Multiple roles for HIV broadly neutralizing antibodies

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Potent broadly neutralizing antibodies may be used to treat or prevent HIV and to help guide HIV vaccine design.

Although great progress has been made in treating and preventing HIV infection with combination antiretroviral drug treatment globally, the epidemic continues with ~38 million people living with AIDS and 1.7 million new infections occurring each year (~4600 infections/day) (www.aidsdatahub. org/unaids-data-2018-unaids-2018). Thus, despite efficacious antiretroviral drug therapy (ART) and other prevention modalities that theoretically could stem the HIV epidemic, the development of new modes of treatment and prevention remains a high priority.

The HIV envelope (Env) trimer mediates virus entry through binding to the CD4 receptor on T cells and is the sole target for neutralizing antibodies. Although remarkably variable, the Env trimer has a number of relatively conserved regions to which broadly neutralizing antibodies (bnAbs) are elicited. These regions include the CD4 binding site, the V3- and V1/V2-glycan sites, the membrane-proximal external region (MPER), the Env silent face, the gp41-gp120 interface, and the fusion domain (1–3). Early on in the epidemic, bnAbs were discovered in individuals chronically infected with HIV (1), although these bnAbs inhibit a narrower range of viruses and are less potent than more recently isolated bnAbs. Studies mapping virus and bnAb coevolution in acutely infected individuals over time demonstrated that bnAbs only arise after years of infection and viral diversification (4). Over the past decade, new techniques have led to isolation of hundreds of bnAbs, some of which have been found to be extraordinarily potent (1–3). Thus, efforts now focus on developing antibodies for passive therapy for prevention or treatment of HIV. In addition, a major goal of HIV vaccine development is to develop immunogens that can induce bnAbs. In this 10th installment of *Science Translational Medicine*'s anniversary Focus series, we review the status of progress in both areas of research.

PASSIVE ADMINISTRATION OF BNABS FOR HIV TREATMENT OR PREVENTION

Some HIV bnAbs are now being tested in humans for their ability to promote immune control of HIV in infected individuals and potentially to eliminate HIV-infected cells. These include VRC01, VRC07-523, 3BNC117, and N6 (CD4 binding site–targeting antibodies); 10-1074 and PGT121 (V3-glycan–targeting antibodies); PDGM1400 and CAP256-VRC26 (V1/V2-glycan–targeting antibodies); and 10E8 (MPER-targeting antibody) (1–3, 5). In mice engrafted with human cells (humanized mice), in macaques chronically infected with simian human immunodeficiency virus (SHIV), or in HIV-infected humans, single bnAbs can lower the viral load by ~10- to 100-fold, but antibody-resistant viruses rapidly emerge. Combinations of bnAbs are more effective in preventing the emergence of antibody-resistant virus. Administration of bnAbs early after SHIV infection can lead to persistent viral clearance in some animals (5, 6). This viral clearance may occur because bnAb administration helps preserve helper CD4+ T cells and because the formation of antibody-antigen immune complexes may help stimulate a more effective CD8+ T cell response (6). Similarly, treatment of HIV-infected humans with the CD4 binding site–directed bnAb 3BNC117 was associated with a modest increase in host neutralizing antibodies to HIV (5). In SHIV-infected macaques, treatment with bnAbs prolonged viral control in a subset of animals after discontinuation of ART (5, 6). In humanized mice, bnAb treatment promoted enhanced clearance of HIV-infected cells (5). Future clinical studies will include modified forms of antibodies, such as bispecific molecules, which may improve the potency and duration of antiviral activity. It remains to be seen if antibodies can reach HIV virions or infected cells in protected sites such as the brain. Nonetheless, antibodies that target HIV-infected cells could be combined with latency reactivating agents (LRAs) to reduce or eliminate infected cells and contribute to curing individuals infected with HIV. An LRA could be used to activate latently infected CD4 T cells, followed by addition of bnAbs or multispecific antibodies, such as bispecific antibodies that can bind to both Env and CD8 T cells. Such strategies have been shown to result in reduction of replication-competent HIV in vitro and in a decrease in the virus reservoir in a humanized mouse model.

