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What Are the Most Powerful Immunogen Design Vaccine Strategies?

A Structural Biologist's Perspective

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The ability of structure-based design to control the shape and reactivity—the atomic-level chemistry—of an immunogen argues for it being one of the "most powerful" immunogen-design strategies. But antigenic reactivity is only one of the properties required to induce a protective immune response. Here, a multidimensional approach is used to exemplify the enabling role atomic-level information can play in the development of immunogens against three viral pathogens, respiratory syncytial virus, influenza A virus, and human immunodeficiency virus (HIV), which have resisted standard approaches to vaccine development. Overall, structure-based strategies incorporating B-cell ontogenies and viral evasion mechanisms appear exceptionally powerful.

GREAT DEBATES

What are the most interesting topics likely to come up over dinner or drinks with your colleagues? Or, more importantly, what are the topics that *don't* come up because they are a little too controversial? In ***Immune Memory and Vaccines: Great Debates***, Editors Rafi Ahmed and Shane Crotty have put together a collection of articles on such questions, written by thought leaders in these fields, with the freedom to talk about the issues as they see fit. This short, innovative format aims to bring a fresh perspective by encouraging authors to be opinionated, focus on what is most interesting and current, and avoid restating introductory material covered in many other reviews.

The Editors posed 13 interesting questions critical for our understanding of vaccines and immune memory to a broad group of experts in the field. In each case, several different perspectives are provided. Note that while each author knew that there were additional scientists addressing the same question, they did not know who these authors were, which ensured the independence of the opinions and perspectives expressed in each article. Our hope is that readers enjoy these articles and that they trigger many more conversations on these important topics.

Editors: Shane Crotty and Rafi Ahmed
Additional Perspectives on Immune Memory and Vaccines: Great Debates available at www.cshperspectives.org

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With less than 30 pathogens with vaccines licensed for human use, the list of desired vaccines is long and ever growing (www.who.int/immunization/diseases/en). The semi-yearly occurrences of viral outbreaks—with H1N1 influenza A virus (2009), Middle East respiratory syndrome coronavirus (2012), Ebola virus (2014), and Zika virus (2016) dominating headlines—emphasize the need for additional vaccines. All the while, more established pathogens such as respiratory syncytial virus (RSV) and human immunodeficiency virus (HIV) continue to kill millions (Murray and Lopez 1997), and a divergent airborne-transmitted influenza A virus such as the 1918 "Spanish flu," which killed an estimated 50 million worldwide (Webster et al. 1992), remains on the watch list of most feared pandemics.

What are the most powerful immunogen design strategies? The three "I"s of classical vaccinology—"Isolate, Inactivate, and Inject"—represent a strategy that has afforded the development of more than half the currently licensed vaccines (Hilleman 1998). But "most powerful" does not seem to be concerned with how successful nor how efficacious a particular strategy might be, but rather, which strategy succeeds, when others fail. Thus it seems reasonable to consider a strategy "most powerful" if it is able to induce a protective response against pathogens, for which other strategies have failed. Here I describe recent results from the Vaccine Research Center against three viral pathogens that have resisted vaccine development. These involve the aforementioned enveloped viruses, RSV, influenza A virus, and HIV, their exposed type 1 fusion machines (Colman and Lawrence 2003), and structure-based strategies that have recently yielded encouraging results.

STRUCTURE-BASED CONFORMATIONAL STABILIZATION STRATEGY FOR RSV

RSV is a member of the paramyxoviradae family of viruses. It infects most children during their first year of life, is responsible for 7% of deaths 1 month to 1 year of age, and is the greatest cause of hospitalization for children under 5 years of age (Shay et al. 1999; Nair et al. 2010; Hall 2012). In the elderly, it induces the same degree of excess mortality as influenza virus (Thompson et al. 2003) and has been on the list of "most desired vaccines" by large pharma for >20 years. A failed vaccine trial in the 1960s induced a number of deaths (Ottolini and Hemming 1997), and decades of RSV-vaccine research achieved an increase of only two- to fourfold in RSV-neutralizing titers in healthy adults (Paradiso et al. 1994; Tristram et al. 1994; Langley et al. 2009; Swanson et al. 2011; Karron et al. 2013; Glenn et al. 2016).

