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NOVEL VACCINE IMMUNOGENS FROM RARE HIV-1 CONTROLLERS THAT POSSESS BROADLY NEUTRALIZING ANTIBODIES

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Abstract

Novel Vaccine Immunogens from Rare HIV-1 Controllers that Possess Broadly Neutralizing Antibodies

Jennie M. Hutchinson

After over 35 years of research, a prophylactic HIV vaccine remains elusive. However, the discovery of potent broadly neutralizing antibodies (bNAbs) in some individuals (elite neutralizers) and other individuals that control HIV infection without anti-retroviral drugs (controllers) have renewed optimism that an effective vaccine may be possible. The production of bNAbs does not correlate with improved disease outcomes because they take too long to develop and the virus typically outpaces the antibody response. However, passive transfer of bNAbs prior to infection is protective in non-human primate models. Thus, a vaccine capable of eliciting bNAbs prior to infection may provide protective immunity. The sole target of bNAbs is the viral spike, a trimer of envelope glycoproteins (Env). Although it is now possible to produce recombinant Envs with epitopes that bind bNAbs, these have failed to elicit bNAbs. Epitopes recognized by bNAbs are inherently poor immunogens due to the densely packed glycan shield that covers the majority of the surface. Previous HIV vaccines were based on Envs without regard for the immune response in virus donors. Here, for the first time, we studied Envs from two rare individuals that controlled viral load and produced bNAbs. From each individual, *envs* were cloned and used to construct 20 pseudotype viruses. These were then screened for neutralization sensitivity against a panel of prototypic monoclonal

bNAbs. Comparative sequence analysis revealed two features that may have contributed to the robust immune response: (1) a major reduction (35%) in the number of potential N-linked glycosylation sites in gp120 and (2) two extra cysteine residues in the V1/V2 region. One *env* from each individual was expressed and used for antibody binding assays and rabbit immunization studies. Antibody responses to the novel immunogens were compared to MN rgp120, used in the only clinical trials that has shown efficacy in humans. A minimally glycosylated immunogen elicited an improved cross-reactive neutralization response compared to other clade B Envs, including MN. This work demonstrated that Envs from individuals with the elite neutralizer/controller phenotype possess unusual structural features that may have enhanced the immune response and represent a new source of HIV vaccine immunogens.

We stand on the shoulders of giants.

I count my parents among them

Introduction

In 1983, human immunodeficiency virus (HIV) was isolated and identified as the pathogen causing acquired immunodeficiency syndrome (AIDS)(Barre-Sinoussi et al., 1983). After over 35 years of global prevention and treatment efforts, HIV remains a significant public health crisis. Since the start of the pandemic, 75 million people have been infected with HIV and 32 million have died from AIDS-related illnesses (UNAIDS, 2019a). Like all infectious diseases, HIV/AIDS disproportionately impacts the most disadvantaged members of society. Globally, AIDS-related illnesses are the leading cause of death in women of reproductive age (UNAIDS, 2019a). In the United States, black Americans face an increased burden primarily due to socio-economic status and mass incarceration (Shrage, 2016), composing 41% of new HIV infections despite representing only 12% of the population (CDC, 2018). While black American women tend to have the same, if not safer sex practices (Hallfors et al., 2007; Reece et al., 2010), they are twenty-times as likely to become infected with HIV than their white counter parts due to social injustices (CDC, 2018). Disparities across the continuum of care make a prophylactic vaccine the most viable solution for HIV eradication and research efforts should focus on the most affected communities (Fauci, 2017).

Challenges to developing an HIV-1 envelope subunit vaccine

A primary goal of HIV-1 vaccine design is to produce an immunogenic HIV-1 envelope glycoprotein (Env), the sole target of neutralizing antibodies. This has

proved a formidable challenge due to viral adaptations to evade the immune system. The HIV virion surface is primarily composed of host-derived plasma membrane with a sparse number (7 – 14) of conformationally dynamic viral spikes (Zhu et al., 2006). The viral spike is a homotrimer of gp160s (Envs) that have been cleaved by furin into non-covalently linked heterodimers of gp120 and gp41 (Seabright et al., 2019). The gp41 portion is membrane-bound and also present on the virion surface as gp41 "stumps," which act as a decoy for immune recognition (Seabright et al., 2019). Similarly, non-functional monomeric gp120 are shed by the virus (Seabright et al., 2019). The extracellular gp120 is described by five constant regions (C1 – C5) and five variable regions (V1 – V5). All published crystal structures of Env show gp120 with 18 cysteine residues that form 9 disulfide bonds and typically have 25 – 26 N-linked glycans (Behrens et al., 2016; Lasky et al., 1986; Wei et al., 2003). These host-produced glycans compose half the mass of gp120 and form a "glycan shield" that is poorly immunogenic (Behrens et al., 2016; Lasky et al., 1986; Wei et al., 2003). Env glycans are not fully processed due to steric hindrance (Doores et al., 2010) resulting in high-mannose glycoforms. Variations in glycoforms and glycan occupancy significantly alter antigenic structures (Rudd and Dwek, 1997).

HIV exhibits unprecedented viral diversity. There are two major types, HIV-1 and HIV-2, which likely emerged from distinct zoonosis events from chimpanzee viruses (CPZ) and simian immunodeficiency virus (SIV), respectively (Korber et al., 2001). HIV-1 is substantially more prevalent and further divided into groups M, N, and O. More than 90% of HIV-infections are from HIV-1 group M, which includes

clades A – K and circulating recombinant forms (CRF)(Korber et al., 2001). There is wide variability even within one person at a single timepoint, where genetic diversity is comparable to global influenza in a given year (Korber et al., 2001). HIV's extraordinary genetic heterogeneity is due to error-prone reverse transcription, fast replication, and frequent recombination (Perelson et al., 1996; Preston et al., 1988; Zhang et al., 2010). In addition, viral RNA is integrated into the host genome, creating a latent reservoir that can reseed infection with ancestral variants.

Antibody response to HIV infection

HIV infection is initiated by one or a few transmitter/founder viruses (T/F)(Derdeyn et al., 2004; Keele et al., 2008). Cell entry requires gp120 binding to the CD4 receptor and a co-receptor, either CCR5 and/or, later in disease progression, CXCR4 (Seabright et al., 2019). Non-neutralizing antibodies targeting gp41 develop 1 – 2 weeks after infection (Mikell et al., 2011), followed by gp120-specific antibodies that often target the V3 loop (Tomaras et al., 2008) and CD4-binding site (Lynch et al., 2012). These initial non-neutralizing antibodies elicit cell-mediated responses, including antibody-dependent cellular cytotoxicity and other antiviral activity (Baum et al, 1996). The first neutralizing antibodies that appear are strain-specific and can neutralize some, but not all, autologous virus (Wei et al., 2003). The virus evolves quickly, escaping the antibody response and driving formation of antibodies recognizing new epitopes. There are continual rounds of viral escape and antibody maturation, with the virus usually outpacing the immune response (Bhiman et al.,

2015; Bonsignori et al., 2016; Doria-Rose et al., 2014; Euler et al., 2010; Freund et al., 2017; Wu et al., 2010; Zhou et al., 2013). After 1 – 3 years, the virus usually undergoes extensive diversification and broadly neutralizing antibodies (bNAbs) emerge in ~30% of people (Sather et al., 2009).

There are significant challenges to developing bNAbs. Antibodies must penetrate or bind to the glycan shield (Doria-Rose et al., 2009; Euler et al., 2010; Hraber et al., 2014; van Gils et al., 2009). Production of glycan-targeting antibodies requires bypassing a negative selection process designed to prevent auto-reactive antibodies (Wardemann et al., 2003). Binding affinity to glycans tends to be lower (Cohen, 2015) and bNAbs usually require multivalent interactions (Kong et al., 2013). The extensive antibody maturation process prior to neutralization breadth can result in antibodies with unusual characteristics, including long CDRH3 regions and high level of somatic hypermutation (Andrabi et al., 2015; Klein et al., 2013). Despite these obstacles, half of individuals produce antibodies with significant cross reactivity (Hraber et al., 2014), suggesting that it is possible that a vaccine could elicit bNAbs.

Less than 1% of individuals are elite neutralizers, defined by having sera with highly potent cross-reactive neutralization activity, able to neutralize viruses from a least four different clades at an average dilution of 1:300 or greater (Simek et al., 2009). However, even in these individuals, neutralization breadth does not correlate with improved disease outcomes (Bhiman et al., 2015; Bonsignori et al., 2016; Doria-Rose et al., 2014; Euler et al., 2010; Freund et al., 2017; Piantadosi et al., 2009; Wu et al., 2010; Zhou et al., 2013). While bNAbs do not provide protection in natural

infection, passive immunization studies in non-human primates have demonstrated that bNAbs can provide protective immunity (Baba et al., 2000; Hessell et al., 2010, 2009; Mascola et al., 2000, 1999; Moldt et al., 2012; Parren et al., 2001; Shibata et al., 1999). Thus, a vaccine capable of eliciting potent bNAbs, like those found in elite neutralizers, remains a primary goal.

Viral control

Some individuals control HIV infection and maintain low viral loads, which correlate with slow disease progression (Okulicz et al., 2009). There are multiple biological mechanisms that contribute to viral control but vary from patient to patient (Lambotte et al., 2009; Pereyra et al., 2008). Human leukocyte antigen alleles that encode for the major histocompatibility complex proteins account for ~15% of individuals with low viral load (Fellay et al., 2009) through improved presentation of immunogenic structures and interactions with natural killer cells (Tomescu et al., 2012). Cell-mediated responses also play a significant role, primarily through CD8+ T cell responses (Leitman et al., 2017b; Pereyra et al., 2014) and antibody-mediated cellular cytotoxicity (Ackerman et al., 2016; Baum et al., 1996; Johansson et al., 2011; Lambotte et al., 2009; Madhavi et al., 2017). Virological factors can lead to viral control, such as infection with attenuated strains (Lassen et al., 2009; Pereyra et al., 2008). Ongoing research efforts seek to further elucidate pathways to viral control, the closest manifestation of natural immunity.

Current strategies for developing HIV envelope subunit vaccine

To date, vaccine candidates have failed to consistently elicit the production of bNAbs and multiple strategies are being explored to enhance immunogenicity (Andrabi et al., 2018; Gilbert et al., 2010; Kwong and Mascola, 2018; Lian et al., 2005; Mascola et al., 1996; Montefiori et al., 2012). Extensive research has led to the production of trimeric Envs with antigenic structures capable of binding to all major classes of bNAbs, i.e., those that target V2-glycans, V3-glycans, CD4-binding site, membrane proximal external region (MPER), and gp120-gp41 interface (Julien et al., 2013a; Sok and Burton, 2018). However, these trimeric Envs have so far failed to consistently elicit bNAbs in immunization studies. Sequential immunizations strategies are being employed to improve immunogenicity and direct the neutralization response to sites of vulnerability. One strategy is based on longitudinal studies examining the co-evolution of Envs and antibody maturation pathway of bNAbs, where immunogens are designed to mimic viral evolution in an attempt to recapitulate the corresponding bNAb ontogeny (de Taeye et al., 2016). An alternative strategy is to use deglycosylated Env trimers, which have been shown to increase immunogenicity, followed by boosts with Envs containing additional glycans (Dubrovskaya et al., 2019). Another approach is using properly folded and glycosylated Env scaffolds in order to focus the antibody response to neutralizing epitopes and limit responses to non-neutralizing immunodominate regions (Chung et al., 2012; Hoffenberg et al., 2013; Morales et al., 2014a, 2016; Ofek et al., 2010, 2010; Yu et al., 2012).

Here, we explore a new source of potentially immunogenic Envs, those isolated from ART-naïve viral controllers that produce bNAbs. The majority of Envs that have been studied were derived from individuals without regard for disease phenotype in the virus donor. Envs isolated from elite neutralizers may share immunogenic features that induced the production of potent bNAbs but antigen selection is challenging due to viral diversification. To overcome this, we looked for elite neutralizers that maintained viral control, reasoning that the smaller viral population with less Env diversity may result in the identification of Envs that are more closely related to those that induced bNAbs. In addition, Envs from viral controllers may have other immunogenic features that contributed to antiviral activity. However, it was unclear if we could obtain samples from ART-naïve individuals with the elite neutralizer/controller phenotype. ART-naïve samples are increasingly rare because the current standard of care includes ART shortly after diagnosis. Also, it has previously been reported that viral controllers have less neutralization breadth (Doria-Rose et al., 2010). Identification of individuals with the elite neutralizer/controller phenotype required extensive screening for neutralization breadth in 136 archival samples from ART-naïve viral controllers in the SCOPE and WIHS longitudinal studies. For the first time, we have characterized Envs from individuals with the elite neutralizer/controller phenotype and found structural features that may have contributed to their robust immune response. These studies demonstrate that Envs isolated from the elite neutralizer/controller phenotype are a promising new source for HIV immunogens.

Chapter 1: SCOPE cohort study

Unusual Cysteine Content in V1 Region of gp120 From an Elite Suppressor That Produces Broadly Neutralizing Antibodies

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Abstract

Although it is now possible to produce recombinant HIV envelope glycoproteins (Envs) with epitopes recognized by the 5–6 major classes of broadly neutralizing antibodies (bNAbs), these have failed to consistently stimulate the formation of bNAbs in immunized animals or humans. In an effort to identify new immunogens better able to elicit bNAbs, we are studying Envs derived from rare individuals who possess bNAbs and are able to control their infection without the need for anti-retroviral drugs (elite supressors or ES), hypothesizing that in at least some people the antibodies may mediate durable virus control. Because virus evolution in people with the ES only phenotype was reported to be limited, we reasoned the Env proteins recovered from these individuals may more closely resemble the Envs that gave rise to bNAbs compared to the highly diverse viruses isolated from normal progressors. Using a phenotypic assay, we screened 25 controllers and identified two for more detailed investigation. In this study, we examined 20 clade B proviral sequences isolated from an African American woman, who had the rare bNAb/ES phenotype. Phylogenetic analysis of proviral envelope sequences demonstrated low genetic diversity. Envelope proteins were unusual in that most possessed two extra cysteines within an elongated V1 region. In this report, we examine the impact of the extra cysteines on the binding to bNAbs, virus infectivity, and sensitivity to neutralization. These data suggest structural motifs in V1 can affect infectivity, and that rare viruses may be prevented from developing escape.

Introduction

Despite the widespread availability of anti-retroviral drugs, recent studies suggest that an HIV vaccine will still be required to control and eliminate the spread of this virus (Fauci, 2017; Medlock et al., 2017). Over the last decade, evidence has accumulated from passive immunization studies to suggest that a vaccine that elicits broadly neutralizing antibodies (bNAbs) to the HIV envelope glycoprotein (Env), prior to infection, can provide protective immunity against HIV (Barouch et al., 2013; Bournazos et al., 2014; Caskey et al., 2015; Jacobson et al., 1993; Klein et al., 2014; Ledgerwood et al., 2015; Shingai et al., 2014, 2013; Stiegler et al., 2002). Although considerable progress has been made in developing immunogens, such as properly folded trimeric Envs (Derking et al., 2015; Georgiev et al., 2015; Julien et al., 2013a; McLellan et al., 2011; Pejchal et al., 2011; Sanders et al., 2015; Sok et al., 2014; Yang et al., 2018) that accurately replicate the antigenic structures of the 5–6 major epitopes recognized by bNAbs (Kwong and Mascola, 2012; van Gils and Sanders, 2013; Walker et al., 2010), none have been effective in eliciting protective neutralizing antibodies (Andrabi et al., 2018; Gilbert et al., 2010; Kwong and Mascola, 2018; Lian et al., 2005; Mascola et al., 1996; Montefiori et al., 2012). Thus, while the immunogens developed to date possess the proper antigenic structure, the epitopes themselves appear to be poorly immunogenic, and we have not yet replicated the immunogenic structure required to elicit bNAbs.

A number of different strategies are being pursued to improve recombinant Env immunogenicity. One approach involves immunization with properly folded and

glycosylated fragments of Env proteins (Chung et al., 2012; Hoffenberg et al., 2013; Morales et al., 2014a, 2016; Yu et al., 2012) in hopes of selectively stimulating the formation of bNAbs, while precluding the possibility of stimulating antibodies to immunodominant non-neutralizing epitopes. A second approach to this problem has been to employ guided immunization strategies, using a prime-boost series that reconstruct the ontogeny of bNAb evolution (Andrabi et al., 2018; Bonsignori et al., 2016; Escolano et al., 2016; Gao et al., 2014; Kwong and Mascola, 2018; Liao et al., 2013; Pancera et al., 2017). A third approach considers the possibility that Envs recovered from rare individuals with high levels of bNAbs, termed elite neutralizers (ENs), may be more effective in eliciting bNAbs than Envs from normal progressors that failed to produce bNAbs. However, the task of antigen selection from ENs is complicated by the fact that bNAbs are typically detected 2 years or more after infection (Gray et al., 2011; Landais et al., 2016). By this time, Env sequences have diversified considerably and most circulating plasma viruses are enriched for neutralization resistant variants (Albert et al., 1990; Wei et al., 2003). Thus, finding the precursor envelope sequences likely to have elicited bNAbs in an EN is a formidable challenge.

Here, we consider whether Env proteins closely resembling those that stimulated the formation of bNAbs can be recovered from rare individuals where virus evolution has been restricted or slowed, but still possess bNAbs. Previous studies have reported that virus evolution is considerably limited in individuals termed elite suppressors (ES) that are defined by their ability to limit virus replication

without the need for antiretroviral drugs (Bailey et al., 2006; Bello et al., 2007, 2004; Casado et al., 2010; Lassen et al., 2009; Roy et al., 2017; Sandonís et al., 2009; Scutari et al., 2018; Smith et al., 2013; Wang et al., 2003). Less than 1% of HIV-positive individuals possess the ES phenotype, meaning their viral load is less than detectable levels, i.e., <50 or <75 RNA copies/ml, for more than a year without anti-retroviral treatment (Okulicz et al., 2009). The ability to control viral load correlates with improved disease outcomes but the mechanism remains unclear. ESs exhibit heterogeneous immune responses but virus-specific CD8+ T cell responses appear to be a dominant feature (Lassen et al., 2009; Leitman et al., 2017a, 2017b; Pereyra et al., 2014, 2008; Walker and Yu, 2013). ESs typically have viral populations with both limited genetic diversity and lower replication rates than normal progressors, including ENs (Bailey et al., 2006; Bello et al., 2007, 2004; Casado et al., 2010; Lassen et al., 2009; Roy et al., 2017; Sandonís et al., 2009; Scutari et al., 2018; Smith et al., 2013; Wang et al., 2003). In addition, HIV in ESs may be constrained due to their fitness landscape, where mutational escape is limited due to the high fitness cost of mutations (Bailey et al., 2006; Brumme et al., 2011; Fernàndez et al., 2007; Lassen et al., 2009; Lobritz et al., 2011; Miura et al., 2009; Mwimanzi et al., 2013; Zaunders et al., 2011). Although most ESs fail to produce bNAbs, rare individuals possess bNAbs with neutralization breadth and exhibit the ES phenotype (bNAbs/ES phenotype). The restrictions on virus evolution in these individuals raised the possibility that Envs from individuals with the bNAb/ES phenotype may be more closely related to those that elicited the production of bNAbs than normal progressors

with the EN phenotype. Therefore, we wondered if Env proteins from ESs that produce bNAbs might be enriched for unusual structural features, possibly related to epitope stabilization, antigen processing, or antigen presentation, that may have enhance immunogenicity leading to the formation of bNAbs. Here, we describe the recovery of 20 functional proviral sequences isolated from an individual who is an elite suppressor (viral load <75 HIV RNA copies/ml for over 4 years) and possesses bNAbs. We show that the majority of these sequences possess an unusual number of cysteines that may form an additional disulfide bond.

Materials and Methods

Clinical Specimens

Twenty-nine blinded archival plasma specimens (0.5–2.0 ml) from anti-retroviral therapy (ART) naïve men and women were obtained from the SCOPE study cohort (University of California, San Francisco, San Francisco, CA). Twenty-five of the specimens were from individuals previously identified as elite supressors (<75 HIV RNA copies/ml for 12+ months without ART). Four specimens were from normal progressors. Specimens were collected according to an Institutional Review Board (IRB) approved protocol for which study participants provided written consent and were seen at regular intervals. At each visit, subjects took a confidential questionnaire, were invited to participate in a medical exam for additional studies, and a blood sample was taken. After screening plasma for virus neutralizing antibodies (described below) an archival contemporaneous sample of non-viable

PBMCs was obtained from an individual (designated as "EN3") with the dual ES and broadly neutralizing antibody phenotype for sequence analysis.

Samples from a separate cohort, which included patient "EN1," were collected by a physician under an Institutional Review Board (IRB) approved protocol from volunteers attending a regional center for recruitment in the San Francisco Bay Area. Inclusion criteria stipulated HIV-positive men and women 18–65 years of age, HIV ELISA or Western Blot positive for at least 1 year prior to screening, and who have never received anti-retroviral therapy (including post-exposure prophylaxis). An initial 10 mL of blood was collected into two EDTA tubes and plasma were aliquoted into cryovials and tested for bNAbs (described below). Four individuals with high titers of bNAbs were asked to participate in a 500 mL blood draw in a clinical setting. The blood was processed by a commercial laboratory using standard techniques and the PBMCs and plasma were separated, aliquoted, and cryopreserved under conditions that preserved plasma virus RNA and cell associated provirus DNA. The specimens were stored at −80°C until further analysis.

Screen for Neutralization Breadth

Plasma samples were tested for virus neutralizing activity at Monogram Biosciences (South San Francisco, CA) in a standard pseudotype virus neutralization assay (PhenoSense®) using a panel of either 22 or 26 international isolates widely used in HIV vaccine research. Briefly, pseudotype viruses were prepared by cotransfecting HEK293 cells (American Type Culture Collection [ATCC], Manassas, VA) with an

Env expression vector and an Env-deficient HIV-1 genomic vector carrying a luciferase reporter gene. Serial dilutions of mAbs or plasmas/sera were incubated with pseudotype viruses for 1 h prior to addition of U87 cells expressing CD4, CCR5, and/or CXCR4. The Z23 serum was used at an initial dilution of 1/100 and was included as an internal control in all experiments. Neutralization data are reported as 50% inhibitory dilutions (ID50s) for serum or plasma calculated from 5 point serum dilution curves. The virus controls included pseudoviruses prepared from the neutralization-sensitive HIV-1 isolate NL4-3 and the less neutralization-sensitive primary isolate JRCSF. The negative-control virus consisted of pseudotype viruses prepared from the envelope of the amphotropic murine leukemia virus (aMLV). HIV-1 neutralization titers were considered significant only if they were >3 times higher than the aMLV titers. The neutralization assays were performed according to good laboratory practice (GLP) and using protocols approved under Clinical Laboratory Improvement Amendments (CLIA). Each assay included acceptability criteria to ensure that inter-assay variation between ID50s, measured with reference standards, fell within 2.5-fold 95% of the time (Petropoulos et al., 2000).

Recovery of Env Sequences and Tropism Assay

An attempt was made to recover virus sequences from plasma and PBMCs from EN3. Due to the low copy number, the PhenoSense® assay system of Monogram Biosciences was unable to recover full-length functional clones of HIV envelope genes from plasma. However, full-length functional clones of HIV envelope genes

were recovered from provirus DNA in PBMCs. A unique feature of Monogram Biosciences' assay system is a selection step for functional Envs that eliminates defective and noninfectious envelope sequences common in proviral specimens. Viral tropism was determined using the Trofile® DNA assay system from Monogram Biosciences. The DNA sequences of the resulting clones were determined by Sanger chain termination sequencing and analyzed for genetic clade using the Recombinant Identification Program (RIP) HIV clade assignment tool (Siepel et al., 1995).

Phylogenetic Analyses

The gp160 sequences were aligned in Geneious v5.6.7 (Kearse et al., 2012) using the MUSCLE algorithm (Edgar, 2004). The sequence of the JRCSF isolate of HIV was designated as the outgroup. The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000). The tree with the highest log likelihood (−8098.9704) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.3273)]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 32 proviral nucleotide sequences (20 from EN3, 11 from EN1, an elite neutralizer with normal progression,

and JRCSF). All positions with <95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 2505 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

Expression of Recombinant gp120

Codon-optimized gene sequences were used for the expression of gp120. Synthetic gp120 genes (Invitrogen Inc., Waltham, MA) were inserted into an in-house plasmid expression vector (pCF1) containing a CMV promoter using standard techniques. PCR site-directed mutagenesis was performed to create variants of gp120 using a standard protocol for Gibson Assembly (New England Biolabs, Inc, Ipswich, MA). Recombinant plasmid sequences were confirmed by Sanger sequencing (University of California Sequencing Facility, Berkeley, CA). DNA was extracted and purified with a maxiprep kit (Qiagen, Redwood City, CA). For gp120 expression studies, envelope genes were transiently transfected into a HEK293 cell variant lacking the enzyme N-acetylglucosaminyltransferase I (HEK293S GnTI-, ATCC® CRL-3022TM)(Morales et al., 2014a). The cells were transfected using polyethylenimine, grown in serum free cell culture medium (FreeStyle TM 293F; Invitrogen, Inc., Waltham, MA), and harvested after 5 days of growth. The gp120 proteins were expressed as fusion proteins that possessed an N-terminal flag epitope of 27 amino acids from herpes simplex virus 1 glycoprotein D (gD-1) as described previously (Berman et al., 1999; Rerks-Ngarm et al., 2009).

Immunoblots

Growth conditioned cell culture supernatants with and without 25 mM DTT were analyzed for identity and molecular mass on 8–12% NuPage SDS-PAGE gel in MES running buffer and transferred to a PDVF membrane using standard protocol for iBlot (ThermoFisher Scientific, Waltham, MA). Membrane was blocked in 5% milk overnight on shaker at room temperature then washed 3 times with phosphate buffer saline with 0.01% Tween 20 (PBST; Sigma-Aldrich, St. Louis, MO) for 10 min. Membrane was probed with 5 μg/ml rabbit polyclonal anti-rgp120 antibody from previous immunization study (PB94)(Berman et al., 1999) in 5% milk for 2 h on shaker at room temperature, washed, then probed with 1:5,000 dilution of HRP-conjugated anti-rabbit secondary antibody (Jackson ImmunoResearch, West Grove, PA) in 5% milk for 2 h on shaker at room temperature and washed again. Antibody was detected using WesternBright reagents (Advansta, Menlo Park, CA) and visualized using an Innotech FluoChem2 system (Genetic Technologies Grover, MO).

Antibody Binding Assays

A fluorescent immunoassay (FIA) was used to measure antibody binding. Briefly, 96 well plates (Greiner, Bio-One, USA) were coated with 60 μl per well of 2 μg/ml of mouse anti-gD antibody (34.1) in phosphate buffer saline (PBS) overnight. Plates were blocked by adding 100 μl per well of 1% bovine serum albumin (BSA) in PBS on a shaker for 2 h at room temperature. Plates were then washed four times with 100

µl per well of PBST. Sixty microliter of supernatant was added and incubated overnight at 4°C then washed again. Serial 1:3 dilutions of monoclonal human antibody in PBS were added to each well and incubated for 90 min on a rocker at room temperature then washed again. Plates were then probed with 100 µl per well of Alexa Fluor 488 goat anti-human IgG secondary antibody (ThermoFisher Scientific, Waltham, MA) at a 1:5,000 dilution in 1% BSA PBS for 90 min on a rocker at room temperature then washed again. Antibodies were detected by adding 50 µl per well of PBS and visualizing at 495 nm. FIA was performed in triplicate.

Virus Neutralization Assays

Envelope gene sequences recovered from provirus clones were chemically synthesized *de novo*. Synthetic wild type (WT) non-codon-optimized gp160 gene sequences (Invitrogen Inc., Waltham, MA) were inserted into an in-house plasmid expression vector (pCF1) containing a CMV promoter using standard techniques. PCR site-directed mutagenesis was performed to create variants of gp160 using a standard protocol for Gibson Assembly (New England Biolabs, Inc, Ipswich, MA). Recombinant plasmid sequences were confirmed by Sanger sequencing (University of California Sequencing Facility, Berkeley, CA). DNA was extracted and purified with a maxiprep kit (Qiagen, Redwood City, CA). The plasmids were transferred to Monogram Biosciences (South San Francisco, CA) for testing in a pseudotype virus neutralization assay (PhenoSense®). The pseudotype viruses were tested for sensitivity and resistance to neutralization by antibodies in autologous

contemporaneous plasma, as well as a panel of broadly neutralizing antibodies provided by the NIH AIDS Reagent Program, Polymun Scientific (Vienna, Austria), and Dr. Dennis Burton (Scripps Clinic and Research Institute, La Jolla, CA). The neutralizing antibody titer (IC50) for monoclonal antibodies is defined as the concentration of purified mAb (μg/L) that produces a 50% reduction in target cell infection.

Results

Identification of a Treatment-Naïve Elite Suppressor With Neutralization Breadth

Twenty-nine plasma specimens from ART-naïve individuals were obtained from the SCOPE Study cohort (University of California, San Francisco) and screened for neutralization breadth with a panel of 22 international virus isolates from five different genetic clades (Table 1.1). Twenty-five of the plasma specimens were from individuals previously identified as elite supressors (ESs) and four specimens were from normal progressors. One specimen from a normal progressor (Z23) from a different cohort previously identified as possessing broad and potent neutralizing antibodies was used as a positive control. The panel included 5 viruses reported by Simek et al. (Simek et al., 2009) previously used to screen for elite neutralizers. Two specimens isolated from elite suppressors, EN2 and EN3, were effective in neutralizing 17 or 18 of the 22 viruses in the panel (respectively). Based on this result and the availability of clinical specimens, we selected one individual (EN3) for

further study. EN3 is an African American woman who was 47 years old and HCV negative at the time of collection. Clinical data showed EN3 possessed < 75 HIV RNA copies/ml for over 4 years. Plasma from EN3 was tested against an expanded panel of viruses and was capable of neutralizing 69% of viruses tested from clades A, B, C, D, and AE, with some ID50 titers above 300 (Table 1.2).

	Virus	Clade	ID50 (1/dilution)																													
			Elite Suppressors																									Normal Progressors				Neg Ctrl	Pos Ctrl
			ES01	ES02	ES03	ES04	ES05	ES06	EN2	ES08	EN3	ES10	ES11	ES12	ES13	ES14	ES15	ES16	ES17	ES18	ES19	ES20	ES21	ES22	ES23	ES24	ES25	NP01	NP02	NP03	NP04	TB	Z23
Simek Panel	94UG103	A	<20	<20	26	22	<20	29	1,292	41	302	81	23	41	23	67	<20	111	<20	45	23	64	32	<20	30	60	92	82	99	41	41	22	222
	92BR020	B	144	106	<20	<20	<20	61	1,282	216	249	46	21	208	72	590	152	425	47	221	151	122	133	<20	251	<20	123	388	170	78	179	<20	347
	93IN905	C	55	41	<20	<20	<20	73	227	288	81	113	26	111	87	253	93	147	44	78	102	113	108	<20	82	136	421	299	98	136	59	<20	388
	M-C-026	C	30	<20	<20	26	<20	24	34	39	27	63	32	50	<20	68	<20	63	21	68	53	58	<20	<20	27	127	573	444	154	100	62	<20	242
	92TH021	AE	25	26	<20	24	<20	23	37	77	65	86	32	43	39	106	74	51	<20	43	30	55	88	<20	44	38	148	76	34	71	32	<20	230
	M-A-002	A	46	21	<20	<20	<20	<20	25	26	92	51	<20	22	<20	29	<20	22	<20	27	<20	41	25	<20	<20	26	86	75	30	34	<20	27	<100
	M-A-006	A	21	21	<20	23	<20	27	92	32	505	36	25	25	<20	42	23	49	<20	65	55	78	32	<20	39	52	54	406	48	38	36	<20	329
	SF162	B	438	20,489	25	29	123	6,290	16,506	37,146	541	3,484	136	21,922	3,468	54,261	12,511	8,361	6,499	8,622	966	4,189	5,885	<20	2,937	743	12,564	22,893	699	41,029	9,408	<20	24,534
	1196	B	84	100	<20	<20	<20	59	5,410	132	131	144	34	203	87	500	125	218	49	66	33	144	90	<20	52	24	182	230	582	255	207	<20	487
	TRO	B	22	25	<20	<20	<20	54	707	67	1,011	209	33	29	29	188	106	448	<20	616	<20	76	<20	<20	21	36	257	64	195	56	89	25	488
	JRFL	B	24	24	<20	20	<20	28	2,333	<20	178	91	31	48	80	108	66	67	<20	39	<20	79	29	<20	<20	<20	56	35	435	64	127	<20	661
	BG1168	B	<20	<20	<20	<20	<20	24	70	<20	45	80	39	20	<20	36	41	30	<20	96	<20	75	<20	<20	27	<20	64	78	120	46	60	<20	163
	QHO692	B	28	23	<20	<20	<20	37	448	<20	71	127	31	29	24	144	40	76	<20	<20	<20	34	<20	<20	<20	<20	77	53	108	34	45	<20	187
	REJO	B	33	32	<20	<20	<20	51	171	110	178	87	28	53	30	177	133	129	23	39	<20	53	32	<20	37	32	246	62	141	59	101	<20	607
	M-SC-B-006	B	<20	<20	<20	<20	<20	<20	96	<20	164	66	36	28	<20	<20	<20	81	<20	76	<20	75	<20	<20	<20	27	20	57	140	73	31	32	153
	APV-16	B	39	21	<20	<20	<20	43	92	45	443	98	31	32	23	135	40	64	<20	<20	<20	51	70	<20	34	80	159	65	135	90	77	<20	826
	M-Chr-B-013	B	26	<20	21	23	<20	22	44	21	123	84	38	34	<20	22	<20	50	<20	21	<20	31	<20	<20	21	29	<20	64	112	54	51	31	297
	PVO	B	<20	<20	<20	<20	<20	<20	2,875	33	403	66	30	23	<20	22	20	46	<20	<20	<20	43	<20	<20	40	<20	42	47	88	64	29	<20	456
	M-C-003	C	38	<20	<20	<20	<20	<20	<20	26	45	29	<20	<20	<20	35	20	22	<20	21	<20	29	27	<20	<20	<20	<20	32	40	<20	22	<20	<100
	M-C-020	C	22	34	26	32	<20	51	2,398	46	22	73	35	56	24	52	33	52	<20	26	31	40	35	<20	33	<20	46	75	25	77	42	23	<100
	M-D-006	D	<20	<20	<20	<20	<20	<20	793	<20	282	51	<20	<20	<20	35	<20	73	<20	<20	<20	28	<20	<20	<20	<20	35	41	<20	28	25	<20	240
	M-D-009	D	144	107	<20	<20	<20	40	664	175	170	286	22	59	117	372	174	195	49	86	145	115	137	<20	49	76	178	157	106	144	202	<20	250
Controls	aMLV (neg.)	N/A	<20	<20	<20	<20	<20	<20	<20	<20	<20	23	<20	<20	<20	<20	<20	24	<20	<20	<20	20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<100
	JRCSF (pos.)	B	<20	68	<20	24	<20	33	3,149	71	556	123	28	111	29	161	79	229	<20	65	39	110	115	<20	67	76	165	260	344	82	46	<20	610
	NL43 (pos.)	C	1,722	1,354	27	<20	47	856	2,109	3,305	9,908	231	48	1,269	1,764	5,241	2,650	1,114	1,057	2,485	2,195	1,249	1,368	<20	722	1,164	611	2,081	2,509	3,465	1,678	<20	4,795

Table 1.1. Screening of 25 Elite Suppressors and 4 Normal Progressors for Neutralization Breadth.

Patient samples exhibiting control of virus replication without anti-retroviral therapy were obtained from the SCOPE cohort (University of California, San Francisco) and were screened using Monogram Biosciences' PhenoSense® neutralization assay for breadth against a panel of 22 internationally recognized viruses. Each assay included acceptability criteria to ensure that inter-assay variation between IC50s, measured with reference standards, fell within 2.5-fold 95% of the time. The neutralizing antibody titer (IC50) is defined as the reciprocal of the plasma dilution that produces a 50% inhibition in target cell infection. Values in grey represent neutralization titers that are at least three times greater than those observed against the negative control (aMLV). The clade B NL4-3 and JRCSF viruses were included as CXCR4- and CCR5-dependent positive controls, respectively. Z23 is a reference serum possessing broadly neutralizing antibodies.

Table 1.2. Expanded pseudovirus panel to define neutralization breadth in elite suppressor (EN3).

	Virus	Clade	ID50 (1/dilution)	
			EN3 plasma	Positive control (Z23)
Simek panel	94UG103	A	**439**	**112**
	92BR020	B	**268**	**226**
	93IN905	C	168	**253**
	M-C-026	C	84	**234**
	92TH021	AE	128	**157**
	JRCSF (Pos. Control)	B	**444**	**225**
	NL43 (Pos. Control)	B	**3,637**	**1,288**
	aMLV (Neg. Control)	N/A	69	<100
Expanded panel	92RW008	A	**728**	**636**
	92RW020	A	**419**	**414**
	M-A-002	A	**102**	**198**
	M-A-009	A	**378**	**308**
	6535.3	B	26	**359**
	Bal	B	**145**	**726**
	MN	B	**790**	**12797**
	PV04	B	**344**	**319**
	REJO	B	**294**	**397**
	TRO.11	B	**660**	**306**
	98IN022	C	51	**207**
	M-C-020	C	40	**103**
	TV1	C	36	<100
	92UG005	D	**247**	**396**
	94UG114	D	**129**	**162**
	M-D-006	D	**412**	**441**
	M-D-009	D	48	**270**
	3017_E10_113035_081	AE	**1,763**	**372**
	3178_E10_142902_080	AE	**298**	**493**
	CM244/A244	AE	**68**	**526**
	TH023	AE	**93**	**262**
	JRCSF (Pos. Control)	B	**208**	**217**
	NL43 (Pos. Control)	B	**2,734**	**1,137**
	aMLV (Neg. Control)	N/A	<20	<100

EN3, an elite suppressor, was screened using Monogram Biosciences' PhenoSense® assay for neutralization breadth against a panel of 26 viruses. Each assay included acceptability criteria to ensure that interassay variation between ID50s, measured with reference standards, fell within 2.5-fold 95% of the time. The neutralizing antibody titer (ID50) is defined as the reciprocal of the plasma dilution that produces a 50% inhibition in target cell infection. Values in bold represent neutralization titers that are at least three times greater than those observed against the negative control (aMLV). The clade B NL4-3 and JRCSF viruses were included as CXCR4- and CCR5-dependent positive controls, respectively. Z23 is a reference serum possessing broadly neutralizing antibodies.

