Published in final edited form as:
Expert Rev Vaccines. 2014 April ; 13(4): 449–452. doi:10.1586/14760584.2014.894469.

Targeting B-cell germlines and focusing affinity maturation: the next hurdles in HIV-1-vaccine development?

Max Medina-Ramírez[1], **Rogier W. Sanders**[1,2], and **Per Johan Klasse**[2,3]

[1]Department of Medical Microbiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands [2]Department of Microbiology and Immunology, Weill Medical College of Cornell University, New York, New York, United States of America

Abstract

Vaccines that protect against viral infection usually elicit neutralizing antibodies, but HIV-1 vaccine candidates have failed to induce broad and potent such responses. Broadly active neutralizing antibodies (bNAbs) do, however, slowly emerge in a minority of HIV-1-infected subjects; and passive immunization with bNAbs protects against viral acquisition in animal models of HIV-1 infection. New techniques have made it possible to interrogate human B cells and thereby to isolate highly potent bNAbs to uncharted epitope clusters. Furthermore, recent high-resolution structure determinations of near-native soluble envelope glycoprotein trimers in complex with different bNAbs reveal the molecular basis for neutralization. Such trimer structures may serve as blueprints for vaccine design. Here we discuss how a vaccine might bridge a reactivity gap from germline antibody to bNAb and simulate the intricate stimuli of affinity maturation that sometimes prevail in chronic infection.

Targeting the right epitopes

The HIV-1 Env trimer comprises three protomers, each a hetero-dimer consisting of a receptor-binding membrane-distal subunit, gp120, non-covalently attached to the transmembrane protein, gp41, which mediates fusion of the viral and cellular membranes - the culmination of the viral entry process. Viral entry is blocked by neutralizing antibodies (NAbs). Recently, the structure of a near-native soluble HIV-1 envelope glycoprotein (Env) trimer in complex with different bNAbs was determined to almost atomic-scale resolution by cryo-electron microscopy and crystallography (1, 2). Native, functional Env trimers on the surface of virions are the only relevant targets for NAbs. And all antibodies that reach a certain occupancy on functional trimers will neutralize viral infectivity. But the virus has evolved a number of defenses against the induction and binding of NAbs, particularly those directed to the less variable regions: extensive N-linked glycosylation, variable loops (V1-V5), quaternary interactions, and conformational flexibility shield conserved epitopes. Nevertheless, the epitopes of many broadly neutralizing (bNAbs) involve residues in variable regions (V1-5) as well as glycans (3–6).

[3]Corresponding author: pek2003@med.cornell.edu.

Four clusters of bNAb epitopes have emerged so far: the CD4-binding site, the V2 loop with its glycans, the V3 and V4 bases with associated glycans, and the membrane-proximal external region (MPER) in gp41 (3–5). Why don't antibody responses to recombinant Env hone in on these epitopes? A problem with such Env immunogens is that they differ from functional Env; and many non-neutralization epitopes are exposed only on non-functional forms of Env, such as precursors, which are uncleaved between gp120 and gp41, disassembled oligomers, and denatured or degraded Env (5, 7). The non-neutralization epitopes are often strongly immunogenic both in vaccination and infection and may thus act as decoys, diverting from neutralizing responses (3, 4).

Germline reactivity of Env?

There are further obstacles to bNAb elicitation. Poor reactivity of Env with the germline ancestors of bNAbs may be one. Antibody specificity arises from the blending of germline diversity in immunoglobulin genes with somatic recombination and mutations in variable regions (3, 4). But germline antibodies differ in their propensity to develop into HIV-1 bNabs: e.g., the most potent CD4bs-directed bNAbs (such as NIH45-46 and 3BNC117) have the gene segment of the germline variable heavy chain V_H1-2 or V_H1-46 in common. The structural features of these V_H variants favor mimicry of CD4 (4, 8).

Recombinant Env proteins often do not bind germline versions of known bNAbs (3, 4, 9–15). Several potential explanations may account for such a deficit in reactivity. The forms of Env used as probes may be structurally deficient: whether cleaved stabilized trimers that better mimic native Env spikes also fail to bind to unmutated ancestors of bNAbs deserves to be systematically investigated. Furthermore, the genetic make-up of the Env tested may not sufficiently match that of the original Env stimulus. Or, alternatively, something other than Env started the selection process, and along the way Env reactivity arose. In this regard, it is notable that bNAbs are more often poly-reactive than are average antibodies (3, 4, 16), although many bNAbs are not (6); and polyreactivity is possibly augmented during HIV-1 infection. Determinants of germline-reverted antibody binding to Env are actively dissected with the aid of computational methods for inferring unmutated common ancestors (3, 13). Indeed, some Env constructs, such as the outer domain of gp120, glycosylation mutants, V1V2 glycopeptides, multimerized forms, and founder-virus variants, do react with germline antibodies (3, 10–12, 14, 17, 18).