Vaccine interest in the type 1 fusion machine of RSV, its fusion (F) glycoprotein, has been heighted by the advent of palizumab (Synagis), a humanized monoclonal whose passive infusion in high-risk infants reduces the incidence of disease (The IMpact-RSV Study Group 1998; Homaira et al. 2014). Over the last 10 years, antibodies of substantially higher potency than palivizumab have been identified that recognize F in its prefusion conformation (Beaumont et al. 2012; McLellan et al. 2013b). Analysis of human serum, moreover, indicates neutralization responses elicited by natural RSV infection to target preferentially the prefusion state of F (Ngwuta et al. 2015). We determined the prefusion structure of RSV F, in complex with the D25 antibody (McLellan et al. 2013b), and used structure-based design to stabilize the metastable prefusion state (McLellan et al. 2013a) by addition of a disulfide bond (DS) and cavity-filling mutations (Cav1). Immunization indicated the prefusion F-stabilized "DS-Cav1" variant to elicit highly protective titers in mice and macaques, a finding hailed as one of the top breakthroughs of 2013 (Cohen 2013). Phase I clinical trials with DS-Cav1 have begun in 2016, and second-generation versions with up to 20-fold increased stability and fourfold increased immunogenicity have recently been developed through a strategy of iterative structure-based improvement (Joyce et al. 2016a).

Altogether, these results provide a paradigm for immunogen design based on conformational stabilization and iterative structure-based optimization (Fig. 1).

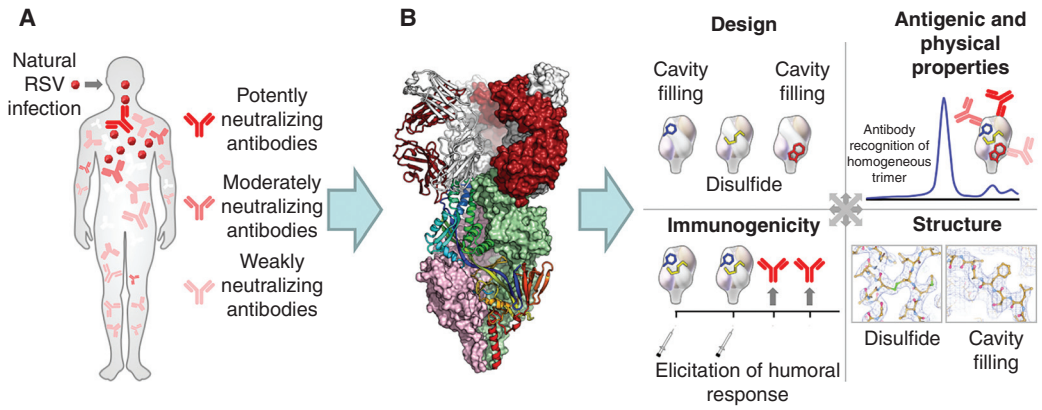

Figure 1. A structure-based paradigm for vaccine development. (*A*) Natural infection induces a variety of responses. In the first step of structure-based vaccine design, a potently neutralizing and frequently elicited response is selected as a template for vaccine design. (*B*) The second step involves the structural determination of the selected response, in complex with the eliciting antigen. Shown is the apex-directed antigen-binding fragment of the D25 antibody (white and red) binding to the trimeric RSV-fusion glycoprotein (pink and green surface representation, with one protomer in rainbow-colored ribbon). (*C*) An information matrix comprising immunogen design, antigenic and physical properties, atomic-level structures, and immunogenicity is used to determine properties that correlate with improved immunogenicity, and these properties are iteratively optimized (Joyce et al. 2016b). (Panel from McLellan et al. 2013a; adapted, with permission, from The American Association for the Advancement of Science © 2013.)

STRUCTURE-BASED B-CELL-ONTOGENY STRATEGY FOR INFLUENZA A VIRUS

While the structure-based strategies developed for RSV may succeed with viruses that evade by conformational change, many viruses do not use structural rearrangements to evade the humoral immune response. Unlike the metastable RSV F glycoprotein, which rearranges to its postfusion conformation even on infectious virus (Liljeroos et al. 2013), the type 1 fusion machine of influenza A virus, its hemagglutinin (HA) glycoprotein, is stable under physiological conditions; as long as the influenza HA glycoprotein does not encounter acidic pH, it resides in a stable prefusion state. Immune evasion by the HA glycoprotein appears not to involve conformational change, but sequence diversity: either antigenic drift (Carrat and Flahault 2007), responsible for the ability of seasonal strains, such as the H1 and H3 subtypes of influenza A that currently cocirculate, to infect an estimated 10% of the human population each year; or antigenic shift (Alexander and Brown 2000), potentially involving zoonotic crossovers from a highly diverse reservoir of influenza subtypes, which reside in domesticated livestock and migratory birds.

