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Progress in HIV-1 vaccine development

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1. To update the knowledge of current advances on HIV vaccine development.
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3. To appreciate future directions for effective HIV vaccine development.

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The past 2 years have seen a number of basic and translational science advances in the quest for development of an effective HIV-1 vaccine. These advances include discovery of new envelope targets of potentially protective antibodies, demonstration that CD8$^+$ T cells can control HIV-1 infection, development of immunogens to overcome HIV-1 T-cell epitope diversity, identification of correlates of transmission risk in an HIV-1 efficacy trial, and mapping of the coevolution of HIV-1 founder envelope mutants in infected subjects with broad neutralizing antibodies, thereby defining broad neutralizing antibody developmental pathways. Despite these advances, a promising HIV-1 vaccine efficacy trial published in 2013 did not prevent infection, and the HIV-1 vaccine field is still years away from deployment of an effective vaccine. This review summarizes what some of the scientific advances have been, what roadblocks still remain, and what the most promising approaches are for progress in design of successful HIV-1 vaccine candidates. (J Allergy Clin Immunol 2014;134:3-10.)

Key words: *HIV-1, vaccine, T cells, B cells, broadly neutralizing antibodies*

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Development of a safe and effective HIV-1 vaccine is a global priority.[1] The HIV-1 vaccine field is 30 years into the effort, yet there is no effective vaccine currently available. However, recent breakthroughs in the HIV-1 vaccine field have buoyed hopes that progress can now be made toward an effective vaccine. These advances include discovery of new envelope (Env) targets of

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Abbreviations used
ADCC: Antibody-dependent cellular cytotoxicity
BCR: B-cell antigen receptor
bnAb: Broad neutralizing antibody
Env: Envelope
gp: Glycoprotein
HC: Heavy chain
HCDR3: Immunoglobulin third heavy chain complementarity determining region
KYNU: Kynureninase
SIV: Simian immunodeficiency virus
UCA: Unmutated common ancestor antibody

potentially protective antibodies,[2,3] demonstration in proof-of-concept studies that $CD8^+$ T cells can control HIV-1 infection,[4,5] development of immunogens to overcome HIV-1 T-cell epitope diversity,[6-9] identification of correlates of transmission risk in the first HIV-1 efficacy trial to show any protection,[10-13] and mapping of the evolution of the founder Env mutants in subjects with broad neutralizing antibodies (bnAbs), thereby defining bnAb development pathways.[14]

Current roadblocks to HIV-1 vaccine development are the inability to induce antibody responses to desired conserved bnAb Env regions[3] and difficulty in overcoming HIV-1 diversity.[9] Nonetheless, as outlined below, progress is being made in understanding the nature of the roadblocks and in devising strategies for overcoming these roadblocks.

NEW EVENTS IN HIV-1 VACCINE RESEARCH

This year, the field of HIV-1 vaccine research had a major disappointment in the announcement of the lack of vaccine efficacy seen in a DNA prime recombinant adenovirus type 5 boost HIV-1 vaccine trial developed by the National Institutes of Health Vaccine Research Center.[15] This vaccine was designed primarily to test the hypothesis that high levels of $CD8^+$ cytotoxic T cells could either protect against transmission or lead to control of plasma HIV-1 viral load. Although a majority of vaccinees had T-cell responses and measureable Env-binding antibody levels, the trial showed no efficacy against HIV-1 acquisition.[15] Although additional efficacy trials with a new generation of vaccines are likely in the future, the 2 tested HIV-1 vaccine efficacy trial candidates that were primarily targeted to eliciting $CD8^+$ T cells cytotoxic for HIV-infected $CD4^+$ T cells have both failed to demonstrate protective efficacy. The second failed trial, the Merck recombinant adenovirus type 5 trial, not only lacked vaccine efficacy but also appeared to enhance infection in those vaccinees seropositive for Ad5.[16] However, even HIV-1 efficacy trials that lack protective efficacy can provide information on the types of immune responses that are unlikely to be protective.[17,18]

