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Modern affinity reagents: Recombinant antibodies and aptamers

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ABSTRACT

Affinity reagents are essential tools in both basic and applied research; however, there is a growing concern about the reproducibility of animal-derived monoclonal antibodies. The need for higher quality affinity reagents has prompted the development of methods that provide scientific, economic, and time-saving advantages and do not require the use of animals. This review describes two types of affinity reagents, recombinant antibodies and aptamers, which are non-animal technologies that can replace the use of animal-derived monoclonal antibodies. Recombinant antibodies are protein-based reagents, while aptamers are nucleic-acid-based. In light of the scientific advantages of these technologies, this review also discusses ways to gain momentum in the use of modern affinity reagents, including an update to the 1999 National Academy of Sciences monoclonal antibody production report and federal incentives for recombinant antibody and aptamer efforts. In the long-term, these efforts have the potential to improve the overall quality and decrease the cost of scientific research.

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1. Introduction

There is a strong desire within the scientific community to see an improvement in the reproducibility of biomedical research. Last year, Francis Collins M.D., Ph.D., Director of the United States National Institutes of Health (NIH), and Lawrence Tabak, D.D.S., Ph.D., Principal

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Deputy Director of the NIH, wrote that the NIH is concerned about the lack of reproducibility in biomedical research and shared actions the NIH was exploring to address this problem (Collins and Tabak, 2014). In response, a workshop organized by the NIH, Science, and Nature Publishing Group was convened to identify principles to increase reproducible, robust, and transparent research (McNutt, 2014). Among these principles is the recommendation to establish best practice guidelines for reporting on antibodies used in research, including the source, dilution used, and how the antibody was validated (NIH, 2015).

Similarly, there is a growing awareness within the scientific community of the need to improve the quality of commercial antibodies, which often show poor specificity or fail to recognize their targets. Recent publications cite documented evidence of the lack of quality and reproducibility of animal-derived antibodies and describe how their use has wasted tremendous amounts of money, time, and experimental samples (Baker, 2015; Bradbury and Plückthun, 2015). One study found that only 49% (2726 out of 5436) of commercial, animal-derived antibodies could be validated to recognize only their targets (Berglund et al., 2008). It has been estimated that half of the $1.6 billion spent worldwide on protein-binding reagents is used on unreliable antibodies and that these antibodies may be the laboratory tool most commonly contributing to irreproducible research (Baker, 2015; Bradbury and Plückthun, 2015).

Alternative affinity reagents offer increased quality, speed of production, and return on investments in research. The existence of aptamers and recombinant antibodies (rAbs), two much-discussed modern non-animal affinity reagents, makes the replacement of conventional animal-based monoclonal antibody (mAb) production methods an attractive and achievable goal. One of the impediments to the replacement of animal-derived antibodies has been that the research community is largely unaware of the benefits associated with rAb and aptamer technologies. This review aims to familiarize antibody users with the state-of-the-science of these non-animal-based methods, how rAbs and aptamers can be incorporated into protocols that require affinity reagents, and how to gain momentum in the transition to these reagents. Greater awareness of the technical advantages of these non-animal alternatives among academia, industry, regulators, and funding bodies will help to facilitate wider funding, development, and use.

1.1. Background on antibodies

In their native role as components of the adaptive immune system, antibodies—also called immunoglobulins (Ig)—are large, complex glycoproteins capable of binding substances, termed antigens, that may elicit a larger immune system response. Antibodies recognize small structural elements, or epitopes, on an antigen, thereby marking them for phagocytosis or other biological processes. Epitopes recognized by antibodies are typically short amino acid sequences within foreign proteins.

There are five mammalian antibody classes: IgA, IgD, IgE, IgG, and IgM. Antibodies belonging to the IgG class are the predominant immunoglobulin in human serum and the most important from a research perspective. They are generally represented as Y-shaped molecules consisting of two heavy and two light chains (Fig. 1). The shorter light chains interact with the N-terminus of the heavy chains to form the two "arms," or antigen-binding (Fab) domains, which are composed of both constant and variable regions. Six variable amino acid loops at the termini of the Fab domains, also called the complementarity determining regions (CDRs), are responsible for binding to the antigen (Kierny et al., 2012). The tail of the Y-shape, the Fc domain, mediates the antibody's interaction with macrophages and other cells expressing Fc receptors.

