Structures of Broadly Neutralizing Anti-HIV Antibodies that Target the CD4 Binding Site on the HIV Envelope

Strategies to combat HIV require structural knowledge of how antibodies recognize HIV envelope proteins and how they are used by the immune system to eliminate viruses and virally-infected cells. A few years after infection, some HIV-infected patients develop broadly neutralizing antibodies (bNAbs), which neutralize across many HIV strains and confer protection against simian immunodeficiency virus (SIV) infection in non-human primates when delivered by passive immunization (i.e. purified Abs were injected). Despite the fact that HIV-infected individuals can produce bNAbs, a vaccine that can elicit such Abs has yet to be identified or designed. One problem in understanding how to elicit bNAbs is that the structural correlates of broad and potent neutralization of HIV are incompletely understood, largely due to the small number of purified and characterized human bNAbs available to researchers. Until recently, only a small number of bNAbs against HIV had been characterized. This issue was resolved by new methods for HIV antibody isolation from B cells of patients who produce high titers of broad neutralizing activity. Several of the antibodies recently isolated by Dr. Michel Nussenzweig's laboratory, including NIH45-46, 3BNC117 and 3BNC60 [1], show superior potency and breadth to VRC01, previously the best bNAb [2].

We initiated a systematic effort to define the structural correlates of broad and potent neutralization of HIV by bNAbs by solving crystal structures of bNAbs alone and complexed with the HIV spike protein gp120. We have solved structures using diffraction data collected at SSRL Beam Line 12-2. This beam line offers two unique features that facilitate data collection from rare, small, and weakly diffracting crystals (which are often the norm for complexes involving highly glycosylated gp120 proteins):

- A PILATUS detector, a 2-D hybrid pixel array detector that operates in single-photon counting mode, which allows read-out of the number of detected x-rays per pixel with no read-out noise or dark current. PILATUS detectors are superior to the CCD and imaging plate detectors used at other beamlines because they have a better signal-to-noise ratio and a read-out time of only 2 msec, which enables a shutter-less data collection.
- A microfocus beam to allow data collection from very small (e.g., ~5 μm) crystals.

In two recent publications, we described structures of the antigen-binding fragments (Fabs) of two new bNAbs that recognize the host receptor (CD4) binding site on gp120: NIH45-46, alone and complexed with a core HIV gp120 spike protein [3], and isolated 3BNC60 Fab [1]. Both Fab structures include canonical immunoglobulin variable domains with complementarity determining regions (CDRs) arranged as loops within the antigen binding site at the center of the variable heavy (VH) and variable light (VL) domains. NIH45-46, a more potent clonal variant of VRC01 that was isolated from the same donor [1], contains a four-residue insertion (residues 99a–99d in CDRH3) relative to VRC01, and there is an unusual insertion of four residues in a loop within 3BNC60's framework region 3 (Fig. 1). As NIH45-46, 3BNC60, and VRC01 are clonally related and

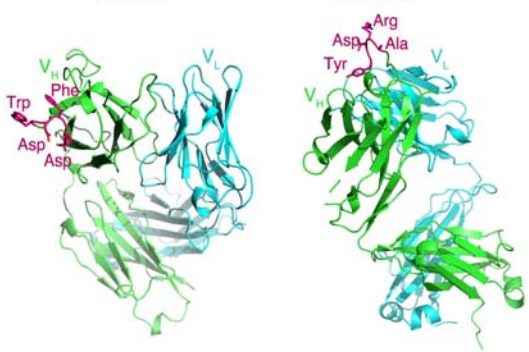

Fig. 1. 3BNC60 and NIH45-46 structures. The insertions are highlighted in pink.

share the same germline gene, these insertions arose during somatic hypermutation. Mutagenesis and structural analyses demonstrate that the NIH45-46 insertion is required for its increased potency compared with VRC01 [3]; unpublished mutagenesis/modeling studies demonstrate the same for the 3BNC60 insertion.

