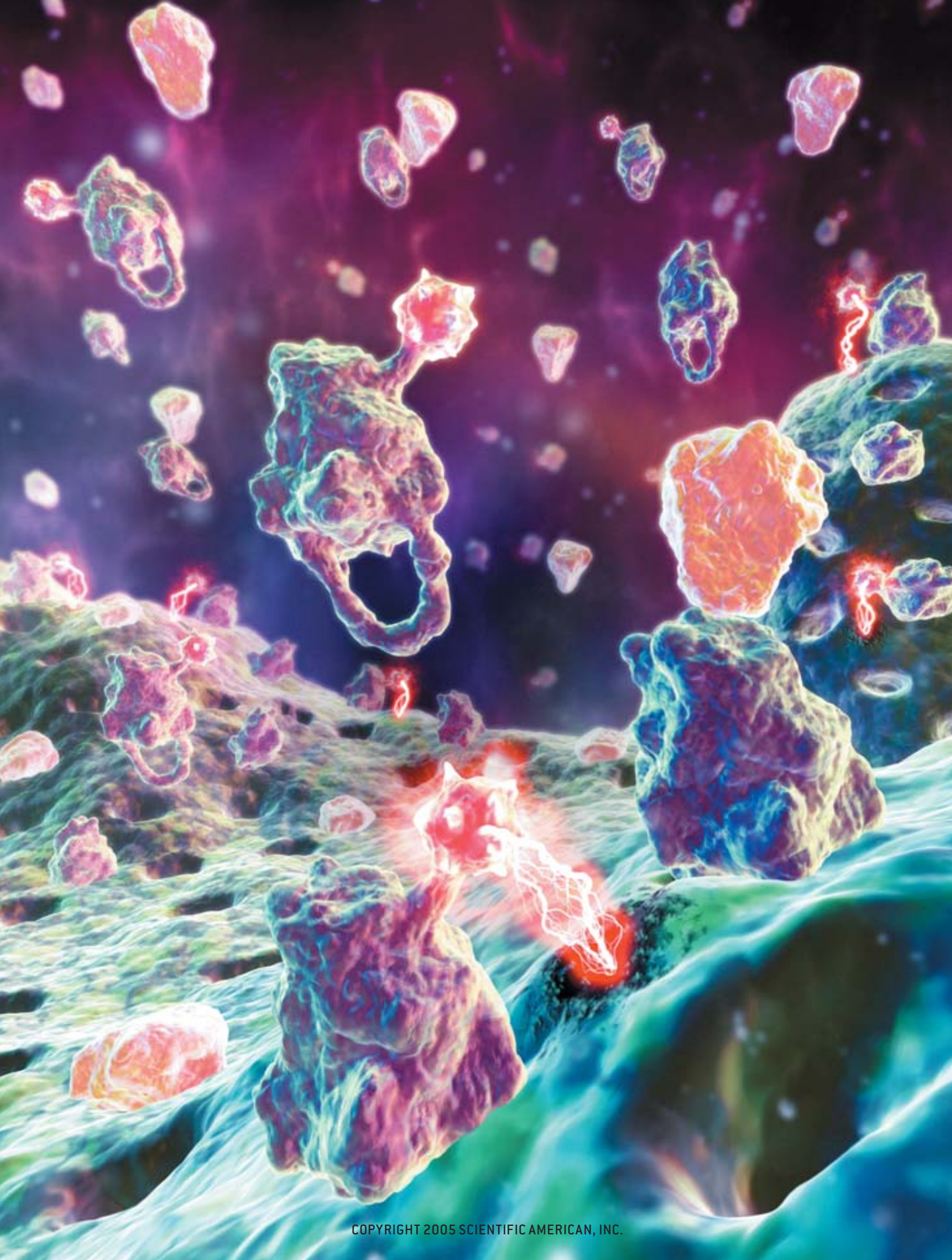


NANOBODIES of several kinds (*purple*) could descend on a cancerous cell (*blue-green*). Some nanobodies might be designed to attach to receptors on the cell, preventing pro-growth signals (*orange*) from reaching the cell. Other nanobodies could deliver radioactive payloads (*clublike appendages*) to cancer-specific targets.