A new set of studies by Hansen et al[4,5] have demonstrated in rhesus macaques that a replicating cytomegalovirus vector expressing simian immunodeficiency virus (SIV) antigens could eradicate early SIV infection in 50% of SIV-challenged rhesus macaques. Moreover, SIV-infected cell eradication was associated with an unusual form of $CD8^+$ T-cell killing in which $CD8^+$ T cells recognized SIV peptides presented in the context of MHC class II molecules instead of the classical MHC class I molecules.[5] Thus the search is on to find safe cytomegalovirus-like vectors that might recreate this activity in human subjects exposed to HIV-1, and intense research is ongoing to explain why the protective effect was only seen in 50% of rhesus macaques. Nonetheless, these data have demonstrated that $CD8^+$ T cells are associated with control and eradication of early retrovirus infections.

The single trial of an HIV-1 vaccine that showed any efficacy was the RV144 canarypox-prime glycoprotein (gp) 120 protein boost vaccine trial carried out in Thailand, which reported an estimated vaccine efficacy of 31.2%.[11] This level of efficacy was not sufficient for deployment of the vaccine but was encouraging to the field because it suggested that a preventive vaccine could be made.[19] An immune correlates study of the RV144 trial demonstrated that plasma antibodies to the second variable region of the gp120 Env correlated with decreased HIV-1 transmission risk. In addition, plasma Env IgA responses correlated with decreased HIV-1 vaccine efficacy.[10] Follow-up correlates analyses demonstrated the robustness and breadth of the IgG correlate of risk across multiple subtypes of V1V2 antigens.[13] A genetic analysis of RV144 breakthrough viruses in vaccinees and placebo-treated subjects demonstrated the site of immune pressure to be a single lysine residue (K169) in the second variable (V2) region of Env.[20] Isolation of V2 mAbs demonstrated that antibodies that bound to K169 neither broadly bound transmitted/founder virions nor neutralized difficult-to-neutralize (tier 2) viruses but did neutralize the vaccine strain virus 92Th023,[21] mediated low-level virion capture,[21-23] and mediated antibody-dependent cellular cytotoxicity (ADCC).[21,24] RV144 induced V1V2 IgG_3 antibody responses correlated with decreased risk of HIV-1 infection[25] and correlated with ADCC in the RV144 trial.[25,26] The Env IgG_3 response decreased quickly after vaccination,[25] as did overall vaccine efficacy,[27] raising the question of whether the quantity of antibody after vaccination might have contributed to decreased vaccine efficacy.

Studies to understand correlates of HIV-1 risk in RV144 have also focused on understanding the mechanisms of specific Env IgA in decreasing HIV-1 vaccine efficacy. We found that HIV-1 Env IgA to a conformational C1 region in gp120 blocked IgG-mediated ADCC, thus providing a rationale that vaccine-induced plasma IgA responses that bind to the same epitope on infected target cells as IgG could block IgG natural killer–mediated effector function.[17] Consequently, new vaccine candidates are now being designed to increase the breadth of induced FcR-mediated IgG anti-HIV activity and to optimize the vaccine-induced antibody subclass (ie, IgG_3) and isotype profile.[25] Moreover, efforts are being made to increase antibody durability by incorporating a new adjuvant into the regimen to determine whether efficacy induced by an canarypox prime gp120 boost vaccine can be improved to the point of being clinically useful. However, the specific roles of ADCC-mediating antibodies and other FcR-mediated antibody functions in prevention of HIV-1 remains to be shown directly. Roederer et al[28] have recently shown that current vaccines can induce antibodies that neutralize a subset of SIVs. These data suggested that partial efficacy in vaccine trials might be due to vaccine-induced neutralization of a small subset of sensitive viral quasispecies.