One potential approach to overcoming sequence diversity involves clues from antibodies identified from natural infection: an antibody to vaccine approach (Burton 2002). In such an approach, antibodies with broad neutralization activity unhindered by sequence diversity would serve as templates for vaccine development. Structure-based approaches can be used to define the epitopes recognized by broadly neutralizing antibodies and to create immunogens that focus the immune response these sites of viral vulnerability. At the Vaccine Research Center, we have proposed the expansion of this approach to include considerations of the B-cell ontogeny by which effective antibodies are generated (Kwong and Mascola 2012). In such an expanded approach, it is critical to understand not only what antibodies are made of and which sites of viral vulnerability they target, but also the immunological pathways by which antibodies are generated. In this approach, one seeks to elicit effective antibodies by replicating

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the pathway or pathways by which the template antibodies were generated.

One potential flaw in this approach: antibodies are created by stochastic processes of recombination and somatic hypermutation, with an estimated diversity of greater than 10^{12} (Boyd et al. 2009). If protective immunity occurs through different mechanisms of recognition or are created by different immunological processes, then B-cell ontogeny strategies based on "re-eliciting" similar antibodies in the general population may flounder.

The identification of multidonor antibodies, with the same mechanism of recognition and the same B-cell development pathway, however, raises hope for B-cell ontogeny strategies (Scheid et al. 2011; Wu et al. 2011; Zhou et al. 2013; Jackson et al. 2014; Truck et al. 2015). Such reproducible, "convergent," or "public" clonal types have been observed against a number of different pathogens, and—for influenza Avirus—the most well-known example involves HA-stem directed antibodies derived from the VH1-69 germline gene (Throsby et al. 2008; Ekiert et al. 2009; Sui et al. 2009; Kashyap et al. 2010). These VH1-69-derived antibodies, however, generally neutralize only group 1 subtypes of influenza A (Dreyfus et al. 2012), and it has not been clear how to induce greater neutralization breadth.

In examining cross-reactive B cells from a vaccine trial (VRC 310) involving immunizations with a divergent H5 strain of influenza A (Ledgerwood et al. 2011, 2013), however, we identified three multidonor classes of antibodies capable of neutralizing diverse strains of influenza A from both group 1 and group 2 subtypes (Fig. 2A) (Joyce et al. 2016a). Two of these multidonor classes, one derived from the VH6-1 germline gene with a D3-3 recombination and a second from the VH1-18 germline gene with a (Q-x-x-V) motif appeared to be substantially induced by both seasonal and divergent H5 immunization (Joyce et al. 2016a). Antibodies from both of these multidonor classes recognize overlapping epitopes in the conserved HA stem, thereby enabling structure-based strategies involving stem-only immunogens (Impagliazzo et al. 2015; Yassine et al. 2015), chimeric immunogens (Krammer et al. 2015), or immunization with diverse HAs (Ledgerwood et al. 2011). Notably, the VRC 310 regimen appeared to increase the frequency of the VH1-18 (Q-x-x-V) class by over 1000-fold. B-cell ontogeny-based immunogens—specific for the VH6-1 + D3-3 germline or the VH1-18 (Q-x-x-V) germline—may induce even higher frequencies, as has been shown for VRC01-class antibodies with knockin mice (Dosenovic et al. 2015; Jardine et al. 2015). Potential solutions to the universal vaccine can thus be found at select B-cell ontogenies in multiple donors (Fig. 2B). Overall, the identification and quantification of multidonor flu antibodies, coupled with structure-based strategies of germline priming, provides an exciting approach to developing a universal influenza A vaccine.

STRUCTURE-BASED EVASION-MECHANISM STRATEGY FOR HIV

What about viruses for which antibody templates from natural infection are exceedingly difficult to elicit? This appears to be the case for HIV, wherein antibodies from natural infection have been identified that neutralize over 90% of circulating HIV isolates, but ontogeny-based analyses indicate these antibodies may take years to mature (Liao et al. 2013; Bonsignori et al. 2016; MacLeod et al. 2016) or to require unusual recombination (Briney et al. 2012; Doria-Rose et al. 2014; Gorman et al. 2016). Would it be possible for immunization to induce antibodies through pathways distinct or more efficient than natural infection?

