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Rational Antibody-based HIV-1 Vaccine Design: Current Approaches and Future Directions

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Abstract

Many anti-viral vaccines elicit neutralizing antibodies as a correlate of protection. For HIV, given the huge variability of the virus, it is widely believed that the induction of a *broadly* neutralizing antibody (bNAb) response will be crucial in a successful vaccine against the virus. Unfortunately, despite many efforts, the development of an immunogen that elicits bNAbs remains elusive. However, recent structural studies of HIV-1 Env proteins, generation of novel bNAbs, maturation of technologies for the isolation of further antibodies, insights into the requirements for antibody-mediated protection, and novel vaccination approaches are providing grounds for renewed optimism.

Introduction

The hallmark of many successful anti-viral vaccines is the ability to induce neutralizing antibodies [1]. One method of showing that antibodies can provide protection is by passive administration followed by virus challenge in animal models. For many viruses, this approach shows good correlation between protection *in vivo* and neutralizing activity *in vitro* [2]. For HIV-1, passively administered NAbs provide protection after intravenous, vaginal, rectal, and oral virus challenge in non-human primate models [3•,4•,5,6,7]. Importantly, several studies demonstrate that vaccine-induced NAb responses can confer complete protection against homologous SHIV challenge in macaques [8,9••,10], indicating that a vaccine capable of eliciting sufficient levels of NAb against HIV-1 could prevent the establishment of infection. For many viruses, extra-neutralizing mechanisms, such as those dependent on interaction of antibody with Fc receptors, e.g. antibody-dependent cellular cytotoxicity (ADCC), or on interaction with complement, also contribute to protection [2,11,12]. For HIV-1, experiments in the macaque model suggest the importance of the interaction of antibody with Fc receptors [11]. Although non-neutralizing antibodies can mediate extra-neutralizing activities, these types of antibodies provide little or no protection against SHIV challenge in non-human primates [5,13], suggesting that a vaccine should focus on the induction of NAbs. Overall, given the observations in animal models, it seems highly likely that neutralizing antibodies to HIV-1 induced by a vaccine would provide benefit on exposure to the virus.

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There are, however, major challenges in the development of immunogens that induce bNAbs. These challenges include the extraordinary genetic diversity of the virus, the relative inaccessibility of conserved epitopes that are targeted by bNAbs, the instability of the envelope glycoprotein (Env, the only known target for neutralizing antibodies), and difficulties sustaining NAb titers following vaccination. Optimism in the field has risen following recent studies in humans and non-human primate models. First, a series of serum mapping studies show that 10–30% of HIV-1 infected individuals develop moderate to broadly neutralizing sera over time, demonstrating that the human immune system is capable of generating bNAb responses against HIV-1 [14]. Studies underway on how these bNAb responses develop may prove valuable in vaccine design. Second, broadly neutralizing monoclonal antibodies with outstanding potency have recently been isolated from infected donors [15••, GJ Nabel *et al.*, personal communication]. Third, recent passive immunization studies in non-human primate models indicate that bNAbs can provide benefit against SHIV challenge at much lower serum neutralizing titers than originally considered obligatory for protection [3•,16••]. Fourth, several studies show that productive HIV-1 infection may often be mediated by one or a few virions, providing a vulnerability of the virus to antibody neutralization early in infection [17].

Initial attempts to generate a protective vaccine against HIV-1 focused on the elicitation of Env-specific humoral immune responses using gp120 subunit immunogens. Unfortunately, the results of Phase 1 clinical trials indicated that the antibodies elicited by monomeric gp120 failed to neutralize HIV-1 primary isolates (representative of circulating viruses as opposed to laboratory adapted strains) [18,19]. The results of two Phase 3 efficacy trials showed that the vaccine failed to prevent HIV-1 infection, reduce viral loads, or delay disease progression [20,21]. Over the ensuing years, HIV-1 researchers have pursued many different approaches to the generation of an antibody-based vaccine, but none of the immunogens generated to date have induced NAb responses of the required breadth and potency. Much effort has focused on rational vaccine design. The Env proteins, more specifically the functional Env heterotrimer $(gp120)_3(gp41)_3$, and bNAbs are the two molecular species that are at the heart of such rational HIV-1 vaccine design. Insight into the molecular structures of Env proteins, both alone and in complex with bNAbs, is crucial for attempts to, in effect, reverse engineer vaccine candidates [22] (Fig 1). This review will discuss recent advances in HIV vaccine design with a focus on rational approaches.

