High resistance of HIV-1$_{CH040}$ envelope glycoproteins to bnAbs without loss of efficient viral entry

307

Durgadevi Parthasarathy[1], Stephanie Pickthorn[1], Shamim Ahmed[1], Dimitry Mazurov[1], Jeffy Jeffy[1], Rajni Kant Shukla[2], Amit Sharma[2] & Alon Herschhorn[1]*

[1]Division of Infectious Diseases and International Medicine, Department of Medicine, University of Minnesota, Minneapolis, Minnesota 55455;

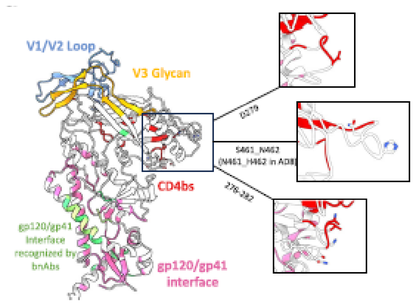
[2]Division of Veterinary Biosciences, Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH 43210, USA.

BACKGROUND

- HIV-1 Envs are critical for viral entry and the main target of neutralizing antibodies.
- Envs must balance immune evasion and efficient viral entry. Envs evolve to be robust, tolerating mutations without losing functionality.
- This study shows that CH040 Envs resist most bnAbs without apparently affecting efficient HIV-1 entry.
- CH040 Envs mediate entry as effectively as other primary Envs.

METHODS

- Sensitivity of recombinant luciferase-expressing viruses displaying CH040 Envs to different antibodies was measured.
- Site-directed mutagenesis. We engineered changes in regions of CH040 Envs presumably important for bnAb antiviral activity.
- We used cell-cell transmission, cell-cell fusion, western blot, and cold sensitivity assays to assess viral entry efficiency, protein expression, and transmission.
- SHIV replication kinetics and viral-cell fusion were analyzed in macaque lymphocytes.



Target bnAb Epitopes: Mutations were focused on specific regions:

gp120 CD4bs: Loop D (residues 276-282) and V5 (residues 461-462).

gp41 MPER: Residues W672 and W680.
Introduced Mutations:

gp120: D279E, S461N+N462D, and 276/282 changes.

gp41: W672L, W680G.