We hypothesized that an effective vaccine might be identified through a structure-based mechanistic strategy involving (1) identification of the evasion mechanism(s) the target pathogen uses to confound the humoral immune response, (2) use of structural biology to create immunogens that overcome each of these evasion mechanisms, and (3) combination of individual solutions into a single immunogen or immunization regime.

With HIV, the Env glycoproteins, gp120 and gp41, comprise the type 1 fusion machine that

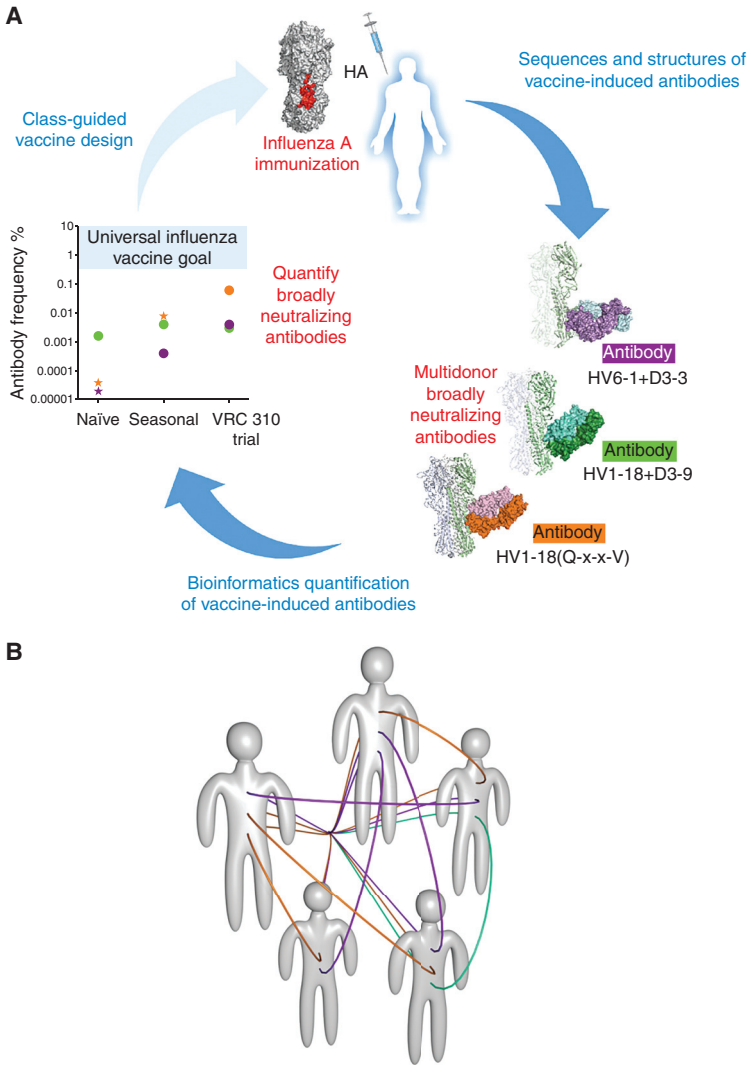

Figure 2. A B-cell ontogeny-based paradigm for vaccine development. (*A*) Reproducible antibodies, observed in multiple donors, represent vaccine solutions potentially available to the general population. Three multidonor classes of antibody (purple, orange, and green) were identified in subjects from the VRC 310 trial, which involved immunization with a diverse H5 influenza strain. Bioinformatics-delineated sequencing signatures allowed for the quantification of transcripts corresponding to these signatures, which should aid in the class-guided elicitation of these antibodies. (Panel from Joyce et al. 2016a; adapted, with permission, from Elsevier © 2016.) (*B*) Schematic of five humans with reproducible classes of broadly neutralizing antibodies, as represented by purple, orange, and green lines.

makes up the Env spike, the sole virion component to extend beyond the protective viral membrane. These assemble into a trimeric closed state recognized by most neutralizing antibodies (Munro et al. 2014). Viral mechanisms of immune evasion that shield the HIV envelope (Env) spike from antibody-mediated neutralization involve sequence variation (Starcich et al. 1986), conformational change (Kwong et al. 2002), and *N*-linked glycosylation (Fig. 3, left) (Wei et al. 2003). Structure-based solutions to each of these viral mechanisms of evasion have

