

# Accessing the human repertoire for broadly neutralizing HIV antibodies

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**Abbreviations:** HIV, human immunodeficiency virus; EBV, epstein-barr virus; bNAb, broadly neutralizing antibody; TERT, telomerase reverse transcriptase; CDR, complementarity determining regions; ELISA, enzyme-linked immunosorbent assay; HTP, high throughput; PEG, polyethylene glycol; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; IFN $\alpha$ , interferon alpha; GM-CSF, granulocyte macrophage colony-stimulating factor

The human antibody response has special significance in the ongoing efforts to develop a protective HIV vaccine. The observation that a subset of HIV infected individuals, who do not develop AIDS, have a broadly neutralizing antibody response has drawn attention to deciphering the nature of this response. It is hoped that an understanding of these protective antibodies, developed over time in response to the ongoing accumulation of mutations in the infecting virus, will facilitate the development of a vaccine that can elicit a similar response. This strategy will be greatly aided by the identification of broadly neutralizing monoclonal HIV antibodies from infected individuals. Several methods have been utilized to isolate and characterize individual antibodies from the human repertoire and each of these methods has been applied to the generation of broadly neutralizing HIV antibodies, albeit with differing rates of success. This review describes several of these methods including human hybridoma; EBV transformation; non-immortalized B cell culture; clonal sorting; and combinatorial display. Key considerations used in the comparison of different methods includes: efficiency of interrogation of an individual's entire repertoire; assay formats that can be used to screen for antibodies of interest (i.e., binding versus biological assays); and the ability to recover native antibody heavy and light chain pairs.

## Introduction

The development of a vaccine that will protect against HIV infection has proven difficult. The recent failure of a vaccine designed to induce T-cell mediated immunity has given increased impetus to efforts aimed at developing a vaccine that induces an additional protective antibody response. To be effective, such a vaccine will need to elicit antibodies capable

of neutralizing the many circulating strains generated by rapid viral mutation, and only a very few such broadly neutralizing antibodies (bNAbs) have thus far been discovered. A path to the development of a protective antibody-inducing vaccine can be traced by following questions: Can antibodies protect against HIV infection? If so, what epitope do these antibodies recognize? Can similar antibodies be elicited by an appropriate vaccine immunogen?

## Can Antibodies Protect Against HIV Infection?

Several lines of evidence indicate that antibodies can indeed protect against HIV infection.<sup>1</sup> Studies in non-human primates have demonstrated that the passive transfer of antibodies can confer protection against subsequent HIV challenge. Although similar data are not available for efficacy in humans, it is noteworthy that a subset of HIV-positive individuals do not develop AIDS despite receiving no treatment. These patients appear to be capable of immunological control of their HIV infection and one potential component of that control is the presence of a neutralizing antibody response.<sup>2</sup>

## What Epitopes do Protective Antibodies Recognize?

Several recent studies have examined the neutralizing antibody response of some of these non-progressing HIV infected individuals through careful characterization of serum antibodies.<sup>3-5</sup> These studies have included examination of the neutralization of various HIV strains, as well as binding, depletion and enrichment of serum antibodies using proteins and peptides corresponding to various regions of the HIV env protein. The findings have indicated the likely presence of bNAbs in non-progressors. However, serological evaluation is a relatively crude tool for exploring the specificity of individual antibodies, and the broad reactivity of a single antibody is indistinguishable from the aggregate reactivity of a polyclonal response. Accurate determinations of antibody specificity require the isolation of the individual monoclonal antibodies (mAbs) that comprise the overall response. Prior to publications in the latter half of 2009, only four monoclonal bNAbs had been described (b12, 2F5, 4E10 and 2G12).<sup>6-8</sup> The mAbs

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2F5 and 4E10 both recognize epitopes in the membrane-proximal region of the gp41 subunit of HIV env, monoclonal bNAb b12 recognizes the CD4 binding site of gp120, while bNAb 2G12 recognizes an oligomannose structure on gp120.

