A Blueprint for HIV Vaccine Discovery

Dennis R. Burton,1,2,12,14* Rafi Ahmed,4,14 Dan H. Barouch,5,12,14 Salvatore T. Butera,1,14 Shane Crotty,6,14 Adam Godzik,7,14 Daniel E. Kaufmann,12,14 M. Juliana McElrath,8,14 Michel C. Nussenzweig,9,10,14 Bali Pulendran,4,14 Chris N. Scanlan,11,14 William R. Schief,1,2,14 Guido Silvestri,4,13,14 Hendrik Streeck,12,16 Bruce D. Walker,12,14 Laura M. Walker,1,15 Andrew B. Ward,3,14 Ian A. Wilson,3,14 and Richard Wyatt1,2,14

1Department of Immunology and Microbial Science
2AVI Neutralizing Antibody Center
3Department of Molecular Biology and Skaggs Institute for Chemical Biology
The Scripps Research Institute, La Jolla, CA 92037, USA
4Emory Vaccine Center, Emory University, Atlanta, GA 30322, USA
5Division of Vaccine Research, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA
6Division of Vaccine Discovery, La Jolla Institute of Allergy and Immunology, La Jolla, CA 92037, USA
7Graduate School of Biomedical Sciences, Sanford Burnham Medical Research Institute, La Jolla, CA 92037, USA
8Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA
9Howard Hughes Medical Institute
10Laboratory of Molecular Immunology
The Rockefeller University, New York, NY 10065, USA
11Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK
12Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard University, Boston, MA 02114, USA
13Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329, USA
14Scripps Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery, The Scripps Research Institute, La Jolla, CA 92037, USA
15Present address: U.S. Military HIV Research Program, Silver Spring, MD 20910, USA
*Correspondence: burton@scripps.edu
http://dx.doi.org/10.1016/j.chom.2012.09.008

Despite numerous attempts over many years to develop an HIV vaccine based on classical strategies, none has convincingly succeeded to date. A number of approaches are being pursued in the field, including building upon possible efficacy indicated by the recent RV144 clinical trial, which combined two HIV vaccines. Here, we argue for an approach based, in part, on understanding the HIV envelope spike and its interaction with broadly neutralizing antibodies (bnAbs) at the molecular level and using this understanding to design immunogens as possible vaccines. BnAbs can protect against virus challenge in animal models, and many such antibodies have been isolated recently. We further propose that studies focused on how best to provide T cell help to B cells that produce bnAbs are crucial for optimal immunization strategies. The synthesis of rational immunogen design and immunization strategies, together with iterative improvements, offers great promise for advancing toward an HIV vaccine.

The HIV Vaccine Problem: Why Have Past Approaches to Develop an HIV Vaccine Failed?

It is nearly 30 years since HIV was identified as the causative agent of AIDS, and yet no vaccine is approaching licensure. This is intensely disappointing. It is due partly to the fact that the process of vaccine development typically takes a long time period, partly to the failure of classical viral vaccination strategies when applied to HIV, and partly to the many barriers to immune recognition evolved by the virus. For acute viruses such as smallpox and polio, natural infection leads to a large fraction of individuals who are immune to reinfection. Hence, vaccination strategies to mimic natural infection without adverse sequelae can be designed. For persistent viruses, immunity to reinfection cannot be readily determined, but nevertheless, in some cases such as human papillomavirus (HPV), a relatively straightforward approach in which vaccines elicit neutralizing IgG is effective (Day et al., 2010). However, for the highly variable retrovirus HIV, the classical vaccination strategies have failed, and it is worth considering why this is the case.

Immunogen Design Strategies Have Failed to Elicit Broadly Neutralizing Antibody Responses to Circulating HIV

As for other viruses, animal model studies, including the macaque, provide an abundance of evidence for protection against HIV or SHIV (chimeric HIV/SIV with envelope (Env) of HIV) challenge by neutralizing antibodies. However, to protect against the huge diversity of global circulating HIVs, the neutralizing antibody response should be broad, and traditional vaccine approaches have failed to induce such a response. We note that although there are reports of some degree of antibody protection in the absence of serum-neutralizing antibodies, including the RV144 trial, the protection is generally not particularly strong, and so we focus here on neutralizing antibodies.

