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Amino Acid Changes in the HIV-1 gp41 Membrane Proximal Region Control Virus Neutralization Sensitivity

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Abstract

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Most HIV-1 vaccines elicit neutralizing antibodies that are active against highly sensitive (tier-1) viruses or rare cases of vaccine-matched neutralization-resistant (tier-2) viruses, but no vaccine has induced antibodies that can broadly neutralize heterologous tier-2 viruses. In this study, we isolated antibodies from an HIV-1-infected individual that targeted the gp41 membrane-proximal external region (MPER) that may have selected single-residue changes in viral variants in the MPER that resulted in neutralization sensitivity to antibodies targeting distal epitopes on the HIV-1 Env. Similarly, a single change in the MPER in a second virus from another infected-individual also conferred enhanced neutralization sensitivity. These gp41 single-residue changes thus transformed tier-2 viruses into tier-1 viruses that were sensitive to vaccine-elicited tier-1 neutralizing antibodies. These data demonstrate that Env amino acid changes within the MPER bnAb epitope of naturally-selected escape viruses can increase neutralization sensitivity to multiple types of neutralizing antibodies, and underscore the critical importance of the MPER for maintaining the integrity of the tier-2 HIV-1 trimer.

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1. Introduction

HIV-1 neutralization sensitivity occurs on a continuum, with some virus strains highly neutralization sensitive (tier-1 viruses) while others are more neutralization-resistant (tier-2 viruses). Transmitted/founder (T/F) HIV-1 strains are uniformly tier-2 viruses (Seaman et al., 2010; Derdeyn et al., 2014; Haim et al., 2011). The sensitivity of T/F viruses is shaped by easy-to-induce antibodies that can neutralize tier-1 viruses, thus selecting for neutralization-resistant founder viruses (Moody et al., 2015; Moore et al., 2009; Richman et al., 2003; Wei et al., 2003). The structural correlate of neutralization sensitivity is the exposure of epitopes such as the second (V2) and third (V3) variable loops on tier-1 viruses that are not exposed on tier-2 viruses (Mascola and Monteﬁori, 2010; McCoy and Weiss, 2013). FRET analysis has demonstrated that the HIV-1 envelope can oscillate between an “open” neutralization-sensitive state (tier-1) and a “closed” more neutralization-resistant (tier-2) state (Munro et al., 2014). However, our understanding of the contribution of specific Env sequences to overall trimer conformation is incomplete.

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HIV-1 Env is comprised of three gp120 monomers, each non-cova-
ently linked with a transmembrane gp41 subunit. The gp120 subunits
interact with target cell surface CD4 and a co-receptor to mediate viral
entry (Berger, 1997; Wyatt and Sodroski, 1998). The membrane-proxi-
mal external region (MPER) is a highly conserved 23 amino acid stretch
that is located in the heptad region-2 (HR-2) at the base of gp41, prox-
imal to the transmembrane domain. The MPER plays a critical role in
membrane fusion during viral entry into the host cell (Monteiro et al.,
2008). The high sequence diversity and glycan shield of HIV-1 Env
limit the breadth of most neutralizing antibodies, but broadly neutraliz-
ing antibodies (bNAbs) targeting the gp120, MPER and gp120–gp41
bridging regions have been identified (de Taeye et al., 2016; Burton
and Mascola, 2015). BNAbs take years to develop during natural HIV-1
infection, have attributes that are disfavored by the host immune sys-
tem, and have yet to be generated by any vaccine strategy (Haynes et
al., 2012; Mascola and Haynes, 2013). One fundamental challenge has
been the development of native-like Env trimers capable of expressing
bnAb epitopes while shielding non-neutralizing epitopes. Recently,
examples of native-like Env trimers, stabilized soluble gp140 SOSIP tri-
ers that have truncated gp41 ectodomains (Sanders et al., 2015),
have been developed and these trimers are able to induce autologous
tier-2 neutralizing antibodies but have yet to elicit bnAbs. SOSIP trimers
do not include the MPER (Sanders et al., 2015), and therefore would not
be expected to elicit antibodies against that epitope.

CAP206 is a South African CAPRISA 002 cohort participant, who was
infected by a clade C virus and at 81 weeks post-infection developed
neutralization breadth that was mediated by MPER-reactive antibodies
in the plasma (Gray et al., 2009). We previously isolated an MPER-react-
ive neutralizing mAb from CAP206 by single memory B cell sorting
(Morris et al., 2011). This mAb, CAP206-CH12 utilized the same VH
and Vκ gene segments, Vκ1-1-69 and Vκ3-20, and had a similar binding
footprint as bnAb 4E10. Study of Env sequences in CAP206 from soon
after infection to over 2.5 years revealed accumulation of amino acid
changes within the CAP206 MPER (K677 N, W680R, and K683Q). We
have also identified a similar gp41 MPER change in another clade C
African individual, CH505, who developed bnAbs against the CD4-bind-
ing site (CD4bs) of gp120 (Liao et al., 2013b). Here, we show that these
MPER changes determine HIV-1 neutralization sensitivity in both
infected individuals. MPER antibodies isolated early during infection
from CAP206 did not neutralize the viruses with MPER changes that
displayed enhanced neutralization sensitivity, indicating that early
autologous MPER-targeting antibodies could have selected for the MPER
amino acid changes.

2. Materials and Methods

2.1. Study Subjects

Plasma and PBMCs were isolated from serial blood samples that
were collected from subtype C HIV-1 infected, antiretroviral therapy-
naïve individuals CAP206 and CH505 (Liao et al., 2013b; Gray et al.,
2009; Morris et al., 2011). Plasma and PBMC samples were stored at
−80 °C and in liquid nitrogen tanks, respectively. HIV-1 viral envelope
sequences were obtained from plasma over the course of infection
(L. Morris, Unpublished). Ethical approval for studies using CAP206
specimens was obtained from the Universities of KwaZulu-Natal and the
Witwatersrand. All work related to human subjects was in compli-
ance with Institutional Review Board protocols approved by the Duke
University Institutional Review Board and the local ethics boards
where the individuals were recruited.

