Opening fronts in HIV vaccine development

The challenges posed by the biology of the virus remain a barrier to obtaining a complete cure using current antivirals and to developing a working vaccine that will protect from infection and clear disease. Researchers are keenly working to uncover how to elicit long-term broadly neutralizing antibody responses in humans that will protect against infection from all HIV clades and to ensure that people already infected will also have a chance at clearing the virus and being cured. In "Bedside to Bench," Florian Klein and Henning Gruell discuss a recent study that tracked how broadly neutralizing antibodies developed in an HIV-infected individual during the natural course of the disease. The findings will help create a roadmap to identify the necessary steps to induce antibody maturation for achieving a broad and potent protective humoral response. Another important aspect that defies HIV eradication in infected individuals is the existence of viral reservoirs that allow the virus to hide from antiviral killing. In "Bench to Bedside," Robert Siliciano peruses recent advances in animal models providing evidence that eliciting effector memory cellular responses to HIV may help eradicate—or prevent the establishment of—latent reservoirs. This strategy could contribute to clearing HIV in treated infected individuals and add to the protection of a humoral vaccine response.

■ BEDSIDE TO BENCH

Tracking the development of broadly neutralizing antibodies

Henning Gruell & Florian Klein

Advances in antibody cloning have uncovered highly potent HIV-1–neutralizing antibodies in a subset of infected individuals[1] that protect against SHIV infection in nonhuman primates at low concentrations[2]. It has been proposed that an immunization strategy that could elicit such antibodies would be protective in humans[3]; however, to date there is no vaccine that induces their production. In an attempt to understand how such antibodies develop in HIV-1–infected individuals, Doria-Rose *et al.*[4] have now followed a single individual from the time of infection until the development of broad and potent anti–HIV-1 serologic activity[4,5]. By investigating the interplay between HIV-1 and the antibody response, they were able to reveal the developmental steps toward a broadly neutralizing antibody (bNAb) that targets the V1-V2 loop on the HIV-1 envelope (Env) spike[4]. This region is of great interest as it is a frequent target of potent HIV-1–neutralizing antibodies[6], and of V1-V2–binding antibodies correlated with a reduced risk of HIV-1 infection in a recent vaccine trial[7].

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Whereas it has been shown that the human immune system is able to generate bNAbs targeting several epitopes[1], these antibodies develop in only a minority of HIV-1–infected subjects[8], reflecting the challenge of mounting an effective response against a pathogen that is notorious for its immune evasion. Most bNAbs harbor one or more uncommon characteristics, which probably pose obstacles to their generation, including high levels of somatic mutations, long heavy-chain complementarity-determining regions 3 (CDRH3s), frequent insertions or deletions, and higher levels of polyreactivity[1]. Moreover, when the immunoglobulin sequences of bNAbs are experimentally reverted to their germline precursors, as they are found on naive B cells, binding to HIV-1 Env is often significantly diminished or even completely abrogated[9]. Thus, understanding what mediates the development of bNAbs, including which HIV-1 Env proteins initiate B cell maturation and how antibodies develop in the context of the autologous viral reservoir, is crucial. To this end, longitudinal samples of subjects that have been followed from the onset of their HIV-1 infection are a valuable resource[10,11].

Doria-Rose *et al.*[4] have studied the developmental pathway of a bNAb in a subject (CAP256) who was infected with HIV-1 subtype C followed by superinfection with an unrelated subtype C virus 15 weeks later[4,5]. Broadly neutralizing serum activity developed 1 year after infection and gradually increased to almost 80% of breadth after 3 years. Functional screening and single B cell cloning of samples obtained at weeks 59, 119 and 206 after infection allowed the isolation of 12 members (01–12) of a bNAb clone termed CAP256-VRC26 (ref. 4). Immunoglobulin sequencing of B cells encoding this clone revealed a long (35–37 amino acids) CDRH3 and moderate levels of somatic mutations. Although clonal members displayed different neutralizing activities, they recapitulated serum neutralization when assayed in combination. Epitope mapping identified a quaternary structure on the V1-V2 loop, overlapping with previously identified epitopes of bNAbs, which also carried a long CDRH3 that has been shown to penetrate the glycan shield of the HIV-1 envelope[1,12].

The authors of the study found that the CAP256-VRC26 lineage emerged as early as 30–38 weeks after infection, and by constructing the phylogenetic tree of this antibody clone, they could infer the unmutated common ancestor that already carried a CDRH3 of 35 amino acids, demonstrating

