Multi-Envelope HIV-1 Vaccine Development: Two Targeted Immune Pathways, One Desired Protective Outcome

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Abstract

In 2016, there were more than 30 million individuals living with HIV-1, ~1.8 million new HIV-1 infections, and ~1 million HIV-1-related deaths according to UNAIDS (unaids.org). Hence, a preventive HIV-1 vaccine remains a global priority. The variant envelopes of HIV-1 present a significant obstacle to vaccine development and the vaccine field has realized that immunization with a single HIV-1 envelope protein will not be sufficient to generate broadly neutralizing antibodies. Here we describe two nonmutually exclusive, targeted pathways with which a multi-envelope HIV-1 vaccine may generate protective immune responses against variant HIV-1. Pathways include (i) the induction of a polyclonal immune response, comprising a plethora of antibodies with subset-reactive and cross-reactive specificities, together able to neutralize diverse HIV-1 (termed Poly-nAb in this report) and (ii) the induction of one or a few monoclonal antibodies, each with a broadly neutralizing specificity (bnAb). With each pathway in mind, we describe challenges and strategies that may ultimately support HIV-1 vaccine success.

Keywords: HIV-1, multi-envelope HIV-1 vaccine, broadly neutralizing antibodies

B Cells and Antibodies: A First Line of Defense Against Virus Infections

ANTIBODIES ARE ATTRACTIVE and sophisticated components of the mammalian adaptive immune system that can provide a first line of defense against invading pathogens. Antibodies circulate through blood, lymph nodes, and tissues, potentially for decades after an immunization, and are well positioned to tackle viruses at their point of entry.

The antibody binding capacity of a B cell is first determined by somatic rearrangements that occur in the germ line, juxtaposing a particular variable segment, diversity segment, and joining segment (V-D-J) in the heavy chain locus, and a particular variable and joining segment (V-J) in the light chain locus. V-D-J heavy chain and V-J light chain sequences define the antigen binding region of the B cell's antibody. Because virtually every developing B cell rearranges genes differently and because N- and P-region additions can be added to segment junctions, the number of unique antibody binding sites in an individual is vast (67). The sophisticated gene rearrangement process that drives combinatorial diver-

sity provides an extraordinary number of antigen binding sites and a formidable protective barrier against invading pathogens.

HIV-1, the Pandemic

HIV-1 was discovered approximately 35 years ago, and according to UNAIDS (unaids.org), the virus has now been responsible for more than 70 million infections and ~35 million deaths. Despite extensive progress in the development of highly active antiretroviral therapies, there remain significant obstacles to disease control. These include the following: (i) failure to diagnose and treat all infected individuals, (ii) failure to overcome stigma associated with HIV-1 infections, (iii) financial and logistical difficulties surrounding drug distribution, (iv) drug adherence difficulties due to complex schedules or intolerable side effects, (v) failure of drugs to eliminate latent virus (65), and (vi) the emergence of escape mutants post-treatment, supported by the extensive diversity of HIV-1.

HIV-1 diversity is of particular concern, because the virus encompasses an error-prone reverse transcriptase that can

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introduce mutations during virtually every cycle of virus replication (91). In 2016, there were ~1.8 million people newly infected with HIV-1 and ~1 million deaths (un-aids.org), demonstrating that infection control has not been achieved. For each of these reasons, the development of a vaccine for HIV-1 remains a top priority in the healthcare field.

Many HIV-1 Vaccines Have Been Tried, with One Partial Clinical Success

Vaccine research has been ongoing for decades since the discovery of HIV-1 (89). Vaccines have utilized a plethora of formulations and vehicles, including purified proteins and adjuvants, killed virus, recombinant virus-like particles, recombinant replication-competent viruses, and recombinant bacteria, to name a few. HIV-1 antigens have included internal and external proteins, or fragments thereof. In some cases, protein sequences were presented in their native form, and in other cases, sequences were mutated, truncated, scaffolded, and/or scrambled (96,98).

