by electron microscopy, the complex of the neutralizing mAbs with the AMC008 trimer showed binding to the gp41 subunit with unusual approach angles and we observed that their neutralization ability depended on their capacity to induce Env trimer dissociation. Thus, AMC008 SOSIP trimer immunization induced clade B neutralizing mAbs with unusual approach angles with neutralizing effects that involve trimer destabilization. Optimizing these responses might provide an avenue to the induction of trimer dissociating bNAbs.

Keywords

HIV-1, vaccine, monoclonal antibodies, AMC008 SOSIP, trimer destabilization, approach angle

IMPORTANCE

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Roughly 32 million people have died as a consequence of HIV-1 infection since the start of the epidemic and still 1.7 million people get infected with HIV-1 annually. Therefore, a vaccine to prevent HIV-1 infection is urgently needed. Current HIV-1 immunogens are not able to elicit the broad immune responses needed to provide protection against the large variation of HIV-1 strains circulating globally. A better understanding of the humoral immune responses elicited by immunization with state-of-the-art HIV-1 immunogens should facilitate the design of improved HIV-1 vaccine candidates. We identified antibodies with the ability to neutralize multiple HIV-1 viruses by destabilization of the envelope glycoprotein. Their weak but consistent cross-neutralization ability indicates the potential of this epitope to elicit broad responses. The trimer-destabilizing effect of the neutralizing mAbs combined with detailed characterization of the neutralization epitope can be used to shape the next generation of HIV-1 immunogens to elicit improved humoral responses after vaccination.

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INTRODUCTION

The ongoing HIV-1 epidemic, in spite of effective HIV-1 medication, highlights the need for an HIV-1 vaccine. To achieve this goal, knowledge on the immune responses elicited by state-of-the-art HIV-1 immunogens is important. Such knowledge will allow the further optimization and development of these immunogens. Many immunogens that are being explored as subunit vaccines are based on the HIV-1 envelope glycoprotein (Env) trimer (1–6). The Env trimer is the only viral protein expressed on the outside of the HIV-1 particle and therefore the only target for neutralizing antibodies (NAbs). Because circulating HIV-1 viruses have extremely diverse Env sequences, in order to provide protection, an HIV-1 vaccine needs to induce broadly NAbs (bNAbs), i.e. NAbs that can cope with Env diversity(7). Extensive research has provided the field with soluble, stable, and native-like versions of Env, including SOSIP trimers(8). So far, SOSIP trimers have generally elicited strong autologous NAb responses, but only sporadic, inconsistent, and weak cross-NAb responses(9–12). It is imperative to study these Ab responses to understand precisely which improvements are needed to consistently broaden the response. Iterative vaccine design based on monoclonal Abs (mAbs) isolated from vaccinated animals is a valuable way to overcome the limitations of the current HIV-1 immunogens(13, 14).

Previous studies characterizing mAbs and bulk serum of SOSIP Env trimer immunized rabbits and macaques showed that the Ab responses frequently target strain-specific glycan holes(15–17). Indeed, the immunodominance of glycan holes was confirmed by re-direction of vaccine induced Ab responses towards *de novo* glycan holes when the original strain-specific glycan hole was filled(18). Env trimers from different virus isolates probably have their own specific immunodominant glycan holes, which would explain why Env-trimer immunized

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animals develop very limited neutralization breadth. Another immunodominant region after immunization is the unprotected base of the soluble Env trimer (17, 19, 20). This region of the Env trimer is, in its natural display, concealed by the viral membrane and in no need of heavy glycosylation to evade the immune system. However, on soluble Env trimers the base forms a large glycan hole that is easily accessed by the immune system, and induces Abs that cannot recognize the full length Env trimer, i.e. are non-NAbs.

Many vaccine induced NAbs target epitopes that overlap those of non-NAbs(11, 15, 16). Yet, it is unclear what exactly determines whether an Ab will have neutralizing ability. For some Ab families binding kinetics might influence neutralization ability(21, 22). Indeed, it was shown that some Ab families elicited during natural infection have kinetics of Ab binding that correlate with their neutralization ability. In particular a high off-rate constant was associated with absent or less effective neutralization. The on-rate constant and overall affinity appeared to be of less influence on the ability to neutralize(21, 22).

SOSIP-induced NAbs have been discovered that target the same epitope as well-known bNAbs elicited after natural HIV-1 infection, but without displaying the same potency or breadth(17, 19, 23–25). Some studies have shown that the approach angle is relevant in this respect. For instance, for CD4-binding site (CD4-bs) directed Abs the correct approach angle is essential for their ability to neutralize. The right approach angle allows bNAbs to reach the CD4bs while circumventing the dense Env glycan shield(23).

Several vaccines involving state-of-the-art Env trimer immunogens(1, 10) or specific Env epitope scaffolds(26, 27) have induced sporadic NAb responses against heterologous viruses in addition to autologous NAbs. Although such heterologous neutralization can be broad, spanning many clades, it is usually not very potent. Interestingly, two bNAbs have been

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isolated from a rabbit immunized with Env trimers on liposomes(1). The serum of this rabbit serum exhibited broad neutralization, and the isolated bNAbs recapitulated that activity. The development of breadth in this one rabbit was exceptional, as none of the other rabbits receiving the same immunogens developed this remarkable neutralization breadth, but the detailed characterization of such immune responses through the isolation of mAbs helps to understand why the development of neutralization breadth is rare, and how it can be improved.

In this study we isolated mAbs from four rabbits immunized with the clade B Env trimer immunogen AMC008 SOSIP. We identified an immunodominant area on the gp41 subunit of the AMC008 SOSIP trimer. Interestingly, these NAbs could cross-neutralize other clade B viruses. Negative-stain electron microscopy (NS-EM) revealed that these NAbs bound with an unusual approach angle that would be expected to be incompatible with binding to virus-associated Env trimers because of a clash with the viral membrane. Contrary to expectations and despite the unusual angle of approach, these mAbs were able to bind and neutralize, demonstrating remarkable flexibility of virus-associated Env trimers in their interaction with Abs(28). We further showed that the neutralization capacity of these NAbs depended on their ability to dissociate the Env trimer, similarly to what has been described previously for bNAbs isolated from both humans and rabbits(1, 29). The information gathered from this study helps to elucidate mechanistic aspects of virus neutralization and may help to tailor immunogens to elicit trimer dissociating NAbs.

RESULTS

AMC008 SOSIP immunization induces NAbs and non-NAbs

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In a previous study by our group, fifteen rabbits (animal IDs: 1594-1608) were immunized with the clade B Env trimer AMC008 SOSIP(10). This immunogen was based on the viral sequence from an individual enrolled in the Amsterdam Cohort Studies (ACS) that showed broad serum neutralization(10). All AMC008 SOSIP trimer-immunized rabbits showed consistent autologous neutralization as well as low cross-neutralization of the clade B viruses BaL, REJO, WITO, and SHIV162p3(10). We further investigated serum neutralization of animals 1605-1608 and observed cross-neutralized at low titers by two and four rabbits of the clade B viruses AMC009 and AMC018, isolated from different HIV-infected individuals (**figure 1A**). To better understand the cross-neutralization in these animals and its limitations, mAbs were isolated from these four AMC008 SOSIP trimer-immunized rabbits. PBMCs were obtained from these animals at week 21, one week after the third immunization (**figure 1A**). Single B cells expressing IgG, and with the ability to bind two distinctly labeled, fluorescent AMC008 SOSIP trimers were selected by fluorescence-activated cell sorting (FACS). On average, 5% of total live B cells were AMC008 SOSIP trimer-reactive. B cell receptor (BCR) sequences were subsequently amplified and cloned into expression vectors to generate mAbs that were tested for binding to autologous AMC008 SOSIP trimers in ELISA. A total of 17 mAbs bound the AMC008 SOSIP trimer. Rabbits 1605 and 1607 yielded 7 and 8 mAbs respectively, while from the other two rabbits (1606 and 1608) only one mAb each was generated (**figure 1B**).

Sequence analysis of the heavy chain variable region showed the expected polyclonal immune response within each rabbit (**figure 1C**). From these sequences we determined the length of the complementary determining region 3 of heavy and light chains (CDRH3 and CDRL3, respectively). The CDRH3 is important as it interacts directly with the immunogen and is often elongated in human bNAbs to cope with the extensive glycan shield surrounding the

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Env trimer(30, 31). We also analyzed the CDRL3 because rabbit Abs, in contrast to human Abs, usually interact with the immunogen predominantly through the CDRs of their light chain(32). The average length of the CDRH3 of all AMC008 SOSIP trimer-reactive mAbs was 15 amino acids, which agrees with previous studies on CDRH3 lengths of BCR sequences in naïve rabbits(32, 33) (**figure 1D**). However, the individual CDRH3 length varied greatly, ranging from 10-22 amino acids. The average CDRL3 length was increased with 2 amino-acid residues to an average of 14 residues in AMC008 SOSIP trimer-reactive mAbs from 12 residues in BCR sequences from naïve rabbits(32, 33). The distribution of the individual CDRL3 lengths in the rabbits was more limited than that of the CDRH3 region, ranging from 11-16 amino acids (**figure 1D**).

We then tested the isolated mAbs for their ability to neutralize the autologous AMC008 virus (**figure 1E)**. Four of the seventeen mAbs were able to neutralize the AMC008 virus although with relatively low potency (IC_{50} values ranging from 16-19 µg/mL). Three of the identified four NAbs were isolated from rabbit 1605 and belonged to the same clonal family; these were designated 05A1, 05A2, and 05A3. The fourth NAb was isolated from rabbit 1607 and named 07A1. Alignment of heavy and light chain variable regions revealed 93% sequence similarity between the CDRL3 sequences of the 05A family (**figure 1C**). In addition, we found no evidence of gene conversion, a common feature for rabbit antibodies, within this family of 3 mAbs. Interestingly, NAb 07A1 CDRL3 region similar to that of 05A1-3 (79% sequence similarity). This shared CDRL3 was relatively long with a length of 14 amino acids. The heavy chain CDR sequences of the 05A family and 07A1 did not show any similarities (**figure 1C**).

AMC008 SOSIP-induced NAbs cross-neutralize some clade B viruses

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The cross-binding ability of the NAbs was tested against a panel of 12 heterologous SOSIP Env trimers (**table 1**). This panel included four clade B SOSIP Env trimers (AMC009, AMC011, AMC016, and AMC018) based on virus sequences from individuals enrolled in the ACS(34). The Env sequences from these four clade B SOSIP trimers have >83% sequence identity with the AMC008 Env protein. Additional Clade B SOSIP Env trimers derived from SHIV162p3 and REJO, 79% and 84% sequence identity with AMC008 respectively, were also included in the panel, as neutralization of these viruses by the corresponding rabbit sera was observed previously (10). Furthermore, we included a selection of non-clade B SOSIP trimers from a representative global panel i.e. CNE55 (clade CRF01_AE), BJOX002000.03.2 (clade CRF07_BC), Ce1176_A3 (clade C), 25710-2.43 (clade C)(35), as well as BG505 (clade A)(5) and finally the ConM SOSIP trimer, a consensus sequence protein based on the consensus sequences of each individual HIV-1 Group M clade(36). All four NAbs displayed cross-reactivity with five out of the six clade B SOSIP Env trimers (**table 1**). The NAb family 05A1-A3 also bound to the clade C SOSIP trimer Ce1176_A3 but there was no binding to other SOSIP trimers, suggesting that the target epitope is fairly conserved in clade B isolates but not across different clades.

All four NAbs were then tested for neutralization breadth against a panel of 17 heterologous viruses consisting of a subpanel representing the global diversity of HIV-1 supplemented with a number of clade B viruses(35). Two of the viruses in this heterologous panel, the clade B tier 2 viruses SHIV162p3 and AMC009, were cross-neutralized by all four NAbs (**table 2**). SHIV162p3 was neutralized relatively weak with IC_{50} values ranging from 5.6-19 µg/mL, i.e. similar to those against the autologous AMC008 virus. Heterologous AMC009 neutralization by the NAbs was much weaker, with IC_{50} values ranging from 58-177 µg/mL.

