Learning How to Bottle the Immune System

A new way of generating specific antibody molecules may render current methods of producing monoclonal antibodies obsolete

IF RICHARD LERNER is right, current methods for making monoclonal antibodies may soon be passé. On page 1275 of this issue of *Science*, Lerner and his colleagues describe a novel cloning system that may permit researchers to produce the entire antibody repertoire of an animal in the bacterium *Escherichia coli*. The new technique, says Lerner, "opens the way to doing in a bottle exactly what the immune system would do." Researchers could then pull out the specific antibodies they need very rapidly.

That would be the answer to many an immunologist's prayer. Ever since monoclonal antibody technology was invented 15 years ago, the pure, highly specific antibodies have found widespread application in basic research and medical diagnosis, and they have won a Nobel Prize for their discoverers Georges Köhler and Cesar Milstein of the Medical Research Council Laboratory in Cambridge, England. Monoclonals may also be useful therapeutically, to destroy cancer cells, for example.

But making monoclonal antibodies can be a tedious business. The antibodies are produced by cells, called hybridomas, that have to be grown first in laboratory dishes, usually for several months, and then in mice. And after all that effort, a researcher may end up with only one, or at most a few, monoclonal antibodies with the desired specificity. Researchers have also had a great deal of difficulty in making human monoclonal antibodies, which may be essential for many potential therapeutic applications.

The new technology now being developed by Lerner's group at the Research Institute of Scripps Clinic in La Jolla, California, and independently by Greg Winter and his colleagues at the MRC Cambridge laboratory may solve these problems. It is very fast, taking only a few days to get specific antibody clones, and it can be used to make human monoclonals. "I think it's going to be a useful technique for getting out antibodies," says immunologist Elvin Kabat of Columbia College of Physicians and Surgeons. "It avoids the use of mammalian cells and you can grow *E. coli* by the ton."

Lerner and his colleagues began looking for a better way to make monoclonal antibodies when they ran into difficulty in obtaining monoclonals by the standard methods in numbers sufficient for one of their research programs. Their work is aimed at producing antibodies that can catalyze biochemical reactions, much as enzymes do. The goal is to tap the essentially unlimited diversity of antibody structures to make enzymes with specificities that Mother Nature was not considerate enough to provide.

The Lerner group has successfully produced catalytic antibodies by standard hybridoma technology, but only in very small numbers. The probability of finding an antibody with high catalytic activity depends, he points out, on the number of variants produced. "For some time, we've been concerned about how good hybridomas are for our purposes," Lerner says. "If you wind up with a few good antibodies, you're lucky." He also wanted to be able to modify antibody structures systematically as a way of improving their catalytic efficiency.

So Lerner and his colleagues set out to transplant antibody-producing genes from animals into *E. coli*. The engineered bacteria, they hoped, would churn out a complete array of antibodies, which researchers would be able to screen for those with the specificities they want. Moreover, it would be easy to alter the antibody structures by mutating the cloned genes.

But the researchers had set themselves a daunting task. One problem was the antibody structure itself. An individual antibody consists of four proteins—two identical heavy chains and two identical light chains—that together form a single Y-shaped molecule. The arms of the Y form the sites at which an antigen binds to the antibody. These sites consist of segments from both the light and heavy chains. But that means that genes for both a heavy and a light chain must be introduced into an *E. coli* cell if the bacterium is to make a complete antibody.