In 2009, the analysis of the RV144 phase III clinical trial demonstrated partial protection from HIV-1 acquisition, and the RV144 trial remains to date the only HIV-1 vaccine trial to demonstrate efficacy against HIV-1 acquisition (56,79). When the outcome of vaccinations was evaluated by a modified intention-to-treat analysis, results showed 31.2% fewer infections in the vaccinated group compared to the controls. Despite incomplete protection in this study, results gave the most compelling evidence that a preventive HIV-1 vaccine could be formulated, and constituted a benchmark for the development of a better vaccine.

Harnessing Immune Responses Toward Diverse HIV-1 Envelope Proteins

Envelope proteins vary among HIV-1 isolates, both by sequence and structure, and thus present a significant obstacle to successful HIV-1 vaccine development. Can the human immune system respond to these variant HIV-1 envelope proteins? In 1994, Wrinn *et al.* described the natural acquisition of immune breadth toward HIV-1 envelopes among humans who were HIV-1 infected (later confirmed by Richman *et al.*) (80,105). Researchers found that shortly after an exposure to HIV-1, human immune responses were limited in breadth. B cells responded specifically to the founder virus with neutralizing antibody activities. Then, due to immune pressure on the autologous founder virus, together with the emergence of envelope mutations (supported by the virus' error-prone reverse transcriptase), there was selection of escape mutants from the autologous virus quasispecies in the infected host. Eventually, the immune system was exposed to, and responded to, a variety of HIV-1 variants. At the completion of these processes, neutralizing responses were evident both toward the founder virus and the emerging viral escape mutants.

During the course of chronic infection, most people living with HIV-1 are eventually capable of making a polyclonal antibody response with at least moderate, but significant, breadth of coverage. Hraber *et al.* reported that ~50% of samples from chronically HIV-1-infected individuals neutralized at least 50% of 219 envelope-pseudotyped viruses, and Hu *et al.* reported that 53% of samples from chronically

HIV-1-infected individuals neutralized more than 50% of 30 envelope-pseudotyped viruses (41,43). Although immune responses in chronically infected patients cannot clear HIV-1 infections, the induction of similar responses by vaccination before an HIV-1 exposure is expected to be protective.

Here we discuss how two nonmutually exclusive pathways lead to immune recognition of diverse HIV-1 and how the exploitation of each of these pathways may assist HIV-1 vaccine design. In the first pathway, multiple diverse B cells are activated that secrete neutralizing antibodies with diverse specificities (Poly-nAb). In the second pathway, there are single B cell lineages that generate monoclonal antibodies with cross-neutralizing activities (bnAbs).

Targeted Pathway 1: Polyclonal B Cell Populations Express Multiple, Diverse, Neutralizing Antibodies (Poly-nAb)

The concept

The concept behind targeted Pathway 1 is that the immune system responds to multiple HIV-1 envelopes by activation of multiple distinct B cell clones (78,85). In an infected host, each time HIV-1 evolves and mutates its envelope antigens, naive B cells with new specificities are activated. B cells that are activated either by the founder virus or by virus escape mutants may be sustained for a lifetime, in part, due to the establishment of long-term antibody-producing cells in the bone marrow (49,86). Individual antibodies expressed by these B cell populations will exhibit different specificities, some able to neutralize only a few HIV-1 isolates and some able to neutralize many (defined by the particular epitope bound by each antibody). Binding sites may span both variable and constant regions of envelope proteins. The B cells responsive to the first virus and B cells responsive to the evolved viruses may combine as a population to provide the breadth of activity exhibited in infected persons. We note that the composite of antibodies that combine to confer immune protection against variant HIV-1 in each person need not be the same. We propose that if a vaccine can safely mimic the processes described above, by presenting multiple envelopes to the immune system, protective immunity toward HIV-1 might be induced.

