Guiding the Long Way to Broad HIV Neutralization

David Peterhoff[a], Ralf Wagner[a,b*]

[a]Institute of Medical Microbiology and Hygiene, University Regensburg, Regensburg, Germany
[b]Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Regensburg, Germany

david.peterhoff@ur.de

ralf.wagner@ur.de

*corresponding author:
Ralf Wagner
Molecular Microbiology (Virology), Institute of Medical Microbiology and Hygiene, University Regensburg
Franz-Josef-Strauß-Allee 11
93053 Regensburg, Germany
e-mail: ralf.wagner@ur.de
phone: +49 941 944 6452

Purpose of review

It has been demonstrated that extensive virus diversification and antibody co-evolution is necessary to give rise to broadly neutralizing antibodies (bnAbs) targeting the envelope protein (Env) of HIV-1. Here we discuss recent progress of vaccine design approaches aiming on strategies to initiate and guide B cell development towards this outcome, as well as their evaluation in mouse models engineered to express human antibodies.

Recent findings

Several specially tailored transgenic mouse strains have been developed in order to test the concept of engaging and guiding B cell development by sequential immunizations. Currently available models display pre-rearranged or non-rearranged germline or mature VDJH and VJL loci of CD4-binding-site- (VRC01, 3BNC60) and high-mannose-patch-specific (PGT121) bnAbs, or even the complete human V(D)J segments. Data generated in these knock-in mouse models elegantly prove the feasibility of the concept when using a carefully selected panel of engineered envelope proteins.

Summary

Recent studies in knock-in transgenic mouse models provide a proof-of-concept that germline B cell receptor targeting followed by sequential immunization can engage the respective naïve precursor B cells and guide B cell receptor development towards broadly neutralizing reactivity.

Keywords

HIV, broadly neutralizing antibodies, germline targeting immunogens, guiding B cell development, germline bnAb knock-in mouse models

Introduction

Several studies in non-human primates (NHPs) clearly demonstrated that passive immunization using broadly neutralizing monoclonal antibodies can provide sterilizing protection against viral challenges [1,2]. Furthermore, a first-in-man dose escalation phase 1 clinical trial of 3BNC117, a potent human CD4 binding site antibody, reduced the viral load in HIV-1-infected individuals by 0.8-2.5 log_{10} and viremia remained significantly reduced for 28 days [3]. Hence, the induction of bnAbs at sufficiently

high titers could presumably protect humans against HIV infection [4*,5]. Therefore, there is generally agreed that the capability to elicit a broad cross-neutralizing antibody (bnAb) response is a desirable feature of an effective HIV vaccine.

Despite encouraging results of RV144 in mediating transient and partial protection from acquisition of HIV (vaccine efficacy of 31%) [6], it became quickly clear that these effects could not be attributed to neutralizing antibodies. Neither vaccination regimens using recombinant and adjuvanted Env proteins as the sole vaccine component, nor regimens combining vectored Env delivery with an adjuvanted Env protein administration were capable of eliciting bnAbs [7–9]. This has been attributed to (i) the immunodominance of highly variable surface regions in the gp120 monomer or the non-stabilized, open conformation gp140 Env trimer, (ii) extensive glycan shielding, conformational masking and steric occlusion of neutralization-sensitive epitopes (see contribution of M. Ramirez and colleagues in this issue), (iii) a generally low affinity of germline receptors of bnAb producing B-cell-progenitors to the available immunogens and ultimately (iv) the fact, that selection mechanisms in the germinal centers of the secondary lymphatic organs are not sampling for functional (neutralizing) antibodies but instead for B cells presenting immunoglobulin receptors with high affinity [10,11]. Lessons learned from prospective virus and B cell co-evolution studies [12–18] on the rare HIV-1 infected individuals who developed bnAbs [19,20] led to the hypothesis, that different versions of the antigen may be necessary to shepherd the B-cell development from germline precursors to mature bnAb-producing species [21–31,22*,25*]. If those should be administered in a cocktail format (as mixture), by sequential immunization or with a combined strategy is currently being investigated by several groups [32,33].

First- and second-generation bnAbs

The magic bullet of successful vaccination against HIV would be an antigen eliciting broad antibody mediated sterilizing immunity against the majority of circulating HIV-1 strains. However, all vaccination trials performed in humans and using Env proteins have so far failed to induce antibody responses with the desired neutralization potency and breadth [34]. Therefore, part of HIV vaccine research during the past decade progressively focused on studying plasma- and lymph node-derived B cells [35] as well as their development in HIV-infected individuals [36], currently resulting in what has been termed "reverse vaccinology 2.0" [37]. Data generated during that period until today not only revealed various neutralization-sensitive sites on the viral envelope [4*] but also led to current considerations regarding envelope immunogen design [38*] (see contribution by M. Ramirez and colleagues in this issue), vaccine formulation and delivery as well as vaccination regimens [39].

Isolation and characterization of broadly neutralizing antibodies has been pioneered already in the late 90ies by H. Katinger, D. Burton, S. Zolla-Pazner and their colleagues, respectively, with the identification of specific neutralizing antibodies such as 2F5 and 4E10 (gp41 MPER), 2G12 (gp120 outer domain, glycan-dependent), b12 (CD4 binding-site) and 447-52D (V3 crown), thus shedding light on the first identified vulnerable sites of Env (thoroughly reviewed by Burton and Hangartner [4*]). Identification of a larger number of "second-generation" bnAbs from a limited number of selected HIV infected individuals (CATNAP database [40]) was enabled by the development of converging platform technologies. These platforms allowed for efficient high-throughput culturing, activation and immortalization of B cells followed by screening of supernatants from individual memory B cells for neutralization or, alternatively, B cell sorting, molecular re-cloning of genes encoding antibody fragments and functional screening of the expressed antibodies [4*]. A broad range of bnAb epitopes was identified, mapped and characterized thoroughly which shaped our view of the vulnerability of the viral envelope and, at the same time, revealed mechanisms which enable the virus to reduce the opportunities for the bnAb development [41]. The extended availability of sequence data furthermore revealed, that the level of somatic hypermutation (SHM) as well as the length of heavy-chain complementarity determining region 3 (HCDR3) in bnAbs is generally extremely high making them to real "freaks of nature" [42].

