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Advances in structure-based vaccine design

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Abstract

Despite the tremendous successes of current vaccines, infectious diseases still take a heavy toll on the global population, and that provides strong rationale for broadening our vaccine development repertoire. *Structural vaccinology*, in which protein structure information is utilized to design immunogens, has promise to provide new vaccines against traditionally difficult targets. Crystal structures of antigens containing one or more protection epitopes, especially when in complex with a protective antibody, are the launching point for immunogen design. Integrating structure and sequence information for families of broadly neutralizing antibodies (bNAbs) has recently enabled the creation of germline-targeting immunogens that bind and activate germline B-cells in order to initiate the elicitation of such antibodies. The contacts between antigen and neutralizing antibody define a structural epitope, and methods have been developed to transplant epitopes to scaffold proteins for structural stabilization, and to design minimized antigens that retain one or more key epitopes while eliminating other potentially distracting or unnecessary features. To develop vaccines that protect against antigenically variable pathogens, pioneering structure-based work demonstrated that multiple strain-specific epitopes could be engineered onto a single immunogen. We review these recent structural vaccinology efforts to engineer germline-targeting, epitope-specific, and/or broad coverage immunogens.

Introduction

Vaccines are a cost effective way to protect against infectious disease [1], but infectious diseases remain a major cause of loss of life and economic harm in the world today. According to the Global Burden of Diseases, Injuries, and Risk Factors Study 2010 [2], infectious diseases were responsible for 18.5% of all human deaths and 40% of years of life lost in 2010. Infectious diseases hit the very young especially hard — among children of ages 28 days to four years, infectious disease caused 61% of all deaths in 2010. A substantial proportion of this burden could potentially be eliminated by new vaccines. Therefore, increased effort should be devoted to discovering and developing new and improved vaccines for human health.

Nearly all licensed vaccines are thought to protect against infection by inducing antibodies that bind antigen and cause neutralization and/or complement fixation and/or opsonophagocytosis [3–6]. In most cases this requires induction of CD4⁺ T cells to assist B cell expansion and differentiation, class switching, and maturation. And for some pathogens, CD4⁺ or CD8⁺ cellular immunity may contribute directly to protection by vaccination. To induce these responses, current FDA-licensed human vaccines [7,8] are based on only a few key strategies: nearly all viral vaccines derive from live, live-attenuated or whole inactivated viruses, and bacterial vaccines are based on purified bacterial surface proteins, detoxified toxins, or capsular polysaccharides conjugated to a carrier protein. Among recently licensed vaccines, recombinant, virus-like particle vaccines have been developed for human papillomavirus (HPV) and hepatitis B virus (HBV). Redoubled efforts with these proven approaches and development of new vaccine strategies are needed to reduce the burden of infectious disease on the human population.

Vaccine research is multi-faceted, and exciting progress is being made on multiple fronts. A variety of nucleic-acid based methods to deliver antigen have shown promise recently, including improved RNA methods [9] and novel self-amplifying RNA vaccines [10], as well as viral vectors [11,12*,13], DNA [14,15], and heterologous prime-boost regimens [11,12*,16]. Adjuvants, particularly toll-like receptor (TLR) agonists, are under intense investigation to identify vaccine formulations that harness innate immunity to induce optimal antibody and cellular immunity [17,18]. New or newly recognized adjuvants [19–21], combinations of adjuvants [22] and particulate platforms with embedded adjuvants as well as CD4 T help epitopes in some cases [23–25], have recently shown promise to enhance the magnitude, quality and duration of antibody responses. Systems biology approaches are now being employed to examine in detail how the immune system responds to vaccines down to the level of changes in protein expression levels and protein–protein interaction networks within specific, localized cell types [26,27]. This work, called ‘systems vaccinology’, can elucidate molecular signatures of protective vaccines and in some cases can predict immunogenicity and efficacy [28]; such information should prove invaluable for the design and evaluation of new vaccines.