Cocktail vaccine lessons from the past

A review of lessons from historical vaccine successes may assist the development of a vaccine against HIV-1. In several instances, successful vaccines have been formulated against variant pathogens by combining antigenically distinct membrane antigens into cocktails. For example, in the mid-1900s, Jonas Salk, with assistance from David Bodian, combined three antigenically distinct polio viruses into a successful polio vaccine (9,10,53,70). Similar strategies were used to develop papillomavirus, influenza virus, rotavirus, and pneumococcus vaccines. The selection of vaccine antigens relied on the testing of antibody-antigen reactivity patterns using panels of antibodies (or serum samples) and whole pathogens or membrane antigens (7). Antigens were categorized based on shared antibody reactivity patterns, after which representative antigens from each of the most distant categories were selected for combination in the

vaccine cocktail. The assays for reactivity pattern analyses have varied among vaccine fields, and have included neutralization assays, hemagglutination-inhibition assays (for influenza virus), and enzyme-linked immunosorbent assays (2,19,23,29,30,74,76,90,94,101).

Of note, successful cocktail vaccines were historically formulated based on antigenicity, not sequence. Antigenic analyses may in these cases prove superior to sequence analyses, because two proteins that are closely related by amino acid sequence need not be bound by the same antibodies, and two proteins that are distantly related by sequence may share key antibody binding sites (27,33,34,59).

Historical successes of cocktail vaccines encourage the use of similar strategies to combat HIV-1. Remarkably, small cocktails of membrane antigens were proven sufficient to represent a vast array of protein sequences (e.g., for the influenza virus vaccine) (94), although an overly small cocktail (e.g., 7-valent vaccines in the pneumococcus vaccine field) could sometimes fail to represent full pathogen diversity (106).

Previous testing of HIV-1 envelope vaccines in research animals and humans

Lessons also derive from previous tests of HIV-1 envelope-based vaccines.

Single- and double-envelope vaccines. Some of the first envelope-based HIV-1 vaccines were produced by VaxGen in the 1980s as soluble recombinant viral antigens, formulated with adjuvant. These vaccines were protective from virus challenges in nonhuman primates (4,5). Another important nonclinical study was reported in 1992 by Hu *et al.* In this case, envelope proteins were delivered to naive macaques in the form of a recombinant vaccinia virus prime and an envelope protein boost. This experiment demonstrated the concept of utilizing more than one delivery system and proved that protection against SIV could be conferred when envelope proteins in vaccine and challenge viruses were matched (42).

Between 1998 and 2003, the first two HIV-1 vaccine phase III clinical trials were conducted with VaxGen products (Vax003 and Vax004), which tested two clinical-grade bivalent gp120 subunit vaccines adjuvanted in alum. The envelopes were selected based on sequence, to match predominant clades in target populations. Either two clade B isolates (MN gp120 and GNE8 gp120) were combined for study in North America and the Netherlands (Vax004) or a clade B and a clade E envelope (MN gp120 and the CRF01_AE A244 gp120) were combined for study in Thailand (Vax003). No efficacy for prevention of acquisition or modification of HIV-1 infection was detected in either trial (5,6,89). Several explanations for these vaccine failures have been proposed. For example, Karnasuta *et al.* performed a comparative analysis of the quality of vaccine-induced antibody responses in the Vax003, Vax004, and RV144 phase III efficacy trials. Authors suggested that a skewing of the elicited antibody response toward higher levels of IgA and IgG4 envelope-specific antibodies, and lower levels of envelope-specific antibodies of the IgG3 subclass, may have contributed to the lack of efficacy observed in the Vax003 and Vax004 trials (54). In addition, the number of protein boosts, the duration of rest intervals between vaccinations, and the

limited number of vaccine antigens may have influenced the overall quality of the elicited antibody responses in terms of maturation, specificity, and breadth.