2.2. Site-Directed Mutagenesis

Specific amino acid changes to HIV-1 envelopes were introduced
using QuikChange Site-Directed Mutagenesis Kit and Quik Change
Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, Santa
Cara, CA). Mutations were confirmed by sequence analysis.

2.3. Neutralization Assays

Neutralizing antibody assays in TZM-bl cells were performed as
described (Montefiori, 2005). Recombinant monoclonal antibodies were
tested against autologous and heterologous HIV-1 Env-pseudotyped
viruses in eight serial threefold dilutions starting at 100 μg/ml or 50
μg/μl as described (Montefiori, 2005; Seaman et al., 2010). IC50
values were calculated using the five-parameter logistic nonlinear re-
gression model. The virus subtypes in the panel were selected to be con-
sistent with previous publications (Huang et al., 2012; Seaman et al.,
2010; Wu et al., 2010). Rhesus plasma neutralization titers were tested
against the same HIV-1 Env-pseudotyped viruses in serial dilutions
using non-heat inactivated plasma and titer was calculated as the recipro-
cal plasma dilutions causing a 50% reduction of relative light units (ID50).

2.4. Isolation and Expression of VnDnDnH and VκL Genes

The VκDκDκH and VκL gene-segment pairs of the isolated antibodies
were amplified by reverse transcription followed by semi-nested PCR
(RT-PCR) (Liao et al., 2009; Tiller et al., 2008) performed on flow-sorted
or limited dilution memory B cell cultures (Bonsignori et al., 2011). Anti-
gen-specific flow sorting was performed using HIV-1 Env CON-5 gp140,
an HIV-1 envelope known to react with all clades of HIV-1 positive sera
(Tomaras et al., 2008), or MPR.03 peptide (KKKNEQELLELDKWASL
WNWFDTINWLVIRKKK) tetramers as described (Morris et al., 2011).
Initial screening of memory B cell cultures was performed with CAP206
T/F gp140 Env. Antibodies were produced in bulk cultures by transient
transfection of Exp293Fs cells (Life technologies, Grand Island, NY) with
1 μg of each heavy- and light-chain genes synthesized in pcDNA plas-
mids (GeneScript, Piscataway, NJ) per 1 L transfection as described
(Liao et al., 2009). Rhesus macaque memory B cell sorting, gene amplifica-
tion and antibody production was performed as described (Bradley et al.,
2016; Wiebe et al., 2014).

2.5. Single Cell PCR Sequencing, Next-Generation Sequencing and Sequence
Annotation

A PCR purification kit (Qiagen, Valencia, CA) was used to purify all
single cell PCR products of Ig VκDκDκH and VκL genes. PCR products
were sequenced in forward and reverse directions using ABI 3700 in-
strument and BigDye sequencing kit (Applied Biosystems).

Base calling for each sequence was performed using Phred (Ewing
and Green, 1998). Forward and reverse strands of the Ig genes were as-
sembled into one final nucleotide sequence based on quality scores at
each base position and genetic information was inferred by using
SoDA (Munshaw and Kepler, 2010).

For high throughput DNA sequencing of Ig V(D)J genes, genomic
DNA samples were isolated from 9 serial aliquots of PBMCs from
CAP206 sampled from the following weeks post HIV-1 transmission:
4, 15, 22, 33, 68, 120, 146, 198 and 254 weeks using Using QIAamp
DNA mini kit. Heavy chain V gene-segment pairs of the isolated antibodies
were ampli
ed by reverse transcription followed by semi-nested PCR
(RT-PCR) (Liao et al., 2009; Tiller et al., 2008) performed on flow-sorted
or limited dilution memory B cell cultures (Bonsignori et al., 2011). Anti-
gen-specific flow sorting was performed using HIV-1 Env CON-5 gp140,
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mids (GeneScript, Piscataway, NJ) per 1 L transfection as described
(Liao et al., 2009). Rhesus macaque memory B cell sorting, gene amplifica-
tion and antibody production was performed as described (Bradley et al.,
2016; Wiebe et al., 2014).

2.6. Identification of Clone Members and Inference of UCA

Clonal relatedness of VκDκDκH and VκL sequences was determined
using an algorithm and the UCAs were inferred as described (Kepler et
al., 2014; Munshaw and Kepler, 2010).
2.7. CH82 Antibody Blocking Assay

Antibody blocking assays were performed with CH82_UCA, CH82, CH133, P9, PG16, PGT121, PGT125, PGT128 and 2G12. Three hundred eight-four-well ELISA plates (Costar #3700) were coated with CAP206 month 0 T/F gp140 overnight at 4 degree C and blocked with assay diluent (PBS containing 4% (weight/volume) whey protein/ 15% Normal Goat Serum/0.5% Tween20/ 0.05% Sodium Azide) for 1 h at room temperature (RT). Antibodies CH82_UCA, CH82, CH133, P9, PG16, PGT121, PGT125, PGT128 and 2G12, starting at 100 μg/ml and diluted two-fold, were incubated in triplicate wells. Biotinylated mAb CH82 was added at (0.1 μg/ml), the ECL50 determined by a direct binding of biotinylated-CH82, for 1 h at RT. Biotin-CH82 binding was detected with streptavidin–HRP (Thermo Scientific; Waltham, MA) at 1:30,000 (1 hour RT) followed by SureBlue Reserve TMB Microwell Peroxidase Substrate (Kirkegaard & Perry Laboratories, Inc.; Gaithersburg, MD). Reaction was stopped with 0.33 N HCl and plates were read at 450 nm. After background subtractions, percent inhibition was calculated as follows: 100-(mAb triplicate mean / no inhibition control mean) × 100.

