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Therapeutic antibody discovery in infectious diseases using single-cell analysis

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Abstract

Since the discovery of mouse hybridoma technology by Kohler and Milstein in 1975, significant progress has been made in monoclonal antibody production. Advances in B cell immortalization and phage display technologies have generated a myriad of valuable monoclonal antibodies for diagnosis and treatment. Technological breakthroughs in various fields of ‘omics have shed crucial insights into cellular heterogeneity of a biological system in which the functional individuality of a single cell must be considered. Based on this important concept, remarkable discoveries in single-cell analysis have made in identifying and isolating functional B cells that produce beneficial therapeutic monoclonal antibodies. In this review, we will discuss three traditional methods of antibody discovery. Recent technological platforms for single-cell antibody discovery will be reviewed. We will discuss the application of the single-cell analysis in finding therapeutic antibodies for human immunodeficiency virus and emerging Zika arbovirus

1. Introduction

The humoral adaptive immunity elicits a protective immunological response against a pathogen. Unlike the innate response, humoral immunity is dependent on the extensive diversity of antigen recognition repertoires of the receptors expressed on B cells. Governed by allelic exclusion, each B cell should express solely one heavy chain and one light chain allele of immunoglobulin, therefore should produce an antibody that binds to one specific antigen. Some of these antigen-specific B cells differentiate into plasma cells to produce potent monoclonal antibodies (mAbs) and some develop into memory B cells to be reactivated for subsequent pathogen exposure. Plasma cells are short-lived, whereas memory B cells are rare and difficult to reactivate in culture. Major technological advances have been attempted to harness the therapeutic power of adaptive immunity, specifically the effector function of mAbs. Since the discovery of mouse hybridoma technology by Kohler and Milstein in 1975, where immortalized myeloma and spleen cells were fused to produce anti-

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sheep red blood cell antibodies[1], the field of antibody discovery and application has evolved into a thriving industry comprised of basic research, diagnoses, and therapy. Global sale of monoclonal antibody products has increased dramatically from $39 billion in 2008 to $75 billion in 2013[2]. Global sales revenue is expected to grow to $122.6 billion in 2019[3]. The impetus for antibody development is the high specificity and affinity to an antigenic target that can either activate, inhibit, or block the target. Furthermore, the continued interest for antibody products is driven by the technological advancement of genomics, transcriptomics, proteomics, and metabolomics which identifies new targets of specific biological pathways that can be utilized to mitigate the disease process.

The Food and Drug Administration approved the first mouse mAb specific against CD3 (known as orthoclone OKT3; Ortho Biotech) for treatment of acute rejection of cadaveric renal transplantation. OKT3 was highly effective for acute renal-allograft rejection in a prospective randomized multicenter trial [4]. However, antibodies of mouse origin have not been successful due to human anti-mouse immune response in patients. To circumvent these challenges, a number of engineering approaches have been undertaken. For example, chimeric antibodies with mouse variable domain regions fused to human constant regions were tested[5,6]. Another approach is antibody humanization in which by grafting mouse complementary determining regions (CDRs) that were evolved to bind to specific antigen into human immunoglobulin (Ig) backbone[7]. Other approaches have been attempted including human hybridoma technology and humanized transgenic animals in which the mouse Ig repertoires are replaced with human Ig repertoires. These technological variations have helped expand the therapeutic mAb product market in which 36 FDA-approved therapies constitute nearly 40% of the biologics market and 350 mAbs are currently in clinical trials [8-10].

Hybridoma technology and immortalization of antigen-specific B cells have been the traditional methods of mAb production. Sorting of desired B cell subset using fluorescence activated cell sorting (FACS), recombinant phage display technologies, and application of humanized transgenic mice have remarkably advanced the field. Some of these methods only capture average measurement from bulk or whole cell population undermines the heterogeneity or the autonomy of individual cells[11,12]. Recent developments in microfluidic chamber devices and microfabrication of nanowells designed to identify antigen-specific single cell have revolutionized the process of antibody discovery. In this review, we will discuss the traditional methods of monoclonal antibody production, specifically immortalization of antigen-specific human B cell by Epstein-Barr virus, hybridoma generation, and phage display. We will focus on current platforms for single-cell antibody discovery including fluorescence activated cell sorting, microfluidic devices, and single-cell antibody nanowells. Lastly, we will discuss the application of the single-cell analysis in finding therapeutic antibodies for human immunodeficiency virus and emerging Zika arbovirus.

