Recombinant DNA Technology as a Viable Alternative to Animal-based Antibody Production Methods

Michelle M. Echko, Samantha K. Dozier, Ph.D.
People for the Ethical Treatment of Animals (PETA), Norfolk, VA, USA

INTRODUCTION

Monoclonal antibodies (mAbs) are ubiquitous in biomedical research and medicine. They are used to fight, diagnose and research disease and have emerged as effective therapeutic treatments for cancer, various auto-immune disorders, and other illnesses. In the area of toxicology, mAbs are frequently used as capture reagents to detect and measure protein and drug levels in biological fluids and to register changes in cellular proteins after exposure to a chemical agent.

The mouse ascites method of mAb production is now widely discouraged due to the pain and distress it causes. Unfortunately, the equivocally named "*in vitro*" method that has become standard today still involves the immunization and extraction of spleen cells from mice and presents serious animal welfare concerns and methodological problems. Creating antibodies for every variation of human protein using these methods would entail the use of hundreds of thousands of animals—if not more.

Fortunately, alternatives to animal-based mAbs exist. **Aptamers and recombinant antibodies (rAbs)** can be created without using animals or animal tissue and they can be used in all of the same applications in which traditional monoclonal antibodies are used.

Applications of Aptamers and rAbs

- Detection reagents
  - Blots
  - Affinity Chromatography
  - Histochemical staining
  - Fluorescence staining
  - Flow Cytometry
- Therapeutic drugs
- Diagnostic tools

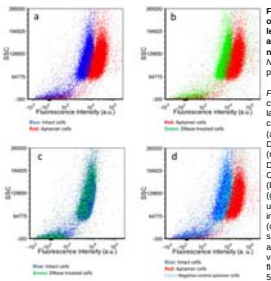

Figure 1. Fluorescence intensity of human acute lymphoblastic leukemia cells labeled with Qdot-aptamer and treated with nuclease. Terazono *et al. Journal of Nanobiotechnology* 2010. Used with permission [1]

From the text: "Fluorescence changes following Qdot-aptamer labelling of intact cells (blue: intact cells, red: aptamer labelled cells) (a). Fluorescence changes following DNase treatment of aptamer cells (red: aptamer labelled cells, green: DNase treated cells) (b). Comparison of unlabelled cells (blue) and DNase treated cells (green) (c). Comparison between unlabelled cells (blue), cells incubated with an irrelevant aptamer (cyan) and cells labelled with cell-specific aptamer (red) (d). Vertical axes show Side Scatter (SSC) values, and horizontal axes show fluorescence intensity measured at 525 nm."

PROBLEMS WITH HYBRIDOMA TECHNOLOGY

Figure 2. Mouse showing swollen abdomen typical of ascites. McArdle J. 1998. [3]

Animal welfare concerns associated with hybridoma-based antibody production methods

Monoclonal antibodies (mAbs) are traditionally created by immunizing a mouse with an antigen of interest and then fusing the antibody-producing spleen cells of the mouse with cells from an immortal cell line. The fusion creates hybridomas which can be expanded either by injection into the abdomen of a second mouse (ascites method) or by culturing the hybridoma cells in flasks or bioreactors (*in vitro method).*

- It is well established that **the ascites method of mAbs production "causes discomfort, distress, or pain"** to animals. [4]
- The National Institutes of Health's Office of Laboratory Animal Welfare encourages the use of *in vitro* methods as the default procedure for producing monoclonal antibodies.[4] Australia, Germany, Switzerland, the Netherlands, and the United Kingdom have effectively banned ascites in favor of *in vitro* methods.[5-6]
- However, like the ascites method, **the "*in vitro*" method still requires the caging, handling, immunization and death of animals**, which presents animal welfare concerns.

Technical disadvantages of hybridoma technology

Like playing the lottery

- No control over the epitopes to which antibodies are formed
- Antibodies must be screened extensively after they are created in the hope that one has been created with characteristics that are desirable to the investigator

Antigen limitations

- Sensitive antigens (e.g. membrane proteins and nucleic acids) could be destroyed inside an animal before antibodies are created
- Toxic antigens may kill the host animal before antibodies are produced
- Proteins highly conserved between species may not elicit an immune response

DNA encoding the antibody is not provided

- Hybridoma derived antibodies cannot be improved until they are first converted into recombinant antibodies

Time

- Hybridoma derived antibodies can take between 4 and 6 months to create

RECOMBINANT ANTIBODIES

Description

Recombinant antibodies (rAb) are created using recombinant DNA. Chimeric antibodies, humanized antibodies, and antibodies from gene libraries can all be considered recombinant antibodies. Only rAbs from synthetic or human antibody gene libraries are entirely animal-free.

