Antibody-Mediated Mechanics on HIV-1 Envelope Protein gp41 at the Membrane Interface Defined by EPR Spectroscopy

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Introduction

A vaccine capable of stimulating protective anti-viral antibodies is needed to curtail the global AIDS epidemic. HIV enters human T cells through the fusion of viral and host-cell membranes. This fusion process is mediated by a surface protein, gp41, and the platform provided by the cholesterol-rich viral membrane. The membrane proximal ectodomain region (MPER) of gp41 plays a critical role in this fusion process and is a major target of vaccine design and anti-gp41 antibodies, including a newly identified potent antibody - 10E8 [1] . How these antibodies bind to the MPER segment and mediate anti-viral activity are unclear. Here, electron paramagnetic resonance (EPR) techniques were used to define the manner in which these antibodies recognize the L-shaped helix-hinge-helix MPER segment.

Experimental

The MPER peptides with a single cysteine substitution at various positions were synthesized at the Tufts Core Facility, and subsequently labeled with MTSL spin labels (R1). EPR experiments were performed on a Bruker E680 spectrometer at the NHMFL.

Results and Discussion

10E8-induced MPER conformational changes at the membrane interface were determined by EPR solvent accessibility and immersion depth measurements. Membrane immersion depths of MPER R1 residues were determined by the ratio of the collision accessibility values of relaxant reagents, oxygen and NiEDDA, measured via power saturation techniques. Upon 10E8 binding, residues 5R1 and 13R1 were lifted out of the membrane surface and exposed to the aqueous phase (Fig. 1A). 10E8 also moves residues 8R1, 9R1, 17R1 and 20R1 towards the membrane surface. Taken together, 10E8 lifts up MPER N-helix and hinge region as indicated by the model built based on x-ray crystallography structure and EPR immersion depth data (Fig. 1B).

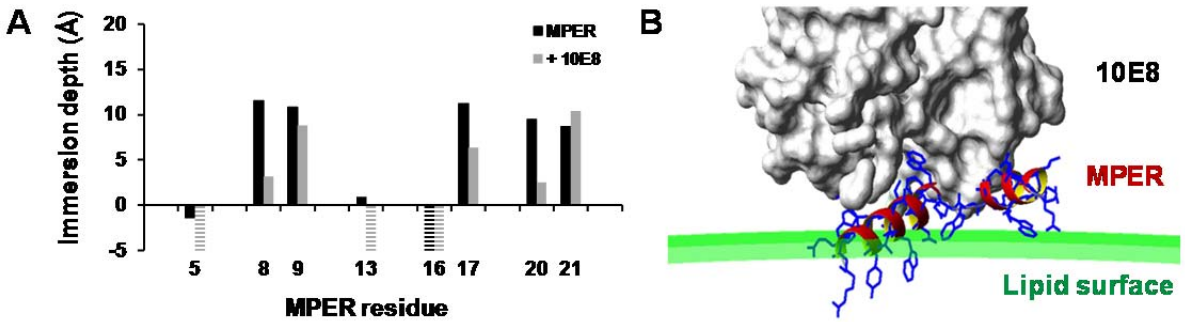

Fig. 1. (A) Immersion depth changes of MPER R1 residues upon 10E8 binding. Depth values between -5 Å to 0 Å and larger than 0 Å correspond to lipid headgroup region and acyl chain region, respectively. Lipid surface exposed residues are indicated by stripped bars. (B) Model of MPER segment in complex with 10E8 antibody as viewed from the side.

Conclusions

10E8 induces large conformational changes in the MPER relative to the membrane, lifting up the hinge region and extracting buried residues from the lipids. Thus, anti-HIV antibodies disrupt virus entry into human T cells by perturbing a facile MPER hinge function during membrane fusion. These findings have revealed important features for targeted vaccine design, the implications of which extend to antibody targets on other infectious diseases.

Acknowledgements

This work was supported by NIH grants RO1AI84785 / U19AI91693 (to E.L.R.), a grant from the Gates Foundation (to E.L.R.), and an UCGP grant 5080 (to L.S.).

References

[1] Huang, J., *et al.*, Nature, **491**, 406-412 (2012).