Multi-envelope vaccines with increasing complexity based on sequence. In 2003, the RV144 phase III efficacy trial started in Thailand. The RV144 trial tested the prime-boost combination of two vaccines: ALVAC-HIV (vCP1521), used as prime, and AIDSVAX B/E, used to boost. ALVAC-HIV (vCP1521) was a recombinant canarypox vaccine engineered to express Gag and Pro of the LAI strain (subtype B) and the subtype E CRF01_AE 92TH023 gp120 envelope linked to the transmembrane anchoring portion of gp41 (LAI), whereas AIDSVAX B/E contained A244 (subtype E) and MN (subtype B) envelope proteins formulated in alum (79). As stated above, the RV144 trial showed an estimated efficacy of 31.2% using a modified intention-to-treat analysis. Vaccine recipients did not develop broad plasma antibody neutralization as defined by *in vitro* assays; instead, the combination of low plasma anti-HIV-1 envelope-specific IgA antibodies and high levels of antibody-dependent cellular cytotoxicity (ADCC) inversely correlated with infection risk, and ADCC-mediating antibodies that preferentially targeted the C1 and V2 regions of envelope were recognized (16,61,75). Multiple trials are now in progress to build on this result, including a phase I/IIa clinical trial (see ClinicalTrials.gov identifier NCT02915016) in the United States and South Africa in which three different envelopes are being used, one in the context of a DNA plasmid (DNA-HIV-PT123), and two as purified proteins (bivalent subtype C gp120s). Envelopes were selected to match the prevalent clade in Africa (clade C). Specifically, the DNA vaccine is a cocktail of three DNA plasmids that, respectively, express clade C 96Zm651 Gag, clade C 96Zm651 gp140, and clade C CN54 Pol-Nef, administered intramuscularly. The bivalent subtype C protein vaccine includes clade C TV1.C gp120 and clade C 1086.C gp120. Protein adjuvants are either MF59 or AS01B.

In 2004, the phase I clinical trial DP6-001 was initiated (ClinicalTrials.gov identifier NCT0061243) to test a Gag and 5-envelope HIV-1 vaccine using a DNA and protein prime-boost regimen. The five gp120 proteins were selected by sequence to represent clades A, B (2×), C, and E (26,55). Development of this multi-envelope vaccine concept continues in the clinical arena.

In 2009, a phase IIb trial (HVTN 505) was initiated to test a three-envelope vaccine for which protection had been observed in *Macaca mulatta*, driving the clinical study of the vaccine concept (ClinicalTrials.gov identifier NCT00865566). Three envelope glycoproteins were selected based on sequence to represent clades A, B, and C and they were administered using a DNA prime/recombinant adenovirus 5 (Ad5) boost regimen. Specifically, there were three DNA primes expressing a mixture of six DNA plasmids (*gag*, *pol*, *nef*, and *env* from clades A, B, and C), one Ad5 boost expressing envelope proteins from clades A, B, and C, and a Gag-Pol fusion protein from clade B. While the vaccine stimulated robust HIV-1 antibody responses, no protection was detected and the trial was prematurely terminated in 2013. Analysis of the vaccine-elicited antibodies by Williams *et al.* demonstrated that 93% of HIV-1 reactive antibodies isolated from memory B cells (identified and sorted from peripheral blood based on binding to fluorophore-labeled recombinant envelope proteins) were directed against

gp41 envelope. Non-neutralizing gp41-reactive antibodies were frequently polyreactive with intestinal microbiota, and pre-existing envelope-microbiota cross-reactive B cells were engaged (103).

In 2017, Bradley *et al.* (18) reported the testing of a pentavalent envelope vaccine in macaques. The vaccine components were selected using a mosaic design tool to include naturally occurring envelope sequences with diversity in the V2 sequence. Clades B and E were represented. The concept was tested using a pox-protein prime boost regimen and the animals were challenged intrarectally with low-dose SHIV. There were improved antibody activities and protective responses in animals that received the pentavalent envelope vaccine compared with those that received the bivalent envelope vaccine, suggesting that breadth was improved when the envelope cocktail size was increased.

Multi-envelope vaccines based on antigenic diversity. Several research groups have categorized envelopes by antigenicity for the purpose of vaccine development rather than by clade. For example, Nyambi *et al.* identified several HIV-1 immunotypes based on antibody reactivity patterns (72,110) and Binley *et al.* used neutralization assays to assign viruses to five antigenically distinct groups (8).