### **Can Similar bNAbs be Elicited by an Appropriate Vaccine Immunogen?**

It has been proposed that a new vaccine may be designed by attempting to reproduce, as isolated immunogens, the structures of the bNAb epitopes.<sup>1</sup> This approach has been variously referred to as knowledge/structure-based vaccine design or “reverse vaccinology,” and is facilitated by co-crystallization of the native antigen and antibody-binding fragment, particularly when the epitope is dependent on higher order protein structure. Unfortunately, to date no effective vaccine immunogen has been developed for HIV using this or other approaches. Attempts to generate antibody responses corresponding to the isolated bNAbs have been unable to duplicate the native response. The epitopes recognized by 2F5 and 4E10 are distinct from each other, but are both found in a spatially constrained region on gp41. Failure to recapitulate their activity is likely due to the difficulty in generating a similarly constrained epitope construct. Despite the observation that gp120 (the target for b12) is highly immunogenic, most antibodies directed against it are not broadly reactive and only recognize the immunizing strain.<sup>9</sup> Finally, in the case of 2G12, a highly unusual domain-swap structure is adopted that is unlikely to be readily duplicated.<sup>10,11</sup>

It is hoped that the discovery of additional broadly neutralizing antibodies will lead to the identification of new epitopes more amenable to structure-based vaccine design. Two recent publications reveal new bNAb antibodies (PG9, PG16 and VRC01) for which efforts to develop a vaccine are only just beginning.<sup>12,13</sup>

### **The Human Repertoire**

This review will describe various methods by which the human antibody repertoire has been accessed in the search for HIV bNAbs, as well as their relative advantages and limitations. In an infectious disease such as HIV, the human humoral immune response is of particular importance because of the unique and dynamic circumstances under which the human immune system is exposed to the infectious organism. Although neutralizing antibodies generally arise in most individuals shortly after HIV infection, these initial antibodies have very narrow specificity and react only with the infecting strain and, accordingly, no significant protection is afforded against other variants that arise during the course of infection. Patients may be infected for many years with the immune system continually countering the ongoing accumulation of mutations by actively replicating virus. This constant evolution of viral strains is countered by the development, over time, of antibodies which show greater breadth of reactivity and neutralization activity. Indeed, a longitudinal study comparing breadth of neutralizing activity to the duration of infection showed a significant positive correlation between the two parameters.<sup>4</sup>

All of the monoclonal bNAbs against HIV reported to date have been isolated from human repertoires, none from immunized mice. This finding is not surprising given the aforementioned dynamic evolution of the immune response during infection. Immunization of an animal with a single strain of virus will produce many antibodies specific for that strain, but, in the absence of any ongoing replication and selective pressure, the development of significant broad cross-reactivity is unlikely. Human bNAb antibodies represent the immune response of the cognate host in response to the evolution of a natural infection; an immune repertoire that is distinct and more relevant than that of a murine or synthetic repertoire.

When interrogating the human repertoire, it is feasible to identify by serological means the sought after broadly neutralizing anti-HIV activities. However, extensive sampling of many individuals may be required if an activity is rare. Indeed, Walker et al. screened more than 1,800 samples, obtained based on medical history from a much larger population of HIV positive patients, to identify the donor used in their study. However, a broadly neutralizing serological reactivity may be due either to the aggregate activity of multiple antibodies, to the presence of a single broadly neutralizing antibody, or some combination. To determine which the case is, the isolation of individual mAbs is required.

### **Key Considerations for Comparing Methods of Isolating Human Monoclonal Antibodies**

**How many B cells need to be examined in order to find a desired antibody specificity?** It has been reported that in humans the frequency of B cells producing antibody to a specific antigen, in this case tetanus toxin, are in the range of  $1 \times 10^{-3}$  to  $1 \times 10^{-4}$ .<sup>14,15</sup> However, the natural immune response is polyclonal and the relative frequency of B cells in circulation that express any particular specificity is certain to be much lower. At these frequencies, it is clearly desirable to screen tens of thousands of B cells in order to have confidence that a serologically observed specificity will be represented in the sample. The ability to effectively survey the entire immune repertoire of any individual will thus be dependent on the efficiency of the method used with respect to the percentage of the B cell repertoire for which the antibody specificity can be assayed and recovered. The memory B cell compartment provides a history of immune responses in an individual and may be maintained through polyclonal activation.<sup>15</sup> In the absence of ongoing antigen stimulation, the serum concentration of specific antibody is correlated with the frequency of the corresponding antigen-specific B cells. Thus, antibody specificities observed serologically should be recoverable from circulating memory B cells.