2.8. Antibody Autoreactivity

The polyreactivity of mPER-reactive antibodies was assayed in the Athena multi-lyte system (Zeus Scientific).

2.9. Recombinant HIV-1 Proteins

HIV-1 Env proteins for ELISA and SPR assays included HIV-1 MN recombinant gp41 (Immunodiagnostik), HIV-1 group M consensus gp120 (CON-S) (Liao et al., 2006), HIV-1 clade C consensus (ConC) gp120, ConC gp120 N332A mutant, Env immunodominant region peptide sp400 (RVLAVERYLRD-QQLLGIWGCSG-KLICTTAVPWN-ASWSNKSLNK), gp41 MPER region peptide SP62 (QQEKNEQELLELDKWASLWN) and GCN4 gp41 Inter (Frey et al., 2010). CAP206 autologous transmitted/founder env and 6 additional envs from the first 30 months of infection were obtained from serial blood samples by single genome amplification (Keele et al., 2008), codon optimized (Andre et al., 1998) and de novo synthesized (GeneScript) as gp140 or gp120, and cloned into pcDNA3.1(hygromycin (Invitrogen). Recombinant Env glycoproteins were produced in 293F cells in serum-free media transfected with HIV-1 gp140 or gp120 expressing pcDNA3.1 plasmids, purified from the supernatant of transfected 293F cells using Galanthus nivalis lectin-agarose (Vector Labs) column chromatography, and stored at −80 °C. ELISA was performed as described (Liao et al., 2011; Liao et al., 2013a).

2.10. Rhesus Macaque Immunizations

12 Indian origin Macaca mulatta were immunized every 6 weeks either sequentially (n = 6) or with a swarm (n = 6) of 100 μg of 7 gp140 Envs isolated from the first 30 months of infection from CAP206 (T/F, 2 month, 6 month, 12 month, 21 month, 24 month and 30 month Envs) formulated with adjuvant MF59 (Novartis) in a 1:1 ratio in 1 mL. Blood samples were collected 2 weeks after each immunization. All rhesus macaques were housed at BioQual. All rhesus macaques were maintained in accordance with the Association for Assessment and Accreditation of Laboratory Animals with the approval of the Animal Care and Use Committees of the NIH and Harvard Medical School. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition.

3. Results

3.1. Amino Acid Changes in the gp41 MPER Increase Global Neutralization Sensitivity

We performed sequencing of HIV-1 env soon after infection through 2.5 years in CAP206 using single-genome amplification (SGA) (Bradley et al., 2016). Examination of the C-terminal MPER sequences revealed three amino acid changes (K677 N, W680R, and K683Q) in HIV-1 Envs isolated at 12 months and beyond (Fig. 1A). The 21 month and 21 month 30 viruses with the W680R mutation were resistant to neutralization by the MPER bnAb 4E10 (Fig. 1A). Analysis of 5129 HIV-1 Env sequences from the Los Alamos National Laboratory (LANL) HIV-1 sequence database revealed limited variability at positions 677 and 683, and conservation of the tryptophan at MPER position 680 with 99.4% of the sequences having W680 (Fig. 1B). In contrast, this position varied in sequences isolated longitudinally from CAP206, with tryptophan or arginine at position 680; only 87.8% of CAP206 sequences had W680 (Fig. 1B; lower panel). In all CAP206 sequences that had the W680R change, there were also coincidental changes of positions 677 and 683 to neutral amino acids, indicating that preserving the charge of the C-terminus of the MPER may be important for interactions with the viral membrane (Fig. 1C). Although rare, there were 51 Env sequences in the LANL database that had W680 changes. Like the change in CAP206, the majority of changes at position 680 were to positively charged amino acids (34 of 51), but there were select examples of changes to neutral or negatively charged residues (Fig. 1D). In the MPER sequences that changed to a positive residue at position 680 there was also an increase in a neutral change at position 683, but variation at positions 677 and 683 was observed (Fig. 1D).

Changes in the MPER have been shown to alter virus sensitivity to neutralizing antibodies and fusion inhibitors (Blish et al., 2008; Nakamura et al., 2010; Shen et al., 2010; Ringe and Bhattacharya, 2012), so to examine the role of these changes in HIV-1 immune escape, we tested sensitivity of all 7 CAP206 pseudoviruses to neutralization by purified IgG from 5 HIV-1 clade C infected individuals (Table 1). All 7 CAP206 viruses, including viruses with the W680 changes, were resistant to neutralization with geometric mean titers >100 μg/ml and remained classified as tier-2, difficult-to-neutralize, viruses (Table 1). We used site-directed mutagenesis to introduce the 3 individual naturally occurring changes alone and in combination (NRQ) into the CAP206 T/F virus. We also altered the highly conserved tyrosine in this region (Y681D) to examine its role in antibody escape; this residue was not a natural viral variant present in CAP206. All of the MPER mutant viruses demonstrated increased neutralization sensitivity to a panel of 5 HIV-1-infected plasma IgG samples, and all MPER mutant viruses, with the exception of CAP206 T/F W680R, exhibited a transition from a tier-2 to a tier-1 phenotype (Table 1). Although the CAP206 T/F W680R virus remained classified as tier-2, this virus demonstrated over a 3-fold enhancement in neutralization sensitivity as measured by the geometric mean titer of neutralization compared to the CAP206 T/F virus (Table 1). Relative infectivity of the CAP206 T/F and 5 mutant viruses was assessed by determining the tissue culture infectious dose 50 (TCID50) which is the viral stock dilution that results in approximately 150,000 RU in TZM-bl cells. Only the CAP206 T/F Y681D mutant virus showed reduced infectivity by this measure, having a 2-log reduction in TCID50 (Fig. 1S).