Fig 1: Rational Design of Mutations in CH040 Envs

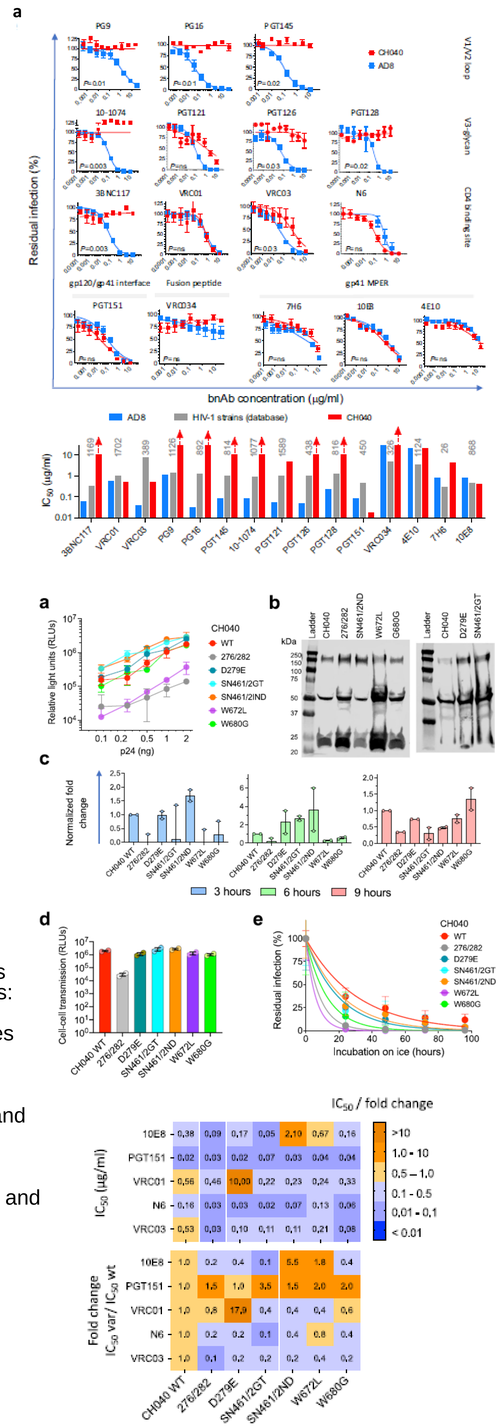


Fig 2: Pre-existing resistance of transmitted/founder CH040 Envs to bnAbs.

(a) Effects of increasing concentrations of different bnAbs on viral entry mediated by HIV-1$_{CH040}$ or the control HIV-1$_{AD8}$ Envs.
(b) Comparison of the sensitivity of HIV-1 CH040 Envs and the average sensitivity of between 26 and 1589 Envs to different bnAbs. We calculated average sensitivity of different Envs using available data in HIV-1 databases [(HIResist and www.hiv.lanl.gov)].

Fig 3: Efficiency of HIV-1 entry mediated by engineered variants of CH040 Envs.

(a) Relationship between number of pseudovirus particles (p24) and viral entry into Cf2-Th/CD4 + CCR5+target cells mediated by viruses pseudotyped with WT HIV-1$_{CH040}$ or indicated engineered Env variants
(b) Western blot analysis of viruses pseudotyped with WT CH040 Envs or with CH040 Env variants.
(c–e) Phenotypic characterization of CH040 Env variants. Cell–cell fusion (c), cell–cell transmission (d), and cold sensitivity (e) of viruses pseudotyped with WT HIV-1$_{CH040}$ Envs or indicated engineered Env variants.

Fig 4: Sensitivity of the specified CH040 Env variants to bnAbs.

Top, IC_{50} values
Bottom, fold change of IC_{50}

Summary.
CH040 Envs can efficiently enter target cells and still highly resist multiple bnAbs.

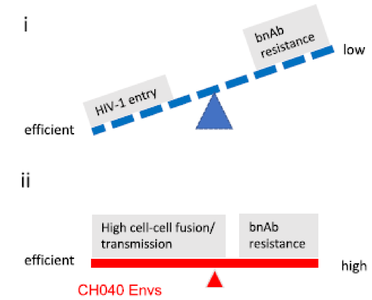


Fig 5: Graphical summary of CH040 Env phenotype.

Many HIV-1 strains exhibit efficient entry and low bnAb-resistance (i); but some strains, such as CH040, can very efficiently enter target cells and still highly resist multiple bnAbs (ii).

CONCLUSIONS

- CH040 Envs can sustain their viral competency while resisting bnAbs.
- Most specific mutations engineered in CH040 Envs did not significantly change bnAb sensitivity.
- Future research could focus on understanding how bnAb-resistant strains evolve and creating effective bnAbs or vaccines to target these resistant HIV-1 strains.

REFERENCES

1. Parthasarathy, D., Pickthorn, S., Ahmed, S. *et al.* Incompletely closed HIV-1$_{CH040}$ envelope glycoproteins resist broadly neutralizing antibodies while mediating efficient HIV-1 entry. *npj Viruses* **3**, 3 (2025).
2. Parthasarathy, D., Pothula, K.R., Ratnapriya, S. et al. Conformational flexibility of HIV-1 envelope glycoproteins modulates transmitted/founder sensitivity to broadly neutralizing antibodies. Nat Commun 15, 7334 (2024).

ACKNOWLEDGEMENT

This work was supported by the Avenir Award 1DP2DA049479 (NIH) Director's New Innovator Award) from NIH/NIDA as well as the U01 grant 1U01AI169587 and R01 1R014AI167653, both from the National Institute of Allergy and Infectious Diseases.