**What characteristics of the repertoire can be assayed in initial screening? Can both binding and biological function be tested?** As noted above, very large numbers of B cells must be screened to adequately assess the repertoire of antibody reactivities. These assessments will therefore generally require high-throughput (HTP) screening methods. All methods that will be considered provide the opportunity to assess binding and assays such

as ELISA have predominated in this regard; however, binding assays require the a priori selection of what is to be bound and do not necessarily allow for the discovery of novel targets with neutralizing epitopes. Because the HIV env is comprised of only gp120 and gp41, for which recombinant constructs are available, one might expect that in this case binding assays alone would be sufficient and finding new targets unlikely, but assays for binding can be surprisingly limited as the proteins are generally expressed and presented in a non-native context such as ELISA. The HIV env complex is a trimeric structure and recapitulating potentially critical quaternary or allosterically induced epitopes may not be possible outside the virus or cell envelope. Recent efforts have mapped the specificity of neutralizing antibody activities in individual sera by selectively depleted antibodies using recombinant protein and synthetic peptide constructs.<sup>9,16</sup> Although neutralization of sensitive viruses was accomplished by depletable antibodies, a significant proportion of the broadly neutralizing activity against resistant viruses came from antibodies of unknown (non-depleted) specificity. These serological results are borne out by the very broadly neutralizing antibodies isolated by Walker et al. that did not bind to recombinant proteins used in ELISA, but did bind to a natively expressed HIV env complex.<sup>12</sup>

Thus, the ability to assess function, e.g., neutralization, in the absence of binding assays (due to constraints on the generation of a suitable binding assay reagent) can result in the identification of novel reactivities. However, assays for neutralization of infection are likely to require much higher concentrations of antibody than assays for binding. Functional inhibition of HIV infection may require concentrations in the range of 10–100 µg/mL or more, while binding assays can detect levels in the range of pg/mL.<sup>3</sup> Therefore, the yield of antibody is a key parameter for consideration in developing initial screening strategies and assays must be compatible with the concentration of antibody produced.

#### **Are the recovered antibodies indicative of the native response?**

Antigen recognition is generally dependent on the CDR regions of both heavy and light chains of an antibody. Although antibody specificities are usually dominated by CDR3 of the heavy chain, the fine specificity may be composed of contributions by any or all of the CDRs of either heavy or light chain.<sup>17</sup> To faithfully recapitulate binding specificities or activities observed in serological screening, native heavy and light chain pairings, i.e., as expressed by human B cells, are likely to be required. In addition, examination of native heavy and light chain pairings may provide valuable information regarding the evolution of the humoral immune response. Antibodies that are isolated as high-affinity binders have generally undergone significant somatic mutagenesis. However, antigen recognition by naïve B cells precedes maturation and vaccines may need to be optimized to ensure that this recognition is preserved. If native pairings are not a priority, the mispaired heavy and light chain pairs that dominate combinatorial libraries may be sufficient, and may in fact produce additional specificities not seen in the native repertoire.

#### **How can the human immunoglobulin repertoire be accessed?**

The following major approaches that have been employed for the generation of neutralizing HIV antibodies include human hybridoma, EBV transformation, non-immortalized B cell culture,

combinatorial display and clonal sorting. All of these methods have been utilized for the generation of neutralizing HIV antibodies and examples will be cited. As previously noted, a broadly neutralizing anti-HIV immunoglobulin response is a challenge that only human sources have thus far been able to address.

## **Human Hybridoma**

In the same way that murine mAbs are generated by fusing splenocytes with a myeloma cell line, human monoclonals can be created from human B cells. Peripheral blood lymphocytes (PBL) from a human subject and a suitable immortalized cell line are fused to form a new hybrid cell. This effectively confers immortality on the B cell and provides a stable source for antibody expression. Because antibodies are expressed by the original B cell, native chain pairings are preserved.

Fusion is usually stimulated by either polyethylene glycol (PEG), electrofusion, or a combination of the two. Typical fusion efficiencies are generally in the range of  $10^{-5}$  to  $10^{-6}$ . Although recent improvements have increased fusion efficiency significantly, to 0.43% ( $4 \times 10^{-4}$ ), it remains well below that achievable by other methods.<sup>18</sup> Considering the relative scarcity of specific B cells discussed above, fusion efficiencies this low are inadequate to allow a comprehensive analysis of the human B cell repertoire.