JEFF JOHNSON *Hybrid Medical Animation*

Nanobodies

Antibodies, often described as magic bullets, are actually more like tanks: big, complicated and expensive. Tinier "nanobodies," derived from camels and llamas, may be able to infiltrate a wider range of diseases at lower cost. That is the hope, at least, of one small start-up in Belgium

BY W. WAYT GIBBS

Like many biotech companies, Ablynx emerged from the confluence of a serendipitous discovery, an open window of opportunity and an unreasonable ambition. Housed on two floors in a nondescript gray laboratory on a technology campus outside the university town of Ghent, Belgium, the three-year-old company employs just 45 people, 33 of them scientists and bioengineers. It is a minimal staff with a simply stated mission: find the tiniest sliver of protein that will do the job of a full-size antibody, then turn it into a billion-dollar medicine—or better yet, into the first of a whole new class of "nanobody" drugs against cancer, rheumatoid arthritis, inflammatory bowel disease, perhaps even Alzheimer's disease.

Despite being backed by $40 million of venture capital and partnerships with Genencor, Procter & Gamble and the National Research Council of Canada, Ablynx faces long odds. Its ambitious goal might seem altogether futile were it not for the recent surge in antibody therapies, the problems that still nag these sophisticated drugs, and the insights that Ablynx scientists have into the peculiar biology of the camel family.

Aside from the brain, the most complicated part of the human body is undoubtedly the immune system—and thank goodness. It's a bacteria-eat-man world out there, filled with a nearly endless variety of germs that see us as spawning grounds. Defending against this onslaught are antibodies, which are manufactured by B cells in an equally impressive panoply of models. Antibodies are huge Y-shaped proteins that float about in the blood and the fluid between cells, arms extended, using a chemical sense of touch to interrogate other molecules they encounter. Each model of antibody has its own mission; it patrols for a distinct chemical signature of a certain microbe, allergen or toxin.

Yet despite the sophistication of our immune defenses, we still get sick. No police force is perfect. The immune system is sometimes too slow or complacent in its reaction—for example, to cancers or to infection by respiratory syncytial virus. Other times it overreacts, as happens in organ-transplant rejection and asthma. And when it mistakenly attacks the body's own cells, the immune response can itself cause a degenerative disease such as rheumatoid arthritis.

For years, drugmakers sought to create artificial antibodies that can correct—or at least moderate—these immunological failures. But most early attempts ended in failure and financial disaster. In the two decades following the 1975 invention of a way to produce large batches of antibodies that are identical, or "monoclonal," just two such therapies survived review by the U.S. Food and Drug Administration.

The logjam finally broke in 1997, and by the end of 2004 the FDA had ap-

proved 17 therapeutic antibodies, including promising treatments for all the ailments mentioned above [see "Magic Bullets Fly Again," by Carol Ezzell; SCIENTIFIC AMERICAN, October 2001]. Pharmaceutical firms reaped $11.2 billion in sales of these medicines in 2004, the consultancy AS Insights reports.

And the market for monoclonal antibodies (usually abbreviated, idiosyncratically, as MAbs) is still in a formative stage of rapid growth. Dozens more MAbs are now in development or clinical trials, and last year Janice M. Reichert of the Center for the Study of Drug Development at Tufts University projected that 16 of them will gain FDA licenses within the next three years. In 2008, she forecast, MAbs will command roughly $17 billion in worldwide sales.

