Cutting Out the Middle Man:
GlycoFi’s Recent Success in Using Yeast to Synthesize Glycosylated Human Antibodies

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Today, half of the world’s supply of recombinant insulin is produced by yeast cells (1). Many believe that the future of pharmaceutical protein production lies in the genetic engineering of non-human cells to produce therapeutic proteins that are adapted to the human immune system. In 2003, Professor Tillman Gerngross of the Thayer School of Engineering at Dartmouth and his colleagues from the GlycoFi biotechnology company manipulated yeast to generate a complex, human-like glycoprotein. In January of 2006, GlycoFi announced its success in using yeast to produce monoclonal antibodies bearing human sugar residues. By engineering yeast cells to supplement the human immune system, GlycoFi has produced revolutionary new technology – a promising development in the long and often problematic history of therapeutic antibodies.

Injections of antibodies have long been used to help immunodeficient individuals battle infections. The great potential of using antibodies to combat disease lies in the high specificity that antibodies have for particular antigens. They can be used to target disease-causing toxins or microorganisms with less collateral damage to human tissue than results from more generalized therapies. Early experiments in the late 1800s involved the injection of a toxin or other antigen into a particular animal, such as a mouse or rabbit. After the animal had time to mount an immune response to the antigen, the scientist would collect the animal’s antibodies and inject these into a human infected with the same antigen. This transfer of passive immunity was a step in a new direction for the treatment of disease, but was frustrated by inefficiency. The human immune system tended to recognize the transferred antibodies as foreign antigens themselves because of their xenogeneic origin. This often resulted in a reaction against the antibodies. Another setback was that the ability to harvest antibodies lasted only as long as the animal lived. Thus, the researchers were incapable of creating a large supply of antibodies for future use. Additionally, the collected antibodies from the animal would be a conglomerate of all of the animal’s antibodies, rather than just those that were formed in response to the injected antigen. This meant that only a small percentage of the collected antibodies would be useful when injected into a human, while all of them would likely provoke an immune response (2).

The means of selectively producing an antibody reactive to a particular antigenic determinant was discovered much later. Monoclonal antibodies were first developed in 1975 by Georges Köhler and César Milstein. This was accomplished by the fusion of an isolated antibody-producing B cell with an immortal myeloma, a bone marrow tumor cell. The joining of the two cells produces a veritable antibody factory, known as a hybridoma, with a single antibody specificity (3). When injected into a human with a particular infection, monoclonal antibodies designed to fight that infection can bind to a specific antigenic determinant peculiar to the infectious organism or toxin, or to a cell marker on an infected cell. For the treatment to be effective, the cell marker must be an accurate indicator of a cell’s infection. The antibodies can then activate the human immune system to destroy the cell to which they are bound, generally by antibody-dependent cellular cytotoxicity or antibody-mediated phagocytosis (4). According to GlycoFi, “Monoclonal antibodies constitute the majority of therapeutic proteins currently in clinical and preclinical
development…” (5). While this new technology solved a few of the problems associated with polyclonal antibodies, the complication of the foreignness of non-human antibodies remained (6).

The next step would be to humanize the antibodies. This involves genetic manipulation of non-human antibodies so that the human immune system will fail to recognize them as foreign antigens. This can involve “replacing the [foreign] immunoglobulin structures with human counterparts” (7). In 1984, the first hybrid of mouse and human antibody was formed, called “chimeric antibody.” Part of a mouse antibody molecule was attached to a human antibody molecule via DNA recombination. The Frankenstein-like antibody was about 30% mouse and 70% human. While this decreased the recipient’s immune response to the injected antibody, the antibody’s foreign character still provoked a reaction. In 1986, Greg Winter and his colleagues at the University of Cambridge went a step further and attached only the binding region of the mouse antibody to a human antibody and further decreased the human response. This humanized antibody contained only 5-10% mouse character. The lower the mouse character in the hybrid antibody, the milder the reaction from the human recipient and the greater the possibility for multiple treatments. These antibodies have been experimentally successful in clinical trials (8).

Professor Gerngross and his colleagues at GlycoFi sought to genetically manipulate yeast cells such that the cells would produce human antibodies outright. In 2003, they successfully altered the DNA of *Pichia pastoris* such that the yeast produced a human-like glycoprotein. Glycoproteins are a diverse group of proteins bearing sugar residues. The sugars affect the function and localization of the glycoproteins in the human body. As antibodies are an important subclass of glycoproteins, the ability to derive glycoproteins from yeast cells could mean a faster and cheaper way to produce effective pharmaceuticals. Typically, glycoproteins must be harvested from mammalian cells after a difficult, costly, and inefficient cultivation process, which produces low yields of product. GlycoFi’s experimental results showed high yields of uniform, human-like glycoproteins (9).

Recently, GlycoFi made another technological leap. Its latest success has been in producing glycosylated, human-like antibodies from yeast cells. There are several advantages associated with this form of synthesis. Unlike antibodies made from mammalian cells, the yeast-produced antibodies allow for greater control of glycosylation – manufacturers can manipulate the types and numbers of sugar residues that attach to the antibody. According to GlycoFi, by controlling the glycosylation, “therapeutic potency can be significantly improved” (10). The glycosylation could also be used to impact the “solubility, half-life, or tissue distribution” of the antibody (11). The primary author of the study, Dr. Huijuan Li, associate director of Analytical Development at GlycoFi stated, “By controlling the sugar structures on antibodies we have shown that the antibodies’ ability to kill cancer cells can be significantly improved and that therapeutic proteins can be optimized by controlling their sugar structures” (12). By altering the yeast cell’s DNA, the scientists ensured that the yeast cell would produce the antibody fully-formed, so that little to no extra manipulation would be required (13). This incredible achievement allows for an assembly-line production of human antibodies, with little human involvement.

GlycoFi is currently at work developing new yeast cell lines to add to their catalog of glycosylated antibodies (14). The successful bioengineering of yeast opens a new door in pharmaceutical production and may revolutionize the way disease is treated. After decades of research dedicated to harnessing the power and specificity of the human antibody, GlycoFi’s recent achievements may allow the great potential of therapeutic antibodies to be more fully realized.

References