Another long-standing limitation of fusion methodology has been the lack of an optimal drug-resistant myeloma cell line as the fusion partner. Recent advances in the understanding of molecular mechanisms of cell immortalization have led to improved and more viable cell lines. For example, a significant improvement in human mouse heterohybridomas was achieved by using a murine cell line ectopically expressing murine IL-6 and the protein subunit of human telomerase (TERT).<sup>19</sup>

Despite these limitations, the use of hybridoma formation has enabled the identification of the broadly neutralizing HIV antibodies 2F5, 2G12 and 4E10;<sup>6,7</sup> however, the shortcomings of the process are also apparent in these studies. Due to the low efficiency of immortalization, a total of more than  $3 \times 10^9$  B cells, obtained from several individuals, were fused with a yield of 361 HIV-specific binding wells of which 10% yielded a stable cell line resulting in a net efficiency of  $10^{-8}$ . Cultures containing anti-HIV hybridomas were identified by ELISA using recombinant proteins and immunofluorescent staining of infected cells. Perhaps because B cells from many donors were used, no correlation of specific donor with antibody has been cited, and each antibody may be derived from a different donor. Deciphering an individual serological response requires that the donor source of any specific antibody be known.

## **EBV Transformation**

Infection with EBV leads to a generalized activation of B cells and the immortalization of a subset of these cells. Activation causes increased Ig expression with or without concomitant transformation.

Defining the subpopulation that is susceptible to transformation has yielded conflicting results that depend on the method

of subpopulation enrichment and the assay used to assess transformation efficiency. Although a broad range of B cells can be infected by EBV, only those in a resting state (~40–60% of cells harvested from human blood) are susceptible to transformation.<sup>20</sup> In addition, it appears that a virus-independent activation of infected cells is required for eventual transformation. This observation has been borne out by the development of an improved method of EBV transformation that includes nonspecific stimulation of cells with CpG oligonucleotide.<sup>21</sup> As with hybridomas, native chain pairings are preserved in the expression from B cells.

Because EBV transformation can lead to immortalization, cultures have generally been initiated with many B cells (tens to thousands) in each well of a culture plate. Once an assay of culture supernatants has identified wells that produce the desired antibody specificity, these can be sub-cultured to produce a monoclonal cell line. EBV immortalized cells grow and divide relatively slowly and may produce limited quantities of antibody. Indeed, cell culture times of 4–8 months were required to produce sufficient antibody to stain SARS-CoV spike.<sup>21</sup> The ability to generate a stable clonal cell line can be a boon in that a single specificity can be isolated from a complex mixture through repeated rounds of subculture; however, there is inevitably a loss of many clones during this process, as truly long-term stability is achieved by only a fraction of initially proliferating B cells. To combat this problem, EBV activation has often been followed by hybridoma formation.<sup>22,23</sup> Another means of improving the proliferation of EBV infected cells is by conducting B cell culture in the presence of a large excess (typically >1,000-fold) of irradiated feeder cells. Both human and murine cell lines have been used as feeders, as well as irradiated allogeneic mononuclear cells.<sup>21</sup>

Values as high as 30–100% have been reported for the efficiency of EBV transformation,<sup>21</sup> but in many cases the use of polyclonal cultures makes accurate scoring of transformation efficiencies impossible; limit dilution experiments are required for this purpose. While transformation efficiencies of 20% or higher can be measured by individual cellular expression of the nuclear antigen EBNA, only a fraction of these cells become immortalized with typical efficiencies in the range of 1–3%.<sup>24</sup>

Many antibodies to HIV have been obtained by EBV transformation of human B cells, although none fall in the category of broadly neutralizing.<sup>25–28</sup> Complicating the already low efficiency of EBV transformation, B cells from HIV-infected subjects have a lowered proliferative potential.<sup>29</sup> The ramifications of B cell exhaustion are evident in a report in which only seven HIV reactive clones were isolated after EBV transformation of >10<sup>9</sup> PBMC from 58 subjects.<sup>30</sup>

### Non-Immortalized B Cell Culture

B cells are a naturally proliferative cell type. In response to infection, memory B cells undergo rapid expansion and differentiation to IgG-secreting plasma cells to produce the corresponding antibody response. This *in vivo* expansion has been replicated to some degree for production of antibodies in culture without immortalization.<sup>31,32</sup> Under appropriate culture conditions, B

cells have been reported to expand as much as 100-fold or more over the course of two weeks, after which no further growth occurs. High concentrations of antibody, up to >1 µg/mL in a polyclonal culture, are produced in this proliferative burst.

