Antibodies for Sale

In the first major impact of biotechnology on clinical procedure, specialized antibodies are diagnosing a variety of medical conditions

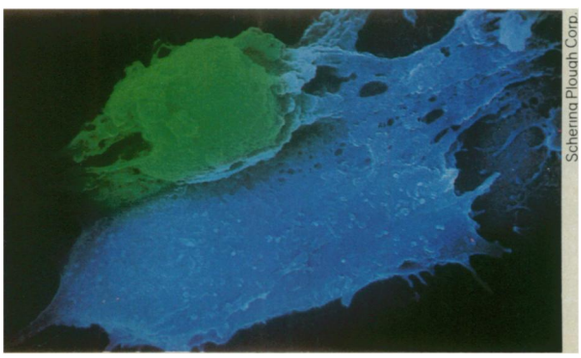
By JULIE ANN MILLER

It all began as an experiment in basic immunology in a cluttered laboratory in England. Now people talk of a new era in medicine and a billion dollar market. Only seven years after the discovery of a method to produce large amounts of a specific antibody, the first fruits of the technique are appearing on the market. They are medical diagnostic tests.

These new tests, which detect microorganisms, hormones and tumor-associated substances, take advantage of the ability of individual antibodies to seek and bind to a specific target. Many tests had already used antibodies to detect disease, but those were uncharacterized mixtures of antibodies taken from animals injected with the target substance. Every time an animal is injected with foreign material, a different mixture of antibodies results. But with the new technique, large amounts of a single antibody can be made at will.

The technique for making single, specific antibodies came out of efforts by Cesar Milstein and colleagues at the Medical Research Council in Cambridge, England, to understand the genetic organization and control of proteins with antibody activity. In the course of this work, they fused some mouse tumor cells with other mouse cells that make antibodies. The result was a malignant cell that can proliferate indefinitely under laboratory conditions but that also makes antibody. Each cell and all its descendants (clones) make identical antibody that binds to the same target, usually just a portion of a molecule. The cells are called hybridomas and their product is called a monoclonal antibody (SN: 12/23&30/78, p. 444).

Spherical antibody-producing cell fuses with flatter cancerous cell to give a source of monoclonal antibodies.



Schering Plough Corp.

More than 20 clinical tests using monoclonal antibodies have now been approved by the Food and Drug Administration, and many are already on the market. Some of these tests are versions of earlier diagnostic methods based on conventional antisera, the mixtures of antibodies an animal produces when injected with a foreign substance.

"It is easier for us and for the FDA to evaluate in a clinical situation markers and [targets] already well understood," says Cole Owen of Hybritech, Inc., in La Jolla, Calif., one of the companies specializing in monoclonal antibodies. In some cases the monoclonal antibody tests, compared with conventional tests, offer greater sensitivity, specificity and speed and can avoid the use of costly instruments or radioactive isotopes.

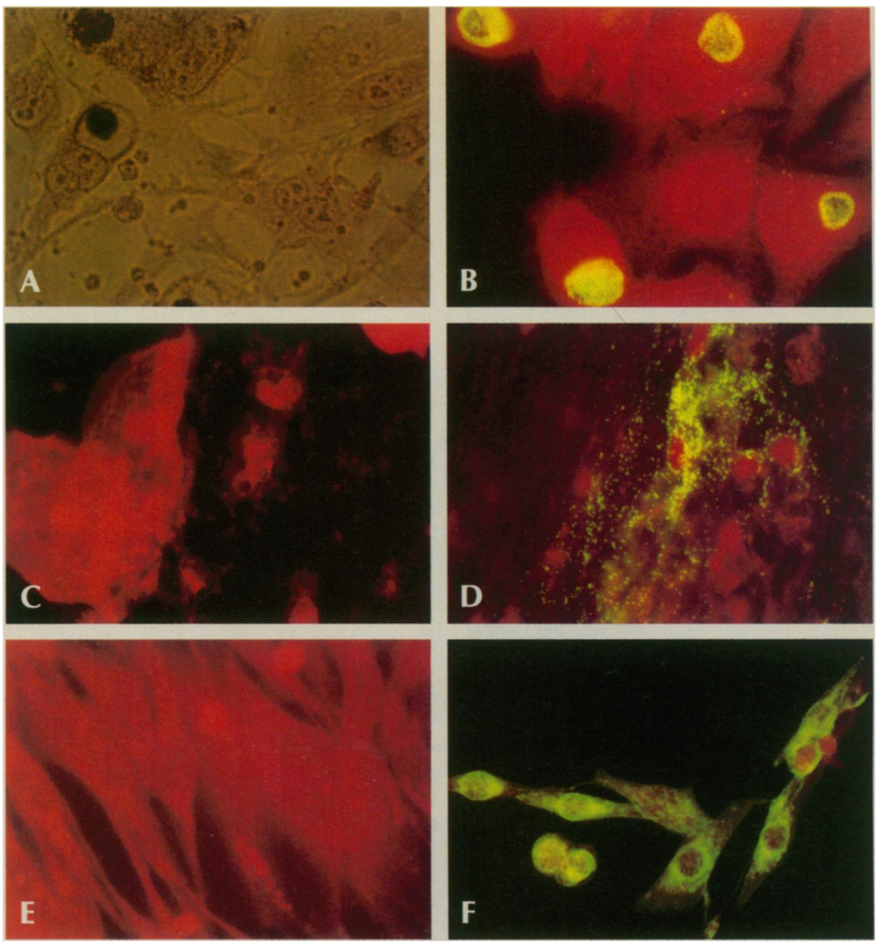
An example of greater sensitivity is the several pregnancy tests using monoclonal antibodies. These tests are being sold to physicians and hospitals. Like the tests that depend on conventional mixtures of antibodies, the new tests detect increased levels of the hormone human chorionic gonadotropin (HCG). Monoclonal Antibodies, Inc., another specialty company, in Palo Alto, Calif., claims its pregnancy test is 3 to 20 times more sensitive than conventional urine tests. Therefore it can detect pregnancy earlier—10 days after conception and before the first missed menstrual period.