Germline targeting

Insights into the complex co-evolution process of the virus and virus-neutralizing antibodies (nAbs) [14–17], the finding that Env trimers have low affinities to the inferred germline (gl) B cell receptors of bnAb-producing precursor B cells [43–45] and, conversely, higher affinities to non-neutralizing gl B cell receptors [46,47] support the hypothesis, that priming of the key germline B cells by germline-targeting immunogens would be a necessary first step in the process eventually leading to bnAbs [44,48]. A number of studies have recently presented immunogens designed to specifically target the gl receptors of bnAb B cells [24,25*,49,50*]. BnAb's inferred germline precursors are generally not broadly cross-reactive [15] and high levels of SHM in bnAbs imply the selection of the B cells that produce them in multiple rounds of affinity maturation. Hence, a number of guide immunogens representing milestone variants of Env during viral escape, have to be identified, pushing the development of B cells to the desired destination. Several recent publications have described such immunogen-collections and methods for their design and selection.

Immunogens to guide B cell responses

The design of Env-based immunogens started early with Env-peptides and gp120 molecules [51] and went a long way until a major breakthrough with the introduction of the stabilized SOSIP by R. Sanders and J. P. Moore (reviewed by Sliepen and Sanders [38*]). Particularly the BG505 SOSIP.664 gp140 (from a subtype A virus isolated from a 6-weeks old, HIV-1-infected infant) gave insights into Env structure, structural states, rearrangements and glycosylation [52–57,54*]. Based on these findings, it was hypothesized that native-like, closed conformation Env trimers are to be preferred over substructures like gp120-variants because (i) Env immunogens need to present sites of vulnerability and at the same time hide non-neutralizing epitopes [46,58], (ii) quaternary epitopes (targeted by e.g. PGT145, PG9, PGT151, 35O22) are ideally properly presented, and because (iii) the trimers allow for cross-linking of multiple B cell receptors which facilitates the germinal center reaction. Yet, despite these improvements, immunization studies with stable and hyperstable [59–62] Env trimers have to date merely shown the elicitation of autologous tier 2 neutralization responses [63,64]. Therefore, the efforts on designing optimized Env-based vaccines are currently focusing on targeting the immune reaction on vulnerable sites (e.g by scaffolding or by glycan shielding, (see contribution of Ramirez and colleagues in this issue) and on modifying Env to enable binding of gl versions or intermediates in the developmental pathway to bnAbs.

To this end, rational protein design [27] or computational design methods combined with directed evolution approaches like high-throughput yeast cell surface display [24] or mammalian cell display [65**,66] led to a new generation of sequential immunogens with narrowly defined binding properties and the capacity to guide B cell development. In all cases, affinity to mature bnAbs and their inferred gl variants as well as partially reverted intermediates guided the design and helped for the selection of proteins. In parallel, avidity of the immunogens in particular with regard to the engagement of gl B cell receptors with low affinity was optimized via different particle-based presentation formats (see contribution by B. Asbach in this issue).

Immunization studies in transgenic mice

To analyze the capacity of the developed (serial) immunogens to engage and expand the respective gl B cell receptors *in vivo*, different genetically-engineered transgenic mouse models have been established which try to compensate for the lack of orthologues of naïve human B cells capable of developing into bnAb-producing B cells (see table 1).

All recent transgenic mouse models were generated by stem cell gene targeting [71]. Depending on the complexity of the model, pre-rearranged or non-rearranged germline or mature VDJH and VJL loci have been introduced. The knocked-in human antibody transgenes are active due to allelic exclusion [72] and the B cells express primarily the knocked-in human B cell receptor (see figure 1 and table 1). Since lambda and kappa light-chain genes and heavy-chain genes are not genetically linked and hence

segregate independently, pre-rearranged VDJH and VJL genes can be studied independently from each other. Thus, pre-rearranged light- or heavy-chain genes can be expressed separately with the endogenous mouse repertoire or alternatively can be combined by crossbreeding, leading to a mouse primarily expressing the complete transgenic B cell receptor. Latest developments yielded mice (referred to as Kymab mice) carrying the complete naïve human Ig heavy- and light-chain repertoire (mouse constant regions intact) thereby approaching a more comparable situation to the human setting [73].