Live Attenuated and Killed Viruses: Direct Pathogen Mimicry

Many highly successful vaccines rely on direct mimicry of the pathogen. Live-attenuated viruses (e.g., measles, mumps, yellow fever), inactivated viruses (e.g., poliovirus), or virus-like particles (e.g., HPV) have been used to imitate natural infection
and imprint immunological memory. However, for many reasons these approaches have been unsuccessful for HIV vaccine development (Burton et al., 2005; Kong and Sattentau, 2012; Kwong et al., 2011; Overbaugh and Morris, 2012; Pantophlet and Burton, 2006; Verkoczy et al., 2011; Wyatt and Sodroski, 1998). The challenges in this approach are largely related to the properties of the HIV envelope (Env) spike, which is a heterotrimer of the glycoproteins gp120 and gp41 that forms trimers on the virion surface and mediates entry by binding to CD4 and CCR5 or CXCR4 on the target cell surface. First, the variable regions of the functional HIV Env trimer spike (Figure 1), the trimer being the sole target of neutralizing antibodies, are typically immunodominant relative to conserved regions of the spike and, thus, the neutralizing antibody response against HIV is generally highly strain specific. Second, the extraordinary variability in antigenic regions of the Env spike means that the number of circulating HIV strains is extremely high and that conventional concepts of viral serotypes are rendered irrelevant. Third, because of the instability of the HIV spike, most viral particle-based vaccines tend to express immunodominant, nonfunctional forms of Env on the virion surface (Poignard et al., 2003), which favor the induction of nonneutralizing antibodies (Crooks et al., 2007). Fourth, there is a relatively low copy number of Env molecules on HIV particles, leading to expectations of rather poor activation of B cells as compared to viruses with dense Env coating, such as influenza. Fifth, other complicating factors, such as the induction of antibodies against cellular proteins, have contributed to difficulties with viral particle-based immunogens (Chan et al., 1992; Crooks et al., 2007; Hammonds et al., 2005). Although a number of strategies to overcome these hurdles have been employed, including pseudotyping HIV with heterologous envelopes (Kuate et al., 2006; Marsac et al., 2002) and generating VLPs with cleavage-defective or disulfide-shackled Env to prevent gp120-gp41 dissociation (Crooks et al., 2007), none of these approaches have yet induced potent heterologous antibody responses in nonhuman primate (NHP) models. Finally, it should be noted that, because of the risk of mutation and reversion to a pathogenic form, the use of live attenuated HIV vaccines in humans raises formidable safety and liability issues.

**Subunit Vaccines: Recombinant Env**

Using the same paradigm that formed the basis for successful development of a vaccine against hepatitis B, initial attempts to generate a protective vaccine against HIV focused on the elicitation of Env-specific humoral immune responses using gp120 subunit immunogens. Unfortunately, the results of clinical trials indicated that the antibodies elicited by monomeric gp120 failed to neutralize HIV primary isolates, prevent HIV infection, reduce viral loads, or delay disease progression (Flynn et al., 2005; Pittsuttithum et al., 2006). Therefore, over the ensuing years, more attention has been focused on the generation of soluble, recombinant trimers as immunogens to better simulate the structure of the native HIV Env spike. Indeed, given that antibody binding to the trimer is both necessary and sufficient for neutralization (Pantophlet et al., 2009; Parren and Burton, 2001; Roben et al., 1994; Sattentau and Moore, 1995; Wallace and Stamatatos, 2009; Yang et al., 2006), a recombinant native trimer represents an excellent starting point for the elicitation of bnAb responses. However, the instability of the functional HIV spike has presented challenges to the development of recombinant trimers that mimic the structure of the native spike (Phogat and Wyatt, 2007). Various strategies, including the introduction of disulfide bonds to covalently link gp120 and gp41, the deletion of the furin cleavage site in gp160 so that furin-mediated proteolysis to yield covalently linked gp120 and gp41 subunits does not occur, and the incorporation of trimerization motifs into the gp41 ectodomain, have been employed to stabilize recombinant trimers (Phogat and Wyatt, 2007). However, none of the recombinant trimers developed to date display antigenic profiles that truly mimic the native HIV spike, and these