Soluble trimers

The native functional HIV-1 trimer (either prior to or during the fusion process) is the sole target for neutralizing antibodies, and it seems that antibody binding to the trimer is necessary and sufficient for neutralization [2]. Therefore, in principle, a recombinant native trimer represents an ideal immunogen for the elicitation of NAb responses. However, the inherent instability of the functional HIV-1 spike has presented challenges to the development of native recombinant trimers [23]. Various strategies, including the introduction of disulfide bonds to covalently link gp120 and gp41, deletion of the furin cleavage site in gp160, and the incorporation of a number of trimerization motifs into the gp41 ectodomain, have been employed to stabilize recombinant trimers [23]. Although none of these recombinant trimers display antigenic profiles that accurately mimic the native HIV-1 spike, some elicit antibodies that neutralize heterologous isolates with very modest potency [24,25,26,27]. For example, both YU2 gp140 GCN4 and KNH1144 gp140 SOSIP trimers induce NAbs with increased breadth and potency relative to those elicited by monomeric gp120 [25,26], although the improvements are small. On a positive note, recent cryoelectron tomographic structures of native HIV-1 trimers [28••,29,30], as well as the isolation of new broadly neutralizing trimer-specific antibodies [15••], will likely aid the design of recombinant trimers that better mimic the native HIV-1 spike.

Immunogens based on the epitopes recognize by bNAbs

HIV-1 tends to elicit a high abundance of NAbs against variable regions of the virus, whereas NAbs that target conserved regions are rare and only develop in a subset of individuals [14]. Nonetheless, a small number of broadly neutralizing human monoclonal antibodies have been isolated, and the epitopes targeted by these antibodies have served as templates for immunogen design (Figure 1 and Figure 2) [23]. Recent crystallographic, cryoelectron tomographic, and molecular modeling studies have provided valuable insights into the molecular surfaces recognized by the antibodies and assisted the rational design of immunogens [31].

The CD4 binding site (CD4bs) is a prime target for the elicitation of bNAbs due to its high degree of conservation and the requirement for accessibility, at least to CD4. Indeed, the well-characterized bNAb b12, as well as several broadly neutralizing sera, have been shown to target this site [14,32]. Furthermore, two new CD4bs-directed bNAbs have recently been reported [33, GJ Nabel *et al.*, personal communication]. One of these, HJ16, exhibits similar breadth and potency as b12 but shows a different pattern of neutralization sensitivity [33]. The other bNAb, VRC01, displays greater breadth and potency compared to b12 [GJ Nabel *et al.*, personal communication]. Until the recent findings, b12 was the most broad and potent CD4bs-directed mAb, and therefore a variety of strategies were employed to focus the immune response on its epitope. In a series of studies, gp120 and gp140 were engineered using alanine substitutions and hyperglycosylation to maintain b12 binding while reducing the binding of most non-neutralizing mAbs [34,35,36]. However, several designs failed to induce b12-like antibodies in rabbits [37]. This may be due to the inherent conformational flexibility of recombinant gp120 and gp140 that militates against uniquely fixing the b12 epitope. Interestingly, a recent crystal structure of b13, a non-neutralizing CD4bs-directed antibody, bound to gp120 revealed a remarkably similar footprint to that of b12 [38•]. This result suggests that either extremely precise targeting induced by the immunogen or presentation of the b12 epitope in the context of the functional trimer may be required for the elicitation of b12-like antibodies. Since b12 and 2G12 primarily interact with the outer domain (OD) of gp120 [39], this surface also represents an attractive target for immunogen design. The OD is poorly immunogenic in the context of gp120, and therefore researchers have focused on the generation of isolated OD constructs that expose the b12 and 2G12 (see below) binding sites. However, when the OD of YU2 gp120 was expressed independently, it bound 2G12 and V3 loop-directed antibodies with high affinity but failed to bind b12 [40]. More recently, the crystal structure of the b12-gp120 complex was used to guide the design of a membrane-anchored OD construct that specifically bound to b12 but not most other non-neutralizing CD4bs-directed antibodies [41]. This construct is currently being tested as a vaccine immunogen.