We sequenced the transmitted/founder virus and evolved Envs of another clade C HIV-1-infected individual, CH505, who produced bnAbs targeting the CD4 binding site. We identified a viral variant of the CH505 T/F virus with a single change (W680G) four weeks after transmission (w4.3) with no other amino acid changes when compared to the predominant T/F virus (Gao et al., 2014; Liao et al., 2013b). This was a single variant identified from 53 SGA sequences from week 4 post-transmission. We determined that the CH505 T/F virus was a tier-2 virus, whereas the CH505 w4.3 mutant with W680G was a
more neutralization-sensitive tier-1 virus (Table 1). These data demonstrate that MPER mutations within CAP206 and CH505 T/F virus backbones enhanced the global neutralization sensitivity and transform the tier-2 T/F virus into an easy-to-neutralize tier-1 virus.

3.2. Isolation of HIV Env-Targeting Antibodies during Infection from CAP206

We previously isolated a neutralizing MPER-reactive antibody, CAP206-CH12, from CAP206 (Morris et al., 2011). To identify additional antibodies that targeted the HIV-1 Env in CAP206, we performed single-cell PCR on HIV-1-specific memory B cells sorted from peripheral blood memory B cells 4–254 weeks post transmission using MPRI.03 tetraters and group M consensus Env Con-S. We also utilized limiting dilution cultures (Bonsignori et al., 2011) of single memory B cells and sequenced wells that exhibited CAP206 T/F gp140 reactivity. Using these methods, we isolated 41 monoclonal antibodies (mAbs) from CAP206 and confirmed HIV-1 Env reactivity by ELISA (Table S1). Fifteen of the 41 mAbs utilized the heavy chain variable gene segment (VH) 1–69 and 9 also used the kappa chain variable gene segment (Vk) 3–20; these are the same V-gene segments utilized by the neutralizing MPER-reactive mAb CAP206-CH12 and the broadly neutralizing MPER antibody 4E10 (Table S1; Fig. 2A). The reactivity of other mAbs that used VH1–69 and V3–20 was not limited to MPER but included additional gp41 and gp120 epitopes (Fig. 2A).

Two of the mAbs isolated by single-cell PCR that utilized Vh 1–69 and Vk 3–20, CH82 and CH82.2, have clonally related VhDjH and VkJc sequences (Table S1). We pyrosequenced genomic DNA isolated from 9 PBMC samples over the first 3 years of infection of CAP206, and identified additional members of this clonal lineage from multiple time points 33–146 weeks post transmission, from which we inferred the unmutated common ancestor (UCA, CH82 UCA (Fig. 2B). CH82 UCA, CH82.2 and CH82 all bound the consensus C (ConC) gp120 protein and binding was also blocked when the critical glycan site within the V3 loop at position 332 was mutated (N332A; Fig. 2C). To compare the binding epitope of CH82 to glycan dependent bnAbs, we tested variable loop 2 (V2)-reactive bnAbs, PG9, PG16, and V3-glycan bnAbs PGT121, PGT125, and PGT128 for their ability to block CH82 binding to the CAP206 T/F gp140 (Fig. 2D). V3-glycan dependent bnAb PGT128 blocked 73% of CH82 binding at 100 μg/ml, binding was also blocked by other V3-glycan dependent bnAbs, PGT121 and PGT126, 53% and 46%, respectively (Fig. 2D). V1/V2-glycan dependent mAbs PG9 and PG16 did not block CH82 binding by >35%.

The CAP206 evolved viruses with the W680R change were detected 21 months post-infection. For this reason, we sought to isolate MPER antibodies at earlier time points that may have provided selective pressure in this region. Two antibodies (DH643, CH12.2) that reacted with gp41, GCN4-inter (a gp41 intermediate state mimic), and MPER peptides were isolated 17 weeks post infection from CAP206 by single-cell PCR after sorting memory B cells from CAP206 that were positive for MPER binding (Fig. 2A).
for MPR03 peptides (Fig. 2E). CH12.2 used the same antibody gene segments (VH1-69, V3–20) utilized by MPER-targeting bnAbs CAP206–CH12 and 4E10, and was determined to be an early member of the CH12 bnAb clonal lineage (Morris et al., 2011). Antibody DH643 used unrelated gene segments (Table S1). DH424, DH425, and DH426 neutralized the CAP206 T/F Y681D mutant virus that displayed enhanced neutralization sensitivity. Only sporadic weak neutralization of the tier-2 CAP206 T/F could be detected in the immunized animals, but much higher titers of neutralizing antibodies to the tier-1 CAP206 Y681D virus was detected in all animals (Fig. 4C). We isolated 3 gp120 reactive mAbs DH423–DH425 from macaque 5096 2 weeks after the 4th immunization, and a 4th gp120 reactive mAb, DH426, was isolated from animal 5160 after the 3rd immunization. All 4 mAbs were IgG isotype and used a V3 gene segment from Vg family 4 (Table S3). DH424–426 reacted with a linear epitope in V3 and DH423 recognized a conformation-dependent epitope in gp120 (Fig. S3B).

The isolated antibodies were unable to neutralize the CAP206 T/F virus, but DH424, DH425 and DH426 neutralized the CAP206 T/F W680R virus, and all 4 isolated mAbs neutralized the CAP206 T/F Y681D virus in the TZM-bl neutralization assay (Fig. 4D). DH423–DH426 also neutralized the heterologous CH505 w4.3 virus, but lacked the ability to neutralize the CH505 T/F virus (Fig. 4E). Additionally, these antibodies neutralized the heterologous tier-1 virus C. MW965 and DH423 could also neutralize B. MN (Fig. 4E). These data demonstrated that viruses with MPER changes are sensitive to autologous CAP206 tier-1 neutralizing antibodies and easy-to-induce vaccine-elicited tier-1 gp120-targeting antibodies.