**Figure 1. Structure and Antibody Recognition of the HIV Envelope Spike**

The molecule is a heterotrimer of composition (gp120)3(gp41)3. Gp41 is a transmembrane protein, and gp120 is the receptor molecule for CD4 and CCR5 or CXCR4. The model (Schief et al., 2009) is adapted from a cryo-electron tomographic structure of the HIV trimer (Liu et al., 2008). The crystal structure of the b12-bound monomeric gp120 core (red) has been fitted into the density map (Zhou et al., 2007). Glycans are shown in purple. The CD4 binding site is shown in yellow. The approximate locations of the epitopes targeted by existing bnMabs are indicated with arrows, and the number of MAbas targeting each epitope is shown in red boxes. A small selection of bnMabs targeting each epitope is included.
In summary, the challenge for epitope-based vaccine design is that only broadly conserved and exposed epitopes are suitable for vaccine targeting, but these epitopes, in their natural context, tend to elicit poor antibody responses. When bnAb responses are elicited, the corresponding bnMAbs tend to have unusual features such as high levels of somatic mutations, insertions/deletions, long CDRH3 loops, posttranslational modifications, polyreactivity, and rare structural motifs such as domain exchange.

Overall, these observations indicate that successful vaccination may require considerable ingenuity in immunogen design and immunization protocols that go far beyond current norms.

**Sterilizing Immunity, or Close to It, Will Likely Be Necessary for Protective Immunity against HIV**

The ultimate goal of vaccination is to provide protective immunity against disease. For most successful vaccines (e.g., measles, polio, smallpox, etc.), protection against disease can be achieved in the absence of sterilizing immunity. Indeed, under most circumstances, immunization does not induce sufficiently high and persistent titers of antibodies to prevent infection (Plotkin, 2008). For example, in the case of measles, very high antibody titers of $\geq 1{,}000$ mIU/ml are generally required to protect against infection, but titers of $\geq 200$ mIU/ml are sufficient to protect against disease (Chen et al., 1990). In contrast, for HIV, because of the establishment of persistent latent infection in which viral DNA is integrated into host genomic DNA, the window of opportunity to clear virus may close permanently once a pool of latently infected cells is in place (Haase, 2010, 2011), although the results of Picker and colleagues, as noted below, do suggest that, in some cases, a cellular response can control and suppress virus replication to very low levels (Hansen et al., 2011).

**Cellular Immune Responses Generally Do Not Prevent Acquisition of Infection**

It is clear from several animal studies that vaccines that induce only CD8+ T cells (also known as cytotoxic T lymphocytes [CTLs]), and no virus-specific antibodies, can efficiently control subsequent challenge with a viral pathogen, as shown for lymphocytic choriomeningitis virus (LCMV) (Klavinskis et al., 1989), influenza virus (Ulmer et al., 1993), and respiratory syncytial virus (RSV) (Kulkarni et al., 1995). However, these “CTL-only vaccines” typically prevent severe disease, but not infection itself, and can allow significant viral replication. Indeed, recent studies suggested that cellular responses alone could not block acquisition of SIV infection in the macaque model; the inclusion of Env in the vaccine was required for complete prevention of infection (Barouch et al., 2012). In the context of HIV, CTL vaccines might be expected to permit considerable viral seeding of lymphoid tissue during acute infection and the establishment of a significant pool of latently infected cells with concomitant adverse consequences (Liu et al., 2009). However, recent studies (Hansen et al., 2011) have described a Cytomegalovirus (CMV) vector-based SIV vaccine that appears to function via CD8+ T cell immunity and restricts SIV replication to very low levels in a significant fraction of vaccinated animals. The induction of such a response could contribute to vaccine protection against HIV and serve as a second line of defense to contain
infection at the portal of entry, should humoral responses fail to fully prevent initial infection of target cells.

