



## Commentary

## Non-cognate ligands of Procrustean paratopes as potential vaccine components



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Animals immunized with specific antibodies - idiotypes - produce anti-antibodies, some of them directed to the idiotypic antigen-binding sites, i.e., the paratopes [1,2]. When the paratopes of one idio-type ligate B-cell receptors, they elicit anti-idiotypes and in a subse-quent round anti-anti-idiotypes that might resemble the first idiotypes [3]. With this rationale, anti-idiotypic paratopes were propounded as substitutes for pathogen epitopes in immunization. The aim is to elicit anti-anti-idiotypic antibodies that mimic the idio-type in anti-microbial effects [4], e.g., neutralization of virus infectivity [5]. Such strategies have been applied to the persistent problem of inducing antibodies that neutralize HIV-1 [6].

That problem is so momentous, because broadly active neutralizing antibodies (bNAbs) are needed to protect against HIV-1 infection; it is so thorny, because their target - the envelope glycoprotein (Env) trimer - hampers the induction and eludes the action of bNAbs by multiple means: extreme genetic variability, a dense and variable glycan shield, and conformational shielding of crucial receptor-binding sites [7]. Ac-cordingly, the bNAbs that eventually do develop in some HIV-1-in-fected persons tend to have unusual features, such as long heavy-chain (IgH) complementarity determining regions (CDRs) and extreme somatic hypermutation, including deletions, insertions, and mutations in the framework regions [7]. And even from such bNAbs the virus es-capes [5]. In addition, the germline precursors of bNAbs often recognize the trimers too feebly to initiate the painstaking development towards neutralization breadth [7].

Many strategies are devoted to overcoming these formidable hur-dles [7]. In this article in *EBioMedicine*, Kosztyu et al. explore a radical concept related to immunization with anti-idiotypic antibodies [8]. They chose a paragon of HIV-1 bNAbs, VRC01, which is highly potent, broadly active, and directed to the binding site of the main receptor for the virus, CD4 [5,7]. Instead of antibodies reactive with its paratope, however, they selected immunogen candidates from display libraries of mutants of the unrelated albumin-binding domain from the streptococ-cal Protein G. Thereby they identified ligands that bound to immobilized VRC01 IgG and Fab in a dose-dependent manner. Gp120, the receptor-binding subunit of the Env trimer, competed with certain putative paratope ligands for binding to VRC01 IgG and Fab; the reciprocal com-petitions were confirmed for the strongest VRC01 binder. These findings

suggest at least an overlap of the binding site of the ligands with the paratope. Furthermore, molecular modeling indicated shape and non-covalent-bond complementarity between the selected ligands and the VRC01 paratope. Mice were then immunized with the strongest gp120 competitors, and antibodies were elicited that recognized gp120 and competed specifically with VRC01 binding to gp120. Although bNAbs are particularly hard to induce in wild-type mice, partly because of short murine IgH CDRs, the ensuing sera in the best immunization group modestly neutralized several resistant HIV-1 isolates (6/12 classi-fied as Tier 2, with reciprocal mean titers >50; sera from naïve animals and those immunized with unmutated ligand neutralized none). To put this into perspective, whereas native-like Env trimers in immuniza-tion also elicits sporadic and modest bNAb responses, these are not fo-cused on the CD4-binding site, and gp120 alone yields none. Therefore, when the immunogen is not homologous to the neutraliza-tion antigen, Env, any genuine neutralization of Tier-2 isolates may war-rant further probing: to isolate monoclonal bNAbs, to determine the structure of their complexes with Env trimers and with the non-cognate paratope ligands, to measure the affinities of these complex for-mations, to improve the immunogenicity of the paratope ligands, and to optimize the immunization regimens in wild-type, humanized, and germline-antibody-knock-in mice as well as other animal models.

HIV-1-specific antibodies are more polyreactive than those in the general repertoire, a propensity shared by germline precursors of HIV-1 bNAbs (9). Several causes of this polyreactivity are conceivable: dur-ing infection, intrinsically low affinity for the sparse Env trimers on the surface of virions can be compensated for by co-ligating B-cell re-ceptors to one of the abundant host antigens on the virion surface [5, 9]. The priming stimulus of certain bNAb responses may even have been a non-Env epitope, the responses subsequently having been boosted and redirected by Env. Now, VRC01 is not polyreactive and co-ligating host antigens is not relevant to paratope-ligand immuniza-tion, but the potential priming by a non-cognate epitope is. Hypotheti-cally, a broader repertoire of germline antibodies might be recruited for shepherding towards bNAb responses by non-cognate stimuli than by the weak and rare Env interactions with germline-precursors.

Future development of paratope-ligand immunogens might select for simultaneous cross-binding to other CD4-binding-site bNAbs, some of which are even more broadly active and more potent than VRC01. Such antibodies can make ancillary contact with a second gp120 subunit in the trimer [10]; the trimerization also affects access to this epitope cluster [5,7]. Certain other bNAb epitopes strictly depend

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on trimerization [5,7]. Indeed, ligands for paratopes of all known bNAb epitope clusters on the trimers might be selected and tested in immunization: soluble trimers instead of gp120 as paratope-ligand competitors might also extend and improve the selection of immunogens. Eventually, experiments might define or refute roles for non-cognate bNAb-paratope ligands in vaccine regimens. Could paratope ligands prime responses to be boosted by Env-based immunogens or by series of different non-cognate paratope-ligands? Could they complement Env by broadening specificities and engaging more of the B-cell repertoires? And could they enhance long-term vaccine efficacy by closing loopholes for viral escape? Coming work may build on Kosztyu et al.'s encouraging pilot study in answering these questions [8]. New light may then be shed on the old problem of whether other occupants than pathogen epitopes of the idiotypic Procrustean bed can benefit vaccination.

#### Author contributions

P.J. Klasse wrote this commentary.

#### Declaration of Competing Interest

The author has no conflicts of interest.

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