2. Traditional methods of antibody discovery

2.1. Hybridoma technology and immortalization of antigen-specific human B cells

As mentioned, the hybridoma technique was first introduced to make mouse mAbs[1]. The technique, which has been refined over the years, took sheep red blood cell (SRBC) as immunogen and immunized BALB/c mice. The splenocytes of immunized mice were collected and fused with myeloma cells (Sp-1) to produce hybridoma cells. Immortalized hybridoma cells were selected in the presence of hypoxanthine-aminopterin-thymidine (HAT) selection medium. Unfused cells lack the hypoxanthine-guanine phosphoribosyltransferase (*HGPRT*) gene which makes them sensitive to the HAT selection. The aminopterin blocks the *de novo* DNA nucleotide synthesis pathway, therefore cells must alternatively utilize the salvage pathway to replicate in the presence of hypoxanthine and thymidine. However, the myelomas deficient in *HGPRT* are unable to replicate. As a result, only fused cells inherit a functional *HGPRT* gene from B cells can proliferate and produce antibodies. Antibody-producing B cells are further cloned and expanded by limited dilution using 96- or 384-well plates. The cloning is typically performed in multiple rounds to possibly obtain expanded clones from a single cell. Supernatants are screened by enzyme-linked immunosorbent assay (ELISA) to identify antigen-specific B cell clones (Figure 1). The process is efficient, but it can be time-consuming and labor intensive. Additionally, the resultant antibodies are of mouse origin, thereby preventing direct therapeutic translation to humans. To avoid some of these obstacles, the Epstein-Barr virus (EBV) has also been utilized to help immortalize B cells. The transformation is achieved by the activation of EBV-encoded nuclear proteins (EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, EBNA-LP) and the latent membrane proteins (LMP1, LMP2A, LMP2B) in latently infected B cells. These proteins have multiple functions, but mainly induction of survival, proliferation, and inhibition of apoptosis by upregulating expression of the anti-apoptotic proteins[13]. The advantages of EBV-transformed B cells are the more rapid and efficiency of screening for antigen-specific B cells in comparison to hybridoma method. Additionally, human B cells can be directly transformed to obtain antibodies, therefore there is little concern for anti-human antibody reaction. While the EBV-transformed B cells produce immunoglobulins, they yield lower quantities, which is sub-optimal for application purposes; these cells are notoriously difficult to clone and propagate[14]. The hybridoma technology and EBV transformation have shown promise and are methods that research and industry have adapted and improved, leading to several approved monoclonal antibodies[15-17]. Furthermore, significant advances in antibody engineering have been made to avoid adverse effects like acute anaphylaxis in patients, when treated with hybridoma derived mAbs[18].

2.2. Phage display

Phage display was first introduced in 1985[19], and has proven an effective method for mAb production, in addition to quicker production time when compared to hybridoma technology. The phage display technology is dependent on libraries naïve, immune, semi-synthetic, or synthetic of antibodies[20-22], which represent non-infected, cleared infection or immunized, random sequences paired with those naturally occurring, and purely generated sequences, respectively. Since a naïve library has antibodies which have not undergone a maturation

process, many will have poor binding affinity[23], however high affinity mAbs have been generated[24,25]. In contrast, the immune library is taken from individuals that are immune to the disease, the library is inherently biased for antibodies that are mature and specific to the disease in question[20,26]; hence, a higher frequency of high affinity antibodies can be obtained[20], but new immune libraries must be created for a specific infection or disease, resulting in a limited repertoire. Both semi-synthetic and synthetic libraries use synthetic oligonucleotides to generate diversity within the library, however may generate sequences that negative selection may have removed[20,27]. While semi-synthetic libraries use some natural sequences, both types of libraries are devoid of a natural maturation process[22]. These libraries can be utilized to design phages which present antibodies or fragments of antibodies as part of their protein coat. Diversity is key to the success of a library, however frame-shift mutations and transformation efficiency or the ability for cells to take up extracellular DNA and encode it can be a major concern[20]. Original cloning methods presented a multitude of technical difficulties, which resulted in several other innovations, including PCR-based assemblies[28]. Furthermore, in an effort to increase diversity, molecular methods, such as mutagenesis and sequence evolution[29-33], have been utilized to help increase the diversity in libraries; this can be done at various intervals in the development process.

After diverse libraries are created, the phages expressing various fragmented or entire antibodies must be selected[34] via a process called “panning”. The antigen-antibody complex is put through a series of parameters –e.g. toxicity to host[35]– then the phages are eluted, often via ELISA, amplified, and sequenced[20,36]. Panning can be performed against cells or nanoparticles (semi-automated), which ensures the reactivity/selectivity of the antibodies. However, both are labor intensive and the latter must be restricted to a lesser number of samples, to remain manageable[20,37]. Nevertheless, semi-automated is a robust, reproducible, and efficient method of panning[38]. Panning can be enhanced via the use of next generation sequencing (NGS), which can help eliminate unwanted clones, identify frequent sequences, and the reveal the evolution of the phages; this helps to reduce the number of rounds of panning[39-41]. However, binding affinities are not taken into account in this process. These methods can be used to select desirable phages from diverse libraries, producing sequences that can be tested in a much shorter timeframe than hybridomas, but this method is still tedious and laborious[3].