What Goals Should Be Set to Move toward a Rationally Designed HIV Vaccine?

Given the challenges identified above, we propose a number of goals that should be targeted in order to move toward an HIV vaccine based on neutralizing antibody, B cell, and CD4+ T cell studies.

Fully Define the Antibodies and Epitopes Associated with Broad Neutralization of HIV

NAbS are the best correlate of protection for many viral vaccines (Amanna and Sifftka, 2011; Plotkin, 2010), and for HIV, nAbs have been shown to provide robust protection against mucosal challenge in the macaque model as described above. Therefore, a major goal of HIV vaccine research should be the discovery of immunogens and immunization strategies that can elicit nAbs, or more specifically broadly nAbs (bnAbs), given high sequence variation in HIV Env. As part of this discovery effort, it is important to fully map the landscape of bnAb recognition of the HIV Env spike, the sole target of nAbs, as described above. The recent generation of larger numbers of potent bnMAbs (Figure 2) using single B cell technologies (Burton et al., 2012; Corti et al., 2010; Haynes et al., 2012; Klein et al., 2012; Kong and Sattentau, 2012; Moir et al., 2011; Overbaugh and Morris, 2012; Scheid et al., 2009, 2011a; Tiller et al., 2008; Walker et al., 2011a; Wu et al., 2010, 2011) has begun to reveal new bn epitopes and defined “sites of vulnerability” (Kyong and Wilson, 2009) on the Env spike with much greater accuracy (Figure 1). However, ideally one would like to generate and characterize enough bnMAbs with enough redundancy in epitope recognition to be confident that HIV Env bnAb space has been fully covered. A full complement of bn epitopes can then be exploited for generating immunogens with optimal precision.

The bnMAbs are, in themselves, valuable tools for guiding vaccine discovery. The isolation of multiple bnMAbs that recognize similar epitopes is revealing the extent to which certain structural features (e.g., long CDRH3s, polyreactivity, domain exchange, high levels of somatic hypermutation, posttranslational modifications, etc.) are required for recognition. For example, recently described CD4bs-directed antibodies exhibit remarkably broad and potent activity but, unlike b12, do not require a long CDRH3 for gp120 binding (Scheid et al., 2009; Wu et al., 2010; Zhou et al., 2010). Therefore, the elicitation of bnMAbs against the CD4bs may not necessitate the design of immunization protocols that aim to preferentially select antibodies with longer CDRH3s. Also, the isolation of multiple bnMAbs against single antigenic regions helps delineate whether the use of certain germline genes can facilitate epitope recognition. In support of this notion, it has been suggested that the use of two closely related VH germline genes (VH1-2’02 and VH1-46), which encode the variable regions of Abs that contribute to immunogen specificity, allows for a conserved mode of epitope recognition by certain CD4bs-directed bnMAbs (Scheid et al., 2011; West et al., 2012; Wu et al., 2011, 2012; Zhou et al., 2010). The bnMAbs described to date have high levels of somatic hypermutation (Scheid et al., 2011; Walker et al., 2009, 2011a; Wu et al., 2010), a process in which mutations are introduced into the antibody variable regions to generate diversity. This may dictate that immunogens and/or immunization protocols should be designed to increase antibody affinity maturation (e.g., adjuvants, viral vectors, etc.). Alternatively, the high levels of somatic hypermutation may simply reflect the outcome of chronic antigen stimulation resulting from long-term HIV infection. Indeed, anti-Env Abs from chronic infection in general, whether neutralizing or not, tend to have high levels of somatic hypermutation (Barbas et al., 1993; Breden et al., 2011; Scheid et al., 2009). Therefore, it may be possible to generate immunogens and/or immunization protocols that shortcut the route to generation of bnAbs and result in antibodies with much less somatic hypermutation.