In terms of identifying appropriate antigens and/or engineering antigens to obtain optimal vaccine responses, considerable work has focused on two broad areas: first, reverse vaccinology, in which novel immunogens are discovered via whole genome bioinformatics [29,30] and second, structural vaccinology, in which immunogens are rationally engineered using available structural information [31–35]. We will review recent studies in structure-based vaccine design, in which immunogens present one or more key epitopes or immunogenic domains, with the goal of inducing epitope-specific antibodies and/or yielding a broad coverage antibody response.

Germline-targeting immunogens

The high antigenic diversity of viruses such as human immunodeficiency virus 1 (HIV-1), influenza virus, and hepatitis C virus (HCV) poses major challenges for vaccine design because large portions of the surfaces of envelope glycoproteins are variable and covered by gly-cans [33]. The discovery of broadly neutralizing antibodies (bNAbs) that neutralize

diverse strains of HIV, influenza, or HCV gave rise to a strategy in which a bNAb is employed to guide design of immunogens to induce responses similar in structural recognition, breadth and potency [36]. In many such cases structural information about the bNAb epitope is essential for immunogen design. This strategy has been termed ‘reverse vaccine engineering’ but is perhaps more clearly labeled as ‘antibody-guided’ or ‘epitope-focused’ vaccine design.

Recently, a highly potent class of HIV bNAbs (VRC01-class bNAbs) was identified that uses the antibody framework to target the CD4 receptor binding site (CD4bs) on the HIV envelope protein (Env) [37–41] (Figure 1a). These bNAbs are highly mutated from germline, and have been produced by HIV-infected individuals only after two to three years of infection [42]. Hence it is expected that elicitation of similar bNAbs by vaccination will be very difficult and may require a lengthy and complex immunization regimen. A further difficulty is that the predicted germline precursor antibodies for these bNAbs lack detectable affinity for wild-type Env from a wide range of different HIV-1 strains [38,40,43,44^{••}]. Thus vaccines based on wild-type envelope proteins may fail to reliably activate the appropriate germline B cells, so may fail to begin the maturation process necessary to produce bNAbs.

This germline-binding hurdle was addressed recently by Jardine *et al.* [44^{••}] using an integrated structure-based and *in vitro* screening approach. Initial modeling of the interaction between the predicted-germline-VRC01 (GL-VRC01) and gp120 identified a potentially clashing glycan on gp120. Upon removal of that glycan and a neighboring one from an engineered outer domain (eOD) of gp120, barely detectable binding was obtained ($K_D \approx 1$ mM). Further modifications to the epitope were devised by a combination of computational interface design using Rosetta [45] with yeast display screening of computation-guided and random-mutagenesis libraries [46^{••}]. This produced an immunogen, eOD-GT6, that bound tightly to GL-VRC01 ($K_D = 44$ nM) and several other GL-VRC01-class antibodies, and in most cases bound with even higher affinity for the corresponding mature VRC01-class bNAbs. The authors hypothesized that if eOD-GT6 were employed as an immunogen, its higher affinity for the mature antibodies might serve to guide early somatic mutation toward the bNAbs. Antibody neutralization of HIV via the CD4bs is widely thought to require antibodies that not only target appropriate conserved epitopes but also approach those epitopes within a narrow range of angles so as to avoid steric clash with other parts of the Env trimer [32]. To address this question and others, three crystal structures were solved, for the bound complex eOD-GT6 + GL-VRC01 (Figure 1b) and for the two unbound components (not shown). These structures showed that GL-VRC01 binds to eOD-GT6 in a similar orientation as mature VRC01 binding to gp120 (Figure 1a), suggesting that in a hypothetical vaccine setting, VRC01-class germline antibodies initially activated in response to an eOD-GT6 vaccine might start off with the correct ‘angle of approach’. To optimize immunogenicity, virus-like nano-particles displaying 60 copies of eOD-GT6 were produced by genetically fusing eOD-GT6 to a self-assembling protein (Figure 1d). To test function *in vitro*, separate B-cell lines were created to express mature or germline VRC01-like antibodies, and immunogens were tested for their ability to activate the B cells as detected by calcium flux. The nanoparticles could activate B cells expressing

either mature or germline versions of three different VRC01-class bNAbs, whereas similar nanoparticles displaying a non-germline-targeting eOD immunogen were only able to activate mature VRC01-class B-cells, and monomeric eOD-GT6 failed to activate B cells entirely.