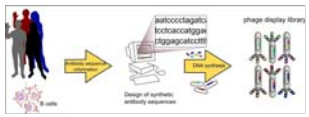

Figure 3. Production of a synthetic antibody library displayed on phage

Generation

- Libraries of antibody genes are created and displayed on the surfaces of cells, phages or ribosomes
- Displayed libraries are incubated with antigen during iterative rounds of "panning" to enrich for antigen-specific antibodies

Bind → Wash → Elute → Amplify → Repeat

- Selected antibodies are screened for desirable characteristics
- Genes for the selected antibodies are sequenced and undergo affinity maturation to further increase antibody function
- Genes for highest performing antibodies are transferred into protein expression systems for larger scale antibody production

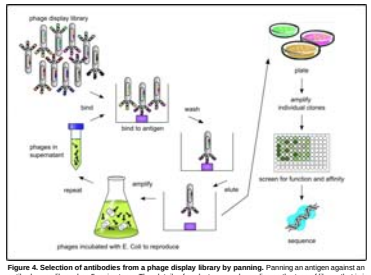

Figure 4. Selection of antibodies from a phage display library by panning. Panning an antigen against an antibody gene library has 5 main steps. The details of each step vary depending on the type of library that is in use. Phage libraries , for example, require bacteria for amplification. Yeast and mammalian cell libraries do not. Antibodies selected from a library are screened for secondary characteristics and then sequenced.

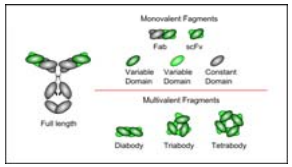

Figure 5. Recombinant Antibody Formats. Full length antibodies are Y-shaped, divalent molecules with variable and constant domains. rAbs can be converted into mono- and multivalent fragments in addition to the full length format.

Library Display Platforms

- Phage display
- Bacterial display
- Yeast display
- Mammalian cell display
- Ribosome display

Figure 6. Saccharomyces cerevisiae yeast cell. Wittrup D. 2006.[7]

Advantages of rAbs

- No animals used if antibodies come from synthetic or human antibody libraries
- Less purified antigen is required compared to hybridoma technology [8]
- Production process gives complete control over the state of the antigen
- rAbs to toxic, fragile, or highly conserved antigens can be generated
- Production time is weeks instead of months
- Nucleic acid sequence of rAb is easily accessible for further manipulation
- rAb fragments can be produced cheaply in bacterial and yeast expression systems

Disadvantages

- Technically challenging
- Improved methods of generating antibody libraries are protected intellectual property [9]
- High-throughput equipment to automate selection procedures can be expensive
- Most libraries available to researchers are made of antibody fragments. An extra step is required to convert these fragments to full length antibodies if full length antibodies are required.

APTAMERS

Description

An aptamer is a single-stranded nucleic acid macromolecule that is engineered to bind a specific target. It can be RNA or DNA; peptide aptamers also exist but their production and applications are beyond the scope of this poster.

Generation

- Pools, or libraries, of random oligonucleotides are designed and synthesized
- Libraries go through iterative rounds of SELEX (Systematic Evolution of Ligands by Exponential Enrichment) to enrich for target-specific oligonucleotides

Bind → Partition → Elute → Amplify → Condition

- Conditioning prepares the oligonucleotides for the next SELEX round
- After several SELEX rounds, the original library is reduced to only target binding oligonucleotides. These oligonucleotides are cloned into bacterial expression vectors for sequence determination and modification.
- Post-SELEX aptamers may be modified to make them more stable *in vivo* or to optimize the way in which they bind to their target.

Figure 7. Schematic representation of cell-based aptamer selection. Aptamers can theoretically be created against any target. This schematic illustrates aptamer selection using whole cells as targets. Shangguan D et al. 2006. Used with permission © 2006 PNAS [10]

Figure 8. 3-Dimensional molecular dynamic model of single-stranded RNA aptamer. Protein Data Bank ID: 1byj.