The bNAb 2G12, which adopts an unusual domain-exchanged structure to recognize a conserved cluster of oligomannose residues on the OD of gp120, has provided a basis for the design of immunogens to target the HIV-1 glycan shield. Attempts to generate immunogens based on the 2G12 epitope initially focused on the multivalent display of chemically synthesized oligomannose-containing glycoconjugates [42,43]. 2G12 recognized the glycoconjugates weakly, and they were poorly immunogenic in rabbits and predominately elicited linker-specific antibody responses. The next generation of synthetic glycoconjugates better mimicked the oligomannose cluster that comprises the 2G12 epitope on the HIV-1 trimer and bound 2G12 in the nM affinity range. Some of these approaches, including the construction of oligomannose dendrons [44], Man_4 containing neoglycoconjugates [45], and cyclic glycopeptides [46], successfully induced anti-carbohydrate antibody responses, but to date these responses have failed to cross-react with gp120 or neutralize HIV-1. A second approach to the design of immunogens for the elicitation of 2G12-like antibodies has been to identify heterologous glycoproteins that express carbohydrates structures that mimic the clustered high mannose glycans on the HIV-1 trimer [47,48]. Recently, Luallen *et al.* engineered a *S.*

cerevisiae triple mutant that exclusively produced homogenous high mannose glycans [49•]. Since 2G12 efficiently bound to the triple mutant, but not wild-type *S. cerevisiae*, whole yeast cells were used in preliminary immunization studies. Although the triple mutant-immunized rabbit sera cross-reacted with a diverse range of HIV-1 Env proteins in a glycan-specific manner, the sera failed to neutralize the corresponding HIV-1 isolates. These results suggest that the glycan epitopes recognized by these antibodies differ from that of 2G12, and/or that the titer of 2G12-like antibodies was too low to observe potent neutralization activity.

The bNAbs 2F5, 4E10, and Z13e1 bind to a conserved tryptophan rich region on gp41 referred to as the membrane-proximal external region (MPER), and this region has attracted considerable interest as a vaccine target. This interest is enhanced by the recent demonstration that both 2F5 and 4E10 can protect against mucosal SHIV challenge [4•]. Of note, some reports suggest that 4E10, and controversially 2F5, cross-react with lipids, and it has been proposed that these types of antibodies may be difficult to elicit by vaccination due to B cell tolerance mechanisms [50,51]. The crystal structures of 2F5, 4E10, and Z13e1 bound to their cognate peptides reveal that 2F5 recognizes an extended loop structure, 4E10 recognizes a helical conformation, and Z13e1 binds to an elbow in the MPER [52,53,54•]. These structural studies, as well as complementary biochemical studies [55,56••,57], also suggest that the viral membrane may play a role in formation of the 2F5 and 4E10 epitopes. Notably, recent studies illustrate the importance of hydrophobic residues at the tip of the 4E10 CDRH3 loop for interaction with the viral membrane and potent neutralization activity [57,58,59]. The crystal structure data has been used to rationally design constrained peptides that mimic the conformations recognized by 2F5 and 4E10 [60,61] and/or to present the 2F5 and 4E10 peptides in the context of a lipid membrane [62,63,64]. However, none of these immunogens have to date elicited 4E10 or 2F5-like antibodies.

Recently, two new broad and potent NAbs, PG9 and PG16, were isolated from a clade A infected donor using a high-throughput functional screening approach [15••,65]. These somatically related antibodies bind to conserved residues in the V1/V2 and V3 loops of gp120 and their epitopes are preferentially expressed on trimeric HIV-1 Env. Both antibodies neutralize a diverse range of HIV-1 isolates at concentrations (sub-μg/ml range) about 10- to 100-fold lower than the previously identified bNAbs. Such concentrations might readily be achieved through vaccination. Vaccination strategies are currently being explored to focus the immune response on conserved regions of the variable loops in the context of the trimeric spike. The identification of potent trimer-specific bNAbs again underscores the limitations of monomeric gp120 as an immunogen and emphasizes the importance of generating trimers that closely resemble the functional spike.

Virus-like particle based immunogens

Although most successful anti-viral vaccines have relied on the use of live-attenuated viruses [66], they are not currently considered safe for HIV-1 vaccine development due to the risk of mutation and reversion to a pathogenic form [67,68]. As an alternative, researchers have turned their attention to the use of virus-like particles (VLPs). VLPs resemble infectious virions but are non-pathogenic because they lack a viral genome. Since viruses display multivalent structures, VLPs are usually highly immunogenic and induce antibody responses in the absence of adjuvant. Another attraction of VLP-based immunogens is the presence of native trimers on the VLP surface. However, due to the instability of the HIV-1 spike, non-functional forms of Env are also expressed on the surface of VLPs [69]. Indeed, in a comparative immunogenicity study of VLPs bearing various forms of HIV-1 Env, the binding activity of the VLP immunized sera was primarily focused on non-functional forms of Env [70]. Furthermore, there are relatively few native spikes on the surface of HIV-1, which is likely to reduce the elicitation of antibodies against native structures. Other complicating factors, such as the induction of

