The Road Less Traveled During Anti-HIV Antibody Maturation

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The quest to develop a vaccine against HIV represents a major effort in viral immunology, but has met with limited success. While there are naturally occurring antibodies, such as 4E10, that neutralize a wide range of HIV strains, they occur in few patients and have not been elicited through any standard vaccine development approach. To determine why 4E10 is difficult to generate through standard vaccine development regimes, graduate student Kathryn Finton and colleagues in the laboratory of Dr. Roland Strong (Basic Sciences Division) traced its path to maturity by determining the structure of 4E10 alone and in complex with part of its target, the HIV Env protein, as well as the structures of several of its mostly likely germline-encoded precursors (GEPs). This analysis showed that mature 4E10 gained both host reactivity and structural flexibility, but lost stability. These findings run counter to expectations of standard vaccination protocols, which assume reduced host reactivity and structural stability upon maturation. "These results help show why it's so difficult to develop an HIV vaccine: not only does HIV do everything conceivable to evade immune responses, but the uncommon antibodies that do slip through the virus's defenses may have properties that make them inherently exceedingly difficult to generate through vaccination," said Dr. Strong.

The researchers first identified a group of candidate GEPs for 4E10. As they did not have access to the donor from which 4E10 was derived, the precursors were predicted using known features of DNA sequence rearrangements leading to antibody diversity. This analysis led to an ensemble of eight GEPs chosen for further study. These GEPs, each encoding a variable antigen-recognition region (Fv), were then expressed in bacteria. All but one GEP Fv displayed proper folding and, notably, higher thermal stability than 4E10 Fv. However, GEP Fvs showed very weak HIV neutralization when compared to 4E10.

To determine changes in HIV-binding properties during the maturation of 4E10, the authors analyzed the binding of 4E10 and GEP Fvs to HIV Env proteins. GEP Fvs displayed unquantifiable but detectable weak binding to Env proteins, and a 100- to 10,000-fold reduction in affinity for engineered antigens relative to 4E10 Fv. Structural comparisons of 4E10 and GEP Fvs bound to the engineered substrate T117 revealed a high degree of binding site conservation, suggesting that the increased affinity of 4E10 for T117 is influenced by indirect effects outside of the antigen binding

site. Further structural analysis showed that T117 makes extensive contacts with 4E10 outside of the targeted epitope, potentially explaining the increased affinity of 4E10 for this molecule.

The authors assessed the polyspecificity (that is, the ability of an antibody to specifically recognize multiple antigens) and the autoreactivity of 4E10 and GEPs using a library of over 400,000 36-amino acid peptides. Both 4E10 and GEP Fvs displayed similar degrees of polyspecificity, though their profiles of autoreactivity varied. This is a striking and counterintuitive result result, as a decrease in polyspecificity is part of the consensus model of antibody maturation.

This study shows that the highly HIV-reactive 4E10 antibody developed through a pathway distinct from that of the current paradigm. The results suggest that 4E10 is unlikely to be elicited through conventional vaccination strategies and thus highlights the need for understanding of unconventional antibody maturation pathways.

Finton KAK, Friend D, Jaffe J, Gewe M, Holmes MA, Larman HB, Stuart A, Larimore K, Greenberg PD, Elledge SJ, Stamatatos L, Strong RK. 2014. Ontogeny of Recognition Specificity and Functionality for the Broadly Neutralizing Anti-HIV Antibody 4E10. PLOS Pathog 10(9): e1004403.

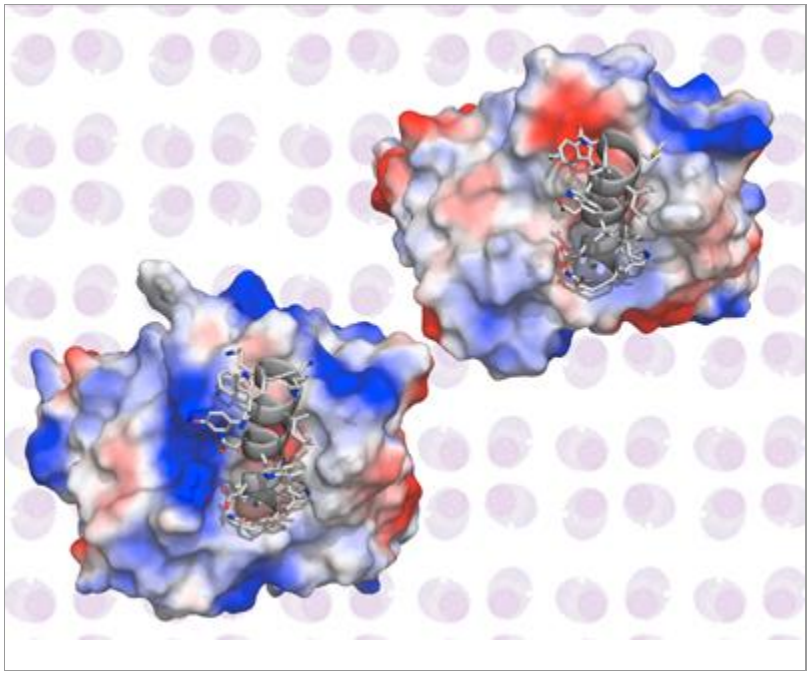

Image provided by Dr. Roland Strong

Structures of the antigen-recognition portion of 4E10 (left) and one of its antecedents (right) bound to the targeted peptide of the HIV Env protein (grey corkscrews).