Determine the bnMAbs that Provide the Best Protection against SHIV in the NHP Model

Clearly, the property of antibodies that interests most in terms of vaccine discovery is protection in humans. Neutralization is simply a property that can be readily measured in vitro and which has a good pedigree for qualitative prediction of protection against SHIV in the NHP model and against many other viruses in humans. Ideally, we would like to correlate protection in humans with neutralization assays, but this likely requires either large-scale human passive immunization studies or a vaccine that has clear efficacy, although superinfection and mother-to-child transmission studies may also provide useful correlative data (Blish et al., 2008; Chohan et al., 2010; Dickover et al., 2006; Guevara et al., 2002; Lathey et al., 1999; Scarlatti et al., 1993a, 1993b; Smith et al., 2006). The best animal model is probably SHIV infection in macaques, and titration of protection for several of the newer bnMAbs in high- and low-dose SHIV vaginal challenge models is important to better define the relationship between protection and both in vivo neutralization and antibody specificity. Finally, investigation of passive protection in animal models expressing human antibody repertoires, e.g., the humanized BLT (bone marrow/liver/thymus) mouse (Brainard et al., 2009; Wheeler et al., 2011), is highly desirable.
Design, Engineer, and Produce a Pure Stable Env Preparation that Mimics the Antigenic Profile of the Functional Env Spike

The functional HIV Env spike is the sole target of neutralizing antibodies, as discussed above. Remarkably, because of the instability of the spike, it is likely that no immunization with pure native spikes has yet been carried out; infectious virions express multiple Env species so that even natural infection presents a complex mix of Env molecules to the immune system (Moore et al., 2006; Poignard et al., 2003). A number of strategies to generate a pure stable Env preparation that mimics the antigenic profile of the functional Env spike are ongoing. First, cocrysrtallization of recombinant Env trimers with a variety of bnMAbs is being attempted and hopefully will eventually generate a high-resolution structure that will allow for rational approaches to stabilizing a recombinant trimer. Second, the resolution and methodologies of cryo-electron microscopy (cryo-EM) and cryo-electron tomography are being enhanced and may similarly provide enough molecular detail to allow the rational design of stable trimers (Liu et al., 2008; Mao et al., 2012). Third, various molecular display and selection strategies, including positive selection with bnMAbs and negative selection with nonneutralizing Abs, are being studied. Fourth, gp120/gp41 sector analyses (Dahirel et al., 2011) are being used to identify potential gp120-gp41 crosslinking stabilization sites. It should be noted, though, that even if stable trimer immunogens can be designed, engineering strategies will still likely be required to dampen responses to immunodominant variable regions of the Env trimer molecule to favor elicitation of bNAb responses.

Define Glycosylation on the Env Trimer

Approximately half of the molecular mass of gp120 is comprised of N-linked glycans that shield the protein backbone. Considering that these carbohydrate structures are critical for Env folding, binding to lectin receptors that may mediate transmigration, antigenicity, and immunogenicity, a complete understanding of the identity and heterogeneity of glycans expressed on native trimers may be crucial for the development of HIV vaccine candidates. Although the glycosylation profiles of recombinant Env proteins and native viral Env have been analyzed by mass spectrometry (Bonomelli et al., 2011; Doores et al., 2010; Go et al., 2008, 2009, 2011; Mizuochi et al., 1988; Zhu et al., 2000), a full definition of site-specific glycosylation on these Env has not yet been possible. Such an analysis would provide insight into the role of specific glycans in forming or shielding antibody epitopes in the context of gp120 monomers and native HIV Env trimers, which may inform immunogen design efforts. Of note, recent studies suggest that functional Env trimers are substantially resistant to mannosidase, which hydrolyzes mannosyleglycans, and express a higher abundance of oligomannose glycans that are often found on monomeric gp120 (Bonomelli et al., 2011; Doores et al., 2010; Eggink et al., 2010) (Figure 3). Additionally, even the same recombinant gp120—when expressed in different, commonly used cell lines—can show widely varying abundances of oligomannose-type glycans as well as exhibiting different varieties of complex-type glycans (Raska et al., 2010). These differences in glycan composition are of direct immunological significance: enzymatic modification of gp120 glycans (Banerjee et al., 2009), or even direct occlusion of gp120 glycans by protective lectin (Banerjee et al., 2012), can dramatically alter the antibody response to gp120. These observations, coupled with the fact that recombinant gp120 has been used extensively in animal studies and human vaccine trials with very limited success, necessitate that consideration be given to the impact of differing glycosylation patterns on the antigenicity and immunogenicity of Env. In contrast to the variation seen between expression systems, the overall glycosylation pattern of native envelope glycoproteins derived from peripheral blood mononuclear cells (PBMCs), even from highly divergent HIV clades, is remarkably well conserved (Bonomelli et al., 2011).