A simpler germline-targeting effort for VRC01-class antibodies was carried out by McGuire *et al.* [43*]. The authors identified a particular strain of HIV in which the removal of one to three glycans on gp140 (analogous to those removed from eOD-GT6) conferred high avidity for GL-VRC01 and GL-NIH4546 (another member of the VRC01-class) but not to predicted germlines for other members of the VRC01-class. This glycan-edited gp140 also activated B cells expressing germline and mature versions of VRC01 and NIH4546. The next step for germline-targeting immunogens from Jardine *et al.* [44**] and McGuire *et al.* [43*] will be to evaluate immune responses *in vivo*, in mice engineered to produce human germline antibodies, or in humans.

Careful examination of bNAb maturation and concomitant viral evolution in rare patients that develop bNAbs can open alternative avenues for identifying germline-targeting immunogens, and further can suggest sequences of immunogens to select appropriate antibody maturation. To this end, Liao *et al.* [47**] followed an African donor from the time of HIV-1 infection through to the development of the CH103 bNAb that targets the CD4bs. The authors sequenced the virus envelope and antibody genes at numerous time points along their evolutionary trajectories, including the transmitted-founder virus thought to initiate the eventual elicitation of CH103. At a late time point (136 weeks after infection), CH103 and other bNAbs specific for the CD4bs were isolated by antigen-specific B cell sorting using RSC3, a CD4bs probe built using structure-based design [37]. Bioinformatics analyses were used to construct a phylo-genetic lineage for CH103, including the bona fide heavy-light paired bNAb sequences as well as artificial pairs of heavy and light chains obtained from pyrosequencing of antibody gene transcripts at other time points. The authors observed that the unmutated common ancestor (UCA) antibody — the predicted starting point for the CH103 lineage — recognized the transmitted-founder envelope protein ($K_D = 37.5$ nM), but showed no detectable affinity for envelopes isolated at later time points. Inferred intermediate antibodies occurring early in the lineage also bound with high affinity to the transmitted-founder envelope but had decreased or undetectable affinity for envelopes isolated at later time points. These observations implied that the virus had escaped the early antibodies in the CH103 lineage. The authors also observed that significant cross-neutralizing breadth in CH103 lineage antibodies arose after significant diversification in viral envelope sequences, implying that viral escape from early antibodies selected for later antibodies with increased breadth. Indeed, mutations were detected within the CH103 epitope (Figure 1c). These results suggest that vaccination regimens to induce broadly cross-reactive antibodies may need to incorporate similar epitope diversity. While CH103 was able to neutralize ~55% of HIV-1 isolates, the UCA ‘germline precursor’ antibody bound to the transmitted-founder envelope but did not bind detectably to any of several heterologous HIV envelope proteins tested. The authors therefore proposed a vaccine concept in which HIV Env or Env subunits for the transmitted-founder, intermediate and late viruses be used in sequential or cocktail immunization regimens to induce a CH103-like bNAb response.

