Recombinant Antibodies and Aptamers are Viable Alternative to Animal-based Antibody Production Methods

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INTRODUCTION

Antibodies are a component of the vertebrate adaptive immune system that recognize and disable foreign material and/or organisms.

The precise specificity and strong affinity of binding that makes antibodies an irreplaceable component of adaptive immunity are the characteristics that are harnessed in molecular biology, clinical medicine, and multiple other scientific disciplines to identify and label proteins of interest.

Because antibody-based affinity reagents are so widely used and the historically-used production process is painful and distressing for the animals used, their replacement with completely non-animal derived technologies should be a priority. Fortunately, viable alternatives that can be incorporated into most protocols that require affinity reagents are available.

Because these non-animal alternatives actually have several technical advantages over traditionally produced antibodies, we are working to make researchers aware of their options in order to facilitate wider use of these reagents and greater compliance with the spirit of federal animal welfare policies.

REASONS TO MOVE AWAY FROM HYBRIDOMA TECHNOLOGY

Animal welfare concerns associated with hybridoma-based antibody production methods

mAb generation in mice
Historically, mice have been used to generate and produce mAbs for laboratory and clinical use. Monoclonal antibodies were generated by immunizing an animal with the target antigen, allowing time for the animal's immune system to produce antibodies against the foreign substance, and then dissecting the animal's spleen or lymph nodes to isolate the lymphocytes producing antibodies to that antigen. Isolated specific antibody-producing cells were then fused to immortalized myeloma cells. These "hybridomas" would then grow and divide indefinitely.

mAb amplification in vivo
To produce large quantities of an antibody, the hybridoma cells were injected into the abdomen of mice where the cells multiplied and produced antibody-filled fluid (ascites) in the animal's abdomen. This method is known as the "mouse ascites method" of antibody production.

mAb amplification "in vitro"
Advances in tissue culture technology made possible the large-scale amplification of mAbs *in vitro* as an alternative to the mouse ascites method. In the *in vitro* amplification methods, hybridomas that were derived from animals, are cultured in petri dishes and antibodies are collected from the medium. This process avoids the ethical and scientific shortcomings of using mice to produce ascites but still requires the formation of hybridomas *in vivo*, which continues to present ethical concerns and leads to the suffering and death of countless mice.

◆It is well established that the ascites method of mAbs production "causes discomfort, distress, or pain" to animals.

◆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.

◆The use of *in vivo* ascites mAb amplification is so painful to the animals used that it has been banned or restricted in Australia, Germany, Switzerland, the Netherlands, and the United Kingdom. The United States National Institutes of Health and U.S. Department of Agriculture encourage the use of *in vitro* methods as the default procedure for mAb amplification

Scientific disadvantages of hybridoma technology

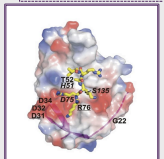
- lack of immunogenicity of some protein antigens due to non-recognition by the host
- inability to raise antigens to molecules that do not activate an immune response (typically only proteins and some carbohydrates induce immune responses *in vivo*)
- inability to raise antibodies against toxic molecules
- many hybridomas cannot be raised to high concentrations (titres) by *in vivo* ascites production
- *in vivo-generated antibodies* can also have experimental limitations during application, including non-specific binding that can lead to cross-reactivity and high background.
- sensitive antigens (e.g. membrane proteins and nucleic acids) are often destroyed inside an animal before antibodies are created
- Proteins that have been highly conserved between species may not elicit an immune response
- hybridoma-derived antibodies cannot be modified/optimized until they are first converted into recombinant antibodies
- hybridoma derived antibodies can take between 4 and 6 months to create

RECOMBINANT ANTIBODIES

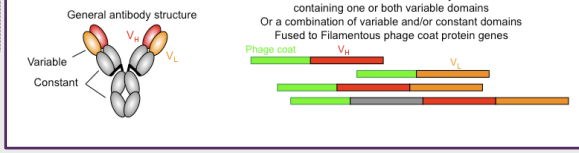
Recombinant antibody engineering involves the use of viruses or yeast to create antibodies, rather than using mice. Advances in molecular biology have lead to the ability to synthesize antibodies *de novo in vitro* – completely without the use of animals.

These techniques rely on rapid cloning of immunoglobulin gene segments to create libraries. Recombinant antibodies are translated from recombinant DNA and displayed on the surfaces of cells or phage particles.