The test has three simple steps that, with incubation periods, take about an hour. The concentration of hormone is revealed by a visible color change from clear to blue. The company says the test provides, without requiring radioactivity or expensive laboratory instruments, almost as great sensitivity and accuracy as the radioimmunoassays done in hospital laboratories only in special circumstances.

A monoclonal antibody test that measures levels of a drug in a patient's blood demonstrates how a method has been made more specific. Theophylline is used to treat lung problems and has a narrow range of effective concentration. Because each patient breaks down the drug and excretes it at a slightly different rate, physicians need to measure theophylline concentration in the blood to adjust the dosage. A problem with previous methods of measuring theophylline is that they cannot distinguish between that drug and caffeine, which is very similar and is found in most diets. But the monoclonal antibody test can measure as little as 1 microgram of theophylline and is unaffected by the presence of as much as 250 micrograms of caffeine, according to Beckman Instruments, Inc. of Fullerton, Calif., which markets the assay.

Another advantage that can be achieved by monoclonal antibody tests is a diagnosis that misses fewer cases. Research by scientists at Albert Einstein College of Medicine and Harvard Medical School had demonstrated that some patients with active or chronic hepatitis, and Australian aborigines in an isolated group with a high exposure to the hepatitis B virus, were not detected by conventional antibody tests. These studies suggested there are virus-associated substances in human blood that can be detected by monoclonal antibody but not by the conventional tests. The monoclonal antibody assay, subsequently developed by Centocor, Inc. of Malvern, Pa., is expected to have a large market among blood banks, which screen samples for hepatitis B virus before using them for transfusions.

In addition, the time savings that can arise from monoclonal antibody tests are critical in treating some serious infectious diseases like meningitis. Becton Dickinson and Co. of Paramus, N.J., is introducing a diagnostic test for *Neisseria meningitidis* group B that can detect the bacterium in body specimens of cerebrospinal fluid, blood or urine without the necessity of having the bacteria grown in laboratory culture. In the previous methods specimens had to be tended under special conditions for up to two days before the bac-



Genetic Systems Corp./Science

Fluorescence reveals antibodies used in detecting infections. Chlamydia can be identified by iodine stain (A) or monoclonal antibodies and fluorescence (B) in cells infected in the laboratory. Chlamydia is present in cervical smear of one patient (D) but not of another (C), confirming results of the conventional culture test. Monoclonal antibody to herpesvirus 1 distinguishes between cells infected with that virus (F) and with herpesvirus 2 (E).

teria would be present in high enough concentration for detection. In contrast, the new test gives results in 15 minutes.

Although there may be large earnings for companies in producing improved versions of widely used tests, the most exciting prospects for monoclonal antibody applications are the forays into the frontiers of medical practice. There are already on the market tests for microorganisms for which previously there were no practical and reliable diagnostic methods. There are also new tests to determine the body's immune system status and to detect cancers.