New progress has been made in overcoming HIV-1 diversity through induction of cross-reactive T-cell responses to HIV-1 by vaccines designed *in silico* (called conserved and mosaic

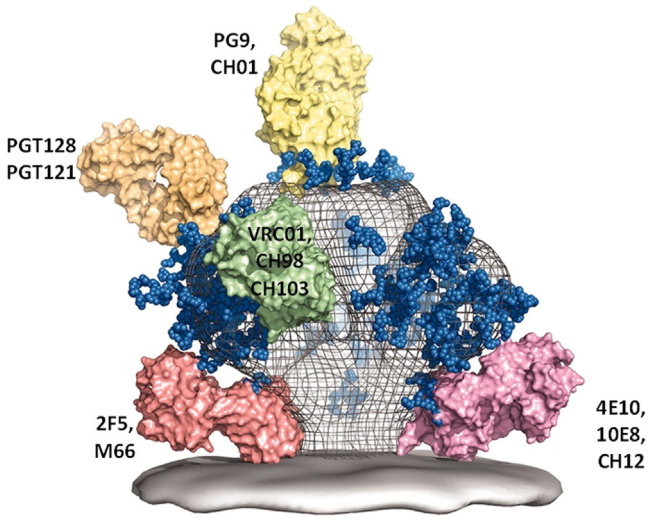

FIG 1. Model of the HIV-1 Env spike with select bnAb Fab molecules bound to bnAb sites. Used with permission from Burton et al.[2] bnAbs that bind to conserved regions of the HIV-1 envelope are shown.

vaccines).[8,29,30] These *in silico*–designed immunogens are constructed to increase the coverage across both $CD4^+$ and $CD8^+$ T-cell epitopes, and studies in nonhuman primates have demonstrated that this is the case. Clinical trials with the conserved gene inserts are ongoing, and phase I clinical trials with mosaic vaccines are planned to begin this year.

The holy grail of HIV-1 vaccine development continues to be the induction of HIV-1 broadly neutralizing antibodies (bnAbs).[3,31] Although the HIV-1 Env does have conserved regions to which neutralizing antibodies can bind,[32] no current vaccine candidates have been able to induce high bnAb levels.[2,31,32] The recent development of methods for generating recombinant antibody from single cells,[33] the efficient isolation of individual plasmacytes and antigen-specific B cells by means of flow cytometric sorting,[34-36] and the establishment of high-throughput clonal memory B-cell cultures[37,38] has permitted a host of new bnAbs to be recovered from HIV-1–infected subjects. HIV-1 bnAbs define 4 conserved Env targets for HIV[31,32] neutralization (Fig 1).[2,3] More than 30 bnAbs specific for conserved neutralizing Env epitopes have been isolated and characterized.[3] It has become clear that all bnAbs share 1 or more unusual characteristics: extraordinary levels of somatic hypermutation (Fig 2),[38-40] autoreactivity for host molecules, and long antibody heavy chain complementarity determining region 3 (HCDR3) sequences.[31,32,41] All of these traits are associated with direct or indirect control by host tolerance and immunoregulatory mechanisms, raising the hypothesis that a major regulator of HIV-1 bnAb generation is immune tolerance.[31,42,43]

In 2005, the observation was made[42] that 2 human recombinant bnAbs, called 2F5 and 4E10, that bind near the virion membrane to Env gp41 were reactive in human autoantibody assays. In a subsequent study 2F5 was shown to avidly bind the human protein kynureninase (KYNU), and 4E10 was shown to react with the mammalian RNA splicing factor 3B3.[44] For 2F5 reactivity with KYNU, the molecular mimicry is striking: the nominal gp41 epitope of the 2F5 bnAb is the linear peptide ELDKWAS, and an identical 6-residue sequence is present in KYNU (ELDKWA). This ELDKWA motif in KYNU is conserved in nearly all mammalian species and absent in all proteins other than the HIV Env.[44] Thus the autoantigens for these 2 bnAbs, 2F5 and 4E10, have been identified, suggesting that expression of these bnAbs is limited by host tolerance mechanisms.