The efficiency reported for this method was by far the highest with 20–90% of B cells proliferating as determined by limiting dilution.<sup>31,33</sup> Although the antibodies produced by this approach will consist of native chain pairings, the lack of an immortalized cell line presents challenges in the identification of corresponding individual gene sequences. However, the use of limit dilution or flow cytometry to produce monoclonal cultures, coupled with advances in molecular biology tools, have eliminated what were once barriers to its routine use for antibody discovery.

As previously noted, Walker et al. recently reported using non-immortalized B cell culture in the identification of two new antibodies to HIV that are both of high affinity and broadly neutralizing.<sup>12</sup> The efficiency of the method allowed for the screening of antibodies from approximately 30,000 IgG positive memory B cells. In addition the high levels of antibody production made possible a screen for antibody activity in a functional assay for neutralization of HIV infection using two different HIV strains, SF162 and JR-CSF.<sup>3</sup> Unexpectedly, of the antibodies that neutralized strain SF162, 46.5% were ELISA negative while 97.7% of those that neutralized JR-CSF were similarly ELISA negative. Indeed, the two new broadly neutralizing HIV antibodies (PG9 and PG16) did not bind to either recombinant gp120 or gp41 in ELISA. These antibodies were found exclusively by their ability to neutralize HIV infection, and their discovery underscores the importance of a strategy that employs viral neutralization as a primary screen.

### Combinatorial Libraries from B Cells

The general methods of antibody identification from combinatorial display libraries are well developed and documented elsewhere for phage display and yeast display.<sup>34,35</sup> In brief, a repertoire of antibody heavy and light chain mRNA sequences derived from a human donor are separately amplified using RT-PCR. Heavy and light chains are then assembled in a combinatorial fashion as linked constructs producing either single chain (scFv) or Fab structures, and then recombinantly expressed as a fusion with a phage coat protein or yeast cell surface protein. Such libraries have been made from blood samples from both naïve and antigen-exposed subjects, and it is the latter that are most relevant in the context of this review. The resulting library is subjected to successive rounds of enrichment for antibody fragment members that bind to a specific target antigen followed by expansion of the binding fraction. Identification of specific antibodies therefore requires the *a priori* selection of a target protein that, in turn, must be suitably derivatized or immobilized. Enrichment from phage libraries generally entails binding the phage to immobilized antigen and washing away unbound phage (panning), whereas enrichment from yeast libraries is accomplished by cell sorting. After several rounds of enrichment, the great majority of the remaining library consists of fragments that bind to the target. Once constructed, a combinatorial library can be propagated and repeatedly probed using different targets or enrichment criteria.

Unlike other methods being considered in this review, display technologies employ antibody fragments rather than native full-length antibodies, and the former may have altered binding characteristics.<sup>36</sup> In addition, such fragments are produced in a heterologous system (*E. coli* or yeast) rather than a human B cell. Heterologous expression of human antibody fragments may result in a partial loss of the repertoire.<sup>36</sup>

The efficiency of combinatorial display libraries in recovering antibody specificities is considerably more difficult to assess than that of any of the other methods described above. Although a phage library can theoretically represent the entire human repertoire ( $\sim 2 \times 10^{12}$ ), in reality such representation is very difficult to accomplish. This is in no small measure due to the number of host cells that must be transformed in order to express the library. The completeness of any given library will also depend on the primer sets chosen for amplification. For example, in the case of the light chains, separate kappa and lambda reactions are required due to low sequence homology between the respective genes. In actuality, the size of a library is generally limited by practical considerations with many libraries in the range of  $10^8$  members. Such a library is the theoretical combinatorial product of  $10^4$  heavy and  $10^4$  light chains. Combinatorial libraries will presumably contain the native pairings of heavy and light chains among a background of many non-native pairings, correspondingly, for a  $10^8$  library, which would be  $10^4$  native pairings and  $9.999 \times 10^7$  mispairings. Further, if a  $10^8$  library is constructed starting from more than  $10^4$  cells, native chain pairs will be correspondingly under-represented. However, many of the non-native pairings are still capable of binding to the target antigen.<sup>37,38</sup> Indeed this ability to readily substitute light chains has been used as a method of in vitro affinity maturation.<sup>39</sup>