Phylogenetic Analysis of an Elite Suppressor and a Normal Progressor Illustrates Significant Difference in Genetic Variation

Phylogenetic analysis compared proviral sequences isolated from the elite suppressor, EN3, and a normal progressor, EN1, which both possessed broadly neutralizing antibodies. These were analyzed with MEGA6 to generate a maximum likelihood tree using the General Time Reversible (Tamura et al., 2013) model with 1,000 bootstrap replicates. EN3 sequences had less intra-patient genetic variation compared to proviral sequences from EN1, who showed similar neutralization breadth (Fig 1.1). EN3 had 126 (4.8%) sites with polymorphisms whereas EN1 had 396 (14.8%) sites with polymorphisms. The result conformed to previous observations, reporting less genetic variation and slower viral evolution in an elite suppressor (EN3) compared to a normal progressor (EN1).

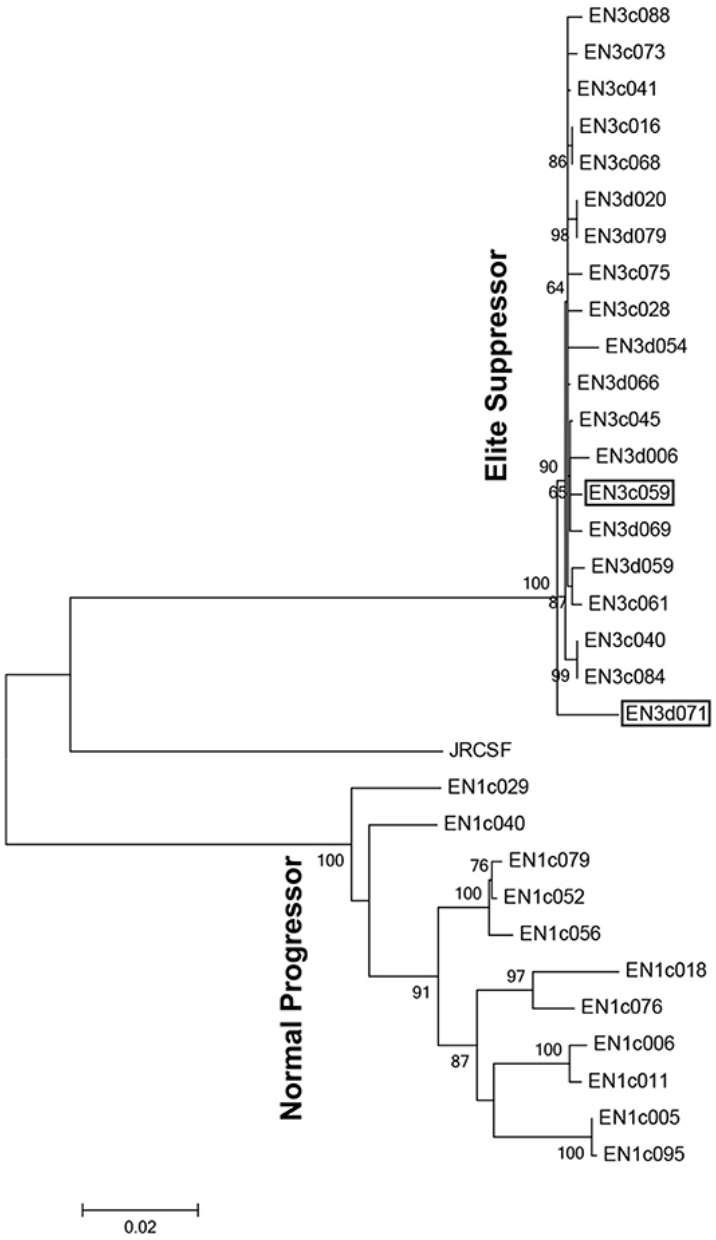

Fig 1.1. Phylogenetic analysis of proviral sequences isolated from an elite suppressor (EN3) and a normal progressor (EN1) that both possess bNAbs. Proviral sequences were recovered from two individuals (EN1 and EN3) using the Monogram PhenoSense® assay system. Evolutionary analyses were conducted in MEGA6 using a Maximum Likelihood method with 1000 bootstrap replicates and a discrete Gamma distribution to model evolutionary rate. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Sequences used in mutagenesis studies, EN3d071 and EN3c059, are shown with a black rectangle.

Characterization of Proviral gp160 Sequences Isolated From EN3

The physical characteristics of the gp160 sequences were analyzed for amino acid length, the number of cysteines, and location of potential N-linked glycosylation sites in each region (Table 1.3). Sequences were compared to the Los Alamos National Laboratory database ("HIV sequence database main page," n.d.) filtered for clade B subtype envelope sequences, with one sequence per individual. EN3 had a significantly longer V1 region (37–39 amino acids; Kruskal-Wallis test, $p < 0.0001$) and rare cysteines in the V1 hypervariable region (3–4 cysteines; Kruskal-Wallis test, $p < 0.0001$) compared to the dataset from LANL (average V1 length is 27 amino acids; average number of cysteines in V1 is 2). When the recovered sequences were aligned (Fig 1.2), all of the sequences with the exception of clone d071 possessed two additional N-linked glycosylation sites and two extra cysteines (Cys) in the V1 region. The extra Cys residues occurred at fixed locations (C134+1 and C136 using HXB2 numbering), resulting in a total of 20 Cys in the gp120 fragment compared to the normal 18 Cys typically found in the major (M) class of HIV envelope sequences. The EN3d071 Env possessed a Cys at position 134+1, like all of the other viruses from this individual, but had the polymorphism C136R, which is adjacent to a 15 amino acid insertion between HXB2 positions 134 and 135 (Fig 1.2).

Table 1.3. Physical characteristics of 20 functional proviral sequences isolated from EN3.

Region	HXB2 numbering	Length (aa)		PNGS		Cysteines	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Signal	1–30	29.0 ± 0.0	29–29	0.0 ± 0.0	0 – 0	1.0 ± 0.0	1–1
C1	31–130	100.0 ± 0.0	100–100	1.9 ± 0.2	1–2	4.0 ± 0.2	4–5
V1	131–157	**37.1 ± 0.4***	**37–39***	4.0 ± 0.0	4–4	**4.0 ± 0.2***	**3–4***
V2	158–196	37.0 ± 0.0	37–37	2.0 ± 0.0	2–2	1.0 ± 0.0	1–1
C2	197–295	99.0 ± 0.0	99–99	6.0 ± 0.0	6–6	5.0 ± 0.0	5–5
V3	296–331	35.0 ± 0.0	35–35	1.0 ± 0.0	1–1	2.0 ± 0.0	2–2
C3	332–384	53.0 ± 0.2	52–53	3.0 ± 0.0	3–3	1.0 ± 0.0	1–1
V4	385–418	38.0 ± 0.0	38–38	5.0 ± 0.3	4–6	2.0 ± 0.0	2–2
C4	419–459	41.0 ± 0.0	41–41	1.0 ± 0.0	1–1	1.0 ± 0.0	1–1
V5	460–469	11.0 ± 0.0	11–11	1.0 ± 0.0	1–1	0.0 ± 0.0	0–0
C5	470–511	42.0 ± 0.0	42–42	0.0 ± 0.0	0–0	0.0 ± 0.0	0–0
gp41 extracellular	512–678	167.0 ± 0.0	167–167	3.0 ± 0.0	3–3	2.0 ± 0.0	2–2
gp41 transmembrane	679–699	21.0 ± 0.0	21–21	0.0 ± 0.0	0–0	0.0 ± 0.0	0–0
gp41 cytoplasmic tail	700–856	157.0 ± 0.0	157–157	1.0 ± 0.0	1–1	1.0 ± 0.0	1–1
gp120	31–511	493.1 ± 0.2	493–494	24.9 ± 0.4	24–26	20.0 ± 0.3	19–21
gp41	512–856	345.0 ± 0.0	345–345	4.0 ± 0.0	4–4	3.0 ± 0.0	3–3
gp160	31–856	838.0 ± 0.2	838–839	28.9 ± 0.4	28–30	23.0 ± 0.3	22–24

The number of amino acids, potential N-linked glycosylations sites, and cysteines in each region were compared to clade B sequences (Los Alamos National Laboratory HIV database). As indicated in bold, EN3 had a significantly longer V1 region (37–39 amino acids) and had rare cysteines in the V1 hypervariable region (3–4 cysteines) compared to the dataset from LANL (average V1 length is 27 amino acids; average number of cysteines in V1 is 2).

**$p < 0.0001$ for the Kruskal-Wallis test comparing features to the Los Alamos National Laboratory dataset.*

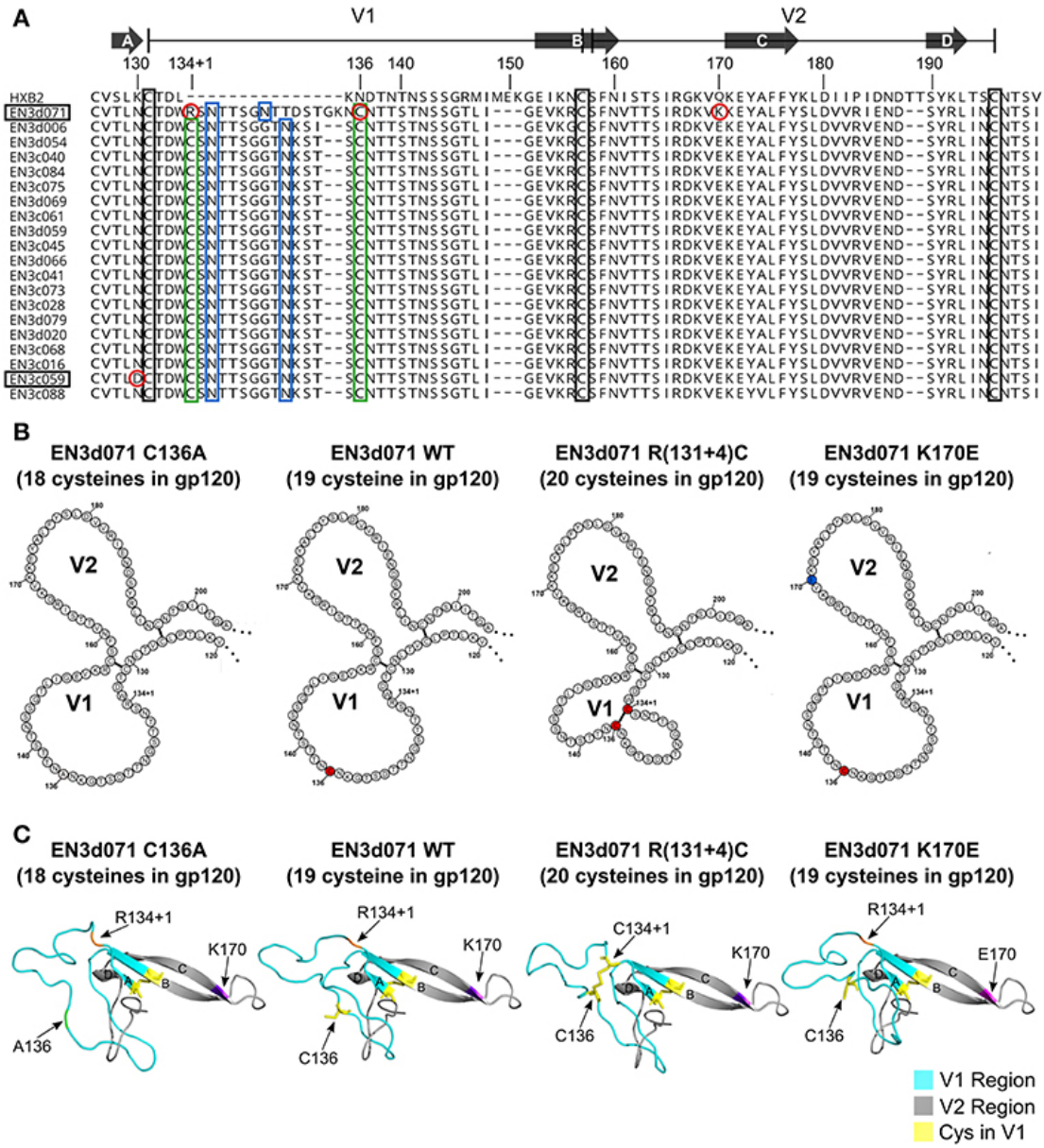
A
V1
V2
A
B
C
D
130
134+1
136
140
150
160
170
180
190
HXB2
EN3d071
EN3d006
EN3d054
EN3c040
EN3c084
EN3c075
EN3d069
EN3c061
EN3d059
EN3c045
EN3d066
EN3c041
EN3c073
EN3c028
EN3d079
EN3d020
EN3c068
EN3c016
EN3c059
EN3c088
B
EN3d071 C136A
(18 cysteines in gp120)
EN3d071 WT
(19 cysteine in gp120)
EN3d071 R(131+4)C
(20 cysteines in gp120)
EN3d071 K170E
(19 cysteines in gp120)
V2
V1
C
EN3d071 C136A
(18 cysteines in gp120)
EN3d071 WT
(19 cysteine in gp120)
EN3d071 R(131+4)C
(20 cysteines in gp120)
EN3d071 K170E
(19 cysteines in gp120)
R134+1
K170
A136
C136
C134+1
E170
V1 Region
V2 Region
Cys in V1

Fig 1.2. V1/V2 regions of proviral sequences isolated from an elite suppressor (EN3).
(A) Alignment of functional proviral sequences isolated from an elite suppressor (EN3). V1 region is defined as amino acids 131–157 and V2 region is defined as amino acids 158–196 using sequence numbering relative to HXB2 reference standard. Arrows above the alignment show the location of four beta strands described in McLellan et al. (McLellan et al., 2011). Black rectangles show the location of canonical cysteines in the V1/V2 region. Green rectangles show aberrant cysteines in functional sequences isolated from EN3. Blue rectangles show potential N-linked glycosylation sites that are part of the 13 or 15 amino acid insertion in V1. Residues circled in red were targeted in mutagenesis experiments. **(B)** Primary structure of EN3d071 wild type (WT) and mutants. Single point mutations of EN3d07 WT were introduced to create three different mutants of Env (EN3d071 C136A with no extra cysteines in V1, EN3d071 R(134+1)C with two extra cysteines in V1, and K170E with one extra cysteine in V1). Non-canonical cysteines are shown in red. K170E mutation is shown in blue. **(C)** Homology models of gp120s EN3d071 wild type (WT) and mutants. Homology models of EN3d071 C136A (18 cysteines), EN3d071 WT (19 cysteines), EN3d071 R(134+1)C (20 cysteines), and EN3d071 K170E (19 cysteines) were built using Modeller v9.21 with 5fykG template. V1 regions are shown in cyan. V2 regions are shown in gray. Cysteines located in the V1 region are shown in yellow. Polymorphisms are labeled with A136 shown in green, R(134+1) shown in orange, K170 shown in purple, and E170 shown in magenta.

Sensitivity to Neutralization by Polyclonal Antibodies in Autologous Plasma and Broadly Neutralizing Monoclonal Antibodies (bN-mAbs)

Pseudotype viruses representing the 20 envelope clones from EN3 were constructed and tested for sensitivity to neutralization by contemporaneous autologous plasma and by a panel of prototypic bN-mAbs (Table 1.4). We observed that all viruses were sensitive to neutralization by autologous plasma (range 1:322–1:53,788) with a mean neutralization titer of 1:3,376. However, one clone, EN3c059, was exceedingly sensitive to neutralization by autologous plasma with a neutralization titer of 1:53,788. We observed that this clone was also particularly sensitive to neutralization by the positive control Z23.

Table 1.4. EN3 pseudotype virus neutralization titers from autologous sera and a panel of bN-mAbs.

Proviral clones	ID50 (1/dil'n)	IC50 (ug/ml)								ID50 (1/dil'n)
	Autologous sera	PG9	PG16	PGT145	PGT121	PGT128	VRC01	4E10	35022	Positive control (Z23)
EN3c016	**525**	>25	>25	**3.5588**	>25	**0.0255**	**1.7871**	>25	>25	**296**
EN3c028	**534**	>25	>25	**3.4674**	>25	**0.0277**	**1.6674**	**17.3799**	>25	**400**
EN3c040	**322**	>25	>25	**5.0631**	>25	**0.0277**	>25	**13.9435**	>25	**212**
EN3c041	**500**	>25	>25	**3.1332**	>25	**0.0219**	**1.3681**	**23.1582**	>25	**303**
EN3c045	**881**	>25	>25	**0.9347**	>25	**0.0158**	**0.5813**	**2.627**	**3.585**	**1026**
EN3c059	**53788**	>25	>25	>25	>25	**0.1172**	**21.4415**	**0.5471**	>25	**90980**
EN3c061	**1151**	>25	>25	**1.2681**	>25	**0.0143**	**0.6382**	**2.6453**	**0.0058**	**1171**
EN3c068	**479**	>25	>25	**2.5685**	>25	**0.024**	**2.0384**	>25	>25	**446**
EN3c073	**600**	>25	>25	**6.194**	>25	**0.0257**	**1.3944**	**12.2846**	>25	**503**
EN3c075	**392**	>25	>25	**3.1324**	>25	**0.0289**	**2.005**	>25	>25	**335**
EN3c084	**426**	>25	>25	**4.7513**	>25	**0.0327**	>25	**11.5505**	>25	**230**
EN3c088	**513**	>25	>25	**6.7844**	>25	**0.0315**	**1.3092**	**15.1361**	>25	**393**
EN3d006	**509**	>25	>25	>25	>25	**0.0182**	**1.0039**	**22.4744**	>25	**437**
EN3d020	**574**	>25	>25	**3.2216**	>25	**0.0313**	**2.0042**	**16.9443**	>25	**336**
EN3d054	**1647**	>25	>25	**1.248**	>25	**0.0473**	**0.9561**	**3.4162**	>25	**611**
EN3d059	**1787**	>25	>25	**13.606**	>25	**0.0241**	**2.8993**	**11.2629**	**7.5214**	**1598**
EN3d066	**545**	>25	>25	**2.0665**	>25	**0.0186**	**0.3923**	>25	>25	**309**
EN3d069	**949**	>25	>25	**1.5781**	>25	**0.0182**	**0.8521**	**2.8472**	**23.4584**	**1152**
EN3d071	**886**	**3.5967**	**0.3081**	**0.1229**	>25	**0.0095**	**7.4507**	>25	**0.0038**	**323**
EN3d079	**510**	>25	>25	**2.8251**	>25	**0.0277**	**2.2708**	**9.4933**	>25	**317**

Pseudotype viruses for each EN3 sequence were tested against autologous sera and a panel of bn-mAbs with Monogram Biosciences' PhenoSense® assay. Each assay included acceptability criteria to ensure that interassay variation between ID50s, measured with reference standards, fell within 2.5-fold 95% of the time. The neutralizing antibody titer (ID50) for autologous sera and Z23 (a reference serum possessing broadly neutralizing antibodies) is defined as the reciprocal of the plasma dilution that produces a 50% inhibition in target cell infection. The neutralizing antibody titer (IC50) for monoclonal antibodies is defined as the concentration of purified mAb (μg/ml) that produces a 50% reduction in target cell infection. Values in bold indicated neutralization sensitivity defined as ID50 values that are at least three times greater than those observed against the negative control (aMLV) or IC50 values that are <25 μg/ml. EN3c059 and EN3d071 were selected for further study and are shown in gray.

When we examined the sensitivity of the EN3 viruses to prototypic bN-mAbs, we found that all but clone EN3d071 were resistant to neutralization by the PG9 and PG16 broadly neutralizing monoclonal antibodies (bN-mAbs) known to bind to glycan-dependent epitopes in the V1/V2 domain (McLellan et al., 2011). When we examined the sensitivity of these Envs to bN-mAbs that recognized glycan-dependent epitopes at the base of the V3 domain (Pejchal et al., 2011), we found that all of the clones were sensitive to neutralization by the PGT128 bN-mAb but all of the clones were resistant to neutralization by the PGT121 bN-mAb. We found that 18 of 20 clones were sensitive to neutralization by the VRC01 bN-mAb specific for the CD4-

binding site, and the PGT145 bN-mAb that recognizes a trimer-specific epitope involving the V1/V2 domain. In addition, 15 of 20 clones were sensitive to neutralization by the 4E10 bN-mAb specific for the membrane proximal external domain (MPER). Finally, 5 of 20 clones were sensitive to the 35022 bN-mAb specific to the gp120 and gp41 interface.

Effect of Extra Cysteine Residues and Polymorphisms at Positions 130 or 170 on Sensitivity to Neutralization by bN-mAbs

Because the EN3d071 clone was the only envelope with 19 Cys residues and the only virus that was sensitive to neutralization by the PG9 and PG16 bN-mAbs, we carried out mutagenesis studies to examine the effect of 18, 19, and 20 Cys residues on virus infectivity and sensitivity to neutralization. In addition to lacking C(134+1), EN3d071 is the only sequence that had a lysine (K) at position 170 where the other 19 sequences had a glutamic acid (E) at this position (Fig 1.2). Previous studies have suggested that K170 can be important for PG9 binding (Doria-Rose et al., 2012). To examine potential effects of the aberrant V1 cysteines and K170, we used site-directed mutagenesis to create three variants of EN3d071 gp160. These included: (1) the EN3d071 C136A variant with 18 cysteines in gp120, (2) the EN3d071 R(134+1)C with 20 cysteines in gp120, and (3) the EN3d071 K170E variant with 19 cysteines in gp120 (Fig 1.2). The wild type and mutated gp160 genes were then used to create pseudoviruses and tested for sensitivity to neutralization by a panel of bN-mAbs, including PG9, and autologous sera.

When expressed as pseudoviruses, we found that the wild type (WT) EN3d071 Env (19 Cys in gp120) and the EN3d071 C136A variant (18 Cys in gp120) retained infectivity and both possessed the CCR5 chemokine receptor (R5) tropic phenotype (Table 1.5). In contrast, the R(134+1)C variant (20 Cys in gp120) that contained two non-canonical cysteines was non-infectious. When the 19 Cys variant possessing the K170E variant was examined, we observed that it had reduced infectivity but still retained the R5 receptor phenotype.

Table 1.5. Infectivity of wild type and mutant EN3d071 pseudoviruses.

Proviral clones	Number of Cys in gp160	Tropism	R5 infectivity (Rank)	X4 infectivity (Rank)
EN3d071 WT	22	R5	2	0
EN3d071 R(134+1)C*	23	N/A	0	0
EN3d071 C136A	21	R5	2	0
EN3d071 K170E	22	R5	0.5	0

Rank to relative light units (RLU) Key

0 Rank < 5,000 RLU (no infectivity detected)
0.5 Rank < 5,000 RLU (infectivity detected)
1 Rank = 5,001 to 15,000 RLU
2 Rank = 15,001 to 150,000 RLU
3 Rank = 150,001 to 1,000,000 RLU
4 Rank = >1,000,000 RLU

Viral tropism was determined using the Trofile® DNA assay system from Monogram Biosciences. CD4+/U87 cells expressing CXCR4 or CCR5 coreceptor were inoculated with pseudoviruses. Infected cells produced luciferase. Tropism was determined by the relative light units (RLU) in lysed cells 72 h after inoculation.
**Infectivity too low for use in the neutralization assay.*

We next examined the sensitivity to neutralization by autologous plasma, and the panel of bN-mAbs directed to different epitopes (Table 1.6). The loss of the cysteine in EN3d071 C136A may have increased resistance to neutralization by VRC01 but the difference in IC50 values is within the margin of error. The K170E mutation caused resistance to neutralization by PG9 and PG16 and surprisingly increased sensitivity to 4E10. Neutralization sensitivity to the PGT145, PGT128, 35022 bN-mAbs, and autologous plasma was preserved in all testable variants.

Table 1.6. EN3 wild type and mutant pseudoviruses sensitivity to neutralization by bN-mAbs and autologous sera.

Proviral clone	Mutation	Number of Cys in gp160	ID50 (1/dil'n)	IC50 (μg/ml)								ID50 (1/dil'n)
			Autologous sera	PG9	PG16	PGT145	PGT121	PGT128	VRC01	4E10	35,022	Z23
EN3d071	None	22	**995**	**8.8474**	**0.1002**	**0.1687**	>25	**0.0071**	**21.3912**	>25	**0.0019**	**229**
	R(134+1)C*	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C136A	21	**754**	**10.9931**	**0.2234**	**0.2041**	>25	**0.0070**	>25	>25	**0.0030**	154
	K170E	22	**1,024**	>25	>25	**0.5077**	>25	**0.0041**	**11.0866**	**4.3028**	**0.0035**	**378**
EN3c059	None	23	**64,097**	>25	>25	>25	>25	**0.0983**	**20.385**	**0.1318**	**8.5338**	**55,331**
	D130N	23	**57,182**	>25	>25	**21.2259**	>25	**0.2095**	**23.6354**	**0.16**	**21.5527**	**56,567**

The neutralization sensitivity of EN3d071 wild type (WT) was compared to three variants with single point mutations. Pseudotype viruses for each EN3 sequence were tested against autologous sera and a panel of bn-mAbs with Monogram Biosciences' PhenoSense® assay. Each assay included acceptability criteria to ensure that interassay variation between measured with reference standards, fell within 2.5-fold 95% of the time. The neutralizing antibody titer (ID50) for autologous sera and Z23 (a reference serum possessing broadly neutralizing antibodies) is defined as the reciprocal of the plasma dilution that produces a 50% inhibition in target cell infection. The neutralizing antibody titer (IC50) for monoclonal antibodies is defined as the concentration of purified mAb (μg/ml) that produces a 50% reduction in target cell infection. Values in bold indicated neutralization sensitivity defined as ID50 values that are at least three times greater than those observed against the negative control (aMLV) or IC50 values that are <25 μg/ml.

**Infectivity too low for use in the neutralization assay.*

The EN3c059 clone was highly sensitive to neutralization by contemporaneous autologous sera (ID50 above 53000) and differed by only 3 point mutations, E32Q, N130D, and A533V from the EN3 consensus sequence. We wondered if the N130D polymorphism, which eliminates a potential N-linked glycosylation site, could account for the increased neutralization sensitivity of this variant. We used site-directed mutagenesis to express EN3c059 WT and a variant with the D130N mutation (EN3c059 D130N) and tested for sensitivity to neutralization by a panel of bN-mAbs and autologous sera. Surprisingly, we found that the D130N polymorphism did not affect sensitivity to neutralization by contemporaneous autologous sera (Table 1.6).

Effect of Extra Cysteine Residues and K170E on bN-mAb Binding to gp120

Additional studies were carried out to characterize bN-mAb binding to recombinant gp120s derived from EN3 envelope proteins. Because multiple bN-mAbs recognize epitopes that are dependent on mannose-5 (Man5) for binding, the Envs were expressed in HEK293 cells lacking N-acteylglucosaminyl transferase 1 (HEK293S GnTI- cells). The absence of N-acetylglucosaminyl transferase 1 disrupts the glycosylation pathway, resulting in the production of monomeric gp120s that contain a high mannose (Man5) glycan found on native HIV virions. The recombinant proteins predominately migrated as monomeric proteins and were not degraded by proteolysis often observed for Envs from clade B viruses (Fig 1.3). The EN3d071 R(134+1)C variant expressed well even though the pseudotype viruses made with this envelope were not infectious.

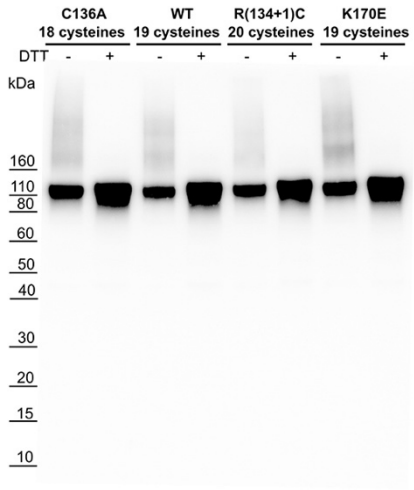

Fig 1.3. HEK293 GnTI- expression of recombinant gp120 EN3d071 WT and mutants.
Recombinant gp120 sequences EN3d071 C136A (18 cysteines), EN3d071 WT (19 cysteines), EN3d071 R(134+1)C (20 cysteines), and EN3d071 K170E (19 cysteines) were transiently transfected in HEK293 GnTI- cells. HEK293 GnTI- cells lack N-acetylglucosaminyltransferase 1, disrupting the glycosylation pathway and resulting in Man5 glycans which have a smaller molecular weight. Supernatant with and without 25 mM DTT was run on SDS-PAGE gel, transferred to a PDVF membrane, and probed with purified rabbit polyclonal anti-gp120 antibodies (PB94) from a previous immunization study. EN3d071 WT and mutants showed similar expression levels with a small amount of aggregation detected in unreduced samples.

A fluorescence immunoassay (FIA) was used to characterize the binding of a panel of bN-mAbs to immunoaffinity purified variants of the EN3d071 Env protein. We found that the envelopes with 18, 19, and 20 cysteines all bound to PG9, PGT128, and VRC01 and did not bind to PGT121 (Fig 1.4). They also did not bind to PG16, which was expected because the gp120s were monomeric and lacked the hybrid complex glycans required for PG16 binding (McLellan et al., 2011). Thus, all three proteins possessed the epitopes recognized by the PG9, PGT128, and VRC01 bN-mAbs but lacked the epitopes recognized by the PG16 and PGT121 bN-mAbs.

EN3 K170E had a similar binding profile but did not bind to PG9. This provides additional evidence that the inability of PG9 to neutralize 19 of the 20 Envs recovered from EN3 appear to be attributable the E170K polymorphism in the V2 region rather than the extra pair of Cys residues in the V1 region.

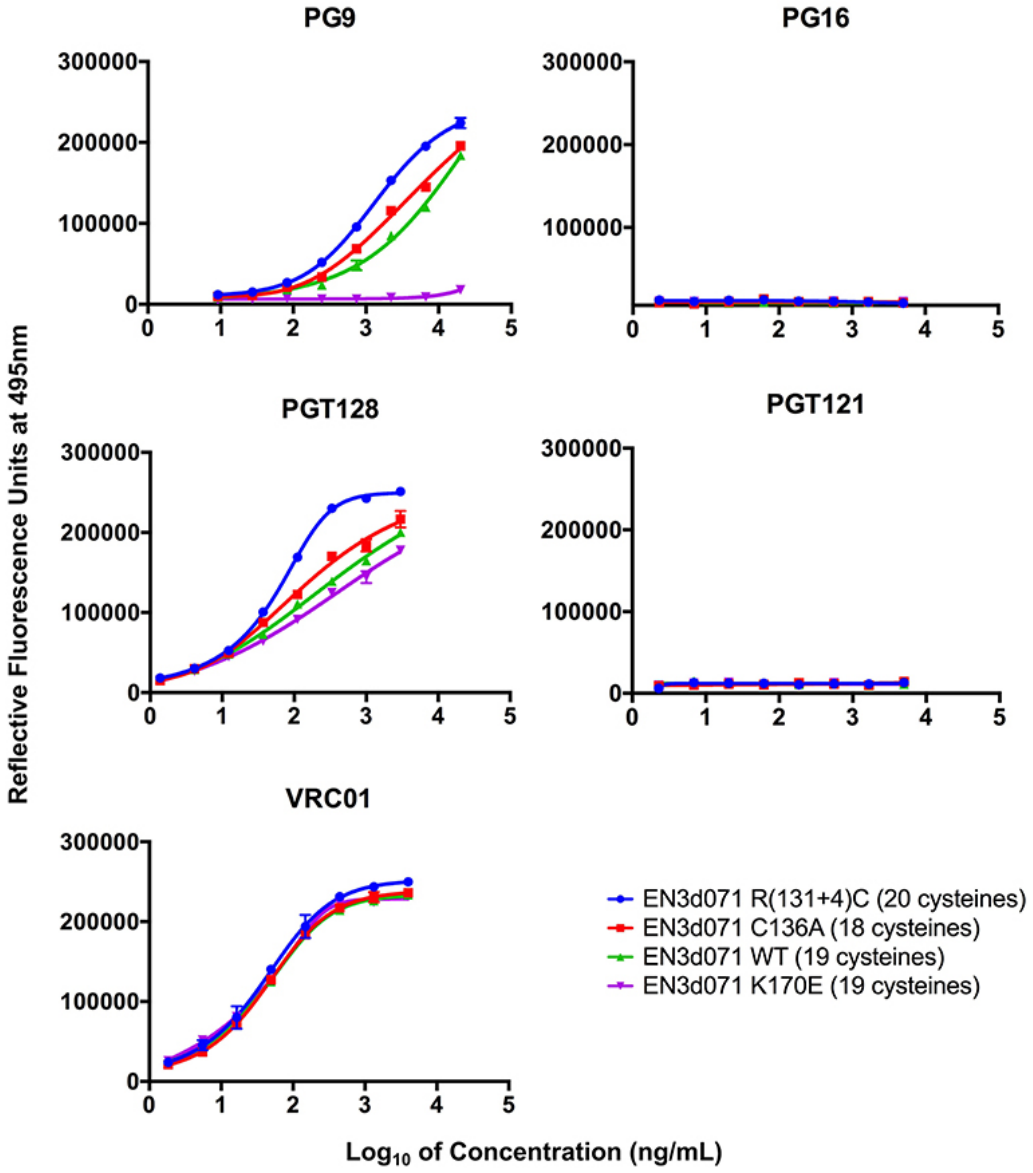

Fig 1.4. bN-mAb binding to recombinant gp120 EN3d071 WT and mutants by fluorescent immunoassay.
Recombinant gp120 sequences EN3d071 C136A (18 cysteines), EN3d071 WT (19 cysteines), EN3d071 R(134+1)C (20 cysteines), and EN3d071 K170E (19 cysteines) were produced with an N-terminal gD tag and captured onto microtiter plates with a mouse monoclonal anti-gD antibody. The captured proteins were then incubated with a panel of broadly neutralizing monoclonal antibodies that included PG9, PGT121, PGT128, and VRC01. After washing, the wells were incubated with Alexa Fluor 488 labeled goat anti-human IgG (ThermoFisher Scientific, Waltham, MA) and washed as described in Materials and Methods.

Discussion

In this paper, we have analyzed proviral Env sequences from a rare elite suppressor (EN3) with the bNAb/ES phenotype. As reported in previous studies (Bailey et al., 2006; Bello et al., 2007, 2004; Casado et al., 2010; Lassen et al., 2009; Roy et al., 2017; Sandonís et al., 2009; Scutari et al., 2018; Smith et al., 2013; Wang et al., 2003), we found that proviral sequences from EN3 were highly homogeneous compared to proviral sequences recovered from a normal progressor. This result supports the idea that virus evolution is slower in ENs due to a reduced rate of virus replication. Because of this slow rate of virus evolution, we reasoned that it might be easier to recover Envs more closely related to those that elicited bNAb from individuals with the bNAb/ES phenotype than from normal progressors that exhibit much higher levels of proviral Env sequence diversity. Moreover, we postulated that analysis of sequences from individuals with bNAb/ES phenotype might provide clues regarding structural features that enhance for the formation of bNAbs compared to Envs from normal progressors that exhibit higher levels of virus diversification. The possibility that HIV has evolved structural features to modulate the immunogenicity of epitopes recognized by bNAbs is well supported by the unusually high degree of N-linked glycosylation resulting in a "glycan shield" (Wei et al., 2003), indels (insertions and deletions) in variable regions (Pinter et al., 2004), and the observation that cleavage sites for antigen processing enzymes occur in close proximity to epitopes recognized by bNAbs (Yu et al., 2010).

In the present study, we found that Envs sequences recovered from EN3 were highly homogenous (99.1% pairwise identity) and 19 of 20 sequences displayed an unusual insertion in the V1 region that added a pair of extra cysteine residues, which may form an extra disulfide bond, and two additional N-linked glycosylation sites. The V1 region has long been known to be highly immunogenic and has previously been termed the "global regulator" of neutralization sensitivity (Pinter et al., 2004). It is the first target of autologous neutralizing antibodies (Granados-Gonzalez et al., 2009; Rong et al., 2009, 2007; Walker et al., 2010). Evolution of glycosylation sites in the V1 region is thought to be a key factor in driving the evolution of bNAbs that normally occurs 2 years or more post infection (Chackerian et al., 1997; Frost et al., 2005; Moore et al., 2009; Rong et al., 2007; Sagar et al., 2006; Wei et al., 2003). Additionally, the V1 region is located at the apex of the virus spike and is in close proximity to several epitopes recognized by bNAbs (McLellan et al., 2011; Simek et al., 2009; Walker et al., 2009). Based on these observations, the three changes we have documented in the V1 region are all features of the type expected to affect either the antigenic structure or the immunogenicity of the Env protein.

In principle, 19 of 20 Envs from this individual could form 10 disulfide bridges compared to the 9 disulfide bridges found in most gp120 sequences. HIV-1 gp120 has 18 conserved cysteines that form 9 disulfide bridges present in all published crystal structures. Approximately 7% of clade B viruses from newly infected individuals also have 20 cysteines (Jobes et al., 2006) and 5.5% of clade B viruses have 2 extra cysteines in the variable 1 (V1) region (van den Kerkhof et al.,

2016). The EN3d071 sequence was unusual in that it possessed only 19 Cys and therefore contained an unpaired Cys that could only form 9 disulfide bridges. Free Cys residues are unusual in most secreted proteins and often lead to the formation of dimers or aggregates. The prevalence of these extra cysteines raised the possibility that EN3 envelope proteins might provide a functional advantage perhaps related to enhanced virus infectivity, neutralization escape, or increased immunogenicity. Additionally, they provided for the possibility of rearrangements of disulfide structures.

Additional N-linked glycosylation sites and insertions in the V1/V2 domain are well documented immune escape mechanisms known to prevent the binding of bNAbs (Chackerian et al., 1997; Frost et al., 2005; Moore et al., 2009; Rong et al., 2007; Sagar et al., 2006; Wei et al., 2003). Surprisingly, neither the extra glycosylation sites, the 13 amino acid insertion, nor the extra cysteines in the V1 region affected binding or sensitivity to neutralization by bN-mAbs PG9, PG16, PGT121, PGT128, PGT130, 35022, or 4E10. Therefore these changes may have evolved to serve another function, perhaps related to virus infectivity or stability. When this possibility was considered, it was interesting that the R(134+1)C variant of EN3d071, possessing 20 Cys residues, had little or no infectivity compared to the WT Env that possessed 19 Cys. All other clones possessing 20 Cys and the C(134+1) polymorphism were infectious, indicating epistatic mutations are required to compensate for the presence of the additional Cys. Similarly, the K170E variant of EN3d071 had reduced infectivity, despite the presence of E170 in all other clones.

Thus, epistatic mutations are also required to preserve infectivity for the K170E polymorphism. Two of the four point mutations altering amino acids to match the consensus sequence reduced infectivity, perhaps indicating the fitness landscape is restricted.