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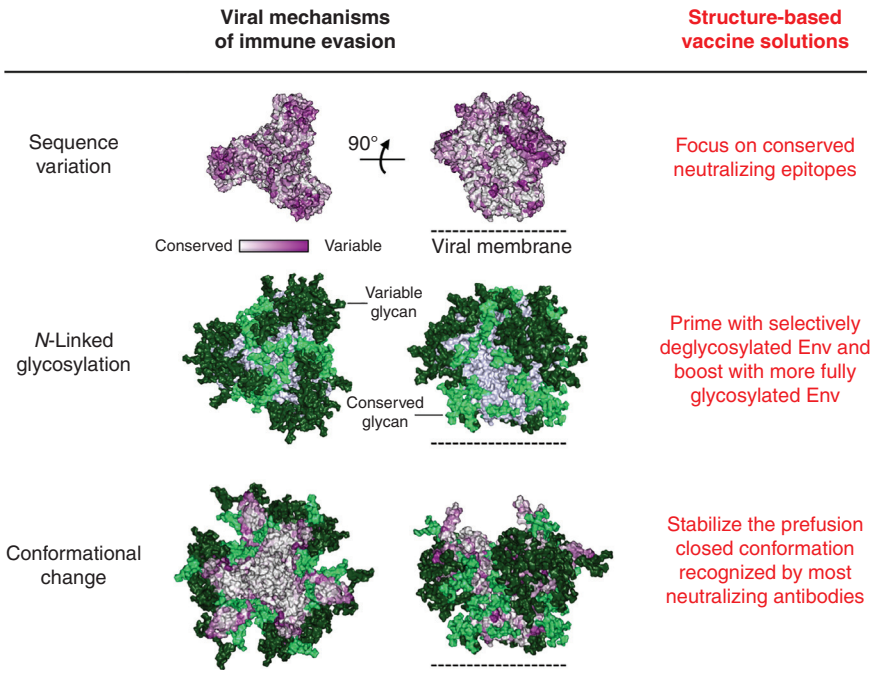

Figure 3. A structure-based mechanistic approach to HIV vaccine development. HIV evades the humoral immune response by Env mechanisms of sequence variation, *N*-linked glycosylation, and conformational change (*left*). Solutions to each of the mechanisms of evasion have been identified by structure-based design (*right*). (Figure from Pancera et al. 2014; adapted, with permission, from Nature Publishing Group © 2014.)

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been proposed including a focus on conserved neutralization epitopes, conformational fixation of the critical prefusion closed conformation of Env, and epitope-specific deglycosylation (Fig. 3, right). However, the combination of all of these approaches into a single immunogen or immunization regimen has only recently been attempted (Kwong 2016). It remains to be seen whether such a combination structure-based mechanistic approach will succeed in eliciting HIV-neutralizing antibodies effective on diverse viral strains.

CONCLUDING REMARKS

In attempting to identify the "most powerful" immunogen design strategy, it seems pertinent to inquire as to the efficiency of various search strategies, which can involve hypothesis-driven searches as well as game theory, resolution-enhancing strategies, and multidimensional searching (Nabel 2009). In truth, hypotheses can always be formulated to include all solutions (e.g., the union of a hypothesis and its null hypothesis), but such inclusiveness says little about the efficiency of the hypothesis in identifying a solution. Similarly, multidimensional searching can be all-encompassing, but this says little about which dimensions provide the most efficient means to find a solution. For example, a 600-residue glycoprotein might be the solution to the HIV vaccine problem; however, it may be exceedingly difficult to identify this solution in the 20^{600} potential solutions that comprise the sequence space for a 600-residue protein made up of the 20 naturally occurring amino acids. Rather, other dimensions such as those that comprise neutralization epitopes, evasion mechanism, or B-cell ontogenies might allow for more efficient identification of vaccine solutions (Fig. 4).