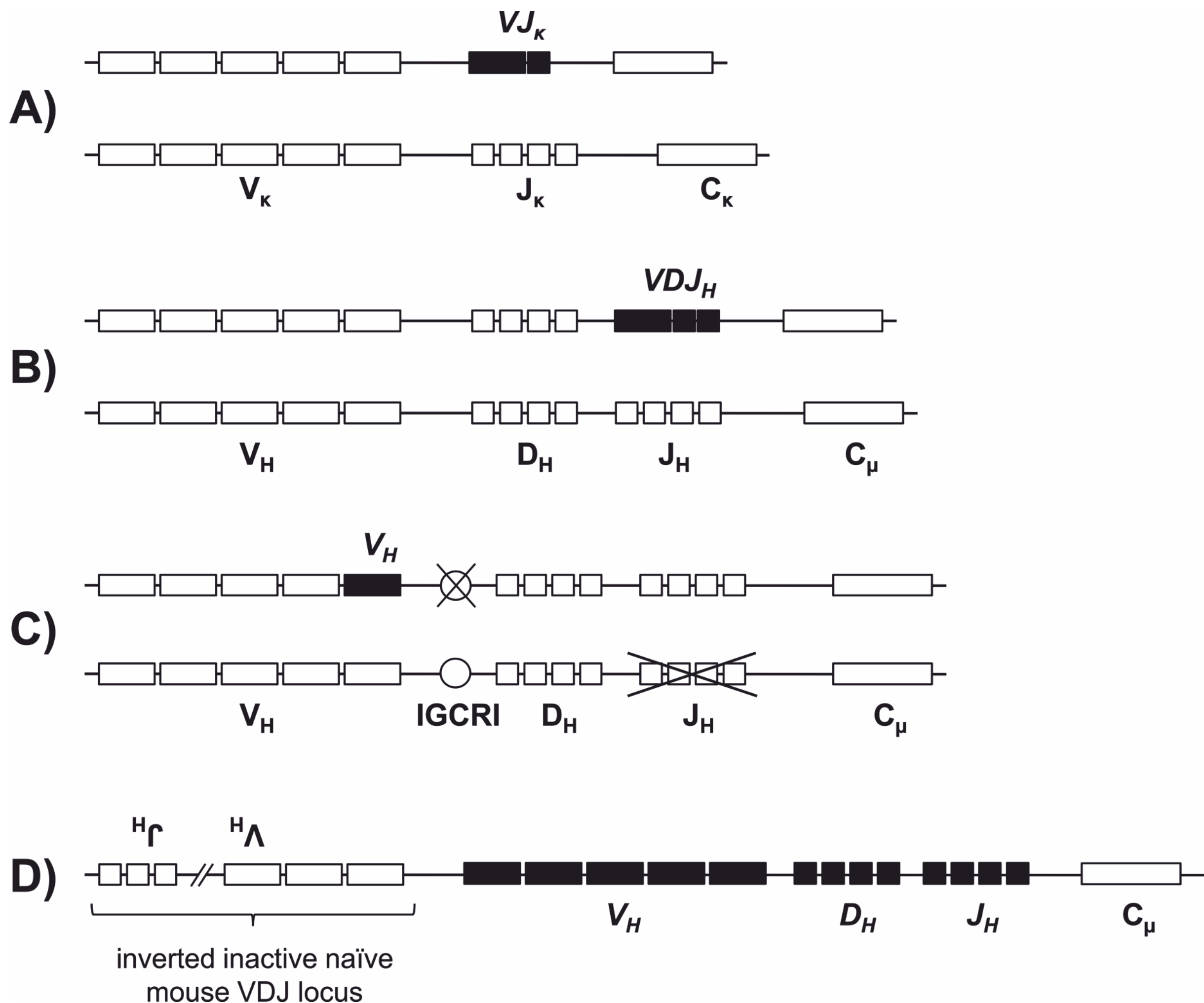

Figure 1) Schematic representation of modifications in genetically-engineered mice described in this review.

Transgenic elements are given in black and with italic labels. Unrearranged alleles are inactive due to allelic exclusion. A) Light-chain modifications. B) Heavy-chain modifications. C) Heavy-chain modifications generated by Tian et al. [67**] D) Heavy-chain modifications in mice carrying the entire human Ig V(D)J gene repertoire. IGCRI, intergenic control region I.

Initial studies explored the conditions of the elicitation of CD4bs-specific bnAbs. Transgenic mice expressed the gl heavy-chain gene (human VH1-2*02 allele in combination with reconstructed gl DJ alleles) of VRC01 and 3BNC60 (both anti-CD4 binding site specific antibodies) either in combination with the mouse LC repertoire [22*,25*] or the associated gl LC [50*]. Engineered outer domain variants (eOD-GT6 and -GT8, optimized to bind the inferred gl version of CD4bs directed bnAbs) from Schief and colleagues as well as modified multimerized 426c (TMΔV1-3, deletions of variable loops 1-3 to

allow binding of several CD4 binding site directed bnAbs and their gl versions [46,50*]) from Stamatatos and colleagues could engage and expand B cells expressing the respective CD4-binding-site-specific heavy-chain alleles *in vivo*. Moreover, mice expressing the VRC01 gl heavy-chain combined with the naïve mouse light-chain repertoire expanded B cells encompassing naturally underrepresented pairs containing 5 amino acid light-chain CDR-loops, thereby reflecting the preferred combination found/calculated in the human context [22*]. On the contrary, binding of native trimers to the selected chimeric antibodies was not observed and native-like trimers could not expand bnAb precursor responses in this model.

Antibody	Heavy chain			Light chain		Study
knock-in	V	D	J	V	J	
3BNC60	germline	mature		mouse		*Dosenovic et al., 2015* [22*]
	mature	mature		mouse		
VRC01	germline		mature	mouse		*Jardine et al., 2015* [25*]
3BNC60	germline	mature		germline		*McGuire et al., 2016* [50*]
VRC01	germline	mouse		mouse		*Tian et al., 2016* [67**]
	germline	mouse		germline		
VRC01	germline		mature	mouse		*Briney et al., 2016* [68**]
PGT121	germline			mature		*Escolano et al., 2016* [69**]
	germline			germline		
human	complete human			complete human		*Sok et al., 2016* [70*]

Table 1) Modifications of immunoglobulin loci in knock-in mouse models used to characterize immunogens.