3. New platforms for single-cell antibody discovery

3.1. Fluorescence activated cell sorting

Fluorescence activated cell sorting (FACS) is an engineering adaption of flow cytometry, in which, cells are obtained or “sorted” based on fluorescent markers. The markers are commonly fluorescent-labeled antibodies against cell-specific proteins/receptors. While flow cytometry has only developed since the 1960s, when the first Coulter counter was produced, it has become a standard for identification of cell subsets. The original Coulter counter was based on the principle that the movement of a cell could be detected via changes in electrical signals as it passed through a microchannel. This has evolved over time, to the current flow cytometer which detects defraction of signal through a series of

detectors when a fluorescently labeled cell passes through a microchannel[42]. The number of detectors available for a flow cytometer (and therefore the number of different fluorescent wavelengths it can distinguish) varies widely, with the high end being able to distinguish nearly 20 signals. As shown in Figure 2, as the cell passes through the microchannel, a laser excites the fluorescent molecule at its specific wavelength(s). The emitted signal passes through a series of bandpass filters (BP) and is able to be distinguished via the detectors. Sorting by FACS is initiated by applying a pulse of electricity to disrupt the droplet containing the cell, so that it is diverted into an appropriate receptacle. Sorting can be used restrict cell populations to those desired for subsequent experiments. Sorting for single cell is becoming a popular tool. For example, sorting of individual antigen-specific B cells were used to isolate HIV-specific antibodies. The high-throughput feature of FACS expedites the downstream applications, such as sequencing VDJ heavy and VJ light chains of single cell and, in this case, via transfection of these amplified DNA sequences into human kidney epithelial (HEK293) cells, produced monoclonal antibodies against an antigen[43]. This technique is heavily translational and can be used for a variety of diseases.

3.2. Microfluidic devices

Microfluidic devices, or chips, started being used in biological applications in the late 1990s, where it was often used as a new immunoassay. The design of these early chips allowed for controlled release of specific reagents, in conjunction with the ability to mix reagents directly on the chip. Pore sizes/lengths and electrolytic buffers control flow rates of each of the components[44,45] (Figure 3). For example, if two reagents need to be mixed on the chip at a disproportionate ratio, the pore size of the lesser component can be made smaller, physically limiting the amount that can be mixed at a time. This protocol is useful when performing whole cell ELISAs, especially when a cell population is limited. While with a conventional ELISA, a significantly larger number of cells and more reagents are necessary. As it is common to replicate samples in duplicate or triplicate, the amounts of reagents can become astronomically higher, making this device an optimal example of high content cell screening (HSC)[46]. With microfluidic devices, the human error is removed; there is no pipetting error nor inconsistencies in plated densities, since these are significantly smaller sample sizes.

In the same fashion as the flow cytometer, this can be used to collect a pool of, or single cells; it may also be used to detect the response or endogenous state of individual cells, e.g. cytokines produced. It should be noted that microfluidic devices encompass a wide range of processes and many different chips can be used. While the chip above is a basic schematic, many variations on this can be used based on the application. In fact, many laboratories design their own. For example, circulating tumor cells (CTCs) are rare, difficult to detect cells which are hypothesized to be the cause of metastatic cancers. A chip was designed for this which is composed simply of a series of posts coated with antibody against epithelial cell adhesion molecule (EpCAM), a common cancer marker. This antibody then captures any cancer cells which come into contact with them on the chip[47]. The CTC chip can be used to enumerate and evaluate this specific cell type, enabling purification from whole blood in a single step. Notably, while this is a fairly simple design for a chip, there were still many calculations and experiments necessary to optimize how cells can be adhered on

this chip. The most important factors here were layout of the posts (including diameter and distance apart), the flow rate, where too high a rate would result in loss of cells, and shear stress, in which the cells would be lysed rendering them useless. By using this same layout, antibodies could be used for a myriad of capture chips. Once a device is optimized, a simple, streamlined process for isolation and/or characterization of single cells has been achieved. This technique allows the user to save both time and money, as the volumes of reagents necessary are quite small. The malleability of this technique to a specific protocol makes it one of the most useful devices, however the time and manpower necessary to establish a single technique may not be practical for some labs.

3.3. Single-cell antibody nanowells (SCAN)

The technology was developed by Christopher Love and colleagues at MIT[8]. SCAN is a soft lithographic technique that uses a dense array of nanowells (50 x 50μm or 30 x 30μm, holding a volume of 0.1–1 nl each) fabricated of polydimethylsiloxane (PDMS) to isolate individual cells for printing of corresponding molecules secreted by each cell. The array of nanowells is fabricated on standard 1”x 3” glass slides containing 84,672 wells for 50μm size nanowells or 248,832 wells for 30μm size. A capture slide coated, for example, with immunoglobulins (Igs) can be hybridized by placement on the top of the nanochip to capture the antibodies being secreted by the corresponding individual live B cells that are seeded in the nanowells (Figure 4). Earlier works have shown that the nanowells with the rapid and high-throughput features were able to identify antigen-specific antibodies[48,49]. With the capability that the single *ex-vivo* cell can be cultured and confined in each nanowell for an extended amount of time, it facilitates the recovery and clonal expansion of cells with specific engraved phenotype[50-52]. Using the arrays of nanowells with multiplexing capability, the Love group was able to examine the isotypes of the secreted antibodies, the specificity and relative affinity for HIV antigens, identify the reactive subset of B cells (memory and plasma B cells), and sequence/identify the genes encoding the heavy and light chains[53]. Using this method, the group isolated HIV-specific neutralizing antibodies in colon biopsies[53]. This method is uniquely able to profile and isolate rare or low frequency B cells. The recent study by Tsioris et al. identified four novel West Nile virus (WNV) neutralizing antibodies in recently infected and post-convalescent subjects[54]. The most interesting aspect of the study was that given a low frequency of WNV-specific B cells (mean <24 events per 100,00 peripheral blood mononuclear cells), the group was able to identify some rare and potent neutralizing antibodies.