Chlamydia is an example of a widespread disease for which there has been no simple and inexpensive diagnostic test. It is considered the most common sexually transmissible disease (SN: 11/21/81, p. 333), with about 10 million cases occurring annually in the United States. Chlamydia can be effectively treated with antibiotic, but if untreated the bacterial infection can cause such serious complications as pelvic inflammatory disease, which can result in infertility. In a newborn of an infected mother, chlamydia can cause eye and respiratory infections.

Genetic Systems Corp. of Seattle, Wash., and Syntex Corp. of Palo Alto, Calif., have developed a test using monoclonal antibodies that identifies more than a quarter more positive specimens than conventional culture tests and that takes two days, instead of six days, to perform. Later this year they plan to introduce an even faster test, performed directly on urethral or cervical smears, which takes only 20 minutes.

Perhaps the most alluring possibilities for monoclonal antibody tests are in diagnosing and monitoring cancers. One test already on the market detects prostate cancer, which had been difficult to diagnose. Both Abbott Laboratories in Chicago and Hybritech have developed monoclonal antibodies to prostatic acid phosphatase, usually called PAP. This enzyme is present at high levels in men with the disease.

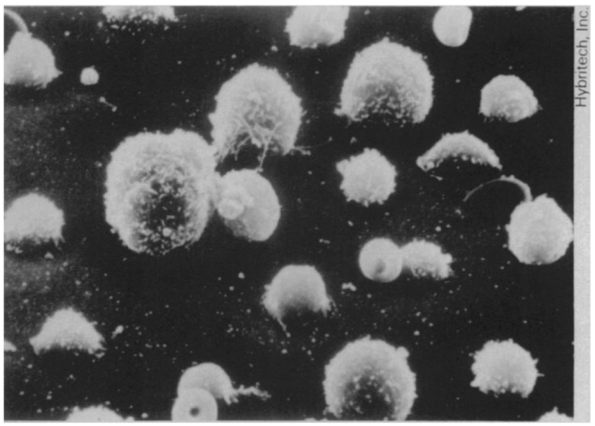
Monoclonal antibodies are also being used to assess patients' immune systems. Both Hybritech and Ortho Diagnostic of Raritan, N.J., have produced monoclonal antibodies that bind to specific types of white blood cells and allow them to be counted. "These can be used to evaluate disease states including cancer," says James A. Murray of Johnson and Johnson Co., the parent company of Ortho. They may also be important in diagnosing immune system diseases, such as leukemia and acquired immune deficiency syndrome (AIDS).

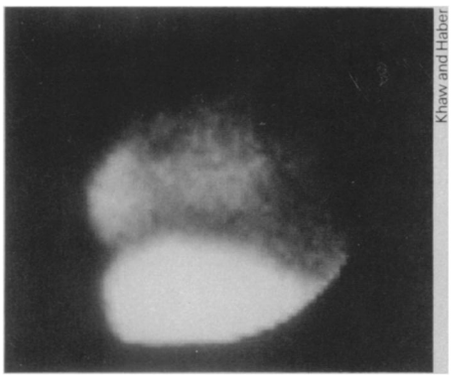
Individual monoclonal antibodies distinguish between different types of white blood cells. The Ortho product works with a special automated instrument to count one type of white blood cell, called a T cell. The Hybritech product requires only a microscope. The companies are developing panels of antibodies with which they expect to be able to identify and count more types of white blood cells than are currently distinguishable.

Cole Owen of Hybritech describes the research behind the new technology: "We screen an incredible number of clones to get the monoclonal antibodies we need. We go through 10,000 cell lines per week looking for an antibody we want. We do as many as 100,000 screens to find the antibody required for a particular application. Sometimes it's quick, but sometimes it takes years."

Choosing antibodies for commercial procedures is more difficult than selecting them for laboratory research, Owen says. He points out that there are more parameters to be considered in developing a successful commercial test, such as stability of the components, the time the assay takes and the number and complexity of steps included.

Four small biotechnology companies, each specializing in the production of monoclonal antibodies, are leading in the development of diagnostic products. Hybritech Inc., founded in 1978, has the greatest number of test kits approved by the FDA. They include monoclonal antibodies to detect and measure ferritin (as an indicator of anemia), HCG for pregnancy testing, human growth hormone for pituitary insufficiency, the protein IgE to assess allergic responses, prolactin for fertility studies and detection of pituitary tumors and thyroid stimulating hormone to measure thyroid function.





Top: Antibody-producing and cancerous cells fuse to create hybridomas. Below: Radioactive tag attached to an antibody reveals tissue damage in dog after an induced heart attack.