As Ablynx lines up for its first clinical trials in late 2006, it is aiming for a small slice of that large pie, says Mark Vaeck, the company's chief executive. Nanobodies—relatively simple proteins about a tenth the size of antibodies and just a few nanometers in length—may one day yield new medicines for Alzheimer's and other diseases beyond the reach of current antibodies, but that is not the opening strategy Vaeck chose. Instead he directed his scientists to create nanobodies that do what some of the best-selling antibodies do, only better.

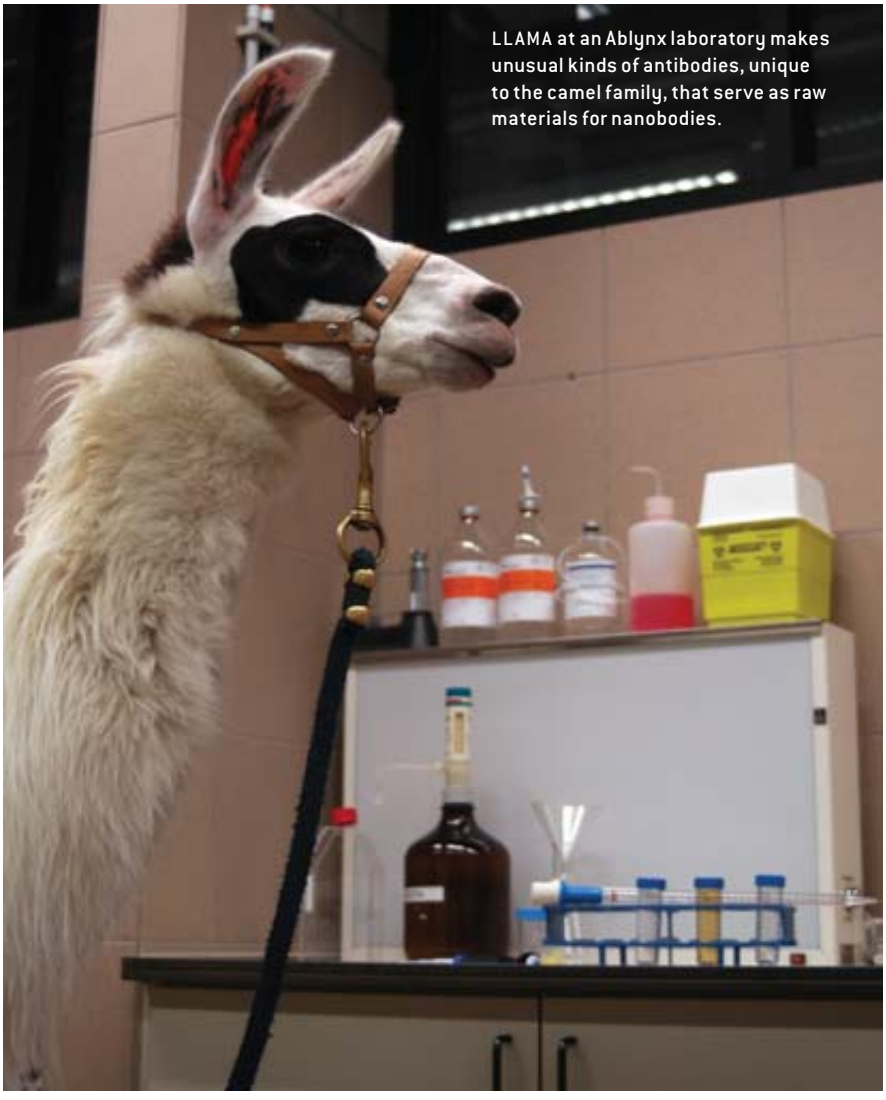
The Trouble with Antibodies

CERTAINLY THERE IS ROOM for improvement. For all their promise, points out Hans de Haard, scientific director at Ablynx, monoclonal antibodies still make pricey and troublesome medicines. According to Medco Health Solutions, treating an asthmatic patient with the antibody Xolair costs about $11,000 a year for the drug alone. Remicade, for rheumatoid arthritis, runs about $4,600 for eight shots. A year's course of Herceptin, an antibody cancer therapy, soars over $38,000.