Although many anti-HIV antibodies have been identified from combinatorial phage libraries only one, b12, is broadly neutralizing.<sup>8,40,41</sup> The library from which b12 was generated was an IgG1-kappa library of  $\sim 10^7$  members and no corresponding lambda library was reported.<sup>40</sup> For comparison, a similar yeast library contained both kappa and lambda chains with a combined library size of  $\sim 4\text{--}5 \times 10^7$ .<sup>36</sup> b12 has a very long CDR3 and blocks the CD4 binding site of gp120. Interestingly, the crystal structure of b12 in complex with gp120 shows no contacts between the light chain and gp120.<sup>42</sup> This unusual finding may be a result of the isolation of a mispaired heavy and light chain. Given that no contacts are made by the light chain, selection of this particular chain pair from the combinatorial library may be due primarily to a minimal impact of the light chain on binding.

### Clonal Sorting

In addition to producing soluble antibody, B cells express the corresponding B cell receptor on their surface. Therefore, incubation of a population of B cells with a fluorescently labeled antigen will selectively stain cells that express antibodies recognizing that antigen. These antigen-specific B cells can then be sorted from the larger population by flow cytometry and placed individually in microtiter wells. Subsequent single-well PCR and sequencing

enables the identification of a large number of antibodies that recognize the antigen.<sup>43</sup> As with combinatorial display libraries, this technique is limited by a priori determination of the target antigen. Likewise, functional assessment of any individual antibody requires the subsequent recombinant expression of the corresponding heavy and light chains after single-cell RT-PCR. While cloning and expression of large numbers of heavy and light chain genes can be laborious and rate-limiting, the use of linear expression cassettes may greatly simplify and expedite this step.<sup>44</sup> As with the various direct culture methods, the native heavy and light chain pairings are preserved using this technique.

Direct clonal sorting was recently exploited to identify individual B cells recognizing an artificially trimerized construct of the gp140 antigen of HIV.<sup>45</sup> The resultant clones represented 0.5–1.9% of the total CD19<sup>+</sup> B cells. Of the 502 recombinant clonal products tested for binding to recombinant antigen, 433 exhibited specific antigen binding and were found to represent 134 unique antibodies. The very high number of clones that can be assessed using this methodology makes it amenable to queries regarding the frequencies of individual antibodies and reactivities. Although none of the identified antibodies was found to be broadly reactive across various HIV strains, the collective reactivity of the individual antibodies corresponded to a broadly neutralizing response. A similar approach was recently reported to have produced broadly neutralizing HIV antibodies, but few details are yet available.<sup>13</sup>

### Production of Recombinant Human Antibody

Although both hybridoma technology and EBV immortalization both result in immortalized cell lines, neither can achieve the culture yields of antibody typically observed using recombinant mammalian cell lines designed for antibody production, e.g., CHO cell derivatives. For all methods, the recombinant production of antibody is an eventual necessity to produce the significant quantities required for purification and characterization of functional attributes in vitro and in vivo. In the case of clonal selection by antigen binding, the cloning of both heavy and light chain sequences is an explicit part of the process. For the B cell culture methods, the antibody sequences are recovered after the antibodies of interest have been identified by screening. The tools of modern molecular biology make this process relatively straightforward and methods for expression cloning of immunoglobulin chains, including primer sequences, are published for both phage display and single B cells.<sup>46,47</sup>

### Summary

Although all of the methods described above can provide access to the human antibody repertoire, there are clearly differences in the relative merits of each depending on specific goals, Table 1. For comprehensive analyses and recovery, the optimal method should: (1) have a high efficiency to enable characterization of the entire repertoire, (2) provide sufficient antibody to allow for assessment of function as well as binding specificity and (3) retain native chain pairings to recapitulate the biology of the