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198 The relative cross-neutralization of autologous AMC008 and heterologous SHIV162p3 and
199 AMC009 is consistent with the cross-neutralization titers of the serum of the rabbits 1605 and
200 1607(10).
201
202 *AMC008 NAbs target an epitope on the gp41 subunit*
203 Because the four NAbs had nearly identical CDRL3 sequences and three of them were
204 clonal family members, we hypothesized that they might target a shared epitope. To test this,
205 we performed competition assays between these four NAbs using Bio-layer interferometry
206 (BLI). All NAbs showed strong and reciprocal competition with each other, suggesting that
207 their epitopes overlap (**figure 2A**). Differences in percentage of residual binding were
208 observed depending on the directionality of the assay. For instance, we observed 54% residual
209 binding of 05A2 after pre-incubation with competitor 07A1 mAb. However, only 9% residual
210 binding of 07A1 was measured when 05A2 was used as the competitor. These differences
211 could be due to differences in affinity of the NAbs. All mAbs showed self-competition (46-27%
212 residual binding) (**figure 2A**).
213 We then performed competition assays with known human bNAbs using ELISA and
214 Surface Plasmon Resonance (SPR) to specify the epitope targeted by these four NAbs. We
215 tested competition with bNAbs VRC01, PGT121, 35O22, 3BC315, PGT151, and ACS202. These
216 bNAbs target diverse regions on the Env trimer enabling us to define a potential binding area
217 of NAbs 05A1-A3 and 07A1. All four NAbs competed significantly in ELISA with bNAbs 35O22,
218 3BC315, and VRC34 which all target gp41. Competition of the four NAbs 05A1, 05A2, 05A3
219 and 07A1 with 35O22 was weakest with residual binding being 58%, 69%, 73%, and 67%,
220 respectively (**figure 2B**). The four NAbs competed more efficiently with VRC34 and 3BC315

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221 with residual binding between 25% and 60% (**figure 2B**). Weak but not statistically significant
222 competition was observed with gp41-gp120 targeting bNAb ACS202 and no statistically
223 significant competition was detected with gp41-gp120 targeting bNAb PGT151, or bNAbs
224 VRC01 or PGT121, which bind to the CD4bs and the V3-N332 glycan epitope, respectively.
225 SPR analyses strengthened the evidence for competition of the 05A family with gp41-
226 gp120-targeting bNAbs 35O22 (~25% residual binding) and ACS202 (~60% residual binding),
227 and also revealed weak competition with PGT151 (~80% residual binding) (**figure 2B**). The
228 competition with ACS202 was enhanced when the SPR assay set-up was reversed. In this
229 reversed set-up, where ACS202 was allowed to bind first and 05A1-3 second, the residual
230 binding dropped to ~10% (**figure 2C**). No significant competition of the rabbit NAbs with the
231 bNAbs VRC01 and PGT121 was observed in the SPR assays in both set-ups (**figure 2B**). These
232 competition results suggest that the AMC008 SOSIP induced NAbs that most likely target an
233 epitope at or near the gp41-gp120 interface area.
234 We also tested competition of the AMC008 SOSIP trimer induced NAbs with the non-
235 NAbs elicited against the same immunogen using bio-layer interferometry (BLI). The binding
236 of the four NAbs to the AMC008 SOSIP trimers was completely or partially abrogated when
237 either of the five non-NAbs 06A1, 07B1, 07D2, 07E1, or 08A1 was present. These competition
238 results suggest that these five non-NAbs have overlapping epitopes with the NAbs (**figure 2D**).
239 The remaining five non-NAbs did not compete with the NAbs, suggesting these target different
240 epitopes on the Env trimer.
241 Negative stain electron microscopy (NS-EM) was performed to further delineate the
242 NAb epitope and to confirm its location at or near the gp41-gp120 interface. Binding of Fab
243 fragments from the four identified NAbs to the AMC008 SOSIP trimer was visualized through

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3D reconstructions. The NAb Fabs interacted with the gp41 subunit of the AMC008 SOSIP trimer, possibly interacting with residues S528-A532, N616-N625, and Q658-D644 in the HR2 region (**figure 3A**). Additional NS-EM analysis carried out with 05A3 revealed that it bound with a predominant stoichiometry of two Fabs to one Env trimer, although full occupancy of three Fabs binding to one trimer could be detected in a minority of cases. Interestingly, the NAbs interacted with the Env trimer with unusual approach angles that would *a priori* be expected to lead to a clash of the NAbs with the viral membrane.

AMC008 NAbs target residues 620 and 624 in HR2

The NS-EM analysis enabled us to make informed changes to identify amino-acid residues important for binding and neutralization. Within predicted interaction regions pinpointed by the NS-EM analysis, we searched for differences between the sequences of Env trimers that the NAbs were able to cross-bind and/or neutralize, and those that they could not bind or neutralize (**figure 3B**). Divergent amino acids were mutated in the context of AMC008 SOSIP trimer and corresponding pseudovirus to the most prevalent amino acid in the non-binding sequences, resulting in three variants with single amino acid substitutions, i.e., D620N, N624D, and E662A. In addition, considering that Env glycans might influence NAb binding, we modified the glycan shield near or on gp41 of the AMC008 trimer. Accordingly, knock-out (KO) mutants of the potential N-linked glycosylation sites (PNGS) at position 88, 611, 616, and 637 were created. Finally, since previous studies showed that SOSIP-induced NAbs often target strain specific holes in the glycan shield(15–17, 25), we knocked-in (KI) the PNGS at positions 230 and 234 of the AMC008 sequence, as the absence of PNGS at these positions in the natural AMC008 sequence is expected to create a strain-specific glycan hole

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(**figure 3C**). Substitution of amino acids 620 and 624 abolished neutralization ability of all four NAbs, but not of the control Ab VRC01, consistent with the NS-EM data (**figure 3D and 3E**). Furthermore, neutralization by 07A1 was also affected by introducing simultaneously the N230 and N234 PNGS, although these substitutions did not completely abrogate activity, as neutralization of the virus still occurred at higher Ab concentrations. This suggests that glycans at N230 and N234 restrict access to the 07A1 epitope. Three out of four NAbs were also affected by removing the N611 and N637 PNGS: these glycans might therefore contribute to (the presentation of) the NAb epitope. The K232T and K236T single KI mutations did not affect neutralization for any of the four NAbs and neither did the N88, and N616 KO mutations, or the E662A amino acid substitution. When residues 230, 234, 611, 620, 624, or 637 were mutated in the AMC008 SOSIP trimer context, it did not detectably affect binding of NAbs 05A1-A3 in ELISA, while the effect on NAb 07A1 was only observed when residues 620 and 624 were changed (**figure 4A**). Differential effects of single mutations in neutralization versus binding assays have been observed in other cases(37, 38), and probably relate to affinity and Env protein conformation and stability.

We were also able to define the epitopes for the majority of the non-NAbs. mAbs 07E1 and 07D2 also showed dependence on the 620, 624, and/or 662 amino acids in ELISA (**Figure 4A**), confirming their epitope overlap with the NAbs as suggested by the competition experiments. NS-EM further confirmed this epitope overlap for non-NAb 07D2 (**figure 4B**). Other non-NAbs bound a variety of epitopes on the gp41 subunit of the Env trimer such as the area around the N637 glycan (07F1) (**Figure 4A**), and an epitope at the base of the Env trimer (07B1). The non-NAb 05D1 was able to bind a linear V3 peptide in ELISA (**Figure 4A**).

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290 *NAbs and non-NAbs targeting the 620/624 site cannot be differentiated based on affinity for*
291 *soluble trimer*

292 We were intrigued by the identification of NAbs and non-NAbs that targeted
293 overlapping epitopes. A first hypothesis would be that binding affinity might explain the
294 differences in neutralization ability between NAbs and non-NAbs. To test this, we subjected a
295 subset of AMC008 SOSIP-induced non-NAbs and all four NAbs, to kinetic binding experiments
296 with AMC008 SOSIP Env trimers. No significant differences were observed in affinity between
297 the NAbs and the non-NAbs ($P = 0.057$ for KD, $P = 0.057$ for Ka and $P > 0.999$ for Kd; Mann-
298 Whitney U test) (**figure 5A**).

299 We also noted that all four NAbs were able to cross-bind certain SOSIP Env trimers, but
300 were unable to neutralize their corresponding virus. We asked whether this observation could
301 be explained by differences in affinity as well. We tested all four of the NAbs in kinetic binding
302 experiments using AMC008 SOSIP and AMC016 SOSIP Env trimers. The latter was selected in
303 addition to the autologous trimer because the AMC016 virus was not neutralized by our NAbs,
304 while they were able to bind to the corresponding AMC016 SOSIP trimer (**table 2**). AMC016
305 SOSIP trimers contain the amino acids essential for neutralization (D620 and N624) but also
306 the N230 and N234 PNGS that are absent from the AMC008 Env sequence. Kinetic analysis did
307 not reveal significant differences in NAb binding kinetics between the AMC008 SOSIP and
308 AMC016 Env trimers ($P = 0.343$ for KD, $P = 0.114$ for Ka and $P = 0.486$ for Kd; Mann-Whitney
309 U test) (**figure 5A**), suggesting that binding kinetics are not the main cause for the (in)ability
310 of these NAbs to neutralize these viruses.

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NAbs and non-NAbs targeting the 620/624 site cannot be differentiated based on binding to membrane associated Env trimer

The next hypothesis posits that the discrepancy between binding and neutralization ability of the autologous AMC008 SOSIP Env trimer-reactive mAbs might be due to an inability of the non-NAbs to bind full length-surface displayed Env trimers, possibly related to the observed unusual approach angle. To test this, we transfected HEK293 cells with AMC008 SOSIP gp160 Env constructs(39), which resulted in surface expressed AMC008 SOSIP trimers, that were subsequently analyzed by FACS to detect mAb binding (**figure 5B**). In contrast to what we expected, we found that NAbs and non-NAbs bound similarly to full-length, cell-surface-displayed AMC008 SOSIP gp160 trimers. Surprisingly, we found that the NAb 05A3 bound weakly, and NAb 07A1 was unable to bind cell-surface-displayed SOSIP gp160 trimers, which is somewhat inconsistent with their ability to neutralize.

Neutralization activity of mAbs against the 620/624 epitope is determined by their ability to disrupt the trimer

A third hypothesis argues that the strength of neutralization is strongly influenced by the capability to induce trimer dissociation. Indeed, gp41 targeting human bNAbs 3BC315 and 3BC176 neutralize by inducing dissociation of the Env trimer and so does rabbit bNAb 1C2 derived from an immunization study(1, 29). We noticed that the 05A3 fab fragments caused the trimer to dissociate into monomers during sample preparation for NS-EM (**figure 6A**). Dissociation of the AMC008 SOSIP Env trimer did not occur spontaneously when the trimer was left overnight at RT in the absence of 05A1 or 05A3, suggesting that the fab was responsible for the observed trimer dissociation.

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335 To corroborate these findings, we performed a neutralization assay with prolonged
336 pre-incubation times (29). This assay determines the neutralization ability of an Ab after pre-
337 incubation over a 24-hour time at 37 ^{o}C period to measure irreversible trimer destabilization.
338 An increase of neutralization with longer incubation times is indicative of trimer
339 destabilization as more trimers are destabilized by the Ab over time(1, 29). The bNAbs PGT126
340 and 3BC315 were tested in this assay as negative and positive controls, respectively, and
341 showed an increase in neutralization potency of 1.5-fold and 10-fold, respectively, after 24 vs
342 1 hour incubation. The potency of our NAbs against the autologous AMC008 virus increased
343 by ~10-fold after 24 hours incubation (**figure 6B**), indicative of trimer dissociation and similar
344 to the effect of 3BC315. The non-NAb 6A1, targeting a similar epitope as our NAbs, remained
345 unable to neutralize the AMC008 virus even after 24 hours of incubation. The results were
346 even more pronounced with the heterologous SHIV162p3 virus, showing increases in potency
347 of up to nearly 30-fold after 24 hours. Interestingly, the NAb 05A1 did show an increase in
348 potency against the AMC008 virus over time, but no significant increase against the
349 heterologous SHIV162p3 virus. Nevertheless, these data indicate that the four NAbs dissociate
350 Env trimers of both autologous and heterologous viruses and that this largely contributed to
351 their neutralization capacity.
352
353 **DISCUSSION**
354 To overcome viral diversity an HIV-1 vaccine should induce bNAbs. However, the
355 currently used immunogens have been unable to consistently elicit such responses. In this
356 study we tried to better understand the humoral immune responses after HIV-1 SOSIP Env
357 trimer immunization in rabbits. We set out to determine which epitopes on the clade B

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AMC008 SOSIP Env trimer were targeted, and whether the observed specificities could explain the low-titer heterologous neutralization that was observed after immunization with this SOSIP trimer. We isolated and characterized 17 mAbs from four AMC008 SOSIP Env trimer-immunized rabbits. The vast majority of the isolated mAbs targeted a similar area in the HR2 region. One antibody family (05A) and the mAb 07A1 were found to neutralize the autologous and two heterologous viruses through destabilization of the Env trimer. A mechanism of neutralization also used by NAbs induced by natural infection or vaccination(1, 29). Additionally, we identified important contact residues for these NAbs. Such knowledge may help to shape next generation SOSIP Env trimer immunogens to induce trimer destabilizing NAbs.