Although we did observe a mutation that improved sensitivity to neutralization by PG9 binding, this mutation occurred in only one virus clone and was distinct from the insertion in the V1 region found in the 19 other viruses. The glutamic acid (E) at position 170 accounts for the resistance to neutralization by the PG9 bN-mAb in all of the viruses except EN3d071 that possesses a lysine (Lys) at this position. Previous studies reported that the K168E, K169E, and K171E polymorphisms disrupt PG9 binding and cause resistance to neutralization by PG9. This is likely due to the change in charge on the "C" beta strand in the V2 region, which is part of the PG9 epitope (McLellan et al., 2011). It is consistent that K170E, located on the "C" beta strand, also disrupts binding and confers resistance to neutralization by PG9.

Based on these studies, the possibility remains that the insertion in the V1 region, the extra pair of cysteines, and the extra glycosylation sites may have contributed to the formation of bNAbs in EN3. It is important to note that the gp160 sequences studied here are a sample of the virus population taken at a single time point and may not include the actual viral sequences that stimulated the production of the bN-mAbs in contemporaneous plasma. However, variation within the 20 proviral sequences was minimal, supporting previous reports suggesting that viral evolution

may be slower in this elite suppressors (Bello et al., 2007, 2004; Casado et al., 2010; Okulicz et al., 2009; Pereyra et al., 2008; Roy et al., 2017; Sandonís et al., 2009; Scutari et al., 2018; Smith et al., 2013; Wang et al., 2003). These sequences may be more similar to their bN-mAb-inducing predecessor than viruses recovered from normal progressors, and thus better vaccine candidates. The envelopes described in this paper provide the basis for future immunization studies where the immunologic potential of individuals possessing the rare bNAb/ES phenotype can be examined in greater detail. To this end, efforts are in progress to create trimeric gp140s and DNA vectors as well as the monomeric gp120s described in this paper to further define the immunogenic properties of these Env proteins.

Data Availability

This manuscript contains previously unpublished data. The name of the repository and accession number are not available.

Author Contributions

JH, KM, and PB contributed to study design. JH and KM contributed to construct design and phylogenetic analysis. JH performed sequence alignments, protein expression and binding assays. DA and BY assisted in protein production. KL and TW performed neutralization assays. SD provided patient samples. All authors contributed to manuscript revision.

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Chapter 2: WIHS cohort study

Vaccine immunogens from HIV-1 elite neutralizers with viral control

[Manuscript in preparation]

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Abstract

A major goal in HIV vaccine research is to identify envelope glycoproteins (Envs) capable of eliciting broadly neutralizing antibodies (bNAbs) similar to those found in rare individuals termed elite neutralizers (ENs). To this end, we are studying Envs derived from an unusual subgroup of ENs that control viral load without antiretroviral therapy (ART). Since Env diversity is often limited in controllers, we reasoned that Envs from individuals with the unusual EN/controller phenotype might have retained immunogenic features that promoted the induction of bNAbs and other effective antiviral immune responses. In a cohort of 53 ART-naïve African American women exhibiting varying levels of HIV control, 11 controllers were found to have bNAb activity. An EN with viremic control for over 17.5 years was selected for further characterization. Plasma virus sequences collected several years after infection coded for functional Envs with only 17 potential N-linked glycosylation sites (PNGS) in the gp120 sequence compared to the normal 26 PNGS. Pseudovirus and recombinant gp120 of a low PNGS Env were prepared to investigate antigenic structure and immunogenic potential. This minimally glycosylated immunogen possessed multiple epitopes recognized by broadly neutralizing monoclonal antibodies including those targeting the V1/V2-domain (PG9 and PG16) and the CD4-binding site (VRC01). Monomeric gp120 was administered to rabbits and compared to MN gp120, previously used in the RV144 clinical trial. The low PNGS Env elicited an improved cross-clade tier-1 neutralizing response with increased binding to linear epitopes in the V3 region. Our results suggest that Envs found in this individual may have

contributed to the formation of bNAbs by creating glycan holes that exposed regions of the Env surface normally occluded by the glycan shield. These studies provide the foundation for additional immunologic studies of naturally occurring Envs with minimal glycosylation.

Introduction

The global pandemic of HIV/AIDS continues to be a significant global health crisis and remains the leading cause of death among women of reproductive age (15 – 49 years old)(UNAIDS, 2019b). In the United States, few studies have focused on viruses and immune responses in African American women who represent the most prevalent high-risk group outside of men who have sex with men (CDC, 2018). While HIV transmission can now be prevented with pre-exposure prophylaxis (PreP)(Rodger et al., 2019), persistent disparities across the continuum of care make a prophylactic vaccine necessary for the eradication of HIV/AIDS and research efforts should focus on the most affected communities (Fauci, 2017). Here, we introduce a study focused on 53 HIV-positive African American women.

A primary goal of HIV-1 vaccine design is the development of an immunogenic envelope glycoprotein (Env) able to elicit protective immune responses, including broadly neutralizing antibodies (bNAbs). This has proved a formidable challenge due to the extraordinary diversity of env, which is attributable to HIV's error-prone reverse transcriptase, frequent recombination events, and the preservation of viral variants in the host genome (Chun et al., 1997; Preston et al.,

1988; Zhang et al., 2010). The Env spike mediates attachment and entry into the host cell and is a trimer of heterodimers. The env gene is transcribed as a precursor protein, envelope glycoprotein 160 (gp160), which is then cleaved into two subunits, glycoprotein 41 (gp41) and glycoprotein 120 (gp120)(Moulard and Decroly, 2000). The extracellular subunit, gp120, possesses an average of 26 N-linked glycans (Cao et al., 2018). The dense packing of host-derived glycans constitutes a glycan shield that protects functionally important regions of gp120 from recognition by the humoral immune system. Insertions and deletions as well as shifts in the location of glycosylation sites provide an additional mechanism of antigenic variation (Behrens et al., 2016; Lasky et al., 1986; Wei et al., 2003). Despite these challenges, bNAbs eventually develop in a fraction of HIV-positive individuals, often targeting glycans at conserved locations which initially evaded immune recognition (Doria-Rose et al., 2009; Hraber et al., 2014; Julien et al., 2013b; McLellan et al., 2011; Pejchal et al., 2011; Walker et al., 2009; Zhou et al., 2010). Because bNAbs typically develop two years post infection and viral evolution typically outpaces the contemporaneous antibody response, the production of bNAbs does not correlate with improved disease outcomes (Bhiman et al., 2015; Bonsignori et al., 2016; Doria-Rose et al., 2014; Euler et al., 2010; Freund et al., 2017; Wu et al., 2010; Zhou et al., 2013). However, passive immunization studies in nonhuman primates have demonstrated that bNAbs can provide protection if present prior to infection (Baba et al., 2000; Hessell et al., 2010, 2009; Mascola et al., 2000, 1999; Moldt et al., 2012; Parren et al., 2001; Shibata et al., 1999).

There has been substantial effort to design Envs capable of eliciting the production of bNAbs. Native-like trimers have been engineered with antigenic structures recognized by the major classes of bNAbs, i.e., those that target V2-glycans, V3-glycan supersite, CD4-binding site, membrane proximal external region (MPER), and gp120-gp41 interface (Julien et al., 2013a; Sok and Burton, 2018). However, these trimers have so far failed to consistently elicit the production of bNAbs in immunization studies (Landais et al., 2016). From these studies, it is clear that while it is now possible to create a variety of recombinant Envs that possess the epitopes recognized by bNAbs, these epitopes are poorly immunogenic. This may resemble the situation that occurs in natural infection where most people are infected with viruses possessing the epitopes recognized by bNAbs but few people actually make these antibodies (Simek et al., 2009).

One current vaccination strategy to improve Env immunogenicity and direct the antibody response to sites of vulnerability is to remove and alter the location of PNGS (Crooks et al., 2017; Dubrovskaya et al., 2019; Klasse et al., 2018; Li et al., 2008; Ringe et al., 2019; Zhou et al., 2017). Previous studies have demonstrated that the deletion of PNGS can improve immunogenicity (Back et al., 1994; Crooks et al., 2015; Koch et al., 2003; Li et al., 2008; Liang et al., 2016; McCaffrey et al., 2004; Pancera et al., 2014; Wang et al., 2015; Zhou et al., 2017) with one study showing ID50s proportional to the exponential surface area of accessible polypeptide (Zhou et al., 2017). Neutralizing antibodies often target glycan holes and the introduction of PNGS can confer resistance (Crooks et al., 2017; Klasse et al., 2018; Ringe et al.,

2019; Wagh et al., 2018). Envs have been engineered with PNGS introduced at immunodominant regions and removed at other locations in order to re-direct neutralizing antibodies to the CD4-binding site (Crooks et al., 2017; Klasse et al., 2018; Ringe et al., 2019). Based on these studies, deglycosylated Env trimers have been used in rabbits to prime the immune system, increasing the initial neutralizing antibody response, followed by boosts that introduce PNGS, in an effort to direct neutralizing antibodies to sites of vulnerability (Dubrovskaya et al., 2019). This mirrors the course of natural infection, where early transmitter/founder (T/F) viruses typically have fewer PNGS and viruses evolve additional PNGS over time to escape immune selection pressure (Chohan et al., 2005; Dacheux et al., 2004; Derdeyn et al., 2004; Keele et al., 2008; Liu et al., 2011; Moore et al., 2012). This pattern has also been observed in longitudinal studies examining the co-evolution of viral population and bNAb maturation, with the introduction of PNGS as a common mode of viral escape (LaBranche et al., 2018). Thus, less glycosylation not only increases immunogenicity, they may also mimic the T/F phenotype and Envs that engage with germline bNAb precursors.

It is important to note that few of the vaccine immunogens used to date were derived from people who made bNAbs, and those that have been made, were derived from people who exhibited normal disease progression (Landais et al., 2016). In this study, we have begun to characterize Envs from rare individuals who possessed bNAbs and developed effective antiviral immune responses. Using these Envs, we examine if they have incorporated yet to be defined structural features that affect the

formation of effective humoral and cellular immune responses. In principal, such features may enhance immune recognition through exposure to regions normally shielded from the immune system by glycosylation or conformational masking, increase susceptibility or resistant to proteases that mediate antigen processing and presentation, or modulate binding to co-receptors such as α4β7 or DC-SIGN that facilitate interactions with antigen presenting cells (e.g. follicular dendritic cells)(Cicala et al., 2009; Geijtenbeek et al., 2000; Kwong et al., 2002; López et al., 2000).

Here, we examine Envs derived from an individual with the rare EN/controller phenotype, i.e., produced potent bNAbs (elite neutralizer or EN) and controlled viral load in the absence of antiretroviral therapy (ART). This individual was identified in the course of a study of 53 HIV-positive African American women who exhibited varying levels of viral control without ART. We found that 26% were capable of neutralizing pseudoviruses from four out of five clades tested. Of these, one viremic controller possessing particularly potent bNAbs, EN6, was selected for additional characterization. Envs from this individual possessed two unusual structural features that may have contributed to the development of protective antibody responses. The plasma viruses were remarkable in that they possessed 35% fewer PNGS than most other reported gp120s. Additionally, proviral Env had elongated V1/V2 regions with two additional non-conserved cysteine residues, potentially able to form unusual disulfide loops. Both plasma and proviral Envs were infectious in pseudotype assays and possessed multiple epitopes recognized by bN-mAbs. A minimally glycosylated

recombinant gp120 was expressed in a cell line that restricted N-linked glycosylation to high-mannose glycoforms and purified protein was used in rabbit immunization studies. These studies are consistent with the possibility that Envs from individuals with the EN/controller phenotype have unusual structural features that may have affected the quality and specificity of the protective immune response.

Results

Identification of five elite neutralizers

Data provided by the WIHS cohort (Bacon et al., 2005; Barkan, 1998) was used to identify HIV-positive, ART-naïve African American women exhibiting some level of virus control. Forty-three individuals were selected with periods of virus control in longitudinal studies that lasted 3 – 17.5 years (μ = 12 years). These were divided into two clinical categories, elite controllers (n = 28) and viremic controllers (n = 15), based on viral loads (<100 RNA copies per ml or 100 – 1,000 RNA copies per ml, respectively). Additionally, we selected a control group of ten individuals including long-term non-progressors (no periods of viral control and >500 CD4+ cells per ml for 7+ years; n = 4), and normal progressors (n = 6). From each individual, plasma samples from two visits were screened for neutralization potency and breadth against a standard panel of five viruses representing international strains routinely used to screen for elite neutralizers (Simek et al., 2009)(S1-S4 Tables). Sera from 26% of individuals were capable of neutralizing at least four out of five viruses in the initial screening panel (two elite controllers, nine viremic controllers, one long-term non-progressor, and two normal progressors). On average, elite controllers had significantly lower neutralization titers and neutralization breadth compared to viremic controllers ($p = 0.0004$, Mann-Whitney $U = 454$; $p < 0.0001$, Mann-Whitney $U = 425.5$) and normal progressors ($p = 0.0132$, Mann-Whitney $U = 184$; $p = 0.0021$, Mann-Whitney $U = 154.5$)(Table 2.1).

	Elite controllers	Viremic controllers	Long-term nonprogressors	Normal progressors
Number of individuals	28	15	4	6
Neutralization potency (ID50s in 1/dilution)	**29.1***	60.51	37.30	44.32
Neutralization breadth (percent neutralized)	**26%****	59%	45%	57%

*p = 0.0013, KW statistic = 15.69

**p = 0.0002, KW statistic = 20.16

Table 2.1. Neutralization potency and breadth of 53 HIV-positive ART-naïve African American women by clinical category.
Neutralization potency and breadth were determined by neutralization titers at two time points assayed against the Simek panel of five pseudotype viruses (Simek et al., 2009). Neutralization potency was defined as the geometric mean of ID50 titers. Neutralization breadth was defined as the arithmetic mean of the percentage of pseudoviruses neutralized at each visit. Bold values indicate statistically significant differences in neutralization potency and breadth in elite controllers.

Plasma from the five individuals with the highest neutralization potency and breadth (geometric mean of ID50s > 150 1/dilution; neutralized ≥ 80% of viruses tested) were selected for additional characterization. One sample from each individual was assayed against an expanded panel of 22 viruses. All five samples were capable of neutralizing clade A, B, C, D, and CRF01_AE viruses (Table 2.2A). Of these, plasma identified as EN6 had the highest neutralization titers, with an average ID50 > 430 1/dilution for all five clades and neutralized 100% of viruses tested (Table 2.2B).

A

			ID50 (1/dilution)					
			Viremic controllers				NP	Pos ctrl
	Virus	Clade	VC04	VC10	VC14	EN6	EN7	Z23
Panel 1	94UG103	A	**189**	**94**	**27**	**376**	**120**	**112**
	92BR020	B	**935**	**1812**	**147**	**365**	**404**	**207**
	93IN905	C	**988**	**3011**	**129**	**900**	**828**	**254**
	M-C-026	C	**585**	**2482**	**110**	**754**	**415**	**215**
	92TH021	AE	**108**	**52**	**164**	**165**	20	**152**
Panel 2	92RW008	A	**1195**	**4001**	**94**	**566**	**1390**	**563**
	92RW020	A	**593**	**841**	20	**521**	**679**	**445**
	M-A-002	A	**275**	**3246**	33	**167**	20	**183**
	M-A-009	A	**671**	**364**	**84**	**905**	**196**	**372**
	6535.3	B	**979**	**3993**	**117**	**600**	**864**	**394**
	Bal	B	**5824**	**2632**	**1046**	**859**	**739**	**874**
	MN	B	**37910**	**7906**	**13402**	**6442**	**5249**	**10258**
	PV04	B	**95**	**112**	**133**	**228**	**44**	**406**
	REJO	B	**738**	**203**	**150**	**576**	**159**	**407**
	TRO.11	B	**420**	**669**	42	**504**	**480**	**326**
	93IN905	C	**1047**	**2522**	**98**	**614**	**761**	**297**
	98IN022	C	**101**	**310**	**89**	**338**	**319**	**240**
	M-C-020	C	**82**	**51**	33	**264**	**81**	**112**
	TV1	C	**63**	**139**	**49**	**175**	**111**	126
	92UG005	D	**194**	**747**	34	**494**	**58**	**391**
	94UG114	D	59	**66**	20	**762**	**47**	**157**
	M-D-006	D	**229**	**480**	20	**547**	34	**415**
	M-D-009	D	**149**	**139**	**63**	**469**	**71**	**216**
	113035_081	AE	**379**	**112**	**237**	**558**	20	**416**
	142902_080	AE	**121**	**120**	**176**	**467**	20	**529**
	CM244/A244	AE	**1318**	**3829**	**115**	**1099**	**877**	**538**
	TH023	AE	**288**	**160**	**74**	**397**	**44**	**254**
Ctrls 1	JRCSF	B	**425**	**2253**	**67**	**559**	**115**	**257**
	NL43	B	**2844**	**1111**	**4073**	**2271**	**296**	**1412**
	aMLV	N/A	<20	<20	<20	<20	<20	<100
Ctrls 2	JRCSF	B	**358**	**2313**	**60**	**456**	**100**	**286**
	NL43	B	**3699**	**1042**	**5176**	**2502**	**327**	**1441**
	aMLV	N/A	<20	<20	<20	<20	<20	<100

B

	Clade	VC04	VC10	VC14	EN6	EN7	Z23
Geometric mean of ID50 Titers	A	**477**	**821**	43	**442**	**214**	**286**
	B	**1290**	**1126**	**297**	**697**	**463**	**640**
	C	**261**	**589**	**76**	**432**	**302**	**207**
	D	**140**	**239**	30	**558**	**51**	**259**
	AE	**1171**	**807**	**491**	**2080**	**133**	**1496**
	All	**409**	**536**	**95**	**514**	**174**	**335**
Neutralization breadth	All	96%	100%	74%	100%	81%	96%

Table 2.2 Identification of individuals with high levels of neutralizing antibodies found in cohort of HIV controllers.

Bold indicates neutralization titers with at least 50% inhibition and three-fold greater than the specificity control (aMLV). (A) Neutralization titers from four viremic controllers (VC) and a normal progressor (NP) screened against two panels of pseudoviruses. Average titers for positive control viruses (JRCSF and NL4-3) and specificity control virus (aMLV) are shown for each panel with corresponding labels. The far-right column shows average titers of positive control plasma, Z23. (B) The results from both pseudovirus panels are summarized.

Unusual features in functional gp160 sequences from a viremic controller with elite neutralizing activity (EN6)

The most potent neutralizer from the cohort, EN6, was selected for further analysis. An *env* library was generated from EN6 samples, expressed as pseudoviruses, and screened for infectivity. Full-length *envs* from 10 plasma-derived and 10 PBMC-derived functional pseudoviruses were sequenced. All sequences were confirmed to be clade B by the Recombinant Identification Program (RIP) HIV clade assignment tool (Siepel et al., 1995). Envs were assayed to determine coreceptor usage; all 10 proviruses and 3 plasma viruses were R5-tropic, while the remaining 7 plasma viruses were dual-tropic.

The amino acid sequence length, cysteine content, and number of potential N-linked glycosylation sites (PNGS) were determined for each region of gp160 and compared to Los Alamos National Laboratory Clade B reference sequences (Table 2.3). Strikingly, the V1 regions were highly unusual in both the provirus sequences and the plasma virus sequences. All provirus sequences had an unusually long V1 region containing two non-canonical cysteines, features found in only 7.4% and 6.7% of LANL clade B reference sequences, respectively. Proviral sequences had a 15 amino acid insert that introduced two cysteines and four PNGS, while all plasma sequences lacked this insert (Fig 2.1).

The most unusual finding was observed in plasma virus sequences, which had very low numbers of PNGS. The plasma virus sequences had only one PNGS (out of an expected four PNGS) in the V1 region at position 156 (Fig 2.1; Table 2.3). Plasma

virus also had fewer than average PNGS in C1, C2, C3, and V5 regions, resulting in a total of 17 PNGS in gp120 and 23 PNGS in gp160 (Fig 2.1; Table 2.3). Only three of the 1,936 (0.0015%) Clade B reference sequences had the same or fewer PNGS in the V1 region, gp120, or gp160, demonstrating the minimal number of PNGS in EN6 plasma virus sequences is an exceptionally rare finding.

The location of PNGS in plasma virus included key residues targeted by potent bN-mAbs in the V1/V2 region (N156 and N160) and the V3 region (N301 and N332)(McLellan et al., 2011; Pejchal et al., 2011; Walker et al., 2011; Zhou et al., 2010). In addition, plasma viruses had PNGS at position 241 and 289, which have been engineered into Envs in multiple rabbit and macaque studies (Klasse et al., 2018; McCoy et al., 2016; Ringe et al., 2019). Glycan holes at positions 241 and 289 can elicit a strain-specific immunodominant response and introducing PNGS at these locations has re-directed the antibody response to sites of vulnerability (Klasse et al., 2018; McCoy et al., 2016; Ringe et al., 2019). PNGS were absent at residue 360, proximal to the CD4-binding site, which has been shown improve immunogenicity (Crooks et al., 2017; Dubrovskaya et al., 2019; Zhou et al., 2017).

Region	HXB2 #	Source	Amino acids (median)		PNGS (median)		Cysteines (median)	
			EN6	Ref	EN6	Ref	EN6	Ref
Signal	1 – 30	PBMCs	**36***	29	0	0	1 – 2	1
		Plasma	29		0		1	
C1	31 – 130	PBMCs	100	100	**1***	2	4	4
		Plasma	100		**0***		4	
V1	131 – 157	PBMCs	**40***	29	**6***	4	**4***	2
		Plasma	25		**1***		2	
V2	158 – 196	PBMCs	41	40	3	2	1	1
		Plasma	**38***		2		1	
V1/V2	131 – 196	PBMCs	**81***	71	**9***	6	**5***	3
		Plasma	**63***		**3***		3	
C2	197 – 295	PBMCs	99	99	6	7	5	5
		Plasma	99		**5***		5	
V3	296 – 331	PBMCs	35	35	1	1	2	2
		Plasma	35		1		2	
C3	332 – 384	PBMCs	52	52	3	3	1	1
		Plasma	52		**2***		1	
V4	385 – 418	PBMCs	**29***	32	4	5	2	2
		Plasma	31		4		2	
C4	419 – 459	PBMCs	41	41	1	1	1	1
		Plasma	41		1		1	
V5	460 – 469	PBMCs	10	11	2	2	0	0
		Plasma	11		**1***		0	
C5	470 – 511	PBMCs	42	42	0	0	0	0
		Plasma	42		0		0	
gp120	31 – 511	PBMCs	489	484	27	26	**20***	18
		Plasma	**474***		**17***		18	
gp41	512 – 856	PBMCs	345	345	**4***	5	3	3
		Plasma	345		**6 ***		3	
gp160	31 – 856	PBMCs	834	829	31	31	**23***	21
		Plasma	**819***		**23***		21	

*p < 0.0001

Table 2.3. Physical characteristics of functional proviral and plasma gp160 sequences isolated from EN6.

The number of amino acids, potential N-linked glycosylation sites (PNGS), and cysteines were counted in each region of functional gp160 sequences isolated from EN6 PBMC and plasma samples. Location of PNGS was defined as the location of N in NX(S/T) motif (where X is any amino acid except proline). The distribution of each measure was compared to clade B sequences from the Los Alamos National Laboratory 2017 Filtered Web Alignment.

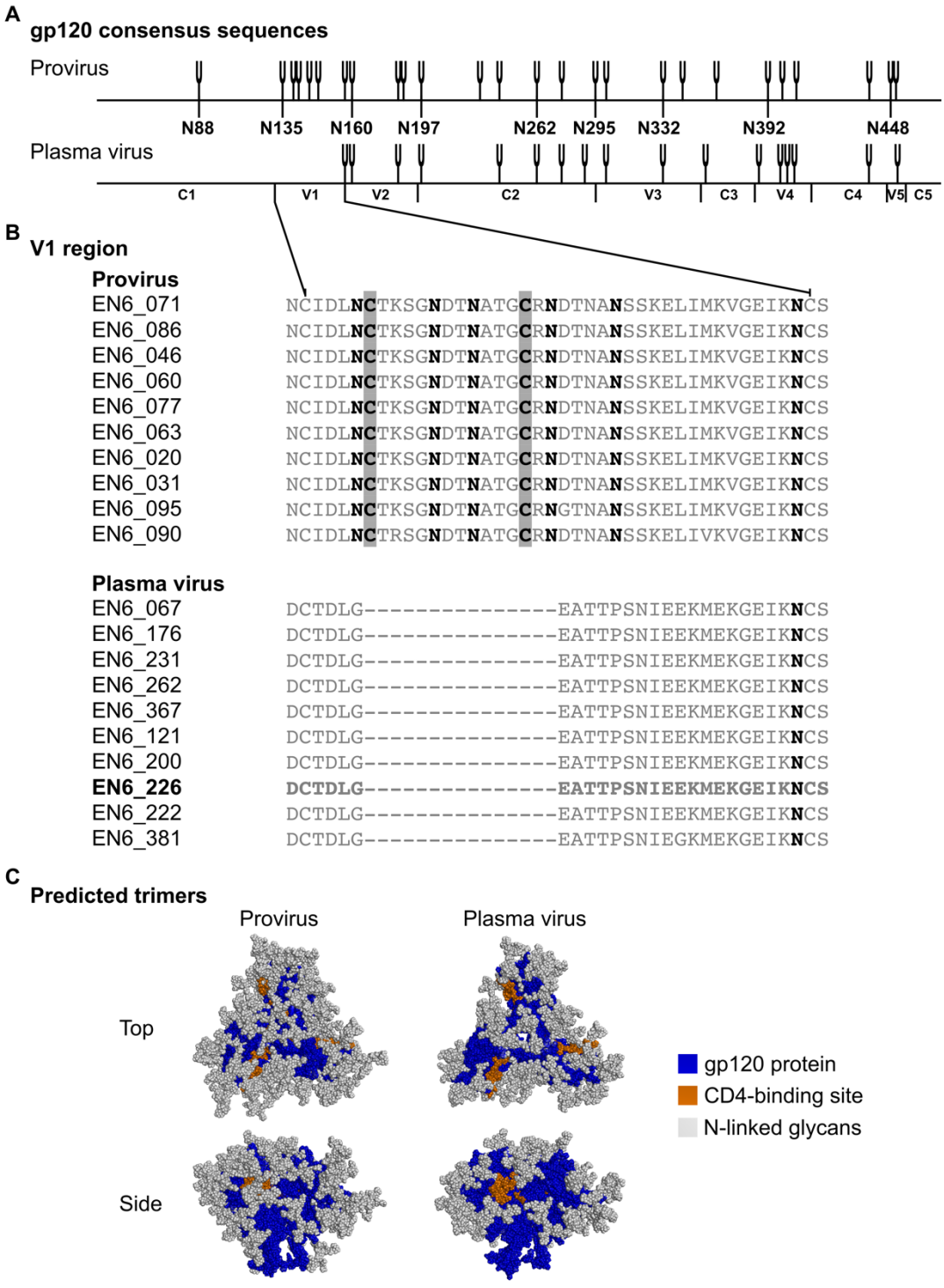

A
gp120 consensus sequences
Provirus
Plasma virus
N88
N135
N160
N197
N262
N295
N332
N392
N448
C1
V1
V2
C2
V3
C3
V4
C4
V5
C5
B
V1 region
Provirus
EN6_071 NCIDLNCTKSGNDTNATGCRNDTNANSSKELIMKVGEIKNCS
EN6_086 NCIDLNCTKSGNDTNATGCRNDTNANSSKELIMKVGEIKNCS
EN6_046 NCIDLNCTKSGNDTNATGCRNDTNANSSKELIMKVGEIKNCS
EN6_060 NCIDLNCTKSGNDTNATGCRNDTNANSSKELIMKVGEIKNCS
EN6_077 NCIDLNCTKSGNDTNATGCRNDTNANSSKELIMKVGEIKNCS
EN6_063 NCIDLNCTKSGNDTNATGCRNDTNANSSKELIMKVGEIKNCS
EN6_020 NCIDLNCTKSGNDTNATGCRNDTNANSSKELIMKVGEIKNCS
EN6_031 NCIDLNCTKSGNDTNATGCRNDTNANSSKELIMKVGEIKNCS
EN6_095 NCIDLNCTKSGNDTNATGCRNGTNANSSKELIMKVGEIKNCS
EN6_090 NCIDLNCTRSGNDTNATGCRNDTNANSSKELIVKVGEIKNCS
Plasma virus
EN6_067 DCTDLG----------EATTPSNIEEKMEKGEIKNCS
EN6_176 DCTDLG----------EATTPSNIEEKMEKGEIKNCS
EN6_231 DCTDLG----------EATTPSNIEEKMEKGEIKNCS
EN6_262 DCTDLG----------EATTPSNIEEKMEKGEIKNCS
EN6_367 DCTDLG----------EATTPSNIEEKMEKGEIKNCS
EN6_121 DCTDLG----------EATTPSNIEEKMEKGEIKNCS
EN6_200 DCTDLG----------EATTPSNIEEKMEKGEIKNCS
EN6_226 DCTDLG----------EATTPSNIEEKMEKGEIKNCS
EN6_222 DCTDLG----------EATTPSNIEEKMEKGEIKNCS
EN6_381 DCTDLG----------EATTPSNIEGKMEKGEIKNCS
C
Predicted trimers
Provirus
Plasma virus
Top
Side
gp120 protein
CD4-binding site
N-linked glycans

Fig 2.1. Location of potential N-linked glycosylation sites and non-canonical cysteines in EN6.
(A) Schematic shows position of potential N-linked glycosylation sites using aligned provirus and plasma virus consensus sequences. Select asparagine locations are labeled using HXB2 reference numbering. Variable and constant regions are also shown for reference. (B) Alignment of the V1 region of functional provirus and plasma virus Envs from EN6 samples. Non-canonical cysteines are highlighted in grey and PNGS are shown in black. EN6_226, shown in bold, was expressed and used as a rgp120 immunogen in this study. (C) Structure prediction models indicating exposed and glycosylated surfaces of gp120 in trimeric Env proteins. Homology models of gp120 for provirus and plasma virus consensus sequences were built using Modeller v9.21 (Webb and Sali, 2016) with PDB ID 5aco template (Pancera et al., 2014) and N-linked glycans were added with GLYCAM Web Glycoprotein Builder (Woods Group, 2005). Surfaces occupied by N-linked glycans are shown in light grey; exposed rgp120 residues are shown in blue; CD4-binding site is shown in orange.

Sensitivity and resistance to neutralization of EN6-derived Envs by autologous plasma and bN-mAbs

Envs derived from PBMCs and plasma were used in a recombinant pseudovirus assay to assess sensitivity and resistance to neutralization by polyclonal antibodies in autologous plasma and prototypic bN-mAbs. Both proviral and plasma viral Envs, with the notable exceptions of EN6_226 and EN6_031, were resistant to neutralization by contemporaneous plasma (Table 2.4; Fig 2.2A). Both the prototypic elite neutralizer plasma (Z23) and EN6 sera were unable to neutralize all but one of the EN6 proviral Envs with the V1 insert (Table 2.4; proviral). However, the Z23 plasma differed from the EN6 plasma in that it was effective in neutralizing 9 of 10 plasma viruses whereas only 2 were weakly neutralized by autologous plasma. This result suggests that the shorter V1 domain and the lack of PNGS facilitated neutralization by the Z23 plasma. The lack of neutralization by the EN6 plasma appears to reflect the evolution of viruses resistant to autologous neutralization as has been described previously (Euler et al., 2010; Frost et al., 2005; Richman et al., 2003).

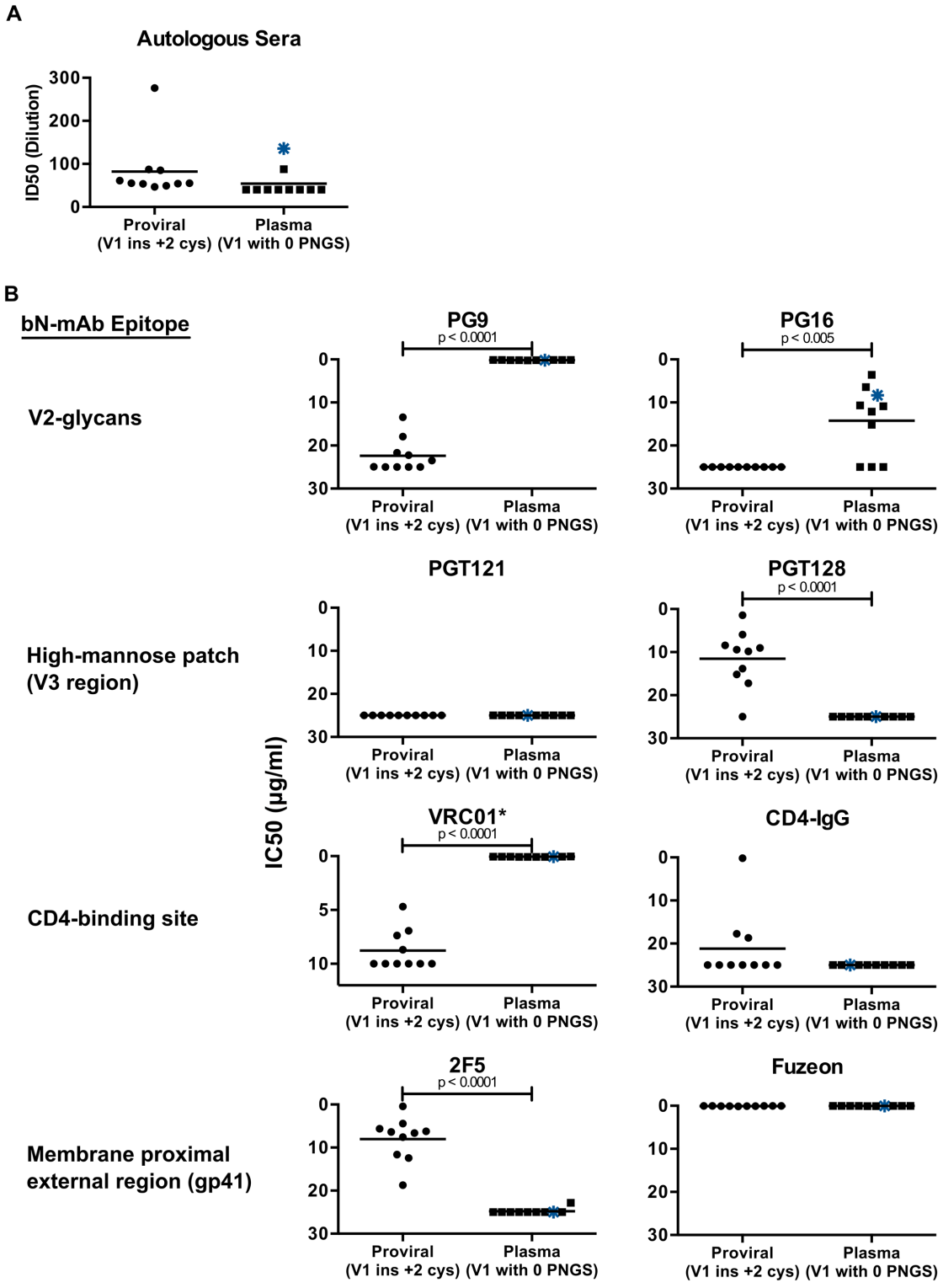
When the sensitivity to neutralization by bN-mAbs was examined (Fig 2.2), the plasma viruses with the low numbers of PNGS and lacking the V1 insertion were markedly more sensitive to neutralization by the PG9, PG16, and VRC01 bN-mAbs compared to provirus (Fig 2.2B). Conversely, the provirus sequences were significantly more sensitive to neutralization by 2F5 bN-mAb targeting the MPER domain in gp41, which correlates with the polymorphism of A667E/G in the 2F5

epitope (Ofek et al., 2004). In control experiments, all of the viruses were found to be sensitive to the antiviral drug Fuzeon (enfuvirtide).

Most EN6 Envs were resistant to neutralization by PGT121 and PGT128, despite having glycosylation at key residues in the V3 region, N332, N301, and IGDIR$^{323\text{-}327}$ (Pejchal et al., 2011). Plasma Envs are likely resistant to neutralization by PGT121 and PGT128 due to polymorphism E(321+1)R, which substituted into a PGT128-gp120 crystal structure (PDB 5C7K) introduces a charge repulsion and steric hindrance with heavy chain residue R100 (Kong et al., 2015). While proviral Envs have E(321+1), they also have a 15 amino acid insert and five additional PNGS in the V1 region which have been shown to alter binding to PGT121 and PGT128 (Garces et al., 2014; Sok et al., 2014). The approach angle of PGT128 allows for better accommodation of V1 glycans (Kong et al., 2015; Pejchal et al., 2011). In contrast, V1 glycans, such as N137, have been shown to interfere with PGT121 binding (Garces et al., 2014). Thus, it is unsurprising that proviral Envs are resistant to neutralization by PGT121 but show modest sensitivity to PGT128.