To determine directly whether expression of 2F5-like antibody is indeed controlled by immune tolerance, Verkoczy et al[45-47] constructed knock-in mouse strains carrying the 2F5 bnAb genes. BnAb knock-in mice exhibited a severe block in B-cell development at the transition between pre-B and immature B cells. This developmental blockade represented the first tolerance checkpoint and was consistent with physiologically significant autoreactivity by both the mature and germline forms of the 2F5 antibody (Fig 3).[31,41,46,48,49] The 2F5 knock-in mouse strain also offered potentially good news for vaccine development. Although the vast majority (95%) of B cells expressing the 2F5 antibody were deleted at the first tolerance checkpoint, a small but significant fraction (5%) of $2F5^+$ B cells escaped this checkpoint but were functionally silenced (anergic).[45-47] Remarkably, these anergic B cells could be activated by an immunogen that mimicked the membrane proximal region of gp41 to elicit plasma 2F5 bnAbs.[47,50] Recently, it has been shown that the 4E10 HIV-1 bnAb is similarly controlled by tolerance deletion and anergy control mechanisms.[48,50] A naturally occurring 2F5-like mAb in an HIV-1–infected subject has been isolated as well,[51,52] lending plausibility for gp41 neutralizing antibody induction by a vaccine.

BnAbs specific for the HIV-1 Env gp120 V1V2 glycan bnAb Env region uniformly carry unusually long antibody HCDR3 sequences that appear to be necessary for neutralization.[3] It is likely that these rare HCDR3 motifs are necessary for the bnAb paratope to reach in and around glycans to produce avid binding at the variable loops of HIV-1 Env.[53-60] In human subjects the population of B cells expressing antigen receptors with exceptionally long antibody HCDR3 sequences are controlled by tolerance mechanisms, and this population is commonly reduced by deletion at the first tolerance checkpoint in bone marrow.[61] Therefore the precursors of V1V2 glycan antibodies are similarly derived from a rare pool of B cells controlled by tolerance mechanisms.

As mentioned, all HIV-1 bnAbs have been shown to carry significantly higher frequencies of V(D)J mutations than non–HIV-1 antibodies (Fig 3).[3] Among the known bnAbs, CD4 binding site antibodies of the VRC01 type (a type of bnAb shaped like the CD4 molecule itself) have the highest levels of somatic hypermutation, often reaching approximately 30%. Many of these antibodies are also autoreactive; interestingly, the most common self-antigen recognized by several CD4 binding site bnAbs are ubiquitin ligases (G. Kelsoe, unpublished observations).[14,62] The extraordinary frequency of point and insertion/deletion mutations in HIV-1 bnAbs is both puzzling and perhaps a significant clue toward determining why bnAbs are so difficult to induce. Whereas V(D)J mutations and selection in germinal centers are necessary to increase antibody affinity and specificity, B cells that become heavily mutated (>5% to 8%) often exhibit reduced fitness by either decreased affinity or the acquisition of autoreactivity. In both instances these mutant B cells are selected against and become a minor component of the humoral response or disappear altogether.[31] The strong association of bnAbs with properties that are typically rare and, in other types of antibodies, disfavored is consistent with the