Even though rabbits are a widely used animal model in the early stages of HIV-1 vaccine testing, we realize there are caveats to using this model. In contrast to humans, rabbits interact with the antigen predominantly through their light chain(32) and use one heavy V-gene in the initial Ab recombination process, therefore, diversity in the heavy chain is much more restricted compared to their light chains. Additionally, gene conversion and somatic hypermutation (SHM) are the major drivers of Ab diversity in rabbits, the former is a process that rarely takes place in humans(32, 40). Nonetheless, numerous isolated rabbit Abs show that similar epitopes on the Env trimer are targeted as seen in macaques and humans after natural infection(41).

Although the NAbs that we describe do not have the desired breadth for a protective HIV-1 vaccine, they are rare examples of mAbs with consistent, albeit weak, cross-neutralizing ability, in particular after only three immunizations with only one immunogen. Other experiments have yielded only autologous neutralizing mAbs with the notable exception of

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381 the isolation of two bNAbs from one Env-immunized rabbit(1, 16, 19, 25). Valuable
382 information from these previous studies led to the development of new immunogens, such as
383 Env trimers displayed on nanoparticles, germline trimers, and specific epitope scaffolds that
384 were expected to induce more broad and consistent neutralization (2, 27, 42, 43).
385 Nonetheless, neutralization breadth in rabbits after HIV-1 immunization remains sporadic and
386 usually of low titer(9–12). The isolation of heterologous NAbs from multiple rabbits using the
387 same neutralization mechanism and targeting similar epitopes highlights the possibility for
388 immune focusing towards this epitope cluster. The identification of bNAbs isolated from both
389 humans and rabbits targeting similar epitopes, with the shared capacity to disrupt trimers,
390 marks this epitope as a very interesting target to be further exploited(1, 29).

391 bNAb 1C2 that exhibited an extraordinary broad NAb response (~85%) was isolated
392 from an Env-immunized rabbit(1). The mechanism of 1C2 neutralization involved
393 destabilization of the Env trimer similar to the effects of bNAbs 3BC315 and 3BC176(29), and
394 NAb family 05A and 07A1 described here. It was proposed that these bNAbs destabilize the
395 trimer by disruption of the stabilizing tryptophan clasp formed by amino acids W623, W628,
396 and W631(1, 44). The CDRH3 loops of both rabbit bNAb 1C2 and human bNAb 3BC315 are
397 close to residue W623 of this clasp. Because the NAbs we describe here have contacts with
398 residues 620 and 624, neighboring this tryptophan clasp, it is not unlikely that a similar
399 disruption mechanisms is utilized by these rabbit NAbs. The description of multiple (b)NAbs
400 directed to this epitope, with the same potent mechanism of action would indicate this
401 epitope as a potential vaccine target. Nonetheless, the isolation of both bNAbs, NAbs, and
402 non-NAbs directed to this epitope shows that adaptations to the immunogen are necessary
403 to amplify this desirable response and guide the responses to neutralization potency and

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breadth. One strategy involves glycan modifications to enhance accessibility of the tryptophan clasp. Another complementary strategy could involve immune focusing on conserved amino acids within this region as opposed to ones that are more variable such as residues 620 and 624.

The AMC008 SOSIP Env trimer lacks the N230 and N234 PNGS, generating a strain-specific glycan hole. The absence of these glycans might dictate the angle of approach for the NAbs identified in this study, and thereby restrict the breadth of the response. Filling the 230/234 glycan hole negatively impacted neutralization by the NAb 07A1. However, this was not the case for the NAbs isolated from animal 1605. One explanation might be that the footprint of the 07A1 epitope is larger and/or in closer proximity to the 230/234 glycan hole. The ability of these NAbs to neutralize viruses containing the 230 and 234 PNGS, such as the SHIV162p3 virus, is encouraging as the 234 PNGS is widely conserved.

The NS-EM revealed an improbable approach angle for the NAbs that should result in a clash with the viral membrane. Possibly the angles by which the NAbs approach soluble Env trimers and virus-associated Env might vary, in particular at the trimer stem. For instance, the MPER bNAb 10E8 Fab approaches soluble SOSIP trimers with an angle similar to that of 05A1-A3 and 07A1, but this angle changes to facilitate the presence of the membrane when 10E8 binds to membrane-anchored Env(45). Moreover, viral membrane-associated envelope proteins can have remarkable flexibility. This flexibility was visualized for the SARS-CoV-2 spike protein which is able to tilt by up to ~60˚ relative to a position perpendicular to the membrane(46). Also, HIV-1 Env trimers on nanodiscs displayed the ability to tilt by ~20˚ when bound to gp41 directed bNAbs(28).

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426 Previous studies suggested that a slower dissociation was associated with stronger
427 neutralization(21, 22). However, this did not apply to the gp41-directed mAbs described here.
428 One difference is that these mAbs originated from different clonal families, whereas the
429 previous studies analyzed clonal family members. Furthermore, we determined binding
430 kinetics using stabilized soluble Env SOSIP trimers. It cannot be excluded that Env stabilization
431 in the SOSIP construct and the observed destabilization of the Env trimer by the Nabs might
432 have affected the affinities we measured. Nevertheless, it is likely that a slow dissociation and
433 the ability to induce trimer dissociation are distinct properties that affect both neutralization
434 potency and efficacy(47).

435 In summary, characterizing humoral immune responses at the mAb level after
436 immunization can inform future immunization studies and strategies. We found that gp41-
437 directed NAbs with an unusual approach angle, predicted to clash with the viral membrane,
438 were able to weakly neutralize autologous and heterologous clade B viruses by inducing Env
439 trimer destabilization, reminiscent of bNAbs that induce trimer dissociation by disrupting the
440 tryptophan clasp in gp41. This knowledge highlights that destabilization of the trimer might
441 be a more important neutralization mechanism than previously appreciated. Inducing trimer
442 destabilizing NAbs that target the tryptophan clasp should be considered in future
443 immunization strategies.

444

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462

463 **CONFLICT OF INTERESTS**

464 No conflict of interests.

465

466 **DATA AVAILABILITY**

467 Antibody sequences will be available on request to the corresponding author. Variable domain
468 sequences of heavy and light chains were uploaded in Genbank under accession numbers
469 MZ170096, MZ170095, MZ170094, and MZ170097 for NAb 05A1, 05A2, 05A3, and 07A1
470 respectively.

471

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METHODS

Isolation of rabbit Peripheral blood mononuclear cells

Blood of immunized rabbits was used for Peripheral blood mononuclear cells (PBMC) isolation through ficoll separation. In short, blood was diluted 1:1 with PBS, loaded on a ficoll layer, and centrifuged for 30 min at room temperature (RT) at 400xg with an acceleration speed of 7 and a deceleration speed of 0. PBMCs were isolated, washed with PBS and subsequently re-suspended in 1-2 mL of ACK buffer (Thermo Fisher Scientific) to remove red blood cells. PBMCs were counted, suspended in fetal calf serum with 10% DMSO and directly frozen at -150°C.

Env protein design, production, and purification

All SOSIP trimers contained the previously described SOSIP mutations(8) and were further stabilized with additional mutations yielding SOSIP.v4.2 (E66K + A316W) trimers(10). ConM and SHIVp3 SOSIP trimers contained additional mutations (SOSIP.v9) described elsewhere(48–50). All SOSIP constructs were cloned into a pPPI4 expression vector(51). Mutant variants were generated by the use of the Q5 site-directed mutagenesis kit (New England Biolabs) with specifically designed primer sets and adapted annealing temperatures for the mutations N88Q, K232T, K236T, K232T+K236T, N611Q, N616Q, N637Q, D602N, N624D and E662A. D7324 tags were incorporated directly C-terminally of residue 664 in each of the plasmids(8). Additionally we replaced the D7324 tag C-terminally of residue 664 with an Avi-tag in the AMC008 SOSIP.v.4.2 construct for biotinylation (16). For BLI and SPR experiments a His-tag was incorporated into AMC008 SOSIP.v4.2, and the AMC008 SOSIP.v4.2 D620N, N624D, and E662A mutant plasmids, replacing the D7324 tag(2).

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All SOSIP trimer variants were produced as described before(6, 8, 10, 52, 53). In short, SOSIP trimers were transiently expressed together with furin from a separate expression plasmid (ratio 4:1) in HEK293F cells (Invitrogen, catalog number R79009). The SOSIP trimers were harvested by spinning for 20 min at 4000xg. The supernatant was 0.22 µM steritop-vacuum-filtered before purification by gravity-driven chromatography on a PGT145 antibody-conjugated Sepharose column. Env proteins were eluted with 3M Mg_2Cl_2 pH 7.8, directly into neutralization buffer (20 mM TrisHCl pH8.0, 75mM NaCl). After purification, Env proteins were concentrated with Vivaspin 100kD filters (GE healthcare), to a final volumes of <500 µL. AMC008 SOSIP.v4.2-Avi was biotinylated to allow conjugation for FACS analysis. BirA biotin protein ligase (Avidity) was used for the biotinylation.

Antibody production and purification

HEK293F cells (Invitrogen, catalog number R79009) were transfected to produce mAbs as described previously(16). In short, 62.5 µg of each heavy and light chain Ab DNA were co-transfected transiently in 250 mL HEK293F cells. Cell supernatant was harvested at 5 days by spinning down for 20 min at 4000xg and subsequent vacuum filtration with a 0.22 µM steritop filter. Abs were purified from the culture supernatant by gravity-driven chromatography on a Protein G (Thermo Fisher Scientific) affinity column. After addition of the supernatant, columns were washed twice with PBS before elution with 9 mL 0.1 M glycine, pH 2.0. Eluted Abs were collected in 1 mL 1 M Tris-HCl pH 7.8. After elution Abs were concentrated, by the use of 100kD vivaspin (GE healthcare) filters, to a final volume of <200 µL.

We named the isolated mAbs as described by McCoy *et al*, 2016(16): in this system each mAb is numbered according to their rabbit ID followed by a unique alphabetical lineage

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identifier (A, B, C, etc.). Distinct lineage members received an additional number (A1, A2, A3, etc.).

Single B cell sorting

Single B cells were selected and sorted as previously described(16). Briefly, biotinylated AMC008 SOSIP.v4.2-Avi Env proteins were conjugated to Strep-APC and Strep-FITC (both Thermo Fisher Scientific). Mouse-anti-rabbit IgG Ab conjugated to PE stained IgG on memory B cells (Southern Biotech). Conjugated Env protein was mixed with PBMCs to allow binding. We first selected IgG^+ cells and within this population sorted single B cells, positive for both APC and FITC conjugated AMC008.v4.2 Env trimer into a lysis buffer (RNAse inhibitor (20U) (Thermo Fisher Scientific), 5X First strand superscript III buffer (Invitrogen), 0.1 M DTT (Invitrogen), and MQ). Sorted cells were immediately frozen at -80°C.

Single cell Ab RT-PCR, variable region gene amplification and cloning

6 µL RT-PCR reaction mixture (random hex primers (200 ng) (Thermo Fisher Scientific), dNTP mix (2 mM each) (New England Biolabs), 50U Superscript III RTase, and MQ) was added directly to the sorted single B cells. The following PCR program was used to convert RNA into cDNA: 42°C 10 min; 25°C 10 min; 50°C 60 min; 95°C 5 min. RT-PCR plates were used directly for variable region gene amplification using two subsequent PCR reactions. For both PCR reactions 13 µL of PCR mix (MQ, 10x PCR reaction buffer, dNTPs (10 mM), 0.25U Hotstar Plus polymerase (Qiagen), forward primer (25 mM), reverse primer (25 mM)) was added subsequently to the RT-PCR plate and the PCR1 plate. For PCR1, 2 µL of RT-PCR product was added to this mix. PCR 1 was run at 95°C for 5 min (50 cycles of: 94°C 30 seconds; 58°C 30 sec;

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72°C 1 min) 72°C 10 min. For the heavy chain amplification, the annealing temperature was adapted to 48°C. For PCR2, 2 µL of PCR1 product was added to the PCR mix and DNA was amplified at 95°C for 5 min; (50 cycles: 94°C 30 sec; 55°C 30 sec; 72°C 1 min) 72°C 10 min. A final PCR3 reaction using 1 µL of PCR2 product was performed in MQ, 5x Phusion PCR buffer, dNTPs (10 mM), forward primers (25 mM), reverse primers (25 mM), 0.2U Phusion high fidelity polymerase (New England Biolabs). The following PCR program was run; 98°C 30 sec; (35 cycles: 98°C 5 sec; 68°C 15 sec; 72°C 20 sec); 72°C 5 min.

Heavy and light chain amplified variable regions were cloned into vectors expressing respectively the heavy or light chain constant region of rabbit Abs by Gibson cloning. In short, 1 µL vector (45 ng) was incubated with 1 µL PCR3 product and 2x Gibson mix (T5 exonuclease (0.2U) (Epibio), Phusion polymerase (12.5U) (New England Biolabs), Taq DNA ligase (2000U) (New England Biolabs), gibson reaction buffer (0.5 grams PEG-8000 (Sigma Life Sciences), 1 M Tris/ HCl pH 7.5, 1 M MgCl2, 1 M DTT, 100 mM dNTPs, 50 mM NAD (New England Biolabs), MQ)) for 60 min at 50°C.

