Retrovirology

BioMed Central

Poster presentation

P04-04. The specific phospholipid binding and neutralizing activities of the anti-HIV antibody 4E10 are separable

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from AIDS Vaccine 2009
Paris, France. 19–22 October 2009

Published: 22 October 2009

Retrovirology 2009, **6**(Suppl 3):P32 doi:10.1186/1742-4690-6-S3-P32

This abstract is available from: http://www.retrovirology.com/content/6/S3/P32

Background

Human 4E10 is one of the broadest-specificity, HIV-1 neutralizing, monoclonal antibodies described to date, recognizing a linear, membrane-proximal epitope on gp41. Although the apparent rarity of 4E10-like antibody responses in HIV infections is suggested to result from their elimination through B cell tolerance mechanisms to self-antigens, the lipid cross-reactivity of 4E10 has also been suggested to derive from virus-specific membrane binding outside of the MPER, rather than auto-reactivity, that contributes to neutralization potency.

Methods

To investigate how 4E10 interacts with membrane components (particularly phospholipids like cardiolipin), and whether such interactions contribute to neutralization mechanisms, we have introduced two mutations into 4E10 Fv constructs: W(H100)A and G(L50)E. The binding of 4E10 and these mutants to liposomes of varying composition, to 4E10 epitope peptides and to soluble monomeric and trimeric HIV Env gp140 was then assayed by surface plasmon resonance.

Results

These analyses revealed that wild type and mutant 4E10 Fvs all bind with the same affinity to epitope peptides and monomeric and trimeric gp140s, but that the affinities for gp140s are uniformly ten-fold weaker than to peptides. Addition of cardiolipin to liposomes increases wild-type, but not mutant, 4E10 Fv SPR binding responses, suggesting that the mutations individually and cleanly attenuate specific 4E10/lipid interactions. However, 4E10 Fv binding to lipid bilayers of any composition is extremely weak. While the W(H100)A affects neutralization significantly, the G(L50)E mutation, selected to disrupt a possible phosphate-binding site on 4E10, does not decrease neutralization efficiency against diverse HIV-1 isolates.

Conclusion

This dichotomy suggests that specific lipid interactions and neutralization by 4E10 are formally separable. Additional EPR experiments suggest an alternate explanation for the effect of the W(H100)A on neutralization: this mutation reduces the ability of 4E10 to wedge the MPER out of the membrane.