A more recent study explored the requirements for elicitation of glycan-dependent V3-specific antibodies like PGT121 [69**]. Here, the mice expressed the gl (VH4-59*07, DH3-3 and JH6*03 allele) or mature heavy-chain gene in combination with the gl light-chain gene (VL3-21*02 and JL3*02 allele). 10MUT, a gl-targeting antigen [65**], proved to induce B cell responses in these mice which were, however, not neutralizing. Similar to the results from the CD4bs-directed bnAb models, native-like trimers failed to engage the gl B cell receptor and did not induce B cell responses in these mice.

To investigate the possibility of targeting specific precursor B cells with certain gl receptors in a human-like B cell repertoire setting, the above mentioned eOD-GT8 was used to immunize Kymab mice [73] displaying the entire human immunoglobulin variable-gene repertoire [70*]. The theoretical frequency of gl VRC01 genes in such mice is as few as 0.2-1.3 cells per mouse due to a smaller number of B cells compared to humans. After immunization, 29 % of the mice responded to the immunization and 1 % of CD4-binding-site-specific B cells were indeed VRC01 precursors (1.6 cells per mouse).

Sequential immunization studies

Taking into account that (i) the development of bnAbs usually requires extensive antigen exposure over a long period of time [74,75], that (ii) clonal bnAb producing B cell development in HIV infected individuals co-evolves with the diversification of the virus [12–18] and that (iii) gl B cell receptors are unable to bind native Env, it is fair to hypothesize that a gl-targeting priming immunization requires booster immunizations with one or more Env variants to shape the B cell maturation towards mature bnAbs. This approach – to shepherd the immune response toward bnAbs – has been adopted as "sequential immunization" strategy [48,76,26]. A proof of this concept came from the above described study which investigated knock-in mice carrying the mature heavy-chain 3BNC60 gene together with the naïve mouse light-chain repertoire representing an intermediate stage in the developmental route from the naïve B cell receptor to the bnAb-expressing B cells [22*]. Immunization of the mice with

germline-targeting antigens (eOD-GT8 60-mer or multimerized 426c TM4ΔV1-3) generated antibodies that only neutralized HIV-1 strains with incomplete glycosylation (position 276) while immunization with native-like trimers (BG505 SOSIP or YU2 SOSIP) elicited heterologous tier 2 neutralizing antibodies (with short 5 amino acid CDR3 loops, avoiding steric hindrance with glycan 276).

Consequently, if gl targeting immunogens can activate naïve bnAb precursors and if, on the other hand, intermediates (like the mature heavy-chain 3BNC60 paired with short mouse CDR3 loops) can be selected by native trimers, the goal would be to induce the intermediates by a certain number of intermediate-stimulating immunogens. Three recent studies followed this strategy, either targeting the CD4 binding-site [67**,68**] or the high-mannose patch/V2 loop [69**]:

Briney et al. [68**] used gl VRC01 mice generated earlier by Jardine et al. [25*] for their study. The mice express the gl heavy-chain V (VH1-2*02) and J (IGHJ1*01) gene in conjunction with the mature VRC01 CDR3 loop as surrogate for the unknown germline D gene, V-D and D-J junctions, paired with the naive mouse light-chain repertoire. The mice were primed by immunization with gl-targeting multimerized eOD-GT8, boosted with BG505-GT3 (a gp120 core which has been selected by yeast-display methods exhibiting low affinity to gl and high affinity to mature VRC01) followed by two further boosts with native trimer (based on BG505 SOSIP). The trimers used for the final boosts were lacking the N276 glycan which generally inhibits gl VRC01 binding but is present in 95% of all viruses. In consequence, the resulting sera had some breadth but were unable to neutralize virus strains comprising the N276 glycan. The authors state, that the elicited antibodies may be partially mature VRC01-class antibodies. Hence one or more additional antigens are needed to guide maturation of CD4bs directed antibodies towards sufficient affinity for fully glycosylated Env.

A more sophisticated mouse model was used by Tian et al. [67**]. Here, the human VH1-2*02 sequence was recombined into the mouse germline locus, replacing the most D-proximal V-segment (see figure 1, C). Furthermore, the intergenic control region 1 was deleted, favoring recombination using the VH1-2*02 segment (along with deletion of the J region from the IgH allele *in trans*). The authors showed by high-throughput antibody repertoire sequencing that a larger proportion (approximately 40 %) of the B cells expressed IGHV1-2*02, when in parallel a predicted precursor of the VRC01 light-chain was knocked in. The mice were immunized with gl-targeting eOD-GT6 60mer followed by a series of Env gp120s and SOSIP trimers (based on 426c) with increasing number of CD4-binding-site-surrounding glycans. This protocol simulates an incrementally growing glycan fence [77] which generally hampers the binding of antibodies to the CD4 binding-site. Sera from these mice showed broad neutralization of a panel of viruses in which the N276 glycan was deleted (N276A). It remains to be shown if and how the final adaption of VRC01 precursors may be accomplished. Probably, further maturation-guiding immunogens will prove valuable here.

In an analogous study [69**], mice expressing the inferred germline heavy- and light-chain of PGT121 were immunized with a series of affinity-maturation-guiding antigens [65**]. In this case, homozygote mice solely expressing gl heavy- and light-chain receptors (confirmed by single B cell sequencing) were generated. As initial gl-targeting antigen, a trimeric BG505-SOSIP derivate with deleted glycans at positions N137 and N133 (10MUT SOSIP), previously shown to bind gl PGT121, was administered. Prior to a final boost with native-like BG505-SOSIP.664 trimer (a mixture of variants differing in the variable loops), a number of boosting variants were administered which were designed rationally and then selected from targeted randomization libraries using an elegant mammalian display system and FACS sorting procedure [65**]. These variants were initially qualified *in vitro* by measuring K_D values against gl-reverted and intermediate PGT121 variants which contained an increasing number of mature PGT121 mutations. For the following *in vivo* experiments, assorted immunogens were chosen which showed increasing affinities for the mature and decreasing affinity for the gl version of PGT121. The applied immunization regimen yielded PGT121-like antibodies in the mice that featured high levels of somatic mutations and resembled key mutations of the human bnAb, thus showing significant potency and breadth against heterologous tier 2 isolates.