Mutant virus construction and production

The infectious molecular clone (IMC) encoding for replication-competent virus with AMC008 Env has been described before(10). First, the AMC008 *env* fragment was transferred to pUC18 by traditional cloning methods using restriction enzymes SalI and BamHI (New England Biolabs). Mutations were then generated using the Q5 mutagenesis kit (New England Biolabs). Mutated AMC008 *env* fragments were cloned back into the original IMC by Gibson reactions. To produce virus stocks, HEK293T cells (ATCC, CRL-11268) were transfected with

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563 the IMCs using lipofectamin2000 (Invitrogen) and supernatants containing viruses were
564 harvested 3 days later. Supernatants were directly frozen at -80˚C.
565
566 *Neutralization assays*
567 Neutralization assays were executed as described previously(8, 15). In short, 1 in 3
568 dilution series were made of the various mAbs starting at end-concentrations varying from 50
569 µg/mL to 100 µg/mL. Virus of interest was added to the diluted mAb, and incubated for 1 h at
570 RT. After incubation the mixture was added to TZM-bl reporter cells (obtained through the
571 NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH from Dr. John C. Kappes, and Dr.
572 Xiaoyun Wu) and incubated for 3 days at 37°C. IC_{50} values were determined as the
573 concentration at which infectivity was inhibited by 50%.
574 For the decay neutralization assays, virus and mAb mixtures were incubated for 1 h, 3
575 h, 6 h, and 24 h at 37°C before addition to the TZM-bl reporter cells.
576
577 *Negative stain electron microscopy*
578 Complex formation was performed by incubating AMC008 SOSIP.v4.2 trimers with a 6-
579 fold molar excess of fab for 3 h at RT. Complexes were subsequently diluted to 0.03 mg/mL in
580 1X TBS pH 7.4 to achieve optimal particle density. Copper mesh grids were plasma cleaned for
581 20s using a mix of Argon and Oxygen gas and samples were stained with 2% uranyl formate
582 for 50s. For each dataset, a FEI Tecnai Spirit (120 kEV) with a Tietz (4k x 4k) camera was used
583 in conjunction with the automated data collection software package, Leginon(54). Data
584 collection parameters include: a magnification of 56,000x, a defocus of -1.5 µm, a pixel size of
585 2.05 Å per pixel, and a dose of 25 $e^-/Å^2$. Resulting images were stored in Appion(55), particles

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were picked with DoGPicker(56), stacked with a box size of 192 pixels, and processed using RELION(57). UCSF Chimera(58) was used for map segmentation and map/model docking.

Binding and competition ELISAs

Binding ELISA experiments were performed as previously described(2, 4). In short, ELISA plates were coated overnight with 100 µg Ghalanthus Nivalis Lectin (GNL) in 0.1 M NaHCO3 pH 8.6 and blocked using casein (Thermo Fisher Scientific) at RT for 1 h after washing off the GNL with Tris Buffered Saline (TBS). Env trimers were added at 2 µg/mL and incubated at RT for 2 h. Subsequently, Ab 3-fold serial dilutions were added for 2 h at RT at starting concentrations ranging from 1 µg/mL up to 10 µg/mL. After washing with TBS a 1:3000 dilution of goat-anti-human or goat-anti-rabbit Horseradish Peroxidase (HRP) (SeraCare, 1 µg/mL) conjugated Ab was added and incubated at RT for 1 h. After washing with TBS+0.05% Tween-20, ELISA plates were developed using 1% 3,3',5,5'-tetranethylbenzidine (Sigma-Aldrich), 0.01% H2O2, 100 mM sodium acetate, and 100 mM citric acid and reactions were stopped after 3 min using 0.8 M H_2SO_4.

For HEK293T supernatant ELISAs the same protocol was followed however, 50 µL of HEK293T supernatant containing unpurified mAb was added instead of purified Abs.

For competition ELISA, His-tagged Env trimers were added at 2 µg/mL to pre-coated Ni-NTA plates (Qiagen) and left at RT for 2 h. Blocking was achieved in 2% milk in TBS at RT for 1 h. The primary Ab (competitor) was added at excess (10 µg/mL) in 50 µl and incubated for 30 min at RT before the analyte Ab was added at a previously determined EC_{70} concentration in 50 µL and left at RT for another 1.5 h. Statistical significance was determined using an one-

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608 way ANOVA multiple comparisons test (Prism) comparing the sample without competitor to
609 the corresponding sample with competitor present.
610
611 *Octet K2 Bio-Layer Interferometry (BLI) kinetics and binding experiments*
612 All assays were conducted on the Octet K2 system (Bioforte). 6 µg/ml of his-tagged
613 AMC008 SOSIP.v4.2-His Env trimer was captured on NiNTA sensors (Bioforte) for 600s after
614 baseline determination in PBS with 0.01% Bovine Serum Albumine and 0.002% Tween for 60s.
615 Association of a serial dilution of rabbit mAbs starting at 15 µg/mL was measured for 600s by
616 dipping the AMC008 SOSIP.v4.2 trimer loaded sensor into a well containing mAb.
617 Subsequently dissociation was measured in PBS 0.01% BSA 0.002% Tween buffer for 600s.
618 Binding kinetics (Kd, ka and kd) were determined using a 1:1 fit model with independent fitting
619 of Rmax (Octet Data Analysis software, Bioforte). Regeneration of the sensors was achieved
620 through alternating cycles of 5s in low pH glycine buffer and a neutralization buffer (PBS,
621 0.01% BSA, 0.002%Tween). After regeneration sensors were used to re-capture Env protein.
622 For competition analysis, after association of the competitor mAb to the SOSIP trimer
623 bound to NiNTA sensors, a second 600s association step was incorporated for the analyte
624 mAb. % residual binding was calculated as follows; shift in nm at 600s of analyte
625 binding*100)/nm shift of analyte in the absence of competitor binding.
626
627 *Surface plasmon resonance (SPR)*
628 Surface Plasmon Resonance (SPR) experiments were done for kinetic and competition
629 analysis of rabbit mAbs, as previously described (34). All assays were conducted on Biacore
630 3000 at 25^{o}C. In all assays HBS-EP (10mM HEPES [pH7.4], 150 mM NaCl, 3 mM EDTA, 0.002%

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P20 surfactant) was used as running buffer (GE Healthcare). Briefly, Anti-his Ab was covalently immobilized (15,000 RU) in all flow cells of a CM5 sensor chip, by standard amine-coupling. AMC008 SOSIP.v4.2-His Env trimer was captured on the anti-His-CM5 surface for both and competition analysis.

Competition assays were carried out with mAbs binding to the CD4bs (VRC01), gp120 V3-glycan (PGT121), and the gp120-gp140 interface (PGT151, 35O22, and ACS202). In the competition assays, mAbs were sequentially injected in the same cycle: the first Ab was injected for 200s immediately followed by the second Ab, at a flow rate of 30 µl/min. Dissociation was followed for 300s during the second injection. In addition, the second mAb was injected alone in a separate cycle.

The trimer-immobilization levels and mAb concentrations were optimized earlier to yield maximum self-competition. Rabbit mAbs (05A1, 05A2, 05A3), PGT121, PGT151, and ASC202 showed 5-15% residual binding at a concentration of 1 µM and trimer density of 500 RU, whereas, VRC01 and 35O22, self-competed (15-20% residual binding) at a concentration of 1.5 µM and a density of ~250 RU of AMC008 SOSIP.v4.2 trimer. Those mAb concentrations were used thereafter in the cross-competitions; and for rabbit mAbs and VRC01 or 35O22, the trimer was captured to a level of 250 RU, whereas the other Abs (PGT121, PGT151, and ACS202) were done at a trimer capture level of 500 RU. The residual binding was calculated as (Response difference at 200s for the second Ab)/(Response difference at 200s for the same, second Ab when injected as a single Ab in a separate cycle)*100 (%). Significance was determined by an one-way ANOVA multiple comparisons test (prism) comparing the sample without competitor to the corresponding sample with competitor present.

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Full length SOSIP binding experiment

Surface expressed full length AMC008.v4.2 SOSIP Env trimers were obtained by co-transfection of 10 µg gp160 SOSIP expression plasmid(39) and 2.5 µg Furin expression plasmid into $1.75x10^6$ cells/mL HEK293F cells as described above. 60-65 h post transfection cells were spun down at 4000xg. $1*10^5$ 293F cells were added to each well of serially diluted mAbs and incubated for 2 h on ice. Cells were washed twice with PBS and subsequently stained in a volume of 20 µl containing 1:70 alexa-647 (2 mg/mL) conjugated mouse anti-human IgG (for 2G12 and PGT145 controls) (Invitrogen), or 1:70 PE (0.1 mg/mL) conjugated mouse anti-rabbit IgG (Southern Biotech). Cells were stained for 45 min on ice and covered in foil and subsequently washed with PBS and re-suspended in 100 µL PBS and analyzed using a FACS canto II analyzer (BD). Maximum mean fluorescent intensity (MFI) was calculated and plotted for each of the samples.

References:

1. Dubrovskaya V, Tran K, Ozorowski G, Guenaga J, Wilson R, Bale S, Cottrell CA, Turner HL, Seabright G, O'Dell S, Torres JL, Yang L, Feng Y, Leaman DP, Vázquez Bernat N, Liban T, Louder M, McKee K, Bailer RT, Movsesyan A, Doria-Rose NA, Pancera M, Karlsson Hedestam GB, Zwick MB, Crispin M, Mascola JR, Ward AB, Wyatt RT. 2019. Vaccination with Glycan-Modified HIV NFL Envelope Trimer-Liposomes Elicits Broadly Neutralizing Antibodies to Multiple Sites of Vulnerability. Immunity 51:915-929.e7.
2. Brouwer PJM, Antanasijevic A, Berndsen Z, Yasmeen A, Fiala B, Bijl TPL, Bontjer I, Bale JB, Sheffler W, Allen JD, Schorcht A, Burger JA, Camacho M, Ellis D, Cottrell CA, Behrens AJ, Catalano M, del Moral-Sánchez I, Ketas TJ, LaBranche C, van Gils MJ, Sliepen K, Stewart LJ, Crispin M, Montefiori DC, Baker D, Moore JP, Klasse PJ, Ward AB, King NP, Sanders RW. 2019. Enhancing and shaping the immunogenicity of native-like HIV-1 envelope trimers with a two-component protein nanoparticle. Nat Commun 10.
3. Rutten L, Lai YT, Blokland S, Truan D, Bisschop IJM, Strokappe NM, Koornneef A, van Manen D, Chuang

Downloaded from https://journals.asm.org/journal/jvi on 14 June 2021 by 193.60.240.99.

Accepted Manuscript Posted Online

679 GY, Farney SK, Schuitemaker H, Kwong PD, Langedijk JPM. 2018. A Universal Approach to Optimize the
680 Folding and Stability of Prefusion-Closed HIV-1 Envelope Trimers. Cell Rep 23:584–595.
681 4. Derking R, Ozorowski G, Sliepen K, Yasmeen A, Cupo A, Torres JL, Julien JP, Lee JH, van Montfort T, de
682 Taeye SW, Connors M, Burton DR, Wilson IA, Klasse PJ, Ward AB, Moore JP, Sanders RW. 2015.
683 Comprehensive Antigenic Map of a Cleaved Soluble HIV-1 Envelope Trimer. PLoS Pathog 11:1–22.
684 5. Sanders RW, Vesanen M, Schuelke N, Master A, Schiffner L, Kalyanaraman R, Paluch M, Berkhout B,
685 Maddon PJ, Olson WC, Lu M, Moore JP. 2002. Stabilization of the Soluble, Cleaved, Trimeric Form of the
686 Envelope Glycoprotein Complex of Human Immunodeficiency Virus Type 1. J Virol
687 https://doi.org/10.1128/jvi.76.17.8875-8889.2002.
688 6. Sanders RW, Van Gils MJ, Derking R, Sok D, Ketas TJ, Burger JA, Ozorowski G, Cupo A, Simonich C, Goo
689 L, Arendt H, Kim HJ, Lee JH, Pugach P, Williams M, Debnath G, Moldt B, Van Breemen MJ, Isik G,
690 Medina-Ramírez M, Back JW, Koff WC, Julien JP, Rakasz EG, Seaman MS, Guttman M, Lee KK, Klasse PJ,
691 LaBranche C, Schief WR, Wilson IA, Overbaugh J, Burton DR, Ward AB, Montefiori DC, Dean H, Moore
692 JP. 2015. HIV-1 neutralizing antibodies induced by native-like envelope trimers. Science (80-) 349.
693 7. Hladik F, McElrath MJ. 2008. Setting the stage: Host invasion by HIV. Nat Rev Immunol.
694 8. Sanders RW, Derking R, Cupo A, Julien JP, Yasmeen A, de Val N, Kim HJ, Blattner C, de la Peña AT,
695 Korzun J, Golabek M, de los Reyes K, Ketas TJ, van Gils MJ, King CR, Wilson IA, Ward AB, Klasse PJ,
696 Moore JP. 2013. A Next-Generation Cleaved, Soluble HIV-1 Env Trimer, BG505 SOSIP.664 gp140,
697 Expresses Multiple Epitopes for Broadly Neutralizing but Not Non-Neutralizing Antibodies. PLoS Pathog
698 9.
699 9. Torrents de la Peña A, de Taeye SW, Sliepen K, LaBranche CC, Burger JA, Schermer EE, Montefiori DC,
700 Moore JP, Klasse PJ, Sanders RW. 2018. Immunogenicity in Rabbits of HIV-1 SOSIP Trimers from Clades
701 A, B, and C, Given Individually, Sequentially, or in Combination. J Virol 92.
702 10. de Taeye SW, Ozorowski G, Torrents de la Peña A, Guttman M, Julien J-P, van den Kerkhof TLGM,
703 Burger JA, Pritchard LK, Pugach P, Yasmeen A, Crampton J, Hu J, Bontjer I, Torres JL, Arendt H,
704 DeStefano J, Koff WC, Schuitemaker H, Eggink D, Berkhout B, Dean H, LaBranche C, Crotty S, Crispin M,
705 Montefiori DC, Klasse PJ, Lee KK, Moore JP, Wilson IA, Ward AB, Sanders RW. 2015. Immunogenicity of
706 Stabilized HIV-1 Envelope Trimers with Reduced Exposure of Non-neutralizing Epitopes. Cell 163:1702–

Journal of Virology
JVI
Journal of Virology
Downloaded from https://journals.asm.org/journal/jvi on 14 June 2021 by 193.60.240.99.