4. Application in infectious diseases

4.1. Human immunodeficiency virus (HIV)

According to UNAIDS / WHO, since the start of the HIV epidemic in the 1980s, worldwide 78 million people have become infected with HIV and 36 million people have died from HIV and AIDS-related diseases. As of 2016, 36.7 million people live with HIV. Combinations of highly active antiretroviral therapy (HAART) have been effective since their introduction in1996 and HIV-related mortality has been reduced since then, but remains above one million per year (1.1 in 2015 compared to 2 million in 2005), mainly due to insufficient access to screening and antiretroviral therapy in economically challenged

countries which are often the most affected by the infection[55]. A recent meta-analysis shows that HIV-infected patients without access to HAART have a 2-year survival probability of progression from AIDS to AIDS-related death at 48% and the 6-year survival probability is 18%, whereas this life expectancy is 87% for the 2-year survival probability and 61% for 10-years survival probability for patients who received HAART[56]. An effective vaccine must achieve a production of protective antibodies against vaccine viral proteins. Due to an extensive genetic diversity of HIV, a prophylactic vaccine must provide global protection against all strains[57,58]. Currently there are three principal research directions on HIV treatment and vaccine development using neutralizing antibodies: 1) activation of B cells by sequential immunogens for expression neutralizing antibodies, 2) development of novel neutralizing antibodies due to passive administration, and 3) vector-mediated gene transfer using adeno-associated virus vectors for delivery of HIV broadly neutralizing antibodies (bNAbs) and antibody-like proteins[59-61].

Human hybridoma, EBV transformation, FACS sorting of HIV-specific B cells, and combinatorial display technologies have been utilized in screening for single B cells that produce potent bNAbs. The interest in single cell antibody cloning has increased in the last few years due to advances in high-efficiency and throughput sequencing, which has reinvigorated studies on bNABs to obtain HIV-1 envelope-reactive antibodies[58,62-64]. Initially, cloning from single cells was introduced to examine the development and silencing of autoreactive B cells[65]. This method was performed for identification of single B cells expressing antibodies[62,65,66] or to screen cultured B cells for the production of neutralizing activity[58,67]. Single cells from HIV infected patients are isolated by FACS, then sequences of immunoglobulin genes isolated from each cell are cloned into a vector for protein expression. Obtained bNAbs are analyzed to understand their specificity, protective capacity, binding conformation, and reactivity breadth and potency. Usually, screening of monoclonal antibodies is utilized to elicit a clonal assessment of specificities present in HIV infected patients[68].

Passive administration of bNABs is advised for prevention and therapy of HIV infection. Studies on humans have proven safe and efficacious administration of monoclonal antibodies, yielding a promising approach of total control of HIV infection due to direct engagement in host immunity[69]. These bNABs must have high potency for HIV treatment with a capacity to reduce HIV viral load and minimize or prevent the risk of viral reactivation[59]. Pre-exposure prophylactic treatment has been studied in experiments with untreated non-human primate models infected with simian-human immunodeficiency virus (SHIV). Passive transfer or injection of HIV-1 bNABs protects host against viral infection[70-74]. A single bNABs infusion prevents virus acquisition with a single high dose[72,75,76] or repeated low doses SHIV infection; this protection can be up to 23 weeks depending on antibody potency and half-life[74]. Furthermore, introduction of a mutation in the fragment cytallizable (Fc) domain extends the antibody half-life median protection[74].

4.2. Emerging arboviruses: Zika

Zika virus (ZIKV) infections are an emerging health pandemic of significant medical importance. The current outbreak has garnered attention by exhibiting unique characteristics

of devastating neurodevelopmental defects in newborns of infected pregnant women[77,78]. Over the past year, doctors in Brazil have documented over 4,000 cases of microcephaly in which infants are born with abnormally small heads[79]. Detection of ZIKV in fetal brain tissues and anti-ZIKV antibodies in these mothers and/or infants established a possible causal link between ZIKV infection and this birth defect[80]. Typical symptoms of ZIKV infection include joint pain, fever, and rash. In addition, there is emerging a potential link to the dramatic increase in the reported cases of Guillain-Barré syndrome, another rare disorder of the peripheral nervous system characterized by muscle weakness and paralysis[81,82]; in severe cases, Zika patients require life support. The spread of ZIKV has reached an alarming rate, particularly in the state of Florida. The influx of international travelers or tourists from ZIKV-infected areas, together with the warm tropical climate of the state, promotes the survival of the ZIKV-carrying mosquitoes, thus accelerating the spread of the virus. Responding to the Zika outbreak has been more than challenging. Unlike other well-known flaviviruses like Dengue, West Nile, Yellow Fever, and Japanese encephalitis viruses, there are no treatments or vaccinations, and diagnostic reagents are very limited. Although many investigations using immune-based therapies for arboviral infection have been pursued and have shown promise, there are no commercially available immune-based products for ZIKV. A better alternative would be to develop effective broadly neutralizing antibodies (bNAbs) as passive protection against ZIKV infection and more importantly prevent maternal-fetal transmission, reducing the likelihood of developing microcephaly in the newborns. As an emerging disease, there is a limited number of ZIKV monoclonal antibodies that are currently still at the testing phase (Table 2). Using EBV–immortalized memory B cells that were reactive to ZIKV NS1 or E proteins, Stettler et al. have identified 119 bNAbs capable of neutralizing ZIKV. The authors have shown that the most potent neutralizing antibodies were ZIKV-specific and targeted EDIII or quaternary epitopes[83]. Using tradition hybridoma technology in the mouse, Zhao et al. isolated six mAbs that recognized ZIKV evelope (E) protein after screening more than 2,000 hybridomas[84]. A recent study by Sapparapu et a. demonstrated that EBV-transformed ZIKV-specific B cells exhibited potent neutralizing capacity. Epitope mapping using X-ray crystallography indicated that the most effective bNAb recognized a unique quaternary epitope on the E protein dimer–dimer interface. Further studies showed the therapeutic efficacy in pregnant and non-pregnant mice in which mAb treatment markedly reduced tissue pathology, placental and fetal infection, and mortality in mice[85]. Future studies using single cell selection as proposed in Figure 5 will generate a complete repertoire of ZIKV-specific antibodies, develop better bNAbs and reveal essential epitopes for future structure-based vaccine design.