Hurwitz *et al.* began testing envelope cocktails based on antigenicity in the 1990s. Delivery vehicles for these cocktails first included pox virus recombinants and later included DNA recombinants and purified recombinant gp140 proteins (22,46,47,81,87,92,95). Studies were originally conducted in mice, cotton rats, rabbits, and chimpanzees, demonstrating that binding and neutralizing antibody activities could be generated by cocktail vaccines, and that the envelopes recognized by antibodies in immunoassays did not need to match vaccine components by sequence (62,77,81,95). Vaccine envelopes were assembled to include as a composite (i) sequences selected longitudinally from infected persons (78), (ii) sequences that represented more than one clade, and (iii) sequences that were characterized as antigenically distinct by antibody–antigen reactivity studies. Studies in macaques tested cocktails comprising dozens of envelopes using DNA-pox virus-protein “prime-boost-boost” regimens and demonstrated that disease caused by challenge with a heterologous SHIV was inhibited in vaccinated animals (52,109). Phase I clinical studies demonstrated safety with the chosen vectors (45,93) and again illustrated that antibody binding and neutralizing responses toward heterologous viruses could be induced using the envelope cocktail approach (20,48,87).

Future prospects for envelope cocktail vaccine development for the induction of Poly-nAb

As of yet, large HIV-1 envelope cocktails and/or cocktails based primarily on antigenicity rather than sequence have not yet progressed to advanced clinical testing, but the concept is gaining traction in the field [reviewed in Korber *et al.* (57)]. Future studies may show that better vaccine successes may be attainable when formulations are based on antigenicity (using analyses of antibody–antigen reactivity patterns and/or viral escape mutants) rather than sequence.

Is it possible to represent HIV-1 diversity in a cocktail vaccine? The number of unique envelope protein sequences among HIV-1 variants is extraordinary in size, but this need

not thwart the development of envelope cocktail vaccines. The number of mutually exclusive antigenic variants is limited, because functional requirements restrict the envelope’s structural diversity. Envelope must bind a conserved cluster of differentiation 4 (CD4) molecule and conserved coreceptor molecules to support infection of the target cell. An envelope that has altered its structure to the extent that it cannot bind the host cell’s membrane is not a threat to humans and need not be represented in a vaccine. Therefore, the level of diversity that a vaccine needs to cover is limited to different antigenic conformations of specific regions, hence reducing the amount of variability required. A systematic investigation of mutations and conformational variations of these key regions may guide rational design of vaccine immunogens.

Today, both polyclonal and monoclonal antibodies from humans and research animals can be used to characterize and categorize HIV-1 envelope proteins (88). Given that pathogen recognition by antibodies is often dependent on the three-dimensional structures of viral proteins (34), antibody–antigen reactivity patterns may provide a better measure of pathogen variability for the purpose of vaccine design, than a list of linear virus sequences.

High-throughput technologies may allow integration of a large amount of data and identification of differences across large numbers of isolates [reviewed in Kwong *et al.* (58)]. An iterative and rapid process of analyzing the immune responses elicited in basic research studies and small-scale clinical trials may then guide the design of new immunogen compositions and “fill the gaps” to accelerate successful vaccine development. Perhaps the attainment of full coverage of diverse HIV-1 will be a work-in-progress (as in other fields), but perhaps (as in other fields) initial, imperfect vaccines will save a vast number of human lives.

Targeted Pathway 2: One B Cell: One Broadly Neutralizing Antibody

The concept

The second approach to vaccine design relies on the observation that the presentation of antigenically distinct but genetically related envelopes during the course of natural infection can result in the development of monoclonal bnAbs and aims at recapitulating a similar sequence of events by vaccination (38). In recent years, the HIV-1 vaccine development field realized that immunization with a single HIV-1 envelope would not be successful at inducing bnAbs (66), and substantial effort has since been directed at better understanding the pathways by which bnAbs are generated in infected patients.