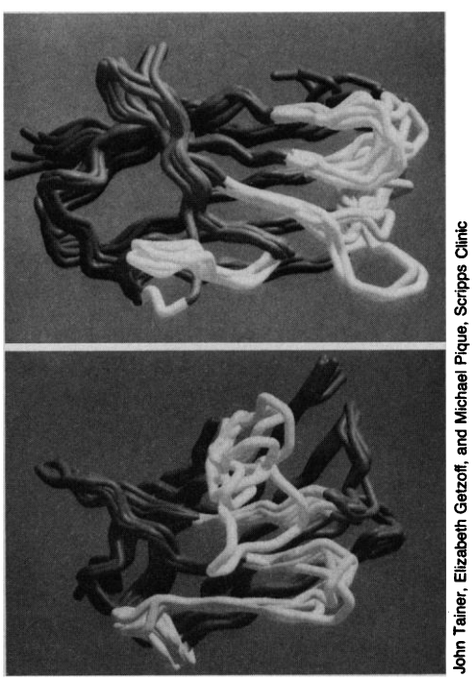
The Winter group took a key step toward achieving this goal earlier this year. They showed that they could obtain antibody-producing genes from hybridoma cells by using the polymerase chain reaction (PCR) to amplify the genes. The amplified genes could then be cloned in *E. coli* so that the bacteria make the antibody proteins, thereby eliminating the need to grow hybridomas.

Subsequently, both the Cambridge workers and Lerner and his colleagues used PCR-assisted cloning to make "libraries" of the extensive repertoire of gene segments encoding the antigen-binding portions of mouse heavy chain proteins. A library is essentially a depository of gene clones from which individual clones can be withdrawn, much as books are taken out of real libraries.

For some purposes, Winter says, having just the antigen-binding segment of an antibody heavy chain may be sufficient. He and his colleagues found that some of the clones from their library made heavy chain fragments that bound protein antigens almost as well as whole antibodies do.

But Lerner didn't think that the heavy chain fragments by themselves would be adequate for his purposes. X-ray crystallographic studies had indicated that both the light and heavy chains are needed to bind the small antigens used for his catalytic antibody work. So Lerner undertook the next step of combining the genes for the antigen-binding portions of the heavy and light chain proteins in the same clones.

He and his colleagues made separate libraries of the gene segments encoding the antigen-binding portions of light and heavy chains. Next, they combined the heavy and light chain gene segments in the DNA of a virus that infects *E. coli*. An infected bacteri-



John Tainer, Elizabeth Getzoff, and Michael Pique, Scripps Clinic

Building an antibody. *In these computer simulations, the white areas show the loops of an antibody light chain (above) and heavy chain (below) that together form an antigen-binding site.*

um would then contain genes to make both components of the antigen-binding portion of an antibody, and a very large number of light-heavy chain combinations could be produced. "Now you can make a library with 10^{12} members," Lerner says. "You have as much diversity as the animal has, or even more."

Lerner and his colleagues also devised a rapid method for screening this multitude of clones to pick out just those that produce antibody fragments with the desired specificity. In this case, they were looking for antibody fragments that bind the chemical *p*-nitrophenyl phosphonamidate. They found them in only about 2 days—approximately 100 in 1 million clones screened. Although this may seem like a low success rate, the number of positive clones obtained was a big improvement over what might be expected from standard monoclonal antibody technology, Lerner says.

"Richard was very brave in taking the total combinatorial approach," Winter remarks. "I'm very glad that it worked out. It shows that the system may be even easier to manage than we thought."

So far the researchers have not produced complete antibody molecules, but for some applications that may not be necessary. As long as the fragments produced bind antigens specifically, as they do, they can be used in purifying proteins or for catalyzing chemical reactions. But Lerner says that the viral vector he is using to transplant the antibody genes into the bacteria can accommodate longer gene segments, so it should be possible to make full-size antibodies if they are needed. He notes, incidentally, that a patent application on the vector is being filed, but he intends to make it available to any researcher who wants it.

Also, the light and heavy chain combinations produced by the cloning method are probably not going to be the same as those produced by antibody-producing cells, but that may not matter as long as the desired specificities are produced.