Accepted Manuscript Posted Online

15.

11. Pauthner M, Havenar-Daughton C, Sok D, Nkolola JP, Bastidas R, Boopathy A V., Carnathan DG, Chandrashekar A, Cirelli KM, Cottrell CA, Eroshkin AM, Guenaga J, Kaushik K, Kulp DW, Liu J, McCoy LE, Oom AL, Ozorowski G, Post KW, Sharma SK, Steichen JM, de Taeye SW, Tokatlian T, Torrents de la Peña A, Butera ST, LaBranche CC, Montefiori DC, Silvestri G, Wilson IA, Irvine DJ, Sanders RW, Schief WR, Ward AB, Wyatt RT, Barouch DH, Crotty S, Burton DR. 2017. Elicitation of Robust Tier 2 Neutralizing Antibody Responses in Nonhuman Primates by HIV Envelope Trimer Immunization Using Optimized Approaches. Immunity 46:1073-1088.e6.

12. Bale S, Martiné A, Wilson R, Behrens AJ, Fourn V Le, Val N de, Sharma SK, Tran K, Torres JL, Girod PA, Ward AB, Crispin M, Wyatt RT. 2018. Cleavage-independent HIV-1 trimers from CHO cell lines elicit robust autologous tier 2 neutralizing antibodies. Front Immunol 9.

13. Burton DR. 2017. What are the most powerful immunogen design vaccine strategies?: Reverse vaccinology 2.0 shows great promise. Cold Spring Harb Perspect Biol https://doi.org/10.1101/cshperspect.a030262.

14. van Haaren MM, van den Kerkhof TLGM, van Gils MJ. 2017. Natural infection as a blueprint for rational HIV vaccine design. Hum Vaccines Immunother 13:229–236.

15. Klasse PJ, Ketas TJ, Cottrell CA, Ozorowski G, Debnath G, Camara D, Francomano E, Pugach P, Ringe RP, LaBranche CC, van Gils MJ, Bricault CA, Barouch DH, Crotty S, Silvestri G, Kasturi S, Pulendran B, Wilson IA, Montefiori DC, Sanders RW, Ward AB, Moore JP. 2018. Epitopes for neutralizing antibodies induced by HIV-1 envelope glycoprotein BG505 SOSIP trimers in rabbits and macaques. PLoS Pathog 14.

16. McCoy LE, van Gils MJ, Ozorowski G, Messmer T, Briney B, Voss JE, Kulp DW, Macauley MS, Sok D, Pauthner M, Menis S, Cottrell CA, Torres JL, Hsueh J, Schief WR, Wilson IA, Ward AB, Sanders RW, Burton DR. 2016. Holes in the Glycan Shield of the Native HIV Envelope Are a Target of Trimer-Elicited Neutralizing Antibodies. Cell Rep 16:2327–2338.

17. Cottrell CA, van Schooten J, Bowman CA, Yuan M, Oyen D, Shin M, Morpurgo R, van der Woude P, van Breemen M, Torres JL, Patel R, Gross J, Sewall LM, Copps J, Ozorowski G, Nogal B, Sok D, Rakasz EG, Labranche C, Vigdorovich V, Christley S, Carnathan DG, Sather DN, Montefiori D, Silvestri G, Burton DR, Moore JP, Wilson IA, Sanders RW, Ward AB, van Gils MJ. 2020. Mapping the immunogenic landscape of

Journal of Virology

JVI

Journal of Virology

Downloaded from https://journals.asm.org/journal/jvi on 14 June 2021 by 193.60.240.99.

Accepted Manuscript Posted Online

near-native HIV-1 envelope trimers in non-human primates. PLoS Pathog 16:e1008753.

18. Ringe RP, Pugach P, Cottrell CA, LaBranche CC, Seabright GE, Ketas TJ, Ozorowski G, Kumar S, Schorcht A, van Gils MJ, Crispin M, Montefiori DC, Wilson IA, Ward AB, Sanders RW, Klasse PJ, Moore JP. 2018. Closing and Opening Holes in the Glycan Shield of HIV-1 Envelope Glycoprotein SOSIP Trimers Can Redirect the Neutralizing Antibody Response to the Newly Unmasked Epitopes. J Virol 93.

19. Nogal B, Bianchi M, Cottrell CA, Kirchdoerfer RN, Sewall LM, Turner HL, Zhao F, Sok D, Burton DR, Hangartner L, Ward AB. 2019. Mapping polyclonal antibody responses in non-human primates vaccinated with HIV Env trimer subunit vaccines. bioRxiv 833715.

20. Bianchi M, Turner HL, Nogal B, Cottrell CA, Oyen D, Pauthner M, Bastidas R, Nedellec R, McCoy LE, Wilson IA, Burton DR, Ward AB, Hangartner L. 2018. Electron-Microscopy-Based Epitope Mapping Defines Specificities of Polyclonal Antibodies Elicited during HIV-1 BG505 Envelope Trimer Immunization. Immunity https://doi.org/10.1016/j.immuni.2018.07.009.

21. Yasmeen A, Ringe R, Derking R, Cupo A, Julien JP, Burton DR, Ward AB, Wilson IA, Sanders RW, Moore JP, Klasse PJ. 2014. Differential binding of neutralizing and non-neutralizing antibodies to native-like soluble HIV-1 Env trimers, uncleaved Env proteins, and monomeric subunits. Retrovirology 11:41.

22. Steckbeck JD, Orlov I, Chow A, Grieser H, Miller K, Bruno J, Robinson JE, Montelaro RC, Cole KS. 2005. Kinetic Rates of Antibody Binding Correlate with Neutralization Sensitivity of Variant Simian Immunodeficiency Virus Strains. J Virol 79:12311–12320.

23. Wibmer CK, Gorman J, Anthony CS, Mkhize NN, Druz A, York T, Schmidt SD, Labuschagne P, Louder MK, Bailer RT, Abdool Karim SS, Mascola JR, Williamson C, Moore PL, Kwong PD, Morris L. 2016. Structure of an N276-Dependent HIV-1 Neutralizing Antibody Targeting a Rare V5 Glycan Hole Adjacent to the CD4 Binding Site. J Virol 90:10220–10235.

24. Pan R, Sampson JM, Chen Y, Vaine M, Wang S, Lu S, Kong X-P. 2013. Rabbit Anti-HIV-1 Monoclonal Antibodies Raised by Immunization Can Mimic the Antigen-Binding Modes of Antibodies Derived from HIV-1-Infected Humans. J Virol 87:10221–10231.

25. Voss JE, Andrabi R, McCoy LE, de Val N, Fuller RP, Messmer T, Su CY, Sok D, Khan SN, Garces F, Pritchard LK, Wyatt RT, Ward AB, Crispin M, Wilson IA, Burton DR. 2017. Elicitation of Neutralizing Antibodies Targeting the V2 Apex of the HIV Envelope Trimer in a Wild-Type Animal Model. Cell Rep 21:222–235.

Journal of Virology

JVI

Journal of Virology

Downloaded from https://journals.asm.org/journal/jvi on 14 June 2021 by 193.60.240.99.

Accepted Manuscript Posted Online

26. Duan H, Chen X, Boyington JC, Cheng C, Zhang Y, Jafari AJ, Stephens T, Tsybovsky Y, Kalyuzhniy O, Zhao P, Menis S, Nason MC, Normandin E, Mukhamedova M, DeKosky BJ, Wells L, Schief WR, Tian M, Alt FW, Kwong PD, Mascola JR. 2018. Glycan Masking Focuses Immune Responses to the HIV-1 CD4-Binding Site and Enhances Elicitation of VRC01-Class Precursor Antibodies. Immunity 49:301-311.e5.

27. Hessell AJ, Powell R, Jiang X, Luo C, Weiss S, Dussupt V, Itri V, Fox A, Shapiro MB, Pandey S, Cheever T, Fuller DH, Park B, Krebs SJ, Totrov M, Haigwood NL, Kong XP, Zolla-Pazner S. 2019. Multimeric Epitope-Scaffold HIV Vaccines Target V1V2 and Differentially Tune Polyfunctional Antibody Responses. Cell Rep 28:877-895.e6.

28. Rantalainen K, Berndsen ZT, Antanasijevic A, Schiffner T, Zhang X, Lee WH, Torres JL, Zhang L, Irimia A, Copps J, Zhou KH, Kwon YD, Law WH, Schramm CA, Verardi R, Krebs SJ, Kwong PD, Doria-Rose NA, Wilson IA, Zwick MB, Yates JR, Schief WR, Ward AB. 2020. HIV-1 Envelope and MPER Antibody Structures in Lipid Assemblies. Cell Rep 31:107583.

29. Lee JH, Leaman DP, Kim AS, Torrents De La Penã A, Sliepen K, Yasmeen A, Derking R, Ramos A, De Taeye SW, Ozorowski G, Klein F, Burton DR, Nussenzweig MC, Poignard P, Moore JP, Klasse PJ, Sanders RW, Zwick MB, Wilson IA, Ward AB. 2015. Antibodies to a conformational epitope on gp41 neutralize HIV-1 by destabilizing the Env spike. Nat Commun 6.

30. Kong L, Lee JH, Doores KJ, Murin CD, Julien JP, McBride R, Liu Y, Marozsan A, Cupo A, Klasse PJ, Hoffenberg S, Caulfield M, King CR, Hua Y, Le KM, Khayat R, Deller MC, Clayton T, Tien H, Feizi T, Sanders RW, Paulson JC, Moore JP, Stanfield RL, Burton DR, Ward AB, Wilson IA. 2013. Supersite of immune vulnerability on the glycosylated face of HIV-1 envelope glycoprotein gp120. Nat Struct Mol Biol 20:796–803.

31. Pejchal R, Doores KJ, Walker LM, Khayat R, Huang PS, Wang SK, Stanfield RL, Julien JP, Ramos A, Crispin M, Depetris R, Katpally U, Marozsan A, Cupo A, Maloveste S, Liu Y, McBride R, Ito Y, Sanders RW, Ogohara C, Paulson JC, Feizi T, Scanlan CN, Wong CH, Moore JP, Olson WC, Ward AB, Poignard P, Schief WR, Burton DR, Wilson IA. 2011. A potent and broad neutralizing antibody recognizes and penetrates the HIV glycan shield. Science (80-) 334:1097–1103.

32. Lavinder JJ, Hoi KH, Reddy ST, Wine Y, Georgiou G. 2014. Systematic characterization and comparative analysis of the rabbit immunoglobulin repertoire. PLoS One 9:1–11.

Journal of Virology

JVI

Journal of Virology

Downloaded from https://journals.asm.org/journal/jvi on 14 June 2021 by 193.60.240.99.

Accepted Manuscript Posted Online

33. Kodangattil S, Huard C, Ross C, Li J, Gao H, Mascioni A, Hodawadekar S, Naik S, Min-debartolo J, Visintin A, Almagro JC. 2014. The functional repertoire of rabbit antibodies and antibody discovery via next-generation sequencing. MAbs 6:628–636.

