Chemistry & Biology *13*, 1243–1244, December 2006 ©2006 Elsevier Ltd All rights reserved DOI 10.1016/j.chembiol.2006.12.003

Innovations

Ablynx Makes Nanobodies from Llama Bodies

Antibodies, the sentinels of the immune system, are "Y" shaped, globular proteins with sites that bind to specific antigens. Antibodies are produced by B lymphocytes in response to stimulation by an antigen. Antibodies are versatile; they can bind to proteins, nucleic acids, carbohydrates, and lipids, making them eminently useful in the development of diagnostics and drugs for the treatment of autoimmune, inflammatory, and infectious diseases, as well as cancers.

Antibodies comprise two heavy and two light protein chains studded by sugars. An antibody's ability to bind specifically to an antigen is dictated by its variable antigen binding region, which is comprised of both a heavy (VH) and light (VL) protein chain. An antibody binds noncovalently, like a lock and key, to a corresponding antigen binding site, called an epitope. B cells produce antibodies that recognize different epitopes on an antigen (for example, different domains of a protein). Antibodies derived from a population of B cells are polyclonal.

Monoclonal antibodies, or mAbs, are produced by immortalized B cells derived from a single parent cell. The team of César Milstein, Georges Köhler, and Niels Kaj Jerne developed the techniques to create mAbs in 1975 and earned the Nobel Prize in Physiology or Medicine in 1984 for their accomplishment. Researchers and clinicians were excited about mAbs for therapeutics because they are so specific; they can be tailored to recognize and bind predictably to a particular epitope on an antigen.

The enthusiasm over therapeutics waned because mAbs were largely produced in mice, and the mouse proteins can cause immunogenic reactions in humans. In the late 1980s, a team at the Medical Research Council Laboratory for Molecular Biology (MRC-LMB), led by Sir Gregory Winter, used recombinant DNA approaches to "humanize" the mouse-produced antibodies; i.e., grafting elements of rodent mAbs onto human antibody sequences as well as developing synthetic antibodies that could be expressed in bacteria and phage. (Another method of humanizing antibodies is to genetically engineer mice to express human DNA.)

[Camelids] produce fully functional miniature antibodies, dubbed "nanobodies" by Ablynx, which are a tenth of the size of a conventional antibody.

Therapeutic antibodies are now a reality. "If we take monoclonal antibodies, they have been very successful," said Edwin Moses, CEO of Ablynx. "There are roughly 18 antibody-based products already on the market. There are predictions that potential sales in the antibody area will be $10 billion in 2008." According to Moses, the high selectivity and high affinity of mAbs to their targets reduces the chances of side effects.

But the large size and complex structures of antibody drugs excludes some attractive therapeutic targets, such as most enzyme active sites and deep receptor clefts; binding to those regions is the realm of small molecule drugs. They are also costly to produce in mammalian cells. Moses noted that Wyeth's antibody-derived drug Enbrel cost $1000 a gram to produce (according to 2001 figures). Additionally, mAbs must be injected and don't store well.

Thinking Small

Ablynx (www.ablynx.com), an 81 person Belgian company, could solve both problems that have bedeviled antibody engineers by producing fully functional antibodies with a compact structure. Ablynx capitalizes on an evolutionary peculiarity of camels and their cousins, the llamas (and certain species of sharks). Members of the camelid family are known for their ability to carry heavy loads and their propensity to curse and spit. They can also produce fully functional miniature antibodies, dubbed "nanobodies" by Ablynx, which are a tenth of the size of a conventional antibody.

Nanobodies comprise the antigen binding fragment of the heavy chain of an antibody. They are comparatively small molecules, between 12,000 to 15,000 Da in molecular weight. Nanobodies can be encoded by a single gene and produced in bacteria and yeast for a fifth of the cost of a mAb. "The other big advantage nanobodies have over monoclonal antibodies is … their stability." said Moses. "They are very resistant to treatment with heat and acids."

Antibody engineers are also attempting to solve the size problem by producing fragmentary antibodies called Fab fragments that are made up of a light chain and part of a heavy chain, or by developing single chain variable region fragments (sFv), which are heavy and light variable regions knit together by a short chain of about 15 amino acids. Moses commented that it is challenging to artificially engineer protein scaffolds and fragments to get the specificity and affinity of naturally derived antibodies. "Developing artificial antibodies is difficult. They tend not to be stable. They tend to aggregate."

"The other difficulty is that each fragment tends to have a different half life in vivo," said Ian Wilson, professor, Department of Molecular Biology and the Skaggs Institute for Chemical Biology at The Scripps Research Institute. "In general, the smaller the fragment, the shorter the half-life." According to Wilson, other drawbacks to antibody fragments can be the lack of Fc regions; hence, some of the usual clearance and effector mechanisms are not utilized.