5. Conclusion

Single-cell analysis is a powerful tool in examining a comprehensive repertoire of antigen-specific Abs from the most abundant to the least abundant B cells that are highly specific. Single-cell antibody discovery is critically important in selecting the few potent B cells with important capacity to produce the most competent therapeutic mAbs and broadly capable of neutralizing pathogens in infected individuals. Diseases in which vaccines are not readily available or effective, therapeutic mAbs can provide significant protection as passive immunity. The two quintessential examples are HIV and Zika as discussed. These

technologies, while strong and important tools currently, have the potential to become widely utilized and even more powerful. They have the potential to be used in diagnostics and beyond that, these techniques are currently being used to develop treatments for other infectious diseases and cancer. In conjunction with shotgun mutagenesis and X-ray crystallography, antigenic epitopes can be mapped and the structural interactions between Abs and antigens can be examined. On a more fundamental level, single-cell analysis will be an essential player in creating immune-therapeutics and eventually vaccines.

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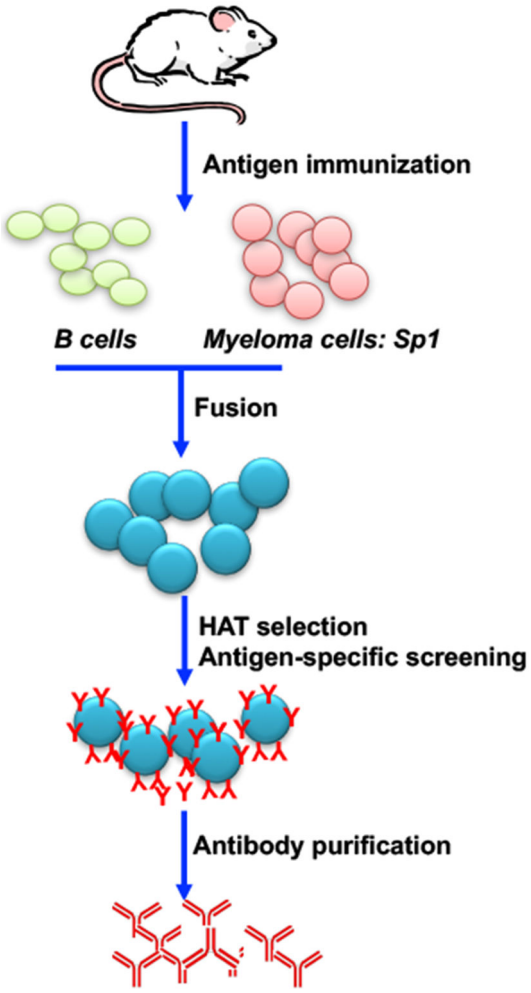

Figure 1. Schematic of monoclonal antibody production by hybridoma technology.

Laboratory mice are immunized with antigen of interest. Spleen cells are isolated and fused with immortalized myeloma cells such as SP1 cell line. Transformed fused cells are selected under HAT media. Antigen-specific B cells are screened using protein analytic methods (ELISA, Western blotting, flow cytometry). Once B cells of interest are identified, serial dilution will be performed to select for single cells which will be clonal expanded. Lastly, antigen-specific B cell clones will be cultured and antibodies will be purified.