Determine Where, When, How, and Which HIV Antigens Are Engaged by B Cells

Understanding how the immune system recognizes and processes viral antigens for durable humoral immunity is of
fundamental importance for vaccine design (Cyster, 2010). It is now clear that B cells can encounter and respond to antigen through many different mechanisms depending on the nature and size of the antigen itself, as well as on the cellular context and location in which antigen presentation occurs. For example, within the context of the lymph node, small soluble antigens access the B cell follicle, the primary site where B cell activation occurs, through a follicular conduit system (Roozendaal et al., 2009), whereas particulate antigens, such as viruses and large immune complexes, are captured by subcapsular sinus macrophages for presentation to follicular B cells (Gonzalez et al., 2011). In the case of HIV Env, particularly considering the dramatic instability of the native Env spike, it is unclear which Env antigens are presented to B cells and how and where these encounters occur after infection. For example, how often, if ever, do B cells engage functional Env spikes in vivo? Additionally, what are the predominant Env species presented to B cells: monomeric gp120, gp41, proteolyzed Env fragments, and/or uncleaved gp160? Although the above questions concern viral Env in the context of HIV/SIV infection, an equally important issue is how soluble vaccine immunogens are processed and presented to B cells. The answers to these questions will have important implications for the design of improved HIV vaccine candidates. We believe these questions can be addressed in the macaque model and in mice, particularly in bnMAB knockin mice.

**Explanation and Why a Subset of HIV-Infected Individuals Makes Potent bnAb Responses**

A series of studies (Sather et al., 2009; Simek et al., 2009; Stamatatos et al., 2009; van Gils et al., 2009) have shown that 10%–30% of HIV-infected donors develop moderate to potent broadly neutralizing serum responses over time, providing support for the notion that bnAbs can be elicited in humans and providing a window into how to elicit such responses. Although serum-mapping experiments (Binley et al., 2008; Gray et al., 2009, 2011; Li et al., 2007, 2009; Mikell et al., 2011; Moore et al., 2011; Stamatatos et al., 2009; Walker et al., 2010) have largely defined the antibody specificities that mediate broad serum neutralization, there is currently limited information on how and why broad responses develop within select individuals. A few longitudinal studies (Euler et al., 2012; Gray et al., 2011; Sather et al., 2009; van Gils et al., 2009) have examined the factors associated with the development of breadth, and although there are some inconsistencies, it has been suggested that broad neutralization correlates with time postinfection, plasma viremia levels, CD4+ T cell count at the viral set point, and binding avidity to the envelope protein. In a recent longitudinal study (Gray et al., 2011), breadth was found to emerge 2 years postinfection and plateaued at year four, which corroborates earlier estimates that bnAbs develop after 1–3 years (Gray et al., 2011; Sather et al., 2009; Stamatatos et al., 2009; van Gils et al., 2009). While all of these studies have provided valuable insight into the factors associated with the development of bnAbs, more extensive longitudinal studies (e.g., isolation of mAbs and viral Env from serial time points) in both humans and NHP models will be required to understand the evolution and maturation of broad responses. A major question is the relative contribution of the virus and the host immune system to the evolution of these responses. Key issues include the influence of the founder virus, the possibility of viral motifs associated with broad neutralization, clinical correlates of broad neutralization, and the roles of B cell dysfunction and CD4+ T cell help in developing bnAb responses. Antibody deep sequencing of donors from whom bnMAbs have been isolated may shed light on how bnAb responses evolve over time (Wu et al., 2011). Of interest, it is expected that deep sequencing methods that maintain correct pairing of the heavy and light chains that comprise antibodies will become available in the near future to allow for functional characterization of antibody responses. The unexpected observation of the development of a highly potent bnAb response in a SHIV-infected macaque only 40 weeks postinfection (Walker et al., 2011b) suggests that the role of the infecting virus and the genetics of the host in bnAb responses could be readily addressed in NHP studies. Of note, retrospective, nested case-controlled studies that utilize systems biological approaches to identify early signatures that correlate with and predict the later development of bnAbs should be useful in providing insights about the mechanisms that control bnAb induction (Querec et al., 2009).