34. Van Gils MJ, Van Den Kerkhof TLGM, Ozorowski G, Cottrell CA, Sok D, Pauthner M, Pallesen J, De Val N, Yasmeen A, De Taeye SW, Schorcht A, Gumbs S, Johanna I, Saye-Francisco K, Liang CH, Landais E, Nie X, Pritchard LK, Crispin M, Kelsoe G, Wilson IA, Schuitemaker H, Klasse PJ, Moore JP, Burton DR, Ward AB, Sanders RW. 2016. An HIV-1 antibody from an elite neutralizer implicates the fusion peptide as a site of vulnerability. Nat Microbiol 2:423–426.

35. deCamp A, Hraber P, Bailer RT, Seaman MS, Ochsenbauer C, Kappes J, Gottardo R, Edlefsen P, Self S, Tang H, Greene K, Gao H, Daniell X, Sarzotti-Kelsoe M, Gorny MK, Zolla-Pazner S, LaBranche CC, Mascola JR, Korber BT, Montefiori DC. 2014. Global panel of HIV-1 Env reference strains for standardized assessments of vaccine-elicited neutralizing antibodies. J Virol 88:2489–507.

36. Sliepen K, Han BW, Bontjer I, Mooij P, Garces F, Behrens AJ, Rantalainen K, Kumar S, Sarkar A, Brouwer PJM, Hua Y, Tolazzi M, Schermer E, Torres JL, Ozorowski G, van der Woude P, de la Peña AT, van Breemen MJ, Camacho-Sánchez JM, Burger JA, Medina-Ramírez M, González N, Alcami J, LaBranche C, Scarlatti G, van Gils MJ, Crispin M, Montefiori DC, Ward AB, Koopman G, Moore JP, Shattock RJ, Bogers WM, Wilson IA, Sanders RW. 2019. Structure and immunogenicity of a stabilized HIV-1 envelope trimer based on a group-M consensus sequence. Nat Commun https://doi.org/10.1038/s41467-019-10262-5.

37. Falkowska E, Le KM, Ramos A, Doores KJ, Lee JH, Blattner C, Ramirez A, Derking R, vanGils MJ, Liang CH, Mcbride R, von Bredow B, Shivatare SS, Wu CY, Chan-Hui PY, Liu Y, Feizi T, Zwick MB, Koff WC, Seaman MS, Swiderek K, Moore JP, Evans D, Paulson JC, Wong CH, Ward AB, Wilson IA, Sanders RW, Poignard P, Burton DR. 2014. Broadly neutralizing HIV antibodies define a glycan-dependent epitope on the prefusion conformation of gp41 on cleaved envelope trimers. Immunity 40:657–668.

38. Blattner C, Lee JH, Sliepen K, Derking R, Falkowska E, delaPeña AT, Cupo A, Julien JP, vanGils M, Lee PS, Peng W, Paulson JC, Poignard P, Burton DR, Moore JP, Sanders RW, Wilson IA, Ward AB. 2014. Structural delineation of a quaternary, cleavage-dependent epitope at the gp41-gp120 interface on intact HIV-1 env trimers. Immunity 40:669–680.

39. Torrents de la Peña A, Rantalainen K, Cottrell CA, Allen JD, van Gils MJ, Torres JL, Crispin M, Sanders

Journal of Virology JVI Journal of Virology

Downloaded from https://journals.asm.org/journal/jvi on 14 June 2021 by 193.60.240.99.

Accepted Manuscript Posted Online

RW, Ward AB. 2019. Similarities and differences between native HIV-1 envelope glycoprotein trimers and stabilized soluble trimer mimetics. PLoS Pathog 15.

40. Mage RG, Lanning D, Knight KL. 2006. B cell and antibody repertoire development in rabbits: The requirement of gut-associated lymphoid tissues. Dev Comp Immunol 30:137–153.

41. Klasse PJ, Ketas TJ, Cottrell CA, Ozorowski G, Debnath G, Camara D, Francomano E, Pugach P, Ringe RP, LaBranche CC, van Gils MJ, Bricault CA, Barouch DH, Crotty S, Silvestri G, Kasturi S, Pulendran B, Wilson IA, Montefiori DC, Sanders RW, Ward AB, Moore JP. 2018. Epitopes for neutralizing antibodies induced by HIV-1 envelope glycoprotein BG505 SOSIP trimers in rabbits and macaques. PLoS Pathog 14.

42. Medina-Ramírez M, Garces F, Escolano A, Skog P, de Taeye SW, Del Moral-Sanchez I, McGuire AT, Yasmeen A, Behrens AJ, Ozorowski G, van den Kerkhof TLGM, Freund NT, Dosenovic P, Hua Y, Gitlin AD, Cupo A, van der Woude P, Golabek M, Sliepen K, Blane T, Kootstra N, van Breemen MJ, Pritchard LK, Stanfield RL, Crispin M, Ward AB, Stamatatos L, Klasse PJ, Moore JP, Nemazee D, Nussenzweig MC, Wilson IA, Sanders RW. 2017. Design and crystal structure of a native-like HIV-1 envelope trimer that engages multiple broadly neutralizing antibody precursors in vivo. J Exp Med 214.

43. Hessell AJ, Malherbe DC, Pissani F, McBurney S, Krebs SJ, Gomes M, Pandey S, Sutton WF, Burwitz BJ, Gray M, Robins H, Park BS, Sacha JB, LaBranche CC, Fuller DH, Montefiori DC, Stamatatos L, Sather DN, Haigwood NL. 2016. Achieving Potent Autologous Neutralizing Antibody Responses against Tier 2 HIV-1 Viruses by Strategic Selection of Envelope Immunogens. J Immunol 196:3064–3078.

44. Ozorowski G, Pallesen J, De Val N, Lyumkis D, Cottrell CA, Torres JL, Copps J, Stanfield RL, Cupo A, Pugach P, Moore JP, Wilson IA, Ward AB. 2017. Open and closed structures reveal allostery and pliability in the HIV-1 envelope spike. Nature 547:360–361.

45. Lee JH, Ozorowski G, Ward AB. 2016. Cryo-EM structure of a native, fully glycosylated, cleaved HIV-1 envelope trimer. Science (80-) 351:1043–1048.

46. Ke Z, Oton J, Qu K, Cortese M, Zila V, McKeane L, Nakane T, Zivanov J, Neufeldt CJ, Cerikan B, Lu JM, Peukes J, Xiong X, Kräusslich HG, Scheres SHW, Bartenschlager R, Briggs JAG. 2020. Structures and distributions of SARS-CoV-2 spike proteins on intact virions. Nature https://doi.org/10.1038/s41586-020-2665-2.

47. Klasse PJ. 2014. Neutralization of Virus Infectivity by Antibodies: Old Problems in New Perspectives. Adv

Journal of Virology

JVI

Journal of Virology

Downloaded from https://journals.asm.org/journal/jvi on 14 June 2021 by 193.60.240.99.

Accepted Manuscript Posted Online

Biol 2014:1–24.

48. Torrents de la Peña A, Julien JP, de Taeye SW, Garces F, Guttman M, Ozorowski G, Pritchard LK, Behrens AJ, Go EP, Burger JA, Schermer EE, Sliepen K, Ketas TJ, Pugach P, Yasmeen A, Cottrell CA, Torres JL, Vavourakis CD, van Gils MJ, LaBranche C, Montefiori DC, Desaire H, Crispin M, Klasse PJ, Lee KK, Moore JP, Ward AB, Wilson IA, Sanders RW. 2017. Improving the Immunogenicity of Native-like HIV-1 Envelope Trimers by Hyperstabilization. Cell Rep https://doi.org/10.1016/j.celrep.2017.07.077.

49. Guenaga J, Dubrovskaya V, de Val N, Sharma SK, Carrette B, Ward AB, Wyatt RT. 2016. Structure-Guided Redesign Increases the Propensity of HIV Env To Generate Highly Stable Soluble Trimers. J Virol https://doi.org/10.1128/jvi.02652-15.

50. Kulp DW, Steichen JM, Pauthner M, Hu X, Schiffner T, Liguori A, Cottrell CA, Havenar-Daughton C, Ozorowski G, Georgeson E, Kalyuzhniy O, Willis JR, Kubitz M, Adachi Y, Reiss SM, Shin M, De Val N, Ward AB, Crotty S, Burton DR, Schief WR. 2017. Structure-based design of native-like HIV-1 envelope trimers to silence non-neutralizing epitopes and eliminate CD4 binding. Nat Commun https://doi.org/10.1038/s41467-017-01549-6.

51. Binley JM, Sanders RW, Clas B, Schuelke N, Master A, Guo Y, Kajumo F, Anselma DJ, Maddon PJ, Olson WC, Moore JP. 2000. 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 74.

52. Julien JP, Lee JH, Ozorowski G, Hua Y, De La Peña AT, De Taeye SW, Nieusma T, Cupo A, Yasmeen A, Golabek M, Pugach P, Klasse PJ, Moore JP, Sanders RW, Ward AB, Wilson IA. 2015. Design and structure of two HIV-1 clade c SOSIP.664 trimers that increase the arsenal of native-like env immunogens. Proc Natl Acad Sci U S A 112.

53. Pugach P, Ozorowski G, Cupo A, Ringe R, Yasmeen A, de Val N, Derking R, Kim HJ, Korzun J, Golabek M, de los Reyes K, Ketas TJ, Julien J-P, Burton DR, Wilson IA, Sanders RW, Klasse PJ, Ward AB, Moore JP. 2015. A Native-Like SOSIP.664 Trimer Based on an HIV-1 Subtype B env Gene . J Virol 89.

54. Suloway C, Pulokas J, Fellmann D, Cheng A, Guerra F, Quispe J, Stagg S, Potter CS, Carragher B. 2005. Automated molecular microscopy: The new Leginon system. J Struct Biol 151.

55. Lander GC, Stagg SM, Voss NR, Cheng A, Fellmann D, Pulokas J, Yoshioka C, Irving C, Mulder A, Lau PW,

Journal of Virology JVI Journal of Virology

Downloaded from https://journals.asm.org/journal/jvi on 14 June 2021 by 193.60.240.99.

Accepted Manuscript Posted Online

875 Lyumkis D, Potter CS, Carragher B. 2009. Appion: An integrated, database-driven pipeline to facilitate
876 EM image processing. J Struct Biol 166.
877 56. Voss NR, Yoshioka CK, Radermacher M, Potter CS, Carragher B. 2009. DoG Picker and TiltPicker:
878 Software tools to facilitate particle selection in single particle electron microscopy. J Struct Biol 166.
879 57. Scheres SHW. 2012. RELION: Implementation of a Bayesian approach to cryo-EM structure
880 determination. J Struct Biol 180.
881 58. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. 2004. UCSF
882 Chimera - A visualization system for exploratory research and analysis. J Comput Chem 25.
883 59. Wagh K, Kreider EF, Li Y, Barbian HJ, Learn GH, Giorgi E, Hraber PT, Decker TG, Smith AG, Gondim M V.,
884 Gillis L, Wandzilak J, Chuang GY, Rawi R, Cai F, Pellegrino P, Williams I, Overbaugh J, Gao F, Kwong PD,
885 Haynes BF, Shaw GM, Borrow P, Seaman MS, Hahn BH, Korber B. 2018. Completeness of HIV-1
886 Envelope Glycan Shield at Transmission Determines Neutralization Breadth. Cell Rep
887 https://doi.org/10.1016/j.celrep.2018.09.087.

888

889 **Figure legends:**

890 **Fig 1: Characteristics of mAbs isolated from AMC008 SOSIP trimer immunized rabbits.** (A)
891 Immunization and sample collection scheme (left) and autologous and heterologous serum
892 neutralization titers at week 22 (right) for four AMC008 SOSIP trimer immunized rabbits.
893 Serum neutralization ID_{50} is shown for each animal. (B) Absolute number of mAbs isolated per
894 animal. (C) Individual CDR3 lengths and CDR amino acid junction sequence of heavy and light
895 chains of all isolated mAbs. (D) Individual CDR3 amino acid lengths of the isolated mAbs. (E)
896 Autologous neutralization ability of the isolated mAbs. Neutralization IC_{50} values are shown
897 for each of the four NAbs in the accompanying table.