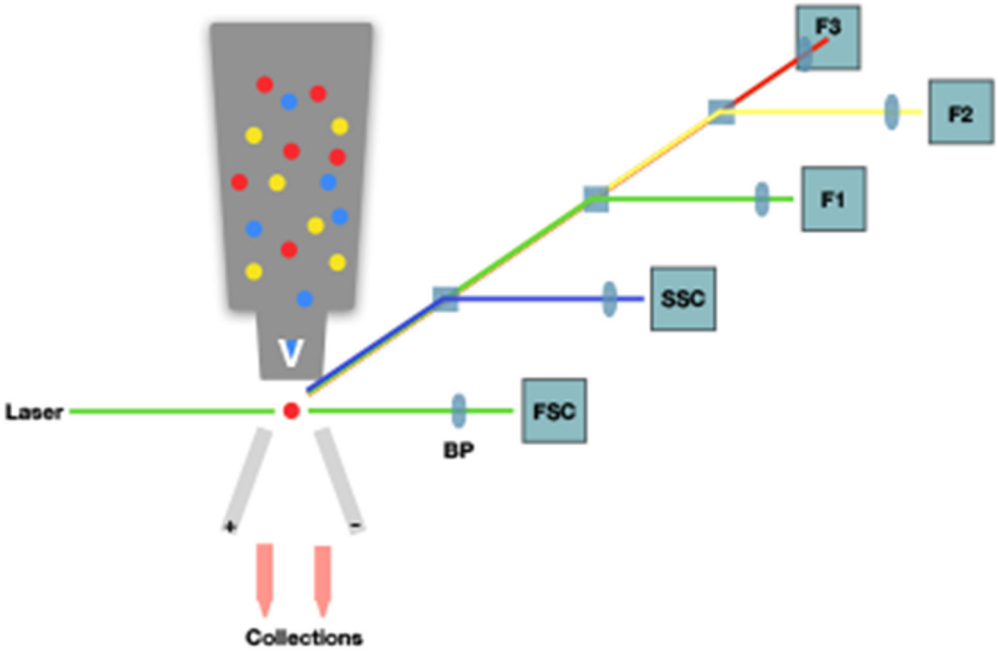

Figure 2. Principles of fluorescence activated cell sorting (FACS).

Fluorescence activated cell sorting (FACS) diagram. Yellow, blue, and red circles represent fluorescently-labelled cells in the flow cytometer, where, upon exiting, they are excited by a laser. Diffraction of the beam is measured through a series of detectors. Forward scatter (FSC) and side scatter (SSC) represent the size and complexity of the cell, respectively. Here, F1-F3 are the detectors and the tubes indicate user-defined positive and negative selection criteria.

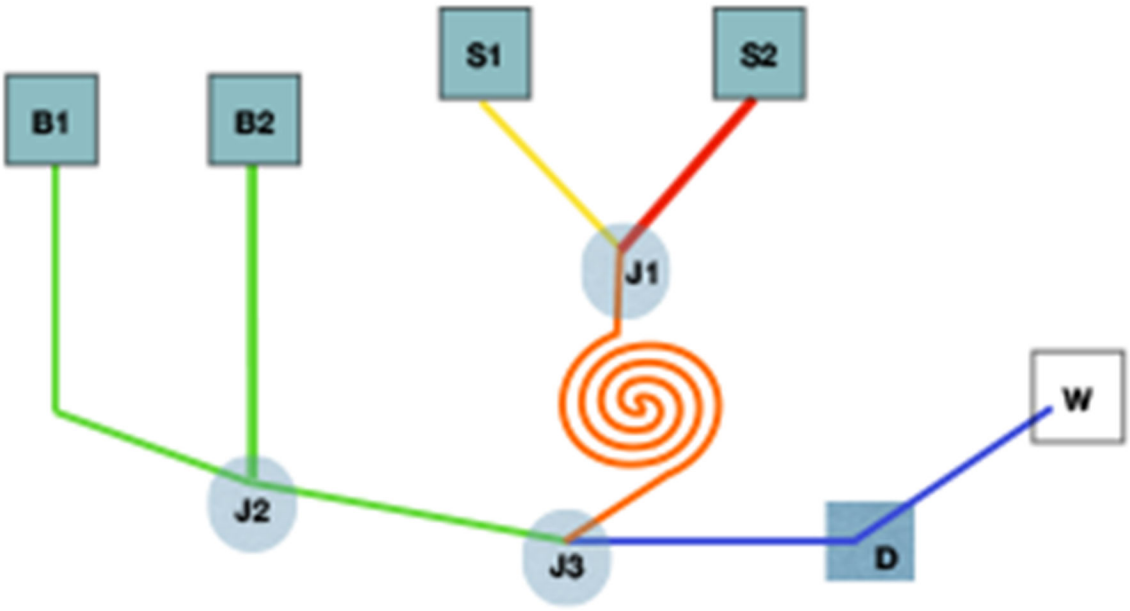

Figure 3. Illustration of microfluidic device for antibody selection.

Basic schematic of a microfluidic device. “B” indicates a buffer, “S” a sample, “J” a junction, “W” is waste, and “D” is a detector. Here, two samples join at junction 1, they then continue through a coil to mix. After exiting the coil, the cells pass through a separation microchannel where cells are further diluted by the buffers (at junction 3). Here they form a single cell suspension for detection before proceeding to waste.