antibodies against cellular proteins and the elicitation of strain-specific NAbs, also contribute to the limited success of VLPs as immunogens. Although a number of strategies to overcome these hurdles have been employed, including pseudotyping HIV-1 with heterologous envelopes [71,72], truncating the cytoplasmic tail of gp41 to increase Env expression [73], and generating VLPs with cleavage-defective or disulfide-shackled Env to prevent gp120-gp41 dissociation [70], so far none of these approaches have induced potent heterologous antibody responses in non-human primate models.

Prime-boost strategies

DNA vaccines are typically poorly immunogenic in non-human primates and humans; multiple immunizations are required to elicit even moderate titers of NAbs and these titers rise and fall with successive immunizations. On the other hand, results from numerous studies suggest that DNA priming followed by Env protein boosting induces NAbs with increased titer and persistence relative to either approach alone. In proof-of-concept studies, DNA priming and Env protein boosting induced high titers of NAbs that correlated with protection against homologous SHIV challenge in rhesus macaques [8,74,75]. In a recent Phase I clinical trial, polyvalent Env antigens were delivered to healthy human volunteers in a DNA prime-boost approach [76]. Despite the weak neutralizing activity in the sera of the vaccinated individuals, a single Env protein boost elicited higher titers of anti-Env antibodies than has been previously achieved with multiple immunizations of recombinant Env proteins alone [76,77]. Thus, although further modifications of the vaccine formulation may be required to elicit NAbs with increased breadth and potency, the results of this study demonstrate the potential of the DNA prime-protein boost strategy for vaccine development. Indeed, the DNA prime-boost approach is currently being employed for many ongoing and planned Phase I and II clinical trials.

DNA priming followed by viral vector boosting has also been shown to improve the magnitude of the NAb response. For example, results from a non-human primate study demonstrate that a DNA prime-recombinant serotype 5 adenovirus boost strategy elicits higher levels of NAbs than either approach alone [78]. Viral vectors have also been used to prime B cells for higher titer NAb responses after boosting with recombinant Env proteins or heterologous viral vectors. For instance, several studies in human volunteers show that ALVAC priming and Env protein boosting elicits higher NAb titers than vaccination with Env protein alone [79,80,81]. Recently, a Phase III HIV-1 vaccine clinical trial in Thailand (RV 144) tested a heterologous prime-boost regimen using a canary pox-HIV vector (ALVAC-HIV) prime and a recombinant gp120 boost (AIDSVAX B/E) [82••]. To the surprise of many HIV researchers, the vaccine showed modest efficacy in preventing HIV-1 infections. Interestingly, the modest protective effect appeared limited to low-risk individuals, and there was suggestion that the effect was confined to the first year following the commencement of vaccination. Also, in contrast to the results of many protection studies in non-human primate models, vaccinated individuals who became infected did not have lower viral loads or decreased loss of $CD4^+$ T cells compared to the placebos. Efforts are currently focused on evaluating the immune responses induced by the vaccine to establish potential correlates of protection.

Genetic approaches

The difficulties faced in eliciting broad and potent NAbs using the approaches described above have led researchers to develop innovative genetic strategies that essentially bypass immunization. In a proof-of-concept study, Lewis *et al.* used an adeno-associated virus (AAV) vector to deliver the IgG1 b12 gene into mouse muscle and discovered that the antibody molecules imparted neutralization activity in the sera for over 6 months [83]. Encouraged by this result, this approach was then tested in a non-human primate model by delivering neutralizing immunoadhesins (antigen-binding variable fragment domains of Fabs fused to the

Fc fragment of a rhesus IgG2 molecule) into macaques [84••]. The immunoadhesins were expressed in the macaque muscle myofibers, and serum neutralization activity was sustained for over 1 year. More importantly, sterilizing protection against SIV challenge was achieved in six out of nine immunized monkeys, and all nine monkeys were protected from AIDS. In another genetic approach, lentiviral vectors were used to engineer human hematopoietic stem cells to produce IgG1 b12 after *in vitro* maturation into B cells [85••]. This study substantiates that human B cells can be "programmed" to secrete antibodies of pre-defined specificity in a tissue culture system.