MAbs are so dear in large part because they are so complex. By molecular standards antibodies are giants, each one a conglomerate of two heavy protein chains and two light chains, intricately folded and garnished with elaborate sugars [*see box on opposite page*]. To make a MAb medicine, scientists usually begin with an antibody isolated from mice. They then "humanize" the molecule by tinkering with the genes that encode it to replace some or all of the protein with amino acid sequences copied from human antibodies. (Alternatively, a few companies have genetically engineered mice so that they produce nearly human antibodies directly.)

The humanization reduces the potentially dangerous side effects that antibody therapies often generate when the patient's body perceives the MAbs as foreign invaders and launches an immune attack on the medicine. But the humanization process often consumes many months of highly technical work. And the resulting macromolecules are so complicated that they cannot be synthesized from chemical building blocks, as conventional drugs are. Instead they must be grown in bioreactor vats of mammalian cells that have been engineered to carry the multiple genes needed to make a single antibody.

Cell cultures of this kind do not scale easily for mass production. MAb factories are much more expensive to build and operate than are similarly sized chemical or microbial biosynthesis plants. Drug companies must ensure, for example, that their vats do not take sick with a virus that might ruin the valuable cells or contaminate the antibodies. A recent analysis by Mark C. Via, published by Cambridge Healthtech Advisors, concluded that demand for monoclonal antibodies most likely will far

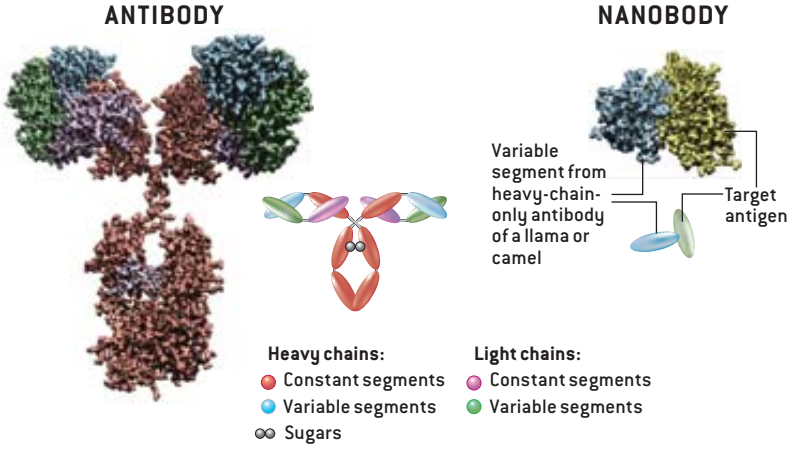


LLAMA at an Ablynx laboratory makes unusual kinds of antibodies, unique to the camel family, that serve as raw materials for nanobodies.

INA JOHANNA GECKE

ANATOMY OF AN ANTIBODY

The millions of kinds of human antibodies all share the same basic structure: two larger (or heavy) protein chains linked with two smaller (or light) chains. The pair of variable segments at the tips of the arms are unique for each model of antibody and determine the target to which it will bind. A nanobody is the variable part of a camel antibody that lacks light chains; it is about one tenth the size of an antibody.



outstrip production capacity for years to come. All these factors conspire to drive up the price of antibody therapies.

The great size of the proteins also imposes practical and medical limitations. High temperatures or extremes of pH make MAbs unravel. They typically expire in weeks unless stored near freezing temperatures. Antibodies are digested quickly in the gut, blocked from entering the brain and held to the periphery of solid tumors. Many illnesses are thus unreachable by monoclonals, and patients who can use MAb therapies must receive them by injection at a clinic.