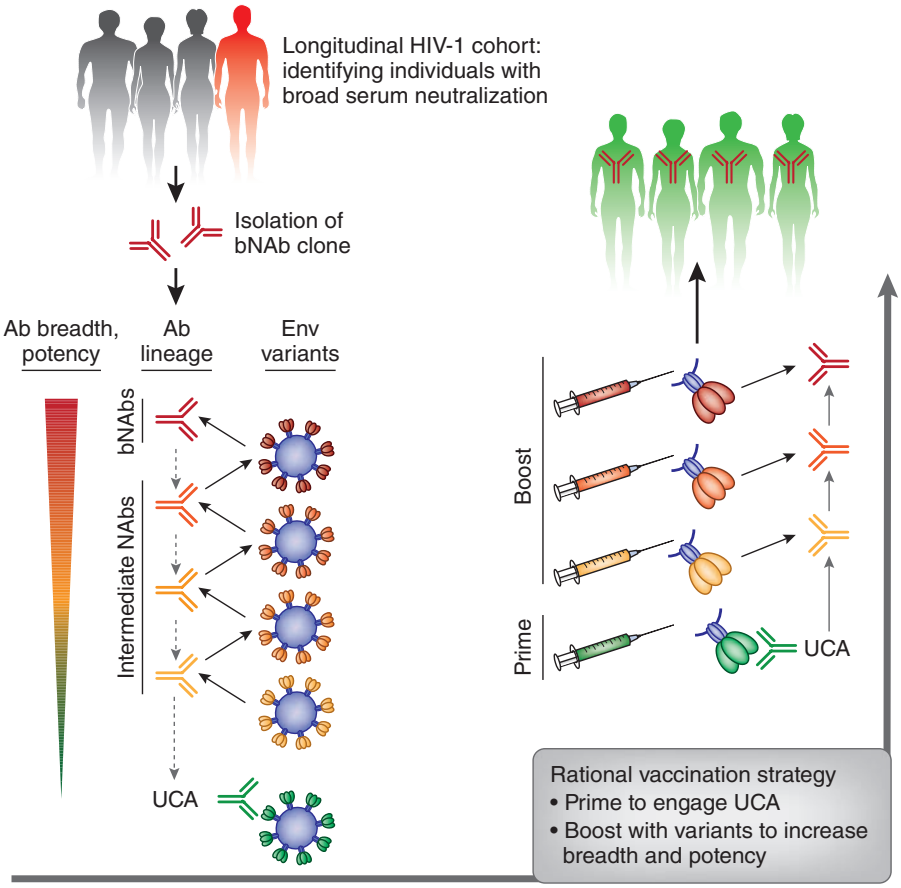

Figure 1 Deciphering bNAb development in an HIV-1–infected subject to guide vaccine strategies. Individuals with acute HIV-1 infection are identified and followed up for the development of broad neutralizing serum activity. Antibody (Ab) cloning, next-generation sequencing and computational analysis of longitudinal samples can be used to reconstruct the development of a bNAb clone, identifying intermediate neutralizing antibodies (NAbs) and the unmutated common ancestor (UCA). Information on the corresponding evolution of HIV-1 Env could then be applied in reverse to vaccination strategies: HIV-1 Env variants are used to engage the UCA, initiating B cell maturation, and to steer antibody development toward the desired bNAb[4,10,14].

that somatic $V_HD_HJ_H$ recombination was responsible for the development of this crucial feature. Notably, the unmutated common ancestor bound and weakly neutralized the superinfecting virus, indicating that this virus initiated the development of the CAP256-VRC26 lineage. Moreover, single-genome analysis of the circulating HIV-1 Envs showed that the emergence of broad serum neutralization followed a shift of the viral repertoire toward sequences associated with the superinfecting virus. Interestingly, at the time the CAP256-VRC26 clone appeared, a mutation in the superinfecting virus occurred (K169I), rendering it resistant to an early member of the antibody clone (VRC26.01). However, clonal members that developed later (VRC26.02–VRC26.11) neutralized this virus. This suggests that VRC26.01 or VRC26.01-like antibodies exerted selective pressure on the virus that resulted in viral escape, to which later clonal members successfully adapted and therefore maintained neutralizing activity. Finally, nucleotide changes in the antibody target site of the virus co-evolved with changes in the antigen-binding site of the antibody, supporting earlier findings of a direct virus-antibody interplay that drives antibody maturation[4,10].

The results of this study provide important information about how a bNAb that targets a quaternary V1-V2 epitope can develop. Most notably, the inferred unmutated common ancestor already harbored the long CDRH3 and bound to the autologous HIV-1 Env. Moreover, the bNAb clone described in this individual required rather moderate levels of somatic mutation (up to around 15%) that might be more readily achievable by vaccination than the exceptionally high rates of mutation (up to around 30%) seen in some other bNAbs[9]. However, the very low frequency of immunoglobulin rearrangements resulting in such an extraordinary long CDRH3 in naive B cells might be an important hurdle for the induction of such bNAbs[13].

The study by Doria-Rose *et al.*[4] complements recent efforts that delineated the development of a bNAb targeting the CD4-binding site[10]. In this previous study, the inferred unmutated common ancestor also bound to the autologous HIV-1 Env of the founder virus but not to heterologous Env, emphasizing the importance of specific Env variants for the initiation of bNAb development. Similar to the findings in subject CAP256, considerable viral diversification at the bNAb target site was detected before the emergence of neutralizing breadth in serum, likewise indicating that viral evolution was the driving force for the generation of broadly neutralizing activity in this individual[10].

The findings of these two studies[4,10] have implications for the design of immunogens for proposed lineage-based vaccine strategies that aim to recapitulate the natural development of bNAbs[4,10,14] (**Fig. 1**). This process would be initiated by priming the antibody response through binding of HIV-1 Env to a naive B cell followed by sequential immunizations that imitate the viral evolution of HIV-1 Env, resulting in increased antibody breadth and potency[4,10,14]. Thus, the identification of pathways leading to potent and broad HIV-1–neutralizing antibodies demonstrates the high value of the detailed investigation of the natural processes in single HIV-1–infected individuals.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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