	Name	Tropism	ID50 (1/dilution) EN6 Sera	ID50 (1/dilution) Z23 (pos ctrl)	IC50 (µg/mL) PG9	PG16	PGT121	PGT128	VRC01	2F5	CD4-IgG	Fuzeon
Provirus (V1 insert with 2 cys and 5 PNGS)	EN6_020	R5	**87**	<100	>25	>25	>25	**15.165**	>10	**7.561**	>25	**0.052**
	EN6_031	R5	**276**	**427**	**13.424**	>25	>25	**1.435**	**4.684**	**0.401**	**0.195**	**0.079**
	EN6_046	R5	54	<100	**22.230**	>25	>25	**8.448**	**7.361**	**6.641**	>25	**0.116**
	EN6_060	R5	54	<100	**23.461**	>25	>25	**13.817**	>10	**11.646**	>25	**0.049**
	EN6_063	R5	85	<100	>25	>25	>25	**17.203**	**8.700**	**5.627**	**17.740**	**0.039**
	EN6_071	R5	55	<100	>25	>25	>25	**9.825**	>10	**18.725**	>25	**0.057**
	EN6_077	R5	47	<100	**24.922**	>25	>25	**5.930**	>10	**4.435**	>25	**0.027**
	EN6_086	R5	61	<100	>25	>25	>25	**9.030**	>10	**6.221**	>25	**0.038**
	EN6_090	R5	49	<100	**17.946**	>25	>25	>25	**6.925**	**6.367**	>25	**0.033**
	EN6_095	R5	55	<100	**21.690**	>25	>25	**9.445**	>10	**12.405**	**18.657**	**0.052**
Plasma virus (V1 del and min glycosylation)	EN6_067	R5	<40	**130**	**0.112**	**3.568**	>25	>25	**0.036**	>25	>25	**0.017**
	EN6_121	Dual	**88**	**299**	**0.153**	**10.874**	>25	>25	**0.052**	>25	>25	**0.054**
	EN6_176	Dual	<40	**139**	**0.137**	**10.651**	>25	>25	**0.059**	>25	>25	**0.003**
	EN6_200	R5	<40	**196**	**0.138**	**6.419**	>25	>25	**0.071**	>25	>25	**0.050**
	EN6_222	Dual	<40	**131**	**0.130**	**15.165**	>25	>25	**0.073**	22.812	>25	**0.032**
	EN6_226	R5	**136**	**576**	**0.167**	**8.321**	>25	>25	**0.041**	>25	>25	**0.046**
	EN6_231	Dual	<40	<100	**0.186**	>25	>25	>25	**0.058**	>25	>25	**0.030**
	EN6_262	Dual	<40	**132**	**0.206**	**12.121**	>25	>25	**0.060**	>25	>25	**0.038**
	EN6_367	Dual	<40	**154**	**0.143**	>25	>25	>25	**0.067**	>25	>25	**0.024**
	EN6_381	Dual	<40	**142**	**0.185**	>25	>25	>25	**0.073**	>25	>25	**0.028**
Provirus controls	JRCSF	R5	**302**	**196**	**0.004**	**0.002**	**0.098**	**0.009**	**0.317**	**5.932**	>25	**0.144**
	NL43	X4	**1981**	**1684**	**0.430**	N/A	>25	>25	**0.279**	**7.370**	**0.422**	**0.401**
	aMLV	N/A	<40	<100	>25	>25	>25	>25	>10	>25	>25	>25
Plasma controls	JRCSF	R5	**234**	**232**	**0.006**	**0.002**	**0.878**	**0.004**	**0.325**	**5.269**	**5.712**	**0.056**
	NL43	X4	**2537**	**1905**	**1.056**	**0.916**	>25	>25	**0.188**	**6.162**	**0.037**	**0.264**
	aMLV	N/A	<40	<100	>25	>25	>25	>25	>10	>25	>25	>25

Table 2.4. Sensitivity of EN6 Envs to neutralization by polyclonal antibodies in autologous plasma and broadly neutralizing monoclonal antibodies.
Envs recovered from EN6 PBMCs and plasma were used to make pseudoviruses and tested for sensitivity to neutralization by autologous plasma and a panel of prototypic bN-mAbs. Neutralization titers represent the reciprocal of the plasma dilution (ID50) or monoclonal antibody concentration (IC50) with 50% inhibition and three-fold greater than the specificity control (aMLV) are shown in bold. A standard reference plasma with broadly neutralizing antibodies, Z23, is included as a positive control. Controls for each assay are shown at the bottom of the table. Coreceptor usage was determined for each Env-recombinant pseudovirus and shown in the second column.



*Maximum VRC01 concentration was 10 µg/ml (all other mAbs assayed up to 25 µg/ml)

Fig 2.2. Comparison of EN6 provirus and plasma virus sensitivity to neutralization by autologous sera and bN-mAbs.
Comparison of provirus and plasma virus EN6 clones to neutralization by (A) autologous plasma (ID50) and (B) bN-mAbs (IC50). Graphs are organized in rows by region targeted by bN-mAbs. Mann-Whitney U test showed statistically significant differences between proviral and plasma viral titers to PG9 ($p < 0.0001$; Mann-Whitney U = 0), PG16 ($p < 0.005$; Mann-Whitney U = 15), PGT128 ($p < 0.0001$; Mann-Whitney U = 5), VRC01 ($p < 0.0001$; Mann-Whitney U = 0), and 2F5 ($p < 0.0001$; Mann-Whitney U = 0). Blue star symbol shows neutralization titers for Env EN6_226, used in immunization study.

Selection, expression, and bN-mAb binding to recombinant gp120 from the EN6_226 clone

Of the twenty gp120 sequences identified in the elite neutralizer, EN6, one (EN6_226) was selected for expression and immunization studies. This sequence was selected because it was sensitive to neutralization by autologous plasma, possessed only 17 PNGS, lacked the V1 region insertion with extra cysteine residues, and possessed epitopes recognized by multiple bN-mAbs. For these studies, codon-optimized rgp120 gene expressed with a gD-1 purification tag protein was created and expressed in HEK293S GnTI$^-$ cells. These cells are deficient in N-acetylglucosaminyltransferase-1 and produce proteins that lack the N-linked sialic acid that inhibits the binding of some bN-mAbs (Byrne et al., 2018; Doran et al., 2018; Morales et al., 2014b; Reeves et al., 2002). The altered glycosylation pathway in HEK293S GnTI$^-$ cells yields proteins primarily with mannose-5 glycans, which have previously been shown to enhance binding of several potent bN-mAbs, including PG9 (Doran et al., 2018; Morales et al., 2014b).

A fluorescent immunoassay (FIA) described previously (Byrne et al., 2018; Doran et al., 2018; Hutchinson et al., 2019; Morales et al., 2014b; O'Rourke et al., 2019) was used to measure EN6_226 rgp120 binding to bN-mAbs. We found that the EN6_226 protein bound to PG9, PGT128, and VRC01, but not PG16 and PGT121 (Fig 2.3). It was surprising that the EN6_226 rgp120 bound to PGT128 since EN6_226-recombinant pseudovirus was resistant to neutralization by PGT128. This result suggests PGT128 can bind to monomeric gp120 despite E(321+1)R, the

putative cause of neutralization resistance to the EN6_226-recombinant pseudovirus. While EN6_226 was sensitive to neutralization by PG16, binding to monomeric rgp120 was not expected, as the recombinant protein lacked the complex sialic acid containing glycans and quaternary structure thought to be required for binding (McLellan et al., 2011). PGT121 binding was also not expected, since EN6_226 was resistant to neutralization by PGT121.

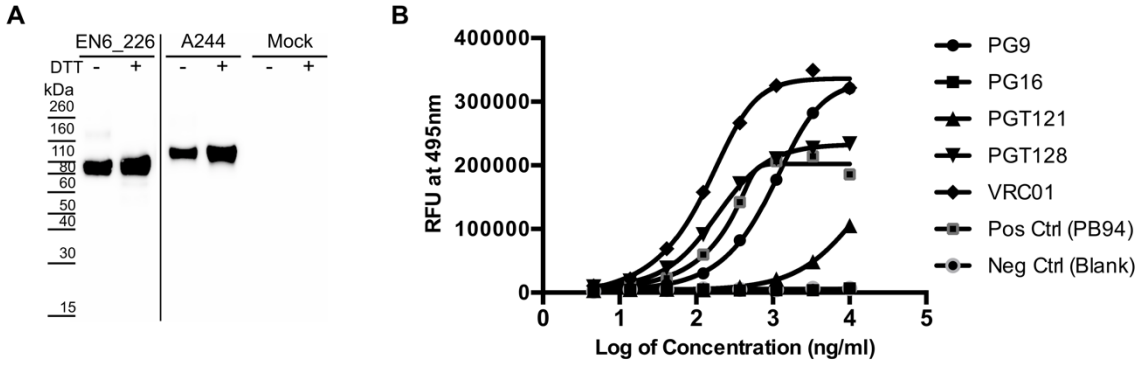


Fig 2.3. Expression and binding of broadly neutralizing monoclonal antibodies to EN6_226 rgp120.
Recombinant EN6_226 gp120 was produced by transient transfection of HEK293S GnTI$^-$ cells and used for antibody binding assays. Recombinant gp120 from the A244 isolate of gp120 was used as a positive control. (A) 12µl of supernatant from HEK293S GnTI$^-$ cells transfected with EN6_226 rgp120, A244 rgp120 (positive control), or "mock" (negative control; no *env*) were analyzed by SDS-PAGE (4-12% gel) with and without DTT reduction, blotted, and probed with a mAb (34.1) targeting the N-terminal epitope tag. HEK293S GnTI$^-$ expressed protein were enriched for mannose-5 glycans and thus smaller than sialic acid-enriched proteins produced in other cell lines. EN6_226 rgp120 is smaller than A244 rgp120 due to less glycosylation and shorter amino acid length. (B) Purified EN6_226 rpg120 was captured with 34.1 mAb (34.1) targeting the N-terminal tag and probed with bN-mAbs PG9, PG16, PGT121, PGT128, and VRC01 by FIA. Purified rabbit polyclonal antibodies raised against MN and A244 rgp120s from a previous immunization study, PB94, were included as a positive control. Blank (no antigen) was used as a negative control.

EN6_226 rgp120 elicits stronger neutralization response in rabbits compared to two other clade B rgp120s

Five rabbits were immunized with EN6_226 rgp120. Post-immunization rabbit sera were compared to rabbit sera from similar immunization studies using EN3_071 rgp120 and MN rgp120. EN3_071 rgp120 was derived from a previously described elite controller with bNAb activity and normal glycosylation (Hutchinson et al., 2019). MN rgp120 was used in the RV144 clinical trials, which elicited protective non-neutralizing antibody responses, and serves as a clade B immunogen to improve upon (Berman et al., 1999, 1996; Doran et al., 2018). All three immunizations elicited rgp120-specific antibody binding with comparable IC50s (S5 Fig).

EN6_226 rgp120 elicited the production of polyclonal antibodies with the strongest neutralization response, capable of neutralizing clade B (MN.3 and SF162.LS), clade C (MW965.26), and clade CRF01_AE (TH023.6) pseudoviruses (Table 2.5). Compared to the other two immunogens, EN6_226 rgp120-raised antibodies neutralized SF162.LS, MW965.26, and TH023.6 at higher titers (Table 2.5). As expected, MN rgp120-raised antibodies neutralized cognate Env MN.3 with the highest titers (Table 2.5). EN3_071 rgp120 did not consistently elicit neutralizing antibodies (Table 2.5). The strength of the immune response corresponded to the amount of glycan holes on the immunogen, with EN6_226 rgp120 eliciting the strongest response.

		ID50 (1/dilution)									
			Clade B				Clade C			Clade A	Clade AE
		Ctrl	Tier 1	Tier 1	Tier 2	Tier 2	Tier 1	Tier 2	Tier 2	Tier 2	Tier 1
Immunogen	ID	SVA-MLV	MN.3	SF162.LS	JR-FL	TRO.11	MW965.26	25710-2.43	Ce1176_A3	BG505/T332N	TH023.6*
EN6_226	R1*	<20	**342**	**94**	<40	<40	**57**	<40	<40	<40	<20
	R2	<20	**280**	**36**	<20	<20	<20	<20	<20	<20	**349**
	R3	<20	**7527**	**541**	<20	<20	**244**	<20	<20	<20	**572**
	R4	<20	**395**	**225**	<20	<20	**33**	<20	<20	<20	**209**
	R5	<20	<20	**60**	<20	<20	**31**	<20	<20	<20	**39**
	Avg	<20	**1709**	**191**	<20	<20	**73**	<20	<20	<20	**234**
EN3_071	R1	<20	<20	<20	<20	<20	**32**	<20	<20	<20	**21**
	R2	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
	R4	<20	<20	**1653**	<20	<20	<20	<20	<20	<20	<20
	R5*	<20	<20	<40	<40	<40	<40	<40	<40	<40	<20
	Avg	<20	<20	**413**	<20	<20	8	<20	<20	<20	5.25
MN	R1*	<20	**>43740**	**45**	<40	<40	<40	<40	<40	<40	<20
	R2*	<20	**12673**	**76**	<40	<40	<40	<40	<40	<40	**86**
	Avg	<20	**28207**	**61**	<40	<40	<40	<40	<40	<40	**43**
CH01-31	ctrl	>25	0.69	0.43	0.04	0.16	1.30	0.16	0.11	0.03	0.23
CH01-31*	ctrl	nt	0.59	0.28	0.02	0.29	2.32	0.27	0.17	0.04	0.23

Table shows results from two neutralization assays performed on separate days
*Results from second neutralization assay

Table 2.5. Neutralization of viruses from clades B, C, and CRF01_AE by rabbit antibodies raised against EN6_226, EN3_071, and MN rgp120s.
Rabbits were immunized with EN6_226, EN3_071, or MN rgp120s. Sera were tested against a panel of nine Env-recombinant pseudoviruses in a standard neutralization assay using TZM-bl cells. Neutralization antibody titers (ID50s) were calculated as the reciprocal dilution conferring 50% inhibition. Reactions with neutralization activity are shown in grey. Arithmetic mean of titers was calculated with ID50s below detection level as zero. CH01-03 and SVA-MLV were included as positive and negative controls, respectively.

Pepscanning of antisera to EN6_226 rgp120

Epitope mapping of polyclonal antibodies raised against EN6_226 or MN rpg120 was performed by peptide microarray. Because bNAbs bind to conformation and glycosylation-dependent epitopes, these studies do not provide information on epitopes recognized by neutralizing antibodies. However, they define the spectrum of linear epitopes recognized by non-neutralizing antibodies and how they differ when elicited by different immunogens. Antibody binding was measured in a peptide microarray assay (Gottardo et al., 2013; Zolla-Pazner et al., 2013) in the laboratory of Dr. Georgia Tomaras (Duke Medical School, Durham, NC). This assay measured antibody binding to 2,058 peptides from eight consensus sequences and five strains representing worldwide gp120 sequences. These studies were undertaken with three objectives in mind. First, we wanted to define the strongest epitopes and see if they differed between EN6_226 rgp120 from a rare elite controller that possessed broadly neutralizing antibodies and MN rgp120, a prototypic lab adapted isolate from a normal progressor. Second, we were also interested in comparing the immunogenicity of EN6_226 rgp120 that possessed only 17 N-linked glycosylation sites compared to MN rgp120 that possessed 20 N-linked glycosylation sites. Third, we wanted to see the extent to which antibodies were directed to linear peptides in the vicinity of sites of vulnerability defined by neutralizing antibodies to the V1/V2 domain (e.g. PG9), the V3 domain (e.g. PGT128) and the CD4-binding site (e.g. VRC01). In particular, we were interested in whether non-glycosylated peptides from locations that were targeted by glycan-dependent broadly neutralizing antibodies were immunodominant

or immunorecessive. The gross summary of the results of these studies are shown in Figure 2.4. We found that both immunogens elicited antibodies with the strongest binding to multiple linear epitopes in the C1, C2, C4, and V5 regions (Fig 2.4A). Of these, both the C4 domain and the V5 domain are known to possess residues recognized by monoclonal antibodies that block CD4 binding. The EN6_226 rgp120-raised antibodies showed significantly stronger binding to peptides in the V3 region compared to those raised against MN rgp120 (Fig 2.4A). The V3 domain is known to possess contact residues recognized by multiple neutralizing antibodies. The enhanced binding to the V3 region may be due to a reduction in glycan shielding attributable to the absence of glycosylation sites at residues 295 and 402 in EN6_226 rgp120, which are present in MN rgp120 (Fig 2.4B).

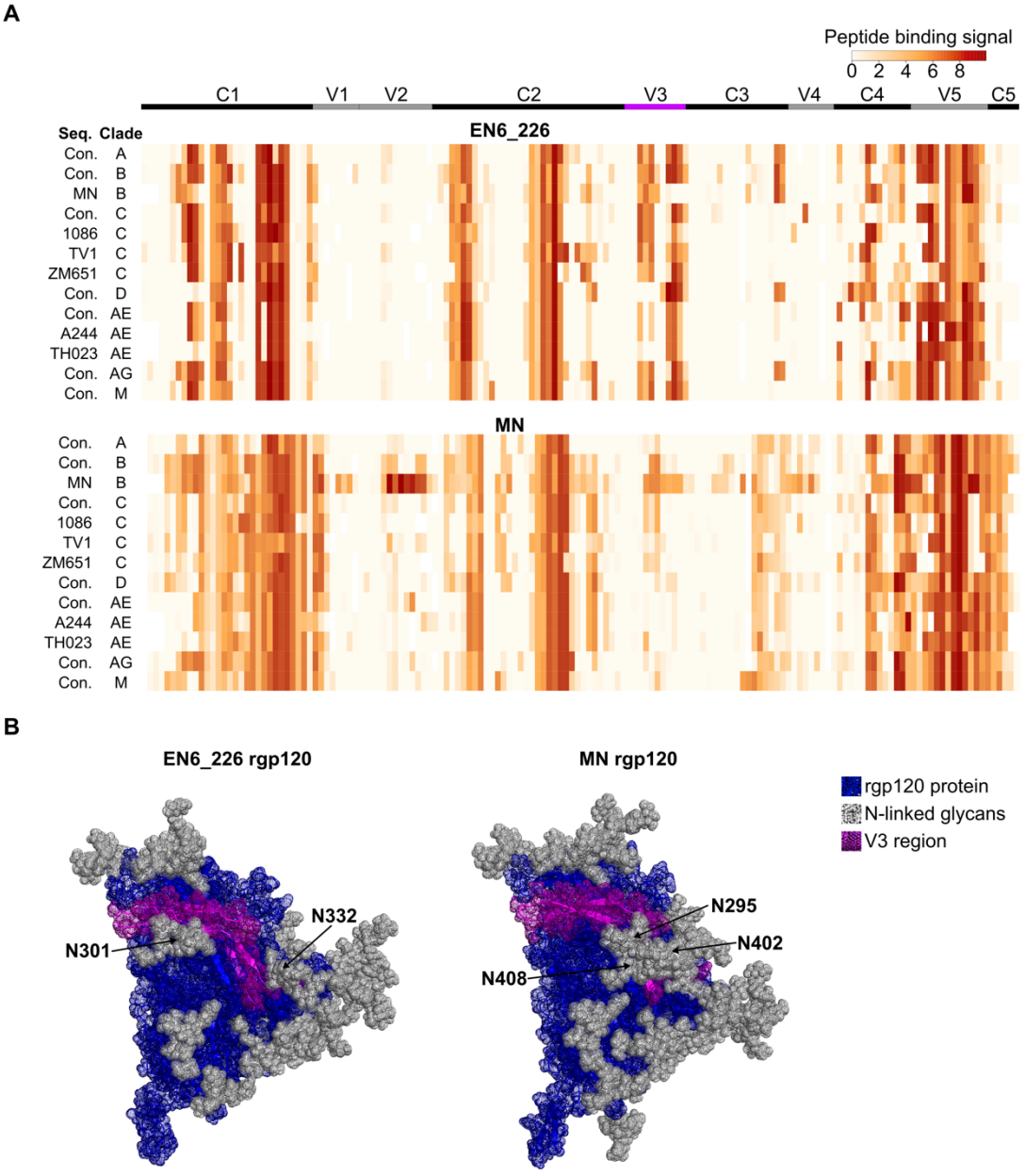

A
Peptide binding signal
0 2 4 6 8
C1 V1 V2 C2 V3 C3 V4 C4 V5 C5
Seq. Clade
EN6_226
Con. A
Con. B
MN B
Con. C
1086 C
TV1 C
ZM651 C
Con. D
Con. AE
A244 AE
TH023 AE
Con. AG
Con. M
MN
Con. A
Con. B
MN B
Con. C
1086 C
TV1 C
ZM651 C
Con. D
Con. AE
A244 AE
TH023 AE
Con. AG
Con. M
B
EN6_226 rgp120
MN rgp120
rgp120 protein
N-linked glycans
V3 region
N332
N301
N295
N402
N408

Fig 2.4. Pepscanning to compare linear epitopes targeted by rabbit antibodies raised against EN6_226 or MN rgp120s.
Pepscanning of polyclonal antibodies raised against rgp120 immunogens was performed by peptide microarray as described previously (Shen et al., 2015). (A) Heatmap shows median magnitude of peptide binding (signal) for rabbit antibodies raised against EN6_226 or MN rgp120s. Peptide library was composed of 15-mers that overlapped by 12 amino acids for thirteen different gp120 sequences, shown by row. Schematic above the heatmap shows peptide locations in gp120, aligned for each column. (B) Homology models of rgp120 immunogens were built using Modeller v9.21 (Webb and Sali, 2016) with PDB ID 5aco template (Pancera et al., 2014) and N-linked glycans were added with GLYCAM Web Glycoprotein Builder (Woods Group, 2005). Amino acid rgp120 residues are shown in blue; N-linked glycans are shown in light grey.

Next, we ranked peptides with the highest antibody binding signals to determine immunodominant regions. EN6_226 rgp120-raised antibodies showed the strongest binding to clade B peptides in non-neutralizing regions (C1, C2, and C5) but the fifth highest response was to a V5 peptide located in the CD4-binding site and the seventh and ninth highest responses were to V3 peptides in the PGT128 epitope (Table 2.6A). MN rgp120-raised antibodies showed the strongest binding to clade B peptides from the CD4-binding site (Table 2.6B). Similar results were observed for antibody binding to peptides from all clades (Table 2.7).

Strongest antibody binding signals to clade B peptides

A

gp120 region	Clade B peptides	HXB2 range	anti-EN6 sera (median signal)
C1	NNMVEQMHEDIISLW	98 - 112	9.66
C2	PVVSTQLLLNGSLAE	253 - 267	9.38
C1	MHEDIISLWDQSLKP	104 - 118	9.12
C5	SELYKYKVVKIEPLG	481 - 495	8.83
V5*	**NTTETFRPGGGDMRD**	**463 - 477**	**8.65**
C1	TDPNPQEVVLENVTE	77 - 91	8.43
V3**	**YATGDIIGDIRQAHC**	**318 - 332**	**8.34**
C1	VEQMHEDIISLWDQS	101 - 115	8.28
V3**	**RAFYATGDIIGDIRQ**	**315 - 329**	**8.24**
V5	NNNNTTETFRPGGGD	92 - 106	8.01

B

gp120 region	Clade B peptides	HXB2 range	anti-MN sera (median signal)
C5*	**MRDNWRSELYKYKVV**	**475 - 489**	**9.74**
C4*	**QIRCSSNITGLLLTR**	**442 - 456**	**9.35**
C5*	**GGDMRDNWRSELYKY**	**472 - 486**	**9.06**
C4*	**CSSNITGLLLTRDGG**	**445 - 459**	**8.97**
C5*	**ETFRPGGGDMRDNWR**	**466 - 480**	**8.75**
C2	PVVSTQLLLNGSLAE	253 - 267	8.22
C1	NNMVEQMHEDIISLW	98 - 112	8.05
C5*	**NWRSELYKYKVVKIE**	**478 - 492**	**7.99**
C1	MHEDIISLWDQSLKP	104 - 118	7.96
C2	STQLLLNGSLAEEEV	256 - 270	7.80

*CD4-binding site (VRC01 contacts)
**V3 region (PGT128 contacts)

Table 2.6. Strongest antibody binding signals to clade B peptides.
Pepscanning of polyclonal antibodies raised against rgp120 immunogens was performed by peptide microarray. Peptide locations were compared to previously published data to identify sites of interest, including functional regions and bN-mAb epitopes. Peptides from sites of interest are shown in bold. (A) Highest median clade B peptide binding signals for rabbit antibodies raised against EN6_226 rgp120. (B) Highest median clade B peptide binding signals for rabbit antibodies raised against MN rgp120.

Strongest cross-reactive antibody binding signals to peptides from all major clades

A

gp120 region	All peptides	HXB2 range	anti-EN6 sera (median signal)
C1	NNMVEQMHTDIISLW	98 - 112	9.69
C1	VEQMHEDIISLWDES	101 - 115	9.68
C1	NNMVEQMHEDIISLW	98 - 112	9.66
C5	SELYKYKVVRIEPLG	481 - 495	9.61
V3**	**QALYTTRIIGDIRQ**	**315 - 329**	**9.51**
V5*	**TNNETFRPGGGNIKD**	**463 - 477**	**9.44**
C5	NELYKYKVVQIEPLG	481 - 495	9.43
C2	PVVSTQLLLNGSLAE	253 - 267	9.38
V5*	**TNNTNNETFRPGGGN**	**460 - 474**	**9.31**
C1	NDMVEQMHEDIISLW	98 - 112	9.27

B

gp120 region	All peptides	HXB2 range	anti-MN sera (median signal)
C5*	**MRDNWRSELYKYKVV**	**475 - 489**	**9.74**
C4*	**LIRCSSNITGLLLTR**	**442 - 456**	**9.68**
C5*	**MKDNWRSELYKYKVV**	**475 - 489**	**9.67**
C4*	**NITGILLTRDGGATN**	**448 - 462**	**9.66**
C4*	**EIRCESNITGLLLTR**	**442 - 456**	**9.49**
C5*	**IKDNWRSELYKYKVV**	**475 - 489**	**9.40**
C4*	**QIRCSSNITGLLLTR**	**442 - 456**	**9.35**
C4*	**NITCKSNITGLLLTR**	**442 - 456**	**9.34**
C5*	**GGDMRDNWRSELYKY**	**472 - 486**	**9.06**
C4*	**CSSNITGLLLTRDGG**	**445 - 459**	**8.97**

*CD4-binding site (VRC01 contacts)
**V3 region (PGT128 contacts)

Table 2.7. Strongest cross-reactive antibody binding signals to peptides from all major clades.
Pepscanning of polyclonal antibodies raised against rgp120 immunogens was performed by peptide microarray. Peptide locations were compared to previously published data to identify sites of interest, including functional regions and bN-mAb epitopes. Peptides from sites of interest are shown in bold. (A) Highest median peptide binding signals for rabbit antibodies raised against EN6_226 rgp120. (B) Highest median peptide binding signals for rabbit antibodies raised against MN rgp120.

Since previous studies have shown that neutralizing antibodies are directed to glycan holes, we analyzed antibody binding signals to non-glycosylated peptides with sequences encoding potential N-linked glycosylation sites. Overall, locations lacking PNGS did not consistently show higher antibody responses when compared to the other immunogen containing a PNGS at the same location, suggesting that these regions are inherently immunorecessive (Table 2.8).

Antibody binding to peptides at PNGS locations

	PNGS		Clade B peptides		All peptides	
HXB2 #	EN6_226	MN	EN6	MN	EN6	MN
88		N	1.06	1.22	0.06	1.37
130		N	3.43	5.84	0.54	5.75
136		N	0.00	0.00	0.00	0.00
142		N	0.00	0.14	0.00	0.00
142+5		N	6.80	0.00	0.00	0.00
156	N	N	2.00	0.00	0.00	0.00
160	N	N	0.00	0.00	0.00	0.00
186	N	N	0.00	4.08	0.00	0.00
197	N	N	0.00	0.00	0.00	0.00
241	N	N	0.00	0.49	0.00	0.00
262	N	N	2.73	3.86	0.58	1.69
276	N		0.00	1.00	0.00	4.25
289	N		2.32	0.85	0.00	0.00
295		N	0.00	0.00	0.00	0.00
301	N		0.00	0.00	0.00	0.00
332	N		0.00	0.00	0.00	0.00
339		N	0.00	0.00	0.00	0.00
356	N	N	0.00	0.35	0.00	0.00
386	N	N	0.00	0.00	0.00	0.00
397	N	N	0.00	0.07	0.00	0.00
405	N		0.00	0.00	0.00	0.00
406		N	0.00	0.00	0.00	0.00
409	N		0.00	0.00	0.00	0.00
413		N	0.00	0.00	0.00	0.00
448	N	N	2.19	9.35	1.10	8.24
463	N	N	7.64	6.17	7.72	5.92

Table 2.8. Antibody binding to peptides at PNGS locations.
Pepscanning of polyclonal antibodies raised against rgp120 immunogens was performed by peptide microarray. EN6_226 rgp120 and MN rgp120 sequences were aligned to HXB2 reference with MUSCLE (Edgar, 2004). All EN6_226 and MN rgp120 PNGS locations are listed in columns 2 and 3. Median antibody binding signals are shown for peptides containing residues at PNGS locations.

Next, we compared antibody binding signals to determine which immunogen elicited stronger responses to sites of interest, including the V2 peptide that correlated with protection in the RV144 study. MN rgp120-raised antibodies showed stronger binding to V2 peptides from each clade and the aggregated of all clades, with the exception of clade A (Table 2.9). EN6_226 rgp120-raised antibodies elicited a stronger response to the clade A V2 peptide (Table 2.9), which was the only sequence in this region that did not correlate with protection (Gottardo et al., 2013).

Antibody binding to V2 peptides (RV144 correlate of protection)

Source	Sequence	anti-EN6 sera (binding signal)	anti-MN sera (binding signal)
A.con	TELRDKKQKVYSLFY	1.23	0.82
A244/TH023	TELRDKKQKVHALFY	0.00	0.00
CRF01.AE/CRF02_AG	TELRDKKQKVYALFY	0.79	0.88
B.con	TSIRDKVQKEYALFY	1.37	3.98
M.con/C.con	TEIRDKKQKVYALFY	0.98	0.99
D.con	TEVRDKKKQVYALFY	0.48	0.65
Aggregate	N/A	0.79	0.88

Table 2.9. Antibody binding to V2 peptides (RV144 correlate of protection). Pepscanning of polyclonal antibodies raised against rgp120 immunogens was performed by peptide microarray. Median binding signals are shown for antibodies raised against EN6_226 or MN rgp120s to V2 peptides from each major clade and an aggregate of all clades tested.

To determine which immunogen elicited a stronger response to sites of vulnerability, we compared antibody binding to peptides from the PG9, PGT128, and VRC01 epitopes, which target the V1/V2 region, V3 region, and CD4-binding site, respectively. EN6_226 rgp120-raised antibodies had higher binding signals to clade B

peptides from the V3-targeting PGT128 epitope. Conversely, MN rgp120-raised antibodies showed stronger binding to clade B peptides in the V2-targeting PG9 epitope and CD4-binding site. Binding signal from an aggregate of all clades showed the same results.

A

Antibody binding to sites of vulnerability (peptides from clade B consensus sequence)

Site of vulnerability	Peptide	HXB2 range	anti-EN6 sera (binding signal)	anti-MN sera (binding signal)
V2 region (PG9 contacts)	IKNCSFNITTSIRDK	154 - 168	0.00	0.00
	CSFNITTSIRDKVQK	157 - 171	0.00	0.11
	NITTSIRDKVQKEYA	160 - 174	0.00	2.75
V3 region (PGT128 contacts)	RPNNNTRKSIHIGPG	298 - 312	5.36	0.00
	NNTRKSIHIGPGRAF	301 - 315	5.50	5.61
	RKSIHIGPGRAFYAT	304 - 318	7.00	6.11
	IHIGPGRAFYATGDI	307 - 321	2.50	1.23
	GPGRAFYATGDIIGD	312 - 326	0.00	0.35
	RAFYATGDIIGDIRQ	315 - 329	8.24	0.92
	YATGDIIGDIRQAHC	318 - 332	8.34	0.00
	GDIIGDIRQAHCNIS	321 - 335	6.95	0.00
CD4-binding site (VRC01 contacts)	SENFTDNAKTIIVQL	274 - 288	0.00	0.00
	FNQSSGGDPEIVMHS	361 - 375	1.28	3.15
	SSGGDPEIVMHSFNC	364 - 378	0.51	1.74
	KQIINMWQEVGKAMY	421 - 435	0.00	0.46
	GLLLTRDGGNNNNTT	451 - 465	0.00	3.44
	ETFRPGGGDMRDNWR	466 - 480	6.63	8.75

B

Cross-reactivity to sites of vulnerability
(aggregate antibody binding signal from 12 sequences)

Site of vulnerability	HXB2 range	anti-EN6 sera (binding signal)	anti-MN sera (binding signal)
V2 region (PG9 contact sites)	154 - 168	0.00	0.00
	157 - 171	0.00	0.00
	160 - 174	0.00	0.00
V3 region (PGT128 contact sites)	298 - 312	5.36	0.62
	301 - 315	3.68	0.00
	304 - 318	4.80	2.33
	307 - 321	0.00	0.00
	312 - 326	0.00	0.00
	315 - 329	5.70	0.00
	318 - 332	7.07	0.00
	321 - 335	6.61	0.00
CD4-binding site (VRC01 contact sites)	274 - 288	0.23	0.00
	361 - 375	0.00	5.20
	364 - 378	0.00	1.33
	421 - 435	0.00	0.00
	451 - 465	0.00	3.31
	466 - 480	7.51	8.49

Table 2.10. Antibody binding to sites of vulnerability.
Pepscanning of polyclonal antibodies raised against rgp120 immunogens was performed by peptide microarray. Binding signals are shown for antibodies raised against EN6_226 or MN rgp120s. (A) Median antibody binding signals to clade B peptides from PG9, PGT128, and VRC01 contact sites. (B) Median antibody binding signals to all peptides from PG9, PGT128, and VRC01 contact sites.

Discussion

In these studies, we consider the possibility that Envs from rare individuals who produced potent bNAbs and controlled their infection without ART (EN/controller phenotype) might possess unusual features that contributed to the formation of effective antiviral immune responses. To identify EN/controllers for this study, we first screened a cohort of ART-naïve controllers for neutralization breadth. Consistent with previous studies, elite controllers as a group exhibited lower neutralization potency and breadth compared to viremic controllers in the cohort (Bailey et al., 2006; Doria-Rose et al., 2010, p., 2009; González et al., 2018; Lambotte et al., 2009; Pereyra et al., 2008). Because elite controllers, by definition, possess low or undetectable viral loads (<50 HIV RNA copies/ml)(Okulicz et al., 2009), the amount of circulating immunogen is usually insufficient to drive the antibody affinity maturation process that appears necessary to develop bNAbs (Corti et al., 2010; Scheid et al., 2009; Walker et al., 2009, p. 2; Wu et al., 2010; Xiao et al., 2009a, 2009b). In contrast, viremic controllers (100 – 1,000 HIV RNA copies/ml) have viral loads that dip and rise, providing antigenic stimulation that leads to neutralization breadth at similar rates to non-controllers (Bailey et al., 2006; Doria-Rose et al., 2010). From the EN/controllers identified, we further characterized EN6, who exhibited elite neutralizing activity (found in <1% of HIV-positive individuals (Simek et al., 2009)) and maintained viremic control for over 17.5 years without antiretroviral therapy (found in <2% of HIV-positive individuals (Okulicz et al., 2009)).

Our study enabled us to identify two major structural features that may have enhanced the protective immune response in EN6. The first was a highly unusual and statistically significant decrease in the number of PNGS (17) compared to the normal number of PNGS (26) found in most gp120s. A reduction in the number of N-linked glycosylation sites would be expected to expand glycan holes, exposing regions of protein surface that are normally shielded from the immune system by glycans, and increase immunogenicity (Zhou et al., 2017). Notably, the Env retained PNGS at conserved sites targeted by several prototypic bN-mAbs, including N156, N160, N301, and N332 (Julien et al., 2013b, p. 121; Kong et al., 2015, p. 16; McLellan et al., 2011, p. 9; Pejchal et al., 2011). The region of Env with the greatest reduction of PNGS was V1, which has been called the "global regulator" of neutralization (Pinter et al., 2004). The V1/V2 domain is known to be the initial target for autologous neutralizing antibodies as well as bN-mAbs, such as PG9 and PG16 (McLellan et al., 2011). Additionally, changes in the V1/V2 domain affect CD4 binding to monoclonal antibodies that facilitate ADCC, such as A32 (Prévost et al., 2018).

The second unusual structural feature we observed in this individual was the presence of two non-conserved cysteine residues in an elongated V1 region, which is found in only 5.5% of clade B Env sequences (van den Kerkhof et al., 2016). While the vast majority of HIV Env proteins possess 18 cysteines that form 9 disulfide bridges, the proviral sequences from EN6 possessed 20 cysteines. The two additional cysteines can potentially form a disulfide loop in a conventional thumb and mitt structure or contribute to a wide variety of alternative disulfide bonds by pairing with

other cysteine residues that contribute to the normal disulfide structure described by Leonard et al (Leonard et al., 1990). In previous studies, we identified Env proteins with 20 Cys in subjects that participated in the VAX004 clinical trials (Jobes et al., 2006). The extra Cys residues frequently occurred as an insertion in the V1 region between positions 131 and 144 (Jobes et al., 2006). Aberrant cysteines in the V1 region were recently found in two elite neutralizers (S6 Table)(van den Kerkhof et al., 2016) and an elite controller (Silver et al., 2019). Significantly, we also identified Envs possessing 20 Cys in another elite controller with broadly neutralizing activity (EN3) from the SCOPE cohort (Hutchinson et al., 2019). In these studies, there is some evidence that epistatic mutations are required to accommodate the unusual cysteines in order to preserve infectivity, suggesting a functional role (Hutchinson et al., 2019; Silver et al., 2019; van den Kerkhof et al., 2016). However, it remains unclear if cysteines are introduced stochastically, possibly from a duplication event as described in van den Kerkhoff et al, 2016, or if they are a result of selection pressure. The effect of non-canonical cysteines in V1, separate from length and glycosylation, warrants further study.

Two distinct Env variants were identified in EN6, proviral Envs with long, heavily glycosylated V1 regions with additional cysteines and plasma Envs with minimal glycosylation and short V1 regions. These two variants may reflect dual pressure to mediate immune escape and transmission fitness, respectively. The long, heavily glycosylated V1 regions in proviral Envs is a mechanism for early viral escape from strain-specific neutralizing antibodies and several known bN-mAbs

(Bunnik et al., 2008; Krachmarov et al., 2005, 2006; Liu et al., 2011; Mikell et al., 2011; O'Rourke et al., 2015; Richman et al., 2003; Rong et al., 2007; Sagar et al., 2006; van den Kerkhof et al., 2016; van Gils et al., 2011). In contrast, the plasma Envs share similar features to the transmitter/founder phenotype, where the short, unglycosylated V1 region may improve infectivity at the expense of exposing neutralizing epitopes (Chohan et al., 2005; Dacheux et al., 2004; Derdeyn et al., 2004; Wu et al., 2006). Indeed, plasma-derived Envs were sensitive to neutralization by V2-glycan targeting bN-mAbs (PG9 and PG16) and CD4-binding site-targeting bN-mAb (VRC01) while the proviral-derived Envs were resistant.

The primary goal of this study was to identify an Env that may elicit the production of bNAbs. Since bNAbs usually do not appear until two years post infection (Sather et al., 2009) and with virus rapidly escaping the antibody response, contemporaneous Envs are unlikely to have stimulated the production of bNAbs. One approach to identify the species likely to have elicited bNAbs is to carry out long term longitudinal studies (Doria-Rose et al., 2014; Gray et al., 2011; Landais et al., 2016) or evolutionary reconstructions (Mesa et al., 2019) to identify species that occurred immediately prior to the appearance of bNAbs. Here we describe another approach to this problem where we take advantage of sequences from rare individuals able to control virus replication and may exhibit lower Env diversity. Analysis of the first individual that we studied with the unusual EN/controller phenotype showed a low level of Env sequence variation (98.5% sequence identity) and a low number of PNGS in gp120 (17 PNGS) from the plasma virus. When expressed, we found that

Envs were infectious and elicited antibody responses that appeared to be superior to antibody responses elicited by two other clade B Envs, including MN rgp120 used in previous vaccine studies. Taken together, our results suggest that further studies of the Envs from individuals with the dual EN/controller phenotype are warranted. The immunological potential of the Envs we identified here will require comparing the immunogenicity of alternate immunogen structures, such as trimeric gp140s (Sanders et al., 2015), and alternate immunization strategies, such as prime boosting with virus vectors (Hansen et al., 2013) or guided immunization (Haynes et al., 2012).