For certain conditions in which MAbs do not work well, and even for some in which they currently do, simpler, smaller proteins might perform better and be easier to make, easier to handle, easier to take and thus more affordable. This idea predates the invention of nanobodies by many years. In the 1980s protein engineers began experimenting with antibody fragments created by chopping off the stem of the Y, or sometimes the stem and an arm, leaving just one "hand" to do the chemical duty of the antibody.

Like full-size MAbs, these antibody fragments (nicknamed Fabs) can treat illnesses by binding to toxins, pathogens or aberrant cell signals—or alternatively to the cell receptors to which those undesirable molecules dock. But antibody fragments cannot recruit other components of the immune system, such as killer T cells, in the same way that full-size antibodies do, because they lack the protein stem that performs that task.

In their favor, Fabs can be manufactured by bacteria, yeast or fungi, which are less expensive than the mouse or hamster cells needed to synthesize antibodies. Fabs can sneak into the center of tumors, and molecular engineers can rig them to tow toxic payloads—such as radioactive isotopes or chemotherapy drugs—directly to diseased tissue.

On the other hand, Fabs tend to fall apart or filter out of the bloodstream quickly, and so their active half-life typically amounts to mere hours rather than the weeks that full-size antibodies can persist within the body. Fast clearance may be just what is wanted for delivering a toxin, but for many medicines it is a disadvantage. So far only one therapeutic Fab has made it to market in the U.S., and that more than a decade ago.

Some companies, such as Domantis in Cambridge, Mass., have trimmed Fabs further, stripping away all but the tip of one of the two chains. This segment, which is unique to each model of antibody, contains the critical chemical fingers known as complementarity determining regions (CDRs), that determine what target an antibody will recognize—its antigen—and how tightly the two will bind when they meet. The resulting domain antibodies, as Domantis calls its proteins, are similar in size to the nanobodies that Ablynx makes.

But domain proteins evolved as segments of much larger, double-chained antibodies, and that has made them inherently sticky, explains Serge Muyldermans, a protein biologist at the Free University of Brussels. The fragments thus agglomerate together inside the bacteria that make them, as well as inside the patients that take them. The stickiness of the molecules lowers their production yields and hinders them in their work.

From Dromedary to Drug

WHILE BIOCHEMISTS continue trying to reengineer antibody fragments to solve these problems, Ablynx is exploiting an alternative offered by nature. In 1989 Muyldermans was among a group of biologists led by Raymond Hamers at the Free University that investigated an odd observation handed in as part of a student project on how dromedary camels (the one-humped, Arabian variety) and water buffalo fight off parasites. One of the tests for antibodies in the dromedary blood seemed to show an error: in addition to normal four-chain antibodies, it indicated the presence of simpler antibodies composed solely of a pair of heavy chains.

