IDENTIFICATION OF HIV BROADLY NEUTRALIZING MONOCLONAL ANTIBODIES TARGETING MPER

Poster 303

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BACKGROUND

Broadly neutralizing monoclonal antibodies (bNAbs) target sites of vulnerability on the HIV envelope, such as the gp41 membrane proximal external region (MPER)[1]. MPER-directed bNAbs of the 4E10-class, such as VRC42.01 and PGZL1, have been previously isolated from chronically subtype CRF_01 AE and subtype B infected donors, respectively [2,3]. Plasma from donor RV149.1027 was characterized prior via neutralization, fingerprinting, binding, and biolayer interferometry (BLI) and indicated that 4E10-like MPER directed antibodies were present. From this chronically infected subtype B donor we report the isolation of another 4E10-class bNAb, MHRP01. MHRP01 and members of its class represent a public clonotype that has the potential to be targeted through vaccination efforts.

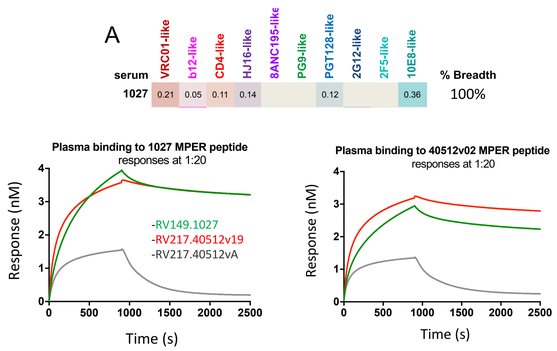


Fig 1. MPER directed antibodies detected in RV149.1027 plasma. (A) Predicted neutralization fingerprints of RV149.1027 plasma to VRC.01, b12, CD4, HJ16, PGT128, and 10E8-like antibodies. This analysis shows predictive potential when the score is >0.25 indicating the plasma from this donor likely has 4E10-like MPER directed antibodies [4]. **(B)** BLI analysis via Octet of plasma antibodies from donor RV149.1027 and donor RV217.40512 at pre-infection (v.A) and chronic infection (v.19) showing binding to RV149.1027 (left) and RV217.40512 MPER (right) peptides. Plasma antibodies that bound to donors 1027 and 40512 MPER peptides were detected in donor 1027 plasma at response rates similar to donor 40512, a donor previously described to have potent neutralizing MPER directed mAbs.

METHODS

- Monoclonal antibodies (mAbs) were isolated from donor RV149.1027 using two parallel methods: high-throughput B-cell culture followed by microneutralization assays, and fluorescently labeled MPER peptide to capture antigen-specific B-cells by cell sorting.
- B-cell receptors were sequenced, and heavy and light chain sequences were cloned into expression vectors and expressed in Expi293F cells [2].
- MAbs were then characterized for binding, epitope mapping, and neutralization. MAbs with potent cross-neutralization were further characterized by crystal structure determination.