Conclusion

Traditional vaccination approaches have thus far failed to elicit NAb responses against HIV-1 of sufficient breadth and potency, and therefore the field has turned to alternative, particularly rational structure-based, vaccine design strategies. Although these approaches have provided insight into the link between Env antigenicity and immunogenicity, immunogens that focus the antibody responses to conserved epitopes still remain elusive. However, recent technological and scientific advances have reignited the field, and hopes for an antibody-based vaccine are notably higher than in previous years.

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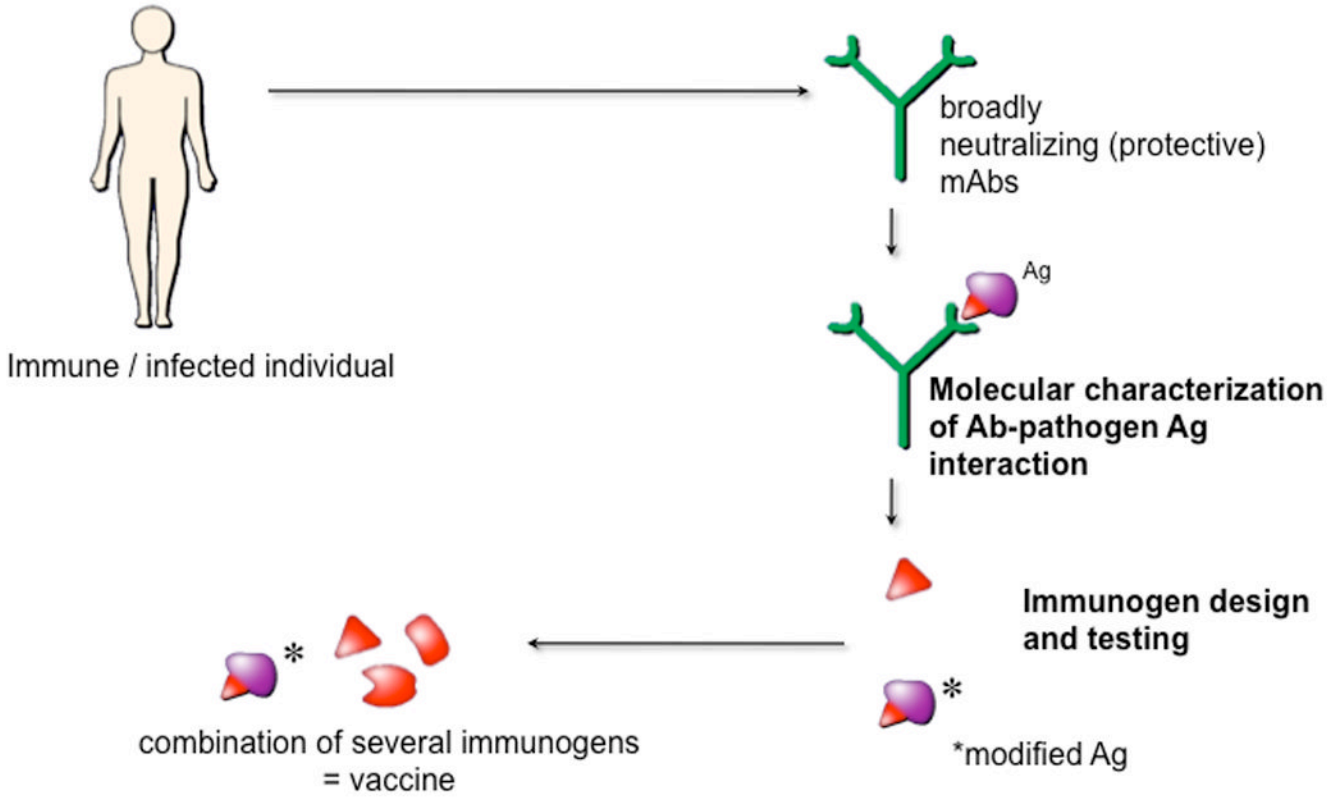

Figure 1. An approach to vaccines for highly mutable pathogens

For these pathogens, e.g. HIV, classical vaccine approaches are problematic but a subset of individuals do produce the types of antibody response that, if they could be elicited by vaccination, would likely provide benefit on exposure to the pathogen. Isolation of monoclonal antibodies (mAbs) constituting these responses together with a molecular characterization of the interaction of the mAbs with their pathogen target antigens (Ag) is proposed as a route to the design of immunogens that can elicit protective responses. For HIV, Ag is the envelope spike.