In summary antibody-guided, epitope-focused vaccine designs [78*] combined with sequential immunizations demonstrated for the first time the feasibility of eliciting bnAbs *in vivo*. Noteworthy, some of these advances were made in mouse models where no polyclonal response with competition between the desired naïve B cells and high affinity B cells against off-target epitopes are present.

Conclusion

Eliciting HIV-1 broadly neutralizing antibodies in humans by a vaccine would certainly mark a turning point in the fight against AIDS. The complex nature of such antibodies, however, emphasizes the need for more elaborate vaccination strategies than those that had been pursued in the past. Recent work has shown unprecedented progress towards the identification of optimal immunogen designs and successful sequential immunization schemes in a variety of humanized mouse models displaying increasingly complex B cell responses. Two vulnerable sites, the CD4 binding site and the high mannose patch have been investigated in detail as potent epitopes selected for targeted B cell stimulation. Further efforts will be undertaken to engage gl B cell receptors and to stimulate B cell lineage development towards bnAbs targeting other vulnerable epitopes. Despite these very elegant experiments and the increasingly encouraging results, it has to be emphasized that these advances were made in low bar *in vivo* models where no polyclonal response with competition between the desired naïve B cells and high affinity B cells against off-target epitopes are present. It is therefore largely unclear to which extent B cell responses can be guided in an outbred situation, to what extent host genetics will influence the outcome of sequential immunizations, whether sufficiently high titers of bnAbs will be reached and how long lasting those responses will be. Although arguing against the above reported recent findings, simplified immunization regimens would be highly desirable in particular in view of the primary target population for a prophylactic vaccination in poor countries. Whether or not advanced adjuvants, clever depot/release formulations, optimized immunogen cocktails or prime/boost regimens may add to overcome some of these obstacles remains to be elucidated.

Key points

- Extensive virus/antibody co-evolution is necessary to elicit bnAbs.
- Sequential immunization with a panel of carefully designed envelope immunogens may be required to elicit bnAbs.
- Several transgenic mouse models displaying pre-rearranged germline and mature heavy- and light-chain genes are available to study the concept of sequential immunization.
- Sets of sequential immunogens are capable of eliciting antibody responses that neutralize a broad panel of native or near-native tier 2 viruses.

Acknowledgements

We thank Benedikt Asbach, Anne Rescle and Rogier Sanders for critically reading the manuscript.

Financial support and sponsorship

This work has received funding from the European Union's horizon 2020 research and innovation program under grant agreement No 681032 (EHVA) and from the Bill & Melinda Gates Foundation Poxvirus T-Cell Vaccine Discovery Consortium (PTVDC, grant 38599) to R.W.. The authors also acknowledge the support by the University of Regensburg.

Conflicts of interest

There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest, ** of outstanding interest

References

1. Kramer V, Siddappa N, Ruprecht R. Passive Immunization as Tool to Identify Protective HIV-1 Env Epitopes. Curr HIV Res. 2007; 5(6):642–55.

2. Moldt B, Rakasz EG, Schultz N, et al. Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. Proc Natl Acad Sci. 2012; 109(46):18921–5.

3. Caskey M, Klein F, Lorenzi JCC, et al. Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. Nature. 2015; 522(7557):487–91.

*4. Burton DR, Hangartner L. Broadly Neutralizing Antibodies to HIV and Their Role in Vaccine Design. Annu Rev Immunol. 2016; 34(1):635–59.

A detailed review of discovery and features of HIV broadly neutralizing antibodies with special focus on how their characteristics and properties influence current developments in HIV vaccine design.

5. Mascola JR, Haynes BF. HIV-1 neutralizing antibodies: understanding nature's pathways. Immunol Rev. 2013; 254(1):225–44.

6. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand. N Engl J Med. 2009; 361(23):2209–20.

7. Montefiori DC, Metch B, McElrath MJ, et al. Demographic Factors That Influence the Neutralizing Antibody Response in Recipients of Recombinant HIV-1 gp120 Vaccines. J Infect Dis. 2004; 190(11):1962–9.

8. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-Correlates Analysis of an HIV-1 Vaccine Efficacy Trial. N Engl J Med. 2012; 366(14):1275–86.

9. Montefiori DC, Karnasuta C, Huang Y, et al. Magnitude and Breadth of the Neutralizing Antibody Response in the RV144 and Vax003 HIV-1 Vaccine Efficacy Trials. J Infect Dis. 2012; 206(3):431–41.

10. Victora GD, Nussenzweig MC. Germinal Centers. Annu Rev Immunol. 2012; 30(1):429–57.

11. Chan TD, Brink R. Affinity-based selection and the germinal center response: Affinity selection in the GC response. Immunol Rev. 2012; 247(1):11–23.

12. Bhiman JN, Anthony C, Doria-Rose NA, et al. Viral variants that initiate and drive maturation of V1V2-directed HIV-1 broadly neutralizing antibodies. Nat Med. 2015; 21(11):1332–6.

13. Bonsignori M, Hwang K-K, Chen X, et al. Analysis of a Clonal Lineage of HIV-1 Envelope V2/V3 Conformational Epitope-Specific Broadly Neutralizing Antibodies and Their Inferred Unmutated Common Ancestors. J Virol. 2011; 85(19):9998–10009.