3.5. MPER Amino Acid Changes Increase Viral Neutralization Sensitivity to Antibodies against Multiple Epitopes and Alter gp120 Conformational Preference

Next, we tested neutralization of the CAP206 T/F and CAP206 mutant viruses by antibodies that targeted different Env epitopes and that were known to neutralize heterologous tier-1 or limited numbers of tier-2 pseudoviruses (Fig. 5A). Antibodies that targeted gp41 outside of the MPER did not neutralize the CAP206 T/F virus or any of the MPER mutants (DH628, DH629, and DH645). V2-targeting antibodies
CH58 and CH59 isolated from RV144 vaccines also did not neutralize CAP206 T/F or any mutants; in contrast, the V2 antibody 697D was able to neutralize the CAP206 W680R and Y681D mutant viruses but it failed to neutralize the CAP206 T/F virus. Antibodies that target the V3 loop (CH14, CH48 and 19B) also exhibited enhanced neutralization of the MPER mutant viruses—when compared with the CAP206 T/F virus, CH14 more potently neutralized K677N, W680R, and Y681D mutant viruses; CH48 more potently neutralized W680R and Y681D mutant viruses; and 19B was only able to neutralize the Y681D mutant virus. The CD4-binding site targeting mAb F105 was unable to neutralize any of the viruses, but CH13, which also targets the CD4-binding site, potently neutralized the CAP206 Y681D mutant virus. Lastly,
antibody 17B, which binds preferentially to the CD4-induced, CCR5 coreceptor binding site epitopes on Env, could neutralize the W680R virus, and even more potently, the Y681D mutant virus. Thus, changes at positions 680 and 681 result in greater binding of antibodies that have been shown to bind to more open Env conformations, suggesting that these changes in the MPER allow Env to sample more open states that resemble the CD4-bound conformation where the CCR5 binding site is exposed (Fig. 5A). Similar enhanced neutralization sensitivity to V3 (CH14, CH48 and 19B), CD4bs (CH13) and CD4-induced coreceptor (17B) targeting antibodies were observed for the CH505 w4.3 virus that had a W680G change in the MPER (Fig. 5B). We also tested neutralization of the MPER mutant viruses by bnAbs that target gp41 and gp120 epitopes on HIV Env. Among the CAP206 mutant viruses, only the Y681D mutant showed enhanced neutralization sensitivity to MPER bnAb 4E10 when compared to neutralization of the T/F virus (Fig. 5C). The CH505 w4.3 virus also exhibited enhanced neutralization sensitivity to 4E10, and was more sensitive to the CD4bs bnAbs b12 and CH103 (Fig. 5D). These data demonstrate that the CAP206 W680R and Y681D viruses and the CH505 w4.3 virus, which has a W680G change, exhibit enhanced neutralization sensitivity to weakly-neutralizing heterologous antibodies that target distal epitopes (V2, V3 and CD4bs) in gp120. In contrast, there was minimal impact of these mutations on sensitivity to bnAbs.

Next, we tested the ability of small molecules that mimic CD4 to inhibit infection of the viruses (Fig. 5E) (Melillo et al., 2016). The CAP206 T/F virus was inhibited by CD4 mimetics BNM-III-170 and BNM-IV-147 at IC_{50}s of 55.4 μM and 11.2 μM, respectively. The CAP206 T/F Y681D mutant virus exhibited increased sensitivity to both molecules with IC_{50} values of 2.9 μM and 2.5 μM. The CAP206 T/F W680R mutant had a more modest two-fold increase in sensitivity to both molecules. The CH505 T/F virus was resistant to BNM-III-170 but was inhibited by BNM-IV-147 with an IC_{50} of 77.1 μM. The CH505 w4.3 virus with a single W680G change became sensitive to BNM-III-170 and was over 30 times more sensitive to BNM-IV-147 (Fig. 5E). These results suggest that changes in the gp41 MPER can confer enhanced Env reactivity to CD4-mimetic compounds, consistent with a greater propensity for Envxs with these mutations to sample more open trimer conformations, which results in enhanced neutralization sensitivity.

4. Discussion

In this study, we identified amino acid changes in the gp41 MPER of CAP206 Env that modulate neutralization sensitivity by antibodies that target multiple HIV-1 Env epitopes. Changes in the MPER of the CAP206 Env can be detected as early as 12 months after infection, and when introduced into the CAP206 T/F virus, they converted the virus
neutralization phenotype from a neutralization-resistant tier-2 virus to a more easy-to-neutralize tier-1 virus. Additionally, we isolated MPER-targeting antibodies during the first 6 months of infection that could have selected these viral escape mutants. This includes antibody CH12.2 which was an early member of the CH12 bnAb lineage (Morris et al., 2011). Similarly, a T/F viral variant (W680G) from another bnAb individual, CH505, also conferred enhanced antibody neutralization sensitivity. Using bnAbs and narrow neutralizing antibodies, we demonstrated that antibodies that target the V2, V3 and CD4bs displayed enhanced neutralization of MPER-mutant viruses.

Changes in the gp41 and cytoplasmic domain outside of the MPER have been demonstrated to increase resistance to neutralization by gp120 antibodies (Watkins et al., 1996; Back et al., 1993; Haim et al., 2011) and modulate sensitivity to MPER-reactive bnAbs (Shen et al., 2010; Blish et al., 2008; Shen et al., 2009; Ringe and Bhattacharya, 2012). Natural polymorphisms are extremely rare for W680; however, naturally occurring variants resistant to the MPER bnAb 4E10 have been isolated and shown to be capable of mother-to-child transmission (Blish et al., 2008; Nakamura et al., 2010). The precise mechanisms of how the MPER changes enhance neutralization sensitivity remains unknown.