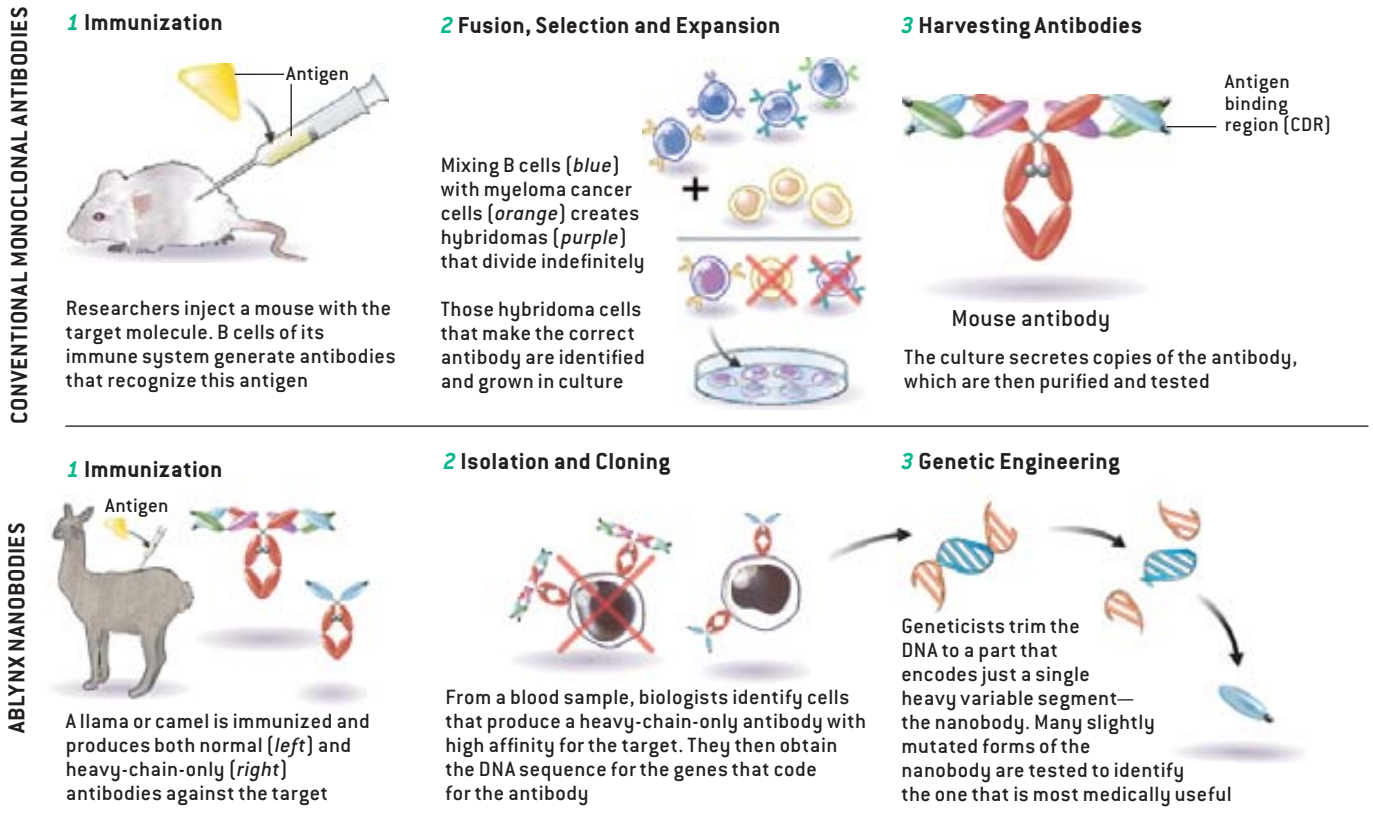
After several years of investigation, Hamers, Muyldermans and their colleagues published their serendipitous discovery in *Nature* in 1993. In dromedaries—and also in two-humped Asian camels and South American llamas—about half the antibodies circulating in the blood lack a light chain. Equally surprising, they found, these "incomplete" antibodies are able to grasp their targets just as firmly as normal antibodies do, despite having only half as many CDRs. And unlike Fabs, the heavy-chain-only antibodies do not stick to one another.

Why species in the camel family differ from all other mammals in this respect remains a mystery, but evolution may have handed scientists a work-