Providing structural information to guide such vaccine development, the crystal structure of the CH103 bNAbs in complex with gp120 was solved and revealed that, like most antibodies, CH103 complementarity-determining region (CDR) loops engage the epitope (Figure 1c). This finding implies that the CH103 UCA ‘germline precursor’ may be quite rare, as it might require several particular antibody genes (V_H , D_H , and J_H in heavy chain; V_L and J_L in light chain) as well as several particular recombination events (V_H – D_H and D_H – J_H junctions in the heavy chain and V_L – J_L junction in the light chain). This raises the question of how often naïve B cells in human vaccinees will harbor potential precursors to the CH103 lineage. In contrast, the VRC01-class germline precursors are thought to be present in most individuals [44^{**}]. Future experiments will be needed to determine the frequencies of ‘vaccine-targetable’ germline precursors for both classes of bNAbs. (Indeed, the frequency of vaccine-targetable germline precursors is a major concern for the germline-targeting strategy applied to any antibody.) Finally, CH103 is less mutated than VRC01-class bNAbs but also considerably less potent and less broadly neutralizing, suggesting that further mutations in CH103 could possibly generate higher potency and breadth comparable to VRC01. It will be fascinating to see if either of these bNAbs classes can be recapitulated by vaccination in engineered mice or humans, and perhaps if even more potent and broadly neutralizing antibodies in these classes can be induced.

Recent discovery of influenza bNAbs targeted toward the conserved stem region of hemagglutinin (HA) has renewed hope for the development of a universal flu vaccine [48–56], and some of the work suggests that consideration of germline-targeting may be important in that quest. A large class of HA stem-directed bNAbs derives from the human VH1-69 antibody gene, including the recently discovered CR9114 antibody that has extraordinary breadth spanning both group 1 and 2 of Influenza type A, as well as type B [56]. Crystal structures have revealed that antibodies in this class contact HA exclusively with the heavy chain, and that many HA-contacting residues are germline-encoded [49,50,54–56]. These VH1-69 bNAbs are analogous to the VRC01-class bNAbs against HIV in their reliance on a particular VH gene, but are considerably less mutated from germline and also lack rare indels in VRC01-class bNAbs [50,56], so the VH1-69 bNAbs against influenza may be more readily induced by vaccination with appropriate immunogens. Lingwood *et al.* studied the structure, binding and B-cell activation of several members of this class of anti-influenza bNAbs [57]. The VH1-69 germline ancestors of bNAbs CR6261 [50], FE53 [51], and 1009-3B05 [53] did not bind HA in soluble IgG form, but could bind when produced as IgM on the surface of 293 cells. Further, a proteoliposome-arrayed HA construct was able to activate B-cells expressing the IgM version of the germline CR6261 antibody to a similar level seen with IgM cross-linking. It would be exciting to see if modified forms of HA could be engineered to bind VH1-69 germline precursors with higher affinity, analogous to the engineering of eOD-GT6 [44^{**}], or glycan-edited gp140 [43^{*}], to bind germline precursors of VRC01-class bNAbs. Corti *et al.* identified a different bNAbs targeting a similar stem epitope but with a different mode of binding that uses both heavy and light chains — the FI6 bNAbs binds to HAs and neutralizes pseudoviruses and viruses from multiple subtypes spanning influenza groups 1 and 2 [55]. The inferred germline for FI6 bound and neutralized a reduced set of viruses only belonging to group 1, suggesting that vaccines to induce FI6-like responses may require a prime based

on group 1 and a boost that at least includes antigens from group 2. It seems plausible that other types of bNAb responses might be induced using a reversed scheme — priming with group 2 and boosting with group 1. The authors solved two crystal structures of FI6v3, a FI6 variant, in complex with HA molecules from group 1 and group 2, and these structures explained how FI6 employs mutations in multiple CDRs to confer binding to group 2.

While the head domain of HA is typically immunodominant, two recent reports indicated that stem-directed neutralizing antibodies have been elicited by vaccination [12*,14]. These encouraging results, taken together with the crystallographic definition of epitopes for multiple classes of bNAbs targeting the stem, should provide strong impetus for use of germline-targeting and other structure-based approaches to optimize antibody responses on these important stem epitopes.