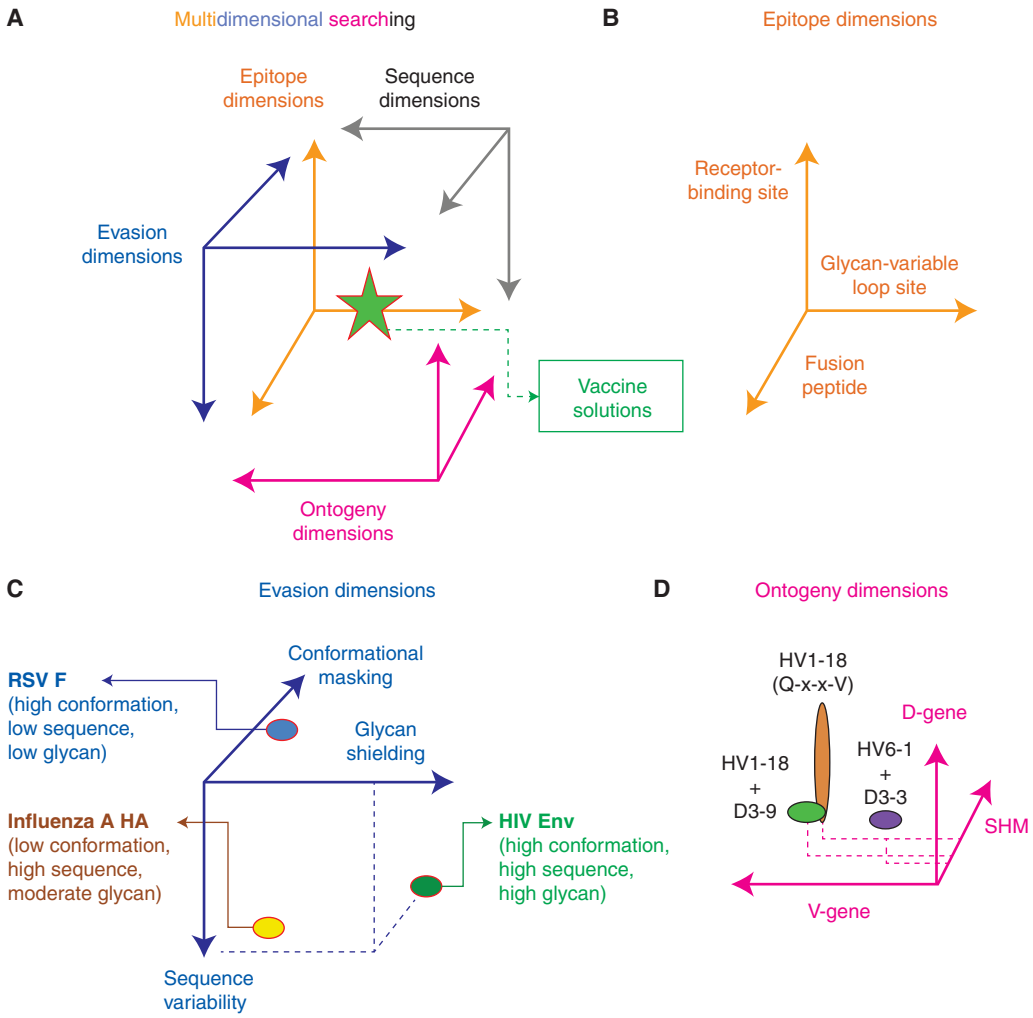

Figure 4. Coordinates of effective vaccines. (*A*) The search for an effective vaccine may involve multiple dimensions, each of which provide a different representation of the vaccine solution (Nabel 2009). (*B*) Promising targets for vaccine design include known epitopes of broadly neutralizing antibodies, such as fusion peptide (Kong et al. 2016) or supersites of vulnerability represented by the clusters of epitopes around the receptor-binding site (Zhou et al. 2015) or a glycan-variable loop site (Kong et al. 2013). (*C*) Searches of evasion dimensions may more efficiently allow for the identification of vaccine roadblocks and their solutions. Evasion mechanisms used by type 1 fusion machines of RSV, influenza A virus, and HIV are shown. (Structure-based solutions to evasion mechanisms for RSV are shown in Fig. 1 and for HIV in Fig. 3.) (*D*) Ontogeny dimensions comprising immunoglobulin-origin genes and SHM of multidonor-antibody classes represent reproducible vaccine solutions available to the general population (Joyce et al. 2016a), as described in the text and in Figure 2.

How important are combination approaches? In searching for efficient strategies to identify solutions to another multidimensional problem, that of protein crystallization, we observed (1) that the most important parameter governing protein solubility was the concentration of the precipitant, and (2) that combinations of mechanistically distinct precipitants identified crystallization conditions capable of inducing lattice formation for proteins that had otherwise resisted crystallization (Majeed et al. 2003).

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Similarly, the most important parameter governing the elicitation of neutralizing antibody against a particular pathogen is likely to be the immune-evasion parameter preventing antibodies from being elicited by that pathogen. Combinations of solutions to mechanistically distinct immune-evasion parameters might similarly provide a means to identify vaccine solutions for pathogens such as HIV, which otherwise resist typical strategies of vaccine design. It will be fascinating to determine which immunogen design strategy allows for the elicitation of broadly neutralizing antibodies against HIV. In light of the difficulty of the HIV vaccine problem, whichever strategy succeeds will likely be deemed most powerful.

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