898

899 **Fig 2: Competitive assays of Nabs with bNAbs and non-NAbs to specify an epitope.** (A)
900 Percentage residual binding of NAbs to AMC008 SOSIP trimers in the presence of a competing

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NAb. The % residual binding was calculated as follows: (shift in nm at 600s of 2nd mAb binding*100)/nm shift of 2nd mAb in the absence of 1st mAb binding. (B) Competition ELISA and SPR data of all NAbs with human bNAbs. The percentage of residual binding was calculated as follows, for ELISA: (average OD450 of a triplo in presence of the 2nd mAb*100)/average OD450 of a triplo in absence of the 1st mAb binding, for SPR: (response difference at 200s for the second Ab)/(Response difference at 200s for the same, second Ab when injected as a single Ab in a separate cycle) * 100 (%). Significant results are highlighted by stars (**=p<0.005 ***=p<0.0001). SPR was only performed for the 05A family of NAbs. (C) SPR binding curves of AMC008 SOSIP trimer binding competition between NAbs and the bNAb ACS2020 that shows the influence of assay directionality. Ab binding is recorded in real-time; the x-axis indicates the time in seconds. The y-axis shows the response (response units, RU), proportional to the mass bound. The dissociation starts at 0s. The top three graphs show binding of ACS202 in presence of competitors 05A1-05A3 (dark red, red, and purple lines), compared to ACS202 binding in absence of these NAbs (blue lines). The lower three graphs show a reverse assay set-up, showing NAb 05A1-05A3 binding ability in presence of competitor ACS202 (blue lines) and in the absence of ACS202 (dark red, red, and purple lines). (D) Competition results of the four rabbit NAbs with the isolated non-NAbs measured by BLI. % residual binding was calculated as stated for figure 3A.

Fig 3: Epitope mapping of NAbs to the gp41 subunit of Env. (A) NS-EM 3D reconstructions of the NAbs fabs in complex with the AMC008 SOSIP trimer. The AMC008 SOSIP trimer structure is modeled as the ribboned density. The NAbs are shown as white densities. Side and bottom views are depicted. (B) Alignment of viral sequences tested for neutralization and binding by

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924 05A1-05A3 and 07A1. AMC008, AMC009 and SHIV162p3 could be neutralized by the NAbs,
925 whereas the viruses below the line were not neutralized by the NAbs. HXB2 amino acid
926 numbering is indicated on top and lines indicate the NAb binding sites as predicted by NS-EM
927 imaging. Red boxes show which amino acid residues were mutated in the predicted binding
928 sites to specify the epitope; D620N, N624D, and E662A. (C) Model of the glycan shield present
929 on the AMC008 SOSIP trimer with a strain-specific glycan hole indicated in cyan and yellow
930 due to the absence of PNGS 230 and 234. Image was created with the glycan shield mapping
931 tool on the Los Alamos database(59). (D) Neutralization ability of NAbs to multiple viral
932 variants. Fold decrease in IC_{50} values is plotted for each of the NAbs and the VRC01 control,
933 each represented by different colors. The dotted lines indicate a 3-fold threshold compared
934 to AMC008 WT neutralization IC_{50} values. (E) Neutralization ability of the NAbs for various
935 AMC008 mutants. IC_{50} values are indicated in µg/ml. The bNAb VRC01 is taken along as a
936 positive control.
937
938 **Fig 4: Epitope mapping of the non-neutralizing antibodies**
939 (A) Binding ability of all isolated mAbs to various AMC008 mutants and linear V3 peptide. Fold
940 change in area under the curve is displayed relative to AMC008.v4.2 binding. Binding to the
941 V3 peptide is indicated as yes or no.
942 (B) NAbs 05A1-05A3 (purple and pink) and 07D2 (grey) were complexed with the AMC008
943 SOSIP Env and analyzed by NS-EM with the AMC008 SOSIP trimer to show differences in their
944 binding angle.
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946 **Fig 5: Analysis of neutralizing and non-neutralization antibody characteristics that might**
947 **influence neutralization** (A) Binding kinetics of NAbs and non-NAb to the autologous AMC008
948 SOSIP and heterologous AMC016 SOSIP Env trimers. K*D*, on- (K*a*), and off-rate constants (K*d*)
949 are shown. NAbs were tested for binding to AMC008 and AMC016 SOSIP Env trimers. Non-
950 NAbs were only tested for binding to the autologous AMC008 SOSIP trimer. Results were fitted
951 to a 1:1 kinetics model for analysis. (B) Binding to full length surface expressed AMC008 SOSIP
952 gp160 Env by mAbs. Maximum median MFI is plotted for each mAb tested. The left panel
953 shows the binding and expression controls (2G12 and PGT145 respectively). The middle panel
954 shows binding of the NAbs to full length AMC008 gp160 Env trimers. The right most panel
955 shows the binding of a selection of non-NAbs to full length AMC008 gp160 Env trimers.
956
957 **Fig 6: NAbs destabilize the trimer as a mechanism of neutralization.** (A) Negative Stain-
958 Electron Microscopy images of AMC008 SOSIP trimers incubated overnight with the mAb
959 05A3. The majority of the images displayed the mAb binding with a stoichiometry of 2 mAbs
960 per trimer (middle image). mAbs binding the monomeric form of the trimer were also
961 observed (right image). (B) Pre-incubation neutralization experiment to determine the *in vivo*
962 destabilization ability of the isolated Abs. A neutralization assay was performed in which virus
963 and Ab mix were incubated for different time periods, up to 24 hours. The fold change in IC_{50}
964 is shown for each of the Abs. The autologous AMC008 virus and heterologous SHIV162p3
965 viruses were tested. 3BC315 and PGT126 were tested as positive control and negative control
966 Abs, respectively. Increased neutralization potency is seen for most of the NAbs and 3BC315
967 but not 06A1 and PGT126 after the 24 hour incubation step. NN = non-neutalizing.
968

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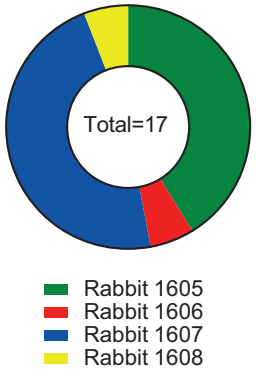
Fig 1

A

week 22 ID_{50}	Rabbit ID			
	1605	1606	1607	1608
AMC008 virus	299	262	272	178
AMC009 virus	22	<20	47	<20
AMC018 virus	44	40	30	54

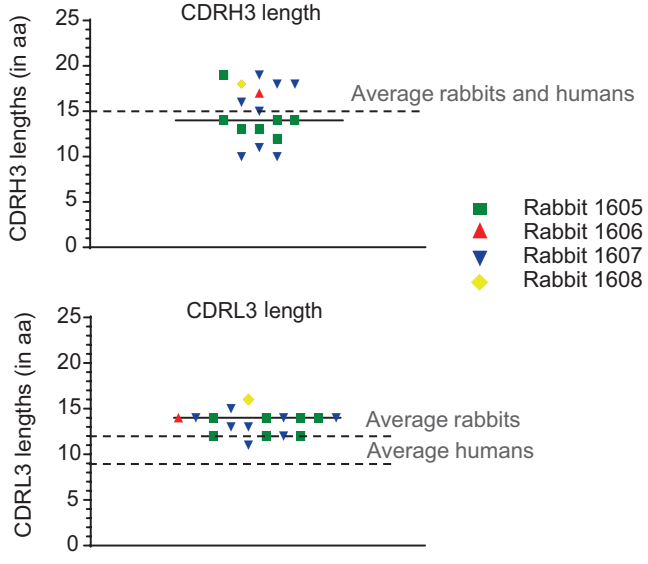
B



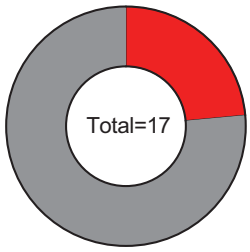
C

Ab ID	CDR3 length heavy	CDR3 length kappa	CDR AA junction heavy	CDR AA junction Kappa
05A1	14	14	CARDPADGLNNYYDLW	CQSTYGTYISSYGGAF
05A2	14	14	CARDPADGLYNYYDLW	CQSTYGTYISSYGGAF
05A3	14	14	CARDPADGLTNYYDLW	CQCTYGTYISSYGGAF
05B1	13	12	CARGNSNYYNGFNI*	CQQTVRGTNVVISF
05B2	13	14	CAEGIVIIIMDLI#W	CQSNVASRDSVYGLGF
05D1	12	12	CARENSNGYVYDIW	CQQAYDHSNIDNAF
05G1	19	12	SVETVLMVVGCW*CPQTITLG	CQGYYLGTDYDNGF
06A1	17	14	CARDGGYGSAWGMSYFNLW	CQSNFYSSSDSVGFAF
07A1	18	14	ARSDIGYDADEYVSYGYTLW	CQCTYGNNINSYGGAF
07B1	16	12	CTRDHGENTAYAF#LW	CQLIYDWKNVDIPL
07C1	11	13	CAKDGAGDSYNMW	CQQGYSYSNVGQNVF
07D1	10	14	CQINYYRSGSIYFFAF	CQINYYRSGSIYFFAF
07D2	10	15	CARYSDSDINLW	CQCTYGSNIIIYYGDAV
07E1	19	11	CARAYARSYGILMRPYYFDLS	WAGSYIIISDIAL
07F1	15	14	CARGSSRGHYIDFFDHW	CQNYYGSSSSTYGNAF
07G1	18	13	CARGVGYDGYGTATWYFDLW	CQSYCGDYSSTYVTF
08A1	18	16	CANSPGYSGFSYVNVYFRLW	CQSGYYSVSYDRVAF

D



E



NAbs	IC_{50} (µg/ml)
05A1	20.5
05A2	14.0
05A3	15.1
07A1	16.2

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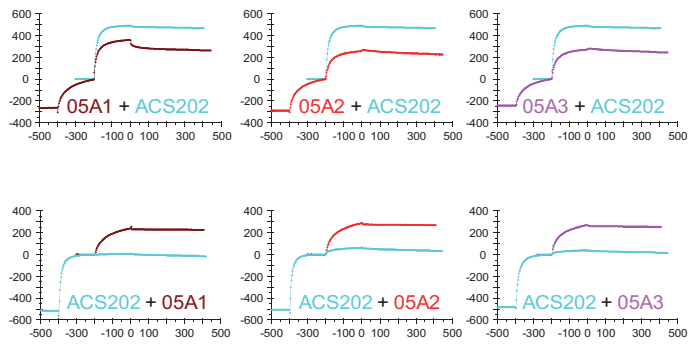
Fig 2

A

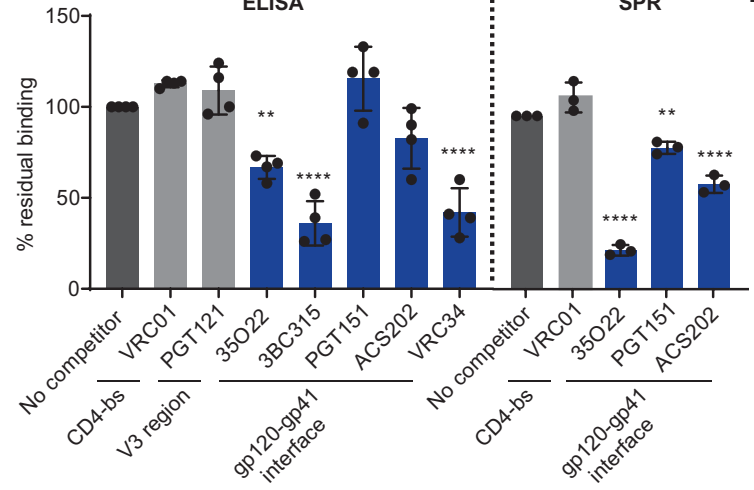
% residual binding		2nd mAb			
		05A1	05A2	05A3	07A1
1st mAb	05A1	35	65	63	56
	05A2	43	27	45	9
	05A3	22	34	39	16
	07A1	46	54	57	46

<25 25-49 50-74 >74

C



B



D

% residual binding		2nd mAb			
		05A1	05A2	05A3	07A1
1st mAb	05B1	92	82	100	88
	05B2	101	96	101	87
	05G1	ND	ND	ND	95
	06A1	62	78	66	19
	07B1	25	39	35	39
	07C1	113	90	98	79
	07D2	0	21	18	16
	07E1	27	34	36	32
	07F1	93	86	97	77
	08A1	70	61	55	57