14. Bonsignori M, Zhou T, Sheng Z, et al. Maturation Pathway from Germline to Broad HIV-1 Neutralizer of a CD4-Mimic Antibody. Cell. 2016; 165(2):449–63.

15. Doria-Rose NA, Schramm CA, Gorman J, et al. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. Nature. 2014; 509(7498):55–62.

16. Liao H-X, Lynch R, Zhou T, et al. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature. 2013; 496(7446):469–76.

17. MacLeod DT, Choi NM, Briney B, et al. Early Antibody Lineage Diversification and Independent Limb Maturation Lead to Broad HIV-1 Neutralization Targeting the Env High-Mannose Patch. Immunity. 2016; 44(5):1215–26.

18. Wu X, Zhang Z, Schramm CA, et al. Maturation and Diversity of the VRC01-Antibody Lineage over 15 Years of Chronic HIV-1 Infection. Cell. 2015; 161(3):470–85.

19. Richman DD, Wrin T, Little SJ, Petropoulos CJ. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc Natl Acad Sci. 2003; 100(7):4144–9.

20. Wei X, Decker JM, Wang S, et al. Antibody neutralization and escape by HIV-1. Nature. 2003; 422(6929):307–12.

21. Dimitrov DS. Therapeutic antibodies, vaccines and antibodyomes. mAbs. 2010; 2(3):347–56.

*22. Dosenovic P, von Boehmer L, Escolano A, et al. Immunization for HIV-1 Broadly Neutralizing Antibodies in Human Ig Knockin Mice. Cell. 2015; 161(7):1505–15.

One of two pilot studies on germline targeting immunogens (eOD-GT8) in genetically engineered mice (carrying gl or mature 3BNC60 heavy chain combined with the naive mouse light chain repertoire). Immunogens specifically designed to activate bnAb-gl-B cells could activate appropriate B cells, but did not elicit bnAbs. Results suggested the need for sequential immunizations with closely related immunogens.

23. Haynes BF, Kelsoe G, Harrison SC, Kepler TB. B-cell–lineage immunogen design in vaccine development with HIV-1 as a case study. Nat Biotechnol. 2012; 30(5):423–33.

24. Jardine J, Julien J-P, Menis S, et al. Rational HIV Immunogen Design to Target Specific Germline B Cell Receptors. Science. 2013; 340(6133):711–6.

*25. Jardine JG, Ota T, Sok D, et al. Priming a broadly neutralizing antibody response to HIV-1 using a germline-targeting immunogen. Science. 2015; 349(6244):156–61.

One of two pilot studies on germline targeting immunogens (eOD-GT8) in genetically engineered mice (VRC01 germline heavy chain combined with the naive mouse light chain repertoire). The induced antibodies showed VRC01-class signatures. Control native-like immunogens failed to activate VRC01-class precursors.

26. Klein F, Mouquet H, Dosenovic P, et al. Antibodies in HIV-1 Vaccine Development and Therapy. Science. 2013; 341(6151):1199–204.

27. McGuire AT, Hoot S, Dreyer AM, et al. Engineering HIV envelope protein to activate germline B cell receptors of broadly neutralizing anti-CD4 binding site antibodies. J Exp Med. 2013; 210(4):655–63.

28. Mouquet H, Scharf L, Euler Z, et al. Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies. Proc Natl Acad Sci. 2012; 109(47):E3268–77.

29. Pancera M, McLellan JS, Wu X, et al. Crystal Structure of PG16 and Chimeric Dissection with Somatically Related PG9: Structure-Function Analysis of Two Quaternary-Specific Antibodies That Effectively Neutralize HIV-1. J Virol. 2010; 84(16):8098–110.

30. Scheid JF, Mouquet H, Feldhahn N, et al. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. Nature. 2009; 458(7238):636–40.

31. Zhou T, Georgiev I, Wu X, et al. Structural Basis for Broad and Potent Neutralization of HIV-1 by Antibody VRC01. Science. 2010; 329(5993):811–7.

32. Shaffer JS, Moore PL, Kardar M, Chakraborty AK. Optimal immunization cocktails can promote induction of broadly neutralizing Abs against highly mutable pathogens. Proc Natl Acad Sci. 2016; 113(45):E7039–48.

33. Klasse PJ, LaBranche CC, Ketas TJ, et al. Sequential and Simultaneous Immunization of Rabbits with HIV-1 Envelope Glycoprotein SOSIP.664 Trimers from Clades A, B and C. Desrosiers RC, editor. PLOS Pathog. 2016; 12(9):e1005864.

34. Lelièvre J-D, Lévy Y. HIV-1 prophylactic vaccines: state of the art. J Virus Erad. 2016; 2(1):5–11.

35. Havenar-Daughton C, Carnathan DG, Torrents de la Peña A, et al. Direct Probing of Germinal Center Responses Reveals Immunological Features and Bottlenecks for Neutralizing Antibody Responses to HIV Env Trimer. Cell Rep. 2016; 17(9):2195–209.

36. Simek MD, Rida W, Priddy FH, et al. Human Immunodeficiency Virus Type 1 Elite Neutralizers: Individuals with Broad and Potent Neutralizing Activity Identified by Using a High-Throughput Neutralization Assay together with an Analytical Selection Algorithm. J Virol. 2009; 83(14):7337–48.

37. Rappuoli R, Bottomley MJ, D'Oro U, et al. Reverse vaccinology 2.0: Human immunology instructs vaccine antigen design. J Exp Med. 2016; 213(4):469–81.

*38. Sliepen K, Sanders RW. HIV-1 envelope glycoprotein immunogens to induce broadly neutralizing antibodies. Expert Rev Vaccines. 2016; :1–17.