Hybritech has been using a method of "tandem assays," which it has patented. The scientists select a pair of monoclonal antibodies to bind to two regions of the target molecule, like a sandwich, Owen says. They also plan to market up to three versions of their tests, matching one pair of antibodies with different detection methods. The pregnancy test, for example, comes in two forms where an enzyme label on the antibody gives a color change that can be detected visually or with a spectrophotometer, and in one form where a radioisotope label allows the antibody to be detected by measuring radioactivity.

Diagnostic tests for sexually transmitted diseases are the first goal of Genetic Systems, which was started in 1980. It is currently marketing the test for chlamydia and has two more tests, for herpes and cytomegalovirus, now in clinical trials. Researchers at Genetic Systems have also developed monoclonal antibodies to such infectious diseases as Legionnaire's disease and walking (mycoplasma) pneumonia. These are among the most difficult respiratory ailments to diagnose. In Legionnaire's disease the course of illness is rapid and often fatal. So it is important to reduce the time required to perform the diagnostic test, which currently takes one to two weeks.

Recently, Genetic Systems formed a partnership with Syntex, the parent corporation of Syva, to work out monoclonal antibodies to diagnose and treat cancer. According to Monica S. Krieger of Genetic Systems, the joint venture, called Oncogēn, will initially focus on leukemia, breast, prostate, colon and lung cancers.

Genetic Systems is also developing automated procedures using monoclonal antibodies to determine cell and tissue types (called HLA typing) for use in matching tissues for transplants and transfusions. They already have monoclonal antibodies to two blood cell components that are part of the HLA typing.

Centocor, Inc., a biotechnology company begun in 1979, is marketing the monoclonal antibody test for hepatitis B and another for the detection of rabies. Centocor scientists are currently completing clinical trials of a monoclonal antibody that binds to a substance found in the blood of patients with such gastrointestinal malignancies as pancreatic and colorectal cancers. It not only can detect cancer but it also appears useful in predicting the recurrence of colorectal cancer in patients after surgery. The company also has developed a monoclonal antibody, not yet released for sale, that is expected to be useful in diagnosing ovarian cancer. "We hope to have a whole panel of cancer markers," says Ellen M. Lucas of Centocor.

Finally, Monoclonal Antibodies, Inc., started in 1979, has a pregnancy test on the market and is planning to introduce this year tests that detect when ovulation occurs. The company expects these tests to be useful both in treating human infertility problems and in animal breeding. Monoclonal Antibodies is also doing research on differentiating among types of cancer.

In addition to their own projects, these specialty companies and several even younger ones are signing agreements with the large pharmaceutical manufacturers for joint programs. The large companies are also doing their own work in this area, sometimes devoting entire research facilities to monoclonal antibody development. Becton Dickinson, for example, plans to put on the market this year a test kit to detect thyroid disorders. It uses a monoclonal antibody that binds to the hormone thyroxine.

Will monoclonal antibodies in time phase out the conventional antisera now used in many diagnostic tests? Some companies envision using monoclonal antibodies in all new products, while others are more conservative. Becton Dickinson spokesman Michael Flynn says, "Our strategy is not to force monoclonals on the market where they are not needed. They are more expensive and difficult to manufacture [than are conventional antisera]." Peter Schwartz of Becton Dickinson adds, "For some tests monoclonal antibodies are inappropriate because they are too specific."

Researchers at Genetic Systems, however, pooled several monoclonal antibodies into a defined mixture when they found a single antibody too specific to identify the entire spectrum of bacteria causing gonorrhea. They report that a mixture of three antibodies successfully identified 99.6 percent of 719 samples taken from patients with the disease. And the mixture did not bind to any closely related bacteria. "Thus in the case of gonorrhea, the construction of an antibody mixture proved to be a satisfactory method to overcome the limited specificity observed with individual antibodies," Robert Nowinski and colleagues at Genetic Systems reported in the February 11, 1983, SCIENCE.

Still, there are reservations about the extent of usefulness of the monoclonal antibody technique. Barry S. Cohen of Abbott says, "We haven't found it has been the answer in every case. I think a lot has been made that it's the be-all and the end-all. It's just one piece of the puzzle."

Related to diagnostics, but more difficult to develop, are monoclonal antibodies' use as medical imaging agents. Imaging is the localization of a disease-related substance in the human body. It can be considered an intermediate between diagnostics and therapeutics. As in diagnostics, the antibody seeks out and binds to a target substance, for instance a microorganism, injured tissue or cancer cell. An attached tag, most likely a radioactive material, allows clinicians to locate the antibody. Although in imaging the antibody is not intended to destroy its target, some of the problems of therapeutics are also met in developing imaging agents. The antibody and its tag enter the patient's body and therefore must be demonstrated to be safe.