Numerous broadly neutralizing antibody lineages against multiple envelope regions have now been isolated, and a wealth of structural, functional, and immunogenetic data are being acquired that, in conjunction with computational analyses, detail the evolution of bnAb lineages [(12,13,17, 31,35,102,107) and reviewed in Bonsignori *et al.* (14)]. These studies have shown that bnAb epitopes have conserved features and shapes that allow limited solutions for bnAbs to fit them, either restricting bnAb usage of selected gene segments or requiring reproducible structural features (or “signatures”): for example, V_H gene segment usage is restricted to V_H3-15 and V_H3-20 among distal MPER

bnAbs; CD4 mimic CD4bs bnAbs use exclusively V_H1-2 or V_H1-46 and adopt the same angle of approach to envelope and mode of antigen recognition; V2 glycan bnAbs use long, protruding, anionic, and often tyrosine-sulfated CDR H3 to penetrate the HIV-1 envelope glycan shield; and V3 glycan bnAbs, while having the greatest degree of variability, need to adopt functionally similar solutions to recognize a four amino acid lineage motif (GDIR) at the base of the HIV-1 V3 loop C-terminus and multiple high-mannose glycans, including the N-linked glycan at position 332.

The “B cell lineage-based immunogen design” is a strategy that leverages such information and aims at eliciting bnAbs through vaccination by recapitulating the key events that lead to increasing neutralization breadth during chronic HIV-1 infection between evolving bnAb lineages and coevolving autologous envelope proteins (38). The phylogeny and maturation pathways of one or more bnAbs from HIV-1-infected individuals are used as templates to select envelopes from the coevolving autologous virus quasispecies that participated in the maturation of a specific bnAb lineage (38).

The goal: instructing affinity maturation

A naïve B cell will express germ line-encoded antibodies on its surface so that engagement of antibody with a target foreign antigen can drive cell activation and division. A natural process, somatic hypermutation, then introduces mutations in coding sequences and further improves sequence heterogeneity. If a new sequence encodes an antibody that has improved binding affinity for the activating antigen, the B cell may have an amplification advantage over other cells and may increase its representation in the responding B cell population (affinity maturation). B cell populations that are sequentially exposed to antigenically distinct HIV-1 envelopes will repeatedly experience somatic hypermutation and selection.

While somatic hypermutation is a stochastic process governed by the activation-induced (cytidine) deaminase (AID) enzyme, and can target nucleotides at AID hot spots and cold spots with varying degrees of probability, the goal here is to select maturing antibodies with antigens (immunogens) that drive clonal B cell evolution toward the expression of high-affinity bnAb. The strategy to identify such immunogens is to map the functional coevolution of autologous virus and bnAb clones over time in infected persons, to select the envelopes with optimal affinity for bnAb precursors (starting with the unmutated common ancestor), and to administer these envelopes sequentially to naïve individuals in the context of a vaccine (1,11,13–15,17,50,84). To mimic the naturally occurring progression of maturation of bnAb lineages, each selected envelope should engage a bnAb precursor with affinity sufficient to trigger the B cell, hence providing an evolutionary advantage to the maturation pathway leading to acquisition of neutralization breadth [(13,17) and reviewed in Bonsignori *et al.* (14)].

Immunogen design for sequential vaccination

Individual and small combinations of bnAbs have been identified that reach near-pan-neutralization *in vitro* with large multiclade panels of diverse HIV-1 strains (15,17,102). However, HIV-1 can escape control from a single bnAb *in vivo*, both in the settings of chronic infection and on infusion (64). Therefore, it can be argued that a vaccine that will elicit a

monovalent bnAb may select for a specific set of escape mutations at the population level that will render the vaccine ineffective. For this reason, a vaccine should optimally induce multiple bnAbs against different bnAb epitopes (15) and/or against antigenic variants of a single bnAb epitope (31).

The selection of candidate immunogens relies on the integration of functional, immunogenetic, and structural data, and algorithms have been designed to assist envelope selection, such as the longitudinal antigenic sequences and sites from intra-host evolution (LASSIE) program that evaluates longitudinal processes in infected individuals and identifies immune-selected HIV-1 variants (40,57). While the precise identification of mutations, insertions, and deletions in the candidate immunogens is of paramount importance, the induced lineages do not necessarily need to mimic the genetic evolution—or one specific somatic hypermutation pathway—or be limited to one, and only one, specific V(D)J rearrangement; rather the sequentially administered immunogens are predicted to elicit lineages with functionally equivalent solutions in response to a predetermined sequence of antigenic variations.