There is also strong evidence that bnAbs can prevent HIV acquisition in animal models. For example, passive administration can protect against SHIV infection of macaques, including repetitive mucosal challenges, with some antibodies protecting at in vivo serum inhibitory concentrations of less than 1 ug/ml (6). Modification of the Fc antibody region for increased half-life conveys the ability to prevent low-dose SHIV infection for more than 6 months (5). Although animal model data demonstrate protection, it remains to be proven that bnAbs can protect against HIV acquisition in humans. Thus, the NIAID HIV Vaccine Trials Network (HVTN) and HIV Prevention Trials Network (HPTN) are carrying out the Antibody Mediated Prevention (AMP) efficacy trials with intravenous administration of the CD4 binding site bnAb, VRC01 (NCT 02716675 and NCT02568215). With clinical trial sites in Africa, South America, Europe, and the United States, 4600 volunteers are already enrolled. The AMP trials are designed to assess if a single bnAb can prevent HIV acquisition in humans and to determine how much serum antibody is needed for protection. The results of the trials will likely be available in 2020. Based on the animal model studies, it is expected that bnAbs can prevent HIV acquisition in humans by sensitive strains, but, due to the antigenic diversity of HIV, a

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two- or three-bnAb combination may be required to protect against globally diverse strains.

INDUCTION OF BNABS BY ENV IMMUNIZATION

Generating bnAbs through Env vaccination continues to be challenging. BnAbs induced as a result of chronic viral replication during HIV-1 infection are the result of B cells undergoing extensive affinity maturation in germinal centers. HIV Env antibodies associated with protection from HIV acquisition in animal models include bnAbs and non-neutralizing Env antibodies (nnAbs) (4, 7). The former have been shown directly to mediate protection in nonhuman primates. In vitro, HIV Env Abs can act antivirally by direct neutralization and by Fc-mediated effector functions such as antibody-dependent cellular cytotoxicity (ADCC), although the relative contributions of these activities in vivo to protection is unclear and may differ between bnAbs. nnAbs rely solely on Fc-mediated activities for antiviral function, and evidence for protection is less direct than for bnAbs. HIV nnAb responses are readily induced by Env vaccination, but bnAb responses have not been robustly induced as yet in humans or nonhuman primate models. Many bnAbs have long heavy-chain third complementarity-determining regions that have low frequencies in the human naïve B cell repertoire and may subject such antibodies to deletion by immune tolerance. Some bnAbs share characteristics of autoantibodies such as auto- or poly-reactivity (4, 7) and thus may be excluded by immune tolerance mechanisms from productive immune responses. We now know that development of bnAbs in HIV infection is associated with high viral loads. Moreover, bnAbs are unusually mutated (affinity matured) by the enzyme activation–induced cytidine deaminase, indicating long periods of germinal center development induced by persistent antigen contact. Thus, there are multiple factors working against the elicitation of bnAbs, including host control roadblocks (4, 7).

The Env structure presents multiple issues as an antigen. Induction of bnAbs is hindered by instability of the native fusion-competent Env and by non-native forms that induce nnAbs. Moreover, HIV Env consists of ~50% glycans by mass, and thus, almost all bnAbs must bind to or accommodate these glycans to bind the native trimer and neutralize viral infection of host cells. Protein-glycan interactions are typically of relatively low binding affinity, further disfavoring bnAb development. In addition, HIV Env has five variable loops that may increase in length and vary in glycosylation with virus evolution over time, and bnAbs must evolve to accommodate such Env loops. Thus, in addition to host controls and repertoire restrictions, Env structural constraints conspire to make bnAb B cell lineages difficult to induce (1–3).

Although our ability to induce bnAbs in animal models and humans remains a challenge, there has been substantial progress in structural definition of neutralization epitopes on the native Env trimer, leading to new vaccine design approaches. Given that binding to the native Env trimer is required for virus neutralization, our ability to stabilize and express soluble mimics of Env as vaccine antigens is a major step forward (8). However, Env trimers alone do not induce bnAbs, in part because most of the glycoprotein is not highly immunogenic and because extensive antibody affinity maturation is required for neutralization. Thus, it is likely necessary to use informed immunogen design to stimulate the appropriate B cell precursors and then guide maturation pathways to yield bnAbs. In addition, use of strong adjuvants may be needed to overcome immune tolerance mechanisms and to support prolonged bnAb germinal center responses.