Comparisons of the NIH45-46–gp120 structure (Fig. 2) to the structure of VRC01 bound to the same gp120 indicate that the Abs target the initial CD4 binding site on gp120 using a similar architecture. Analyses of the NIH45-46 insertion reveal interactions with gp120 that are not present in the VRC01–gp120 complex: three of the four residues within the insertion contribute to NIH45-46 binding to gp120. Consistent with the co-crystal structure, deletion of the insertion results in reduced neutralization potencies [3].

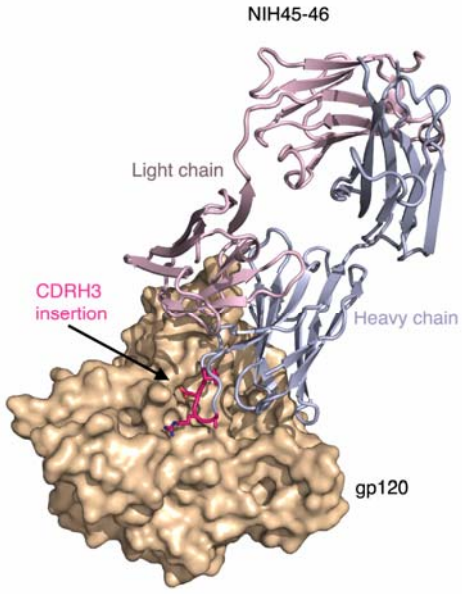

Fig. 2. NIH45-46–gp120 complex structure

NIH45-46, like VRC01, fails to fill a hydrophobic pocket within gp120 to mimic the burying of $Phe43_{CD4}$, a critical residue within the host receptor for binding gp120s. However, the Cα of $Gly54_{NIH45\text{-}46\ HC}$ is only ~1.4Å from the Cα of $Phe43_{CD4}$ in a superimposition of CD4 and NIH45-46 bound to gp120, suggesting that this important interaction might be mimicked by substituting $Gly54_{NIH45\text{-}46}$ with a large hydrophobic residue. We constructed a series of NIH45-46 mutants in which we substituted $Gly54_{NIH45\text{-}46}$ with residues having large hydrophobic side chains and evaluated their neutralization activity using a panel of viruses chosen to include NIH45-46– resistant and transmitted founder strains [3]. The most promising mutant, NIH45-46^{G54W}, showed uniformly increased neutralization potencies (2- to 2000-fold) compared to NIH45-46 and gained activity against viruses that were resistant to NIH45-46 [3]. Our analyses demonstrate NIH45-46^{G54W} is the most potent and broadly neutralizing Ab described to date even when evaluated using a difficult to neutralize panel of viruses [3].

The combination of structural biology and protein design that led to the development of NIH45-46^{G54W} is relevant to efforts to develop passive delivery reagents to combat HIV. Passive delivery is already used to prevent over a dozen infectious diseases, including hepatitis A and B and respiratory syncytial virus. Passively delivered Abs could be injected or delivered via gene therapy approaches in which long-term delivery of a neutralizing reagent is achieved after a single injection of a recombinant viral vector directing expressing of a bNAb. Studies using adeno-associated virus (AAV) to deliver anti-SIV immunoadhesin proteins to rhesus macaques [4] and whole anti-HIV IgGs to mice [5] demonstrate efficacy for protection from HIV challenge. A recent human clinical trial using AAV to provide factor IX to permanently treat hemophilia B demonstrated that AAV delivery methodology is potentially safe and effective [6]. Gene therapy methods to deliver bNAbs can be regarded as a sort of "reverse vaccination" strategy—rather than injecting an immunogen that elicits protective antibodies, long-term delivery of bNAbs is accomplished with a single injection of an engineered virus. Because individuals who naturally produce broad and potent anti-HIV antibodies are rare, this sort of reverse vaccination strategy may ultimately be more effec-

tive than a conventional vaccine in which the host raises bNAbs, especially given that identification of an immunogen that elicits bNAbs has yet to be accomplished. As gene delivery methods begin to be explored in humans for protection against HIV infection, there is an urgent need to identify the best set of bNAbs and improve upon their potencies and breadth, which requires understanding their modes of neutralization.

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