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Screening and isolation of broadly neutralizing HIV-1 antibodies

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Abstract

Passive immunization for prevention and treatment of HIV-1 infection still plays important roles in fighting against HIV/AIDS. Recently, a panel of potent broadly neutralizing antibodies (bnAbs) against HIV-1 has been isolated. These potent antibodies are considered of promise in passive therapeutic treatment and the guidance of vaccine design. In this brief review, current techniques in monoclonal antibody screening and isolation have been discussed. Screening and isolation of broadly neutralizing HIV-1 antibodies can provide insights in both efficient vaccine design and therapeutic drug design, as well as the knowledge for antibody screening against other pathogens.

Introduction

HIV/AIDS has become a worldwide pandemic; however, there is no effective HIV-1 vaccine which has been fully developed. In this situation, passive immunization for prevention and treatment of HIV-1 infection still plays important roles in fighting against HIV/AIDS. Broadly neutralizing antibodies (bnAbs) against HIV-1, which have attracted considerable attention, are considered of holding the promise of passive therapeutic drug design and the guidance of vaccine design. The observation that 20% HIV-1 infected individuals generated cross-reactive neutralizing antibodies two to four years after infection has lighten the interest of HIV-1 specific broadly neutralizing antibody isolation. Current techniques in broadly neutralizing monoclonal antibody isolation can be described in four dominant ways, including hybridoma technique, B cell immortalization, display techniques, and flow cytometry based single cell sorting and cloning [1].

Hybridoma technique, B cell immortalization are traditional methods in monoclonal isolation [2-8]. Hybridoma technique requires effective fusion of B cells and partner cells followed by large scale screening of individual antibody producing cells by measurement of supernatant. Similarly, there is another way to immortalize human B cells via Epstein-Barr Virus (EBV) mediated transformation [9-14]. EBV-binding receptor positive B cells can be immortalized by EBV infection to generate antibodies. Application of CpG DNA during EBV infection can increase the rate of B cell transformation, which can be further used to isolate monoclonal antibodies secreted by transformed B cells. In conventional method of EBV transformation, the B cell immortalization efficiency is quite low (1%). Lu et al. 2017 optimized the EBV transformation by multiple aspects including viral concentration, cytokines, co-culture with feeder cells, cell density [15,16]. The estimated efficiency of the method can get

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7.8% (0.6%–20%), which is much higher than that of the conventional method. Epstein–Barr Virus (EBV) transformation has become a useful tool in generating immortalized B cells. Both hybridoma cells and immortalization of human B cells can provide a substantial resource of human B cells for the subsequent screening.

Display technique is a library construction based method which allows the screening of antibodies from a large recombinant library [17-22]. Antibodies can be displayed in the form of either single chain variable fragments (ScFv) or antigen-binding fragments (Fab), or full length IgG, or any other creative formats [23-27]. Display techniques include phage display, yeast display, mammalian cells display, and ribosome display. Recombinant antibody library can increase the diversity of antibodies in B cell repertoires, which promotes the screening of antibodies with novel properties. Antibody libraries are generally constructed by random-assembling of antibody heavy and light chain variable regions, and to further increase the diversity through gene shuffling of the heavy and light chains.

Development of single cell sorting and cloning is an important advance [28-30]. This methodology is efficient in cloning antibody heavy and light chain from extremely rare and highly discrete antigen specific single memory B cells or plasma cells. This method requires accurate design of probes in the procedure of sorting single B cells. Generation of RSC3 and ΔRSC3 is one successful example of computer-assisted probe design in fishing potent broadly neutralizing HIV-1 monoclonal antibodies. The Resurfaced Stabilized Core 3 (RSC3) is a functional structure core with preserved antigenic structure of the CD4 binding site and substitution of other antigenic regions with Simian Immunodeficiency Virus (SIV) residues. ΔRSC3 contains one amino acid deletion at position 371 in RSC3, which can knock out the function of binding with CD4 binding site antibodies. VRC01 is an antibody isolated by fishing out the single memory B cell which can positive bind RSC3 and negative bind ΔRSC3. VRC01 can neutralize up to 70% of the tested pseudo viruses with an IC50 below 1ug/ml [31-33]. Another successful example is the design of an engineered crystal structure of the HIV-1 Env trimer with an exposed native glycan shield of high-mannose and complex-type N glycans. This design results the definition of IOMA, a new CD4-binding site (CD4bs) antibody [34].

With the recent development of monoclonal antibody cloning and isolation techniques, an increasing number of Human Immunodeficiency Virus type 1 (HIV-1)-specific broadly neutralizing antibodies have been defined. In some infected patients, Unmutated Common Ancestors (UCA) of broadly neutralizing HIV-1 antibodies has been identified. And this Unmutated Common Ancestors (UCA) is eventually matured to potent broadly neutralizing antibodies. Knowledge regarding these antibodies maturation *in vivo* is limited. An HIV-1-specific naive B-cell line was established from screening of immortalized naïve B-cell libraries derived from healthy PBMC donors. This artificial naive cell line acquires a lymphoblastic phenotype, and no expression of activation-induced cytidine deaminase was observed [15]. This cell line provides a model for antibody maturation by the stimulations of different antigens *in vitro*.

In most of HIV-1-infected individuals which can develop high titers of broadly neutralizing HIV-1 antibodies, the monoclonal antibodies (mAbs) isolated do not, in most cases, depict the serum IgGs in neutralizing the virus. One possible reason could be HIV-1-specific antibodies in infected subjects may work in a population manner in containing the virus *in vivo*; the other possible reason could be that the current techniques are not sensitive enough to fishing out the potent mAb-generating B cells. Most of methods for isolation of broadly neutralizing antibodies are rely on binding affinity, which results in the loss of antibodies with low binding affinity to antigen but high potency in neutralization. Sun et al. described a way of antibody isolation by neutralization directly, but this method also has limitations, including the low efficiency, and the requirement of high efficiency in cell transfection [35]. Another way of increasing the antibody isolation efficiency is to engineer the fishing agents used for sorting or panning. A recent successful example is the success of rapid elicitation of broadly neutralizing antibodies to HIV-1 by immunization of BG505 SOSIP in cows [36]. BG505 SOSIP is a next-generation HIV-1 Env Trimer, which can expresses multiple epitopes for broadly neutralizing as the concept was previously described [37,38].

In recent decades, the technology advances allow for human monoclonal antibodies to be isolated efficiently. These monoclonal antibodies can be used for therapeutic purpose, and also provide the knowledge for vaccine design, for they are serving as a potential source of discovering neutralizing epitopes that can be targeted. All these observations highlight the roles of broadly neutralizing antibodies in HIV-1 prevention and treatment. Altogether, screening and isolation of broadly neutralizing HIV-1 antibodies, can not only help to guide efficient vaccine design or therapeutic drug design, but also provides the knowledge for antibody screening against other pathogens.

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