Materials and methods

Clinical samples

Plasma and non-viable peripheral blood mononuclear cell (PBMC) samples were provided by the Women's Interagency HIV Study (WIHS, Baltimore, MD) after review and approval of this study. The WIHS is a multicentered longitudinal study of HIV-infected women in the United States (Bacon et al., 2005; Barkan, 1998). All WIHS participants provided written informed consent, including permission to store and use specimens for research. The University of California, San Francisco Institutional Review Board approved consent forms and protocols for the Northern California location. The initial recruitment interview included self-identification of race and sex. After enrollment, participants visited a research site every six months for an interview, clinical examination, and blood draws. Blood specimens from each

visit were used for laboratory testing, which included viral load and CD4+ cell count, and stored in the WIHS repository.

A subset of 53 HIV-positive ART-naïve African American women was selected based on their ability to control viral load and CD4+ cell count for prolonged periods without ART. Individuals were categorized into four groups, elite controllers (<100 HIV RNA copies/ml for three consecutive visits over >12 months), viremic controllers (100 – 1,000 HIV RNA copies/ml for three consecutive visits over >12 months), long-term nonprogressors (no periods of elite or viremic control and >500 CD4+ cells/µl for at least 7 years), and normal progressors (no periods of elite or viremic control nor 7+ year period with >500 CD4+ cells/µl). From each individual, de-identified cryopreserved plasma samples from two visits were selected and assayed for neutralization potency and breadth against Env-recombinant viruses. Five individuals capable of neutralizing pseudoviruses from at least 4/5 clades were selected and specimens (plasma and PBMCs) from the earlier of the two visits were used for subsequent analyses.

Neutralization assays

Initial virus neutralization assays were performed at Monogram Biosciences (South San Francisco, CA) with a previously described panel of five viruses useful for detecting elite neutralizers (Simek panel)(Frost et al., 2005; Richman et al., 2003; Simek et al., 2009). This assay utilized Env-recombinant pseudoviruses generated from patient samples or a panel of well-characterized viruses. A second panel of

twenty-two pseudoviruses representing all major subtypes of HIV-1 (clades A, B, C, D, and AE) was used to characterize the antibodies in plasma from individuals possessing bNAbs as indicated by the Simek panel. A panel of broadly neutralizing monoclonal antibodies (bN-mAbs) was also assembled that contained bN-mAbs PG9, PG16, PGT121, PGT128, and VRC01 that were provided by the NIH AIDS Reagent Program, the NIH Vaccine Research Center Polymun Scientific (Vienna, Austria), or synthesized in our lab based on published sequences. Neutralization antibody titers were calculated as the reciprocal plasma dilution (ID50) or reciprocal mAb concentration (IC50) conferring 50% inhibition, as measured by luciferase activity. Positive neutralization calls required an ID50 or IC50 that is at least three-fold greater than the specificity control, a pseudovirus made with the amphotropic murine leukemia virus (aMLV) envelope protein. Pseudoviruses with clade B NL4-3 and JRCSF rgp160s were included as CXCR4- and CCR5-dependent positive controls, respectively. Broadly neutralizing plasma (Z23) was included in all assays as an additional positive control. This assay has been validated and widely used in HIV research (Simek et al., 2009; Walker et al., 2011, 2009).

Rabbit sera to recombinant Env proteins was tested with a standard TZM-bl neutralization protocol and performed on two separate days at Duke University (Durham, NC). Positive neutralization calls required 50% inhibition. SVA-MLV was used as a specificity control and CH01-31 was used as a positive control. This assay has been optimized and validated for accurate result, as previously described (Montefiori, 2005; Sarzotti-Kelsoe et al., 2014).

Sequencing and coreceptor usage

Sequencing and coreceptor usage assays were performed at Monogram Biosciences (South San Francisco, CA) with a previously described protocol (Whitcomb et al., 2007). Briefly, *env* libraries generated from patient plasma or PBMCs were used to make pseudoviruses. Pseudoviruses were then grown and screened for infectivity. Full-length *envs* from 10 plasma-derived and 10 PBMC-derived infectious pseudoviruses were sequenced. Chemokine receptor usage of the 20 sequenced pseudoviruses was determined by inoculating U87 expressing CD4+ and either CCR5+ or CXCR4+ and measuring luciferase activity. This coreceptor usage assay has been validated in accordance with Clinical Laboratory Improvement Amendments (CLIA) regulations.

Datasets and statistical analysis

Patient-derived gp160s were compared to the Los Alamos National Laboratory HIV Sequence Database Filtered web alignment for clade B Env proteins curated in 2017 ("HIV sequence database main page," n.d.). Statistical analyses were performed in GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA) using the nonparametric Kruskal-Wallis and Mann-Whitney U tests.

Expression and purification of recombinant gp120

Codon-optimized gp120 fusion genes were designed with an N-terminal purification tag gD-1, herpes simplex virus 1 glycoprotein D (O'Rourke et al., 2019). Genes were synthesized (Invitrogen, Inc., Carlsbad, CA) and inserted into a pCF vector with a cytomegalovirus promoter using Gibson Assembly® (New England Biolabs, Inc., Ipswich, MA). Recombinant plasmids were verified with Sanger sequencing (UC Berkeley, Berkeley, CA), transformed into 5-alpha Competent E. coli cells (New England Biolabs, Inc., Ipswich, MA), then purified (QIAGEN, Hilden, Germany). HEK293S GnTI$^-$ cells (ATCC® CRL-3022™), that lack N-acetylglucosaminyltransferase-1 (Reeves et al., 2002), were transfected using Maxcyte electroporation system HEK293 protocol (MaxCyte STX®, MaxCyte, Inc., Gaithersburg, MD). The cells were grown in shake flask cultures (FreeStyle™ 293 Expression Medium, Invitrogen Inc., Waltham, MA) and harvested five days after transfection. Secreted gp120 was purified by immunoaffinity chromatography (O'Rourke et al., 2019) followed by size exclusion chromatography (Sephacryl S-200, GE Healthcare Life Sciences, Marlborough, MA).

Growth conditioned cell culture supernatants with and without 25mM dithiothreitol (DTT) were run on a NuPAGE™ 4-12% Bis-Tris precast gel (Invitrogen™, Carlsbad, CA) in MES running buffer (Invitrogen™, Carlsbad, CA). Protein was transferred to a polyvinylidene fluoride (PDVF) membrane using standard protocol for iBlot (ThermoFisher Scientific, Waltham, MA). Membrane was blocked in 5% milk, probed with 0.2 μg/ml mouse anti-gD-1 monoclonal antibody

34.1 (lot A16.02, UC Santa Cruz, Santa Cruz, CA)(O'Rourke et al., 2019), then 1:5,000 dilution of HRP-conjugated anti-mouse secondary antibody (Jackson ImmunoResearch, West Grove, PA). Antibody was detected using WesternBright reagents (Advansta, Menlo Park, CA) and visualized using Innotech FluoChem2 system (Genetic Technologies, Grover, MO).

Antibody binding assays

Antibody binding to rgp120 was assessed using fluorescent immunoassay (FIA). 96 well black microplates (Greiner Bio-One, Monroe, NC) were coated with 2 μg/ml of purified mouse anti-gD-1 mAb 34.1 (lot A69, UC Santa Cruz, Santa Cruz, CA) in PBS overnight at 4°C. Plates were blocked with 2.5% BSA in PBS for 4 h on a shaker at room temperature. Purified EN6_226 rgp120 at 2 μg/ml in PBST was captured overnight on a shaker at room temperature. Primary antibodies were added in duplicate serial 1:3 dilutions starting with either undiluted rabbit sera or 10 μg/ml of bN-mAbs PG16 (lot 130229, NIH AIDS Reagent Program, Germantown, MD), PG9, PGT121, PGT128, VRC01 (lots A62, A9, A32, A27, respectively, UC Santa Cruz, Santa Cruz, CA) in PBST and incubated on a shaker at room temperature for 1.5 h. Purified rabbit polyclonal antibodies raised against MN and A244 rgp120s from a previous immunization study, PB94 (lot A19.3, UC Santa Cruz, Santa Cruz, CA), were included as a positive control. Blank (no antigen) or pre-immune rabbit sera were used as negative controls. Dilutions of 1:5000 goat anti-human Alexa Flour® 488-conjugated IgG Fcγ polyclonal Ab (lot 137248, Jackson ImmunoResearch

Laboratories, Inc, West Grove, PA) or goat anti-rabbit Alexa Flour® 488-conjugated IgG (H+L) polyclonal Ab (lot 1981125, Invitrogen, Inc., Carlsbad, CA) were incubated on a shaker at room temperature for 1 h. Between each step, plates were washed four times in PBST. Plates were visualized using EnVision™ Multilabel Plate Reader (PerkinElmer, Inc., Waltham, MA) with FITC 485 excitation and 535 emission filters.

Rabbit immunization studies

Rabbit immunization studies were conducted in accordance with the Animal Welfare Act at Pocono Rabbit Farm & Laboratory (Canadensis, PA), a facility fully accredited by Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC 925) and assured by the National Institute of Health (NIH) and Office of Laboratory Animal Welfare (OLAW A3886-01). The Pocono Rabbit Farm & Laboratory Institutional Animal Care and Use Committee (IACUC) approved the immunization protocol (PRF2A).

For immunogens rgp120 EN6_226 and EN3_071, five New Zealand White rabbits were immunized with 200 µg/dose produced in HEK293S GnTI$^-$ cells (ATCC® CRL-3022™), delivered intradermally on days 0, 28, 56, and 140. The primary immunization was formulated with Complete Freund's Adjuvant (CFA) while the subsequent three boosts were formulated with Incomplete Freund's Adjuvant (IFA). Bleeds were taken from each rabbit on days 0 (pre-immune), 42, 70, and 147. Control rabbit sera to MN rgp120 was produced in two New Zealand White

rabbits immunized according to a standard protocol provided by Pocano Labs designed to elicit high affinity antibodies (PRF2A).

Peptide microarray

Peptide microarray was performed at Duke University (Durham, NC). Serial dilutions of rabbit sera starting at 1:50 were tested against 15-mer peptides overlapping by 12 and covering consensus gp160s for clade A, B, C, D, group M, CRF1, and CRF2 and vaccine gp120s 1.A244, 1.TH023, MN, C.1086, C.TV1, and C.ZM651 (library 2738, JPT Peptide Technologies, Berlin, Germany). Binding was detected with 1:500 dilution of goat anti-rabbit IgG Alexa Fluor® 647 polyclonal Ab and visualized with InnoScan function XDR (Stevanato Group, Padua, Italy). Microarray data was processed using R package pepStat (Imholte et al, 2013) to determine intensity for each peptide. For EN6_226, the magnitude of binding (signal) was measured as $\log_2$ (intensity of the post-immunization sample / intensity of the pre-immunization sample). For MN, the magnitude of binding was measured as $\log_2$ (intensity of the post-immunization sample / 50^{th} percentile value).

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

Sequences described in this study have been deposited in GenBank with accession numbers MN516423-MN516442 (EN3).

Author Contributions

PB designed study. JH and KM contributed to construct design. JH performed sequence alignments, predictive modeling, cloning, binding assays, and data analysis. SO and JH produced protein. BY and LY purified protein. TW and YL performed virus screening, initial sequencing, tropism and gp160 neutralization assays. DM and CL performed rabbit sera neutralization assays. GT and SS performed peptide microarray.

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Supplementary information

Patient info		Neutralization titer (ID50 in 1/dilution)							
UCSC ID	Visit	Clade A 94UG-103	Clade B 92BR-020	Clade C 93IN-905	Clade C M-C-026	Clade AE 92TH-021	Pos ctrl JRCSF	Pos ctrl NL43	Neg ctrl aMLV
EC01	A	<20	40	<20	23	<20	<20	**62**	<20
	B	<20	**45**	<20	<20	<20	<20	**70**	<20
EC02	A	<20	**84**	**90**	**122**	25	**59**	**330**	<20
	B	21	**120**	**72**	42	22	**52**	**490**	<20
EC03	A	<20	27	<20	<20	<20	<20	**48**	<20
	B	<20	25	26	22	<20	<20	**63**	<20
EC04	A	<20	<20	<20	<20	<20	<20	28	<20
	B	<20	26	<20	<20	<20	<20	**250**	<20
EC05	A	<20	**46**	24	30	<20	<20	**458**	<20
	B	<20	**87**	<20	<20	<20	21	**920**	<20
EC06	A	<20	**41**	21	<20	<20	<20	**152**	<20
	B	<20	43	25	<20	21	24	**113**	<20
EC07	A	32	45	61	26	36	21	**762**	26
	B	21	**41**	**46**	<20	<20	<20	**628**	<20
EC08	A	<20	<20	<20	<20	21	<20	37	<20
	B	<20	<20	<20	<20	<20	<20	**40**	<20
EC09	A	28	**79**	**254**	25	53	23	**555**	23
	B	22	**124**	**298**	21	47	29	**732**	24
EC10	A	30	**103**	48	29	24	**61**	**752**	<20
	B	25	**195**	**76**	<20	29	**91**	**1124**	<20
EC11	A	<20	<20	<20	<20	<20	<20	<20	<20
	B	<20	<20	<20	<20	<20	<20	<20	<20
EC12	A	<20	<20	<20	<20	<20	<20	<20	<20
	B	<20	<20	<20	<20	<20	<20	<20	<20
EC13	A	<20	41	35	<20	<20	<20	**274**	<20
	B	28	54	45	35	25	25	**268**	<20
EC14	A	<20	**25**	<20	<20	<20	<20	**26**	<20
	B	<20	**63**	**27**	<20	<20	<20	**47**	<20
EC15	A	<20	**56**	<20	<20	<20	**63**	**369**	<20
	B	<20	**53**	<20	<20	<20	**72**	**289**	<20
EC16	A	27	**55**	32	<20	20	23	**116**	<20
	B	<20	**70**	<20	<20	<20	**37**	**270**	<20
EC17	A	<20	<20	<20	<20	<20	<20	<20	<20
	B	<20	20	**25**	<20	<20	<20	**84**	<20
EC18	A	<20	**34**	**31**	**23**	<20	<20	**334**	<20
	B	<20	**28**	**24**	**26**	20	<20	**421**	<20
EC19	A	<20	<20	**24**	<20	<20	<20	**111**	<20
	B	<20	<20	<20	<20	<20	<20	**166**	<20
EC20	A	<20	**96**	27	<20	<20	37	**92**	<20
	B	<20	**151**	**28**	<20	<20	**40**	**142**	<20
EC21	A	<20	<20	<20	<20	<20	**24**	**86**	<20
	B	<20	<20	<20	<20	<20	<20	**55**	<20
EC22	A	**58**	**123**	**27**	<20	<20	**84**	**147**	<20
	B	<20	**122**	21	<20	<20	**51**	**144**	<20
EC23	A	<20	<20	**59**	<20	<20	34	**382**	<20
	B	28	25	**61**	27	26	**66**	**367**	<20
EC24	A	<20	**150**	**27**	**164**	<20	<20	**188**	<20
	B	<20	**167**	**31**	**119**	<20	**26**	**163**	<20
EC25	A	22	**138**	**172**	**51**	<20	**42**	**686**	<20
	B	<20	**159**	**169**	**34**	**26**	**37**	**1330**	<20
EC26	A	**28**	**348**	**36**	<20	**48**	**95**	**363**	<20
	B	<20	**232**	**29**	<20	<20	**48**	**203**	<20
EC27	A	**26**	**76**	**65**	20	<20	**66**	**368**	<20
	B	25	**41**	31	<20	<20	34	**151**	<20
EC28	A	<20	**51**	**79**	**50**	<20	<20	**462**	<20
	B	<20	**141**	**230**	**84**	<20	30	**1173**	<20
Pos ctrl Z23	N/A	**136**	**241**	**244**	**226**	**162**	**258**	**1383**	<100

2.S1 Table. Neutralization potency and breadth in 28 elite controllers.
Cryopreserved, de-identified plasma from 28 elite controllers were obtained and tested for the presence of virus neutralizing antibodies using a panel of pseudotype viruses. Elite controllers are defined as HIV-positive individuals with <100 HIV RNA copies/ml for three consecutive visits over >12 months. For each individual, samples from two time points were assayed against pseudoviruses from a standard panel of five Envs representing global strains. Assays included positive control Z23, plasma containing broadly neutralizing antibodies, and average titers are shown in the table. ID50 titers are defined as the reciprocal of the plasma dilution that inhibits 50% luciferase activity in U87 cells. Bold values indicate ID50s at least three times greater than the negative control (aMLV).

Patient info		Neutralization titer (ID50 in 1/dilution)							
UCSC ID	Visit	Clade A 94UG-103	Clade B 92BR-020	Clade C 93IN-905	Clade C M-C-026	Clade AE 92TH-021	Pos ctrl JRCSF	Pos ctrl NL43	Neg ctrl aMLV
VC01	A	<20	**64**	**43**	<20	<20	**22**	**197**	<20
	B	22	**66**	**53**	<20	22	29	**256**	<20
VC02	A	<20	32	<20	<20	<20	<20	**86**	<20
	B	<20	**24**	<20	<20	<20	<20	**99**	<20
VC03	A	**64**	**64**	**78**	33	20	36	**826**	<20
	B	**28**	**94**	**113**	**28**	<20	**51**	**948**	<20
VC04	A	**189**	**935**	**988**	**585**	**108**	**425**	**2844**	<20
	B	**84**	**515**	**345**	**121**	**72**	**263**	**3307**	<20
VC05	A	21	**144**	**119**	**91**	22	**49**	**600**	<20
	B	25	**109**	**164**	**142**	**42**	**58**	**905**	<20
VC06	A	<20	<20	35	<20	<20	**115**	**271**	<20
	B	29	<20	42	<20	36	**77**	**322**	22
VC07	A	<20	<20	22	<20	20	<20	**58**	<20
	B	24	37	46	<20	31	31	**190**	24
VC08	A	<20	**61**	**41**	<20	**32**	**35**	**382**	<20
	B	**29**	**88**	**134**	**21**	**60**	**81**	**838**	<20
VC09	A	<20	**145**	**32**	<20	<20	**38**	**1207**	<20
	B	<20	**267**	**23**	<20	**27**	**36**	**1679**	<20
VC10	A	**94**	**1812**	**3011**	**2482**	**52**	**2253**	**1111**	<20
	B	**117**	**1544**	**2557**	**2107**	**54**	**2016**	**1343**	<20
VC11	A	**52**	**85**	**120**	**149**	**29**	**83**	**582**	<20
	B	**71**	**80**	**76**	**136**	23	**72**	**580**	<20
VC12	A	<20	**84**	<20	<20	<20	<20	**145**	<20
	B	<20	**69**	**21**	<20	<20	<20	**128**	<20
VC13	A	<20	<20	**28**	<20	<20	<20	**510**	<20
	B	**27**	**33**	**25**	**24**	<20	**26**	**997**	<20
VC14	A	**27**	**147**	**129**	**110**	**164**	**67**	**4073**	<20
	B	**74**	**237**	**373**	**337**	**585**	**191**	**4433**	<20
EN6	A	**376**	**365**	**900**	**754**	**165**	**559**	**2271**	<20
	B	**95**	**168**	**582**	**373**	**81**	**215**	**891**	<20
Pos ctrl Z23	N/A	**136**	**241**	**244**	**226**	**162**	**258**	**1383**	<100

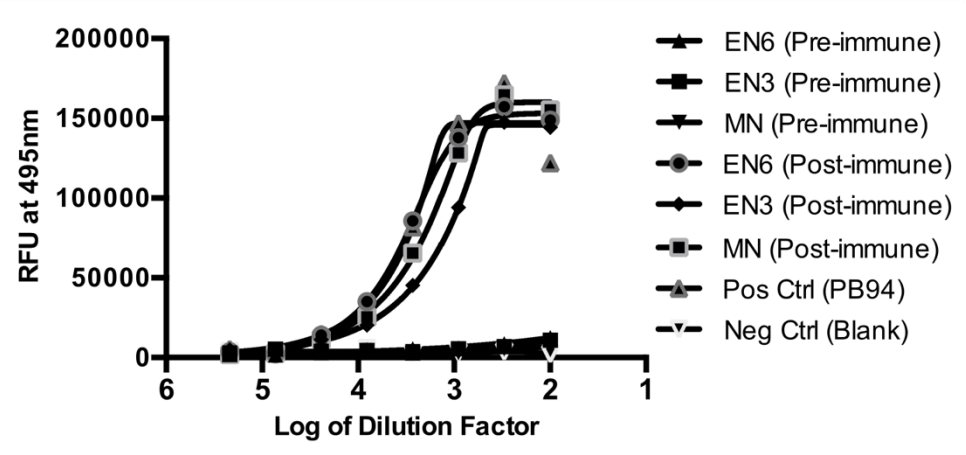
2.S2 Table. Neutralization potency and breadth in 15 viremic controllers. Cryopreserved, de-identified plasma from 15 viremic controllers were obtained and tested for the presence of virus neutralizing antibodies using a panel of pseudotype viruses. Viremic controllers are defined as HIV-positive individuals with 100 - 1,000 HIV RNA copies/ml for three consecutive visits over >12 months. For each individual, samples from two time points were assayed against pseudoviruses from a standard panel of five Envs representing global strains. Assays included positive control Z23, plasma containing broadly neutralizing antibodies, and average titers are shown in the table. ID50 titers are defined as the reciprocal of the plasma dilution that inhibits 50% luciferase activity in U87 cells. Bold values indicate ID50s at least three times greater than the negative control (aMLV).

Patient info		Neutralization titer (ID50 in 1/dilution)							
UCSC ID	Visit	Clade A 94UG-103	Clade B 92BR-020	Clade C 93IN-905	Clade C M-C-026	Clade AE 92TH-021	Pos ctrl JRCSF	Pos ctrl NL43	Neg ctrl aMLV
LTNP01	A	21	**189**	**99**	25	<20	35	**527**	<20
	B	31	**200**	**114**	22	<20	**43**	**481**	<20
LTNP02	A	<20	<20	**32**	<20	<20	<20	**144**	<20
	B	<20	**21**	<20	<20	<20	<20	**228**	<20
LTNP03	A	**32**	**60**	**96**	**115**	**29**	23	**418**	<20
	B	36	51	**120**	**125**	36	27	**461**	<20
LTNP04	A	20	**99**	**24**	<20	<20	<20	**177**	<20
	B	**35**	**92**	**48**	<20	<20	**24**	**270**	<20
Pos ctrl Z23	N/A	**136**	**241**	**244**	**226**	**162**	**258**	**1383**	<100

2.S3 Table. Neutralization potency and breadth in 4 long-term nonprogressors. Cryopreserved, de-identified plasma from 4 long-term nonprogressors were obtained and tested for the presence of virus neutralizing antibodies using a panel of pseudotype viruses. Long-term nonprogressors are defined as HIV-positive individuals with no periods of elite or viremic control and > 500 CD4+ cells/μl for at least 7 years. For each individual, samples from two time points were assayed against pseudoviruses from a standard panel of five Envs representing global strains. Assays included positive control Z23, plasma containing broadly neutralizing antibodies, and average titers are shown in the table. ID50 titers are defined as the reciprocal of the plasma dilution that inhibits 50% luciferase activity in U87 cells. Bold values indicate ID50s at least three times greater than the negative control (aMLV).

Patient info		Neutralization titer (ID50 in 1/dilution)							
UCSC ID	Visit	Clade A 94UG-103	Clade B 92BR-020	Clade C 93IN-905	Clade C M-C-026	Clade AE 92TH-021	Pos ctrl JRCSF	Pos ctrl NL43	Neg ctrl aMLV
NP01	A	21	**75**	<20	<20	<20	**92**	**245**	<20
	B	<20	**68**	**29**	<20	<20	**47**	**183**	<20
NP02	A	<20	**48**	**61**	**73**	<20	<20	**252**	<20
	B	<20	**77**	**96**	**89**	21	23	**362**	<20
NP03	A	23	**55**	32	<20	<20	<20	**113**	<20
	B	<20	**57**	30	<20	<20	<20	**117**	<20
NP04	A	**31**	**44**	**68**	**31**	20	**65**	**263**	<20
	B	**78**	**79**	**86**	**53**	**53**	**85**	**243**	<20
NP05	A	<20	**45**	**23**	<20	<20	**21**	**389**	<20
	B	35	**78**	31	<20	**39**	27	**284**	<20
EN7	A	**120**	**404**	**828**	**415**	<20	**115**	**296**	<20
	B	**81**	**185**	**432**	**286**	<20	**67**	**262**	<20
Pos ctrl Z23	N/A	**136**	**241**	**244**	**226**	**162**	**258**	**1383**	<100

2.S4 Table. Neutralization potency and breadth in 6 normal progressors.
Cryopreserved, de-identified plasma from 5 normal progressors were obtained and tested for the presence of virus neutralizing antibodies using a panel of pseudotype viruses. Normal progressors are defined as HIV-positive individuals with no periods of elite or viremic control nor a 7+ year period with > 500 CD4+ cells/µl. For each individual, samples from two time points were assayed against pseudoviruses from a standard panel of five Envs representing global strains. Assays included positive control Z23, plasma containing broadly neutralizing antibodies, and average titers are shown in the table. ID50 titers are defined as the reciprocal of the plasma dilution that inhibits 50% luciferase activity in U87 cells. Bold values indicate ID50s at least three times greater than the negative control (aMLV).



2.S5 Fig. Comparison of rabbit antibody titers elicited by rgp120s from controllers (EN6_226 and EN3_071) and MN rgp120.
Recombinant gp120s (EN6_226 and EN3_071) expressed in HEK293 GnTI$^-$ cells were purified and used to immunize rabbits. The resulting sera were tested for antibody binding to antisera prepared against MN rgp120. Purified EN6_226 rgp120s fusion protein was captured with mouse anti-gD-1 mAb (targeted the N-terminal tag). Purified rabbit polyclonal antibodies raised against MN and A244 rgp120s from a previous immunization study, PB94, were included as a positive control. Pre-immune blood sera and no primary antibody served as a negative control. Antibodies raised against EN6_226, EN3_071, MN rgp120 or positive control showed similar binding to purified EN6_226 rgp120 (IC50s = 3.1μl/ml, 1.3μl/ml, 2.0μl/ml, and 3.0μl/ml, respectively).

Region	HXB2 #	Source	Amino acids (median)		PNGS (median)		Cysteines (median)	
			EN6	Ref	EN6	Ref	EN6	Ref
Signal	1 – 30	PBMCs	29	29	0	0	1	1
		Plasma	29		0		1	
C1	31 – 130	PBMCs	100	100	**3***	2	4	4
		Plasma	100		**3***		4	
V1	131 – 157	PBMCs	**40***	29	5	4	**4***	2
		Plasma	**43***		**6.5***		2	
V2	158 – 196	PBMCs	39	40	2	2	1	1
		Plasma	39		2		1	
V1/V2	131 – 196	PBMCs	**79***	71	7	6	**5***	3
		Plasma	**82***		**8.5***		3	
C2	197 – 295	PBMCs	99	99	7	7	5	5
		Plasma	99		6.5		5	
V3	296 – 331	PBMCs	35	35	1	1	2	2
		Plasma	35		1		2	
C3	332 – 384	PBMCs	52	52	4	3	1	1
		Plasma	52		3.5		1	
V4	385 – 418	PBMCs	34	32	**3***	5	2	2
		Plasma	34		3.5		2	
C4	419 – 459	PBMCs	41	41	1	1	1	1
		Plasma	41		1		1	
V5	460 – 469	PBMCs	10	11	2	2	0	0
		Plasma	10		2		0	
C5	470 – 511	PBMCs	42	42	0	0	0	0
		Plasma	42		0		0	
gp120	31 – 511	PBMCs	**492***	484	28	26	**20***	18
		Plasma	**494***		29		18	
gp41	512 – 856	PBMCs	345	345	4	5	**4***	3
		Plasma	345		5		**4***	
gp160	31 – 856	PBMCs	**837***	829	32	31	**24***	21
		Plasma	**839***		33.5		**22***	

*p < 0.0001

2.S6 Table. Physical characteristics of functional proviral and plasma gp160 sequences isolated from EN7.

The number of amino acids, potential N-glycosylation sites (PNGS), and cysteines were counted in each region of functional gp160 sequences isolated from EN7 PBMC and plasma samples. Location of PNGS was defined as the location of N in NX(S/T) motif (where X is any amino acid except proline). The distribution of each measure was compared to clade B sequences from the Los Alamos National Laboratory 2017 Filtered Web Alignment. EN7 functional proviral sequences had an elongated V1 region with two unusual cysteines.

Chapter 3: Targeted amplicon sequencing – application of Tn5-enabled and molecular identifier-guided amplicon assembly (TMIseq) to *env* V1 – V3 region and immunoglobulin heavy chain (IGH) VDJ region.

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Contributions: TMIseq conceptualized by Christopher Vollmers. Library preparation protocol developed by Sumedha Dharmadhikari and Camille Scelfo-Dalbey and modified for *env* by Jennie Hutchinson. Assembly pipeline developed by Charles Cole and Roger Volden. Samples were provided by Phillip W. Berman. Sara M. O'Rourke assisted with processing samples. Data analysis performed and summarized by Jennie M. Hutchinson.

Introduction

A new targeted amplicon sequencing method (TMIseq) has been developed that overcomes major obstacles for sequencing critical region in the highly diverse *env* and IGH genes. TMIseq improves accuracy and increases read length to >800bp on the Illumina platform by using molecular identifiers and a custom Tn5 reaction to guide amplicon assembly. Previous methods were limited to shorter reads (~450bp) on the Illumina platform or lower accuracy with 454-pyrosequencing (Di Giallonardo et al., 2014; Li et al., 2014). The application of TMIseq to immunogenic regions of *env* controls for sampling error that could account for unusual sequence variants, such as the extra disulfide bonds described in Chapter 1 and Chapter 2. TMIseq can also be used to sequence the IGH VDJ region, including a portion of the C region for identification of antibody isotypes. Characterization of antibody repertoires in elite neutralizers and/or viral controllers could help elucidate the adaptive immune response that leads to the production of bNAbs or improved disease outcomes. Here, we present initial results demonstrating TMIseq can successfully be applied to the most variable region of *env* and used to explore antibody repertoires for different disease phenotypes.

Materials and methods

Whole blood sample from one HIV-positive elite neutralizer (GSID001), one HIV-positive non-elite neutralizer (GSID007), and two HIV-negative individuals (Control 1 and Control 2) were processed by Ficoll gradient (GE Healthcare). Plasma and PBMCs were stored at -80°C. Proviral *env* V1 – V3 region was sequenced from GSID007 sample. All four samples were used for antibody repertoire sequencing.

For proviral sequences, DNA was extracted from PBMCs with QIAamp DNA Mini Blood kit using standard QIAcube protocol (Qiagen) and gp160 was pre-amplified in a 15-cycle PCR reaction with Phusion polymerase (Thermo Fisher Scientific). For IGH sequences, RNA was extracted from plasma with QIAamp Viral RNA Mini kit and reverse transcribed with SuperScript III (Thermo Fisher Scientific). In a 2-cycle PCR reaction, V1 – V3 region or IGH VDJ region was amplified with primers containing unique 16-base barcodes and partial Nextera sequences.

PCR products >300bp were purified with Select-a-Size DNA Clean & Concentrator (Zymo Research). In a 30-cycle PCR reaction, PCR products were amplified with primers that completed the partial Nextera sequences. PCR products >300bp were purified with Select-a-Size DNA Clean & Concentrator (Zymo Research) and run on 1% agarose gel.

Tn5 enzymes were loaded with either Nextera A adapters or Nextera B adapters. Separate transposase reactions randomly cleaved and tagged double-stranded DNA with either Nextera A or B adapter overhangs (referred to as “A cut”

and “B cut”). Tagmentation products were PCR amplified using Nextera primers. Products were purified with DNA Clean & Concentrator (Zymo Research) and run on 2% EX gel (Life Technologies). DNA ranging from 300bp – 900bp was extracted from the gel and purified using QIAquick Gel Extraction kit (Qiagen). Uncut DNA, Tn5 A cut, and Tn5 B cut libraries were pooled and sequenced according to standard Illumina protocols on an Illumina MiSeq.

Results and discussion

Proviral TMIseq of gp120 V1 – V3 regions

Targeted amplicon sequencing can detect the frequency of unusual variants and provide information on the evolution of immunologically significant regions, such as the V1 – V3 region. However, high-throughput sequencing introduces PCR errors and requires assembly, which are challenging problems to overcome in the highly diverse HIV population. Here, we applied TMIseq to improve accuracy over an ~800bp region. To correct for PCR errors, we attached molecular barcodes (16 random nucleotides) prior to amplification with out-nested primers (Bhiman et al., 2015; Cole et al., 2016; Jabara et al., 2011). Unique barcodes attached to each end of the DNA template allowed us to correct for recombination, allelic skewing, and sequencing errors. Molecular barcodes were also used to assemble Illumina reads with a modified version of the protocol described in Cole et al, 2016. While 454 pyrosequencing reads are long enough to cover the V1 – V3 region without assembly, 454 pyrosequencing has lower yields, is less sensitive and has a higher false positive

rate than Illumina sequencing (Di Giallonardo et al., 2014; Li et al., 2014). To use the Illumina platform, we designed three separate library preps. The first library prep followed the standard Illumina protocol after adding the molecular barcodes (Figure 3.1). This yielded high coverage at the ends (but insufficient coverage in the middle) and allowed us to match the 5' and 3' barcodes that came from each template. The other 2 library preps used custom transposase reactions to manipulate Illumina's tagmentation step. Transposase reactions randomly cleaved and tagged double-stranded DNA with either Illumina Nextera A or Illumina Nextera B adapters, resulting in varying lengths of DNA that ensured high coverage over the entire V1 – V3 region (Figure 3.2). Uncut DNA, DNA cut and tagged with Nextera A adapters, and DNA cut and tagged with Nextera B adapters were pooled and sequenced according to standard Illumina protocols on an Illumina MiSeq (Figure 3.3). The reads were assembled with custom scripts using the molecular barcodes with high coverage throughout the V1 – V3 region (Figure 3.3 and Figure 3.4).

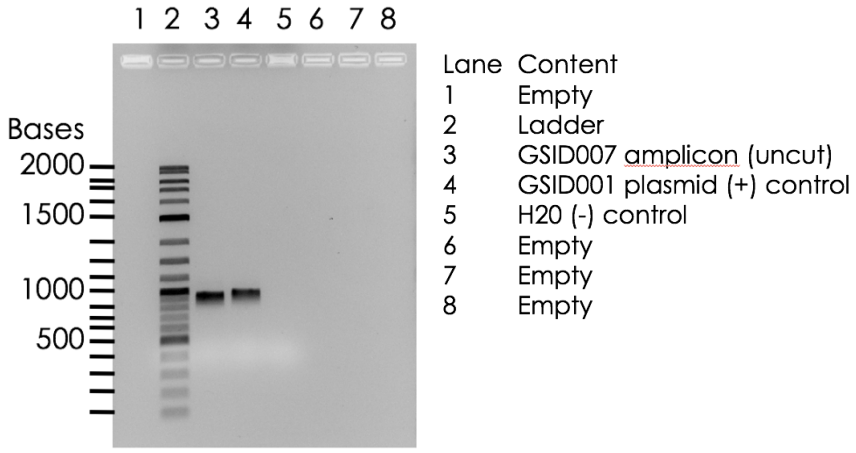


Fig 3.1. Amplification of gp120 V1-V3 region for TMIseq.
DNA was extracted from sample GSID007. V1 – V3 region was PCR amplified with unique molecular identifiers, purified, and run on 2% EX gel (Life Technologies).

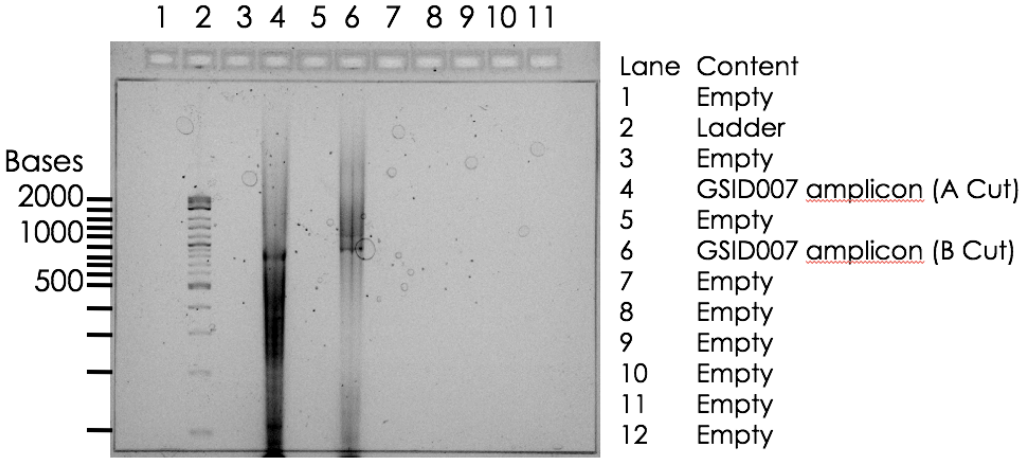


Fig 3.2. Transposase digest of gp120 V1 – V3 region library for TMIseq.
Tn5 enzymes randomly cleave GSID007 V1 – V3 amplicons. Tagmentation products of different sizes were amplified, purified, and run on 2% EX gel (Life Technologies).