Advantages of Aptamers

- No animals used during production
- Less purified target molecule is required compared to hybridoma technology
- Production process gives complete control over the state of the target molecule
- Aptamers to toxic, fragile, or highly conserved targets can be generated
- Production time is weeks instead of months
- Nucleic acid sequence of aptamer is easily accessible for further manipulation
- Aptamers can be produced in large scale through PCR and *in vitro* transcription [11]
  - Cheap, highly reproducible, ensures same quality for different batches

Disadvantages

- Aptamers are cleared rapidly from the blood stream owing to their low molecular weight
- Rapid clearance can be an advantage in some applications such as diagnostic imaging. Aptamers need to be modified for other in vivo applications that require a longer half-life.
- Targets need to be highly pure to reduce unspecific binding
- Aptamer selection is more difficult if the target is negatively charged or largely hydrophobic
  - Targets with positively charged groups, aromatic compounds, and hydrogen bonding capabilities facilitate aptamer selection[12]

ALTERNATIVE BINDERS IN RESEARCH & MEDICINE

Therapeutics

- In 2002 the FDA approved the first fully human mAb derived from phage display technology, Humira. Humira (generic name Adalimumab) was initially approved to treat patients with moderate to severe rheumatoid arthritis. Since then, the drug has been approved to relieve symptoms associated with other autoimmune disorders including Crohn's disease, ankylosing spondylitis, and psoriatic arthritis.[13]
- In 2004, the first aptamer-based therapeutic agent, Macugen, was approved by the FDA for clinical use. Macugen is approved to treat macular degeneration, an eye disorder that affects the center of the retina or macula.[14]

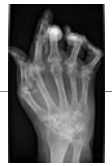

Figure 9. Typical x-ray of rheumatoid arthritis. Brägelmann B. 2008. [16]

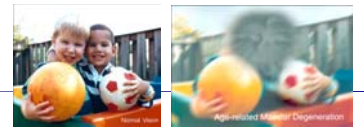

Figure 10. Two children as they might be viewed by a person with normal vision and age-related macular degeneration. National Eye Institute, National Institutes of Health. 2010. [17]

Diagnostics

- Blood testing company, Phadia, incorporated non-animal antibodies into its Varelisa™ and EliA™ products that test for autoimmune disorders.[15]

Research

- The Ellington Lab at the University of Texas put together a searchable aptamer database "to collect, organize and distribute all the known information regarding aptamer selection."
- Antibody vendors such as AbDSerotec and Axxora.com have begun to offer non-animal, recombinant antibodies from phage-display platforms alongside the traditional hybridoma-based antibodies.
- Proteomic initiatives including the ProteomeBinders Consortium and the Antibody Factory use phage display technology in an attempt to create antibodies against all the proteins encoded in the human proteome. ProteomeBinders partners also use cell display, ribosome display, aptamers and other affinity reagent technologies.

Structural Genomics Consortium (SGC) pilot study

- The SGC initiated a study to help evaluate the methods and economics involved in the systematic generation of affinity reagents against proteins in the human proteome
- Participants generated polyclonal, monoclonal, and recombinant antibodies to 25 different human SH2 domains
- At the end of the exercise the cheapest and fastest approach to achieving highly specific antibodies was determined.
- Publications summarizing the study are still in preparation but two teams have already determined phage display to be "the method of choice" for projects requiring rapid generation of antibodies to a large number of proteins." [18-19]

CONCLUSIONS

Aptamers and rAbs are scientifically validated technologies that have inherent benefits not available in animal-based antibodies, which present a host of methodological and ethical concerns. Aptamers and rAbs are sufficiently advanced to allow for their immediate evaluation and implementation in laboratories.

The letter and spirit of animal welfare laws governing animal experimentation in the U.S., E.U. and elsewhere stress the importance of seeking, considering and implementing modern alternatives to the use of animals. Aptamers and recombinant antibodies from synthetic or human antibody libraries are a viable and, in many applications, a methodologically-superior alternative to the animal-based methods of monoclonal antibody production. Unfortunately, these methods are not being used as frequently as they could be.

While it is true that there are some obstacles to the wide spread use of this technology, these obstacles are not insurmountable. In the interest of upholding the principles of the 3Rs (replacement, reduction, and refinement of the use of animals), researchers must make a greater effort to familiarize themselves with and employ non-animal research methods like those offered by aptamer and recombinant antibody technology whenever possible.

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