Most HIV infections are caused by one or a few transmitted/founder viruses. Antibody-virus co-evolution studies have led to investigation into how bnAb lineages arise and develop during the course of HIV infection, demonstrating an "arms race" between the evolving viral quasi-species and host neutralizing antibody responses. In some individuals, virus Env proteins evolve that eventually select B cell receptors encoding bnAb activity (4, 7). Reproducing the results of these pathways via immunization is one approach for vaccine development. The concept of using bnAbs as the scaffold for vaccine design has been termed reverse vaccinology or epitope-based vaccine design (9, 10). Here, vaccine immunogens are designed to mimic known bnAb epitopes. Additionally, using information about immunological pathways leading to development of antibody lineages with broad neutralizing activity has been termed "lineage-based" vaccine design (7). Knowledge of the unmutated common ancestor (UCA) of a bnAb lineage is paired with knowledge of the optimal Env immunogen to trigger the appropriate precursor B cell expressing the UCA antibody on its surface (7). Combining structural and immunological information for some antibodies, such as the CD4 binding site antibodies of the VRC01 class, has led to an approach termed "germline targeting" (7, 8). Germline targeting begins with the estimation that critical bnAb precursor features are sufficiently common within and among different individuals to make them targetable by appropriately templated immunogens. For the CD4 binding site, an immunogen (eOD-GT8) was templated from VRC01 class bnAb precursors from multiple HIV-infected individuals. Additional immunizations (boosts) must then be designed to drive affinity maturation toward mature bnAbs.

DEVELOPING IMMUNOGENS FOR INDUCTION OF BNABS IN CLINICAL STUDIES

Notably, many of the above immunization approaches have advanced to phase 1 clinical trials. This includes vaccines comprising soluble Env trimers that mimic native Env, lineage-based sequential immunogens for inducing CD4 binding site antibodies, or germline targeting for CD4 binding site VRC01 class or gp41 MPER antibodies. Over the next 2 years, several additional immunogens in each of these categories will enter clinical testing, including lineage-based and germline-targeting approaches for the V3-glycan and V1/V2-glycan region and epitope-based vaccines to target the fusion peptide region of Env. Immunization strategies likely will require sequential immunizations to prime and then boost bnAb lineages with the necessary affinity maturation to recognize Env and potently neutralize HIV. Once early-stage antibody lineages are stimulated and expanded, intermediate immunogens could foster further affinity maturation, and native trimer Env boosts could lead to final development of systemic bnAbs (Fig. 1). This level of immunogen design and sequential immunization has no precedent in vaccinology and will require iterative phase 1 experimental medicine clinical studies to assess, modify, and improve initial immunogens and immunization strategies.

MAKING A PROTOTYPE HIV BNAB VACCINE

The challenges posed by the antigenic diversity, glycosylation, and immune evasion

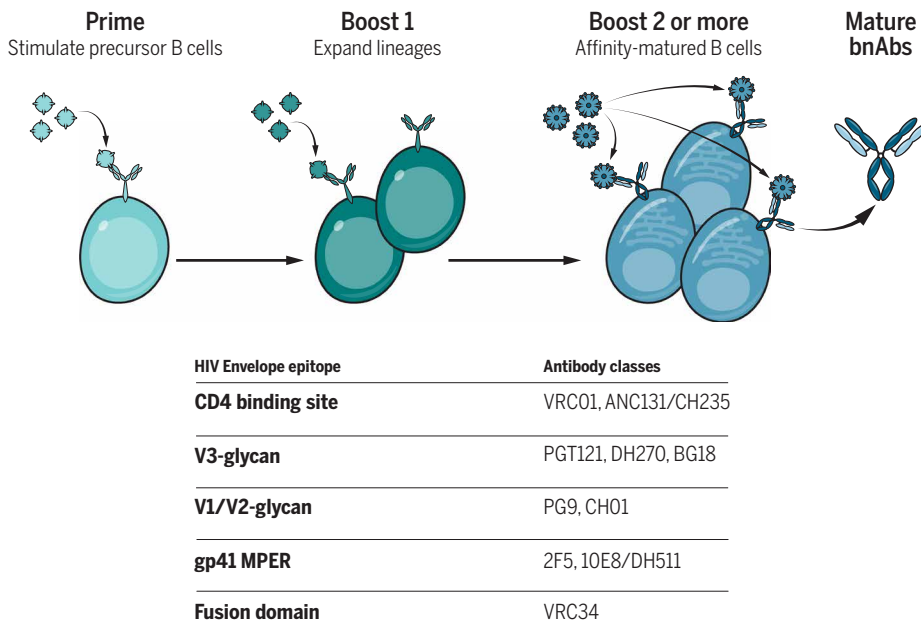| HIV Envelope epitope	Antibody classes
CD4 binding site	VRC01, ANC131/CH235
V3-glycan	PGT121, DH270, BG18
V1/V2-glycan	PG9, CH01
gp41 MPER	2F5, 10E8/DH511
Fusion domain	VRC34