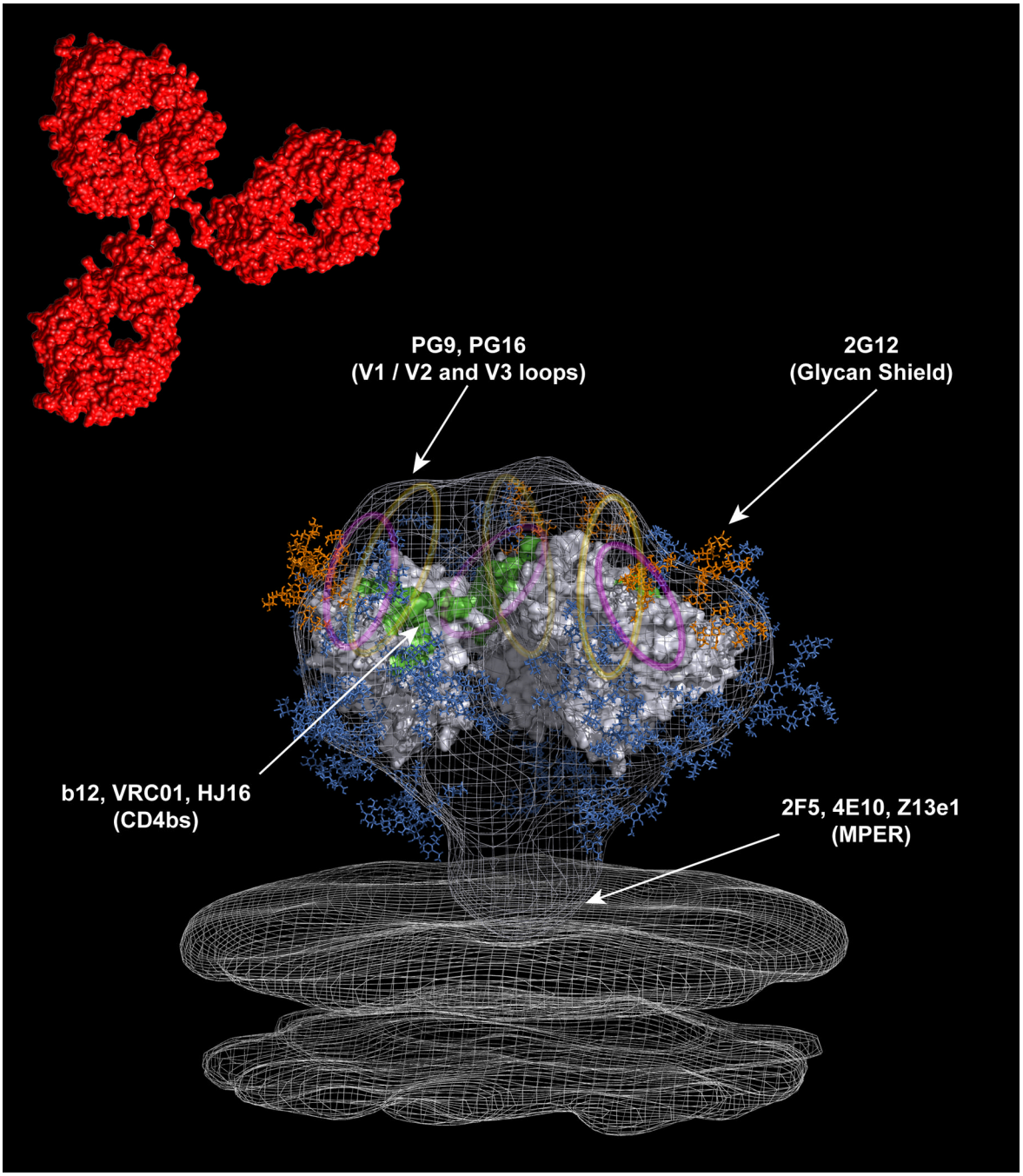

Figure 2. Modeling the epitopes recognized by bNAbs onto the HIV-1 trimer

The above model is adapted from a recent cryo-electron tomographic structure of the HIV-1 trimer [28••,86]. The crystal structure of the b12-bound monomeric gp120 core has been fitted into the density map [39]. The V1/V2 and V3 loops, which are not resolved in the structure, are represented as yellow and magenta ovals, respectively. The red structure located above the trimer is representative of a human IgG1 molecule. The approximate locations of the epitopes targeted by the existing bNAbs are indicated with arrows. Carbohydrate chains are shown in blue, and the oligomannose cluster targeted by mAb 2G12 is shown in orange.