RESULTS

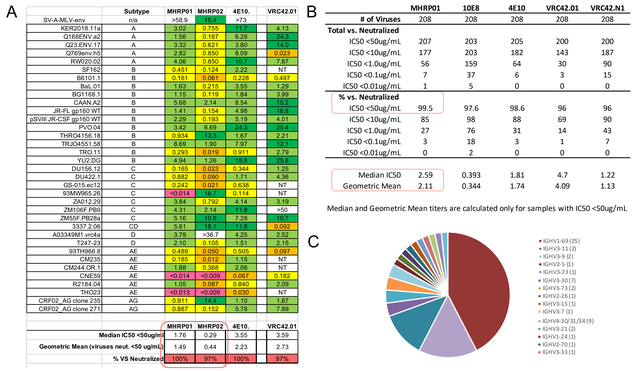


Fig 2. Heterologous neutralization of mAbs isolated from donor RV149.1072. (A) MHRP01 and MHRP02 isolated from donor RV149.1027 were tested for heterologous neutralization in a panel of 35 diverse pseudoviruses from different subtypes with the IC50 (μg/mL) values listed. MHRP01 and MHRP02 neutralized 100% and 97% of the panel, indicating potential breadth and potency for both mAbs. **(B)** MHRP01 is both potent and broad against much larger heterologous neutralization panel of 208 pseudoviruses from different subtypes with the IC50 (μg/mL) values listed. MHRP01 was able to neutralize 99.5% of the 208 pseudoviruses with an IC50 <50μg/mL. **(C)** Pie chart showing the variable heavy chain gene usage from the antibodies isolated from donor RV149.1072. The most frequent usage is IGHV1-69 shown in red, which is the same exact variable heavy chain gene usage as other MPER directed broadly neutralizing mAbs such as 4E10 and VRC42 [5].

Donor	Antibody	% Breadth	VH α	% SMH β	HCDR3 Γ	VK/L δ	% SMH	LCDR3 ε
PG13	PGZL1	84	VH1-69	21	15	VK3-20	13	9
Unknown	4E10	98.6	VH1-69	13	18	VK3-20	7	9
CAP206 (C)	CH12	6	VH1-69	12	15	VK3-20	5	11
RV217.40512 (AE)	VRC42.1	96	VH1-69	11	15	VK3-20	6	9
RV149.1027 (B)	MHRP01	99.5	VH1-69	19	16	VK3-20	16	9
RV149.1027 (B)	MHRP02	97	VH4-34	14	17	VL3-19	14	11
CH0210	DH517	50	VH4-30-4	23	22	VL3-19	14	12

Table 1. Heavy and light chain gene usage MHRP01, MHRP02, and other MPER directed mAbs. MHRP01 has identical heavy and light chain usage to 4E10 and VRC42, other MPER directed mAbs isolated from donors of different subtypes. The HCDR3 length and percent somatic hypermutation is also similar to other MPER directed mAbs. α: variable heavy chain. β: % somatic hypermutation Γ: heavy chain CDR3 length. δ: gene usage for the variable light chain ε: light chain CDR3 length.

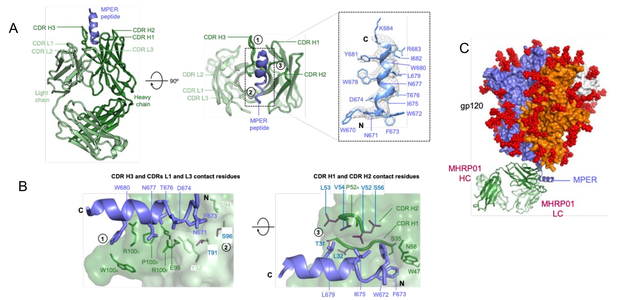


Fig 4. Crystal structure of MHRP01 reveals residues important for binding. (A) MHRP01 Fab is shown in ribbon representation colored dark green (heavy chain) and light green (light chain) in complex with MPER peptide (residues 670-684) in two orientations. MPER peptide is shown in ribbon and stick representation in purple and contact residues shown in a zoomed in view of the MPER peptide with 2fo-fc electron density contoured at 1σ (gray). CDR residues are numbered using the Kabat format. **(B)** MHRP01 contact residues (1) CDR L1; (2) CDR H3; (3) CDR H1 and H2. **(C)** Model of MHRP01 Fab showing heavy and light chains bound to Env trimer gp120 (PDB: 5FUU; EMD-3312).