Hence, this strategy postulates that, given equivalent antigenic exposure in two individuals, antibody development will follow functionally comparable maturation pathways. It will be important to determine if antibody evolution is indeed a deterministic event in animal models.

Protection Beyond Neutralization

For the evaluation of new vaccines, neutralization remains the gold standard (21,39,63,100). However, in the wake of the RV144 trial, the importance of additional effector functions and assays has been emphasized. Clinical trials of new vaccines now assess Fc-mediated effector functions, including ADCC, antibody-dependent cell-mediated virus inhibition (ADCVI), and phagocytosis (16,25,37,44,56,60,61,79,82,99). While the definitive evaluation of efficacy is protection against infection and disease in phase III clinical trials, *in vitro* assays may influence “go/no go” decisions at earlier stages of vaccine production. Screening by multiple assays provides a comprehensive understanding of the spectrum of distinct antibody functions elicited by vaccine candidates, while potentially defining new correlates of protection (69,108).

Protection Beyond Vaccines: Will Genomic Manipulations Ever Supersede Vaccines? A Future Strategy?

The discovery of rare, monoclonal antibodies with broadly neutralizing activities has encouraged passive transfer strategies (68), which demonstrate infection prevention. Will the design of custom antibodies or cells supersede vaccines one day? Antibodies and customized T cells are at the forefront of immune-mediated tumor control strategies (e.g., anti-CD20, anti Her2, and chimeric antigen receptor [CAR] T cells) (28,71,73). For example, CAR T cells are now targeted toward particular tumor epitopes and can be designed to carry drug-sensitivity markers so that cells may be deliberately manipulated *in vivo*.

As a substitute for HIV-1 vaccination, Saunders *et al.* have introduced AAV recombinants expressing broadly neutralizing antibodies into mice and macaques and demonstrated stable antibody expression and protection (83).

Technologies for manipulations of genomes are advancing (e.g., the CRISPR/Cas9 technologies) (3,32,51,97,104). Cheong *et al.* recently demonstrated that CRISPR/Cas9 can be used to drive class switch recombination in B cells and thereby instruct the antibody isotype expressed by those cells (24). Perhaps the next step will be the construction of precise V-D-J and V-J heavy and light chain antibody sequences in human B cells, or the design of “off-the-shelf” lymphoid cell lines expressing sequences of interest.

There are obvious limitations to such strategies in the context of protection from virus infections. Hurdles concern safety, the logistics of providing antibodies to large populations, cost, durability of immune responses, and the potential for induction of autoimmune complications. The latter potential is of particular concern because some epitopes recognized by broadly cross-reactive antibodies are also exhibited on “self” (36). While autoreactive antibodies that result in autoimmune diseases are rare due to tolerance control, the introduction of genetically modified cells in healthy individuals requires careful scrutiny. The general use of custom antibodies or B cells can be logistically difficult today, but will perhaps constitute an important, future tool for HIV-1 protection.

Conclusions

The need for a successful HIV-1 vaccine remains, and the multiantigen cocktail approach, which has been proven successful in numerous other vaccine fields, might now take the lead in HIV-1 vaccine design. Several new developments may improve vaccine design, including (i) an increased understanding of bnAb development, the ability to monitor and reconstruct the evolution of individual lineages in the context of polyclonal responses and the concept of B cell lineage immunogen design (15,17), (ii) the progressively tighter integration of information obtained from functional, structural, and immunogenetic studies, and (iii) renewed attention to the complex interplay of multiple antibody specificities and functions. These may all lend to the selection of HIV-1 envelope cocktails and vaccination regimens that better represent HIV-1’s natural diversity, and better protect humans from HIV-1 infections. Fine-tuning of vaccines will surely be required on the road to perfection, but millions of human lives might be saved along the way.

Author Disclosure Statement

No competing financial interests exist.

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