The ability of antibodies to precisely bind their target antigen is the principal characteristic making antibodies an irreplaceable component of the immune system and particularly useful in research

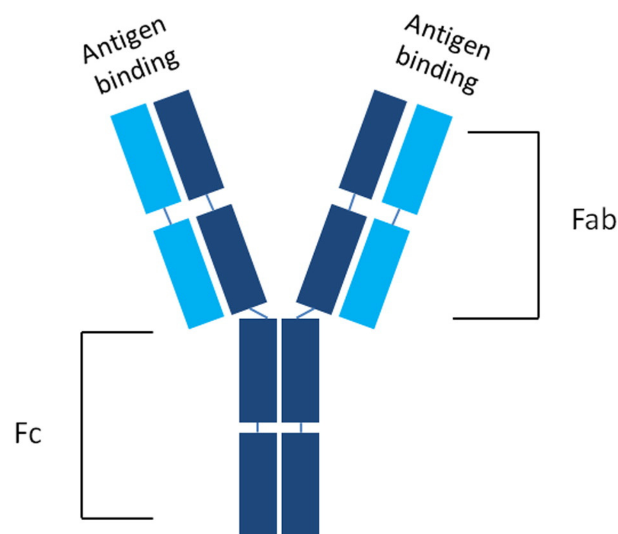

Fig. 1. General structure of an IgG antibody showing the heavy and light chains, the Fab, and Fc domains, and antigen binding sites.

applications. Both polyclonal (derived from multiple lines of antibody-producing cells) and monoclonal (derived from a single line of antibody-producing cells) antibodies are used in research. Monoclonal antibodies are defined by their capacity to selectively bind a single antigen.

1.2. Historical methods of monoclonal antibody discovery and production

Monoclonal antibodies are generated using either animal or recombinant DNA methods. Many technical advances have been made in mAb production technology in the four decades since Köhler and Milstein published their manuscript on hybridoma technology in 1975 (Köhler and Milstein, 1975). Their report describes the hybridization of antibody-producing B cells from the spleens of immunized mice with an immortal mouse myeloma tumor cell line, enabling the production of mouse mAbs for use as an investigational tool. The two general ways to discover and produce mAbs in animals, the ascites method and the "*in vitro*" method, share initial discovery steps. First, an animal (usually a mouse) is immunized with an antigen of interest. The mouse is often immunized multiple times over several weeks and, ultimately, killed to extract the spleen. Antibody-producing spleen cells from the mouse (immunocompetent B cells, which have a limited life span) are fused with immortalized myeloma tumor cells *in vitro* to produce a hybridoma. Hybridomas can be expanded in two ways: (1) by injection into the peritoneal cavity of a second mouse (called the *in vivo* ascites method) or (2) by culturing the hybridoma cells *in vitro* (called the "*in vitro*" method). While both methods use animals in the initial immunization step, the ascites method uses additional animals in procedures recognized to cause considerable pain and distress (Fig. 2) (Animal Welfare Division of OPRR, 1997; Marx et al., 1997; NRC, 1999). Historically, the ascites method produced more concentrated antibodies than the "*in vitro*" method without the need for expertise in cell culture methods; however, technological advancements have led to the "*in vitro*" production of more concentrated antibodies and non-animal affinity reagents (Hendriksen, 2006; Marx and Merz, 1995).

More specifically, ascites antibody production often involves injecting animals' abdominal linings with a priming solution (such as Pristane or Freund's Incomplete Adjuvant) to induce inflammation and interfere with drainage of peritoneal fluid. Priming is followed by injection of the hybridoma cell suspension. Hybridoma cells multiply and produce antibody-containing fluid, which accumulates in the abdominal cavities of the mice. As tumors grow, animals' abdomens distend as they fill with antibody-containing fluid;

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