Epitope-focused immunogens

One general approach to focus responses on protective epitopes is to generate stable domains of larger viral proteins that contain the epitopes of interest but lack other non-protective or potentially undesirable epitopes. This type of ‘antigen minimization’ work is most effectively done with knowledge of the relevant protein structure to guide selection of stable domains and/or to guide design of stabilizing features that compensate for domain isolation. One example of this is the eOD employed for germline-targeting as discussed above. This approach has been widely used in immunogen design for lyme disease [58], influenza [59*,60,61], HIV [44**,62–66], Ricin [67], and Chlamydia [68]. Nuccitelli *et al.* [69**] used a related structure-based approach to design an immunogen for Group B *Streptococcus* (GBS). The crystal structure of the BP-2a pilus protein from the 515 strain of GBS was determined and used to identify four potential immunogenic domains. An isolated domain, D3, was used to immunize mice, and was found to induce antibodies with potent bactericidal activity against the homologous strain. To induce broad GBS protection, a novel chimeric immunogen was constructed by fusing D3 domains from the six PI-2A GBS strains end-to-end. This hexameric immunogen elicited broad coverage opsonophagocytosis antibodies in mice that protected against challenge by the six different GBS strains (Figure 2).

In another approach to induce epitope-specific responses, a crystal structure of a peptide or protein in complex with an antibody can be employed as a starting point for design of epitope-scaffold immunogens, in which epitopes are transplanted to unrelated scaffold proteins for conformational stabilization (Figure 3). Ofek *et al.* [70], Correia *et al.* [71], and Azoitei *et al.* [72] implemented this strategy using computational design to create epitope-scaffolds presenting the HIV-1 gp41 epitopes 2F5 and 4E10. In all cases, the epitope-scaffolds bound with high affinity (nM or sub-nM) to the cognate antibody, and both Correia *et al.* and Azoitei *et al.* demonstrated that epitope-scaffolds bound antibody with significantly higher affinity than the corresponding epitope peptide alone. The increased affinity was thought to be due to the scaffolds pre-configuring the epitope in the bound conformation. Indeed, in all three cases crystal structures of epitope-scaffolds revealed precise epitope mimicry. Both Ofek *et al.* and Correia *et al.* demonstrated that epitope-scaffolds could induce epitope-structure-specific antibodies from small animals. Ofek *et al.*

further solved crystal structures of scaffold-elicited mouse monoclonals bound to 2F5 peptide and showed that these mouse mAbs bound to a very similar conformation of the peptide epitope as the 2F5 antibody itself [73], thus providing crystallographic proof that scaffolds had elicited epitope-structure-specific antibodies.

Despite these successes, the sera from animals vaccinated with 2F5 or 4E10 epitope-scaffolds did not neutralize HIV-1 as assessed by *in vitro* neutralization assays. One likely factor preventing the induction of neutralizing antibodies was that the scaffolds in Ofek *et al.* and Correia *et al.* did not contain the complete epitope for either 2F5 or 4E10, and that the missing portions of the epitope are probably essential for inducing potent neutralizing antibodies. Separate studies on the 2F5 and 4E10 antibodies have revealed that hydrophobic residues on the tip of the HCDR3 loop on each antibody are essential for neutralization potency, and in both cases there is evidence to suggest that these HCDR3 residues may interact with lipids in the viral membrane and/or with other portions of gp41 [74–78]. The epitope-scaffolds in Ofek *et al.* and Correia *et al.* contained no features to interact with those important HCDR3 residues, because the crystal structures of 2F5 and 4E10 in complex with their peptide epitopes revealed no such contacts. Thus in these cases further advances in scaffold immunogen design and testing must probably await structural elucidation of the additional HCDR3 interactions. Another potential difficulty may be that small animals rarely if ever make antibodies with the very long HCDR3 loops as found in 2F5 and 4E10, so testing in non-human primates or mice engineered to produce human antibodies may be necessary. Inducing antibodies highly similar to 2F5 or 4E10 may be further restricted by tolerance mechanisms [79], but it is plausible that different antibodies targeting similar epitopes could avoid polyreactivity or autoreactivity. Recently a significantly more potent bNAb targeting a similar epitope as 4E10 was reported [80]. This bNAb, called 10E8, lacks autoreactive characteristics of 4E10. 10E8 also employs a very long HCDR3 to contact its epitope, but it appears that most if not all of the critical epitope contacts are visible in the crystal structure, so the 10E8 epitope will provide an important test for epitope-scaffold immunogen design. Owing to steric barriers to antibody access of the 10E8 epitope by glycans, membrane, and other portions of gp41 and gp120 [32,80,81], inducing antibodies that target the 10E8 epitope with appropriate angles of approach is likely to be of critical importance for eliciting cross-neutralizing activity.