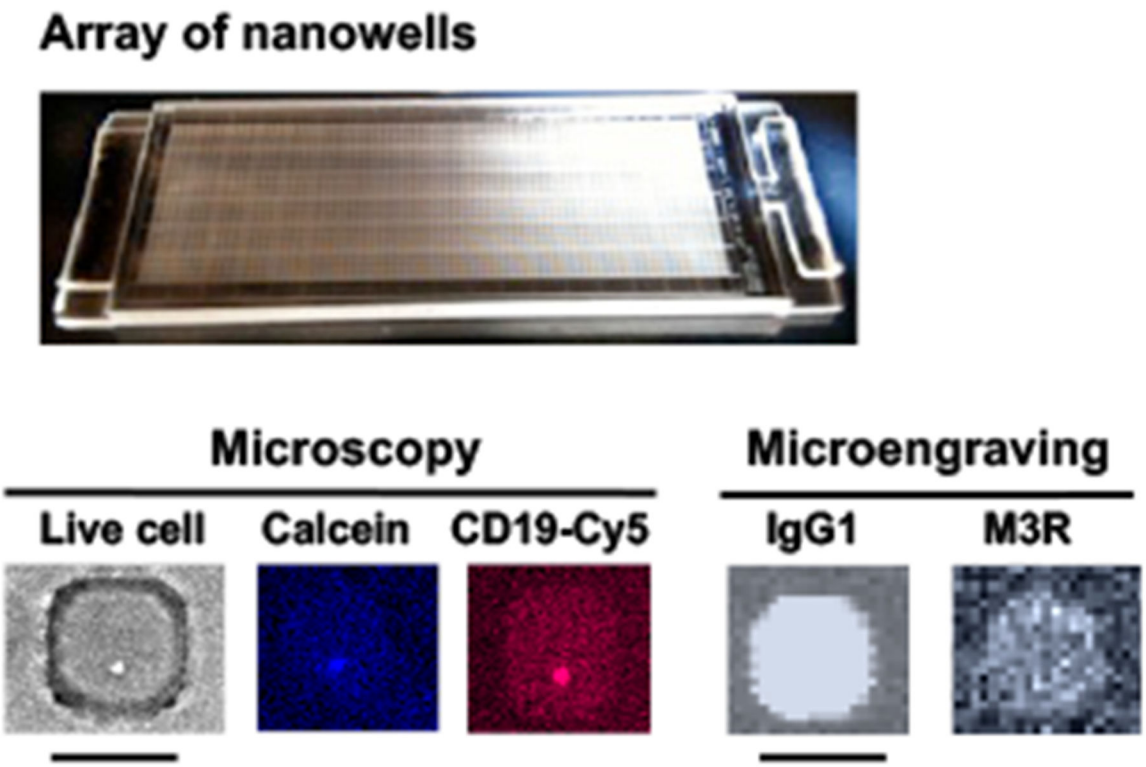

Figure 4. An application of SCAN to identify anti-muscarinic acetylcholine receptor type-3 (M3R) producing B cells.

Representative array of nanowells with microscopic micrographs showing a live cell in brightfield, calcein dye for live cell marker, and CD19 for B cell marker. Microengraving microarrays show the secretion of IgG1-isotypic anti-M3R antibody. Scale bar: 50 μm.

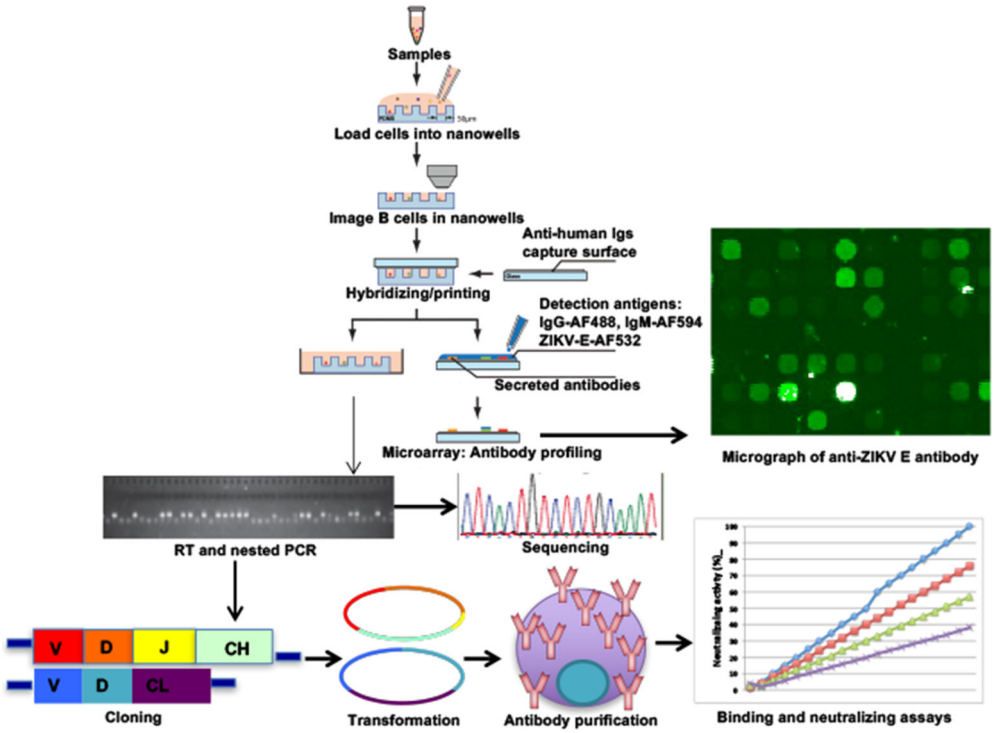

Figure 5. Screening for ZIka virus neutralizing antibodies using SCAN.