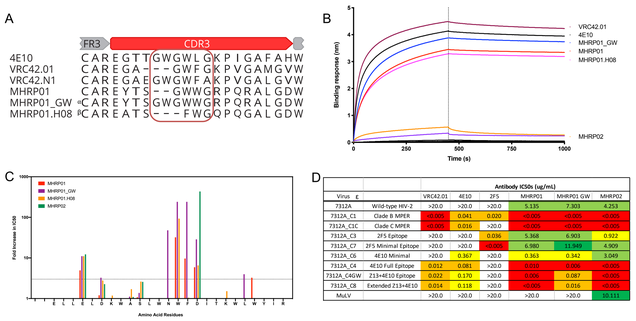


Fig. 3. MHRP01 and MHRP02 have specificity for MPER. (A) Sequence alignment of broadly neutralizing MPER directed mAbs and MHRP01, MHRP01_GW, and MHRP01.H08. The red box indicates a possible bNAb signature that includes "GW" amino acids which was utilized in MHRP01_GW. **(B)** BLI analysis via Octet of MPER mAbs against the MPER peptide from RV149.1027. Slightly more robust binding to MPER peptide is seen using the GW MHRP01 variant compared to the parent MHRP01. MHRP02, despite high neutralization potency and breadth, does not bind to the MPER peptide very well. **(C)** Fine epitope mapping was conducted using a panel of alanine scanning mutants along the MPER of BG168. The fold increase in IC50 (μg/mL) indicates decreased neutralization when residues are mutated to alanine. MHRP01_GW mutant appeared to expand the number of critical residues for neutralization. **(D)** MHRP01, MHRP01_GW mutant, and MHRP02 show directed neutralization against HIV-1/HIV-2 MPER chimeras. MHRP01 and MHRP02 mAbs recognize and are directed towards MPER due to neutralization of specific chimera's C1 and C1C, which come from clade B and C MPER respectfully, specifically at the 4E10, Z13+4E10, and extended Z13+4E10 epitopes. ε: Chimeras contain HIV-2 backbone scaffolding which display HIV-1 grafted epitopes such as clade B and C MPER, 2F5, and 4E10.

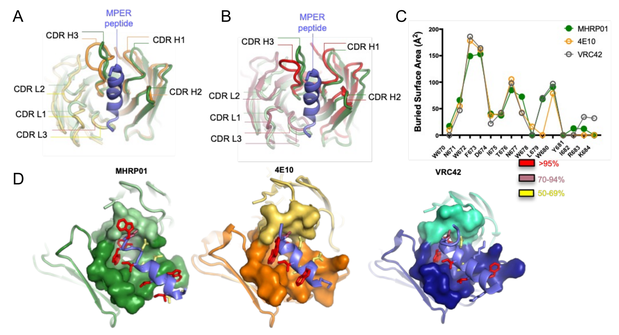


Fig 5. Structural comparison of MHRP01 with other HIV-1 MPER targeting antibodies. (A) Structural superimposition of MHRP01 and 4E10 antibodies indicating overlap in binding to the MPER peptide with similar buried surface interface and CDR3 loops indicated. **(B)** Structural superimposition of MHRP01 and VRC42 antibodies with the same conditions as (A). **(C)** Total buried surface area (BSA)(A2) for the MPER residues contacting with heavy and light chain residues of MHRP01, 4E10, and VRC42 antibodies. **(D)** Antibody-MPER contact regions for antibodies MHRP01 (green), 4E10 (orange), and VRC42 (indigo) are shown in surface representation. MPER peptide is colored blue, and residue side chains are colored based on HIV-1 sequence conservation.

CONCLUSIONS

- mAbs targeting MPER have been isolated from **multiple individuals infected with different HIV subtypes.**
- While the MHRP01_GW mutation appeared to increase the number of critical residues for neutralization, it did not improve overall neutralization compared to MHRP01. MHRP01_GW did appear to have more robust binding capabilities compared to parent MHRP01 (*Fig. 3*).
- MHRP01 exhibits **breadth and potency** directed towards MPER (*Fig. 3*).
- VRC42.01 (subtype CRF01_AE), PGZL1 (subtype B) and now MHRP.01 (subtype B) have **identical gene usage**, **targeted epitope**, **angle of binding**, and **neutralization profiles** (*Table 1, Fig. 2, Fig. 3, Fig. 4*).
- MHRP01 crystal structure indicates **strong structural similarities** between other MPER directed mAbs 4E10 and VRC42 (*Fig. 5*).
- Suggests that individuals from different demographic areas could elicit B-cell clones targeting this epitope through vaccination efforts.
- This public clonotype targeting the MPER epitope may be **easier for mAb production** compared to CD4 or V3 epitopes that require much more mutation.

ADDITIONAL KEY INFORMATION

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The views expressed are those of the authors and should not be construed to represent the positions of the U.S. Army or the Department of Defense.

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