**Develop Model Systems for Immunogen Evaluation**

A classical initial approach to immunogen evaluation is to use serum antibody responses in small animals, such as mice and rabbits, as a “gatekeeper” measurement to determine whether to proceed with the immunogen in humans. An example of such a strategy is to create knockin mice that carry germline forms of the different human bnMAbs. The mice could be used to screen a wide variety of Env antigens for their abilities to induce bnMAb+ murine B cells to enter germinal centers (GCs) where B cells proliferate and develop and to mature into B cells producing fully active antibodies. A comparison of B cells carrying germline and mutated versions of bnMAbs could be used to determine whether an antigen that initiates immunity from “naïve” B cell receptors (BCRs) also stimulates antibody secretion from somatically mutated “mature” BCRs. Further examples of potentially interesting model systems for high-throughput immunogen evaluation include transgenic mice with human repertoires (Legrand et al., 2009; Rathinam et al., 2011) and the mouse BLT model (Brainard et al., 2009; Wheeler et al., 2011), as described above. Another approach is to generate immunogens that bind to NHP germline versions of bnMAbs, as well as the corresponding mature human bnAbs, based on modeling studies and on library selection. Determination of the molecular interactions between germline NHP Abs and immunogen would allow iterative improvement of the immunogens, which could then be investigated as candidate vaccines in NHPs. Whether in knockin mice or NHPs, it is anticipated that iterative cycles of vaccination followed by analysis of B and CD4+ T cell responses to guide redesign of immunogens and immunization strategies will be needed to generate optimal vaccines.

**Accurately Identify and Functionally Characterize HIV/SIV-Specific T Follicular Helper Cells in Humans**

T follicular helper (Tfh) cells are newly appreciated cells, distinct from other CD4+ T cell subsets, that are specialized for B cell
Tfh cells provide different signals to B cells to control different B cell fates, such as plasma cell (antibody secreting cell) differentiation, memory B cell differentiation, death, or repeated rounds of somatic hypermutation and GC B cell proliferation. Generation of high-affinity neutralizing antibodies is generally a multistep, iterative process that is dependent on affinity maturation via somatic hypermutation and extensive signaling from Tfh cells (Crotty, 2011).
In summary, the central hypotheses that we advocate are that a successful HIV vaccine should elicit protective antibodies, and that the combination of B cell and CD4+ T cell responses is critical for the induction and long-term maintenance of vaccine protection. We believe that it is crucial to define immunogens and immunization regimens that induce protective B cell and CD4+ T cell responses in preclinical models and thereby guide product development strategies for a preventive human HIV vaccine. We note that such a vaccine may well also need to induce HIV-specific CD8+ T cell responses for maximal efficacy, as considered elsewhere (Barouch and Korber, 2010; Johnston and Fauci, 2007; McElrath and Haynes, 2010; Walker and Burton, 2008; Watkins, 2008). We propose here integrated efforts focused on two areas: (1) B cell and antibody research to guide the development of immunogens and immunization regimens that elicit protective HIV antibody responses, and (2) CD4+ T cell research, taking advantage of key preliminary data to maximize the T cell help offered to B cell responses through immunization and to harness the direct antiviral activity of CD4+ T cells (Figure 5). Overall, we view this as an extremely exciting period in the field of HIV vaccine research and one that engenders more hope than has been noted for a long time.

REFERENCES


REFERENCES