One of the grand challenges in scaffolding is to transplant and stabilize discontinuous epitopes involving two or more segments of protein backbone, because the majority of antibody epitopes are indeed discontinuous. Azoitei *et al.* [46**] developed a hybrid computational-experimental approach for scaffolding discontinuous epitopes and succeeded to transplant a two-segment portion of the epitope for the anti-HIV bNAb b12 [82,83] to a scaffold protein (reviewed in [84], Figure 3). The best epitope-scaffold had a b12 affinity comparable to that of HIV gp120 itself ($K_D = 10$ nM), and a crystal structure of one such scaffold in complex with b12 demonstrated precise mimicry of the b12–gp120 interaction. It will be fascinating to see how these and other scaffolds can be employed as reagents to probe serum specificities and as immunogens to induce epitope-specific activity.

Broad-coverage immunogens

Vaccines are often required to elicit protective responses against diverse strains of a pathogen. The group B serotype of *Neisseria meningitis* (MenB) is a bacterial pathogen without a vaccine, due in part to the high antigenic variation. Reverse vaccinology of MenB aided the discovery of a protective antigen, the lipid-anchored surface protein, fHBP [85,86]. There are three main sequence groups for the 300 variants of fHBP, and cocktails of immunogens from two of the three groups are currently being pursued in clinical trials [87]. Scarselli *et al.* [88**] used sequence and structure analysis to create a single hybrid immunogen that contains relevant surface patches from all three MenB sequence groups. The authors first realized that immunodominant regions from groups 1, 2 and 3 were spatially separated on the fHBP crystal structure surface. This led to identification of 11 partially overlapping surface patches of typical antibody footprint size [89], where sequence diversity could be introduced. Starting from fHBP of group 1, amino acids in these patches were mutated to the corresponding residues from groups 2 and 3 in a manner intended to recapitulate the full variation observed in the natural bacterial population. A total of 54 such chimeric immunogens were designed. Mice were immunized separately with each immunogen, and the sera was tested *in vitro* for bactericidal activity against a panel of diverse MenB subvariants from groups 1, 2 and 3. Eight immunogens were selected for biochemical and biophysical investigation based on their increased activity and diverse mutation profiles. These immunogens were stable, soluble proteins with similar structure to the base fHBP protein as assessed by size-exclusion chromatography, circular dichroism and NMR. The panel of eight immunogens was tested for their ability to cross-protect. As a control, un-mutated fHBP proteins from groups 1 and 2 showed group-specific protection. However, the designed immunogens had cross-group activity, and the immunogen with the highest bactericidal antibody titers across nine MenB subvariants was identified as the lead vaccine candidate. Thus, the authors were able to engineer a single immunogen that contains immunodominant regions from all three MenB sequence groups, and which is able to elicit protective antibodies against all the tested variants of the MenB serotype. This was a remarkable achievement, and it will be fascinating to see if this type of design can be employed successfully for other epitopes and pathogens.