No imaging agents using monoclonal antibodies have reached the market, but some are in clinical tests. Ban An Khaw and Edgar Haber of Massachusetts General Hospital, in work in conjunction with Centocor, are using monoclonal antibodies to pinpoint the size and position of damage done by a heart attack. Unlike thallium, the agent currently used for heart disease imaging, monoclonal antibodies distinguish between heart tissue that has been destroyed and areas just lacking blood flow. Other monoclonal antibodies are being used to localize tumors that produce characteristic proteins.

In the work on heart attack assessment, Khaw and Haber made monoclonal antibodies to a contraction protein in the heart. In healthy heart cells this protein is not exposed to the outside of the cell, and thus to antibody circulating in the blood. But death of the heart cell reveals the protein. A monoclonal antibody to the protein, called cardiac myosin, binds to heart muscle in the region damaged by a heart attack. A variety of radioactive tags are being examined for use in this imaging.

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Khaw and Haber now use technetium-99m to give a signal detectable by standard scanners. Their initial experiments were done on dogs, but recent clinical studies show that detection and visualization of human heart attack damage is feasible.

Cancer is the target of most of the imaging projects. Hybritech is working on monoclonal antibodies specific for each of three types of solid tumor — breast, lung and liver. "The use of a radioactive label to identify a tumor will help the surgeon to define where the tumor is and the extent of surgery necessary," says Owen. "It will also confirm that the tumor is not anywhere else—that is, whether it is localized or metastasized."

In addition to the work Centocor is supporting on heart imaging, the Pennsylvania company also is attempting to image colorectal cancer using two monoclonal antibodies. "Those studies have been very, very promising up to this point," says Stewart Rosenberg of Centocor. "Tumor imaging has been demonstrated in terms of feasibility." He says that Centocor is currently looking at monoclonal antibodies that bind to other gastrointestinal, breast and ovarian tumors.

"We now stand at the threshold of a new era of highly selective antibody imaging," say Khaw and Haber. "Antibodies to 'tumor-specific' or 'tumor-associated' antigens can now be produced in large quantities by monoclonal antibody technology. As labeling methods undergo still further improvement, new radionuclides become available, and more tumor- or organ-specific antibodies are generated, these methods will play increasingly important roles in diagnosis and therapy."

It may be a short step from imaging with monoclonal antibodies to using them in therapy. If an antibody will seek out a tumor and bind to it, that same antibody may reduce the tumor size. However, if antibodies alone will not correct a medical condition, scientists predict that chemicals attached to the antibodies will make them better able to attack microorganisms or malignancies.

Experiments are already under way testing monoclonal antibodies as therapy in a variety of cancers. One problem anticipated with using monoclonal antibodies produced in rodents is that a patient's immune system will reject them as foreign substances. To get around this problem, techniques are being developed to produce monoclonal antibodies starting with human blood cells. Genetic Systems has filed a patent application for one such technology; Damon Corp. of Needham Heights, Mass., recently announced another (SN: 4/2/83, p. 215).

Scientists at Genetic Systems have produced human monoclonal antibodies against three bacterial infections that are serious problems for patients with depressed immune system function, such as burn victims and cancer patients undergoing chemotherapy. The company is beginning animal studies to determine the therapeutic effectiveness of these human antibodies against the bacteria pseudomonas, *Escherichia coli* and klebsiella. These studies are considered to be the start of a long-term project "because the process of getting FDA approval for a therapeutic agent is such a lengthy one," says Krieger of Genetic Systems.

Another therapeutic use of monoclonal antibodies is in suppressing in a very specific way the immune response of a patient receiving an organ or tissue transplant. With antibodies to specific white blood cells the patient may be unable to reject the transplant, but still have the immune system functioning to fight infections. Monoclonal antibodies against T cells also have been used successfully in a bone marrow transplant to prevent graft-versus-host disease (SN: 10/16/82, p. 245).

Finally, there is promise that monoclonal antibodies will specifically counter human cancer. Clinical trials are underway for a variety of cancers including leukemias and colorectal carcinoma.

In a sense, it is ironic that this antibody production technique, which starts with a cancer cell, is supplying new diagnostic, imaging and therapeutic tools to fight malignancy diseases. The technique thus tames a cancer in the laboratory and employs its prolific growth to supply previously unobtainable amounts of the natural agents for fighting disease. □

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