Profound review of developments and efforts on HIV envelope immunogen design.

39. Gilbert PB, Huang Y, Janes HE. Modeling HIV vaccine trials of the future: Curr Opin HIV AIDS. 2016; 11(6):620–7.

40. Yoon H, Macke J, West AP, et al. CATNAP: a tool to compile, analyze and tally neutralizing antibody panels. Nucleic Acids Res. 2015; 43(W1):W213–9.

41. Mouquet H. Antibody B cell responses in HIV-1 infection. Trends Immunol. 2014; 35(11):549–61.

42. Kwong PD, Wilson IA. HIV-1 and influenza antibodies: seeing antigens in new ways. Nat Immunol. 2009; 10(6):573–8.

43. Sliepen K, Medina-Ramírez M, Yasmeen A, et al. Binding of inferred germline precursors of broadly neutralizing HIV-1 antibodies to native-like envelope trimers. Virology. 2015; 486:116–20.

44. Hoot S, McGuire AT, Cohen KW, et al. Recombinant HIV Envelope Proteins Fail to Engage Germline Versions of Anti-CD4bs bNAbs. Lifson J, editor. PLoS Pathog. 2013; 9(1):e1003106.

45. McGuire AT, Glenn JA, Lippy A, et al. Diverse Recombinant HIV-1 Envs Fail To Activate B Cells Expressing the Germline B Cell Receptors of the Broadly Neutralizing Anti-HIV-1 Antibodies PG9 and 447-52D. J Virol. 2014; 88(5):2645–57.

46. McGuire AT, Dreyer AM, Carbonetti S, et al. Antigen modification regulates competition of broad and narrow neutralizing HIV antibodies. Science. 2014; 346(6215):1380–3.

47. Xiao X, Chen W, Feng Y, et al. Germline-like predecessors of broadly neutralizing antibodies lack measurable binding to HIV-1 envelope glycoproteins: Implications for evasion of immune responses and design of vaccine immunogens. Biochem Biophys Res Commun. 2009; 390(3):404–9.

48. Haynes BF, Kelsoe G, Harrison SC, Kepler TB. B-cell–lineage immunogen design in vaccine development with HIV-1 as a case study. Nat Biotechnol. 2012; 30(5):423–33.

49. Jardine JG, Sok D, Julien J-P, et al. Minimally Mutated HIV-1 Broadly Neutralizing Antibodies to Guide Reductionist Vaccine Design. Trkola A, editor. PLOS Pathog. 2016; 12(8):e1005815.

*50. McGuire AT, Gray MD, Dosenovic P, et al. Specifically modified Env immunogens activate B-cell precursors of broadly neutralizing HIV-1 antibodies in transgenic mice. Nat Commun. 2016; 7:10618.

A study in which mice, carrying both the heavy and light chains of gl 3BNC60, were immunized with trimeric and highly multimerized immunogens. Gl 3BNC60 B cells displayed an autoreactive phenotype and did only respond to multimerized immunogens.

51. McCoy LE, Weiss RA. Neutralizing antibodies to HIV-1 induced by immunization. J Exp Med. 2013; 210(2):209–23.

52. Pancera M, Zhou T, Druz A, et al. Structure and immune recognition of trimeric pre-fusion HIV-1 Env. Nature. 2014; 514(7523):455–61.

53. McCoy LE, van Gils MJ, Ozorowski G, et al. Holes in the Glycan Shield of the Native HIV Envelope Are a Target of Trimer-Elicited Neutralizing Antibodies. Cell Rep. 2016; 16(9):2327–38.

*54. Behrens A-J, Vasiljevic S, Pritchard LK, et al. Composition and Antigenic Effects of Individual Glycan Sites of a Trimeric HIV-1 Envelope Glycoprotein. Cell Rep. 2016; 14(11):2695–706.

The authors describe the site-specific glycan composition of a BG505 SOSIP.664 trimer and reveal clusters of oligomannose glycans on its outer domain as well as the prevalence of complex glycans at its N and C terminus.

55. Behrens A-J, Harvey DJ, Milne E, et al. Molecular architecture of the cleavage-dependent mannose patch on a soluble HIV-1 envelope glycoprotein trimer. J Virol. 2016; :JVI.01894-16.

56. Pritchard LK, Vasiljevic S, Ozorowski G, et al. Structural Constraints Determine the Glycosylation of HIV-1 Envelope Trimers. Cell Rep. 2015; 11(10):1604–13.

57. Crispin M, Doores KJ. Targeting host-derived glycans on enveloped viruses for antibody-based vaccine design. Curr Opin Virol. 2015; 11:63–9.

58. Guttman M, Cupo A, Julien J-P, et al. Antibody potency relates to the ability to recognize the closed, pre-fusion form of HIV Env. Nat Commun. 2015; 6:6144.

59. Guenaga J, Dubrovskaya V, de Val N, et al. Structure-Guided Redesign Increases the Propensity of HIV Env To Generate Highly Stable Soluble Trimers. Sundquist WI, editor. J Virol. 2016; 90(6):2806–17.

60. van Gils MJ, van den Kerkhof TLGM, Ozorowski G, et al. An HIV-1 antibody from an elite neutralizer implicates the fusion peptide as a site of vulnerability. Nat Microbiol. 2016; 2:16199.

61. de Taeye SW, Ozorowski G, Torrents de la Peña A, et al. Immunogenicity of Stabilized HIV-1 Envelope Trimers with Reduced Exposure of Non-neutralizing Epitopes. Cell. 2015; 163(7):1702–15.