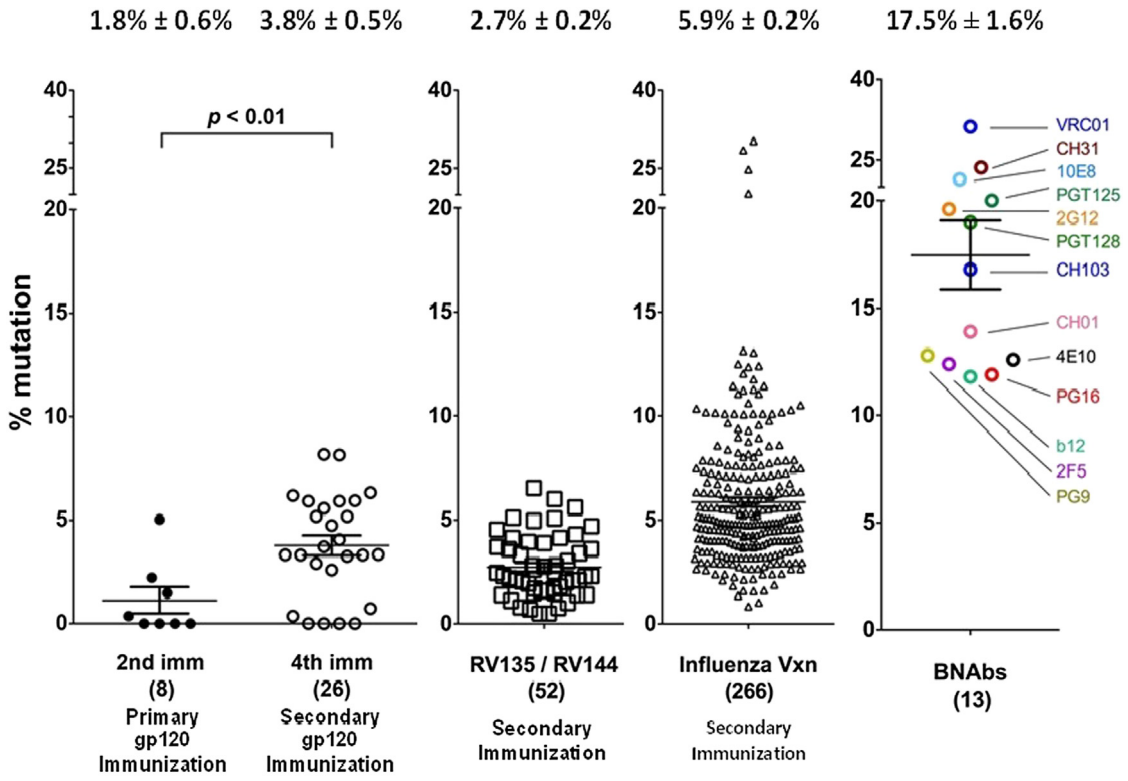

FIG 2. Comparison of heavy chain mutation frequency in HIV-1 immunization, influenza immunization, and HIV-1 bnAbs. Heavy chain mutation frequencies were determined for 3 different vaccine studies and compared with those of well-characterized bnAbs. The *left 2 columns* show heavy chain mutation frequencies induced by 2 or 4 immunizations of a gp120 immunogen.[39] There was an increase in mutation observed with repeated immunization. The *third column* shows an intermediate degree of mutation frequencies observed among antibodies isolated from the canarypox-prime Env boost RV144 regimen in phase II and III trials.[38] The *fourth column* is the mutation frequency observed for influenza vaccine recipients[40]; mutation frequencies after repeated exposure to influenza are higher than those for HIV-1 vaccines. The *last column* shows 13 well-characterized bnAbs, all of which show an exceptional degree of mutation.