Peripheral blood cells of Zika infected patients will be isolated. Purified single-cell suspension will be labeled with anti-CD20-FITC and Calcein violet-405 for live cell and plated onto fabricated nanowells. Labeled cells in the nanowells will be imaged for surface markers and locations on the chips. Capture slide coated with anti-human immunoglobulins will be hybridized. Detection antibody mixture containing IgG-AF-488, IgM-AF594, and ZIKV E-AF532 will be added. Micrograph of anti-ZIKV E-secreting B cells will be generated. Individual ZIKV E-secreting B cells will be picked and performed RT/nested PCR for heavy/light chain sequences. Both chains will be cloned into an expression vector and expressed in 293T cell line. Secreted antibodies will be purified and screened for binding and neutralizing activity against ZIKV. AF: Alexa Fluor

Table 1.

Characterization of HIV broadly neutralizing antibodies

Broadly neutralising antibody	Envelope subunit	Epitope	Breadth (%)	Potency (μg/mL)	Development stage
4E10	gp41	Gp41 membrane-proximal external region	13 (32)	3.41 (32)	Phase I/II clinical trial (NCT00219986)
2FS	gp41		19(33)	2.30 (33)	Phase I/II clinical trial (NCT00219986)
10E8	gp41		72 (35)	0.35 (35)	*In-vivo* (rhesus macaque) (62)
b12	gp120	CD4 binding site	10 (32)	2.82 (32)	*In-vivo* (rhesus macaque) (105)
VRC01	gp120		74 (32)	0.33 (32)	Phase II clinical trial (NCT02664415)
VRC01-N	gp120				
VRC07	gp120		83 (41)	0.11 (41)	Phase I clinical trial (NCT03015181)
3BNC117	gp120		77 35)	0.11 (35)	Phase II clinical trial (NCT02446847)
NIH45-46	gp120		76 (35)	0.2 (35)	*In-vivo* (rhesus macaque) (106)
N6	gp120		96 (43)	0.038 (43)	In vitro (43)
ECD4-Ig	gp120		100 (100)	0.05 (100)	*In-vivo* (rhesus macaque) (100)
PG9	gp120	V1/V2 domain	54 (32)	0.23 (32)	Phase I clinical trial (NCT01937455)
PGDM1400	gp120		83 (42)	0.003 (42)	*In-vitro* (42)
PGT145	gp120		52 (32)	0.2 (32)	*In-vitro* (32)
PG16	gp120		59 (35)	0.15 (35)	*In-vitro* (35)
10-1074	gp120	V3 domain	54 (66)	0.4 (66)	Phase I clinical trial (NCT02824536)
PGT121	gp120		57 (32)	0.03 (32)	Phase I clinical trial (NCT02960581)
PGT 128	gp120		60 (32)	0.02 (32)	In vivo (rhesus macaque) (107)

Breadth is the percentage of viruses neutralized at IC50 > 1 μg/mL in a panel of 100–200 pseudoviruses. Potency is the measure by the IC50 (μg/mL) in a panel of 100–200 pseudoviruses.

Table 2.

Neutralizing antibodies against Zika virus

Neutralizing antibody	Viral unit	Epitope	Results
ZV-54	Envelope subunit DIII	Lateral ridge	No cross-reactivity with DENV and/or JEV. Neutralization of 4 ZIKV strains *in-vitro*. Potency: 0.087-0.582 μg/mL
ZV-67	Envelope subunit DIII	Lateral ridge	No cross-reactivity with DENV and/or JEV. Neutralization of 4 ZIKV strains *in-vitro*. Potency: 0.143-0.511 μl/mL
VH3-23/VK1-5	Envelope subunit DIII	Lateral ridge	Recognition and neutralization of DENV-1 and ZIKV Potency: 0.7–4.6 ng/mL
ZV-64	Envelope subunit DIII	C-C' loop	No cross-reactivity with DENV and/or JEV. Reduced inhibitory activity *in-vitro* against African and American strains
zEDIII	Premembrane-envelope EDIII		Recognition and neutralization of ZIKV. No exacerbation of DENV infection
c10	Envelope subunits DI, DII (near from fusion loop), DIII	Intradimer	Recognition and neutralization of ZIKV *in-vitro* and *in-vivo*
ZV-2	Envelope subunit DIII	ABDE sheet	No cross-reactivity with DENV and/or JEV. Neutralization of 4 ZIKV strains *in-vitro*
ZV-13	Envelope subunit DI-II	Fusion loop	Cross-reactivity with DENV-1, 2, 3, 4, WNV, and JEV. No inhibitory activity *in-vitro*
ZIV-117	Envelope subunit DII,	Dimer-dimer interface. Quaternary epitope	Broad neutralization of African, Asian, and American ZIKV strains. Potency: 5-25 ng/ml

Potency is the measure by the IC50 (μg/mL) in a panel of 100–200 pseudoviruses. DENV: Dengue virus. JEV: Japanese encephalitis virus. ABDE :