**Figure 4.** Tier-1 neutralizing gp120-targeting antibodies from CAP206 and CAP206 Env-immunized rhesus macaques neutralized viruses with MPER changes. (A–B) CH82 lineage and CH259 neutralization of (A) CAP206 T/F and CAP206 T/F MPER mutant viruses and (B) of heterologous CH505 T/F and variant w4.3 that has a W680 mutation in the MPER in the TZM-bl neutralization assay displayed as IC50 values. Simian virus amphotropic murine leukemia virus (SVA) was tested as a negative control. (C) Plasma neutralization at 8 timepoints post-immunization of 12 rhesus macaques immunized with a sequential (group 1) or swarm (group 2) of CAP206 T/F virus, CPA206 T/F Y681D virus and heterologous viruses B.MN and C. MW965 in the TZM-bl assay measured by ID50 (y-axis). Animals were immunized at weeks 0, 6, 12, 18, 24, 30 and 36. (D–E) Neutralization profiles of mAbs isolated from CAP206 Env immunized rhesus macaques (DH423-DH424) against (D) CAP206 T/F, CAP206 T/F MPER mutants, (E) CH505 T/F and CH505 w4.3 viruses. Measured as IC50 in the TZM-bl neutralization assay. Simian virus amphotropic murine leukemia virus (SVA) was tested as a negative control.
unclear. Prolonged epitope exposure due to changes in fusion kinetics, Env dissociation, Env expression and changes in infectivity may play a role in global enhancement of neutralization sensitivity, but were not sole factors for neutralization sensitivity in previous studies (Nakamura et al., 2010; Blish et al., 2008; Ringe and Bhattacharya, 2012; Vishwanathan and Hunter, 2008; Munoz-Barroso et al., 1999). MPER residue W680R and Y681D changes in CAP206 and W680G change in CH505 increase neutralization sensitivity to CD4-induced antibody 17b indicated that these two mutations acted by exposing the coreceptor binding site and other gp120 epitopes.

Consistent with previous studies we found that the MPER polymorphisms have different neutralization sensitivities depending on the genetic background of the virus. The CAP206 month 21 and month 30 viruses with the NRQ mutations remained difficult-to-neutralize tier-2 viruses, but when this mutation was introduced in the T/F virus it converted it to tier-1 (Nakamura et al., 2010). Moreover, we showed that two clade C T/F viruses have varying degrees of sensitivity to MPER changes; these changes in sensitivity are likely due in part to the fact that the conserved amino acid tryptophan is large and hydrophobic. Replacement of this tryptophan at position 680 in CH505 T/F with the less hydrophobic residue glycine confers greater neutralization sensitivity than we observed with the larger charged arginine residue as we observed in the CAP206 T/F. These results indicated that the properties of the amino acid changes in the MPER and compensatory mutations elsewhere in the Env can determine the magnitude of the neutralization phenotype change.

Fig. 5. MPER changes enhanced neutralization sensitivity to heterologous HIV antibodies and altered Env conformational preference. (A–D) Neutralization profiles of non-bnAbs (gp41 mAbs (DH628, DH629, DH645), V2 mAbs (CH58, CH59, 697D), V3 mAbs (CH14, CH18, 19B), CD4bs mAbs (CH13, F105) and conformational epitope mAb 17B; A–B) and bnAbs (C–D), measured as IC50 in TZM-bl neutralization assays, against transmitted founder viruses isolated from clade C infected individuals CAP206 and CH505, compared to T/F viruses containing MPER mutations that confer enhanced neutralization sensitivity (A,C) CAP206 viruses. (B,D) CH505 viruses. (E) Neutralization by two compounds the mimic CD4 and induce Env conformations similar the CD4-bound state (BNM-III-170 and BNM-IV-147) measured in the TZM-bl neutralization assay displayed as IC50 concentration (Yellow, <100; Red, <10).
Changes at amino acid position 680 in CAP206 were always associated with changes at positions 677 and 683, and in the LANL database, when position 680 is mutated to a positively charged residue there is higher prevalence of a neutral (Q) change at position 683. These 3 residues are solvent exposed within the second amphipathic helix and the coordinated changes of these positions may be important for interactions with distal Env regions or fusion dynamics and this mutation pattern has been observed in other viral sequences (Nakamura et al., 2010; Sun et al., 2008). Furthermore, amino acid residues 679–683 of the HIV-1 gp41 MPER have a cholesterol recognition amino acid consensus motif and disruption of this motif may inhibit viral fusion or stabilization of the Env within the viral membrane (Vishwanathan and Hunter, 2008). The motif WLWYIK overlaps with epitopes recognized by MHC class I alleles, but WLGYIK or WLRYIK do not. Thus, in addition to neutralizing antibody escape, changes at W680 could result in escape from cytotoxic T-lymphocyte responses early in infection before a potent neutralizing antibody response (Kundu et al., 1998; Colletto et al., 2009; Reins et al., 2007).

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The enhanced sensitivity of these MPER mutant viruses to gp120 antibodies and CD4-mimetic molecules demonstrate the importance of gp41 MPER residues near the viral membrane in maintaining the stability of the closed native trimer state. Structural studies of viruses containing these changes may provide insights into how these gp41 alterations affect Env conformations and present conserved epitopes more effectively. Moreover, eliciting MPER-targeting antibodies, like DH463 and CH12.2 that arose early and did not require high somatic hypermutation, that selected MPER changes that induce more open, easy-to-neutralize, trimer conformations by vaccination may contribute to viral clearance and protection. In particular, this scenario could be important in the setting of maternal-to-child transmission (MTCT) of HIV-1 where inducing these types of MPER-targeting antibodies that select MPER-changes that transform tier-2 viruses to tier-1 viruses in pregnant women could lead to lower transmission rates. Indeed, a previous study characterized a W680R mutation that was transmitted by MTCT that was resistant to the bnAb 4E10 (Nakamura et al., 2010), and a recent analysis of a W680R mutation that was transmitted by MTCT that was characterized a W680R mutation that was transmitted by MTCT that was resistant to the bnAb 4E10 (Nakamura et al., 2010), and a recent analysis of a W680R mutation that was transmitted by MTCT that was resistant to the bnAb 4E10 (Nakamura et al., 2010), and a recent analysis of a W680R mutation that was transmitted by MTCT that was resistant to the bnAb 4E10 (Nakamura et al., 2010), and a recent analysis of a W680R mutation that was transmitted by MTCT that was resistant to the bnAb 4E10 (Nakamura et al., 2010).