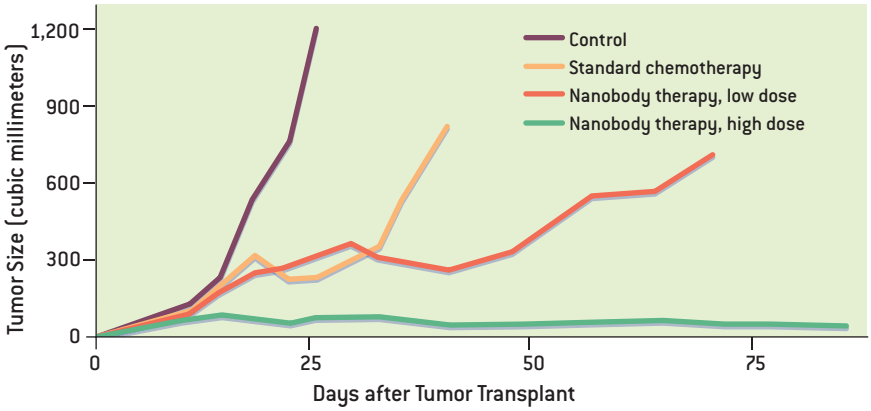
W. WAYT GIBBS AND LUCY READING-IKKANDA

CONSTRUCTING ANTIBODIES AND NANOBODIES

Creating an effective nanobody takes less time and money than a therapeutic antibody requires, according to scientists at Ablynx. In both cases, the immune system of a live animal performs the initial "design" of a protein that can latch onto the target molecule. Geneticists then tweak the DNA encoding that protein to add the properties desired in a medicine.



around for some of the thorniest problems with antibodies and antibody fragments. When Muyldermans's group pared these novel molecules down to just their distinctive, variable segments, the segments retained amazingly strong affinity for their targets, virtually equal to a full antibody 10 times their size. These shortened proteins were also more chemically agile, able to engage targets—including the active sites of enzymes and clefts in cell membranes—too small to admit an antibody. Nanobodies were born, and Ablynx soon followed.



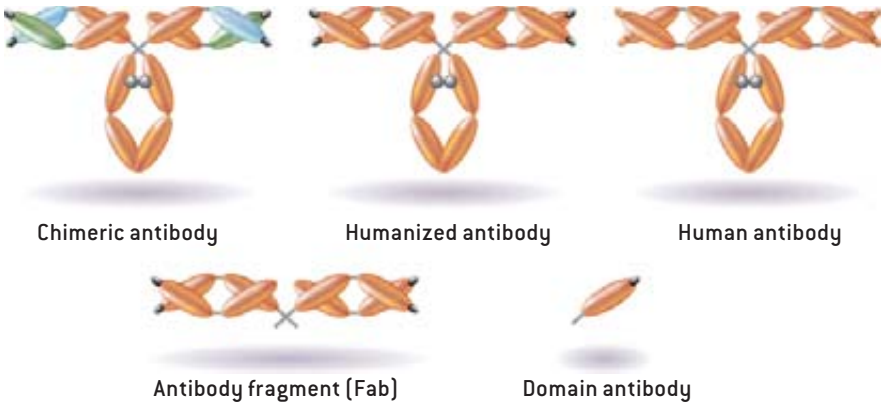
NANOBODIES AGAINST CANCER were tested on mice after they were injected with human tumor cells. Standard chemotherapy merely delayed the growth of the cancers. High doses of nanobody-directed chemotherapy, however, knocked the tumors into remission.

Because nanobodies are so much smaller than antibodies and are not chemical hydrophobes (as are domain antibodies), they are more resistant to heat and pH, Muyldermans says. Pieter Rottiers and Hilde Revets of the Flanders Institute for Biotechnology (VIB) in Belgium have shown that the compounds retain their activity as they pass through the gastrointestinal tract of mice, raising the prospect of nanobody pills to treat inflammatory bowel disease, colon cancer and other disorders of the gut.

Because nanobodies are so much simpler than antibodies in chemical composition and shape, they can be encoded by a single gene and are easier for microbes to synthesize. In 2002 biologists at Unilever Research in the Netherlands brewed more than a kilogram of nanobodies from a standard 15,000-liter tank of yeast (a yield of 67 milligrams per liter), whereas Ablynx scientists re-