Library Display Platforms
- Phage display
- Bacterial display
- Yeast display
- Mammalian cell display
- Ribosome display



Depiction of engineered rAb designed to inhibit mosquito-borne viruses, like West Nile virus, and others. Described in: Shiyaev, et al. Biochem J. 2010, 427 (3):369-76



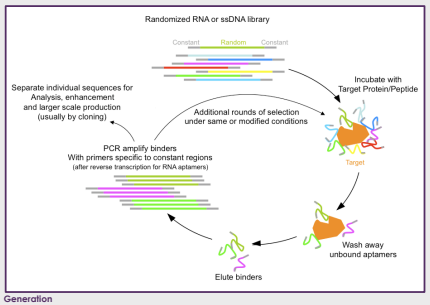
Generation
Libraries are created and displayed on the surfaces of cells
Displayed libraries are incubated with antigen during iterative rounds of SELEX to enrich for target-specific antibodies
Bind → Wash → Elute → Amplify → Repeat
Selected antibodies are screened for desirable characteristics
Selected antibodies are sequenced and undergo affinity maturation to further increase antibody function
Highest performing antibodies are transferred into protein expression systems for larger scale antibody production

APTAMERS

Description:

Aptamers are short single-stranded DNA, RNA, or peptide oligomers that bind targets with high affinity and specificity by folding into tertiary structures, much in the way an antibody binds an antigen.

Comparable to the binding of both traditional and recombinant antibodies, aptamer binding also occurs through combination of hydrogen bonding, electrostatic interactions, van der Waals forces and stacking interactions. Aptamer-target binding affinities are comparable to and can surpass those of traditional mAbs.



Generation
Pools, or libraries, of random oligonucleotides are designed and synthesized
Libraries go through iterative rounds of SELEX to enrich for target-specific aptamers
Bind → Partition → Elute → Amplify → Condition
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 or to optimize the way in which they bind to their target.

BENEFITS of rAbs AND APTAMERS

Decreased time to produce rAb/aptamers: an antigen-specific antibody suitable for research purposes can be produced in as little as 8 weeks –significantly less time than the months required for hybridoma-based methods.
Exceptional avidity: *in vitro* selection for high affinity binding is high-throughput and robust, and can produce antibodies with affinities unobtainable *in vivo*
Reproducibility: batch-to-batch variation is avoided due to a tightly controlled chemical synthesis process
Specific Binding Recognition: recognition of modified versus unmodified protein is possible and precise with the use of aptamers and rAbs
Conditional Binding: a high degree of control is possible, making selection of aptamers and rAbs that bind in a particular pH, salinity, or in other specific buffer conditions precise and practical
Unlinked to Immune Response: the process is independent of the biological immune response
Isotype Switching Possible: once a desirable antibody fragment is found it can be easily converted into any antibody isotype (e.g. IgA, IgM IgG etc.) from any species by adding the appropriate constant domain, making these methods highly adaptable and very practical.
rAbs/aptamers to highly toxic/non-immunogenic proteins possible: antibodies to highly toxic or non-immunogenic antigens can be created using library methods, unlike animal immunization technologies.
No animal welfare concerns or costs related to animal care and use: eliminating the use of animals throughout the process also eliminates animal welfare concerns

Additional benefits of aptamers:

Regeneration: can be stored denatured and then regenerated quickly.
Easy Transport: can be transported in ambient temperatures without harm
Changeable selection conditions: binding conditions can be changed so that the aptamer has desirable qualities for differing assays (i.e., binding in non-physiological, extreme pH buffers)
Kinetic parameters changeable: on/off rates can be changed on demand
Reporter molecules: the use of reporter molecules can be used at precise locations without interfering with binding avidity
Exceptional target discrimination: allow recognition of chirality or the presence or absence of a single methyl group

APPLICATIONS

Detection reagents
- **Western Blot**
- **Affinity Chromatography**
- **Histochemical staining**
- **Fluorescence staining**
- **Flow Cytometry**

Therapeutic drugs
Diagnostic tools

SUMMARY

Antibodies derived from animals present a host of methodological and ethical concerns that are not an issue with aptamers or rAbs that are not derived from animals. 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. However, these newer methods are not being used as frequently as they could be.

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 such as those offered by aptamer and recombinant antibody technology.