References

André, S., Seed, B., Eberle, J., Schraut, W., Bultmann, A., Haas, J., 1998. Increased immune response elicited by DNA vaccination with a synthetic gp120 sequence with optimized codon usage. J. Virol. 72, 1497–1503.
Back, N.K., Smitt, L., Schutten, M., Nara, P.L., Tersmette, M., Goudsmit, J., 1993. Mutations in human immunodeficiency virus type 1 gp41 affect sensitivity to neutralization by gp120 antibodies. J. Virol. 67, 6897–6902.
Berger, E.A., 1997. HIV entry and tropism: the chemokine receptor connection. AIDS 11 (Suppl A), S3–16.
Blish, C.A., Nguyen, M.A., Overbaugh, J., 2008. Enhancing exposure of HIV-1 neutralization epitopes through mutations in gp41. PLoS Med. 5, e9.
Bonignori, M., Hwang, K.K., Chen, X., Tsao, C.Y., Morris, L., Gray, E., Marshall, D.J., Crump, J.A., Kapija, S.H., Sam, N.E., Singhal, F., Pancrea, M., Yongpring, Y., Zhang, B., Zhu, J., Kwong, P.D., O’Dell, S., Mascola, J.R., Wu, L., Nabel, G.J., Phogat, S., Seaman, M.S., Whitesides, J.F., Moody, M.A., Kelsoe, G., Yang, X., Sodroski, J., Shaw, G.M., Montefiori, D.C., Kepler, T.B., Tomaras, G.D., Alam, S.M., Liao, H.X., Haynes, B.F., 2011. Analysis of a clonal lineage of HIV-1 envelope V2/V3 conformational epitope–specific broadly neutralizing antibodies and their inferred unmutated common ancestors. J. Virol. 85, 9998–10009.
Boyd, S.D., Marshall, E.L., Merker, J.D., Maniar, J.M., Zhang, L.N., Sahaf, B., Jones, C.D., Simen, B.B., Hanczaruk, B., Nguyen, X.D., Kau, M., Egholm, M., Milikis, D.B., Zehnder, J.L., Fire, A.Z., 2009. Measurement and clinical monitoring of human lymphocyte cholinergic activity by massively parallel VDJ pyrosequencing. Sci. Transl. Med. 1, 12ea23.
Bradley, T., Fera, D., Bihman, J., Eslamizar, L., Lu, X.Z., Anasti, K., Zhang, R.J., Sutherland, L.L., Sancear, R.M., Bowman, C.M., Stolaruch, C., Lloyd, K.E., Parks, R., Eaton, A., Foulger, A., Nie, X.Y., Karim, S.S.A., Barnett, S., Kelsoe, G., Kepler, T.B., Alam, S.M., Montefiori, D.C., Moody, M.A., Liao, H.X., Morris, L., Santra, S., Harrison, S.C., Haynes, B.F., 2016. Structural constraints of virus-induced tier-2 autologous HIV neutralizing antibodies targeting the receptor-binding site. Cell Rep. 14, 43–54.
Burton, D.R., Marcela, J.R., 2015. Antibody responses to envelope glycoproteins in HIV-1 infection. Nat. Immunol. 16, 571–576.
Colletto, B.A., Huang, X.L., Melhem, N.M., Fan, Z., Borowski, L., Rappocciolo, G., Rinaldo, C.R., 2009. Primary human immunodeficiency virus type 1-specific CD8+ T-cell responses induced by myeloid dendritic cells. J. Virol. 83, 6288–6299.
de Teye, S.W., Moore, J.P., Sanders, R.W., 2016. HIV-1 Envelope Trimer Design and Immunization Strategies to Induce Broadly Neutralizing Antibodies Trends Immunol. Dennison, S.M., Stewart, S.M., Stempel, K.C., Liao, H.X., Haynes, B.F., Alam, S.M., 2009. Stable docking of neutralizing human immunodeficiency virus type 1 gp41 membrane-proximal external region monoclonal antibodies 2F5 and 4E10 is dependent on the membrane immersion depth of their epitope regions. J. Virol. 83, 10211–10223.
Derdeyn, C.A., Moore, P.L., Morris, L., 2014. Development of broadly neutralizing antibodies from autologous neutralizing antibody responses in HIV infection. Curr. Opin. HIV AIDS 9, 210–216.
Ewing, B., Green, P., 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 8, 186–194.
Frey, G., Chen, J., Rits-Volloch, S., Freeman, M.M., Zolla-Pazner, S., Chen, B., 2010. Distinct epitopes of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2016.08.045.
Neutralizing antibodies to HIV-1 induced by immunization.

Mascola, J.R., Monte

Lu, X., Yu, J.S., Hwang, K.K., Gao, F., Markowitz, M., Heath, S.L., Bar, K.J., Goepfert, P.A., T., Moody, M.A., Haynes, B.F., 2009. High-throughput isolation of immunoglobulin genes from single human B cells and expression as monoclonal antibodies. J. Virol. 83, 4137–4146.

Monte

Monte

Munoz-Barroso, I., Salzwedel, K., Hunter, E., Blumenthal, K.R., 1999. Role of the membrane proximal external domain of gp41 in the initial viremia. J. Virol. 82, 12449–12460.

