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A Co-receptor-mimicking Antibody Stabilizes the Displaced V1V2 Loops in a Partially Open sCD4-bound HIV-1 Envelope Complex

Christopher Barnes[1], *Haoqing Wang*[1], *Zhi Yang*[1], *Michel Nussenzweig*[2,3], *Pamela Bjorkman*[1]

[1]California Institute of Technology, United States, [2]Rockefeller University, United States, [3]Howard Hughes Medical Institute, United States

Background: HIV-1 envelope glycoprotein (Env), a trimer of gp120-gp41 heterodimers, mediates membrane fusion after binding host receptor CD4. Receptor binding to closed Env displaces the V1V2 loops from Env's apex, allowing co-receptor binding and opening Env to enable gp41-mediated fusion.
Methods: Using single-particle cryoEM methods, we solved the sCD4-bound structure of a clade B B41 SOSIP trimer in complex with 21c, a CD4-induced (CD4i) coreceptor-mimicking antibody, and with 8ANC195, a broadly neutralizing antibody (bNAb) that recognizes the gp120-gp41 interface, to a resolution of 4.05 Å.
Results: Despite binding of the 8ANC195 Fab that partially closes the open, sCD4-bound Env conformation, our structure shows rearrangements in gp120, including displacement of V1V2, exposure of V3, formation of the 4-stranded bridging sheet, and formation of the α0 helix. In addition, unlike the V1V2 regions in similar sCD4-bound Env structures complexed with the CD4i antibody 17b, the displaced V1V2 loops in the B41-sCD4-21c-8ANC195 structure exhibited ordered density allowing the structure of the displaced V1V2 to be determined for the first time. Comparing partially- and fully-open Envs with closed Envs shows that gp41 rearrangements are independent of CD4-induced gp120 bridging sheet formation and V1V2 displacement, suggesting an order of conformational changes before co-receptor binding: (*i*) gp120 opening inducing side chain rearrangements and a compact gp41 central helix conformation, and (*ii*) bridging sheet formation, and V1V2 displacement.
Conclusions: Analyses of these results further our understanding of HIV-1 Env conformational changes leading to fusion and provide templates for designing agents to disrupt HIV-1 entry into target cells.

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A Newly Developed B Cell Immunoglobulin Phenotyping Panel to Facilitate More Efficient HIV-1 Antibody Discovery

Leonard Nettey[1], *Thomas Liechti*[1], *Mario Roederer*[1], *Rosemarie Mason*[1]

[1]Vaccine Research Center (VRC), National Institute of Allergy and Infectious Disease (NIAID), National Institutes of Health (NIH), United States

Background: Antibody discovery research has been instrumental in facilitating the isolation and characterization of HIV-specific broadly neutralizing antibodies. With growing interest in mining the antibody repertoire in order to identify additional HIV-specific antibodies and characterize their multiple effector functions, there is a clear need to capture antibody subclass information at the single cell level.
Methods: We tested over 100 antibody reagents to design and optimize a flow cytometry panel that could characterize the phenotypic diversity of circulating HIV-specific memory B cells with an emphasis on discriminating immunoglobulin classes and subclasses.
Results: Pan-IgA-, pan-IgG-, IgD-, IgM- and IgE-specific antibodies were included to define immunoglobulin isotypes of individual B cells while IgA and IgG subclasses were delineated using IgA1-, IgA2-, IgG1-, IgG2-, IgG3- and IgG4-specific antibodies. We verified that immunoglobulin isotypes are exclusively expressed, that each subclass within the IgA and IgG isotypes is exclusively expressed and that all isotype cells are accounted for by the sum of the subclasses (e.g. IgA1 + IgA2 = IgA; IgG1 + IgG2 + IgG3 + IgG4 = IgG). The latter validation measure allows anti-pan-IgA and anti-pan-IgG antibodies to be removed and substituted with fluorescently labeled HIV-1 peptide or protein antigens, thus linking antigen-specificity of each individual B cell with its immunoglobulin subclass. More importantly, combining this newly developed panel with indexed single cell sorting methods for HIV antibody discovery (such as antigen-specific single B cell sorting and unbiased single B cell culture) will facilitate studies to thoroughly interrogate HIV-specific B cell effector functions.
Conclusions: The ability to characterize both epitope specificity and IgA/IgG subclass profile at the single B cell level will enable a better understanding of the link between HIV-1 antibody specificities, effector functions and protective efficacy against HIV-1 infection.