Conclusion

The structure-based vaccine design tool kit is full of innovative solutions to engineer new or improved candidate immunogens and vaccines. Structure determination of viral and bacterial antigens containing protective epitopes, and often discovery of broadly neutralizing or otherwise protective antibodies, provide essential starting information for this type of work. Here, selected recent work was reviewed on germline-targeting, nanoparticle antigen display, epitope-transplant scaffolding, antigen minimization, and design of multi-strain chimeras for broad coverage. Looking ahead, we can expect that computational design, bioinformatic analysis, and *in vitro* screening and evolution methods will be integrated with biophysical, structural and immunological evaluation in iterative design cycles to develop vaccines with highly optimized and specifically tailored immune responses. Indeed, increasingly detailed analysis of immune responses to structurally defined vaccines, for example by pyrosequencing of expressed antibody genes or by B cell sorting to isolate

monoclonals for crystallographic and other analysis, should provide a wealth of data to guide further improvements in immunogen design. We are entering an exciting era for structural vaccinology.

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Figure 1.

Structures related to recent germline-targeting work in the field of HIV vaccine design. **(a)** The crystal structure of anti-HIV bNAb VRC01 bound to gp120 shows how VRC01 binding utilizes the heavy chain (PDBID: 3NGB [38]). Key heavy chain framework positions, most of which are germline-encoded, are indicated with spheres. Coloring: VRC01 heavy chain, blue but with heavy chain CDR3 loop in wheat; VRC01 light chain, pink; gp120, green. **(b)** The crystal structure of germline VRC01 bound to germline-targeting eOD-GT6 shows that germline VRC01 binds with a similar angle of approach as VRC01 (PDBID: 4JPK [44^{''}]). Coloring as in (a). **(c)** The newly discovered bNAb CH103 engages the CD4 binding site of gp120 like VRC01, but approaches with a different angle and relies on variable regions for binding, especially HCDR3 and LCDR3 loops (PDBID: 4JAN [47^{''}]). Liao *et al.* monitored viral evolution during the development of CH103 and found that mutations in or near the CH103 epitope occurred first in loop D (yellow), later in the region of β 23-V5 loop- β 24 (orange), and still later in the CD4-binding loop (red). Other mutations occurred in the V1 loop (not shown). **(d)** The eOD-GT6 immunogen was genetically fused to a self-assembling protein, to create a virus-like nanoparticle (d). (a)–(c) are shown with the gp120 CD4-binding site in the same orientation.

Figure 2.

Immunogen minimization and chimerization strategies employed in structural vaccinology for Group B *Streptococcus* (GBS). **(Left)** The crystal structure of GBS protein BP-2a is depicted in cartoon representation for the three structurally defined domains (D2, red; D3, yellow; D4, blue) (PDBID: 2XTL [69**]) and a green shape represents the one domain (D1) that was cleaved during crystallization. **(Middle left)** Mice immunized with single D3 or D4 domains, or with full length BP-2a, were protected against strain 515. **(Middle right)** A chimeric immunogen, 6xD3, was created by linking together D3 domains from six different GBS strains. **(Right)** Mice immunized with 6xD3 were protected against all six strains.

Figure 3.

Examples of epitope-scaffold design for continuous and discontinuous epitopes. The crystal structure of the anti-HIV bNAb 2F5 (heavy chain Fv, red; light chain Fv, purple) in complex with its epitope (yellow) is shown on the top left panel (PDBID: 1TJI [73]). The crystal structure of 2F5 in complex with an epitope-scaffold (blue) is shown on the top-right (PDBID: 3LEV [70]), with the epitope structural mimicry illustrated by aligning the starting epitope (yellow) onto the epitope-scaffold. In the bottom-left panel, the crystal structure of the anti-HIV bNAb B12 (heavy chain Fv, red; light chain Fv, purple) in complex with gp120 (green) is shown (PDBID: 2NY7 [83]). Two gp120 loops that are essential portions of the epitope, the CD4-binding loop and the outer-domain exit loop, are colored in yellow. In the bottom-right panel, the crystal structure of the epitope-scaffold (blue) in complex with B12 is shown, with the two transplanted loops in yellow (PDBID: 3RU8 [46**]).