Unusual affinity maturation

After specific uptake of antigen and encounters with cognate T-helper cells, naïve B-cells enter germinal centers of secondary lymphoid organs where they proliferate, diversify, and express antigen-binding B-cell receptors. The better the B-cell receptors bind, the more antigen the B cells internalize and present, thereby getting reinforcing stimuli from follicular T-helper cells (19). But the affinity increase has a ceiling set by diffusion and endocytosis rates, and therefore B-cells usually exit the germinal center after ~10 mutations in the V_H. Human IgG has on average only 10–20 such mutations, but strain-specific HIV-1 NAbs have twice as many, and bNAbs ~80. This degree of somatic hyper-mutation (SHM) would arise from iterated germinal-center cycles, in which viral escape mutants with reduced

affinity continually trigger affinity restoration: SHM, potency, and breadth are all correlated (17).

Apart from deletions and insertions in the complementarity-determining regions (CDRs), which are rare in regular antibodies (3, 4), bnAbs display mutations even in the normally conserved framework regions (FWR), modifications that are sometimes crucial to their neutralizing capacity (20, 21). Highly potent CD4bs bNAbs have short CDRL1 and 3, conferring tight binding (4, 13). In contrast, CDRH3s of these antibodies are long, and a requirement for that feature would skew germline recruitment (see above). These unusual traits reflect how the antibody response has co-evolved with the many intricate viral defenses against neutralization that characterize the native Env spike. The unusual demands on germline reactivity and antibody evolution would counteract - but apparently not preclude - the elicitation of potent bNAbs. Other factors, such as disadvantageous Env properties for T-cell help, probably exacerbate the situation further (3).

Vaccine design

The considerations so far suggest a multi-pronged reverse-vaccinological strategy. High-resolution structures of Env-bNAb complexes would serve as templates for immunogen development. The optimal form of Env should bind bNAbs well and non-NAbs as little as possible, thereby potentially focusing the antibody response on conserved neutralization epitopes and minimizing immuno-dominant decoy effects. Stabilized, cleaved Env trimers might come close to meeting these criteria (5, 7).

Some strains of HIV-1 apparently promote bNAb responses better than others do, and if the structural basis for such differences were identified, that could inform immunogen design (12). One contributing factor might be that Env proteins from certain isolates react with favorable germline B-cell receptors. Therefore, the next strategic step would be to explore how the immunogen template reacts with germline antibodies that have been reverted from bNAbs based on computational ancestral analysis. When it is not germline-reactive, the immunogen may need to be selected among alternative viral isolates or else modified in order to bridge the gap to reactivity with precursor antibodies. In one study the founder-virus Env, unlike later evolved variants, was germline-reactive (17). Deletion of glycosylation sites can confer germline reactivity (3, 14), and glycopeptides, single domains, or scaffolded epitopes would potentially bind precursor antibodies when the corresponding entire Env trimer does not (10, 18). As a remote possibility, non-Env priming immunogens might be identified that can promote lineages prone to bNAb evolution. Ideally, a priming immunogen to start the bNAb development would be tailored towards V(D)J recombinations with the greatest potential for potency and breadth.

The antibody response thus initiated, the next goal would be to guide the affinity maturation towards the impressive breadth and potency that sometimes arise in chronic infection. One approach would be to simulate the immense antigenic variation in the surface-exposed loops of gp120 by sequential boosting with variants of Env. Whether each immunization should also contain several variants remains to be determined, but sequential exposure gave better maturation of the response to Env in rabbits than did a mixture of variants (22). This scheme

to shepherd antibody maturation might learn specifically from the continual waves of NAb responses and viral escape that presage the development of bNAbs in response to chronic infection (17, 23). Thus, select mutants of Env might be introduced consecutively as signposts for the affinity maturation. Hypothetically, the high degree of mutation in CDRs and even FWRs would ensue. Other stratagems might prove superior or complementary, e.g., deletions in variable loops on Env, in order to circumvent the first waves of type-specific antibodies.

Original antigenic sin or novel immunogenic virtue?

According to the doctrine of original antigenic sin, the first encounter with a changeable antigen traps the immune response into focusing on certain dominant epitopes, thereby depriving subsequent responses to mutated antigens of their full efficacy. Later responses will then cross-react sub-optimally with the mutated versions of the dominant epitopes rather than yield potentially more effective reactivities with alternative targets. Maybe constraints to this effect contribute to the lack of bNAb responses in the majority of HIV-1-infected persons. But then, conversely, in the minority who do mount bNAb responses, maybe the extreme variability of the most exposed Env epitopes dilutes the boosting of the immune response to those sites and by default focuses it on conserved elements, making for breadth, and with ever iterated boosting, for potency.

The new structural information (1, 2, 24), together with the collated mapping of bNAb responses (3, 4, 6, 8, 23), will eventually answer how much of the conserved external surface of the Env trimer remains immuno-silent. Dormant bNAb epitopes may exist that never get a chance to elicit responses because of their dominant neighbors; perhaps some silent potential epitopes are not germline-reactive, but could be rendered immunogenic when presented in isolated, scaffolded form. Hypothetical strategies of that kind might broaden the NAb response and narrow the loopholes of viral escape.

Acknowledgments

MMR is a recipient of a fellowship from the Consejo Nacional de Ciencia y Tecnología (CONACyT) of Mexico. RWS is a recipient of a Vidi grant from the Netherlands Organization for Scientific Research (NWO) and a Starting Investigator Grant from the European Research Council (ERC-StG-2011-280829-SHEV). The authors' work in this area is supported by NIH grants P01 AI82362 and R37 AI36082.

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