Fig. 1. Strategy for induction of broadly neutralizing antibodies. Shown here is the vaccine strategy of sequential prime and boost to induce mature broadly neutralizing antibodies (bnAbs) against HIV. The initial immunogen would engage the precursor naïve B cell of bnAb B cell lineages, and subsequent boosts would select for neutralizing members of the antibody lineage. The classes or types of bnAbs and representative bnAbs are shown in the bottom panel. A successful vaccine will likely need to induce a polyclonal antibody response against multiple bnAb epitopes to cover the full diversity of HIV virus quasi-species to which a vaccinee could be exposed and to avoid transmitted/founder virus escape.

of HIV Env, together with the unusual antibody characteristics required to achieve virus neutralization, call for a highly coordinated approach to phase 1 vaccine studies in order to achieve the key goal of eliciting bnAbs. As shown in Fig. 1, sequential Env immunogens for each bnAb B cell lineage, of necessity, must be coordinated in their design such that one can follow the other to select for desired bnAb B cell receptor mutations in intermediate and mature bnAbs. This will in effect keep bnAb lineages "on track" in germinal centers to acquire the required mutations necessary for neutralization. Thus, the biology of bnAbs with disfavored B cell lineages, resulting in the need to direct or shepherd the lineages to full maturation with sequential immunization regimens, coupled with the need for good manufacturing practice (GMP) production of a number of Env vaccine candidates, necessitates unprecedented cooperation and communication among HIV vaccine investigators. Such cooperation will allow optimization of clinical trial design by taking advantage of available immunogens with the aim of finding sequential immunization regimens that are able to induce durable cross-reactive serum neutralizing antibody responses.

The NIH, NIAID Consortia for HIV/AIDS Vaccine Development (CHAVD) centered at Duke University and The Scripps Research Institute, the NIAID intramural Vaccine Research Center (VRC), and the HVTN have come together to form the Collaborative HIV Immunogen Project (CHIP) to coordinate logistics and priorities for preclinical testing, GMP manufacturing, and entry into human phase 1 clinical trials. The coordination provided by the CHIP will both optimize the utilization of resources and speed the development of a broadly protective HIV vaccine. The CHIP will both invite and welcome all groups of investigators engaged in HIV vaccine development with the goal of inducing bnAbs. Examples of the efforts of other groups that would be important to include are the two European Vaccine Consortia, other HIV vaccine development teams funded by the NIAID, the Bill & Melinda Gates Foundation, and the International AIDS Vaccine Initiative.

In summary, the isolation of bnAbs has provided a new category of biologic antiretroviral drugs for treatment and prevention and provided hope that bnAbs could be induced by vaccination. However, the discovery of the complex biology of bnAbs and their response to HIV Env conformations has necessitated a far more complicated vaccination strategy than has been used for any currently approved vaccine. Preclinical data suggest that we are on the verge of major progress, although ultimate success in humans will require closely tied scientific and clinical studies. We hope that with the complexity of the problem will come unprecedented collaboration and cooperation in the HIV vaccine field that will soon result in a safe and effective bnAb-based vaccine.

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