Peripheral Membrane Interactions Boost the Engagement by an Anti HIV-1 Broadly Neutralizing Antibody

P01-05

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Abstract:

The 4E10 antibody displays an extreme breadth of HIV-1 neutralization and therefore constitutes a model system for structure-guided vaccine design and immunotherapeutics. In this regard, the relevance of auto-reactivity with membrane lipids for the biological function and development of this antibody is still subject of controversy. To address this issue, here we have compared membrane-partitioning capacities of the 4E10 antibody and several of its variants, which were mutated at the paratope surface in contact with the membrane-interface. We have first used a physical separation approach (vesicle flotation), and subsequently carried out quantitative fluorescence measurements in an intact system (spectroscopic titration), using 4E10 Fab labeled with the polarity-sensitive 4-Chloro-7-Nitrobenz-2-Oxa-1,3-Diazole (NBD) probe. Moreover, recognition of epitope peptide in membrane has been probed by photo-cross linking using a Fab that incorporated the genetically encoded unnatural amino acid p-benzoylphenylalanine (pBPA). The experimental data rule out stereospecific recognition of viral lipids as a requirement for function, but support nonspecific electrostatic interactions between 4E10 basic residues and acidic phospholipids in membranes. Membrane-partitioning energetics indicates that 4E10 behaves as a peripheral membrane protein, using in concert interactions mediated by solvent-exposed hydrophobic and basic residues for enhancing its ability to binding viral membrane-associated ligand epitope. The implications of these findings for the natural production and biological function of this antibody are discussed.