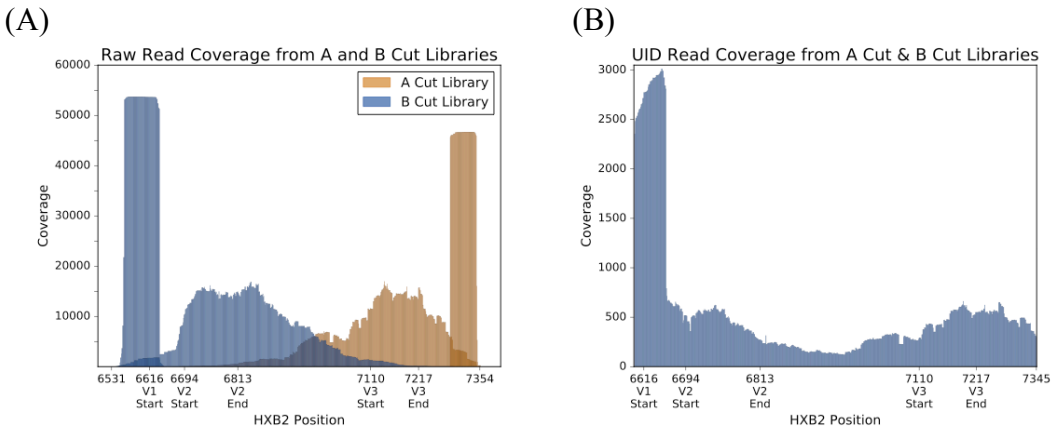


Fig 3.3. Read coverage of gp120 V1 – V3 region.
Histograms of (A) trimmed raw read coverage and (B) unique identifier (UID) coverage mapped to HXB2 reference.

```
GSID007_Monog      1 LWDQSLKPCVKLTPLCVTLNCNNVNVTNNSTSTNNSLTLEEGEMKNCSFN     50
                     |||||||||||||||||||||||||||||||||.|.||.|||||||||||||
GSID007_Illum      1 LWDQSLKPCVKLTPLCVTLNCNNVNVTNNSTSNNASLKLEEGEMKNCSFN     50

GSID007_Monog     51 ITTNIGDKRKEEYALFYKLDVVQIDNGNN--NNSTSYRLVSCNTSVLTQA     98
                     ||.|||||||:||||||||||||||.||   ||||:||||||||||:|||
GSID007_Illum     51 ITRNIGDKRKQEYALFYKLDVVQIDNDNNSTNNSTNYRLVSCNTSVITQA    100

GSID007_Monog     99 CPKISFEPIPIHYCTPAGFALLKCNDKKFSGKGPCTNVSTVQCTHGIRPV    148
                     ||||||||||||||||||||||||||||||.|:||||||||||||||||||||
GSID007_Illum    101 CPKISFEPIPIHYCTPAGFALLKCNDKNFNGKGPCTNVSTVQCTHGIRPV    150

GSID007_Monog    149 VSTQLLLNGSLAEEEVIIRSENFTNNAKTIIVHLNESVEINCTRPNNNTR    198
                     ||||||||||||||||:||||||||||||||||::||||||||||||||
GSID007_Illum    151 VSTQLLLNGSLAEEEVVIRSENFTNNAKTIIVHLNKAVEINCTRPNNNTR    200

GSID007_Monog    199 KSIPIGPGSAFYATGEIIGDIRQAHCNISKERWHDTLQQIVAKLREQFTN    248
                     ||||||||.||||||||||||||||||||||.|||||:||||||||||:|
GSID007_Illum    201 KSIPIGPGKAFYATGEIIGDIRQAHCNISKENWHDTLKQIVAKLREQFSN    250

GSID007_Monog    249 KTIAFKSSSGGDPEIVM    265
                     :|||||||||||||||
GSID007_Illum    251 RTIAFKSSSGGDPEIVM    267
```

Fig 3.4. Proviral V1 – V3 region TMIseq results.
Proof of concept study yielded proviral consensus sequence for patient GSID007. V1 – V3 sequences from plasma (Monog) and proviral (Illum) are shown.

Antibody repertoires from an elite neutralizer and HIV-positive individual that did not produce bNAbs

A proof of concept study was conducted on an elite neutralizer and non-elite neutralizing normal progressor. Both HIV-positive individuals had a high proportion of IgG antibodies compared to controls (Fig 3.5) with significant somatic hypermutation (Fig 3.6), an expected result in response to HIV infection. Interestingly, the non-elite neutralizing individual showed highly mutated IgD antibodies (Fig 3.6). Longer CDRH3s were found in both HIV-positive subjects compared to controls (Fig 3.7). Further investigation is needed to characterize the antibody repertoires by disease phenotype, which may elucidate features that led to neutralization breadth. Current immunization strategies involve a series of vaccinations, where some mimic the evolution of the virus in attempt to shape antibody maturation (Liao et al., 2013). Characterization of antibody repertoires from different disease phenotypes may help resolve whether sustained selection pressure is necessary for the development of broadly neutralizing antibodies.

Fig 3.5. Proportion of antibody isotypes in the antibody repertoires of an HIV-positive elite neutralizer and a non-elite neutralizer.

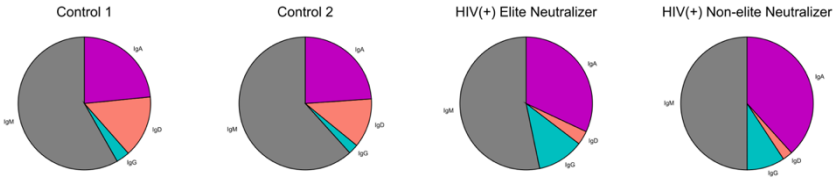


Fig 3.6. Somatic hypermutation in the antibody repertoires of an HIV-positive elite neutralizer and a non-elite neutralizer.

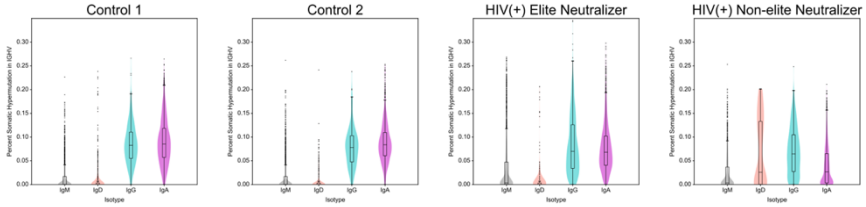
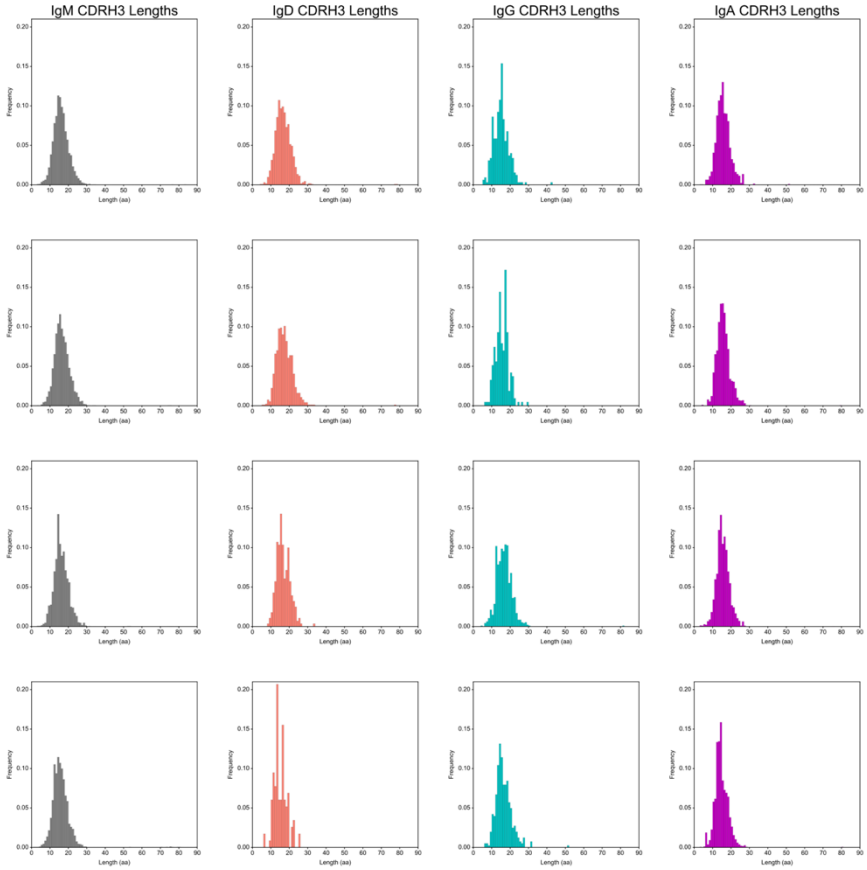


Fig 3.7. CDRH3 lengths in the antibody repertoires of an HIV-positive elite neutralizer and a non-elite neutralizer.



Conclusion

A primary focus of HIV vaccine research is to engineer immunogenic Env proteins. However, properly folded and glycosylated Env trimers have so far failed to elicit bNAbs. Envs are inherently poor immunogens and new strategies are needed in order to elicit an effective immune response. Here, we use Envs isolated from individuals with the elite neutralizer/controller phenotype and found unusual, potentially immunogenic structural features that may have contributed to the production of bNAbs and viral control.

We identified individuals with the rarely reported elite neutralizer/controller phenotype from the SCOPE and WIHS longitudinal studies. In the WIHS cohort, we demonstrated that elite controllers as a group had lower neutralization breadth and potency compared to viremic controllers and normal progressors. The low level of viremia in elite controllers is less likely to provide sufficient antigenic stimulation to produce bNAbs but there was not a linear relationship between neutralization breadth and viral load. While elite controllers are less likely to produce bNAbs, we identified an elite controller with broadly neutralizing activity from the SCOPE cohort, one of the few individuals that have been reported with this phenotype.

From the 78 individuals screened, two controllers with broadly neutralizing activity, EN3 and EN6, were selected for in depth analysis in an effort to identify Envs that may recapitulate their immune response. We studied viral controllers in part because they may have restricted evolution (Bailey et al., 2006; Bello et al., 2007, 2004; Casado et al., 2010; Lassen et al., 2009; Roy et al., 2017; Sandonís et al., 2009;

Scutari et al., 2018; Smith et al., 2013; Wang et al., 2003) and, therefore, contemporaneous Envs may be more closely related to ancestral variants that elicited the production of bNAbs. We found low genetic diversity in both EN3 and EN6 Env populations (99.1% and 99.6% pairwise identity, respectively), however, longitudinal studies and high-throughput sequencing (e.g., TMIseq) are needed to determine if evolution was in fact limited. We also reasoned that viral controllers may have features that enhance immunogenicity, such as those that contribute to improved antigen presentation. Both EN3 and EN6 had Envs with highly unusual features in immunodominant regions.

EN3 and EN6 had proviral Envs with elongated V1 regions containing two non-conserved cysteines, a feature found in only 5.5% of clade B viruses (van den Kerkhof et al., 2016). Mutagenesis experiments in EN3 demonstrated that mutations are necessary to accommodate the cysteine in order to preserve infectivity, suggesting a functional role. It appears the additional cysteines may arise from a duplication event and provide viral escape from V3-targeting antibodies but further investigation is needed. During these studies, we discovered the K170E polymorphism was a mode of escape from V2-glycan targeting bN-mAbs, PG9 and PG16.

Envs isolated from EN6 plasma had a 35% reduction in potential N-linked glycosylation sites. The absence of glycans has been associated with the T/F phenotype, bNAb-inducing Envs, and increased immunogenicity (Chohan et al., 2005; Dacheux et al., 2004; Derdeyn et al., 2004; Wu et al., 2006; Zhou et al., 2017). These previous findings have led to substantial effort in engineering Envs with fewer

glycans, which have improved neutralization response in rabbits and macaques (Koch et al., 2003; Li et al., 2008; Liang et al., 2016). Here, we identified a native, minimally glycosylated Env that elicited an improved cross-clade neutralization response in rabbits when compared to EN3 Env and reference Env, MN.

Future research can build on this work by using the minimally glycosylated EN6 Env in alternate modes of immunization, including trimeric gp140s (Dey et al., 2018; Sanders et al., 2013; Sliepen et al., 2019) in sequential immunization regimens (Dubrovskaya et al., 2019; Jardine et al., 2013; Steichen et al., 2016), and vector prime with rgp120 boosts (Barouch and Picker, 2014; Stephenson et al., 2016). The early results described in these studies provide evidence that elite neutralizer/controllers have unusual structural features that may have enhanced their immune response. Furthermore, this work demonstrated that individuals with the elite neutralizer/controller phenotype are a promising new source of HIV vaccine immunogens.

References

Ackerman, M.E., Mikhailova, A., Brown, E.P., Dowell, K.G., Walker, B.D., Bailey-Kellogg, C., Suscovich, T.J., Alter, G., 2016. Polyfunctional HIV-Specific Antibody Responses Are Associated with Spontaneous HIV Control. PLoS Pathog 12. https://doi.org/10.1371/journal.ppat.1005315

Albert, J., Abrahamsson, B., Nagy, K., Aurelius, E., Gaines, H., Nyström, G., Fenyö, E.M., 1990. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. AIDS 4, 107–112.

Andrabi, R., Bhiman, J.N., Burton, D.R., 2018. Strategies for a multi-stage neutralizing antibody-based HIV vaccine. Current Opinion in Immunology 53, 143–151. https://doi.org/10.1016/j.coi.2018.04.025

Andrabi, R., Voss, J.E., Liang, C.-H., Briney, B., McCoy, L.E., Wu, C.-Y., Wong, C.-H., Poignard, P., Burton, D.R., 2015. Identification of common features in prototype broadly neutralizing antibodies to HIV envelope V2 apex to facilitate vaccine design. Immunity 43, 959–973. https://doi.org/10.1016/j.immuni.2015.10.014

Baba, T.W., Liska, V., Hofmann-Lehmann, R., Vlasak, J., Xu, W., Ayehunie, S., Cavacini, L.A., Posner, M.R., Katinger, H., Stiegler, G., Bernacky, B.J., Rizvi, T.A., Schmidt, R., Hill, L.R., Keeling, M.E., Lu, Y., Wright, J.E., Chou, T.-C., Ruprecht, R.M., 2000. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian–human immunodeficiency virus infection. Nat Med 6, 200–206. https://doi.org/10.1038/72309

Back, N.K.T., Smit, L., De Jong, J.-J., Keulen, W., Schutten, M., Goudsmit, J., Tersmette, M., 1994. An N-Glycan within the Human Immunodeficiency Virus Type 1 gp120 V3 Loop Affects Virus Neutralization. Virology 199, 431–438. https://doi.org/10.1006/viro.1994.1141

Bacon, M.C., von Wyl, V., Alden, C., Sharp, G., Robison, E., Hessol, N., Gange, S., Barranday, Y., Holman, S., Weber, K., Young, M.A., 2005. The Women's Interagency HIV Study: an Observational Cohort Brings Clinical Sciences to the Bench. Clin Diagn Lab Immunol 12, 1013–1019. https://doi.org/10.1128/CDLI.12.9.1013-1019.2005

Bailey, J.R., Lassen, K.G., Yang, H.-C., Quinn, T.C., Ray, S.C., Blankson, J.N., Siliciano, R.F., 2006. Neutralizing Antibodies Do Not Mediate Suppression of Human Immunodeficiency Virus Type 1 in Elite Suppressors or Selection of

Plasma Virus Variants in Patients on Highly Active Antiretroviral Therapy. J Virol 80, 4758–4770. https://doi.org/10.1128/JVI.80.10.4758-4770.2006

Barkan, S.E., 1998. The Women's Interagency HIV Study. WIHS Collaborative Study Group. Epidemiology (cambridge, Mass.) 9, 117–125.

Barouch, D.H., Picker, L.J., 2014. Novel vaccine vectors for HIV-1. Nature Reviews Microbiology 12, 765–771. https://doi.org/10.1038/nrmicro3360

Barouch, D.H., Whitney, J.B., Moldt, B., Klein, F., Oliveira, T.Y., Liu, J., Stephenson, K.E., Chang, H.-W., Shekhar, K., Gupta, S., Nkolola, J.P., Seaman, M.S., Smith, K.M., Borducchi, E.N., Cabral, C., Smith, J.Y., Blackmore, S., Sanisetty, S., Perry, J.R., Beck, M., Lewis, M.G., Rinaldi, W., Chakraborty, A.K., Poignard, P., Nussenzweig, M.C., Burton, D.R., 2013. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. Nature 503, 224–228. https://doi.org/10.1038/nature12744

Barre-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W., Montagnier, L., 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220, 868–871. https://doi.org/10.1126/science.6189183

Baum, L.L., Cassutt, K.J., Knigge, K., Khattri, R., Margolick, J., Rinaldo, C., Kleeberger, C.A., Nishanian, P., Henrard, D.R., Phair, J., 1996. HIV-1 gp120-specific antibody-dependent cell-mediated cytotoxicity correlates with rate of disease progression. The Journal of Immunology 157, 2168–2173.

Behrens, A.-J., Vasiljevic, S., Pritchard, L.K., Harvey, D.J., Andev, R.S., Krumm, S.A., Struwe, W.B., Cupo, A., Kumar, A., Zitzmann, N., Seabright, G.E., Kramer, H.B., Spencer, D.I.R., Royle, L., Lee, J.H., Klasse, P.J., Burton, D.R., Wilson, I.A., Ward, A.B., Sanders, R.W., Moore, J.P., Doores, K.J., Crispin, M., 2016. Composition and Antigenic Effects of Individual Glycan Sites of a Trimeric HIV-1 Envelope Glycoprotein. Cell Reports 14, 2695–2706. https://doi.org/10.1016/j.celrep.2016.02.058

Bello, G., Casado, C., García, S., Rodríguez, C., del Romero, J., Borderia, A.V., López-Galíndez, C., 2004. Plasma RNA viral load is not associated with intrapatient quasispecies heterogeneity in HIV-1 infection. Arch. Virol. 149, 1761–1771.

Bello, G., Casado, C., García, S., Rodríguez, C., del Romero, J., Carvajal-Rodriguez, A., Posada, D., López-Galíndez, C., 2007. Lack of temporal structure in the

short term HIV-1 evolution within asymptomatic naïve patients. Virology 362, 294–303. https://doi.org/10.1016/j.virol.2006.11.039

Berman, P.W., Huang, W., Riddle, L., Gray, A.M., Wrin, T., Vennari, J., Johnson, A., Klaussen, M., Prashad, H., Köhne, C., deWit, C., Gregory, T.J., 1999. Development of Bivalent (B/E) Vaccines Able to Neutralize CCR5-Dependent Viruses from the United States and Thailand. Virology 265, 1–9. https://doi.org/10.1006/viro.1999.0031

Berman, P.W., Murthy, K.K., Wrin, T., Vennari, J.C., Cobb, E.K., Eastman, D.J., Champe, M., Nakamura, G.R., Davison, D., Powell, M.F., Bussiere, J., Francis, D.P., Matthews, T., Gregory, T.J., Obijeski, J.F., 1996. Protection of MN-rgp120-Immunized Chimpanzees from Heterologous Infection with a Primary Isolate of Human Immunodeficiency Virus Type 1. J Infect Dis 173, 52–59. https://doi.org/10.1093/infdis/173.1.52

Bhiman, J.N., Anthony, C., Doria-Rose, N.A., Karimanzira, O., Schramm, C.A., Khoza, T., Kitchin, D., Botha, G., Gorman, J., Garrett, N.J., Abdool Karim, S.S., Shapiro, L., Williamson, C., Kwong, P.D., Mascola, J.R., Morris, L., Moore, P.L., 2015. Viral variants that initiate and drive maturation of V1V2-directed HIV-1 broadly neutralizing antibodies. Nat Med 21, 1332–1336. https://doi.org/10.1038/nm.3963

Bonsignori, M., Zhou, T., Sheng, Z., Chen, L., Gao, F., Joyce, M.G., Ozorowski, G., Chuang, G.-Y., Schramm, C.A., Wiehe, K., Alam, S.M., Bradley, T., Gladden, M.A., Hwang, K.-K., Iyengar, S., Kumar, A., Lu, X., Luo, K., Mangiapani, M.C., Parks, R.J., Song, H., Acharya, P., Bailer, R.T., Cao, A., Druz, A., Georgiev, I.S., Kwon, Y.D., Louder, M.K., Zhang, B., Zheng, A., Hill, B.J., Kong, R., Soto, C., Mullikin, J.C., Douek, D.C., Montefiori, D.C., Moody, M.A., Shaw, G.M., Hahn, B.H., Kelsoe, G., Hraber, P.T., Korber, B.T., Boyd, S.D., Fire, A.Z., Kepler, T.B., Shapiro, L., Ward, A.B., Mascola, J.R., Liao, H.-X., Kwong, P.D., Haynes, B.F., 2016. Maturation Pathway from Germline to Broad HIV-1 Neutralizer of a CD4-Mimic Antibody. Cell 165, 449–463. https://doi.org/10.1016/j.cell.2016.02.022

Bournazos, S., Klein, F., Pietzsch, J., Seaman, M.S., Nussenzweig, M.C., Ravetch, J.V., 2014. Broadly neutralizing anti-HIV-1 antibodies require Fc effector functions for in vivo activity. Cell 158, 1243–1253. https://doi.org/10.1016/j.cell.2014.08.023

Brumme, Z.L., Li, C., Miura, T., Sela, J., Rosato, P.C., Brumme, C.J., Markle, T.J., Martin, E., Block, B.L., Trocha, A., Kadie, C.M., Allen, T.M., Pereyra, F., Heckerman, D., Walker, B.D., Brockman, M.A., 2011. Reduced replication capacity of NL4-3 recombinant viruses encoding RT-Integrase sequences

from HIV-1 elite controllers. J Acquir Immune Defic Syndr 56. https://doi.org/10.1097/QAI.0b013e3181fe9450

Bunnik, E.M., Pisas, L., Nuenen, A.C. van, Schuitemaker, H., 2008. Autologous Neutralizing Humoral Immunity and Evolution of the Viral Envelope in the Course of Subtype B Human Immunodeficiency Virus Type 1 Infection. Journal of Virology 82, 7932–7941. https://doi.org/10.1128/JVI.00757-08

Byrne, G., O'Rourke, S.M., Alexander, D.L., Yu, B., Doran, R.C., Wright, M., Chen, Q., Azadi, P., Berman, P.W., 2018. CRISPR/Cas9 gene editing for the creation of an MGAT1-deficient CHO cell line to control HIV-1 vaccine glycosylation. PLoS Biol 16. https://doi.org/10.1371/journal.pbio.2005817

Cao, L., Pauthner, M., Andrabi, R., Rantalainen, K., Berndsen, Z., Diedrich, J.K., Menis, S., Sok, D., Bastidas, R., Park, S.-K.R., Delahunty, C.M., He, L., Guenaga, J., Wyatt, R.T., Schief, W.R., Ward, A.B., Yates, J.R., Burton, D.R., Paulson, J.C., 2018. Differential processing of HIV envelope glycans on the virus and soluble recombinant trimer. Nat Commun 9, 1–14. https://doi.org/10.1038/s41467-018-06121-4

Casado, C., Colombo, S., Rauch, A., Martínez, R., Günthard, H.F., Garcia, S., Rodríguez, C., del Romero, J., Telenti, A., López-Galíndez, C., 2010. Host and Viral Genetic Correlates of Clinical Definitions of HIV-1 Disease Progression. PLoS One 5. https://doi.org/10.1371/journal.pone.0011079

Caskey, M., Klein, F., Lorenzi, J.C.C., Seaman, M.S., Jr, A.P.W., Buckley, N., Kremer, G., Nogueira, L., Braunschweig, M., Scheid, J.F., Horwitz, J.A., Shimeliovich, I., Ben-Avraham, S., Witmer-Pack, M., Platten, M., Lehmann, C., Burke, L.A., Hawthorne, T., Gorelick, R.J., Walker, B.D., Keler, T., Gulick, R.M., Fätkenheuer, G., Schlesinger, S.J., Nussenzweig, M.C., 2015. Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. Nature 522, 487–491. https://doi.org/10.1038/nature14411

CDC, 2018. HIV Surveillance Report, 2017; vol. 29.

Chackerian, B., Rudensey, L.M., Overbaugh, J., 1997. Specific N-linked and O-linked glycosylation modifications in the envelope V1 domain of simian immunodeficiency virus variants that evolve in the host alter recognition by neutralizing antibodies. J. Virol. 71, 7719–7727.

Chohan, B., Lang, D., Sagar, M., Korber, B., Lavreys, L., Richardson, B., Overbaugh, J., 2005. Selection for Human Immunodeficiency Virus Type 1 Envelope Glycosylation Variants with Shorter V1-V2 Loop Sequences Occurs during

Transmission of Certain Genetic Subtypes and May Impact Viral RNA Levels. J Virol 79, 6528–6531. https://doi.org/10.1128/JVI.79.10.6528-6531.2005

Chun, T.-W., Carruth, L., Finzi, D., Shen, X., DiGiuseppe, J.A., Taylor, H., Hermankova, M., Chadwick, K., Margolick, J., Quinn, T.C., Kuo, Y.-H., Brookmeyer, R., Zeiger, M.A., Barditch-Crovo, P., Siliciano, R.F., 1997. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 387, 183–188. https://doi.org/10.1038/387183a0

Chung, N.P.Y., Matthews, K., Klasse, P.J., Sanders, R.W., Moore, J.P., 2012. HIV-1 gp120 Impairs the Induction of B Cell Responses by TLR9-Activated Plasmacytoid Dendritic Cells. The Journal of Immunology 189, 5257–5265. https://doi.org/10.4049/jimmunol.1201905

Cicala, C., Martinelli, E., McNally, J.P., Goode, D.J., Gopaul, R., Hiatt, J., Jelicic, K., Kottilil, S., Macleod, K., O'Shea, A., Patel, N., Ryk, D.V., Wei, D., Pascuccio, M., Yi, L., McKinnon, L., Izulla, P., Kimani, J., Kaul, R., Fauci, A.S., Arthos, J., 2009. The integrin α4β7 forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1. PNAS 106, 20877–20882. https://doi.org/10.1073/pnas.0911796106

Cohen, M., 2015. Notable Aspects of Glycan-Protein Interactions. Biomolecules 5, 2056–2072. https://doi.org/10.3390/biom5032056

Cole, C., Volden, R., Dharmadhikari, S., Scelfo-Dalbey, C., Vollmers, C., 2016. Highly Accurate Sequencing of Full-Length Immune Repertoire Amplicons Using Tn5-Enabled and Molecular Identifier–Guided Amplicon Assembly. The Journal of Immunology 196, 2902–2907. https://doi.org/10.4049/jimmunol.1502563

Corti, D., Langedijk, J.P.M., Hinz, A., Seaman, M.S., Vanzetta, F., Fernandez-Rodriguez, B.M., Silacci, C., Pinna, D., Jarrossay, D., Balla-Jhagjhoorsingh, S., Willems, B., Zekveld, M.J., Dreja, H., O'Sullivan, E., Pade, C., Orkin, C., Jeffs, S.A., Montefiori, D.C., Davis, D., Weissenhorn, W., McKnight, Á., Heeney, J.L., Sallusto, F., Sattentau, Q.J., Weiss, R.A., Lanzavecchia, A., 2010. Analysis of Memory B Cell Responses and Isolation of Novel Monoclonal Antibodies with Neutralizing Breadth from HIV-1-Infected Individuals. PLOS ONE 5, e8805. https://doi.org/10.1371/journal.pone.0008805

Crooks, E.T., Osawa, K., Tong, T., Grimley, S.L., Dai, Y.D., Whalen, R.G., Kulp, D.W., Menis, S., Schief, W.R., Binley, J.M., 2017. Effects of partially dismantling the CD4 binding site glycan fence of HIV-1 Envelope

glycoprotein trimers on neutralizing antibody induction. Virology 505, 193–209. https://doi.org/10.1016/j.virol.2017.02.024

Crooks, E.T., Tong, T., Chakrabarti, B., Narayan, K., Georgiev, I.S., Menis, S., Huang, X., Kulp, D., Osawa, K., Muranaka, J., Stewart-Jones, G., Destefano, J., O'Dell, S., LaBranche, C., Robinson, J.E., Montefiori, D.C., McKee, K., Du, S.X., Doria-Rose, N., Kwong, P.D., Mascola, J.R., Zhu, P., Schief, W.R., Wyatt, R.T., Whalen, R.G., Binley, J.M., 2015. Vaccine-Elicited Tier 2 HIV-1 Neutralizing Antibodies Bind to Quaternary Epitopes Involving Glycan-Deficient Patches Proximal to the CD4 Binding Site. PLoS Pathog 11. https://doi.org/10.1371/journal.ppat.1004932

Dacheux, L., Moreau, A., Ataman-Önal, Y., Biron, F., Verrier, B., Barin, F., 2004. Evolutionary Dynamics of the Glycan Shield of the Human Immunodeficiency Virus Envelope during Natural Infection and Implications for Exposure of the 2G12 Epitope. Journal of Virology 78, 12625–12637. https://doi.org/10.1128/JVI.78.22.12625-12637.2004

de Taeye, S.W., Moore, J.P., Sanders, R.W., 2016. HIV-1 envelope trimer design and immunization strategies to induce broadly neutralizing antibodies. Trends Immunol 37, 221–232. https://doi.org/10.1016/j.it.2016.01.007

Derdeyn, C.A., Decker, J.M., Bibollet-Ruche, F., Mokili, J.L., Muldoon, M., Denham, S.A., Heil, M.L., Kasolo, F., Musonda, R., Hahn, B.H., Shaw, G.M., Korber, B.T., Allen, S., Hunter, E., 2004. Envelope-Constrained Neutralization-Sensitive HIV-1 After Heterosexual Transmission. Science 303, 2019–2022. https://doi.org/10.1126/science.1093137

Derking, R., Ozorowski, G., Sliepen, K., Yasmeen, A., Cupo, A., Torres, J.L., Julien, J.-P., Lee, J.H., van Montfort, T., de Taeye, S.W., Connors, M., Burton, D.R., Wilson, I.A., Klasse, P.-J., Ward, A.B., Moore, J.P., Sanders, R.W., 2015. Comprehensive Antigenic Map of a Cleaved Soluble HIV-1 Envelope Trimer. PLoS Pathog 11. https://doi.org/10.1371/journal.ppat.1004767

Dey, A.K., Cupo, A., Ozorowski, G., Sharma, V.K., Behrens, A., Go, E.P., Ketas, T.J., Yasmeen, A., Klasse, P.J., Sayeed, E., Desaire, H., Crispin, M., Wilson, I.A., Sanders, R.W., Hassell, T., Ward, A.B., Moore, J.P., 2018. cGMP production and analysis of BG505 SOSIP.664, an extensively glycosylated, trimeric HIV-1 envelope glycoprotein vaccine candidate. Biotechnol Bioeng 115, 885–899. https://doi.org/10.1002/bit.26498

Di Giallonardo, F., Töpfer, A., Rey, M., Prabhakaran, S., Duport, Y., Leemann, C., Schmutz, S., Campbell, N., Joos, B., Lecca, M., 2014. Full-length haplotype

reconstruction to infer the structure of heterogeneous virus populations. Nucleic Acids Research 42, gku537. https://doi.org/10.1093/nar/gku537

Doores, K.J., Bonomelli, C., Harvey, D.J., Vasiljevic, S., Dwek, R.A., Burton, D.R., Crispin, M., Scanlan, C.N., 2010. Envelope glycans of immunodeficiency virions are almost entirely oligomannose antigens. PNAS 107, 13800–13805. https://doi.org/10.1073/pnas.1006498107

Doran, R.C., Tatsuno, G.P., O'Rourke, S.M., Yu, B., Alexander, D.L., Mesa, K.A., Berman, P.W., 2018. Glycan modifications to the gp120 immunogens used in the RV144 vaccine trial improve binding to broadly neutralizing antibodies. PLoS One 13. https://doi.org/10.1371/journal.pone.0196370

Doria-Rose, N.A., Georgiev, I., O'Dell, S., Chuang, G.-Y., Staupe, R.P., McLellan, J.S., Gorman, J., Pancera, M., Bonsignori, M., Haynes, B.F., Burton, D.R., Koff, W.C., Kwong, P.D., Mascola, J.R., 2012. A Short Segment of the HIV-1 gp120 V1/V2 Region Is a Major Determinant of Resistance to V1/V2 Neutralizing Antibodies. Journal of Virology 86, 8319–8323. https://doi.org/10.1128/JVI.00696-12

Doria-Rose, N.A., Klein, R.M., Daniels, M.G., O'Dell, S., Nason, M., Lapedes, A., Bhattacharya, T., Migueles, S.A., Wyatt, R.T., Korber, B.T., Mascola, J.R., Connors, M., 2010. Breadth of Human Immunodeficiency Virus-Specific Neutralizing Activity in Sera: Clustering Analysis and Association with Clinical Variables. Journal of Virology 84, 1631–1636. https://doi.org/10.1128/JVI.01482-09

Doria-Rose, N.A., Klein, R.M., Manion, M.M., O'Dell, S., Phogat, A., Chakrabarti, B., Hallahan, C.W., Migueles, S.A., Wrammert, J., Ahmed, R., Nason, M., Wyatt, R.T., Mascola, J.R., Connors, M., 2009. Frequency and Phenotype of Human Immunodeficiency Virus Envelope-Specific B Cells from Patients with Broadly Cross-Neutralizing Antibodies. Journal of Virology 83, 188–199. https://doi.org/10.1128/JVI.01583-08

Doria-Rose, N.A., Schramm, C.A., Gorman, J., Moore, P.L., Bhiman, J.N., DeKosky, B.J., Ernandes, M.J., Georgiev, I.S., Kim, H.J., Pancera, M., Staupe, R.P., Altae-Tran, H.R., Bailer, R.T., Crooks, E.T., Cupo, A., Druz, A., Garrett, N.J., Hoi, K.H., Kong, R., Louder, M.K., Longo, N.S., McKee, K., Nonyane, M., O'Dell, S., Roark, R.S., Rudicell, R.S., Schmidt, S.D., Sheward, D.J., Soto, C., Wibmer, C.K., Yang, Y., Zhang, Z., Mullikin, J.C., Binley, J.M., Sanders, R.W., Wilson, I.A., Moore, J.P., Ward, A.B., Georgiou, G., Williamson, C., Abdool Karim, S.S., Morris, L., Kwong, P.D., Shapiro, L., Mascola, J.R., 2014. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. Nature 509, 55–62. https://doi.org/10.1038/nature13036

Dubrovskaya, V., Tran, K., Ozorowski, G., Guenaga, J., Wilson, R., Bale, S., Cottrell, C.A., Turner, H.L., Seabright, G., O'Dell, S., Torres, J.L., Yang, L., Feng, Y., Leaman, D.P., Vázquez Bernat, N., Liban, T., Louder, M., McKee, K., Bailer, R.T., Movsesyan, A., Doria-Rose, N.A., Pancera, M., Karlsson Hedestam, G.B., Zwick, M.B., Crispin, M., Mascola, J.R., Ward, A.B., Wyatt, R.T., 2019. Vaccination with Glycan-Modified HIV NFL Envelope Trimer-Liposomes Elicits Broadly Neutralizing Antibodies to Multiple Sites of Vulnerability. Immunity. https://doi.org/10.1016/j.immuni.2019.10.008

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32, 1792–1797. https://doi.org/10.1093/nar/gkh340

Escolano, A., Steichen, J.M., Dosenovic, P., Kulp, D.W., Golijanin, J., Sok, D., Freund, N.T., Gitlin, A.D., Oliveira, T., Araki, T., Lowe, S., Chen, S.T., Heinemann, J., Yao, K.-H., Georgeson, E., Saye-Francisco, K.L., Gazumyan, A., Adachi, Y., Kubitz, M., Burton, D.R., Schief, W.R., Nussenzweig, M.C., 2016. Sequential Immunization Elicits Broadly Neutralizing Anti-HIV-1 Antibodies in Ig Knockin Mice. Cell 166, 1445-1458.e12. https://doi.org/10.1016/j.cell.2016.07.030

Euler, Z., van Gils, M.J., Bunnik, E.M., Phung, P., Schweighardt, B., Wrin, T., Schuitemaker, H., 2010. Cross-Reactive Neutralizing Humoral Immunity Does Not Protect from HIV Type 1 Disease Progression. J Infect Dis 201, 1045–1053. https://doi.org/10.1086/651144

Fauci, A.S., 2017. An HIV Vaccine Is Essential for Ending the HIV/AIDS Pandemic. JAMA 318, 1535–1536. https://doi.org/10.1001/jama.2017.13505

Fellay, J., Ge, D., Shianna, K.V., Colombo, S., Ledergerber, B., Cirulli, E.T., Urban, T.J., Zhang, K., Gumbs, C.E., Smith, J.P., Castagna, A., Cozzi-Lepri, A., Luca, A.D., Easterbrook, P., Günthard, H.F., Mallal, S., Mussini, C., Dalmau, J., Martinez-Picado, J., Miro, J.M., Obel, N., Wolinsky, S.M., Martinson, J.J., Detels, R., Margolick, J.B., Jacobson, L.P., Descombes, P., Antonarakis, S.E., Beckmann, J.S., O'Brien, S.J., Letvin, N.L., McMichael, A.J., Haynes, B.F., Carrington, M., Feng, S., Telenti, A., Goldstein, D.B., Immunology (CHAVI), N.C. for H.V., 2009. Common Genetic Variation and the Control of HIV-1 in Humans. PLOS Genetics 5, e1000791. https://doi.org/10.1371/journal.pgen.1000791

Fernàndez, G., Clotet, B., Martínez, M.A., 2007. Fitness Landscape of Human Immunodeficiency Virus Type 1 Protease Quasispecies. J. Virol. 81, 2485–2496. https://doi.org/10.1128/JVI.01594-06

Freund, N.T., Wang, H., Scharf, L., Nogueira, L., Horwitz, J.A., Bar-On, Y., Golijanin, J., Sievers, S.A., Sok, D., Cai, H., Cesar Lorenzi, J.C., Halper-Stromberg, A., Toth, I., Piechocka-Trocha, A., Gristick, H.B., van Gils, M.J., Sanders, R.W., Wang, L.-X., Seaman, M.S., Burton, D.R., Gazumyan, A., Walker, B.D., West, A.P., Bjorkman, P.J., Nussenzweig, M.C., 2017. Coexistence of potent HIV-1 broadly neutralizing antibodies and antibody-sensitive viruses in a viremic controller. Sci Transl Med 9. https://doi.org/10.1126/scitranslmed.aal2144

Frost, S.D.W., Wrin, T., Smith, D.M., Pond, S.L.K., Liu, Y., Paxinos, E., Chappey, C., Galovich, J., Beauchaine, J., Petropoulos, C.J., Little, S.J., Richman, D.D., 2005. Neutralizing antibody responses drive the evolution of human immunodeficiency virus type 1 envelope during recent HIV infection. Proc Natl Acad Sci U S A 102, 18514–18519. https://doi.org/10.1073/pnas.0504658102

Gao, F., Bonsignori, M., Liao, H.-X., Kumar, A., Xia, S.-M., Lu, X., Cai, F., Hwang, K.-K., Song, H., Zhou, T., Lynch, R.M., Alam, S.M., Moody, M.A., Ferrari, G., Berrong, M., Kelsoe, G., Shaw, G.M., Hahn, B.H., Montefiori, D.C., Kamanga, G., Cohen, M.S., Hraber, P., Kwong, P.D., Korber, B.T., Mascola, J.R., Kepler, T.B., Haynes, B.F., 2014. Cooperation of B Cell Lineages in Induction of HIV-1-Broadly Neutralizing Antibodies. Cell 158, 481–491. https://doi.org/10.1016/j.cell.2014.06.022