Moody, M.A., Gao, F., Curely, T.C., Amos, J.D., Kumar, A., Hora, B., Marshall, D.J., Whitesides, J.F., Xia, S.M., Parks, R., Lloyd, K.E., Hwang, K.K., Liu, X., Bonsignori, M., Finzi, A., Vazquez, K.A., Shin, H., Xiang, Y., Chen, H.R., Huang, C., Cohen, M.S., Sam, N.E., Kapiga, S., Gray, E.S., Tumba, N.L., Morris, L., Lopes, P., Gorny, M.K., Mascola, J.R., Hahn, B.H., Shaw, G.M., Sodroski, J.G., Liao, H.X., Montefiori, D.C., Crabtree, P.T., Horber, K.T., Butte, B.F., 2015. Strategic-specific V3 and CD4 binding site neutralizing antibodies select neutralization-resistant variants. Cell Host Microbe 18, 356–362.

Moore, P.L., Rancho, N., Lamson, B.E., Gray, E.S., Cave, E., Chambers, M.R., Bandawre, G., Mascola, R., Kozink, L., Debnath, G., Moldt, B., Van Breemen, M.J., ISIK, G., Medina-Ramirez, M., Back, M.E., Scearce, R.M., Vanleeuwen, S., Alam, S.M., Xie, S.M., Brock, M.E., Alam, S.M., Mclellan, J.S., Tomaras, G.D., Moody, M.A., Kozink, L.A., JR., 2009. Broad neutralization of HIV-1 by a gp41-specific human antibody. Nature 491, 406–412.

Keele, B.F., Giorgi, E., Elazar-Zon, J., Crunch, J., Hwang, S.C., Tshabangu, F., Wang, L., Courter, J.R., Princiotto, A., Mcgee, K., Stuart, N., Staszewski, S., Self, S.G., Zolla-Pazner, S., Gao, F., Montefiori, D.C., Shaw, G.C., Alam, S.M., Margolis, D.M., Denny, T.N., Boyd, S.D., Alam, S.M., Moody, M.A., Xia, S.M., Brock, M.E., Scearce, R.M., Vanleeuwen, S., Alam, S.M., Mclellan, J.S., Tomaras, G.D., Moody, M.A., Kozink, L.A., JR., 2009. Broad neutralization of HIV-1 by a gp41-specific human antibody. Nature 491, 406–412.

Monte

Moody, M.A., Gao, F., Curely, T.C., Amos, J.D., Kumar, A., Hora, B., Marshall, D.J., Whitesides, J.F., Xia, S.M., Parks, R., Lloyd, K.E., Hwang, K.K., Liu, X., Bonsignori, M., Finzi, A., Vazquez, K.A., Shin, H., Xiang, Y., Chen, H.R., Huang, C., Cohen, M.S., Sam, N.E., Kapiga, S., Gray, E.S., Tumba, N.L., Morris, L., Lopes, P., Gorny, M.K., Mascola, J.R., Hahn, B.H., Shaw, G.M., Sodroski, J.G., Liao, H.X., Montefiori, D.C., Crabtree, P.T., Horber, K.T., Butte, B.F., 2015. Strategic-specific V3 and CD4 binding site neutralizing antibodies select neutralization-resistant variants. Cell Host Microbe 18, 356–362.

Moore, P.L., Rancho, N., Lamson, B.E., Gray, E.S., Cave, E., Chambers, M.R., Bandawre, G., Mascola, R., Kozink, L., Debnath, G., Moldt, B., Van Breemen, M.J., ISIK, G., Medina-Ramirez, M., Back, M.E., Scearce, R.M., Vanleeuwen, S., Alam, S.M., Mclellan, J.S., Tomaras, G.D., Moody, M.A., Kozink, L.A., JR., 2009. Broad neutralization of HIV-1 by a gp41-specific human antibody. Nature 491, 406–412.

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Vishwanathan, S.A., Hunter, E., 2008. Importance of the membrane-perturbing properties of the membrane-proximal external region of human immunodeficiency virus type 1 gp41 to viral fusion. J. Virol. 82, 5118–5126.

Watkins, B.A., Buge, S., Aldrich, K., Davis, A.E., Robinson, J., Reitz JR., M.S., Robert-Guroff, M., 1996. Resistance of human immunodeficiency virus type 1 to neutralization by natural antisera occurs through single amino acid substitutions that cause changes in antibody binding at multiple sites. J. Virol. 70, 8431–8437.

Weili, 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.

Weihe, K., Easteoff, D., Luo, K., Nicola, N.L., Bradley, T., Jaeger, F.H., Denison, S.M., Zhang, R.J., Lloyd, K.E., Stolarachuk, C., Parks, R., Sutherland, L.L., Scearce, R.M., Morris, L., Kaewkungval, J., Niyazaphan, S., Pitisuttithum, P., Berks-Ngarm, S., Sinangil, F., Phogat, S., Michael, N.L., Kim, J.H., Kelsoe, C., Montefiori, D.C., Tomaras, G.D., Bonsignori, M., Santra, S., Kepler, T.B., Alan, S.M., Moody, M.A., Liao, H.X., Haynes, B.F., 2014. Antibody light-chain-restricted recognition of the site of immune pressure in the RV144 HIV-1 vaccine trial is phylogenetically conserved. Immunity 41, 309–318.

Wu, X., Yang, Z.Y., Li, Y., Hogerkoop, 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.

Wyatt, R., Sodroski, J., 1998. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science 280, 1884–1888.

Zhang, R., Verkoczy, L., Wiehe, K., Munir Alam, S., Nicely, N.L., Santra, S., Bradley, T., Pemble, C.W.T., Zhang, J., Gao, F., Montefiori, D.C., Bouton-Verville, H., Kelsoe, C., Larimore, K., Greenberg, P.D., Parks, R., Foulger, A., Peel, J.N., Luo, K., Lu, X., Trama, A.M., Vandergriff, N., Tomaras, G.D., Kepler, T.B., Moody, M.A., Liao, H.X., Haynes, B.F., 2016. Initiation of immune tolerance-controlled HIV gp41 neutralizing B cell lineages. Sci. Transl. Med. 8, 336ra62.