<25 25-49 50-74 >74

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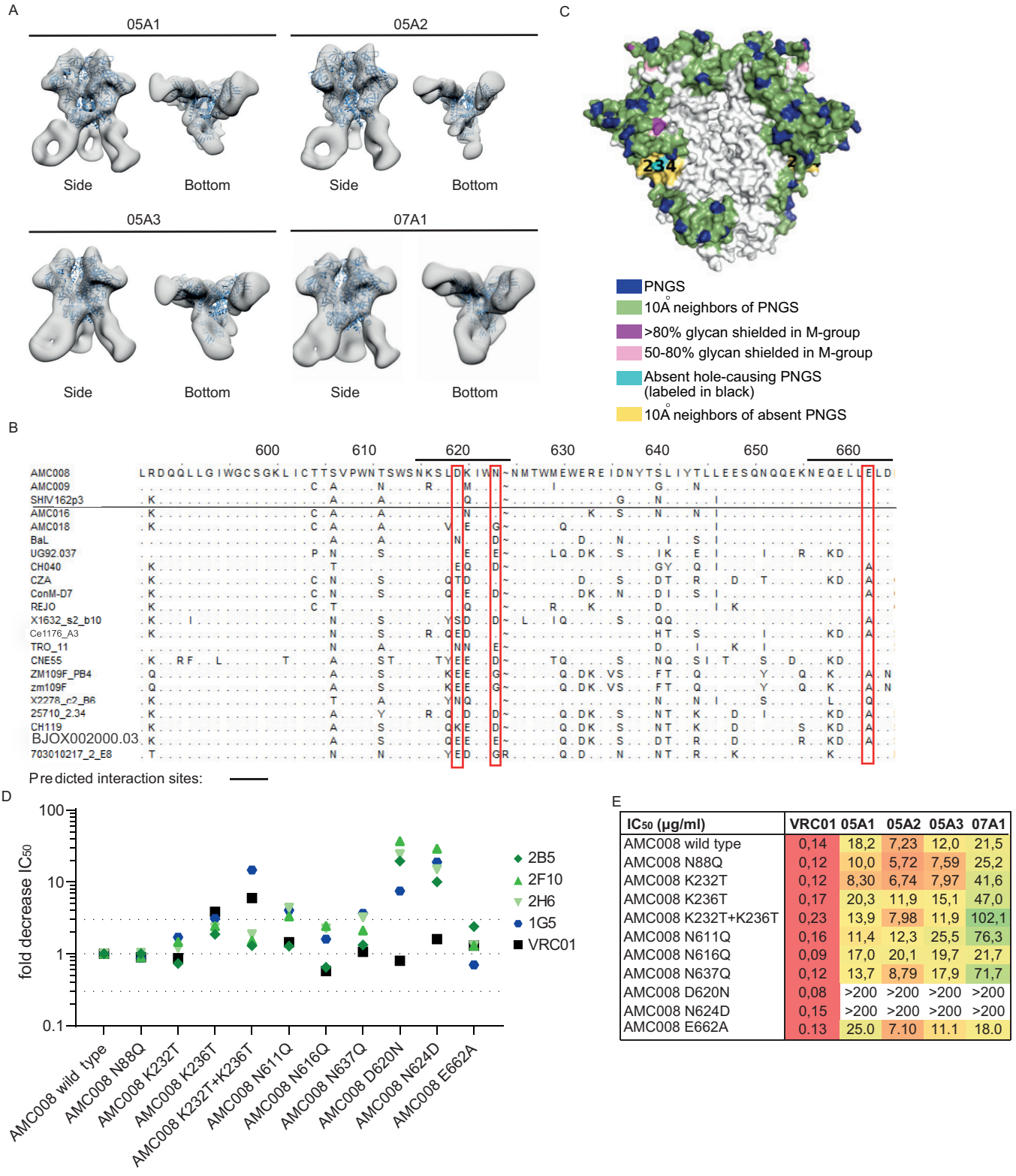
Fig 3



E

IC50 (µg/ml)	VRC01	05A1	05A2	05A3	07A1
AMC008 wild type	0,14	18,2	7,23	12,0	21,5
AMC008 N88Q	0,12	10,0	5,72	7,59	25,2
AMC008 K232T	0,12	8,30	6,74	7,97	41,6
AMC008 K236T	0,17	20,3	11,9	15,1	47,0
AMC008 K232T+K236T	0,23	13,9	7,98	11,9	102,1
AMC008 N611Q	0,16	11,4	12,3	25,5	76,3
AMC008 N616Q	0,09	17,0	20,1	19,7	21,7
AMC008 N637Q	0,12	13,7	8,79	17,9	71,7
AMC008 D620N	0,08	>200	>200	>200	>200
AMC008 N624D	0,15	>200	>200	>200	>200
AMC008 E662A	0.13	25.0	7.10	11.1	18.0

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Fig 4

A

	Fold change AUC											
Ab ID	AMC008 v4.2	AMC008 gp120	K232T	K236T	K232T+ K236T	N88Q	N611Q	N616Q	N637Q	D620N	N624D	E662A	linear V3 peptide
2G12	1.0	1.0	0.9	0.9	0.9	0.8	0.8	0.9	0.9	0.9	0,8	0,9	No
PGT145	1.0	ND	1.0	1.0	0.9	0.8	0.8	0.9	0.8	1.5	0,9	1,1	ND
05A1	1.0	0.1	0.8	0.7	0.7	0.8	0.8	0.8	0.9	0.7	0,6	0,8	No
05A2	1.0	0.1	0.9	0.9	0.9	0.3	0.8	0.9	0.9	0.9	1,0	1,0	No
05A3	1.0	0.1	0.9	0.9	1.0	0.9	1.0	1.0	0.9	1.0	0,8	0,9	No
05B1	1.0	ND	ND	ND	ND	ND	ND	ND	ND	1.0	1,0	1,0	ND
05B2	1.0	0.1	0.9	0.9	0.9	0.9	1.1	1.0	1.7	0.7	0,8	1,1	No
05D1	1.0	0.1	0.9	0.8	1.0	0.8	0.5	0.8	0.8	0.8	0,7	0,9	Yes
05G1	1.0	0.0	0.9	0.9	0.9	0.9	0.8	0.9	0.7	0.6	0,4	0,9	ND
06A1	1.0	0.0	0.8	0.8	0.8	0.9	0.5	0.8	1.0	1.0	0,8	1,0	ND
07A1	1.0	0.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.1	0,6	1,0	ND
07B1	1.0	1.4	1.0	1.0	1.0	1.0	1.0	1.1	1.0	0.9	1,0	1,2	No
07C1	1.0	0.1	1.0	0.9	1.0	0.9	1.1	1.1	1.1	0.7	0,9	1,1	No
07D1	1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
07D2	1.0	0.0	1.0	0.7	0.7	0.7	0.6	1.2	1.2	0.1	0,1	0,1	ND
07E1	1.0	0.1	1.2	0.8	1.0	0.8	1.4	1.7	0.9	0.8	0,4	0,6	ND
07F1	1.0	0.1	1.0	1.0	0.8	0.9	1.0	1.0	0.3	0.9	0,9	0,9	No
07G1	1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
08A1	1.0	ND	0.9	1.0	1.0	0.9	0.7	0.9	0.7	1.0	0,8	0,9	ND
			Strain specific glycan hole			**gp41 glycans**				**NAb Interaction site**			**V3**

0.1-0.3
0.4-0.6
0.7-1.0
>1.0

B

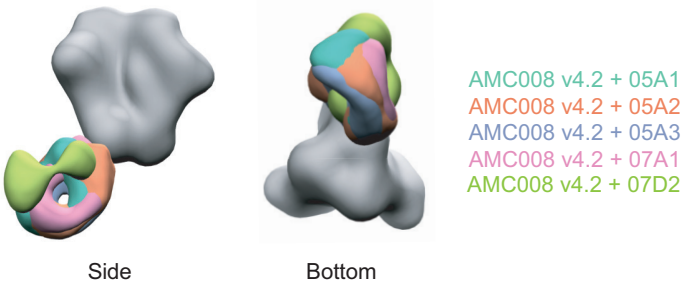

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Fig 5

A

Neutralizing mAbs

AMC008 SOSIP Env

Sample ID	KD (nM)	Ka (1/Ms)	Kd (1/Ms)
05A1	7.74	1.57E+04	1.21E-04
05A2	12.0	2.23E+04	2.69E-04
05A3	10.0	1.07E+04	1.74E-04
07A1	8.35	1.83E+04	1.53E-04

AMC016 SOSIP Env

Sample ID	KD (nM)	Ka (1/Ms)	Kd (1/Ms)
05A1	7.58	2.39E+04	1.81E-04
05A2	3.91	3.36E+04	1.31E-04
05A3	18.0	1.74E+04	3.19E-04
07A1	7.65	2.61E+04	2.00E-04

Non-neutralizing mAbs

AMC008 SOSIP Env

Sample ID	KD (nM)	Ka (1/Ms)	Kd (1/Ms)
05D1	1.95	9.72E+04	1.90E-04
07D2	1.75	1.16E+05	2.03E-04
07E1	0.7	1.15E+05	8.04E-05

B

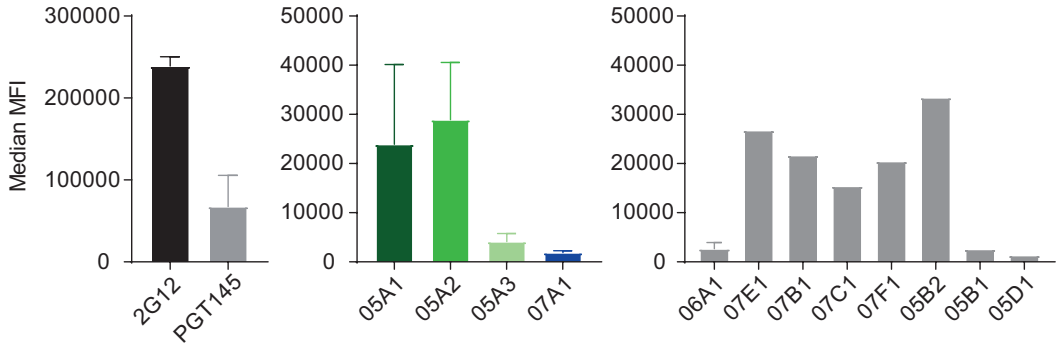

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Fig 6

A

3 fabs bound (minority) | 2 fabs bound (majority) | Monomer+fab

B

AMC008 autologous virus

Fold change IC_{50}							
Time (h)	05A1	05A2	05A3	07A1	06A1	3BC315	PGT126
1	1.0	1.0	1.0	1.0	NN	1.0	1.0
3	1.2	1.5	1.5	1.6	NN	1.9	0.8
6	1.9	2.3	2.1	2.4	NN	2.4	0.9
24	12.4	7.9	10.2	11.4	NN	9.6	1.5

SHIV162p3 heterologous virus

Fold change IC_{50}						
Time (h)	05A1	05A2	05A3	07A1	3BC315	PGT126
1	1.0	1.0	1.0	1.0	1.0	1.0
3	1.7	4.8	3.6	11.5	9.1	0.5
6	2.3	6.8	6.2	12.5	11,3	1.5
24	4.8	18.5	15.9	28.1	21.4	4.5

>10
5-10
<5

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Table 1: Heterologous binding ability of the isolated NAbs. (A) ELISA cross-binding ability (EC50 in µg/ml) of the isolated AMC008 SOSIP trimer-reactive NAbs to various clade B and non-clade B SOSIP Env trimers.

	Antibody ID	Clade	Tier	05A1	05A2	05A3	07A1
Clade B SOSIPs	AMC008	B	1B	++	++	++	++
	AMC009	B	2	++	++	++	++
	AMC011	B	2	-	-	+/-	-
	AMC016	B	3	++	++	++	+
	AMC018	B	2	++	++	++	+
	SHIV162p3	B	2	++	+	++	+/-
	REJO	B	2	++	++	++	++
non-clade B SOSIPs	BG505	A	2	-	-	-	-
	ConM	M	1A/1B	-	-	-	-
	CNE55	CRF01_AE	2	-	-	-	-
	BJOX002000.03.2	CRF07_BC	2	-	-	-	-
	Ce1176_A3	C	2	++	++	++	-
	HIV_25710-2.43	C	2	-	-	-	-

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Table 2: Neutralization ability (IC50 in µg/ml) of the autologous NAbs to neutralize a panel of 17 heterologous viruses.

IC_{50} (µg/ml)	Clade	Glycan hole	05A1	05A2	05A3	07A1	05D1
AMC008	B	230 + 234	20,5	14	15,1	16,2	>50
SHIV162p3	B	230	8,8	5,6	18	6,1	0,72
AMC009	B	230	177	58	170	114	>200
AMC016	B	-	>200	>200	>200	>200	>200
AMC018	B	234	>200	>200	>200	>200	>200
BaL	B	230 + 234	>200	>200	>200	>200	>200
WITO4160.33	B	234	>200	>200	>200	>200	>200
CH040	B	230 + 234	>200	>200	>200	>200	>200
CZA97.012	C	234	>200	>200	>200	>200	>200
ZM109F	C	234	>200	>200	>200	>200	>200
25710-2.43	C	230	>200	>200	>200	>200	>200
Ce1176_A3	C	-	>200	>200	>200	>200	>200
X2278	B	230	>200	>200	>200	>200	>200
BJOX002000.03.2	CRF07_BC	230	>200	>200	>200	>200	>200
Ce703010217_B6	C	-	>200	>200	>200	>200	>200
X1632-S2-B10	G	230	>200	>200	>200	>200	>200
CNE55	CRF01_AE	230	>200	>200	>200	>200	>200
CH119.10	CRF07_BC	230	>200	>200	>200	>200	>200

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