Garces, F., Sok, D., Kong, L., McBride, R., Kim, H.J., Saye-Francisco, K.F., Julien, J.-P., Hua, Y., Cupo, A., Moore, J.P., Paulson, J.C., Ward, A.B., Burton, D.R., Wilson, I.A., 2014. Structural evolution of glycan recognition by a family of potent HIV antibodies. Cell 159, 69–79. https://doi.org/10.1016/j.cell.2014.09.009

Geijtenbeek, T.B.H., Kwon, D.S., Torensma, R., van Vliet, S.J., van Duijnhoven, G.C.F., Middel, J., Cornelissen, I.L.M.H.A., Nottet, H.S.L.M., KewalRamani, V.N., Littman, D.R., Figdor, C.G., van Kooyk, Y., 2000. DC-SIGN, a Dendritic Cell–Specific HIV-1-Binding Protein that Enhances trans-Infection of T Cells. Cell 100, 587–597. https://doi.org/10.1016/S0092-8674(00)80694-7

Georgiev, I.S., Joyce, M.G., Yang, Y., Sastry, M., Zhang, B., Baxa, U., Chen, R.E., Druz, A., Lees, C.R., Narpala, S., Schön, A., Galen, J.V., Chuang, G.-Y., Gorman, J., Harned, A., Pancera, M., Stewart-Jones, G.B.E., Cheng, C., Freire, E., McDermott, A.B., Mascola, J.R., Kwong, P.D., 2015. Single-Chain Soluble BG505.SOSIP gp140 Trimers as Structural and Antigenic Mimics of

Mature Closed HIV-1 Env. J. Virol. 89, 5318–5329. https://doi.org/10.1128/JVI.03451-14

Gilbert, P., Wang, M., Wrin, T., Petropoulos, C., Gurwith, M., Sinangil, F., D'Souza, P., Rodriguez-Chavez, I.R., DeCamp, A., Giganti, M., Berman, P.W., Self, S.G., Montefiori, D.C., 2010. Magnitude and breadth of a non-protective neutralizing antibody response in an efficacy trial of a candidate HIV-1 gp120 vaccine (AIDSVAX™ B/B). J Infect Dis 202, 595–605. https://doi.org/10.1086/654816

González, N., McKee, K., Lynch, R.M., Georgiev, I.S., Jimenez, L., Grau, E., Yuste, E., Kwong, P.D., Mascola, J.R., Alcamí, J., 2018. Characterization of broadly neutralizing antibody responses to HIV-1 in a cohort of long term non-progressors. PLoS ONE 13. https://doi.org/10.1371/journal.pone.0193773

Gottardo, R., Bailer, R.T., Korber, B.T., Gnanakaran, S., Phillips, J., Shen, X., Tomaras, G.D., Turk, E., Imholte, G., Eckler, L., Wenschuh, H., Zerweck, J., Greene, K., Gao, H., Berman, P.W., Francis, D., Sinangil, F., Lee, C., Nitayaphan, S., Rerks-Ngarm, S., Kaewkungwal, J., Pitisuttithum, P., Tartaglia, J., Robb, M.L., Michael, N.L., Kim, J.H., Zolla-Pazner, S., Haynes, B.F., Mascola, J.R., Self, S., Gilbert, P., Montefiori, D.C., 2013. Plasma IgG to Linear Epitopes in the V2 and V3 Regions of HIV-1 gp120 Correlate with a Reduced Risk of Infection in the RV144 Vaccine Efficacy Trial. PLoS One 8. https://doi.org/10.1371/journal.pone.0075665

Granados-Gonzalez, V., Piedrahita, L.D., Martinez-Gutierrez, M., Lawrence, P., Saoudin, H., Zapata, X., Lucht, F., Pinter, A., Genin, C., Urcuqui-Inchima, S., Riffard, S., 2009. Neutralizing inter-clade cross-reactivity of HIV-1 V1/V2-specific secretory immunoglobulin A in Colombian and French cohorts. AIDS 23, 2219. https://doi.org/10.1097/QAD.0b013e328329d134

Gray, E.S., Madiga, M.C., Hermanus, T., Moore, P.L., Wibmer, C.K., Tumba, N.L., Werner, L., Mlisana, K., Sibeko, S., Williamson, C., Abdool Karim, S.S., Morris, L., 2011. The Neutralization Breadth of HIV-1 Develops Incrementally over Four Years and Is Associated with CD4+ T Cell Decline and High Viral Load during Acute Infection ▿. J Virol 85, 4828–4840. https://doi.org/10.1128/JVI.00198-11

Hallfors, D.D., Iritani, B.J., Miller, W.C., Bauer, D.J., 2007. Sexual and Drug Behavior Patterns and HIV and STD Racial Disparities: The Need for New Directions. Am J Public Health 97, 125–132. https://doi.org/10.2105/AJPH.2005.075747

Hansen, S.G., Jr, M.P., Ventura, A.B., Hughes, C.M., Gilbride, R.M., Ford, J.C., Oswald, K., Shoemaker, R., Li, Y., Lewis, M.S., Gilliam, A.N., Xu, G., Whizin, N., Burwitz, B.J., Planer, S.L., Turner, J.M., Legasse, A.W., Axthelm, M.K., Nelson, J.A., Früh, K., Sacha, J.B., Estes, J.D., Keele, B.F., Edlefsen, P.T., Lifson, J.D., Picker, L.J., 2013. Immune clearance of highly pathogenic SIV infection. Nature 502, 100–104. https://doi.org/10.1038/nature12519

Haynes, B.F., Kelsoe, G., Harrison, S.C., Kepler, T.B., 2012. B-cell–lineage immunogen design in vaccine development with HIV-1 as a case study. Nature Biotechnology 30, 423–433. https://doi.org/10.1038/nbt.2197

Hessell, A.J., Poignard, P., Hunter, M., Hangartner, L., Tehrani, D.M., Bleeker, W.K., Parren, P.W.H.I., Marx, P.A., Burton, D.R., 2009. Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. Nat Med 15, 951–954. https://doi.org/10.1038/nm.1974

Hessell, A.J., Rakasz, E.G., Tehrani, D.M., Huber, M., Weisgrau, K.L., Landucci, G., Forthal, D.N., Koff, W.C., Poignard, P., Watkins, D.I., Burton, D.R., 2010. Broadly Neutralizing Monoclonal Antibodies 2F5 and 4E10 Directed against the Human Immunodeficiency Virus Type 1 gp41 Membrane-Proximal External Region Protect against Mucosal Challenge by Simian-Human Immunodeficiency Virus SHIVBa-L. Journal of Virology 84, 1302–1313. https://doi.org/10.1128/JVI.01272-09

HIV sequence database main page [WWW Document], n.d. URL https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html (accessed 8.6.18).

Hoffenberg, S., Powell, R., Carpov, A., Wagner, D., Wilson, A., Pond, S.K., Lindsay, R., Arendt, H., DeStefano, J., Phogat, S., Poignard, P., Fling, S.P., Simek, M., LaBranche, C., Montefiori, D., Wrin, T., Phung, P., Burton, D., Koff, W., King, C.R., Parks, C.L., Caulfield, M.J., 2013. Identification of an HIV-1 Clade A Envelope That Exhibits Broad Antigenicity and Neutralization Sensitivity and Elicits Antibodies Targeting Three Distinct Epitopes. J. Virol. 87, 5372–5383. https://doi.org/10.1128/JVI.02827-12

Hraber, P., Seaman, M.S., Bailer, R.T., Mascola, J.R., Montefiori, D.C., Korber, B.T., 2014. Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection. AIDS 28, 163–169. https://doi.org/10.1097/QAD.0000000000000106

Hutchinson, J.M., Mesa, K.A., Alexander, D.L., Yu, B., O'Rourke, S.M., Limoli, K.L., Wrin, T., Deeks, S.G., Berman, P.W., 2019. Unusual Cysteine Content

in V1 Region of gp120 From an Elite Suppressor That Produces Broadly Neutralizing Antibodies. Front. Immunol. 10. https://doi.org/10.3389/fimmu.2019.01021

Jabara, C., Jones, C., Roach, J., Anderson, J., Swanstrom, R., 2011. Accurate sampling and deep sequencing of the HIV-1 protease gene using a Primer ID. Proceedings of the National Academy of Sciences of the United States of America 108, 20166–71. https://doi.org/10.1073/pnas.1110064108

Jacobson, J.M., Colman, N., Ostrow, N.A., Simson, R.W., Tomesch, D., Marlin, L., Rao, M., Mills, J.L., Clemens, J., Prince, A.M., 1993. Passive immunotherapy in the treatment of advanced human immunodeficiency virus infection. J. Infect. Dis. 168, 298–305.

Jardine, J., Julien, J.-P., Menis, S., Ota, T., Kalyuzhniy, O., McGuire, A., Sok, D., Huang, P.-S., MacPherson, S., Jones, M., Nieusma, T., Mathison, J., Baker, D., Ward, A.B., Burton, D.R., Stamatatos, L., Nemazee, D., Wilson, I.A., Schief, W.R., 2013. Rational HIV immunogen design to target specific germline B cell receptors. Science 340, 711–716. https://doi.org/10.1126/science.1234150

Jobes, D.V., Daoust, M., Nguyen, V., Padua, A., Michele, S., Lock, M.D., Chen, A., Sinangil, F., Berman, P.W., 2006. High incidence of unusual cysteine variants in gp120 envelope proteins from early HIV type 1 infections from a Phase 3 vaccine efficacy trial. AIDS Res. Hum. Retroviruses 22, 1014–1021. https://doi.org/10.1089/aid.2006.22.1014

Johansson, S.E., Rollman, E., Chung, A.W., Center, R.J., Hejdeman, B., Stratov, I., Hinkula, J., Wahren, B., Kärre, K., Kent, S.J., Berg, L., 2011. NK Cell Function and Antibodies Mediating ADCC in HIV-1-Infected Viremic and Controller Patients. Viral Immunology 24, 359–368. https://doi.org/10.1089/vim.2011.0025

Julien, J.-P., Cupo, A., Sok, D., Stanfield, R.L., Lyumkis, D., Deller, M.C., Klasse, P.-J., Burton, D.R., Sanders, R.W., Moore, J.P., Ward, A.B., Wilson, I.A., 2013a. Crystal structure of a soluble cleaved HIV-1 envelope trimer. Science 342. https://doi.org/10.1126/science.1245625

Julien, J.-P., Sok, D., Khayat, R., Lee, J.H., Doores, K.J., Walker, L.M., Ramos, A., Diwanji, D.C., Pejchal, R., Cupo, A., Katpally, U., Depetris, R.S., Stanfield, R.L., McBride, R., Marozsan, A.J., Paulson, J.C., Sanders, R.W., Moore, J.P., Burton, D.R., Poignard, P., Ward, A.B., Wilson, I.A., 2013b. Broadly Neutralizing Antibody PGT121 Allosterically Modulates CD4 Binding via

Recognition of the HIV-1 gp120 V3 Base and Multiple Surrounding Glycans. PLoS Pathog 9, e1003342. https://doi.org/10.1371/journal.ppat.1003342

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Keele, B.F., Giorgi, E.E., Salazar-Gonzalez, J.F., Decker, J.M., Pham, K.T., Salazar, M.G., Sun, C., Grayson, T., Wang, S., Li, H., Wei, X., Jiang, C., Kirchherr, J.L., Gao, F., Anderson, J.A., Ping, L.-H., Swanstrom, R., Tomaras, G.D., Blattner, W.A., Goepfert, P.A., Kilby, J.M., Saag, M.S., Delwart, E.L., Busch, M.P., Cohen, M.S., Montefiori, D.C., Haynes, B.F., Gaschen, B., Athreya, G.S., Lee, H.Y., Wood, N., Seoighe, C., Perelson, A.S., Bhattacharya, T., Korber, B.T., Hahn, B.H., Shaw, G.M., 2008. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. PNAS 105, 7552–7557. https://doi.org/10.1073/pnas.0802203105

Klasse, P.J., Ketas, T.J., Cottrell, C.A., Ozorowski, G., Debnath, G., Camara, D., Francomano, E., Pugach, P., Ringe, R.P., LaBranche, C.C., Gils, M.J. van, Bricault, C.A., Barouch, D.H., Crotty, S., Silvestri, G., Kasturi, S., Pulendran, B., Wilson, I.A., Montefiori, D.C., Sanders, R.W., Ward, A.B., Moore, J.P., 2018. Epitopes for neutralizing antibodies induced by HIV-1 envelope glycoprotein BG505 SOSIP trimers in rabbits and macaques. PLOS Pathogens 14, e1006913. https://doi.org/10.1371/journal.ppat.1006913

Klein, F., Disking, R., Scheid, J.F., Gaebler, C., Mouquet, H., Georgiev, I., Pancera, M., Zhou, T., Incesu, R.-B., Fu, B.Z., Gnanapragasam, P.N.P., Oliveira, T.Y., Seaman, M.S., Kwong, P.D., Bjorkman, P.J., Nussenzweig, M.C., 2013. Somatic mutations of the immunoglobulin framework are generally required for broad and potent HIV-1 neutralization. Cell 153, 126–138. https://doi.org/10.1016/j.cell.2013.03.018

Klein, F., Nogueira, L., Nishimura, Y., Phad, G., West, A.P., Halper-Stromberg, A., Horwitz, J.A., Gazumyan, A., Liu, C., Eisenreich, T.R., Lehmann, C., Fätkenheuer, G., Williams, C., Shingai, M., Martin, M.A., Bjorkman, P.J., Seaman, M.S., Zolla-Pazner, S., Hedestam, G.B.K., Nussenzweig, M.C., 2014. Enhanced HIV-1 immunotherapy by commonly arising antibodies that target virus escape variants. Journal of Experimental Medicine 211, 2361–2372. https://doi.org/10.1084/jem.20141050

Koch, M., Pancera, M., Kwong, P.D., Kolchinsky, P., Grundner, C., Wang, L., Hendrickson, W.A., Sodroski, J., Wyatt, R., 2003. Structure-based, targeted deglycosylation of HIV-1 gp120 and effects on neutralization sensitivity and antibody recognition. Virology 313, 387–400. https://doi.org/10.1016/S0042-6822(03)00294-0

Kong, L., Lee, J.H., Doores, K.J., Murin, C.D., Julien, J.-P., McBride, R., Liu, Y., Marozsan, A., Cupo, A., Klasse, P.-J., Hoffenberg, S., Caulfield, M., King, C.R., Hua, Y., Le, K.M., Khayat, R., Deller, M.C., Clayton, T., Tien, H., Feizi, T., Sanders, R.W., Paulson, J.C., Moore, J.P., Stanfield, R.L., Burton, D.R., Ward, A.B., Wilson, I.A., 2013. Supersite of immune vulnerability on the glycosylated face of HIV-1 envelope glycoprotein gp120. Nature Structural & Molecular Biology 20, 796–803. https://doi.org/10.1038/nsmb.2594

Kong, L., Torrents de la Peña, A., Deller, M.C., Garces, F., Sliepen, K., Hua, Y., Stanfield, R.L., Sanders, R.W., Wilson, I.A., 2015. Complete epitopes for vaccine design derived from a crystal structure of the broadly neutralizing antibodies PGT128 and 8ANC195 in complex with an HIV-1 Env trimer. Acta Crystallogr D Biol Crystallogr 71, 2099–2108. https://doi.org/10.1107/S1399004715013917

Korber, B., Gaschen, B., Yusim, K., Thakallapally, R., Kesmir, C., Detours, V., 2001. Evolutionary and immunological implications of contemporary HIV-1 variation. Br Med Bull 58, 19–42. https://doi.org/10.1093/bmb/58.1.19

Krachmarov, C., Pinter, A., Honnen, W.J., Gorny, M.K., Nyambi, P.N., Zolla-Pazner, S., Kayman, S.C., 2005. Antibodies That Are Cross-Reactive for Human Immunodeficiency Virus Type 1 Clade A and Clade B V3 Domains Are Common in Patient Sera from Cameroon, but Their Neutralization Activity Is Usually Restricted by Epitope Masking. J Virol 79, 780–790. https://doi.org/10.1128/JVI.79.2.780-790.2005

Krachmarov, C.P., Honnen, W.J., Kayman, S.C., Gorny, M.K., Zolla-Pazner, S., Pinter, A., 2006. Factors Determining the Breadth and Potency of Neutralization by V3-Specific Human Monoclonal Antibodies Derived from Subjects Infected with Clade A or Clade B Strains of Human Immunodeficiency Virus Type 1. Journal of Virology 80, 7127–7135. https://doi.org/10.1128/JVI.02619-05

Kwong, P.D., Doyle, M.L., Casper, D.J., Cicala, C., Leavitt, S.A., Majeed, S., Steenbeke, T.D., Venturi, M., Chaiken, I., Fung, M., Katinger, H., Parren, P.W.I.H., Robinson, J., Van Ryk, D., Wang, L., Burton, D.R., Freire, E., Wyatt, R., Sodroski, J., Hendrickson, W.A., Arthos, J., 2002. HIV-1 evades

antibody-mediated neutralization through conformational masking of receptor-binding sites. Nature 420, 678–682. https://doi.org/10.1038/nature01188

Kwong, P.D., Mascola, J.R., 2018. HIV-1 Vaccines Based on Antibody Identification, B Cell Ontogeny, and Epitope Structure. Immunity 48, 855–871. https://doi.org/10.1016/j.immuni.2018.04.029

Kwong, P.D., Mascola, J.R., 2012. Human Antibodies that Neutralize HIV-1: Identification, Structures, and B Cell Ontogenies. Immunity 37, 412–425. https://doi.org/10.1016/j.immuni.2012.08.012

LaBranche, C.C., McGuire, A.T., Gray, M.D., Behrens, S., Zhou, T., Sattentau, Q.J., Peacock, J., Eaton, A., Greene, K., Gao, H., Tang, H., Perez, L.G., Saunders, K.O., Mascola, J.R., Haynes, B.F., Stamatatos, L., Montefiori, D.C., 2018. HIV-1 envelope glycan modifications that permit neutralization by germline-reverted VRC01-class broadly neutralizing antibodies. PLOS Pathogens 14, e1007431. https://doi.org/10.1371/journal.ppat.1007431

Lambotte, O., Ferrari, G., Moog, C., Yates, N.L., Liao, H.-X., Parks, R.J., Hicks, C.B., Owzar, K., Tomaras, G.D., Montefiori, D.C., Haynes, B.F., Delfraissy, J.-F., 2009. Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. AIDS 23, 897–906. https://doi.org/10.1097/QAD.0b013e328329f97d

Landais, E., Huang, X., Havenar-Daughton, C., Murrell, B., Price, M.A., Wickramasinghe, L., Ramos, A., Bian, C.B., Simek, M., Allen, S., Karita, E., Kilembe, W., Lakhi, S., Inambao, M., Kamali, A., Sanders, E.J., Anzala, O., Edward, V., Bekker, L.-G., Tang, J., Gilmour, J., Kosakovsky-Pond, S.L., Phung, P., Wrin, T., Crotty, S., Godzik, A., Poignard, P., 2016. Broadly Neutralizing Antibody Responses in a Large Longitudinal Sub-Saharan HIV Primary Infection Cohort. PLOS Pathogens 12, e1005369. https://doi.org/10.1371/journal.ppat.1005369

Lasky, L.A., Groopman, J.E., Fennie, C.W., Benz, P.M., Capon, D.J., Dowbenko, D.J., Nakamura, G.R., Nunes, W.M., Renz, M.E., Berman, P.W., 1986. Neutralization of the AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. Science 233, 209–212. https://doi.org/10.1126/science.3014647

Lassen, K.G., Lobritz, M.A., Bailey, J.R., Johnston, S., Nguyen, S., Lee, B., Chou, T., Siliciano, R.F., Markowitz, M., Arts, E.J., 2009. Elite Suppressor–Derived HIV-1 Envelope Glycoproteins Exhibit Reduced Entry Efficiency and Kinetics. PLoS Pathog 5. https://doi.org/10.1371/journal.ppat.1000377

Ledgerwood, J.E., Coates, E.E., Yamshchikov, G., Saunders, J.G., Holman, L., Enama, M.E., DeZure, A., Lynch, R.M., Gordon, I., Plummer, S., Hendel, C.S., Pegu, A., Conan-Cibotti, M., Sitar, S., Bailer, R.T., Narpala, S., McDermott, A., Louder, M., O'Dell, S., Mohan, S., Pandey, J.P., Schwartz, R.M., Hu, Z., Koup, R.A., Capparelli, E., Mascola, J.R., Graham, B.S., 2015. Safety, pharmacokinetics and neutralization of the broadly neutralizing HIV-1 human monoclonal antibody VRC01 in healthy adults. Clin Exp Immunol 182, 289–301. https://doi.org/10.1111/cei.12692

Leitman, E.M., Thobakgale, C.F., Adland, E., Ansari, M.A., Raghwani, J., Prendergast, A.J., Tudor-Williams, G., Kiepiela, P., Hemelaar, J., Brener, J., Tsai, M.-H., Mori, M., Riddell, L., Luzzi, G., Jooste, P., Ndung'u, T., Walker, B.D., Pybus, O.G., Kellam, P., Naranbhai, V., Matthews, P.C., Gall, A., Goulder, P.J.R., 2017a. Role of HIV-specific CD8+ T cells in pediatric HIV cure strategies after widespread early viral escape. J Exp Med 214, 3239–3261. https://doi.org/10.1084/jem.20162123

Leitman, E.M., Willberg, C.B., Tsai, M.-H., Chen, H., Buus, S., Chen, F., Riddell, L., Haas, D., Fellay, J., Goedert, J.J., Piechocka-Trocha, A., Walker, B.D., Martin, J., Deeks, S., Wolinsky, S.M., Martinson, J., Martin, M., Qi, Y., Sáez-Cirión, A., Yang, O.O., Matthews, P.C., Carrington, M., Goulder, P.J.R., 2017b. HLA-B*14:02-Restricted Env-Specific CD8+ T-Cell Activity Has Highly Potent Antiviral Efficacy Associated with Immune Control of HIV Infection. J Virol 91. https://doi.org/10.1128/JVI.00544-17

Leonard, C.K., Spellman, M.W., Riddle, L., Harris, R.J., Thomas, J.N., Gregory, T.J., 1990. Assignment of intrachain disulfide bonds and characterization of potential glycosylation sites of the type 1 recombinant human immunodeficiency virus envelope glycoprotein (gp120) expressed in Chinese hamster ovary cells. J. Biol. Chem. 265, 10373–10382.

Li, J.Z., Chapman, B., Charlebois, P., Hofmann, O., Weiner, B., Porter, A.J., Samuel, R., Vardhanabhuti, S., Zheng, L., Eron, J., Taiwo, B., Zody, M.C., Henn, M.R., Kuritzkes, D.R., Hide, W., Team, and the A.A.S., 2014. Comparison of Illumina and 454 Deep Sequencing in Participants Failing Raltegravir-Based Antiretroviral Therapy. PLOS ONE 9, e90485. https://doi.org/10.1371/journal.pone.0090485

Li, Y., Cleveland, B., Klots, I., Travis, B., Richardson, B.A., Anderson, D., Montefiori, D., Polacino, P., Hu, S.-L., 2008. Removal of a Single N-Linked Glycan in Human Immunodeficiency Virus Type 1 gp120 Results in an Enhanced Ability To Induce Neutralizing Antibody Responses. J Virol 82, 638–651. https://doi.org/10.1128/JVI.01691-07

Lian, Y., Srivastava, I., Gómez-Román, V.R., Megede, J. zur, Sun, Y., Kan, E., Hilt, S., Engelbrecht, S., Himathongkham, S., Luciw, P.A., Otten, G., Ulmer, J.B., Donnelly, J.J., Rabussay, D., Montefiori, D., Rensburg, E.J. van, Barnett, S.W., 2005. Evaluation of Envelope Vaccines Derived from the South African Subtype C Human Immunodeficiency Virus Type 1 TV1 Strain. J. Virol. 79, 13338–13349. https://doi.org/10.1128/JVI.79.21.13338-13349.2005

Liang, Y., Guttman, M., Williams, J.A., Verkerke, H., Alvarado, D., Hu, S.-L., Lee, K.K., 2016. Changes in Structure and Antigenicity of HIV-1 Env Trimers Resulting from Removal of a Conserved CD4 Binding Site-Proximal Glycan. J Virol 90, 9224–9236. https://doi.org/10.1128/JVI.01116-16

Liao, H.-X., Lynch, R., Zhou, T., Gao, F., Alam, S.M., Boyd, S.D., Fire, A.Z., Roskin, K.M., Schramm, C.A., Zhang, Z., Zhu, J., Shapiro, L., Mullikin, J.C., Gnanakaran, S., Hraber, P., Wiehe, K., Kelsoe, G., Yang, G., Xia, S.-M., Montefiori, D.C., Parks, R., Lloyd, K.E., Scearce, R.M., Soderberg, K.A., Cohen, M., Kaminga, G., Louder, M.K., Tran, L.M., Chen, Y., Cai, F., Chen, S., Moquin, S., Du, X., Joyce, G.M., Srivatsan, S., Zhang, B., Zheng, A., Shaw, G.M., Hahn, B.H., Kepler, T.B., Korber, B.T.M., Kwong, P.D., Mascola, J.R., Haynes, B.F., 2013. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature 496, 469–476. https://doi.org/10.1038/nature12053

Liu, L., Cimbro, R., Lusso, P., Berger, E.A., 2011. Intraprotomer masking of third variable loop (V3) epitopes by the first and second variable loops (V1V2) within the native HIV-1 envelope glycoprotein trimer. PNAS 108, 20148–20153. https://doi.org/10.1073/pnas.1104840108

Lobritz, M.A., Lassen, K.G., Arts, E.J., 2011. HIV-1 replicative fitness in elite controllers. Curr Opin HIV AIDS 6, 214–220. https://doi.org/10.1097/COH.0b013e3283454cf5

López, D., Gil-Torregrosa, B.C., Bergmann, C., Val, M.D., 2000. Sequential Cleavage by Metallopeptidases and Proteasomes Is Involved in Processing HIV-1 ENV Epitope for Endogenous MHC Class I Antigen Presentation. The Journal of Immunology 164, 5070–5077. https://doi.org/10.4049/jimmunol.164.10.5070

Lynch, R.M., Tran, L., Louder, M.K., Schmidt, S.D., Cohen, M., DerSimonian, R., Euler, Z., Gray, E.S., Abdool Karim, S., Kirchherr, J., Montefiori, D.C., Sibeko, S., Soderberg, K., Tomaras, G., Yang, Z.-Y., Nabel, G.J., Schuitemaker, H., Morris, L., Haynes, B.F., Mascola, J.R., 2012. The Development of CD4 Binding Site Antibodies during HIV-1 Infection. J Virol 86, 7588–7595. https://doi.org/10.1128/JVI.00734-12

Madhavi, V., Wines, B.D., Amin, J., Emery, S., Lopez, E., Kelleher, A., Center, R.J., Hogarth, P.M., Chung, A.W., Kent, S.J., Stratov, I., 2017. HIV-1 Env- and Vpu-Specific Antibody-Dependent Cellular Cytotoxicity Responses Associated with Elite Control of HIV. J Virol 91. https://doi.org/10.1128/JVI.00700-17

Mascola, J.R., Lewis, M.G., Stiegler, G., Harris, D., VanCott, T.C., Hayes, D., Louder, M.K., Brown, C.R., Sapan, C.V., Frankel, S.S., Lu, Y., Robb, M.L., Katinger, H., Birx, D.L., 1999. Protection of Macaques against Pathogenic Simian/Human Immunodeficiency Virus 89.6PD by Passive Transfer of Neutralizing Antibodies. J Virol 73, 4009–4018.

Mascola, J.R., Snyder, S.W., Weislow, O.S., Belay, S.M., Belshe, R.B., Schwartz, D.H., Clements, M.L., Dolin, R., Graham, B.S., Gorse, G.J., Keefer, M.C., McElrath, M.J., Walker, M.C., Wagner, K.F., McNeil, J.G., McCutchan, F.E., Burke, D.S., 1996. Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary isolates of human immunodeficiency virus type 1. The National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group. J. Infect. Dis. 173, 340–348.

Mascola, J.R., Stiegler, G., VanCott, T.C., Katinger, H., Carpenter, C.B., Hanson, C.E., Beary, H., Hayes, D., Frankel, S.S., Birx, D.L., Lewis, M.G., 2000. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat. Med. 6, 207–210. https://doi.org/10.1038/72318

McCaffrey, R.A., Saunders, C., Hensel, M., Stamatatos, L., 2004. N-Linked Glycosylation of the V3 Loop and the Immunologically Silent Face of gp120 Protects Human Immunodeficiency Virus Type 1 SF162 from Neutralization by Anti-gp120 and Anti-gp41 Antibodies. J Virol 78, 3279–3295. https://doi.org/10.1128/JVI.78.7.3279-3295.2004

McCoy, L.E., van Gils, M.J., Ozorowski, G., Messmer, T., Briney, B., Voss, J.E., Kulp, D.W., Macauley, M.S., Sok, D., Pauthner, M., Menis, S., Cottrell, C.A., Torres, J.L., Hsueh, J., Schief, W.R., Wilson, I.A., Ward, A.B., Sanders, R.W., Burton, D.R., 2016. Holes in the glycan shield of the native HIV envelope are a target of trimer-elicited neutralizing antibodies. Cell Rep 16, 2327–2338. https://doi.org/10.1016/j.celrep.2016.07.074

McLellan, J.S., Pancera, M., Carrico, C., Gorman, J., Julien, J.-P., Khayat, R., Louder, R., Pejchal, R., Sastry, M., Dai, K., O'Dell, S., Patel, N., Shahzad-ul-Hussan, S., Yang, Y., Zhang, B., Zhou, T., Zhu, J., Boyington, J.C., Chuang,

G.-Y., Diwanji, D., Georgiev, I., Do Kwon, Y., Lee, D., Louder, M.K., Moquin, S., Schmidt, S.D., Yang, Z.-Y., Bonsignori, M., Crump, J.A., Kapiga, S.H., Sam, N.E., Haynes, B.F., Burton, D.R., Koff, W.C., Walker, L.M., Phogat, S., Wyatt, R., Orwenyo, J., Wang, L.-X., Arthos, J., Bewley, C.A., Mascola, J.R., Nabel, G.J., Schief, W.R., Ward, A.B., Wilson, I.A., Kwong, P.D., 2011. Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. Nature 480, 336–343. https://doi.org/10.1038/nature10696

Medlock, J., Pandey, A., Parpia, A.S., Tang, A., Skrip, L.A., Galvani, A.P., 2017. Effectiveness of UNAIDS targets and HIV vaccination across 127 countries. Proc Natl Acad Sci U S A 114, 4017–4022. https://doi.org/10.1073/pnas.1620788114

Mesa, K.A., Yu, B., Wrin, T., Petropoulos, C.J., Pogson, G.H., Alexander, D.L., Perez, G., O'Rourke, S.M., Sinangil, F., Robinson, J., Conant, M.A., Berman, P.W., 2019. Ancestral sequences from an elite neutralizer proximal to the development of neutralization resistance as a potential source of HIV vaccine immunogens. PLoS One 14. https://doi.org/10.1371/journal.pone.0213409

Mikell, I., Sather, D.N., Kalams, S.A., Altfeld, M., Alter, G., Stamatatos, L., 2011. Characteristics of the Earliest Cross-Neutralizing Antibody Response to HIV-1. PLoS Pathog 7. https://doi.org/10.1371/journal.ppat.1001251

Miura, T., Brockman, M.A., Brumme, Z.L., Brumme, C.J., Pereyra, F., Trocha, A., Block, B.L., Schneidewind, A., Allen, T.M., Heckerman, D., Walker, B.D., 2009. HLA-Associated Alterations in Replication Capacity of Chimeric NL4-3 Viruses Carrying gag-protease from Elite Controllers of Human Immunodeficiency Virus Type 1. J Virol 83, 140–149. https://doi.org/10.1128/JVI.01471-08

Moldt, B., Rakasz, E.G., Schultz, N., Chan-Hui, P.-Y., Swiderek, K., Weisgrau, K.L., Piaskowski, S.M., Bergman, Z., Watkins, D.I., Poignard, P., Burton, D.R., 2012. Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. Proc Natl Acad Sci U S A 109, 18921–18925. https://doi.org/10.1073/pnas.1214785109

Montefiori, D.C., 2005. Evaluating neutralizing antibodies against HIV, SIV, and SHIV in luciferase reporter gene assays. Curr Protoc Immunol Chapter 12, Unit 12.11. https://doi.org/10.1002/0471142735.im1211s64

Montefiori, D.C., Karnasuta, C., Huang, Y., Ahmed, H., Gilbert, P., de Souza, M.S., McLinden, R., Tovanabutra, S., Laurence-Chenine, A., Sanders-Buell, E., Moody, M.A., Bonsignori, M., Ochsenbauer, C., Kappes, J., Tang, H.,

Greene, K., Gao, H., LaBranche, C.C., Andrews, C., Polonis, V.R., Rerks-Ngarm, S., Pitisuttithum, P., Nitayaphan, S., Kaewkungwal, J., Self, S.G., Berman, P.W., Francis, D., Sinangil, F., Lee, C., Tartaglia, J., Robb, M.L., Haynes, B.F., Michael, N.L., Kim, J.H., 2012. Magnitude and Breadth of the Neutralizing Antibody Response in the RV144 and Vax003 HIV-1 Vaccine Efficacy Trials. J Infect Dis 206, 431–441. https://doi.org/10.1093/infdis/jis367

Moore, P.L., Gray, E.S., Wibmer, C.K., Bhiman, J.N., Nonyane, M., Sheward, D.J., Hermanus, T., Bajimaya, S., Tumba, N.L., Abrahams, M.-R., Lambson, B.E., Ranchobe, N., Ping, L., Ngandu, N., Karim, Q.A., Karim, S.S.A., Swanstrom, R.I., Seaman, M.S., Williamson, C., Morris, L., 2012. Evolution of an HIV glycan–dependent broadly neutralizing antibody epitope through immune escape. Nat Med 18, 1688–1692. https://doi.org/10.1038/nm.2985

Moore, P.L., Ranchobe, N., Lambson, B.E., Gray, E.S., Cave, E., Abrahams, M.-R., Bandawe, G., Mlisana, K., Karim, S.S.A., Williamson, C., Morris, L., Immunology (CHAVI), the C. 002 study and the N.C. for H.V., 2009. Limited Neutralizing Antibody Specificities Drive Neutralization Escape in Early HIV-1 Subtype C Infection. PLOS Pathogens 5, e1000598. https://doi.org/10.1371/journal.ppat.1000598

Morales, J.F., Morin, T.J., Yu, B., Tatsuno, G.P., O'Rourke, S.M., Theolis, R., Mesa, K.A., Berman, P.W., 2014a. HIV-1 Envelope Proteins and V1/V2 Domain Scaffolds with Mannose-5 to Improve the Magnitude and Quality of Protective Antibody Responses to HIV-1. J. Biol. Chem. 289, 20526–20542. https://doi.org/10.1074/jbc.M114.554089

Morales, J.F., Morin, T.J., Yu, B., Tatsuno, G.P., O'Rourke, S.M., Theolis, R., Mesa, K.A., Berman, P.W., 2014b. HIV-1 Envelope Proteins and V1/V2 Domain Scaffolds with Mannose-5 to Improve the Magnitude and Quality of Protective Antibody Responses to HIV-1. J Biol Chem 289, 20526–20542. https://doi.org/10.1074/jbc.M114.554089

Morales, J.F., Yu, B., Perez, G., Mesa, K.A., Alexander, D.L., Berman, P.W., 2016. Fragments of the V1/V2 domain of HIV-1 glycoprotein 120 engineered for improved binding to the broadly neutralizing PG9 antibody. Molecular Immunology 77, 14–25. https://doi.org/10.1016/j.molimm.2016.07.003

Moulard, M., Decroly, E., 2000. Maturation of HIV envelope glycoprotein precursors by cellular endoproteases. Biochim. Biophys. Acta 1469, 121–132. https://doi.org/10.1016/s0304-4157(00)00014-9

Mwimanzi, P., Markle, T.J., Martin, E., Ogata, Y., Kuang, X.T., Tokunaga, M., Mahiti, M., Pereyra, F., Miura, T., Walker, B.D., Brumme, Z.L., Brockman, M.A., Ueno, T., 2013. Attenuation of multiple Nef functions in HIV-1 elite controllers. Retrovirology 10, 1. https://doi.org/10.1186/1742-4690-10-1

Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics, 1 edition. ed. Oxford University Press, Oxford ; New York.