Moreover, once an antibody with the desired specificity is identified, a researcher will be able to tinker with it to improve its antigen-binding or catalytic activity. This can be done by introducing mutations into the cloned antibody genes or by varying light and heavy chain combinations. "Someday," Lerner says, "we'll be sitting around reminiscing about monoclonal antibody technology and saying 'remember when we used to do *that*.' " ■ JEAN MARX

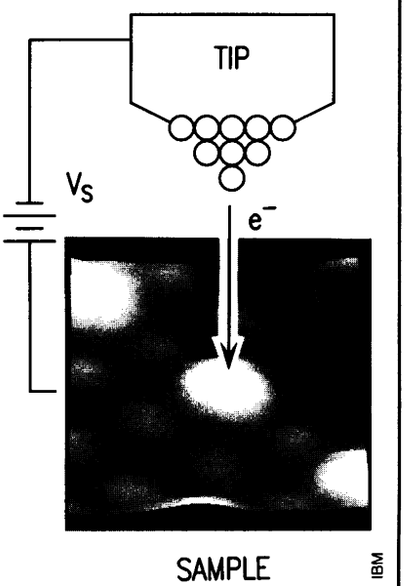
ADDITIONAL READING

R. Orlandi, D. H. Güssow, P. T. Jones, G. Winter, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3833 (1989).
L. Sastry *et al.*, *ibid.*, p. 5728.
E. S. Ward *et al.*, *Nature* **341**, 544 (1989).

A Small, Small, Very Small Diode

Two recent experiments have shown that it may someday be possible to build electronic devices as small as a few atoms across. "This is really the ultimate limit on size," says physicist Peter Bedrossian. "Devices will never get smaller than atomic sizes."

Bedrossian's team at Harvard University's Lyman Laboratory in Cambridge, Massachusetts, and a group at IBM's T.J. Watson Research Center in Yorktown Heights, New York, have independently reproduced the essential features of a tunnel diode in structures that consist of only a few atoms. However, although the existence of these structures shows that atomic-scale electronics may be feasible, the researchers caution that it will be many years before practical development catches up with laboratory demonstrations.



Microswitch. *As the tip of a scanning tunneling microscope is placed near a defect site on the silicon surface (bright area), it creates a tunnel diode effect.*

Normal-sized tunnel diodes are important in a number of applications that require high-speed circuitry, such as fast switches used in digital signal processing and high-frequency oscillators used in microwave instruments. Their value depends on an unusual property called negative differential resistance: As the voltage across the diode increases, the induced current goes down, at least for a certain range of voltages. A diode switch can be turned on by a low voltage and off by a high one. And tunnel diodes are very fast because electrons need to travel only a short distance in them.

As with all electronic devices, trying to diminish the size of a tunnel diode by simply decreasing its dimensions soon runs into a fundamental limit. Once a device gets small enough, quantum effects start to dominate its electronic behavior, and it begins to act very differently. So the two groups did not actually shrink a tunnel diode; they mimicked it with a new, atom-sized structure in which negative differential resistance is produced by quantum effects instead of electronic effects.

In-Whan Lyo and Phaedon Avouris of IBM reported their work in the 22 September *Science*. Lyman Lab's Bedrossian, Dongmin Chen, Klaus Mortensen, and Jene Golovchenko, who performed their experiment at the Rowland Institute for Science in Cambridge, announced the result in the 16 November *Nature*.

Both groups created the atomic-scale tunnel diode effect by bringing the tip of a scanning tunneling microscope down near the surface of a boron-doped silicon crystal. A voltage applied between the tip of the scanning tunneling microscope and the crystal created a tunneling current between them. When the microscope was positioned over certain sites on the surface, the tunnel diode effect appeared—the current dropped as the voltage increased.

The spots on the surface that exhibit this negative differential resistance seem to be isolated defect sites. "Our interpretation is that the detailed nature of the sites does not appear to be important," IBM's Avouris says. Instead, the essential requirement for the tunnel diode effect, he says, is the presence of localized quantum states on the surface of the sample and on the probe of the scanning tunneling microscope. Such localized states, he says, are usually found over defect sites on the surface and at the tip of the probe, which consists of only one or a few atoms. "It's a general effect—you can probably cook up many combinations that work," Avouris adds.

Golovchenko warns that although they have shown how to produce an atomic-scale tunnel diode effect, this doesn't mean practical applications are around the corner. "The interesting parts of the system are very small—a couple of atoms on one side and a couple of atoms on the other—but the surrounding hardware is enormous," he says. What it does mean is that researchers now have something to shoot for in the quest to make electronic devices as small as possible. ■ ROBERT POOL