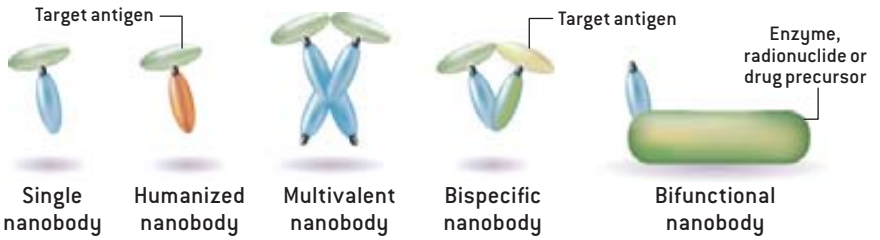
LUCY READING-IKKANDA

4 Humanization



Genetic engineers can replace pieces of the mouse antibody with human segments (*orange*) and can also trim the antibody to create fragments of various sizes

4 Construction of Nanobody Medicine



Nanobody genes can be spliced with genes for other nanobodies or other biochemicals to create medicines that are then produced in bacteria, fungi or yeast cultures

port recent yields exceeding a gram of nanobodies per liter of yeast culture—production rates that far exceed those typical for full-size antibodies. "Plus, our nanobodies are stable at room temperature and have a long shelf life without refrigeration," asserts Tim Van Hauwermeiren, who manages business development for the company.

The creation of new kinds of nanobodies is less difficult—and thus faster and less costly—than it is for antibodies, Van Hauwermeiren claims [*see box above*]. By immunizing llamas with the target antigen and then extracting heavy-chain-only antibodies from their blood, he says, "we can go from isolated target antigen to high-affinity nanobodies within four months." For some conditions, such as rheumatoid arthritis, the nanobody may serve unadorned as a medicine by jamming harmful cellular signals, either by attaching to the signal molecule or by clogging up the receptors for the signal on the surface of cells.

One of the most powerful advantages of nanobodies, however, is the relative ease with which the proteins can be joined to one another or to different kinds of compounds, de Haard says. His team has attached anti-albumin nanobodies to target-specific nanobodies to extend their half-lives in the bloodstream to weeks, he says. They have linked up to four nanobodies to create "multivalent" assemblies that can sop up more antigen per molecule or bind to either, or both, of two different targets.

Recently Revets, Muyldermans and Patrick De Baetselier of VIB published impressive results from an experiment in which they designed nanobodies to bind to a receptor on cancer cells, thus sticking to any tumors the molecules encounter. The researchers tailored a group of such nanobodies to be bifunctional by connecting each protein to an enzyme; the enzyme converts another chemical, called a prodrug, from its normal harmless form into a toxic chemotherapy that kills cells in the immediate vicinity.

The "patients" were mice that the scientists injected with human cancer cells, which soon grew into marble-size tumors. Revets treated some of the mice with the chemotherapy alone; those animals got sick and lost weight, just as happens in all chemotherapies. Their tumors shrank only a little. But the doctors gave another group of mice a high dose of the bifunctional nanobody with its attached enzyme. They waited a bit to give the unbound nanobodies time to filter out of the body, then injected the prodrug. As hoped, the nanobodies focused the chemotherapy on the cancer, sparing healthy tissues while completely driving back the tumors.

Until nanobodies make it through clinical trials, no one knows whether they will work as well in people as they do in mice. But if nanobodies do have an Achilles' heel, it is very likely to be the immune system itself. Ablynx scientists have worked out ways to humanize nanobodies, and studies with baboons have found that they raise no immune response to the tiny llama proteins. But de Haard acknowledges that nanobodies might not be able to evade the more sophisticated web of cellular surveillance that protects humans. The results of next year's clinical safety trials will determine whether nanobodies continue advancing at the recent breakneck pace or get tripped up by the complexities of the human immune system. SA

W. Wayt Gibbs is senior writer.

MORE TO EXPLORE

New Directions in Monoclonal Antibodies. Mark C. Via. Cambridge Healthtech Advisors, October 2004. Available at **www.chadvisors.com**

Nanobodies as Novel Agents for Cancer Therapy. Hilde Revets, Patrick De Baetselier and Serge Muyldermans in *Expert Opinion on Biological Therapy,* Vol. 5, No. 1, pages 111–124; January 2005.