Ofek, G., Guenaga, F.J., Schief, W.R., Skinner, J., Baker, D., Wyatt, R., Kwong, P.D., 2010. Elicitation of structure-specific antibodies by epitope scaffolds. Proc Natl Acad Sci U S A 107, 17880–17887. https://doi.org/10.1073/pnas.1004728107

Ofek, G., Tang, M., Sambor, A., Katinger, H., Mascola, J.R., Wyatt, R., Kwong, P.D., 2004. Structure and Mechanistic Analysis of the Anti-Human Immunodeficiency Virus Type 1 Antibody 2F5 in Complex with Its gp41 Epitope. J Virol 78, 10724–10737. https://doi.org/10.1128/JVI.78.19.10724-10737.2004

Okulicz, J.F., Marconi, V.C., Landrum, M.L., Wegner, S., Weintrob, A., Ganesan, A., Hale, B., Crum-Cianflone, N., Delmar, J., Barthel, V., Quinnan, G., Agan, B.K., Dolan, M.J., 2009. Clinical Outcomes of Elite Controllers, Viremic Controllers, and Long-Term Nonprogressors in the US Department of Defense HIV Natural History Study. J Infect Dis 200, 1714–1723. https://doi.org/10.1086/646609

O'Rourke, S.M., Sutthent, R., Phung, P., Mesa, K.A., Frigon, N.L., To, B., Horthongkham, N., Limoli, K., Wrin, T., Berman, P.W., 2015. Glycans Flanking the Hypervariable Connecting Peptide between the A and B Strands of the V1/V2 Domain of HIV-1 gp120 Confer Resistance to Antibodies That Neutralize CRF01_AE Viruses. PLOS ONE 10, e0119608. https://doi.org/10.1371/journal.pone.0119608

O'Rourke, S.M., Yu, B., Morales, J.F., Didinger, C.M., Alexander, D.L., Vollmers, C., Berman, P.W., 2019. Production of a recombinant monoclonal antibody to Herpes Simplex Virus glycoprotein D for immunoaffinity purification of tagged proteins. Journal of Immunological Methods 465, 31–38. https://doi.org/10.1016/j.jim.2018.11.015

Pancera, M., Changela, A., Kwong, P.D., 2017. How HIV-1 entry mechanism and broadly neutralizing antibodies guide structure-based vaccine design. Curr Opin HIV AIDS 12, 229–240. https://doi.org/10.1097/COH.0000000000000360

Pancera, M., Zhou, T., Druz, A., Georgiev, I.S., Soto, C., Gorman, J., Huang, J., Acharya, P., Chuang, G.-Y., Ofek, G., Stewart-Jones, G.B.E., Stuckey, J., Bailer, R.T., Joyce, M.G., Louder, M.K., Tumba, N., Yang, Y., Zhang, B., Cohen, M.S., Haynes, B.F., Mascola, J.R., Morris, L., Munro, J.B., Blanchard, S.C., Mothes, W., Connors, M., Kwong, P.D., 2014. Structure and immune recognition of trimeric pre-fusion HIV-1 Env. Nature 514, 455–461. https://doi.org/10.1038/nature13808

Parren, P.W.H.I., Marx, P.A., Hessell, A.J., Luckay, A., Harouse, J., Cheng-Mayer, C., Moore, J.P., Burton, D.R., 2001. Antibody Protects Macaques against Vaginal Challenge with a Pathogenic R5 Simian/Human Immunodeficiency Virus at Serum Levels Giving Complete Neutralization In Vitro. Journal of Virology 75, 8340–8347. https://doi.org/10.1128/JVI.75.17.8340-8347.2001

Pejchal, R., Doores, K.J., Walker, L.M., Khayat, R., Huang, P.-S., Wang, S.-K., Stanfield, R.L., Julien, J.-P., Ramos, A., Crispin, M., Depetris, R., Katpally, U., Marozsan, A., Cupo, A., Maloveste, S., Liu, Y., McBride, R., Ito, Y., Sanders, R.W., Ogohara, C., Paulson, J.C., Feizi, T., Scanlan, C.N., Wong, C.-H., Moore, J.P., Olson, W.C., Ward, A.B., Poignard, P., Schief, W.R., Burton, D.R., Wilson, I.A., 2011. A Potent and Broad Neutralizing Antibody Recognizes and Penetrates the HIV Glycan Shield. Science 334, 1097–1103. https://doi.org/10.1126/science.1213256

Perelson, A.S., Neumann, A.U., Markowitz, M., Leonard, J.M., Ho, D.D., 1996. HIV-1 Dynamics in Vivo: Virion Clearance Rate, Infected Cell Life-Span, and Viral Generation Time. Science 271, 1582–1586. https://doi.org/10.1126/science.271.5255.1582

Pereyra, F., Addo, M.M., Kaufmann, D.E., Liu, Y., Miura, T., Rathod, A., Baker, B., Trocha, A., Rosenberg, R., Mackey, E., Ueda, P., Lu, Z., Cohen, D., Wrin, T., Petropoulos, C.J., Rosenberg, E.S., Walker, B.D., 2008. Genetic and Immunologic Heterogeneity among Persons Who Control HIV Infection in the Absence of Therapy. J Infect Dis 197, 563–571. https://doi.org/10.1086/526786

Pereyra, F., Heckerman, D., Carlson, J.M., Kadie, C., Soghoian, D.Z., Karel, D., Goldenthal, A., Davis, O.B., DeZiel, C.E., Lin, T., Peng, J., Piechocka, A., Carrington, M., Walker, B.D., 2014. HIV Control Is Mediated in Part by CD8+ T-Cell Targeting of Specific Epitopes. J Virol 88, 12937–12948. https://doi.org/10.1128/JVI.01004-14

Petropoulos, C.J., Parkin, N.T., Limoli, K.L., Lie, Y.S., Wrin, T., Huang, W., Tian, H., Smith, D., Winslow, G.A., Capon, D.J., Whitcomb, J.M., 2000. A Novel Phenotypic Drug Susceptibility Assay for Human Immunodeficiency Virus

Type 1. Antimicrob. Agents Chemother. 44, 920–928. https://doi.org/10.1128/AAC.44.4.920-928.2000

Piantadosi, A., Panteleeff, D., Blish, C.A., Baeten, J.M., Jaoko, W., McClelland, R.S., Overbaugh, J., 2009. Breadth of Neutralizing Antibody Response to Human Immunodeficiency Virus Type 1 Is Affected by Factors Early in Infection but Does Not Influence Disease Progression. Journal of Virology 83, 10269–10274. https://doi.org/10.1128/JVI.01149-09

Pinter, A., Honnen, W.J., He, Y., Gorny, M.K., Zolla-Pazner, S., Kayman, S.C., 2004. The V1/V2 Domain of gp120 Is a Global Regulator of the Sensitivity of Primary Human Immunodeficiency Virus Type 1 Isolates to Neutralization by Antibodies Commonly Induced upon Infection. Journal of Virology 78, 5205–5215. https://doi.org/10.1128/JVI.78.10.5205-5215.2004

Preston, B.D., Poiesz, B.J., Loeb, L.A., 1988. Fidelity of HIV-1 reverse transcriptase. Science 242, 1168–1171. https://doi.org/10.1126/science.2460924

Prévost, J., Richard, J., Ding, S., Pacheco, B., Charlebois, R., Hahn, B.H., Kaufmann, D.E., Finzi, A., 2018. Envelope glycoproteins sampling states 2/3 are susceptible to ADCC by sera from HIV-1-infected individuals. Virology 515, 38–45. https://doi.org/10.1016/j.virol.2017.12.002

Reece, M., Herbenick, D., Schick, V., Sanders, S.A., Dodge, B., Fortenberry, J.D., 2010. Condom Use Rates in a National Probability Sample of Males and Females Ages 14 to 94 in the United States. The Journal of Sexual Medicine, Findings from the National Survey of Sexual Health and Behavior (NSSHB) Center for Sexual Health Promotion. Indiana University 7, 266–276. https://doi.org/10.1111/j.1743-6109.2010.02017.x

Reeves, P.J., Callewaert, N., Contreras, R., Khorana, H.G., 2002. Structure and function in rhodopsin: High-level expression of rhodopsin with restricted and homogeneous N-glycosylation by a tetracycline-inducible N-acetylglucosaminyltransferase I-negative HEK293S stable mammalian cell line. PNAS 99, 13419–13424. https://doi.org/10.1073/pnas.212519299

Rerks-Ngarm, S., Pitisuttithum, P., Nitayaphan, S., Kaewkungwal, J., Chiu, J., Paris, R., Premsri, N., Namwat, C., de Souza, M., Adams, E., Benenson, M., Gurunathan, S., Tartaglia, J., McNeil, J.G., Francis, D.P., Stablein, D., Birx, D.L., Chunsuttiwat, S., Khamboonruang, C., Thongcharoen, P., Robb, M.L., Michael, N.L., Kunasol, P., Kim, J.H., 2009. Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand [WWW Document]. http://dx.doi.org.oca.ucsc.edu/10.1056/NEJMoa0908492. https://doi.org/10.1056/NEJMoa0908492

Richman, D.D., Wrin, T., Little, S.J., Petropoulos, C.J., 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc Natl Acad Sci U S A 100, 4144–4149. https://doi.org/10.1073/pnas.0630530100

Ringe, R.P., Pugach, P., Cottrell, C.A., LaBranche, C.C., Seabright, G.E., Ketas, T.J., Ozorowski, G., Kumar, S., Schorcht, A., van Gils, M.J., Crispin, M., Montefiori, D.C., Wilson, I.A., Ward, A.B., Sanders, R.W., Klasse, P.J., Moore, J.P., 2019. 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. https://doi.org/10.1128/JVI.01656-18

Rodger, A.J., Cambiano, V., Bruun, T., Vernazza, P., Collins, S., Degen, O., Corbelli, G.M., Estrada, V., Geretti, A.M., Beloukas, A., Raben, D., Coll, P., Antinori, A., Nwokolo, N., Rieger, A., Prins, J.M., Blaxhult, A., Weber, R., Eeden, A.V., Brockmeyer, N.H., Clarke, A., Guerrero, J. del R., Raffi, F., Bogner, J.R., Wandeler, G., Gerstoft, J., Gutiérrez, F., Brinkman, K., Kitchen, M., Ostergaard, L., Leon, A., Ristola, M., Jessen, H., Stellbrink, H.-J., Phillips, A.N., Lundgren, J., Coll, P., Cobarsi, P., Nieto, A., Meulbroek, M., Carrillo, A., Saz, J., Guerrero, J.D.R., García, M.V., Gutiérrez, F., Masiá, M., Robledano, C., Leon, A., Leal, L., Redondo, E.G., Estrada, V.P., Marquez, R., Sandoval, R., Viciana, P., Espinosa, N., Lopez-Cortes, L., Podzamczer, D., Tiraboschi, J., Morenilla, S., Antela, A., Losada, E., Nwokolo, N., Sewell, J., Clarke, A., Kirk, S., Knott, A., Rodger, A.J., Fernandez, T., Gompels, M., Jennings, L., Ward, L., Fox, J., Lwanga, J., Lee, M., Gilson, R., Leen, C., Morris, S., Clutterbuck, D., Brady, M., Asboe, D., Fedele, S., Fidler, S., Brockmeyer, N., Potthoff, A., Skaletz-Rorowski, A., Bogner, J., Seybold, U., Roider, J., Jessen, H., Jessen, A., Ruzicic, S., Stellbrink, H.-J., Kümmerle, T., Lehmann, C., Degen, O., Bartel, S., Hüfner, A., Rockstroh, J., Mohrmann, K., Boesecke, C., Krznaric, I., Ingiliz, P., Weber, R., Grube, C., Braun, D., Günthard, H., Wandeler, G., Furrer, H., Rauch, A., Vernazza, P., Schmid, P., Rasi, M., Borso, D., Stratmann, M., Caviezel, O., Stoeckle, M., Battegay, M., Tarr, P., Christinet, V., Jouinot, F., Isambert, C., Bernasconi, E., Bernasconi, B., Gerstoft, J., Jensen, L.P., Bayer, A.A., Ostergaard, L., Yehdego, Y., Bach, A., Handberg, P., Kronborg, G., Pedersen, S. s, Bülow, N., Ramskover, B., Ristola, M., Debnam, O., Sutinen, J., Blaxhult, A., Ask, R., Hildingsson-Lundh, B., Westling, K., Frisen, E.-M., Cortney, G., O'Dea, S., Wit, S.D., Necsoi, C., Vandekerckhove, L., Goffard, J.-C., Henrard, S., Prins, J., Nobel, H.-H., Weijsenfeld, A., Eeden, A.V., Elsenburg, L., Brinkman, K., Vos, D., Hoijenga, I., Gisolf, E., Bentum, P.V., Verhagen, D., Raffi, F., Billaud, E., Ohayon, M., Gosset, D., Fior, A., Pialoux, G., Thibaut, P., Chas, J., Leclercq, V., Pechenot, V., Coquelin, V., Pradier, C., Breaud, S., Touzeau-Romer, V., Rieger, A., Geit, M.K.-M., Sarcletti, M., Gisinger, M., Oellinger, A., Antinori,

A., Menichetti, S., Bini, T., Mussini, C., Meschiari, M., Biagio, A.D., Taramasso, L., Celesia, B.M., Gussio, M., Janeiro, N., 2019. Risk of HIV transmission through condomless sex in serodifferent gay couples with the HIV-positive partner taking suppressive antiretroviral therapy (PARTNER): final results of a multicentre, prospective, observational study. The Lancet 393, 2428–2438. https://doi.org/10.1016/S0140-6736(19)30418-0

Rong, R., Bibollet-Ruche, F., Mulenga, J., Allen, S., Blackwell, J.L., Derdeyn, C.A., 2007. Role of V1V2 and Other Human Immunodeficiency Virus Type 1 Envelope Domains in Resistance to Autologous Neutralization during Clade C Infection. Journal of Virology 81, 1350–1359. https://doi.org/10.1128/JVI.01839-06

Rong, R., Li, B., Lynch, R.M., Haaland, R.E., Murphy, M.K., Mulenga, J., Allen, S.A., Pinter, A., Shaw, G.M., Hunter, E., Robinson, J.E., Gnanakaran, S., Derdeyn, C.A., 2009. Escape from Autologous Neutralizing Antibodies in Acute/Early Subtype C HIV-1 Infection Requires Multiple Pathways. PLOS Pathogens 5, e1000594. https://doi.org/10.1371/journal.ppat.1000594

Roy, A., Banerjee, R., Basak, S., 2017. HIV Progression Depends on Codon and Amino Acid Usage Profile of Envelope Protein and Associated Host-Genetic Influence. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.01083

Rudd, P.M., Dwek, R.A., 1997. Glycosylation: Heterogeneity and the 3D Structure of Proteins. Critical Reviews in Biochemistry and Molecular Biology 32, 1–100. https://doi.org/10.3109/10409239709085144

Sagar, M., Wu, X., Lee, S., Overbaugh, J., 2006. Human Immunodeficiency Virus Type 1 V1-V2 Envelope Loop Sequences Expand and Add Glycosylation Sites over the Course of Infection, and These Modifications Affect Antibody Neutralization Sensitivity. J Virol 80, 9586–9598. https://doi.org/10.1128/JVI.00141-06

Sanders, R.W., Derking, R., Cupo, A., Julien, J.-P., Yasmeen, A., Val, N. de, Kim, H.J., Blattner, C., Peña, A.T. de la, Korzun, J., Golabek, M., Reyes, K. de los, Ketas, T.J., Gils, M.J. van, King, C.R., Wilson, I.A., Ward, A.B., Klasse, P.J., Moore, J.P., 2013. A Next-Generation Cleaved, Soluble HIV-1 Env Trimer, BG505 SOSIP.664 gp140, Expresses Multiple Epitopes for Broadly Neutralizing but Not Non-Neutralizing Antibodies. PLOS Pathogens 9, e1003618. https://doi.org/10.1371/journal.ppat.1003618

Sanders, R.W., van Gils, M.J., Derking, R., Sok, D., Ketas, T.J., Burger, J.A., Ozorowski, G., Cupo, A., Simonich, C., Goo, L., Arendt, H., Kim, H.J., Lee, J.H., Pugach, P., Williams, M., Debnath, G., Moldt, B., van Breemen, M.J.,

Isik, G., Medina-Ramírez, M., Back, J.W., Koff, W., Julien, J.-P., Rakasz, E.G., Seaman, M.S., Guttman, M., Lee, K.K., Klasse, P.J., LaBranche, C., Schief, W.R., Wilson, I.A., Overbaugh, J., Burton, D.R., Ward, A.B., Montefiori, D.C., Dean, H., Moore, J.P., 2015. HIV Neutralizing Antibodies Induced by Native-like Envelope Trimers. Science 349, aac4223. https://doi.org/10.1126/science.aac4223

Sandonís, V., Casado, C., Alvaro, T., Pernas, M., Olivares, I., García, S., Rodríguez, C., del Romero, J., López-Galíndez, C., 2009. A combination of defective DNA and protective host factors are found in a set of HIV-1 ancestral LTNPs. Virology 391, 73–82. https://doi.org/10.1016/j.virol.2009.05.022

Sarzotti-Kelsoe, M., Bailer, R.T., Turk, E., Lin, C., Bilska, M., Greene, K.M., Gao, H., Todd, C.A., Ozaki, D.A., Seaman, M.S., Mascola, J.R., Montefiori, D.C., 2014. Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1. J. Immunol. Methods 409, 131–146. https://doi.org/10.1016/j.jim.2013.11.022

Sather, D.N., Armann, J., Ching, L.K., Mavrantoni, A., Sellhorn, G., Caldwell, Z., Yu, X., Wood, B., Self, S., Kalams, S., Stamatatos, L., 2009. Factors Associated with the Development of Cross-Reactive Neutralizing Antibodies during Human Immunodeficiency Virus Type 1 Infection. Journal of Virology 83, 757–769. https://doi.org/10.1128/JVI.02036-08

Scheid, J.F., Mouquet, H., Feldhahn, N., Seaman, M.S., Velinzon, K., Pietzsch, J., Ott, R.G., Anthony, R.M., Zebroski, H., Hurley, A., Phogat, A., Chakrabarti, B., Li, Y., Connors, M., Pereyra, F., Walker, B.D., Wardemann, H., Ho, D., Wyatt, R.T., Mascola, J.R., Ravetch, J.V., Nussenzweig, M.C., 2009. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. Nature 458, 636–640. https://doi.org/10.1038/nature07930

Scutari, R., Faieta, M., D'Arrigo, R., Fabeni, L., Mussini, C., Cossarizza, A., Casoli, C., Perno, C.F., Svicher, V., Alteri, C., Aquaro, S., 2018. The degree of HIV-1 amino acid variability is strictly related to different disease progression rates. Virus Genes 54, 493–501. https://doi.org/10.1007/s11262-018-1571-2

Seabright, G.E., Doores, K.J., Burton, D.R., Crispin, M., 2019. Protein and Glycan Mimicry in HIV Vaccine Design. J Mol Biol 431, 2223–2247. https://doi.org/10.1016/j.jmb.2019.04.016

Shen, X., Duffy, R., Howington, R., Cope, A., Sadagopal, S., Park, H., Pal, R., Kwa, S., Ding, S., Yang, O.O., Fouda, G.G., Le Grand, R., Bolton, D., Esteban, M., Phogat, S., Roederer, M., Amara, R.R., Picker, L.J., Seder, R.A., McElrath,

M.J., Barnett, S., Permar, S.R., Shattock, R., DeVico, A.L., Felber, B.K., Pavlakis, G.N., Pantaleo, G., Korber, B.T., Montefiori, D.C., Tomaras, G.D., 2015. Vaccine-Induced Linear Epitope-Specific Antibodies to Simian Immunodeficiency Virus SIVmac239 Envelope Are Distinct from Those Induced to the Human Immunodeficiency Virus Type 1 Envelope in Nonhuman Primates. J Virol 89, 8643–8650. https://doi.org/10.1128/JVI.03635-14

Shibata, R., Igarashi, T., Haigwood, N., Buckler–White, A., Ogert, R., Ross, W., Willey, R., Cho, M.W., Martin, M.A., 1999. Neutralizing antibody directed against the HIV–1 envelope glycoprotein can completely block HIV–1/SIV chimeric virus infections of macaque monkeys. Nat Med 5, 204–210. https://doi.org/10.1038/5568

Shingai, M., Donau, O.K., Plishka, R.J., Buckler-White, A., Mascola, J.R., Nabel, G.J., Nason, M.C., Montefiori, D., Moldt, B., Poignard, P., Diskin, R., Bjorkman, P.J., Eckhaus, M.A., Klein, F., Mouquet, H., Cetrulo Lorenzi, J.C., Gazumyan, A., Burton, D.R., Nussenzweig, M.C., Martin, M.A., Nishimura, Y., 2014. Passive transfer of modest titers of potent and broadly neutralizing anti-HIV monoclonal antibodies block SHIV infection in macaques. J Exp Med 211, 2061–2074. https://doi.org/10.1084/jem.20132494

Shingai, M., Nishimura, Y., Klein, F., Mouquet, H., Donau, O.K., Plishka, R., Buckler-White, A., Seaman, M., Piatak, M., Lifson, J.D., Dimitrov, D., Nussenzweig, M.C., Martin, M.A., 2013. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. Nature 503, 277–280. https://doi.org/10.1038/nature12746

Shrage, L., 2016. African Americans, HIV, and mass incarceration. The Lancet 388, e2–e3. https://doi.org/10.1016/S0140-6736(16)30830-3

Siepel, A.C., Halpern, A.L., Macken, C., Korber, B.T. m., 1995. A Computer Program Designed to Screen Rapidly for HIV Type 1 Intersubtype Recombinant Sequences. AIDS Research and Human Retroviruses 11, 1413–1416. https://doi.org/10.1089/aid.1995.11.1413

Silver, Z.A., Dickinson, G.M., Seaman, M.S., Desrosiers, R.C., 2019. A Highly Unusual V1 Region of Env in an Elite Controller of HIV Infection. Journal of Virology 93, e00094-19. https://doi.org/10.1128/JVI.00094-19

Simek, M.D., Rida, W., Priddy, F.H., Pung, P., Carrow, E., Laufer, D.S., Lehrman, J.K., Boaz, M., Tarragona-Fiol, T., Miiro, G., Birungi, J., Pozniak, A., McPhee, D.A., Manigart, O., Karita, E., Inwoley, A., Jaoko, W., DeHovitz, J., Bekker, L.-G., Pitisuttithum, P., Paris, R., Walker, L.M., Poignard, P., Wrin,

T., Fast, P.E., Burton, D.R., Koff, W.C., 2009. Human Immunodeficiency Virus Type 1 Elite Neutralizers: Individuals with Broad and Potent Neutralizing Activity Identified by Using a High-Throughput Neutralization Assay together with an Analytical Selection Algorithm. Journal of Virology 83, 7337–7348. https://doi.org/10.1128/JVI.00110-09

Sliepen, K., Han, B.W., Bontjer, I., Mooij, P., Garces, F., Behrens, A.-J., Rantalainen, K., Kumar, S., Sarkar, A., Brouwer, P.J.M., Hua, Y., Tolazzi, M., Schermer, E., Torres, J.L., Ozorowski, G., Woude, P. van der, Peña, A.T. de la, Breemen, M.J. van, Camacho-Sánchez, J.M., Burger, J.A., Medina-Ramírez, M., González, N., Alcami, J., LaBranche, C., Scarlatti, G., Gils, M.J. van, Crispin, M., Montefiori, D.C., Ward, A.B., Koopman, G., Moore, J.P., Shattock, R.J., Bogers, W.M., Wilson, I.A., Sanders, R.W., 2019. Structure and immunogenicity of a stabilized HIV-1 envelope trimer based on a group-M consensus sequence. Nat Commun 10, 1–16. https://doi.org/10.1038/s41467-019-10262-5

Smith, S.A., Wood, C., West, J.T., 2013. HIV-1 Env C2-V4 Diversification in a Slow-Progressor Infant Reveals a Flat but Rugged Fitness Landscape. PLoS One 8. https://doi.org/10.1371/journal.pone.0063094

Sok, D., Burton, D.R., 2018. Recent progress in broadly neutralizing antibodies to HIV. Nat Immunol 19, 1179–1188. https://doi.org/10.1038/s41590-018-0235-7

Sok, D., Doores, K.J., Briney, B., Le, K.M., Saye-Francisco, K.F., Ramos, A., Kulp, D.W., Julien, J.-P., Menis, S., Wickramasinghe, L., Seaman, M.S., Schief, W.R., Wilson, I.A., Poignard, P., Burton, D.R., 2014. Promiscuous glycan site recognition by antibodies to the high-mannose patch of gp120 broadens neutralization of HIV. Sci Transl Med 6, 236ra63. https://doi.org/10.1126/scitranslmed.3008104

Steichen, J.M., Kulp, D.W., Tokatlian, T., Escolano, A., Dosenovic, P., Stanfield, R.L., McCoy, L.E., Ozorowski, G., Hu, X., Kalyuzhniy, O., Briney, B., Schiffner, T., Garces, F., Freund, N.T., Gitlin, A.D., Menis, S., Georgeson, E., Kubitz, M., Adachi, Y., Jones, M., Mutafyan, A.A., Yun, D.S., Mayer, C.T., Ward, A.B., Burton, D.R., Wilson, I.A., Irvine, D.J., Nussenzweig, M.C., Schief, W.R., 2016. HIV Vaccine Design to Target Germline Precursors of Glycan-Dependent Broadly Neutralizing Antibodies. Immunity 45, 483–496. https://doi.org/10.1016/j.immuni.2016.08.016

Stephenson, K.E., D'Couto, H.T., Barouch, D.H., 2016. New concepts in HIV-1 vaccine development. Current Opinion in Immunology, Vaccines * Special

section: Cancer immunology: Genomics & biomarkers 41, 39–46. https://doi.org/10.1016/j.coi.2016.05.011

Stiegler, G., Armbruster, C., Vcelar, B., Stoiber, H., Kunert, R., Michael, N.L., Jagodzinski, L.L., Ammann, C., Jäger, W., Jacobson, J., Vetter, N., Katinger, H., 2002. Antiviral activity of the neutralizing antibodies 2f5 and 2g12 in asymptomatic Hiv-1-infected humans: a phase I evaluation. Aids 16, 2019–2025.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30, 2725–2729. https://doi.org/10.1093/molbev/mst197

Tomaras, G.D., Yates, N.L., Liu, P., Qin, L., Fouda, G.G., Chavez, L.L., Decamp, A.C., Parks, R.J., Ashley, V.C., Lucas, J.T., Cohen, M., Eron, J., Hicks, C.B., Liao, H.-X., Self, S.G., Landucci, G., Forthal, D.N., Weinhold, K.J., Keele, B.F., Hahn, B.H., Greenberg, M.L., Morris, L., Karim, S.S.A., Blattner, W.A., Montefiori, D.C., Shaw, G.M., Perelson, A.S., Haynes, B.F., 2008. Initial B-Cell Responses to Transmitted Human Immunodeficiency Virus Type 1: Virion-Binding Immunoglobulin M (IgM) and IgG Antibodies Followed by Plasma Anti-gp41 Antibodies with Ineffective Control of Initial Viremia. Journal of Virology 82, 12449–12463. https://doi.org/10.1128/JVI.01708-08

Tomescu, C., Duh, F.-M., Hoh, R., Viviani, A., Harvill, K., Martin, M., Carrington, M., Deeks, S., Montaner, L., 2012. Impact of protective killer inhibitory receptor/human leukocyte antigen genotypes on natural killer cell and T-cell function in HIV-1-infected controllers. Aids 26, 1869–1878. https://doi.org/10.1097/QAD.0b013e32835861b0

UNAIDS, 2019a. Global HIV & AIDS statistics — 2019 fact sheet [WWW Document]. URL https://www.unaids.org/en/resources/fact-sheet (accessed 10.14.19).

UNAIDS, 2019b. Women and HIV — A spotlight on adolescent girls and young women. UNAIDS.

van den Kerkhof, T.L.G.M., de Taeye, S.W., Boeser-Nunnink, B.D., Burton, D.R., Kootstra, N.A., Schuitemaker, H., Sanders, R.W., van Gils, M.J., 2016. HIV-1 escapes from N332-directed antibody neutralization in an elite neutralizer by envelope glycoprotein elongation and introduction of unusual disulfide bonds. Retrovirology 13. https://doi.org/10.1186/s12977-016-0279-4

van Gils, M.J., Bunnik, E.M., Boeser-Nunnink, B.D., Burger, J.A., Terlouw-Klein, M., Verwer, N., Schuitemaker, H., 2011. Longer V1V2 Region with Increased

Number of Potential N-Linked Glycosylation Sites in the HIV-1 Envelope Glycoprotein Protects against HIV-Specific Neutralizing Antibodies. J Virol 85, 6986–6995. https://doi.org/10.1128/JVI.00268-11

van Gils, M.J., Euler, Z., Schweighardt, B., Wrin, T., Schuitemaker, H., 2009. Prevalence of cross-reactive HIV-1-neutralizing activity in HIV-1-infected patients with rapid or slow disease progression: AIDS 23, 2405–2414. https://doi.org/10.1097/QAD.0b013e32833243e7

van Gils, M.J., Sanders, R.W., 2013. Broadly neutralizing antibodies against HIV-1: Templates for a vaccine. Virology, 2013 Reviews Issue 435, 46–56. https://doi.org/10.1016/j.virol.2012.10.004

Wagh, K., Kreider, E.F., Li, Y., Barbian, H.J., Learn, G.H., Giorgi, E., Hraber, P.T., Decker, T.G., Smith, A.G., Gondim, M.V., Gillis, L., Wandzilak, J., Chuang, G.-Y., Rawi, R., Cai, F., Pellegrino, P., Williams, I., Overbaugh, J., Gao, F., Kwong, P.D., Haynes, B.F., Shaw, G.M., Borrow, P., Seaman, M.S., Hahn, B.H., Korber, B., 2018. Completeness of HIV-1 Envelope Glycan Shield at Transmission Determines Neutralization Breadth. Cell Rep 25, 893-908.e7. https://doi.org/10.1016/j.celrep.2018.09.087

Walker, B.D., Yu, X.G., 2013. Unravelling the mechanisms of durable control of HIV-1. Nature Reviews Immunology 13, 487–498. https://doi.org/10.1038/nri3478

Walker, L.M., Huber, M., Doores, K.J., Falkowska, E., Pejchal, R., Julien, J.-P., Wang, S.-K., Ramos, A., Chan-Hui, P.-Y., Moyle, M., Mitcham, J.L., Hammond, P.W., Olsen, O.A., Phung, P., Fling, S., Wong, C.-H., Phogat, S., Wrin, T., Simek, M.D., Koff, W.C., Wilson, I.A., Burton, D.R., Poignard, P., 2011. Broad neutralization coverage of HIV by multiple highly potent antibodies. Nature 477, 466–470. https://doi.org/10.1038/nature10373

Walker, L.M., Phogat, S.K., Chan-Hui, P.-Y., Wagner, D., Phung, P., Goss, J.L., Wrin, T., Simek, M.D., Fling, S., Mitcham, J.L., Lehrman, J.K., Priddy, F.H., Olsen, O.A., Frey, S.M., Hammond, P.W., Kaminsky, S., Zamb, T., Moyle, M., Koff, W.C., Poignard, P., Burton, D.R., 2009. Broad and Potent Neutralizing Antibodies from an African Donor Reveal a New HIV-1 Vaccine Target. Science 326, 285–289. https://doi.org/10.1126/science.1178746

Walker, L.M., Simek, M.D., Priddy, F., Gach, J.S., Wagner, D., Zwick, M.B., Phogat, S.K., Poignard, P., Burton, D.R., 2010. A Limited Number of Antibody Specificities Mediate Broad and Potent Serum Neutralization in Selected HIV-1 Infected Individuals. PLOS Pathogens 6, e1001028. https://doi.org/10.1371/journal.ppat.1001028

Wang, B., Mikhail, M., Dyer, W.B., Zaunders, J.J., Kelleher, A.D., Saksena, N.K., 2003. First demonstration of a lack of viral sequence evolution in a nonprogressor, defining replication-incompetent HIV-1 infection. Virology 312, 135–150. https://doi.org/10.1016/S0042-6822(03)00159-4

Wang, W., Zirkle, B., Nie, J., Ma, J., Gao, K., Chen, X.S., Huang, W., Kong, W., Wang, Y., 2015. N463 Glycosylation Site on V5 Loop of a Mutant gp120 Regulates the Sensitivity of HIV-1 to Neutralizing Monoclonal Antibodies VRC01/03. J Acquir Immune Defic Syndr 69, 270–277. https://doi.org/10.1097/QAI.0000000000000595

Wardemann, H., Yurasov, S., Schaefer, A., Young, J.W., Meffre, E., Nussenzweig, M.C., 2003. Predominant Autoantibody Production by Early Human B Cell Precursors. Science 301, 1374–1377. https://doi.org/10.1126/science.1086907

Webb, B., Sali, A., 2016. Comparative Protein Structure Modeling Using MODELLER. Curr Protoc Bioinformatics 54, 5.6.1-5.6.37. https://doi.org/10.1002/cpbi.3

Wei, X., Decker, J.M., Wang, S., Hui, H., Kappes, J.C., Wu, X., Salazar-Gonzalez, J.F., Salazar, M.G., Kilby, J.M., Saag, M.S., Komarova, N.L., Nowak, M.A., Hahn, B.H., Kwong, P.D., Shaw, G.M., 2003. Antibody neutralization and escape by HIV-1. Nature 422, 307–312. https://doi.org/10.1038/nature01470

Whitcomb, J.M., Huang, W., Fransen, S., Limoli, K., Toma, J., Wrin, T., Chappey, C., Kiss, L.D.B., Paxinos, E.E., Petropoulos, C.J., 2007. Development and Characterization of a Novel Single-Cycle Recombinant-Virus Assay To Determine Human Immunodeficiency Virus Type 1 Coreceptor Tropism. Antimicrob Agents Chemother 51, 566–575. https://doi.org/10.1128/AAC.00853-06

Woods Group, 2005. GLYCAM Web.

Wu, X., Parast, A.B., Richardson, B.A., Nduati, R., John-Stewart, G., Mbori-Ngacha, D., Rainwater, S.M.J., Overbaugh, J., 2006. Neutralization Escape Variants of Human Immunodeficiency Virus Type 1 Are Transmitted from Mother to Infant. J Virol 80, 835–844. https://doi.org/10.1128/JVI.80.2.835-844.2006

Wu, X., Yang, Z.-Y., Li, Y., Hogerkorp, C.-M., Schief, W.R., Seaman, M.S., Zhou, T., Schmidt, S.D., Wu, L., Xu, L., Longo, N.S., McKee, K., O'Dell, S., Louder, M.K., Wycuff, D.L., Feng, Y., Nason, M., Doria-Rose, N., Connors, M., Kwong, P.D., Roederer, M., Wyatt, R.T., Nabel, G.J., Mascola, J.R., 2010. Rational Design of Envelope Identifies Broadly Neutralizing Human

Monoclonal Antibodies to HIV-1. Science 329, 856–861. https://doi.org/10.1126/science.1187659

Xiao, X., Chen, W., Feng, Y., Dimitrov, D.S., 2009a. Maturation Pathways of Cross-Reactive HIV-1 Neutralizing Antibodies. Viruses 1, 802–817. https://doi.org/10.3390/v1030802

Xiao, X., Chen, W., Feng, Y., Zhu, Z., Prabakaran, P., Wang, Y., Zhang, M.-Y., Longo, N.S., Dimitrov, D.S., 2009b. Germline-like predecessors of broadly neutralizing antibodies lack measurable binding to HIV-1 envelope glycoproteins: implications for evasion of immune responses and design of vaccine immunogens. Biochem Biophys Res Commun 390, 404–409. https://doi.org/10.1016/j.bbrc.2009.09.029

Yang, L., Sharma, S.K., Cottrell, C., Guenaga, J., Tran, K., Wilson, R., Behrens, A.-J., Crispin, M., de Val, N., Wyatt, R.T., 2018. Structure-Guided Redesign Improves NFL HIV Env Trimer Integrity and Identifies an Inter-Protomer Disulfide Permitting Post-Expression Cleavage. Front. Immunol. 9. https://doi.org/10.3389/fimmu.2018.01631

Yu, B., Fonseca, D.P.A.J., O'Rourke, S.M., Berman, P.W., 2010. Protease Cleavage Sites in HIV-1 gp120 Recognized by Antigen Processing Enzymes Are Conserved and Located at Receptor Binding Sites. Journal of Virology 84, 1513–1526. https://doi.org/10.1128/JVI.01765-09

Yu, B., Morales, J.F., O'Rourke, S.M., Tatsuno, G.P., Berman, P.W., 2012. Glycoform and Net Charge Heterogeneity in gp120 Immunogens Used in HIV Vaccine Trials. PLoS One 7. https://doi.org/10.1371/journal.pone.0043903

Zaunders, J., Dyer, W.B., Churchill, M., 2011. The Sydney Blood Bank Cohort: implications for viral fitness as a cause of elite control. Curr Opin HIV AIDS 6, 151–156. https://doi.org/10.1097/COH.0b013e3283454d5b

Zhang, M., Foley, B., Schultz, A.-K., Macke, J.P., Bulla, I., Stanke, M., Morgenstern, B., Korber, B., Leitner, T., 2010. The role of recombination in the emergence of a complex and dynamic HIV epidemic. Retrovirology 7, 1–15. https://doi.org/10.1186/1742-4690-7-25

Zhou, T., Doria-Rose, N.A., Cheng, C., Stewart-Jones, G.B.E., Chuang, G.-Y., Chambers, M., Druz, A., Geng, H., McKee, K., Kwon, Y.D., O'Dell, S., Sastry, M., Schmidt, S.D., Xu, K., Chen, L., Chen, R.E., Louder, M.K., Pancera, M., Wanninger, T.G., Zhang, B., Zheng, A., Farney, S.K., Foulds, K.E., Georgiev, I.S., Joyce, M.G., Lemmin, T., Narpala, S., Rawi, R., Soto, C., Todd, J.-P., Shen, C.-H., Tsybovsky, Y., Yang, Y., Zhao, P., Haynes, B.F.,

Stamatatos, L., Tiemeyer, M., Wells, L., Scorpio, D.G., Shapiro, L., McDermott, A.B., Mascola, J.R., Kwong, P.D., 2017. Quantification of the Impact of the HIV-1-Glycan Shield on Antibody Elicitation. Cell Reports 19, 719–732. https://doi.org/10.1016/j.celrep.2017.04.013

Zhou, T., Georgiev, I., Wu, X., Yang, Z.-Y., Dai, K., Finzi, A., Kwon, Y.D., Scheid, J.F., Shi, W., Xu, L., Yang, Y., Zhu, J., Nussenzweig, M.C., Sodroski, J., Shapiro, L., Nabel, G.J., Mascola, J.R., Kwong, P.D., 2010. Structural Basis for Broad and Potent Neutralization of HIV-1 by Antibody VRC01. Science 329, 811–817. https://doi.org/10.1126/science.1192819

Zhou, T., Zhu, J., Wu, X., Moquin, S., Zhang, B., Acharya, P., Georgiev, I.S., Altae-Tran, H.R., Chuang, G.-Y., Joyce, M.G., Kwon, Y.D., Longo, N.S., Louder, M.K., Luongo, T., McKee, K., Schramm, C.A., Skinner, J., Yang, Y., Yang, Z., Zhang, Z., Zheng, A., Bonsignori, M., Haynes, B.F., Scheid, J.F., Nussenzweig, M.C., Simek, M., Burton, D.R., Koff, W.C., Mullikin, J.C., Connors, M., Shapiro, L., Nabel, G.J., Mascola, J.R., Kwong, P.D., 2013. Multi-donor Analysis Reveals Structural Elements, Genetic Determinants, and Maturation Pathway for Effective HIV-1 Neutralization by VRCO1-class Antibodies. Immunity 39, 245–258. https://doi.org/10.1016/j.immuni.2013.04.012

Zhu, P., Liu, J., Bess, J., Chertova, E., Lifson, J.D., Grisé, H., Ofek, G.A., Taylor, K.A., Roux, K.H., 2006. Distribution and three-dimensional structure of AIDS virus envelope spikes. Nature 441, 847–852. https://doi.org/10.1038/nature04817

Zolla-Pazner, S., deCamp, A.C., Cardozo, T., Karasavvas, N., Gottardo, R., Williams, C., Morris, D.E., Tomaras, G., Rao, M., Billings, E., Berman, P., Shen, X., Andrews, C., O'Connell, R.J., Ngauy, V., Nitayaphan, S., de Souza, M., Korber, B., Koup, R., Bailer, R.T., Mascola, J.R., Pinter, A., Montefiori, D., Haynes, B.F., Robb, M.L., Rerks-Ngarm, S., Michael, N.L., Gilbert, P.B., Kim, J.H., 2013. Analysis of V2 Antibody Responses Induced in Vaccinees in the ALVAC/AIDSVAX HIV-1 Vaccine Efficacy Trial. PLoS One 8. https://doi.org/10.1371/journal.pone.0053629