**Table 1.** Summary table of method characteristics

	Efficiency <sup>a</sup>	Bioassay <sup>b</sup> compatible	Native chain pairings	Stable expression <sup>c</sup>
Human Hybridoma	Low	Yes	Yes	Immortalized cell
EBV Transformation	Low-Moderate	Yes	Yes	Immortalized cell
Non-immortalized B cell culture	High	Yes	Yes	Recombinant
Combinatorial Display	Low	No	Unlikely	Recombinant
Clonal Sorting	High	No	Yes	Recombinant

<sup>a</sup>Percentage of input B cells for which the corresponding antibody specificity can be assessed. <sup>b</sup>Generally cell-based assays, not including binding assays for which all methods are suitable. <sup>c</sup>Potential source for long-term expression of antibody after identification.

native repertoire. The only method excelling in each of these key areas is non-immortalized B cell culture.

Although both human hybridoma and EBV transformation provide the convenience of producing cell lines capable of relatively long-term expression of antibody, low efficiencies of transformation may limit their ability to adequately examine the antibody repertoire. Non-immortalized cell culture has the highest efficiency of the culture methods and the clonal interrogation of antibody from tens of thousands of B cells is feasible.

Each of the methods allows the assessment of binding specificity; however, only the culture methods produce full-length native antibody without the prior determination of binding, thus making primary functional screening possible. Indeed, combinatorial display and clonal sorting rely exclusively on high affinity binding for the identification of clones, thus requiring that some prior knowledge of the target be available. Any determination of function can only be secondarily tested using recombinantly produced antibody.

Native chain pairings should be unambiguously identified by all methods except combinatorial display. Although a native chain pairing may be among selected clones, conclusive identification of a native pair will not be possible.

### Beyond HIV: Cancer, Autoimmune Disease, Healthy Donors

In addition to the importance of the human immune repertoire in the development of an effective vaccine for HIV, there are many other applications for antibodies from the human immune repertoire. In the context of cancer, an immune response can be generated to self proteins that are specific to the cancer.<sup>48,49</sup> Antigens may be expressed by cancer cells that are not present or present only at low levels on normal cells. Such antigens may therefore be cancer-specific, and provide potential targets for cancer immunotherapy. One means of identifying such antigens is through screening human antibody repertoires from cancer patients to identify tumor-specific antibodies and working backward to find the cognate antigen.<sup>50</sup>

In the development of autoimmune disease, antibodies to self-proteins may be pathological in the disease process. However,

among the antibody reactivities reported are those for which therapeutic potential has been observed, including GM-CSF, IFN $\alpha$ , IL-12 and IL-6.<sup>51-53</sup> If such antibodies can be identified and produced recombinantly, they may provide effective therapies for autoimmune diseases in which the overexpression of the targeted protein is pathological.

The therapeutic use of intravenous (IV) IG is clinically efficacious in several diseases including, among others, Alzheimer's disease (AD) and systemic lupus erythematosus (SLE).<sup>54,55</sup> Although the exact mechanism by which IVIG works is still in dispute, the efficacy in both these diseases may be attributable to specific antibody reactivities. For AD and SLE, potential therapeutic mechanisms have been ascribed to reactivity with  $\beta$ -amyloid and IFN $\alpha$ , respectively.<sup>56,57</sup> However, in IVIG any single specificity will be rare amidst the polyclonal mix of mostly irrelevant antibodies. Even among antibodies that bind to the intended target, there may be both antibodies that are protective and non-protective, as well as non-protective antibodies that block the activity of protective ones.<sup>58,59</sup> Clonal identification of the particular antibodies responsible for the beneficial effects may provide new and more optimal therapies for these poorly treated diseases.

One reason often cited for generation of human antibodies is minimization of the potential risk of immunogenicity should an antibody reach clinical testing. Despite the relatively widespread use of mice expressing human immunoglobulin genes, and the many methods of humanization that have been described, this remains an area of concern. An antibody derived from a human repertoire will not only be fully vetted for immunogenicity by the donor, but potentially also for harmful cross-reaction to other targets.

With the recent advances seen in the methods described above, the human antibody repertoire is more accessible than ever before. Perhaps the initial promise of the human antibody repertoire, subsequently supplanted by various murine antibody sources, will now be more effectively realized in the discovery of new human antibodies.

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