From llama to Antibody

Ablynx maintains a revolving herd of about 20 llamas, which it buys from pet dealers and later resells. Llamas can be immunized a number of times. Why llamas? While llamas are easier to handle than camels, "…llamas spit as well." said Moses. "There are also differences in antibodies produced by llamas than by camels." According to Moses, after immunizing the llamas with a target protein, it takes about 6–12 weeks to isolate the first nanobodies against the target. Then to optimize, humanize, and format the nanobodies takes another 6–12 months. Ablynx can configure linked nanobodies to target two different targets/epitopes on an antigen.

To avoid the immunogenic reactions created by using natural products, Ablynx does not derive its antibodies directly from the llama. They isolate the nanobodies and then determine their amino acid sequences. From that information, the company can predict the gene sequence, enabling them to produce them artificially. So far, Ablynx's antibodies have been shown effective against 100 different targets in vitro.

"Dromedaires" to the Rescue

The discovery of the miniature camel antibody "was a pure coincidence," said Dr. Serge Muyldermans, one of the original researchers and a cofounder of Ablynx. A group of biology students at the Free University of Belgium in 1990 were asked to draw blood from each other but refused for fear of HIV. They also refused to kill a mouse to obtain blood. "So we gave them blood from a dromedaire that we had in the lab, as there was another group working on parasitic diseases," Muyldermans recalled.

The students purified the camel blood serum and noticed curious antibodies. "We were trying to clone antibodies from hybridomas (artificial cells created by fusing a tumor cell with a B-lymphocyte) and to express [them] in bacteria," said Muyldermans. "There was always something going wrong. We noticed in camels [that] we didn't have antibodies with the light chain. That immediately solved part of the problem."

While the general concept of nanobodies was patented in 1992, and the discovery was published in Nature in 1993, it wasn't until 2001 that Ablynx was founded with the aid of the university tech transfer office. Last year alone, Ablynx raised $50 million (making the total funds the company has raised from investors $87 million). Ablynx collaborates with pharma companies including Wyeth Pharmaceuticals, Novartis, Centocor, Kirin Breweries, and P&G Pharmaceuticals.

Sending Nanobodies into the Fray

Ablynx is targeting some areas that are currently being treated by small molecule drugs as well as those covered by other antibody applications. "The most obvious thing for us to do is to look at targets where antibodies have already been shown to work," said Moses. "If nothing else, we will have the cost-of-goods advantage, though to date we have also seen improved efficacy and safety profiles."

Ablynx's most advanced program is in thrombosis. Their reagent has been tested in animal models, and they expect to file an IND in Europe to begin volunteer clinical trials in the first quarter of 2007. Ablynx has a preclinical TNFα-blocker nanobody, licensed to Wyeth for rheumatoid arthritis. Wyeth currently markets the multi-billion dollar drug Enbrel for this indication.

U.K.-based Domantis, Ltd., (www.domantis.com) is probably Ablynx's closest rival. Sir Gregory Winter cofounded Domantis, Ltd., in 2000 with Dr Ian Tomlinson to commercialize the domain antibodies that they developed. Domain antibodies are the smallest functional binding units of antibodies, corresponding to either heavy or light protein chains of human antibodies. Domantis says its human dAbs are stable for therapies in inflammatory diseases, cancers, and autoimmune diseases. Domantis' collaborators include Abbott Laboratories, and they raised $83 million prior to being purchased in early December by GlaxoSmithKline for $454 million in cash.

Other players in the antibody field are looking beyond the basic antibody structure to develop new reagents for specific recognition. These approaches typically involve replacing the heavy and light chain scaffolds with other biology-inspired structures. Pieris (www.pieris-ag.com) in Germany is using lipocalins as a scaffold (named anticalins). Affibody (www.affibody.com) in Sweden is using the protein A domain as a scaffold to focus on cancer diagnostics. Molecular Partners, AG, (Switzerland) (www.molecularpartners.com) uses a synthetic ankyrin as a scaffold. Their DARPins (designed ankyrin repeat proteins) bind to targets like antibodies but have better stability and expression, according to the company. Molecular Partners is now evaluating repeat proteins for the tumor market.

Worth the Trek

Ablynx is still an early stage company, but its venture capitalists tout its strong IP position and promising animal data. "It is not very often you come across what is a potentially a new class of therapeutics," said, Katya Smirnyagina, Ph.D., venture partner at San Francisco-based Alta Partners. "The proof of concept is of course, going to man."

It is an ambitious venture, but a reliable team of llamas can carry a company far.

Wendy Wolfson (wendywolfson@nasw.org) is a science technology writer based in Oakland, CA.