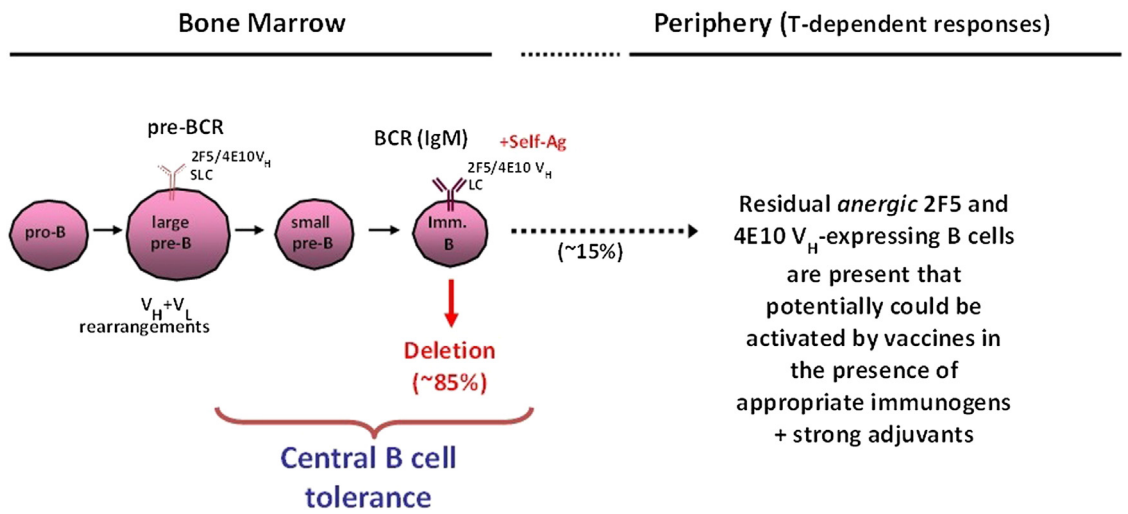

FIG 3. Central deletion of B cells expressing gp41 broadly neutralizing antibodies. Highlighted is the pre-B to immature B-cell transition, the stage of B-cell development in the bone marrow at which most B cells expressing 4E10 or 2F5 bnAbs (as BCRs) have been demonstrated in knock-in mice to be profoundly impaired.[46,48,49] This stage also coincides with the first general checkpoint at which B-cell tolerance mechanisms, including apoptotic deletion, begin to occur.[31,41]