62. Do Kwon Y, Pancera M, Acharya P, et al. Crystal structure, conformational fixation and entry-related interactions of mature ligand-free HIV-1 Env. Nat Struct Mol Biol. 2015; 22(7):522–31.

63. Hessell AJ, Malherbe DC, Pissani F, et al. Achieving Potent Autologous Neutralizing Antibody Responses against Tier 2 HIV-1 Viruses by Strategic Selection of Envelope Immunogens. J Immunol. 2016; 196(7):3064–78.

64. Sanders RW, van Gils MJ, Derking R, et al. HIV-1 neutralizing antibodies induced by native-like envelope trimers. Science. 2015; 349(6244):aac4223-aac4223.

**65. Steichen JM, Kulp DW, Tokatlian T, et al. HIV Vaccine Design to Target Germline Precursors of Glycan-Dependent Broadly Neutralizing Antibodies. Immunity. 2016; 45(3):483–96.

Excellent description of state-of-the-art immunogen design by means of rational considerations and directed evolution methods combined with mammalian cell display. The immunogens presented by the authors are optimized to target germline-reverted precursors of PGT121-class supersite bnAbs and guide their maturation to the mature bnAbs. The Immunogens were studied in a gl PGT121 knock-in mouse and published back-to-back.

66. Bruun T-H, Mühlbauer K, Benen T, et al. A Mammalian Cell Based FACS-Panning Platform for the Selection of HIV-1 Envelopes for Vaccine Development. Pizzo SV, editor. PLoS ONE. 2014; 9(10):e109196.

**67. Tian M, Cheng C, Chen X, et al. Induction of HIV Neutralizing Antibody Lineages in Mice with Diverse Precursor Repertoires. Cell. 2016; 166(6):1471–1484.e18.

One of two recent studies on the elicitation of CD4bs directed bnAbs by sequential immunization. A mouse model that allows a germline human IGHV1-2*02 segment to undergo normal V(D)J recombination was used to sequentially immunize with glycan modified gp120s to engage VRC01 germline and intermediate B cell receptors. The sera of the sequentially immunized mice displayed broad neutralization of a panel of viruses where the N276 glycan was deleted.

**68. Briney B, Sok D, Jardine JG, et al. Tailored Immunogens Direct Affinity Maturation toward HIV Neutralizing Antibodies. Cell. 2016; 166(6):1459–1470.e11.

One of two recent studies on the elicitation of CD4bs directed bnAbs by sequential immunization. Mice, expressing gl VRC01 heavy chain VJ in conjunction with mature VRC01 CDR3 loop were sequentially immunized with nanoparticle presented gl targeting and intermediate immunogens and finally boosted with a N276 glycan deleted BG505 SOSIP.664 trimer. A panel of viruses where the N276 glycan was deleted was broadly neutralized by the sera.

**69. Escolano A, Steichen JM, Dosenovic P, et al. Sequential Immunization Elicits Broadly Neutralizing Anti-HIV-1 Antibodies in Ig Knockin Mice. Cell. 2016; 166(6):1445–1458.e12.

Regimens of immunogens from the study published by Steichen et al. [65] were used to immunize mice expressing the predicted PGT121 germline receptor. Serum ELISAs guided the design of a sequence of immunogens that could elicit heterologous tier-2-neutralizing responses.**

*70. Sok D, Briney B, Jardine JG, et al. Priming HIV-1 broadly neutralizing antibody precursors in human Ig loci transgenic mice. Science. 2016; 353(6307):1557–60.

The authors immunize mice transgenic for the entire human immunoglobulin loci for germline-targeting analyses (Kymab mice). VRC01-class bnAb germline-targeting immunogen eOD-GT8 60mer was used to test activation of VRC01-class precursors. The Frequency of precursor cells in mice is as few as 1 cell. Yet, a third of the mice produced a VRC01-class memory response.

71. Bouabe H, Okkenhaug K. Gene Targeting in Mice: A Review. In: Bailer SM, Lieber D, editors. Virus-Host Interactions [Internet]. Totowa, NJ: Humana Press; 2013 [cited 2017]. p. 315–36. Available from: http://link.springer.com/10.1007/978-1-62703-601-6_23

72. Nussenzweig M, Shaw A, Sinn E, et al. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin mu. Science. 1987; 236(4803):816–9.

73. Lee E-C, Liang Q, Ali H, et al. Complete humanization of the mouse immunoglobulin loci enables efficient therapeutic antibody discovery. Nat Biotechnol. 2014; 32(4):356–63.

74. Sather DN, Armann J, Ching LK, et al. Factors Associated with the Development of Cross-Reactive Neutralizing Antibodies during Human Immunodeficiency Virus Type 1 Infection. J Virol. 2009; 83(2):757–69.

75. Mikell I, Sather DN, Kalams SA, et al. Characteristics of the Earliest Cross-Neutralizing Antibody Response to HIV-1. Trkola A, editor. PLoS Pathog. 2011; 7(1):e1001251.

76. Doria-Rose NA, Joyce MG. Strategies to guide the antibody affinity maturation process. Curr Opin Virol. 2015; 11:137–47.

77. Binley JM, Ban YEA, Crooks ET, et al. Role of Complex Carbohydrates in Human Immunodeficiency Virus Type 1 Infection and Resistance to Antibody Neutralization. J Virol. 2010; 84(11):5637–55.

*78. Lanzavecchia A, Frühwirth A, Perez L, Corti D. Antibody-guided vaccine design: identification of protective epitopes. Curr Opin Immunol. 2016; 41:62–7.

Reviews the characteristics of analytic, structure-based and epitope-based vaccinology by discussing recent developments in HCMV, HRSV, Influenza, HIV vaccine research.