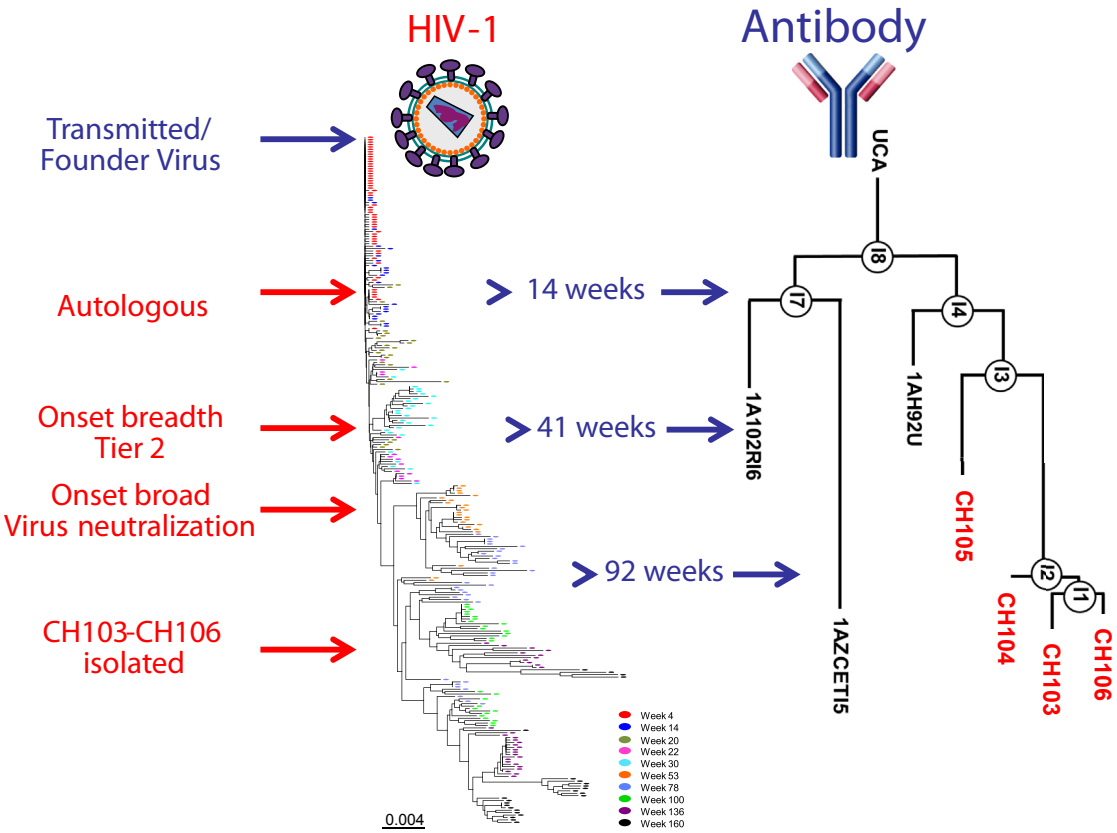

FIG 4. Coevolution of virus and a single antibody lineage in an HIV-1 seroconverter. Mature $CD4^+$ binding site antibodies CH103 to CH106 were isolated from circulating memory B cells at week 136 after infection. Longitudinal sampling allowed inference and reconstruction of the evolution of the infecting viral sequence and of the specific neutralizing antibody lineage. B-cell gene sequencing and bioinformatics analyses were used to infer early intermediate antibodies and the UCA. The *left part of the figure* displays a phylogenetic tree of Env sequences derived from week 4 through week 160. The UCA and intermediate antibody heavy chain sequences of the CH103 antibody lineages are shown alongside viral evolution. This antibody lineage evolved to gain high-affinity Env binding, and virus neutralization evolved from strain-specific autologous virus activity to cross-reactive neutralization of heterologous viruses.[14] Used with permission from Mascola and Haynes.[3]

absence or rarity of unmutated naive B cells capable of founding clonal lineages leading to bnAb production.[31] This tolerance hypothesis[31,43] explains not only why bnAb production is uncommon but also why bnAb B-cell antigen receptors (BCRs) are so characteristically atypical.

B-cell lineage immunogen design is a strategy that has been proposed to overcome the disfavored status of HIV-1 bnAb clonal lineages (Fig 3).[31] B-cell lineage immunogen design is based on the survival advantage exhibited by germinal center BCRs with the highest affinity for antigen.[63-65] By defining optimal immunogens to guide clonal evolution in germinal centers, B-cell lineages that would normally be disfavored can be promoted. Briefly, the process of B-cell lineage design for HIV-1 bnAb production is as follows: (1) bnAb clonal lineages from a patient or vaccine are isolated or inferred; (2) the recovered bnAbs are expressed as recombinant antibodies for Env-binding assays; (3) panels of recombinant Envs are expressed to screen for their binding affinities to each crucial branch in the bnAb lineage; and (4) those Envs or Env fragments that bind the highest affinity to the BCR at critical lineage branches are selected as immunogens. The serial administration of the selected Env immunogens would favor those V(D)J mutations and evolutionary trajectories that are necessary to generate bnAbs. The Env immunogens that optimally bind to the germline or unmutated common ancestor antibody (UCA) become the vaccine prime, those that bind to intermediate antibodies become the first boosts, and those that bind to mature antibodies become the final boosts.[31]

Screening with heterologous Envs for B-cell lineage immunogen design can be effective, but heterologous Envs frequently do not react with the UCAs of bnAb lineages.[66,67] This is likely because bnAbs lineages arise from the initial autologous Env antibody responses, which are exquisitely specific for the infecting founder virus Env.[14,68] Consequently, the ideal immunogens for initiating bnAb responses will likely be autologous founder virus Envs from those subjects who make bnAbs during the course of infection[14] or Env immunogens specifically engineered to bind to specific UCA BCRs.[69]

In the Center for HIV/AIDS Vaccine Immunology-Immunogen Discovery program at Duke, 17 subjects followed from the time of transmission of HIV-1 to the development of bnAbs are being studied for the coevolution of virus and immunity. The

TABLE I. Major new directions in HIV-1 vaccine research

Induction of protective T-cell responses	Induction of protective B-cell responses
Defining strategies for overcoming T-cell immunity (mosaic, conserved immunogens)	Defining pathways of broadly neutralizing antibodies in HIV-infected subjects
Defining new conserved T-cell epitopes for incorporation into T-cell immunogens	Selecting immunogens that can bind to the naive B-cell receptors of broadly neutralized antibodies
Defining replicating vectors for T-cell immunogens (attenuated cytomegalovirus, replicating adenovirus, poxviruses)	Selecting sequential and "swarm" immunogens that can recapitate bnAb induction with vaccination
Design immunogens to induce T follicular helper cells to drive protective antibody responses	Design immunogens to improve on efficacy seen in RV144 canarypox-prime clade B/E gp120 boost Thai trial

first of these subjects, CH505, has been extensively studied, and the coevolution of founder virus and bnAb clonal lineage maturation has been meticulously mapped (Fig 4).[14] In doing so, the evolution of HIV-1 Env in response to antibody-mediated selection has been elucidated in unprecedented detail.[14] Indeed, in subject CH505 a complete history of the sequential Env mutants that elicited bnAb production was demonstrated, and now Envs and their sequence of administration can be recreated as serial immunogens to attempt to induce bnAb production by vaccination.

The studies in subject CH505 revealed that bnAbs emerged only after the extensive diversification of the founder virus Env in successive waves of virus escape from the serial production of autologous neutralizing antibodies (the HIV-1 arms race, Fig 4). Thus CH505 sequential Envs are being produced for trials in rhesus macaques and in human subjects to determine whether similar bnAb lineages can be driven in a vaccination setting. Because it takes approximately 2 years for bnAbs to develop in chronically HIV-1–infected subjects,[35,51,70,71] we expect that multiple immunizations in macaques and human subjects will be necessary to drive bnAb development.

Various HIV-1 Env immunogen types are now being developed that express epitopes for bnAbs and their precursors. There are 3 new structures proposed for the HIV-1 Env trimer,[72-75] and the hope is that having the structure of a native Env trimer will be more antigenic and more immunogenic than previously used immunogens. In general, 3 categories of Env immunogens are in development: minimal immunogens that are fragments or scaffolded portions of HIV-1 Env neutralizing epitopes[69,76,77]; intermediate Env immunogens, such as monomeric Env gp120,[11] and various forms of Env trimers.[78] To date, however, not even those single immunogens with near-native structures have been capable of inducing the immune system to generate bnAbs after vaccination.

It has been the view of researchers in this field that only approximately 10% to 20% of subjects with chronic HIV-1 infection are capable of making bnAbs.[35,51,70,71] More recently, however, screens of large numbers of infected subjects for their breadth of plasma neutralization has demonstrated that there are not polar extremes of neutralizing capacity but rather a gradation of neutralization breadth in chronically infected populations.[79] What is consistent is that those subjects who do make bnAbs require years to do so. Early in HIV-1 infection, all infected subjects make autologous neutralizing antibodies that are not different from those of patients who never generate bnAbs. Thus, for vaccine trials, the concept is emerging that a successful vaccination for HIV-1 and induction of bnAbs will require repetitive immunizations over a longer period of time than for currently licensed vaccines. This type of immunization poses the difficult question of how to design practical immunizations to replicate the evolution of the transmitted founder virus Env over time of bnAb development.

THE WAY FORWARD

The way forward for HIV-1 vaccine development is centered on development of new immunogens to overcome diversity for T-cell responses (mosaic and conserved immunogens), the induction of greater breadth and durability of immune responses induced in RV144 to improve on the minimal efficacy seen in RV144, and the development of vaccine strategies to recreate the Env swarms that generate bnAbs when they do occur in the setting of infection (Table I). In essence, the job of the HIV-1 vaccine development field is to convert subdominant immune responses to become dominant responses, a task never before required of or successfully accomplished by an infectious disease vaccine. Thus HIV-1 vaccine work is breaking new ground in vaccinology, and success in the development of an HIV-1 vaccine will herald success for other difficult-to-make broadly reactive vaccines, such as for influenza and hepatitis C.

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